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Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis

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Telomere shortening and telomerase activation both occur in human tumors. Telomere shortening has been proposed to have two conflicting roles in tumorigenesis: tumor suppression and initiation of chromosomal instability. Similarly, while telomerase activation is suggested to be necessary for tumor growth, telomerase may help to stabilize genomic instability. Here we review what is known about these conflicting roles and propose a framework to understand the role of telomerase in cancer progression.
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Introduction

Telomere dysfunction and telomerase activation play paradoxical roles in tumorigenesis. It has been argued that telomere shortening in human somatic cells where telomerase is absent suppresses tumor formation by limiting the number of times that cells can divide (Harley *et al*., 1990). However, telomere shortening has been shown to increase genetic instability and tumor formation in mice (Blasco *et al*., 1997; Rudolph *et al*., 1999). Telomerase activation in cancer adds another level of complexity. Telomerase is activated in 90% of tumors and this activation is thought to confer immortal growth properties on the tumor cells (Kim *et al*., 1994; Shay and Bacchetti, 1997). Telomerase activation is thought to occur late in tumorigenesis (Chadeneau *et al*., 1995; Tang *et al*., 1998). In contrast, chromosomal instability is thought to be initiated early in tumorigenesis (Shih *et al*., 2001). It has been argued that telomerase activation occurs after the initiation of genetic instability and is necessary to inhibit further instability by stabilizing chromosome ends (DePinho, 2000; Rudolph *et al*., 2001). However, it is unclear whether telomerase activation in tumors facilitates tumor growth by circumventing checkpoints that recognize dysfunctional telomeres or by stabilizing chromosome rearrangements. Therefore, telomere shortening and telomerase activation appear to have both tumor suppressive and oncogenic roles: the apparent tumor suppressive role of telomere shortening may help to initiate genetic instability, and the apparent oncogenic role of telomerase activation may help to stabilize genetic instability (Figure 1). Defining under what circumstances each of these mechanisms operate is essential to an understanding of the complex roles of telomeres and telomerase in cancer.

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Telomeres are protected from being recognized by the cell as a DNA break

Hermann J Muller was the first to begin to define the specialized functions of telomeres. While irradiating *Drosophila* with X-rays to produce mutations, Muller noticed that the ends of the chromosomes had properties that distinguished them from the ends of the chromosome fragments produced by radiation (Muller, 1938). While ends produced by irradiation could be rejoined with other broken ends to produce chromosomal rearrangements, the natural ends of the chromosomes were not fused to other ends. Muller proposed that the telomeres have the special property of sealing the ends of chromosomes. This characterization of telomere function was augmented by the work of Barbara McClintock (McClintock, 1941). She engineered the formation of dicentric chromosomes in maize by allowing rearranged chromosomes to recombine during meiosis. She demonstrated that the force of the movement of the two centromeres to opposite spindle poles during cell division broke the dicentric chromosome at a variable location. In addition she showed that the broken chromosome ends in the daughter cells could fuse with the ends of their broken sister chromatids prior to mitosis. Fusion of these broken DNA ends propagated a breakage-fusion-bridge (BFB) cycle that resulted in either gene deletion or gene amplification. She proposed that telomeres protected natural DNA ends from chromosome fusion. Therefore, Muller and McClintock defined the ends of linear chromosomes as structures that are distinct from DNA breaks and provide protection from processes that fuse broken chromosomes.

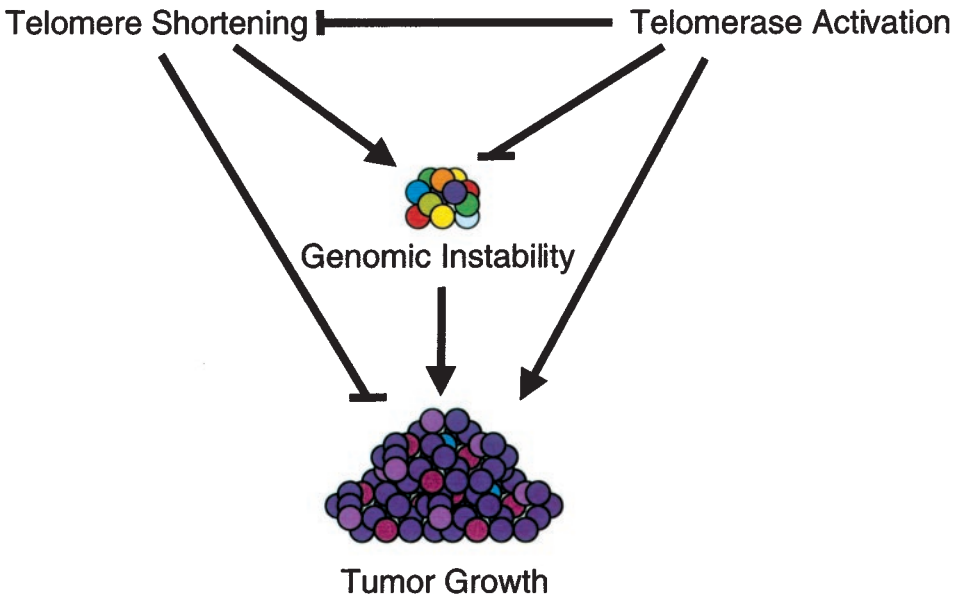

Figure 1 Dual roles for telomerase and telomere dysfunction in genetic instability and tumor growth. Telomere shortening can potentially contribute to the genetic instability that drives tumorigenesis. Early stage tumors with ongoing genetic instability are represented by the cluster of multicolored balls. However, telomere shortening may also limit tumor growth by activating checkpoints. Telomerase activation can facilitate tumor growth, but telomerase can also help to control genetic instability. Late stage tumors in which certain cells have been selected from early stages in tumor progression are represented by the large cluster of blue and purple balls

Telomeres are composed of repetitive TG-rich sequences and the proteins that bind them. *S cerevisiae* telomere sequence contain ~300 base pairs of an irregular TG_{1-3} repeats (Zakian, 1989). Mammalian telomeres are much longer, ~40 kb in inbred strains of mice and 10–15 kb of TTAGGG repeats in humans. Telomeres are elongated by the ribonucleoprotein enzyme telomerase that contains essential reverse transcriptase and RNA template components (Greider and Blackburn, 1989). Telomere shortening occurs in the absence of telomerase and results in loss of telomere function, as described below.

In addition to telomere length, telomere function is conferred by proteins that bind telomeric DNA (reviewed in Gasser, 2000). Loss of protein binding at telomeres causes end-to-end chromosome fusions. In yeast, Rap1 binds along the length of telomeric DNA and acts as a negative regulator of telomere length (Conrad *et al*., 1990; Lustig *et al*., 1990). Mutating the Rap1 binding site in *K lactis* through telomerase RNA template mutations can cause either telomere shortening or telomere elongation, depending on the specific mutation. Both types of mutants show chromosome circularization with retention of telomere sequence at the junction (McEachern *et al*., 2000). In similar experiments, telomerase RNA template mutations have been introduced into human cells. Cells expressing mutant telomerase RNA have a reduced growth rate, reduced viability, and an abnormal cell cycle (Guiducci *et al*., 2001). The cells also have abnormal nuclei and large numbers of chromosome fusions. These fusions retain telomere sequence at the junction. Expression of a mutant template alters the binding sites for telomere binding proteins that maintain telomere function; loss of this binding results in loss of telomere function and chromosome fusion.

In addition to the general deregulation of telomere function observed when mutant RNA templates are expressed, the loss of several specific telomere-binding proteins can alter telomere structure and cause chromosome fusions. The ciliates *Oxytrichia* and *Euplotes* have a telomere end-binding protein, the $\alpha\beta$ heterodimer, that caps the ends of their telomeres, preventing degradation (Gray *et al*., 1991; Hicke *et al*., 1990; Wang *et al*., 1992). A homologue of the α subunit, Pot1, was recently identified in *S pombe* and humans and was shown to bind G-strand telomeric DNA (Baumann and Cech, 2001). In *S pombe*, $Pot1^+$ deficiency causes rapid loss of telomere sequence and chromosome circularization. Pot1 is proposed to function as a cap at the end of linear chromosomes in yeast and humans that is essential for the maintenance chromosome integrity.

A specialized telomere structure, the t-loop, has also been implicated in the maintenance of telomere function in humans (Griffith *et al*., 1999). In a t-loop, the single stranded G-tail of the telomere loops around and base pairs with a more proximal region of telomeric DNA, causing displacement of one strand. TRF2 localizes to this displacement loop and has been proposed to be necessary for establishing or maintaining t-loop structure in conjunction with the Mre11/Rad50/Nbs1 complex (Zhu *et al*., 2000). Consistent with TRF2 playing a role in t-loop and telomere function, expression of a dominant negative TRF2 protein in human cells results in loss of TRF2 binding

to telomeres and loss of G-strand overhangs and an increase in the number of anaphase bridges and chromosome fusions. These fusions often have telomere sequence at the junction (van Steensel *et al*., 1998). Finally the dominant negative TRF2 protein induces an increase in apoptosis mediated by ATM and p53, consistent with loss of TRF2 binding making telomeres resemble a double strand break (Karlseder *et al*., 1999).

Telomere shortening increases genetic instability

Telomere shortening is associated with an increased tumor formation rate in the telomerase knockout ($mTR^{-/-}$) mouse. There is a 4–6-fold increase in spontaneous tumor incidence in late generation $mTR^{-/-}$ mice that have short telomeres and the age of tumor onset is younger than wildtype (Rudolph *et al*., 1999). Tumors arise from highly proliferative tissues that may have particularly short telomeres due to the large number of cell divisions. A possible explanation for this increase in tumor formation is the increase in genetic instability that occurs in late generation $mTR^{-/-}$ mice. $mTR^{-/-}$ cells have increased frequencies of signal-free ends (telomeres without detectable telomere repeats by FISH) and end-to-end chromosome fusions (Blasco *et al*., 1997; Hemann *et al*., 2001). Chromosome ends that most frequently lack a FISH signal preferentially participate in end-to-end fusions, thus providing a direct link between short telomeres and end-to-end fusions (Hemann *et al*., 2001). Significantly, $mTR^{-/-}$ tumors have a 3–18-fold increase in chromosome fusions and a twofold increase in aneuploidy compared to $mTR^{+/+}$ tumors (Rudolph *et al*., 1999). Therefore, chromosomal instability might help to initiate tumorigenesis in $mTR^{-/-}$ mice. In support of this hypothesis, p53 deficiency increases the frequency of chromosome fusions and aneuploidy and decreases apoptosis in late generation $mTR^{-/-}$ cells (Chin *et al*., 1999). Moreover, reintroduction of *mTR* on a vector in G6 $mTR^{-/-}p53^{-/-}$ cells inhibits their ability to form foci in a Myc/RAS transformation assay (Chin *et al*., 1999). Introduction of *mTR* has no effect in an $mTR^{+/+}$ background. This suggests that telomere dysfunction cooperates with p53 deficiency to initiate malignant transformation. Telomere dysfunction in late generation $mTR^{-/-}$ mice also results in tumor formation at an earlier age in a $p53^{-/-}$ or $p53^{+/-}$ background (Artandi *et al*., 2000).

Direct evidence that telomere shortening can cause genomic instability comes from the study of *S cerevisiae*. *est1*Δ yeast exhibit progressive telomere shortening accompanied by a loss of growth rate after a lag period (Lundblad and Szostak, 1989). The measurement of the rate of loss of a marker gene in haploid *est1*Δ yeast revealed an increase in mutation rate as telomeres shortened (Hackett *et al*., 2001). This increase in mutation rate resulted from an increased frequency of terminal, but not internal deletions. The broken chromosome ends could initiate nonreciprocal, but not reciprocal translocations. The presence of nonreciprocal translocations and the fact that internal deletions did not increase suggest that specifically the distal portion of the chromosome with telomere dysfunction is lost. This might be caused either by resection of terminal DNA or by end-to-end chromosome fusions and breakage during mitosis. Importantly, the production of a broken chromosome with the loss of distal sequence is mutagenic. If the cell does not die, the single broken end can lead to chromosome loss or it can initiate a nonreciprocal translocation, or possibly fuse to another dysfunctional telomere, creating a new dicentric chromosome. The least mutagenic outcome in a haploid is *de novo* addition of telomere repeats onto the broken end, a process that requires telomerase (Kramer and Haber, 1993). In diploid cells, a broken chromosome can also be repaired by the copying of the DNA from its homologue, another potential mechanism for loss of heterozygosity (Bosco and Haber, 1998; Malkova *et al*., 1996).

In *est1*Δ yeast, the increase in terminal deletions was inversely proportional to the decrease in growth rate due to telomere dysfunction (Hackett *et al*., 2001). To measure the mutation rate in cultures where telomere shortening decreases growth rate and colony formation, telomerase was reactivated when cells were plated. This reactivation prevented a decrease in colony number and size. Because mutants were selected based on resistance to a toxic drug, marker loss would have to occur before plating the cells. This suggests that, at least in yeast, the decrease in cell growth due to telomere dysfunction does not completely prevent the initiation of chromosomal instability. The initial chromosomal instability may have occurred in cells that were still dividing, or in cells that 'repaired' dysfunctional telomeres through end-to-end fusion, or other mechanisms. Similarly, an induced chromosome break can persist in a yeast colony for a number of cell divisions before arrest or chromosome loss occurs (Sandell and Zakian, 1993).

A precedent for chromosome fusion in cells with dysfunctional telomeres is found in *S pombe*. Strains with progressive telomere shortening due to loss of telomerase reverse transcriptase (trt^+) or both ATM homologues ($tel1^+$ and $rad3^+$) exhibit a progressive loss of growth rate until survivors emerge that have circularized all three chromosomes (Naito *et al*., 1998; Nakamura *et al*., 1998). Therefore, telomere dysfunction in *S pombe* or *S cerevisiae* can initiate chromosomal instability in the presence of functional DNA damage checkpoints. However, this situation in yeast may not occur in mammalian cells where more stringent responses to dysfunctional telomeres may exist.

How does telomere dysfunction lead to end-to-end chromosome fusions?

Because telomere function distinguishes the ends of chromosomes from DNA breaks, it would be predicted

that loss of this function should allow double-strand break repair to act upon telomeres. Although a number of factors that prevent chromosome fusion have been identified, the mechanism involved in fusing dysfunctional telomeres is poorly understood. Chromosome fusion junctions from cells with dysfunctional telomeres have been isolated from *S cerevisiae* (Hackett *et al*., 2001) and mice (Hemann *et al*., 2001). The structures of these fusion junctions are strikingly similar, suggesting the possibility of a common mechanism for their formation. Fusion junctions from both organisms contain 0–10 bp of microhomology, characteristic of a nonhomologous end-joining mechanism. These fusions involve the loss of all telomeric and some subtelomeric sequence, as is seen for circularized chromosomes of *trt*$^-$ or *pot1*$^-$ *S pombe* (Baumann and Cech, 2001; Nakamura *et al*., 1998). This loss of sequence suggests that the mechanism of fusion might be an error-prone DNA repair pathway.

The role of Ku and other NHEJ proteins at telomeres (Lewis and Resnick, 2000) has been puzzling since telomeres are normally protected from end-joining. Ku binds the ends of telomeric DNA *in vitro* and *in vivo* (Bianchi and de Lange, 1999; Hsu *et al*., 1999). However, several experiments suggest that *Ku*/*Lig4*-dependent NHEJ is not essential for chromosome fusion. In *S pombe*, chromosome circularization in response to dysfunctional telomeres occurs in *trt*$^-$, *trt*$^-$*pku70*$^-$, *trt*$^-$*lig4*$^-$, and *trt*$^-$*rad22*$^-$ strains (*rad22*$^+$ is the *S pombe RAD52* homologue) (Baumann and Cech, 2000). Therefore, chromosome fusion does not appear to be mediated by homologous recombination or NHEJ. Further, the rate of formation of terminal deletions in response to dysfunctional telomeres in *S cerevisiae* is not decreased in *rad52*Δ or *lig4*Δ backgrounds (Hackett *et al*., 2001). Interestingly, in mice, deletion of the NHEJ proteins Ku, Lig4, or DNA-PK$_{cs}$ increases the number of telomere fusions and chromosomal rearrangements (Bailey *et al*., 1999; Ferguson *et al*., 2000; Hsu *et al*., 2000; Sekiguchi *et al*., 2001). While it is unclear whether the telomere fusions are the result of specific telomere defects or general genomic instability, the fact that these fusions can occur in the absence of NHEJ proteins suggests that another mechanism is capable of fusing telomeres. There may be particular conditions, however, under which telomere fusion can be mediated by NHEJ. The chromosomes of *S pombe taz1*$^-$ mutants, which have extremely long telomeres, undergo *Ku*, *Lig4*-dependent end-to-end fusion under specific cell growth conditions (Ferreira and Cooper, 2001).

Telomere shortening inhibits tumor growth

The idea that telomere shortening may suppress the growth of tumors originated from the model of primary human cell culture. In primary cells, telomeres shorten progressively prior to senescence, when the growth of the culture declines and reaches a plateau (Figure 2a) (Harley *et al*., 1990). It was proposed that telomere shortening provides a mechanism for cells to count the number of times that they have divided. If the senescence checkpoint is bypassed by transfection with SV40 T-antigen, the culture continues to grow for a limited number of cell divisions until all cells have stopped dividing at a point called crisis. Infrequently, some cells can escape from crisis and gain the ability to divide indefinitely (immortalization) (Wright and Shay, 1992). At crisis, there is a peak in dicentric chromosomes and telomere associations. After crisis, telomerase activity is detected, the number of dicentrics stabilized, and telomere length is stabilized after a few cell divisions at a length shorter than the initial telomere length (Counter *et al*., 1992; Ducray *et al*., 1999).

It has been proposed that the senescence that occurs in human cell culture might parallel a similar barrier to growth as a result of short telomeres in tumorigenesis (Harley *et al*., 1990). In fact, telomeres are shorter in human tumors compared to surrounding normal tissue (de Lange *et al*., 1990). Parallels have also been drawn between the increased genetic instability just prior to crisis and telomerase activation and the presence of genetic instability followed by telomerase activation in human cancer (DePinho, 2000).

Despite the insights gained from cell culture, it has not yet been definitively established that telomere shortening limits the growth of human tumors or that telomere dysfunction contributes to genetic instability in human tumorigenesis. Differences in telomere length, cell culture profile, and tumor spectrum in mice and humans have contributed to the continued ambiguity of the role of telomeres in human cancer. Significantly, the cell culture profile for human primary cells is not recapitulated in mice. Primary mouse cells lack a true senescence checkpoint and immortalize spontaneously (Figure 2b) (Todaro and Green, 1963). This difference is not an effect of the much longer telomere lengths in mice compared to humans, but is likely due to the absence of a specific checkpoint in the mouse (Greider, 2002). Mouse cells with very short telomeres do not undergo senescence and immortalize spontaneously with kinetics similar to wildtype cells (Blasco *et al*., 1997).

The difference between the mouse and human cell culture models may be significant to the difference in tumor profile in mice and humans. Adult human tumors are predominantly epithelial carcinomas while pediatric human tumors and mouse tumors are predominantly sarcomas and lymphomas (reviewed in DePinho, 2000). DePinho has proposed that this difference in tumor spectrum is due to differences in telomere length and regulation in mice. Extremely long telomere lengths and somatic telomerase expression in mice would prevent dysfunctional telomeres from playing a role in tumorigenesis. In support of this argument, mice with short telomeres and p53 deficiency develop human-like epithelial tumors (Artandi *et al*., 2000). While mice with short telomeres do seem to provide better models of the early stages of human epithelial carcinomas, these mice lack the telomerase

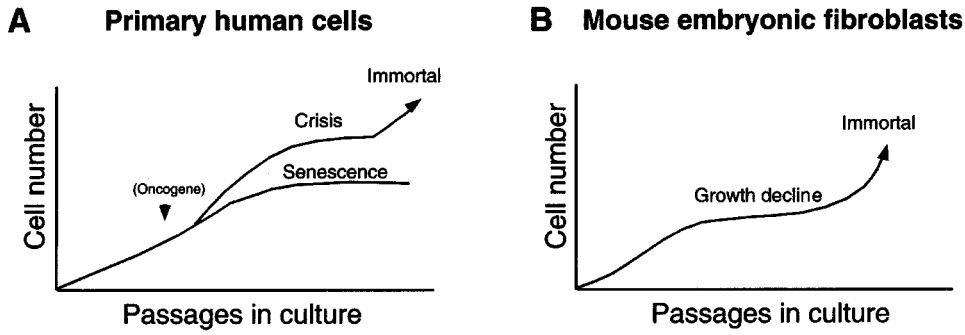

Figure 2 Growth of primary human and mouse cells in culture. (**a**) Growth of primary human cells in culture. The growth of cells initially increases and then plateaus at senescence. Senescence can be bypassed by prior introduction of SV40 T-antigen or other oncogenes (oncogene). If senescence is bypassed, cells can continue to divide until crisis. At crisis, rare cells can become immortalized and continue to grow indefinitely. (**b**) Growth of primary mouse cells in culture. Mouse cells lack a senescence checkpoint. The inflection point in the growth curve is only observed when cells are grown at low cell densities. Rather than stopping growth at a senescence point, mouse cells immortalize spontaneously

RNA, so they can never reactivate telomerase as is seen in later stages of human tumorigenesis.

Experiments in the $INK4a^{-/-}$ ($INK4a^{\Delta 2\Delta 3}$) mouse provide evidence that short telomeres do inhibit tumorigenesis. The $INK4a^{-/-}$ mouse is null for $p16^{INK4a}$ and $p19^{ARF}$, which have roles in the pRB and p53 pathways, but DNA damage recognition is intact. When tumor formation is induced in $INK4a^{-/-}$ $mTR^{-/-}$ mice by treatment with DMBA and UVB, short telomeres reduce the tumor incidence (Greenberg *et al*., 1999). Short telomeres also impair focus formation in $INK4a^{-/-}mTR^{-/-}$ cells in a Myc/RAS cotransformation assay. When transformed cells from late generation $INK4a^{-/-}mTR^{-/-}$ mice are injected into *SCID* mice, there is a significant reduction in the number of signal free ends and an increase in the number of fusions/metaphase in the tumor compared to the parental culture. Therefore, there is selection against signal free ends and selection for chromosome fusions in a situation where cell growth control is lost, but DNA damage recognition is intact. To assess the role of telomerase in this process, *mTR* is reintroduced during Myc/RAS transformation. Significantly, in tumors derived from these cells, there is a reduction in signal-free ends, but no reduction in end-to-end fusions.

Similar results are obtained in human cancer cell lines in which telomerase function was suppressed by expression of a dominant negative telomerase reverse transcriptase (TERT). These cells exhibit telomere shortening and loss of growth capacity after a lag period (Hahn *et al*., 1999; Zhang *et al*., 1999). Dominant negative TERT expression eliminates the ability of these cells to form tumors in nude mice. Therefore, short telomeres can suppress tumorigenesis.

An optimal degree of telomere dysfunction for tumorigenesis?

Insight into the relationship between genomic instability and tumor suppression in response to short telomeres comes from studies of telomere dysfunction in the Apc^{Min} mouse (Rudolph *et al*., 2001). These mice all develop multiple intestinal neoplasias and die at 4–6 months of age. Apc^{Min} mice have deregulated cell growth due to buildup of ß-catenin in the absence of APC (reviewed in Peifer and Polakis, 2000). Apc^{Min} cells also show increased chromosomal instability, with gross changes in ploidy and some chromosomal rearrangements (Fodde *et al*., 2001; Kaplan *et al*., 2001). In the $mTR^{-/-}$ background used to generate Apc^{Min} $mTR^{-/-}$ mice, generation 4 (G4) mice were the last generation of mice in the absence of telomerase that could be generated. Survival decreased in G2 and G3 Apc^{Min} $mTR^{-/-}$ mice, however, it increased again to the wildtype level in G4 mice. This phenotype may result from increased genetic instability in G2 and later telomerase null mouse generations but poorer tumor growth in response to dysfunctional telomeres in G4. Tumors in the Apc^{Min} mouse can be quantitated and microadenomas can be distinguished from the later staged macroadenomas. Microadenoma numbers peaked in G3 and was still fairly high in G4. In contrast, macroadenoma number peaked in G2 and was almost zero in G4. Additionally, macroadenoma size decreased progressively from wildtype to G4. Therefore more adenomas were initiated in G4, but fewer progressed to the macroadenoma stage. In macroadenomas, the frequency of anaphase bridges increased from wildtype to G4. All tumors were p53 positive, but macroadenomas in late generation mice showed increased p53 expression and increased apoptosis (Rudolph *et al*., 2001).

The presence of p53 activity in Apc^{Min} $mTR^{-/-}$ tumors suggests that chromosomal instability can be initiated by short telomeres in mice prior to inactivation of p53. Therefore, in mice, it seems that dysfunctional telomeres can initiate genomic instability at lengths that do not trigger checkpoints resulting in tumor suppression, or that a small number of dysfunctional telomeres can initiate instability and not trigger a checkpoint. However, the cell culture models suggest that this may not be the case in human cancer. The lack of a senescence checkpoint in late generation mouse

$mTR^{-/-}$ cells indicates that mice may not respond to short telomeres during tumorigenesis with the same timing or severity as human cells. It may be that in humans, short telomeres trigger checkpoint arrest before they shorten to a point where they can initiate genetic instability. It will be interesting to determine whether telomere dysfunction can provide a primary initiating step in tumorigenesis, or whether it can only contribute to chromosomal instability in tumors after loss of a checkpoint. Perhaps triggering of a checkpoint can sometimes facilitate the fusion of dysfunctional telomeres by stimulating DNA repair pathways.

Telomerase activation facilitates tumor growth

Several lines of evidence suggest that activation of telomerase can facilitate tumor progression. The presence of telomerase activity in greater than 85% of cancers suggests that telomerase activity stimulates tumorigenesis by increasing cell proliferation (Kim *et al*., 1994; Shay and Bacchetti, 1997). Typically, telomerase activation occurs late in tumorigenesis (Chadeneau *et al*., 1995; Tang *et al*., 1998). Maintenance of telomere length is a common feature of tumors late in tumorigenesis; even in those tumors without detectable telomerase activity, telomere lengthening occurs through an alternative mechanism (ALT) (Bryan *et al*., 1997). In mice, reintroduction of telomerase increases the tumor forming ability of late generation $mTR^{-/-}$ $INK4a^{-/-}$ fibroblast cultures. This reintroduction eliminates the signal-free ends in these cells, indicating that telomere function is restored (Greenberg *et al*., 1999). In yeast, reintroduction of telomerase can efficiently restore growth potential in cells with critically short telomeres, including cells with some degree of ongoing chromosomal instability (Hackett *et al*., 2001). Thus in a number of systems telomerase activity can facilitate cell growth. However, in human cancer cells, where the checkpoints that recognize dysfunctional telomeres may have been inactivated, the exact role of telomerase activation is still not clear.

Telomerase inhibits genomic instability

In addition to the possible role of telomerase in preventing the initiation of chromosomal instability by maintaining telomere function, telomerase might also suppress mutagenic chromosome fusions that facilitate breakage-fusion-bridge (BFB) cycles by allowing telomere addition to repair broken ends. The role of telomerase in halting BFB cycles is supported by the work of Barbara McClintock. When a broken chromosome is transmitted to the endosperm in maize, BFB continues during the development of the endosperm. However, if a broken chromosome is transferred to the zygote, the broken end is healed and no BFB occurs (McClintock, 1941). The presence of BFB in the endosperm, but not in the embryo may result from the presence of significant telomerase activity in the embryo, but not in the endosperm (Killan *et al*., 1998) which allows telomerase to add a new telomere to broken ends in the embryo. Telomerase-mediated *de novo* telomere addition has been demonstrated as a mechanism for healing broken chromosomes in yeast (Kramer and Haber, 1993). In support of the role of telomere addition in limiting BFB, yeast that undergo gross chromosomal rearrangements are more likely to contain chromosome fusions and rearrangements involving multiple chromosomes in the absence of telomerase than when telomerase is present (Hackett *et al*., 2001; Myung *et al*., 2001).

Despite a possible role for telomerase in limiting BFB, the significance of a decrease in genetic instability following telomerase activation in the process of tumorigenesis is unclear. Interestingly, nonreciprocal translocations are clonally stable in $p53^{-/-}$ $mTR^{-/-}$ tumors (Artandi *et al*., 2000). This suggests that stabilization of genomic instability can occur even in the absence of telomerase. In these cells telomere function may be restored though the ALT pathway. It remains possible that telomerase activation may be significant for tumor growth simply by increasing the growth potential of cells with short telomeres rather than by limiting genomic instability. It is also possible that telomerase activation *per se* does not always confer a selective advantage, but is merely the byproduct of other changes in the tumor such as Myc activation. However, this explanation seems unlikely given the presence of either telomerase activity or the ALT mechanism in tumors.

Future directions

The apparently conflicting effects of telomere dysfunction and telomerase activation in tumorigenesis can be understood in terms of the timing of these processes. Telomere dysfunction probably contributes to relatively early stages of tumorigenesis (Figure 3). It is not yet clear whether telomere dysfunction inhibits tumor growth before or after the initiation of genetic instability. Telomerase activation seems to occur late in tumorigenesis and may contribute to the growth potential of the tumor after genetic instability has been established (Figure 3).

Telomerase inhibitors have been proposed as chemotheraputic agents (Counter *et al*., 1992; Harley *et al*., 1990) and are currently being studied by pharmaceutical companies. Reintroduction of telomerase into tissues undergoing high turnover-rate due to disease has also been proposed as a possible tumor-suppressive therapy (DePinho, 2000). The antagonistic nature of these two therapies reveals possible problems with attempting to target telomeres or telomerase to fight cancer. Telomerase inhibitors function by reducing telomeres to a critical length. While telomerase inhibition may decrease the growth of late stage tumors, it may also select for more malignant subclones from the tumors by enhancing genomic instability. Therefore, care should be taken when using these drugs. It may be that the risk

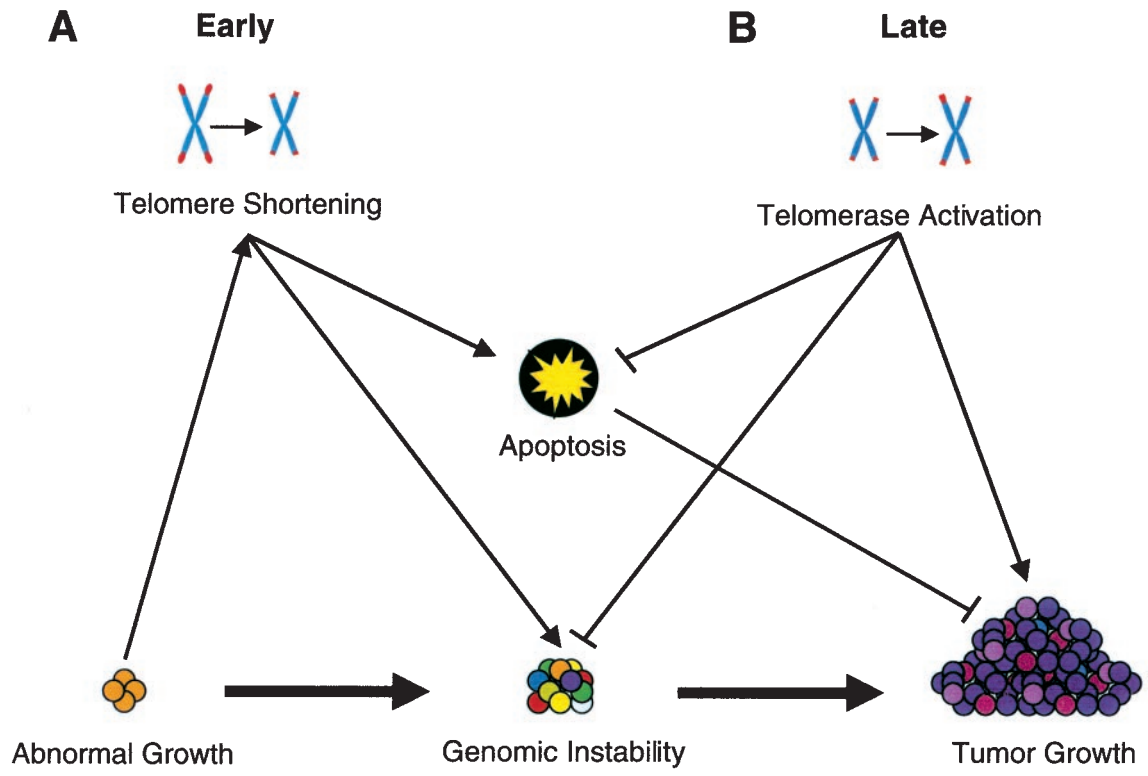

Figure 3 Model for the roles of telomere dysfunction and telomerase activation in terms of the stage of tumor progression. A schematic depicting stages of tumor progression are shown along the bottom. The initial growth of a clone of cells is shown by the small cluster of orange balls. Early stage tumors with ongoing genetic instability are represented by the cluster of multicolored balls. Late stage tumors in which certain cells have been selected from early stages in tumor progression are represented by the large cluster of blue and purple balls. Apoptosis is represented by the yellow star in the center of the diagram. (**a**) Telomere shortening is depicted by the loss of the red telomere signal from the ends of the blue chromosomes. Short telomeres can either induce genetic instability or inhibit tumor growth through apoptosis. It is unclear how frequently or in what order these two pathways operate. Short telomeres may trigger apoptosis through the induction of a DNA damage checkpoint. (**b**) Telomerase activation is depicted by the restoration of red telomere signal to the ends of the blue chromosomes. Telomerase activation can both limit genetic instability and facilitate tumor growth

of outgrowth of a resistant subclone could be minimized by combination therapy.

An important area of future research will be to establish how frequently short telomeres trigger genomic instability versus apoptosis during tumor initiation. Is there actually a link between telomere dysfunction and chromosomal instability in human tumors? Can dysfunctional telomeres initiate genomic instability in the absence of other mutations in checkpoint genes, or is the main role of telomere dysfunction in ongoing chromosomal instability after the loss of checkpoints?

The mechanism that mediates the fusion of dysfunctional telomeres are also largely unknown. Knowledge of this mechanism could suggest possible ways to minimize chromosome fusion and maximize apoptosis in response to dysfunctional telomeres. Such an approach would have the advantage of directly targeting dysfunctional telomeres, rather than altering telomerase expression.

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