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CHROMOSOME END PROTECTION BY TELOMERES

Telomeres are protein-DNA complexes that protect natural chromosome ends from being treated as damaged DNA. Telomeres progressively shorten with every cell division until they become too short to function properly. The subsequent recognition of chromosome ends as broken DNA has important consequences for cellular and organismal life span but also for tumor development, and telomere maintenance is therefore target of several recently developed anti-cancer strategies. Our main aim is to increase our understanding of how mammalian cells precisely perceive and respond to loss of telomere function, how telomere maintenance is controlled and how factors involved in the response to telomere dysfunction affect cellular transformation and tumor development.

Telomere-induced cellular senescence Loss of telomere function triggers a DNA damage-like response that causes cells to die or stop proliferating indefinitely (senescence). This response limits the replicative life span of cells and thereby contributes to organismal aging. In addition, it represents an important tumor suppressor mechanism as it prevents unlimited outgrowth of potentially cancerous cells. To investigate the consequences of loss of telomere protection we have performed micro-array analysis of the gene expression changes induced by loss of telomere function upon TRF2 inhibition. Next to genes involved in cancer, cell death and cell cycle, genes involved in inflammatory/immune responses represented gene groups with the most significant changes in expression upon telomere dysfunction. We have subsequently focused on common upstream transcriptional regulators of these genes and have investigated whether these regulators respond directly to telomere dysfunction and contribute to telomere-induced senescence.

As an alternative unbiased approach to identify novel factors involved in DNA damage and/or telomere damage response activation, we performed an siRNA screen to find factors that are involved in ATM activation following DNA damage. Out of 254 different chromatin remodelers, modifiers or DNA helicases, we found 26 genes that upon knockdown led to reduced activation of ATM in a primary screen. We are in the process of validating and characterizing several of these genes.

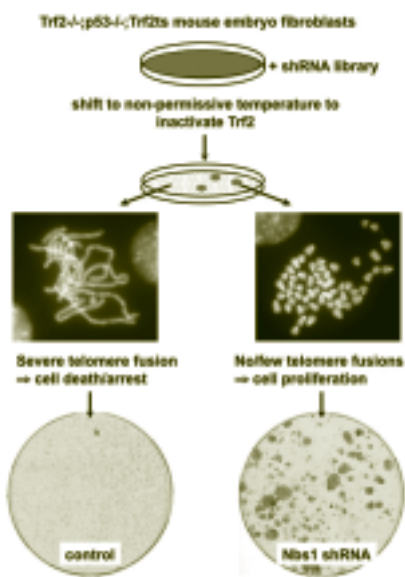


Figure 6: Loss-of-function genetic screening strategy aimed at identifying genes that contribute to telomere-induced genome instability. Mouse embryo fibroblasts with genetic disruption of the telomere component Trf2, but expressing a temperature sensitive Trf2 allele, lose telomere protection on all their chromosome ends when cultured at a non-permissive temperature that inactivates Trf2. This results in severe fusion of chromosome ends and cells are unable to divide or die. However, inhibition of Nbs1 by a shRNA isolated in this screen, allows cells to survive and divide in the presence of uncapped telomeres.

Telomere maintenance and telomere-induced chromosome instability

If cells with uncapped telomeres fail to senesce or die and continue proliferating in the absence of a mechanism that replenishes telomeric repeats, DNA repair activities acting on chromosome ends cause chromosome fusions, anaphase bridges and nonreciprocal translocations. Such cells are at high risk of developing into cancer cells. Although telomere dysfunction is thought to be a major mechanism underlying chromosomal instability in human cancers, little is known about the precise structure of an uncapped telomere, how it is recognized, what precise processing events occur and how these events are controlled. To identify novel factors that contribute to telomere-induced genome instability we have developed an RNAi loss-of-function genetic screen in mouse cells in which we can instantly and reversibly uncap telomeres (see figure 6). The degree of telomere fusion induced in this system is so severe that cells stop proliferating or die. In this screen we have obtained multiple cell clones that survived severe telomere uncapping. The isolation and characterization of shRNA vectors is ongoing, but has already led to the discovery of several genes whose inhibition by RNAi is able to confer resistance to lethal telomere-induced genome instability. One of these is Nbs1, a component of the MRN complex which has recently also been shown by others to indeed contribute to the processing of uncapped telomeres by non-homologous end-joining (see figure), and which validates our screen. The other genes identified so far have not previously been implicated in the response to uncapped telomeres. They include genes that encode for proteins involved in protein ubiquitination and methylation. Their mechanism of action in the telomere damage response is now being investigated, as well as their potential roles in the response to DNA double-strand breaks and in cellular transformation and tumor formation.