CELL SENESCENCE AND CANCER

Nicole F. Mathon and Alison C. Lloyd

Historically, the senescent state has been associated with, and was named after, the cell-cycle arrest that occurs after cells have undergone an intrinsically defined number of divisions *in vitro*. More recently, however, it has been shown that extrinsic factors, including those encountered in normal tissue-culture environments, can prematurely induce an indistinguishable senescent phenotype. In this review, we discuss the pathways of cell senescence, the mechanisms involved and the role that these pathways have in regulating the initiation and progression of cancer.

MRC Laboratory for Molecular Cell Biology and the Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, UK.
Correspondence to A.C.L.
e-mail: alison.lloyd@ucl.ac.uk

Forty years ago, Leonard Hayflick first suggested that there was a "finite limit to the cultivation period of diploid cell strains" and that this was "attributable to intrinsic factors which are expressed as senescence at the cellular level"[1]. Stated more simply, Hayflick proposed that normal cells cannot divide indefinitely because they are programmed for a set proliferative lifespan. The replicative lifespan of a cell should permit sufficient divisions for the proliferative requirements during the lifespan of an animal, but act as a brake to excessive proliferation to protect against cancer. The balance required to achieve these aims has been postulated to contribute to organismal ageing as cells at the end of their proliferative lifespan accumulate in the body.

Human diploid fibroblasts (HDFs) were the cell type initially used to define the mechanisms underlying replicative lifespan[2]. These cells can undergo 60–80 population doublings in culture, after which they cease division and develop a senescent phenotype that is characterized by a large, flat morphology, a high frequency of nuclear abnormalities and a positive stain for β-galactosidase activity at pH 6.0 (REF. 3). This metabolically active, senescent state is extremely stable and can be maintained in culture for several years. The cell-cycle arrest that is imposed in these cells is maintained by signals that activate the tumour suppressors p53 and retinoblastoma (RB)[4–6]. Abrogation of these pathways — by, for example, the expression of tumour viruses — bypasses the senescence-associated cell-cycle arrest and the cells continue to proliferate beyond their normal lifespan. These cells still cannot divide indefinitely — they are not immortal. Following 20–30 additional population doublings, they enter a state that is termed 'crisis'. At crisis, the cells continue to proliferate but show high rates of apoptosis — triggered by gross chromosomal abnormalities — so there is no net increase in cell number. Rare cells emerge from crisis and these are immortal (FIG. 1).

The molecular mechanisms that determine the intrinsic replicative lifespan of HDFs seem to be controlled by a single process — telomere shortening. Telomeres are the repetitive DNA sequences, bound by a complex of proteins, at the ends of linear chromosomes (BOX 1). Telomerase, a ribonucleoprotein enzyme, is required for complete replication of the DNA ends (BOX 1). HDFs, like most human somatic cells, do not express telomerase, so the telomeres progressively shorten at each division[7]. Once a telomere reaches a critical length, a damage signal triggers the senescent state through mechanisms that are incompletely understood (BOX 2). If this damage signal is abrogated by inactivation of p53 and RB, the cells can continue to proliferate, but the telomeres continue to shorten until they cause the chromosomal instability that is responsible for crisis (FIG. 1). That telomere shortening is responsible for a limit to the lifespan of HDFs has been generally accepted, as expressing telomerase in HDFs is sufficient to bypass both senescence and crisis, and confer immortality[8,9]. So, telomere shortening acts as a mitotic counter that determines the proliferative lifespan of HDFs and will ultimately limit the proliferative capacity of any cell type that lacks telomerase activity.

However, other cell types that maintain telomere length in culture, either as a result of endogenous telomerase activity or by experimental expression of telomerase, still acquire a senescent phenotype in

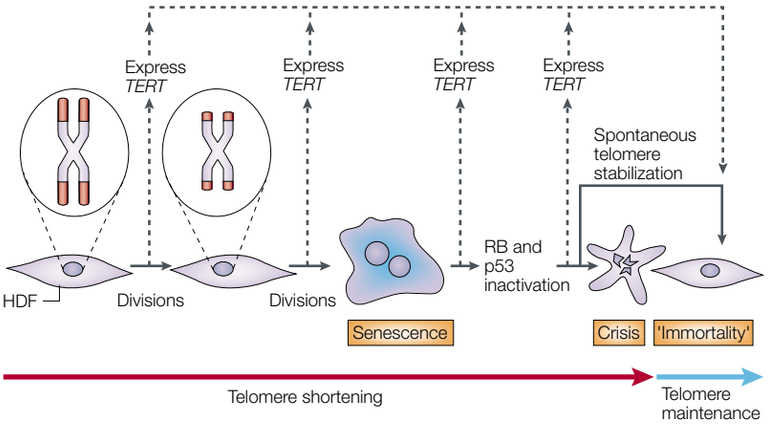


Figure 1 | **Telomere shortening determines the proliferative lifespan of human diploid fibroblasts.** After 60–80 population doublings in culture, short telomeres in human diploid fibroblasts (HDFs) trigger a damage response that leads to senescence. Overcoming senescence, by abrogation of the retinoblastoma (RB) and p53 tumour-suppressor pathways, allows continued division until critically short telomeres trigger crisis. Rare cells emerge from crisis by activating mechanisms for telomere stabilization. Exogenous expression of telomerase reverse transcriptase (*TERT*) at any stage in the replicative history allows immortalization of these cells.

culture. For example, human keratinocytes and mammary epithelial cells are not immortalized by telomerase expression alone[10]. Similarly, rodent cells express telomerase and maintain very long telomeres, yet senesce in culture. This indicates that there are additional, telomere-independent, mechanisms that limit the proliferative lifespan of certain cell types.

Recent findings, however, have led many to question the existence of telomere-independent replication-induced senescence. It has been known for some time that 'damage' signals can induce a senescent phenotype in cells, independent of their replicative history. For example, young HDFs become senescent if exposed to agents that induce cellular damage, such as DNA-damaging agents[11,12] or oxidative stress[13,14]. In addition, inappropriate activation of mitogenic signalling pathways by expression of oncogenic *RAS* will also trigger entry into senescence in these cells[15]. These pathways to senescence have been termed premature senescence, as they cut short an intrinsic replicative lifespan (FIG. 2).

It has been proposed that the telomere-independent mechanisms that limit replicative lifespan — seen in some human epithelial cells and rodent cells — are a form of premature senescence, triggered by the cumulative trauma of culturing cells *in vitro*[16,17]. Evidence for this hypothesis has come from recent findings that some cell types seem not to have a limited replicative capacity, and that by altering culture conditions it is possible to change the onset of senescence. Studies of two rat cell types — SCHWANN CELLS and OLIGODENDROCYTE PRECURSOR CELLS, which both normally express telomerase — showed that they can be cultured indefinitely, but a change in the culture conditions could subsequently trigger entry into senescence[18–20]. Similarly, altering the culture conditions of human epithelial cells — by growing them on feeder layers, rather than on plastic — allowed these cells to bypass senescence and, when expressing exogenous telomerase, proliferate indefinitely[21] (FIG. 2). These results show that, in the presence of telomerase activity, there is no intrinsic mechanism that limits the lifespan of these cell types, but that extrinsic factors can limit their proliferative capacity. This might indicate that the culture conditions of other cell types are responsible for the apparent limits to their proliferative lifespan, and that any cell expressing telomerase could proliferate indefinitely if suitable culture conditions were found.

Is replicative senescence tumour suppressive?

Most human somatic cells do not express telomerase, so would be predicted to have a limited replicative lifespan. This inbuilt mechanism has been proposed to protect against the development of cancer by limiting the proliferation required for the formation of a clonal tumour. One criticism of this hypothesis has been that, at first glance, the proliferative capacity of normal cells *in vitro* seems to vastly exceed the number of divisions that are required to form a tumour. For example, 40 population doublings are equivalent to one cell giving rise to 10^{12} cells, whereas a detectable tumour rarely contains more than 10^{9} cells. However, recent studies have shown that although the early stages of tumour development are highly proliferative, expansion of the tumour is stalled by correspondingly high rates of apoptosis. It is therefore difficult to determine the number of divisions required for the development of a human tumour and to assess how a limit to replicative capacity might contribute as an impediment to the process.

What is clear is that telomere stabilization is an important step in tumour development. Most human malignant tumours express telomerase; those that don't, stabilize their telomeres by a different mechanism — alternative lengthening of telomeres (ALT)[22,23]. Telomeres are often shorter within tumours than in the surrounding tissues, indicating that telomere shortening occurs at some point during tumour development[24,25]. Moreover, telomerase expression in tumour cells is essential for their continued proliferation, as expression of DOMINANT-NEGATIVE telomerase reverse transcriptase (*TERT*) results in apoptosis[26]. This requirement for telomerase activation has been reproduced *in vitro*. Starting with a normal human fibroblast, telomerase expression is a key requirement — in addition to oncogenic *RAS* and SV40 LARGE T ANTIGEN expression — for the production of transformed cells that are capable of forming tumours *in vivo*[27].

So, there seemed to be a simple story. Human somatic cells do not express telomerase. The proliferation required for a tumour to develop is sufficient to trigger replicative senescence, and so acts to protect us against cancer. Rarely, cells bypass this checkpoint by the activation of telomerase or stabilization of telomeres, and tumour development can then proceed. This would make telomerase a perfect target for new cancer drugs, as most tumour cells need telomerase activity, whereas normal cells do not.

SCHWANN CELL
Glial cell of the peripheral nervous system.

OLIGODENDROCYTE PRECURSOR CELL
Glial cell precursor of the central nervous system.

ALT
(Alternative lengthening of telomeres). A recombination-based mechansim that allows telomere length maintenance in the absence of telomerase activity.

DOMINANT NEGATIVE
A defective protein that inhibits wild-type function by retaining interaction capabilities that result in distortion or competition with normal proteins.

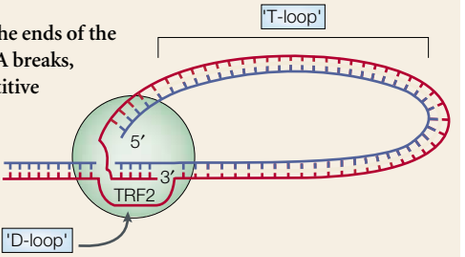
SV40 LARGE T ANTIGEN
A large multifunctional protein that is encoded by the Simian virus 40 (SV40). SV40 large T can bind and inhibit functions of p53- and RB-family members.

Box 1 | **Telomeres and telomerase**

Telomeres

Telomeres are tandem repeats ((TTAGGG/CCCTAA)$_n$ in vertebrates) that are found at the ends of linear eukaryotic chromosomes[80]. Their length varies between chromosomes and species, but can range from a few to tens of kilobase pairs. Telomeres have several roles: they prevent the ends of linear chromosomes from appearing as DNA breaks, they protect chromosome ends from degradation and fusion, they allow complete chromosome replication and they position the chromosomes within the nucleus.

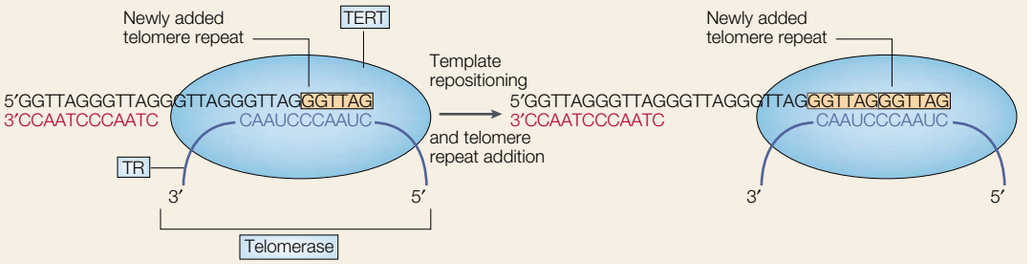
Telomere structure



Telomeres are, through necessity, dynamic structures. Although the ends of the chromosomes need to be camouflaged to avoid being seen as DNA breaks, DNA replication requires remodelling of this structure. The repetitive sequences of telomeres are bound by both duplex-DNA-binding proteins, which include TRF1 and TRF2 (REF. 81), and single-stranded DNA-binding proteins[80]. Telomeres end in a 3′ single-strand overhang. The telomere end folds back on itself, forming a protective 'T-loop', to hide the vulnerable 3′ overhang. This single-stranded DNA invades and hybridizes with a region of the double-stranded telomere repeat and the displaced DNA forms a small 'D-loop'. Telomere-binding proteins — for example, TRF2, which binds the T-loop juncture — are important in maintaining the stability of this structure[82,83].

Telomerase and the end-replication problem

Replication of the DNA lagging strand occurs by extension of primers in the 5′→3′ direction. At the ends of the chromosomes, the terminal primer on the lagging strand (3′ end) leaves a stretch of DNA that cannot be replicated. As a result of this, the telomeres shorten by 50–200 base pairs with each cell division. Telomerase is a telomere-specific ribonucleoprotein reverse transcriptase that adds single-stranded telomeric repeats to the chromosomal 3′ ends, preventing continual telomere shortening. The telomerase ribonucleoprotein complex contains TR (the integral RNA template) and TERT (telomerase reverse transcriptase; the protein catalytic subunit)[80].



Lessons from mice

However, life is never that simple. To address the role of telomere shortening in cancer and ageing, a telomerase knockout mouse was developed in which the RNA component, mTR, was deleted[28] (reviewed in REF. 29). Due to the extremely long telomeres of the laboratory mouse, successive breeding of the knockout animals was required to generate telomeres that approach the lengths seen in humans, but by the fourth generation, various effects of telomere shortening could be observed. As would be predicted, the earliest effects of telomere shortening were defects in the most highly proliferative tissues[30]. But the surprise was that these late-generation mice had an increased frequency of tumour development, as compared with wild-type or early-generation animals[31]. This indicated that, in these mice, telomere shortening was increasing the frequency of cancer, rather than protecting against it (BOX 3).

A likely explanation for this anomalous finding is that shortened telomeres have a dual effect: they initiate checkpoint signals that provoke a cell-cycle arrest or trigger apoptosis, but they can also cause chromosomal instability[32,33]. So, whereas the checkpoint signals protect against tumour formation, increased genetic instability is likely to speed up the multistep tumorigenic process. It has been shown, both *in vitro* and *in vivo*, that shortened telomeres provoke chromosomal instability. HDFs approaching crisis show gross chromosomal abnormalities with end-to-end fusions, resulting in MULTICENTRIC CHROMOSOMES with breaks occuring at mitosis[34]. In yeast, the chromosomal abnormalities induced by short telomeres lead to an increase in the mutation rate[35]. Similarly, cells isolated from proliferating tissues of late-generation, telomerase knockout mice show evidence of similar chromosomal abnormalities, and it is in these tissue types that the tumours arise[28]. The fact that the mice have a higher incidence of tumour formation shows that the more rapid accumulation of deleterious mutations as a result of shortened telomeres outweighs any protective advantage of a proliferative checkpoint.

MULTICENTRIC CHROMOSOMES
Chromosomes with more than one centromere.

CDKN2A
This locus, which is commonly mutated in cancer, encodes two transcripts with overlapping reading frames — *Ink4a* and *Arf*. Both protein products are involved in negative regulation of the cell cycle.

INK4A
A 16-kDa cyclin-dependent kinase inhibitor that is expressed from the *Cdkn4a* locus. It acts on cyclinD–CDK4/6 activity to inhibit cell-cycle progression.

ARF
A 19-kDa protein (14 kDa in humans) that is expressed from the *Cdkn2a* locus in response to oncogenic stimuli. It functions by stabilizing p53.

Box 2 | **Telomere signalling**

Telomeres are complex structures that protect the ends of linear chromosomes. A telomere is considered to be 'capped' when it is sufficiently stable to signal continued proliferation to the cell. An 'uncapped' telomere triggers a cell-cycle arrest or apoptosis. In addition, 'uncapped' telomeres can lead to an increase in genetic instability, resulting in an increased mutation rate[84].

In the absence of telomerase, telomeres will shorten at each division. Analysis of individual chromosome ends has shown that it is the length of the shortest telomere that determines replicative capacity rather than the average telomere length[85]. These short telomeres are seen as 'uncapped'. Telomeres can also become uncapped by the disruption of telomere structure. Expression of a dominant-negative version of *TRF2* (see BOX 1) disrupts the normal structure and results in the rapid onset of either senescence or apoptosis, depending on the cell type[86,87]. Disruption of telomeres by the expression of dominant-negative *TRF2* activates a damage pathway that is known to be activated in response to double-stranded DNA breaks. This involves activation of the ATM kinase that signals to the cell-cycle machinery and apoptotic pathways in a p53-dependent manner[86].

Telomerase expression has also been proposed to increase genetic stability in a manner that is independent of regulating telomere length[84]. It has been suggested that telomerase expression increases the overall stability of the 'capped state'. The idea that the telomere state is dynamic and that even long telomeres can become uncapped fits with the stochastic onset of senescence in telomerase-deficient cells. A second suggestion is that, following DNA damage, *de novo* telomere addition by telomerase to broken chromosomal ends can change the spectrum and decrease the severity of the resultant chromosomal rearrangements[35].

This effect is amplified in a p53-null background, probably because of the increased survival of genetically damaged cells[36,37].

If the mutation rate is increased by applying carcinogens, or the mutation requirement is reduced by crossing the telomerase-negative mice to mice that already carry oncogenic mutations, the opposing protective role of telomere shortening on the incidence of tumour formation can be observed. For instance, CDKN2A knockout mice have a much higher frequency of tumour formation because they lack the two tumour suppressors that are encoded by the *Cdkn2a* gene — INK4A (also known as p16) and ARF (also known as p19)[38]. When crossed to the telomerase knockouts and treated with carcinogens, the late-generation mice have a reduced tumour frequency compared with the early-generation mice that maintain long telomeres[39]. Therefore, in this situation, limits to replicative capacity seem to protect against tumour formation. Similarly, short telomeres protect against the formation of epithelial tumours when carcinogens are applied to the skin of telomerase-knockout mice[40] (BOX 3).

The most dramatic illustration of the opposing effects that telomere shortening can have on tumour incidence is seen in Apc^{Min} mice[41]. These have a truncating mutation in the adenomatous polyposis coli tumour-suppressor gene *Apc* and, as a result, develop multiple intestinal neoplasia. Humans who harbour a similar mutation are also predisposed to develop multiple polyps and have an increased risk of developing colon carcinomas (FAMILIAL ADENOMATOUS POLYPOSIS)[42]. Apc^{Min} mice with short telomeres show increased frequencies of microscopic adenomas compared with animals that have long telomeres, presumably as the increased genetic instability increases the likelihood of loss of the second *Apc* allele. However, the number progressing to form macroscopic adenomas is reduced and the mice live longer than Apc^{Min} mice with long telomeres[41]. This indicates that replicative capacity limits the progression of microadenomas into malignant tumours and so, in this context, telomere shortening acts to protect against tumour formation (BOX 3).

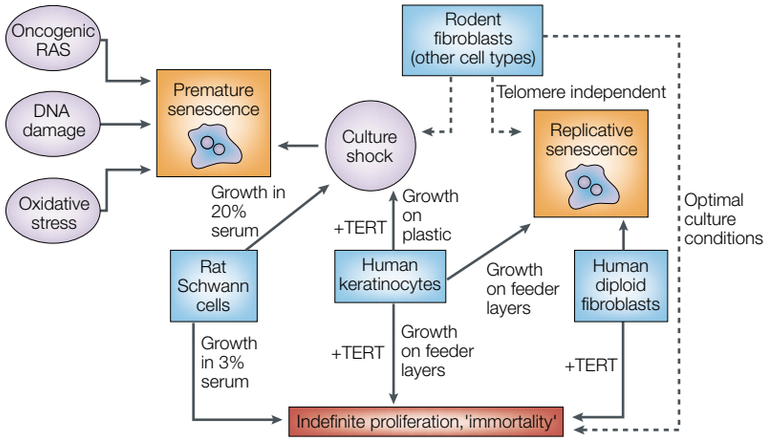


Figure 2 | **Pathways to senescence.** Cellular senescence can be triggered by different pathways. Replicative senescence is the term used to describe senescence induced as a result of the countdown of an intrinsic mitotic counter. The only known mitotic counter mechanism is telomere shortening. This mechanism seems to be responsible for the induction of replicative senescence in human diploid fibroblasts, as expression of telomerase reverse transcriptase (*TERT*) allows these cells to proliferate indefinitely. Premature senescence is the term used to describe senescence induced by extrinsic factors that can act at any point in a cell's replicative history. The senescent phenotype is indistinguishable, irrespective of the inducer. Factors known to induce premature senescence are oncogenic RAS, DNA damage and oxidative stress. The cumulative trauma of *in vitro* culture ('culture shock') can also trigger premature senescence. This is shown in rat Schwann cells and human keratinocytes, as changing culture conditions alters their proliferative lifespan. For other cell types that show telomere-independent senescence (e.g. rodent fibroblasts), it remains to be established whether this arrest is induced by an unknown counting mechanism or by culture shock. If culture shock is responsible, optimization of conditions might allow unlimited proliferation.

From mice to man

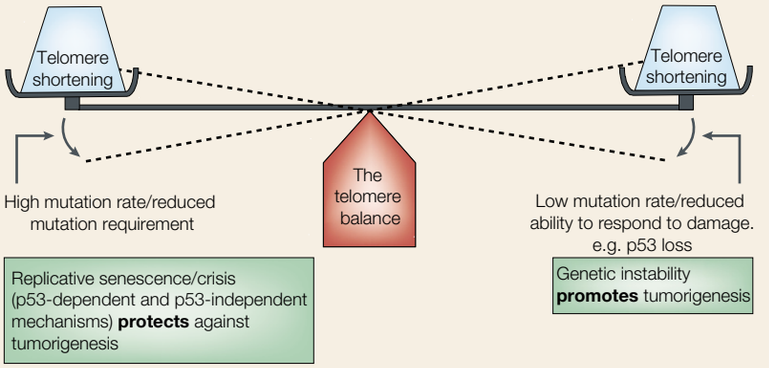
So, telomerase expression protects against genetic instability and, as the phenotype of the telomerase knockout mice shows, the absence of telomerase activity can promote tumorigenesis. Why, then, is telomerase switched off in human somatic tissue? Wouldn't this result in an

Box 3 | **Telomere knockout mice**

Knocking out the RNA component of telomerase — mTR — in mice does not cause defects for the first few generations[28]. Phenotypes first become evident at generation 4 (G4) and become pronounced by G6 (REFS 30,31). In a mouse strain with naturally shorter telomeres, the phenotype is apparent at earlier generations[88]. Mice lacking telomerase reverse transcriptase (Tert) — the protein catalytic unit — also show progressive telomere shortening[89,90]. The late-generation *mTR*$^{-/-}$ mice have a reduced lifespan, which is associated with increased greying of hair, alopoecia and skin lesions; decreased body weight, secondary to abnormalities in the gastrointestinal tract; impaired wound healing; and defects in the reproductive system (infertility) and haematopoietic system[31,91].

The telomere balance

The role of telomere shortening in tumorigenesis is finely balanced — depending on genetic context, it might either promote or inhibit tumour formation. Telomere shortening triggers replicative senescence and causes genetic instability. When intrinsic mutation rates are low, induction of instability outweighs the protective role of senescence against tumorigenesis and the net outcome of telomere shortening is to promote cancer. In backgrounds in which the requirement for oncogenic mutations is reduced (high mutation rate/reduced mutation requirement), the balance is tipped and the protective role of telomere shortening predominates. The balance in human cells is unknown.



Mouse models for effects of telomere shortening on cancer incidence

mTR$^{-/-}$ (REF. 31)

- **Increased frequency of spontaneous tumours compared with wild-type mice. Tumours arise from proliferative cell types — for example, teratocarcinomas, lymphomas and squamous-cell carcinomas.**
- **Decreased frequency of mutagen-induced skin tumours compared with wild-type mice[40].**

Cdkn2a$^{-/-}$*mTR*$^{-/-}$ (REF. 39)

- **Decreased frequency of mutagen-induced tumours compared with *Cdkn2a*$^{-/-}$ mice (which lack two tumour suppressors — Ink4a and Arf).**

Apc^{Min}*/mTR*$^{-/-}$ (REF. 41)

- **Increased frequency of tumour initiation (microscopic adenomas).**
- **Decreased frequency and size of macroscopic adenomas compared with *Apc*^{Min} mice.**

Trp$^{53-/-}$*/mTR*$^{-/-}$ (REFS. 36,37)

- **Increased frequency of tumorigenesis compared with *Trp53*$^{-/-}$ mice. Shift in tumour spectrum indicates that short telomeres might promote epithelial carcinogenesis. This shift (to tumour types most commonly seen in ageing humans) has led to the proposal that telomere shortening might have an important role in tumorigenesis in ageing humans[37,92].**

increase in the incidence of cancer? One possible explanation is that the balance in human cells is different from that in mice — the benefits of a limited proliferative capacity might outweigh the cost of increased genetic instability. However, as human cells have a lower rate of spontaneous mutation *in vitro* than rodent cells[43] and require more genetic changes to achieve tumorigenicity, based on the studies in mice this would seem unlikely.

Another issue to consider is that although human somatic cells are generally described as being telomerase negative, this is not always the case (see BOX 4). In fact, in proliferating tissues, telomerase expression is quite widespread[44]. Expression, however, is confined to precursor cells, such as the basal layers of epithelia in the skin[45] and gut, or is tightly regulated to ensure expression only when proliferation of a particular cell type is required, such as during lymphocyte activation. It therefore seems, with a few possible exceptions, that telomerase activity correlates tightly with the proliferative requirements of cells. So, humans cannot be considered to be the equivalent of telomerase-negative mice in the sense that human tumours might be arising from cells that normally express telomerase.

If it is important to express telomerase in proliferating cells, particularly those that require a large replicative capacity, why is telomerase not expressed in all our cells? Why have we developed mechanisms

FAMILIAL ADENOMATOUS POLYPOSIS
Genetic disorder that is characterized by an increased predisposition to colorectal cancer, associated with germline mutations of the *APC* gene.

Box 4 | **Telomerase expression**

The laboratory mouse expresses telomerase in almost all cell types. There is, however, tissue-specific differential regulation, which is reflected by differences in telomere length[48,49,93,94]. Activity is high in the testes and liver, lower in the kidney and spleen, and undetectable in the brain.

Telomerase activity has been detected in a variety of human somatic tissues, including haematopoietic cells, basal keratinocytes and epithelial cells in the endometrium, oesophagus, prostate and pancreas[44].

Telomerase activity correlates with the proliferative requirements of cells at various stages throughout life: the endometrium undergoes significant replication throughout the menstrual cycle and is telomerase positive. By contrast, telomerase activity in endometrial tissues from menopausal women is very low[95].

Haematopoietic cells are required to undergo massive proliferation during development and following immune responses. Mitogenic stimulation of T cells (for example, by antigen application) results in a 500–1000-fold increase in telomerase activity[96].

The lobular epithelium of the breast is required to proliferate at various stages throughout life — for example, during pregnancy — and maintains active telomerase[44].

Downregulation of telomerase following exit from the cell cycle has been shown by inducing quiescence in tumour cell lines and differentiation of stem cells in culture[97,98].

for regulating telomerase activity? One possibility is that telomerase has additional functions that are deleterious to the normal behaviour of some cells. This explanation has gained some credence from recent experiments. If *Tert* is overexpressed in the basal keratinocytes of mice, the mice develop normal skin structure, but treatment with tumour promoters or wounding of the skin results in a large increase in the proliferation rate of the keratinocytes[46]. These results indicate that high levels of telomerase can actually promote cell proliferation — a function that seems to be distinct from the maintenance of telomeric ends. In a second series of experiments, *Tert* was expressed in the heart, a tissue that in adult mice does not normally express telomerase. This resulted in cardiac hypertrophy and promoted myocyte survival following injury, again indicating that telomerase can have other cellular effects[47]. Additional functions for telomerase could explain why high levels of telomerase expression are progressively selected for during human tumour development[44]. It would also provide an explanation for why mice show tissue-specific regulation of telomerase[48,49] and select for high levels of telomerase expression during tumour progression[50,51], despite having telomeres more than ten times longer than those in humans.

A second possibility is that the regulation of telomerase reflects the benefits or costs to a particular cell type of the expression of telomerase, which can vary according to the proliferative requirements and functions of a cell. This might explain the intriguing findings that telomerase activation in some cell types — for example, lymphocytes — seems to be insufficient to maintain telomere length[52,53]. Possibly for these cells, telomerase expression is set at levels that provide protection for the normal proliferative requirements of the cell, but the progressive shortening of the telomeres would still provide a proliferative barrier to protect against malignancy.

Telomerase regulation in tumour development

Telomerase activity is present in most human tumours, and telomeres are stabilized by ALT in the remainder. In addition, it seems that increased levels of telomerase expression are selected for during the malignant progression of tumours[44]. These observations indicate that telomere stabilization is a requisite step for tumour development, with higher levels of telomerase expression offering a selective advantage to the tumour cell. Somewhat surprisingly, considering the importance of telomerase expression in tumour development, little is known about the mechanisms involved.

The *TERT* gene is amplified in a proportion of tumours, indicating that a direct mutational mechanism can be responsible for upregulating telomerase activity[54]. Interestingly, the expression of certain oncogenes — for example, *MYC*[55–57] and the tumour virus protein E6 (REFS 58,59) — can directly upregulate telomerase expression. This ability seems to be cell-type specific, and correlates with the ability of these oncogenes to immortalize cells. It is therefore possible that telomerase activation

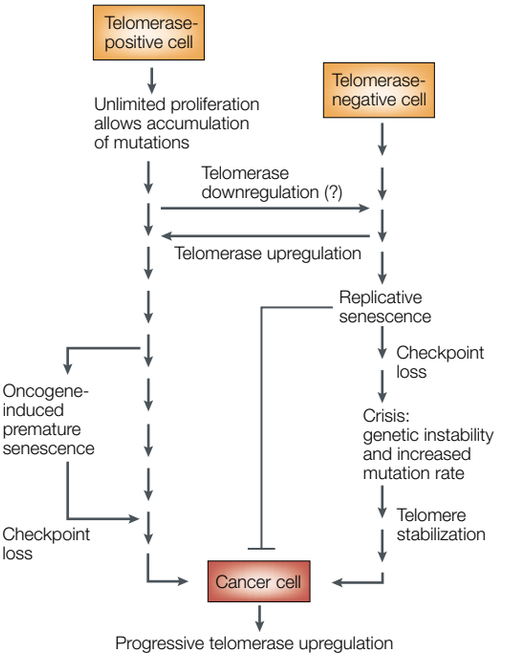


Figure 3 | **Telomerase regulation during tumorigenesis.** Tumours might arise from both cells that express telomerase (e.g. stem cells) and cells that do not. In a telomerase-negative cell, short telomeres can induce replicative senescence that should protect against cancer. Alternatively, shortened telomeres might lead to an increase in genetic instability, and so act to promote tumour development. Subsequent telomere maintenance by telomerase upregulation could then stabilize the transformed genome, allowing tumour expansion. Conversely, in a telomerase-positive cell, switching off telomerase might be one mechanism to induce genetic instability. Oncogene-induced premature senescence might also protect against tumorigenesis. Telomerase upregulation that occurs as tumours become more aggressive could be indicative of the selection for high levels of telomerase expression that confer a proliferative advantage.

Box 5 | **The role of replicative senescence in ageing**

In considering a role for replicative senescence in the ageing process, we can first assume that it is only a contributory factor. Lower organisms, consisting mostly of post-mitotic cells, still age, as do the post-mitotic tissues of higher organisms. Moreover, mice have long telomeres and telomerase knockout mice have no phenotype for several generations, arguing against a role for telomere shortening and replicative senescence in the ageing process.

Do cells reach their replicative limit during a normal human lifespan? An inverse correlation between the lifespan of cells in culture and donor age indicated that replicative capacity is depleted as we age[99,100], but another study indicates that this correlation is not significant[101]. Attempts to measure telomere length to look for evidence of telomere shortening in older people has also proved inconclusive as there seems to be no clear link between telomere repeat number and donor age in certain cell types. Whether replicative senescence occurs as we age therefore remains controversial.

Accelerated ageing of late-generation telomerase knockout mice has been used to argue in favour of an important role for replicative senescence in ageing[102], as does the discovery that some human premature ageing syndromes are associated with telomere defects[103]. The phenotype of the ageing disorder dyskeratosis congenita closely mirrors that of the telomerase knockout mice[104–106] — cells from these patients have reduced telomerase activity and accelerated telomere shortening. Severe telomeric dysfunction therefore leads to certain signs of ageing, but does this actually occur during a normal human lifespan? What is interesting about the human diseases is that, unlike the mouse, defects in telomerase activity have an effect during a single lifetime, arguing that human telomeres are critically poised at a length that is sensitive to attrition.

during tumour development can also occur indirectly, as a result of oncogene activation or a change in the proliferative or differentiation status of the cell.

Our knowledge of the effects that the absence of telomerase activity can have on the propensity to develop tumours allows us to envisage several scenarios in which telomerase regulation can contribute to tumour formation (FIG. 3). In a telomerase-negative cell, telomere shortening, as a result of multiple divisions in the early stages of tumour development, might protect against tumorigenesis owing to replicative senescence. Presence of telomerase activity would permit the bypass of this replicative block. Alternatively, shortened telomeres might result in increased genetic instability and enhance the likelihood of additional oncogenic mutations and progression to malignancy. Subsequent activation of telomerase might act to stabilize the malignant genotype, allowing expansion of the resulting cells. For tumours that originate from telomerase-positive cells, loss of genetic stability might be required to drive tumorigenesis, which could involve repression of telomerase activity. Telomerase would then need to be switched on again at a later stage to restabilize the genome. For many tumours, as tumorigenesis proceeds, cells that express high levels of telomerase activity are likely to be selected for, reflecting the proliferative advantage of these cells[46].

Is premature senescence tumour suppressive?

Damage signals from shortened telomeres are not the sole route to a senescent phenotype. Oncogenic RAS activation[15], other damage signals — such as γ-irradiation[11] and oxidative stress[13] — and inappropriate culture conditions[18,19,20] all trigger premature entry into senescence in young cells, at least *in vitro* (FIG. 2). It has been speculated that premature senescence can also act protectively, as a tumour-suppressor mechanism, by removing damaged cells from a proliferative state (FIG. 3).

It has been known for some time that normal cells *in vitro* are refractory to transformation by a single oncogene[60]. Cells that contain these oncogenic changes tend not to be maintained in the population, but are triggered to either exit the cell cycle (RAS) or die (MYC). RAS signalling pathways are constitutively activated in a large proportion of human tumours, so RAS activation seems to be an important pathway to tumorigenesis[61]. If inappropriate activation of *RAS* triggered entry into senescence, this could be an important mechanism of limiting tumour formation. *In vitro*, it is quite clear that the cell-cycle arrest induced in primary cells is an efficient barrier to cellular transformation by RAS, and that genetic changes that overcome senescence are required for the production of tumour cells[62]. These genetic changes — such as loss of *CDKN2A* or *TP53* (the gene that encodes p53 in humans), or *MYC* expression — are often found in concert with RAS activation in primary human tumours, and will cooperate with Ras to result in more rapid tumour formation in transgenic mice.

Whether premature senescence as a result of RAS activation acts as a barrier to tumour formation *in vivo* has been more difficult to address. Transgenic animals that express activated Ras throughout a tissue would indicate that they do not, as these tissues seem to proliferate normally, or even hyperproliferate in response to oncogenic *Ras* expression[63]. However, these mice also express activated Ras throughout development, a situation that might permit adaptation to occur. Recently, a new transgenic mouse model of tumour development has been developed that might allow these questions to be addressed. In these mice, spontaneous recombination events trigger the production of activated Kras at a low frequency in cells throughout the body as the animal develops[64]. Interestingly, these mice are predisposed to the development of a number of cancer types, but predominantly lung cancers. Analysis of the early stages of lung tumour formation identified small areas of hyperplasia, which indicate that Ras activation is proliferative, though even at this early stage it is possible that additional mutations have occurred. Intriguingly, many tissues do not develop tumours. One possibility is that in these tissues, activating Ras is, indeed, antiproliferative. Cell-specific differences in the effects of Ras activation on the proliferation of normal cells have been seen *in vitro*: activation of Ras in fibroblasts[15], Schwann cells[65] and keratinocytes[66,67] leads to cell-cycle arrest, whereas expression in thyroid epithelial cells[68] results in enhanced proliferation. Marking and following the fate of recombined cells in these transgenic mice should offer insights into the cell-specific effects of oncogenic Ras.

The observation that many of the checkpoint-mediating proteins that control replicative capacity or induce premature senescence are oncogenes or tumour suppressors has been used to argue that overcoming these proliferative checkpoints is an important step in

Box 6 | **Clinical applications of telomeres and cancer**

Diagnostics

Telomerase activity might be useful as a diagnostic and prognostic marker for cancer as telomerase activity is detected in >90% tumours. Sensitive assays for telomerase activity and expression are easy to carry out and can be used on fresh/frozen tumour biopsies, fluids and secretions. Assays include the following:

- TRAP (telomere repeat amplification protocol), a polymerase chain reaction (PCR)-based enzymatic assay[22].
- Immunohistochemical detection of telomerase reverse transcriptase (TERT), which correlates well with telomerase activity and allows detection at the single-cell level[44].
- RT/PCR (reverse transcriptase/PCR) analysis, which can be used to detect expression of TERT mRNA[107,108].

Therapeutics

Inhibiting telomerase in cancer cells might also be of therapeutic benefit. TERT (the reverse transcriptase) and TR (the RNA template) have been identified as valid targets *in vitro*: dominant-negative TERT induces apoptosis in tumour cell lines[26], and treatment of tumour cell lines with antisense oligodeoxynucleotides to TR results in a crisis-like response after the lag required for telomere shortening[109]. Promising therapeutic strategies based on these approaches include:

- Inhibiting TERT: small-molecule screens using standard assays for telomerase activity have identified isothiazolone derivatives as specific, efficient inhibitors of TERT [110].
- Inhibiting TR: antisense peptide nucleic acids (PNAs) to TR, delivered *in vitro* by a carrier[109,111], inhibit tumour-cell proliferation.
- Telomerase might also be a useful tumour-specific antigen. Effective antitumour T-lymphocyte responses against *TERT*-expressing cells have been generated[112], showing the potential for immunotherapy. This strategy circumvents the time lag required for telomere shortening.

tumour development. However, as proteins such as MYC and p53 are multifunctional and have important roles in other cellular processes, including differentiation and apoptosis, it makes it difficult to pinpoint their precise role during the development of a tumour.

Human mammary epithelial cells senesce in culture after approximately 20 population doublings, and the arrest is associated with the induction of INK4A[69], but the senescence checkpoint is bypassed at high frequency (one cell in 10^5)[70] as expression of the *INK4A* transcript is lost owing to promoter methylation. In different culture conditions, INK4A levels remain low, so the proliferative capacity of the epithelial cells is extended[21]. It has therefore been argued that the induction of senescence *in vitro* is a result of 'culture' shock — extrinsic, rather than intrinsic, mechanisms are responsible for determining the apparent proliferative capacity of this cell type. However, the development of human mammary carcinomas is associated with the induction of INK4A to levels higher than those in surrounding normal tissue, although its expression is then repressed as a result of *de novo* methylation of the promoter[71]. This indicates that INK4A is induced during the development of the tumour, but continued proliferation requires lower levels, a situation that parallels continual proliferation *in vitro*. Although highly speculative, it is possible that extrinsic conditions found in the tumour microenvironment trigger premature senescence in a manner similar to those induced by sub-optimal conditions *in vitro*.

Cell senescence, ageing and cancer

Ageing is associated with a progressive decline in bodily function, ultimately resulting in disease and death. The mechanisms responsible for ageing are poorly understood, but the accumulation of cell damage, as a result of oxidative damage to macromolecules, is generally thought to be an important contributor[72]. Replicative senescence has been proposed to be one component of the ageing process — as we age, an accumulation of cells at the end of their proliferative lifespan could decrease regenerative potential and increase genetic damage. An accumulation of senescent cells within the body could also contribute to ageing because of their distinct properties — a result of altered gene expression. For example, senescent fibroblasts secrete elevated levels of collagenase, which could account for the decreased elasticity of aged skin[73].

Evidence for a role of replicative senescence in the ageing process remains controversial (BOX 5). Perhaps the most direct evidence for the occurence of cell senescence *in vivo* would be the detection of increased numbers of senescent cells within the aged body. β-galactosidase staining shows that senescent keratinocytes accumulate in aged skin, and this has been used to argue that replicative senescence occurs *in vivo* as we age[3]. However, as the senescent phenotype induced by replicative and premature senescence pathways is indistinguishable, it is possible that senescence-associated-β-galactosidase-positive cells in aged skin are not the result of cells reaching the end of their replicative capacity in response to telomere attrition, but are induced in response to other damage signals. There is increasing evidence that oxidative stress and other forms of cell damage are responsible for the ageing process[14,72,74]. *In vitro*, oxidative stress, and other damage signals, including those induced by inappropriate culture conditions, trigger premature cell senescence. It would therefore not be surprising that senescent cells accumulate in the body in response to these signals through pathways that are independent of telomere shortening.

CYCLIN-DEPENDENT KINASE INHIBITORS (CDKIs). Proteins that inhibit cell-cycle progression by binding to and inhibiting cyclin-dependent kinases.

The incidence of cancer increases with age. This has generally been assumed to reflect the time required to accumulate sufficient mutations for tumour development. This idea is reinforced by the observation that humans carrying a genetic mutation in a cancer-promoting gene develop cancer at an earlier age. But, in addition, it has also been proposed that an increase in genetic instability might also contribute[24,75]. As increasing numbers of cells approach the end of their replicative lifespan, it has been proposed that the resultant telomere shortening might increase genetic stability. Certainly, increased genetic abnormalities are observed in cells from aged individuals, but whether this is the result of telomeric shortening requires further study.

A second suggestion has been that the accumulation of senescent cells as we age creates an environment that is more sustainable for tumour growth[75]. This idea stems from observations that other non-transformed cell types are important for the successful development of a tumour. In particular, the stromal environment seems to be critically important for the development of epithelial cancers[76,77]. Again, we propose that this might not reflect the accumulation of cells at the end of their replicative lifespan as a result of telomere shortening, but is likely to be due to the accumulation of senescent cells triggered by other damage pathways.

Implications for cancer therapy

The barrier imposed on proliferation by telomere shortening has been proposed to act as a tumour-suppressor mechanism. However, the complexities of telomerase regulation and telomere stability indicate that the role of telomerase is not so straightforward. Depending on context, telomere shortening seems to either promote or protect against cancer. Furthermore, expression of telomerase activity in normal human cells is more widespread than was originally thought.

So, how can our limited understanding of this complex situation be applied to cancer treatment and prevention (BOX 6)? Whatever the cause or mechanism of telomerase expression, the finding that at least 90% of human tumours express high levels of telomerase activity, whereas most normal somatic cells do not, makes telomerase activity a potentially useful diagnostic marker, as well as a therapeutic target.

Inhibiting telomerase activity with dominant-negative versions of TERT has shown that this activity is essential for continued proliferation of tumour cells *in vitro*. This finding has led to the proposal that telomerase inhibitors could provide effective treatment for advanced cancers. However, this type of therapy is only likely to be useful for tumours that have short telomeres, because of the time lag required for sufficient attrition of long telomeres. Moreover, this therapy would be ineffective for the low percentage of tumours that maintain their telomeres using the ALT mechanism. Another concern is that telomerase inhibition might also affect normal cells that express telomerase, perhaps decreasing regenerative potential and increasing genetic instability. Nevertheless, despite these drawbacks, targeting telomerase activity remains a potentially useful, new line of attack for cancer therapy.

Signals other than shortening telomeres can trigger a senescent phenotype. These include signals from RAS, which is active in a high proportion of human tumours. One potential therapeutic route could be to re-establish the signalling pathways that mediate RAS signalling to senescence in tumour cells and irreversibly block the cell cycle. Promisingly, *in vitro* experiments have shown that expression of CYCLIN-DEPENDENT KINASE INHIBITORS (CDKIs) in tumour cell lines results in a senescent phenotype, showing that some tumour cells retain this potential. *In vitro*, the cell-cycle arrest induced by oncogenic RAS is dependent on functional p53. Reactivating p53 in tumour cells — by, for example, the use of small molecules that have been shown to 'normalize' mutant p53 activity[78,79] — might therefore be a therapeutic route to the induction of senescence in tumour cells. Hopefully, in the future, we will be able to exploit a greater understanding of the pathways to senescence and manipulate this cell-cycle arrest as an effective cancer treatment.

1. Hayflick, L. The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25**, 585–621 (1961).
2. Hayflick, L. The limited *in vitro* lifetime of human diploid cell strains. *Exp. Cell Res.* **37**, 614–636 (1965).
3. Dimri, G. P. *et al.* A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. *Proc. Natl Acad. Sci. USA* **92**, 9363–9367 (1995).
4. Artandi, S. E. & DePinho, R. A. A critical role for telomeres in suppressing and facilitating carcinogenesis. *Curr. Opin. Genet. Dev.* **10**, 39–46 (2000).
5. Hara, E., Tsurui, H., Shinozaki, A., Nakada, S. & Oda, K. Cooperative effect of antisense-Rb and antisense-p53 oligomers on the extension of life span in human diploid fibroblasts, TIG-1. *Biochem. Biophys. Res. Commun.* **179**, 528–534 (1991).
6. Shay, J. W., Pereira-Smith, O. M. & Wright, W. E. A role for both RB and p53 in the regulation of human cellular senescence. *Exp. Cell Res.* **196**, 33–39 (1991).
7. Harley, C. B., Futcher, A. B. & Greider, C. W. Telomeres shorten during ageing of human fibroblasts. *Nature* **345**, 458–460 (1990).
8. Bodnar, A. G. *et al.* Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**, 349–352 (1998).
9. Vaziri, H. & Benchimol, S. Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr. Biol.* **8**, 279–282 (1998).
References 8 and 9 show how telomerase expression immortalizes human cells.
10. Kiyono, T. *et al.* Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* **396**, 84–88 (1998).
Human epithelial cells seem to have, in addition to telomere shortening, a second checkpoint that determines replicative lifespan.
11. Di Leonardo, A., Linke, S. P., Clarkin, K. & Wahl, G. M. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev.* **8**, 2540–2551 (1994).
12. Robles, S. J. & Adami, G. R. Agents that cause DNA double strand breaks lead to $p16^{INK4A}$ enrichment and the premature senescence of normal fibroblasts. *Oncogene* **16**, 1113–1123 (1998).
13. Chen, Q. & Ames, B. N. Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. *Proc. Natl Acad. Sci. USA* **91**, 4130–4134 (1994).
14. Finkel, T. & Holbrook, N. J. Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247 (2000).
15 Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. & Lowe, S. W. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and $p16^{INK4A}$. *Cell* **88**, 593–602 (1997).
Oncogenic RAS expression in normal cells triggers premature senescence.
16. Sherr, C. J. & DePinho, R. A. Cellular senescence: mitotic clock or culture shock? *Cell* **102**, 407–410 (2000).
17. Wright, W. E. & Shay, J. W. Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology. *Nature Med.* **6**, 849–851 (2000).
18. Mathon, N. F., Malcolm, D. S., Harrisingh, M. C., Cheng, L. & Lloyd, A. C. Lack of replicative senescence in normal rodent glia. *Science* **291**, 872–875 (2001).

19. Tang, D. G., Tokumoto, Y. M., Apperly, J. A., Lloyd, A. C. & Raff, M. C. Lack of replicative senescence in cultured rat oligodendrocyte precursor cells. *Science* **291**, 868–871 (2001).
20. Shay, J. W. & Wright, W. E. Aging: when do telomeres matter? *Science* **291**, 839–840 (2001).
21. Ramirez, R. D. *et al.* Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. *Genes Dev.* **15**, 398–403 (2001).
References 18, 19 and 21 show that culture conditions, rather than an intrinsic clock, limit the proliferative lifespan of many cell types.
22. Kim, N. W. *et al.* Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**, 2011–2015 (1994).
23. Bryan, T. M., Englezou, A., Dalla-Pozza, L., Dunham, M. A. & Reddel, R. R. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nature Med.* **3**, 1271–1274 (1997).
24. Hastie, N. D. *et al.* Telomere reduction in human colorectal carcinoma and with ageing. *Nature* **346**, 866–868 (1990).
25. Sommerfeld, H. J. *et al.* Telomerase activity: a prevalent marker of malignant human prostate tissue. *Cancer Res.* **56**, 218–222 (1996).
26. Hahn, W. C. *et al.* Inhibition of telomerase limits the growth of human cancer cells. *Nature Med.* **5**, 1164–1170 (1999).
Shows that cancer cells need to maintain telomerase activity.
27. Hahn, W. C. *et al.* Creation of human tumour cells with defined genetic elements. *Nature* **400**, 464–468 (1999).
How many genetic changes to transform a normal human cell?
28. Blasco, M. A. *et al.* Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* **91**, 25–34 (1997).
Mouse cells lacking telomerase senesce at the same rate, are immortalized and are as transformable as wild-type cells.
29. Artandi, S. E. & DePinho, R. A. Mice without telomerase: what can they teach us about human cancer? *Nature Med.* **6**, 852–855 (2000).
30. Lee, H. W. *et al.* Essential role of mouse telomerase in highly proliferative organs. *Nature* **392**, 569–574 (1998).
31. Rudolph, K. L. *et al.* Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* **96**, 701–712 (1999).
Documents the unexpected finding that telomerase-deficient mice have an increased predisposition to cancer.
32. Hande, M. P., Samper, E., Lansdorp, P. & Blasco, M. A. Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J. Cell Biol.* **144**, 589–601 (1999).
33. Niida, H. *et al.* Severe growth defect in mouse cells lacking the telomerase RNA component. *Nature Genet.* **19**, 203–206 (1998).
34. Counter, C. M. *et al.* Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J.* **11**, 1921–1929 (1992).
35. Hackett, J. A., Feldser, D. M. & Greider, C. W. Telomere dysfunction increases mutation rate and genomic instability. *Cell* **106**, 275–286 (2001).
36. Chin, L. *et al.* p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**, 527–538 (1999).
37. Artandi, S. E. *et al.* Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* **406**, 641–645 (2000).
38. Serrano, M. *et al.* Role of the *INK4A* locus in tumor suppression and cell mortality. *Cell* **85**, 27–37 (1996).
39. Greenberg, R. A. *et al.* Short dysfunctional telomeres impair tumorigenesis in the INK4A(δ2/3) cancer-prone mouse. *Cell* **97**, 515–525 (1999).
40. Gonzalez-Suarez, E., Samper, E., Flores, J. M. & Blasco, M. A. Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nature Genet.* **26**, 114–117 (2000).
41. Rudolph, K. L., Millard, M., Bosenberg, M. W. & DePinho, R. A. Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. *Nature Genet.* **28**, 155–159 (2001).
References 39–41 show that replicative senescence can protect against tumorigenesis when the frequency of tumour initiation is increased.
42. Su, L. K. *et al.* Multiple intestinal neoplasia caused by a mutation in the murine homolog of the *APC* gene. *Science* **256**, 668–670 (1992).
43. Hart, R. W. & Setlow, R. B. Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species. *Proc. Natl Acad. Sci. USA* **71**, 2169–2173 (1974).
44. Kolquist, K. A. *et al.* Expression of *TERT* in early premalignant lesions and a subset of cells in normal tissues. *Nature Genet.* **19**, 182–186 (1998).
45. Harle-Bachor, C. & Boukamp, P. Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. *Proc. Natl Acad. Sci. USA* **93**, 6476–6481 (1996).
46. Gonzalez-Suarez, E. *et al.* Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. *EMBO J.* **20**, 2619–2630 (2001).
Indicates that high levels of telomerase might have an additional proliferative effect.
47. Oh, H. *et al.* Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc. Natl Acad. Sci. USA* **98**, 10308–10313 (2001).
48. Prowse, K. R. & Greider, C. W. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl Acad. Sci. USA* **92**, 4818–4822 (1995).
49. Greenberg, R. A., Allsopp, R. C., Chin, L., Morin, G. B. & DePinho, R. A. Expression of mouse telomerase reverse transcriptase during development, differentiation and proliferation. *Oncogene* **16**, 1723–1730 (1998).
50. Blasco, M. A., Rizen, M., Greider, C. W. & Hanahan, D. Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. *Nature Genet.* **12**, 200–204 (1996).
51. Chadeneau, C., Siegel, P., Harley, C. B., Muller, W. J. & Bacchetti, S. Telomerase activity in normal and malignant murine tissues. *Oncogene* **11**, 893–898 (1995).
52. Vaziri, H. *et al.* Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc. Natl Acad. Sci. USA* **91**, 9857–9860 (1994).
53. Vaziri, H. *et al.* Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *Am. J. Hum. Genet.* **52**, 661–667 (1993).
54. Zhang, A. *et al.* Frequent amplification of the telomerase reverse transcriptase gene in human tumors. *Cancer Res.* **60**, 6230–6235 (2000).
55. Greenberg, R. A. *et al.* Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. *Oncogene* **18**, 1219–1226 (1999).
56. Wu, K. J. *et al.* Direct activation of TERT transcription by c-MYC. *Nature Genet.* **21**, 220–224 (1999).
57. Wang, J., Xie, L. Y., Allan, S., Beach, D. & Hannon, G. J. Myc activates telomerase. *Genes Dev.* **12**, 1769–1774 (1998).
58. Oh, S. T., Kyo, S. & Laimins, L. A. Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and GC-rich Sp1 binding sites. *J. Virol.* **75**, 5559–5566 (2001).
59. Klingelhutz, A. J., Foster, S. A. & McDougall, J. K. Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* **380**, 79–82 (1996).
60. Weinberg, R. A. Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis. *Cancer Res.* **49**, 3713–3721 (1989).
61. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
62. Lloyd, A. C. Ras versus cyclin-dependent kinase inhibitors. *Curr. Opin. Genet. Dev.* **8**, 43–48 (1998).
63. Adams, J. M. & Cory, S. Transgenic models of tumor development. *Science* **254**, 1161–1167 (1991).
64. Johnson, L. *et al.* Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature* **410**, 1111–1116 (2001).
65. Lloyd, A. C. *et al.* Cooperating oncogenes converge to regulate cyclin/cdk complexes. *Genes Dev.* **11**, 663–677 (1997).
66. Roper, E., Weinberg, W., Watt, F. M. & Land, H. $p19^{ARF}$-independent induction of p53 and cell cycle arrest by Raf in murine keratinocytes. *EMBO Rep.* **2**, 145–150 (2001).
67. Lin, A. W. & Lowe, S. W. Oncogenic ras activates the ARF–p53 pathway to suppress epithelial cell transformation. *Proc. Natl Acad. Sci. USA* **98**, 5025–5030 (2001).
68. Lemoine, N. R. *et al.* Partial transformation of human thyroid epithelial cells by mutant H-ras oncogene. *Oncogene* **5**, 1833–1837 (1990).
69. Foster, S. A., Wong, D. J., Barrett, M. T. & Galloway, D. A. Inactivation of p16 in human mammary epithelial cells by CpG island methylation. *Mol. Cell Biol.* **18**, 1793–1801 (1998).
70. Romanov, S. R. *et al.* Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. *Nature* **409**, 633–637 (2001).
71. Hui, R. *et al. INK4a* gene expression and methylation in primary breast cancer: overexpression of $p16^{INK4a}$ messenger RNA is a marker of poor prognosis. *Clin. Cancer Res.* **6**, 2777–2787 (2000).
72. Johnson, F. B., Sinclair, D. A. & Guarente, L. Molecular biology of aging. *Cell* **96**, 291–302 (1999).
73. West, M. D., Pereira-Smith, O. M. & Smith, J. R. Replicative senescence of human skin fibroblasts correlates with a loss of regulation and overexpression of collagenase activity. *Exp. Cell Res.* **184**, 138–147 (1989).
74. Ames, B. N., Shigenaga, M. K. & Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl Acad. Sci. USA* **90**, 7915–7922 (1993).
75. DePinho, R. A. The age of cancer. *Nature* **408**, 248–254 (2000).
76. Olumi, A. F. *et al.* Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res.* **59**, 5002–5011 (1999).
77. Krtolica, A., Parrinello, S., Lockett, S., Desprez, P. Y. & Campisi, J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc. Natl Acad. Sci. USA* **98**, 12072–12077 (2001).
78. Foster, B. A., Coffey, H. A., Morin, M. J. & Rastinejad, F. Pharmacological rescue of mutant p53 conformation and function. *Science* **286**, 2507–2510 (1999).
79. Bullock, A. N. & Fersht, A. R. Rescuing the function of mutant p53. *Nature Rev. Cancer* **1**, 68–76 (2001).
80. Collins, K. Mammalian telomeres and telomerase. *Curr. Opin. Cell Biol.* **12**, 378–383 (2000).
81. Smogorzewska, A. *et al.* Control of human telomere length by TRF1 and TRF2. *Mol. Cell Biol.* **20**, 1659–1668 (2000).
82. Griffith, J. D. *et al.* Mammalian telomeres end in a large duplex loop. *Cell* **97**, 503–514 (1999).
83. Greider, C. W. Telomeres do D-loop–T-loop. *Cell* **97**, 419–422 (1999).
84. Blackburn, E. H. Telomere states and cell fates. *Nature* **408**, 53–56 (2000).
85. Hemann, M. T., Strong, M. A., Hao, L. Y. & Greider, C. W. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* **107**, 67–77 (2001).
86. Karlseder, J., Broccoli, D., Dai, Y., Hardy, S. & de Lange, T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science* **283**, 1321–1325 (1999).
87. van Steensel, B., Smogorzewska, A. & de Lange, T. TRF2 protects human telomeres from end-to-end fusions. *Cell* **92**, 401–413 (1998).
88. Herrera, E. *et al.* Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. *EMBO J.* **18**, 2950–2960 (1999).
89. Liu, Y. *et al.* The telomerase reverse transcriptase is limiting and necessary for telomerase function *in vivo*. *Curr. Biol.* **10**, 1459–1462 (2000).
90. Nikaido, R. *et al.* Presence of telomeric G-strand tails in the telomerase catalytic subunit *Tert* knockout mice. *Genes Cells* **4**, 563–572 (1999).
91. Herrera, E., Martinez, A. C. & Blasco, M. A. Impaired germinal center reaction in mice with short telomeres. *EMBO J.* **19**, 472–481 (2000).
92. Chang, S., Khoo, C. & DePinho, R. A. Modeling chromosomal instability and epithelial carcinogenesis in the telomerase-deficient mouse. *Semin. Cancer Biol.* **11**, 227–239 (2001).
93. Blasco, M. A., Funk, W., Villeponteau, B. & Greider, C. W. Functional characterization and developmental regulation of mouse telomerase RNA. *Science* **269**, 1267–1270 (1995).
94. Martin-Rivera, L., Herrera, E., Albar, J. P. & Blasco, M. A. Expression of mouse telomerase catalytic subunit in embryos and adult tissues. *Proc. Natl Acad. Sci. USA* **95**, 10471–10476 (1998).
95. Brien, T. P. *et al.* Telomerase activity in benign endometrium and endometrial carcinoma. *Cancer Res.* **57**, 2760–2764 (1997).
96. Bodnar, A. G., Kim, N. W., Effros, R. B. & Chiu, C. P. Mechanism of telomerase induction during T cell activation. *Exp. Cell Res.* **228**, 58–64 (1996).
97. Holt, S. E., Wright, W. E. & Shay, J. W. Regulation of telomerase activity in immortal cell lines. *Mol. Cell Biol.* **16**, 2932–2939 (1996).
98. Sharma, H. W. *et al.* Differentiation of immortal cells inhibits telomerase activity. *Proc. Natl Acad. Sci. USA* **92**, 12343–12346 (1995).
99. Schneider, E. L. & Mitsui, Y. The relationship between *in vitro* cellular aging and *in vivo* human age. *Proc. Natl Acad. Sci. USA* **73**, 3584–3588 (1976).
100. Allsopp, R. C. *et al.* Telomere length predicts replicative capacity of human fibroblasts. *Proc. Natl Acad. Sci. USA* **89**, 10114–10118 (1992).
101. Cristofalo, V. J., Allen, R. G., Pignolo, R. J., Martin, B. G. & Beck, J. C. Relationship between donor age and the

replicative lifespan of human cells in culture: a reevaluation. *Proc. Natl Acad. Sci. USA* **95**, 10614–10619 (1998).
102. Kipling, D. & Faragher, R. G. Telomeres: ageing hard or hardly ageing? *Nature* **398**, 191, 193 (1999).
103. Klapper, W., Parwaresch, R. & Krupp, G. Telomere biology in human aging and aging syndromes. *Mech. Ageing Dev.* **122**, 695–712 (2001).
104. Marciniak, R. A. & Johnson, F. B. & Guarente, L. Dyskeratosis congenita, telomeres and human ageing. *Trends Genet.* **16**, 193–195 (2000).
105. Mitchell, J. R., Wood, E. & Collins, K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* **402**, 551–555 (1999).
106. Vulliamy, T. *et al.* The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* **413**, 432–435 (2001).
107. Ito, H. *et al.* Detection of human telomerase reverse transcriptase messenger RNA in voided urine samples as a useful diagnostic tool for bladder cancer. *Clin. Cancer Res.* **4**, 2807–2810 (1998).
108. Takakura, M., Kyo, S., Kanaya, T., Tanaka, M. & Inoue, M. Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer. *Cancer Res.* **58**, 1558–1561 (1998).
109. Shammas, M. A., Simmons, C. G., Corey, D. R. & Shmookler Reis, R. J. Telomerase inhibition by peptide nucleic acids reverses 'immortality' of transformed human cells. *Oncogene* **18**, 6191–6200 (1999).
110. Hayakawa, N. *et al.* Isothiazolone derivatives selectively inhibit telomerase from human and rat cancer cells *in vitro*. *Biochemistry* **38**, 11501–11507 (1999).
111. Hamilton, S. E., Simmons, C. G., Kathiriya, I. S. & Corey, D. R. Cellular delivery of peptide nucleic acids and inhibition of human telomerase. *Chem. Biol.* **6**, 343–351 (1999).
112. Minev, B. *et al.* Cytotoxic T cell immunity against telomerase reverse transcriptase in humans. *Proc. Natl Acad. Sci. USA* **97**, 4796–4801 (2000).

Acknowledgements
We thank M. Raff and A. Hall for critical reading of the manuscript. A.C.L. is a Cancer Research Campaign Senior Cancer Research Fellow. N. F. M. is funded by the Medical Research Council graduate programme.

Online links

DATABASES
The following terms in this article are linked online to:
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
Apc | β-galactosidase | *Cdkn2a* | *CDKN2A* | *MYC* | p53 | *RAS* | RB | *TERT* | *Tert* | TRF1 | TRF2
Access to this interactive links box is free online.