# Telomere Elongation During Pre-Implantation Embryo Development


**Hyuk-Joon Jeon, Mia T. Levine, and Michael A. Lampson**

## Contents



**Abstract** The primary mechanism of telomere elongation in mammals is reverse transcription by telomerase. An alternative (ALT) pathway elongates telomeres by homologous recombination in some cancer cells and during pre-implantation embryo development, when telomere length increases rapidly within a few cell cycles. The maternal and paternal telomeres in the zygote are genetically and epigenetically distinct, with differences in telomere length and in chromatin packaging. We discuss models for how these asymmetries may contribute to telomere regulation during the earliest embryonic cell cycles and suggest directions for future research.

**Keywords** Telomere · Preimplantation development · ALT

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## 1 Telomere Regulation

Each time a cell divides, chromosomes shorten. DNA replication fails to completely copy the terminal DNA sequence, while nucleases and reactive oxygen species (ROS) further degrade terminal DNA (Kawanishi and Oikawa 2004; Olovnikov 1973). However, self-renewing cells, such as stem cells and tumor cells, can maintain and even elongate telomeres (Okamoto and Seimiya 2019). The primary mechanism of elongation is reverse transcription by the telomerase holoenzyme. Telomerase is a ribonucleoprotein that consists of a reverse transcriptase (TERT), an RNA template (TERC), and other protein subunits (Shay and Wright 2019) (Fig. 1a).

Many factors promote or inhibit telomerase-based elongation, including Shelterin, a multi-protein complex that binds chromosome ends and protects them from inappropriate DNA repair into lethal end-to-end fusions (Fig. 1b) (Rai et al. 2016). Shelterin consists of six subunit proteins, including the DNA-binding factors TRF1 and TRF2. Shelterin inhibits telomerase activity by promoting a telomere loop (t-loop) that prevents TERC binding to the single-stranded DNA overhang (Smogorzewska and de Lange 2004; Sobinoff and Pickett 2017).

Not all telomere elongation arises from telomerase activity. A second, ancient mechanism of telomere elongation relies instead on homologous recombination. This pathway, called Alternative Lengthening of Telomeres (ALT), has been well-documented in both telomerase-negative budding yeast cells and in cancer cells. Yeast lacking the telomerase RNA component undergo lethality as telomeres shorten; however, 1% survive and ultimately proliferate using ALT (Lundblad and Blackburn 1993). Similarly, 10–15% of tumors do not rely on telomerase

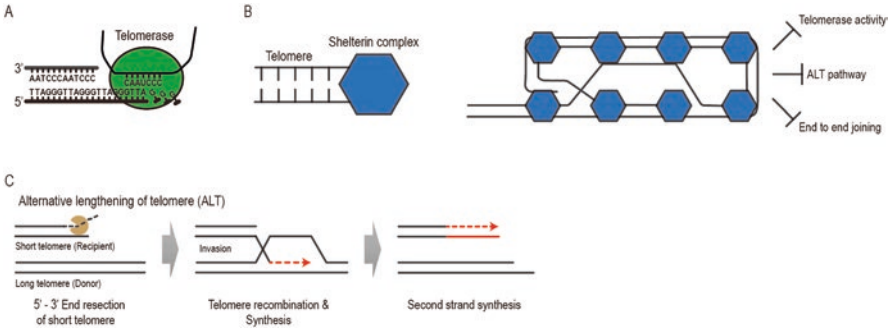


**Fig. 1** Telomere regulation mechanisms. (**a**) Telomerase is a ribonucleoprotein that consists of a reverse transcriptase and an RNA component that, together with other subunits, elongate telomere ends. Telomerase RNA (5'-CCCUAA-3′) binds single stranded telomeric DNA, 5′-TTAGGG-3′. (**b**) Shelterin is a protein complex that binds to the telomeric DNA sequence. This nucleoprotein complex forms a telomere loop (t-loop) that protects telomeric DNA sequences from telomerase activity, the alternative lengthening of telomere (ALT) pathway, and end-to-end chromosome fusions. (**c**) ALT is initiated through end-resection of a short telomere that cannot make a t-loop. The resulting single-stranded telomere (recipient) invades a long telomere (donor) to recombine with the homologous template and initiate DNA synthesis. The second strand of the recipient is synthesized after resolving telomere recombination

reactivation to achieve immortality but instead rely on ALT. When telomeres from telomerase-negative tumor cells lose sufficient telomere sequence, DNA repair proteins are recruited to the chromosome end. These repair proteins, including Rad51, Rad52, and RPA, counteract telomere degradation by promoting recombination between sister chromatids, homologous chromosomes, or non-homologous chromosomes (Sobinoff and Pickett 2017) (Fig. 1c). This mechanism of elongation appears to lengthen telomeres faster than reverse transcription by telomerase: most ALT-dependent tumor cells have longer telomeres than telomerase-dependent tumor cells (Toubiana et al. 2021; Wang et al. 2013).

## 2 ALT during Normal Development

### *2.1 Rapid Telomere Elongation during Pre-Implantation Embryo Development Depends on ALT*

Telomerase ensures that actively dividing, pluripotent cells maintain a critical telomere length, but telomerase activity is low in the actively dividing cells of mammalian pre-implantation development (Liu et al. 2007). This observation is paradoxical because cells in the early embryo go on to differentiate into all other cell types, including those cells that rely on telomerase to maintain their telomeres. Moreover, maternal telomeres are dangerously short after fertilization. The oocyte arrests in prophase of meiosis I prior to birth. During this extended cell cycle arrest, which can last for years in mice or decades in humans, reactive oxygen species (ROS) in primordial follicles shorten oocyte telomeres (Jeon et al. 2022; Passos and Von Zglinicki 2005; Yamada-Fukunaga et al. 2013). Consequently, oocyte telomere length is about 16–18 kilobase pairs (kb) in laboratory mouse strains compared to 30–200 kb in somatic cells, although telomeres can be shorter in wild-derived strains (Hemann and Greider 2000; Keefe et al. 2007; Liu et al. 2007; Starling et al. 1990). Such reduced oocyte telomere length is similar to estimates from aged blood cells from mice (Vera et al. 2012). These aged blood cells will soon senesce or undergo apoptosis in response to DNA damage signaling from their critically shortened telomeres, yet the pre-implantation embryonic cells go on to establish all cell lineages in the developing organism. These observations suggest that, during the earliest embryonic cell cycles, rapid telomere elongation is necessary to avoid cell death. Indeed, telomere length increases by approximately 20% (3 kb) during the first six embryonic cell cycles in mouse embryos, with most elongation during the first two cell cycles (Liu et al. 2007) (Fig. 2). In comparison, telomerase is expected to elongate telomeres by only ~60 nucleotides per cell cycle under length maintenance conditions, or approximately 120 nucleotides per cell cycle in cells where telomeres are elongating (Zhao et al. 2011). Thus, canonical telomerase-based elongation cannot account for the telomere elongation rates in the pre-implantation embryo.

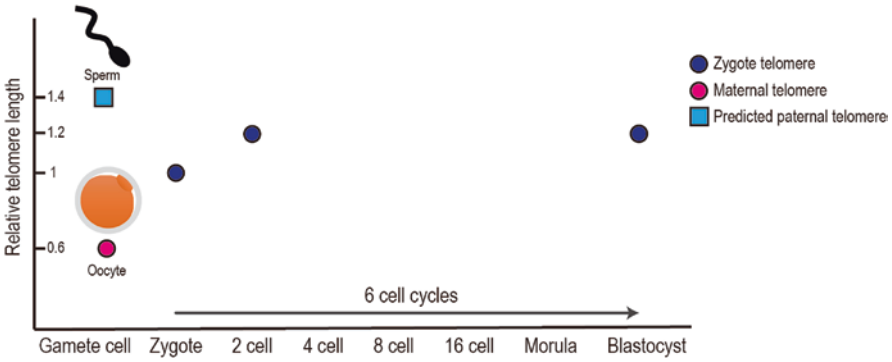


**Fig. 2** Telomere lengthening during preimplantation embryo development. In the CD1 mouse strain, q-PCR data (Liu et al. 2007) show that telomere length of MII oocytes is ~40% shorter than the average telomere length in zygotes, suggesting that telomeres in sperm are 40% longer than in zygotes. Telomere length increases by ~20% after one cell cycle and then remains constant until the blastocyst stage

Several lines of evidence support the idea that ALT, rather than telomerase, is the dominant mechanism of telomere lengthening during pre-implantation embryo development. First, a classic hallmark of ALT tumor cells, called telomere sister-chromatid exchange (T-SCE), has been observed in zygotes and 2-cell embryos by chromosome-oriented fluorescence *in situ* hybridization (CO-FISH). Second, the recombination protein Rad50 localizes to the early embryonic telomeres (Liu et al. 2007). Localization of recombination proteins to telomeres is another hallmark of ALT, though classic ALT markers in tumor cells, like Rad51 and Rad52, have not yet been studied in the early embryo (Chen et al. 2001; Zhang et al. 2019). A third compelling piece of evidence that ALT elongates the telomeres of pre-implantation embryos is the rapid elongation of these embryonic telomeres even in telomerase null embryos (Liu et al. 2007). After the first 6 cell cycles, T-SCE and Rad50 levels drop while telomerase activity increases (Liu et al. 2007) .

Although these striking observations suggest that ALT likely plays an essential role in early embryonic telomere regulation, many questions about telomere regulation during these critical divisions remain. Below we consider the genetic and epigenetic asymmetry of maternal and paternal telomeres that meet for the first time during this early phase of embryonic development. We integrate several disparate observations made over the past two decades to generate several predictions of how the asymmetry of maternal and paternal telomeres may promote ALT and, ultimately, rapid telomere elongation during pre-implantation development.

## 2.2 Maternal and Paternal Telomere Asymmetry in the Pre-Implantation Embryo

Although telomere regulation across preimplantation embryos and telomerase-negative tumor cells share many common features, the telomeres of these two cell types exhibit an important difference. The maternal and paternal telomeres deposited by the egg and sperm, respectively, are genetically and epigenetically distinct. The maternal chromosomes, including maternal telomeres, are packaged into a canonical chromatin state, including genome-wide histones and telomere-restricted Shelterin. The paternal chromosomes carried by the sperm, in contrast, are packaged tightly by protamines and appear to lack Shelterin components (Jenkins and Carrell 2012; McLay and Clarke 2003). Consequently, paternal telomeres must undergo a rapid and radical chromatin unpackaging and repackaging to match their maternal counterparts before undergoing replication, telomere elongation, and mitosis. In addition to this **epigenetic asymmetry** at fertilization, maternal and paternal telomeres are expected to exhibit **genetic asymmetry** at fertilization. Telomeres degrade during the extended oocyte arrest (see above) but self-renew during spermatogenesis (De Frutos et al. 2016; Lin 1997; Oakberg 1971; Tardat and Déjardin 2018). Consequently, maternal telomeres are shorter than paternal telomeres at fertilization. Yet there is no evidence of maternal—paternal telomere asymmetry in somatic cells. These data suggest that initially asymmetric maternal and paternal telomeres must achieve symmetry during preimplantation embryo development. The relative importance of epigenetic and genetic asymmetry for telomere regulation, the underlying mechanism(s) of achieving symmetry, and the impact of asymmetry itself on maternal telomere rescue from a dangerously short length have not been explored.

## 2.3 Proposed Models for Asymmetric Regulation of Telomere Elongation

We propose that ALT promotes biased elongation of the shorter maternal telomeres by the longer paternal telomere donors, thus resolving maternal-paternal genetic asymmetry. Our model is based on the idea that telomeres can have three distinct states: fully capped, intermediate, and uncapped (Cesare and Reddel 2010) (Fig. 3a). The "fully capped" state has sufficient telomere length and Shelterin complex to form a t-loop, which prevents both a DNA damage response and chromosome end-to-end fusions (Van Ly et al. 2018). The "intermediate" state can prevent end-to-end fusions but not the DNA damage response observed in ALT cells. The details of the intermediate state are unclear but may reflect insufficient telomere length and/or insufficient Shelterin to form a t-loop, resulting in recombination-mediated telomere elongation. Finally, the "fully uncapped" state cannot prevent end-to-end fusion because telomeres are too short to bind sufficient Shelterin. We propose that

the shorter maternal telomeres of the pre-implantation embryo are more likely to adopt the intermediate state whereas the longer paternal telomere are more likely in the closed state. Based on this asymmetry of telomere states, combined with previous observations that homology directed repair (HDR) is promoted at short telomeres but not at long telomeres in yeast (Graf et al. 2017), we predict that maternal telomeres elongate via ALT by preferentially using paternal telomeres as templates (Fig. 3b). If during ALT paternal telomeres more frequently act as donors and maternal telomeres more frequently act as recipients, then maternal telomeres will elongate faster and promote telomere symmetry (Fig. 3c).

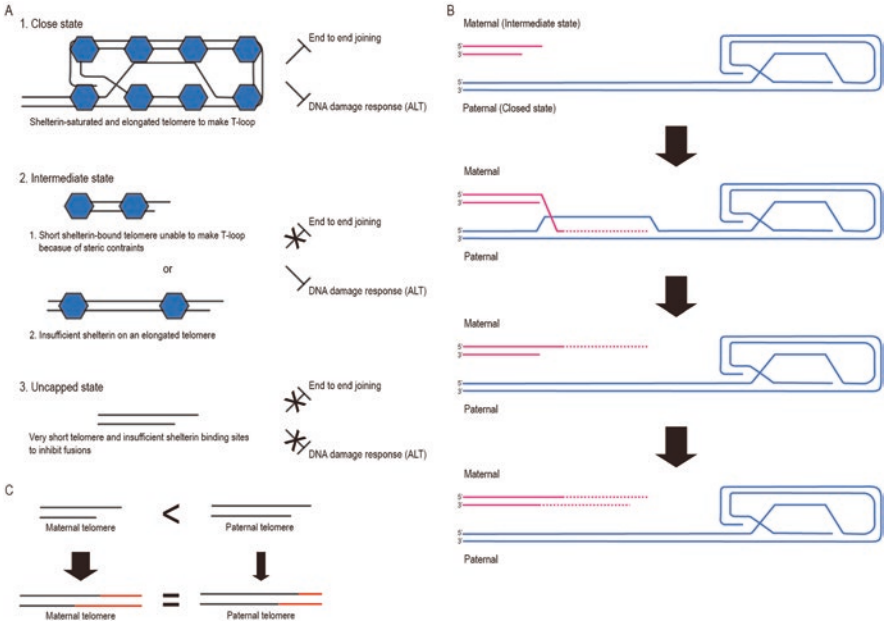


**Fig. 3** Hypothesis for preferential lengthening of maternal telomeres during preimplantation development. (**a**) Three states of telomere ends have been proposed (Cesare and Reddel 2010) that depend on telomere length and Shelterin binding. The closed state (1) has sufficient telomere length and Shelterin to make a t-loop. The t-loop prevents chromosome end-to-end fusions, DNA damage response, and ALT. The intermediate state (2) lacks a t-loop due to either insufficient telomere length or insufficient Shelterin binding. The intermediate state can prevent end-to-end fusions but not a DNA damage response. The uncapped state (3), with short telomeres and insufficient Shelterin, undergoes a DNA damage response and potentially lethal end-to-end chromosome fusions. (**b**). Since maternal telomeres are shorter than paternal telomeres in preimplantation embryos, we propose that maternal telomeres are more likely than paternal telomeres to be in an intermediate state, while paternal telomeres are more likely in a closed state. This difference in state makes maternal telomeres more likely to participate in ALT as recipients. The schematic shows a maternal telomere invading a paternal telomere and using it as a template for ALT (as in Fig. 1c). A cross between a long telomere mother and short telomere father would result in the opposite effect in the early embryo. (**c**). Both maternal and paternal telomeres elongate during preimplantation development but maternal telomeres elongate faster because they preferentially act as recipients during ALT, leading to equal telomere lengths

Alternatively, **epigenetic asymmetry** may determine the preferential elongation of maternal telomeres. Maternal chromosomes are packaged into a canonical chromatin state, whereas sperm chromatin is initially packaged with protamines and without Shelterin, and so must rapidly undergo a radical unpackaging and repackaging in the zygote (Tardat and Déjardin 2018). The naïve chromatin state of paternal telomeres, rather than telomere length asymmetry, may promote asymmetric telomere elongation. Consistent with this possibility, a known ALT regulator, ATRX, is enriched on maternal chromosomes during the earliest embryonic cycles (De La Fuente et al. 2015).

## 3 New Directions

Telomere length is a heritable trait that varies significantly among individuals (Lansdorp et al. 1996). Scores of studies link telomere length variation with individual genes that regulate telomeres, including CTC1, TERC, RTEL, and POT1 (Codd et al. 2010, 2013; Li et al. 2020; Mangino et al. 2012). However, the impact of divergence in parental telomere length on early embryonic telomere regulation has received virtually no attention. Our model suggests that the longer gametic telomeres, which are typically paternal, may act as ALT donors, thereby determining early embryonic telomere length. Moreover, sperm telomere length varies not only between genetically distinct individuals but also within individuals (Antunes et al. 2015), with contributing factors including age, diet, and oxidative stress (Mishra et al. 2016; Sharqawi et al. 2022). This variation in sperm telomeres may propagate to the embryo. Alternatively, telomere length may be determined maternally because oocytes initially provide the molecules for telomere regulation before zygotic genome activation. TRF1 and TRF2, for example, decrease in quantity when oocyte age, which can affect the regulation of telomere length in offspring (Jeon et al. 2022). Such age-dependent effects can be investigated by comparing telomere lengths of pre-implantation embryos from young and old female mice.

The pre-implantation embryo is the only known healthy physiological context where ALT is the primary mechanism of telomere elongation in mammals. This context offers the opportunity to probe mechanisms of ALT activation or suppression during development. Specifically, the early embryo can be harnessed to study molecular details of ALT initiation as well as the regulatory switch between ALT and telomerase. Given the dangerously short length of oocyte telomeres, the impacts of these early developmental transitions are profound. Mis-regulation of ALT-dependent elongation may be an unappreciated source of early embryonic lethality that results in miscarriage.

**Compliance with Ethical Standards**

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**Conflict of interest:** All authors declare that they have no conflicts of interest.

**Ethical approval:** This chapter is a review of previously published accounts, as such, no animal or human studies were performed.

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