Cell, Vol. 117, 279–283, April 30, 2004, Copyright ©2004 by Cell Press

Telomerase Regulation at the Telomere: A Binary Switch

Telomerase is known to preferentially elongate the shortest telomeres in a cell. Using an elegant yeast assay, Texeira et al. (2004 [this issue of *Cell*]) address what aspect of telomerase action is regulated by telomere length: the frequency or the extent of telomere elongation. They show that short telomeres are elongated more frequently than long telomeres, arguing that telomeres switch between two states, one that allows telomere extension and one that does not.

Telomere maintenance by telomerase is critical for chromosome integrity and stability. Telomerase-deficient cells undergo gradual telomere shortening and eventually enter a senescent state. Reintroduction of telomerase leads to recovery of normal telomere maintenance and infinite replicative potential.

It has long been known that telomere length is regulated by a negative feedback loop (reviewed in Smogorzewska and de Lange, 2004). Long telomeres contain more negative regulators that limit further telomere elongation by telomerase. Because of this, the enzyme preferentially acts on the shortest telomeres in the cell (Hemann et al., 2001; Zhu et al., 1998). Much is known about the telomere binding proteins that execute this homeostasis pathway, but it was not known what aspect of telomerase action was regulated. Perhaps both long and short telomeres recruit telomerase in every S phase but the shorter telomeres allow the enzyme to add more telomeric repeats. Alternatively, one could imagine that telomerase only acts on a subset of telomeres in each cell division, showing preference for the shortest ends and leaving some telomeres—the longer ones—untouched. The latter case would be indicative of telomeres switching between two states, one extendible by telomerase, the other not.

Teixeira et al. (2004) use a beautiful genetic trick to distinguish between the two models in *Saccharomyces cerevisiae*: they start with a yeast strain deficient for telomerase and reintroduce the enzyme by mating with a wild-type strain. The telomeres of a marked chromosome end are then PCR amplified, cloned, and sequenced. Using this method, they can follow the elongation of a single telomere at nucleotide resolution literally within minutes after reintroduction of telomerase. This approach works in budding yeast because of an interesting peculiarity of its telomerase that produces slightly different repeats of $TG_{1\text{-}3}$ every time it synthesizes telomeric DNA. This variability is sufficient to distinguish the old telomeric sequence already in place at the marked telomere and those repeats that were added after mating.

The results obtained by the Lingner group are very clear. Their sequencing data show that telomerase does not elongate all telomeres at the same time. Within one cell cycle, the enzyme only acts on a subset of telomeres ($<$40%) and shows a strong preference ($\sim$6-fold) for the shortest ones. Thus, some telomeres are "open" for telomere addition, and some others are "closed" and the setting of this binary switch depends on telomere length. Short telomeres are more frequently in the open, extendible state, and long telomeres tend to switch to the closed state. But what regulates this state of the telomere, and ultimately their length?

Extensive research in budding yeast, fission yeast, and human cells has revealed a common theme in telomere length regulation (reviewed in Smogorzewska and de Lange, 2004). The critical factors that govern telomere length are proteins that bind to the telomeric repeats, in amounts that are proportional to the number of cognate binding sites present at the chromosome end. In budding yeast for example, the $TG_{1\text{-}3}$ repeat binding protein, Rap1, was long known to be critical for regulating the length of telomeres. Formal proof of *cis*-acting regulation at the telomere was shown by tethering Rap1-Gal4 fusions to Gal4 DNA binding sites of various lengths integrated in close proximity to a telomere (Marcand et al., 1997). The artificially recruited Rap1p acted as a negative regulator of telomere length (i.e., telomeres shortened) to an extent proportional to the amount of Rap1 fusion protein present at chromosome ends. Thus, Rap1 is involved in a counting mechanism, transmitting information about the length of the telomere to telomerase and determining the probability of elongation. Further work has established that the "counting" of repeats performed by bound Rap1 is mediated by two Rap1-interacting proteins, Rif1 and Rif2 (Hardy et al., 1992; Wotton and Shore, 1997). It is unknown how these factors act in inhibiting telomerase.

The work by Teixeira et al. now tells us what is regulated by these homeostasis pathways. Their results show that proteins like Rif1 and Rif2 affect the frequency of telomere elongation by telomerase. When Rif1 or Rif2 is absent, all telomeres (long and short) have a greater tendency to switch to the extendible state. Thus, the 'protein counting' pathway ultimately determines the state of the telomere. The extent to which telomeres get elongated is not different in *rif1*Δ or *rif2*Δ cells, but the telomeres get elongated much more often. As a consequence, the overall length of all the telomeres becomes reset to a new, longer length when Rif1 or Rif2 is deleted.

The implication is that the telomere length homeostasis pathway acts by controlling a binary switch. When a telomere is long and recruits a lot of the Rap1/Rif1/Rif2 complex, the telomere switches preferentially to a state that cannot be elongated by telomerase. When telomeres get very short and lose most of the Rap1 complex, they more frequently adopt an extendible state.

A similar telomere length homeostasis pathway is at play in human cells. Long human telomeres recruit a larger amount of TRF1, which binds to double-stranded telomeric DNA, and long telomeres contain more POT1, a protein recruited by TRF1. POT1 also binds to the single-stranded 3′ overhang of the telomere, effectively

leading to the downregulation of telomerase at the 3′ end (Loayza and de Lange, 2003). Does POT1 also affect the state of the telomere in a binary manner? Are long human telomeres visited less often by telomerase, as in yeast? Or are long telomeres more likely to limit the extent of their elongation? Given that human cells lack the nifty features that the Lingner group used to address this problem, it may be a while before we know.

The next challenge in telomere length regulation in yeast will be to define the nature of the nonextendible state: Is the telomere folded so that the 3′ end of chromosomes is hidden from telomerase? Is telomerase always at the telomere, but gets locally inhibited as they get longer? In any event, we are now much closer to seeing the light at the end of the (chromosome) tunnel.

Diego Loayza and Titia de Lange
Laboratory for Cell Biology and Genetics
The Rockefeller University
1230 York Avenue
New York, New York 10021

Selected Reading

Hardy, C.F., Sussel, L., and Shore, D. (1992). Genes Dev. *6*, 801–814.

Hemann, M.T., Strong, M.A., Hao, L.Y., and Greider, C.W. (2001). Cell *107*, 67–77.

Loayza, D., and de Lange, T. (2003). Nature *424*, 1013–1018.

Marcand, S., Gilson, E., and Shore, D. (1997). Science *275*, 986–990.

Smogorzewska, A., and de Lange, T. (2004). Annu. Rev. Biochem. *73*, 177–208.

Teixeira, M.T., Arneric, M., Sperisen, P., Lingner, J. (2004). Cell *117*, this issue, 323–335.

Wotton, D., and Shore, D. (1997). Genes Dev. *11*, 748–760.

Zhu, L., Hathcock, K.S., Hande, P., Lansdorp, P.M., Seldin, M.F., and Hodes, R.J. (1998). Proc. Natl. Acad. Sci. USA *95*, 8648–8653.

Hitchhiking without Covalent Integration

In eukaryotes, many latent viruses attach to mitotic chromosomes noncovalently for effective partitioning in dividing cells. For different viruses, the *cis* and *trans* elements encoded by the episomes have been effectively defined but the chromosomal "receptors" for such tethering have remained elusive. In this issue of *Cell*, You et al. (2004) give us a first insight into the cellular protein machinery important for animal papillomavirus retention.

In a historically important work, Lwoff and Gutman (Lwoff and Gutmann, 1950) showed that with lysogenic strains of bacteria, each cell harbored within a noninfective structure, a prophage, that enabled the organism to give rise to virus particles without exogenous infection. It was Alan Campbell who first proposed that the prophage came about by genetic recombination between a vegetative phage and the host chromosome (Campbell, 1962). This paradigm system provided a marvelous springboard for the study of among other things site-specific recombination, gene expression, and DNA replication. It also provided models for thinking about latent viral infections in higher eukaryotes. I can remember being inspired by the papers of Renato Dulbecco as a graduate student while studying phage λ molecular biology in Hatch Echols' class. Dulbecco, with his students, had used the prophage ideas to attack the question of how the small DNA viruses such as Polyoma and SV-40 managed to infect certain mammalian cells and essentially disappear although persistently changing the growth properties of the infected cell. The analogy to the classical prophage was not a very good one in the end and by and large covalent integration is a dead end for all nondefective animal DNA viruses so far studied.

The exception to this generalization seems to be provided by the defective or so called "dependoviruses" such as the human adeno-associated virus (AAV) that encode for a site-specific endonuclease/helicase required for viral replication and establishment of latency. AAV can establish a latent infection by integrating into chromosome 19 as tandem head to tail copies. The provirus then waits for subsequent rescue by a superinfecting helper adenovirus (Muzyczka and Berns, 2001).

A large number of very successful viruses—in that there are many different evolutionary variants infecting many different cell types—including the papillomaviruses (Pv) and the γ-herpesviruses do establish a natural latency as plasmids in the nuclei of dividing cells. During the initial infection phases, these viruses attempt to get their nucleoproteins into the nucleus and once there, an essential function is to keep the viral episomes in the nuclear space. This is especially important upon the breakdown of the nuclear membrane during the mitotic phase of the cell cycle. This maintenance is established by hooking onto another structure that has the ability to persist in the nuclear space even without the membrane. Mutations in the viral factors that mediate this function lead to catastrophic loss of the plasmids, even for high copy number plasmids such as those maintained in Bovine papillomaviral (BPV-1) transformed cells. As this viral plasmid can amplify its genome after segregation mistakes, it would seem that this rapid curing reflects the inefficient re-entry of the genome back into the nucleus if the hitchhiking fails.

The mechanism used by the Epstein-Barr virus, the human herpesvirus-8, and BPV-1 is attachment to the cellular chromosomes. All of these viruses encode for site-specific DNA binding proteins that recognize repeated DNA motifs near the viral origins of plasmid replication. Further, these viral site-specific binding proteins play dual functions that are critical for plasmid replication during interphase and for tethering during mitosis. In the case of BPV-1, the enhancer protein E2 serves as a matchmaker, bringing the DNA replication initiator/helicase E1 to the viral origin during S phase. During mitosis, an intriguing sandwich is made. Cytological approaches reveal that E2 must be bound to both the viral plasmid and the cellular chromosome throughout mitosis. The E1 protein likely competes for E2 activation domain binding with a cellular protein(s) important for tethering as overexpression of E1 pulls E2 off the chromosomes (Voitenleitner and Botchan, 2002). How does this tethering mechanism work, is it regulated, and what