the cycling of the *PDK4* gene, which has a periodicity of 60 min. The ability to explain this phenomenon by using the association and disassociation rates of the individual components is remarkable in its simplicity.

Another important question addressed in the manuscript is the relationship between the timing of transcription factor binding and function. Can the temporal binding patterns of transcription factors and other events at the promoter be used to gain insight into their function during the transcription cycles? To answer this question, Degenhardt et al. performed hierarchical clustering analysis on the DNA-binding kinetic profiles of each of the components of the transcriptional machinery tested. Notably, the transcription factors fall into three distinct groups correlating with their roles in the transcription process: a "deactivation group" consisting of HDAC1 and HDAC2; an "activation group" consisting of SMARCA2, H3K3me3, BRG1, AcH3, CARM1, PPARδ, and CBP; and an "initiation group" consisting of TBL1, pPolII, TRAP220, RAC3, and acH3K9. Of special interest is the clustering of RAC3 with PolII as RAC3 is known to be involved in chromatin remodeling. Thus, the temporal association of RAC3 with PolII suggests that RAC3 plays a previously unknown role in transcriptional initiation.

Taken together these and other data raise a number of important questions that need to be addressed. For example, why are genes transcribed in bursts? As many more genes are studied at the single-cell level it becomes apparent that cyclic gene expression is more common than originally thought. This is due to oscillatory molecular interactions in the cytoplasm and nucleus that are integrated somehow with promoters to produce periodicity in mRNA expression. The obvious question is why are genes expressed in a cyclic and not continuous fashion? What are the advantages of such a complex mode of transcription? We propose that transcriptional bursts have been selected to better control gene transcription. As activation and deactivation of a cycling promoter occur many times during its expression phase, there are numerous windows of opportunity for transcriptional silencing complexes to generate a nearly irreversible (nonactivatable) chromatin environment, thus establishing long-term epigenetic repression. This hypothesis predicts that complexes that repress transcription can exist at low cellular concentrations and act in a stochastic manner even in the presence of strong activators. In the alternative scenario, a gene that is constantly transcribed can only be turned off when the complexes that repress transcription outcompete those that activate transcription. Our ability to visualize and quantitate transcriptional processes in individual cells should clarify the effects of transcriptional bursting and its roles in the regulation of gene expression.

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Human Telomerase Caught in the Act

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Based on prior work, it was expected that telomerase would preferentially elongate the shortest telomeres in a cell, extending the telomeric G-rich strand through a process that is coupled to the synthesis of the complementary strand. Contrary to this view, Zhao et al. (2009) now show that telomerase in human cancer cells extends most telomeres during every S phase and that complementary strand synthesis does not immediately follow telomerase action.

Telomere length depends on the balance between telomere synthesis and resection. Telomere elongation occurs through the addition of G-rich repeats by the enzyme telomerase, followed by synthesis of the C-rich complementary strand (C strand) of DNA. Meanwhile, incomplete lagging strand synthesis and resection of the C strand contribute to telomere shortening. Attempts to modulate telomere dynamics in treating diseases of aging and cancer depend on understanding these fundamental processes.

In this issue of *Cell*, Zhao et al. (2009) report that under conditions of telomere maintenance in cultured human cancer cells, telomerase acts indiscriminately at most chromosome ends during each S phase (Figure 1). In addition, whereas the extension of the telomeric G-rich strand (G strand) is coupled to telomere replication throughout S phase, the authors find that synthesis of the C strand is delayed and occurs through a stepwise process distinct from the conventional mechanism of lagging strand replication (Figure 1). These observations are particularly exciting because telomerase was expected to preferentially elongate the shortest telomeres in a cell, and C strand synthesis was thought to immediately follow G strand extension.

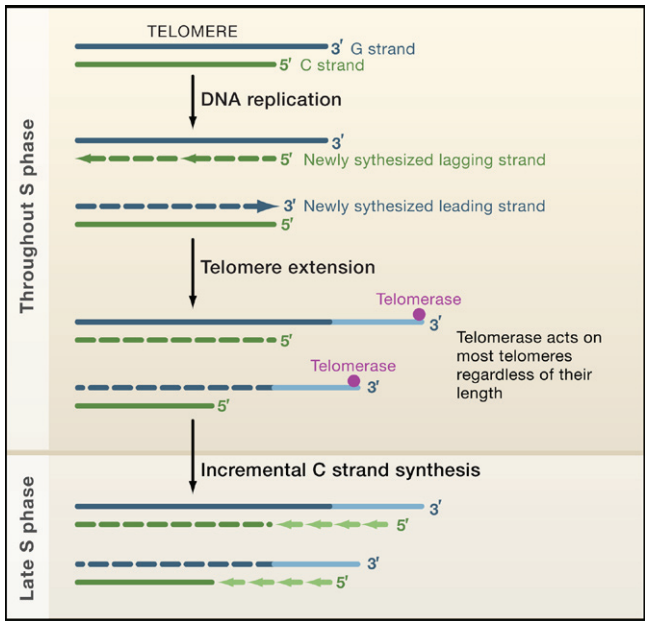

Figure 1. Telomere Elongation in Human Cancer Cells
Zhao et al. (2009) show that in cultured human cancer cells, telomerase acts on most telomeres regardless of length, including the products of both lagging and leading strand replication. While extension of the telomeric G-rich strand (G strand) by telomerase is temporally coupled to telomere replication throughout S phase, synthesis of the complementary C-rich strand (C strand) is substantially delayed until late S phase. Furthermore, C strand synthesis occurs incrementally through a process that is distinct from conventional lagging strand synthesis.

The prevailing model for telomerase action originates from studies in yeast and ciliates, model organisms in which telomeric sequences and telomerase were first identified. In budding yeast, telomere replication, telomerase action, and synthesis of the complementary C strand all occur during late S phase in a series of tightly coupled steps. During each cell cycle, telomerase adds G-rich repeats to the 3′ ends of a subset of telomeres. Several studies have shown that yeast telomerase preferentially associates with short telomeres (reviewed in Bianchi and Shore, 2008) and, consequently, that the shortest telomeres have the greatest probability of being extended (Marcand et al., 1999; Teixeira et al., 2004). Telomere elongation requires DNA polymerases α and δ, as well as primase, the enzyme that synthesizes the RNA primer during lagging strand replication, suggesting that telomerase action is coupled to the semiconservative replication of telomeric DNA (Diede and Gottschling, 1999). Similarly, in the ciliate *Euplotes crassus*, the C and G strands of telomeres are coordinately synthesized in a process that depends on DNA polymerases (Fan and Price, 1997).

Despite progress in understanding telomerase action in model organisms, much remains unknown about the biogenesis of the human telomerase complex and its mechanism of action. Human telomerase localizes to telomeres during S phase (Tomlinson et al., 2006). The active telomerase complex assembles in Cajal bodies and has many components that have only recently been identified (for example, see Venteicher et al., 2009). Although inactive in most adult somatic tissues, telomerase endows immortal cells, including germ cells and most cancer cells, with the ability to divide indefinitely. Although compounds that inhibit telomerase appear to be promising for treating cancer, the development of therapeutics with greater specificity and efficacy requires an understanding of the mechanistic details of telomerase biogenesis and action in human cells.

Zhao and colleagues take advantage of the fact that human telomeres replicate throughout S phase to distinguish between processes that are coupled to replication and those that are delayed. They synchronize cells at the G1/S transition and analyze the telomeres at different time points after the cells are released into S phase. Their initial analyses include measurement of the G- and C-rich strands on individual telomeres, separation of leading and lagging strand telomeres by bromodeoxyuridine (BrdU) pulse labeling, and assays to detect the G strand overhang. These sophisticated techniques reveal the time intervals during which the overhang is elongated by telomerase, as well as when the complementary strand is synthesized.

To more carefully examine the timing of telomere replication, telomerase action, and C strand synthesis, Zhao et al. pulse label replicating telomeres with BrdU and analyze the G strand overhang at different time points in S phase. They isolate the single-stranded telomeric overhangs by digesting genomic DNA with a nuclease specific for double-stranded DNA (duplex-specific nuclease) and use cesium chloride (CsCl) density gradients to separate the unlabeled overhangs of lagging strands from the BrdU-labeled overhangs of leading strands. The authors detect and plot the telomeric signal in each fraction as a function of density, revealing different patterns of low, intermediate, and high-density peaks at different time points in S phase, corresponding to the various steps of telomere synthesis. Low-density peaks represent overhangs on the lagging strand, whereas high-density peaks represent the BrdU-containing leading strand overhangs. The intermediate peaks represent lagging strand overhangs that were extended by telomerase during the BrdU pulse. Zhao et al. observe that over the course of S phase, the intermediate peak disappears because C strand synthesis converts part of the extended overhangs on the lagging strands into double-stranded DNA, which is removed by duplex-specific nuclease digestion before the density gradient analysis.

Through this laborious experimental procedure and careful analysis, Zhao and colleagues determine that ~70%–100% of chromosome ends, including those of both leading and lagging strands, are extended by 50 nucleotides during each cell cycle. This contrasts with yeast telomerase, which extends only ~7% of telomeres by 44 nucleotides during each S phase. Importantly, completion of C strand synthesis in human cells is delayed until 6–8 hr into S phase and occurs in incremental steps. This conclusion is based on direct measurement of the lengths of both the telomeric C strand and the G strand overhang at different time points during S phase, as well as the observed disappearance of the intermediate density peak in late S phase.

These findings have implications for cancer therapies that target cellular life span. Strategies to modify telomere length and telomerase activity must account for the fact that human telomerase acts on all chromosome ends and does not appear to preferentially elongate only the shortest of the telomeres. Also, the observation that C strand synthesis appears both mechanistically and temporally distinct from DNA replication provides a promising new avenue for research in potential therapeutics. It suggests that this step in DNA synthesis may be unique to telomeres and thus can be disrupted without affecting general lagging strand synthesis during DNA replication.

Why are these aspects of telomere synthesis not conserved between different organisms? As mammals evolved ways to accelerate telomere shortening in order to suppress tumor growth, telomerase may have coevolved to counteract this faster rate of telomere loss. Indeed, the interspecies differences in the fraction of telomeres at which telomerase acts is consistent with the different rates of telomere shortening observed in the absence of telomerase. Whereas yeast telomeres shorten at ~3 base pairs per chromosome end per cell division, human telomeres lose ~50 base pairs per chromosome end per cell division. In order to maintain telomere length in the germline and in other immortal cell types, mammalian telomerase must either add more nucleotides per telomere or act on more telomeres per cell cycle in comparison to yeast. Regarding the coupling of C strand synthesis to telomerase action seen in yeast, it has been suggested that this coregulation may prevent the harmful formation of excess single-stranded DNA that could activate DNA damage checkpoints (Diede and Gottschling, 1999). However, the long G strand overhangs that are generated because of delayed C strand synthesis may not greatly affect mammalian cells, because normal mammalian telomeres contain longer overhangs that are protected from activating DNA damage responses. Overall, the findings of Zhao et al. highlight the increasing awareness that although model organisms have been exceedingly useful in implicating pathway components and providing conceptual frameworks, findings in these systems may not be globally applicable. As tumor suppression gained priority in multicellular organisms with longer life spans, mechanisms of telomere length maintenance in different organisms may have diverged.

The elegant elucidation of the action of human telomerase by Zhao and colleagues raises important questions for future research. The machinery that completes C strand synthesis, as well as the regulation of this delayed step, requires elucidation. Because many telomere binding proteins regulate telomerase in *cis* (Smogorzewska and de Lange, 2004), it will also be interesting to determine whether these proteins affect the ability of telomerase to act on all chromosome ends and whether they regulate C strand synthesis. Finally, as the mechanism of telomere elongation becomes clearer, there remains the equally important problem of understanding telomere shortening in human cells by characterizing the as yet unknown nucleases that resect the telomeric C strand.

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