normally sequestered in heterochromatin may indirectly have detrimental effects on both genome integrity and gene expression. On the other hand, reduced DNMT3B activity could have a more direct effect on gene expression and, perhaps, somatic recombination, in lymphocytes.

Some of the key players of the methylation machinery are now in hand, offering an opportunity to resolve contentious issues in the field of methylation. Perhaps the most controversial question is whether DNA methylation is involved in developmental control of gene expression. Those of us with an interest in imprinting mechanisms will explore whether these or additional *de novo* methyltransferases are involved in introducing methylation imprints in germ cells. Given the perils associated with the lack of appropriate methylation, the challenge will be to understand why the mammalian embryonic genome is globally demethylated, whereas those of other vertebrates are not. □

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At the end of the millennium, a view of the end

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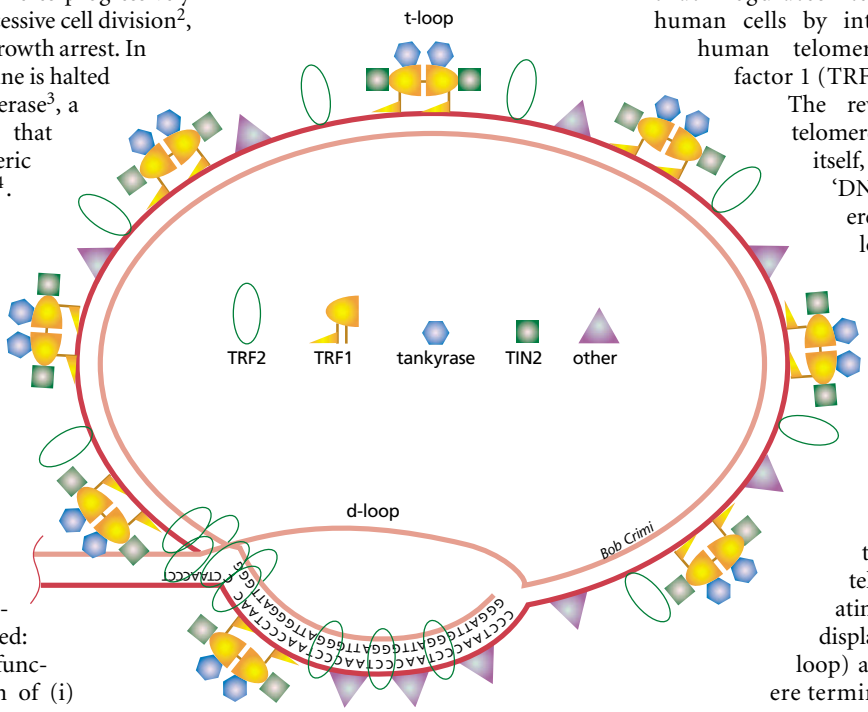
The ends of human chromosomes, telomeres, contain long stretches of a tandemly arranged hexameric sequence, TTAGGG, that is bound by specific proteins[1]. In normal human cells, telomeres progressively shorten with each successive cell division[2], ultimately leading to growth arrest. In tumour cells, this decline is halted by activation of telomerase[3], a reverse transcriptase that extends the telomeric TTAGGG repeat[4]. Telomere length in nearly all tumour cells is stably maintained, suggesting that regulatory mechanisms limit telomere elongation by telomerase. But what is the molecular nature of the mechanism that governs telomere length? To address this question, two overlapping strategies are being pursued: identification and functional characterization of (i) the proteins that localize to the telomere, and (ii) those that associate with the telomerase holoenzyme complex. On page 405 of this issue, Judith Campisi and colleagues[5] advance the former strategy in describing a new protein that regulates telomere length in human cells by interacting with the human telomeric-repeat binding factor 1 (TRF1).



The revelation[6] that the telomere loops back on itself, forming a large 'DNA lariat' or telomere duplex loop (t-loop) structure, has effected a paradigm shift in our view of the mammalian telomere terminus (see figure). It is believed that the long 3′ G-rich single-stranded overhang of mammalian cells invades the double-stranded telomeric repeats, creating a single-stranded displacement loop[6] (d-loop) and masking telomere termini.

A new view of the mammalian telomere. At the end of the chromosome, the mammalian double-stranded telomere bends back on itself[6] forming a large telomere loop (t-loop). The 3′ G-rich single-stranded overhang at the end of the t-loop invades the double-stranded telomere and produces a displacement loop (d-loop). The sequestration of the G-rich single-stranded overhang may prevent inappropriate DNA damage checkpoint responses. The telomere-specific DNA-binding proteins TRF1 and TRF2 are required for normal telomere function along with the TRF1-associated proteins tankyrase and TIN2. Homodimerization of TRF1 is required to form a stable complex with DNA in mammalian cells. Whereas tankyrase may only associate with TRF1 transiently, TIN2 appears to be recruited to TRF1, and this complex, together with TRF2, may make the 3′ G-rich overhang inaccessible to telomerase. Thus, TRF1 and its associated proteins (TIN2 and tankyrase), TRF2, the telomerase holoenzyme, and perhaps as yet unidentified proteins, are important regulators of human telomere length.

A TRFic pair

Human telomeres require at least two telomere-specific DNA-binding proteins, TRF1 (ref. 7) and TRF2 (ref. 8), to regulate telomere length approximately. TRF1 binds duplex TTAGGG repeats and has some similarities to yeast Rap1p (refs 1,4). Both TRF1 and Rap1p contain a Myb-like DNA-binding motif; TRF1 must homodimerize to mediate stable complex formation and telomeric DNA looping. The binding of TRF1 (ref. 9) and Rap1p to telomeric repeats induces a shallow bend in telomeric duplex DNA, indicating an architectural role in DNA looping. Binding of Rap1p molecules to duplex telomeric DNA may provide a feedback mechanism that regulates telomere length in yeast[10]. TRF1 may have a similar function in mammalian cells; overexpression of normal TRF1 in telomerase-expressing tumour cells results in gradual telomere shortening, whereas overexpression of mutant TRF1 results in telomere elongation[11].

It seems that TRF2 maintains chromosomal stability; overexpression of mutant TRF2 in immortalized cells induces end-to-end chromosome fusion and growth arrest, as seen in replicative senescence[12]. It binds along the telomeric repeats in a similar fashion to TRF1, but its localization is biased toward the loop-tail junction[6], possibly stabilizing the G-rich single-stranded telomeric overhang at the d-loop. It could therefore facilitate strand invasion and prevent chromosome fusion, and thereby protect single-stranded telomeric DNA from initiating a DNA damage response[13]. Senescent growth arrest may be triggered by reduction of telomers to a critical threshold at which t-loops cannot form.

Teasing apart the telomere

Although we lack a complete understanding of how TRF1 regulates telomere length, progress has been made in identifying other components of the telomere complex. Tankyrase, a protein with homology to ankyrins and the catalytic domain of poly(ADP-ribose) polymerase (PARP), was identified using a yeast two-hybrid TRF1 screen[14]; it co-localizes with telomeres and can ribosylate both itself and TRF1 *in vitro*. Ribosylation of TRF1 inhibits its binding to telomeric DNA, and may permit the telomerase complex to gain access to the telomere. Using a similar strategy, Campisi and colleagues have now identified another TRF1-binding protein—TIN2—which co-localizes with TRF1 on metaphase chromosomes. They found that expression of mutant TIN2 (with an N-terminal truncation) causes elongation of telomeres in a telomerase-dependent manner, suggesting that TIN2 mediates TRF1 function and negatively regulates telomere length. A model consistent with these observations involves TRF1 recruiting TIN2 to the telomere, where it regulates telomerase-mediated telomere elongation.

How does TIN2 regulate telomere elongation? We know that it neither inhibits telomerase activity *in vitro* nor interacts directly with the catalytic protein subunit of telomerase. It may promote compaction of telomeric structure and limit telomerase access to the single-stranded 3′ end, or alternatively, stimulate TRF1-mediated parallel pairing of telomeric DNA tracts[17]. The constitutive expression of both TRF1 and TIN2 indicates that they may act together in regulating telomere length, perhaps by stabilizing the t-loop and rendering the G-rich overhang less accessible to telomerase. In normal cells, progressive telomere shortening may lead to "loss of telomeric" TRF1, TIN2 and tankyrase, signalling growth arrest when telomeres reach a critical length. Perhaps a parallel telomere loss in tumour cells might initiate a signalling pathway that recruits telomerase, thus maintaining stable telomere length. How tankyrase and TIN2 modulate TRF1 function is unknown, but both may recruit other, possibly unidentified, proteins to the telomere which in turn regulate the activity of telomerase or other proteins critical to telomere structure or function.

There are no obvious TIN2 or tankyrase homologues in yeast, so the mammalian proteins may provide insights into the differences between yeast and mammalian cells. It is not yet known whether t-loops and d-loops form in yeast, which have much shorter telomeres and G-rich overhangs than mammalian cells[1,4]. The mammalian t-loop structure may therefore represent an alternative to end-binding protein complexes that have been identified in some model organisms.

With increasing evidence that telomeres and telomerase are critical to human replicative senescence and cancer, the elucidation and regulation of telomere function has become a central question in biology. Identifying telomere-associated proteins is the first priority, but the long-term goal is to understand how the telomerase complex and telomere interact and how the interaction is affected by immediate and distant environmental signals. As the millennium closes, the end—of uncovering the chromosome end—may soon be in sight. □

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