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TELOMERES

Preventing unauthorized entry

To ensure genomic stability, cells have checkpoints that detect DNA double-strand breaks (DSBs) and block entry into mitosis. Uncapped telomeres are also recognized as DNA damage, leading to checkpoint activation, and, as Tarsounas and colleagues now show, G2-to-M arrest, albeit through a distinct mechanism.

Detection of DNA DSBs induced by exogenous sources such as ionizing radiation (IR) leads to activation of ataxia telangiectasia mutated (ATM) and ATR, which phosphorylate p53, ultimately preventing cell cycle progression. Previous studies had shown that artificial telomere uncapping through removal of the shelterin protein TRF2 (which activates ATM) blocks mitotic entry in a p53-dependent manner. Similarly, the authors found that deletion of the shelterin protein POT1 (which activates ATR) in human cells leads to p53 phosphorylation and G2-to-M arrest, with loss of p53 in TRF2- or POT1-depleted cells allowing normal mitotic entry. This phosphorylation event was ATM and ATR dependent, occurring specifically at Ser15, as cells carrying p53 mutated at this residue progressed to mitosis following telomere uncapping.

In addition to p53 phosphorylation, ATR and ATM respond to DNA DSBs by phosphorylating checkpoint kinase 1 (CHK1) and CHK2, respectively, which mediate cell cycle arrest by inactivating the mitotic phosphatases CDC25A and CDC25C. POT1 and TRF2 depletion in mouse cells is known to promote CHK1 and CHK2 phosphorylation, respectively, and the authors observed that this is also true in human cells. Importantly, depletion of POT1 or TRF2 blocked mitotic entry, but this effect was restored with concomitant loss of CHK1 or CHK2.

So, is the mechanism by which CHK1 and CHK2 block mitotic entry after telomere uncapping similar to the one occurring in response to IR-induced DNA DSBs? Interestingly, although POT1 depletion decreased both CDC25A and CDC25C levels, TRF2 inhibition reduced only CDC25C levels. As CDC25A degradation is sufficient to arrest cell cycle progression following IR, these findings suggest that the checkpoint responses to loss of TRF2 and to IR-induced DSBs are distinct. Specifically, the authors observed that TRF2 induced CHK2-mediated phosphorylation of CDC25C at Ser216, which resulted in nuclear export and proteasomal degradation of CDC25C. Indeed, proteasome inhibition stabilized CDC25C, leading to normal mitotic entry of cells carrying uncapped telomeres. By contrast, proteasome inhibition had no effect in IR-treated cells, confirming that the mechanism blocking mitotic entry is different from the one triggered by uncapped telomeres.

Finally, the authors identified an additional mechanism of regulating CDC25C levels following telomere uncapping that is independent of CHK1 and CHK2. Human CDC25C is thought to be regulated by p53-mediated transcriptional repression. Consistent with this, CDC25C levels were decreased in TRF2-depleted cells carrying intact p53 but were restored with concomitant depletion of p53.

So, it seems that, in addition to regulating CDC25A levels, cells use two distinct pathways to regulate CDC25C and thus mitotic entry following telomere uncapping, thereby ensure genomic stability.

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ORIGINAL RESEARCH PAPER Thanasoula, M. *et al.* ATM/ATR checkpoint activation downregulates CDC25C to prevent mitotic entry with uncapped telomeres. *EMBO J.* 27 Jul 2012 (doi:10.1038/emboj.2012.191)

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