TELOMERE REPLICATION

Capping protein takes the lead

Linear chromosomes wear a snugly fitting nucleoprotein cap, which prevents degradation of the chromosome ends and protects against inappropriate recombination. In mammalian cells, this cap — the telomere — comprises a repetitive G-rich sequence bound by a number of proteins, including Ku70, Ku80, the catalytic subunit of the DNA-dependent protein kinase (DNA-PK_{cs}) and the telomeric-repeat binding factor 2 (TRF2). Goodwin and colleagues have now investigated how capping occurs and, reporting in Science, they discuss how the processing of telomeres is linked to their mode of replication.

To investigate the capping mechanism, the authors used a cell line containing a dominant-negative mutant of TRF2 ($TRF2^{\Delta B\Delta M}$), which removes endogenous TRF2 from telomeres. They expressed $TRF2^{\Delta B\Delta M}$ for five days in HTC75 human fibrosarcoma cells, and found that 44 of 154 mitotic cells showed end-to-end chromosomal fusions. These fusions — dubbed telomeric chromatid concatenates (TCCs) by the authors — involved just one sister chromatid from each of the two fusing chromosomes (see image), indicating that fusion, and hence TRF2 capping, must have occurred after telomere replication.

Telomere replication poses special challenges. The cap must not only disassemble for replication to occur, but it must also re-form afterwards. Replication involves the generation of two new telomeres — one produced by leading-strand DNA synthesis, the other through lagging-strand synthesis.

To study this process further, Goodwin and colleagues used a technique known as chromosome-orientation fluorescence *in situ* hybridization, which produces different hybridization patterns depending on the type of fusion that has occurred — that is, lagging–lagging, lagging–leading or leading–leading strand fusions. The authors then asked whether the impaired capping seen with $TRF2^{\Delta B\Delta M}$ is limited to telomeres synthesized by one mode of replication, or whether it is a random process. They found that, in 133 out of 135 cases, TCCs were produced by fusion between leading-strand telomeres.

Why does capping fail to occur only on the leading-strand telomeres? Goodwin and colleagues speculate that this might be due to the ends generated by the two modes of replication — leading strands are blunt ended, so have an absolute requirement for TRF2 and DNA-PK_{cs} to fashion 3′ overhangs prior to the formation of a t-loop at the chromosome end. Lagging-strand telomeres, on the other hand, already have a 3′ overhang after replication. Alternatively, they say, there may be other differences in how the two types of telomere are capped.

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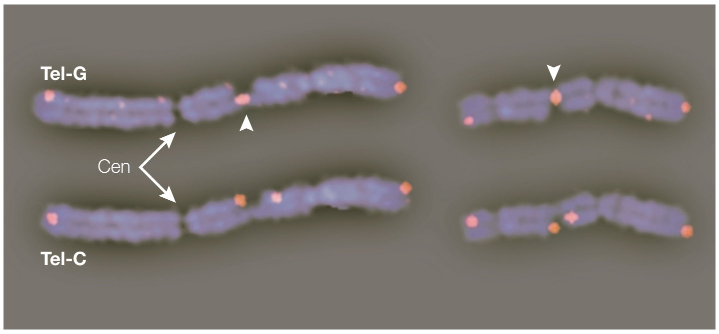
References and links

ORIGINAL RESEARCH PAPER Bailey, S. M. *et al*. Strand-specific postreplicative processing of mammalian telomeres. *Science* **293**, 2462–2465 (2001)



Two TCCs in HTC75 cells. The tel-G probe (which detects leading-strand telomeres) was hybridized and photographed, then the probe removed by denaturation and the tel-C probe hybridized and photographed. Yellow arrowheads indicate the point of fusion. Image courtesy of Michael Cornforth, University of Texas. (Cen, centromere.)

IN BRIEF

ANTIGEN PRESENTATION

Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells.

Kleijmeer, M. *et al. J. Cell Biol.* **155**, 53–63 (2001)

The maturation of dendritic cells — antigen-presenting cells that initiate primary T-cell responses — is characterized by the redistribution of MHC II molecules from the internal membranes of multivesicular bodies to the external membranes of these organelles. There, MHC II encounters DM, an accessory molecule required for peptide loading. The mechanism through which mixing of internal and external membranes occurs is not well understood, but this relocalization could prove important in regulating loading of MHC II antigens.

CELL CYCLE

The human decatenation checkpoint.

Deming, P. B. *et al. Proc. Natl Acad. Sci. USA* **98**, 12044–12049 (2001)

Cells actively delay mitosis until daughter chromatids have become decatenated after DNA replication. Here, Kaufmann and colleagues provide evidence for a decatenation checkpoint. Cells that expressed an ataxia-telangiectasia mutated- and rad3-related (ATR) kinase-inactive allele, or BRCA1-mutant cells, failed to undergo mitotic delay — which seems to require nuclear exclusion of cyclin B1/Cdk1 complexes — implicating ATR-mediated signalling and BRCA1 in this G2 checkpoint.

ION CHANNELS

A sperm ion channel required for sperm motility and male fertility.

Ren, D. *et al. Nature* **413**, 603–609 (2001)

Clapham and colleagues describe the cloning of CatSper, a six-transmembrane-spanning cation channel localized to the principal piece of the sperm tail. Sperm from *CatSper*$^{-/-}$ mice couldn't elicit a Ca^{2+} influx in response to either cAMP or cGMP, implying that CatSper comprises a cyclic nucleotide-gated Ca^{2+}-permeant channel. CatSper functions in male fertility, as sperm from mutant mice were poorly motile and could not penetrate the outer layers of the egg.

SIGNAL TRANSDUCTION

Carboxyl-terminal modulator protein (CMTP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane.

Maira, S.-M. *et al. Science* **294**, 374–380 (2001)

Protein kinase B/Akt has well-established functions in insulin signalling, cell survival and transformation. Searching for new PKB/Akt interactions, Hemmings and colleagues identified CTMP — carboxy-terminal modulator protein. By binding to the carboxy-terminal regulatory domain of PKBα at the plasma membrane, CTMP can inhibit phosphorylation of PKB on serine 473 and threonine 308, and thereby negatively regulate PKB activity.