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Balancing Out the Ends during iPSC Nuclear Reprogramming

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DOI 10.1016/j.stem.2009.01.004

In this issue of *Cell Stem Cell*, Marion et al. (2009) report that efficient production of iPSCs requires active telomerase, which allows the rejuvenation of telomeres to a state similar to that observed in embryonic stem cells, even in iPSCs generated from old donor cells.

The recent discovery that differentiated cells can be reprogrammed to an embryonic stem cell (ESC)-like pluripotent state, so-called induced pluripotent stem cells (iPSCs), by the expression of just a few transcription factors in somatic cells from both mice and humans (Yamanaka, 2007) has led to renewed hope for patient-specific stem cell therapies. The successful utilization of iPSCs in therapy will require the maintenance of genomic stability and cell survival to ensure long-term function of iPSC-derived cells following engraftment. A key player for these requirements is the enzyme telomerase, which functions to ensure stability of the ends of chromosomes and to prevent cell senescence by completing the replication of telomeres. Indeed, unlike most differentiated somatic cells, abundant levels of telomerase activity are a well-established feature of ESCs (Thomson et al., 1998). However, nuclear reprogramming of somatic cells to a pluripotent state does not necessarily guarantee reactivation of functional telomerase and extension of telomeres, as evidenced by unusually short telomeres observed in cloned sheep (Shiels et al., 1999). In this issue of *Cell Stem Cell*, Marion, Blasco, and colleagues now report the detailed effects of nuclear reprogramming of somatic cells into iPSCs on telomerase and telomeres (Marion et al., 2009). Their findings demonstrate telomerase-dependent rejuvenation of telomeres in iPSCs, derived from normal cells taken from either young or old mice, with telomeres that eventually reach lengths similar to that observed in ESCs.

The two components of the telomerase holoenzymatic complex that are essential for activity are the telomerase RNA component (Terc) and the catalytic component, telomerase reverse transcriptase (Tert). Thus, the *Terc* knock-out ($^{-/-}$) mouse strain lacks functional telomerase and is characterized by continuous telomere shortening from one generation to the next, eventually leading to telomere dysfunction, premature aging, and a reduced lifespan (Blasco et al., 1997).

However, telomerase-independent mechanisms for telomere lengthening have been observed during very early embryogenesis and could conceivably also come into play to restore telomere length during the generation of iPSCs (Liu et al., 2007). Using the *Terc*$^{-/-}$ mouse strain, Marion et al. (2009) now show that the efficiency of iPSC generation is greatly diminished in the absence of telomerase and that *Terc*$^{-/-}$ iPSCs fail to produce any viable chimeric mice. Furthermore, the authors show that telomere length in *Terc*$^{-/-}$ iPSCs is shorter than that observed in the *Terc*$^{-/-}$ fibroblasts from which they were derived, unlike iPSCs generated from wild-type fibroblasts. Moreover, this reduced efficiency in iPSC generation can be largely compensated for by reintroduction of a wild-type Terc allele back into the *Terc*$^{-/-}$ genome, via breeding to *Terc*$^{+/-}$ mice. Telomere lengthening is once again observed in the iPSCs generated from fibroblasts obtained from the F1 *Terc*$^{+/-}$ embryos, but not the F1 *Terc*$^{-/-}$ embryos, concomitant with a reduction in the frequency of dysfunctional telomeres. These observations demonstrate that telomere length maintenance and long-term proliferative capacity of iPSCs is dependent on telomerase.

Previous studies by Blasco and colleagues (Gonzalo et al., 2006) have shown that epigenetic modification of telomeric chromatin in mice, namely the prevention of methylation of histone H3 and H4 at the telomeres, effects an elongation of telomeres in normal somatic cells from these mice. Interestingly, Marion et al. (2009) also show in the present study that both murine ESCs and iPSCs have reduced levels of histone methylation at telomeres, relative to the primary fibroblasts from which the iPSCs were derived. Furthermore, the exchange of DNA between telomeres has been shown to occur at an elevated frequency in cells in which histone methylation is abrogated (Gonzalo et al., 2006) and also occurs at an elevated frequency in both ESCs and iPSCs (Marion et al., 2009). Together, these observations provide initial evidence that telomeric chromatin is also successfully "re-programmed" in iPSCs, to acquire an epigenetic signature similar to that observed for ESCs. This reprogramming of telomeric chromatin into a more relaxed state may indeed be required to allow access of telomerase to the end of the telomere and subsequent telomere lengthening (Figure 1), although this has yet to be formally demonstrated.

Given the importance of telomerase to the restoration of telomeres during iPSC generation, it is important to understand how this process is regulated. Like ESCs, it has already been demonstrated that iPSCs have elevated levels of telomerase activity relative to the somatic cells from which they derive (Yamanaka, 2007; Marion et al., 2009). Thus, it is quite possible that the expression of Terc and/or Tert is elevated during the nuclear reprogramming of somatic cells to iPSCs, in particular Tert, which has been shown to be the key component that limits telomerase activity in various types of normal somatic cells and adult stem cells (Bodnar et al., 1998; Allsopp et al., 2003). One of the best-characterized transactivators of Tert in some types of somatic cells is the

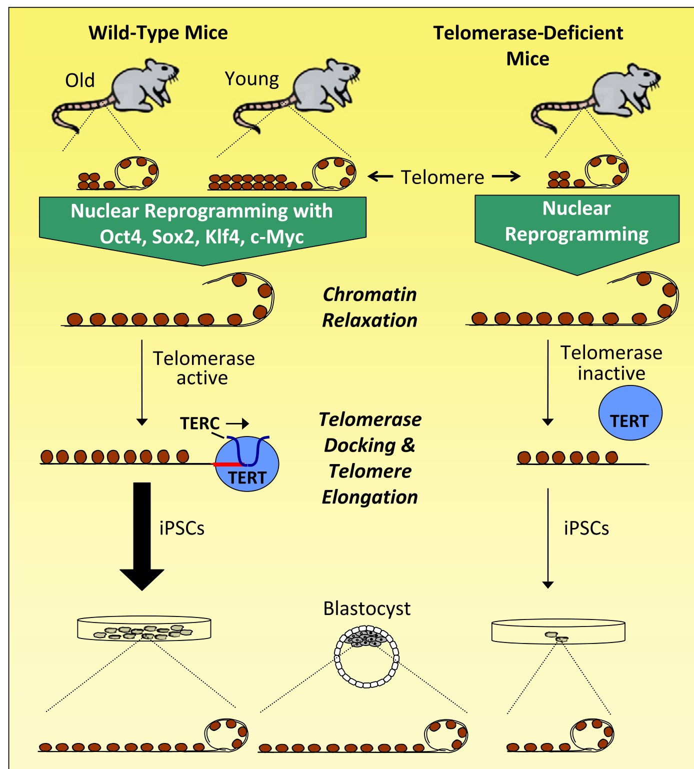

Figure 1. Telomere Length Regulation during Nuclear Reprogramming of Fibroblasts to iPS Cells

Telomeres form a loop structure at the very end of the chromosome, which protects the end and also prevents telomerase from associating with the 3′ terminal end of the telomere. Marion et al. show that telomeres are lengthened by telomerase during 3 or 4 factor reprogramming of fibroblasts, from young or old wild-type mice, to iPS cells (left side), achieving a size similar to that observed in cultured ESCs derived from the blastocyst. Telomeric chromatin is also demethylated during nuclear reprogramming (Marion et al., 2009), which is proposed to facilitate the relaxation of telomeric chromatin, allowing telomerase access to the telomere 3′ end and subsequent synthesis of new telomeric DNA (red line). In telomerase deficient cells, like those from *Terc*$^{-/-}$ mice, telomeric chromatin is presumably demethylated as well; however, telomerase cannot bind to the telomere or synthesize new telomeric DNA (right side). Therefore, critically short telomeres observed in *Terc*$^{-/-}$ cells are not repaired and remain critically short in *Terc*$^{-/-}$ iPSCs (Marion et al., 2009). This, in turn, adversely affects cell proliferation and greatly limits the number of iPSCs obtained.

proto-oncogene c-Myc, which coincidentally is one of the four genetic factors used in the seminal studies demonstrating the development of iPSCs (Knoepfler, 2008). However, Marion et al. (2009) convincingly show that 3-factor iPSCs, generated using only Oct4, Nanog, and Klf4, show similar levels of telomerase activation as observed for 4-factor iPSCs. Thus, the mechanism for telomerase activation during iPSC nuclear reprogramming remains obscure. Possible regulatory events affecting the switch in telomerase activity that should be addressed in future studies include transactivation of the *Terc* and/or *Tert* genes, as well as posttranslational modification of telomerase and modification of telomeric chromatin.

The discovery of iPSCs holds great promise for creating new methods to study mechanisms for different diseases and the development of customized patient-specific therapies. One potential therapy of high interest is the repair of damaged or aged tissue in elderly individuals using "rejuvenated" iPS-derived cells created from cells donated by the patient. If during the nuclear reprogramming stage, the telomeres fail to be regenerated to lengths typically observed in young cells, then the high proliferative stress that the iPS-derived cells encounter, either pre- or post-transplant, could very well cause premature senescence of the iPS-derived cells following engraftment. Marion et al. (2009) provide an encouraging finding in regards to this potential cell therapy, namely, that iPS cells could be readily derived from skin fibroblasts obtained form elderly animals with reduced telomere length and that telomere length was fully restored in these iPS cells, achieving telomere lengths comparable to iPS cells created from skin fibroblasts taken from young animals. Furthermore, they show that reintroduction of functional telomerase into telomerase-deficient cells harboring substantially shortened and dysfunctional telomeres is sufficient to allow efficient iPS cell development, the generation of viable chimeric mice, and restore telomere length. These observations suggest that iPS cell therapy may one day be practical even for individuals with abnormally short telomeres owing to defects in telomerase. Although a number of hurdles must still be cleared before iPS-based cell therapy becomes practical, the results from Marion et al. (2009) suggest that reprogramming of telomerase and telomeres may not be one them.

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