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Telomeres Are Double-Strand DNA Breaks Hidden from DNA Damage Responses

A network of ATM/ATR-mediated events regulates cell cycle checkpoints and genomic integrity and contributes to the processing of DNA double-strand breaks in both genomic DNA and at telomeres. In yeast and in human cells, investigators, including Takata et al. and Herbig et al., published in this issue of *Molecular Cell*, are beginning to decipher the signaling pathways involved at the telomeres.

The ends of linear chromosomes are similar to DNA double-stranded breaks. An important function of normal telomeres is to protect chromosome ends from homologous recombination (HR) and nonhomologous end joining (NHEJ) reactions (de Lange, 2002). This is accomplished by several mechanisms such as single- and double-strand telomere binding factors and by hiding the chromosome ends in a special architectural structure that prevents DNA damage responses. Should these mechanisms that are designed to hide the chromosome ends from being recognized as double-strand breaks fail or be unrestrained, and if HR and NHEJ go unchecked, this could lead to serious consequences such as complete telomere deletion or chromosome fusions (de Lange, 2002). Thus it becomes important to dissect the signaling cascade mediating DNA damage responses when cells have dysfunctional telomeres.

DNA damage responses are an integrated set of events designed to promote cell and organism survival. The phosphatidylinositol 3-kinase like kinases (PIKKs) are a family of kinases that play an important role in these processes (Abraham, 2001; Chan and Blackburn, 2003; Shiloh, 2003). In budding yeast and mammals, Tel1p and Mec1p (ATM and ATR) have been implicated in certain aspects of normal and abnormal telomere integrity. In yeast, deletion of Tel1p and Mec1p causes gradual loss of telomeres, which can be rescued by tethering telomerase to the telomeres. However, the precise mechanism by which Tel1p and Mec1p (or Rad3 in fission yeast) regulate telomere integrity remains unclear.

Takata et al. (2004) examined the associations between Tel1p and Mec1p and telomeres *in vivo*. Using chromatin immunoprecipitation (ChIP) assays, they found that Mec1p associates with structurally altered

telomeres that are formed in late S phase or that are due to defects in telomere metabolism. Tel1p and Mec1p are recruited reciprocally to the telomeres during the cell cycle, and this reciprocity is directed by Mec1 kinase activity, which peaks in late S phase. In yeast it is believed that upon DNA damage Mec1 can signal through Chk1 and/or Rad53/Dun1 to affect cell cycle checkpoint or transcription induction repair. Similarly, upon DNA damage, through the Mre11/Rad50/Xrs2 pathway, Tel1 also signals into the Rad53/Dun1 repair pathway. What was not known before the present work was the role of Mec1p and Tel1p in normal telomere homeostasis. With the ability to cross yeast strains deficient in many of the DNA damage response pathways, Takata et al. (2004) show that Tel1p and Mec1p are recruited to the telomeres at specific times in the cell cycle, in a mutually exclusive manner. In particular, Mec1p interacts with the telomeres during late S phase and is associated preferentially with shortened telomeres. They propose a model in which telomere integrity is maintained by the reciprocal association of PIKKs, and Mec1p acts as a sensor for structural abnormalities in the telomeres. Their investigations suggest a mechanistic similarity between telomere length regulation and DNA double-strand break repair, both of which are achieved by the direct association of PIKKs.

In a related study in human cells, Herbig et al. (2004) provide a description of the ATM/ATR signaling pathway in human replicative senescence at the single cell level, which provides both confirmation and interesting differences from the yeast results. In contrast to the late replication found in yeast, human telomeres replicate asynchronously; thus one cannot do a simple cell cycle CHIP analysis comparing ATM and ATR association to telomeres to observe the reciprocity found above. Herbig et al. (2004) looked at the association of ATM and ATR with the DNA damage foci induced by dysfunctional telomeres (telomere-induced foci) in senescent cells (Takai et al., 2003; d'Adda di Fagnana et al., 2004). They observed colocalization of ATM with most senescent telomere-induced foci, but did not find ATR in these foci. By CHIP analysis, senescent cells showed a 37-fold increase in the amount of ATM associated with telomeres compared to only a 6-fold increase in ATR. Thus, while short dysfunctional telomeres in yeast recruited Mec1 to replace Tel1, it appears that it is primarily ATM (rather than the Mec1 homolog ATR) that is at dysfunctional telomeres at replicative senescence in human cells. Inhibiting ATM with siRNA in senescent cells was also sufficient to cause their re-entry into the cell cycle, confirming its primacy in mediating the DNA damage response from dysfunctional telomeres in repli-

cative aging. Herbig et al. (2004) also showed that ATR could replace ATM at dysfunctional telomeres in ATM^{-/-} cells, becoming colocalized with telomere-induced foci and inducing a G2 (rather than G1) arrest. Although these results might reflect some differences between the function of Mec1 at yeast and ATR at human telomeres, it is important to note that the results could also represent different states of telomere dysfunction. Replicative senescence represents the time when telomeres first become sufficiently short to induce a growth arrest (Shay and Wright, 2002). However, if p53 pathways are blocked then the cells can continue to divide and undergo an additional 10 to 20 doublings until crisis occurs. Additional telomere shortening occurs during this time, so one would expect the amount of telomeric deprotection to increase. This might be both quantitative (more deprotected ends) and qualitative (the extent of deprotection on the shortest telomeres could progress from mild to severe). It could well be that the telomere dysfunction found in yeast cells with *est2* (the telomerase catalytic subunit) or *yKu70* deletions is more equivalent to the severe dysfunction found at crisis rather than what might be a more limited abnormality at senescence.

The Herbig et al. (2004) studies also demonstrate an important independence between telomere-induced and p16-induced growth arrest. Using single cell analysis, they showed that the fraction of p16⁺ cells increased with culture and strongly correlated with growth arrest, but it did not correlate with telomere-induced foci. In contrast, there were strong associations between cells showing telomere-induced foci and increased p21 growth arrest and the ability of siRNA to ATM to induce cell cycle progression. These studies appear to resolve the differences between telomere-based senescence and a non-telomere-dependent growth arrest (sometimes referred to as stasis, premature senescence, and culture shock-induced senescence). Since there are similarities

between the two types of growth arrest, there has been confusion about the functional meaning of the term "senescence" (Shay, 2003; Shay and Roninson, 2004). The analysis at the single cell level by the Sedivy lab (Herbig et al., 2004) clearly demonstrates that p16 is not associated with the classic telomere-based replicative senescence. This in no way diminishes the importance in understanding the stress-induced growth arrest that is often concordant with upregulation of p16. The insights that this present study have provided should now permit the dissection of differences in the signaling pathways that cause cells to stop proliferating.

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