SMADs are feedback regulators of TGF-β signalling opens up the question of how the expression and regulation of these molecules participates in the patterning of TGF-β responses in developing tissues. □

Malcolm Whitman is in the Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, USA.
e-mail: mwhitman@warren.med.harvard.edu

1. Massagué, J., Hata, A. & Liu, F. *Trends Cell Biol.* **7,** 187–192 (1997).
2. Imamura, T. *et al. Nature* **389,** 622–626 (1997).
3. Tsuneizumi, K. *et al. Nature* **389,** 627–631 (1997).
4. Nakao, A. *et al. Nature* **389,** 631–635 (1997).
5. Hayashi, H. *et al. Cell* **89,** 1165–1174 (1997).
6. Topper, J. N. *et al. Proc. Natl Acad. Sci. USA* **94,** 9314–9319 (1997).
7. Alevizopoulos, A. & Mermod, N. *Bioessays* **19,** 581–591 (1997).
8. Neumann, C. & Cohen, S. *Bioessays* **19,** 721–729 (1997).
9. Chen, X. *et al. Nature* **389,** 85–89 (1997).
10. Kim, J., Johnson, K., Chen, H. J., Carroll, S. & Laughon, A. *Nature* **388,** 304–308 (1997).
11. Raftery, L. A., Twombly, V., Wharton, K. & Gelbart, W. *Genetics* **139,** 241–254 (1995).

Telomerase

Cancer and the knockout mouse

David Wynford-Thomas and David Kipling

Telomeres are specialized structures at the ends of linear chromosomes. They usually consist of tandem arrays of a short DNA sequence (TTAGGG in vertebrates) and associated proteins, and they are thought to have at least two essential functions[1]. First, they stop natural chromosome ends behaving as random breaks, which might otherwise generate inter-chromosomal fusions and activate DNA-damage-induced cell-cycle arrest. Second, they provide the structural basis for solving the end-replication problem[2] — the inability of DNA polymerases to completely replicate the end of a DNA duplex (see box).

In the germ line, telomeres are maintained by the compensatory addition of TTAGGG repeats to chromosome ends. These repeats are synthesized by an enzyme called telomerase (Fig. 1, overleaf), and a lack of telomerase activity — such as occurs physiologically in most adult human somatic cells — leads to progressive telomere shortening with every cell cycle[3]. If such cells are forced to grow for long enough, they eventually lose telomere function leading to end-to-end chromosome fusions and cell death. What, then, might be the effect of a constitutional (germline) deficiency of telomerase? An intriguing (although still partial) answer is provided in the latest issue of *Cell*, where Blasco *et al.*[4] report their studies of a telomerase-deficient mouse.

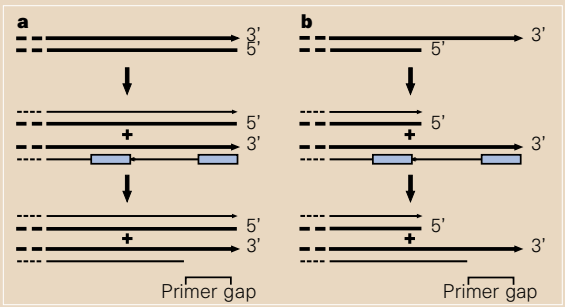
Telomerase contains an essential RNA component that provides the template for the specific synthesis of TTAGGG repeats. Blasco *et al.* used transgenic technology to create a germline deletion of the *mTR* gene, which encodes the RNA component of mouse telomerase. The authors bred homozygous, null $mTR^{-/-}$ mice which turned out to be both viable and fertile, despite having no detectable telomerase activity. Moreover, the mice have now been maintained for six generations (G1–6). The absence of any initial phenotype is striking. So is the fact that the next few generations have also shown no symptoms, suggesting that the telomerase inhibitors that have been envisaged for cancer therapy will not have any acute toxicity. But telomere shortening is occurring: by the latest generation studied (G6), there is clear evidence of telomere erosion, with around five per cent of chromosomes in embryonic fibroblasts lacking detectable TTAGGG, accompanied by increasingly frequent chromosome fusions.

This 'delay' in manifestation of the phenotype almost certainly reflects the unusually long telomere-repeat arrays in the germ line of this mouse species. These repeats are not exhausted until many generations have elapsed — a human 'knockout' would be predicted to show a much more rapid onset. Yet, despite reaching what seems to be a critical state of erosion, cells from generation G6 do not show an obvious reduction in growth capacity in culture, or any reduction in the ability to generate tumours when oncogene-transformed cells are injected into nude (immunodeficient) mice.

It has been suggested that progressive erosion of telomeres in somatic cells (which have physiologically repressed telomerase) represents a barrier to indefinite cell proliferation. Tumour cells are thought to overcome this block by reactivating telomerase. But the apparently undiminished tumorigenic ability of oncogene-transformed $mTR^{-/-}$ cells suggests that telomerase may not be necessary for tumorigenesis. Data showing that, in mice, telomerase can be up-regulated at an early stage in tumour development[5] — before any significant telomere erosion has occurred — leads the authors to hint that the current dogma may be wrong. In other words, telomerase may be nothing more than a passive bystander, rather than facilitating tumour growth[4]. If so, this would have considerable implications for telomerase as a target for cancer therapy (although it does not necessarily detract from its value as a diagnostic marker).

There are, however, good reasons for exercising caution when extrapolating from the mouse model. The main limitation is that the phenotype is probably not yet completely developed. Although telomere failure is occurring at G6, most of the chromosomes retain detectable TTAGGG. The very fact that viable G6 animals exist suggests

The end-replication problem



For lagging-strand DNA replication, short RNA primers (blue) are made by RNA primase. These are then extended by DNA polymerase to form Okazaki fragments. When these RNA primers are removed, there is no way to synthesize lagging-strand sequence that is complementary to the small region at the end of the chromosome (which is at least as large as an RNA primer). So, with continuing cell division, sequence is lost from the ends of linear chromosomes. Some blunt-ended daughter molecules are produced by this scheme, irrespective of whether the starting terminus is blunt-ended (a) or has a 3' extension (b).

Why are such natural blunt ends not recognized as DNA damage? One possibility is that this is because unnatural ends have a slightly different chemical structure; for example, radiation-induced blunt ends can have terminal 3' phosphoglycolate residues whereas physiological ends do not. Human cells may have an additional mechanism to ensure that natural chromosome ends are even more different. This involves a degradative pathway which, even in telomerase-deficient human cells, results in 3' extensions for most chromosome ends[9]. This may be analogous to a degradative pathway that has been described in budding yeast, involving Cdc13p and other proteins[10]. **D.W.-T. & D.K.**

Nature © Macmillan Publishers Ltd 1997

Nature © Macmillan Publishers Ltd 1997

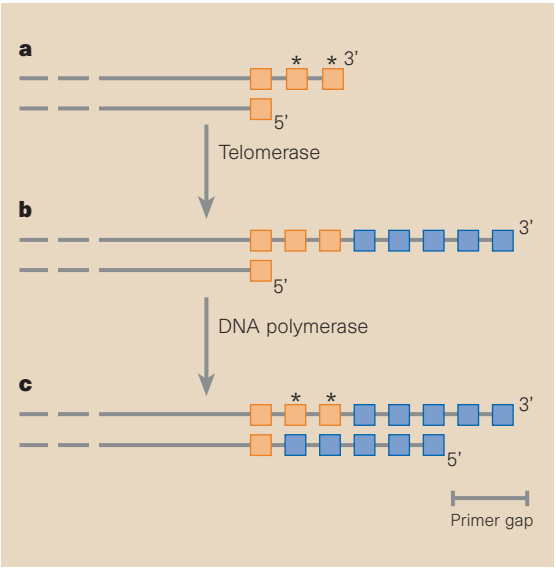


Figure 1 **Synthesis of telomeric DNA by telomerase.** a, **A short region of the upper strand of the telomere (composed of TTAGGG repeats) cannot be copied by normal DNA replication (see box) and is indicated by asterisks.** b, **Telomerase circumvents this by synthesizing new TTAGGG repeats (blue), allowing the ends to be copied by conventional DNA replication** (c). **Mouse telomerase carries an RNA molecule that acts as a template for synthesis of the TTAGGG sequence. Blasco *et al.*[4] have deleted the *mTR* gene (which encodes this RNA molecule) in the mouse germ line.The resultant knockout mice have no telomerase activity, and their telomeres become shorter with continuing cell division.**

that interference with chromosome function may need to be more widespread to compromise cell proliferation severely. It seems improbable that the observed telomere erosion could continue unabated without eventually stopping growth. For example, viral-oncogene-transformed human cells eventually undergo 'crisis' (cell death) during continuous culture, unless telomere erosion is halted by activation of either telomerase[3] or of an alternative pathway[6]. A remarkable counterpart *in vivo* may be the spontaneous tumour regression that occurs in a subset of childhood neuroblastomas (so-called stage IV-S), which apparently fail to up-regulate telomerase[7]. So, if telomere erosion continues, oncogene-transformed $mTR^{-/-}$ cells might be expected, eventually, to lose their ability to generate tumours, once they have undergone enough cell divisions.

It is also risky to extrapolate from the mouse with regard to the timing of telomerase up-regulation in tumour development — not least because telomerase seems to be more tightly regulated in man. Many (but not all) human cancers possess very short telomeres, consistent with the classic model of telomere erosion followed by selection for telomerase reactivation during an *in vivo* equivalent of crisis. Of course, some tumours may acquire telomerase ahead of critical telomere erosion; for example, if telomerase is reactivated as part of a 'package' of changes in gene expression that occurs after some other genetic event (the 'co-selection' hypothesis[4,8]). But such anticipatory up-regulation does not mean that telomerase is not required at a later stage, so this should not dampen enthusiasm for anti-telomerase therapies.

We may also need to revise our ideas about how cells distinguish between unnatural genomic breaks such as those caused by ionizing radiation (which usually lead to immediate cell-cycle arrest or programmed cell death), and natural chromosome ends (which do not). It has been assumed that in mammalian cells, specific terminal DNA sequences and associated proteins somehow 'shield' the natural end of the chromosome and prevent it from activating genome-damage-monitoring systems. The later-generation $mTR^{-/-}$ cells show an apparent loss of telomere-protective function (as judged by end-to-end fusions in metaphase chromosome preparations). But the fact that such cells have reached metaphase suggests that they have passed through at least one damage-sensing checkpoint (at G2–M), despite having critically eroded telomeres.

If the absence of telomere-specific DNA sequences and proteins does not activate cell-cycle arrest, then what does? It is possible that the physical structure of the DNA-end itself is differentially recognized — a break caused by ionizing radiation is not only physiologically abnormal, but it is chemically very different from a natural chromosome terminus formed by DNA replication.

We now await with interest the phenotype of later generations of these mice (if they remain fertile), and later passages of cell cultures derived from them. Whatever the outcome, it is certain that interest in telomeres has not come to an end. □

David Wynford-Thomas and David Kipling are in the Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK.

1. Kipling, D. *The Telomere* (Oxford Univ. Press, 1995).
2. Olovnikov, A. M. *Dokl. Akad. Nauk* **201,** 1496–1499 (1971).
3. Counter, C. M. *et al. EMBO J.* **11,** 1921–1929 (1992).
4. Blasco, M. A. *et al. Cell* **91,** 25–34 (1997).
5. Broccoli, D., Godley, L. A., Donehower, L. A., Varmus, H. E. & de Lange, T. *Mol. Cell. Biol.* **16,** 3765–3772 (1996).
6. Bryan, T. M. & Reddel, R. R. *Eur. J. Cancer* **33,** 767–773 (1997).
7. Hiyama, E. *et al. Nature Med.* **1,** 249–255 (1995).
8. Kipling, D. *Eur. J. Cancer* **33,** 792–800 (1997).
9. Makarov, V. L., Hirose, Y. & Langmore, J. P. *Cell* **88,** 657–666 (1997).
10. Lin, J.-J. & Zakian, V. A. *Proc. Natl Acad. Sci. USA* **93,** 13760–13765 (1996).

Daedalus

Smooth ascent

A solid-fuel rocket is simply an enormous firework. Once lit, it cannot be stopped or controlled. Furthermore, it burns in massively turbulent flow, generating extreme vibration. Everything that has to fly in a rocket must pass a searching vibration test. Daedalus is now devising a less vibrant way to climb into space.

A typical solid fuel is a mixture of aluminium powder and ammonium perchlorate, formed into a resilient solid by a polymeric binder. DREADCO chemists are replacing the aluminium powder by fine aluminium wire, and the binder by a water-based gel. The product is just damp enough so that ignition cannot spread through it spontaneously; but each element can be ignited by passing a current through the local aluminium wire. The charge will be fired controllably, element by element, by passing a programmed sequence of current pulses through the wires.

In this simple form, the idea is clearly impractical. A big rocket motor could have millions of ignitable elements, far too many for each to have its own electrical leads. But Daedalus recalls the old flashbar units for small cameras, which carried ten separate flash-bulbs. As each bulb fired, it carbonized and caused a lead to the next bulb to become conducting. Each time the shutter was depressed, the trigger current set off the next live bulb. This trick, suitably developed, would allow thousands of elements in the motor to be set off controllably by a single set of leads. A few thousand sets of such leads could command the complete motor.

The resulting rocket will be a masterpiece of smooth power. Its thrust will be controlled from instant to instant by the rate at which its elements are fired. Its vibration will be damped by fast-acting acceleration sensors feeding its control computer, which will stagger the firing sequence to the next elements so as to cancel or minimize the instantaneous fluctuations of thrust. If required, the whole motor can be shut off instantly, and started again later; once in space, it can be operated on command at very low thrust for orientation or course correction.

The technology could even be adapted for other purposes, such as blasting, demolition and explosive forming. Instead of many explosives for different jobs, one all-purpose DREADCO product will do. It can be programmed to act as a propellant, a low, medium or high explosive, or even a subtle combination of each.

David Jones

Nature © Macmillan Publishers Ltd 1997