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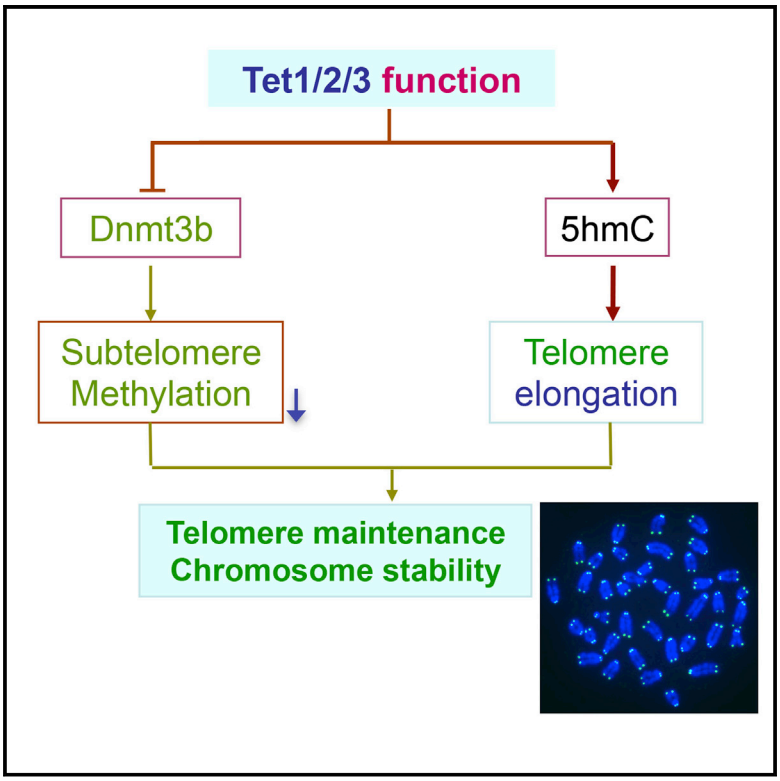
Cell Reports

Tet Enzymes Regulate Telomere Maintenance and Chromosomal Stability of Mouse ESCs

Graphical Abstract



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In Brief

Sub-telomeric DNA methylation negatively regulates telomere recombination and telomere length. Here, Yang et al. report that Tet enzymes maintain telomere length and chromosomal stability by modulating both Dnmt3b expression and methylation levels.

Highlights

- Tet enzymes maintain telomere length
- Tet enzymes maintain chromosomal stability of ESCs
- Dnmt3b and 5hmC are involved in Tet-signaling-mediated telomere maintenance
- Excessive Zscan4 and Dnmt3b lead to heterogeneous telomere elongation and loss

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Tet Enzymes Regulate Telomere Maintenance and Chromosomal Stability of Mouse ESCs

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SUMMARY

Ten-eleven translocation (Tet) family proteins convert 5-methylcytosine to 5-hydroxymethylcytosine. We show that mouse embryonic stem cells (ESCs) depleted of *Tet1* and/or *Tet2* by RNAi exhibit short telomeres and chromosomal instability, concomitant with reduced telomere recombination. *Tet1* and *Tet2* double-knockout ESCs also display short telomeres but to a lesser extent. Notably, *Tet1/2/3* triple-knockout ESCs show heterogeneous telomere lengths and increased frequency of telomere loss and chromosomal fusion. Mechanistically, *Tets* depletion or deficiency increases *Dnmt3b* and decreases 5hmC levels, resulting in elevated methylation levels at sub-telomeres. Consistently, knockdown of *Dnmt3b* or addition of 2i (MAPK and GSK3β inhibitors), which also inhibits *Dnmt3b*, reduces telomere shortening, partially rescuing *Tet1/2* deficiency. Interestingly, *Tet1/2* double or *Tet1/2/3* triple knockout in ESCs consistently upregulates *Zscan4*, which may counteract telomere shortening. Together, Tet enzymes play important roles in telomere maintenance and chromosomal stability of ESCs by modulating sub-telomeric methylation levels.

INTRODUCTION

Ten-eleven translocation (Tet) family proteins oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), an intermediate that can lead to DNA demethylation (Kohli and Zhang, 2013; Tahiliani et al., 2009). TET proteins are implicated in diverse biological processes, including epigenetic regulation of gene transcription, embryonic development, stem cell function and pluripotency, and cancer, but the underlying mechanisms still remain to be defined (review Pastor et al., 2013; Costa et al., 2013; Dawlaty et al., 2013; Ficz et al., 2011; Ito et al., 2010; Koh et al., 2011). Tet proteins regulate genome-wide reprogramming by modulating DNA methylation levels (Wu et al., 2011; Xu et al., 2011) and affect embryonic stem cell (ESC) pluripotency and differentiation (Costa et al., 2013; Ficz et al., 2011; Ito et al., 2010).

Roles of Tets in vivo were tested by generating *Tets* knockout (KO) mice. Intriguingly, if they survive from early development, *Tets*-deficient mice mostly do not show observable phenotypes (Dawlaty et al., 2013; Li et al., 2011). Mice mutant for either *Tet1* or *Tet2* are viable or show only neuronal deficiencies (Dawlaty et al., 2011; Ko et al., 2011; Li et al., 2011; Zhang et al., 2013). Some *Tet1* mutant mice have a slightly smaller body size at birth (Dawlaty et al., 2011), which might reflect a developmental delay, and also are subfertile (Yamaguchi et al., 2012). These enzymes may have overlapping roles in development. KO of both *Tet1* and *Tet2* suggests that *Tet1/2* do not play a significant role in embryonic development (Dawlaty et al., 2013; Hu et al., 2014). This seems to be difficult to reconcile with the roles of *Tets* in self-renewal and pluripotency of ESCs/induced pluripotent stem cells (iPSCs) (Costa et al., 2013; Ficz et al., 2011; Ito et al., 2010).

Telomeres maintain genomic stability and are critical for unlimited self-renewal and pluripotency of ESCs and iPSCs (Huang et al., 2011; Marion et al., 2009). Mouse telomeres are quite long, and telomerase *Terc*- deficient mice following several generations of breeding acquire striking phenotypes associated with telomere dysfunction, including developmental defects, aging, and cancer (Blasco et al., 1997; Herrera et al., 1999; Rudolph et al., 1999). *Tet1* and *Tet2* double-knockout (DKO) mice, if survived from embryonic lethal, show partially penetrant perinatal lethality or reduced fertility with small ovaries (Dawlaty et al., 2013). These defects mimic those of telomere-shortened, late-generation, telomerase-deficient mice that also exhibit small body, defective neurogenesis, and infertility (Hao et al., 2005; Herrera et al., 1999).

Nevertheless, ESCs isolated from the first generation (G1) telomerase-deficient mice already exhibit telomere dysfunction