Short Telomeres Flirt with Stem Cell Commitment

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High expression of telomerase in embryonic stem cells (ESCs) is important for their maintenance, but whether telomere length affects lineage commitment is unknown. In this issue of *Cell Stem Cell*, Pucci et al. (2013) reveal that ESCs with short telomeres exhibit unstable differentiation by inducing altered DNA methylation.

Long term self-renewal of stem cells requires faithful replication of telomeres by the enzyme telomerase. Unlike most proliferating cells, embryonic stem cells (ESCs) express an abundance of telomerase (Kim et al., 1994). The primary consequence of a lack or deficiency of telomerase in proliferating cells is the gradual attrition of telomeres and, ultimately, cell senescence once a sufficient number of telomeres become critically short (Bodnar et al., 1998). Thus, high levels of telomerase in ESCs likely ensure that their in vivo counterparts in the early embryo have long telomeres and sufficient replicative potential to prevent deleterious effects during prenatal and postnatal development. Now, in this issue of *Cell Stem Cell*, Fabio et al. report for the first time that there are additional presenescent consequences of aberrantly short telomeres in ESCs that adversely affect the ability of these cells to retain a differentiated state (Pucci et al., 2013).

The study by Harrington and colleagues (Pucci et al., 2013) used ESCs derived from mice (Liu et al., 2000) that lack the gene encoding the catalytic component of telomerase, telomerase reverse transcriptase (Tert), to query the role of telomeres in ESC biology. They discovered that late-passage Tert$^{-/-}$ ESCs with short telomeres, including a fraction of chromosomal ends that entirely lack detectable telomeric DNA (dysfunctional telomeres), express unusually high levels of the pluripotency-promoting factor Nanog (Pucci et al., 2013). Furthermore, this effect on pluripotency factor expression was specific for Nanog but not for other factors such as Oct4 or Sox2. A more detailed analysis into the mechanism leading to high levels of Nanog expression in these cells revealed a reduction in trimethylated histone H3 lysine 27(H3K27me3) in the Nanog promoter, whose reduction was previously shown to be associated with elevated Nanog expression (Shen et al., 2008). Reparation of telomeres in these cells by reintroduction of Tert both reduced Nanog expression and restored H3K27me3 to wild-type levels in the Nanog promoter, demonstrating a direct consequence of reduced telomere length on Nanog expression in ESCs.

To begin to assess the functional consequences of elevated Nanog levels in Tert$^{-/-}$ ESC, Pucci et al. induced these cells to differentiate by treating them with retinoic acid (RA). While Nanog expression was initially diminished after RA-induced differentiation, akin to what is observed in wild-type ESCs, subsequent addition of leukemia inhibitory factor (LIF) after a few days of growth led to upregulated Nanog expression in the Tert$^{-/-}$ ESC-harboring short telomeres, but not in wild-type ESC. Moreover, this was accompanied by a marked increase in ESC colony formation in these cells, which was not observed in wild-type ESC. Restoration of telomeres by reintroduction of Tert into the Tert$^{-/-}$ ESC prevented the ability of LIF to reactivate Nanog expression following the RA-induced differentiation of these cells, again demonstrating that this observation is a consequence of shortened telomeres. Furthermore, the RA-induced differentiated state could be stabilized by RNA interference-mediated knockdown of Nanog in these cells, showing that this effect is primarily, if not entirely, attributed to elevated Nanog expression.

Further analysis by Pucci et al. into the epigenetic effects of aberrantly shortened telomeres in Tert$^{-/-}$ ESC revealed changes in DNA methylation (methyl-CpG), with global hypomethylation occurring throughout the genome and not solely at the Nanog promoter. This finding prompted Pucci et al. to assess the levels of de novo methyl transferases (Dnmt) in the Tert$^{-/-}$ ESCs. Interestingly, Dnmt3a and Dnmt3b, but not Dnmt1, were reduced compared to wild-type ESC. Furthermore, enforced expression of Dnmt3b was sufficient not only to restore the genome-wide methylation to a level comparable to what is observed in the wild-type ESC genome but also to stabilize the differentiated state of these cells following RA treatment.

Thus, telomerase deficiency and, more specifically, the resulting telomere attrition in ESCs can lead not only to eventual cell senescence and/or cell death (Niida et al., 1998) but also to genome-wide hypomethylation and unstable differentiation of cells. However, the exact mechanism by which critical telomere shortening leads to genome-wide hypomethylation and subsequent aberrant gene expression, such as elevated Nanog, remains to be determined. The reintroduction of Tert into the late-passage telomerase-deficient ESC rescues both the telomere dysfunction phenotype and the hypomethylation-attributed unstable differentiation phenotype of these cells, implying that the presence of dysfunctional, telomere-free ends is somehow signaling a response that involves, perhaps in addition to other things, genome-wide hypomethylation (Figure 1A). However, rescuing the Tert$^{-/-}$ ESC by enforced Tert expression also leads to extensive telomere lengthening (Pucci et al., 2013). Therefore, a possible alternative explanation could be an effect of compromised telomere length, possibly via altered telomeric chromatin or reduced levels of histones (O'Sullivan et al., 2010), on Dnmt3b expression (Figure 1B). Presenescent epigenetic affects of telomere attrition have recently been documented for late-passage human fibroblasts, which do not contain dysfunctional

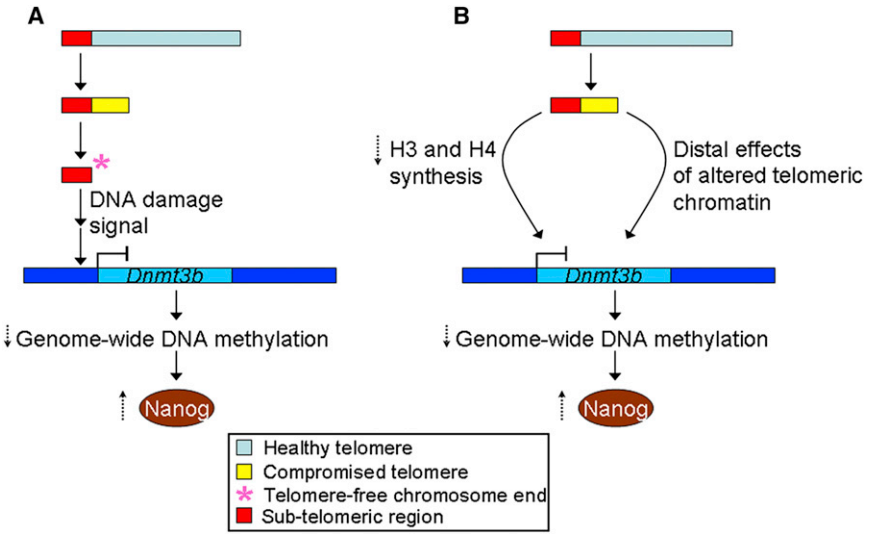

Figure 1. Hypothetical Mechanisms for Upregulation of Nanog in Late-Passage Tert$^{-/-}$ ESC
(A) One possibility is that the accumulation of telomere-free chromosomal ends elicits a DNA damage response, which, in the early stages, causes a reduction in genome-wide methylation. (B) Alternatively, telomeres that are compromised, but still retain some protective capping function, could affect DNA methylation by evoking a decline in histone synthesis or perturbing expression of distal genes due to alterations in telomeric chromatin.

telomeres (i.e., telomere-free chromosomal ends) (O'Sullivan et al., 2010). Additional studies will be required to clearly distinguish between these possibilities.

The effect of shortened telomeres on the differentiated state of ESC reported by Pucci et al. implies that there could be similar effects in other types of stem cells at advanced replicative age. This may be particularly interesting to assess in adult stem cells for highly proliferative tissues, such as hematopoietic stem cells, which undergo extensive telomere shortening during human aging (Vaziri et al., 1994). Furthermore, many different types of tumors have now been shown to rely on cancer stem cells for growth, and many types of human tumors have short, dysfunctional telomeres (Shay and Wright, 2011). Thus, it will also be of interest to assess the stability of the differentiated state for cancer stem cells, as well as the expression of self-renewing factors such as Nanog, particularly in tumors with short telomeres.

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