Modern genome editing meets telomeres: the many functions of TPP1

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The telomeric complex has been analyzed in detail for its role in regulating telomere protection and telomere length. Now, modern genome-editing techniques in human embryonic stem cells reveal TPP1 as the essential recruitment factor for telomerase, with additional functions in telomerase activation and definition of telomere length homeostasis.

Until relatively recently, we knew of just a single mammalian telomeric protein. In 1995, TRF1 (telomeric repeat-binding factor 1) was cloned from HeLa cultures by its ability to tightly bind TTAGGG repeats. Two years later, TRF1 was demonstrated to regulate telomere length homeostasis; overexpression shortened telomeres in telomerase-positive cells, whereas removal of the factor from chromosome ends with a dominant-negative allele led to telomere elongation [van Steensel and de Lange 1997]. TRF2 was also discovered in 1997 by its homology with the TRF1 DNA-binding domain. Removal of TRF2 from the telomere, however, had very different consequences: It led to chromosome fusions, indicating that TRF2 played a major role in protecting natural chromosome ends from being recognized as DNA damage and being repaired [van Steensel et al. 1998]. Thus, TRF1 and TRF2 regulated the two major functions of telomeres: TRF1 controlled telomere length, and TRF2 controlled chromosome end protection. Thus began the discovery of the telomeric complex.

The next factor to emerge was the enzyme Tankyrase, which interacted with TRF1 and had polynucleotide diphosphate-ribose polymerase (PARP) activity. Tankyrase controlled TRF1 binding to telomeric DNA and therefore played a role in telomere length regulation [Smith et al. 1998]. TIN2 [TRF1-interacting nuclear protein 2], another TRF1-binding partner, similarly affected telomere length. In 2000, the first TRF2-interacting factor was identified as the homolog of Saccharomyces cerevisiae RAP1. In yeast, RAP1 directly binds to telomeric DNA, but in humans, it is recruited to chromosome ends by its interaction with TRF2, suggesting different evolutionary pathways of the telomeric complex. Human POT1 was found to interact with the telomeric single-stranded G tail as well as with TRF1 and therefore serves as a transducer of TRF1 telomere length control. Three laboratories independently identified TPP1, a bridging factor between the TRF1 and the TRF2 complexes. The composite of these six factors—TRF1, TRF2, TIN2, RAP1, POT1, and TPP1—was ultimately named shelterin [de Lange 2005].

Functional dissection of the shelterin complex required mouse knockouts. In fact, increasingly complex knockout approaches provided insight into how members of the complex interacted with each other and regulated various DNA damage pathways throughout the cell cycle as well as what roles the factors played in establishing telomere structure and function and preventing fusion of functional telomeres [de Lange 2010]. However, due to inherent differences between mouse and human telomeres, many questions remained unanswered. The laboratory mouse strains used for targeted deletions have very long telomeres, and somatic mouse cells exhibit telomerase activity. As a consequence, telomeres do not shorten enough during the animal’s life span to impact cell division, and thus replicative telomere shortening does not act as a tumor suppressor. Additionally, loss of p53 in mouse cells is sufficient to overcome the growth inhibitory effects of dysfunctional telomeres, whereas, in humans, suppression of both the p53 and pRB pathways is required. Finally, shelterin complex composition in mice differs from the human complex. Mice have two distinct POT1 proteins (POT1a and POT1b), whereas the human genome encodes only one POT1 isoform. Recently developed genome-editing techniques, which enable the targeted manipulation of endogenous human genes, have allowed further delineation of these differences.

TPP1 was originally described as a bridging factor between TRF1 and TRF2 that functioned in a pathway with POT1 as a negative regulator of telomerase-dependent telomere length control [Hockemeyer et al. 2007]. More detailed analysis suggested that TPP1 directly promotes telomerase activity at the telomere through the so-
called TEL patch, a part of the TPP1 oligonucleotide/oligosaccharide-binding (OB) fold that interacts with the catalytic subunit of telomerase, hTERT [Nandakumar et al. 2012; Sexton et al. 2012; Zhong et al. 2012]. However, these studies had limitations, as they relied on either overexpression of mutant proteins in the presence of endogenous wild-type TPP1 or incomplete knockdown of endogenous TPP1. A number of questions were left unanswered: Is the TEL patch essential for telomerase recruitment? Are there redundant ways for telomerase recruitment? What is the composition of the recruitment complex? Do TPP1 or the TEL patch have other functions? Sexton et al. [2014] use sophisticated genome editing of human embryonic stem cells (hESCs) to find some answers.

The Collins and Hockemeyer laboratories [Sexton et al. 2014] collaborated to use zinc finger nucleases in hESCs to heterozygously and homozygously replace the portion of the TEL patch on TPP1 that is required for telomerase interaction. The cells initially tolerated the mutations in the endogenous protein, but long-term culture revealed that the TEL patch mutants eventually displayed shortened telomeres and ultimately died with critically short telomeres. Their rates of telomere shortening and cell death dynamics were identical to hTERT-deleted cells, suggesting that TPP1 is the essential and only pathway required for recruitment of telomerase to chromosome ends.

Expression of wild-type hTERT in hTERT-deficient cells rescued telomere length, but expression of a fusion between the TPP TEL patch mutant and hTERT did not, despite telomeric localization of the fusion protein. Similarly, tethering hTERT to the telomeres in cells harboring the endogenous TEL patch mutation still did not allow telomere elongation and rescue from cell death. These sophisticated approaches clearly identified a novel role for the TEL patch on TPP1: in addition to telomerase recruitment, the TEL patch region is essential for telomerase activation directly at telomeres.

The groups [Sexton et al. 2014] complemented the TPP1 TEL patch-mutated cells with a number of TPP1 point mutation alleles, one of which was Leu104, which resides on the opposite side of the OB fold from the TEL patch. This mutant also localized to telomeres as well as wild-type TPP1 and could rescue the cell death phenotype of TPP1 TEL patch mutants in long-term cultures. Remarkably, telomere length analysis revealed that telomeres were very short, comparable with those in TPP1 TEL patch mutant cells at the time of their death. However, no DNA damage markers were detected at the short telomeres in the L104 mutants, suggesting that the short telomeres were functional and had been maintained—just at a much shorter length setting. Thus, a third function of TPP was discovered: TPP1 not only is required for telomerase recruitment and activation but also defines telomere length homeostasis in hESCs.

Thus, within a few years, aided by ever-increasing sophistication in techniques used to dissect its functions, TPP1 has moved from a simple bridging molecule between TRF1 and TRF2 complexes to a regulator of telomerase recruitment, activation, and regulation. Undoubtedly, our current understanding of the functions of shelterin components is incomplete. Considering TPP1 as an example, we can expect many more exciting and unanticipated discoveries from clever genome editing of telomeric protein-encoding genes in human cells.

References


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