Visualisation of telomeres by telomere fibre-FISH

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Telomeres are nucleoprotein complexes that cap the ends of the linear eukaryotic chromosomes. Telomeres comprise repetitive non-coding DNA sequences (TTAGGG repeats in vertebrates) bound to the hexameric protein complex, shelterin. Telomeric DNA is eroded with each round of cell division, eventually resulting in cellular senescence. To counteract this process and achieve proliferative immortality, cancer cells must activate a telomere maintenance mechanism. The majority of cancers activate the ribonucleoprotein enzyme telomerase, while a smaller proportion engage the Alternative Lengthening of Telomeres (ALT) pathway. ALT is a homology-directed repair pathway that utilises telomeric templates for repeat extension, but the precise protein requirements of this complex multi-component pathway remain elusive

We have established telomere fibre-fluorescence *in situ* hybridisation (TFF) to visualise (i) the distribution of individual telomere lengths in a cell population, and (ii) the frequency and length of telomere extension events at individual telomeres. By employing a constant stretching factor of 2 kb/μm to stretch DNA fibres, followed by hybridisation to a telomere-specific PNA probe, we have measured telomere length in ALT cancer cells stably overexpressing the BLM helicase or the SLX4 structure-specific endonuclease. We identified telomere lengthening in BLM overexpressing ALT cell lines, and telomere shortening in SLX4 overexpressing ALT cell lines, and demonstrated the requirement for the BLM-TOP3A-RMI1-RMI2 (BTR) complex for ALT-mediated telomere synthesis. Our data are consistent with ALT being a conservative DNA replication process, analogous to break"induced replication. We are currently using TFF to investigate other protein components of the ALT pathway.