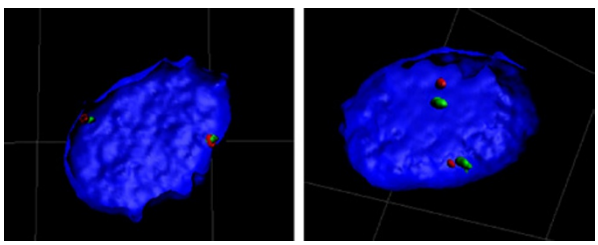
CHROMOSOME BIOLOGY

Short telomeres can't reach

> long, but not short, telomeres are involved in forming chromosome loops

It has long been known that telomeres can silence the expression of nearby genes — a phenomenon known as the telomere position effect (TPE) — and that telomere shortening can affect TPE, albeit by unknown mechanisms. A team led by Shay and Wright had previously found an example in human cells of a gene (*ISG15*) located 1 Mb away from the end of chromosome 1, the expression of which increased as telomeres shortened even though the expression of genes closer to the telomere remained unchanged. They now report that long, but not short, telomeres are involved in forming chromosome loops of up to 10 Mb, and can affect high-order chromatin structure and gene expression.

The enzyme telomerase reverse transcriptase (TERT), which is responsible for maintaining telomere length, is not expressed in most adult cells. By expressing it ectopically for varying durations in human primary myoblasts and fibroblasts, the authors established a collection of isogenic subclones with uniform clonal, but differential interclonal, telomere lengths. Upon TERT clearance from the cells, telomere shortening resumes, and the following experiments were performed before this could lead to replicative senescence or DNA damage signalling. Using subclones with long and short telomeres, the authors performed Hi-C (chromosome conformation capture (3C) followed by high-throughput sequencing) modified to produce a high-resolution map of the chromosomal interactions along the short arm of chromosome 6 (6p), which was chosen because, endogenously, its telomeres are very short. The interaction of the 6p telomeres with two genes (the farthest being 7.5 Mb away) were validated and quantified by 3C and by three-dimensional fluorescence *in situ* hybridization (3D-FISH), and this revealed that as telomeres shorten, telomere-dependent chromosome looping is diminished.



3D-FISH of the subtelomeric region of human chromosome 1p (red) and the ISG15 locus (green) in cells with long telomeres (left) and short telomeres (right). Images courtesy of J. Shay, University of Texas Southwestern Medical Center, USA.

Next, the authors analysed gene expression in myoblasts with long (15 kb) and short (6 kb) telomeres using microarrays, and found that the expression of genes located up to 10 Mb from telomeres of various chromosomes changed when the telomeres shortened. The differential expression of 14 genes (the majority of which showed increased expression) in different subclones of fibroblasts and myoblasts was validated by droplet digital PCR (ddPCR), a technique for quantifying nucleic acids that gives an absolute number of molecules per input. Importantly, the reintroduction of TERT into subclones with short telomeres, and consequently the lengthening of their telomeres, reversed the expression pattern of these genes. Thus, telomere length-dependent, long-range chromosome interactions may enhance or repress gene expression, a phenomenon termed TPE over long distances (TPE-OLD).

The authors then examined telomere-dependent looping in TPE-OLD using 3D-FISH with probes for several TPE-OLD genes located on chromosome 1p. They observed substantial differences in the localization of the loci in cells with long or short telomeres, with the genes being adjacent to the 1p subtelomeric region in cells with long telomeres, but separated from it in cells with short telomeres. Thus, the changes in telomere length-dependent gene expression are accompanied by changes in the high-order organization of the chromatin. How telomere–chromatin loops are formed in human cells awaits further investigation. As the extent and nature of TPE-OLD was found to vary between chromosomes, genes and cell types, it is likely that additional mechanisms are responsible for establishing gene- and cell-specific effects.

Eytan Zlotorynski

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