A Date with Telomerase: Pick You Up at S Phase

S. Hocine[1] and R.H. Singer[1,*]
[1]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
*Correspondence: robert.singer@einstein.yu.edu
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Using the MS2 system for labeling mRNA, in this issue, Gallardo et al. (2011) find that telomere lengthening depends on a stable accumulation of multiple telomerase complexes in late S phase and that this process is temporally regulated by Rif1/2 proteins.

The natural ends of chromosomes require unique processing such that they are protected from degradation and exposure to the DNA damage response machinery. This is achieved by the formation of structures called telomeres. Telomeres progressively shorten as cells divide, and a specialized ribonucleoprotein complex known as telomerase has evolved to offset this phenomenon and preserve genomic integrity (O'Sullivan and Karlseder, 2010). Following the 2009 Nobel prize awarded to Blackburn, Greider, and Szostak for the discovery of telomerase and the cellular consequences of telomere shortening, the timing and mechanism by which telomerase is recruited to telomeres has been a subject of ongoing investigation. A recent study by Gallardo et al. (2011) employs a novel and creative in vivo imaging approach to probe this process in *Saccharomyces cerevisiae*.

Taking advantage of the MS2 system described previously for tagging of *Saccharomyces cerevisiae* mRNAs (Bertrand et al., 1998), Gallardo et al. (2011) engineer *TLC1*, the RNA component of telomerase, with 10× MS2 stem loops. Upon expression of fluorescent MS2 coat protein, telomerase particles become fluorescently labeled while maintaining physiological localization and activity. Since the *TLC1* RNA is the limiting factor in telomerase assembly, its dynamics would reflect the behavior of the active holoenzyme. The authors find that telomerase particles exhibit homogenous fluorescence and diffusive behavior in G1 and G2 cells suggesting that these particles represent unbound preassembled telomerase complexes. Interestingly, tracking of telomere IV_R using site-specific integration of a LacO array demonstrates significantly different dynamics and reveals a much more static behavior as compared to G1/G2 telomerase particles. Therefore, it seems unlikely that telomerase is associated with telomeres in G1 and G2 cells.

Biochemical evidence has suggested that components of the telomerase holoenzyme preferentially associate with telomeres during late S phase (Taggart et al., 2002), coincident with telomere lengthening. If true, a change in telomerase particle dynamics would be expected in S phase cells. Indeed Gallardo et al. (2011) find that velocit, displacement, diffusion coefficient, and mean squared displacement of telomerase particles in S phase resemble that of telomere IV_R. Most surprising is the finding that these static telomerase particles exhibit 6 to 15-fold increased fluorescence in S phase compared to the diffusing population found in G1 and G2. The authors term these high-intensity particles "T-Recs," or telomerase recruitment clusters, and speculate that they represent sites of active telomere elongation (Figure 1). To more directly address this, they perform colocalization studies using fluoresescent Rap1, a marker for telomeres in yeast (Schober et al., 2008). As expected, telomerase particles colocalize transiently with Rap1 in G1 and G2, but stable colocalization is observed only in S phase. This transient behavior in G1/G2 helps to explain previous ChIP results in which telomerase components can be found to associate with telomeres outside of late S phase (Fisher et al., 2004; Taggart et al., 2002). G1/G2 telomerase particles diffuse rapidly, and it is possible that the increased fluorescence intensity of S phase T-Recs is a function of increased dwell times as opposed to (or in addition to) molecule numbers. Even if T-Recs do represent a clustering of telomerase, it is difficult to quantify how many telomerase molecules are actually present. But why would telomere lengthening require the clustering of telomerase complexes? Telomerase is not processive (Chang et al., 2007), and one possibility is that short telomeres in need of lengthening overcome this by recruiting several telomerase complexes, which could act as an assembly line.

Furthermore, deletion or mutation of genes involved in telomerase recruitment (*XRS2*, *TEL1*, and *CDC13*) substantially reduced the frequency of T-Rec formation in S phase cells. Rif1/2 proteins are negative regulators of telomere length thought to inhibit telomerase recruitment. In yeast lacking *RIF1* or *RIF2*, T-Rec occurrence was no longer restricted to S phase and appeared in all phases of the cell cycle. Gallardo et al. (2011) go on to show that elongation of an artificially introduced short telomere in G1-arrested cells is observed in *rif1* and *rif2* strains but not in wild-type cells. Thus, they argue that Rif1/2 proteins control the cell cycle-dependent access to telomeres (Figure 1), consistent with previous findings, which suggest Rif1/2 regulate the frequency of telomere elongation events (Teixeira et al., 2004) and may be involved in rendering telomeric DNA inaccessible (Levy and Blackburn, 2004). From yeast to human cells, much divergence has occurred within telomere binding proteins and their interacting factors, however the overall structure and basic lengthening mechanism of telomeres is conserved. In fact, components of human telomerase have been reconstituted into budding yeast with mixed results (Auriche et al., 2008). A human ortholog of yeast Rif1 protein (hRif1) has been identified, however deletion of hRif1 does not affect telomere length, and it does not localize to normal telomeres (Xu and Blackburn, 2004). Whether the same mechanism for

cell cycle-dependent recruitment of telomerase exists in humans is not completely clear at this time.

The approach of Gallardo et al. (2011) helps to differentiate between two telomere lengthening models. A telomerase-activation model would posit that telomerase is present on telomeres in G1 and S phase but is locally activated on only the shortest telomeres in late S phase. The other possibility is described by a recruitment model in which telomere binding proteins interact with components of the telomerase holoenzyme for preferential recruitment to the shortest telomeres in S phase. Gallardo and colleagues state that the results reconcile these disparate molecular models, however these data seem to point more toward a recruitment model whereby telomere lengthening depends on a temporally-regulated accumulation of several telomerase complexes on a few telomeres (Figure 1). This argument is further supported by the observation that fluorescence intensity of individual T-Recs increases throughout S phase.

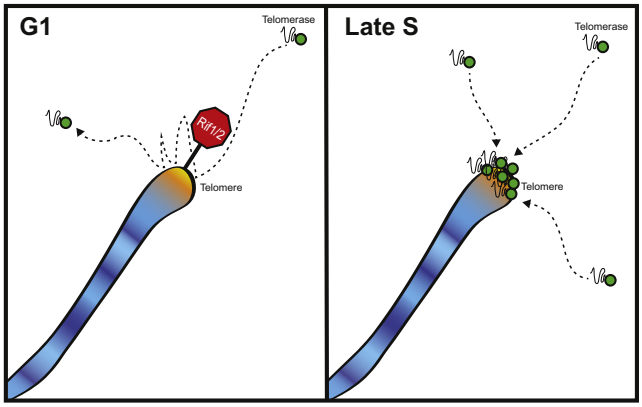

Figure 1. Cell Cycle-Dependent T-Rec Formation
Telomerase complexes do not stably associate with telomeres outside of S phase. During late S phase, telomere elongation occurs via recruitment of multiple telomerase complexes to the shortest telomeres. Rif1/2 proteins are believed to control the cell cycle-dependent nature of this recruitment.

Overall, the occurrence of T-Recs in a given S phase cell is quite rare. While it is believed that only 6%–8% of yeast telomeres are elongated per cell division (Teixeira et al., 2004), it still seems surprising that the vast majority of S phase cells show only 0 or 1 T-Rec. This curiosity is more pronounced in the final experiment described in the Gallardo et al. study. Cells harboring temperature sensitive *yku70*-30 can be used to temporarily shorten telomeres. Upon shifting back to permissive temperatures, the authors observe that the distribution of T-Rec occurrence was shifted to higher numbers, as expected. However, under these conditions, all telomeres would require lengthening, and the authors witness a relatively minor distribution shift. It seems likely that a threshold for telomere shortening must be crossed in order to initiate T-Rec formation, but the details of how and under what conditions this process is initiated remain unresolved.

The specifics of telomerase recruitment and the exact role of the various interacting proteins have yet to be elucidated. However, this study highlights the power of in vivo imaging approaches over ensemble biochemical methods, such as ChIP. The spatiotemporal resolution of such experiments allows the authors to scrutinize telomerase kinetics in real time. A fully comprehensive view of this fundamental cellular process ultimately relies on the marriage of both cell biology and biochemical techniques.

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