

# Mammalian telomere dynamics: healing, fragmentation shortening and stabilization

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Telomeres are essential for stable chromosome maintenance. The simple G-rich sequence motif  $d(\text{TTAGGG})_n$  is all that is required in *cis* for telomere function in mammalian cells, as in other eukaryotes. Using this fact, telomeres have been used to specifically fragment mammalian chromosomes to dissect their structure and function. Telomere length maintenance is altered in cancer cells. *Trans*-acting factors, such as telomerase and telomere-binding proteins, may determine telomere function in both normal and cancer cells. Current experiments are aimed at understanding the role of telomerase and telomere-binding proteins in cellular senescence and immortalization.

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## Introduction

Telomere research is experiencing a boom phase. Biologists in a wide range of fields are beginning to examine the role of telomeres in their favorite biological process. No wonder, then, that the list of processes in which telomeres seem to be important is growing and now includes chromosome length maintenance, chromosome stability, transcriptional silencing, chromosome positioning in the nucleus, chromosome healing, generation of artificial chromosomes, cellular senescence and cancer. A boom has also been seen in the number of reviews of telomere research in the past few years [1–6]. In this review, therefore, I will focus on recent advances in understanding mammalian telomere function. Some aspects that I will not be able to cover, such as telomere positioning in the nucleus, telomere-associated sequences, and karyotype changes associated with human disease and cancer, are covered in other recent reviews [7–9].

The molecular characterization of telomeres began in the 1970s with the identification of tandemly repeated simple sequences at the ends of ciliate chromosomes [10,11]. It proceeded with the identification of similar repeated sequences at yeast chromosomal telomeres and the demonstration that these sequences are sufficient to stabilize linear chromosomes [12–14]. At the molecular level, the first hint that human telomeres might share structural and functional features with those of unicellular organisms came from work on the pseudoautosomal region near the telomeres on the human X chromosome. When this sequence was cloned, Southern analysis revealed a smear of heterogeneously sized DNA fragments [15], this being a characteristic

feature of telomeric DNA from unicellular eukaryotes. Further work indicated that the average length of the human telomere smear differed in sperm and somatic tissue, indicating tissue-specific regulation of telomere length [16].

## Telomere-binding proteins

Although the simple G-rich sequence elements found at telomeres in most eukaryotes seem to be the only DNA elements necessary for telomere function, the proteins that bind telomere sequences *in vivo* are essential for telomere function. The prototypic telomere-binding protein is the  $\alpha/\beta$  heterodimer that was isolated from the ciliate *Oxytricha* [17,18]. This protein complex binds very tightly to the  $d(\text{TTTTGGGG})$  single-stranded overhang at *Oxytricha* chromosome ends and is thought to play an essential role in telomere function. Telomere-binding proteins from a few other ciliates have been found to have analogous properties to this *Oxytricha* protein [19,20]; however, considering the high degree of conservation of telomere G-rich sequence motifs in evolution, telomere-binding proteins appear to be much less conserved. Over the past 4–5 years, several research groups have been looking for mammalian proteins analogous to those of *Oxytricha*, with limited success.

To identify mammalian homologs of the *Oxytricha* single-stranded end binding proteins, researchers have used gel mobility shift assays to purify specific  $d(\text{TTAGGG})_n$ -binding proteins. McKay and Cooke [21] have reported an activity from mouse liver extracts

## Abbreviations

hGH—human growth hormone; hnRNP—heterogeneous nuclear ribonucleoprotein; HPRT—hypoxanthine phosphoribosyltransferase; XTEF—*Xenopus* telomere end factor.

that shifts a  $d(\text{TTAGGG})_6$  single-stranded probe. Using  $d(\text{TTAGGG})_n$  affinity chromatography, they showed that a highly abundant protein co-purified with this activity. This protein was sequenced and shown to correspond to the heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 protein [22\*]. Consistent with its identification as an hnRNP, the purified fraction efficiently bound a synthetic  $r(\text{UUAGGG})_n$  RNA, and the purified 37 kDa protein was recognized on western blots by a monoclonal antibody directed against hnRNP proteins. These same antibodies did not stain metaphase chromosomes, however, suggesting that the hnRNPs do not have a dual role in RNA metabolism and telomere function.

Independently, Ishikawa *et al.* [23\*\*] isolated hnRNPs in a study designed to purify human single-stranded  $d(\text{TTAGGG})_n$ -binding proteins. Careful analysis of the binding specificity showed that RNA  $r(\text{UUAGGG})_4$  was bound at a much higher affinity than the corresponding DNA  $d(\text{TTAGGG})_4$  probe. Mutational analysis of each nucleotide position in the  $r(\text{UUAGGG})_4$  repeat showed that the first four nucleotides are necessary for recognition, whereas mutations in the 5' GG residues were tolerated. This sequence requirement matches the consensus sequence for mammalian splice sites, indicating that these hnRNPs may be involved in splice site binding. Purification and protein sequence analysis revealed the presence of co-purified A2/B1 protein, as in the study by McKay and Cooke [21], as well as nucleolin and three additional hnRNPs, A1, D, and E. These studies by McKay and Cooke, and Ishikawa *et al.* indicate that it may be quite difficult to identify candidates for specific mammalian single-stranded telomere binding proteins. Human telomeres are present at a copy number of 92 per cell; therefore, one would not expect telomere-binding proteins to be abundant. As the hnRNP proteins are present at a copy number of about  $10^7$  per cell, it may be difficult to remove these from extracts isolated in experiments aimed at identifying the authentic telomere-binding proteins.

Another protein that binds single-stranded  $d(\text{TTAGGG})_n$  repeats has been isolated from avian cells [24]. Methylation interference and inosine-substitution experiments have suggested that this activity is specific for binding to Hoogsteen G–G base pairs, a structure that has been shown to be formed by telomeric oligonucleotides [25,26]. As the activity described in this study was not purified and the ability to bind RNA was not tested, it is not clear whether this binding activity is related to the mammalian hnRNP proteins isolated by the other groups.

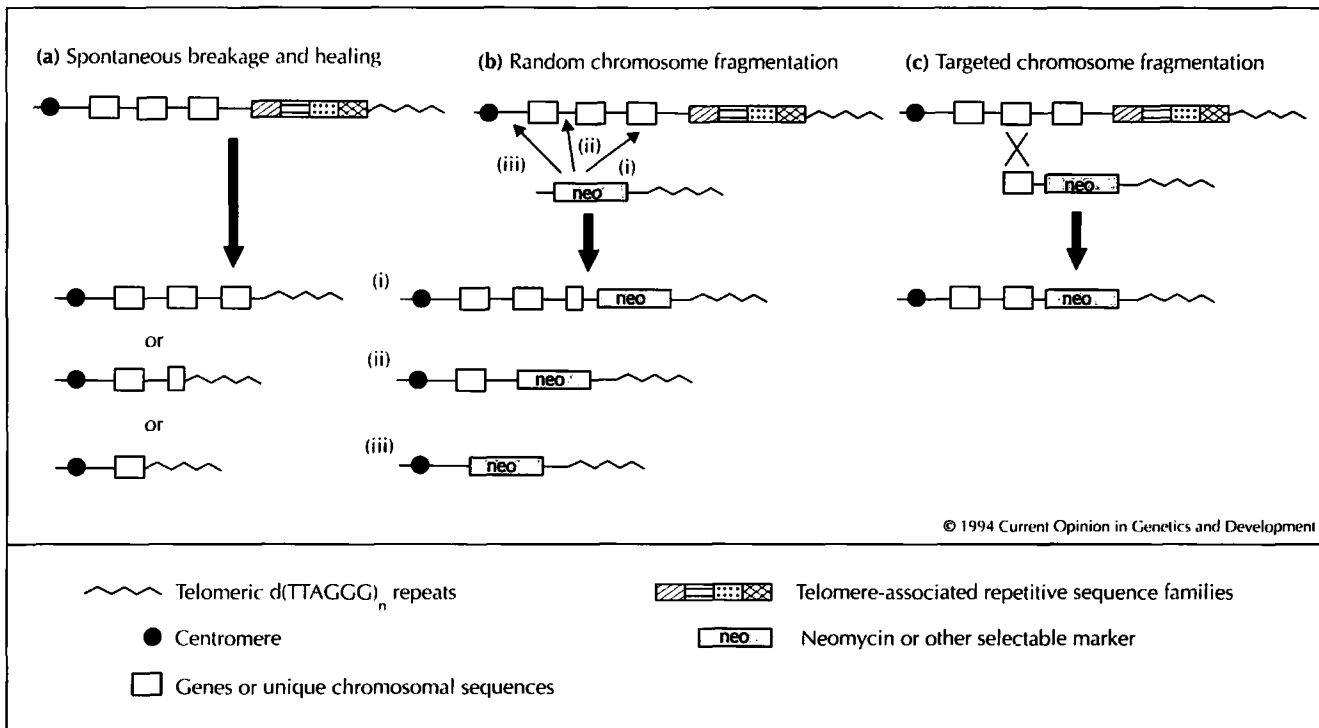
The best candidate so far for a functional homolog of the well-studied *Oxytricha* telomere-binding protein is a DNA-binding activity identified in *Xenopus* oocytes [27\*\*]. This factor binds telomeric primers with single-strand/double-strand junctions, and binding requires a free single-stranded 3' end. The binding of the *Xenopus* telomere end factor (XTEF) is very stable in high salt, like the *Oxytricha* proteins, and is highly spe-

cific for  $d(\text{TTAGGG})_n$  sequences. Telomere sequence repeats from other organisms are not bound. XTEF is distinct from the single-stranded telomere binding proteins characterized in human and mouse cells, in that it does not bind to RNA  $r(\text{UUAGGG})_n$  repeats. It is much more abundant in oocytes and ovaries than in somatic cell nuclei, suggesting a stockpiling of factors necessary for rapid embryonic cell divisions. The properties of the XTEF suggest that it may be the protein that binds to the terminal regions of *Xenopus* chromosomes *in vivo* and may function as a cap to protect the chromosome end.

Proteins that bind to the double-stranded regions along telomere length represent a different class of telomere-binding factors. Such factors have been isolated from yeast and the slime mold *Physarum* and may play a role in the unique chromatin structure found at telomeres in some organisms [28–31]. Recent experiments have identified a highly specific factor that binds to mammalian telomeric sequences [32\*\*]. This activity is present in various mammalian species and will bind to six contiguous  $d(\text{TTAGGG})$  repeats, but will not bind  $d(\text{TAGGG})_n$ ,  $d(\text{TTGGGG})_n$  or other telomere-like repeated sequences. The binding to telomere sequences does not require proximity to a molecular end. Purification of the binding activity and recovery from an SDS–polyacrylamide gel indicates that a 50 kDa polypeptide is responsible for the binding activity. As  $d(\text{TTAGGG})_n$  sequence repeats have been shown to associate preferentially with the nuclear matrix fraction [33], this protein may mediate those associations.

### Spontaneous chromosome healing

Barbara McClintock's classic work on chromosome structure in maize has shown that telomeres are essential for stability, but also that unstable chromosomes undergoing repeated breakage and fusion events are sometimes spontaneously healed. Molecular evidence from many organisms has suggested that (with the notable exception of *Drosophila*) this healing is most often a result of the addition of G-rich telomere repeats onto the broken ends [34,35] (reviewed in [36]). The first evidence that  $d(\text{TTAGGG})_n$  is sufficient for human telomere function came from studies on a chromosome truncation in an  $\alpha$ -thalassemia patient [37]. In a terminal deletion, a large region of the  $\alpha$ -globin locus, the most distal marker on chromosome 16, was lost and  $d(\text{TTAGGG})_n$  repeats were added onto the new end. As this chromosome is stably inherited, the  $d(\text{TTAGGG})_n$  repeats must be sufficient for telomere function (see Fig. 1a). Examination of eight other patients with  $\alpha$ -thalassemia identified a second example of *de novo* telomere addition. The structure of these healed chromosome ends suggests that the  $d(\text{TTAGGG})_n$  repeats were added *de novo* by telomerase [38\*\*].



**Fig. 1.** Possible routes for chromosome breakage and fragmentation. (a) Spontaneous breakage and healing. Mammalian chromosome ends lacking telomeres are usually unstable; they will be degraded or fuse with other parts of the genome. These broken ends can be healed, at a low rate, by the addition of telomeric d(TTAGGG)<sub>n</sub> repeats, probably by the action of telomerase. The broken end that contains the centromere is now stabilized, and the other end is lost. If there are no essential genes distal to the new telomere, the chromosome is stably inherited with a new end. (b) Random chromosome fragmentation. Chromosome fragmentation can be induced in tissue culture by the transfection of a linear fragment containing a selectable marker, such as neomycin (neo); the vector integrates randomly into the genome. During integration, possibly at a double-strand break, the d(TTAGGG)<sub>n</sub> sequences on the vector can 'seed' the addition of more telomere repeats. The healed chromosome fragment containing the centromere will be stably maintained. (c) Targeted chromosome fragmentation. Using gene targeting techniques, a linear fragment containing d(TTAGGG)<sub>n</sub> on one end can be integrated into a specific chromosomal locus. As the frequency of homologous recombination is low, a selection scheme must be used to identify the correctly targeted clones. As with the random chromosome fragmentation, the transfected d(TTAGGG)<sub>n</sub>-containing DNA can 'seed' the addition of d(TTAGGG)<sub>n</sub> repeats and the chromosome end will be stabilized.

Telomerase is the DNA polymerase that maintains telomere length by the *de novo* addition of telomeric repeats onto telomeres *in vitro* and *in vivo* [39,40]. *Tetrahymena* telomerase heals chromosomes fragmented during macronuclear development, by adding G-rich repeats onto ends which lack them *in vivo* [41]. *In vitro*, both the human and *Tetrahymena* telomerases will elongate primers with non-telomeric sequences at their 3' ends. In fact, both human and *Tetrahymena* telomerase will elongate a primer with the sequence found at the site of repeat addition in one  $\alpha$ -thalassemia patient [42,43].

### Chromosome fragmentation

The fact that d(TTAGGG)<sub>n</sub> sequences are sufficient to allow telomere function *in vivo* has led to some interesting chromosome fragmentation experiments. Farr *et al.* [44] first demonstrated that transfection of linearized plasmids containing d(TTAGGG)<sub>n</sub> repeats and a selectable marker leads to chromosome integration, and sometimes to new telomere formation at the integration site [44] (see Fig. 1b). They transfected a

linear construct containing the histidinol gene into Chinese hamster cells, and the histidinol gene was found to be associated with a new chromosome end in 6 of the 27 integrants. During the integration event, a double-stranded break is presumably present, and the d(TTAGGG)<sub>n</sub> sequences are recognized by telomerase and elongated to generate a new stable chromosome end. The remaining part of the chromosome lacks a centromere and is lost during further divisions. The input 500bp of d(TTAGGG)<sub>n</sub> was elongated to more than 1 kb in the transformants. In a more recent study, Farr *et al.* [45•] used this fragmentation method to generate a series of deletions in the human X chromosome. They transfected a telomere fragment containing a hygromycin resistance gene into a hamster-human hybrid cell line containing a histidinol gene on the short arm of the X chromosome. Transfected cells were simultaneously selected for the hygromycin and histidinol resistance genes and against the *HPRT* gene (located on the long arm of the X chromosome). By applying this simultaneous positive and negative selection, cells were generated in which the hygromycin telomere construct replaced the long arm telomere containing the *HPRT* gene. A new telomere was generated in 42 of the 85 transfectants. The deletions could be or-

dered using available probes for the long arm of the X chromosome. On average, a new telomere was created every 1–2 Mb. Surprisingly, many of the telomere fragmentation events were accompanied by large 1–2 Mb terminal duplications. These duplications make it difficult to obtain unique sequence adjacent to the healing site. As other research groups using transfection of  $d(\text{T TAGGG})_n$  plasmids have not seen these duplications ([46\*]; T de Lange, personal communication), they might be specific to procedures used in the study by Fair *et al.* [45\*\*], such as simultaneous drug selections. The approach of telomere-mediated chromosome fragmentation may be useful for both chromosome mapping and for defining the structural requirements for mammalian chromosome function.

Random chromosome fragmentation appears to require the presence of telomerase to generate a new telomere. Barnett *et al.* [46\*] have studied fragmentation events in a number of different human and mouse cell types. When a linear plasmid containing  $d(\text{T TAGGG})_n$  repeats at one end was transfected into HeLa cells, 24 new telomeric sites were generated from 44 random integrations. HeLa cells have been shown to contain telomerase activity [47]. Two other immortalized human cell lines also allowed new telomere formation, but no new telomere generation has been observed after transfection of a primary human cell line. Previous studies have shown that telomeres continually shorten in primary cells and that telomerase activity is either absent or very low (see below). Further evidence for the role of telomerase in new telomere formation has come from embryonic stem cell experiments. Telomere-containing fragments transfected into mouse embryonic stem cells, which have telomerase activity (M Scharring, C Greider, unpublished data), were shown to generate new healed chromosome ends [46\*].

Evidence for telomerase-mediated healing also comes from the structure of healed chromosomes. A DNA construct, similar to one used in yeast chromosome healing experiments, was generated by placing a small amount of non-telomeric DNA distal to the  $d(\text{T TAGGG})_n$  repeats in the fragmentation vector. Using this construct, new telomeres were efficiently generated in HeLa cell transfectants. Five of eight clones retained the non-telomeric sequences between the input  $d(\text{T TAGGG})_n$  repeats and the newly added terminal  $d(\text{T TAGGG})_n$  sequences [46\*]. These results are similar to those obtained in yeast by Murray *et al.* [48] and Kramer and Haber [49]. Because recombination requires homology and  $d(\text{T TAGGG})_n$  repeats were added onto non- $d(\text{T TAGGG})_n$  sequences, telomerase was probably responsible for the *de novo* repeat addition.

### Fragile sites

The chromosome fragmentation experiments described above raise the issue of the consequence of chro-

somal internal  $d(\text{T TAGGG})_n$  repeats. In many fragmentation studies, ~50% of the integration events do not generate a new telomere. These may represent simple integration of the marker plus the  $d(\text{T TAGGG})_n$  repeats that is not accompanied by chromosome breakage. In some cases, loss of the  $d(\text{T TAGGG})_n$  sequences may occur before integration [46\*]. Naturally occurring chromosome internal  $d(\text{T TAGGG})_n$  repeats are found in humans, mice and other vertebrates. [50–53]. Several groups have hypothesized that these repeats may be inherently unstable and prone to breakage, giving rise to fragile sites [50,54,55]. The accumulated evidence, however, argues against tandem  $d(\text{T TAGGG})_n$  repeats being a direct cause of chromosome breakage. First, a fragile site on chromosome 2 maps distal to inverted telomere repeat arrays on that chromosome. Human chromosome 2 contains the ancestral fusion point between two ape chromosomes. This fusion point contains head-to-head inverted telomeric  $d(\text{T TAGGG})_n$ – $d(\text{CCCTAA})_n$  repeats, as well as inverted sub-telomeric sequences; this structure is probably a relic of the ancestral telomeres on the two ape chromosomes [56]. Second, the human fragile X site has recently been cloned and shown not to be made up of telomeric sequences, but is, rather, a region with expanding triplet repeats of the sequence  $(\text{CCG})_n$  [57,58]. Third, internal telomeric repeat arrays are found in yeast and ciliate chromosomes and are not associated with chromosome breakage or instability.

In yeast, an artificial head-to-head telomere construct inserted into a plasmid or chromosome has been shown to be associated with a low level of chromosome breakage. From the very low level of this reaction, it was concluded that it is not normally involved in chromosome metabolism [48]. In mouse chromosomes, a head-to-head telomere repeat is located near a common breakage site associated with acute myeloid leukemia. It has been proposed that irradiation of mice may cause chromosome breakage at telomere-like repeats [55,59]. This study, however, like others that propose inherent  $d(\text{T TAGGG})_n$  instability, did not distinguish between the repeats causing breakage and the repeats allowing the stabilization and recovery of broken chromosomes. Breaks may occur along the whole length of chromosomes, but only those that are stabilized near  $d(\text{T TAGGG})_n$  repeats are recovered. The statistical association between the broken mouse chromosome and acute myeloid leukemia may be real, but it may be the stabilization, not the generation of a break itself, that is the causal event.

In yeast, the generation of artificial constructs containing simple tandem telomere repeats at internal chromosome sites has shown that the repeats are stable under normal growth conditions. However, if a break is induced by a specific endonuclease near the telomere repeats, the repeated sequences will allow stabilization of the broken chromosome, presumably through a telomerase-mediated healing event [48,49,60]. If no telomeric sequences are located near the break, the chromosome is lost [49]. Thus, sites of internal  $d(\text{T TAGGG})_n$  repeats may be associated with

new telomeres under certain conditions, although that does not necessarily mean that these repeats are inherently unstable.

### Targeted chromosome fragmentation

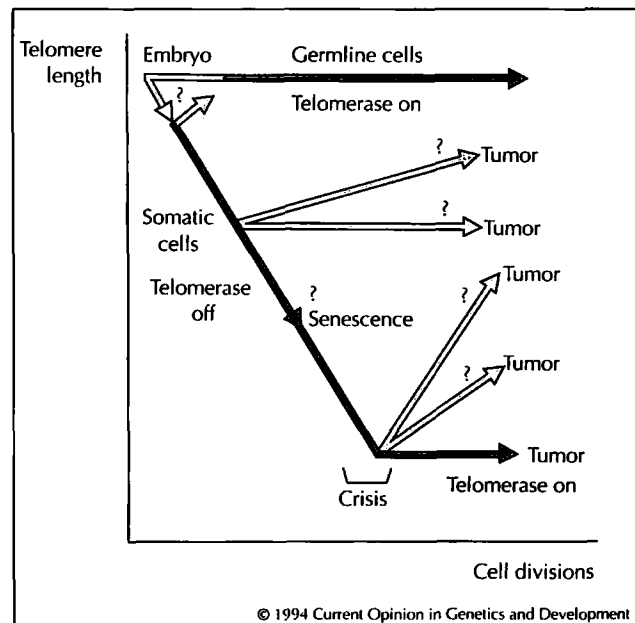
Telomere constructs can also be targeted to specific loci to generate very specific chromosome truncations (see Fig. 1c). Although the frequency of homologous recombination events is low in mammalian cells, it is possible to select for the desired events. Itzhaki *et al.* [61\*\*] have designed a vector to target a telomere construct to the human interferon-induced gene 6-16. A promoterless human growth hormone (hGH) gene was designed to recombine with the endogenous promoter of gene 6-16 — correctly targeted constructs could then be identified by their secretion of hGH in response to interferon. Using this strategy, eight hGH-expressing clones were generated from 12 000 transformants, and one of the clones was found to be associated with a new telomere. The structure of this transformant indicated that the targeting had successfully truncated the chromosome and generated a new telomere at the endogenous 6-16 locus. The input telomere DNA was extended in the clone by ~1 kb in the immortal sarcoma cell line used, suggesting that it was elongated by telomerase. This ability to generate specifically defined chromosomal truncations will be a powerful tool for defining essential chromosome functions. As chromosomal fragmentation also works in embryonic stem cells, specifically engineered chromosomes can also be tested *in vivo* in transgenic mice, in addition to being used in assays for chromosome function in tissue culture cells.

### Telomere hypothesis for cellular senescence and immortalization

In the early 1990s, work from several research groups converged to generate a picture of human telomeres as dynamic structures. Soon after the cloning of the first human telomeres, de Lange *et al.* [62] showed that telomeres in human cancers were shorter than those in normal tissues. This observation was supported by evidence that colon carcinoma telomeres are shorter than normal tissue telomeres [63]. In addition, Harley *et al.* [64] showed that primary cells in culture lose telomere sequence with each round of cell division, suggesting that telomere loss in cancer cells may be due to the increased number of cell divisions they undergo. This telomere shortening also occurs *in vivo*: older individuals have shorter telomeres in somatic tissues than younger people. Germline telomeres, however, do not shorten with age [63,65,66,67\*\*].

A model has been proposed to explain the behavior of telomeres in cellular senescence and in cancer. In

somatic cells, telomeres shorten with each round of cell division due to the lack of telomerase activity, whereas in the germline, telomerase activity keeps telomeres long to ensure faithful chromosome transmission to the next generation [16,68]. Primary cells have a limited life span *in vitro*. As  $d(\text{TTAGGG})_n$  loss is tightly coupled to cell division, telomere shortening could provide the signal for cells to enter senescence. In immortal human cancer cells, telomerase is re-activated and telomeres stably maintained [68,69] (see Fig. 2).



**Fig 2.** Regulation of telomere length during mammalian development and tumor formation. In somatic tissue, telomere length decreases with increasing cell division; this loss may be due to the absence of telomerase. Germline telomeres are maintained at a long length, and telomerase activity is present. The stage of embryogenesis at which telomerase may be turned on or off is not yet clear. In primary human cells which undergo senescence, cells exit the cell cycle and cease dividing with characteristically short telomeres. Telomere length may play a role in signaling this entry into senescence. Tumor viruses cause a bypass of senescence, and telomere length in the extended life span clones continues to shorten. At crisis, telomere length stabilizes and telomerase is detected. In some cell lines, telomere length increases after crisis, in others it is maintained at a short size. In many different tumor tissues, telomere length is shorter than neighboring non-tumor tissue. Telomere length, however, is not always less in large tumors than in small tumors, suggesting that telomerase may be activated earlier in some tumors than in others.

The phenomenon of cellular senescence has been well studied since it was described by Hayflick and Moorhead in the 1960s [70]. Although much is known about the difference between young and senescent cells, it is not clear what mechanism tells the cell how many times it has divided and, thus, when to enter senescence. The life span of cultured fibroblasts correlates very well with telomere length: cells with long telomeres can undergo more rounds of cell division *in vitro* than those with short telomeres [66]. This suggests that telomere length could be used as a measure of replica-

tive history; short telomeres may trigger a checkpoint, signaling entry into senescence.

Established immortal cell lines often have very short telomeres that are maintained during growth, and telomerase activity is present. Primary cells, which lose telomeric repeats and have a limited life span, have undetectable telomerase. During immortalization of human cells in culture, cells that express telomerase may be selected because they can maintain their telomere length [68]. These data suggest that telomerase may be required for the growth of immortalized cells; thus, telomerase has been proposed as a target for anti-cancer drugs.

In the past year, several research groups have begun testing the above hypothesis in both tissue culture systems and human tumors. Telomerase activity has been isolated from 10 different immortalized human and mouse cell types, suggesting that activation may be universal for immortalized cells with stable telomere length ([47,71\*\*]; CB Harley, KR Prowse, personal communication). Three different research groups have followed telomere length after transfection with viruses and selection for immortalized cells [72\*\*,73\*,74]. Before immortalization, telomere length decreased, but after immortalization, telomere length was stabilized. In one case, immortalization with human papilloma virus was associated with lengthening of telomeres after crisis. The average length of telomeres in the clones at late passage was similar to, or even greater than, that in the initial tissue. Clonal variants of HeLa cells containing long telomeres have also been reported [62]. It may be that, in immortalized cells, the regulation of telomere length is not properly controlled. Telomerase may be induced in immortalized cells, but the other factors that regulate length maintenance may be still missing [75].

### Telomeres and telomerase in cancer tissues

If telomerase inhibition is a therapeutic goal for cancer chemotherapy, we need to understand telomere length and telomerase regulation in cancer tissue, not simply in cultured cells. In the past year, several research groups have begun these studies. Telomere reduction has been found when normal tissue is compared with tumor tissue from endometrial adenocarcinoma, colorectal carcinoma, leukemia, renal tumors, neuroblastoma and ovarian carcinoma [76–78,79\*\*,80]. In one study of intracranial tumors, no telomere shortening was reported [81]; however, this study compared telomeres in brain tissue with telomeres in cultured blood samples from the same individual. As blood cell telomeres shorten *in vivo* and *in vitro* [67\*\*], and as brain telomeres are longer than telomeres in other tissues (C Harley, E Chang, personal communication), blood telomere length is not a meaningful substitute for the control normal telomere length in these patients.

Initial work on telomere length in cancer cells had suggested that length correlates with cell divisions and might thus be a useful marker for the staging of various cancers or even for prognosis [77]. However, a recent detailed study of renal cell carcinoma suggests that telomeres may not be a good marker of tumor progression, in this cancer at least [82\*\*]. Telomere restriction fragment length was followed in 10 independent renal cell carcinomas and compared to normal kidney tissue from the same individual. In some cases, two or three independent regions of the tumor were assayed for telomere length, and in all cases, telomere shortening was found. The size of the tumors, however, did not correlate with the length of the telomeres. If telomeres shorten progressively during tumor growth, one would expect large tumors to have shorter telomeres than small tumors. In a tumor measuring 40 mm, telomeres were on average 1.2 kb shorter than normal, whereas in a tumor of 150 mm, only 0.7 kb was lost. The authors suggest that telomerase may be activated in these tumors *in vivo*. Depending on the size of the tumor at activation, telomere length may be stabilized at any of a range of sizes (see Fig. 2). Although this finding suggests that telomere length may not be a reliable measure of tumor progression, it also suggests that if telomerase is active in the tumors, then the affected cells can be targeted by anti-telomerase therapies.

The first report of telomerase in a cancer tissue came from a study of ovarian carcinoma [79\*\*]. Ascites cells taken directly from six independent cases of ovarian carcinoma were assayed directly for telomerase activity. As the peritoneal fluid contains both normal and cancer cells, the cells could be fractionated into normal and transformed populations. The normal cells had long telomeres, while the transformed cells had very short telomeres. Telomerase activity was present in the fractionated cancer cells containing short telomeres. Repeated samples from the same patient taken 8 months apart showed that the short telomere length was maintained over this time. These results suggest not only that telomerase is present in clinical samples, but also that telomeres are maintained at a short length, at least in this cancer. This means that targeting of telomerase may be an effective cancer treatment. The cancer cells which start with very short telomeres would be expected to lose them after only a few rounds of cell division [79\*\*], which could inhibit further tumor progression.

### Conclusions

Just six years ago, we did not even know the human telomeric repeat sequence. Today, human telomere research is the fastest growing area in telomere biology. Cancer biologists have known for years that gross structural changes in chromosomes are associated with cancer progression. Now we can address the subject of structure and function in human chromosome dynamics. The role that telomerase plays in immor-

talized cells was unforeseen when the enzyme was first discovered in *Tetrahymena* cells. Today, human telomerase is being pursued as a target for anti-cancer therapies. Clearly, the impact of telomere biology is being felt in fields previously considered as being unrelated and this ripple effect into new areas of biology will probably continue. The boom phase that telomere research is now experiencing is likely to turn the small town of telomere research into a major bustling metropolis with superb inter-city connections to other major centers of biological research.

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This paper presents a very careful study of telomere lengths in renal cell carcinoma. Although telomere shortening was observed in all tumors, the size of the tumor did not correlate with telomere length. The authors suggest that telomerase may be activated at different times in various tumors leading to telomere stabilization at either long or short lengths.

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