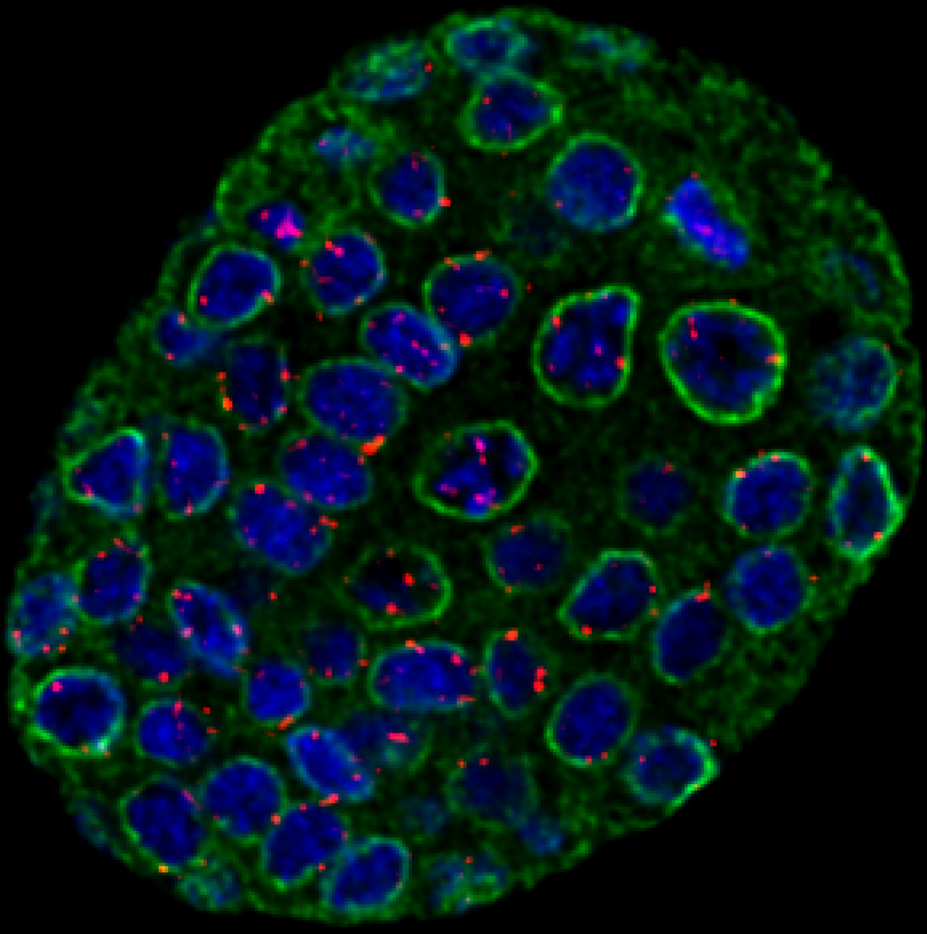
Abstracts of papers presented
at the 2015 meeting on

TELOMERES & TELOMERASE

April 28–May 2, 2015



Cold Spring Harbor Laboratory
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Abstracts of papers presented
at the 2015 meeting on

TELOMERES & TELOMERASE

April 28–May 2, 2015

Arranged by

Julia Cooper, *National Cancer Institute*
Titia de Lange, *The Rockefeller University*
Roger Reddel, *Children's Medical Research Institute, Australia*

This meeting was funded in part by the **National Cancer Institute** and the **National Institute on Aging,** branches of the **National Institutes of Health.**

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Front Cover: Shown is an early stage DAPI-stained C elegans embryo expressing laminGFP (green) in which telomeres are identified by FISH (red). For details see Ferreira et al., JCB 2013.

Back Cover: Live cell imaging of RPE1-hTERT cells experiencing telomere dysfunction. Cells expressing GFP, mCherryH2B, and TurquoiseRPA70 were imaged using a spinning disk microscope. Individual images were stitched together to produce a wide field image at high resolution.
Image provided by John Maciejowski, de Lange lab.

TELOMERES & TELOMERASE

Tuesday, April 28 – Saturday, May 2, 2015

Tuesday	7:30 pm	**1** Telomerase, Telomeres and Cancer
Wednesday	9:00 am	**2** Repair of Dysfunctional Telomeres
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	*Wine and Cheese Party**
Wednesday	7:30 pm	**4** Recombination at Telomeres and the ALT Pathway I
Thursday	9:00 am	**5** Recombination at Telomeres and the ALT Pathway II
Thursday	10:00 am	**6** Chromatin at chromosome ends
Thursday	2:00 pm	**7** Poster Session II
Thursday	7:30 pm	**8** Telomere Replication
Friday	9:00 am	**9** Telomeres, Telomerase RNA and Human Disease
Friday	11:15 am	**10** Telomere Length Homeostasis and Telomerase Recruitment I
Friday	2:00 pm	**11** Telomere Length Homeostasis and Telomerase Recruitment II
Friday	6:00 pm 7:00 pm	Concert Banquet
Saturday	9:00 am	**12** The Structure of Telomeres and Telomerase

* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am

Lunch 11:30 am-1:30 pm

Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

TUESDAY, April 28—7:30 PM

SESSION 1 TELOMERASE, TELOMERES AND CANCER

Chairpersons: **S. Gasser,** Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
C. Greider, Johns Hopkins University, Baltimore, Maryland

WEDNESDAY, April 29—9:00 AM

SESSION 2 REPAIR OF DYSFUNCTIONAL TELOMERES

Chairpersons: **E. Lazzerini Denchi,** The Scripps Research Institute, La Jolla, California
V. Lundblad, Salk Institute for Biological Studies, La Jolla, California

WEDNESDAY, April 29—2:00 PM

SESSION 3 POSTER SESSION I

WEDNESDAY, April 29—4:30 PM

Wine and Cheese Party

WEDNESDAY, April 29—7:30 PM

SESSION 4 RECOMBINATION AT TELOMERES AND THE ALT PATHWAY I

Chairpersons: **T. Bryan,** Children's Medical Research Institute, Westmead, Australia
J. Shay, UT Southwestern Medical Center, Dallas, Texas

SESSION 5 RECOMBINATION AT TELOMERES AND THE ALT PATHWAY II

Chairpersons: **V. Zakian,** Princeton University, New Jersey
L. Rudolph, Leibniz Institute for Age Research, Jena, Germany

SESSION 6 CHROMATIN AT CHROMOSOME ENDS

Chairpersons: **V. Zakian,** Princeton University, New Jersey
L. Rudolph, Leibniz Institute for Age Research, Jena, Germany

SESSION 7 POSTER SESSION II

THURSDAY, April 30—7:30 PM

SESSION 8 TELOMERE REPLICATION

Chairpersons: **J. Lingner,** EPFL, Lausanne, Switzerland
P. Baumann, HHMI, Stowers Institute for Medical Research, Kansas City, Missouri

SESSION 9 TELOMERES, TELOMERASE RNA AND HUMAN DISEASE

Chairpersons: **S. Boulton,** London Research Institute, South Mimms, United Kingdom
A. Bertuch, Baylor College of Medicine, Houston, Texas

Budding yeast telomerase contains a single Tlc1 molecule
Emmanuel Bajon, Nancy Laterreur, Raymund J. Wellinger.
Presenter affiliation: Université de Sherbrooke, Sherbrooke, Canada. 197

Yeast telomerase RNP—Exceptional flexibility as well as new essential structural features of its long noncoding RNA
Kevin J. Lebo, Melissa A. Mefford, Rachel O. Niederer, David C. Zappulla.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland. 198

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SESSION 10 TELOMERE LENGTH HOMEOSTASIS AND TELOMERASE RECRUITMENT I

Chairpersons: **S. Boulton,** London Research Institute, South Mimms, United Kingdom
A. Bertuch, Baylor College of Medicine, Houston, Texas

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Adrian S. Tong, Josh L. Stern, Anthony J. Cesare, Xu-Dong Zhu, Tracy M. Bryan.
Presenter affiliation: Children's Medical Research Institute, NSW, Australia. 199

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Presenter affiliation: Instituto Gulbenkian de Ciência, Lisbon, Portugal. 200

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Stella S. Lee, Bohrson Craig, Sarah J. Wheelan, Carol W. Greider.
Presenter affiliation: Johns Hopkins University School of Medicine, Baltimore, Maryland. 201

SESSION 11 TELOMERE LENGTH HOMEOSTASIS AND TELOMERASE RECRUITMENT II

Chairpersons: **D. Shore,** University of Geneva, Switzerland
M.T. Teixeira, CNRS, Paris, France

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Pingping Jia, Olga Shiva, Chengtao Her, Weihang Chai.
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Amanda Frank, Duy Tran, Roy Qu, Lifeng Xu.
Presenter affiliation: University of California, Davis, Davis, California. 209

Analysis of specific roles of TIN2S vs TIN2L in telomere protection and regulation
Beth A. Cimini, Elizabeth H. Blackburn.
Presenter affiliation: University of California-San Francisco, San Francisco, California. 210

Differentiating the roles of the short and long isoforms of TIN2 at telomeres
Nya D. Nelson, Ivana Mihalek, Alison Bertuch.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 211

FRIDAY, May 1—6:00 PM

CONCERT
Grace Auditorium

Julia Bullock, soprano

Winner of the 2014 Naumburg International Vocal Competition, soprano Julia Bullock has been hailed as an "impressive, fast-rising soprano... poised for a significant career" (The New York Times). Her busy 2014-2015 season begins with a performance of Delage's Quatres poèmes hindous with the Sphinx Symphony Orchestra and a recital at Napa's Festival del Sole. She performs recitals and educational outreach programs at the University of Florida Performing Arts, Alys Stephens Performing Arts Center, the Levine School of Music, and Music for Youth, as well as recitals at the Isabella Stewart Gardner Museum, the National Museum of Women in the Arts, San Francisco Performances, Rockefeller University, the Michael Schimmel Center for the Arts at Pace University, and Carnegie Hall Neighborhood Concerts. She is also featured in the New York Festival of Song's Harlem Renaissance program on tour and at Merkin Concert Hall, as well as in the Mondavi Center's Rising Stars of Opera. She reprises the title role in Henry Purcell's The Indian Queen, directed by Peter Sellars at the Perm Opera House, and at English National Opera later this season. She was acclaimed for her performance of the role last season in Perm and at the Teatro Real in Madrid; a DVD of the Madrid production will be available this season.

FRIDAY, May 1

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SATURDAY, May 2—9:00 AM

SESSION 12 THE STRUCTURE OF TELOMERES AND TELOMERASE

Chairpersons: **T. Cech,** HHMI, University of Colorado, Boulder
D. Rhodes, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

TUMORIGENIC POTENTIAL OF SHELTERIN INACTIVATION IN VIVO

Alexandra Pinzaru[2], Nidhi Nair[1], Angela Beal[1], Agnel Sfeir[2], Eros Lazzerini Denchi[1]

[1]The Scripps Research Institute, Molecular and Experimental Medicine, La Jolla, CA, [2]NYU School of Medicine, Department of Cell Biology, New York, NY

Recent studies have linked mutations in POT1 to the development of different types of human cancers including chronic lymphocytic leukemia (CLL). These mutations cluster in the OB (oligonucleotide /oligosaccharide binding) folds domain of POT1 required for its binding to telomeres. Here, we found that cancer-associated POT1 mutations cause phenotypes previously associated with POT1 inactivation such as ATR-dependent DNA damage activation at chromosome ends and replication stress-associated phenotypes. In order to understand whether POT1 loss of function is sufficient to promote tumorigenesis we generated a mouse model in which POT1a is depleted in common lymphoid progenitor cells (CLPs). Our results show that POT1a inactivation in p53-proficient mice leads to a severe loss of mature B and T cells leading to immunodeficiency. In contrast, concomitant depletion of POT1a and p53 results in the development of aggressive thymic lymphomas that infiltrate several surrounding tissues. Tumor cells derived from POT1a and p53 deficient mice show high levels of genomic instability. Collectively, our data show that loss of end-protection independent of variation in telomere length can promote tumor development.

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A NOVEL MUTATION IN THE POT1 GENE EXPLAINS P53-NEGATIVE LI-FRAUMENI-LIKE FAMILIES WITH CARDIAC ANGIOSARCOMA

Paula Martinez[1], Oriol Calvete[2], Pablo Garcia-Pavia [3], Carlos Benitez-Buelga[2], Beatriz Paumard-Hernandez[2], Victoria Fernandez[2], Miguel Urioste[4], Javier Benitez[2], Maria A. Blasco[1]

[1]CNIO, Molecular Oncology, Madrid, Spain, [2]CNIO, Human Genetics, Madrid, Spain, [3]Hospital Puerta de Hierro, Department of Cardiology, Madrid, Spain, [4] CIBERER, , Madrid, Spain

Cardiac angiosarcoma (CAS) is a rare malignant tumor that represents less than 10% of cardiac malignancies and whose genetic bases are unknown. By whole exome sequencing of a p53-negative Li-Fraumeni-like (LFL) family including CAS cases, we identified a missense variant in POT1 (p.R117C) as responsible of CAS. The study of two new LFL families with CAS showed the same alteration. We extended the study to p53-negative LFL families with no CAS and the same mutation was found in a breast angiosarcoma family. The mutation was not described in any database or found in 1520 Spanish controls. In silico structural analysis showed how the mutation disrupts POT1 structure. We performed several functional and in vitro studies that demonstrate that carriers of the mutation show reduced telomere bound POT1 levels, abnormally long telomeres, and increased telomere fragility. These results may have implications for people at risk in relation to prevention and treatment of this tumor.

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NEW APPROACHES TO TARGETING TELOMERASE

Jerry W Shay, Ilgen Mender, Andrew Ludlow, Wanil Kim, Woodring Wright

UT Southwestern, Cell Biology, Dallas, TX

Nucleoside-based telomerase substrates as potential therapeutic agents
We reasoned that a guanine-based nucleoside analogue (MW <500 Da) of an already approved drug, 6-thioguanine, could be developed that was more toxic for cancer cells expressing telomerase compared to normal telomerase silent cells. We synthesized 6-thio-2'-deoxyguanosine and demonstrated that it is incorporated more efficiently in telomerase expressing cells compared to normal telomerase silent cells. This approach has several advantages: elimination of the "lag" period of typical telomerase inhibitors, rapid induction of telomere dysfunction-induced DNA damage foci, less toxicity to telomerase silent or quiescent stem cells.

Regulation and manipulation of hTERT splicing
The human TERT gene makes several alternative spliced forms with full length catalytically active telomerase being a minor component. Thus developing methods to manipulate TERT splicing that result in less full length TERT could lead to new cancer therapeutic opportunities. We have initiated a series of screens to determine RNA binding proteins and splicing factors that regulate hTERT splicing. From an initial candidate list we have focused on a subset of genes that reduce full length TERT by shifting to more abundant hTERT splicing variants.

Telomere position effect over long distances (TPE-OLD)
We previously reported that genes at long distances from telomeres may be regulated by a modification of the classic telomere position effect (TPE) mechanism. We discovered using 3D co-FISH and a modification of Hi-C (chromosome capture followed by high-throughput sequencing), that the ISG15 gene was regulated by telomere length but genes closer to the telomere were not regulated by classic TPE. We called this phenomenon telomere position effect over long distances (or TPE-OLD) to distinguish it from classic TPE. We now report that the human TERT locus is associated with looped chromatin structures in cells with long telomeres but is reduced in cells with shorter telomeres. Our working hypothesis is that the expression of active telomerase requires permanent or reversible disengagement of telomere looping to make the hTERT locus permissive for active full length transcription and translation. This change in the genome structure at the hTERT locus might provide novel insights into how the tight regulation of human telomerase in somatic cells is reduced during aging, potentially leading to a permissive environment for telomerase activation as part of tumor development. Importantly, this indicates that genes can change in gene expression prior to a DNA damage response from a too short telomere.

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GENETICALLY SHORT TELOMERES DECREASE CANCER RISK AMONG 95 568 INDIVIDUALS FROM THE GENERAL POPULATION

Line Rode[1], Børge G Nordestgaard[1,2,3], Stig E Bojesen[1,2,3]

[1]The Copenhagen General Population Study, Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Copenhagen, Denmark, [2]The Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Copenhagen, Denmark, [3]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Background: Short telomeres have been associated with increased risk of cancer, but the combined evidence from numerous and conflicting epidemiological studies is unclear. On the cellular level, cancer cells overcome senescence and divide indefinitely, often through reactivation or up-regulation of telomerase, suggesting that short telomeres would protect against cancer. With the recent identification of single nucleotide polymorphisms (SNPs) in telomere maintenance genes associated with telomere length, it is possible to perform genetic and therefore largely unconfounded analyses of the association between telomere length and cancer risk. We tested the hypothesis that genetically predicted short telomeres are associated with increased risk of overall and type-specific cancer.

Methods: We studied 95 568 individuals from the Danish general population until December 2011. In the national Danish Cancer Registry, 10 895 individuals developed cancer. All 95 568 individuals had the telomere length-associated genotypes rs7726159 (*TERT*), rs1317082 (*TERC*), and rs2487999 (*OBFC1*) determined, and 65 176 had telomere length measured by Q-PCR in DNA from peripheral blood. The number of telomere shortening alleles from each of the three SNPs were combined to an allele sum, ranging from 0 to 6. Endpoints were any cancer, as well as 25 specific cancer types. We conducted epidemiological logistic and cox regression analyses and instrumental variable analyses using the combined allele sum as genetic instrument.

Findings: Telomeres shortened 17 and 67 base-pairs per year increase of age and per allele (both p-values $<10^{-300}$), but not with risk of cancer after multifactorial adjustment; the hazard ratio per 200-base pair telomere decrease was 0•99 (0•99-1•00). The per-allele odds ratio for cancer was 0•95 (95%CI 0•93-0•97) for the allele sum, robust across the three genotypes. A genetically predicted 200-base pair shorter telomere length yielded an odds ratio of 0•86 (0•81-0•91) for cancer, contrasting the epidemiological findings. For specific cancer types, corresponding odds ratios of melanoma and lung cancer were 0•60 (0•50-0•72) and 0•68 (0•56-0•83), both significant after taking the 25 cancer/telomere length comparisons into consideration.

Interpretation: Genetically predicted - and thus unconfounded - short telomeres are associated with decreased cancer risk overall, and in particular melanoma and lung cancer, implying that long rather than short telomeres could be a cause of increased risk of cancer.

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MONOALLELIC VS. BIALLELIC ACTIVATION OF TERT IN CANCER

Franklin W Huang[1,2,3], Gregory V Kryukov[2,3,4], Mikael Rinne[1,2,3], Levi A Garraway[1,2,3]

[1]Dana-Farber Cancer Institute, Medical Oncology, Boston, MA, [2]The Broad Institute of Harvard and MIT, Cancer Program, Cambridge, MA, [3]Harvard Medical School, Medicine, Boston, MA, [4]Brigham and Women's Hospital, Genetics, Boston, MA

We previously reported the presence of highly recurrent mutations in the promoter of the telomerase gene in melanoma and in cancer cell lines from other tumor types. These mutations have subsequently been found to occur at high frequencies in a number of cancers. We hypothesized that these mutations of the *TERT* promoter should drive expression of TERT in an allele-specific manner. We analyzed whole genome sequencing data and RNA-seq data from cell lines from the Cancer Cell Line Encyclopedia to assess the promoter of *TERT*. We were able to differentiate activation of one allele of TERT from both alleles of TERT. We show that cell lines harboring *TERT* promoter mutations exclusively demonstrate allele-specific expression. These results suggest that TERT promoter mutations drive expression of TERT through a cis-regulatory event and suggest that several mechanisms may exist for telomerase reactivation in cancer.

TRANSCRIPTIONAL REGULATION OF ENDOGENOUS hTERT AND REPRESSION UPON DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

Kunitoshi Chiba, Tina Wagner, Joshua Johnson , Dirk Hockemeyer

University of California, Berkeley, Dept. of Molecular and Cell Biology, Berkeley, CA

The transcriptional silencing of the protein component of telomerase (TERT) causes telomere shortening in the majority of human somatic cells. This progressive terminal sequence loss has evolved to function as a tumor suppressor mechanism by limiting the number of divisions a cell can undergo. Most tumor cells override this mechanism and obtain immortality through the aberrant reactivation of TERT expression. Similarly, human stem cells are required to maintain TERT expression to counteract telomere shortening and allow unlimited replicative capacity. Here, we investigate the physiological regulation of TERT in human cells. We employed CAS9 mediated genome editing in human embryonic stem cells (hESCs) to generate an array of genetically precise homozygous deletion mutants within the endogenous TERT promoter. Using this approach we comprehensively determined cis-regulatory elements within the 6kb genomic region upstream of TERT's transcriptional start site in pluripotent stem cells. Furthermore, we differentiated our genetically engineered TERT promoter deletion hESC lines into fibroblasts and neurons to uncover the cis-elements that are required for the repression of TERT during differentiation. We present the insights gained from our analysis in relation to mutations within the TERT promoter that have recently been identified by GWAS analysis to be highly enriched in a subset of human tumor cells.

HOW *TERT* PROMOTER MUTATIONS DRIVE TELOMERASE EXPRESSION IN HEPATOCELLULAR CARCINOMA

Josh L Stern[1], Nick Papadopoulos[2], Bert Vogelstein[2], Thomas R Cech[1]

[1]Department of Chemistry and Biochemistry, BioFrontiers Institute, Howard Hughes Medical Institute, University of Colorado, Boulder, CO, [2]Ludwig Center, Howard Hughes Medical Institute, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center , Baltimore, MD

A non-coding, highly recurrent somatic mutation was recently discovered in the promoter of the gene for telomerase reverse transcriptase (TERT) in a wide range of cancers including melanoma, hepatocellular carcinoma, glioblastoma and urothelial cancers. This C>T transition, predominantly at 124 bp upstream of the TERT start codon, is the most common mutation yet observed in some cancers, and published data indicate that it is associated with higher TERT and telomerase levels (e.g., Borah et al. 2015). Using CRISPR-Cas9 to modify the genomic sequence of a tumor-derived cell line, we show that the mutation is directly responsible for an increase in TERT expression. Furthermore, we provide evidence for the following model. An epigenetically silenced TERT gene gains a mutation at position -124, which recruits a pioneer transcription factor, switches the status of the chromatin and recruits RNA polymerase II to activate TERT transcription.

S. Borah, L. Xi, A. J. Zaug, N. M. Powell, G. M. Dancik, S. B. Cohen, J. C. Costello, D. Theodorescu and T.R. Cech, Science in press (2015).

TRANSCRIPTIONAL CONTROL OF TELOMERASE IN STEM CELLS IN VIVO

Matthew Pech[1,2], Alina Garbuzov[2], Meena Sukhwani[4], Kyle Orwig[4], Steven E Artandi[1,2,3]

[1]Stanford University, Cancer Biology, Stanford, CA, [2]Stanford University, Medicine, Stanford, CA, [3]Stanford University, Biochemistry, Stanford, CA, [4] University of Pittsburgh, Obstetrics, Gynecology and Reproductive Sciences, Pittsburgh, PA

One of the invariant features of human cancer is unlimited proliferation, a hallmark conferred by telomerase in 90% tumors. Somatic mutations in the telomerase reverse transcriptase (TERT) gene promoter are highly recurrent in human cancers. Telomerase is also critically important in human stem cells, as evidenced by mutations in telomerase, which contribute to degenerative diseases. Despite the importance of telomerase in tissue maintenance, the identity of telomerase-positive cells has remained elusive, owing to low levels of the core telomerase components. The ability to isolate TERT-positive cells in vivo would significantly advance our understanding of telomerase regulation, tissue function and carcinogenesis. To address these issues, we created knock-in transcriptional reporters of TERT expression by replacing the TERT open reading frame with the red fluorescent protein, TdTomato. Among mouse tissues, telomerase activity is most strongly expressed in testis, a tissue in which resident stem cells fuel the continuous generation of male gametes. In human sperm, telomere lengths are preserved with age, although how this is achieved, in contrast to the age-dependent telomere shortening seen in somatic tissues, remains unresolved. Using $TERT^{dTomato/+}$ knock-in reporter mice, we found that only a rare subset of cells in mouse testis expresses high levels of TERT. By double immunostaining, these TERTHigh cells were synonymous with undifferentiated spermatogonia, the primitive cell population in which male germline stem cells reside. By FACS of the germ cells in testis, $TERT^{High}$ cells and $TERT^{Low}$ cells represent discrete populations that were further studied using additional markers. The undifferentiated spermatogonia in the $TERT^{High}$ population were further fractionated into GFRalpha+ and GFRalpha- populations. Cells in the $TERT^{Low}$ population were nearly all cKit+, consistent with their identification as differentiated spermatogonia. Using RNAseq, we established a hierarchy among these populations according to which the $TERT^{High}$ GFRalpha1+ cells give rise to $TERT^{High}$ GFRalpha1- cells, which in turn yield $TERT^{Low}$ cKit+ cells. Surprisingly, in transplantation studies, $TERT^{High}$ GFRalpha1+ cells and $TERT^{High}$ GFRalpha1- cells possess comparable stem cell activity. These data suggest the existence of stem cell plasticity according to which cells in either primitive population retain stem cell potential. In contrast, $TERT^{Low}$ cKit+ cells fail to reconstitute spermatogenesis in transplantation experiments and therefore lack stem cell activity. These studies reveal marked transcriptional regulation of telomerase in vivo and show a strong concordance between stemness and telomerase levels in rare subsets of tissue stem cells in vivo. These findings indicate the existence of innate signaling pathways controlling TERT expression over a surprising dynamic range.

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CYREN: A CELL CYCLE REGULATOR OF TELOMERE FUSION

Nausica Arnoult, Marco Tognetti, Jan Karlseder

The Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, La Jolla, CA

TRF2 depletion causes telomere deprotection and allows the non-homologous end joining (NHEJ) machinery to generate chromosome fusions. These telomere fusions are predominantly chromosome-type fusions where both sister chromatids are fused, pointing at G1 as the cell cycle phase where fusions occur. Until now the mechanism that limits telomere fusions to G1 was not known. Extensive resection of double strand breaks by the homologous recombination machinery prevents the binding of RIF1 and the downstream NHEJ factors in S/G2. Such extensive resection is not observed at deprotected telomeres and is therefore unlikely to explain the inhibition of NHEJ in S/G2, thereby suggesting an active suppression of NHEJ in S phase and G2.
We discovered that the polypeptide CYREN (Cell CYcle REgulator of NHEJ) prevents telomere fusions during S/G2. Concomitant depletion of CYREN and TRF2 increased the frequency of chromatid-type fusions without affecting the rate of chromosome-type fusions. Ligase 4 depletion and the use of a DNA-PK inhibitor during S/G2 suppressed these chromatid-type fusions. We therefore concluded that CYREN prevents classical NHEJ at telomeres during S/G2.
To test whether CYREN is specific to telomeres or whether it also acts at intrachromosomal double strand breaks to suppress the dominance of NHEJ over homologous recombination in G2 where sister chromatids are present, we developed a fluorescent reporter system that allows us to follow and quantify break repair pathway choice specifically during S/G2.

MAD2L2 CONTROLS DNA REPAIR AT TELOMERES AND DNA DOUBLE-STRAND BREAKS BY INHIBITING 5' END-RESECTION

Vera Boersma*[1], Nathalie Moatti*[1], Sandra Segura-Bayona[1], Marieke H Peuscher[1], Jaco van der Torre[1], Brigitte A Wevers[1], Alexandre Orthwein[2], Daniel Durocher[2,3], Jacqueline J Jacobs[1]

[1]The Netherlands Cancer Institute, Division of Molecular Oncology, Amsterdam, Netherlands, [2]Mount Sinai Hospital, The Lunenfeld-Tanenbaum Research Institute, Toronto, Canada, [3]University of Toronto, Department of Molecular Genetics, Toronto, Canada

* authors contributed equally

Appropriate repair of DNA lesions and the inhibition of DNA repair activities at telomeres are critical to avoid genomic instability. By fuelling the generation of genetic alterations and by compromising cell viability, genomic instability is a driving force in cancer and aging. As the mechanisms underlying the control of DNA damage responses and repair activities are not completely understood, we performed functional genetic screens to identify genes and activities that play critical roles in genomic instability triggered by telomere deprotection. In these screens we identified MAD2L2 (also known as MAD2B or REV7) as a novel factor controlling DNA repair activities at mammalian telomeres. While MAD2L2 has a well-established role in translesion synthesis (TLS), we found that MAD2L2 accumulates at uncapped telomeres and promotes NHEJ-mediated fusion of deprotected chromosome ends and genomic instability. Interestingly, MAD2L2 depletion does not impair recognition of uncapped telomeres as damaged DNA but causes elongated 3' telomeric overhangs, implying that MAD2L2 inhibits 5' end-resection. End-resection strongly inhibits NHEJ while committing to homology-directed repair (HDR), and was recently shown to be under control of 53BP1 and its interaction partners RIF1 and PTIP. In line with MAD2L2 promoting NHEJ-mediated telomere fusion by inhibiting 5' end-resection, knockdown of the end-resection nucleases CTIP or EXOI partially restores telomere-driven genomic instability in MAD2L2-depleted cells. Control of DNA repair by MAD2L2 is not limited to telomeres. We found that MAD2L2 also accumulates and inhibits end-resection at irradiation-induced DNA double-strand breaks (DSBs) and promotes end-joining of DSBs in multiple settings, including during immunoglobulin class switch recombination (CSR). DNA damage response factor dependencies and epistasis analyses reveal MAD2L2 as a critical contributor to the control of DNA repair activity that promotes NHEJ by inhibiting 5' end-resection downstream of 53BP1. Furthermore, this activity of MAD2L2 appears to be independent of REV1 and REV3, which act with MAD2L2 in TLS.

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A ROLE FOR *SACCHAROMYCES CEREVISIAE* RIF1P IN THE REGULATION OF NON-HOMOLOGOUS END JOINING AND HOMOLOGY-DEPENDENT REPAIR PATHWAYS

Udo C Obodo, Ahmed Memon, Gabrielle Santiago, Lucy Cox, Katherine L Friedman

Vanderbilt University, Biological Sciences, Nashville, TN

Although identified as a telomere length regulatory factor in Saccharomyces cerevisiae, Rif1p (Rap1p-interacting factor 1) is implicated in the regulation of DNA repair. In mammalian cells, Rif1 inhibits 5' strand resection, limiting homology-dependent DNA repair (HDR) and favoring non-homologous end joining (NHEJ). Surprisingly, mammalian Rif1 does not influence telomere length regulation in somatic cells, suggesting functional divergence. However, Rif1p has recently been reported to stimulate resection in yeast, raising the possibility that DNA repair function(s) are conserved. Here we report that *S. cerevisiae* Rif1p regulates DSB repair through both NHEJ and HDR pathways.

We have utilized a haploid strain in which galactose-induced expression of the HO endonuclease causes a persistent DSB on chromosome V. Only cells in which repair is accompanied by mutation or loss of the HO site survive. Cells that retain the chromosome terminus following repair incur small (1-3 bp) insertions or deletions at the HO site as a result of NHEJ. In cells lacking *RIF1*, the rate at which such errors are produced increases three-fold. Sequencing of repair junctions in WT and *rif1*Δ cells reveals a change in the spectrum of mutational events, suggesting differential processing of the 3' overhang ends generated by HO endonuclease in WT and *rif1*Δ cells. Incompatible 3' overhangs generated in a plasmid-based DSB repair assay are likewise differentially processed in the absence of *RIF1*. Cells lacking the C-terminal domain of Rif1p required for interaction with Rap1p show WT levels of survival on galactose, suggesting that the function of Rif1p during NHEJ is independent of Rap1p. We are currently examining interactions between *RIF1* and genes required for end-processing during NHEJ to elucidate the mechanism of this effect.

In the absence of NHEJ (*yku80*Δ), cells that survive on galactose while retaining the chromosome terminus incur large deletions surrounding the HO cleavage site that are mediated through either microhomology-mediated end joining (MMEJ) and/or single-strand annealing (SSA). Deletion of *RIF1* results in a pronounced shift in both the location and sequence content of the microhomologies present at the repair junctions with increased utilization of 25-30 bp stretches of poly A/T tracts. We are currently utilizing plasmid-based DNA repair substrates to pinpoint the aspect of NHEJ-independent repair that is influenced by *RIF1*.

In addition to its roles at telomeres and in DNA repair, Rif1p regulates several aspects of DNA replication. Our findings indicate that Rif1p function may be more conserved than previously recognized, consistent with its increasing recognition as a master regulator of genome stability.

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END RESECTION OF SHORT TELOMERES PROMOTES STEM CELL AND TISSUE AGING BY MEDIATING 53BP1 DEPENDENT FORMATION OF CHROMOSOMAL FUSIONS

Omid Omrani, Satjavani Ravipati, Tobias Sperka, K. Lenhard Rudolph

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Telomere shortening limits the proliferative capacity of human cells and tissues by induction of DNA damage checkpoints. Experiments in yeast revealed the first experimental evidence that the induction of DNA damage checkpoints in response to telomere shortening is mediated by Exonuclease1 (Exo1) dependent end resection of critically shortened telomeres. Studies on telomerase deficient mice revealed that telomere shortening impairs the maintenance of adult tissue stem cells and organ homeostasis leading to premature aging and a shortened lifespan. Interestingly, Exo1 deletion rescued the activation of p53 dependent DNA damage checkpoints in stem and progenitor cells of aging mice with critically short telomeres resulting in prolonged tissue maintenance and an increased lifespan. This rescue was associated with impaired single stranded DNA formation and reduced RPA/ATR activation at laser induced DNA breaks in cells from Exo1 deficient mice compared to wildtype mice. In addition, Exo1 deletion impaired the formation of chromosomal fusion in tissues with critically short, dysfunctional telomeres. These data indicated that Exo1 dependent end resection of shortened telomeres contributes to both the activation of DNA damage checkpoint and to the formation of chromosomal fusions in response to telomere shortening. The relative contribution of these two effector pathways to stem cell dysfunction and tissue aging in the context of telomere shortening is unknown. Here, we addressed this question in aging late generation telomerase deficient mice with critically short telomeres (G3 mTerc-/-) by co-deleting the DNA repair factors 53BP1, H2AX or MDC1. The homozygous deletions of genes encoding for inhibitors of DNA end-resection (MDC1, H2AX) leads to increased activation of p53 in telomere dysfunctional stem and progenitor cell and an increased number of chromosomal fusions and chromosomal imbalances in tissues. The double mutant mice exhibit aggravated organ atrophy and a shortened lifespan. In contrast, the deletion of 53BP1 – also an inhibitor of end-resection, but simultaneously an important mediator DNA end tethering and ligation – extended the lifespan of G3 mTerc-/- mice. Double mutant mice exhibit a strong decrease in the formation of chromosomal fusions and DNA damage checkpoint activation despite enhanced end resection and formation of single stranded DNA in stem and progenitor cell compartments. Moreover, 53BP1 knockdown is sufficient to rescue the adverse effects of H2AX deletion on proliferation and self renewal of intestinal stem cells with critically short telomeres. Together, these results indicate that end resection of short telomeres requires the induction 53BP1 dependent formation of chromosomal fusions to promote the activation of DNA damage checkpoints, stem cell exhaustion and tissue atrophy in response to telomere shortening.

FUNCTIONAL ANALYSIS OF MAMMALIAN POLΘ REVEALS ITS ROLE IN DOUBLE-STRAND BREAK REPAIR

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Following telomere de-protection chromosome ends are fused either by a Ligase 4-dependent classical NHEJ process (C-NHEJ) or using an alternative NHEJ pathway (alt-NHEJ) mediated by Ligase 3 and PARP1. We recently investigated the differences in sequence fidelity between the two end-joining reactions, and identified Polymerase theta (Polθ; encoded by Polq) as a critical alt-NHEJ factor that introduces random nucleotides at fusion junctions. Polq inhibition suppresses alt-NHEJ at telomeres that have lost shelterin protection, and blocks translocations at non-telomeric loci. In parallel, loss of Polq results in increased rates of homology directed repair (HDR). Interestingly, depleting Polq in cells carrying mutations in the breast-cancer susceptibility genes (Brca1 or Brca2) exacerbates chromosomal aberrancies and reduces cellular survival, suggesting that Polθ-mediated alt-NHEJ compensates when HDR is impaired.

Polθ is an atypical A-family DNA polymerase with an N-terminal helicase-like domain, a large central domain harboring a Rad51 interaction motif, and a C-terminal polymerase domain capable of extending DNA strands from mismatched or even unmatched termini. In vitro experiments identified an evolutionarily conserved loop in the polymerase domain that is essential for synapsing DNA ends during end joining. Deleting this particular loop did not hinder the ability of Polθ to extend primer-templates, which is important for its function in interstrand cross-link and base excision repair. To address the mechanism by which this low-fidelity polymerase orchestrates double-strand break repair we employed CRISPR/Cas9 gene editing and established cell lines with key Polθ mutations (PolqΔhelicase, PolqΔRAD51, PolqΔpolymerase, and PolqΔloop). Here we report on the impact of these mutations on Polθ function in promoting alt-NHEJ and suppressing HDR, and address the importance of Polθ-mediated alt-NHEJ in survival of HDR defective cells.

FATE OF DICENTRIC CHROMOSOMES FORMED THROUGH TELOMERE FUSION

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Telomere shortening acts as a barrier to tumorigenesis by driving cells to senescence in response to unchecked growth. Conversely, telomere crisis can fuel cancer growth by unleashing genome instability through breakage-fusion-bridge cycles (BFB). BFB cycles initiate when dysfunctional telomeres fuse and generate a dicentric chromosome. Dicentric chromosomes are unstable structures that are widely assumed to break during anaphase. However, calculations of the spindle force relative to the tensile strength of DNA indicate that anaphase breakage is unlikely.

To determine the fate of dicentric chromosomes formed by telomere fusion, we used spinning disk microscopy to image H2B-mCherry marked cells with telomere fusions resulting from conditional inactivation of the shelterin protein TRF2. We find that dicentrics do not break in anaphase or during cytokinesis. Instead, dicentric chromosomes form persistent chromatin bridges that connect daughter cell nuclei well into the next cell cycle before their ultimate rupture. Cells can be separated by as much as 300 microns while retaining their connecting chromatin bridge. After mitotic exit, when the daughter cells are still in G1, chromatin bridges accumulate RPA and components of the MRE11-RAD50-NBS1 (MRN) complex, suggesting nucleolytic attack may contribute to the severing of the chromatin bridges. Indeed, preliminary data using the MRE11 nuclease inhibitor Mirin implicate nucleolytic processing in timely bridge resolution.

In addition, we observe that daughter cells connected by chromatin bridges suffer from transient nuclear envelope rupture during interphase (NERDI) until the connecting bridge is resolved. NERDI correlates with lamin depletion from the primary nuclear envelopes, a known cause of nuclear envelope rupture in cancer cell lines. Such NERDI events may further endanger genome integrity by exposing the DNA in the nucleus to cytoplasmic factors.

In sum, our data reveal that dicentric chromosome resolution occurs long after anaphase and cytokinesis and is a more complex process than previously appreciated that may affect genome integrity in multiple ways.

PROCESSING BY MRE11 IS INVOLVED IN THE SENSITIVITY OF TELOMERIC REGIONS TO DNA DOUBLE-STRAND BREAKS

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Double-strand breaks (DSBs) are very hazardous to cells, because they promote chromosome rearrangements leading to cell death or cancer if improperly repaired. We have investigated the consequences of DSBs near telomeres using integrated plasmids that contain an I-*Sce*I endonuclease recognition sequence to selectively introduce DSBs, and transgenes to analyze the consequence of DSBs. We have previously shown that DSBs generated near telomeres in a human cancer cell line show a much higher frequency of large deletions and gross chromosome rearrangements (GCRs), and a lower frequency of nonhomologous end joining (NHEJ). This repair deficiency at telomeres has been proposes as a mechanism by which oncogene-induced replication stress promotes telomere dysfunction, leading to chromosome instability in human cancer cells (Cancer Res. 70:4255, 2010), or senescence in normal human cells (EMBO J, 31:2839, 2012). In addition, the repair deficiency in repair of DSBs has been proposes as a mechanism for ionizing radiation-induced senescence in normal mammalian cells (Nat Cell Biol. 14:355, 2012; Nat Commun. 3:708, 2012). Our previous studies demonstrated that ATM is important in preventing large deletions resulting from DSBs near telomeres (PLoS Genet. e1003386, 2013). Our current results suggest that the ATM inhibits large deletions at interstitial sites by protecting DSBs, while ATM inhibits large deletions at telomeric sites due to its requirement for Classical nonhomologous end joining (C-NHEJ). Our current results also show that inappropriate processing of DSBs by MRE11 is involved in the formation of large deletions and GCRs at both interstitial and telomeric DSBs, although the frequency and extent of this inappropriate processing is greatly increased at DSBs near telomeres. In contrast, MRE11 is not involved in the formation of small deletions, which occur at the same frequency at interstitial and telomeric DSBs, leading us to conclude that small deletions occur through C-NHEJ, and that C-NHEJ functions normally at telomeric DSBs. Combined, our results demonstrate that telomeric regions are proficient in C-NHEJ, but that telomeric DSBs are highly prone to inappropriate processing, which promotes large deletions and GCRs, involving Alternative NHEJ.

DIFFERENTIATING DNA DOUBLE-STRAND BREAKS FROM TELOMERES AT THE NUCLEAR PERIPHERY

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Yeast telomeres at steady-state are clustered in 6-8 foci at the nuclear periphery. Peripheral anchorage involves Sir4-Esc1 and Sir4-Mps3 interactions in G1 phase, while in S phase an additional pathway requiring the telomerase subunit Est1 is active. In absence of telomerase, telomeres were shown to colocalize with nuclear pores (Khadaroo et al 2009). Earlier work also showed that persistent double-strand breaks (DSBs), i.e. those lacking a donor for homologous recombination are relocated to the nuclear periphery, where they also interact either with the nuclear pore or with Mps3. The interaction of a DSB with pores requires Mec1/Tel1 checkpoint activation and the deposition of Htz1 by the SWR1 remodeler (SRCAP in humans). It is striking that telomeres and DSBs share common ligands and anchorage sites despite the fact that they ultimately have opposite fates: DSBs need to be repaired, while telomeres have to be protected from unwanted recombination or ligation events that generate chromosome fusions and genomic instability. We have examined the effect of inserting short and long TG stretches at an internal DSB. We note that long TG stretches (250 bp) are sufficient to relocate the uncut sequence to the nuclear envelope in a Sir4 dependent manner. Consistently, TG250 suppresses both resection and telomerase-mediated elongation. 80 bp of TG repeats, on the other hand, confers a delay in relocation of the DSB to the nuclear envelope, and triggers telomerase-mediated elongation. Intriguingly, resection is strongly reduced and Mec1-dependent checkpoint activation is suppressed. We are examining the recruitment of MRX and/or Rif1 to try to distinguish the critical steps that allow uncapped telomeres to restrict the processing that normally occurs at DSBs. The effect of mps3ΔN or nuclear pore mutants on the processing of TG-flanked breaks, and on Break-induced recombination, which drives the survivor pathway in telomerase deficient cells, is being determined.

TELOMERES ARE PROFICIENT FOR REMOVAL OF UV PHOTOPRODUCTS BY NUCLEOTIDE EXCISION REPAIR

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UV irradiation induces photoproducts in the genome that if left unrepaired can interfere with replication and transcription, and ultimately cause mutations or chromosome breaks. Telomeric repeats are enriched for dipyrimidines that are prone to UV-induced cyclobutane pyrimidine dimers (CPD) and pyrimidine(6-4)pyrimidone photoproduct formation. Nucleotide excision repair (NER) removes photoproducts, but whether this pathway acts at telomeres is unresolved. To address this question, we developed an assay to directly and quantitatively measure photoproduct formation with precision by immuno-spot blot in telomeres isolated from human cells after UVC exposure. This assay revealed that UVC exposure induced approximately 2-fold fewer photoproducts in telomeres compared to the bulk genome, and that 6-4PP removal was completed in both telomeres and the bulk genome by 6 hours. However, complete CPD removal from telomeres required 48 hours, but occurred 1.5-fold faster than in the bulk genome. Importantly, UV lesions were not removed from telomeres in cells lacking the XPA protein, indicating the involvement of the NER pathway. We showed that a CPD lesion disrupted TRF1 binding to telomeric DNA in vitro, suggesting unrepaired lesions may compromise telomere integrity. To test this, we are examining telomere structure and function following UVC exposure in NER proficient and deficient BJ-hTERT cells. To further determine whether telomeres can be repaired by transcription coupled repair (TCR), we are testing telomeric photoproduct removal in cell lines deficient in either global genomic repair (GGR) or TCR pathways. These results, provide new evidence that telomeric DNA is partially shielded from UV irradiation and that NER functions at telomeres to restore damaged DNA.

CRUCIAL ROLES OF SMCHD1 AT UNCAPPED TELOMERES FOR DAMAGE SIGNALING, REPAIR AND TELOMERE ARCHITECTURE

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To understand how changes in telomere associated factors mediate different telomeric states, our laboratory developed a Quantitative Telomeric Chromatin Isolation Protocol (QTIP). When applied to isogenic HeLa cells with different average telomeric lengths (10kb vs 30kb), we detected SMCHD1 (Structural maintenance of chromosomes flexible hinge domain containing protein1) and its partner LRIF1/HBiX1 enriched at long telomeres (30kb). SMCHD1 contains an N-terminal ATPase domain and a C-terminal SMC hinge domain found in the SMC protein family. SMCHD1 has been implicated in compaction and inactivation of the inactive X chromosome, DNA methylation and DNA damage repair.
In addition to being enriched at long telomeres, we discovered that SMCHD1 is also recruited to short telomeres when they are rendered dysfunctional by TRF2 depletion. Co-depletion of SMCHD1 together with TRF2 abrogated γH2AX accumulation. Consistently, SMCHD1 KD also affected the formation of telomere dysfunction induced foci (TIFs) in TRF2-depleted cells diminishing the accumulation of 53BP1 at damaged telomeres. In addition the number of telomere fusion events was strongly reduced upon KD of SMCHD1. Finally, telomere volume measurements by STORM microscopy suggest roles of SMCHD1 in telomere compaction that occurs when TRF2 is depleted. Taken together our data reveal requirements for SMCHD1 at TRF2-depleted telomeres for structural remodeling, damage signaling and DNA damage repair.

QTIP: UNRAVELING CHANGES IN TELOMERIC PROTEIN COMPOSITION ALONG THE CELL CYCLE

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To characterize variations in telomere protein composition during the cell cycle, we adapted the recently developed quantitative telomeric chromatin isolation protocol (QTIP). QTIP allows the comprehensive characterization of telomere protein composition by immunoprecipitation of telomeric chromatin and quantitative mass spectrometry analysis.
We established SILAC culture conditions for human cells that can be grown in suspension. Elutriation sorting was used to isolate synchronous cell populations, avoiding the secondary effects linked to chemical synchronization. QTIP was then applied to compare changes in telomere protein composition between G1, S, and G2 phases of the cell cycle.
Our comprehensive analysis allowed us to monitor variations of known proteins at telomeres during cell cycle progression and to identify new candidates whose association with telomeres varies between the different phases. Currently, we are investigating the function of a subset of newly identified proteins.

BONE MARROW TRANSPLANTATION WITHOUT RADIATION OR DNA ALKYLATING AGENTS FOR PATIENTS WITH SHORT TELOMERE SYNDROMES

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Bone marrow failure (BMF) is a major cause of illness and death in patients with dyskeratosis congenita (DC). Hematopoietic cell transplantation (HCT) cures BMF in DC patients but is associated with a high incidence of graft failure and treatment-related mortality. Radiation and DNA alkylating agents are mainstays of allogeneic HCT preparative regimens: their highly effective myeloablative and immunosuppressive properties provide a niche for donor hematopoietic progenitors and decrease graft rejection. However, exposure to these agents is likely to contribute to the poor outcomes in DC patients undergoing HCT by accelerating pulmonary and liver disease and malignancy, to which they are predisposed. Based on these concerns and a rationale that presumes (1) niche availability in DC patients with BMF, and (2) an intrinsic replicative defect in their hematopoietic cells due to short telomeres, we hypothesized that engraftment might be feasible using a preparative regimen of immunosuppressive agents alone.
Here we report that in a prospective study, 4 consecutive patients with DC have undergone successful HCT using a preparative regimen without radiation and alkylating agents. Patients received alemtuzumab and fludarabine, followed by bone marrow transplantation from unrelated donors. All 4 patients engrafted neutrophils by day +30 and showed full donor myeloid engraftment by day +60. There were no significant, unexpected toxicities or infections. All 4 patients are alive and well with follow-up ranging from 12 months (1 patient) to 30 months (3 patients). By tracking chimerism in the patients, we find evidence for a competitive advantage of the donors' hematopoietic cells compared to the patients' cells, which may reflect their replicative defect due to short telomeres. These results show promising outcomes using an immunosuppression-only preparative regimen in HCT for BMF in DC patients. This approach could enable HCT for patients with severe disease-related co-morbidities that would usually prohibit transplant; spare DC patients the acceleration of non-hematologic complications and malignancies; and improve long-term survival. The results also raise the possibility that telomere length might be useful as a criterion for choosing a similar approach for patients with other genetic or acquired forms of BMF. To our knowledge, this is the first prospective study to demonstrate full donor myeloid engraftment in a series of HCT patients without using radiation or alkylating agents in the preparative regimen.

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TELOMERE DYSFUNCTION ACTIVATES A SOMATIC STRESS RESPONSE PATHWAY IN C. ELEGANS

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Repression of telomerase in somatic cells occurs in mammalian species that have large body mass (1-3), and mammals with shorter telomeres have longer lifespans (1). One reason for the association of telomerase repression and telomere length with longevity is that replicative aging suppresses tumor formation. It is unknown if additional factors aside from tumor suppression might promote longevity in the context of telomere attrition. In fact, somatic telomere dysfunction impairs mitochondria and associated metabolism in mice (4,5), revealing at least one significant cost to replicative aging.

We found that telomere dysfunction in C. elegans telomerase mutants activates a somatic stress response pathway. Although various forms of DNA damage induce the innate immune response in C. elegans (6), we found that telomere dysfunction activates a distinct somatic stress response pathway via DNA damage checkpoint proteins that normally interact with telomeres to promote telomerase activity. Our results point to an unexpected benefit of telomere attrition in the nematode C. elegans, where telomere dysfunction acts in a cell-non-autonomous manner to promote somatic stress resistance.

(1) Gomes, Ryder, Houck, Charter, Walker, Forsyth, Austad, Venditt, Pagel, Shay and Wright (2011). Aging Cell 10:761-768.
(2) Seluanov, Chen, Hine, Sasahara, Ribeiro, Catania, Presgraves, and Gorbunova (2007). Aging Cell 6:45-52.
(3) Seluanov, Hine, Bozzella, Hall, Sasahara, Ribeiro, Catania, Presgraves, Gorbunova (2008). Aging Cell 7:813-821.
(4) Sahin, Colla, Leisa, Moslehi, Muller, Fuo, Chin, DePinho (2011). Nature 470: 359-365.
(5) Moslehi, DePinho, Sahin (2012). Circ Res 110:1226-1237.
(6) Ermolaeva, Segref, Dakhovnik, Ou, Schneider, Utermohlen, Hoppe, Schumacher (2013). Nature 501: 416-420.

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TELOMERE DYSFUNCTION CAUSES ALVEOLAR STEM CELL FAILURE

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Telomere syndromes have their most common manifestation in lung disease that is recognized as idiopathic pulmonary fibrosis and emphysema. In both cases, there is loss of alveolar integrity, but the underlying mechanisms are not known. We tested the capacity of alveolar epithelial and stromal cells from mice with short telomeres to support alveolar organoid colony formation and found that type 2 epithelial cells (AEC2s), the stem cell-containing population, were limiting. When telomere dysfunction was induced in adult AEC2s by conditional deletion of the shelterin component, Trf2, cells survived but remained dormant and showed all the hallmarks of cellular senescence. Telomere dysfunction in AEC2s was sufficient to recruit a robust inflammatory response in the lung. This was associated with up-regulated transcription of cytokine signaling pathways in AEC2s that are known to trigger immune responses. In response to a pulmonary toxin challenge, bleomycin, mice with telomere dysfunction in AEC2s uniformly died underscoring an essential role for intact telomere function in these cells for alveolar repair and recovery after injury. Our data show that telomere dysfunction in alveolar stem cells is sufficient to recapitulate the regenerative defects, inflammatory responses, and susceptibility to injury that are characteristic of telomere-mediated lung disease, and suggest alveolar stem cell senescence may be a driver of this pathology.

SPERM TELOMERE LENGTH INCREASES WITH AGE AND IS ASSOCIATED WITH BLASTOCYST DEVELOPMENT BY THE SIXTH DAY AFTER IN VITRO FERTILIZATION TREATMENT.

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Objective: Segregation errors, embryo arrest, apoptosis, and implantation failure are common during early human development. Oocyte telomere length influences each of these but the impact of sperm telomere length is less understood. Our aims were to study the relationship between sperm telomere length (STL), men's age, semen parameters, embryo morphology and pregnancy outcome in men undergoing in vitro fertilization treatment (IVF).
Design: Prospective observational study.
Materials and Methods: 109 semen samples were collected from consented patients undergoing in vitro fertilization (IVF) treatment at NYU Fertility Center. Clinical information including age, semen parameters, embryo morphology and pregnancy outcome (defined by fetal heart rate) were obtained from medical records. After DNA extraction, STL was measured by semi-quantitative real-time polymerase chain reaction (qPCR), as previously described by Cawthon et al., 2002, with minor modifications (Wang et al., 2013). Mean telomere length (T/S) was determined by comparing the values of telomere DNA (T) and a single copy reference gene (36B4) (S), amplified simultaneously. Mean STL (T/S) was compared to clinical variables using Mann-Whitney test, and compared to pregnancy outcome using Unpaired t test with Welch correction. P value less than 0.05 was considered significant.
Results: Older men (age > 35 years old, n=73) presented significantly longer STL (4.86 ± 0.56) compared to younger men (age ≤ 35 years old: 3.14 ± 0.51, n=36), p=0.047, Mann-Whitney test. STL in abnormal semen specimens (3.64 ± 0.76, n=18) did not differ significantly from that in normal semen specimens (3.99 ± 0.43, n=71), p=0.631, Mann-Whitney test. Embryos which developed into blastocysts by the sixth day after fertilization derived from longer STL (3.92 ± 0.28, n=111) when compared to embryos that remained at cleavage stage (3.67 ± 0.34, n=127), p=0.036, Mann-Whitney test. Sperm resulting in clinical pregnancies trended toward longer telomeres (4.52 ± 1.03, n=19) than sperm not producing pregnancies (3.28 ± 0.53, n=31), though this difference did not reach significance (p=0.295, Unpaired t test) in the sample size studied.
Conclusions: We confirmed prior findings that sperm from older men have longer telomeres than from younger men. We also showed that embryos developing to blastocyst stage came from sperm with significantly longer STL than embryos which did not develop properly, remaining at the cleavage stage after six days of fertilization. We did not find significant differences in STL specimens when comparing abnormal vs. normal semen parameters. Additional studies are needed to establish whether STL affects pregnancy outcome.
Supported by CAPES Foundation, Ministry of Education of Brazil, Brazil and the Stanley H. Kaplan Fund, Department of Obstetrics and Gynecology, New York University Langone Medical Center.

DECIPHERING THE INTERPLAY BETWEEN POT1 AND HP1^{SWI6} IN TELOMERASE-MINUS HAATI SURVIVORS

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In the absence of telomerase, non-telomeric heterochromatin can acquire the ability to perform the telomere-associated function of linear chromosome end-protection. We recently uncovered such Heterochromatin Amplification-mediated And Telomerase Independent (HAATI) survivors in S.pombe (Jain et al., Nature 2010). HAATI survivors replace canonical telomeric sequences at chromosomal ends with 'generic' heterochromatic repeats, usually the rDNA. While HAATI chromosomes have been extensively analyzed in terms of their DNA rearrangements, chromosome behavior and genetic requirements, in-depth cytological analysis is still missing and can provide important insights about the organization and maintenance of re-arranged HAATI genomes. We are currently pursuing two interesting ideas using single cell analysis techniques.

1.Loss of canonical chromosomal ends as well as spreading of heterochromatic sequences predicts changes in the localization and/or distribution of HP1 (Swi6, a fission yeast Heterochromatin Protein 1 ortholog) in HAATI. While total levels of HP1 remain unchanged in HAATI cells, quantitative live microscopy reveals greater numbers of nuclear HP1 foci. These differences raise the possibility that HP1 becomes limiting in HAATI cells. We are testing the possibility that HP1 binding dynamics change in this setting, and examining the localization of HP1 with respect to that of Pot1.

2.Preliminary analyses suggest that Pot1 appears as bright foci in HAATI cells only for a limited time during cell cycle. This is also true in wild type (telomerase-positive) cells, but the timing of focus appearance differs in WT and HAATI. We speculate that in the absence of its high-affinity binding sites, Pot1 is concentrated at the extreme end of HAATI chromosomes due to amplified heterochromatin along with ssDNA binding stretches. Hence, the timing of Pot1 focus appearance may reflect the differences in replication timing of telomeric versus rDNA sequences, a possibility we will address here. Such replication dependence would link Pot1 localization to the generation of non-telomeric ssDNA overhangs as the replication fork passes.

EVOLUTIONARILY CONSERVED DNA BINDING BY THE OB1 DOMAIN IN *ARABIDOPSIS* POT1A.

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Protection of telomeres 1 (POT1) is implicated in both telomere replication and end protection. In most model organisms, POT1 binds single-strand G-rich telomeric DNA via oligonucleotide/oligosaccharide binding folds (OB folds). However, DNA binding has not been associated with the two POT1 paralogs, AtPOT1a and AtPOT1b from *Arabidopsis thaliana*. Moreover, biochemical and genetic analysis of AtPOT1a failed to reveal an end protection function and instead showed that AtPOT1a physically interacts with the canonical telomerase RNA TER1 where it serves as a positive regulator of telomerase activity *in vivo*. Here we examine the nucleic acid binding properties of AtPOT1a. Since OB1 specifically interacts with telomeric DNA *in vitro* in yeast and vertebrates, we expressed AtPOT1a OB1 in *E. coli*. Under the conditions tested, filter binding experiments showed an apparent binding affinity (K_d) of ~ 4.5×10^{-7} M for AtPOT1a OB1-TER1 interaction. However, competition experiments revealed no specificity for TER1 RNA sequence.

POT1 protein from the moss *Physcomitrella patens* binds single-stranded telomeric DNA *in vitro*; raising the possibility that OB1 of AtPOT1a might have intrinsic DNA binding activity. Therefore, EMSA experiments were performed with telomeric DNA oligonucleotides. AtPOT1a OB1 binds $(TTTAGGG)_5$ with an apparent binding affinity (K_d) of ~ 2.3×10^{-7} M. The minimal binding site (MBS) was defined as a dodecameric sequence '5-TTAGGGTTTAGG-3'. In addition, mutation of F65 to alanine that corresponds to F62A in the OB1 domain of mouse POT1a led to a complete loss of telomeric DNA binding in accordance with analysis of mouse POT1a. These findings indicate that telomeric DNA binding is a conserved feature of POT1 in higher plants, and argue that domains outside OB1 influence OB1-nucleic acid contacts and recognition. In all, the current study reveals an unexpected evolutionarily conserved telomeric DNA binding by the POT1 OB1 domain.

TELOMERE ATTRITION IN SELECTED LYMPHOCYTES SUBPOPULATIONS

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Subpopulations of leukocytes of healthy individuals show differences in telomere loss with age which are most pronounced for memory T cells and NK/fully differentiated T cells1, consistent with the observed functional decline of these cells in the elderly. Our findings in the normal population suggest that defects in cellular immune compartments and immune senescence could result from telomere loss in specific cell types contributing to immune related pathologies.

We used Flow-FISH to measure the median telomere length of leucocytes and leucocyte subsets in human subjects. In order to define the length distribution of specific cell subsets, telomere specific fluorescent PNA probe hybridization is combined with limited immuno-phenotyping allowing for some identification of specific cell types. Applying our established methodology, samples of few human subjects display a bimodal distribution of telomere fluorescence within a particular lymphocyte subset. This reproducible observation suggests that cells within this phenotype have either different replicative histories or that different populations within a given phenotype exist. Our findings point to significant differences in the telomere length between cells within lymphocyte subpopulations that can only be detected by approaches that measure telomere length in populations of individual cells and may play an important role in pathological processes.

Unusual telomere length distributions such as bimodal distribution in specific subsets and their potential implication for immune status and function will be discussed. Further development of multicolor (more than 4 colors) Flow-FISH aimed at refining lymphocyte subset definition by combining new phenotype markers will be valuable in further studies of telomere length heterogeneity and these approaches will also be discussed.

1 Aubert, G., Baerlocher, G. M., Vulto, I., Poon, S. S. & Lansdorp, P. M. Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. PLoS Genet 8, e1002696, doi:10.1371/journal.pgen.1002696 (2012).

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ROLES OF UNIQUE TELOMERE MAINTENANCE: INSIGHTS FROM THE NAKED MOLE RAT.

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In mammals, changes in telomere structure play critical roles in aging and cancer. Genome analysis suggests that this function is uniquely changed in the NMR: Naked Mole Rat (Kim *et al.*, Nature 2011), an emerging model organism characterized by exceptional longevity and extreme resistance to cancer development. Sequence analyses revealed that TRF1 is positively selected in NMRs. Based on this observation, we hypothesize that the telomeres of NMRs have an exceptionally high capacity to protect chromosome ends from genome instability.

Initial observations show that NMR cells under stress condition do not produce TIF (Telomere dysfunction-Induced Foci), suggesting that telomeres become weakly dysfunctional. We examine the biology of NMR telomeres and DNA damage response pathways and compare them to those of humans and mice cells. Also, we determine the consequence of complementation of human/mouse TRF1 with that of the NMR in human/mice cells in order to examine if the NMR version of this gene increases resistance to cancer and delay senescence in human and mouse cells.

STRESS AND TELOMERE SHORTENING AMONG CENTRAL INDIAN CONSERVATION REFUGEES

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Accumulating evidence links a variety of life stresses to accelerated telomere shortening and human aging. However, this association has only been demonstrated in Western contexts (so-called "WEIRD" societies; Western, educated, industrialized, rich, and democratic), where stress is typically lower and life expectancies longer. By contrast, we examined stress and telomere shortening in a non-Western setting, among a highly stressed population with overall lower life expectancies: poor indigenous people—the Sahariya—living in a central Indian wildlife sanctuary. Two representative villages, created merely by the capricious draw of boundaries in order to accommodate introduction of Asiatic lions into the sanctuary, were selected. In one, relocated villagers had been displaced from their ancestral forest homes and traditional ways of life, and in the other, villagers had been isolated and restricted within the sanctuary buffer zone following the forced departure of their fellow Sahariya. Individuals from each village were evaluated for key indicators of stress: salivary analytes cortisol and α-amylase (physical measures); self-assessments of psychosomatic stress and ethnographic observations; and telomere length. Importantly, telomere length was assessed specifically in putative basal stem/progenitor cells (from bucall swabs) using telomere fluorescence in situ hybridization (TEL-FISH) coupled with 3D reconstruction of individual nuclei to facilitate analysis of all signals within the entire extension of each cell. Such a strategy greatly improved the specificity and quantification of telomere length, including the ability to define distributions of the shortest telomeres. Further, it also afforded particularly high-resolution data amenable to multilevel statistical analysis and development of predictive models, without which telomere length results can be misleading. Consistent with the premise that life stress contributes to telomere shortening, we found significant associations between each of our stress measures and telomere length, after adjusting for relevant behavioral, health, and demographic traits. By meticulously tracing and predictively modeling links between stress and telomere maintenance in this highly distressed non-Western population, our research serves to strengthen the case for stress-related telomere shortening as a pancultural biomarker of compromised health and aging.

STRATEGIES FOR TELOMERASE ACTIVATION TO TREAT TELOMERE SYNDROMES AND AGE-ASSOCIATED DISEASES.

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Progressive telomere shortening throughout life is one of the hallmarks of molecular aging and short telomeres are risk factors for age-associated diseases including cardiovascular disease, which is the number one cause of death worldwide. Besides the natural occurring telomere shortening, which may contribute to disease, accelerated telomere attrition by virtue of mutations in the telomere maintenance machinery leads to a broad spectrum of diseases summarized as Telomere Syndromes. This includes amongst other, life-threatening conditions such as pulmonary fibrosis and aplastic anemia. Over the past decade our laboratory developed different telomerase activation strategies (i.e. transgenesis, virus-based gene therapy) with which we demonstrated that organismal aging can be delayed in wild-type mice. More recently we are exploring strategies for telomerase activation in mouse models recapitulating aging-associated diseases and telomere syndromes.

Here, I will present our recent advances in the therapeutic treatment with telomerase in mice after acute myocardial infarction. I will show that telomerase reactivation in the adult mouse heart is cardio protective as indicated by improved survival, smaller infarct scars, improved ventricular function. Moreover, telomerase over-expression induces a shift in the gene expression towards a neonatal signature and enhances the number of cycling cardiomyocytes near the infarct borders.

Furthermore, I will present our progress in the treatment of aplastic anemia in a mouse model where the disease is produced by short telomeres. I will show that sex hormones can activate telomerase expression in *vitro* and that *in vivo* androgen therapy delays the appearance of the aplastic anemia phenotype which is associated with longer telomere length in peripheral blood monocytes and in bone marrow cells compared to untreated control mice. Similarly, *in vivo* reactivation of telomerase by means of AAV9 gene therapy significantly reduces aplastic anemia related morbidity owed to telomere elongation in blood and bone marrow cell which also coincides with higher bone marrow cellularity compared to non-treated mice.

TELOMERE SHORTENING AND MITOCHONDRIAL DYSFUNCTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM MORBID OBESE PATIENTS.

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Introduction: Mitochondrial dysfunction has an important role in the pathophysiology of many diseases including obesity. Consistent associations are documented in peripheral blood from patients with obesity as increased oxidative stress and inflammation. Excess of reactive oxygen species (ROS) cause damage to mitochondrial components, initiate degradative cellular processes that significantly contribute to the aging process and induce accelerated telomere erosion. Current understanding in mitochondrial respiratory function in intact peripheral blood mononuclear cells (PBMC) from patients with obesity providing a dynamic measurement of metabolic rates is limited. In this regard, the main objective of this work is to explore mitochondrial respiration parameters as an integrative measure of the dynamics of complex coupled metabolic pathways in PBMC from patients with morbid obesity that may contribute to telomere shortening.
Methods: 39 patients with morbid obesity (BMI ≥ 35 kg/m2) and 27 healthy controls (BMI 20.0 - 24.9) were recruited. Demographic data and clinical history was recorded. Peripheral blood was collected and PBMC were isolated by density gradient. Genomic DNA was extracted from PBMC and relative mean telomere length (T/S) was measured by real time qPCR. Activity of the mitochondrial respiration in intact fresh PBMC was achieved through high-resolution (Oxygraph-2k) and normalized to number of cells (4x106).
Results: Telomere length was significantly shorter in morbid obese patients when compared to controls (mean = 0.49 95% CI (0.37 – 1.80) versus 1.57 95% CI (0.50 – 1.67), $p = 0.004$). The differences remained significant after adjustment for age as covariate in a multivariate analysis ($p = 0.026$). In the morbid obese group we found significant direct association between mitochondrial ROS production and proton leak ($p < 0.05$) with telomere shortening.
Conclusions: Telomere length is diminished in patients with morbid obesity. Our data suggest that mitochondrial dysfunction contributes to the accelerated phenotype of cellular senescence in morbid obese patients.
Financial support: CAPES, CNPq, FAPERGS.

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EXAMINING NON-CANONICAL ROLES OF TELOMERASE IN FIBROBLASTS EXPRESSING hTERT MUTANTS ASSOCIATED WITH LUNG FIBROSIS

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Aberrant telomerase activity has been observed in multiple human diseases including cancer and certain premature aging disorders such as dyskeratosis congenital, aplastic anemia and Idiopathic Pulmonary Fibrosis (IPF). Characterization of select heterozygous hTERT mutations found in a subset of IPF patients revealed that telomere lengths in patients harbouring those mutations were shorter compared to age-matched controls. Characterization of BJ fibroblasts expressing either one of two IPF associated hTERT mutations, V144M and R865C, did not have elongated telomeres despite the mutant proteins retaining almost wild-type (WT) catalytic activity. The BJ fibroblasts did however, display a prominent growth advantage similar to that seen with the wild-type protein compared to the vector-only control cell lines. We hypothesize that this observed growth advantage is partially due to non-canonical, or telomere length independent functions of telomerase. Recently, telomerase has been shown to function independent of telomere elongation in several biological processes, including roles as a transcriptional regulator in the Wnt/ß-catenin and NF-κB signalling pathways. In order to further delineate the molecular mechanism in which these IPF-associated hTERT mutations may confer a growth advantage in cells, we identified four genes whose expression was upregulated in cells expressing either the mutant or WT-hTERT proteins: CXCL12, CXCR4, c-KIT and JAG1. Each of these gene targets have been implicated in pathways that promote cell growth, survival, migration and invasion.

It has previously been suggested that hTERT expression confers an invasive phenotype and promotes the migration of fibroblasts; this mechanism could potentially contribute to the progression of IPF. We therefore postulated that upregulated CXCR4/CXCL12 signalling, a known axis in tissue migration, could promote migration of our hTERT-expressing fibroblasts. Using a modified Boyden Chamber migration and invasion assays, we have demonstrated that the conditioned media from BJ fibroblasts expressing either WT or mutant hTERT acts as a chemo-attractant promoting the migration and invasion of BJ cells, regardless of the hTERT expression of the migrating fibroblast. This reveals a potential driving mechanism of IPF disease progression, which could play a fundamental role in cancer metastasis. Our evidence suggests that non-canonical roles of telomerase may have critical implications in cellular physiology and play a prominent role in disease progression.

A SINGLE-MOLECULE MICROSCOPY ASSAY TO MEASURE TELOMERE ELONGATION BY HUMAN TELOMERASE

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Telomerase is a reverse transcriptase that, together with its integral RNA, exhibits fascinating and complex nucleoprotein interactions during substrate recognition, catalysis, and template/enzyme translocation. Current assays used to assess telomerase activity rely on ensemble, population-based measurements in which rare events of biological interest may be masked. Additional high-resolution, single-molecule methods to assay telomerase activity would greatly refine our understanding of the inner workings and mechanisms-of-action of this unique enzyme.
Toward that end, we have begun to develop assays employing total internal reflection fluorescence (TIRF) microscopy to track the activity of telomerase with the goal to achieve single-molecule resolution of enzyme function. Using streptavidin-coated coverslips to capture biotinylated single-stranded DNA substrates, we optimized conditions under which a ssDNA-binding dye would elicit a detectable signal whose intensity was dependent on substrate length. We then measured the signal intensity of short telomeric DNA substrates after incubation with reconstituted telomerase (from rabbit reticulocyte lysates). We observed a time-dependent and enzyme concentration-dependent increase in dye signal that depended on the presence of active human telomerase, but was not elicited when catalytically inactive mutants of human TERT were incubated under the same conditions. We are currently further optimizing the image acquisition, quantification, and statistical analysis of our results. This assay will be used to shed insight into how telomerase recognizes and extends its substrates in vitro, and to other, less-explored properties such as the potential for nucleolytic cleavage. In summary, it is our goal to use TIRF single-molecule microscopy to unveil additional interesting properties of telomerase enzymology.

ENGINEERING A TPP1-TEL PATCH DISEASE MUTATION (K170Δ) USING CRISPR/CAS9 TECHNOLOGY TO UNDERSTAND ITS DOMINANT NATURE IN DYSKERATOSIS CONGENITA.

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Dyskeratosis Congenita (DC) is a rare bone marrow failure syndrome characterized by telomere shortening and defective stem cell homeostasis. In collaboration with Dr. Sharon Savage's group, we recently added ACD/TPP1 to the list of DC genes. We demonstrated that a single amino acid deletion (K170Δ) in TPP1-TEL patch causes a severe form of DC known as Hoyeraal-Hreidarsson syndrome characterized by defective telomerase recruitment and telomere shortening. The K170Δ has also been implicated in bone marrow failure by another independent study. In both studies, the proband inherited a heterozygous mutation (+/K170Δ) in the TEL patch of TPP1 and displayed a very short telomere phenotype. These observations suggest that a single wild-type copy of the TEL patch of TPP1 is insufficient to maintain the telomere length in DC patients. However, based on other studies on TPP1, it is unclear how a heterozygous TEL patch mutant genotype results in a deleterious telomere length phenotype.

To test the absolute effect of the K170Δ mutation on telomere length in the absence of any gross change to the TPP1 gene or to TPP1 protein expression, we introduced the K170Δ mutation at the endogenous TPP1 locus in human cell lines. We accomplished this using CRISPR/Cas9-based cleavage of the TPP1 locus, and subsequent repair using mutagenic single-stranded oligonucleotides (ssODN). We successfully isolated +/K170Δ heterozygous clones and performed telomere repeat length analysis. Our results show that the K170Δ mutation results in telomere shortening as a function of increasing population doubling despite the co-existence of a WT TPP1 allele. We are currently examining telomerase recruitment as well as telomerase processivity determinations with these +/K170Δ TEL patch mutant clones to dissect the exact mechanism (e.g. dominant negative versus haploinsufficiency) underlying the dominant TEL patch mutant phenotype. In addition to addressing an important question in telomere biology, our efforts also showcase the usefulness of CRISPR/Cas9 technology when compared with currently pervasive overexpression strategies.

THE ROLE OF TPP1 IN TELOMERE LENGTH HOMEOSTASIS: AN ANALYSIS OF L104.

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Telomere length homeostasis is essential for the long-term survival of stem cells, and its set point determines the proliferative capacity of differentiated cell lineages by restricting the reservoir of telomeric repeats. Our lab has previously reported, in the context of overexpression, that amino acid substitutions at residue 104 of TPP1 results in changes to the homestatic telomere-length set point. Based on these finding we used genome editing to engineer hESC to carry homozygous TPP1 L104 substitutions at the endogenous TPP1 locus. TPP1 L104A/L104A cells are viable and do not reveal a telomere deprotection phenotype. However, telomere length analysis indicates that they have very short telomeres that are maintained at a stable length. This data further demonstrates that TPP1 L104A is competent to recruit telomerase to telomeres, but fails to appropriately communicate telomere length towards telomerase actions on the 3'OH. Next we used overexpression of TPP1 and other shelterin alleles from the AAVS1 safe harbor locus to determine whether L104A functions as a dominant mutation or produces deficiency in TPP1's role in relaying telomere length information from double stranded binding proteins to telomerase or POT1.

REGULATION OF COORDINATION BETWEEN DNA REPLICATION AND TELOMERE ELONGATION MECHANISMS

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Telomeres are processed by a telomere-specific machinery that includes nucleases as well as telomerase and its regulatory units. Telomerase elongates the leading strand, whereas the lagging strand is carried out by the regular lagging strand replication machinery, which is also in charge of replicating the whole genome. The replicative polymerases are held in place by PCNA, a ring or clamp that must be loaded and unloaded. In a very short period (at the very end of the S- phase) the cell must coordinate the activities of the replication and the telomere processing machineries. We aim at dissecting this coordination, by identifying the factors involved, characterizing their function and investigating the interactions between them. So far, we have found that post-translational modifications (such as the addition of SUMO tags) of the PCNA ring, and the function of one of the clamp-loading complexes subunits, Elg1, are important for the regulation of telomere elongation.
Among other results, we have found physical and genetic interactions between Elg1, a component of the lagging strand synthesis machinery, and Stn1, a protein that binds to telomeres. The interactions, as well as the elongation of telomeres observed in the absence of these factors, require SUMOylation of PCNA. Our preliminary results therefore suggest interesting interactions between the lagging strand synthesis proteins and telomere maintenance factors. Our results will have implications for our understanding of genome replication and stability, as well as the development of cancer and cellular aging.

GENERATION OF A TISSUE-MATCHED PANEL OF CELL LINES TO STUDY THE ALTERNATIVE LENGTHENING OF TELOMERES (ALT) PHENOTYPE IN CANCER

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Overcoming the end-replication problem is a major hurdle for cancers. Recently, it has been appreciated that approximately five percent of all cancers use a telomerase-independent telomere maintenance mechanism, termed alternative lengthening of telomeres (ALT)[1,2]. Overall, the underlying molecular mechanisms through which ALT arises in cancer have not been completely elucidated. As such, the identification and validation of a panel of ALT-positive cell lines is necessary. Therefore, we examined cell lines derived from tumor subtypes known to display the ALT phenotype in clinical specimens, including osteosarcomas, rhabdomyosarcomas, gliomas, medulloblastomas, testicular germ cell tumors, rhaboid tumors, and neuroblastomas, as well as the NCI-60 cell line panel. We determined the ALT status of these cell lines using a telomere-specific fluorescent in situ hybridization assay. Furthermore, we assessed the expression of ATRX and DAXX, two proteins thought to confer resistance to ALT[3,4], by immunostaining. We have identified subsets of osteosarcoma, rhabdomyosarcoma, glioma, and neuroblastoma cell lines that are ALT-positive. Of the ALT-positive cell lines, the majority, as expected, have complete loss of nuclear ATRX or DAXX expression. Interestingly, we have identified two cell lines that display a high degree of cell-to-cell heterogeneity for ATRX or DAXX protein expression; cells that have lost either factor display large telomeric foci, consistent with ALT, while neighboring cells that retain expression of these proteins lack these ALT-associated foci. These results suggest a possible epigenetic or alternative mechanism of ATRX and DAXX control. Further characterization and use of these tissue-matched ALT-positive and ALT-negative cells will allow a deeper understanding of ALT biology in these tumor types, as well as the potential to therapeutically target ALT-positive tumors.

1) Heaphy, C.M., et al. (2011) Am J Pathol, 179:1608
2) Cesare, A.J. and Reddel, R.R. (2010) Nat Rev Genet, 11:319
3) Heaphy, C.M., et al. (2011) Science, 333:425
4) Lovejoy, C.A., et al. (2012) PLoS Genet, 8:e1002772

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STUDYING CHROMATIN ORGANIZATION ON A SINGLE TELOMERE

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Several chromatin modifications have been associated with telomere deprotection but the nucleosomal organization and the epigenetic pattern of telomeric chromatin in protected and deprotected states is still ill-defined. Telomere heterogeneous length (about 2-20 kbp in humans) coupled with the uniformly repeated sequence renders hard to establish whether the telomere has a regular structural organization along its overall length and how its structure changes when telomeres shorten and uncap. To address this issue we set up several experimental approaches, including in vitro models of telomeric chromatin and taking advantage of the realization of a cell line containing a seeded telomere with a strong nucleosome positioning sequence in a subtelomeric position. To map telomeric chromatin at a resolution higher than that present in literature, we set up a LM-PCR-based technique; the analysis of the nucleosomal organization of the seeded telomere as a function of telomere length is in progress.

TARGETING TERT HYPERMETHYLATED ONCOLOGICAL REGION FOR GLIOMA STRATIFICATION AND EXHAUSTION OF SELF RENEWAL OF MALIGNANT GLIOMA STEM CELLS.

Pedro Castelo-Branco, Donghyun Lee, Marco Gallo, Tatiana Lipman, Joshua Mangerel, Aryeh Price, Marc Remke, Cindy Zhang, Ricardo Leao, Abolfazl Heidari, Khalida Wani, Michael Taylor, Cynthia Hawkins, Hai Yan, Kenneth Aldape, Peter Dirks, Uri Tabori

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Gliomas are a deadly group of cancers associated with high relapse rate following current therapies. Pathological stratification of gliomas is problematic and many tumors progress from low to malignant at recurrence. Gliomas exhibit multiple telomere maintenance mechanisms including ALT and TERT promoter mutations, however the clinical and biological implications of these are largely unknown. We have recently uncovered a region in the TERT promoter, THOR (TERT Hypermethylated Oncological Region) which is paradoxically hypermethylated in gliomas with telomerase activation. In order to further explore the biological impact of THOR hypermethylation on self renewal and telomere maintenance of gliomas we undertook a stepwise approach.
Mapping of the human TERT promoter reveals that THOR spans 432 BP and comprises 52 CG sites. In contrast, the region where mutations in TERT promoter were uncovered is permanently hypomethylated. Luciferase based promoter assays unveiled a repressive effect of THOR on the the proximal region where the TERT mutations are found.
Analysis of allelic Tert expression reveals that THOR is initially methylated in one allele and throughout tumor progression, the other allele becomes methylated. This correlates with higher TERT expression. Moreover, most gliomas with the THOR hypermethylation also present TERT expression, TERT mutations and differential allelic expression. In contrast, tumors that present the ALT phenotype lack THOR hypermethylation and TERT mutations. Together, 70% of primary non-ALT malignant gliomas (n=111) exhibited THOR hypermethylation and most secondary gliomas utilize ALT to maintain their telomeres.
Demethylation of THOR with epigenetic modifiers results in loss of telomerase activation in glioma cells glioma cancer stem (GCS) cells. In contrast no such effect was observed in normal embryonic stem cells which lack THOR hypermethylation. Combining telomerase inhibition with demethylating agents results in permanent loss of self renewal capacity of GCS cells and lack of tumor formation in vivo. Mice treated with combined therapy had significant improved survival when compared to control ($p<0.0001$).
We offer a novel model of glioma classification based on THOR hypermethylation and alterations in the telomere maintenance pathway. Since THOR hypermethylation is restricted to cancer cells, demethylation can be a safe and viable option for exhausting self renewal capacity of gliomas.

STRUCTURAL BASIS OF TELOMERASE RECRUITMENT IN YEAST

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The action of telomerase to elongate telomeres is regulated through multiple pathways. In budding yeast, the single-stranded G-tail binding protein Cdc13 recruits telomerase to telomeres by direct interaction with the telomerase component Est1. However, the underlying molecular mechanism of telomerase recruitment in yeast still remains unclear and controversial. Our biochemical analysis reveals a minimum Est1-binding motif (EBM) of *Kluyveromyces lactis* Cdc13 that contains ~ 25 amino acids and is the most evolutionarily conserved region within the in vivo defined Cdc13 recruitment domain. The apparent disassociation constant (K(d)) between *Kl*Est1 and *Kl*Cdc13_EBM is ~ 4 μM. We determine the crystal structure of *Kl*Cdc13_EBM in complex with *Kl*Est1. The structure shows that the *Kl*Cdc13_EBM adopts an extended conformation and binds to two separate pockets on the surface of *Kl*Est1. ITC measurements indicate that the N-terminal sequence in *Kl*Cdc13_EBM is essential for the *Kl*Cdc13-*Kl*Est1 interaction. Notably, the C-terminal part of *Kl*Cdc13_EBM that has been extensively studied previously only plays a minor role in the Cdc13-Est1 interaction. In addition, our analysis also reveals a subtle difference in the Cdc13-Est1 interaction between *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. And in consistent with this difference, *K. lactis* charge-swap mutations that are equivalent to *S. cerevisiae* Cdc13(E252K) (*cdc13-2*) and Est1(K444E) (*est1-60*) cannot restore the interaction between *Kl*Cdc13 and *Kl*Est1. These data provide the first structural insight into the recruitment of telomerase by Cdc13 in budding yeast.

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MECHANISM FOR dGTP-DEPENDENT REPEAT ADDITION PROCESSIVITY OF HUMAN TELOMERASE

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Processive DNA repeat synthesis by telomerase relies on a unique, yet poorly understood, mechanism whereby the telomerase RNA template translocates and realigns with the DNA primer following synthesis of each repeat. Many factors affecting telomerase repeat processivity have been identified within TERT, TR and accessory proteins. Interestingly, the concentration of dGTP, and not other dNTPs, has been demonstrated to have a positive effect on telomerase repeat processivity. Despite this phenomenon being reported in ciliates and vertebrates, the underlying mechanism has continued to remain elusive. We recently proposed that the incorporation of the first nucleotide after RNA template-DNA primer realignment is a key determinate for human telomerase repeat processivity (Brown et al., P.N.A.S. 111:11311-6, 2014). We have tested this hypothesis by converting this first nucleotide incorporation from guanosine to adenosine. Our results surprisingly show that the nucleotide stimulation of human telomerase repeat processivity correspondingly shifted from dGTP-dependent to dATP-dependent. Thus increasing the concentration of the first nucleotide to be incorporated after template realignment overcomes the hindrance of telomerase first nucleotide addition and improves template translocation efficiency as well as repeat addition processivity. Our data provide an explanation for the dGTP-dependent stimulation of human telomerase processivity.

SPOTLIGHT ON NON-CANONICAL FUNCTIONS OF TELOMERASE IN PRIMARY CUTANEOUS T CELL LYMPHOMAS

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Cutaneous T-cell lymphomas (CTCL) are a group of lymphoproliferative disorder characterized by localization of neoplastic lymphocytes to the skin with no evidence of extracutaneous disease at the time of the diagnosis. As telomere length and telomerase activity were poorly studied in CTCL, in a first study, we demonstrated that aggressive CTCL are short telomere syndrome, telomerase positive tumors and that telomerase exerts functions beyond telomere maintenance (Chevret et al, Blood 2014). In this context, to better understand the non-canonical functions of Telomerase in CTCL, and to highlight the pathways implicated in these functions, we performed a trancriptomic analysis in CTCL cells over-expressing or not the Telomerase catalytic subunit (hTERT). Hundreds of target genes are modulated by hTERT. However we focused on those implicated in cell proliferation, apoptosis and migration. Interestingly, we observed an up-regulation of cell migration activators, a down-regulation of cell migration inhibitors, and a down-regulation of cell adhesion molecules (CAMs) suggesting a pro-migratory function of hTERT in CTCL. To go forward, transwell assays allowed us to analyze migration capacities: in endogenous CTCL cell lines and in cells over-expressing hTERT. Depending on cell line, hTERT strongly enhances cell migration capacities. We invalidated specific target genes, identified by the transcriptomic analysis, using lentiviral construction or drugs, to highlight the pathway regulated by hTERT in cell migration capacities. This study that explore the non-canonical functions of Telomerase, as well as their mechanism of action, will allowed to design effective therapeutic interventions against short telomere syndromes and telomerase-active cancers as primary cutaneous T cell lymphomas.

MINIMAL TELOMERE LENGTH IS MAINTAINED BY TELOMERASE

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Telomerase, an RNA-dependent DNA polymerase that consists of two essential components, a template RNA (TR) and a catalytic reverse transcriptase (TERT), plays an important function in chromosomal stability by maintaining telomere length. It has been demonstrated that the mutation of human telomerase can result in telomere shortening and telomere-related clinical syndromes. However, the genotypically wild-type child of telomerase-mutant also has shortening telomeres but does not develop telomere-related clinical syndromes. In the previous report, we had established a shortened-telomere $mTERT^{+/+}$ mouse model by intercrossing $mTERT^{+/-}$ mice for multiple generations. Through 18 generations of intercrossing $mTERT^{+/-}$ mice, telomere length shortened progressively, and overall telomere length of the late generations of mTERT+/+ mice as measured by flow-FISH. When crosses were carried out among these short-telomere genotypically wild-type offspring mice, or between these offspring and normal C57BL/6 ($mTERT^{+/+}$) mice, there was no elongation of the shortened-telomeres, even after as many as 6 generations of such crosses. In the present report, we will show telomere length continue to be shortened through 33 generations of intercrossing $mTERT^{+/-}$ mice, however, the shorten rate is decreased form 36 generations of intercrossing $mTERT^{+/-}$ mice. After 36 generations of intercrossing $mTERT^{+/-}$ mice, some $mTERT^{+/-}$ mouse has same telomere length with its littermate $mTERT^{+/+}$ mice. No elongation of the shortened-telomere is also observed in 35th generation $mTERT^{+/+}$ mice for 5 generations. No physiological defect in observed in late (38th) generation $mTERT^{+/+}$, $mTERT^{+/-}$ and $mTERT^{-/-}$ mice. It implicates the average telomere length $mTERT^{+/-}$ mice has been closed to the minimal telomere length after 36 generations of intercrossing $mTERT^{+/-}$ mice, therefore, the shorten rate of telomere length is decreased or ceased.

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MECHANISMS OF INTERCHROMOSOMAL HOMOLOGY SEARCHES DURING ALT TELOMERE RECOMBINATION

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Telomere maintenance by alternative lengthening of telomeres (ALT) requires recombination between telomeric sequences that results in net lengthening of chromosome ends. We have previously reported that this recombination can occur following long-range directional movement and synapsis of non-sister telomeres, and that this process depends on the recombinase Rad51 and Hop2-Mnd1 heterodimer. However, Hop2 regulation and function in ALT telomere recombination remains to be investigated. Here, we identify several potent regulators of Hop2 localization to ALT telomeres. Furthermore, we provide new data demonstrating the function of Hop2 in controlling recombination between non-sister telomeres. These results further define a specialized homology searching mechanism in ALT-dependent telomere maintenance, which frequently relies on non-sister recombination in contrast to canonical homologous recombination.

A GENOME-WIDE SCREEN REVEALS THAT SISTER CHROMATID COHESION, DOUBLE-STRAND BREAK REPAIR AND TELOMERE MAINTENANCE ARE IMPORTANT FOR BYPASS OF THE ESSENTIAL CST COMPONENT CDC13

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CST (Cdc13, Stn1, Ten1) caps the budding yeast telomere to prevent activation of the DNA damage response in budding yeast. Deletion of any of the CST components is normally lethal. We have previously shown that the requirement for Cdc13 can be bypassed in a strain lacking *NMD2* (required for nonsense-mediated mRNA decay) and the exonuclease *EXO1*. However, the other CST proteins Stn1 and Ten1 could not be deleted in the same genetic background. To uncover the mechanisms underlying Cdc13 bypass, we conducted a genome-wide screen using robotic synthetic genetic array (SGA) technology to cross an *nmd2*Δ *exo1*Δ *cdc13*Δ strain with a library of single gene deletion strains. The resultant diploids were sporulated and *nmd2*Δ *exo1*Δ *cdc13*Δ *yfg*Δ haploids were selected by successive pinning to selective media using a robot. A second $CDC13^{+}$ control screen, using an *nmd2*Δ *exo1*Δ strain, was carried out to obtain *nmd2*Δ *exo1*Δ *yfg*Δ haploids. Colony size of the final haploids was measured in both SGA screens and compared. The screens revealed that genes relating to sister chromatid cohesion (*CTF4, CTF8, CTF18, DCC1, TOF1*) and DNA repair (*RAD51* and *RAD55*) were important for bypassing Cdc13. The telomere maintenance protein Rif1 was also essential for Cdc13 bypass, indicating that Rif1 functions in telomere length regulation, checkpoint repression or DNA replication are required.

EVIDENCE FOR TELOMERASE- AND RAD52-INDEPENDENT SEQUENCE ALTERATIONS AT YEAST TELOMERES.

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In the yeast *Saccharomyces cerevisiae*, cells lacking telomerase senesce after 60-80 generations but a small subset of cells can overcome senescence using recombination-mediated mechanisms to become 'survivors'. Two main types of survivors have been described: type I and type II. Both types require Rad52, a protein necessary for almost all recombination events in yeast, and Pol32, which is needed for break-induced replication. Type I survivors involve the amplification of subtelomeric elements, while type II survivors resemble the majority of human ALT cancer cells in that they both exhibit amplification of the terminal telomere repeats. Recombination proteins are also important in pre-senescent cells, before the formation of survivors. Telomerase mutants lacking Rad52 exhibit accelerated senescence, but the shortening rate of telomeres is unchanged. The precise function of recombination proteins in pre-senescent cells is unknown. Using a telomere sequencing approach, telomere recombination events have been detected in pre-senescent cells, although at a much lower frequency than in survivors. Surprisingly, we have found that these events still occur in *rad52Δ* and *pol32Δ* mutants. We are currently studying how these events occur as well as their significance with respect to telomere maintenance.

ELECTRON MICROSCOPY STUDY OF THE HUMAN TELOMERASE ENZYME COMPLEX

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Following our report determining the composition of the core human telomerase enzyme complex, consisting of two molecules each of: i) hTERT; ii) hTR; and iii) dyskerin (*1*), we established an over-expression system in suspension HEK-293T cells that yields ~500-fold greater activity over endogenous levels on the 20-Litre scale. This system has provided sufficient telomerase for negative-stain electron microscopy. Using purified telomerase obtained with our activity-dependent elution (*1*), we collected uranyl formate-stained micrographs and have processed ~25,000 particles to provide a low-resolution (~30 Angstrom) structure. Consistent with a previous report (*2*), the data reveal an elongated bi-lobal dimeric structure that displays significant conformational heterogeneity. Current efforts aim to obtain cryo-EM data to enable a higher-resolution structure.

(*1*) Cohen SB, et al. (2007) *Science* 315, pp 1850-1853.
(*2*) Sauerwald A, et al. (2013) *NSMB* 20, pp 454-460.

PROTECTIVE ROLES OF CDC13 AND RAP1 AGAINST DEGRADATION OF TELOMERIC SINGLE-STRANDED 3' OVERHANGS

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Telomeres are terminal structures in eukaryotic chromosomes composed of stretches of repetitive DNA ending with a TG-rich single-stranded (ss) 3' overhang and its associated proteins. This nucleoprotein cap structure protects the DNA ends from nucleolytic degradation and recognition by DNA repair proteins, and prevents end-to-end chromosome fusions. The budding yeast telosome includes the Rap1 and Cdc13 proteins, which bind to the double-stranded (ds) DNA and ss 3' overhangs, respectively. Cdc13 plays a dual role in regulating the telomere lengths by either recruiting telomerase or by forming the telomerase inhibitory CST complex (Cdc13-Stn1-Ten1 complex).
We have previously defined the minimal binding sites (MBS) for Rap1 and Cdc13 on *Saccharomyces castellii* telomeric DNA and we have determined that a minimal 10 nt 3' overhang length is necessary for a stable Cdc13 binding (Rhodin et al., 2006, 2011). We have also shown that Rap1 can bind over the ds-ss DNA junction and compete with Cdc13 for binding to the ssDNA close to the junction (Gustafsson et al., 2011). In combination with our results showing that the S. castellii 3' overhangs vary drastically in length in the cell cycle, there is a possibility for a loss of Cdc13 binding sites at telomeres with very short 3' overhangs. However, the binding to the ds-ss junction would possibly allow Rap1 to protect such short 3'overhangs where Cdc13 cannot bind stably.
To study the role of Cdc13 and Rap1 in the protection of the ss-3' overhangs against 3'-5' exonucleases, we have developed an in vitro DNA end protection assay (DEPA). We found that Cdc13 on its own was able to provide protection to the single-stranded 3' overhangs against degradation by various exonucleases, when analyzed on a short overhang of 20 nt. Interestingly, Cdc13 bound to its MBS conferred protection to 6 nt beyond the MBS in the 3' overhang. Surprisingly, Rap1 binding over the ds-ss junction showed remarkable protection of the ssDNA beyond its MBS at the ds-ss junction. However, protection of the ss-overhang was dependent on the sequence permutation at the ds-ss junction. Our studies argue that both Rap1 and Cdc13 would be important players in providing protection to short telomeric ss 3' overhangs.

References:
Rhodin J, Astromskas E and Cohn M (2006) J. Mol. Biol. 355, 335-346
Rhodin J, Gustafsson C and Cohn M (2011) Genome Integrity 2, 2
Gustafsson C, Rhodin J and Cohn M (2011) J. Biol. Chem. 286, 45174-85

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INVESTIGATION OF GENES AFFECTED BY TELOMERE SHORTENING IN OSTEOARTHRITIS

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Osteoarthritis is a chronic degenerative joint disorder and a major cause of disability in the elderly, which eventually leads to loss of joint function, pain and immobility. Primary osteoarthritis has a very strong genetic component; however, only a small number of osteoarthritis risk alleles have been identified, and these explain only a small percentage of all osteoarthritis cases. The silencing effect of telomeres on the genes located nearby is well known and is disrupted by shortening of telomeres. We investigated whether telomere shortening contributes to the development of osteoarthritis through accelerated expression of telomere-proximal genes. Our studies were conducted on patients with knee osteoarthritis and our experiments were performed on affected and unaffected cartilage collected from the same joint of each patient. We measured via qPCR relative telomere length in affected and unaffected chondrocytes obtained from fifty patients with knee osteoarthritis during joint replacement surgery and detected severe shortening of telomeres in affected cartilage. Using RNA-Seq, we found an increase in the expression of subtelomeric genes in these osteoarthritis patients. The analysis of telomere shortening effect on over-expression of telomere-proximal genes has not been previously explored in the etiology of osteoarthritis, and it may reveal new factors involved in the initiation and progression of this disorder. This research was funded by NIH (NIGSM), Mentoring Research Excellence in Aging and Regenerative Medicine grant No. P20GM103629:552729 to M. Czarny-Ratajczak.

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EPIGENETIC REGULATION OF 2-CELL GENE ACTIVATION AND TELOMERE LENGTH HOMEOSTASIS IN PLURIPOTENT STEM CELLS

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Maintenance of telomere length is essential for self-renewal and pluripotency of embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. How telomere length and homeostasis are regulated in mouse ES cells remains elusive. Mammalian telomeres and subtelomeres are marked by heterochromatic epigenetic modifications, including repressive DNA methylation and histone methylation (H3K9me3 and H4K20me3). Interestingly, a small subpopulation (1-5%) of mouse ES cells sporadically activate two-cell (2C) embryo genes, including Zscan4, required for telomere elongation. The molecular regulation of this process remains poorly understood. We found that Rif1, which is highly expressed in ES cells, plays a novel role in repressing 2C-genes and Zscan4 by stabilizing H3K9 methylation complex-mediated heterochromatic silencing, preventing terminal hyper-recombination, and thus maintaining telomere length homeostasis and chromosomal stability of ES cells. Tbx3 (a T- box transcription factor), heterogeneously expressed in mouse ES cells, can elongate telomeres. Tbx3 activates Zscan4+/2C genes by reducing Dnmt3b and DNA methylation at subtelomeres.
Recently, we showed that trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, which improves nuclear transfer cloning efficiency, also facilitates telomere reprogramming and elongation. Additionally, another HDAC inhibitor (sodium butyrate, NaB) enhances iPS cell induction and quality. It is unclear whether histone acetylation also regulates telomere length in pluripotent stem cells. Using chemicals with specific effects on histone acetylation, we find that histone hyperacetylation dramatically elongates telomeres in ES cells, but only slightly in Terc-/- ES cells, suggesting that Terc is involved in histone acetylation-induced telomere elongation. Histone hypoacetylation shortens telomeres in both wide-type and Terc-/- ES cells. Additionally, histone hyperacetylation activates Zscan4 and 2C-specific genes, whereas histone hypoacetylation represses Zscan4 and 2C genes. These data suggest that histone acetylation affects the heterochromatic state at telomeres and subtelomeres, and regulates the expression of nearby genes, including Zscan4. Together, epigenetic modifications, including heterochromatic DNA methylation, histone acetylation and repressive histone (e.g. H3K9me3) silencing, coordinate to ensure proper expression of 2C genes and Zscan4 for telomere length maintenance and homeostasis in ES cells, implying an important role for telomere position effect (TPE) in pluripotent stem cells.

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THE ROLE OF METHYLTRANSFERASES IN TELOMERE DAMAGE RESPONSES

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Introduction
Telomeres are complex structures of DNA, RNA and proteins that cap chromosome ends and protect them from being recognized as DNA double-strand breaks. Loss of telomere protection leads to the activation of DNA-damage checkpoints and the processing of deprotected chromosome ends by DNA-repair factors. These repair activities can result in the formation of telomere fusions and dicentric chromosomes and thereby contribute to genomic instability and tumorigenesis. However, the precise mechanisms by which dysfunctional telomeres lead to chromosomal instability and cancer remain largely unknown.

Aim
DNA-damage recognition and repair act in the context of chromatin and are controlled by the post-translational modification of histones, including methylation. We hypothesized that the methylation of histones or other proteins might contribute to repair activities at telomeres and thereby control telomere-driven genomic instability. We addressed this by performing a functional genetic screen in which we inactivated the telomeric protein TRF2 to induce telomere uncapping in combination with shRNA-mediated knockdown of different methyltransferases. Through this approach, I identified several SET-domain containing histone methyltransferases (HMTs) that upon their inhibition alleviate telomere uncapping-induced lethality resulting from severe telomere fusion. We have followed up on one of these HMTs in more detail and found that it is significantly contributes to telomere-induced genomic instability by promoting the formation of chromosome end-to-end fusions. This was dependent on its enzymatic activity enabled by the SET domain. The aim of my project is to further understand the role of this and other HMTs in the telomere damage response.

Future
The identification of multiple SET-domain containing HMTs that contribute to telomere-driven genomic instability indicates a critical role for methylation in the response to telomere deprotection. Further studies on these HMTs will increase our understanding of how telomere uncapping results in telomere fusions and genomic instability.

INDUCED TELOMERE DYSFUNCTION UNCOVERS A SECOND 53BP1/RIF1-INHIBITED DSB 5' RESECTION PATHWAY

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Resection of 5' ends, a critical step in the repair of DNA double-strand breaks (DSBs), is stringently controlled to prevent genome instability. The known DSB resection pathway is stimulated by ATM signaling, initiated by CtIP, and involves the BLM helicase, DNA2 and Exo1. Rif1 bound to 53BP1 blocks inappropriate DSB resection by the ATM/CtIP pathway, whereas at telomeres this resection is primarily inhibited by the shelterin protein TRF2. Here we use engineered dysfunctional telomeres to identify a second 5' end resection pathway. Telomeres lacking the single-stranded (ss) telomeric repeat binding protein POT1 and its TPP1 binding partner are processed by a 5' resection pathway that is stimulated by ATR signaling and involves BLM and Exo1. We show that this ATM-independent pathway can act at sites of replication stress and is blocked by Rif1. Thus, mammalian cells employ 53BP1-bound Rif1 to prevent inappropriate resection by two distinct pathways. At telomeres, these resection pathways are repressed by two different shelterin-based mechanisms.

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TERRA, PGC-1α AND NRF1: A NEW LINK BETWEEN TELOMERES AND METABOLISM

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Telomeres are transcribed into non-coding RNA species dubbed TERRAs. TERRAs appear to play multiple roles at telomeres and have been involved in heterochromatin regulation, T-loop formation and, more generally, in telomere protection. In human cells, TERRA levels oscillate during cell cycle progression, a feature that is likely to be important for the switch between RPA and POT1 at telomeres after completion of S phase. So far, the identity of transcription factors involved in human TERRA production has remained elusive. Here, we report on the role of NRF1 (Nuclear Respiratory Factor 1) in human telomere transcription. NRF1 being activated by the AMPK (5' AMP-activated protein kinase) pathway involving PGC-1α, we showed that both increased AMP/ATP ratio and PGC-1α overexpression are strong up-regulators of TERRA production. We are investigating the role of AMPK and NRF1 in protection of telomeres against damages and in cell cycle regulation of TERRA. Altogether, our findings reveal new links between metabolism and telomeres that we are currently extrapolating to in vivo models. Put in the context of caloric restriction and physical exercise, two activators of the AMPK-PGC-1α axis, these new observations fit within the proposed theories of ageing.

INFLUENCE OF TELOMERE DYNAMICS ON DISEASE PROGRESSION AND THERAPEUTIC RESPONSE IN BONE MARROW FAILURE SYNDROMES.

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Inherited bone marrow failure (IBMF) syndromes are a group of disorders associated with insufficient production of hematopoietic cells and are characterized by a predisposition for malignancies including myelodysplastic syndrome and acute leukemia. A majority of these disorders including aplastic anemia and dyskeratosis congenita are characterized by defects in telomere maintenance and excessively short telomeres. Studies have demonstrated an association between shortened telomeres, advanced disease and increased risk of developing blood cancers. Heterozygous mutations in the gene encoding the telomerase protein component hTERT, are seen in 5-15% of patients with IBMF, resulting in shortened telomeres and advanced disease. Loss of function of one autosomal copy of hTERT is sufficient to reduce telomerase levels and accelerate telomere attrition. The degree of inactivity is variable among mutations as is the subsequent disease phenotype. However, how these telomerase mutations and shortened telomeres impact disease progression and response to therapeutics is not well understood.

To understand the biochemical properties and cellular consequences of mutant hTERT expression we have generated expression constructs that correspond to hTERT mutations found in patients with bone marrow failure syndromes (A202T, H412Y, K570N, P704S, R979W, K1050N, and A1062T). These mutations have been identified in patients with family history or clonal evolution of disease and are all located in functionally distinct regions of the protein. We have demonstrated that the hTERT mutants retain varying levels of telomerase activity *in vitro* and we are investigating the consequences of their expression in BJ fibroblasts and the leukemic cells line THP-1. Expression of mutant hTERT proteins in THP-1 cells results in the expression of varying phenotypes *in vitro* including distinct morphological and cell cycle changes. Most notably, expression of the A202T or A1062T mutant hTERT protein results in a delay of the G1/S transition, which may have profound implications during hematopoiesis, negatively impacting the development and differentiation of mature blood cells. Although THP-1 cells have moderate endogenous telomerase activity and maintain telomere length, expression of our hTERT mutant proteins differentially influences the telomere maintenance. Additionally, specific mutants expressed in THP-1 cells are more resistant to chemotherapeutic agents suggesting that treatment protocols might have differential efficacies depending whether cells express wild-type or mutant hTERT proteins. Additional assays are being performed to characterize biochemical properties of the mutations, and to examine the function in hematopoiesis.
By defining the role of telomeres in hematological disorders, it may be possible to alter treatment strategies based on predicted outcomes from our investigations.

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ARMADILLO/BETA-CATENIN PROTEINS MAY FUNCTION IN TERT RECRUITMENT INTO ITS NON-TELOMERIC PATHWAYS

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In addition to telomeres elongation, telomerase reverse transcriptase (TERT) may be involved in the regulation of a number of cellular processes such as gene transcription, cell cycle or cell proliferation. How these regulatory processes are executed on the molecular level is a challenging question of current telomerase research.

When screening a bimolecular fluorescence (BiFC) cEYFP cDNA library for the protein-protein interactions of the CTE domain of *At*TERT, we identified an armadillo/beta-catenin like repeat containing protein (ARM) interacting in the cytoplasm of the tobacco BY2 protoplasts. Using BiFC and Y2H, we have analyzed the interaction pattern of the ARM protein which turned to interact with chromatin remodeling factors and two groups of Myb-domain containing proteins previously described as telomere-binding proteins and/or transcription factors, suggesting a putative dual role of ARM-TERT interaction in the regulation of gene transcription.

We have further analyzed T-DNA insertion mutants in the arm gene. Analysis of telomeres length and telomerase activity suggests rather a non-telomeric function of the ARM-TERT interaction. Interestingly, RT-qPCR experiments on the mutant plants suggest an involvement of the ARM protein in transcription regulation of genes important for the DNA-damage response and genes driven by a telobox containing promoter that were confirmed using a luciferase assay.

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MECHANISMS OF END-PROTECTION AND THE RESPONSE TO TELOMERE-INTERNAL DOUBLE STRAND BREAKS

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The TRF2 component of shelterin blocks Ku70/80-dependent cNHEJ and MRN-dependent ATM signaling. The t-loop model suggests that TRF2 protects telomeres by forming t-loop structures where the chromosome end is “invisible” to end-loading factors, such as Ku70/80 and MRN, that mediate the DNA Double Strand Break (DSB) response. In agreement with this model, we recently showed that TRF2, but no other component of shelterin is required for t-loop formation. However, TRF2 has also been shown to directly interfere with the DNA damage signaling and repair pathways, suggesting the existence of multiple mechanisms at work to ensure full protection of telomeres.
Here we aim to test the contribution of t-loops to end protection by studying the response to telomere-internal double strand breaks. The t-loop model predicts that a DSB inside the telomeric repeat array, featuring DNA ends that lack the correct terminal structure, will activate the DNA damage response, despite the presence of TRF2 and the rest of shelterin. We used the FokI nuclease tethered to the shelterin factor TRF1 to induce DSBs inside mouse telomeres. Consistent with the t-loop model, Fok1 cleavage generated telomere-internal DSBs that indeed induced the ATM kinase pathway. Thus, shelterin is incapable of fully repressing ATM at these DNA ends. We are currently studying the DSB repair pathways in these conditions.

EVALUATION OF TELOMERASE ACTIVATOR TA-65 IN EARLY MACULAR DEGENERATION.

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Telomere attrition and corresponding cellular senescence of the retinal pigment epithelium (RPE) likely contribute to early age-related macular degeneration (ARMD). Activation of the enzyme telomerase can add telomeres to RPE chromosomes and telomerase activation has been proposed as a treatment for ARMD. We report the use of TA-65, an oral telomerase activator, in early macular degeneration. 38 patients were randomly assigned to a one year, double-blinded, placebo-controlled interventional study with arms for oral telomerase activator TA-65 or placebo. Macular functions via micro-perimetry with the MAIA testing instrument were the primary measured outcomes. In the two macular function parameters tested, the arm receiving the telomerase activator showed significant improvement: average threshold sensitivity improved (p-value 0.02), and percent reduced thresholds lessened (p-value 0.04) at six months compared to the placebo arm. The improved function was maintained at twelve months while in the placebo group it worsened. This study was a pilot and a larger, confirmatory study is being planned.

hTERT REGULATION IN PEDIATRIC MEDULLOBLASTOMA

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Telomerase is present in the majority of human cancers and its activation correlates tightly with the expression of its catalytic subunit hTERT. Transcriptional regulation of the *hTERT* gene is the major determinant of cancer-specific activation of telomerase. The mechanisms of *hTERT* upregulation in carcinogenesis remain unclear. Moreover, hTERT has non-telomeric functions including gene expression regulation, DNA damage repair, cell survival and metabolism. Understanding the mechanisms of *hTERT* expression in cancer is critical to identifying novel therapeutic targets to treat patients with devastating diseases, such as brain tumors.

Medulloblastoma (MB) is the most common malignant brain tumor in children and is comprised of four subgroups with various molecular signatures and variable clinical outcome: Wingless (WNT), Sonic hedgehog (SHH), group 3 and group 4. The prevalence and mechanism of *TERT* regulation and their correlation with survival in MB are unknown. We conducted a multi-institutional retrospective study of telomerase expression and telomere maintenance in newly-diagnosed pediatric MB.

Our data indicate that *TERT* is highly expressed in MB and patients with high levels of *TERT* had a significantly worse overall survival (OS). *c-MYC* amplification was unique to group 3 while overexpression was primarily seen in WNT and Group 3; *c-Myc* amplification and overexpression positively correlated with *TERT* expression. *TERT* promoter mutations were identified and correlated with high levels of *TERT* expression. Methylation of the *TERT* promoter was found in all subgroups and correlated with *TERT* overexpression. Interestingly, we found that a third of patients in our cohort use multiple *TERT* regulation mechanisms.

Consistent with other tumor types, we show a correlation between *TERT* expression and shorter OS in MB. Our data suggest that *TERT* regulation involves multiple mechanisms that are not mutually exclusive. Furthermore, we demonstrate that *TERT* expression is a subgroup-independent prognostic factor. We provide new insight into *TERT* regulation and demonstrate its potential as a therapeutic target across all subgroups of MB in pediatric MB.

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TELOMERECAT: AN ALGORITHM FOR ESTIMATING THE LENGTH OF TELOMERES FROM WHOLE GENOME SEQUENCING SAMPLES.

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Cancer WGS data is being produced at a rate unthinkable even a few years ago. The ability to estimate the length of telomeres from whole genome sequencing WGS data is therefore a hot topic in the study of telomeres and cancer.

All of the previous approaches to estimating the average telomere length in WGS samples have counted reads containing a set threshold of the repeating telomere hexamer. In this way they measure abundance of telomere, but can only relate this to length through fore-knowledge of the number of telomeres. Thus they cannot be applied to cross-species studies or to cancer samples that exhibit aneuploidy.

We introduce telomerecat, a different type of method for estimating average telomere length (TL) from cancer WGS data. Telomerecat is a principled approach that utilises knowledge of sequencing technologies to, not only allow for aneuploidy in cancer samples, but also to account for Interstitial telomeric sequences and GC biases. Additionally, Telomerecat also works on non-human WGS samples. Telomerecat confirms published TL heterogeneity between two strains of wildtype derived inbred mice.

In diploid control samples, Telomerecat shows good correlation with other validated approaches to this problem, but demonstrates that these would be misleading when applied to cancer data. Telomerecat has been used to uncover TL heterogeneity within Prostate cancer samples from the International Cancer Genome Consortium and to show associations with other omics data. It has also been applied to an investigation of the progression of oesophageal adenocarcinma, where a recent publication has implicated telomere-driven aberrations in a third of cases.

SHORT TELOMERES IN KEY TISSUES TRIGGERS LOCAL AND SYSTEMIC AGING IN ZEBRAFISH

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Telomere erosion in aging functions as a tumor suppressor mechanism, but also depletes the stem cells required for tissue homeostasis. It remains unclear if this phenomenon constitutes a primary cause of aging.
Here, we analyze wild type and telomerase mutant zebrafish to determine in which tissues telomeres shorten with age, triggering cellular damage that leads to organ dysfunction. Juvenile *tert-/-* mutant telomeres can be used to predict the telomeric length at which tissue dysfunction should arise in old individuals. We show that accumulation of short telomeres in gut and muscle of WT and *tert-/-* precedes the activation of DNA damage responses, intestinal inflammation and progressive sarcopenia. In contrast, the gonads and kidney marrow fail to shorten their telomeres prior to accumulation of DNA damage markers and onset of tissue function defects. Surprisingly, *tert-/-* mutants also suffer accelerated onset and increased incidence of tumorigenesis. Thus, tissue-specific telomere length is limiting for physiological integrity and leads to tissue degeneration in aging.

ALTERNATIVE LENGTHENING OF TELOMERES RENDERS CANCER CELLS HYPERSENSITIVE TO ATR INHIBITORS

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Cancer cells rely on telomerase or the alternative lengthening of telomeres (ALT) pathway to overcome replicative mortality. ALT is mediated by recombination and is prevalent in a subset of human cancers, yet whether it can be exploited therapeutically remains unknown. Loss of the chromatin-remodeling protein ATRX associates with ALT in cancers. Here, we show that ATRX loss compromises cell-cycle regulation of the telomeric noncoding RNA TERRA and leads to persistent association of replication protein A (RPA) with telomeres after DNA replication, creating a recombinogenic nucleoprotein structure. Inhibition of the protein kinase ATR, a critical regulator of recombination recruited by RPA, disrupts ALT and triggers chromosome fragmentation and apoptosis in ALT cells. The cell death induced by ATR inhibitors is highly selective for cancer cells that rely on ALT, suggesting that such inhibitors may be useful for treatment of ALT-positive cancers.

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CHROMOSOMAL SINGLE STRANDED TELOMERIC TRACTS AND BULKY RNA-DNA–HYBRIDS IN HUMAN TELOMERASE POSITIVE AND ALT CELL LINES

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To evaluate the extent of chromosomal single-stranded DNA (ssDNA) at human telomeres and to reveal telomere maintenance-related RNA-DNA interactions, we performed non-denaturing PNA fluorescence in situ hybridization (ND-FISH) in metaphase preparations from a panel of continuous human cell lines. Without RNAse-A pre-treatment, PNA (Peptide Nucleic Acid analogue) fluorescence signals specific for C-and G-rich telomeric strands were detectable at mitotic telomeres of cells utilizing the alternative lengthening of telomeres (ALT), whereas in telomerase positive cell lines no signals were detected. ND-telomeric PNA FISH after RNAse-A treatment, showed extensive presence of G- and C-rich telomeric ssDNA, in both telomerase positive and ALT cell lines that was increased in frequency and intensity in ALT cells. RNAse-A treatment, had no effects in the ND-FISH hybridization patterns of two PNA centromere specific probes. Stable reconstitution of telomerase activity and suppression of the ALT pathway in VA-13 cells, was associated with suppression of ND telomere fluorescence at C- and G-rich strands, suggesting that continuous cellular growth, is associated with extensive tracts of chromosomal telomeric ssDNA and increased RNA-DNA interactions at both telomere strands, while the abundance of “unmasked” telomeric C-rich ssDNA, may be related to increased telomeric recombination that is a hallmark of ALT. We tested these hypotheses by overexpressing UPF1 that promotes dissociation of TERRA from telomeres in telomerase positive and ALT cells. In both types of telomere maintenance, overexpression of UPF1 was associated with significantly increased C-rich single stranded telomeric DNA in absence of RNAse-A treatment, and with increased rates of sister chromatid exchanges at the ALT telomeres. Hence, in telomerase positive cell lines most of chromosomal telomeric ss-DNA associates with complementary RNAs to form DNA/RNA hybrids and to suppress telomeric recombination, whereas in the ALT pathway, telomere recombination is facilitated by C-rich RNA-free telomeric ssDNA.

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TRF2-INDUCED DNA WRAPPING: A NEW MECHANISM FOR TELOMERE PROTECTION

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Through the years, our team has studied the biochemical properties of the human shelterin protein TRF2 and has unveiled several intrinsic properties of this protein that we believe important for its biological role. In the present study, we focused on the capacity of this protein to condense DNA. We reveal that this condensation is due to the right-handed wrapping of ~90 bp of DNA around the TRFH domain of TRF2. DNA wrapping involves lysines and arginines which mutation severely reduces TRF2 capacity to modify DNA topology and to stimulate single strand invasion. As expected, since these properties do not depend on DNA topology, telomere specific DNA binding and Holliday junction formation/migration are untouched. Expression of this separation-of-function mutant in human cancer cells causes alteration of telomeres topology but protection against NHEJ, telomere length as well as G-overhang length and TERRA levels are untouched. Importantly, we do observe recruitment of DNA damage signaling factors which indicate that the capacity of TRF2 to wrap DNA contributes to telomere capping.

A NEW FACTOR INVOLVED IN 5' END RESECTION AND ATR SIGNALING

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The ends of mammalian chromosomes are protected by shelterin complex to suppress DNA damage and repair pathways. Similar to general double-strand breaks (DSBs), deprotected telomeres are recognized as damage sites and activates DSB response, moreover, these ends are vulnerable to repair pathways, which threatens genome integrity.

Here we report the identification of a novel mediator of DNA end resection, RARE1 (Resection and ATR signaling promoting RPA and EXO1 interacting protein 1), which was identified in the course of searching for factors associated with dysfunctional telomeres. We found that RARE1 localizes to sites of damage and forms nuclei foci at both dysfunctional telomeres after TRF1 or POT1 deletion, as well as at genome-wide lesions that activate ATR signaling. Under both conditions, RARE1 foci co-localized extensively with RPA, suggesting it is involved in ATR signaling pathway. Furthermore, time course experiment of RARE1 foci formation in response to IR suggested RARE1 slowly accumulates to DSBs at the time resection takes place.

Downregulation of RARE1 by shRNA oligos leads to diminished ATR activation, RPA accumulation at sites of DNA damage, and Chk1 phosphorylation. We also show that downregulation of RARE1 diminishes EXO1 dependent resection, and consequently increases radial formation after PARP inhibitor treatment. Through affinity purification of RARE1 protein complex, we identified interaction between RARE1 with RPA and EXO1. GST pull down and *in vitro* reconstitution assay further confirmed the direct binding between RARE1 with RPA complex and EXO1. Consistent with the *in vivo* data, biochemical analysis indicates that RARE1 stimulates EXO1 activity on RPA containing substrates.

Taken together, these results suggest that RARE1 promotes resection through direct interaction and/or recruitment of EXO1 to the small amount of RPA that binds after the first resection step. By stimulating the formation of longer tracts of ssDNA, RARE1 further promotes full ATR activation.

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N-TERMINAL BAT DOMAIN OF RIF2 IS NECESSARY AND SUFFICIENT TO LIMIT TELOMERE ELONGATION

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Maintaining telomere length equilibrium is essential for all eukaryotes. This equlibrium is established though a regulated interaction of telomere binding proteins with telomerase and likely other factors. In yeast, Rap1 binds along the length of the telomeric DNA and recruits Rif1 and Rif2 through protein interactions within its C-terminal domain. Rif1 and Rif2 both negatively regulate telomere length throught distinct genetic pathways (1). When either protein is deleted, telomeres are over-elongated by telomerase. If both are deleted, telomere elongation is additive. The molecular mechanism that underlies this regulation is not yet clear. To further understand the function of Rif2, we carried out scanning mutagenesis of the endogenously expressed protein. The majority of the mutations that resulted in long telomeres were located in the N-terminal region of Rif2. We generated a fusion construct that attached 60 aa of this N terminal region directly to Rap1. This Rap1-Rif260 fusion protein fully rescued the long telomeres in a rif2Δ mutant, indicating this domain is both necessary and sufficient to block telomere elongation. We termed this domain BAT (Blocks Addition of Telomeres) and further found this domain blocks telomere elongation in both rif1Δ and RAP1ΔC-terminal deletion mutants. This suggests the mechanism that blocks elongation is not pathway specific. Surprisingly, mutation of a single amino acid within the BAT domain, a phenylalanine at position 8 to alanine, abrogated function of this domain resulting in long telomeres. Substitution of F8 with either tryptophan or tyrosine restored telomere length regulation suggesting these aromatic amino acids mimicked the wildtype phenylalanine and represent a protein interaction site. These studies establish a minimal functional region that limits telomere elongation and have implications for telomere length in other organisms.

1. Wotton, D., and Shore, D. (1997). A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in Saccharomyces cerevisiae. Genes Dev 11, 748-760

THE SILENT CHROMATIN PROTEIN SIR4 IS REQUIRED FOR KU-MEDIATED TELOMERASE RECRUITMENT TO TELOMERES IN *SACCHAROMYCES CEREVISIAE*

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In *Saccharomyces cerevisiae* and in humans, the RNA subunit of telomerase is bound by the Ku heterodimer, a complex best known for its function in DNA repair. Ku bound to yeast telomerase RNA (TLC1) promotes telomere lengthening and telomerase recruitment to telomeres. Because Ku cannot bind telomeric DNA while bound to TLC1 RNA, Ku likely promotes telomerase recruitment through interaction with a telomere-associated protein. In yeast, Ku is required for telomeric transcriptional silencing and binds to Sir4, a structural component of telomeric silent chromatin. Also, cells without Sir4 have been shown to exhibit shorter telomeres. Here, we report that deleting *SIR4* does not cause further telomere shortening in cells lacking TLC1-bound Ku. Additionally, a TLC1 allele containing three Ku-binding sites, TLC1$(Ku)_3$, causes progressive telomere hyper-lengthening that is *SIR4*-dependent. Telomerase recruitment to telomeres, as measured by performing ChIP on TERT, is as low in *sir4*Δ cells as it is in cells with Ku-binding-defective TLC1. Furthermore, TLC1$(Ku)_3$, which causes a 10-fold increase in telomerase recruitment in a wild type background, does not cause a significant increase in recruitment in a *sir4*Δ background. We also find that tethering Sir4 directly to Ku-binding-defective TLC1 RNA restores otherwise-shortened telomeres to wild type length. Tethering Sir3, another structural component of telomeric silent chromatin not known to bind Ku, to the same RNA, however, does not have this effect. In summary, these results support a model in which Sir4 is the telomeric target of Ku-mediated telomerase recruitment, suggesting a previously unappreciated link between telomeric silent chromatin and telomerase regulation.

ALTERNATIVE-LENGTHENING-OF-TELOMERES ACTIVITY IS INCREASED BY DNA DAMAGE

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Alternative-lengthening-of-telomeres (ALT) is a telomere maintenance mechanism (TMM) used by approximately 10% of cancers. Little is known about the regulation of ALT activity. We have treated ALT+ cancer cell lines with various types of DNA damaging agents and found that both total telomeric DNA and the ALT-specific biomarker, C-Circles, increased after DNA damage. Increases in both were dependent on an active ALT mechanism and DNA synthesis by α-type polymerases. The DNA repair proteins ATM and BLM were also required. The increase in either C-Circles or telomeric DNA is not detectable until six hours after γ-irradiation and then both steadily increased for three days. This indicates that DNA damage in ALT cells resulted in an increase in ALT activity. The same DNA damage in telomerase-positive cell lines did not cause an increase in telomerase activity, indicating that the two TMMs respond differently to DNA damage. The increase in ALT activity by DNA damage was associated with a growth arrest and was also dependent on a component of the cell cycle arrest pathway, p21. However, cell cycle arrest alone did not cause an increase in ALT activity. Further investigation of the pathways involved in regulating ALT activity may help design of ALT-targeted anti-cancer treatments.

BIOCHEMICAL AND STRUCTURAL STUDIES OF TELOMERE BOUQUET COMPLEX IN FISSION YEAST

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Telomeres attach to the inner nuclear envelope and cluster to form the bouquet during meiotic prophase, which is conserved in all eukaryotes. The bouquet formation has been suggested to facilitate homologous chromosomes pairing and synapsis [1,2]. However, the molecular mechanism of telomere clustering and movement on nuclear envelop is poorly understood, partly due to lack of the structure information of bouquet complex. In *Schizosaccharomyces pombe*, the telomere bouquet complex is composed of Bqt proteins (Bqt1/2/3/4) [3,4], the telomere protein Rap1 and a SPB (Spindle Pole Body) protein Sad1 [3,5,6]. Here we identified the minimum Rap1 fragment that increases the solubility and stability of Bqt1-Bqt2 heterodimer, which enables us to purify the stable bouquet complex for structural studies. We have successfully purified ternary complexes containing Bqt1, Bqt2 and Rap1 from three species of fission yeast, and characterized the interaction with the SPB protein Sad1. Meanwhile, Rap1 binds to another nuclear membrane protein Bqt4 using a different interaction interface which is compatible with Bqt1/Bqt2 interaction. We also demonstrated that Bqt4 is a DNA-binding protein and can specifically recognize telomeric dsDNA, indicating a possible role of Bqt4 in recruitment of telomere to nuclear membrane at early stage. Future structural elucidation of the telomere bouquet complex will shed light on the molecular mechanism of telomere clustering during meiosis.

1 Harper, L., Golubovskaya, I., and Cande, W. Z. (2004). A bouquet of chromosomes. Journal of cell science 117, 4025-4032.
2 Scherthan, H. (2001). A bouquet makes ends meet. Nature reviews. Molecular cell biology 2, 621-627.
3 Chikashige Y., Tsutsumi C., Yamane M., Okamasa K., Haraguchi T., and Hiraoka Y. (2006). Meiotic proteins bqt1 and bqt2 tether telomeres to form the bouquet arrangement of chromosomes. Cell 125, 59-69.
4 Chikashige Y., Yamane M., Okamasa K., Tsutsumi C., Kojidani T., Sato M., Haraguchi T., and Hiraoka Y. (2009). Membrane proteins Bqt3 and -4 anchor telomeres to the nuclear envelope to ensure chromosomal bouquet formation. The Journal of cell biology 187, 413-427.
5 Chikashige, Y., and Hiraoka, Y. Telomere binding of the Rap1 protein is required for meiosis in fission yeast. Current biology: CB 11, 1618-1623.
6 Miki F., Kurabayashi A., Tange Y., Okazaki K., Shimanuki M., and Niwa O. (2004). Two-hybrid search for proteins that interact with Sad1 and Kms1, two membrane-bound components of the spindle pole body in fission yeast. Molecular genetics and genomics: MGG 270, 449-461.

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TELOMERASE REGULATION IN HUMAN T LYMPHOCYTES.

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In most human tissues, except some rare proliferating stem-like cells, telomerase activity is usually undetectable. Previous reports have shown that mitogen stimulated T lymphocytes transiently turn on telomerase activity that may reduce the rate of telomere loss during rapid proliferation. However, telomerase activation is transient in T-cells as opposed to cancer cells, and is only maintained for a few days even with continual mitogen stimulation. After approximately 4 days of stimulation, telomerase activity greatly decreased and eventually T-cells stop proliferating. With increased human age, T-lymphocytes show progressive telomere shortening. While almost all cancer cells activate telomerase, it does not turn off, and cells achieve unlimited proliferation and telomere do not further shorten. Currently how telomerase activation is regulated in normal cells (such as in T lymphocytes) and how this regulation is hijacked by cancer cells are unclear.

Recently, our lab has reported evidence that hTERT alternative splicing is a potential mechanism for telomerase regulation. In our recent studies, we found that changes of telomerase activity after T lymphocyte stimulation corresponds with the expression shifts of several hTERT splicing variants. By 72 hrs, the ratio of catalytically active telomerase is increased compared to the non-functional splice variants (minus alpha and minus beta). This observation further emphasizes the potential role of hTERT alternative splicing in telomerase regulation in normal T lymphocytes. Taking advantages of the T lymphocytes stimulation model, we aim to study telomerase regulatory mechanisms in normal cells. The elucidation of how telomerase is regulated reversibly in primary proliferating transiently amplifying cells may facilitate our understanding of the potential mechanism(s) that cancer cells use to maintain telomerase activation.

STRUCTURAL BASIS OF TEMPLATE BOUNDARY DEFINITION IN *TETRAHYMENA* TELOMERASE

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Telomerase is a ribonucleoprotein (RNP) enzyme that maintains telomeres, the repetitive G-rich DNA sequence at the ends of chromosomes. Telomerase is essential in rapidly dividing cell types to combat gradual telomere shortening that occurs with each round of cell division. Telomerase extends telomere substrates by utilizing a unique mechanism in which the telomerase reverse transcriptase (TERT) subunit catalyzes the synthesis of simple DNA repeats, using a small region of the integral telomerase RNA (TER) subunit as a template. A hallmark of telomerase activity is its ability to establish a strict template boundary by limiting which part of the TER subunit may access the TERT active site. In the model organism Tetrahymena thermophila, high affinity protein--RNA contacts between the conserved RNA binding domain (RBD) within the TERT protein and the template boundary element (TBE) within TER are proposed to enforce template boundary definition. Here, we report the first high--resolution structure of the TERT-RBD domain bound to the TBE. TERT-RBD is wedged into the base of the TBE RNA stem-loop and each of the flanking RNA strands wraps around opposite sides of the protein domain. The structure explains existing biochemical data and directly reveals how evolutionarily conserved amino acids mediate the necessary protein- protein and protein-RNA contacts to establish template boundary definition. Homology modeling of our TERT-RBD/TBE structure with existing structural data provides strong constraints on the overall organization of the telomerase RNP complex.

TELOMERE-DRIVEN CHROMOSOME INSTABILITY IMPACTS THE GENETIC PROGRAM OF TRANSFORMED CELLS THROUGH GENOME-WIDE CHROMATIN REMODELING

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Telomere shortening is a major source of chromosome instability (CIN) at early stages during carcinogenesis. However, the mechanisms through which telomere-driven CIN (T-CIN) contributes to the acquisition of tumor phenotypes remain uncharacterized.
We have used human epithelial kidney cells (HEK cells), a well-established in vitro model of progressive telomere instability, to study the impact of T-CIN on the genetic program. We discovered that HEK cells undergoing T-CIN display massive non-coding RNA, including microRNAs (miRs), expression changes. This miR deregulation encompasses a miR-200-dependent epithelial-to-mesenchymal transition (EMT) that confers to immortalized pre-tumoral cells phenotypic traits of metastatic potential. Since deregulation of miRs in HEK CIN+ cells was widespread, orchestrated, and not related with copy number changes we hypothesized that the HEK genetic reprogramming responded to epigenetic cues. A ChIP-seq approach revealed important changes in distribution of chromatin marks genome-wide in CIN+ cells, in direct correlation with gene expression changes. Enrichment analyses for different combinations of histone marks identified significant modifications of bivalent domains associated with developmental genes. Our analyses also point to a major redistribution of heterochromatic domains genome-wide in CIN+ cells. In cells undergoing T-CIN, this redistribution precedes that of active marks.
Our results reveal for the first time that T-CIN profoundly modifies the chromatin landscape genome-wide thereby fueling the transformation process in pre-tumor epithelial cells.

CHARACTERIZING THE ROLE OF 53BP1 PHOSPHORYLATION USING DYSFUNCTIONAL TELOMERES

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53BP1 is a key regulator of mammalian DSB repair, playing an important role in promoting non-homologous end-joining (NHEJ) and repressing homology-directed repair (HDR). Telomeres lacking the shelterin protein TRF2 have proven a versatile system for studying 53BP1 in DSB repair since 53BP1 protects dysfunctional telomeres from resection, promotes their mobility, and is required for telomere fusions formed by c-NHEJ. The N terminus of 53BP1 features 29 S/TQ sites that can be phosphorylated by ATM and/or ATR upon induction of DNA damage. Phosphorylation of the 29 S/TQ sites leads to recruitment of the 53BP1 interacting proteins RIF1 and PTIP. The main function of RIF1 is to block resection at DNA breaks whereas the function of PTIP is not well understood.
To understand the role of these and other potential 53BP1 interacting factors, we created a panel of mutant 53BP1 alleles in which subsets of the 29 S/TQ sites are mutated and determined their ability to block resection and stimulate NHEJ of telomeres rendered dysfunctional through the deletion of TRF2. Our results indicate that RIF1 is the only factor downstream of 53BP1 responsible for blocking resection, as has previously been shown. However, NHEJ is only partially defective in the 53BP1ΔRIF1 mutant indicating other proteins are involved. Loss of S/TQ sites that mediate PTIP binding does not affect the ability of 53BP1 to block resection and results in a very mild NHEJ defect that cannot explain the residual NHEJ in the 53BP1ΔRIF1 mutant. Furthermore, a 53BP1 mutant lacking both the PTIP and RIF1 binding site is still capable of promoting NHEJ to a significant level. Using this mutant, we are mapping the S/TQ sites of 53BP1 that are involved in promoting the mobility of dysfunctional telomeres. Together, these 53BP1 mutants help us understand the mechanisms by which 53BP1 contributes to DSB repair.

CHARACTERIZATION OF POT1c IN ARABIDOPSIS THALIANA

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Telomeres protect chromosome ends from nucleolytic attack and end-to-end fusions through their unique structure and specific telomere binding proteins. One of most conserved telomere binding proteins is Protection Of Telomeres (POT1). Mammalian POT1 is a core component of shelterin, a six member telomere binding complex. POT1 consists of two oligosaccharide/oligonucleotide binding (OB) folds that specifically recognize single strand G-rich telomeric DNA. Mammalian POT1 functions to both protect chromosome ends, and to regulate telomerase access to telomeric DNA.

Gene duplication has shaped telomere biology in the flowering plant Arabidopsis thaliana. A. thaliana has three POT1 paralogs (AtPOT1a, AtPOT1b, and AtPOT1c), as well as two different telomerase RNA (TER) subunits.TER1 assembles into an RNP that functions as a canonical telomerase. TER2, in contrast, forms into an RNP that acts as a negative regulator of telomerase in response to DNA damage. We previously showed that AtPOT1a and AtPOT1b do not bind telomeric DNA, but instead are associated with TER1 and TER2, respectively. AtPOT1a functions as a positive regulator of telomerase. AtPOT1b appears to function in chromosome end protection.

In this study we examine the function of the third POT1 paralog, AtPOT1c. AtPOT1c emerged through a recent partial gene duplication of AtPOT1a. AtPOT1c consists of a single OB fold with only 56% ID to AtPOT1a OB1. Two different splice forms of AtPOT1c were identified. One contains an additional 14 amino acids. The importance of these additional residues is unknown. Unlike AtPOT1a and AtPOT1b, AtPOT1c interacts with both TER1 and TER2 in vitro. Knock down (KD) of AtPOT1c by RNAi leads to increased telomerase activity and decreased TER2, suggesting AtPOT1c is a negative regulator of telomerase and may stabilize TER2. Furthermore, AtPOT1c KD mutants have shorter, more heterogeneous telomeres as well as increased G-overhang signals, consistent with a role for AtPOT1c in chromosome end protection. These results indicate that AtPOT1c is a multifunctional protein that negatively regulates telomerase and contribute to telomere protection. Thus, AtPOT1c has evolved functions distinct from the other POT1 paralogs in A. thaliana.

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CELL CYCLE-DEPENDENT REGULATION OF DNA DAMAGE RESPONSE AND DNA REPAIR AT DYSFUNCTIONAL TELOMERES

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The specialized nucleoprotein complexes at telomeres are required to protect chromosome ends from inappropriate DNA damage response (DDR). Once chromosome ends become unprotected, DDR is activated and induces chromosome ends fusion through non-homologous end-joining (NHEJ).
Previously, we reported that NHEJ at unprotected telomeres was inhibited at S/G2 cell cycle phase due to the high CDK activity. The mechanism of cell cycle-dependent regulation of NHEJ at dysfunctional telomeres is largely unknown.
Our detailed analysis of DDR at dysfunctional telomeres revealed that the recruitment of 53BP1, one of the DDR related factors and required for chromosome ends fusion at dysfunctional telomeres, into the unprotected chromosome ends was largely inhibited at S/G2 phase. Accumulation of 53BP1 at DNA lesions is downstream of the chromatin ubiquitination. The chromatin ubiquitination at dysfunctional telomeres was also largely diminished at S/G2 phase. These findings suggest that the cell cycle-dependent chromatin ubiquitination status regulates NHEJ at unprotected telomeres.
In contrast to dysfunctional telomeres, the chromatin ubiquitylation at DNA damage sites induced by X-ray irradiation was observed throughout interphase, indicating that cell cycle-dependent regulation of chromatin ubiquitylation is specific at telomeres. Telomere protection is abrogated during replication folk progression at telomeres, so that this mechanism is thought to be important for the suppression of the risky NHEJ after telomere DNA replication.
In this meeting, I will discuss the cell cycle-dependent regulation of DDR at dysfunctional telomeres.

VERIFICATION OF DNA INTEGRITY IS IMPERATIVE FOR ACCURATE DOWNSTREAM TELOMERE LENGTH ANALYSIS IN HUMAN SPERM CELLS

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Telomeres in sperm are longer than in somatic cells as a result of telomerase activity in the testes, and in contrast to the age-associated telomere attrition observed during somatic cell expansion, telomeres in the male germ line appear to increase in length with aging. In recent years, the reproductive function and inheritance of these elongated telomeres has gained increasing interest. In our effort to study this phenomenon, our first objective was to confirm the reliability of our telomere length assay when applied to human sperm cells. Spermatozoa possess a number of unique properties which differ from somatic cells, including haploidy, hypercondensation of the nucleus, a reduced nuclear envelope and rigid perinuclear theca. These properties require specialized treatment and consideration when isolating DNA for telomere analysis.

Motile sperm were isolated from 15 semen samples via two-layer density gradient centrifugation. Each sample was divided in half, a DTT (80mM) and proteinase K (250ug/ml) treatment was used to lyse sperm heads, and sperm DNA was isolated with QIAamp DNA Mini Kit (Qiagen) or Genomic Tips (Qiagen). Leukocyte DNA was isolated with both methods as a somatic cell control. The integrity of undigested DNA was assessed by field inversion gel electrophoresis (FIGE) with a high range DNA ladder (10-48 kb) to confirm sperm DNA was >20 kb, the longest telomere length expected in sperm. Average telomere length was measured with terminal restriction fragment (TRF).

TRF results from the QIAamp DNA mini detected telomeres which were shorter than the published range: 2-9 kb vs. 9-21 kb expected. FIGE results revealed non-specific fragmentation in sperm DNA isolated using QIAamp, which explained the shortened telomeres detected by TRF. This effect was unique to sperm, as leukocyte DNA was not fragmented. Sperm DNA isolated using Genomic Tips were of high molecular weight (>48 kb) and homogenous in size. Downstream TRF results yielded telomeres within the expected length range.

Our results demonstrate that the integrity of DNA isolated from sperm cells can vary greatly depending on the DNA isolation method used, and these fragments of DNA include telomeres. In light of these results, we conclude that the evaluation of DNA integrity is imperative for accurate downstream telomere length analysis. It is important to note that abnormal shifts in telomere length can be detected by TRF, but can remain undetected using other methods such as qPCR which measure relative telomeric DNA content.

SHORTENING OF TELOMERE INDUCES LOSS OF SISTER TELOMERES COHESION

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DNA replication is a tightly regulated process. From the beginning of replication until anaphase, sister chromatids are tightly held together by the cohesin complex. Properly established cohesion between sister chromatids protects the cell against genome instability and aneuploidy. Cohesion is established by a tripartite ring composed of Mcd1/Scc1, Smc1 and Smc3 that embraces both chromatids. In anaphase, it is cleaved and removed from chromosomes to allow chromosome segregation. Cohesion establishment and functions have been well studied at centromere and along chromosomes arms, but little is known about sister telomeres cohesion. In mammalian cells, loss of cohesion at telomeres is known to impair telomere length regulation and has been proposed to act as a telomerase regulatory mechanism. Cohesin is also involved in the regulation of telomeric non-coding RNA TERRA expression in human cells.
Our laboratory study TERRA RNA functions and dynamics in budding yeast using live-cell imaging techniques. By doing so, we previously showed that TERRA RNA expression is increased during telomere shortening and TERRA molecules accumulate as a single nuclear focus. Interestingly, we noticed that TERRA-expressing telomeres display a lack of cohesion in S phase. The relationship between telomere shortening and absence of telomere cohesion was assessed by live-cell imaging. Separation of sister telomeres in S phase was observed in a yeast strain containing a short inducible telomere 6R labelled with TetO-TetR-mCherry, but not at the normal telomere 6R. These results were confirmed by chromatin immunoprecipitation using a myc-tagged Mcd1. We identified a specific cohesin binding site near telomere 6R and confirmed a decreased accumulation of Mcd1 on the short telomere 6R. Loss of cohesion at short telomere spans from the telomere's end to several tens of kilobases toward the centromere. This absence of cohesion at a short telomere is not triggered by the telomere elongation pathway, as it was not affected by knockouts of YKU70, MRE11 or TEL1. Preliminary data also suggests that tethering cohesin at a telomere interfere with enhancement of TERRA expression when this telomere is short. Altogether, these results reveal an interplay between sister telomere cohesion, telomere length regulation and TERRA expression. We aim to study the mechanisms regulating this loss of cohesion and how it can affect telomere homeostasis.

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TERT PROMOTER/ENHANCER MUTATIONS IN CHICKENS AND HUMANS

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Avian leukosis virus (ALV) is a simple retrovirus that infects chickens and causes B-cell lymphoma. Tumorigenesis is caused by random proviral integrations that perturb normal host gene expression. High-throughput sequencing for proviral integrations in B-cell tumors revealed a common integration site in the telomerase reverse transcriptase (TERT) promoter/enhancer region. These proviral integrations enhanced TERT transcription by promoter activation and have been shown to be clonal, suggesting that upregulation of TERT expression by promoter activation was an early event in B-cell lymphoma development. To test if early TERT expression promotes tumorigenesis in ALV-infected chickens, chickens have been infected with a TERT-expressing recombinant virus. Recently, somatic mutation in the TERT promoter had been shown to be recurrent across many human cancers, especially, glioblastoma and melanoma. Genome-wide association studies revealed that TERT mutations had a significant association with increased TERT expression across different cancers. To test the frequency of TERT promoter mutations in human B-cell malignancies, conventional sequencing was used to screen different types of human lymphoma and leukemia.

A NEW PLACE FOR KU ON TELOMERES.

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The conserved KU complex, composed of Ku70 and K80, plays a number of important roles in telomere biology and is essential for NHEJ. The herterodimeric, ring shaped complex binds DSBs with high affinity but little sequence specificity. It has been assumed that Ku association with telomeric DNA ends occurs in a similar fashion as on a DSB, but direct evidence for this is lacking. In budding yeast cells, YKu is thought to associate with telomeric DNA ends in such a fashion that the two faces of the ring molecule affect telomere maintenance in different ways. In addition, YKu interacts with the silencing factor Sir4, but it is not clear whether this happens with DNA-bound Yku or not. Finally, while YKu also interacts with the telomerase RNA, it appears that it can only interact with the RNA or DNA, but not both at the same time.
Using a fusion protein formed by Yku70 and micrococcal nuclease (Yku70-MN) and based on MN cutting of the underlying DNA (a technique called ChEC, Chromatin Endogenous Cutting), we show here that YKu not only binds at the very physical ends of chromosomes, but also occurs inside telomeric repeats near the junction between telomeric repeats and subtelomeric elements. Furthermore, we find YKu constitutively associated with subtelomeric repeat tracts that occur between the telomere associated repeat elements (Y' and X in budding yeast). In many eukaryotic species, interstitial telomeric repeats are subject to frequent chromosomal rearrangements. Furthermore, these sequences are described as natural replication barriers leading to replication fork stalling, potentially compromising genome stability and cell viability. We thus speculate that YKu is associated with telomeric repeats at internal sites to ensure DSBs repair by NHEJ. In order to verify this hypothesis, we constructed strains in which excision and circularization of telomeric repeat DNA can be induced. Indeed, via Yku70-MN cutting, we again observe YKu binding to blocks of telomeric repeats on the circular DNA molecule. These observations lead us to propose a new YKu binding mode at telomeres at internal sites of telomeric repeats. In this place, YKu could ensure NHEJ in order to maintain telomeric repeat lengths. It remains unclear why YKu remains associated with DNA at these sites, while at other genomic loci, YKu is removed quite rapidly after completion of NHEJ.

ALTERNATIVE LENGTHENING OF TELOMERES (ALT) IN NON-SMALL CELL LUNG CARCINOMA.

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Lung cancers (of which approximately 84% are classified histologically as non-small cell) are the leading cause of cancer-related death worldwide. Telomerase is the telomere length maintenance mechanism in most lung cancers, whereas a few use the alternative lengthening of telomeres (ALT) mechanism[1]. ALT can be used as a prognostic indicator for some tumor types; here we investigated whether telomere lengthening mechanism is a prognostic factor in non-small cell lung carcinomas (NSCLC). Tumor sections from 288 NSCLC cases diagnosed and treated in the US were assessed for ALT-Associated PML Bodies (APBs). Of these, 11 (3.8%) were found to be positive for APBs, including 7% of the squamous cell carcinoma subtype of NSCLC. The median survival of patients with ALT+ NSCLC was 31 months, not significantly different from those with ALT- NSCLC of 46 months (hazard ratio 95% CI = 0.33 – 1.45). Our data imply that, of the >150,000 lung cancer deaths per year in the US, >5,000 are due to ALT+ NSCLC. This substantially sized group could benefit from the development of ALT-targeted diagnostics and therapeutics.

1. Heaphy CM. et al. (2011). Am J Pathol., **179**(4). 1608-1615.

TETRAHYMENA TELOMERASE SUBCOMPLEX P75-P45-P19 IS A CST COMPLEX

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The Tetrahymena telomerase holoenzym contains eight subunits: three core components (TERT, TER, and P65) and five regulatory factors (P75, P45, P19, P50, and Teb1). Here we present structural and functional analyses of P45 and P19 that together with P75 assemble into a subcomplex with unknown function. Surprisingly, the crystal structure of the N-terminal domain of P45 (P45N) complexed with P19 reveals an Stn1-Ten1-like complex. In both structures, the OB folds of the two components pack against each other through interactions between two C-terminal helices. In addition, we also solve the structure of the C-terminal domain of P45 (P45C) that is composed of two winged helix-turn-helix (WH) motifs and most similar to the WH motifs at the C terminus of Stn1, again supporting the notion that P45 resembles Stn1. These structural analyses strongly indicate that P45-P19 is the tetrahymena Stn1-Ten1 complex. P75 forms a stable complex with P45-P19 and the primary sequence analysis shows some similarity between P75 and Cdc13. Notably, removal of the predicted α helix at the very end of P75 C-terminus disrupts the interaction between P75 and P45-P19. Given that the C-terminal helix in Cdc13 is essential for the assembly of the CST complex, these results suggest that P75-P45-P19 is the CST complex in tetrahymena. Functional analysis shows that over-expression of P45 and P19 mutants that disrupt the P45-P19 interaction exhibit different G-overhang phenotypes, suggesting that P19 and P45 have some distinct functions. Collectively, our structural and functional studies illustrate that the CST complex (P75-P45-P19) is evolutionarily conserved in tetrahymena.

KU PRIMARILY IMPACTS TELOMERE LENGTH IN *SACCHAROMYCES CEREVISIAE* VIA EST1 RECRUITMENT TO THE TELOMERE

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In *Saccharomyces cerevisiae*, telomerase is comprised of the RNA component, TLC1, the reverse transcriptase component, Est2, and Est1 and Est3, which are required for telomerase activity *in vivo*, but not in *vitro*. Est1 recruits telomerase to the telomere in late S phase by interacting with Cdc13, a single-stranded telomeric DNA binding protein. The Ku heterodimer also contributes to telomere length maintenance by associating with TLC1. In contrast to strains lacking telomerase, telomeres are short yet stable and cells do not senesce in the absence of Ku or Ku:TLC1 interaction. Cells lacking Ku or Ku:TLC1 binding, such as the *yku80-135i* mutant, fail to localize TLC1 to the nucleus and have reduced levels of Est2 and Est1 at the telomere. We recently showed that the requirement for Ku in telomere length maintenance could be rescued by tethering Est1 to the telomere via Cdc13 (Williams et al, 2014). In addition, we showed that Ku promotes the association of Est1 to the telomere even when Est2 is telomere-associated. We also found Ku in a complex with Est1. Finally, we showed that Ku's DNA end binding (DEB) activity is required for Ku's influence on telomere length even if TLC1 is localized to the nucleus. Together, these results led us to propose that Ku's primary role in telomere elongation lies in its influence on Est1, and not on Est2 recruitment or TLC1 nuclear localization. Currently, we are exploring multiple hypotheses involving Ku's influence on Est1 recruitment to the telomere. One hypothesis is that Ku binding to TLC1 and/or Ku's DEB activity modulates the interaction between Est1 and Cdc13. Using co-immunoprecipitation assays in asynchronous cells, we unexpectedly found an increase in Est1:Cdc13 interaction in *yku80Δ* or *yku80-135i* strains compared to wild type. We plan to further investigate this role of Ku on Est1:Cdc13 interaction in synchronized cells, in *yku70-R456E* mutants, which cannot bind DNA ends and in various Est1 and Cdc13 mutants. We are also testing whether Ku, when bound to telomeres, modulates Cdc13 phosphorylation. Li et. al previously showed that Est1:Cdc13 interaction at telomeres is promoted by Cdc13 T308 phosphorylation (2009). Additionally, telomeres exhibit the same degree of shortness in *yku70*Δ *cdc13-T308A* and *yku70Δ* mutants suggesting that Ku and Cdc13 T308 phosphorylation function in the same pathway (Tseng et. al, 2009). Thus, Ku might influence Est1:Cdc13 interaction by promoting Cdc13 T308 phosphorylation. However, we detected no difference in Cdc13 phosphorylation status in wild type vs. *yku80Δ* asynchronous cells. We are further exploring this using synchronized strains.

TARGETING TELOMERASE FOR CELL THERAPY

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The generation of human embryonic stem cells and induced pluripotent stem cells (iPSCs) has provided enormous opportunity for cell therapy and disease modeling. However, stem cell-based therapy also brings new safety challenges. One major concern is the tumorigenic potential of the stem cells. The pluripotent stem cells share cellular and genetic similarity with tumor cells: such as unlimited potential for cell proliferation and propensity for genomic instability when cultured in vitro. Such pluripotent stem cells can form teratoma when injected into the immunodeficient mice.

The unlimited proliferation potential is a hallmark of cancer, which is shared by the stem cells. In order to proliferate continuously, the cells need to find a way to maintain their telomere, a special nucleoprotein complex found at the ends of human linear chromosomes. Telomeres are synthesized by telomerase, a reverse transcriptase that contains two core components-the protein catalytic subunit, hTERT, and the RNA subunit, hTER. Although the telomerase RNA subunit-hTER is widely expressed, hTERT and consequently, telomerase activity, are hardly detectable in the majority of human adult somatic cells, except stem cells and germ cells. As a result, normal somatic cells only have a limited proliferation potential that is limited by their telomere length. In contrast, pluripotent stem cells share the unlimited proliferation capacity of cancer cells, and express high telomerase activity for telomere maintenance.

In our lab, we explore the possibility: whether we can functionally separate the two unique properties of human embryonic stem cells: self-renewal and pluripotency. Previous studies in mouse models have shown that inactivation of telomerase activity by inducible knockout of either the mTERT or mTR does not result in dramatic phenotype in the first 2-3 generations, owing to the extremely long telomere in laboratory mice. These results raise a possibility that we may be able to limit the proliferation capacity of human embryonic stem cells, without affecting their pluripotency, by inactivating telomerase activity. We have engineered telomerase inducible knockout human embryonic stem cells using gene targeting. The cellular effects of telomerase inactivation in embryonic stem cells will be presented. These results provide further insights into the regulation of telomere maintenance in vivo and provide a new avenue of targeting telomerase function for cell therapy.

MAMMALIAN DNA2 CLEAVES TELOMERIC G-QUADRUPLEX DNA AND IS REQUIRED FOR GENOME INTEGRITY

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Accurate and efficient replication of telomeric DNA is required for chromosome end protection and genome integrity. Inefficient replication of the repetitive TTAGG telomeric sequences that form the G-quadruplex structure can impair telomere replication and lead to telomere instabilities. However, it is unclear how the telomeric G4 is processed and whether defects in these processing pathways may contribute to genomic instability in vivo. Here we show that telomeric G4 can be recognized and cleaved by mammalian DNA2 in vitro. Heterozygous knockout of DNA2 in mouse leads to telomere replication defects and elevated levels of fragile telomeres (FTs) and sister telomere associations (STAs). Meanwhile these telomere defects are aggravated by chemical stabilization of G4. Moreover, DNA2 deficiency induces telomere DNA damage and chromosome segregation errors that further lead to aneuploidy. Consequently, DNA2-deficient mice develop cancers with a high incident. In conclusion, our results suggest that mammalian DNA2 reduces telomeric DNA replication stress, and thereby preserves genome stability and suppresses cancer development. This is due to, at least in part, efficient processing of telomeric G4.

SINGLE-MOLECULE STUDIES OF THE TPP1-POT1 SEARCH MECHANISM FOR THE TELOMERIC SINGLE-STRANDED DNA TAIL

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The human telomeres are important regions at the ends of the chromosomes that confer genome stability. They consist of long stretches of a repetitive DNA sequence, TTAGGG, that are bound by a group of telomeric proteins known as the shelterin complex. One unresolved question is how the end-capping shelterin sub-complex, TPP1-POT1, is recruited to the telomeric single-stranded tail, given that it has TIN2 binding sites along the telomere. In this work, using a single-molecule experimental setup, we seek to observe how a single TPP1-POT1 complex searches for the telomeric tail along a long telomeric double-stranded DNA. We have engineered a TIN2 fusion protein that consists of a TPP1-interacting domain and a DNA-interacting domain. This fusion protein and TPP1 have been expressed and purified using a bacterial expression system. Both recombinant proteins are then labelled with individual fluorescent-dye for single-molecule total-internal reflection microscopy (smTIRFM) to image the fluorescent proteins moving along a single doubly-tethered artificial telomeric DNA that is stretched on the imaging surface. By studying the diffusion dynamics of TPP1 and the TPP1-POT1 complex along the artificial telomeric DNA (TIN2-coated DNA), we aim to elucidate how TPP1-POT1 locates telomeric ends and its corresponding search mechanism.

TELOMERE COMPACTION DETERMINED BY STORM: SIZE MATTERS

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Using QTIP and ChIP experiments, we found that SMCHD1 is specifically enriched at telomeres when they are very long or when they become uncapped by TRF2-depletion. Stimulated by published work demonstrating that SMCHD1 compacts the inactive X-chromosomes we speculated that SMCHD1 might have roles for the folding of telomeres. To address this question we applied stochastic optical reconstruction microscopy (STORM) to human telomeres. Telomere DNA was stained with fluorescently labeled oligonucleotide probes and signal clusters were analyzed. The size of each telomere was quantified by the radius of gyration of the localized fluorescent signals within a cluster. Normal telomeres had an average gyration radius of 77 nm. Interestingly, although longer telomeres had an increased radius, their volumes did not scale in a linear fashion with the telomere length change indicating that compaction was significantly enhanced at long telomeres. We also find that telomere compaction is influenced by shelterin components. TRF1 depletion led to telomere decompaction whereas depletion of TRF2 reduced telomere size. Importantly, SMCHD1 appears to play crucial roles for reducing telomere size in TRF2-depleted cells. Thus, TRF1 and TRF2 appear to have opposing effects on telomere size and SMCHD1 may contribute to telomere remodeling and collapse, which occurs when telomeres become depleted for TRF2.

SMAD7 DEFICIENCY TRIGGERS TELOMERE DYSFUNCTION AND PULMONARY AGING IN MICE

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Population aging is associated with increases in morbidity and mortality attributable to lung disease but not to other prevalent diseases. Chronic obstructive pulmonary diseases including idiopathic pulmonary fibrosis (IPF) have risen to become the third leading cause of death in the United States. The type II lung alveolar epithelial cells (AECII) play a key role in the damage and repair of lung epithelium, by serving as the progenitor stem cells undergoing renewal, proliferation and differentiation and secretary cells releasing a number of cytokines. Signaling of the cytokine transforming growth factor-beta (TGF-beta) and telomere maintenance represent environmental and genetic factors important in stem cell fate and in IPF, but their mechanisms and relationship remain unclear in aging.

Using mouse models of specific disruption of the gene coding for the inhibitory Smad7 in AECII, we observed for the first time that Smad7 deficiency induces telomere dysfunction, AECII senescence and pulmonary aging. Aging cells in the mouse lung with Smad7 deficiency resembled the molecular pathology induced by mutations of the genes coding telomerase reverse transcriptase (TERT) or telomerase RNA component (TERC). Inhibition of Smad7 resulted in repression of the TERT gene without affecting the genes coding for various other telomere binding proteins, and ectopic expression of recombinant TERT prevented Smad7 inefficiency-induced telomere dysfunction-induced foci (TIFs), unveiling a key role of regulated TERT withdrawal from AECII cells in pulmonary aging. Thus, our novel findings demonstrate a physiologically relevant circuit in which the environmental cue TGF-beta radiates to the genetic element telomeres via a Smad7-regulated TERT mechanism in pulmonary epithelial progenitor AECII in vitro and in mice, indicating a permissible targeting strategy of the TGF-beta receptor-mediated Smad-wired telomere regulatory pathway underlying lung aging.

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CARRIERS OF GERMLINE POT1 MUTATIONS ARE PREDISPOSED TO FAMILIAL CUTANEOUS MALIGNANT MELANOMA

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To underlie genetic factors for melanoma-prone families, we have employed whole-exome sequencing and identified several rare variants in the telomere shelterin gene POT1, including a rare variant that arose as a founder mutation (chromosome 7, g.124493086C>T; p.Ser270Asn) in five unrelated melanoma-prone families from Romagna, Italy. Carriers of this variant had increased telomere lengths and numbers of fragile telomeres, suggesting that this variant perturbs POT1 function and thus telomere maintenance. These variants were not found in public databases or in 2,038 genotyped Italian controls. We also identified two rare recurrent POT1 variants in US and French familial melanoma cases. Our findings suggest that POT1 is a major susceptibility gene for familial melanoma in several populations.

SLX4 CONTRIBUTES TO REGULATED PROCESSING OF TELOMERIC JOINT INTERMEDIATES

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Human SLX4 protein assembles a complex consisting of endonucleases SLX1, MUS81, and XPF, which is recruited to telomeres via direct interaction of SLX4 with the telomeric DNA-binding protein TRF2. Telomeres present an inherent obstacle for the DNA metabolism apparatus due to their high propensity to form branched DNA intermediates. Here we provide novel insight into the mechanism and regulation of the SLX4 complex in telomere metabolism. SLX4 associates with telomeres, peaking in the late S phase and also under replication stress. Disruption of the interaction of SLX4 with TRF2 or SLX1 independently causes telomere fragility, suggesting a requirement of the SLX4 complex at telomeres for nuclease-dependent resolution of branched intermediates during telomere replication. Indeed, we find that in vitro the SLX1-SLX4 complex processes a variety of telomeric joint molecules. The nucleolytic activity of the SLX4-nuclease complex is negatively regulated by telomeric proteins TRF1 and TRF2 and by the helicase BLM in vitro. Furthermore, homologous recombination-mediated telomere maintenance processes are negatively regulated by BLM. We propose that the SLX4-nuclease toolkit is a bona fide telomere accessory complex that, in conjunction with other telomere maintenance proteins ensures unhindered, but regulated progression of telomere maintenance.

TERRA RNA-DNA HYBRIDS PROMOTE RAD52-DEPENDENT REPAIR AT CRITICALLY SHORT TELOMERES IN PRE-SENESCENT CELLS

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Telomeres are transcribed into TElomeric Repeat containing RNAs (TERRAs). We have previously shown that TERRA RNA-DNA hybrids are restricted by RNase H activity at telomeres. Increased hybrid levels, obtained by deleting RNase H, delayed the onset of replicative senescence and was associated with increased telomere length. The increased telomere length was due to Rad52-dependent recombination events occurring at the hybrid-accumulating telomeres.
The experimental generation of a single, critically short, telomere leads to an increase of TERRA levels and hybrids in cis, both of which were reduced upon the overexpression of RNase H1. The association of an elongating form of RNAPII was not increased at the critically short telomere, suggesting that hybrid stability accounts for the increase in TERRA. RNase H1 overexpression reduced Rad52 recruitment to the shortest telomere and accelerated the onset of senescence in this strain. Even at natural telomeres progressive telomere shortening during senescence was associated with increasing TERRA levels, supporting the idea that TERRA RNA-DNA hybrids that accumulate at short telomeres promote Rad52-dependent recombination events. Consistently, overexpression of RNase H1 leads to increased senescence rate. This effect was epistatic with *RAD52* deletion, which completely abolishes Homology-Directed Repair (HDR). RNase H1 overexpression accelerated the senescence rate in both *rad51* and *rad59* backgrounds, which make up the two Rad52-dependent branches of HDR that can act at telomeres. This suggests that RNase H1 overexpression inhibits both pathways of HDR at telomeres. Importantly, Rad52, Rad51 and Rad59 protein levels were not affected by RNase H1 overexpression, excluding that the overexpression phenotype was due to a decreased expression of HDR genes. RNase H1 overexpressing cells were fully capable of repairing internal double strand breaks and were not rendered sensitive to genotoxic agents, indicating a telomere specific effect. Finally, RNase H1 overexpression did not affect telomere length in telomerase-positive cells. We have now extended our previous observation that RNA-DNA hybrids promote HDR in pre-senescent cells and demonstrate that RNA-DNA hybrids are specifically allowed to accumulate at critically short telomeres to promote Rad52 recruitment.
*these authors contributed equally

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53BP1 AND THE LINC COMPLEX PROMOTE MICROTUBULE-DEPENDENT DSB MOBILITY AND DNA REPAIR

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Changes in the dynamic behavior of DNA Double Strand Breaks (DSBs) have been noted in yeast and mammalian cells but it is unclear how DSB signaling elicits this phenomenon and how it contributes to DSB repair. In mammalian cells, 53BP1 induces an increase in the mobility and roaming of telomeres that are rendered dysfunctional through the removal of TRF2. Here we use a telomere-based system to determine the mechanism by which 53BP1 acts and its functional consequences. We show that 53BP1-driven mobility of dysfunctional telomeres is negated by microtubule poisons, implicating a microtubule-dependent process. Using genetic dissection, we identify the SUN1/2 components of the LINC (LInker of the Nucleoskeleton and Cytoskeleton) complex as well as Nesprin-4 and kinesin-1 and -2 as major effectors of the induced chromatin mobility. Importantly, the data establish that the increased mobility of dysfunctional telomeres itself, independent of other effects of 53BP1, promotes their fusion through c-NHEJ. The 53BP1/microtubule/LINC-dependent increase in mobility also occurs at genome-wide DSBs and contributes to the mis-repair of DNA damage in PARPi treated BRCA1-deficient cells. These findings reveal a novel mechanism underlying DNA repair in mammalian cells with potential clinical significance.

LOSS OF ATRX CAUSES A TELOMERE-SPECIFIC COHESION DEFECT

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The Alternative Lengthening of Telomeres (ALT) pathway is a telomerase-independent mechanism of telomere maintenance present in a subset of cancers and immortalized cell lines. This pathway is reliant on homology directed repair (HDR), but the mechanisms through which ALT is activated remain unknown. Mutations in ATRX have been identified in tumors displaying features of ALT, and we have demonstrated that mutations in the ATRX gene and loss of ATRX protein are hallmarks of ALT-immortalized cell lines. Our efforts to determine how a deficiency in ATRX may facilitate ALT have uncovered a role for ATRX in promoting telomere cohesion. FISH staining of interphase cells with probes for the subtelomeric or arm region of two separate chromosomes reveal cohesion defects specifically at the telomeres of ATRX-deficient MEFs. The percentage of telomere doublets observed after deletion of ATRX was similar to that observed after silencing the telomere-specific cohesin subunit SA1. Importantly, the combined loss of ATRX and SA1 led to no further increase in telomere doublets.

Additional evidence for a telomere cohesion defect was observed in MEFs lacking ATRX and TPP1. Deletion of TPP1 alone causes a modest level of non-sister, post-replicative telomere fusions. The combined deletion of ATRX and TPP1 causes a significant increase in non-sister fusions, suggesting telomeres are more readily available to fuse with non-sister partners. Similarly, silencing of SA1 in TPP1-deficient MEFs caused a significant increase specifically in non-sister telomere fusions. No further increase in non-sister fusions was observed with the combined loss of ATRX, SA1, and TPP1. These data indicate ATRX and SA1 function in the same pathway to promote telomere cohesion, and highlight the importance of cohesion in preventing inappropriate telomere-telomere interactions.

Telomere cohesion was also assessed in ALT cell lines by FISH staining of interphase cells with subtelomeric or arm-specific probes. Many of the ALT cell lines tested display a significant increase in telomere doublets compared to cells expressing telomerase. Consistent with a telomere cohesion defect, ALT cells display telomere replication problems and have elevated levels of fragile telomeres compared to telomerase-positive cells. We have further determined that RPA foci are more prominent in ALT cells. We are currently exploring whether the occurrence of replication problems and increased levels of ssDNA, particularly when combined with a telomere cohesion defect, could promote the HDR-mediated repair events with non-sister telomeres that are reminiscent of ALT.

A COMPREHENSIVE GENETIC AND BIOCHEMICAL ANALYSIS OF THE EST1 YEAST TELOMERASE SUBUNIT

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Telomerase from budding yeast consists of the catalytic Est2 protein and two regulatory subunits (Est1 and Est3) in association with the TLC1 RNA, with each of the four subunits essential for in vivo telomerase function. Telomerase is highly regulated, in that only a subset of telomeres are elongated in each cell cycle; however, the mechanism(s) that restrict telomerase activity in vivo are still poorly understood. This deficit may stem from the fact that the surface of yeast telomerase represents a largely unexplored territory; we propose that there are as-yet-unidentified interaction surfaces on the three Est proteins that regulate telomerase function.

To address this, we have developed a mutagenesis protocol designed to identify the rare sub-class of mutations that target functionally important residues on the surface of a protein. In collaboration with Deborah Wuttke's laboratory, we initially applied this protocol to the small Est3 subunit as a proof-of-principle test (Lubin et al., 2013), which was validated once the structure of the Est3 protein was determined by the Wuttke group (Rao et al. 2014). Notably, all of the predicted surface residues identified by our genetic protocol were located on the experimentally determined Est3 protein surface.

We have subsequently applied this mutagenesis strategy to the Est1 telomerase subunit, which has generated a highly curated collection of separation-of-function mutations. By combining this with biochemical analysis, we have correlated these mutations with the two known protein binding partners of Est1 (Cdc13 and Est3). Through a separate approach, we have identified a novel ~90 amino acid domain in the N-terminus of the Est1 protein that mediates RNA binding. By process of elimination, we now have two clusters of residues with no known binding partners and thus define novel functions for Est1; the results of on-going efforts to identify factors that interact with these two novel surfaces will be presented.

REGULATION AND MANIPULATION OF hTERT SPLICING IN CANCER CELLS

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The hTERT gene makes several alternatively spliced forms with full-length hTERT mRNA actually being a minor component. Small molecules to manipulate hTERT splicing may be a viable option to accelerate shortening of telomeres in cancer cells and sensitize cells to traditional chemotherapies. To test this hypothesis we quantified several known and novel hTERT splice variants with droplet digital PCR. We observed that normal diploid fibroblasts and telomerase negative transformed cells (ALT cells) express 1-2 copies of hTERT mRNA/cell while telomerase positive cancer cells express from 1-20 copies/cell. To define the isoforms of hTERT we utilized Pacific Biosciences single molecule long read length sequencing to identify hTERT transcripts in both normal and cancer cells. We identified many common and previously known hTERT transcripts as well as several novel splice variants of hTERT. The majority of the normal cell hTERT transcripts lack the reverse transcriptase domain and thus are not catalytically active. To determine if hTERT isoforms protect cancer cells from apoptosis we disrupted the hTERT ORF (CRISPR/Cas9) and observed that cells lacking hTERT are significantly more sensitive to cisplatin induced apoptosis.

Alternative splicing choice is regulated by RNA binding proteins and splicing factors, however the genes that regulate hTERT splicing choice are unknown. To identify candidate genes that regulate hTERT splicing, we analyzed transcriptomic data from a panel of lung cancer cell lines. We compared telomere length, telomerase activity and hTERT splicing variants to genome wide expression data. This analysis revealed that the four pathways most significantly related to hTERT full-length expression were P70S6K/Cullin, PI3K, RAC/RAS, and NfκB/PRKC/MAPK3K. In a sub-analysis, we found 54 candidate RNA binding/splicing factor genes differentially expressed between the three highest hTERT full-length lines and the three lowest hTERT full-length lines. We performed a forward genetic screen (shRNA) of the candidate genes and identified three lead target genes, NOVA1, CDC40 and PCGF6, that induced at least a 2-fold reduction in telomerase activity and a shift in the expression of hTERT splicing variants. We have knocked out (CRISPR/Cas9) and knocked down (shRNA stable) these lead target genes in lung cancer cell lines and measured telomere length, telomerase activity and hTERT splicing. These data support the concept that shunting hTERT transcripts from full-length to non-functional variants may be a viable direction for identifying new therapeutic options to manipulate telomerase activity.

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THE MECHANISMS AND ASSEMBLY OF THE CDC13-STN1-TEN1 COMPLEX FROM *CANDIDA GLABRATA*

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Studies of the Cdc13-Stn1-Ten1 (CST) complex, which mediates critical functions in telomere maintenance, have been hampered by difficulties in its reconstitution and purification. We recently succeeded in isolating large quantities of the CST complex from *Candida glabrata*, which enabled us to characterize its DNA-binding and primase-pol α (PP) stimulatory activities *in vitro* (1, 2). A particularly interesting finding to date is that CST stimulates the primase-to-polymerase switch, a mode of regulating the PP complex that has not been observed before.

Another important issue concerning the CST complex that has not been fully addressed is its assembly mechanisms. Whereas the interaction between Stn1 and Ten1 is well understood at the structural level, the ones between these two small subunits and Cdc13 have not been analyzed carefully *in vitro*. We investigated the *Candida glabrata* CST subunits in this regard, and found that Stn1, but not Ten1, can form a stable complex with Cdc13. Accordingly we purified all five domains of Cdc13 (OB1, RD, OB2, DBD and OB4), and assessed their interactions with the N-terminal OB fold and the C-terminal winged-helix domain of Stn1. Surprisingly, we detected the strongest interaction between the OB2 domain of Cdc13 and the winged-helix domain of Stn1. Because previous findings in *S. cerevisiae* suggest an interaction between the Cdc13 OB4 and the Stn1 winged-helix domains, the current results raise the intriguing possibility that the assembly mechanism of CST may be malleable in evolution. We have begun to use point mutations to identify surface features of $Cdc13_{OB2}$ and $Stn1_{WH}$ that are responsible for their mutual interaction, and a progress report will be presented.

1. Lue et al. (2013) The telomere capping complex CST has an unusual stoichiometry, makes multipartite interaction with G-tails and unfolds higher order G-tail structures. PLoS Genet, 9, e1003145.

2. Lue et al. (2014) The Cdc13-Stn1-Ten1 complex stimulates Pol α activity by promoting RNA priming and the primase-to-polymerase switch. Nat. Comm. 5:5762.

TRF2 USES A HOLLIDAY JUNCTION (HJ) BINDING FOLD TO REPRESS PARP1 SIGNALING AND T-LOOP CLEAVAGE

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The shelterin component TRF2 promotes the formation of t-loop structures *in vivo*, which is critical for the repression of ATM kinase signaling and c-NHEJ. However, the t-loop configuration may also create challenges because of its structural resemblance to intermediates in homologous recombination and because it contains DNA structures that can activate poly(ADP-ribose) polymerase 1 (PARP1), a potent DNA damage sensor whose activation is detrimental for telomeres. It was previously shown that the N-terminal basic domain of TRF2 is required to prevent cleavage of the t-loop by HJ resolvases such as Mus81 and GEN1. Biochemical experiments have suggested that the basic domain of TRF2 might function as a HJ binding motif. Here, we report that the TRF2 basic domain acts as a HJ binding fold *in vivo* to repress t-loop cleavage and the activation of detrimental PARP1 signaling at telomeres.

To evaluate the role of HJ binding by the basic domain of TRF2 in the repression of t-loop cleavage, we tested the H31A mutation, which diminishes HJ binding of the basic domain *in vitro*. TRF2 bearing the H31A mutation resulted in the same telomere loss phenotype as TRF2 lacking the basic domain (TRF2ΔB). To more definitively test whether HJ binding is the mode of action of the TRF2 basic domain, we substituted it with a genuine HJ binding domain derived from the E. coli RuvC HJ resolvase. Remarkably, this RuvC fusion re-established the ability of TRF2ΔB to repress t-loop cleavage. These data are consistent with the proposal that TRF2, via its HJ binding fold in the N-terminus, masks the site where HJ processing enzymes could act and therefore protects telomeres from t-loop cleavage.

We next determined whether the same mechanism protects telomeres from activating PARP1. Using conditional mouse KO for each of the shelterin proteins, we established that PARP1 is repressed by a combination of TRF2 and TIN2. With regard to the role of TRF2 in repressing PARP1, the TRF2ΔB mutant was deficient, pointing to a second function of the TRF2 basic domain. Our data using TRF2-H31A and the RuvC-fused TRF2ΔB indicate that the TRF2 HJ binding activity is also required for the repression of PARP1 at telomeres.

Collectively, our data establish that TRF2 employs a HJ binding fold in vivo to mask the structure at the base of the t-loop from HJ resolvases and PARP1.

OPPOSING ROLES OF HOLLIDAY JUNCTION RESOLUTION AND DISSOLUTION IN ALT-MEDIATED TELOMERE SYNTHESIS

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Alternative lengthening of telomeres (ALT) is an homologous recombination (HR)-mediated DNA synthesis mechanism that accounts for telomere maintenance in some of the most aggressive cancer subtypes. A number of genetic and epigenetic changes that contribute to ALT have been characterised, however very little is known regarding the underlying mechanism of telomere synthesis. We demonstrate that the SLX4/SLX1 and BTR protein complexes play opposing roles at ALT telomeres. SLX4 is a Holliday junction (HJ) resolvase, which processes HJs within ALT telomeres to produce crossover events. This results in telomeric exchange with no net increase in telomeric DNA, and suppresses ALT-mediated telomere lengthening. In contrast, the BTR complex, which comprises BLM, TOP3A, RMI1 and RMI2, acts as a HJ dissolvase at ALT telomeres, catalysing HJ dissolution by producing non-crossover events. Our data support a model whereby BTR promotes template-driven telomere synthesis by branch migration, followed by HJ dissolution. This results in a net increase in telomere length in the absence of telomeric exchange.

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TELOMERE TETHERING TO THE NUCLEAR PORE COMPLEX AND SUMOYLATION OF TELOMERE-BOUND PROTEINS MODULATES ERODED TELOMERE RECOMBINATION.

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Homologous recombination (HR)-dependent telomere maintenance mechanism is activated in a fraction of human cancers. This mechanism was originally identified in telomerase-negative yeast which can escape replicative senescence via two Rad52-dependent pathways, which require Rad51 and Rad59, respectively, and result in type I and type II survivors with distinct telomere organization. Previously, we have shown that a fraction of eroded telomeres highlighted by Cdc13/Rad52 foci in telomerase-deficient cells localizes to the nuclear pore complex (NPC), but the functional significance of this phenomenon was not understood. Now we present evidence that telomeres which cannot be repaired by Rad51-dependent HR localize to the NPC, and this in turn promotes type II survivor formation. We show that tethering of a single telomere to NPC improves type II telomere pattern formation in mutants with compromised type II pathway. We found that the level of telomere-associated SUMO gradually increases as telomeres shorten in the absence of telomerase. Moreover, we observed that delocalization of SUMO protease Ulp1 from NPC resulted in type II survivor formation defect, as well as targeting Ulp1 to a specific telomere resulted in type II pattern formation defect on that telomere. Finally, inactivation of the SUMO-targeted ubiquitin ligase, Slx5-Slx8, also resulted in type II survivor formation defect. Together our results suggest that tethering of unrepaired damaged telomere to the NPC favor a rescue pathway through type II recombination that is regulated by the sumo-state of telomere-bound proteins.

SLX4-INTERACTING PROTEIN SLX4IP: ROLES IN DNA REPAIR AND TELOMERE HOMEOSTASIS

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SLX4, also known as FANCP, participates in diverse genome maintenance pathways, including Holliday junction resolution, restoration of stalled replication forks, repair of DNA interstrand crosslinks and telomere homeostasis. As such, SLX4 is a key factor in preserving genome integrity. The physiological importance of SLX4 is underscored by the finding that biallelic mutations in the human *SLX4* gene are the underlying cause for Fanconi anemia, a severe genome instability syndrome that is associated with early onset bone marrow failure, congenital abnormalities and predisposition to cancer.
SLX4 is a large, multi-domain protein that acts as a molecular platform and regulator for multiple DNA signaling and repair factors including MSH2-MSH3, PLK1, TRF2 and the structure-specific endonucleases XPF-ERCC1, MUS81-EME1 and SLX1. In addition, SLX4 interacts directly with an uncharacterized protein called SLX4IP, whose function in maintaining genome integrity has remained elusive until now. Here, we show that SLX4IP localizes to telomeric chromatin and APBs, which is particularly evident in ALT cells. SLX4IP$^{-/-}$ U2OS and VA13 ALT cells display a hyper-recombination phenotype associated with elevated T-circles, T-SCEs and APBs. In addition, loss of SLX4IP leads to increased levels of endogenous DNA damage and sensitizes cells to DNA interstrand crosslinking agents and to the DNA polymerase inhibitor Aphidicolin. Collectively, our results indicate that SLX4IP participates in the maintenance of genome integrity, potentially as a regulator of SLX4-mediated DNA recombination during DNA repair and telomere homeostasis. Our current work will be presented.

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DECONDENSATION OF TELOMERIC CHROMATIN INDUCES DELETION OF T-LOOPS AND ACTIVATION OF ALT-MECHANISM IN HUMAN CELLS

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T-loops are ubiquitous in telomeric chromatin and are postulated to contribute significantly to telomere function and/or maintenance in eukaryotic cells. However, the mechanisms that regulate formation and dissolution of t-loops are poorly understood. Here, we employ a novel two-dimensional non-denaturing agarose gel method to detect t-loops and find that t-loops are present throughout the cell cycle and that formation of t-loops is tightly coupled to telomere replication. We also show that less condensed telomeric nucleosomes in ALT cells is correlated with fewer t-loops and more abundant t-circles. Indeed, TSA treatment of telomerase positive cells leads to hyperacetylation and decondensation of telomeric chromatin that are associated with decreased number of t-loops, the formation of telomere-associated PML bodies (APBs) and appearance of t-circles. The loss of t-loops is also accompanied by increased frequency of telomere sister chromatin exchange (T-SCE), rapid telomere attrition, and appearance of 5'-C-rich overhangs. These findings suggest that deletion of t-loops may occur by an HR-dependent process and that chromatin hyperacetylation may be a marker of and/or may activate the alternative lengthening of telomeres (ALT) pathway. This study provides evidence for novel epigenetic regulation of t-loops, and may have implications for understanding, preventing or reversing telomere dysfunction in human cells.

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SUPPRESSION OF THE ALTERNATIVE LENGTHENING OF TELOMERE PATHWAY BY THE CHROMATIN REMODELING FACTOR ATRX

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15% of cancers maintain telomere length independently of telomerase by the homologous recombination (HR) associated Alternative Lengthening of Telomeres (ALT) pathway. A unifying feature of these tumours are mutations in ATRX. Here we show that expression of ectopic ATRX triggers a suppression of the pathway and telomere shortening. Importantly ATRX mediated ALT suppression is dependent on the histone chaperone DAXX. Re-expression of ATRX is associated with a reduction in replication fork stalling, a known trigger for HR and loss of MRN from telomeres. A G-quadruplex stabiliser partially reverses the effect of ATRX, inferring ATRX may normally help facilitate replication through these sequences which, if they persist, promote ALT. We propose that defective telomere chromatinisation through loss of ATRX promotes the persistence of aberrant DNA secondary structures, which in turn present a barrier to DNA replication, leading to replication fork stalling, collapse, HR and subsequent recombination-mediated telomere synthesis in ALT cancers.

ROLE OF HISTONE VARIANT H3.3 IN TELOMERE CHROMATIN ASSEMBLY, AND H3.3 DYNAMIC IN ALT CANCER CELLS.

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The importance of Histone variant H3.3 and its chaperone ATRX in maintaining chromatin repression at the telomeres is implied by recent studies showing a strong link of ATRX mutations to the Alternative Lengthening of Telomeres (ALT) phenotype. It is unclear how ATRX mutations drive ALT, or affects it H3.3 deposition and post-translational modification in the global genome. In this study, we show here that H3.3 is targeted for K9 trimethylation (K9me3) by histone methyltransferases KMT1A/B to establish a heterochromatic state enriched in H3.3K9me3 at the telomeres. In H3f3a-/- and H3f3b-/- mouse embryonic stem (ES) cells, particularly with the loss of H3f3b, H3.3 deficiency results in reduced levels of heterochromatin marks including H3K9me3, H4K20me3 and of ATRX at telomeres. The H3f3b-/- cells show increased levels of telomeric damage and sister chromatid exchange (t-SCE) activity when telomeres are compromised by treatment with a G-quadruplex (G4) DNA structure binding ligand or by siRNA-depletion of ASF1. Overexpression of wild-type H3.3 (but not a K9 mutant of H3.3) in H3.3-deficient H3f3b-/- cells increases H3K9 trimethylation level at the telomeres and represses t-SCE activity induced by a G4 ligand. This is the first report to demonstrate that H3.3 is utilized as a heterochromatic mark, via trimethylation of its K9 residue.

To understand how ATRX mutation affects H3.3 behaviour in ALT cells, we explore the dynamics of phosphorylated H3.3 serine 31 (H3.3S31ph) in human ALT cancer cells. While H3.3S31ph is found only at pericentric DNA repeats during mitosis in most somatic human cells, a high level of H3.3S31ph is detected on the entire chromosome in ALT cells, attributable to an elevated CHK1 activity. Drug inhibition of CHK1 activity and expression of mutant H3.3S31A in these ALT cells result in a decrease in H3.3S31ph levels, accompanied with increased levels of γH2AX on chromosome arms and at the telomeres, and a reduced level of cell viability. Our findings suggest a novel role of CHK1 as a H3.3S31 kinase, and that CHK1-mediated H3.3S31ph plays an important role in the maintenance of chromatin integrity and cell survival in ALT cancer cells.

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LOSS OF ATRX IS SUFFICIENT FOR ALT ACTIVATION WHEN COMBINED WITH TELOMERE STRESS OF CELLS UNDERGOING CRISIS

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All cancer cells must attain replicative immortality to sustain their unlimited growth. This is normally achieved by the reactivation of telomerase. In a small (~10%) subset of cases, telomere elongation occurs in the absence of telomerase by utilizing the Alternative Lengthening of Telomeres (ALT) mechanism. Although much is known about telomerase-mediated telomere maintenance, the molecular mechanism of ALT remains elusive, although telomere elongation by aberrant homologous recombination (HR) between repetitive telomere DNA sequences seems likely. Additionally, in a high percentage of ALT-positive tumors, the chromatin remodeler, ATRX, is mutated. To test for the requirement of ATRX-loss in ALT onset we used a genetic knockout approach with rAAV and Cas9/CRISPR to delete ATRX in primary, transformed, and immortalized human cells. Genetic ablation of ATRX alone was not sufficient to enable ALT in either primary or telomerase-immortalized cells. In stark contrast, the loss of ATRX in transformed, but not immortalized cells, which were subsequently allowed to proceed to crisis resulted in an elevated rate of immortalization where all of the immortalized clones were ALT. We subsequently mimicked telomere crisis in ATRX-null, telomerase-immortalized cells by the over-expression of dominant negative telomerase (DN-hTERT). The expression of DN-hTERT induces a gradual telomere shortening that resembles, to a first approximation, the telomere shortening that accompanies normal cellular aging. In this setting, wild type cells invariably escaped crisis by either overexpression of the endogenous hTERT gene or by generating inactivating mutations in the integrated DN-hTERT gene. In striking contrast, ATRX-null cells expressing DN-hTERT escaped crisis by at least a temporary activation of ALT, which was characterized by the very rapid (within 2 to 3 population doublings) large amplification of their telomeric ends as quantitated by STELA. We are currently undertaking a screen of mutants in DNA double-strand break repair genes to test whether there is a genetic requirement for HR in ALT activation. Taken together, this is strong, albeit preliminary, evidence that the loss of ATRX may be sufficient to promote ALT when cells transit crisis, thus making it potentially an excellent therapeutic target for ALT-associated cancers.

LOSS OF ATRX SUPPRESSES RESOLUTION OF TELOMERE COHESION TO CONTROL RECOMBINATION IN ALT CANCER CELLS

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Ten to fifteen percent of all human cancers rely on a recombination-mediated mechanism of telomere maintenance termed ALT (alternative lengthening of telomeres) for their survival. These include cancers that are poorly understood and have terminal prognoses, hence understanding how the ALT mechanism is sustained and how it can be targeted for cancer therapy is important for human health. The chromatin-remodeling factor ATRX is frequently lost in tumors that use the ALT mechanism for telomere maintenance, but its role in telomere recombination is not known. Here we show that loss of ATRX suppresses resolution of sister telomere cohesion at mitosis. The resulting persistent telomere cohesion promotes chromatid exchange between sister telomeres, while it suppresses inappropriate non-allelic recombination between non-sisters. In the absence of ATRX, the histone variant macroH2A1.1 binds to the poly(ADP-ribose) polymerase (PARP) tankyrase 1, preventing it from localizing to telomeres and resolving cohesion. Forced resolution of sister telomere cohesion by overexpression of tankyrase 1 (or introduction of the macroH2A1.1-binding domain of ATRX) results in rampant telomere recombination between non-homologous chromosomes, genomic instability, and impaired cell growth, indicating that keeping sister telomeres in proximity into mitosis is essential for the ALT cell state. We propose that the newly identified ATRX-macroH2A1.1- tankyrase 1 axis may provide a novel therapeutic target in ALT tumors.

TWO ROUTES TO SENESCENCE IN THE ABSENCE OF TELOMERASE

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Failure to maintain telomeres leads to their progressive erosion at each cell division. This process is heterogeneous but eventually triggers replicative senescence, a pathway shown to protect from unlimited cell proliferation. However, the mechanisms underlying its variability and its dynamics are not characterized. Here, we used a microfluidics-based live-cell imaging assay to investigate replicative senescence in individual *Saccharomyces cerevisiae* cell lineages following telomerase inactivation. We show that most lineages experience an abrupt and irreversible transition from a replicative to an arrested state, contrasting with the idea of a progressive transition. Such a sharp switch is fully consistent with a mathematical model where the first telomere reaching a critical short length triggers senescence onset. Notably, a considerable part of replicative senescence heterogeneity is structurally built in the asymmetrical telomere replication mechanism. However, many lineages also undergo frequent reversible DNA damage checkpoint cell-cycle arrests, beginning soon after telomerase inactivation. Cells with this phenotype persist only at low frequency in bulk cultures, making them undetectable in conventional population-averaged assays. Based on data obtained in *RAD51*, *MEC1* and *POL32* mutant backgrounds, we propose a model where telomere replication fragility, enhanced by telomerase inactivation, initiates both genomic instability and post-senescence survival. These data reveal a cryptic route to senescence and suggest that another source of heterogeneity of senescence onset consists of stochastic telomere damages that require telomerase or homologous recombination for repair.

RAP1 IS A GATEKEEPER TO THE TELOMERASE-INDEPENDENT TELOMERE MAINTENANCE PATHWAY

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Uncontrolled cancer cell proliferation depends on chromosomal escape from telomere attrition due to the DNA "end-replication problem" for most linear eukaryotic chromosomes. In most human cancer cells, hyper-activation of telomerase activity allows chromosomes to be continually extended; however, in 10–15% of cancers, chromosomal escape from shortening is achieved through a telomerase-independent mechanism known as alternative lengthening of telomeres (ALT). Moreover, anti-telomerase cancer therapy provokes ALT to maintain telomeres in the surviving cells. ALT, which is dependent on homologous recombination (HR), is therefore an important primary and secondary target for cancer therapy. In both human cells and fission yeast (*Schizosaccharomyces pombe*), conserved shelterin components bind to telomeric DNA repeats forming a nucleoprotein complex providing telomere structure. Despite recent progress in understanding regulatory roles of shelterin in telomerase-mediated telomere elongation, little is known about how shelterin components control activation and perpetuation of telomerase-independent ALT telomere maintenance pathways. Here, we solved the crystal structure of the BRCT domain of *S. pombe* Rap1 and found that Rap1-BRCT contains the conserved BRCT phosphor-Ser/Thr binding cavity. Our biochemical analyses showed that Rap1-BRCT directly interacts with γH2A (γH2AX in humans), which usually marks DNA double-strand break sites or stalled replication forks to initiate HR-based DNA repair. We further found that the Rap1-BRCT–γH2A interaction is essential for the association of Rap1 with subtelomere but not telomere regions. Disruption of Rap1-BRCT–γH2A interaction does not affect telomerase-mediated telomere elongation, but instead accelerates the switch to the recombinational mode of telomere maintenance when telomerase is compromised or absent. We therefore propose that Rap1 acts as a molecular sensor of telomere length to ensure the switch to the telomerase-independent telomere maintenance mechanism happens when and only when the telomere becomes critically short in the absence of telomerase elongation.

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THE "NATURALLY HUMANIZED" TELOMERES OF THE BASIDIOMYCETE *USTILAGO MAYDIS* OFFER INSIGHTS ON ALT AND THE ROLE OF DNA REPAIR PROTEINS AT TELOMERES

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The basidiomycete *Ustilago maydis* is an attractive model for telomere research because it has the same telomere repeat unit as humans, a recombinational repair system that resembles the mammalian system, and a shelterin-like telomere complex. In support of the relevance of *U.* maydis, we found strong similarities between *U. maydis ku* mutants and human KU80-null cells; both are non-viable and suffer from substantial telomere aberrations (1).

To further characterize the telomere defects of the *U. maydis* mutant, we investigated the development of telomere aberrations over time. Strikingly, the progression of different defects displays distinct kinetics, suggesting that different reactions are triggered at different time points following *ku* depletion. The earliest alteration is the loss of G-strand overhang at ~12 hrs post *ku* depletion. This is followed (at ~18 hrs post depletion) by profound telomere length heterogeneity, high levels of extra-chromosomal telomere repeats, and massive accumulation of unpaired telomere C-strand and C-circles, all of which are characteristic of ALT cancer cells. This "intermediate" ALT stage is eventually superseded by a "terminal" stage (at ~30 hrs post depletion) characterized by loss of long telomeres and even more drastic accumulation short extra-chromosomal telomeres. This terminal phenotype is averted by concurrent *atr1* or *chk1* deletion, implying a requirement for an intact checkpoint pathway.

To identify factors involved in the formation of ALT-like telomeres in *U. maydis*, we depleted *ku70* in a series of DNA processing/repair mutants. The telomere aberrations of the *ku70*-deficient cells are partially suppressed by deletions of *ctip*, *dna2* and *exo1*, and almost fully suppressed by deletions of *mre11* and *blm*. The involvement of these factors, which are all implicated in double strand break (DSB) resections, suggest that aberrant resection of telomeres may be a key step in the genesis of the ALT pathway. In contrast, the abnormal telomeres of *ku70*-deficient cells are exacerbated by mutations in the 9-1-1 complex, *top3* and *rad51*, indicating that these factors function in later stages of the pathway. Our results provide detailed insights on the complex, multi-step nature of telomere metabolism in the *U. maydis* ALT model. Both MRN and BLM have been implicated in the ALT pathway of human cancers, further underscoring the relevance of the *U. maydis* model.

1. de Sena-Tomás et al. NAR (2015). Fungal Ku prevents permanent cell cycle arrest by suppressing DNA damage signaling at telomeres. In press.

HALO-FISH REVEALS THE DYNAMIC LIFE OF ECTR DNA IN ALT HUMAN CELLS

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To better study extrachromosomal telomere-repeat (ECTR) DNA in ALT cells, we developed a single-cell technique called 'Halo-FISH' (Nucleic Acids Res doi: 10.1093/nar/gkv091 in press). In Halo-FISH, agarose-embedded cells are stripped of protein, treated with NaOH to break up aggregated DNA, and subjected to Q-FISH. Telomeres remain in a 'nuclear core', while ECTR DNA diffuse into the agarose surrounding the nucleus ('Halo' region). ECTR DNA molecule number and size, as well as strand composition, is then analyzed in individual cells by deconvolution microscopy.

We find GM847 and VA13 human ALT cells average ~80 G/C-strand ECTR DNA molecules/nucleus. In comparison, U2OS ALT cells have fewer but longer ECTR DNA molecules, while primary and telomerase-positive cells contain <5 ECTR DNA molecules/nucleus. ECTR DNA in ALT cells exhibit striking cell-to-cell variations in number (<20 to >300), and range widely in length within the same cell (<1 to >200kb). ECTR DNA are composed of primarily G- or C-strand telomere-repeat DNA, indicating that individual ECTR DNA molecules do not undergo ligation or catenation events that would covalently link them with other ECTR DNA.

In spontaneously growing ALT populations, ECTR DNA molecules increase 3-5 fold in number but only ~22% in length as ALT cells progress from G1 to G2 of the cell cycle. This indicates that ECTR DNA are maintained by generating new molecules rather than elongating existing ones, ECTR DNA amplification is favored over semi-conservative replication, and that most ECTR DNA molecules are lost during mitosis. In addition, marked cell-to-cell variability in the ratios of G- to C-strand ECTR DNA molecules suggests that G- and C-strand ECTR DNA molecules are amplified independently of each other. Interestingly, S and G2 phase VA13 and GM847 ALT cells typically contain more ECTR DNA molecules than telomeres, suggesting that they are competitive substrates for telomere "sister chromatid exchanges."

Using Halo-FISH, we demonstrate that the number and size of ECTR DNA molecules in ALT cells is highly dynamic and dependent on BLM and FANCJ DNA helicases. By Flow-FISH, depletion of FANCD2 and FANCM causes rapid, ALT-specific, BLM- and FANCJ-dependent increases in total telomeric DNA. Since Halo-FISH enables the simultaneous analysis of ECTR DNA and chromosomal telomeres in a single cell, we show that these effects are primarily caused by marked increases in ECTR DNA number, rather than elongation of telomeres or ECTR DNA molecules. In summary, Halo-FISH provides a powerful tool to investigate the dynamics of ECTR DNA.

NEW ROLES OF THE RNAi PATHWAY IN MAKING TELOMERE-FREE CHROMOSOME ENDS.

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The discovery of a new mode of telomerase minus survival, 'HAATI' (Heterochromatin Amplification-mediated And Telomerase-Independent), has recently shaken the dogma that canonical telomeres are essential to maintain linear chromosomes (Jain et al., Nature 2010). In HAATI cells, telomeric sequences are superseded by blocks of generic heterochromatin, which jump to all chromosome ends and acquire the ability to protect them from fusions and degradation. While they lack of detectable telomeric repeats, these newly acquired termini recruit the canonical end-protection factor Pot1 and guarantee end protection.

The amplified elements in HAATI are repetitive sequences associated with heterochromatin. Most commonly, the ribosomal DNA (rDNA) is the preferred substrate for this spreading to all chromosome ends. However, in a rare subset of cases, the SubTelomeric Elements (STE) can carry out this unconventional end-protective function; yet, unlike rDNA-HAATI, STE-HAATI involves the amplification of STE repeats to internal as well as terminal genomic sites and thus is associated with a drastic genomic disruption.

Here we show that the RNA interference pathway (RNAi) is absolutely required for HAATI-rDNA formation. RNAi is specifically necessary for the jumping of rDNA between different chromosome ends. Moreover, we find that sequence jumping is the sole limiting event in HAATI formation. Intriguingly, we have also identified a new role for Dicer (Dcr1), the RNase component of the canonical RNAi pathway. Dicer actively inhibits the formation of STE-HAATI by repressing the amplification of the telomere-proximal STE repeats. Surprisingly, Dicer acts independently of the rest of the RNAi pathway in carrying out this genome-surveillance function.

Our results disclose the importance of noncoding RNAs in dictating the fates of unprotected chromosome ends and suggest avenues for uncovering mechanisms cancer cells might exploit in escaping the requirement for telomerase activation.

A NOVEL FISSION YEAST TELOMERE FORMATION SYSTEM REVEALS EFFICIENT HEALING OF SUBTELOMERIC BREAKS AND THE SLOW ESTABLISHMENT OF TELOMERIC HETEROCHROMATIN

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The fission yeast *Schizosaccharomyces pombe* has provided a robust model for telomere function in mammals, in part because it possesses a number of evolutionarily-conserved features including the histone H3 lysine 9 dimethylation (H3K9me2) mark for heterochromatin and large, complex subtelomeres composed of diverse repeats. However, *S. pombe* has lacked a rapidly inducible double-strand break (DSB) and telomere formation system. We and others have recently constructed multiple rapidly inducible DSB systems, and we have now leveraged one of these to create a rapidly inducible telomere formation system. Our system consists of an engineered, inducible I-SceI endonuclease and a "proto-telomere" cassette containing an I-SceI site in a unique DNA region adjacent or a control site at an internal chromosomal locus. Continuous I-SceI induction in cells with an internal site blocks cell growth. In contrast, I-SceI induction in cells containing a proto-telomere with 48 nt of telomere repeats placed adjacent to the subtelomere grow normally. The cut 48 nt proto-telomere was not degraded, and instead the repeat tracts were lengthened over several hours. Surprisingly, cutting at the 0 nt proto-telomere in the same location also did not impair growth. The subtelomeric DSB in the 0 nt proto-telomere was rapidly degraded, and then efficiently healed by recombination between homologous repeats in the subtelomere and elsewhere in the genome. Telomere-adjacent chromatin is silenced by inclusion of the H3K9me2 mark, and the stable, newly formed telomere caused increased H3K9me2 levels in the adjacent chromatin for at least 40 kb. As the stability and elongation of the 48 nt proto-telomere indicated the rapid acquisition of telomere function upon cleavage, we determined how rapidly cells acquired the new telomere-adjacent heterochromatin domain. A kinetic analysis of the H3K9me2 silent chromatin mark showed that heterochromatin spread slowly from the newly formed telomere over many cell divisions. Therefore, our rapidly inducible telomere formation system reveals a mechanism for the rapid healing of subtelomeric DSBs and the kinetics of formation of a new chromatin domain.

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MRE11 IS INVOLVED IN THE EPIGENETIC AND HERITABLE REGULATION OF YEAST TELOMERIC CHROMATIN

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The *mre11A470T* sensitive allele of *MRE11*, bypasses replicative senescence in telomerase negative yeast and eliminates of telomere healing in telomerase positive cells. The *mre11A470T* allele confers reduced telomere size through an increase of break-induced replication (BIR) survivors at the cs temperature, 15°C. We found that this allele also displays a unique pattern of telomere size complementation. After introduction of a mutant gene into a wild type background, the wild type telomere size is maintained, as expected for a recessive allele. In striking contrast, when a wild type allele is introduced into *mre11A470T* background, a heritable mutant phenotype is formed. In addition, after a plasmid shuffle between wild type and mutant genes in *mre11Δ* cells, wild type cells carrying shortened telomeres are formed. Thus, this allele is reliant on epigenetic factors. Telomeric sequences in yeast are organized into non-nucleosomal structures composed of multiple Rap1 molecules and associated binding factors. It has previously been shown that micrococcal nuclease digestion of telomeric chromatin form a structure (operationally termed the "telosome") that protects 400-500 bp of telomeric DNA. To examine the relationship of *mre11A470T* with Rap1, we used a temperature sensitive (ts) alleles of *RAP1* (*rap1-5*) that is located upstream of the Rif1 and Rif2 binding sites that are negative regulators of telomere size. We assayed the incubation time of MNase required to release the telosome in wild type, *mre11A470T*, *rap1-5*, and *mre11A470T rap1-5* cells at an identical concentrations of MNase. We found that the structure became more resistant to micrococcal nuclease in *mre11A470T* cells than in wild type or *rap1-5* telosomes. The *rap1-5* allele has little effect on the telosome structure at semi-permissive temperatures. In contrast, *mre11A470T rap1-5* cells increase the time substantially longer than wild type or *mre11A470T* cells. The dominant effect on telosomes is consistent with the in vivo inability of wild type Mre11 to confer a wild type phenotype. Our data suggest the Mre11 plays a critical role in the heritable and epigenetic regulation of the terminal chromatin. We also observed genetic interactions between *rap1-5* and *mre11A470T*. After a brief arrest, the *mre11A470T rap1-5* at the *rap1-5* restrictive temperature, 37°C, confers a premature and rapid increase in BIR suppressors at functionally limiting telomere sizes, suggesting a burst of BIR at subtelomeric and telomere regions, co-incident with loss of telomerase activity. This study demonstrates an epigenetic and heritable dimension to telomere homeostasis at the structural level and links the telosome and Mre11 to the regulation of telomere size control in yeast.

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DEPLETION OF TbRAP1 LEADS TO INCREASED LEVELS OF TERRA AND TELOMERIC R-LOOP

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Trypanosoma brucei causes human African trypanosomiasis, which is fatal without treatment. While proliferating in its mammalian host, *T. brucei* undergoes antigenic variation and regularly switches its major surface antigen, VSG, to evade elimination by host immune responses. Antigenic variation is a key pathogenesis mechanism enabling long-term *T. brucei* infection. There are more than 2,500 *VSG* genes and pseudogenes in the *T. brucei* genome, but VSG is expressed exclusively from subtelomeric VSG expression sites (ESs) in a strictly monoallelic manner. Both coupled activation and silencing of ESs (*in situ* switch) and homologous recombination (HR)-mediated events are involved in VSG switching. We have previously found that depletion of TbRAP1, a telomere protein, led to derepression of all subtelomeric silent VSGs (Yang et al. 2009. *Cell* 137:99; Pandya et al. 2013. *NAR* 41:7673). Recently we also found that a transient depletion of TbRAP1 led to an increased VSG switching frequency and HR-mediated VSG gene conversion is a predominant mechanism of these switching events. *T. brucei* telomeres are transcribed into TERRA of heterogenous sizes (Rudenko & van der Ploeg. 1989. *EMBO J.* 8:2633). Interestingly, we found that depletion of TbRAP1 led to a significant increase in the TERRA amount. TERRA has been shown to form R-loop structures involving the DNA-RNA hybrid, and this R-loop structure has been shown to promote HR (Balk et al. 2014. *RNA Biol.* 11:95). Using R-loop specific antibody, we also detected increased amount of R-loop structure at the telomere when TbRAP1 is depleted, suggesting that increased TERRA and R-loop level in TbRAP1 deficient cells leads to subsequent increased amount of HR at VSG loci that are immediately adjacent to the telomere.

* These authors contributed equally.

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IDENTIFICATION AND CHARACTERIZATION OF NEUROSPORA SHELTERIN

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A telomere-specific protein complex, Shelterin, caps and protects chromosome ends against inappropriate DNA damage, telomeric fusion and telomere length in eukaryotes. However, in any filamentous fungi, the corresponding Shelterin has not been identified. Here we show Neurospora Shelterin which is composed of at least five proteins. The two components, POT-1 and RAP-1, are conserved from yeasts to mammals whereas the others are only conserved in Ascomycota. Interestingly, Neurospora Shelterin does not contain CCQ-1, which is a core Shelterin component in *S. pombe*. By conventional Chromatin Immunoprecipitation (ChIP) and ChIP-seq anaylses, we confirmed that one of Neurospora Shelterin components is specifically localized to telomeres and many interstitial telomeric sequences. Fluorescence microscopic analyses revealed that all the components of Neurospora Shelterin are co-localized to several foci within nuclei. The telomeric foci are mostly associated with nuclear envelope and heterochromatin but not a single centromeric spot. Furthermore, the localization is not dependent of H3K9 methylation that directs heterochromatin and DNA methylation in Neurospora.

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REGULATION OF TELOMERASE GENE EXPRESSION BY TELOMERE LOOPING IN HUMAN CELLS

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The regulation of telomerase expression has been actively investigated since it was originally cloned in 1997. However, detailed mechanisms are still largely unknown or controversial. Here, we suggest a new mechanism of hTERT regulation by the length of telomeres. We previously reported that genes at long distances up to 10Mb from telomeres may be regulated by a modification of the classic telomere position effect (TPE) mechanism. We discovered the ISG15 over 1 MB from the telomere was regulated by telomere length but genes closer to the telomere were not regulated by classic TPE. We called this phenomenon telomere position effect over long distances (or TPE-OLD) to distinguish it from classic TPE. We observed that young human fibroblasts with long telomeres showed a looped chromatin structure between the hTERT locus and a region on 5p close to the telomeric repeats (distance about 1MB). The location of the telomerase gene on the genome was conserved at the end of chromosomes in higher primates, and we hypothesized that the telomerase gene in large long lived animals might be regulated by TPE-OLD. Thus using co-FISH methods we found in young cells with long telomeres that the hTERT gene and the probe near the 5p telomere to be in close proximity/adjacent which was lost when telomere became short (e.g. the co-FISH signal became separated). The looping structure also influenced changes in hTERT transcription, and it was also closely associated with telomerase activity in primary lymphocytes during a mitogenic stimulation. Telomere looping was also related to regulation of hTERT expression in human promyelocytic leukemia cells during differentiation. Therefore, we propose that the expression of active telomerase requires permanent or reversible disengagement of telomere looping in various situations. While the underlying mechanism is being actively investigated, we did observe significant changes in DNA methylation at the hTERT locus related to the length of telomeres. This change in the genome structure at the hTERT locus might provide novel insights into how the tight regulation of human telomerase in somatic cells is reduced during aging potentially leading to permissive environment for telomerase activations as part of tumor development.

ALTERED ACTIVITY AND TELOMERE ASSOCIATION OF DISEASE-ASSOCIATED VARIANTS IN THE HUMAN TELOMERASE "INSERTION IN FINGERS" DOMAIN

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The telomerase holoenzyme confers replicative longevity to eukaryotic cells in which it is active and is known to contribute to the evasion of proliferation regulatory mechanisms in cancer cells. Telomerase reverse transcriptase (TERT), the catalytic subunit is a key biomarker in tumors and a desirable target for cancer therapy. Unique human TERT (hTERT) domains like the "insertion in fingers" domain (IFD) may mediate inherent telomerase functions such as repeat addition processivity (RAP), thus providing an opportunity for specific inhibition of telomerase. RAP is the process by which telomerase extends chromosomal ends through the addition of short telomeric repeats without dissociating from the chromosome. It is crucial for overcoming the end-replication problem in organisms with linear chromosomes and the mechanism by which constitutively active hTERT contributes to replicative immortality in cancer. Moreover, mutations in the IFD have been found to be associated with pre-mature aging diseases like dyskeratosis congenita, which target frequently proliferating cells. Patients with these diseases also sometimes present with increased incidence of cancers like acute myeloid leukemia at later stages in life due to high levels of genomic instability caused by critically shortened telomeres. Developing a greater understanding of the IFD and its disease-associated mutations could lead to future treatment avenues in both cancer and pre-mature aging syndromes. We generated and transiently overexpressed IFD disease-associated telomerase mutant enzymes to examine their ability to assemble with hTR, the level of enzyme activity and processivity including the translocation efficiency of these variants, and telomere localization. When compared with wild-type enzyme, these variants demonstrate ~50% decreased enzyme processivity but no significant defects in assembly with the telomerase RNA template component (hTR) or in enzyme translocation. However, the average instances of co-localization between disease-associated mutant telomerases and telomeres were reduced compared to the average number of wild-type telomerase associations with telomeres. Using telomerase-negative cells with limited lifespan, we introduced wild-type or mutant hTERT to assess ability to confer cellular immortalization. All variants could immortalize the limited lifespan cells, however the cells displayed a slower growth rate and increased apoptosis compared to cells expressing wild-type telomerase. These findings implicate the importance and disease relevance of telomere recruitment in telomerase insufficiency syndromes.

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ANALYSIS OF RDRP PRODUCTS SYNTHESIZED BY TERT

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TERT elongates telomere through an RNA-dependent DNA polymerase activity. We have reported that human TERT also has an RNA-dependent RNA polymerase (RdRP) activity (Maida Y. *et al.*, Nature 2009). In addition, we have reported that the RdRP activity of TERT is enriched in mitotic phase and contributes to proper mitotic progression in part via the transcriptional repression of heterochromatic regions (Maida Y. *et al.*, MCB 2014). Although the RdRPs in several model organisms and viruses produce antisense RNA strands in a primer independent manner (*de novo* synthesis), *de novo* RNA synthesis by TERT had not been demonstrated. Here we report that TERT generates short RNAs *de novo* through the RdRP activity. We performed *in vitro* RdRP assay with TERT immune complexes immunoprecipitated (IP-RdRP assay) from cell lysates or recombinant TERT (rTERT) from insect cells using chemically synthesized RNAs of 34 nucleotides in length as templates. We found the short RNA products corresponding to the template length in the assays with both cellular TERT and rTERT. The production was inhibited by telomerase inhibitors and a viral RdRP inhibitor but not by a Pol II inhibitor, indicating that the RdRP activity of TERT was responsible for the RNA synthesis. We also identified the short RNA products with 5'-triphosphate termini, the specific structure for the RNAs synthesized *de novo*. We further analyzed the profiles of the RdRP products using next generation sequencing method and confirmed that the short RNAs synthesized in the assay are complementary to the template RNAs. Taken together, the data indicate that RdRP of TERT synthesizes the short RNA species using *de novo* synthesis mode.

CHANGES IN TELOMERE PROTEIN COMPOSITION INDUCED BY TUMORIGENIC CONVERSION OF NORMAL HUMAN FIBROBLASTS

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Several lines of evidence suggest that cancer development might be accompanied by reorganization of telomeric chromatin. However, a systematic, comprehensive study of the tumorigenesis-associated changes in telomere protein composition is still missing. Here, we have applied the Quantitative telomeric chromatin isolation protocol (QTIP) [1] to compare telomeric states in isogenic cell lines representing several stages of the transformation process. Using the approach developed by Hahn et al. [2, 3], human embryonic lung fibroblasts were converted into tumorigenic cells in a step-by-step fashion using serial introduction of genes encoding the hTERT subunit of telomerase, the SV40 large T and small T antigens, and the H-RasV12 oncogene.

Ongoing pairwise comparison of the four cell lines has revealed transformation-induced alterations in abundance and/or telomere occupancy of multiple proteins, including some unexpected protein networks. Interestingly, tumorigenic conversion in our cellular model system is associated with an altered shelterin recruitment pattern. The biological relevance of these findings requires further investigation. Besides, several novel telomeric proteins have been identified and are being validated.

All in all, this project may open up novel avenues for investigating the roles of telomeres in cancer.

1. Grolimund L, et al. Nat Commun 4:2848 (2013).
2. Hahn WC, et al. Nature 400:464-8 (1999).
3. Hahn WC, et al. Mol Cell Biol 22:2111-23 (2002).

THE 1213-NT FISSION YEAST TELOMERASE RNA SUBUNIT TER1 IS A FLEXIBLE SCAFFOLD

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Previous research on *Saccharomyces cerevisiae* telomerase RNA, TLC1, has led to a functional miniaturized allele, indicating that two-thirds of this 1157-nt RNA is dispensable for function. TLC1 also acts as a flexible scaffold for holoenzyme protein subunits; the Est1, Ku and Sm7- -binding regions can be repositioned on the RNA with retention of function. The fission yeast *Schizosaccharomyces pombe* is evolutionarily distant from *S. cerevisiae* yet its 1213-nt telomerase RNA, TER1, is similar in size to TLC1. Unlike TLC1, TER1 shares with the human telomerase RNA the requirement for a three-way junction domain for catalytic activity. While several regions of the TER1 structure have been studied and modeled, a complete, well-tested secondary structure model of the RNA does not yet exist. Phylogenetic analysis of TER1 is difficult because telomerase RNAs are evolving very rapidly and only four species of fission yeast have been identified. Therefore, alternative approaches are necessary in order to develop and test a complete model of the RNA. Using an *in vitro* reconstituted activity assay, we have created an active 623-nt Micro-TER1 RNA that contains the catalytic core as well as the essential three-way junction region. We are now working on testing a smaller Micro-TER1 to define the minimal amount of RNA required for catalytic activity. We have also used truncation mutants in genetic complementation tests to determine which regions of the RNA are essential *in vivo*, we find that about 40% of the RNA is dispensable. *In vivo* tests have also shown that the essential three-way junction region can be relocated, providing evidence that TER1, like TLC1, is acting as a flexible scaffold. Lastly, using base-pair compensatory mutants in the core, we are testing models for the TER1 pseudoknot.

RELOCATING THE ENDS OF HUMAN TELOMERASE RNA TO NEW POSITIONS REVEALS INSIGHTS INTO RNP ARCHITECTURE AND MECHANISM

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Human telomerase is upregulated in more than 85% of cancers; thus, understanding the molecular mechanisms of telomerase function can facilitate finding ways to inhibit telomerase activity as anti-cancer therapeutics. To better understand structure-function relationships in human telomerase RNA (hTR), we have tested 46 circular permutations throughout the 451-nt hTR via the direct *in vitro* activity assay. Circular permutations reposition the 5' and 3' ends, thus essentially breaking the phosphate backbone at the new location. Our telomerase activity results reveal several important areas of RNA connectivity within hTR. First, we find that circular permutations 3′ of the template have reduced repeat-addition processivity. This suggests functional similarity with the analogous template-recognition element in *T. thermophila* telomerase RNA, despite the evolutionarily distance, and significant differences with the *S. cerevisiae* core (Mefford et al, EMBO, 2013). Second, we find that circular permutations in the G-rich region at the 5′ end of hTR as well as between the conserved core and the CR4/5 region increase telomerase activity. Third, several circular permutations in and around the base triples of the pseudoknot or the P6.1 helix in the CR4/5 region completely abolish telomerase activity. Together, our comprehensive exploration of RNA connectivity requirements in hTR extends current understanding of telomerase RNA function. Further, the last class of mutants represent attractive candidates for developing anti-cancer therapeutics.

CAP INDEPENDENT SURVIVORS REQUIRE CHECKPOINT INACTIVATION AND DSB ADAPTATION GENES.

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In budding yeast, the telomerase RNP and the Cdc13 protein are two key players acting to assure telomere stability. In the absence of telomerase, yeast cells enter a crisis but some undergo a conserved process to “Survive” this telomerase loss. These cells survive by using homologous recombination-dependent mechanisms to maintain telomeres. Previously, we showed that such “survivor” cells can even bypass the loss of Cdc13 and give rise to Adaptor strains. In this study we show that those adaptor cells (or *cdc13Δ* cells) grow with persistent DNA damage, which does not result in detectable checkpoint activation or a defect in the cell cycle progression. The results show that this lack of checkpoint inactivation is due to the accumulation of mutations in at least two checkpoint genes, namely *RAD24* or *MEC1*. Finally, two genes, *PTC2* and *TID1*, that are required for adaptation to persistent DNA damage are also required for cap-independent cdc13Δ formation. Altogether, these results show that while the capping process can be very malleable and flexible, it takes a very specific genetic setup to allow a change from canonical capping to alternative capping. In the alternative capping mode, genome integrity mechanisms are abrogated, which we hypothesize could cause increased mutation frequencies. These latter characteristics have clear parallels in transformed human cancer cells and our study of this process in yeast should allow deeper insights into genome instability processes in cancerous cells.

TESTING THE ROLE OF TIN2 IN TELOMERASE ACTIVITY AND PROCESSIVITY

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The shelterin protein TIN2 protects telomeres and regulates telomere length. TIN2 depletion results in telomere elongation and loss of end protection. However, a number of characterized TIN2 missense mutations cause telomere shortening in Dyskeratosis Congenita (DC)[1] and pulmonary fibrosis[2] patients. While the genetics underscore TIN2's importance, the mechanism through which TIN2 regulates telomere length remains unclear. Previous work has shown these TIN2 mutations do not affect telomere protection or shelterin binding interactions, but the severity of the mutations in humans indicates that TIN2 has an important function in telomere elongation.

We generated cDNA expression constructs of V5-tagged TIN2 variants with wild-type sequence or DC mutations K280X, K280E, R282S, or R282H in both TIN2S and TIN2L. To dissect the function of TIN2, we tested whether these TIN2 mutant proteins affect either telomerase activity or processivity in a direct telomerase activity assay. To test the effect of these proteins on telomerase activity in cell lysates, we over-expressed TERT, TR, TPP1 and POT1 components in human cells, similar to a previously described assay.[3] Rather than using a multi-plasmid transfection, we generated a cell line that reproducibly expresses each protein at similar levels to assure uniform expression of the necessary components. We engineered a construct that joined FLAG-tagged TERT, TPP1, and POT1 with 2A peptides to form a polycistronic expression cassette that was then stably integrated into a single locus in 293T-REx cells. Western blot analysis showed that these cell lines reproducibly co-express all three proteins at similar levels. Lysates from these cells generate highly processive telomerase activity. Using these cell lines, we are expressing TIN2 variants in both TIN2L and TIN2S to examine the effect of TIN2 on telomerase activity.

These experiments will provide mechanistic insight to the effect of TIN2 and the two TIN2 isoforms on telomerase activity. Elucidating how TIN2 participates in telomere length maintenance is important to understanding telomere length maintenance in human cells.

1. Savage SA, Giri N, Baerlocher GM, et al. TINF2, a component of the shelterin telomere protection complex, is mutated in dyskeratosis congenita. Am J Hum Genet 2008; 82:501-509
2. Alder JK, Stanley SE, Wagner CL, et al. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. Chest 2014
3. Nandakumar J, Bell CF, Weidenfeld I, et al. The TEL patch of telomere protein TPP1 mediates telomerase recruitment and processivity. Nature 2012; 492:285-289

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TELOMERIC G-QUADRUPLEXES ARE A SUBSTRATE AND SITE OF LOCALIZATION FOR HUMAN TELOMERASE

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It is widely accepted in telomere biology that G-quadruplexes sequester the 3′ end of the telomere and prevent it being extended by telomerase. Here we purify and characterize stable, conformationally homogenous human telomeric G-quadruplexes, and demonstrate that human telomerase is able to extend parallel intermolecular conformations in vitro. These G-quadruplexes align correctly with the RNA template of telomerase, demonstrating that at least partial G-quadruplex resolution is required. A highly purified preparation of human telomerase retains this extension ability, establishing that the core telomerase enzyme complex is sufficient for partial G-quadruplex resolution and extension. The parallel-specific G-quadruplex ligand N-methyl mesoporphyrin IX (NMM) causes an increase in telomeric G-quadruplexes during S phase, and we show that telomerase colocalizes with a subset of telomeric G-quadruplexes in vivo. The ability of telomerase to partially unwind, extend, and localize to these structures implies that parallel telomeric G-quadruplexes may play an important biological role.

TELOMERE DE-PROTECTION IN THE BRAIN INDUCES MASSIVE CHROMOSOME FUSIONS BUT LIMITED COGNITIVE IMPAIRMENT.

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Proper telomere function is essential in highly proliferative tissues. However, whether end-protection is required in differentiated cell types is not fully understood. A previous report showed that severe loss of end protection caused by TRF2 depletion is well tolerated in hepatocytes (1). In contrast, telomere de-protection within the brain has been suggested to contribute to neurological disorders (2). In particular a critical role for TRF2 in terminally differentiated neurons has been reported (3). To probe the consequences of telomere de-protection in the context of the central nervous system we used conditional inactivation of the shelterin components TRF2 and POT1 at different stages of differentiation in mice. Our data show that TRF2 (or POT1) inactivation in multipotent neural stem cells (NSCs) leads to lethality and severe impairment in brain development. In contrast, depletion of TRF2 (or POT1) in differentiating and differentiated neurons had no adverse consequences on global brain development and function. Our data supports the hypothesis that proper telomere function is dispensable for differentiated cell types.

1. Lazzerini Denchi E, Celli G, de Lange T. Hepatocytes with extensive telomere deprotection and fusion remain viable and regenerate liver mass through endoreduplication. Genes & development. 2006;20(19):2648-53. Epub 2006/10/04. doi: 10.1101/gad.1453606. PubMed PMID: 17015429; PubMed Central PMCID: PMC1578691.
2. Eitan E, Hutchison ER, Mattson MP. Telomere shortening in neurological disorders: an abundance of unanswered questions. Trends in neurosciences. 2014;37(5):256-63. Epub 2014/04/05. doi: 10.1016/j.tins.2014.02.010. PubMed PMID: 24698125; PubMed Central PMCID: PMC4008659.
3. Zhang P, Dilley C, Mattson MP. DNA damage responses in neural cells: Focus on the telomere. Neuroscience. 2007;145(4):1439-48. Epub 2007/01/09. doi: 10.1016/j.neuroscience.2006.11.052. PubMed PMID: 17207936; PubMed Central PMCID: PMC1924472.

SILENCING OF THE TELOMERIC GENE TPP1 BY A NONCODING RNA DERIVED FROM ITS OWN 3'-UTR

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The protein TPP1 is a unique shelterin component in that it not only protects chromosome ends, but it also recruits telomerase to the same ends. The dual functions of TPP1 at telomeres must mandate strict regulation of TPP1 levels in the cell. Whereas excess TPP1 at telomeres may increase POT1 and/or telomerase at telomeres, excess TPP1 in the nucleoplasm may sequester POT1 and/or telomerase to elicit the opposite effect. Here, we report the serendipitous discovery of a unique form of TPP1 downregulation resulting from base complementarity between regions in the ORF of the TPP1 mRNA and a noncoding RNA derived from its 3'-UTR. To our knowledge, this is the first report of silencing of a gene by an RNA derived from its own 3'-UTR. We propose that this novel 3'-UTR-based gene regulation helps achieve the low levels of TPP1 observed in vivo.

We identified and cloned noncoding RNAs that are expressed from an alternative transcription start site slightly upstream of the 3'-UTR of TPP1 using the CAGE database (which catalogs observed, 5'-capped transcript start sites), and by 5'-RACE (which is used for cloning the 5'-end of RNAs). Transient or stable overexpression of noncoding RNAs containing the 3'-UTR sequence of human TPP1 depletes both TPP1 mRNA and protein. Mutations that disrupt the potential base pairing between the 3'-UTR and the ORF rescue TPP1 protein levels. TPP1 silencing is only observed when the 3'-UTR is expressed as part of a noncoding RNA and not when it is contained within TPP1 mRNA.

Our results provide strong evidence for silencing of the TPP1 gene by a noncoding RNA containing its 3'-UTR sequence. However, our discovery also raises several questions. First, what is the mechanism of silencing? We are currently performing experiments to distinguish between transcription-inhibition versus mRNA-degradation based mechanisms. Second, how conserved is this mode of TPP1 silencing? There is strong conservation of this intragenic base pairing potential in the TPP1 gene from several mammals, but there are interesting exceptions too. Third, what is the importance of this novel regulation of TPP1 in telomere biology? We propose that the 3'-UTR based silencing provides an efficient avenue for reducing TPP1 protein to levels observed at natural telomeres; this will avoid deleterious effects on end protection and/or end replication that could result from aberrant recruitment of POT1 and/or telomerase. Future studies will further reveal the mechanism and biological importance of this unique gene silencing mechanism, and explore whether it is a more generally utilized method for gene regulation.

FUNCTIONAL EVIDENCE THAT ATRX REPRESSES THE ALTERNATIVE LENGTHENING OF TELOMERES MECHANISM

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Normal mortal fibroblasts contain repressors of the ALT mechanism, as demonstrated by somatic cell hybridization of ALT cells with normal mortal fibroblasts. Previous studies indicate that the majority of ALT-positive tumors and cell lines contain mutations in a member of the ATRX/DAXX chromatin remodeling complex. To provide functional evidence that ATRX is indeed an ALT repressor, we conducted immortalization and ATRX over-expression studies. Using SV40-transformed human fibroblasts, we knocked down ATRX in pre-crisis cells and continued to passage the cells through a period of culture crisis. Upon examination of 36 immortal cell lines, we found that knockdown of ATRX was not sufficient to activate ALT, but that it 1) significantly decreased the length of time in crisis prior to immortalization and 2) increased the proportion of immortal lines that activated the ALT mechanism. In seven shRNA control and one shDAXX immortal cell lines, spontaneous loss of ATRX was observed. Upon sequencing ATRX, only two lines were shown to harbor premature stop codons. These data are consistent with ATRX loss facilitating immortalization via the ALT mechanism. To further examine if ATRX is indeed an ALT repressor, we expressed exogenous ATRX in three ALT-positive/ATRX-negative cell lines. Western blotting showed that the ATRX protein level peaked at day 2 and was undetectable by day 8 post-transfection. The presence of C-circles and ALT-associated PML bodies (APBs), as well as telomere length, were assessed until 8 days after ATRX expression. C-circles significantly decreased by up to 50% after 2 days of ATRX expression, which was also accompanied by a significant decrease in the number of APB-positive nuclei. However, telomere length did not change after transient ATRX expression. G418-resistant clones were isolated from ATRX-transfected cultures. Of over 80 clones analyzed from two different ALT-positive/ATRX-negative cell lines, only one expressed ATRX. Further examination of this single clone showed that ATRX expression was maintained until 40 PDs, after which ATRX expression was lost. These results suggest there is selection pressure against expression of ATRX in ALT cells, consistent with the other evidence that ATRX functions as an ALT repressor.

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PROTECTION OF THE DNA 5'-END AT TELOMERIC DS-SS JUNCTIONS BY RAP1 AND CDC13

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Telomeres are specialized chromosomal end structures composed of stretches of repetitive DNA ending with a TG-rich single-stranded 3' overhang and its associated proteins. Presence of a single-stranded overhang creates a double and single-strand (ds-ss) DNA junction at the telomere ends, producing susceptible 3' and 5' DNA ends demanding protection. While the end-replication problem explains the formation of 3' overhangs in the lagging- strand synthesis at telomeres, the 3' overhang formed at the leading-strand telomere is believed to be acquired through a resection of the 5' end. Although the mechanism behind the 5' end resection is still unclear, protection from uncontrolled 5' end resection would be necessary for maintaining genome stability.

The budding yeast Saccharomyces castellii provides ideal settings for detailed studies on telomere binding proteins by virtue of its regular 8 nt telomeric repeats (5'-TCTGGGTG-3'). In budding yeasts telomeric dsDNA is bound by Rap1 and ssDNA 3' overhang is bound by Cdc13, and we have defined the minimal binding sites (MBS) for S. castellii Rap1 and Cdc13 in these respective regions (Rhodin et al., 2006, 2011). However, we have also shown that Rap1 can bind over the ds-ss DNA junction and compete with Cdc13 for binding to the ssDNA close to the junction (Gustafsson et al., 2011).

We have developed a sensitive in vitro assay to analyze the DNA end protective ability of telomere binding proteins against 5'-3' exonucleases. In our DNA end protection assay (DEPA), we use oligos mimicking the telomeric ds-ss DNA junctions with Rap1 or Cdc13 bound to them and analyze the protection of the CA-rich 5' strand against 5'-3' exonuclease degradation.

We find that both Cdc13 and Rap1 are capable of protecting the 5' end from degradation by exonuclease activity. The extent of the protection is depending on where we position the MBS of these respective proteins in relation to the 5' end. Thus, the protective ability of Rap1 versus Cdc13 is depending on the 5' end permutation at the ds-ss junction. We conclude that the two proteins are acting in concert to protect the 5' end, which would be beneficial for limiting the 5' resection in a situation where Rap1 competes with the Cdc13 protein for binding at the ds-ss junction.

References:

Rhodin J, Astromskas E and Cohn M (2006) J. Mol. Biol. 355, 335-346
Rhodin J, Gustafsson C and Cohn M (2011) Genome Integrity 2, 2
Gustafsson C, Rhodin J and Cohn M (2011) J. Biol. Chem. 286, 45174-85

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TELOMERIC DOUBLE STRAND BREAK REPAIR

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The majority of DNA double stranded breaks (DSBs) are repaired with high fidelity within the first few hours following damage. However a subset of breaks go unrepaired, perhaps indefinitely. Unrepaired DSBs contribute to instability and/or trigger cell death or senescence, potent drivers of carcinogenesis and/or degeneration of human tissues with age. Therefore, characterizing differences between reparable and irreparable DSBs is of the upmost importance.

Telomeres, the repetitive and protective ends of eukaryotic chromosomes, must avoid recognition as DSBs to prevent inappropriate processing by the cellular repair machinery. In order to do so, functional telomeres must not only preserve some minimal telomere length, they must also maintain end-capping ability, a feat accomplished by a plethora of proteins, but which is thought to rely heavily on telomere repeat factor 2 (TRF2). This intentional repair avoidance feature of telomeres makes them a particularly attractive candidate for investigating impaired DSB repair capacity. Consistent with recent reports, we find that telomeres represent the majority of unrepaired damage following induction of global DNA damage, as evidenced by residual DNA damage foci co-localizing at telomeres. Importantly, we also find that these persistent, unrepaired telomeres are of normal length (they are not critically shortened), and they are not deficient in TRF2 (they are likely not un-capped), suggesting that they may indeed contain DSBs. However, because telomeres represent such a small fraction of the genome, massive amounts of global damage are required to ensure telomere-specific damage and telomere enriched damage does not become evident for several days, making interpretation difficult. To directly test whether telomeric DSB repair occurs at a slower rate than elsewhere in the genome – or perhaps not at all – we have initiated studies utilizing a recombinant endonuclease that cuts telomeric DNA in human cell lines. Results will facilitate critical comparisons between the kinetics of DSB repair occurring specifically at telomeres, globally (ionizing radiation induced), or at a specific genomic DSB site (I-SCEI induced).

DEFINING THE DISTINCT TRANSCRIPTIONAL RESPONSE TO SHORT TELOMERE-INDUCED SENESCENCE

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Loss of telomerase activity results in the progressive shortening of telomeric DNA and eventually a specific G2/M cell-cycle arrest known as senescence. In yeast, a small subset of cells known as survivors can escape this arrest by initiating a recombination-mediated telomere lengthening pathway. Using a telomerase-negative yeast strain, we have taken several approaches to elucidate the adaptive changes required in senescent and post-senescent cells, including whole-genome sequencing to identify any potential causative mutations as well as RNA-seq to monitor changes in gene expression. We find no mutations correlating with survivor cells and conclude that genetic changes are not a required step in survivor formation. Our transcriptome data reveal several interesting features of the cellular response to telomerase deletion. First, a shared subset of genes shows differential expression at every time point, consistent with previous reports of a telomerase-deletion response. This subset is particularly enriched for genes involved in amino-acid biosynthesis. Second, both the pre-senescent and survivor samples exhibit widespread down-regulation of ribosomal proteins, some of which also have lower expression in cancer cells. Third, in senescing cells we observe differential expression of ~1100 genes. The differentially expressed genes show significant overlap with changes observed during slow growth and the global starvation response, including upregulation of several key autophagy genes and cell-wall components as well as apparent changes in hexose transporter expression. Further bioinformatic analysis revealed a set of 573 genes that are differentially expressed during senescence, but not during the DNA-damage response, slow growth or in G2/M-arrested cells. This indicates that telomere-induced senescence represents a specific and distinct quiescence-like state. The senescence state is characterized by concerted changes in both the meiotic and ribosome biogenesis machinery. We propose a model where senescing cells adopt a starvation-state program to promote survival during cell-cycle arrest until telomeres can be restored, either by telomerase or recombination-mediated lengthening.

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ANALYSIS OF TELOMERE LENGTH VARIATION IN RECOMBINANT INBRED MAGIC LINES OF ARABIDOPSIS THALIANA

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While each eukaryotic species is characterized by a specific size range of telomere tracts, mean telomere length in many species shows considerable inter-individual variation. This telomere length variation is known to be under strong genetic control, but the factors establishing telomere length set point within natural populations are largely unknown. Although several recent genome-wide association studies uncovered a few genetic loci that contribute to telomere length polymorphism in humans and other eukaryotes, the power of natural variation analysis is currently underutilized, especially in terms of identification of novel genes and genetic variants with large effects on telomere length set point. Because of its superior biological and genomic resources and the availability of hundreds of genetically distinct natural populations and recombinant inbred lines, the model plant Arabidopsis thaliana offers a unique opportunity for the analysis of natural telomere length variation in a model multicellular eukaryote. We previously showed that natural Arabidopsis populations display significant telomere size differences, strongly suggesting that identification of casual QTLs is a promising route to understanding genetic diversity controlling telomere length polymorphism.
Telomere length in several hundreds of MAGIC (Multiparent Advanced Generation Inter-Cross) lines was assayed using a standard Telomere Restriction Fragment analysis, and values for mean telomere length, maximum intensity and telomere range were quantified with the TeloTool software. We show that Arabidopsis MAGIC lines display up to 2-3 fold difference in telomere length set points. Telomere length heritability in the 19 parental ecotypes used to construct MAGIC lines is remarkably high, suggesting that environmental influence on the phenotype is minor and that the observed intra-population telomere length polymorphism can be largely explained by genetic factors. Current efforts aim to identify QTL loci involved in the establishment of population-specific telomere length. Overall, better understanding of genetic differences in natural populations with distinct telomere length set points may provide important insights into the molecular basis for different rates of aging among individuals.

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IDENTIFICATION AND CHARACTERIZATION OF SITES OF *DE NOVO* TELOMERE ADDITION IN SACCHAROMYCES CEREVISIAE

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Telomeres are the protein/DNA complexes at the end of many linear eukaryotic chromosomes. Telomerase is a ribonucleoprotein enzyme complex that catalyzes the addition of nucleotides to the ends of chromosomes. Telomerase is also capable of adding telomeric repeats at internal chromosomal sites following a double-strand break. We hypothesize that such *de novo* telomere addition serves as a mechanism for damage control by preventing extreme resection and potential loss of essential genes when other mechanisms of repair fail. *De novo* telomere addition at internal chromosomal sites allows cells to continue dividing at the expense of some terminal sequences. We have defined an 80bp TG-rich region on the left arm of chromosome V as a 'hotspot' for *de novo* telomere addition. To address contextual effects, we have moved a minimal portion of this 'hotspot' onto a non- essential region on chromosome VII and shown that this site stimulates telomere addition in this new location. The hotspot on chromosome V lies telomere-proximal to the last essential gene on the left arm of this chromosome such that further resection would ultimately lead to cell death. We propose that additional hotspots for *de novo* telomere addition will be located telomere-proximal to the last essential gene on the chromosome. We have recently identified a new site of telomere addition on the left arm of chromosome IX that closely resembles the site on chromosome V. Current work aims to identify additional sites and to understand the mechanism and regulation of these events.

TELOMERASE REVERSE TRANSCRIPTASE AS METABOLIC REGULATOR IN TELOMERASE-DEFICIENT MOUSE MODEL

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Among human telomeropathies, liver disease is a common feature, including cirrhosis, non-cirrhotic portal hypertension (NCPH), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and hepatocellular carcinoma (HCC). However, liver injury cannot be entirely explained by proliferative senescence provoked by telomere erosion. To assess this issue, the metabolic alterations in telomerase-deficient mice livers were assessed in WT (with long telomeres), telomerase reverse transcriptase knockout (Tert-/-), and telomerase RNA component knockout (Terc-/-) (with short telomeres) by exposure to liquid high fat diet (HFD) and regular diet (RD) conditions for 15 days. Liver weights increased (P=0.002) and serum ALT levels were elevated (P=0.03) in Tert-/- mice after HFD, compared to Terc-/- and WT animals. HFD also induced higher liver triglycerides levels in Tert-/- (P=0.02) and Terc-/- (P=0.007) but not in WT livers and provoked hepatic steatosis with abundant and large lipid deposits only in Tert-/- hepatocytes. Telomerase inhibition by zidovudine led to fat accumulation in Terc-/- livers upon HFD, whereas WT livers exhibited few changes in similar condition. In Tert-/- and Terc-/- livers, HFD induced the expression of several genes related to glucose and lipid uptake such as Cpt1, Acaca, and Pklr. Gene expression of Tert-/- livers also induced repression of Fabp5, a fatty acid binding protein. In addition, quantitative analysis of metabolites, as determined using capillary electrophoresis mass spectrometry (CE-MS), revealed alterations on tricarboxylic acid cycle (TCA cycle) by decreasing aconitate, malate, fumarate and low NADH/NAD+ ratio in Tert-/- livers. Whereas HFD restored NADH/NAD+ ratio and maintained fumarate and malate levels in Terc-/- mice, in Tert-/- mice the NADH/NAD+ ratio remained low and components of TCA cycle were significantly reduced, suggesting an intrinsic defect in this cycle when telomerase is absent. These results indicate that short telomeres and deficient telomerase enzyme severely blocks the TCA cycle, inducing metabolic senescence and making hepatocytes less adaptive to environmental challenges.
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MODELING TELOMERASE STRUCTURE AND ARCHITECTURE THROUGH HYBRID METHODS

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Telomeres, the repeating DNA sequence at the ends of eukaryotic chromosomes, tend to shorten after consecutive cell division cycles as a result of the end replication problem. The ribonucleoprotein telomerase counteracts this shortening by extending telomeric DNA ends through a reverse transcription reaction. Telomerase utilizes a protein core that resembles a canonical reverse transcriptase, and an internal RNA component that provides the template for reverse transcription, among other functions. Over the past decade, several fragments of the telomerase protein and RNA component have been structurally elucidated, yet models for the high-resolution structure and architecture of an active telomerase complex are lacking. A major barrier to structural studies of active telomerase is the inability to produce large quantities of homogeneous enzyme. To address this challenge, we have utilized single molecule FRET to determine inter-domain distances in the active telomerase complex. Distance constraints from our single molecule assay can be readily integrated with complementary biophysical and biochemical structure probing methods to further illuminate structural and functional properties of the telomerase catalytic core.

CANCER-ASSOCIATED POT1 MUTATIONS LEAD TO TELOMERE DYSFUNCTION AND PROMOTE GENOME INSTABILITY

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Mutations in *p*rotection *o*f *t*elomeres 1 (*POT1*) have emerged repeatedly among top hits identified through whole exome sequencing of patients afflicted by various cancers, including chronic lymphocytic leukemia (CLL), melanoma and glioma. The mutations typically cluster to the OB (oligonucleotide / oligosaccharide binding) folds of *POT1*, which are necessary for the DNA binding activity of the protein. Interestingly, *POT1* mutations appear to be acquired in CLL, but are associated with familial forms of melanoma and glioma. Despite extensive sequencing data, minimal functional analysis has been performed to investigate the POT1 mutants, and the impact of such mutations on cancer initiation and/or progression remains unknown. By combining genome editing tools with biochemical and cell biological approaches, we show that several cancer-associated POT1 mutations impair its binding to telomeric ssDNA, triggering an ATR-dependent DNA damage response and replication stress-associated phenotypes. The resulting telomere aberrancies ultimately lead to genomic instability, which augments cellular transformation.

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STRUCTURE-FUNCTION COMPENSATION WITHIN THE RNA COMPONENT FOR TELOMERASE CATALYSIS

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Telomerase is a ribonucleoprotein enzyme requiring, in addition to the catalytic telomerase reverse transcriptase (TERT), the telomerase RNA (TR) subunit for enzymatic function. In vertebrates, two highly conserved TR structural domains, pseudoknot and CR4/5, are essential for telomerase catalysis. We have previously demonstrated that the distal stem-loop moiety P6/P6.1 in CR4/5 is essential for telomerase activity and pervasive in both vertebrate and filamentous fungal TRs (Qi et al., N.A.R. 41:450-62, 2012). Moreover, we proposed a functional role of the P6/6.1 stem-loop in allosteric folding of TERT protein domains by mapping the RNA-protein binding interface (Bley et al. P.N.A.S. 108:20333-8, 2011). Our recent studies on telomerase from echinoderm, the sister phylum to vertebrates, surprisingly found structural and functional co-evolution between TR structural domains and the TERT protein for maintaining telomerase catalysis. While lacking the ancestral P6/P6.1 structure, the echinoderm pseudoknot and TERT have co-evolved to function in concert and generate telomerase activity. However, a single helix appears to have replaced the ancestral P6/P6.1 stem-loop moiety and moderately stimulates telomerase activity of the pseudoknot-TERT complex. This decreased reliance on a P6/P6.1 stem-loop moiety, and increased telomerase activity with the pseudoknot alone, explains the exclusive rapid evolution and differentiation of the echinoderm TR domains. Additionally, the echinoderm pseudoknot exhibits features chimeric of vertebrate and fungal pseudoknot domains. Thus echinoderm telomerase provides insights into how telomerase is capable of compensating for alterations and loss-of-function within one critical RNA domain by a gain-of-function within another domain. Moreover, the stable and functional echinoderm pseudoknot-TERT complex offers an outstanding model system for structural studies of this poorly understood RNP complex.

THE CHROMATIN REMODELER SMARCAL1 SUPPRESSES TELOMERE INSTABILITY.

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With each round of replication, billions of DNA bases must be replicated accurately and completely to yield two identical copies of the genome. There are several obstacles to this process including damage from endogenous or exogenous sources, nucleotide depletion, and hard-to-replicate sequences in the DNA. To ensure complete replication of the genome in the presence of these challenges, a mechanism to promote replication to the ends of linear chromosomes must exist. SMARCAL1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like1) functions in the replication stress response to promote genome stability. Replication Protein A (RPA) recruits SMARCAL1 to replication forks where the replication checkpoint regulates its activity. Biochemically SMARCAL1 is an annealing helicase that can remodel replication forks, a mechanism of DNA repair in which the fork is converted into a 4-way junction. In vitro SMARCAL1 is able to form these 4-way junctions and also catalyze the reverse reaction to restore the junction into an elongating fork. I have recently identified an RPA-independent function of SMARCAL1 at telomeres. In SMARCAL1-depleted settings, I observed a change in telomere integrity as measured by the presence of extrachromosomal telomere circles. Our studies indicate SMARCAL1 functions to preserve the stability of telomeres and prevent the formation of aberrant telomere structures.

TELOMERE DYSFUNCTION AS DRIVER OF IDIOPATHIC PULMONARY FIBROSIS.

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Idiopathic pulmonary fibrosis (IPF) is a chronic and degenerative disease of the lungs with an average survival post-diagnosis of 2-3 years, for which no approved treatments are available. Thus, new therapeutic targets and effective treatments are necessary. Short telomeres are risk factors for age-associated diseases including IPF. Moreover, mutations in components of the telomere-maintenance enzyme telomerase or in proteins important for telomere protection have been found both in familiar and sporadic IPF cases. The lack of mouse models that recapitulate the molecular features of the human disease has hampered new advances. Here, we generated two independent mouse models, which develop IPF owing to either critically short telomeres (telomerase-deficient mice) or to severe telomere dysfunction in the absence of telomere shortening (mice deficient for the TRF1 shelterin protein). We show that both mouse models recapitulate the pathobiology of human IPF, including abnormal CT pattern, interstitial collagen deposition, chronic inflammation, and pulmonary dysfunction In Trf1lox/lox mice with an inducible CreERT2 under the control of SPC promoter, alveolar type II cells were TRF1-depleted after one week of tamoxifen treatment. Subsequent telomere uncapping triggered a DNA damage response (in a telomere length independent manner) charactherized by increase in both γH2AX and p21 positive cells in the lungs.
Telomerase deficient mice, Tert-/-, were additionally stressed with a low dose of intratracheally administered bleomycin. We show that bleomycin sinergizes with the short telomeres to induce DNA damage that ultimately leads to lung fibrosis development.
In conclusion, we demonstrate that DNA damage in lung cells, stemming from telomeres, and its consequent DNA damage response are sufficient and leads to cell loss and aberrant healing. Thus our mouse models constitute proof of principle of the causal role of telomere dysfunction in IPF development and identifying telomeres as promising targets for new treatments.

DISSECTING THE ROLE OF HUMAN CTC1 IN TELOMERE REPLICATION AND GENOME-WIDE REPLICATION RESCUE

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Mammalian CST (CTC1-STN1-TEN1) is a ssDNA binding complex that functions in multiple aspects of telomere replication and genome-wide replication rescue. During telomere replication, CST facilitates passage of the replication machinery through the telomere duplex and enables C-strand fill-in synthesis. The genome wide roles of CST are not well understood but STN1 depletion results in anaphase bridges in the absence of telomere fusions, sensitivity to a variety of DNA damaging agents and reduced ability to restart replication after HU-induced fork stalling. Mutations in CTC1, but not STN1 or TEN1, cause the disease Coats plus. Since shRNA depletion of CTC1 results in variable phenotypes, we set out to dissect the functions of human CTC1 using a tamoxifen-inducible, gene disruption in HCT116 cells. After tamoxifen addition the initial growth rate was normal, but it then slowed by day 8-10 and by day 21 the cells ceased to divide. The decline in growth was accompanied by sporadic telomere loss with later appearance of abundant chromosome fusions. Thus, the lethality of CTC1 disruption appears to reflect the importance of CST in telomere duplex replication with loss of CST leading to fork stalling, double-strand breaks and chromosome fusion. Analysis of telomere length and G-overhang structure revealed an early dysregulation that did not affect cell growth. Telomere length grew by ~50 bp/PD while G-overhang abundance increased 5-8 fold by day 6 and then stabilized. The striking telomere growth and large increase in overhang abundance contrasts with the modest increases seen after CTC1, STN1 or TEN1 depletion with shRNA, suggesting that only small amounts of CST are needed for telomerase regulation and overhang maintenance. ChIP analysis revealed that DNA pol α association with the telomere is unaffected by CTC1 loss. Given that CST displays RPA-like DNA binding modes, our results suggest a hand-off model where dynamic binding allows CST to unload telomerase and engage pol α for C-strand fill-in. CTC1 disruption also resulted in non-telomeric phenotypes. DAPI stained anaphase bridges were apparent by day 4, long before the appearance of telomere fusions. Staining for PICH revealed bridges in about 40% of anaphases and some showed co-staining with FANCD2. This finding indicates the bridges correspond to unresolved replication intermediates, confirming the role of CST in resolving endogenous replication stress. CST disruption also sensitized cells to some, but not all forms of DNA damage. Cells were more sensitive to camptothecin, MMS and HU but not the DNA cross-linkers cisplatin or MMC. Thus, CST appears to work in many, but not all, DNA repair/replication rescue pathways.

NOVEL TELOMERASE RNA COMPONENT (TERC) PARALOG IN MOUSE BRAIN MODULATS TELOMERASE ACTIVITY.

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Telomerase, a ribonucleoprotein complex, is responsible for the maintenance of telomere length by using telomerase RNA subunit (TERC) as a template. Telomerase facilitates telomere homeostasis using its catalytic subunit, TERT, to reverse transcribed the internal RNA template TERC, thereby replenishing terminal sequences lost during DNA replication. Telomerase is a highly regulated enzyme; its action is largely confined to, and essential for, self-renewing cell populations. Recent study has shown an alternative telomerase RNA component in Arabidopsis that modulate enzyme activity in response to DNA damage. In our lab, we have found a novel mouse TERC paralog sequence in proximity to the TERC gene. We hypothesized that the novel TERC paralog has a role in the regulation of telomerase activity. Brain extracts from ICR mice were used for TERC paralog expression by RNA purification and PCR analysis using specific primers. The interaction of the paralog with TERT was analyzed using Immuno-precipitation with TERT specific antibody and RNA purification from the precipitate following PCR analysis. To examine the paralog effect on telomerase activity we constructed a paralog overexpression model in a neuronal cell-line, from which total protein was extracted and telomerase activity was analyzed via the TRAP assay. In this study, we have shown that the TERC paralog is expressed in the mouse brain and interacts with telomerase. Additionally, overexpression of the paralog gene altered TERT activity in-vitro. We propose that mouse telomerase in the brain is modulated by TERC paralog, a novel noncoding RNA that competes with the canonical TERC to regulate TERT activity.

IN FLASK EVOLUTION OF CHROMOSOME END SEQUENCES

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The evolution of telomere repeat sequence is guided by the many essential roles of telomeres in cellular processes. Sequence diversity is limited by the need for the telomerase RNA template to align to 3' overhangs and contribute to telomerase processivity. Also binding specificities of protective and regulatory telomeric proteins directly affect the ability of cells to divide. Lastly, the organization of telomeric DNA into regulatory structures, like heterochromatin and G-Quadruplexes, may exert additional selective pressure. Yet, despite these constraints, repeat diversity is particularly large among fungi ranging from perfect 25 nucleotide repeats in *K. lactis* and 9 nucleotide repeats in *S.cryophilus*, to heterogeneous repeats in *S. cerevisiae* and *S.pombe*. Repeat heterogeneity in fission yeast results from stuttering, slippage, and a poorly defined boundary element in the telomerase RNA template and signifies diversity at the level of the individual telomere that must be tolerated by the cell.

We have characterized the diversity of telomeric sequences that maintain telomeric functions in *S. pombe*. We replaced the *ter1*template sequence with all 16,384 possible nucleotide combinations and grew cells containing this library competitively in liquid culture. As variant repeats are incorporated into the telomere, cells with dysfunctional telomeres undergo arrest or experience cell cycle delays and ultimately become outgrown, or outcompeted, by cells that maintain capped telomeres. Using Illumina, we followed the changes in abundance of different templates that resulted from differential growth rates of cells with variant telomeres. Most surprisingly, cells expressing the WT *ter1* template did not emerge as the ultimate winners in either rich or minimal media. Instead, we identified eight alternative templates from populations of cells that outcompeted WT in rich media. Interestingly, variant templates maintained linear chromosomes within the range of WT lengths with only one to four WT repeats preceding the alternative sequences. Furthermore, a small subset of templates from the initial library made up 90% of all reads by the end of the time course. Because these templates are from populations of cells that were as successful as WT in liquid culture, they represent a flexible space that can be used to generate variant telomeric repeats that are compatible with cellular survival. An important question remains: Why is WT not the ultimate winner? By addressing this question, we will probe which selective pressures impose the greatest constraints on telomeric repeat sequence under different conditions.

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A NOVEL ALLOSTERIC SITE ON THE THUMB DOMAIN MODULATES TELOMERASE PROCESSIVITY

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BIBR1532 is a widely used small molecule inhibitor of telomerase, however the molecular basis for inhibition is unknown. Here we present the crystal structure of BIBR1532 bound to T. castaneum telomerase (tcTERT) and investigate the mechanistic significance of its binding pocket. BIBR1532 binds to a conserved hydrophobic pocket on the outer surface of the CTE (thumb) domain, which we name the FVYL motif. The FVYL motif is near TRBD residues that bind the CR4/5 domain of hTR, and RNA binding assays show that the human TERT (hTERT) thumb domain binds the CR4/5 in vitro. hTERT mutations that occlude the FVYL pocket disrupt CR4/5 binding and cause telomere attrition in cells. Furthermore, the hTERT FVYL mutations V1025F, N1028H and V1090M are implicated in dyskeratosis congenita and aplastic anemia, further supporting the biological and clinical relevance of this novel motif.

DDRNAS, A NOVEL CLASS OF SMALL NON-CODING RNA, REGULATE THE DNA DAMAGE RESPONSE AT DYSFUNCTIONAL TELOMERES

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We have previously shown that small non-coding RNAs, named DDR RNA or DDRNA, are novel components of the DNA damage response (DDR) machinery. These small RNAs are generated by the endoribonucleases DROSHA and DICER and have the sequence of the damaged locus. Upon DNA double-strand break (DSB) generation by ionizing radiation, endonuclease cleavage, or oncogene-induced DNA replication stress, DDR activation and maintenance is dependent on DDRNA (Francia et al., Nature 2012). DROSHA and DICER have also been proposed to be involved in DSB repair (Wei et al., Cell 2012).
We investigated the potential role of DDRNA in DDR activation at dysfunctional telomeres. Following TRF2 loss, deprotected telomeres are recognized as DSBs and thus activate DDR signaling and DNA repair events. In TRF2 knock out mouse cells, we show that DDR foci maintenance at telomeres is dependent on RNA: transient RNase A treatment of permeabilized cells impairs DDR signaling, which can be restored by reintroducing cellular RNA. In contrast, the rescue is not observed using RNA purified from cells lacking DROSHA or DICER. Consistently, DROSHA or DICER knock down prevents DDR activation at uncapped telomeres and reduces telomeric fusions upon telomere uncapping. Together, these results suggest that DDR activation at deprotected telomeres is RNA-dependent and is controlled by DROSHA and DICER. Indeed, small RNA species with a telomeric sequence, and with a size compatible with a DROSHA and DICER dependent biogenesis, can be detected upon telomere uncapping. Finally, telomeric DDRNA activities can be inhibited by the use of sequence-specific antisense oligonucleotides, preventing the accumulation of DDR markers at telomeres and senescent-associated cell-cycle arrest in human cells. These data strongly indicate that telomeric DDRNA are necessary for the full activation of the DDR cascade at dysfunctional telomeres.

THE ROLE OF PARP1 IN TELOMERE STRUCTURE REGULATION

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The telomere length and structure maintenance is one of the programs involved in genome stability providing systems. The one of the major participant of the DNA repair system is PARP1. PARP1 is activated by DNA breaks and involved in protein activity and specificity regulation by the posttranslational modification. PARP activity has been implicated in many aspects of genome integrity and cell survival regulation, like repair, transcription, DNA replication, cellular differentiation, mitosis and mitotic organization, cell death pathways, vesicle trafficking and telomere length regulation. It is known that telomere proteins TRF1 and TRF2 interact with PARP family proteins and controversial data about telomere length changes caused by PARP inhibition were obtained by independent investigations. We generated cell lines with stable expression of Parp1 and stable expression of short hairpin RNA counteracts PARP1 mRNA. The PARP1 influences to telomere length and structure, telomerase activity and shelterin components were revealed.

A *SCHIZOSACCHAROMYCES POMBE* TRANSPOSON INSERTION LIBRARY FOR HIGH-THROUGHPUT GENOME-WIDE STUDIES

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The fission yeast *S. pombe* has proven to be powerful system for telomere biology, and has similarities in chromatin modification, structure and the DNA damage response with human cells that are absent from the more popular yeast *Saccharomyces cerevisiae*. Unfortunately, the genetic resources for *S. pombe* are not as well developed. A set of *S. pombe* haploid deletion strains has been constructed, but this set lacks mutations in essential genes and only contains loss of function of alleles. Mutations causing altered expression or partial protein function may reveal new roles for essential and non-essential genes. We have therefore used a modified *Hermes* transposon to construct a random, sequenced, barcoded *S. pombe* insertion library that is compatible with the current genetic tools for the deletion strain set. To construct our library, we developed a novel three-dimensional pooling strategy and a multiplexed high-throughput sequencing analysis pipeline to sequence the transposon insertion sites and DNA barcodes from thousands of samples at once. The library consists of 4,095 strains with 4,391 insertions stored in 96-well plates. The insertions are in open reading frames, 5' and 3' regulatory regions of genes, in essential genes and in genes for non-coding RNAs. To test our prediction that some of the insertion mutants would possess novel phenotypes compared to the analogous deletion mutants, we examined the phenotypes of insertions mutants in genes classified as being required for growth on a non-fermentable carbon source or whose loss causes hypersensitivity to the topoisomerase I inhibitor CPT. Many insertion mutants had the predicted phenotypes, while one insertion caused a novel CPT resistant phenotype. This library therefore provides an important, complementary resource for the *S. pombe* deletion strain set, and establishes a valuable approach for the construction and analysis of insertion mutant libraries in a wide variety of model systems.

A ROLE FOR *HDA1* IN TELOMERE LENGTHENING IN *USTILAGO MAYDIS*

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Chromatin modification constitutes a sophisticated way of encrypting molecular signals into chromatin and contributes to the genetic regulation of many genes. Among such modifications, histone acetylation/deacetylation represents one of the most important post-translational modifications involved in the epigenetic regulation of gene expression. Histone deacetylases (HDACs) Rpd3 from yeast and HDAC5 from humans have roles in regulating telomere length. In humans, HDAC inhibition by trichostatin enhances telomere lengthening via ALT pathways in telomerase-negative cancer cells; HDACs in normal cells contribute to controlling the expression of the telomerase reverse transcriptase (TERT) subunit by modulating the binding of heterodimers of either c-Myc or Mnt with Max to E-boxes in the *hTERT* promoter. In fungal cells, trichostatin treatment increases the expression of genes residing in subtelomeric regions. *Ustilago maydis* is a model organism that undergoes dimorphic transitions from haploid sporidial to dikaryotic hyphae. *U. maydis* has been employed for studies on telomere maintenance; the gene encoding the telomerase catalytic subunit (*trt1*) was recently described, and its function in the haploid sporidial stage of life was assessed. However, the role of *trt1* in dikaryotic hyphae remains to be elucidated, as *U. maydis* in this stage of its life cycle is an obligate parasite of maize plants and thus unsuitable for telomere-telomerase studies. As many genes normally expressed only in the dikaryotic phase of life are derepressed in *hda1Δ* strains, null *hda1* mutants were constructed for use in this work. The *hda1Δ* strains exhibited changes in the terminal restriction fragments (TRFs) of chromosomes: either slight lengthening; shortening to half the length, with homogenization of telomere smears, as occurs in human cells; and no change in telomere length. The TRF length was stable over 500 doubling times. Transcriptional expression of *trt1* and that of subtelomeric sequence *UTASa* were increased between 2 to 5-fold, with no significant difference from wild type. Mechanisms involved in TRF length changes in *U. maydis hda1Δ* mutants are currently under study, rendering this fungus an interesting model system for telomere research.

INDIVIDUAL FUNCTIONAL DOMAINS OF *TRYPANOSOMA BRUCEI* RAP1 CONTRIBUTE TO TELOMERIC SILENCING

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Trypanosoma brucei is a protozoan parasite that causes sleeping sickness in humans and nagana in animals. The main reason for persistent infection of *T. brucei* in its mammalian host is that *T. brucei* undergoes antigenic variation and regularly switches its major surface antigen, Variant Surface Glycoproteins (VSG), to evade the host's immune response. VSGs are exclusively expressed in a monoallelic manner from VSG expression sites (ESs) located at subtelomeric loci. We and others have shown that telomeres play important roles in VSG expression and switching regulation. So far, we have identified three conserved telomere proteins in *T. brucei:* TbTRF (ortholog of TRF2; Li et al. 2005. MCB 25:5011), TbRAP1 (ortholog of RAP1; Yang et al. 2009 Cell 137:99), and TbTIF2 (ortholog of TIN2; Jehi et al. 2014. Cell Res. 24:870). Studies from our lab have established that TbRAP1 is essential for VSG silencing, as depletion of TbRAP1 leads to derepression of all subtelomeric VSGs and expression of multiple VSG proteins on *T. brucei* cell surface simultaneously (Yang et al. 2009. Cell 137:99; Pandya et al. 2013. NAR 41:7673). However, the mechanism by which TbRAP1 regulates VSG silencing is not completely clear. TbRAP1 contains three conserved functional domains: BRCA1 C-terminus (BRCT), Myb, and RAP1 C-terminus (RCT). In order to characterize functions of individual domains of TbRAP1, we deleted different functional domains of TbRAP1. We have established a number of TbRAP1 conditional knockout strains expressing various TbRAP1 mutants and examined their effects on VSG silencing. We will provide data about functions of different TbRAP1 domains.

DONOR LEUKOCYTE TELOMERE LENGTH IN HEMATOPOIETIC CELL TRANSPLANTATION OUTCOMES

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Clinical outcomes after allogeneic hematopoietic cell transplant (HCT) have improved over the last several decades. However, HCT survivors remain at high risk of serious complications such as graft versus host disease (GvHD), infection, and cancer. HCT requires rapid expansion of transplanted donor cells to achieve engraftment in the recipient. Post-HCT telomere shortening appears to occur in parallel with donor cell proliferation. Shorter telomeres in the transplanted HCT cells have been associated with older donor age, female donors, and chronic GvHD.

Telomere length in recipients of HCT for acquired aplastic anemia has been associated with relapse, clonal evolution, and survival. Acquired aplastic anemia is typically immune-mediated whereas inherited bone marrow failure is usually caused by germline defects in telomere biology, ribosomal function, or DNA repair.

In order to better understand the role of telomere biology in HCT, we evaluated the association between recipient and donor pre-transplant leukocyte relative telomere length (RTL) with outcomes after unrelated donor HCT in patients with severe aplastic anemia. We measured pre-HCT leukocyte RTL by qPCR on 330 patients and their unrelated donors who also had clinical data available at CIBMTR. Patients underwent HCT between 1989 and 2007 and were followed-up until March 2013. RTL was classified into long (3rd tertile) and short (1st and 2nd tertiles combined) based on donor RTL distribution.

Longer donor RTL was associated with higher survival probability (5-year overall survival=56% vs. 40% in the long vs. short RTL, respectively; p=0.009). The association remained statistically significant after adjusting for donor age, disease subtype, Karnofsky performance score, graft type, HLA matching, prior aplastic anemia therapy, race, and calendar year of transplant (hazard ratio=0.61, 95% confidence intervals=0.44-0.86). There was no association between donor RTL and neutrophil engraftment, acute or chronic GvHD. Recipient pre-transplant RTL was significantly shorter than the donor RTL but not associated with post-HCT survival.

In summary, this observational study suggests that donor RTL, irrespective of donor age, may have a role in long-term post-transplant survival in patients with aplastic anemia. Validation studies are underway.

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UNIQUE KINETIC PROPERTY OF HUMAN TELOMERASE HOLOENZYME SUGGESTS A CATALYSIS DEPENDENT BRAKE ON ITS ACTIVITY

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Human telomerase is a ribonucleoprotein (RNP) responsible for telomere length maintenance. The catalytic subunit, hTERT, and the RNA subunit, hTR, are two major components of the telomerase holoenzyme. The enzyme is important for cancer research because approximately 85% of tumors in humans display elevated level of telomerase activity, making telomerase a potential target for anti-cancer therapy. Our current biophysical studies focus on expanding our understanding of the function and enzymology of the telomerase complex. Our working hypothesis is that the dimeric telomerase behaves as a single-pass enzyme, which needs to be reactivated after its processive extension reaction on a substrate. We used different biochemical approaches in conjunction with a highly quantitative activity assay to test our working hypothesis. We first observed that in both gel based TRAP and the digital droplet TRAP (ddTRAP) assays, telomerase exhibited catalysis-dependent inactivation. Our qPCR and ddPCR analysis found that the holoenzyme in our preparations was stable without catalysis. In sequential extension experiments, the telomerase showed both fast-acting and slow-acting sites and these two types of active sites had different substrate affinity and acted in tandem. The sequential action of these two active sites required that both must be harbored by individual dimeric telomerase complexes, suggesting that the two active sites in each enzyme are asymmetrical, one fast and one slow. After two reactions, a dimeric enzyme becomes inactive. Furthermore, we tested the ability of a variety of cell lysates (both telomerase negative normal diploid and transformed) to reactivate/activate our catalytically exhausted telomerase complexes. Taken together, our data support the single-pass hypothesis, provide a new catalytic mechanism for telomerase holoenzyme and suggest an exquisite control of its activity in a catalysis-dependent manner.

TOWARDS A MECHANISTIC UNDERSTANDING OF TELOMERE LOOP STRUCTURES IN *SACCHAROMYCES CEREVISIAE*

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Telomere "end protection" has largely been attributed to the activities of the major telomere binding complexes such as shelterin in humans and the CST complex in yeast. The telomeric lariat structure (t-loop) is likely also adding to end protection, although its contribution has been difficult to discern due to an incomplete understanding of t-loop regulation. Whereas human and mouse t-loops can be visualized via super-resolution- and electron -microscopy, the short length and base composition of yeast telomeres prevent such approaches. Using a combination of ChIP and transcriptional read-outs, yeast telomeres have been demonstrated to loop back into the subtelomeric region where they may be maintained by either protein-protein interactions or DNA base pairing (HR); however, both methods are indirect and unsatisfactory in terms of analyzing the dynamic regulation of loop structures. We have therefore developed a method based on the chromosome conformation capture (3C) technique. With our approach we can directly detect and quantify interactions between a telomere and its subtelomeric region in *S. cerevisiae*. In this manner we can exploit the genetic advantages of the yeast system to understand the mechanistic details of telomere loop formation and maintenance.

Using this approach, we observe a significant looping defect in cells that lack telomerase as well as in other mutants that harbor short telomeres. On the contrary, elongated telomeres were able to maintain the looped structure. This suggests that a critical telomere length is essential to maintain the telomere loop and that telomeres in senescent cells are likely in an open conformation, rendering them susceptible to nucleolytic end resection and unscheduled DNA repair events. Moreover, we have confirmed the *in vitro* prediction that t-loops are stabilized by HR, as *rad52* mutant cells are looping defective.

In addition, we are addressing the relationships that may exist between telomere looping and gene looping as well as the production of the telomeric lncRNA, TERRA. Interestingly, we have observed that both an intact transcription initiation- and termination -machinery are required for the establishment of a telomere loop, suggesting that telomere loops have similar prerequisites as gene loops. Taken together, our 3C approach indicates that yeast telomere loops are established in both a transcription- and recombination-dependent manner and require a minimal telomere length.

BLM HELICASE FACILITATES TELOMERE REPLICATION DURING LEADING STRAND SYNTHESIS OF TELOMERES

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Efficient replication of telomeric DNA is essential for the maintenance of telomere structure and function. The majority of telomere DNA is duplicated by conventional semiconservative DNA replication. Mammalian telomeres present a special challenge to the genomic replication machinery. This is in part due to their G-rich repetitive sequence, which can form stable secondary structures, particularly G-quadruplexes (G4). Based on its robust in vitro unwinding activity on G4 DNA, the Bloom syndrome-associated helicase BLM is proposed to participate in telomere replication by aiding fork progression through the G-rich telomeric DNA. To determine the contribution of BLM helicase to telomere replication, we employed a powerful single molecule approach termed single molecule analysis of replicated DNA (SMARD) to follow replication progression through a specific telomere locus in individual murine chromosomes in BLM-proficient and -deficient cells. We observed that in BLM-deficient cells, replication forks initiating from origins within the telomere, which copy the G-rich strand by leading strand synthesis, moved slower through the telomere compared to the adjacent subtelomere. Fork progression through the telomere was further slowed in the presence of a G4 stabilizer in BLM-deficient cells, indicating involvement of G4 structures as impediments to fork progression. Using a G4-specific antibody, we found that deficiency of BLM, or another G4-unwinding helicase, the Werner syndrome-associated helicase WRN, resulted in increased G4 structures detected in cells. Importantly, deficiency of either helicase led to greater increases in G4 DNA detected in the telomere compared to G4 seen genome-wide, indicating a stronger dependence of telomeres on the helicases for suppression of G4 formation. Collectively, our findings are consistent with BLM helicase facilitating telomere replication by resolving G4 structures formed during copying of the G-rich strand by leading strand synthesis.

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HYPOMETHYLATION OF SUBTELOMERIC REGIONS AND ACCELERATED TELOMERE SHORTENING IN ICF SYNDROME VIA DNA:RNA HYBRIDS.

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Human telomeric regions are packaged as constitutive heterochromatin, and adjacent subtelomeres contain CpG rich repetitive sequences that exhibit extensive methylation. The non-coding RNA TERRA (telomeric repeat-containing RNA) is transcribed from the C-rich telomere strand of at least 10 telomeres and is postulated to play a role in telomere maintenance. DNA:RNA hybrids are predicted to form between the C-rich telomeric strand and the G-rich TERRA transcript.
Mutations in *DNMT3B* lead to ICF (Immunodeficiency, Centromeric instability and Facial anomalies) syndrome, type I, characterized by severe subtelomeric-hypomethylation, abnormally high TERRA levels and accelerated telomere shortening. Telomeres shorten unevenly in ICF syndrome suggesting that CpG density, GC skewing and TERRA expression at each subtelomere may affect the degree of telomere shortening in cis.
Analysis of methylation levels of various subtelomeres in ICF and control cell types reveals that the methylation pattern of a specific subtelomere is similar among all ICF patients, but subtelomeres differ from each other by their degree and pattern of methylation. Quantitative real time PCR analysis of telomere-specific TERRA transcription demonstrates that subtelomeres differ also in their transcription levels of TERRA. In addition we found by combined telomere-FISH and chromosome-painting, non-random telomeric shortening in ICF patients. Notably, we demonstrate that telomeres form DNA:RNA hybrids *in vitro* and are studying the formation of such hybrids *in vivo* at telomeres that highly express TERRA.
Compiling the data from the subtelomeric methylation, TERRA expression and the telomere loss analyses together with the data on telomere-specific DNA:RNA hybrid formation will elucidate whether telomere shortening in ICF syndrome results from TERRA transcription and hybrid formation that may hinder the progression of the replication fork at telomeric regions and lead to telomere loss.

TELOMERE LENGTH ASSESSMENT AFTER WHOLE GENOME AMPLIFICATION (WGA)

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Objective:
Telomere DNA deficiency has been associated with genomic instability and it's measurement alongside other genome assays is desirable. However insufficient DNA often limits these parallel analyses when performed on one or a few cells. Whole genome amplification (WGA) can solve this problem by providing high yields, so long as it provides an accurate representation of original gDNA. However, telomeres have not been assessed for faithful representation after WGA. We assessed the impact of WGA on telomere length measurement in single cells using two commercially available kits, namely Sigma GenomePlex® and Yikon Genomics single cell WGA Kit (PCR and MALBAC technologies respectively).
Materials and Methods:
Previous experiments using a novel single cell Telomere qPCR based method (SCT-qPCR) (1) and Q-FISH (2) showed high (R2>98%) concordance in telomere lengths between polar bodies (PB) and oocytes pairs. To examine the effects of WGA on telomere measurement we studied ten human oocyte-PB pairs and 6 pairs of human cultured cells (hESC and fibroblasts) obtained by micro dissection. Ten polar bodies and six cultured cells were subjected to WGA followed by SCT-qPCR. These were compared to the no-WGA group (oocytes and the six sister cultured cells), which were subjected to the SCT-qPCR alone.
SCT-qPCR assay measures DNA quantity of a Telomere target (T) normalized to DNA quantity of a multi-copy reference gene (R) yielding a T/R ratio. Final values were expressed as fold-change of T/R values relative to a standard placenta gDNA.
Results:
PBs that underwent WGA (Sigma Kit) returned lower average relative telomere lengths than sister oocytes that were measured directly by SCT-qPCR, i.e. that did not undergo WGA (0.65 to 3.83 versus 25 to 1151). Similarly, cultured cells had lower T/R ratios following WGA with MALBAC technology compared with the no-WGA group (0.03-0.27 versus 0.25 to 0.54).
The 2 WGA methods did not correlate to SCT-qPCR :
PCR-WGA (r= -0.1292, p=0.7220) and MALBAC-WGA (r=-0.1292, p=0.1626)

Conclusions:
Telomeres do not amplify reliably and consistently after WGA. Neither PCR nor MALBAC based WGA methods provide reliable estimates of telomere content. Investigators wishing to study telomere DNA content in individual cells should employ methods that do not depend upon WGA. SCT-qPCR provides a reliable alternative to methods employing WGA1.

1. Robust measurement of telomere length in single cells
Proc. Natl. Acad. Sci. USA (2013) 110 (21): E1906-E1912
2. Telomeres and aging-related meiotic dysfunction in women,
" Cellular and Molecular Life Sciences, vol. 64, no. 2, pp. 139–143, 2007

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A NOVEL ROLE FOR RIF1 IN REGULATING THE FINAL STEP OF CHROMOSOME SEGREGATION.

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Telomeres play a vital role in the protection and replication of the ends of eukaryotic chromosomes. We have previously shown that Taz1, the fission yeast orthologue of mammalian TRF1 and TRF2, helps promote passage of the replication fork through repetitive telomeric sequences. Deletion of *taz1*$^{+}$ causes telomeres to suffer replication fork stalling, leading to telomeric entanglements which fail to be resolved at cold temperatures. Therefore, *taz1Δ* cells are cold-sensitive. Live cell microscopy shows that *taz1*Δ cells harbor dynamic streaks of RPA stretching across the midregion at anaphase; these streaks are associated with foci of histones, Rad52 and DNA polymerase α, indicating that DNA processing events occur well into mitosis.

Deletion of the gene encoding the conserved telomere/replication/repair protein, Rif1, rescues *taz1*Δ cold sensitivity. Rif1 has been previously shown to regulate replication origin firing in S-phase in both budding and fission yeast via association with PP1 phosphatases. Mammalian Rif1 has not been shown to be associated with telomeres, but in fact helps regulate S-phase DNA damage responses coordinated by 53BP1.

We find that SpRif1 plays a role in the resolution of telomeric entanglements rather than the fork-stalling events that generate them. Although Rif1 is reported to bind telomeres in a Taz1-dependent manner, we find that Rif1 frequently appears in the mid zone of both wild type and *taz1*Δ dividing cells, indicating Taz1-independent telomere localization and suggesting a role in regulating the final steps of chromosome disentanglement. We find that while Rif1 prevents resolution of *taz1Δ* telomeres, it promotes timely resolution of wt telomeres as well as DNA bridges formed from repeated tetO arrays. Hence, while Rif1 may be a universal regulator of the final steps of chromosome segregation, it may erroneously channel the detangling machinery in inappropriate directions when entangled telomeres are present. We are developing a unifying model that integrates Rif1's apparently widespread roles in regulating chromosome replication and repair from yeast to human.

UNEXPECTED DIVERGENCE AND CONSERVATION OF THE TELOMERE PROTEIN COMPLEX IN PLANTS

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In mammals, the hexameric protein complex shelterin includes the single-strand DNA binding protein POT1 and the double-strand binding proteins TRF1 and TRF2. Shelterin shields chromosome ends from eliciting a DNA damage response. In the flowering plant *Arabidopsis thaliana* single-stranded telomeric DNA is bound by the CST complex (CTC1-STN1-TEN1). Moreover, there are three POT1 paralogs in *A. thaliana*. The best characterized, POT1a, is a telomerase processivity factor and is not implicated in chromosome end protection. In addition, at least six TRF-like genes are encoded by *A. thaliana*. Mutants deficient in several TRFL components exhibit no significant genome instability, suggesting that either the TRFL genes act redundantly or do not contribute to telomere biology in the same fashion as mammalian TRF1 and TRF2. Thus, the function and composition of telomere-associated proteins in *A. thaliana* is not well understood.

The moss *Physcomitrella patens* diverged from *Arabidopsis* more than 300 million years ago and is one of the earliest land plants. We previously showed that the single copy POT1 gene in *P. patens* plays a critical role in telomere length regulation and end protection like its mammalian counterpart. Here we investigate the role of two of the three TRFL paralogs in *P. patens*. We found that the putative DNA binding domain of PpTRFL2 and 3 bind efficiently to double-strand telomeric DNA in vitro, with a Kd value of 220nM, similar to mammalian TRF1. An 11-mer sequence, 5'-NNGGGTTTANN-3', which harbors one complete telomeric DNA repeat, was determined to be the minimal binding sequence for TRFL2/3. Using homologous recombination, we generated *P. patens* mutants containing a deletion in TRFL3. The mutant exhibited shortened telomeres and chromosome end-to-end fusions. Altogether, our findings indicate a conserved function for TRFL and POT1 orthologs in telomere end-protection in early diverging land plants, and argue that these functions were altered and/or lost in higher plants.

CTC1-STN1-TEN1 COMPLEX PLAYS A ROLE IN BASE EXCISION REPAIR IN HUMAN CELLS

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Telomeric DNA is particularly vulnerable to oxidative damage, and such lesion is repaired by the base excision repair (BER) machinery. It has been already shown that telomere repeat binding factor 2 (TRF2) is involved in BER pathway in vitro (Muftuoglu et al. 2006). Previously, we have reported a conserved ssDNA-binding protein complex called the CST complex in mammalian cells (Miyake et al. 2009). The CST complex binds to telomeres in interphase nuclei. However, the association of CST complex and BER is still elusive.

To test the possibility that CST complex is involved in DNA damage repair, we performed immuno-precipitation/mass spectrometry analyses of CST, and identified BER proteins, including NEIL1 and POLB. Immunofluorescence experiments showed that tagged CTC1 co-localized with XRCC1 (X-ray repair cross complementing 1), a protein required for efficient repair of BER in mammalian cells, after UV irradiation of HeLa cells, suggesting that CST complex is involved in BER. We also demonstrated that the EdU incorporation was decreased after H_2O_2 treatment in *STN1*-knockdown cells. These results suggest that CST plays a role in DNA synthesis step of BER pathway.

STRUCTURAL CONSEQUENCES OF A SINGLE AMINO ACID DELETION OF TPP1 THAT IS CAUSATIVE OF DYSKERATOSIS CONGENITA

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The shelterin protein TPP1 has recently been shown to recruit telomerase through an acidic patch of amino acids termed the TEL patch. Recently, in collaboration with others, our laboratory characterized a severe case of dyskeratosis congenita (DC) caused primarily by a single amino acid deletion in TPP1 – K170Δ – which is positioned in the middle of a loop between with two critical TEL patch residues -- E169 and E171. The lysine side chain is probably not involved in directly binding telomerase since it is buried in the structure of the wild type TPP1 protein, and the K170A mutation has very modest effects on telomerase recruitment. Instead, we hypothesize structural changes to this loop upon deletion of this amino acid, impair proper positioning of critical TEL patch contact residues.
Here we set out to use x-ray crystallography to test our hypothesis and determine a structural basis for the deleterious consequences of the DC mutation of TPP1. Our crystallographic analysis of TPP1-OB K170Δ clearly shows the distortion of the peptide backbone spanning the acidic E169 and E171 residues, providing support for our hypothesis. Structural analysis of the K170A mutant will show if these glutamic acid residues are affected by alanine substitution at K170, a result we do not anticipate. Our study, which marks the first crystallographic effort to decode the structural basis of any known DC mutation, also lends impetus to structure-guided efforts to discover drugs against DC.

TELOMERASE MUTATIONS IN SMOKERS WITH SEVERE EMPHYSEMA

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Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the United States. While smoking is its greatest risk factor, a genetic component has been postulated to contribute to disease susceptibility. Mutations in the essential telomerase genes, *TERT* and *TR*, cause familial pulmonary fibrosis; however, in telomerase null mice, short telomeres predispose to emphysema after chronic cigarette smoke exposure. Here, we tested whether telomerase mutations are a risk factor for human emphysema by examining their frequency in smokers with COPD. Across two independent cohorts, COPDGene and the Lung Health Study, we found 3 of 292 severe COPD cases carried deleterious mutations in *TERT* (1%). This prevalence is comparable to the frequency of alpha-1 antitrypsin deficiency, the only known Mendelian cause of emphysema. The *TERT* mutations significantly compromised telomerase catalytic activity and mutation carriers had short telomeres. Telomerase mutation carriers with emphysema were predominantly female, and had an increased incidence of pneumothorax. In families, emphysema showed an autosomal dominant inheritance pattern, along with pulmonary fibrosis and other telomere syndrome features, but manifested only in smokers. Given the public health burden of COPD, our findings suggest that emphysema may be a common manifestation of telomere syndromes in populations where smoking remains prevalent.

COMBINATORIAL RECOGNITION OF A COMPLEX TELOMERE G-STRAND REPEAT SEQUENCE BY THE *CANDIDA PARAPSILOSIS* CDC13AB HETERODIMER--WE ARE HAVING TWINS!

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The telomere repeat units of *Candida* species are substantially longer and more complex than those in other organisms, raising interesting issues concerning the recognition mechanisms and evolution of telomere-binding proteins. Notably, the G-strand binding proteins in this genus of fungi (i.e., Cdc13s) are structurally distinct from those in other closely related organisms. In particular, all *Saccharomyces* and *Kluyveromyces* genomes harbor a single *CDC13* gene that consists of four OB fold domains, whereas all *Candida* species possess two small *CDC13* homologs (named *CDC13A* and *CDC13B*), each consisting of just two OB folds. Previous studies of *C. albicans* and *C. tropicalis* Cdc13 homologs indicate that Cdc13A and Cdc13B can form homo-oligomers as well as heterodimers, and that both the AA and AB complexes are capable of binding telomere G-strand with moderate to high affinities. However, the detailed molecular interactions within the *Candida* Cdc13-DNA complexes remain poorly understood.

In the current study, we seek to broaden and deepen the current understanding by investigating the *C. parapsilosis* Cdc13A and Cdc13B homologs. We found that *Cp*Cdc13A and *Cp*Cdc13B can each form complexes with itself. The two paralogs can also combine to form a heterodimeric complex. Among the different oligomeric forms of Cdc13s, only the heterodimer exhibits high-affinity and sequence-specific binding to the telomere G-tail. EMSA and Crosslinking analysis revealed a combinatorial mechanism of DNA recognition, which entails the A and B subunit making contacts to the 3' and 5' region of the repeat unit, respectively. While both the DBD and OB4 domain of *Cp*Cdc13A can bind to the equivalent domain in *Cp*Cdc13B, only the OB4 complex behaves as a stable heterodimer. The unstable Cdc13ABDBD complex binds G-strand with greatly reduced affinity but the same sequence specificity. Thus the OB4 domains evidently contribute to binding by promoting dimerization of the DBDs. Our investigation reveals a rare example of combinatorial and sequence-specific recognition of single-stranded DNA by a protein dimer. It also illustrates the potential utility of gene duplication and protein dimerization in promoting the rapid co-evolution of functional DNA elements and their cognate binding proteins.

UNDERSTANDING THE EFFECT OF A MUTANT TELOMERE SEQUENCE.

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TLC1 is the *Saccharomyces cerevisiae* telomerase RNA subunit, which contains a short stretch that serves as a template to extend telomeres by adding the sequence $(TG)_{0\text{-}6}TGGGTGTG(G)_{0\text{-}1}$. We are characterizing a *TLC1* template mutant (*tlc1-tm*) where the telomerase RNA template is modified to introduce the sequence $[(TG)_{0–4}TGG]_nATTTGG$ instead of the wild type telomeric sequence, resulting in the incorporation of altered sequences at the distal ends of telomeres. We have found that telomerase-negative cells containing such telomeres exhibit an accelerated senescence, which could be due to an increased 5' to 3' nucleolytic resection of the C-rich strand. To test whether the recruitment of telomere binding proteins could be affected by alterations in the telomere sequence, we examined the localization of the fluorescently tagged dsDNA and ssDNA telomere binding proteins, Rap1 and Cdc13, respectively. We found no change in the number of Rap1 foci but, interestingly, and supporting the increased resection hypothesis, we observed an increase in the number of Cdc13 foci in *tlc1-tm* cells. Altogether, this preliminary result suggests a possible telomere-capping defect in cells with $[(TG)_{0–4}TGG]_nATTTGG$ telomere sequences.
An interesting feature of the *tlc1-tm* mutant telomeres is the absence of the GGG motif of the wild type sequence. This could affect the formation of G-quadruplexes at telomeres, since G-quadruplexes are predicted to be generated when the consensus sequence $G_{\geq 3}N_xG_{\geq 3}N_xG_{\geq 3}N_xG_{\geq 3}$ is present. There is evidence supporting a role for G-quadruplexes in telomere capping when the natural Cdc13-mediated telomere protection is defective. We now plan to confirm that indeed *tlc1-tm* cells exhibit increased telomeric resection and a capping defect and, moreover, to uncover the reason behind this phenotype, which could be due to impaired formation of G-quadruplexes.

ASA1 COLLABORATES WITH TEL2 TO CONFIGURATE PROTEIN KINASES MEC1 AND TEL1

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Two large phosphatidylinositol 3-kinase-related protein kinases (PIKKs), ATM and ATR, play a central role in the DNA damage response pathway. In budding yeast ATM and ATR correspond to Tel1 and Mec1, respectively. The Tel2-Tti1-Tti2 (TTT) complex interacts with and regulates protein stability of PIKKs. The TTT complex has been proposed to promote protein maturation of PIKKs. However, it has not been determined whether the TTT complex configures the protein kinase domain of PIKKs. Moreover, it remains unclear whether the TTT complex controls protein stabilities of Mec1. No apparent checkpoint defect has been observed in available *tel2* mutants. To answer these questions, we examined the effect of Tel2 depletion on protein stability and kinase activity of Mec1 and Tel1 using a modified auxin-induced degradation system. We transiently expressed Mec1 or Tel1 using the *GAL1* promoter and subsequently depleted Tel2 at various time points after *GAL1* promoter shut-off. Tel2 depletion resulted in protein instability of Mec1 and Tel1. Moreover, Tel2 depletion impaired kinase activity of Mec1 and Tel1. In turn Tel2 depletion conferred defects in checkpoint signaling. However, delaying Tel2 depletion restored protein stability of Mec1 and Tel1, supporting a role of the TTT complex in protein maturation. Thus, the TTT complex appears to format the kinase domain of Mec1 and Tel1 in the process of protein maturation.

Rvb1 and Rvb2 form a complex, and further interact with Tah1 (TPR-containing protein associated with Hsp90) and PIHD1/Pih1 (Protein interacting with Hsp90), termed the R2TP complex. In higher eukaryotes several lines of evidence have established a model in which the R2TP complex interacts with the TTT complex, thereby modulating protein stability of PIKKs. We thus tested the effect of *pih1Δ* mutation on expression levels of Mec1 and Tel1. However, no apparent expression defect was observed in *pih1Δ* mutants. Previous studies have shown that Asa1 regulates the Tel1 expression level. We examined the effect of Asa1 depletion on checkpoint signaling and expression levels of Mec1 and Tel1. Similar to Tel2 depletion, Asa1 depletion decreased protein expression of Mec1 and Tel1 and exhibited defects in checkpoint signaling. These results suggest that Asa1-Tel2 interaction, rather than Pih1-Tel2 interaction, plays a major role in protein maturation of Mec1 and Tel1.

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THE USE OF KU SEPARATION-OF-FUNCTION MUTANTS TO PROBE KU'S ASSOCIATION AND FUNCTION AT HUMAN TELOMERES

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Telomeres prevent the natural ends of linear chromosomes from being fully engaged as substrates for DNA repair. The shelterin component, TRF2, plays a crucial role in telomere end protection. It achieves this, in part, by facilitating the assembly of the t-loop structure, thereby shielding the telomeric end from being acted upon by the nonhomologous end joining (NHEJ) pathway, the major pathway of DNA double strand break (DSB) repair in mammalian cells. However, Ku, an integral component of the canonical NHEJ machinery, associates with functional telomeres and contributes to telomere functions.

The Ku70 and Ku80 subunits of the Ku heterodimer form a ring-like structure, which allows it to load onto DNA ends at DSBs, where it contributes several activities to NHEJ. How Ku is repressed from participating in NHEJ at telomeres, particularly when the t-loop is resolved, is not well understood. Data from our lab suggests that this may be achieved, in part, by Ku's interaction with TRF2 (Ribes-Zamora, Cell Rep 2013).

Ku is an essential protein in human cells, not for its role in NHEJ, but to prevent telomere loss mediated by homology-directed repair. Given this essential role in humans, we are capitalizing on separation-of-function mutants to better understand how Ku contributes to telomere maintenance, while its NHEJ activity is repressed. We previously reported the human Ku70-α5 mutant, which is impaired for its interaction with TRF2 and for NHEJ. We have now shown that it is proficient for DNA end binding. In addition, we have generated a human Ku80 mutant that is defective for DNA end binding (Ku80-DEB), but proficient for heterodimerization with Ku70 and for interaction with TRF2. Together, they will allow us to test how the α5 region and end binding contribute to Ku's association with telomeres and its role in telomere maintenance.

HYPERMETHYLATION OF A SPECIFIC AREA IN THE TERT PROMOTER DEFINES A NOVEL RISK STRATIFICATION FOR PROSTATE CANCER

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We previously uncovered a region in the TERT promoter (THOR - TERT Hypermethylated Oncological Region) which is paradoxically hypermethylated and associated with telomerase activation in neoplastic tissues from several childhood cancers. We hypothesized that THOR Hypermethylation is a pan cancer biomarker and can serve as a diagnostic and prognostic biomarker in common adult cancers which activate telomerase.
We first determined THOR methylation status on 11 different cancers (n=3054) using Illumina 450K arrays from the TCGA data. All telomerase dependent cancers including prostate cancer (n=300) revealed high THOR methylation. We then mapped the methylation status of the whole TERT gene using MEDCHIP Seq analysis on 53 benign prostate samples and 51 malignant prostate cancers (PCa) samples and found that THOR is the only region in the whole TERT gene which is differentially methylated between normal and malignant prostate tissue ($p<0.0001$).
In order to define the clinical implications of THOR methylation we assembled 2 cohorts (Discovery Cohort (DC), n=164; Validation Cohort (VC), n=103) of patients submitted to radical prostatectomy and with long-term follow-up data. THOR was significantly hypermethylated in PCa tissues when compared to paired benign tissues ($p<0.0001$).
THOR methylation correlates with Gleason Score ($p=0.0082$) but is independent from other risk factors such as PSA ($p=0.71$). Interestingly, THOR hypermethylation is associated with tumor invasiveness and lymph node invasion ($p=0.0147$ and $p=0.031$, respectively).
THOR status predict recurrence in both DC ($p=0.0146$) and VC ($p=0.0306$). Importantly, THOR can predict recurrence in the problematic early stage PCa. Analysis of low and intermediate risk disease (Gleason 6 and 7) revealed that recurrence is rarely observed in THOR hypomethylated PCa (Gleason 6 and 7 (3+4) subgroups $p=0.0077$). In contrast, THOR hypermethylation was highly prevalent in PCa with Gleason 7(4+3) and higher. Multivariate analysis for low and intermediate risk patients revealed that THOR is an independent risk factor for recurrence (HR: 3.685 $p=0.0247$). Lastly, THOR Hypermethylation doubles the risk of recurrence for each PSA level measured (OR 2.5, $p=0.02$).
Overall, THOR redefines recurrence risk for patients with PCa and adds new dimension to both Gleason and PSA scores. Finally, THOR can identify patients where noninvasive management with active surveillance is recommended.

POT1 MUTATION IN COATS PLUS SYNDROME

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Coats plus syndrome (CP) is a rare, autosomal recessive disorder, the key characteristics of which include; retinal telangiectasia and exudates (Coats disease); intracranial calcification with leukodystrophy and cysts; osteopenia with a tendency to fracture and poor bone healing; and a high risk of developing vasculature ectasias in the stomach, small intestine and liver, leading to gastrointestinal bleeding and portal hypertension. Recently, CP was shown to be due to biallelic mutations in *CTC1*, encoding conserved telomere maintenance component 1 (Anderson et al., 2012; Polvi et al., 2012, Walne et al. 2013). Here we report two siblings demonstrating a clinical phenotype consistent with CP, in whom mutations in *CTC1* were not present, but who posses a homozygous mutation in the shelterin protein POT1 (POT1^{CP}). POT1^{CP} was indistinguishable from wild type POT1 with regard to expression level, TPP1 binding, localization to telomeres, and ability to prevent activation of ATR signaling at telomeres. However, POT1^{CP} fibroblasts from the patient show extended telomeric 3' overhangs, stochastic telomere truncations and an associated proliferative arrest that is partially circumvented by the introduction of SV40 large T antigen, and fully repressed by expression of telomerase or wild type POT1. We propose that CP is caused by mutations in either *CTC1* or *POT1* that disable CST-dependent fill-in at telomere ends.

SUBTELOMERES INFLUENCE TELOMERE SHORTENING-DRIVEN TERRA ACCUMULATION AND REPLICATIVE SENESCENCE IN *SACCHAROMYCES CEREVISIAE*

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In eukaryotes, telomeres determine cell proliferation potential by triggering replicative senescence upon telomere shortening in the absence of telomerase. In *Saccharomyces cerevisiae*, it is likely mediated by the first telomere that reaches a critically short length, which activates a DNA-damage-like response. How the signaling is modulated by the telomeric structure and context is largely unknown. Here we investigated how subtelomeric elements of the shortest telomere in the cell influence the onset of senescence. By comparing strains in which the pre-determined shortest telomere either harbors naturally occurring subtelomeric elements or lacks these elements altogether, we show that removal of subtelomeric regions accelerates the establishment of senescence. This effect is likely not due to differential Rad51-mediated homology directed repair activities at the different (sub)telomere variants. Furthermore, TERRA transcription is induced at both types of critically short telomeres, although levels are elevated in the absence of natural subtelomeric elements. Thus, subtelomeric elements become essential in the absence of telomerase, independently of being at the shortest telomere in the cell. Our results also demonstrate that telomeric transcripts from a telomere-proximal region greatly increase when the shortest telomere reaches a critical length, regardless of the presence of a native subtelomere or a dedicated TERRA promoter.

RARE AND NOVEL DELETERIOUS MUTATIONS IN TERT ARE ENRICHED IN A PAEDIATRIC ACUTE MYELOID LEUKAEMIA AND MYELODYSPLASTIC SYNDROME COHORT, AND ARE ASSOCIATED WITH FEATURES OF DYSKERATOSIS CONGENITA.

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Included among the spectrum of telomere biology disorders (TBDs) are acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). Several studies have shown an enrichment of defects in telomere maintenance genes within adult hematologic malignancy cohorts. We investigated a local paediatric AML/MDS cohort for evidence of similar enrichment, as well as for clinical features of TBDs. Sequence analysis of four telomere maintenance genes most commonly mutated in association with DC compared with racially and ethnically matched controls demonstrated a significant enrichment of novel and rare TERT variants in AML/MDS cases (p=0.01). Direct telomerase extension assays revealed three of eight variants impacted telomerase activity and processivity in vitro. Furthermore, a medical record review blinded to mutational status revealed that the number of clinically recognized DC features was associated with the presence of a novel or rare TERT variant (p=0.04). Thus, novel and rare constitutional telomerase variants are enriched in paediatric AML/MDS cases, are associated with features of TBDs, and, in some cases, are true mutations that impact telomerase catalytic function.

TELOMERE LENGTH AND BILIRUBIN: AN UNEXPECTED COLLABORATION

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Telomeres shorten with age, due to progressive cell division, until they reach a critical point at which they are capable of deciding a cell's fate. Critically short telomeres cannot keep their function as chromosome protectors, thus trigger signals leading to cellular senescence, which at turn might trigger apoptosis, on one hand, or carcinogenesis, on the other. In humans, accumulation of such events is an underlying factor for onset of age-related diseases.

The free radical theory of ageing suggests that reactive species, formed in excess, lead to damage accumulation and accelerated telomere shortening yielding critically short telomeres and chromosomal instability. Bilirubin, a product of heme catabolism, is known to be a potent antioxidant with ability to scavenge free radicals and reduce their deteriorating consequences. In the last years a number of studies proposed a strong inverse correlation of bilirubin with cancer and CVDs incidence. We chose Gilbert's Syndrome (GS), a genetic disorder characterized by mildly elevated unconjugated bilirubin (UCB) levels in serum, as a preferential model for studying the potential effects of bilirubin in humans.

The aims of our present study is to determine whether individuals with GS have longer lymphocyte telomere length (LTL) compared to age- and gender-matched individuals. Furthermore, we are evaluating the overall effects of mildly elevated, physiological levels of UCB on isolated human PBMCs in culture.

Our first preliminary data confirms significantly higher LTLs in GS ($n = 128$, $p = 0.022$), a difference which increases with age. It is still unclear whether this difference is influenced by serum UCB, as a result of its direct antioxidant or anti-inflammatory effect, or it is somehow related to the distinct genetic pattern underlying GS. We suggest, however, that increased telomere length and stability is one of the main factors for the lower incidence of age-related diseases in older GS individuals.

DIFFERENTIAL REGULATION OF TANKYRASE 1 BY K63- AND K48-LINKED POLYUBIQUITINATION

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Tankyrase 1 is a multifunctional poly(ADP-ribose) polymerase (PARP) that PARsylates a number of proteins including itself and the shelterin subunit TRF1. Tankyrase 1 localizes to telomeres in the G2/M window of the cell cycle and promotes resolution of telomere cohesion, dependent on its catalytic PARP activity. Tankyrase 1 is constitutively autoPARsylated and has a rapid turn over due to K48-linked polyubiquitination and proteasomal degradation. The RING-type E3 ubiquitin ligase RNF146 is responsible for tankyrase 1's PARsylation-dependent ubiquitination and degradation. Here we describe a novel post-translational modification of tankyrase 1 that impacts its stability and function. We show that tankyrase 1 is modified by K63-linked polyubiquitination, mediated by the RING-type E3 ligase RNF8. This modification is cell cycle regulated, occurring in late S/G2 phase of the cell cycle. Moreover, while the bulk of tankyrase 1 is found in the cytoplasm (as is RNF146), K63-linked polyubiquitinated tankyrase 1 is found in the nucleus (as is RNF8). We hypothesize that RNF8-mediated K63-linked polyubiquitination protects tankyrase 1 from RNF146-mediated K48-linked polyubiquitination, thereby stabilizing tankyrase 1 for its nuclear/telomere function. In support of this notion, we show that in RNF8-depleted cells, tankyrase 1 K63-linked polyubiquitination is diminished, tankyrase 1 association with chromatin is reduced, and sister telomere cohesion is not resolved. We propose that K63-linked polyubiquitination competes with K48-linked polyubiquitination of tankyrase 1 preventing its rapid degradation by the proteasome and rendering it more stable in mitosis, thereby enabling it to execute its cell cycle regulated telomeric functions.

NON-CANONICAL P53 BINDING TO HUMAN SUBTELOMERES MOUNTS A PROTECTIVE TRANSCRIPTION AND CHROMATIN RESPONSE TO GENOMIC STRESS

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Telomeres and tumor suppressor protein TP53 (p53) function in genome protection, but a direct role of p53 at telomeres has not yet been described. Here, we have identified non-canonical p53 binding sites within the human subtelomeres that suppress the accumulation of DNA damage at telomeres. These non-canonical subtelomeric p53 binding sites conferred transcription enhancer-like functions that include an increase in local histone H3K9 and H3K27 acetylation and subtelomeric transcripts, including TERRA. p53 suppressed formation of gammaH2AX and enhanced subtelomere DNA stability in response to DNA damage. Our findings indicate that p53 provides direct local protection to some of its DNA bound sites, including subtelomeres. This is a previously unrecognized p53-associated tumor suppressor function that may partly account for many genome-wide p53 binding sites of unknown function.

MUTUALLY EXCLUSIVE BINDING OF THE *KLUYVEROMYCES LACTIS* TELOMERASE RNA TEMPLATE AND THREE-WAY JUNCTION BY EST2

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Telomerase RNA includes two functional domains that are conserved across ciliates, yeast and vertebrates: (1) The templating domain, including the template for telomeric repeat synthesis, a template boundary element (TBE), and a triple-helix containing pseudoknot (PK); and (2) an assembly/activation stem-loop domain termed stem-loop IV in ciliates, three-way junction (TWJ) in yeast, and CR4-CR5 domain in vertebrates. Several lines of evidence suggest that these are homologous elements that provide a conserved function in the assembly of an active telomerase RNP complex. In ciliates and vertebrates, the telomerase reverse transcriptase (TERT) has been shown to interact with both of these domains. However, the direct interactions of Est2 (the yeast TERT) with these elements have not been demonstrated previously. We have studied the three dimensional structure of the templating domain and TWJ of *K. lactis* telomerase RNA, and their interactions with Est2 *in vivo* and *in vitro*. In addition, we solved the structure of the *K. lactis* Est2 RNA binding domain (TRBD) by X-ray crystallography. Surprisingly, although the TRBD structure is conserved with other species, it did not appear to bind TWJ, unlike the vertebrate TRBD which binds well to CR4-CR5. Only the full length Est2 bound TWJ, suggesting that the affinity of TRBD to the RNA is not high enough and thus an additional domain of Est2, possibly the C-terminal extension (CTE), is required to sustain stable interaction. The three arms of TWJ, arranged in the correct orientation with respect to each other, are required for the binding of this domain by Est2. Within the templating domain, the template itself with a short 5' flanking sequence is required and sufficient for the binding of Est2. Interestingly, we show that the binding of the template and TWJ by Est2 is mutually exclusive. Altogether, our results suggest that Est2 forms multiple interactions with telomerase RNA and changes its position within the telomerase complex, while telomerase RNA changes its conformation, during the assembly and/or reaction cycle of telomerase.

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CHARACTERIZATION OF *TETRAHYMENA* TELOMERASE HOLOENZYME STRUCTURE AND FUNCTION

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Biologically active *Tetrahymena* telomerase holoenzyme requires association of the catalytic core with accessory proteins consisting of p19, p45, p50, p75, and the RPA-1 related subunit, Teb1. Despite knowledge of the overall architecture and EM structure of the *Tetrahymena* holoenzyme, relationships between the accessory proteins and their specific physiological roles have remained unresolved. We have used a variety of *in vivo* and *in vitro* biochemical techniques to characterize direct binding interactions and their functional importance. Our results show that the high affinity single-stranded DNA binding subunit Teb1 is necessary for telomerase-telomere interaction and sufficient for cell cycle regulated telomere association. Additionally, structural evidence from collaboration with the Laboratory of Ming Lei indicates that p45 and p19 are a telomerase-specific Rpa2/Stn1 and Rpa1/Ten1 complex, respectively. Cellular expression of p45 or p19 variants altered for protein-protein interactions compromises telomere synthesis of one or both DNA strands. This work expands the known functions of telomerase-associated proteins and characterizes the molecular mechanisms of telomere synthesis in *Tetrahymena*.

STRINGENCY OF KU-DNA INTERACTION IS MORE CRITICAL FOR DNA REPAIR THAN FOR TELOMERE PROTECTION

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The Ku heterodimer is known for its function in non-homologous end joining (NHEJ) DNA repair, but it is also critical for telomere protection and stability in virtually all eukaryotes where it has been studied. How Ku protects chromosome ends without triggering NHEJ is still poorly understood. In the initial step of NHEJ Ku loads on broken DNA via positively charged DNA channel and subsequently translocates inwards onto DNA to free the ends for processing and ligation. Studies in budding yeast showed that Ku association with DNA is also required for its telomeric function suggesting that Ku mode of action at telomeres and DSBs may be analogous. To examine requirements for Ku-DNA association in telomere protection in higher eukaryotes, we created human and Arabidopsis Ku complexes with altered DNA interaction properties by systematically mutating amino acid residues in the DNA loading channel. We found a mutant combination that impairs DNA binding, but at the same time it appears to increase retention of Ku at DNA termini. Functional complementation in Arabidopsis revealed that this complex is deficient in DNA repair, but still proficient in telomere protection. This data suggest that telomere protection and DNA repair have distinct requirements for Ku-DNA association and further implies that Ku may employ a different mode of action in the context of telomeres and at DSBs.

IDENTIFICATION OF GENES THAT PLAY A ROLE IN RECOMBINATION-MEDIATED TELOMERE MAINTENANCE IN YEAST

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In telomerase-deficient cells, telomeres shorten progressively during each cell division due to incomplete end-replication. When the telomeres become very short, cells enter a state that blocks cell division, termed senescence. A subset of these cells can overcome senescence and maintain their telomeres using telomerase-independent mechanisms. In *Saccharomyces cerevisiae*, these cells are called 'survivors' and require Rad52-dependent homologous recombination and Pol32-dependent break-induced replication. There are two main types of survivors: type I and type II. Type I survivors require Rad51 and maintain telomeres by amplification of subtelomeric elements, while type II survivors are Rad51-independent, but require the MRX complex and Sgs1 to amplify the TG1-3 telomeric sequences. Rad52, Pol32, Rad51, and Sgs1 are also important to prevent accelerated senescence, indicating that recombination processes are important at telomeres even before the formation of survivors.

The Shu complex, which consists of Shu1, Shu2, Psy3, and Csm2, promotes Rad51-dependent recombination and has been suggested to be important for break-induced replication. It also promotes the formation of recombination intermediates that are processed by the Sgs1-Top3-Rmi1 complex, as mutations in the *SHU* genes can suppress various *sgs1* and *top3* mutant phenotypes. Given the importance of recombination processes during senescence and survivor formation, and the involvement of the Shu complex in many of the same processes during DNA repair, we hypothesized that the Shu complex may also have functions at telomeres. Surprisingly, we have found that this is not the case: the Shu complex does not affect the rate of senescence, does not influence survivor formation, and deletion of *SHU1* does not suppress the rapid senescence and type II survivor formation defect of a telomerase-deficient *sgs1* mutant.

We are now performing a genome-wide screen to find genes that are required for type II survivor formation. We have already identified several genes known to be important (e.g. *RAD52*, *RAD59*, *MRE11*, *RAD50*, *XRS2*, *SGS1*), showing the efficacy of our screening approach. We also identified *NMD2*, which is involved in nonsense-mediated mRNA decay. While *NMD2* has been previously reported to be required for type II survivor formation, it is unclear how and why. We plan to investigate the link between nonsense-mediated mRNA decay and survivors.

ACCELERATING *IN VITRO* NEURAL AGING BY MANIPULATIONS OF TELOMERASE FUNCTION AND ITS APPLICATION FOR MODELING LATE ONSET DISEASE.

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Modeling of late onset disorders such as Parkinson's disease (PD) by conventional differentiation paradigms remains a challenge, as current induced pluripotent stem cells (iPSC) differentiation protocols yield cells that typically show the behavior of fetal-stage cells. These observations prompted us to test whether it would be possible to accelerate aging *in vitro* to obtain a late onset disease phenotype such PD by iPSC disease modeling. In order to manipulate a factor that is directly involved in natural aging and based on the premature aging syndromes associated to mutations in the telomerase complex, we propose to use telomere shortening as an aging inducing tool. Our main objective was to test whether shortened telomeres result in an aging and disease phenotype in human embryonic stem cells (hES) and iPSC derived midbrain dopamine (mDA) neurons. Our novel approach will provide a powerful tool for basic research and drug discovery in late onset diseases while providing critical knowledge to further understand the role of telomere shortening in neuronal aging.

UNG AND MSH2 CONTROL TELOMERE STABILITY IN B-CELLS EXPRESSING ACTIVATION-INDUCED DEAMINASE.

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Immunoglobulin (Ig) class switch recombination (CSR) is an essential mechanism for the diversification of the humoral immune response through efficient generation of antibody isotypes that mediate the elimination of pathogens. CSR is a programmed deletional recombination event between DNA double strand breaks (DSBs) at switch (S) regions in the Ig heavy chain gene locus (Igh). These DSBs are initiated by the mutagenic enzyme activation-induced cytidine deaminase (AID), which converts the cytosine (C) base of deoxycytidine into uracil (U). The guanine-uracil (G:U) mismatches are processed by uracil N-glycosidase (UNG)-dependent base excision repair (BER) and MSH2-dependent mismatch repair (MMR) pathways to yield DSBs that can recombine via end-joining mechanisms. Although AID is preferentially targeted to the Igh locus, it also mutates other genes, including proto-oncogenes, thereby creating a predisposition for B-cell lymphomas. Although off-target AID activity contributes to oncogenic point mutations and chromosomal translocations associated with B-cell lymphomas, the role of downstream AID-associated DNA repair pathways in lymphomagenesis is not well defined. Here we report that AID deaminates the telomeric C-rich strand. Furthermore, B-cells rely on UNG to protect the telomeres from AID activity; however, inhibition of UNG results in the recruitment of MMR proteins to the telomeres and a sudden loss of chromosome ends. Finally, UNG-deficiency decreases the proliferation of normal and tumor B-cells expressing AID.
Based on these results in B-cells, we propose a molecular model where BER is more efficient than MMR for repairing AID-induced deamination at telomeres. However, MMR will become relevant for the processing of the AID-induced G:U mismatches that persist at the telomere in UNG-deficient B-cells. Altogether, our results support a model where the processing AID-induced lesions at the telomeres by BER and MMR control the proliferation capacity of AID-expressing B-cells.

MODELLING THE *LEISHMANIA* SPP. TELOMERASE AND ITS INTERACTIONS WITH THE TELOMERIC DNA AND THE TELOMERASE RNA COMPONENT

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The *Leishmania* genus comprise a group of parasites among which are the causative agents of leishmaniasis, a spectrum of diseases that affect millions of people worldwide and to which there are no effective control program or therapeutics. Therefore, efforts for the establishment of intensive research to better understand the molecular biology of these parasites are encouraged. Due to their role in maintaining genomic stability, telomeres have been proposed as important targets in the development of new drugs to treat leishmaniasis. *Leishmania* telomeres are composed by the conserved TTAGGG repeated sequence which is maintained by the action of telomerase. The two main components of this complex (LaTERT and LeishTER) have been previously cloned and characterized, showing that both the protein and the RNA share conserved domains and motifs with their eukaryote counterparts. In addition, LeishTER co-immunoprecipitates and colocalizes with the telomerase protein component (TERT) in a cell cycle-dependent manner. The interaction dynamics between TERT, TER and the genomic DNA in *Leishmania* is still unknown and very important to understand how telomerase acts in this organism. However, until this moment, there is no structural information about LeishTER and LaTERT as well as any structural information about the interactions among the protein and the nucleic acids. In this work, we created *in silico* models of TRBD and RT domains of LaTERT using molecular modeling and molecular dynamics simulation. The preliminary analysis of these models showed that both domains have significant structural differences in comparison with the telomerase crystal structures of *Tribolium castaneum*, *Tetrahymena termophila* and *Takifugu rubripes*. The main structural differences are located in CP and T motifs, which are involved with nucleic acids interaction. Subsequently, *in silico* models of TRBD domain bound to TER and to the G-rich telomeric DNA are being generated in order to evaluate the impact of these structural differences on nucleic acids binding. This is the first study of the three dimensional structure of a telomerase from a primitive pathogenic protozoa. Our results should give insights on the evolution of telomerase enzyme and the nature of the interactions between the enzyme and the nucleic acids.

DYNAMICS OF HUMAN TELOMERASE HOLOENZYME COMPOSITION OVER THE CELL CYCLE

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Human telomerase has been shown to act on telomeres during S phase of the cell cycle, which is accompanied by intranuclear trafficking of telomerase subunits and telomerase colocalization with telomeres. However, it is unclear whether the in vivo assembly and composition of the telomerase holoenzyme is under cell cycle regulation. We investigated telomerase subunit interaction and exchange over the cell cycle using in vivo crosslinking, co-immunoprecipitation, and reverse transcription quantitative PCR (RT-qPCR) to quantify telomerase subunits bound to the telomerase RNA component (hTR) under several cell cycle synchronization methods. Our in vivo snapshots of the human telomerase holoenzyme composition reveal that the core components of telomerase, hTERT and hTR, remain tightly bound once assembled, regardless of position within the cell cycle. In contrast, we found that the telomerase Cajal Body associated protein, TCAB1, is released and exchanged in a cell cycle specific manner. During and immediately following mitosis, TCAB1 dissociates from hTR. This shift in telomerase subunit equilibrium is uncoupled to changes in telomerase activity, TCAB1 protein levels, or hTR levels. We also report that TCAB1 expression is not limiting the hTERT-hTR association in immortalized cell lines and that increasing the cellular levels of hTERT minimally impacts in vivo TCAB1-hTR levels. Additionally, we found that the H/ACA small nucleolar ribonucleoprotein component, Naf1, can increase the association of TCAB1 with hTR. Our characterization of telomerase subunit composition reveals a stable core complex that changes its interaction partners over the cell cycle.

T CELL QUALITATIVE DEFECTS IN THE TELOMERE SYNDROMES

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T cell diversity and memory are essential for immune surveillance, and telomere function is required for maintaining the self-renewal potential of long-lived cells. Mice with short telomeres have defects in T cell number and survival, and we hypothesized that telomere syndrome patients may similarly have T cell defects that contribute to their increased risk of infection. We first examined the T cell receptor (TCR) repertoire diversity in young adults with telomere syndromes. We studied the TCRβ chain CDR3 region by deep sequencing and found it was significantly restricted in telomere syndrome cases compared to young and elderly controls who had normal telomere length. To test the functional relevance of these deep sequencing results, we examined the TCRβ diversity by flow cytometry and found it was also more significantly restricted in telomere syndrome cases than in the control groups. T cells isolated from both young telomere syndrome patients and elderly controls were prone to apoptosis when challenged in vitro. We queried the functional pathways that underlie the apoptotic defect by examining the gene expression profile of CD8+ CD45RA+ effector memory T cells (known as T_{EMRA}). T_{EMRA} CD8+ T cells accumulate with age and have been hypothesized to contribute to immune aging. We found that T_{EMRA} cells from telomere syndrome cases showed an altered gene expression signature relative to young and elderly controls. In the telomere syndrome cases, the pathways that were most differentially expressed included the DNA damage response, cell cycle regulation and the p53 pathway. In contrast, the profile in elderly controls was notable for apoptotic pathways that were p53-independent. These data indicate that short telomeres cause qualitative defects in the T cell compartment of telomere syndrome patients; these defects likely contribute to an increased risk of infection in this population.

A POOLED shRNA SCREEN TO IDENTIFY NOVEL REGULATORS OF TELOMERE LENGTH

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Telomere length is maintained at an established equilibrium in mammalian cells with active telomerase. Telomere shortening causes disease in the human telomere syndromes [1]. The interaction between telomerase and telomere binding proteins plays an integral role in defining this equilibrium, but the full details of this regulation have not been elucidated.

To identify novel regulators of telomere length, we designed an shRNA screen to identify genes that alter telomere length. In a pilot experiment, we transduced mammalian cells with a pool of 3 lentiviral shRNAs with known effects on telomere length: TERT, POT1 and non-silencing control. As expected, shRNA Knockdown of TERT shortened telomeres, while knockdown of POT1, a telomere binding protein, lengthened them. Fifty population doublings after transduction, we sorted cells with the 7% shortest and 7% longest telomeres as determined by flow-FISH. Flow-FISH is a flow cytometry based assay that measures telomere length in intact cells by hybridization with fluorescent probes, allowing us to sort cells based on telomere length. We amplified shRNA inserts from the long and short telomere cell populations and analyzed insert abundance by deep sequencing. We found POT1 shRNA inserts were enriched in the long telomere fraction, while TERT shRNA insertions were enriched in the short telomere fraction. This pilot experiment demonstrated the utility of this assay in identifying shRNAs that affect telomere length.

In our initial screen, we targeted kinases, due to their established regulatory role, and relative ease of targeting by small molecule inhibitors. Similar to the pilot screen, we transduced 2 different human cell lines with a pool of 4675 lentiviral shRNAs targeting all protein kinases. Transductions were performed in triplicate, and cells were cultured for 50 population doublings. We collected cells with the top and bottom 7% of telomere length by flow-FISH sorting, and analyzed shRNA abundance by deep sequencing. We are in the process of analyzing and validating these results. This screen may identify novel kinases in telomere length regulation, and further our understanding of telomere length homeostasis.

References:
1. Armanios, M., and Blackburn, E. H., Nat Rev Genet 2012. 13(10): 693-704.

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A TERRA-CONTAINING TELOMERIC CHROMATIN COMPLEX FOUND IN EXTRACELLULAR EXOSOME FRACTIONS STIMULATES INFLAMMATORY CYTOKINE PRODUCTION

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Telomeres play a critical role in regulating cellular aging and cancer development. While telomere dysfunction is known to promote chromosomal instability, relatively little is know about its impact on cellular and tumor microenvironment. Here, we report that a cell-free chromatin complex containing telomeric repeat-containing RNA (TERRA) can be found in culture medium and blood plasma. This cell-free TERRA (cfTERRA) complex could be separated from exosome vesicles using sucrose equilibrium density centrifugation. Mass spectrometry and Chromatin immunoprecipitation (ChIP) revealed cfTERRA associated with histones and telomere DNA, which may retain the structure of telomere chromatin. cfTERRA complex is able to activate the transcription of inflammatory cytokines in peripheral blood mononuclear cell (PBMC) from fresh blood. These findings imply a crosstalk between telomere biology and innate immunity, and provide a novel insight of telomeres and TERRA in modulating cellular and tumor microenvironment.

THE TELOMERASE RNA STEM TERMINUS ELEMENT AFFECTS TEMPLATE BOUNDARY ELEMENT FUNCTIONS, TELOMERE SEQUENCE AND SHELTERIN BINDING

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The stem terminus element (STE), which was discovered thirteen years ago in human telomerase RNA, is required for telomerase activity yet its mode of action is unknown. We report that the *S. pombe* telomerase RNA, TER1, also contains a STE, which is essential for telomere maintenance. Cells expressing a partial loss-of-function TER1 STE allele maintained short stable telomeres by a recombination-independent mechanism. Remarkably, the mutant telomere sequence was different from that of wild type cells. The unusual sequence was neither due to recombination nor to short telomeres. Generation of the altered sequence is explained by reverse-transcription into the template boundary element (TBE), demonstrating that the STE helps maintain TBE function. The altered telomeres bound less Pot1 *in vivo* and Taz1 in a bacterial one-hybrid assay. Thus, the *S. pombe* STE, although distant from the template, ensures proper telomere sequence, which in turn promotes proper assembly of the shelterin complex.
An RNA three-hybrid screen for proteins that interact with the STE region identified the multifunctional La protein homolog, Sla1. ChIP and ChIP-SEQ experiments showed that Sla1 bound to multiple loci, including telomeres, and this binding was largely RNA-dependent. As with human La, telomerase activity was Sla1 associated in immuno-precipitates. Cells lacking Sla1 had short telomeres and reduced TER1 RNA. In addition, point mutations that eliminate *sla1* chaperoning activity also have short telomeres, but do not affect TER1 levels. Notably, *sla1Δ* and *sla1* chaperoning mutants partially suppressed the STE mutant telomere length and sequence phenotypes. This result suggests that the STE and Sla1 function together to maintain telomere sequence integrity.

MOLECULAR PHENOTYPES AND SKEWED X-INACTIVATION IN FEMALE CARRIERS OF X-LINKED DYSKERATOSIS CONGENITA MUTATIONS

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Dyskeratosis congenita (DC) manifests with clinical symptoms that include abnormalities in the skin, mucous membranes, and hematopoietic tissues. X-linked recessive (X-DC) is caused by germline mutations in dyskerin (DKC1). Autosomal dominant, and recessive forms of the disease have been described. More than 80% of young males with X-DC develop progressive bone marrow failure by age 30, and this is the major cause of death. Skewed X-inactivation has been reported in female X-DC carriers, implying that after random X-inactivation at gastrulation, female carrier cells expressing the normal DC allele have a survival/growth advantage over cells expressing the mutant allele. The extent of skewed X-inactivation in different cell types is not clear, and it is yet to be determined how skewed X-inactivation affects inheritance of telomere length.

The NCI's prospective cohort study of DC has identified female carriers of X-DC. Clinical and exome sequencing has identified women with features of DC and heterozygous DKC1 mutations in the absence of mutations in other telomere biology genes. Biospecimens including, blood, buccal cells, fibroblast cell lines, and EBV-transformed lymphoblastoid cell lines were derived from an female with heterozygous X-DC, male X-DC probands, and relatives who are unaffected female X-DC carriers. We characterized the extent of skewed X-inactivation in DKC1 heterozygous carriers with the standard androgen receptor CAG-repeat polymorphism assay, and directly determined the coding sequence of the expressed dyskerin mRNA. We also directly compared X-inactivation patterns, using different tissues collected from the same female carriers of the X-DC allele, to discern whether skewed X-inactivation is universal across different tissue compartments. The extent of skewed X-inactivation is not significantly different in peripheral blood mononuclear cells or EBV-transformed lymphoblastoid cells, when compared with skin fibroblasts or buccal epithelial samples. Dyskerin expression levels were comparable between wildtype controls and heterozygous carriers of X-DC alleles.

Female carriers of the X-DC allele are frequently overlooked in biomedical studies of X-DC. Our data indicate that efficient skewed X-inactivation could allow for the maintenance of functional telomerase activity over a carrier's lifespan. The mechanism behind how X-DC clinical complications manifest in female carriers, based on their family pedigree, remains unclear. Mapping the molecular signatures and mechanisms of such inheritance will allow for the implementation of proper clinical preventive measures.

TELOMERASE REVERSE TRANSCRIPTASE EXPRESSION PROTECTS TRANSFORMED HUMAN CELLS FROM DNA-DAMAGING AGENTS, AND INCREASES THE TOLERANCE TO CHROMOSOMAL INSTABILITY

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Reactivation of telomerase reverse transcriptase (TERT) expression in late tumorigenesis permits immortal growth of oncogenic transformed cells. TERT reactivation is responsible for repairing chromosome ends in over 85% of human cancers. The remaining cancers rely on the alternative lengthening of telomeres (ALT), a recombination-based mechanism for telomere length maintenance. Prevalence of TERT reactivation over ALT was linked to secondary TERT function unrelated to telomere length maintenance. To characterize this non-canonical TERT function, we created a panel of ALT cells with recombinant expression of telomerase components. TERT-positive ALT cells showed higher tolerance of genotoxic insults compared with their TERT-negative counterparts. TERT expression significantly improved the kinetics of double-strand chromosome break repair. We identified telomere-synthesis defective TERT variants bestowing similar genotoxic stress tolerance, indicating that telomere synthesis activity is dispensable for this survival phenotype. TERT and TERT variant expression reduces DNA-damage-induced nuclear division abnormalities, a phenotype associated with ALT tumors. Despite this reduction in cytological abnormalities, survival of TERT-positive ALT cells is found with gross chromosomal instability. We sorted TERT-positive cells with cytogenetic changes and followed their growth in cell culture. We found that the chromosome number changes persist, and TERT-positive ALT cells surviving genotoxic events propagated through subsequent generations with new chromosome numbers. Our data confirm that telomerase expression protects against DNA-damaging events, and show that this protective function is uncoupled from its role in telomere synthesis. TERT expression promotes oncogene-transformed cell growth by reducing inhibitory effects of cell intrinsic (telomere attrition) and cell extrinsic (chemically- or metabolism-induced genotoxic stress) challenges. These data provide the impetus to develop new therapeutic interventions for telomerase-positive cancers through simultaneous targeting of multiple telomerase activities.

DDM1 PROTECTS AGAINST TELOMERE RECOMBINATION IN *ARABIDOPSIS THALIANA*

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Telomeres stabilize linear chromosomes by protecting the ends from eliciting DNA damage responses. In addition to telomere-specific protein complexes, recent studies reveal that several epigenetic pathways, including DNA methylation, are crucial for telomere maintenance. Deficient in DNA Methylation1 (DDM1) encodes a nucleosome remodeling protein that is essential for maintaining DNA methylation in *Arabidopsis thaliana*. Although *ddm1* mutants can be propagated, in the sixth generation (F6) hypomethylation leads to rampant transposon activity and infertility. Here we examine the role of DDM1 in *A. thaliana* telomere homeostasis. We report that bulk telomere length remains within the wild type range (2-5kb) in *ddm1* mutants until F6, where it precipitously drops so that telomeres now span 1-2kb. Plants lacking DDM1 exhibit no dysregulation of the known telomere-associated transcripts. Although, telomerase activity decreases in successive generations and by F6 is less than half of the wild type level, this decline in enzyme activity cannot account for the dramatic telomere shortening. Instead, telomere abrupt shortening is associated with a significant increase in extrachromosomal telomeric circles and G-overhang signals, indicating that telomeres lacking DDM1 are subject to deletional recombination. Strikingly, telomere instability coincides with the onset of hypersensitivity to DNA damage in the root apical meristem of F6 *ddm1* mutants.

Previous studies have shown that DNA damage triggers an increase in global homologous recombination in *A. thaliana*. Therefore, our data suggest that the truncation of telomere tracts in F6 *ddm1* mutants is a byproduct of elevated recombination in response to genotoxic stress. Furthermore, we hypothesize that deletional recombination of telomeric DNA in *ddm1* mutants may have a beneficial role by accelerating the elimination of stem cells with extensive DNA damage.

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SUPPRESSION OF STN1 ENHANCES THE CYTOTOXICITY OF CHEMOTHERAPEUTIC AGENTS IN CANCER CELL LINES BY ELEVATING DNA DAMAGES AND TELOMERE INSTABILITY

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DNA damage inducing agents are among the most effective regimen in clinical chemotherapy. However, drug resistance and severe side effects caused by these agents greatly limit the efficacy of these drugs. Sensitizing malignant cells to chemotherapeutic agents has long been a goal of successful chemotherapy. In this study, we describe that suppression of STN1, a novel player important for safeguarding genome stability, sensitizes tumor cells to chemotherapeutic agents.Using various cancer cell lines, we have found that down-regulation of STN1 results in a significant decrease in IC_{50} values of several conventional anti-cancer agents and telomerase inhibitors. When cells are treated with anti-cancer agents, STN1 suppression leads to declined colony formation and diminished anchorage-independent growth. Furthermore, we also find that STN1 knockdown augments the levels of DNA damage caused by damage inducing agents. When cells are treated with telomerase inhibitors, STN1 knockdown drives faster telomere attrition. To the best of our knowledge, this is the first study characterizing that suppression of STN1 enhances the cytotoxicity of chemotherapeutic drugs. Our findings imply that cancer cells with low expression of STN1 protein, either intrinsically or decreased by medical means, may have positive responses to lower doses of DNA damage inducing agents and telomerase inhibitors.

ATM REGULATES RNA-MEDIATED RECRUITMENT OF PHOSPHORYLATED (PT371)TRF1 TO ALT-ASSOCIATED PML BODIES

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Telomeres, heterochromatic structures found at the ends of linear eukaryotic chromosomes, function to protect natural chromosome ends from being recognized as damaged DNA. Telomeres in most human somatic cells shorten every time cells divide, ultimately leading to replicative senescence. A majority (85-90%) of human cancers avoid replicative senescence and gain unlimited growth potential by activating telomerase, an enzyme that is responsible for adding telomeric DNA onto chromosome ends. The remaining human cancers do not activate telomerase but instead maintain their telomere length through a homologous recombination (HR)-based mechanism, referred to as alternative lengthening of telomeres (ALT). Characteristic features of ALT cells include ALT-associated PML bodies (APBs), C-circles and elevated sister chromatid exchanges.

TRF1, a duplex telomeric DNA-binding protein, is a component of the six-subunit shelterin complex essential for maintaining telomere length and integrity. TRF1 is a multifunctional protein that has been implicated in telomere length regulation, cell cycle progression, resolution of sister telomeres as well as DNA double strand break repair. TRF1 undergoes extensive post-translational modifications, which in turn regulate its cellular localization, protein stability and DNA binding activity. Previously we have reported that Cdk1 phosphorylates TRF1 at T371 and that this phosphorylation impairs its interaction with duplex telomeric DNA. We have shown that T371 phosphorylation in mitosis is needed for the resolution of sister telomeres whereas its phosphorylation in interphase facilitates HR-mediated repair of DNA double strand breaks. Since ALT cells rely on HR to maintain their telomere length, we set out to investigate the role of phosphorylated (pT371)TRF1 in ALT cells. We find that phosphorylated (pT371)TRF1 is a component of APBs. Loss of T371 phosphorylation impairs not only the formation of APBs but also the production of C-circles. Although T371 phosphorylation is needed for TRF1 localization to APBs, it becomes dispensable for directing TRF1 to APBs when the Myb-like DNA binding domain of TRF1 is deleted. On the other hand, the Myb-like DNA binding domain of TRF1 is needed to support the recruitment of Nbs1 and other shelterin proteins to APBs. Furthermore, we demonstrate that (pT371)TRF1 localization to APBs is dependent upon RNA and ongoing transcription. Depletion or inhibition of ATM impairs (pT371)TRF1 localization to APBs. Our work suggests that ATM regulates RNA-dependent recruitment of (pT371)TRF1 to APBs to facilitate HR-mediated telomere length maintenance in ALT cells.

CST COMPLEX AND G-QUADRUPLEX

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We have previously reported the mammalian CST complex, as telomere-associated ssDNA-binding proteins. In immunofluorescence (IF) experiments, CST forms punctate foci. While a fraction of the foci overlapped with telomere signals, others did not, suggesting non-telomeric functions of CST. We also found that CST foci do not overlap with the replication foci, and observed the presence of CST foci in quiescent cells, suggesting that CST plays a role in non-replicating cells. Here we show that CST co-localizes with G-quadruplex foci detected with the BG4 antibody in IF experiments. Moreover, immunoprecipitation and mass spectrometry analyses identified base excision repair proteins associated with the CST complex (YS, YW and FI, this meeting). A model for the functional role of CST at G-quadruplex regions will be discussed.

DIFFERENT BINDING MODES OF HUMAN CST MEDIATE SPECIFIC ASPECTS OF TELOMERE REPLICATION AND GENOME-WIDE REPLICATION RESCUE.

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Human CST (CTC1-STN1-TEN1) is a ssDNA-binding complex that was originally identified as a DNA polymerase α stimulatory factor. CST functions in telomere replication first by aiding passage of the replication machinery through the telomere duplex and then enabling fill-in synthesis of the telomeric C-strand following telomerase action. CST also has genome wide roles in the resolution of replication stress. CST bears striking resemblance to RPA, the ssDNA binding protein responsible for moderating key transactions in DNA replication, recombination and repair. STN1 and TEN1 contain OB fold domains and are structurally similar to RPA2 and RPA3 respectively. While CTC1 is much larger than RPA1, the C-terminus is predicted to harbor three OB folds with high structural similarity to the three DNA binding motifs of RPA1 (OB folds A-C). The similarities between CST and RPA suggested that the various functions of CST might utilize subsets of OB folds for different modes of DNA binding. To address this possibility, we generated a CST DNA binding mutant by altering three residues in the STN1 OB fold (STN1-OBM). The equivalent residues in RPA2 contact or lie close to DNA in the crystal structure. In vitro studies indicated that STN1-OBM greatly decreases CST binding to short G-strand oligonucleotides however binding to long telomeric or non-telomeric oligonucleotides is largely unaffected. These results indicate that the STN1 OB fold is responsible for high affinity binding to short stretches of telomeric G-strand DNA. Moreover, CST appears to resemble RPA in exhibiting different DNA binding modes but the trajectory of DNA engagement is different. To determine the in vivo effect of altered DNA binding, we asked if STN1-OBM expression alters telomere replication or genome-wide replication rescue. Interestingly, we found STN1-OBM to be a separation of function mutant. The STN1-OBM cells had increased anaphase bridges and multiple telomeric FISH signals (MTS). However, the length of the telomeric G-overhang and the rate of C-strand fill-in were normal. Likewise, the cells showed wild type sensitivity to hydroxyurea (HU) and the level of new origin firing after release from HU was unaffected. Thus, the ability to bind short stretches of ssDNA appears to be important for replication through natural barriers such as telomeres but is less critical for C-strand fill-in or stress-induced origin firing. Overall our work suggests that CST binds DNA dynamically via multiple OB folds and mediates different transactions via specific DNA binding modes.

THE ESSENTIAL ROLE OF THE YEAST T-RNA COMPLEX IS TO PROMOTE REPLICATION OF DUPLEX TELOMERIC DNA

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In budding yeast, the telomere-specific single-strand DNA (ssDNA) binding protein Cdc13, along with two associated subunits, forms a telomere-dedicated RPA complex (which we have dubbed the t-RPA complex). A long-standing model has proposed that an essential function of this complex is to protect chromosome ends from unregulated resection, based on the appearance of extensive ssDNA in telomeric and sub-telomeric regions, when subunits of this complex are impaired. However, a series of recent observations have challenged this model, including the demonstration that this ssDNA is not terminal (based on resistance to digestion in vitro by terminus-specific exonucleases).

We have therefore investigated an alternative model, by asking whether the essential function of the t-RPA complex instead is to facilitate DNA replication through duplex telomeric DNA. As a direct test of this model, we designed an assay that allowed us to observe spontaneous replication fork collapse at an internal (i.e. interstitial) duplex telomeric tract located either 29 or 56 Kb from the natural chromosome terminus and adjacent to a high efficiency origin of replication (oriented so that the G-rich strand is replicated by lagging strand synthesis). These interstitial telomeric regions behave as fragile sites in response to replication stress, as loss of the distal segment is increased in the presence of HU or in a strain bearing a mutation in the DNA pol alpha/primase complex. Following loss of the distal tract, the remaining telomeric tract is sufficient to allow cells to continue dividing (i.e. recovery is not dependent on telomerase-mediated re-elongation); furthermore, sequence analysis of these events demonstrates that recombination or BIR are not playing a role. Thus, loss of the distal marker provides a read-out for spontaneous replication fork collapse, which we can monitor in single cell divisions. Using this assay, we show the replication fork collapse is increased by as much as 20-fold in several different categories of mutations in the t-RPA complex. Furthermore, the genetics of "end protection" is recapitulated in this replication fork collapse assay; *i.e.* mutations that suppress *cdc13*-ts growth phenotypes and/or increased ssDNA at telomeres also suppress the increased frequency of replication fork collapse in *cdc13* or *stn1* mutants. These, and other observations, lead us to propose that the t-RPA complex performs its primary function at telomeres as a component of the DNA replication machinery, to ensure high fidelity replication of duplex telomeric DNA.

A NOVEL ROLE OF BUB3 PROTEIN COMPLEX IN PROMOTING TELOMERE DNA REPLICATION

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Telomere integrity plays an important role in the preservation of genomic stability and is regulated by the telomerase and telomere-binding proteins. The unique secondary structure of telomeres and special telomere-binding proteins also present a challenge for DNA replication. Although several proteins have been shown to facilitate telomere replication, it remains unclear how telomere replication and replication stress are regulated during S phase of the cell cycle. Here, we show that the BUB3 complex, a key complex in spindle assembly checkpoint (SAC), binds to telomeres during S phase and promotes telomere DNA replication. Loss of the BUB3 complex results in replication fork stalling at telomeres, and leads to telomere replication defects including telomere fragility. The regulatory mechanism of BUB3-dependent telomere replication will be discussed. Our data shed light on a previously unknown function of the BUB3 complex in S phase and an unexplored mechanism of telomere replication signaling.

PROTEIN-DNA COMPLEX HELPS REPLICATION FORK PROGRESSION THROUGH TELOMERES

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In *S. cerevisiae*, replication forks slow as they move through telomeric DNA, whether the DNA is at the telomere or at an internal site on the chromosome (1). Replication slowing occurs even in wild type cells, but is increased about 10-fold in the absence of the Rrm3 DNA helicase. Likewise, replication forks pause in terminal and internal tracts of *S. pombe* telomeric DNA, and this pausing is exacerbated in the absence of the duplex sequence-specific binding protein Taz1 (2), while TRF1 is needed for normal replication through mouse telomeric DNA (3). These data suggest that the duplex telomere binding proteins promote fork progression, possibly by preventing formation of G-quadruplex (G4) structures on the lagging strand template. We are monitoring the impact of telomere structural proteins, DNA helicases, G4 stabilizing drugs, and direction of replication through *S. cerevisiae* telomeric DNA. We avoid the complexities of telomere length effects caused by the absence of telomere proteins by following replication through an ~800 bp internal stretch of telomeric DNA. We use chromatin immuno-precipitation to determine levels of both DNA polymerase occupancy, a measure of replication pausing, and phosphorylated gamma-H2A, a measure of DNA damage at the telomeric tract in different strains and conditions (4). Telomere proteins are depleted either by gene deletion for non-essential genes or by a degron for essential genes. When the telomeric tract is replicated in the same direction as at the telomere (i.e., G-strand as template for lagging strand synthesis), loss of Rrm3, Rap1, and Cdc13 increase replication pausing and DNA damage within the telomeric tract but not at control sequences, while loss of Rif1 or Yku80 does not. As in mammalian cells, fork pausing is strongest as forks entered the telomeric tract. We are currently examining replication in *pif1-m2 and rap1-t* strains (*rap1-t* lacks the COOH end of Rap1 so does not recruit Rif1, Rif2, or Sir proteins) and determining the impact of PhenDC3, a G4 stabilizing drug on replication and DNA damage. The effects of G4 stabilization, Pif1 (a G4 unwinding helicase), and orientation of the telomeric tract relative to the nearest replication origin should help determine if pausing is due to formation of G4 DNA.

References
1. Ivessa et al., 2002. Genes Dev, 2003. Mol Cell
2. Miller, Rog, and Cooper, 2006. Nature
3. Sfeir et al. 2009. Cell
4. Azvolinsky et al. 2009. Mol Cell

AKTIP (FT1), A TELOMERIC PROTEIN THAT INTERACTS WITH LAMIN, IS REQUIRED FOR MOUSE SURVIVAL AND DEVELOPMENT

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We have identified a new telomeric accessory protein named AKTIP, Ft1 in mouse, an ubiquitin E2 variant enzyme, which interacts with TRF1 and TRF2 and immunoprecipitates telomeric DNA. Loss of Ft1 results in fragile telomeres and sister telomere associations. In doubly depleted TRF1/Ft1 cells, the TRF1-induced fragile telomere phenotype is epistatic to that of Ft1, suggesting that TRF1 and Ft1 are involved in a common molecular pathway, likely telomere replication. In line with this hypothesis, we observed that AKTIP interacts with the DNA replication factors PCNA and RPA70, and, in AKTIP depleted cells, telomeric replication is impaired. A further feature of AKTIP that we have more recently investigated is its interaction with lamins. LaminA/C and lamin B1 were identified by mass spectrometry analysis in AKTIP-enriched protein extracts. AKTIP co-localizes with lamin B1 at the nuclear lamina and co-immunoprecipitates lamin A/C and lamin B1. The expression of the premature aging-related mutated lamin A, named progerin, results in AKTIP delocalization from the nuclear rim. Interestingly, AKTIP depletion causes the selective reduction of lamin A, but not of lamin B1 and lamin C. Fifteen days upon AKTIP depletion, primary cells show distorted nuclei and senescence-associated markers, which recalls the phenotype of progeroid cells. In vivo, the depletion of the Ft1 causes premature death and severe abnormalities, including the absence of subcutaneous fat, skeletal and muscle alterations and male sterility. Altogether, our results suggest that the telomeric protein AKTIP/Ft1 plays a crucial role in vitro and in vivo, which intercepts that of lamins.

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YEAST TELOMERE PROTEIN RIF1: ROLES IN DNA REPLICATION AND REPAIR

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The yeast Rif1 (Rap1-interacting factor 1) protein is concentrated at telomeres where it participates, together with the duplex telomere repeat binding protein Rap1 and a second Rap1-interacting factor, Rif2, in the formation of a molecular Velcro-like structure that contributes to the telomere cap and to telomerase regulation. Rif1 protein is highly conserved throughout evolution, yet its mammalian homologues do not appear to localize at native telomeres, but instead function both in DNA repair and temporal control of the chromosomal DNA replication program. However, recent studies, from our labs and others, indicate that yeast Rif1 also plays important roles in DNA repair and replication control at non-telomeric sites. We will discuss our recent genetic, biochemical and structural studies that address molecular mechanisms underlying these non-telomeric functions of yeast Rif1, as well as their relationship to Rif1's role at telomeres.

SPINDLE ASSEMBLY CHECKPOINT PROTEIN SGO2 REGULATES SILENCED CHROMATIN FORMATION AND DNA REPLICATION TIMING AT SUBTELOMERE.

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Telomere is essential for genome inheritance. Recent studies have shown that subtelomere located adjacent to telomere is also important for various chromosomal events, such as heterochromatin formation and replication timing control. However, the molecular mechanisms underlying those events are not fully understood. Here we show the unexpected roles of the shugoshin protein at subtelomere. Shugoshin plays critical roles in chromosome segregation in eukaryotes. Sgo2, one of the shugoshin proteins in the fission yeast *Schizosaccharomyces pombe*, is recruited to centromeres in mitosis and contributes to spindle assembly checkpoint (SAC) activation and kinetochore-microtubule attachment for precise chromosome segregation. Interestingly, Sgo2 is localized at the vicinity of telomeres during interphase of the cell cycle; however, the roles of Sgo2 near telomeres remain unknown.
Our ChIP-chip analyses revealed that the Sgo2 localization spans more than 100 kb at subtelomere region. We found that phosphorylation of histone H2A by Bub1 kinase is required for the localization of Sgo2 at subtelomeres. We also found that Sgo2 is phosphorylated by Cdc2 kinase and that the subtelomeric localization of Sgo2 is regulated by its phosphorylation states. Interestingly, Sgo2 located at subtelomeres is critical for the formation of a highly condensed chromatin structure called "knob" and regulates gene silencing. Furthermore, DNA replication at subtelomeres was prematurely accelerated in the presence of HU in Sgo2-deleted cells, indicating that Sgo2 controls replication timing of the late origins at subtelomeres. Our analyses suggested that Sgo2 limits loading of a replication factor Sld3 onto subtelomeric late origins. Our results demonstrate that the functions of the subtelomere region in interphase are regulated by a multi-functional protein Sgo2 relocated from centromeres after mitosis.

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PATHWAYS THAT HELP DNA POLYMERASES α, δ AND ε COORDINATE CHROMOSOME REPLICATION

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The three major eukaryotic DNA polymerases coordinate their activities to replicate the linear chromosomes. Pol α and Pol δ replicate the lagging-strand and Pol α and Pol ε the leading-strand. To identify factors affecting coordination of DNA replication, we have performed genome-wide Quantitative Fitness Analyses (QFA) of budding yeast cells containing defective polymerases. We combined temperature sensitive mutations affecting the three replicative polymerases, Pol α, Pol δ and Pol ε with genome-wide collections of null and reduced function mutations. We identify large numbers of suppressor and enhancer genetic interactions that inform about the roles that specific proteins play in Pol α, Pol δ and Pol ε function. For example, our genetic interaction data are consistent with biochemical data showing that Pol ε is more dependent on the MCM/GINS helicase complex than either Pol α or Pol δ. We investigated the interplay between DNA replication and telomere protection by analysing the fitness profiles of mutants in DNA polymerase, telomere-defective strains and in the presence of hydroxyurea, the S-phase poison. Interestingly, among many findings, we find that Rif1, a telomere binding protein, and recently reported to be a DNA replication inhibitor, affects the fitness of Pol ε defective strains. This suggests that Rif1 affects leading-strand. To encourage others to engage with the large volumes of data we have generated two novel, interactive visualization tools, DIXY and Profilyzer. Our genome-wide screen datasets are a unique and powerful resource to help understand how leading- and lagging-strand replication is coordinated.

TELOMERIC INTEGRATION AND EXCISION OF HUMAN HERPESVIRUS-6: EVIDENCE OF CI-HHV-6 LOSS FROM TELOMERE IN PRIMARY EFFUSION LYMPHOMA

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The human herpesviruses 6A and 6B (HHV-6A and B) are closely related double-stranded DNA viruses ~162kb in length, terminated by 8kb direct repeats that each contain two regions homologous to human telomere repeats. About 1% of the population inherit a copy of the viral genome integrated into a single telomere (CI-HHV-6). There is increasing evidence that telomeric integration is a form of latency for HHV-6 but the processes of integration, excision and viral reactivation are poorly understood. To address this we have sequenced the entire viral genome from 20 unrelated individuals and shown that two of three donors from a small isolated population in Orkney carry the same viral sequence, presumably inherited from a common ancestor. The remaining 18 individuals carry different viruses indicating independent integration events. All the sequenced viruses contain a full set of ORFs and appear intact, consistent with viral latency not an evolutionary dead end.

Previously we showed that telomeres carrying a CI-HHV-6 can be unstable, often shorter than other telomeres and prone to truncations at virus encoded (TTAGGG)n repeats. In cell lines, the HHV-6 genome can be released from the telomere as a circular molecule, possibly via t-loop formation and excision. However, the significance of viral release is unclear. Here we describe a female patient diagnosed with primary effusion lymphoma (PEL), a B-cell malignancy usually arising in immunocompromised, HIV infected individuals following infection with human herpesvirus-8 (HHV-8). However, the patient was not immune-compromised and she and her two unaffected brothers were all carriers of CI-HHV-6A in a 19q telomere. The HHV-6A genome was absent from the woman's tumour but heterozygosity analysis showed that both copies of chromosome 19 were retained. The specific loss of the CI-HHV-6A from tumour cells is consistent with escape from the telomere via t- loop formation and excision and may have contributed to the pathogenesis of the lymphoma. Using cell lines carrying CI-HHV-6A as a model, we have investigated the relationship between replication dependent telomere erosion and viral excision. This data will be presented.

MUTATIONS IN THE POLY(A)-SPECIFIC RIBONUCLEASE (*PARN*) GENE CAUSE TERC DEFICIENCY IN PATIENTS WITH DYSKERATOSIS CONGENITA

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Mutations in 10 genes associated with telomere biology have been described in dyskeratosis congenita (DC) and related short telomere syndromes, and account for 60-70% of cases, with the remainder genetically uncharacterized. We report compound heterozygous loss-of-function mutations in the poly(A)-specific ribonuclease (*PARN*) gene in 2 families with DC. Probands manifested classic stigmata of DC, bone marrow failure, and very short peripheral blood cell telomere length. The *PARN* lesions were pathogenic based on inheritance pattern, the nature of the genetic defects (e.g. gene deletion; diminished accumulation of transcripts), and *in silico* predictions and biochemical studies of missense variants. PARN has no known role in telomere biology. Based on its m7G-cap recognition and poly(A) deadenylase functions, PARN's primary role is considered to be in regulating mRNA metabolism. Recent studies implicate PARN in snoRNA processing via the deadenylation of oligo-adenylated intermediates. We hypothesized that PARN plays a similar role in the biogenesis of the telomerase RNA component (*TERC*), based on its shared 3' H/ACA domain architecture. In support of this possibility, we find diminished steady-state levels of *TERC* in somatic cells and reprogrammed cells from patients with PARN mutations. In addition, PARN depletion by RNA interference in immortalized human cells results in decreased levels of TERC. In *PARN* mutant cells, we find an increase in oligo-adenylated forms of *TERC*. The diminished steady-state levels and the increased oligo-adenylated forms of *TERC* are normalized by ectopic expression of PARN. We propose that PARN functions in *TERC* biogenesis via deadenylation of oligo-adenylated nascent transcripts, which promotes 3' end maturation. We speculate that this role of PARN is independent of its role in mRNA metabolism; or alternatively, based on the phenotype of patients with *PARN* mutations, that a major cellular role of PARN is in the maturation of *TERC* and other H/ACA RNAs.

POLY(A) SPECIFIC RIBO NUCLEASE DEFICIENCY IMPACT TELOMERE MAINTENANCE CAUSING DYSKERATOSIS CONGENITA

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The clinically and genetically heterogeneous premature aging syndrome dyskeratosis congenita (DC) is characterised by loss of cells in tissues such as the bone marrow that have a rapid turnover. DC can be caused by mutations in genes associated with telomerase function or telomere integrity, however many patients remain genetically uncharacterised. In an attempt to further elucidate the genetic basis of this disease we have performed exome sequencing and have identified biallelic mutations in the poly(A)-specific ribonuclease (PARN) gene in four individuals from three unrelated families. These mutations affect key domains within PARN and are predicted to impact on function. PARN is a deadenylase involved in global mRNA surveillance and therefore regulates the turnover and stability of a large number of transcripts, as well as playing a key role in nonsense mediated mRNA decay. In this study we have shown that PARN also functions in telomere maintenance, as individuals with biallelic PARN mutations and cells that are depleted of PARN both have reduced RNA levels for several key genes associated with telomere maintenance (TERC, DKC1, RTEL1 and TERF1). MMqPCR and flow-FISH analysis also revealed critically short telomeres in these cases. Studies on patient lyphoblastoid cells show reduced deadenylation activity, an abnormal DNA damage response, and G2/M cell cycle arrest upon UV damage. In summary, our results demonstrate that biallelic mutations in PARN impact on telomere maintenance and the DNA damage response, and are disease-causing in a subset of patients with severe DC.

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TELOMERASE RNA PROCESSING AND QUALITY CONTROL

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Telomerase is a key enzyme that maintains and replenishes telomeric DNA by using part of an RNA subunit as a template for reverse transcription. Previously we have identified telomerase RNA (TER1) from fission yeast and demonstrated that its biogenesis pathway involves sequential binding of Sm and Lsm proteins and the first transesterification reaction of a splicing reaction. Precursor and spliced forms of TER1 RNA fail to support telomere maintenance and accumulate to much lower levels than the mature form even when maturation is blocked. Characterization of stability and processing of these isoforms revealed a quality control pathway that selectively destabilizes inactive forms of the RNA. A series of deletion mutants identified an RNA motif that is critical for destabilizing the precursor and spliced forms of TER1 RNA and lead to the identification of the factors that mediate degradation. Examination of telomerase RNA processing in other fungi and mammals revealed intriguing similarities as well as differences in how mature forms are produced and inactive variants are degraded.

THE NONCANONICAL TELOMERASE RNA TER2 IS A REGULATORY SWITCH THAT PROMOTES GENOME INTEGRITY AND REPRODUCTIVE FITNESS IN ARABIDOPSIS

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Telomerase is a highly regulated reverse transcriptase composed of a catalytic subunit, TERT, and an RNA template, TER. *Arabidopsis thaliana* is unusual as it harbors three TER isoforms: TER1, a canonical TER required for telomere maintenance; TER2, a novel negative regulator of telomerase induced in response to double-strand breaks (DSBs); and TER2s, a processed form of TER2 with unknown function. Here we further investigate the role of TER2 in promoting genome integrity. Data from the 1,001 Arabidopsis genomes project revealed two versions of the TER2 gene amongst *A. thaliana* accessions. The most abundant class (784/853) (e.g. Col-0) contains a transposable element (TE), that bisects two conserved regions in TER1 and TER2. A less abundant class (69/853) (e.g. Ler-0) lacks the TE and encodes a smaller RNA transcript termed TER2Δ. We found that DSBs do not induce TER2Δ or alter telomerase activity. Unlike TER2, which is a highly unstable transcript ($t_{1/2}$= 13min), TER2Δ is a stable RNA ($t_{1/2}$=240min). These observations indicate that exaptation of a TE within TER2 has generated a sensitive regulatory switch for telomerase that modulates enzyme activity in response to genotoxic stress. Unexpectedly, we found that TER2 peaks during early floral development and further that *ter2* mutants exhibit decreased pollen viability, reduced seed set, and seed abortion, consistent with a role for TER2 in reproductive development. In addition, we discovered that TER2 contributes to telomere maintenance. Plants lacking TER2 have extended G-overhangs. Moreover, telomere shortening is dramatically accelerated in plants doubly deficient in TER2 and the telomerase processivity factor POT1a, indicating that TER2 functions in a telomerase-independent pathway to promote telomere integrity. Altogether, our findings define TER2 as a novel multifunctional regulatory lncRNA that promotes genome stability and reproductive fitness.

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BUDDING YEAST TELOMERASE CONTAINS A SINGLE TLC1 MOLECULE

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Telomerase assembly and maturation are essential steps for RNP functionality at telomeres. Yet, although several components of the enzyme have been identified in different eukaryotic species, the stoichiometry of the subunits in the complex is still debated. Human telomerase may be dimeric as assessed by cryo-EM and co-IP experiments, but in ciliates, activity of the enzyme does not require multiple copies of the RT subunit. However, this question by and large is not resolved for *Saccharomyces cerevisiae* telomerase. Biochemical pull-down experiments suggested a dimeric RNP, while results from IP and *in vivo* telomere extension experiments are not compatible with that hypothesis.
We set to study budding yeast telomerase and its core components Est2 and Tlc1 RNA *in vivo*, using tagged but functional versions of the subunits. In diploid heterozygous cells co-expressing a wt and a tagged Tlc1-MS2 allele, no wt Tlc1 RNA could be detected through MS2-ProA-IgG IP pellets. Similarly, co-IP experiments with co-expressed ProA-Est2/Myc-Est2 proteins failed. In order to get more direct insight into the issue, we decided to assess the stoichiometry of the Tlc1 RNA *in vivo* by quantitative multi-colour FISH experiments. For this purpose, we used differentially tagged Tlc1 alleles (with either or both 10xMS2 and 24xPP7 tags) in diploid strains and used tag-specific FISH probes with different fluorophores. All constructs are able to maintain telomeres and are expressed at a similar level, as measured by foci number in our assay system. We first used colocalisation assays to determine the frequency of potential Tlc1 dimers, as expected if telomerase contains two Tlc1 molecules. However, the obtained frequencies were inconsistent with two molecules/RNP, but fit rather well a single molecule/RNP modelisation. In order to measure the absolute number of Tlc1 RNA in each of the foci, we used the characterised PP7-tagged Mdn1 mRNA as internal control for one molecule and acquired absolute fluorescence intensities for PP7-tagged Tlc1 RNA in the same cells. The results show that the signal intensity detected in these Tlc1 foci represents a single RNA molecule. These data demonstrate that the telomerase RNP contains only one Tlc1 RNA, but could not exclude the possibility that there is a functional dimerization of telomerase on its substrate. However, we also determined that telomerase can be active even in cells arrested in G1, a window of the cell cycle during which no telomere elongation occurs *in vivo* and no telomere replication clusters (T-RECs) are detected. Altogether, these data strongly suggest that *Saccharomyces cerevisiae* telomerase contains only one Tlc1 molecule.

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YEAST TELOMERASE RNP: EXCEPTIONAL FLEXIBILITY AS WELL AS NEW ESSENTIAL STRUCTURAL FEATURES OF ITS LONG NONCODING RNA

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Telomerase long noncoding RNA subunit is far more than just a template. First, it has additional active roles in catalytic mechanism, including the two essential structures we have recently discovered in S. cerevisiae TLC1: the Area of Required Connectivity (ARC) and the Second Essential Est1-arm Domain. The ARC connects the four conserved secondary structure elements in the core to permit catalytic activity. Circular permutation analysis that led to discovery of the ARC also showed that the ends of the RNA could be moved to other locations within the core with retention of function in vitro and in vivo. Thus, functional flexibility exists in certain parts of the TLC1 core. These locations are also physically flexible junctions, based on our recent assaying of each nucleotide's chemical reactivity by SHAPE. In addition to having critical roles in catalysis, telomerase RNA is also required to form the RNP enzyme by providing a scaffold for the protein subunits. We have determined that the TLC1 lncRNA tolerates the following perturbations without causing senescence: (1) relocation of the position for each of the enzyme's holoenzyme-specific RNA-binding proteins, (2) deletion of the bulky, rapidly evolving portions of its three long arms, and (3) stiffening of the arms by conversion to uninterrupted double-stranded RNA by deletion of their loops and bulges. With respect to #1 above, we have recently found that the essential Est1 subunit can even be artificially tethered to TLC1 through a heterologous RNA-protein interaction module. This analysis also led to the discovery that there is a Second Essential Est1-arm Domain (SEED) in the highly conserved 108-nt region around where Est1 binds. The SEED acts Est1-independently after telomerase recruitment to the telomere, and can even function in trans. Thus, the SEED has a function beyond scaffolding and may be required for establishing telomere extendibility or promoting telomerase RNP holoenzyme activity. As for perturbations #2 and #3 in TLC1 RNA listed above, these actually improve in-vitro telomerase activity compared to wild type, presumably due to an increased fraction of the RNA molecules forming a natively folded catalytic core. However, both the truncated and stiffened-arm RNAs have reduced length and structure of the long arms that perform the flexible scaffolding role. To create a TLC1 RNA with wild-type length and structure that — unlike TLC1 — provides robust in vitro activity, we mutated A-U and G-U residues in the long arms to G-C in an effort to promote favorability of native-state folding. In vivo, these "determined-arm" (DA-TLC1) alleles maintain telomeres that are nearly wild-type length and, in vitro, reconstitute robust telomerase activity. Thus, synthetically designing telomerase RNAs can improve the free-energy landscape to promote the native structure in vitro. DA-TLC1 will facilitate studying structure and function of telomerase and this approach may be useful for examining other RNAs as well.

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INVOLVMENT OF ATM IN THE RECRUITMENT OF HUMAN TELOMERASE TO TELOMERES

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Human telomerase is known to be present at telomeres in S phase[1] and to elongate telomeres following bulk telomere replication[2], but the mechanistic basis of cell cycle regulation of telomerase recruitment to telomeres in human cells is unknown. The budding yeast and fission yeast homologues of the DNA damage-response protein ATM are critical for telomerase recruitment[3,4], but the involvement of ATM in telomerase recruitment to telomeres has not been demonstrated in human cells. The telomere-binding protein TRF1 has been shown to be phosphorylated at serine 367 by ATM, leading to telomere elongation[5]. We therefore directly tested the hypothesis that ATM mediates telomerase recruitment to the telomere via phosphorylation of TRF1. Using hTR/telomere fluorescence in situ hybridisation, we demonstrated that ATM depletion or kinase inhibition resulted in decreased telomerase recruitment across the cell cycle, while TRF1 knockdown abrogated the removal of telomerase from telomeres in G2 phase. Depletion of TRF1 partially rescued the phenotype of diminished telomerase recruitment resulting from ATM knockdown alone, demonstrating that these two proteins act in the same pathway. A phosphomimetic mutation of TRF1 S367 showed reduced telomere localisation across the cell cycle, and resulted in increased telomerase at the telomere in G2 phase, while TRF1 carrying a mutation preventing phosphorylation at this residue (S367A) was able to localise to telomeres and block telomerase access in S phase. The data support a model in which ATM phosphorylation of TRF1 at S367 is necessary for depletion of TRF1 from telomeres during S phase, and this in turn allows telomerase to access the telomere at the appropriate phase of the cell cycle to counteract replication-dependent telomere shortening. Furthermore, these data suggest that human telomeres trigger a DNA damage-dependent response during S phase that is important for their maintenance by telomerase and telomere length homeostasis.

References

1. Tomlinson, R. L.; Ziegler, T. D.; Supakorndej, T.; Terns, R. M.; Terns, M. P. Mol. Biol. Cell 2006, 17, 955-965.
2. Zhao, Y.; Sfeir, A. J.; Zou, Y.; Buseman, C. M.; Chow, T. T.; Shay, J. W.; Wright, W. E. Cell 2009, 138, 463-475.
3. Moser, B. A.; Subramanian, L.; Khair, L.; Chang, Y. T.; Nakamura, T. M. PLoS Genet. 2009, 5, e1000622.
4. Goudsouzian, L. K.; Tuzon, C. T.; Zakian, V. A. Mol. Cell 2006, 24, 603-610.
5. McKerlie, M. A.; Lin, S.; Zhu, X. D. Nucleic Acids Res. 2012, 40, 3975-3989.

SSU72 PHOSPHATASE REGULATES TELOMERE LENGTH IN *S. POMBE*

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Telomeres are protein-DNA complexes that reside at the ends of eukaryotic chromosomes. Their main functions are to prevent loss of genetic information and inhibit DNA repair at chromosome termini. Maintaining telomeres at a fairly constant length is a critical process. If telomeres become critically short, the DNA damage machinery is activated, leading to cell cycle arrest and end-joining DNA repair. In contrast, if they become too long, they become unstable and recombinogenic, leading to sudden telomere loss events.
Making use of the S. pombe whole genome gene deletion library, we identified the phosphatase Ssu72 as a regulator of telomere length. Both *ssu72*Δ and *ssu72-C13S* (phosphatase-dead mutant) exhibit 3-5x longer telomeres than WT cells. Ssu72 is a highly conserved phosphatase previously identified as a RNA polymerase II C-terminal domain phosphatase from yeast to human. Other roles and targets have been described for this phosphatase, but none of the previous studies described telomere homeostasis defects.
Telomere length in *ssu72Δ* mutants is both *trt1*+ and *rad3*+-dependent, consistent with a role of a negative regulator of telomerase. Interestingly, *ssu72Δ* mutants show defects in Stn1 recruitment, part of the (C)ST ssDNA-binding complex that promotes lagging strand synthesis and telomerase inhibition. Therefore, we hypothesize that *ssu72Δ* mutants have longer telomeres due to a defect in lagging strand fill-in reaction. Accordingly, *ssu72Δ* mutants exhibit longer G-rich overhangs and their telomere defects are epistatic both with of *stn1* and *pol1* (Polα). Importantly, we show that Polα overexpression rescues the telomere length defect of *ssu72Δ*. Thus, our preliminary data strongly supports an unexpected role for Ssu72 in controlling lagging-strand synthesis, and therefore, reducing telomere ssDNA and inhibiting telomerase recruitment. We are currently identifying possible posttranscriptional modifications regulated by Ssu72 in shelterin components.

ATM KINASE IS REQUIRED FOR TELOMERE ELONGATION

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The ATM and ATR kinase-dependent DNA damage response pathways are activated in primary human cells when telomeres are critically short [1]. Induction of telomere dysfunction through the loss of shelterin components also activates ATM or ATR-dependent signaling [2]. While there is a sophisticated understanding of the role of ATM and ATR in signaling telomere dysfunction, less is known about the role of these kinases in regulating telomere elongation. The ATM kinase plays a clear role in regulating telomere length maintenance in yeast [3], yet a role in humans and mice is less clear in part due to infertility of $ATM^{-/-}$ mice.

Given the conserved role of ATM in telomere length regulation in yeast, we wanted to revisit the role of ATM kinase in telomere elongation in mammalian cells. To examine the role of ATM, and other genes, we developed an assay that can rapidly identify effectors of telomere length, even if these regulators are essential genes. This assay was inspired by an assay from yeast [4], and is referred here as ADDIT (Addition of *de novo* initiated telomeres). Briefly, in a stable mouse fibroblast cell line, a single chromosome was modified to contain a unique I-Sce1 restriction site placed adjacent to short telomere 'seed' sequence that can be elongated by telomerase. When I-Sce1 cut is induced *in vivo*, the telomere seed is exposed allowing telomere elongation. We verified telomerase-dependent telomere addition occurs *in vivo* over just one cell cycle.

Using this assay we found that blocking ATM kinase with the specific inhibitor KU33955 or with siRNA, blocks telomere elongation. Inhibition of ATM kinase activity also prevented bulk telomere elongation by telomerase overexpression, assayed on a Southern blot, further supporting the essential role of ATM in telomere length regulation. The ADDIT assay will allow rapid dissection of the ATM pathway of telomere length regulation in addition to identification of new regulators of telomere length. The assay will provide insights into telomere length homeostasis and may identify potential targets for future therapeutics.

1. d'Adda di Fagagna, F., et al., Nature, 2003. 426(6963): p. 194-8.
2. Palm, W. and T. de Lange, Annu Rev Genet, 2008. 42: p. 301-34.
3. Craven, R.J., et al., Genetics, 2002. 161(2): p. 493-507.
4. Diede, S.J. and D.E. Gottschling, Cell, 1999. 99(7): p. 723-33.

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HUMAN RTEL1 IMPACTS TELOMERE LENGTH HOMEOSTASIS BY PROMOTING POT1 BINDING TO TELOMERES

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Telomere maintenance is an important process that protects the cell against genome instability and senescence. Accelerated telomere attrition is a characteristic of premature aging syndromes including Dyskeratosis congenita (DC). Mutations in hRTEL1 were recently associated with a severe form of DC called Hoyeraal-Hreidarsson syndrome (HHS). In addition to very short telomeres, these patients are characterized by telomere loss and increased sister telomere recombination. Here we investigated how hRTEL1 contributes to telomere maintenance in primary as well as telomerase-positive cells. Transient depletion of hRTEL1 resulted in rapid telomere shortening exclusively in the context of telomerase-positive cells with very long telomeres. This rapid shortening was not induced by massive T-loop excision or a defect in telomerase biogenesis and recruitment. Instead, we found that depletion of RTEL1 led to a decrease in the amount of POT1 at telomeres, together with a decrease in single strand telomeric G-rich repeat content and partial telomere uncapping. Interestingly, the overexpression of POT1 rescued the telomere length phenotype but not the loss of the single strand telomeric G-rich repeats, indicating that the latter is the primary defect in RTEL1-depeleted cells. Consistent with the suggested function of the RTEL1 protein in resolving secondary structures accumulating at single strand repeats, we propose that human RTEL1 intervenes in the homeostasis of very long telomeres by stabilizing the G-rich single stranded DNA thereby promoting POT1 binding.

THE ROLE OF RIBONUCLEOTIDE REDUCTASE (RNR) IN TELOMERE LENGTH MAINTENANCE

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Activation of the DNA Damage Response (DDR) in S. cerevisiae affects several processes in the cell, including the inhibition of firing by late origins-of-replication, the prevention of sister chromatid separation in anaphase and the transcriptional induction of the ribonucleotide reductase (RNR) complex, which catalyzes the rate-limiting step in dNTP synthesis. The RNR complex of *S. cerevisiae* has an α2ββ' architecture: it is composed of a homodimer of the large Rnr1 subunit, and a heterodimer of two small subunits (Rnr2 and Rnr4). A second large subunit is encoded by the *RNR3* gene, which is normally expressed at very low levels. Yeast RNR activity is upregulated when cells enter S-phase as well as in response to DNA damage. Activation of the DDR induces both the activity and transcription of the different RNR subunits: the protein kinase Dun1 inactivates the transcriptional repressor Crt1, causing overexpression of *RNR2*, *RNR3* and *RNR4*. Dun1 also phosphorylates the Rnr1 inhibitor Sml1, causing its ubiquitin-dependent degradation and modifies Dif1, causing the cytoplasmatic localization of the small subunits. The net result of these activities is an increase in the levels of available dNTPs in the cell.
We have found that yeast *rnr1* mutants (which are alive due to *RNR3* leakage) have extremely short telomeres, which cannot be elongated even by tethering of telomerase subunits to the telomeres. Mutations in *RNR1* also prevent elongation of telomeres in mutants with elongated telomere phenotype. We dissect the roles of dNTP levels, as well as Dun1, Sml1 and the different RNR subunits in telomere length maintenance.

DYNAMIC TELOMERASE INTERACTION WITH TELOMERES IN FISSION YEAST

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Fission yeast telomerase is recruited to the telomere protein Ccq1, a component of the Pot1 telomere-binding complex, *via* the telomerase subunit Est1. However, we recently demonstrated that this interaction is not sufficient to engage and fully activate telomerase at telomeres (Armstrong *et al.* 2014). After recruitment, association of the telomerase catalytic subunit Trt1 with Tpz1, an ortholog of the human Pot1 binding protein TPP1, is crucial for telomerase activation. The OB fold domain of Tpz1 controls telomerase activity/processivity after association of telomerase with the Pot1 complex. Nevertheless, the molecular link between telomerase and the telomere remains elusive. We have identified Trt1 binding domains in both Tpz1 and Ccq1, and found temporal interactions between telomeric proteins and telomerase. We will discuss how telomerase is engaged at the telomere to promote processivity.

Armstrong *et al.* Curr Biol. 2014 Sep 8;24(17):2006-11

DYNAMICS OF TELOMERE OVERHANGS IN YEAST

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Single-stranded telomeric overhangs are required to provide a substrate for telomerase action and to assemble a functional capping complex at telomere ends. In order to investigate telomere overhang dynamics more precisely in yeast we have developed a PCR-based assay for individual telomeres which measures the length of the G-rich overhang. Using this assay, we show that the G-strand overhangs in fission yeast are about 25 nucleotide long outside S phase, and that their length doubles in late S-phase. Consistent with previous results, mutations in the Ku complex lead to significant increases in overhang length, to about 70 nucleotides.

Using a system that we have developed to induce telomere shortening in wild-type fission yeast cells we find that overhang length increases to about 45 nucleotides at telomeres which are about half the normal length. The increase in overhang length is dependent on the presence of functional telomerase, suggesting that the extra single stranded DNA represents telomere elongation intermediates. The results indicate that there is no increased exonucleolytic processing at shorter telomeres in fission yeast.

Consistent with our finding that short telomeres in fission yeast are not transiently deprotected, we observe increased overhangs in the absence of the Rad3/ATR kinase, which marks short telomeres for elongation by telomerase and which we establish is required for telomere protection independently of Tel1. We propose that, at short telomeres in fission yeast, Rad3/ATR promotes telomerase activity though Ccq1 phosphorylation, as previously shown, and not by promoting overhang formation, which it instead suppresses.

The effect of Rad3/ATR on telomere overhangs in fission yeast thus differs form that of Tel1/ATM in budding yeast, which instead promotes resection. We are currently addressing whether Tel1 at budding yeast telomeres might lead to increased telomerase recruitment at short telomeres by modulating overhang length as a function of overall telomere length.

THE TELOMERASE ASSOCIATED CDC48-NPL4-UFD1 COMPLEX REGULATES EST1 ABUNDANCE AND TELOMERE LENGTH

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We isolated S. cerevisiae telomerase from both G1 arrested (when telomerase is inactive) and G2/M (when telomerase is active) cells and identified associated proteins by mass spectrometry. In addition to the ten known protein subunits of yeast telomerase (Est1, Est2, Est3, and the seven subunits of the TLC1-binding Sm protein complex -- Smd1, Smd2, Smd3, Smb1, Sme1, Smx2 and Smx3), over 100 proteins had high confidence, DNAse-resistant association with telomerase. Although there are multiple interesting telomerase-associated proteins in our complex, we focus here on the three subunits of the essential Cdc48-Npl4-Ufd1 complex. This complex is found from yeasts to humans where it removes ubiquinated proteins from multi-protein complexes and targets them for degradation by the proteosome. All three subunits were among the most prominent telomerase interactions in both G1 and G2/M phase cells. Because the Cdc48 complex targets proteins for degradation, we examined the abundance of six telomere proteins in a ts cdc48 strain. Of the tested proteins, Est1, a telomerase component that is critical for both recruitment and activation of telomerase, was the only one affected: Est1 levels were ~40-fold higher in cells with temperature sensitive alleles in any of the three Cdc48 complex subunits. Given that Est1 activates telomerase, and its over-expression in wild type cells (a finding we confirm in our strain) results in telomere lengthening, we were surprised that telomeres were shorter in cdc48 mutant cells. Est1 is the only telomerase subunit whose abundance is cell cycle regulated. Because Est1 is required to recruit Est3 to telomeres, its cell cycle regulated abundance guarantees that telomerase is active only late in the cell cycle. This cell cycle regulated abundance is also lost in cdc48 cells. We find that Est1 is mono-ubiquinated in both wild type and cdc48 cells, but the fraction of Ub-Est1 is higher in both G1 and G2/M cdc48 cells than in wild type cells. Combined with the telomere length data, this finding suggests that Ub-Est1 is not active or less active than unmodified Est1. Thus, the Cdc48 complex regulates telomerase and hence telomere length by controlling the level and activity of Est1. These, data are explained by a model in which Cdc48-catalyzed removal of Est1 from the telomerase holoenzyme restricts telomerase action and can do so both in G1 phase on prematurely assembled telomerase and at the end of the cell cycle after telomerase acts. This role is similar to the role of the Cdc48 complex in regulating the replisome where it targets Ub-Mcm7 for degradation in both G1 and post S phase cells in budding yeast and vertebrates (Maric et al., 2014 Science; Moreno et al., 2014 Science).

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CDK1 COORDINATES TELOMERE REPLICATION BY REGULATING THE TEMPORAL RECRUITMENT OF TELOMERASE AND CST COMPLEX

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In budding yeast, Cdk1 coordinates the cell cycle-dependent elongation of telomere by telomerase. Cdk1 phosphorylates the telomere-specific ssDNA binding protein, Cdc13, which promotes the recruitment of telomerase to the telomere and thereby, telomere elongation. Cdc13 is also an integral part of the CST (Cdc13-Stn1-Ten1) complex, which provides the protective cap at the telomere and inhibits telomere elongation by telomerase. Therefore, the telomere length homeostasis is balanced between these two telomerase-extendable and CST-unextendable states. However, the cellular signaling that regulates the switch between these two states is largely unknown. Here we show that Cdk1 phosphorylates Stn1 sequentially to that of Cdc13. This Cdk1-dependant phosphorylation of Stn1 is essential for telomere maintenance in vivo, CST stability at the telomeres and the inhibition of telomerase at the telomeres. Additionally, our recent results suggest that the sequential phosphorylation of Cdc13 and Stn1 by Cdk1 is facilitated by the different cyclins that confer substrate specificity to Cdk1. By controlling the timing of Cdc13 and Stn1 phosphorylation during cell cycle progression, Cdk1 coordinates the recruitment of telomerase holoenzyme and CST complex to the telomeres respectively and hence the switch between the telomerase-extendable and CST-unextendable states of the telomeres in budding yeast. With increasing evidence of human CST complex being involved in telomere length maintenance, the results from my study will provide insights into telomere homeostasis in humans and its disruption in cancers. Considering that telomerase is up-regulated in about 90% of human cancers and telomerase-mediated telomere elongation is significantly deregulated in cancers, insights from our study can be used to target the telomere replication machinery for therapeutic intervention in cancer.

A NOVEL FUNCTION OF MLH1 IN TELOMERE MAINTENANCE

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The DNA mismatch repair (MMR) system, consisting of a group of proteins including MLH1, MLH3, MSH2-6, PSM1 and PSM2, corrects and repairs nucleotide errors that are mistakenly incorporated into genome during replication. Defects in MMR increase mutation rate and associate with many types of cancer. In addition to correcting mismatches, MMR proteins also play an important role in DNA damage response and recombination. Telomere is the special DNA-protein structure at the chromosome end that protects genome stability. Faithful duplication of telomeric DNA is critical for maintaining telomere stability and preventing the development of cancer and premature aging syndromes. The repetitive nature of telomeric DNA sequence makes it vulnerable to mismatch incorporation during replication. However, the process of MMR at telomeres is unknown and the function of MMR proteins in telomere maintenance is unclear. In this study, we investigate the function of MLH1, mutations in which lead to more than half of the colorectal cancer cases, in telomere maintenance. Our results show that human MLH1 localizes at telomeres and interacts with shelterin proteins and telomerase. Suppression of MLH1 leads to a moderate elongation of telomeres in telomerase-expressing cancer cells with no apparent telomere dysfunction phenotypes including end-to-end fusions, fragile telomeres, T-SCE, indicating that MLH1 has a nominal role in telomere capping. Interestingly, we observe that MLH1 is correlated to the stability of interstitial telomeric sequences and telomerase activity may be implicated to this correlation. Our findings uncover the novel function of MLH1 in controlling telomerase activity and suggest that MMR proteins may have a unique role in protecting the stability of telomeric sequences.

TIN2 MEDIATES TELOMERASE RECRUITMENT TO TELOMERES

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The shelterin complex protects telomeres from being processed by the DNA damage repair machinery, and also performs a second mechanistically distinct role in regulating telomerase access and activity at telomeres. In humans, mutations in shelterin components result in a heritable multi-organ stem cell failure disorder due to the inability to maintain telomere homeostasis, causing human dyskeratosis congenita (DC) and releated disease syndromes. We recapitulated the most common DC-causing mutation in the shelterin component TIN2 by introducing a TIN2-R282H mutation into cultured telomerase-positive human cells via a zinc finger nuclease-mediated knock-in approach. The resulting heterozygous TIN2-R282H mutation does not perturb occupancy of other shelterin components on telomeres, result in activation of telomeric DNA damage signaling or exhibit other characteristics indicative of a telomere deprotection defect. Using two independent systems for analyzing individual telomeres in cells to examine in vivo telomerase function - a variant telomeric repeat incorporation assay and the co-localization of telomerase RNA to telomeres - we have shown that the TIN2-R282H mutation impairs telomerase recruitment, resulting in a reduction of the frequency of telomere extension by telomerase, which perturbs telomere homeostasis and causes progressive telomere shortening. Our observations demonstrate a direct role for TIN2 in mediating telomere length through telomerase, separable from its role in telomere protection.

ANALYSIS OF SPECIFIC ROLES OF TIN2S VS TIN2L IN TELOMERE PROTECTION AND REGULATION

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The shelterin scaffold protein TIN2 has essential roles in telomere protection and telomerase recruitment (Abreu et al 2010) and its mutations cause severe clinical phenotypes (Savage et al 2008, Walne et al 2008). While TIN2 has been shown to exist as two different splice variants (Kaminker et al 2009), whether these isoforms have different roles in telomere maintenance is unknown. We have tested this by developing shRNAs to remove all endogenous TIN2 expression (both TIN2S and TIN2L) while simultaneously overexpressing exogenous TIN2S or TIN2L. First, acute shRNA-induced knockdown of total TIN2 caused large numbers of telomere-damage induced foci (TIFs) in UM-UC-3 or HeLa cells, as well as in primary human T cells (Gazzaniga and Blackburn 2014). Interestingly, these TIFs did not induce apoptosis or gross defects in cell cycle distribution in UM-UC-3 or HeLa cells, similar to findings for TRF2 acute partial knock-down (Cesare et al 2013). Second, when either TIN2 isoform (S or L) was massively overexpressed in these shRNA-treated cells, the exogenous TIN2 prevented TIF formation, but there was no isoform-specific difference in the ability to protect telomeres. Therefore, we generated lines modestly overexpressing each TIN2 isoform, by using GFP-tagged TIN2S or TIN2L, and FACS sorting for low expression. We then expressed the anti-TIN2 shRNAs in these cell lines under conditions that reduced all TIN2 protein expression but left the exogenously expressed GFP-TIN2S or L isoform as the primary source of TIN2 in the cell. Upon anti-TIN2 shRNA treatment, we consistently saw higher numbers of 53BP1-TIFs in those cells overexpressing GFP-TIN2L, compared to the cells expressing GFP-TIN2S at comparable levels. Thus TIN2L is less efficient in mediating optimal telomere protection than TIN2S. In comparison with a different telomere damaging agent - the 47A mutant telomerase RNA template, which causes ATM-dependent telomere damage foci - the shRNA-induced 53BP1-TIFs were relatively deficient in pATM, consistent with the findings of Frescas and de Lange, 2014 for mouse TIN2. In contrast to the effects of anti-TIN2 shRNA, little differential effect of the TIN2 isoform (S or L) was seen on the ability to induce TIFs via the 47A mutant telomerase RNA template. Given TIN2L's known attachment to the nuclear matrix, and the known connection between telomere damage and telomere speed, we examined telomere motions in these cell lines. Preliminary findings indicate that TIN2S expressing cells have more mobile telomeres than those expressing TIN2L. These findings suggest an exciting new mode of interplay between telomere protection components and telomere movement.

DIFFERENTIATING THE ROLES OF THE SHORT AND LONG ISOFORMS OF TIN2 AT TELOMERES

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Dyskeratosis congenita (DC) is a disorder of telomere biology. DC-associated mutations in *TERT* and *hTR* are frequently inherited and result in progressive telomere shortening and disease anticipation over multiple generations. In contrast, mutations in *TINF2*, which encodes the shelterin component TIN2, are typically *de novo* and result in drastic telomere shortening and severe, childhood onset disease in the first generation. Humans express two TIN2 isoforms, a 354 amino acid (aa) short isoform, TIN2S, encoded by exons 1-6, and a 451 aa long isoform, TIN2L, comprised of all of the residues in TIN2S plus 97 additional C-terminal residues. Strikingly, all of DC-associated *TINF2* mutations cluster in exon 6, which is shared by both isoforms (referred to as the DC-cluster). We hypothesize that some of the deleterious effects of the DC-cluster mutations are specifically on TIN2L function. We have, therefore, examined whether TIN2L can be functionally distinguished from TIN2S with respect to telomere maintenance and whether mutation of the DC-cluster has specific effects on TIN2L.

We found TIN2L, in contrast to TIN2S, was predominantly phosphorylated *in vivo*. Mutation of a conserved CK2 consensus site eliminated phosphorylation *in vivo* and the ability of CK2 to phosphorylate TIN2L *in vitro*. Co-immunoprecipitation assays revealed more TRF2 in TINSL immunoprecipitates (IPs) than in TIN2S IPs. The DC-cluster and phosphorylation of TIN2L cooperated to promote this enhanced TRF2-TIN2L interaction. The impact of the DC-cluster and the CK2 phosphorylation site on enhanced TRF2-TIN2L interaction was similarly observed in protein-fragment complementation assays. Conversely, TRF1 was present in greater amounts in TIN2S than TIN2L IPs and mutation of the DC cluster did not impact the enhanced interaction between TIN2S and TRF1. Finally, TPP1 was found to associate equivalently with TIN2L and TIN2S. TIN2L co-fractionated with TIN2S, TRF1, TRF2, and POT1 over a range of molecular masses larger than 670 kDa from HeLa nuclear lysates, whereas TINS also distributed with lower molecular mass fractions. These results suggest variable distribution of TIN2L and TINS in shelterin and that this distribution may be impacted in patients with *TINF2* mutations.

We have attempted to differentially knock down TIN2S and TIN2L using shRNA. Three shRNAs specifically targeted to TIN2S decreased TIN2S protein expression below that of the scrambled control. In contrast, none of three shRNAs targeting TIN2L preferentially decreased TIN2L expression. Interestingly, an shRNA with sequence homology to both isoforms preferentially decreased TIN2S, suggesting that cells may be resistant to shRNA knock down of TIN2L.

THERAPEUTIC INHIBITION OF TRF1 IMPAIRS THE GROWTH OF P53-DEFICIENT K-RASG12V-INDUCED LUNG CANCER BY INDUCTION OF TELOMERIC DNA DAMAGE

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Telomeres are considered anti-cancer targets, as telomere maintenance above a minimum length is necessary for cancer growth. Telomerase abrogation in cancer-prone mouse models, however, only decreased tumor growth after several mouse generations when telomeres reach a critically short length, and this effect was lost upon p53 mutation. Here, address whether induction of telomere uncapping by inhibition of the Trf1 shelterin protein can effectively block cancer growth independently of telomere length. We show that genetic Trf1 ablation impairs the growth of p53-null K-RasG12V-induced lung carcinomas and increases mouse survival independently of telomere length. This is accompanied by induction of telomeric DNA damage, apoptosis, decreased proliferation, and G2-arrest. Downregulation of Trf1 in established p53-deficient K-RasG12V lung cell lines also impairs tumor growth and metastasis in xenograft models. Importantly, long-term whole-body Trf1 deletion in adult mice did not impact on mouse survival and viability. Moreover, inhibition of TRF1 binding to telomeres by small molecules blocks the growth of already established lung carcinomas without affecting mouse survival or tissue function. Thus, induction of acute telomere uncapping emerges as a potential new therapeutic target for lung cancer.

PROGRESS IN CRYO-ELECTRON MICROSCOPY STRUCTURE OF *TETRAHYMENA* TELOMERASE

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Telomerase is an RNA-protein complex that extends the ends of linear chromosomes, and is a highly regulated determinant of cellular aging, stem cell renewal, and tumorigenesis. We use a combination of NMR spectroscopy, X-ray crystallography, and electron microscopy to study the structure and function of *Tetrahymena thermophila* telomerase. We determined the 3D structure of endogenously assembled *Tetrahymena* telomerase holoenzyme at 25 Å resolution using negative stain electron microscopy (EM). Six of the 7 protein subunits and the stem-loop 2 region of TER were localized in the 3D structure by affinity labeling. Fitting with the available high-resolution structures, including RNA structures determined by NMR and a p65-TER complex determined by combining NMR and X-ray crystallography, revealed the general organization of TERT, TER, and p65 in the RNP catalytic core. Among the other holoenzyme proteins, p50 has an unanticipated role as a hub between the RNP catalytic core, p75-p19-p45 subcomplex, and the DNA-binding Teb1. A complete *in vitro* holoenzyme reconstitution correlates activity with structure. This first physical and functional network architecture of a telomerase holoenzyme provided the first view into the structure of the RNP catalytic core and revealed the organization of holoenzyme subunits. We have now obtained a cryoelectron microscopy (cryoEM) structure of *Tetrahymena* telomerase holoenzyme in which TER secondary structures and some protein secondary structure elements are visible. Fitting the available high-resolution domain structures of the protein and RNA subunits into the cryoEM structure reveals new details of their interactions and implication of their functions.

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SINGLE-MOLECULE INVESTIGATION OF THE TELOMERASE REVERSE TRANSCRIPTASE CONTENT OF DNA-BOUND AND ACTIVE HUMAN TELOMERASE COMPLEXES

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Interactions between TERT subunits have been proposed to be essential for forming a functional human telomerase active site. Furthermore, interactions between telomerase and shelterin components TPP1/POT1 are required for telomerase recruitment to the telomere and for activating telomeric repeat synthesis. We sought to determine the architecture and functional impact of assembly of telomerase subunits to higher-order complexes. Using biochemical and single-molecule imaging techniques, we investigated the relationship between TERT subunit content, DNA-binding and telomeric repeat synthesis of telomerase RNP assembled in cells and in vitro. These experiments revealed the existence of surprisingly heterogeneous subpopulations of RNPs with diverse subunit stoichiometry, which correlate to different properties of substrate binding and extension. Furthermore, we developed new strategies for the specific assembly of telomerase with shelterin as purified recombinant complexes to uncover how these interactions influence subunit content and catalytic function. These studies will lead to greater understanding of the determinants, mechanisms and significance of the interactions within telomerase and of telomerase with shelterin required for telomere elongation.

THE HUMAN TELOMERASE 'INSERTION IN FINGERS DOMAIN' CAN MEDIATE ENZYME PROCESSIVITY AND TELOMERASE RECRUITMENT TO TELOMERES IN A TPP1-DEPENDENT MANNER.

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As a major tumor biomarker, telomerase expression in 85% of human cancer confers cellular immortalization by counteracting telomere erosion. Telomerase has the unique ability to processively add long stretches of DNA repeats onto the telomere using its integral RNA template (TR), a property termed “repeat addition processivity” (RAP). Telomerase-specific inhibition is feasible by targeting domains exclusive to hTERT, such as the ‘Insertion in Fingers Domain’ (IFD) located between the motif A and B’ of the reverse transcriptase (RT) region. Based on the *Tribolium castaneum* TERT crystal structure, the IFD is positioned on the periphery of the TERT ring, making it an attractive candidate for the design of specific and accessible telomerase-based therapies. In our current study, we generated 2 hTERT IFD variants that reconstituted reduced levels of DNA synthesis compared to the wild-type (WT) enzyme. Mutant A showed a dramatic decrease in RAP while mutant B displayed a 1.5 fold increase in RAP. Furthermore, similarly to the previously studied loss of function V791Y-hTERT mutant, variant A also showed a significant decrease in the number of colocalizations with telomeres by fluorescence in situ hybridization and reduced binding to the telomere by ChIP, while mutant B displayed a milder phenotype. Interestingly, overexpression of the telomerase recruitment protein hTPP1, along with hPOT1, partially rescued processivity and telomere localization defects of mutant A but not V791Y-hTERT. Our data suggest that these hTERT mutants are defective in recruitment due to altered interaction with hTPP1. To assess the ability of these variants to confer cellular survival, hTERT-negative HA5 cells expressing mutant A and B were generated, and showed severe growth defects accompanied by increased incidence of DNA damage at the telomeres (telomere-dysfunction induced foci, TIFs) and apoptosis. Furthermore, hTERT mutant-expressing cells also harbor short telomeres, measured as signal free ends (SFEs). We are presently assessing the interaction of associated proteins with our variants, such as the recruitment protein HOT1 and hPif helicase, a negative regulator of telomerase. Our current results suggest for the first time, that the IFD can interact with hTPP1 to regulate enzyme processivity and recruitment to the telomeres, which are crucial determinants of telomere maintenance and cellular survival.

A MODEL FOR TELOMERE SYNTHESIS

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Synthesis of telomere repeats by telomerase has been reconstituted in vitro and requires no additional factors. Telomerase is a nucleoprotein complex composed of a catalytic protein subunit, which is a homolog of reverse transcriptase and known as TERT, and telomerase RNA (TR), which contains sequence complementary to ~1.5 telomeric repeats and serves as a template for reverse transcription. Despite extensive analyses, how the RNA template and DNA primer dissociate and re-anneal between cycles of DNA synthesis in order to make hundreds of telomere repeats at chromosome ends is not fully understood. In our studies of translesion DNA synthesis, we recently observed a looped out and re-aligned primer, which allows DNA synthesis to continue when a template strand is blocked and results in primer expansion. Owing to repetitive telomere sequences, looping out one repeat unit in the DNA primer can occur without a mismatched base pair in the template-primer duplex. After analyzing unique telomere sequences and modeling of various TERT proteins based on the crystal structures of beetle TERT and HIV-1 RT, we propose a model for telomere synthesis involving looping out of the repeat sequence. Looping-out of primer allows re-alignment of telomere DNA when it reaches the 5′-end of RNA template and initiation of a new cycle of repeat synthesis. This model is consistent with a wide variety of biochemical, mutagenic and single-molecule microscopic data. Furthermore, our proposed model is testable and suggests simple experiments to probe the mechanism of telomere synthesis.

STRUCTURAL INSIGHTS OF HUMAN POT1-TPP1 INTERACTION

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Mammalian shelterin proteins POT1 and TPP1 form a stable heterodimer that protects chromosome ends and regulates telomerase-mediated telomere extension. We previously have reported the crystal structures of the N-terminal two OB folds of POT1 bound with single-stranded telomeric DNA and the OB fold of TPP1. These structures revealed that POT1 and TPP1 are the homologues of *O. nova* TEBP α and β subunits, respectively. However, how POT1 interacts with TPP1 to form a heterodimer remains unknown. Here we present the crystal structure of the C-terminal portion of human POT1 complexed with the POT1-binding-domain (PBD) of TPP1 at a resolution of 2.1 Å. The structure reveals that C-terminal half of POT1 contains two domains, an OB-fold (POT1_OB3) and a Holliday-Junction domain (POT1-HJ). Interestingly, the HJ domain is actually a large insertion within POT1_OB3. Structural comparison clearly reveals that POT1_OB3 highly resembles the third OB fold of TEBPα although almost no sequence similarity can be detected between the two proteins. Both the OB3 and HJ domains of POT1 are essential for the binding with TPP1. One rare missense variant of a key POT1 residue that is essential for the stable POT1-TPP1 interaction has been identified in melanoma-prone families. Carriers of this variant had increased telomere lengths and numbers of fragile telomeres, suggesting that this variant perturbs telomere maintenance.

ENHANCED ELECTROSTATIC FORCE MICROSCOPY REVEALS THE MECHANISM OF TRF2-MEDIATED DNA COMPACTION

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Shelterin protein TRF2 modulates telomere structures by promoting dsDNA compaction and T-loop formation. The mechanism of how TRF2 compacts DNA remained largely unknown. Traditional atomic force microscopy (AFM) and electron microscopy (EM) cannot directly visualize DNA paths in large heterogeneous protein-DNA complexes. Since protein and DNA molecules contain charged amino acids and phosphate backbones, scanning Kelvin probe force microscopy (SKPM) and electrostatic force microscopy (EFM) have been used to detect variation in surface electric potentials of these biomolecules. However, the nanometer resolution required for detecting DNA in a protein complex using these techniques has not been previously demonstrated. We recently developed an advanced imaging technique, Dual-Resonance-frequency-Enhanced Electrostatic force Microscopy (DREEM), which permits high-resolution imaging of weak electrostatic potentials Using DREEM imaging technique we show that TRF2 dimers and tetramers form on DNA without facilitating DNA folding. On larger TRF2-DNA complexes that are ~103-104 nm3 in volume and store a DNA contour length longer than their diameters, DREEM imaging establishes that some of the folded DNA appears at the rim of the complexes. Surprisingly, in contrast to histone proteins and a DNA repair protein, hMutSα, TRF2 mediated DNA compaction leads to parts of the DNA apparently protrude from the protein cluster. Supporting coarse-grained molecular dynamics simulations reveal the sequential events during the DNA compaction process and result in similar protein-mediated folded DNA structures. These results provide new mechanistic insight into the telomere maintenance pathway and DNA compaction processes.

COHESIN SUBUNIT SA1 AND SHELTERIN PROTEIN TRF1 SYNERGISTICALLY BIND TO TELOMERIC DNA AND PROMOTE DNA-DNA PAIRING

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The cohesin complex plays a crucial role in accurate chromosome segregation, organization of interphase chromatin, DNA replication, and post replicative DNA repair in part by promoting DNA-DNA pairing. The core cohesin subunits consist of a tripartite ring and the fourth core subunit Scc3/SA. In somatic vertebrate cells, SA can be either SA1 or SA2. Recent work indicates that while SA2 is important for cohesion at the centromere, SA1 plays a specific role in sister telomere association. In addition, SA1 directly interacts with shelterin subunits TRF1 and TIN2. While these results demonstrate a unique sister telomere cohesion process depending on the SA1-TRF1-TIN2 complex without the core cohesin ring, the underlying mechanism is still poorly understood. We applied Atomic Force Microscopy (AFM) and oblique angle Total Internal Reflection Fluorescence Microscopy (TIRFM) imaging (using a DNA tightrope assay and quantum dot labeled proteins) to investigate the structure and dynamics of SA1- and SA1-TRF1-DNA interactions. Single-molecule imaging using DNA substrates containing both telomeric and non-telomeric sequences demonstrated that: 1) SA1 only displays telomere sequence dependent intermittent slow diffusion ($D < 0.005\ \mu m^2/s$) with dwell time of ~3 seconds amid faster 1-D free diffusion ($D = 0.06\ \mu m^2/s$); the total SA1 diffusion range on DNA covers both telomeric and nontelomeric regions. 2) TRF1-SA1 together show higher percentage (32% to 67%) of complexes with persistent slow diffusion ($D = 0.0004\ \mu m^2/s$) over telomeric DNA regions throughout the observation periods; 3) AFM imaging reveals that SA1 promotes longer TRF1 protein tracts during DNA-DNA pairing. These data provide direct experimental evidence for the synergistic roles of SA1 and TRF1 in promoting telomere cohesion.

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LONG RANGE ORGANIZATION OF THE TELOMERE G-RICH STRAND OVERHANG

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Telomeres in nearly all eukaryotes consist of short nucleotide repeats containing 3 to 4 consecutive G residues. Thus, one of the two DNA strands is G-rich while the other is C-rich. The 3' terminus G-rich strand in primates and numerous other species extends several hundred bases beyond the 5' terminus of the C-rich strand to generate what is known as the single stranded G-rich overhang. In humans, this overhang is 150- 200 nucleotides (nt) in length. In order to examine the physical properties of natural length G-rich overhangs, we have developed an in vitro replication system using 120 nucleotide long telomeric circles with pre-formed replication forks that provide excellent substrates for Phi29 DNA polymerase. Using this system it has been possible to generate multi-microgram amounts of both the human G-rich and C-rich single stranded DNAs that are up to 20,000 nt in length. By altering the sequence of the template circle, very long single stranded DNA containing telomeric repeats of plants (TTTAGGGn), tetrahymena (T4G4) as well as t the yeasts S. pombe and Y. lipolytica have been generated.
Examination of these long telomeric single stranded DNAs by electron microscopy revealed a striking difference between the G-rich and C-rich DNAs. While the C-rich DNA is relatively featureless and forms thin filaments, the G-rich DNA of all of the species examined consisted of a repeating chain of relatively large particles (G-beads) spaced by a thinner fiber. For the human G-rich DNA, the G-beads measure 7-9 nm in diameter producing a several fold compaction of the DNA. CD spectral analysis of the DNAs revealed a relatively normal spectrum for the C-rich DNA with a single positive peak at 276 nm. The spectra for all the G-rich DNAs showed a negative peak at 245 nm and a slight positive value at 290 nm. Moreover, the CD spectra revealed no difference in structure in the presence of 150 mM Na+ or K+. This observation is striking because G-rich 88 nt long oligos showed the classic G-quadruplex spectra and a significant change upon addition of 150 mM Na+ or K+ ions. These data together suggest that G-beads are stable quaternary structures that are potentially formed of mixed type of G-quadruplexes.
To provide a more detailed structural analysis of the G-bead motifs, we are currently conducting nuclease digestion studies and the preliminary data has revealed a basic subunit of ~24 nt which is the same size as the repeating units observed in our previous studies of TERRA. In addition larger repeating units likely corresponding to the G-beads are present. A model of the organization of natural sized telomere overhangs will be presented.

* These authors contributed equally

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DIRECT OBSERVATION OF MECHANICALLY INDUCED STRUCTURAL TRANSITIONS AND STRAND INVASION IN SINGLE DUPLEX HUMAN TELOMERE DNA MOLECULES

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Telomeres are specialized chromatin structures that protect chromosome ends from unwanted processing by DNA repair machinery. The foundation of human telomere structure consists of a long array of tandem duplex DNA sequences (TTAGGG) and terminates with a single-stranded 3' end. To protect the chromosome end, telomeres are thought to adopt a lariat structure known as a telomere-loop (T-loop). T-loops are stabilized by DNA displacement loops (D-loops) generated by the invasion of a single-stranded telomeric DNA tail into an adjacent region of duplex telomere. Recent studies suggest that telomere-associated proteins promote strand invasion during telomeric D-loop formation through the application of torque to the DNA. Although the molecular mechanism of T-loop formation has been described using biochemical approaches, the torque response and internal structural equilibrium of duplex telomeric DNA are not well characterized. To probe the mechanical properties of duplex telomeric DNA, we developed a magnetic tweezers assay to detect the response of single telomeric DNA molecules to precisely applied degrees of tension and torque. Rotation-extension curves under varying tension demonstrate that the repetitive telomere DNA sequence is more refractory to torque-induced denaturation than a non-telomeric control molecule of comparable GC content. In addition, force-extension analysis of negatively supercoiled telomeric DNA in the presence of different counter-ions (K+ vs. Li+), reveals that transient torque-induced denaturation of duplex telomeric DNA promotes a structural transition into stable DNA G-quadruplexes. Lastly, using a single molecule DNA topology-based assay, we directly monitor the torque-dependent invasion of single stranded telomere DNA primers into duplex telomeric DNA tethers. Our results reveal unique insight into the mechanical properties of duplex telomere DNA and provide an experimental platform for mechanistic investigation of telomere-associated proteins.

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EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (********) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
***Helpful tips* – Use PIN# 62265** to enter Library after hours.
See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes
Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, east wing, lower level
PIN#: **Press 62265 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($9.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
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KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322