It's getting HOT at telomeres

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How telomerase and telomeres are brought together to alleviate telomere shortening and preserve genome integrity in the cell remains incompletely understood. A study in *The EMBO Journal* now identifies HOT1 as a direct telomere repeat binding protein that contributes to telomerase-mediated telomere elongation, likely by bridging telomere and telomerase interactions.

In contrast to the usually circular DNA of prokaryotes, eukaryotes maintain their genetic information in the form of linear chromosomes, whose extremities assemble complex structures called telomeres, consisting of repetitive DNA sequences and associated proteins. Telomeres serve two major functions: (1) to prevent chromosome ends from being recognized as broken DNA and triggering checkpoint responses, which would lead to deleterious chromosome end-to-end fusions and (2) to protect genetic material from degradation by cellular nucleases. Protection of chromosome

ends from fusion is coordinated by the shelterin complex, which consists of six proteins: TRF1, TRF2, POT1, RAP1, TIN2 and TPP1. Both TRF1 and TRF2 can bind directly to double-stranded telomeric DNA with sequence specificity, while POT1 binds to single-stranded TTAGGG repeats. The shelterin complex acts to suppress DNA damage responses at telomeres, which would result from recognition of the ends of linear chromosomes as double-strand breaks. As this function is required at all telomeres, almost throughout the cell cycle, the shelterin proteins are intrinsic and permanent components of telomeres (reviewed in Martínez and Blasco, 2011).

The second challenge of functional telomeres is to protect the genetic material from degradation. To achieve this, telomeric repeats must be constantly replenished at chromosome ends to prevent attrition of the telomeric tract. This is facilitated by transient telomere uncapping during every S phase of the cell cycle, when telomeres are replicated

Figure 1 Model for recruitment of telomerase to chromosome ends facilitated by HOT1. HOT1 binds to telomeric DNA with sequence specificity and also interacts with coilin, thus mediating telomere translocation into the Cajal bodies. Telomerase holoenzyme assembles into the Cajal bodies where it interacts with HOT1-bound telomeres, which accumulate in the same nuclear compartment. Bringing telomerase and telomeres in close proximity enables telomerase access to telomeric repeats, likely via a direct interaction with TPP1, and initiates the elongation reaction.

and/or elongated by the enzyme telomerase and become prone to degradation by cellular nucleases. Furthermore, active nucleolytic end processing is required to generate a single-stranded 3'-overhang after every round of replication. Although this is a prerequisite for end protection and elongation, end processing can also contribute to telomere shortening. A more substantial threat is the gradual shortening of telomeres with every cell division. This steady loss of telomeric DNA has originally been defined as the 'end replication problem' and is based on the inability of DNA polymerases to fully replicate chromosome ends. Telomere shortening continues until at least one telomere within a cell reaches a critically low length and subsequently triggers a DNA damage signal leading to cellular senescence. In all cases, loss of telomeric DNA is counteracted by active telomere lengthening, most prominently through activation of telomerase, an RNA-templated reverse transcriptase that adds telomeric sequence repeats to the single-stranded telomere overhang (reviewed in Collins, 2006). Telomere elongation by telomerase is highly regulated at several levels, including telomerase recruitment, activity and processivity control. First, the catalytic subunit TERT and the RNA component TERC have to be brought together, which in human cells is believed to take place in Cajal bodies (Jády *et al*, 2006). Telomerase-containing Cajal bodies can then be recruited to telomeres. Second, several studies have shown that the shelterin component TPP1 acts as a telomerase recruitment factor. Indeed, loss of TPP1 leads to reduced association of telomerase with telomeres (reviewed in Nandakumar and Cech, 2013). In addition, TPP1-POT1 complexes enhance telomerase processivity on telomeric substrates *in vitro* (Wang *et al*, 2007), suggestive of a positive regulation of telomere length. Finally, telomerase should act only once per cell cycle on individual telomeres, a feature that has recently been shown to be regulated by the CST complex (Chen *et al*, 2012). However, how exactly telomeres are brought together with telomerase in the Cajal bodies is not well understood.

In this issue of *The EMBO Journal*, Kappei *et al* (2013) identify HOTA1 (homeobox telomere binding protein 1) as a factor that binds to both telomeres and telomerase to bring them in close proximity, and that positively regulates telomere length. HOTA1 had been previously isolated as a telomeric component using proteomics of isolated chromatin segments, but no function had been assigned (Déjardin and Kingston, 2009). Using SILAC-based quantitative mass spectrometry, Kappei *et al* (2013) establish a straightforward screening approach for the identification of proteins that directly bind to double-stranded telomeric DNA. As a proof-of-concept, the assay is validated by detection of all shelterin members. Additionally, the authors identify the protein HOTA1 as a factor with equally strong affinity for double-stranded DNA with telomeric sequences. This stringent and selective assay suggested the possibility that HOTA1 is in fact a direct telomere-binder. In subsequent experiments, the authors demonstrate direct binding of HOTA1 in an *in vitro* binding assay. Finally, by solving the co-crystal structure of the HOTA1 DNA-binding domain in complex with telomeric DNA, they identify residues critical for this interaction. Similarly to TRF1 and TRF2, HOTA1 recognizes telomeres by means of a homeodomain (Court *et al*, 2005). However, unlike the TRF proteins, HOTA1 does not appear to be a constitutive component of the telomere complex and does not associate

with all telomeres in the cell at once. Using immunofluorescence staining, Kappei *et al* (2013) show that the degree of HOTA1-telomere association varies between different cellular settings, from few HOTA1-labelled telomeres in HeLa cells, to an intermediate degree in mouse embryonic stem cells and binding to almost every telomere in mouse spermatocytes. As HOTA1 *per se* can directly bind to telomeric DNA, it seems likely that this differential binding is itself regulated. One simple possibility is that a post-translational modification of HOTA1 (e.g., phosphorylation) could modulate its binding behaviour. Addressing this will clearly be a key avenue to further our understanding of the mechanistic contribution of HOTA1 to telomere maintenance.

The variability in HOTA1 association with the telomeres may reflect dynamic binding and variable degrees of elongation at individual telomeres. The authors propose that HOTA1 preferentially binds to actively processed telomeres or even contributes to the availability of telomeres for elongation by bringing together telomerase and telomeres that need to be elongated (Figure 1). Whether HOTA1 identifies the shortest telomeres in the population remains to be established. Currently, three lines of evidence support a role for HOTA1 in facilitating telomerase translocation to the telomere where the catalytically active holoenzyme is assembled. First, HOTA1 is a positive regulator of telomere length based on both overexpression and knockdown experiments. Second, HOTA1 localizes to the periphery of Cajal bodies, a pattern of striking resemblance to that of telomerase-telomere complexes (Jády *et al*, 2006). Moreover, HOTA1 is found in complex with coilin, a Cajal body structural component, which may mediate recruitment of HOTA1-bound telomeres to Cajal bodies. Third, HOTA1 immunoprecipitation enriches both for active telomerase complex components (DKC1, GAR1, NHP2 and NOP10) and for telomerase activity *per se*. Importantly, using *Hot1*-null mouse embryonic fibroblasts, Kappei *et al* (2013) were able to show that HOTA1 is required for TERT binding to chromatin. The authors propose that HOTA1 is an additional factor contributing to telomerase recruitment. Whether this occurs synergistically with TPP1-dependent recruitment remains to be determined. While this is a very exciting discovery in particular, because the dynamic binding HOTA1 to telomeres resembles the well-characterized telomerase association only with a subset of telomeres, the model needs further testing and refinement. For instance, it will be important to test by triple immunofluorescence staining whether telomeres, telomerase and HOTA1 are indeed found together in foci localized at the periphery of Cajal bodies. The model might further be strengthened by epistasis analysis with members of the active telomerase holoenzyme, and by monitoring the effects of HOTA1 loss of function in human primary, telomerase-negative cells. In this context, it will also be interesting to see whether the *Hot1*-null mice phenocopy the effects and kinetics of telomere erosion seen in telomerase-negative mice.

One intriguing aspect of this study is the lack of interaction between HOTA1 and the shelterin complex. While HOTA1 and shelterin proteins co-localize at telomeres in immunofluorescence staining, Kappei *et al* (2013) have not identified any shelterin component in their HOTA1 immunoprecipitation experiments. Conversely, HOTA1 has not been detected in pull-down reactions with shelterin antibodies in any previous study so far. Conceivably, HOTA1, TRF1 and TRF2 may compete

for the same binding sites at telomeres, as they all share the specificity for double-stranded telomeric DNA sequences. Measuring telomere occupancy for each of these factors using chromatin immunoprecipitation assays and/or determining their distribution along telomeres using super resolution microscopy in wild-type cells or cells lacking HOT1 or shelterin components should help solve this conundrum. As for the relationship with the shelterin complex, it will be particularly relevant to identify any potential functional interactions between HOT1 and TPP1. Both proteins contribute to telomerase recruitment and dissecting their roles relative to each other will be of fundamental importance for our mechanistic understanding of this process. One possible scenario is sequential action of the two factors, that is, dynamic telomere binding by HOT1 promotes telomerase–telomere interaction within the Cajal bodies (Figure 1), whereas TPP1 as a constitutive shelterin component stabilizes telomerase once its interaction with telomeres has been established. Such a model certainly requires rigorous testing, and novel experimental approaches may have to be

developed in order to differentiate between telomerase delivery to telomeres and telomerase retention at telomeres.

In summary, Kappei *et al* (2013) discovered HOT1 as a new key player in telomere length maintenance and telomerase regulator. It is remarkable that more than 15 years after the discovery of the TRF proteins, a third factor sharing their specificity for direct binding to telomeric double-stranded DNA is now isolated. This discovery opens an exciting new angle to several aspects of telomere biology, including how structural, non-shelterin telomere components modulate telomere elongation and subcellular localization. This adds more complexity to our picture of telomeres and supports the emerging view of the telomere as a dynamic and multifaceted chromosomal structure with various (and some yet undiscovered) factors transiently binding to achieve the fine-tuning of telomere functions.

Conflict of interest

The author declares that she has no conflict of interest.

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