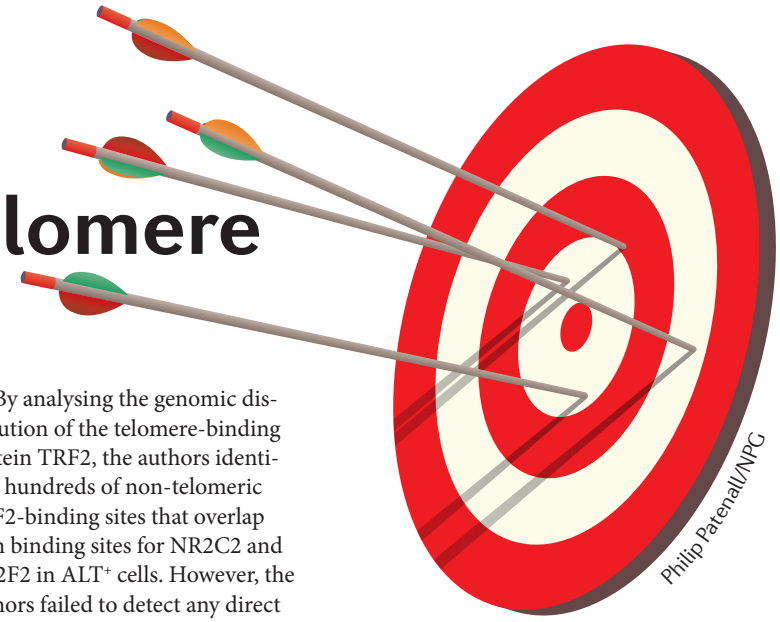
Nature Reviews Molecular Cell Biology | AOP, published online 11 March 2015; doi:10.1038/nrm3969



GENOME INSTABILITY

Targeted telomere insertion

> NR2C/F proteins mediate local rearrangements between telomeric and non-telomeric sites by bridging the loci

When telomeres are disrupted they can fuse to each other and form dicentric chromosomes, which break at random positions during mitosis and cause genome instability. In most cases, this is stopped by the reactivation of telomerase to create functional telomeres on broken chromosomal extremities. However in some tumours, mainly sarcomas, telomeres are maintained by an alternative mechanism known as alternative lengthening of telomeres (ALT). A study by Marzec *et al.* now shows that ALT, in contrast to telomerase activation, leads to further genome instability by adding telomeric DNA to multiple discrete sites in the genome through a mechanism dependent on orphan nuclear receptors of the NR2C and NR2F (NR2C/F) classes.

NR2C/F proteins had been shown previously to associate with telomeres in an ALT$^+$ cell line, and this study confirmed that NR2C2 and NR2F2 bind to telomeres in ALT$^+$, but not ALT$^-$, cell lines. The GGGTCA motif, which is similar to telomeric repeats, is commonly bound by nuclear hormone receptors; this motif was enriched in DNA sequences bound by NR2C2 and NR2F2 and was found to be sixfold more frequent in telomeric DNA from ALT$^+$ cells compared with ALT$^-$ cells. The authors identified the direct repeats DR0, DR6 and DR7 (denoting two motifs in the same orientation and separated by 0, 6 or 7 nucleotides) as being the major binding sites for NR2C/F proteins at telomeres. DR0 repeats were ~80-fold enriched in telomeres from ALT$^+$ compared with ALT$^-$ cells.

By analysing the genomic distribution of the telomere-binding protein TRF2, the authors identified hundreds of non-telomeric TRF2-binding sites that overlap with binding sites for NR2C2 and NR2F2 in ALT$^+$ cells. However, the authors failed to detect any direct interaction between TRF2 and NR2C/F proteins, and the absence of the canonical telomere sequence (GGGTTA) at the dual binding sites excludes direct recruitment of TRF2. Instead, they hypothesized that NR2C/F proteins could bridge TRF2-bound ALT$^+$ telomeric material with genomic NR2C/F-binding sites. In support of this, a fluorescent plasmid containing DR0 repeats was targeted to ALT$^+$ telomeres in an NR2C/F-dependent manner, and a NR2C2–LacI fusion protein tethered to a *LacO* transgene in an ALT$^+$ cell line was extensively colocalized with telomeres.

Physical interactions between loci can trigger chromosomal rearrangements, and this could occur as a result of NR2C/F-mediated bridging. Telomere sequences from ALT$^+$ cells were more degenerated than those from ALT$^-$ cells, and 25% of them could not be aligned with the reference genome. In TRF2-bound genomic libraries of ALT$^+$ cells, these non-aligned, rearranged sequences were composed of unique genomic regions with additions of GGGTTA and GGGTCA motifs in proximity to DR0 sites. The data indicate that NR2C/F proteins mediate local rearrangements between telomeric and non-telomeric sites by bridging the loci, which leads to the addition of telomeric DNA throughout the genome by a process that the authors term targeted telomere insertion (TTI).

As well as potentially affecting the regulation of neighbouring genes, TTI in ALT$^+$ cells may be a source of genomic instability by creating fragile sites that are prone to breakage. Of the translocations resulting from radiation-induced double-strand breaks in an ALT$^+$ cell line, 33% had detectable telomeric signals at the translocation points, of which approximately half (15% of all translocations) did not involve terminal fusions.

The involvement of 'internal' telomeric DNA in 15% of chromosomal translocations suggests that TTI-mediated genomic instability occurs frequently in ALT$^+$ cells. This was supported by the analysis of ALT$^+$ human primary sarcomas, of which ~79% showed telomeric accumulation of NR2C2 and NR2F2, with increased accumulation correlating with higher tumour grade (which is indicative of increased genome rearrangements).

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ORIGINAL RESEARCH PAPER Marzec, P. *et al.* Nuclear-receptor-mediated telomere insertion leads to genome instability in ALT cancers. *Cell* **160**, 913–927 (2015)