Minireview

Telomeres and telomerase: their mechanisms of action and the effects of altering their functions

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Abstract **The molecular features of telomeres and telomerase are conserved among most eukaryotes. How telomerase and telomeres function and how they interact to promote the chromosome-stabilizing properties of telomeres are discussed here.**

Keywords: Telomere; Telomerase

1. Introduction

Telomeres, the DNA-protein complexes at the ends of eukaryotic chromosomes, are protective against genome instability-promoting events. Such events can include degradation of the terminal regions of chromosomes, fusion of a telomere, either with another telomere or with a broken DNA end, or inappropriate recombination. These processes are potentially catastrophic; for example, fusions can lead to the formation of dicentric chromosomes, which are inherently unstable and result in imbalances in the genetic content of dividing cells' progeny, or to loss of genetic information. Telomeric DNA consists of tandemly repeated, simple, often G-rich, sequences specified by the action of telomerase. The tandem repeats form a molecular scaffold containing many binding sites for telomeric proteins, which in turn nucleate the coalescence of a higher order, although still ill-defined, complex of protective telomeric proteins, including the telomeric DNA-sequence-specific binding proteins. The resulting DNA-protein complex at the telomere is dynamic; during interphase, the telomere-bound proteins are exchanged on and off individual telomeres at rates of the order of minutes or less, depending on which protein components are examined [1].

2. Telomerase: polymerizing enzyme and protector

The complete replication of telomeric DNA requires telomerase, a specialized cellular ribonucleoprotein RNP reverse transcriptase (RT). The core enzyme contains the protein TERT, which has a RT homology domain as well as other essential conserved domains, and the RNA component, TER. By copying a short template sequence within its intrinsic RNA moiety, telomerase synthesizes the telomeric DNA strand running 5′ to 3′ toward the distal end of the chromosome, thereby extending it. Regulated extension of the chromosomal DNA termini occurs to compensate for shortening that results from nuclease action and incomplete terminal DNA replication. A multi-component "telomere homeostasis" system prevents, on the one hand, the over-extension of telomeres. Protein–protein interactions among the telomere-associated proteins are important for this function, which acts in *cis* on a telomere [2].

Conversely, the telomeric homeostasis system acts to promote telomeric extension whenever a telomere becomes shortened, thereby keeping the tract of telomeric repeats within a well-defined range in telomerase-containing cells.

Telomerase functions in its capacity as a cellular RT using a mechanism that results in the synthesis of the short, repeated DNA sequence found at telomeres. Only a short region of the telomerase RNA, the templating domain, is copied. The telomerase RNA is bound at high affinity by conserved domains of TERT that lie outside its RT homology domain ([3]; and references therein). These and other RNA-protein interactions between TERT and TER are critical to telomerase function. In various species, telomerase is dimeric and within the dimeric complex, its RNAs interact with each other in a fashion that, under at least some experimental conditions, can be crucially important for enzyme activity [4–6].

TER has a secondary core structure which is conserved among eukaryotes in general. The core contains motifs that not only bind TERT but also include sequences and elements demonstrated to be critical for enzyme function, as reviewed elsewhere [3] (see Fig. 1).

One example of the importance of the RNA structure is seen in experiments changing a structural element in the RNA (whose nature varies from species to species) that acts as a barrier to synthesis beyond the template. RNA mutations in this element can cause read-through synthesis, in which portions of the telomerase RNA beyond the normal template boundary are copied, both in vitro and in vivo. TERT binds to this element; such binding, occurring as it does to a region of the RNA close to the template, may depend on how this barrier function is accomplished. However, various other mutations in the structural and sequence elements of the telomerase RNA core cause effects that have been less easy to reconcile with a mechanism solely involving TERT–TER binding. Some

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of these mutations cause deficiencies in detectable enzymatic activity. For example, specific point mutations in the templating domain can abolish enzymatic activity [7]. Certain base changes in the template, in a fashion that currently appears idiosyncratic, cause incorrect usage of the RNA template, such as DNA base misincorporation and mispair extension, or high rates of slippage synthesis. This has been observed in *Tetrahymena*, *Kluyveromyces lactis* and *Saccharomyces cerevisiae* telomerases. These errors lead to the synthesis of telomeric DNA bearing significant amounts of unpredicted DNA sequences (reviewed in [8]).

In the telomerase of the budding yeast *K. lactis*, some mutations in the conserved pseudoknot diminish or even ablate telomerase enzymatic activity; other pseudoknot mutations cause incomplete copying of the template sequence, such that only a truncated region of the template is used and consequently mutated (truncated) repeats are present at telomeres, causing cell growth defects [9]. Hence, telomerase RNA bases play important roles in enzyme function. The ultimate ability of telomerase to synthesize the correct telomeric DNA sequence is needed not only to counteract telomere terminal attrition, but also to synthesize the correct telomeric DNA binding sites for the sequence-specific protective telomeric proteins.

Telomerase also acts to prevent chromosome fusions. The net elongation of the bulk of the telomeres in a cell by telomerase can be uncoupled from its ability to protect from chromosome fusions, in yeast and human cells [10–13] (see Fig. 1).

Telomerase in yeast cells can be cross-linked to telomeric DNA even during times in the cell cycle when it is not competent to polymerize DNA onto chromosome ends [14–16].

Together, these results raise the possibility that telomerase physically helps cap the chromosomal termini and thereby may help protect telomeres against fusions.

Although it was not initially detected in cells that undergo senescence in culture, telomerase activity can be found even in these cells. This has been observed with such cells after they have been passaged in culture or when they are fresh, and before they have been passaged. Telomerase activity was reported to be readily detectable in primary chicken fibroblasts and in primary human endothelial cells soon after removal from the bird/human, but to become greatly diminished after culturing in vitro has got underway [17,18].

Cultured human fibroblasts eventually undergo senescence, before which their telomeres gradually shorten, and as the cells enter senescence in culture, DNA damage foci accumulate specifically at telomeres [19]. Thus, the low amount of telomerase in these cells is insufficient to prevent their eventual telomere uncapping or cellular senescence. Supplementing the meager amounts of telomerase in primary cultured human cells, by forced overexpression of TERT, can keep the cells multiplying in culture. Even a hypomorphic telomerase allele that does not cause net telomere shortening is able to prolong cell life span in culture [10]. Conversely, effectively swamping out the small amount of endogenous functional telomerase, via overexpression of a catalytically dead point mutant of TERT protein, causes premature senescence and apoptosis of primary human fibroblasts or keratinocytes ([10], and Fig. 2). Apoptosis similarly increases upon overexpression of a catalytically competent but biologically non-functional point mutant of telomerase ([10], and Fig. 2). These results suggest that, despite

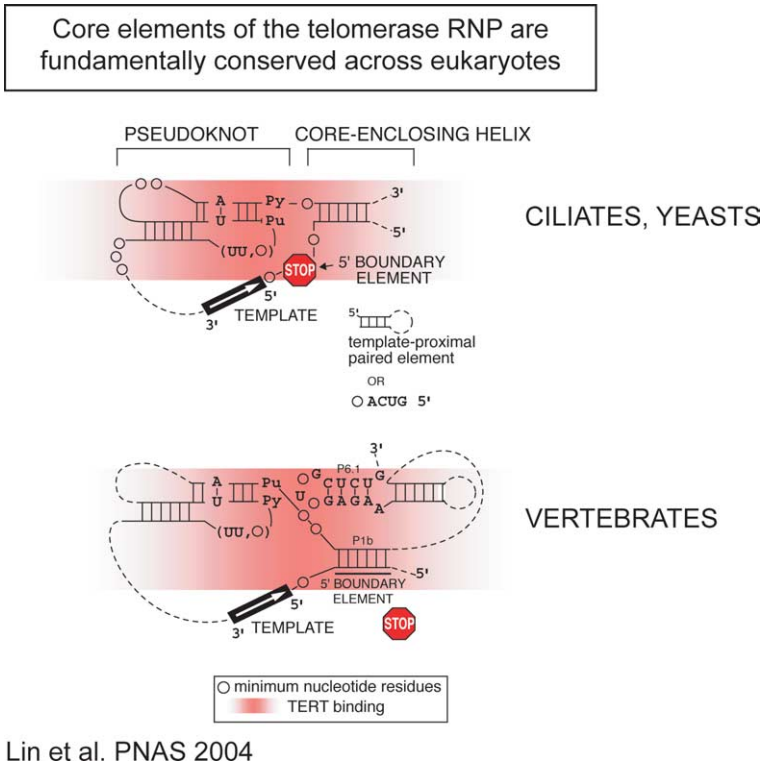

Fig. 1. A universally conserved core in telomerase RNA (from [3]).

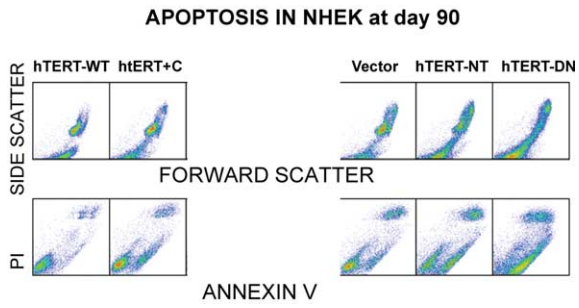

Fig. 2. Apoptosis in normal human epithelial keratinocytes. At day 90 after beginning passaging cells in culture, FACS analysis was performed. Top panels, forward and side scatter of ungated cells; bottom panels, gated whole cells, *y*-axis, propidium iodine uptake (as a measure of fraction of inviable cells) and *x*-axis, Annexin V staining (as a measure of apoptosis) of cells transformed stably with hTERT overexpression vectors carrying different forms of human TERT as described in Kim et al. [10]: WT, wild-type, +C, hTERT construct bearing a 10-amino-acid C-terminal extension; NT, N125A, T126A double point mutant of hTERT; DN, D868A catalytically dead hTERT. Note that hTERT-WT or hTERT +C, both of which are enzymatically active, decrease cell death, while NT-hTERT and especially DN-hTERT dramatically increase cell death.

the eventual telomere shortening, uncapping and senescence that ensues, even the low amount of telomerase in these cells is partially protective. Together, these findings raise the possibility that normal cells may never be truly telomerase-negative in vivo, but, rather, have low amounts of telomerase that serve some telomere-protective function.

3. The hitherto-secret other life of the telomerase TERT subunit

TERT, the catalytic protein subunit of telomerase, is a large (>100 kD) protein containing conserved domains outside its RT domain. An inhibitor of telomerase activity, the human protein PinX1, was first identified in mammalian cells by Lu and colleagues and was shown to inhibit telomerase in vitro and to bind TERT protein in vitro [20].

Extending the analysis of PinX1 to yeast led to the surprising finding that in vivo, TERT can form an alternative, enzymatically inactive complex lacking telomerase RNA. This TERT-containing complex contains, instead, PinX1, which replaces the telomerase RNA by binding to a domain of TERT protein that overlaps with the RNA-binding domain. This TERT-PinX1 complex is unable to synthesize telomeric DNA, since it lacks the telomerase RNA. In yeast, it was found that PinX1 and the telomerase RNA bind to the same domain of TERT. Furthermore, evidence that telomerase RNA and PinX1 both compete for TERT binding in vivo was found [21].

Because PinX1 is a nucleolar protein in yeast, binding TERT to PinX1 is expected to have the effect of sequestering TERT in the nucleolus, away from the telomeric DNA substrate of enzymatic telomerase. Normal human cells have TERT distributed in the nucleolus and nucleoplasm, but in human cancer cells TERT primarily locates in the nucleoplasm, and it is generally not detected in the nucleolus [22]. Evidence that PinX1 is a tumor suppressor has been reported [20] and it is often found to be diminished in amount in human cancers. Hence, we have proposed that the mechanism by which PinX1 acts as a tumor suppressor is to sequester telomerase in the nucleolus, and that loss of this regulatory mechanism promotes tumorigenesis by freeing the available TERT to the nucleoplasm, where it can carry out telomere maintenance functions (see Fig. 3).

The finding that the large TERT protein, with its extensively conserved domains outside its RT domain, can form a complex different from the enzymatically functional RNP opens up the possibility that even more complexes of TERT, or of TER, may exist in cells.

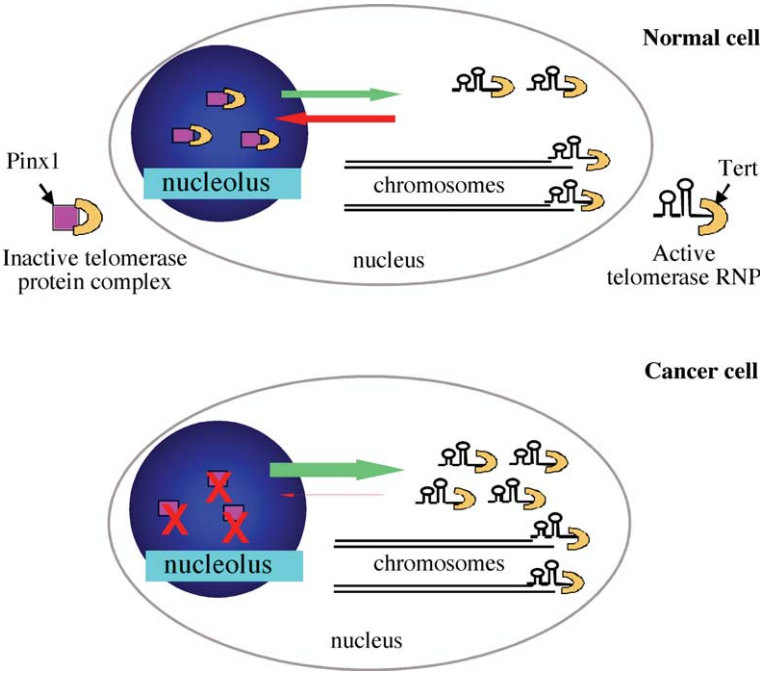

Fig. 3. Model for sequestration of TERT protein of telomerase by the tumor suppressor protein PinX1.

4. Cellular responses to aberrant telomeres

Mutations of the telomerase RNA template can cause correspondingly mutated telomeric DNA sequences to be added to telomeres in vivo. The responses of cells to such mutations do not resemble the responses elicited by DNA damage, such as double-stranded DNA breaks. In *S. cerevisiae*, such mutant telomeres result in a pre-anaphase cell arrest phenotype in which chromosomes do not separate, or show delayed separation. DNA damage foci containing Ddc1 or Ddc2 increase in frequency in such cells over background. Whether these foci reflect chromosome fusions and ensue breakage of the resulting dicentric chromosomes, or whether the foci are formed at the aberrant telomeres themselves, was not distinguished. However, the genetic dependencies for this mutant telomerase template-induced arrest differ from that of a conventional DNA damage response; unlike a DNA damage response, the arrest was not ameliorated by the absence of Mec1, Mec3, Ddc1, Ddc2 or Mad1 [23]. Only when both the DNA damage checkpoint and spindle checkpoint pathways were simultaneously disrupted did the cells fail to elicit the arrest response to mutant-template telomerase (D. Smith and EHB, unpublished). This suggests that both pathways contribute to the yeast cells' arrest in response to mutant telomeric DNA repeats and that the two pathways can function at least somewhat redundantly in this role. In interesting contrast to these results, a mutation in the telomere-protective protein Cdc13p, which binds the single stranded terminal portion of yeast telomeric DNA [24], elicited an arrest with genetic requirements that resembled a DNA damage response; that is, the arrest could be alleviated by deleting just the DNA checkpoint [23].

In human cancer cells, telomere uncapping is caused by expression of a telomerase RNA with a mutant template [25]. In these cells, DNA damage foci can be seen clearly forming at the telomeres themselves, the DNA damage protein p21 is induced, and apoptosis increases. However, again, the genetic dependencies for this cellular response differ from that of a DNA damage response: p53 is not required. This lack of a requirement for p53 for the cellular apoptotic response contrasts with the dependence on p53 that was reported for a different type of telomere aberrancy in human cells: depletion of the telomeric binding protein TRF2 from telomeres by overexpression of a truncated, dominant negative form of TRF2. In that case, apoptosis showed a p53 dependence [26].

In summary, the above results suggest that the different kinds of molecular aberrancies at telomeres may initiate signaling within cells differently from each other, or from double-stranded DNA breaks. Eventually, the ensuing downstream events appear to converge on a common DNA damage response. How these cellular responses to threats to telomere integrity are triggered and coordinated remains an important mechanistic question.

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