

TERRA –A Calling Card for Telomerase

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Transcription of telomeric DNA has long been thought to inhibit telomerase. Using live cell imaging, in this issue, Cusanelli et al. (2013) now reveal a different scenario: telomeric RNA is preferentially generated at short telomeres and delivers telomerase to the chromosome end from which the transcript originated.

Sequences at the ends of chromosomes are lost during normal DNA replication and must be replenished to permit continued proliferation. The guanosine-rich DNA repeats that make up the telomeres in most eukaryotes are synthesized by the enzyme telomerase and were believed to be transcriptionally silent. The discovery of telomeric repeat containing RNA (TERRA) challenged this view (Azzalin et al., 2007) and raised the question of whether the act of transcription or the noncoding RNA product function in the regulation of telomere length and chromosome end capping. Although TERRA was found in yeasts and mammals, the low abundance of the endogenous transcripts and challenges in genetically manipulating TERRA levels have hampered functional characterization of this RNA. In work published in this issue, Cusanelli et al. (2013) use multi-color imaging to simultaneously visualize telomeric DNA, TERRA, and telomerase in living yeast cells. Their experiments suggest an exciting and unanticipated model in which TERRA sequesters and directs telomerase to those telomeres most in need of elongation.

Prior studies have characterized TERRA as RNA polymerase II transcripts that start at a variety of sites in subtelomeric DNA and extend into the telomeric repeat tracts (Azzalin et al., 2007; Porro et al., 2010). A fraction of TERRA is telomere associated and appears to function in maintaining telomere structure and facilitating telomere replication (Deng et al., 2009). Long before naturally occurring telomeric transcripts were first identified, placement of a strong promoter upstream of a yeast telomere suggested that telomere transcription inhibits their elongation (Sandell et al., 1994). Biochem-

ical experiments later confirmed that telomeric RNA oligonucleotides bind to and potentially inhibit human telomerase (Redon et al., 2010). Further support for TERRA inhibiting telomerase came from the observation that TERRA levels are low in wild-type budding yeast but are dramatically increased in cells defective in the Rat1 exonuclease (Luke et al., 2008). These cells have short telomeres, a phenotype that can be rescued by exogenous expression of RNaseH, indicating that TERRA inhibits telomerase via a DNA/RNA hybrid. In contrast, TERRA upregulation in human cancer cells did not impair telomere elongation (Farnung et al., 2012). Instead, telomere elongation repressed TERRA expression through increased H3K9 trimethylation suggesting that telomere shortening is associated with increased transcription (Arnout et al., 2012).

In order to detect TERRA in living yeast cells, Chartrand and colleagues integrated binding sites for the MS2 coat protein directly upstream of a specific telomere. Transcripts from this telomere are thus bound by an MS2-GFP fusion protein expressed in the same cell and can be visualized by fluorescence microscopy. TERRA/MS2-GFP foci were detected in approximately 10% of interphase cells. Simultaneous integration of different tags into TERRA from two telomeres revealed that TERRA foci from different telomeres rarely coexist in the same cell. This suggested that TERRA transcription is regulated in *cis* and prompted the authors to ask whether telomere shortening stimulated transcription. A combination of FISH, qPCR, and live-cell imaging revealed that bulk telomere shortening due to loss of functional telomerase, as well as inducible shortening

of a marked telomere, result in increased TERRA production. Notably, the increase is limited to the shortened telomere.

Previous work had shown that telomerase preferentially elongates the shortest telomere in late S phase (Teixeira et al., 2004), but how telomerase is recruited to the shortest telomere had remained unclear. The observation that TERRA is induced specifically at a short telomere raised the possibility that TERRA is involved in telomere elongation. Drawing on their earlier work visualizing telomerase in living yeast cells, the Chartrand group simultaneously monitored the position of a telomere, its TERRA transcript, and telomerase. They observed accumulation of the transcript in a nuclear focus followed by association with telomerase during S phase. Most intriguingly, as a TERRA cluster moves around the nucleus, it is ten times more likely to associate with the telomere from which it originated than with another telomere. These results suggest that TERRA plays a positive role in telomere elongation—first, by acting as a scaffold that sequesters telomerase, and second, by directing preformed telomerase clusters to the short telomere from which the TERRA cluster originated (Figure 1).

In light of these observations it is tempting to speculate that TERRA transcripts serve as unique identifiers of the chromosome end from which they were transcribed, thereby aiding the recruitment of telomerase to the shortest telomeres. If this “postcode” is encoded in the RNA sequence and recognition is mediated by DNA/RNA interactions, sequence differences in the subtelomeric parts of TERRA would have to mediate targeting—a possibility that can easily be tested. Alternatively, structural changes

that occur at short telomeres may increase the affinity for a TERRA-telomerase cluster independent of the actual origin of the TERRA molecules. A plethora of protein-protein interactions and post-translational modifications have already been implicated in telomerase recruitment and activation, and placing TERRA into this network will be a necessary and exciting next step. The observation that human TERRA can participate in promoting changes in the composition of telomeric complexes that favor telomerase activity suggests one possible mechanism by which TERRA may contribute to telomere elongation (Flynn et al., 2011).

As with any well-executed study this work reveals new and unexpected insights but also raises many questions: If telomerase finds the shortest telomere by virtue of it being transcribed, how does RNA polymerase know which telomere to transcribe? Are structural changes associated with telomere shortening responsible? What is the structure of TERRA complexes? Are G-quadruplexes in play? What mediates the interaction between telomerase and TERRA clusters? With biochemical data supporting direct interactions between TERRA and both telomerase core subunits, does part of the enzyme assembly take place on the TERRA cluster? It will also be important to examine to what extent TERRA expression improves telomerase recruitment and activity. Fifteen percent

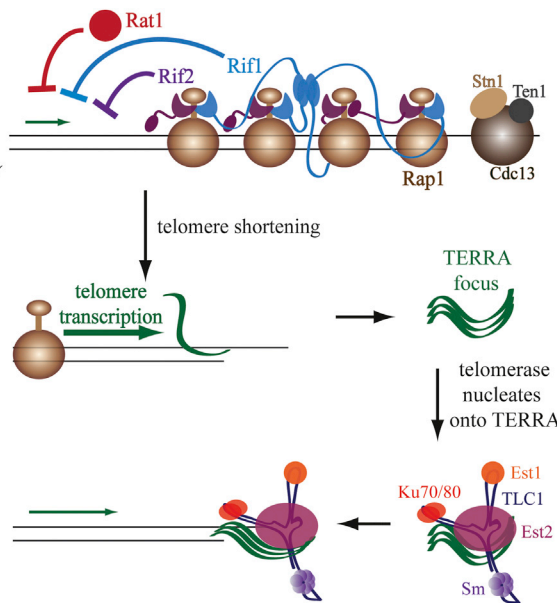


Figure 1. A Schematic Illustrating the Role of TERRA in Telomere Maintenance

On long telomeres transcription of TERRA is inhibited by telomere-associated proteins including Rap1, Rif1, and Rif2 and transcripts are degraded by the RNA exonuclease Rat1. Telomere shortening triggers expression of TERRA, which assembles into a focus in the nuclear periphery. In early S phase TERRA serves as a seed for the formation of telomerase clusters. In late S phase preformed telomerase-TERRA clusters associate with the short telomere from which the TERRA transcripts originated.

of cells that lacked a visible TERRA focus still showed telomerase recruitment to the marked telomere. Is the enzyme just as active in the absence of TERRA?

In light of the new results one wonders why artificially elevating TERRA levels results in telomere shortening in yeast? Is it simply too much of a good thing, or does it take more than fine-tuning expression for TERRA to stimulate telomere elongation? We often wish for pathways to be linear and for effects to be either positive or negative—a wish rarely granted in the context of biology. The sin-

gle-stranded telomeric DNA binding protein Pot1 inhibits and activates telomerase and the major telomere binding protein in budding yeast is aptly named the “Repressor and Activator Protein” (RAP1) for its telomere-independent roles in the regulation of gene expression. We should expect TERRA to be just as interesting and full of surprises.

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