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RESEARCH HIGHLIGHT

# Telomere length and iPSC re-programming: survival of the longest

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**A hallmark feature of pluripotent stem cells is the ability to proliferate indefinitely (immortal phenotype). Despite reactivation of telomerase during iPSC derivation, there exists considerable heterogeneity in telomere length amongst established iPSC cell lines. Both telomerase-dependent and -independent mechanisms have now been shown to be important for continuous self-renewal of iPSC via the maintenance or extension of telomere length.**

The pluripotent stem cells that constitute the inner cell mass of the early blastocyst must have the capacity to go through many rounds of cell division to allow completion of embryonic development. Not surprisingly, these embryonic stem cells exhibit long telomeres and the enzymatic complex telomerase, which functions to complete telomere replication, is present at relatively high levels [1, 2]. The initial reports by Yamanaka and others on transcription factor (Oct4, Sox2, Klf4 and c-Myc) directed re-programming of somatic cells to a pluripotent state showed that this event is accompanied by telomerase activation and telomere length extension [3, 4]. However, as more labs have begun to generate additional induced pluripotent stem cell (iPSC) lines, it has become evident that iPSC can exhibit heterogeneity in the levels of telomerase and telomere length. In their detailed mechanistic analysis of telomere length regulation during iPSC re-programming, Wang *et al*. now propose an explanation for telomere length heterogeneity in iPSCs [5].

The catalytic component of telomerase, telomerase reverse transcriptase (Tert) and the telomerase RNA component (Terc) are both essential for telomerase function. Wang *et al*. show that the expression level of both components is upregulated relatively early during the iPSC re-programming process, preceding the activation of the endogenous *Oct4, Nanog* and *Sox2* pluripotency genes, but not *Klf4*. Klf4 also appears to contribute to the maintenance of telomerase activity in ES cells [6], which, together with the results by Wang *et al*., suggest that Klf4 may be responsible for reactivating Terc and/or Tert, though this remains to be rigorously tested.

Wang *et al*. also report that iPSC lines with reduced proliferative capacity and telomere length had corresponding low levels of Tert and Terc expression. To directly examine the role of telomerase activation in iPSC re-programming, they compared the relative efficiency of re-programming murine tail-tip fibroblasts from Terc heterozygous (+/–) and Terc deficient (–/–) mice. Both $Terc^{-/-}$ and $Terc^{+/-}$ fibroblasts yielded iPSCs upon re-programming, consistent with prior observations [7] and implying that telomerase *per se* may not be necessary for pluripotency. On the other hand, analysis of the potential of $Terc^{-/-}$ iPSCs to form chimeras by injection into 8-cell embryos failed to yield any pups, suggesting that telomerase may be required for true pluripotency [5]. An alternative explanation is that the inability of $Terc^{-/-}$ iPSCs to form chimeras is due to the severely compromised telomere length in these cells, thereby limiting long-term proliferative capacity, since $Terc^{+/-}$ iPSC, with an intermediate telomere length, could form chimeras although at a reduced efficiency relative to wild-type iPSCs [5]. Moreover, telomerase is not required for pluripotency in ES cells as Terc knockout mice are viable for several successive generations [8]. Telomerase defects also do not preclude derivation of iPSCs from human cells [9].

Interestingly, telomere shortening was not observed by Wang *et al*. in early passage $Terc^{-/-}$ or $Terc^{+/-}$ iPSCs, indicating that a telomerase-independent mechanism may be active in iPSCs. Telomere sister chromatid exchange (TSCE) events are indicative of an active recombination-based mechanism for telomere length maintenance (alternative lengthening of telomeres, ALT), and were observed at an elevated frequency in $Terc^{-/-}$ iPSCs. DNA methyltransferase expression was also

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significantly reduced in Terc$^{-/-}$ iPSCs, suggesting that telomeric chromatin exists in a relaxed status in these cells, which is conducive to recombination events. Moreover, the zinc finger protein Zscan4, recently shown to have an important role in recombination-based telomere length maintenance in ES cells [10], was observed to be highly expressed in Terc$^{-/-}$ iPSCs. Perhaps the most compelling evidence by Wang *et al*. for a direct role of ALT in telomere length maintenance in iPSC is the observed telomere attrition induced upon knockdown of Zscan4 in iPSCs, although the effect of Zscan4 knockdown in Terc$^{-/-}$ iPSCs relative to Terc$^{+/+}$ iPSCs was not assessed. Together, these results of Wang *et al*. indicate that both telomerase-dependent and -independent ALT mechanisms play critical roles in telomere length maintenance in iPSCs.

The presence of both active telomerase and the Zscan4-dependent ALT mechanism for telomere length maintenance in ESCs and iPSC indicates that these cells are particularly well protected against the accumulation of short telomeres, in contrast to adult somatic cells (Figure 1). While the reason for this is unknown, it is speculated that enhanced protection of telomeres may be crucial to ensure the generation of healthy embryos. The inner cell mass of the early embryo (blastocyst) is composed of just a few hundred cells and must retain sufficient replicative capacity to undergo many subsequent cell divisions. Furthermore, the accumulation of a single telomere-free end on a chromosome is sufficient to instigate genomic instability via end-to-end chromosome fusion events in normal somatic cells, which could lead to lethal tumors in the developing embryo. It remains to be assessed whether Zscan4 dependent or other ALT mechanisms for telomere length maintenance have a role in telomere length regulation in other types of stem cells in the adult. However, with respect to telomeres in pluripotent stem cells it certainly appears that size matters.

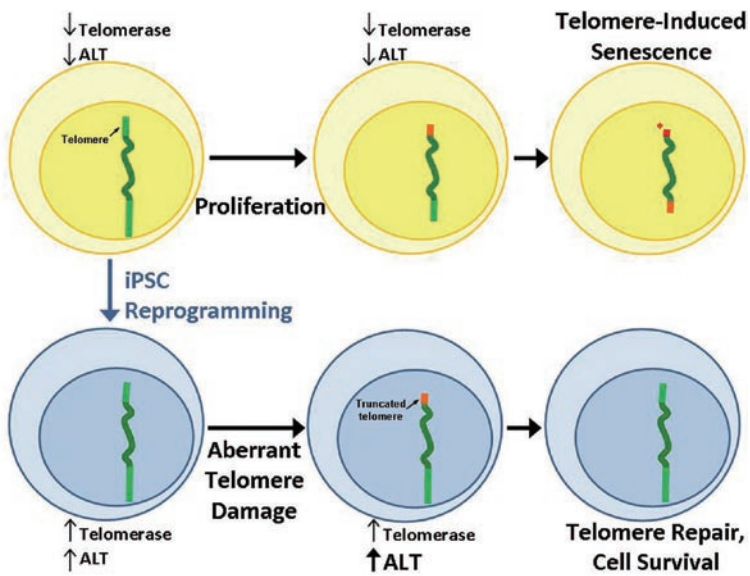


**Figure 1** Comparison of telomere length regulation in somatic cells and derived iPSCs. Most normal human adult somatic cells (shown in yellow, top panel) lack telomerase and ALT recombination-based mechanisms to maintain telomere length. Consequently, telomere attrition occurs as these cells proliferate until one or more telomeres become too short to retain proper capping function (red, marked with asterisk). Re-programming of adult somatic cells to a pluripotent state (shown in blue, lower panel) using Yamanaka transcription factors activates both telomerase and ALT mechanisms for telomere length maintenance or extension. Consequently if one or more telomeres encounter sporadic damage, causing sudden truncation to a dangerously short length (orange), it may be efficiently repaired (ALT is particularly important for iPSC telomere repair), allowing continued cell proliferation and avoiding further genomic damage.

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