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Review article

How stem cells keep telomeres in check

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ABSTRACT

In multicellular organisms, regulation of telomere length in pluripotent stem cells is critical to ensure organism development and survival. Telomeres consist of repetitive DNA that are progressively lost with each cellular division. When telomeres become critically short, they activate a DNA damage response that results in cell cycle arrest. To counteract telomere attrition, pluripotent stem cells are equipped with telomere elongation mechanisms that ensure prolonged proliferation capacity and self-renewal capacity. Excessive telomere elongation can also be deleterious and is counteracted by a rapid telomere deletion mechanism termed telomere trimming. While the consequences of critically short telomeres are well established, we are only beginning to understand the mechanisms that counteract excessive telomere elongation. The balance between telomere elongation and shortening determine the telomere length set point in pluripotent stem cells and ensures sustained proliferative potential without causing chromosome instability.

1. Telomere length regulation

Telomeres are essential nucleoprotein structures required to cap and protect chromosome ends. In mammals, telomeres consist of repetitive [TTAGGG]n sequences that represent the binding site of a protective protein complex called shelterin (de Lange, 2005). Shelterin is a six-protein complex containing two double-stranded binding proteins TRF1 and TRF2 that specifically recruit the rest of the complex (TIN2-TPP1-POT1 and RAP1) to chromosome ends. The shelterin complex shapes telomeric DNA into a lasso–like secondary "t-loop" that results from invasion of the single-stranded 3′ telomeric overhang into the double stranded telomeric region (Doksani et al., 2013; Griffith et al., 1999). T-loop structures as well as binding of the shelterin complex ensure chromosome end protection from nucleolytic degradation and activation of the DNA damage response (Denchi, 2009; Denchi and de Lange, 2007; Doksani et al., 2013; Karlseder et al., 2004; Okamoto et al., 2013; Sfeir and de Lange, 2012). Gradual telomere shortening occurs with each cellular division due to the inability of DNA polymerases to completely replicate a linear template, the so-called "end replication problem" (Olovnikov, 1973; Watson, 1972). As a result, replicating cells undergo progressive telomere attrition that, if not counteracted by telomere elongating mechanisms, results in critically short telomeres that do not recruit sufficient shelterin complex. Telomere length is a major determinant of the proliferation potential in cells that lack telomere lengthening mechanisms such as human somatic cell lines.

Telomere elongation is ensured by telomerase, an enzyme composed of the reverse transcriptase TERT and an RNA template, TERC, as well as associating proteins such as the ribonucleoprotein Dyskerin (Cohen et al., 2007). Telomerase performs *de novo* addition of TTAGGG repeats to chromosome ends, allowing replenishment of terminal sequences lost due to the end replication problem (Blackburn, 1997) (Fig. 1). As a result, telomere elongation in germ cells and stem cells is critical to ensure sufficient cellular divisions for development, tissue turnover and tissue regeneration. In long-lived mammals such as humans, telomerase expression is repressed in most somatic tissues (Gomes et al., 2011). A set of human diseases associated with defective telomere elongation are collectively called Telomere Biology Disorders (TBD), which highlight the importance of proper telomere length regulation (Savage, 2014). Patients affected by these diseases have critically short telomeres and, depending on the severity of the disease, display symptoms associated with defective cellular proliferation (Savage, 2014).

Telomeres can also be extended in a telomerase-independent manner by a recombination-based process termed alternative lengthening of telomeres (ALT) (Bryan et al., 1997) (Fig. 1). A significant fraction of cancer cells (approx. 15%) do not express telomerase and maintain telomeres using the ALT pathway. ALT engages the homologous recombination machinery to use telomeric sequences as a template for telomere extension (Dunham et al., 2000). Interestingly, ALT has also been reported to occur in non-transformed mouse somatic cells (Neumann et al., 2013). Furthermore, it has been reported that ALT-like mechanisms are active during early stages of embryogenesis (Liu et al.). In these cases, ALT-like features co-occur

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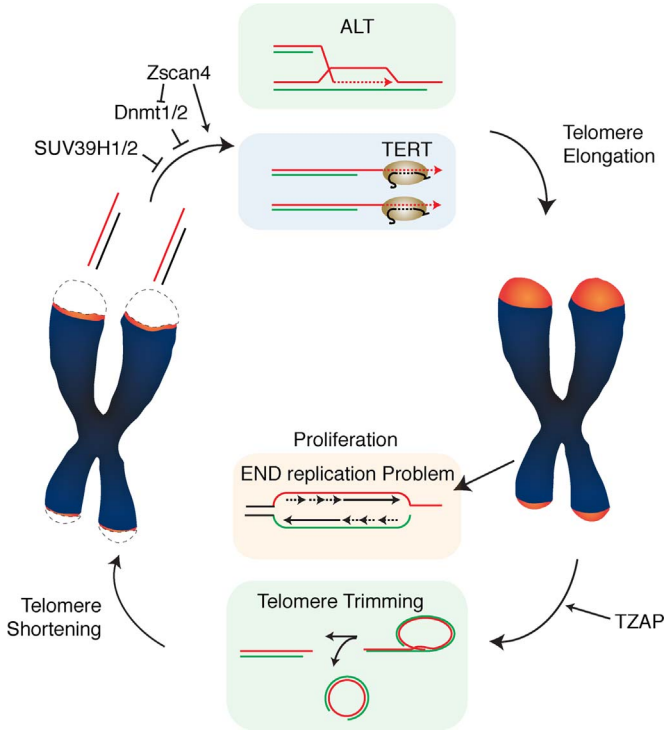

Fig. 1. Balance between telomere elongation and telomere shortening maintain telomere length set-point in pluripotent stem cells. Telomere elongation by telomerase or ALT maintain the lower threshold of telomere length to sustain self-renewal capacity. Telomere trimming by t-loop excision maintains the upper threshold of telomere length.

with telomerase expression. It is currently unclear to what extent these telomerase-independent mechanisms contribute to telomere elongation in normal cells. Finally, telomere elongation is balanced by a process called telomere trimming, which negatively regulates telomere length by actively eliminating excessively long telomeres harmful for genome stability (Pickett et al., 2009) (Fig. 1). While telomere elongation mechanisms that maintain the lower limit of telomere length have been well studied for decades, how the upper limit of telomere length is determined by telomere trimming remains poorly understood. Here we provide an overview of the recent advancements of telomere length control with a particular emphasis on the balance between elongation and trimming in pluripotent stem cells.

2. Telomere elongation in pluripotent stem cells

Pluripotent stem cells such as embryonic stem cells (ESC) are able to self-renew and give rise to virtually any type of somatic cell. ESCs were the first pluripotent stem cells that could be isolated and cultured in vitro (Evans and Kaufman, 1981; Martin, 1981). Mouse ESCs revolutionized the field of mouse genetics based on the fact that they are immortal, can be genetically modified and used to generate any mouse even after months of in vitro culture. Recently, establishment of human ESCs cultures as well as the ability to create “induced” Pluripotent Stem Cells (iPSCs) from somatic cells represent the promise of new transplantation therapies (Takahashi and Yamanaka, 2006; Thomson et al., 1998). ESCs and iPSCs can elongate their telomeres and proliferate indefinitely while maintaining pluripotency and genome stability. Highlighting the importance of telomere homeostasis in these cells is the finding that ESCs with short telomeres show reduced pluripotency and display differentiation defects (Pucci et al., 2013).

Multiple mechanisms ensure telomere elongation in ESCs, including elevated levels of telomerase activity (Thomson et al., 1998). Interestingly, ALT-like activity has been observed in ESCs (Liu et al., 2007). However, given that depletion of telomerase activity ESCs results in critical telomere shortening (Huang et al., 2011; Niida et al., 1998; Pucci et al., 2013) the contribution of ALT-like mechanism to telomere homeostasis in ESCs remains to be established.

A distinguishing feature of ESCs is represented by an “open” telomeric chromatin structure with reduced levels of the heterochromatin markers H3K9me3 and H4K20me3. Lack of these markers suggests that in ESCs telomeric chromatin is “decompacted”, a state that has been linked with increased telomerase-mediated elongation. Indeed, reduction of H3K9me3 by depletion of SUV39H1/H2 or reduction of H4K20me3 by depletion of Suv4–20 h both induce telomere elongation (Benetti et al., 2007; Gomes et al., 2011). Similarly, during mouse development, expression of Zscan4 results in telomere elongation through the reduction of DNA methylation (Dan et al., 2017; Zalzman et al., 2010). In mouse embryonic stem cells, loss of the DNA methyl transferases (Dnmt1, Dnmt3a/3b) results in telomere elongation (Gonzalo et al., 2006). However, in human cells, loss of DNA methyltransferase has a different outcome in terms of telomere length. Patients deficient of DNMT3b have very short telomeres and suffer from the Immunodeficiency, Centromeric instability and Facial anomalies (ICF)-syndrome (Yehezkel et al., 2008). This discrepancy could be due to specific differences between mouse and human cells in terms of telomere length regulation or by additional modifier factors that contribute to telomere shortening in ICF patients.

Interestingly, reduced methylation levels have also been associated with ALT-like activities such as an increase in telomere sister chromatid exchange (TSCE) (Dan et al., 2017; Zalzman et al., 2010). In agreement with this observation, low levels of H3K9me3 and H4K20me3 have also been reported in cancer cells that use the ALT pathway to elongate telomeres (Episkopou et al., 2014). In cancer cells, ALT is correlated with mutations in the ATRX/DAXX remodeling complex and in the histone variant H3.3 (Heaphy et al., 2011; Lovejoy et al., 2012). The ATRX/DAXX complex acts as a chaperone that deposits histone H3.3 at pericentric heterochromatin, telomeres, as well as heterochromatic sites throughout the genome (Voon et al., 2015). Collectively, these data show a clear connection between chromatin status and telomere elongation mechanisms, a topic that has been extensively covered by previous reviews (for further detail see (O'Sullivan and Almouzni, 2014)).

3. Evidence of an upper threshold of telomere length in yeast

While the need to maintain a lower threshold of telomere length has been well established, whether an upper threshold of telomere length is important to maintain genome stability is less clear. Early clues of an upper limit of telomere length came from the analysis of an *S. cerevisiae* strain carrying mutant alleles of Rap1 (Kyrion et al., 1992). Telomeres in this mutant strain were elongated up to 4 kb from the normal size of ~300 bp (Kyrion et al., 1992; Lustig and Petes, 1986). Strikingly, these elongated telomeres were found to be highly unstable, causing elevated rates of chromosome loss. Analysis of the fate of cells carrying these hyper-elongated telomeres revealed a process termed Telomere Rapid Deletions (TRD) that could reset excessively long telomeres back to WT length. Telomere Rapid Deletions occurred through a homologous recombination pathway, mechanistically distinct from the gradual loss of telomeres known as telomere attrition (Li and Lustig, 1996). Genetically, TRD require the homologous recombination factors Mre11 and Rad50 and are suppressed by the non-homologous-end-joining (NHEJ) factor Ku70 (Bucholc et al., 2001; Li and Lustig, 1996; Lustig, 2003). It was later shown that, in *S. cerevisiae*, meiotic cells undergo high rates of precise deletion to maintain wild-type telomere size in a process that resembles TRD (Joseph et al., 2005). In this study, TRDs were 30 fold to 70 fold greater in meiotic cells compared to mitotic cells, suggesting that the control of the upper limit of telomere length is particularly important in this stage in development. In *S. pombe*, depletion of the telomere