

TELOMERES

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(ALT)

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I. TELOMERE STRUCTURE IN MAMMALS

All eukaryotic chromosomes are capped by telomeres, structures composed of DNA and associated proteins comprising the ends of each linear chromosome.

I.1. DNA Sequence

- Ends of linear chromosomes composed of a (TTAGGG) repeat
- Hexamer unit present in as many as 2,000 copies (up to 15 kb of DNA)
- 50-150 bp of terminal DNA lost with each passage through the cell cycle
- Natural "erosion" of telomeres contributes to myriad of physiological processes (see below)

I.2. t-loops, G-loops, D-loops

- Telomeres have a 3' G-rich overhang
- A t-loop is formed when the single-stranded 3' strand is looped back and anneals to the double-stranded hexamer repeats; as the G-rich strand displaces one strand a D, or displacement loop, is created
- t-loop formation confers some protection from exonucleases

I.3. Protein Components

Telomere binding proteins include:

I.3.1. TRF1 (telomeric repeat binding factor 1)

- Expressed ubiquitously throughout the cell cycle
- Binds to TTAGGG repeat as a homodimer (at t-loops) with great specificity
- Functions in cis to inhibit telomerase-dependent elongation
- Participates in regulation of the mitotic spindle
- Regulated in turn by: TIN2, TANK1, TANK2 proteins (see below)
- Negative regulator of telomere length (telomerase-dependent pathway)
- Some data suggest a role for TRF1 in response to DNA double-stranded breaks

I.3.2. TRF2 (telomeric repeat binding factor 2)

- Expressed ubiquitously throughout the cell cycle
- Binds to TTAGGG repeat as homodimer with great specificity
- Localizes to t-loops, involved in their formation
- C-terminal domains homologous to MYB family of protooncogenes
- Might be involved in inhibition of replication fork
- Stabilizes the G-rich strand overhang and inhibits telomere-telomere fusions
- TRF2-negative telomeres are recognized as damaged DNA
- Negative regulator of telomere length; TRF2 overexpression in somatic cells = telomere shortening
- TRF2 inhibition causes apoptosis and non-homologous end joining (NHEJ) of telomeres
- Promotes binding of hRAP1, a telomere associated protein

I.3.3. hRAP1

- Human homologue of yeast protein
- Negative regulator, in cis, of telomere length
- C-terminus mediated interaction with TRF2
- Functions in determining relative telomere length

I.3.4. TIN2 (TRF1-interacting nuclear factor 2)

- Regulates telomere length via NH₂-terminus mediated binding to TRF1
- Mutant TIN2 lacking NH₂-terminus leads to elongated telomeres
- Promotes TRF1-dependent pairing of telomere repeats

I.3.5. TANK1/TNKS (tankyrase, TRF1-interacting ankyrin-related polymerase)

- Poly(ADP-ribose) polymerase (PARP) activity
- Tankyrase-mediated ADP-ribosylation of TRF1 inhibits binding to telomere repeats
- Promotes telomere elongation

I.3.6. TANK2/TNKS2 (tankyrase 2) :

- Related to TANK1
- Overexpression induces necrotic cell death

I.3.7. WRN (Werner syndrome gene product) :

- RecQ subfamily of helicases
- Required for DNA replication
- Involved in control of genomic stability

N.B. Therefore, telomere function can be compromised by affecting telomere-binding protein function(s).

II. TELOMERE FUNCTION

II.1. Confer Stability and Protect Chromosome Ends

- Protection from cellular exonucleases
- Protection from non-homologous end joining
- Allow cells to differentiate between natural chromosome ends and damaged DNA
- Preserves integrity of chromosomes by allowing replication to occur without loss of coding sequences

II.2. Count Number of Cell Divisions

- Judges number of cell divisions that have occurred
- Determines cellular lifespan and when replicative senescence will occur

II.3. Provide Mechanism for Replication of Linear DNA Ends

- Discontinuous replication on lagging strand involves Okazaki fragments and template that must be replicated
- Telomerase (see below) adds hexamer repeats to 3' ends, allowing DNA polymerase to complete synthesis of the opposite strand

III. TELOMERE MAINTENANCE

III.1. Telomerase

III.1.1. RNA Component: hTERC (human telomerase encoded RNA)

- RNA: AAUCCC, encoded by hTERC
- Serves as template for TTAGGG synthesis
- Constitutively expressed

III.1.2. Catalytic Component: hTERT (human telomere reverse transcriptase)

- Synthesizes DNA from an RNA template
- Not expressed in most somatic cells
- Telomerase-associated proteins include hEST2, hTEP1, SSB, DKC1(dyskerin)

III.1.3. Mechanism

- Reverse transcription by hTERT synthesizes telomeric sequences lost during routine DNA replication
- hTERT activity is a critical factor in stabilizing telomeres through addition of TTAGGG repeats

III.1.4. Expression

- >90% of neoplasias reactivate expression of hTERT
- hTERT not expressed in most somatic tissues
- hTERT expressed in germ cells, immortalized cells
- Inactivation of hTERT results in shorter telomeres

III.2. Telomerase independent/alternative lengthening of telomeres (ALT)

III.2.1. Length of telomeres synthesized by ALT is characteristically heterogeneous

III.2.2. Telomere length is dynamic, changes regularly

III.2.3. Active in telomerase negative neoplasias (~10-15% of all neoplasias)

III.2.4. Preferentially active in mesenchymally-derived cells, compared with those of epithelial origin

III.2.5. Repressors of ALT expressed in normal cells and in certain telomerase negative cells (i.e., ALT activity and telomerase activity can co-exist in the same cells)

III.2.6. Proportion of ALT(+) cells are associated with PML bodies (promyelocytic leukemia nuclear body, or PML NB)

- PML NB comprised of telomeric DNA, TRF1, TRF2, and PML proteins
- Telomeric DNA, TRF1, TRF2 and PML proteins all co-localize in ALT(+) cells
- Co-localization not observed in telomerase (+) cells
- Potential role for PML NB in cellular differentiation, cell growth, apoptosis, and an undefined role maintaining telomere integrity

III.2.7. Mechanism of ALT likely involves homologous recombination between telomeres; sequences copied from a single telomere to another by complementary annealing as a means of priming new telomeric DNA

III.2.8. G-loop vs. t-loop, D-loop (see above for description of roles)

III.2.9. Experiments performed in yeast :

- Demonstrate necessity for DNA repair genes such as RAD50, RAD51, RecQ helicases in order for homologous recombination to occur properly
- Inhibition of mismatch repair pathways has been shown to enhance ALT pathway, presumably because homologous recombination requires mismatch repair pathway proteins

IV. SENESENCE AND IMMORTALIZATION

IV.1. Hayflick Limit (1961)

- Demonstration that a limited number of cell doublings are possible in vitro (between 30-50)
- Number of cell doublings counted and recorded by cell
- Exceeding doubling limit results in cellular, or replicative, senescence

IV.2. Telomeres and Telomerase

IV.2.1. Telomeres hold a critical function in cellular senescence

IV.2.2. Telomeres count the number of cell divisions

IV.2.3. Telomerase can reset the cell division counter :

- By repairing shortened or damaged telomeres, and
- Inhibiting telomerase causes loss of telomeric sequences and eventually cellular senescence

IV.2.4. Two biological impediments to extended lifespan of human cells :

- a. M1: replicative senescence, or mortality stage 1 (function is to inhibit cellular immortalization)

b. M2: crisis (cells in crisis usually enter apoptotic pathway, those that can elude crisis stage become immortal). These cells:

1. Express telomerase
2. Show relatively constant telomere lengths
3. Show aneuploidy
4. Show non-reciprocal translocations
5. Together, these data suggest that at crisis stage, telomeres lose protective abilities

IV.2.5. Expression of telomerase in primary (human) cells

- Causes immortalization
- Suggests telomeres are active at both M1 and M2 stages and are central to determining cellular lifespan

IV.2.6. Sufficient damage sustained by telomeres

- Is recognized as DNA damage
- Initiates p53-dependent arrest of the cell-cycle
- Can induce cellular senescence

IV.2.7. Telomere-length threshold capable of initiating senescence

- Can be changed by overexpressing TRF2
- Cells can detect chromosomes with reduced concentration of bound telomere-associated proteins, suggesting
- Senescence determined by both by telomere length and effects of telomere-bound proteins

IV.3. Immortalization

- Immortalization can be achieved through activation of telomerase, such that cells are no longer limited by negative controls on growth
- In vitro expression of enzymatic subunit of telomerase in diploid cells causes immortalization
- Prolonged or constitutive expression of telomerase can also induce immortalization
- M1, M2 phases of senescence both function to impose limited lifespan

V. AGING

V.1. Role of Telomere Length

- Premature aging seen in telomerase null mice can be rescued by reactivation of telomerase expression
- Re-expression of telomerase heals telomeres shortened below a critical length threshold
- Chromosomal stability is regained

V.2. Role of ATM

- Ataxia-telangiectasia mutated gene product (ATM)
- Integral component in pathway that recognizes double-stranded DNA damage
- Involved in telomere length maintenance through direct binding with TRF1; participates in protection of telomeres from NHEJ
- Loss of ATM results in defects of DNA repair (particularly those pathways involving homologous recombination), cell cycle control and increased incidence of cancers
- ATM-/Terc double knockout mice feature shortened telomeres, increased genomic instability reflected in chromosomal fusions, defects in proliferation and early death

V.3. Human Disorders of Premature Aging

Genetic aberrations that increase rates of telomere erosion and inhibit normal DNA repair from occurring at the telomere synergize to cause premature aging, a phenomenon seen in several disorders that feature predisposition to neoplasias.

- Dyskeratosis congenita: defective telomerase with very short telomeres; DKC1 (dyskerin) protein stabilizes hTERC
- Werner syndrome: accelerated telomere erosion
- A-T (ataxia-telangiectasia): accelerated telomere loss (see above)
- Bloom syndrome (BLM): BLM DNA helicase suppresses abnormal improper recombination; binds TRF2 in ALT(+) cells; enables recombination-mediated telomere amplification

V.4. Telomere Position Effect (TPE) in Humans

- Expression of genes mapping near telomere varies according to telomere length
- Reversible suppression of gene expression
- Affected by higher-order chromatin organization
- Mechanism by which age-related gene expression can be regulated

VI. GENOMIC INSTABILITY AND NEOPLASIA

VI.1. Role of Telomere Length

- Genomic instability is facilitated in the presence of shortened telomeres
- Data strongly suggest crucial steps in the development and progression of neoplasia include the dysregulation of both telomerase and telomeres
- When a critical telomere length has been reached, chromosomes begin to fuse end-to-end, bringing cells into crisis

VI.2. Expression of Telomerase

VI.1.1. Reactivation of telomerase expression directly correlates with neoplasias, supporting the notion that telomeres and telomere maintenance are central to the formation of cancers

VI.1.2. Expression of hTERT alone causes immortalization alone; cell transformation requires immortalization accompanied by inactivation of tumor suppressor genes and activation of cellular oncogenes

VI.1.3. Telomere shortening can serve to inhibit early stages of tumor growth; however, telomere shortening, particularly in the context of a dysregulated cell cycle, can facilitate neoplasia by:

- exerting selective pressure favoring immortal clones
- promoting accumulation of subsequent genetic changes

VI.1.4. Recent data suggest telomerase reactivation contributes to neoplasia through pathways independent of telomere maintenance

- stabilizing chromosomal changes
- favoring growth of immortalized clones
- conferring resistance to apoptosis (some data suggest expression of hTERT confers this attribute)

VI.3. Chromosome and Genomic Instability

VI.3.1. Molecular and cytogenetic studies have indicated chromosomes with even a single unprotected chromosome end are genetically unstable until telomere integrity has been restored. During this period of genetic instability, breakage-fusion-breakage (BFB) cycles occur, often culminating in chromosomal aneuploidies

VI.3.2. BFB cycles and chromosomal instability also promote sister chromatid fusions through non-

homologous end joining (NHEJ)

VI.3.3. During mitosis, separation of centromeres in dicentric chromosomes to opposite poles produces an anaphase bridge, followed by chromosome breakage, subsequent fusion of damaged ends, and promotion of additional BFB cycles

VI.3.4. Recurring cycles of gene amplification can arise during acquisition of new telomeres by rearranged chromosomes, suggesting double-stranded DNA breaks are important in promoting amplification of genes closest to a chromosomal break

VI.3.5. In order to survive, genetically unstable cells also must escape detection by cell-cycle regulators, such as p53, which can induce growth arrest or apoptosis in response to damaged DNA

- Critically shortened telomeres can be detected by p53
- p53 binds to the G-rich, single-stranded overhang telomeric DNA and also interacts with the t-loop
- Loss of p53 function and telomere shortening work together to promote tumorigenesis

Contributor : Azra H. Ligon

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