

Telomerase: Dr Jekyll or Mr Hyde?

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The past year has seen the ectopic expression of human telomerase and the consequent increased replicative lifespan of cells, whereas mice lacking telomerase have lived and reproduced for six generations. Core telomerase activity from various organisms was reconstituted *in vitro*, yet how its action is regulated remains largely unknown. Telomerase activation preceded oncogenic transformation in some human cell types, yet was lacking in other transformed cells. These advances highlight the potentials of telomerase-based therapeutics and warn of their pitfalls.

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Abbreviations

HEK	human embryonic kidney cell
HMEC	human mammary epithelial cell
HPV	human papilloma virus
pRb	retinoblastoma protein
RPE-340	human retinal pigmented epithelial cell
RT	reverse transcriptase
TER	RNA subunit of telomerase
TERT	RT protein subunit of telomerase

Introduction

Telomeres, the nucleoprotein complexes at the ends of linear chromosomes, protect against degradation and recombination [1,2]. Telomeres are maintained within a length range that is characteristic of a given organism or cell type but they are not fully replicated by DNA polymerases and gradually shorten with every cell division unless they are actively maintained [3]. As telomeres shorten, chromosomes become mitotically unstable, leading eventually to aneuploidy and sometimes cell death [4,5].

Telomerase, the ribonucleoprotein reverse transcriptase that synthesizes one strand of telomeric DNA, counterbalances such loss of telomeric DNA [6]. Macromolecular components of telomerase, along with enzymatic activity, have been identified in a variety of organisms, including yeasts and mammals [7]. Although telomerase activity is undetectable in most human somatic tissues, it is generally present in highly replicative tissues and in most human cancers [8].

By the early part of this century, it was known that populations of yeasts and other single-celled eukaryotes, if sufficiently nourished, continue to divide indefinitely. Thus, they are effectively 'immortal'. We now know that such eukaryotes normally express telomerase and only

when telomerase expression is disrupted do their telomeres shorten and the cells cease dividing after some tens of divisions [9]. In contrast, Hayflick observed more than forty years ago that normal human somatic cells propagated in primary culture have a limited replicative capacity (see [4,5,10] for review). Cells can be stimulated to bypass this limit, called senescence or M1, if they are transformed by any of a number of oncogenes, such as SV40 T-Ag or HPV E6/E7. Such transformed cells continue to divide, only to reach a second block to proliferation termed crisis (M2). Telomeres progressively shorten throughout this entire replicative cellular 'aging' process, an observation that led to the hypothesis that telomeres act as a 'mitotic clock', counting down the number of replications a cell can endure [11]. This hypothesis was bolstered by the observation that many somatic tissues do not contain detectable telomerase, whereas most immortalized cells or cancers do. Cancerous cells that bypass M1 and then escape crisis generally have lost cell-cycle controls, exhibit genomic instability, can form metastatic tumors *in vivo*, and typically maintain telomeres at relatively stable, though variable, lengths (Figure 1) [3].

In this review, we discuss experiments reported over the past one to two years that test both the telomere hypothesis of mammalian cellular senescence and aging and the role of telomerase in cancer progression.

Reconstitution of telomerase activity *in vitro*

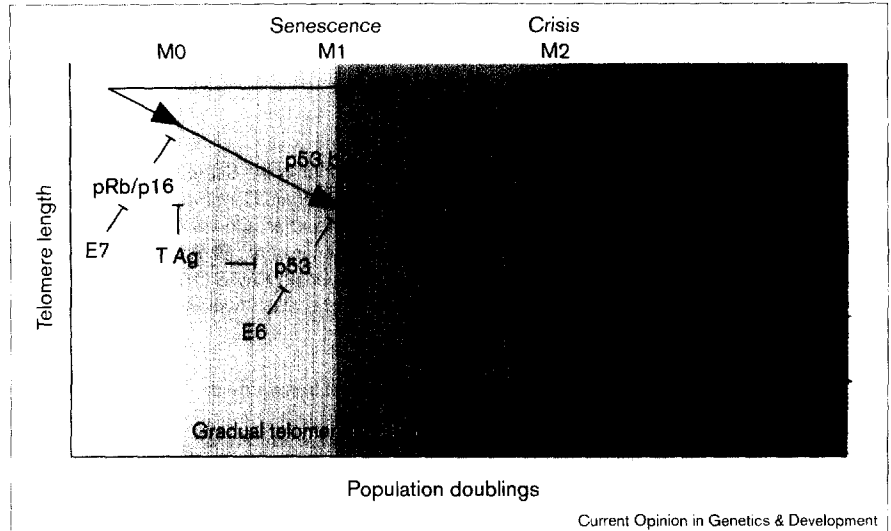
Telomerase is a large enzyme complex. Indeed, in the yeast *Saccharomyces cerevisiae* the essential RNA subunit (TER) is ~430 kDa (1.3 kb), and at least two TER molecules (and probably two 103 kDa TERT molecules) are present in the telomerase complex of this yeast [12,13]. In addition, telomerase is likely to contain numerous other protein subunits. Despite the apparent enormity of this complex (22–24S in *Saccharomyces cerevisiae*), only TER and TERT are required for core catalytic activity [13–15]. It was shown recently that core *in vitro* telomerase activity can be reconstituted from *in-vitro*-synthesized TER and TERT, for both the human and the Tetrahymena telomerases [16,17*,18]. Although the specific activity of the reconstituted systems is not yet high, such systems open the door to delineating which domains of the core subunits are essential for enzymatic activity.

Extension of cellular lifespan with telomerase expression

The sufficiency of TER and TERT alone for basal telomerase activity *in vitro* begged the question of whether ectopic expression of telomerase activity might be accomplished by ectopic expression of exogenously supplied genes for these subunits. Although TER is expressed in most tissue types, its level of expression does not correlate

Figure 1

As human cells proliferate in culture, telomere lengths shorten and the cells are subject to a succession of proliferative blockades. Some cells exhibit a pRb/p16-dependent cell cycle arrest (M0) that stops proliferation in culture after ten or fewer cell doublings. Whether cells exhibit an M0 arrest or not, they are subject to a later p53-dependent arrest (M1) after 40–50 cell doublings. Cells that bypass M1 are then subject to a final block to immortality following an additional 30–40 doublings (M2). These proliferative checkpoints can be bypassed by a number of viral oncogenes E7, T Ag, E6 that inactivate pRb/p16 and/or p53, and by telomere stabilization by either telomerase (TERT) or ALT.



with the level of extractable enzyme activity [19–21]. On the other hand, expression levels of hTERT in several human tissues often correlate with both the level of *in vitro*-measured telomerase activity and the proliferative potential of the tissue [22–24], suggesting that hTERT is limiting *in vivo* for the formation of an active telomerase complex. Capitalizing on this observation, a number of laboratories have demonstrated that constitutive ectopic expression of hTERT is sufficient to induce telomerase activity in cells in which it was otherwise undetectable, allowing for a direct test of the telomere-shortening hypothesis of senescence [25•,26,27•–30•].

Initial results were encouraging: ectopic expression of hTERT in primary cultures of both pre-senescent epithelial cells (RPE-340) and pre-senescent fibroblasts (B) foreskin) was sufficient to induce telomerase activity and allowed for growth well beyond the normal replicative capacity of these cell types (Figure 2) [25•,28•]. Furthermore, these immortalized cell lines showed no signs of the chromosomal abnormalities typically found in post-senescent survivors [31•,32•]. It was soon shown, however, that hTERT is not sufficient to extend the lifespan of all normal cell types. In one study [26], in which hTERT was expressed, such expression by itself was unable to extend the lifespan of human lung fibroblast (IMR90) cells — note, however, that the hTERT construct used contained a carboxy-terminal HA epitope tag, see below — but cell lifespan was extended by the expression of c-Myc which, in turn, induced the endogenous (presumably wild-type) telomerase activity. It was thus suggested that a second transforming event (c-Myc expression) is required for telomerase to extend the lifespan of these cells [26]. Ectopic hTERT expression also was not sufficient to extend the lifespan of untransformed human mammary epithelial cells (HMECs) containing an apparently normal pRb/p16 pathway (that is, pre-M1 [M0], HMECs), nor was

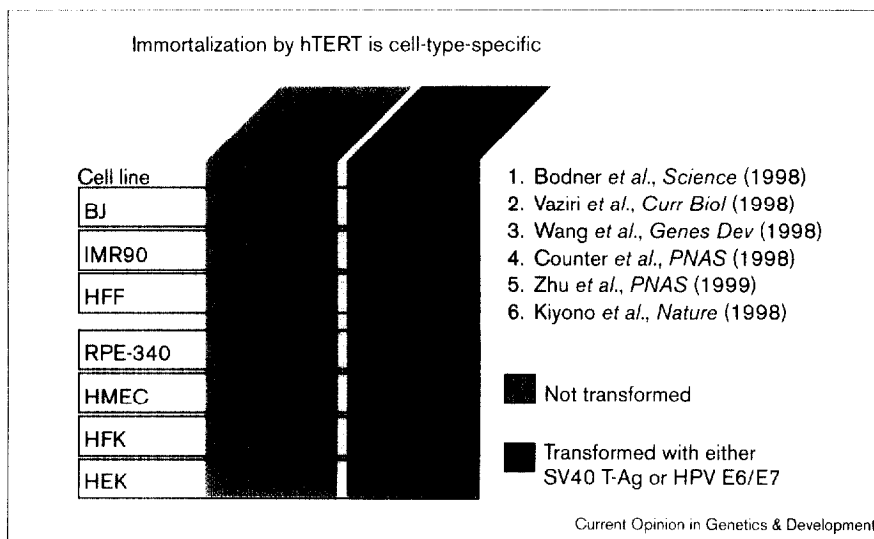
it able to immortalize normal keratinocytes [29•]. However, co-expression of the viral HPV E7 protein, which inactivates pRb, along with hTERT was sufficient to immortalize both HMEC cells and keratinocytes. The fact that most commercially available HMECs are post M0 may explain why, in a previous report [26], hTERT alone immortalized these epithelial cells [29•].

Telomerase activity *in vitro* versus telomere maintenance *in vivo*

In yeast, TER and TERT are not sufficient to maintain telomeres or cellular replicative capacity *in vivo*. The *EST1*, *EST3*, and *EST4/CDC13* gene products of yeast, although not required for enzymatic activity of telomerase *in vitro*, are essential for telomere maintenance *in vivo* [14,15,33,34]. Interestingly, in human cells, an hTERT construct bearing a carboxy-terminal HA epitope tag supported *in vitro* enzymatic activity and immortalized HMEC cells (which may have been post M0), yet was unable to maintain telomeres or avert crisis in human lung (IMR90) and foreskin fibroblasts, and human embryonic kidney cells (Figure 2) [26,27•,30•]. Hence, in both yeast and human cells, a catalytically active telomerase is not necessarily sufficient for telomere maintenance *in vivo*. One possibility is that the HA tag in the hTERT subunit interferes with an interaction between TERT and a putative human homologue of *EST1*, *EST3*, or *EST4/CDC13*.

Telomere maintenance *in vivo* requires the productive interaction of telomerase with the telomeric DNA–protein complex. Altering components of either telomerase or the telomere itself changes the length regulation of telomeres, leading to the important concept that telomere-length homeostasis involves regulating access of telomerase to telomeres [35–39]. Thus, the presence of telomerase in a cell does not guarantee its access to a telomere.

Figure 2



Cells immortalized by exogenous TERT expression exhibit cell-type specificity. This figure is a summary of the results from a number of laboratories in which hTERT was ectopically expressed in various cell lines. A plus sign indicates that cells were immortalized and a minus sign indicates cells not immortalized by TERT. hTERT-HA contains an HA epitope tag at the carboxyl terminus. Cell lines and types tested are BJ and HFF (human foreskin fibroblasts), IMR90 (lung fibroblasts), RPE-340 (retinal pigmented epithelial cells), HMEC (human mammary epithelial cells), HFK (human foreskin keratinocytes), and HEK (human embryonic kidney cells).

Telomere homeostasis is regulated, at least in part, by telomere-binding proteins: the abundant Rap1p in budding yeasts, Taz1p in fission yeast, and TRF1 and TRF2 in humans are all sequence-specific binding proteins that bind duplex telomeric DNA repeats [40–42,43]. Telomere lengthening responds to the number of Rap1p molecules and other structural features of the complex present at a chromosome end ([35–37,44]; CD Smith, J Prescott, EH Blackburn, unpublished data). In addition, mutating the telomeric repeat sequences to reduce the affinity of Rap1p for the telomere can deregulate telomere-length maintenance ([35,37,44]; CD Smith, J Prescott, EH Blackburn, unpublished data). Where tested, such mutations did not alter the level of telomerase activity measured *in vitro* (T Boswell-Fulton, J Prescott, EH Blackburn, unpublished data). Similarly, mutations in TRF1 that disrupt its telomere binding lead to telomere lengthening [42]. Interestingly, the presence of TRF1 on telomeric DNA may be regulated in cells by Tankyrase (TRF1-interacting ankyrin-related ADP ribose polymerase), a recently identified enzyme that adds poly(ADP-ribose) to both itself and TRF1 *in vitro*, thereby decreasing the affinity of TRF1 for telomeric DNA [45]. Such modification might be a signal for telomerase to elongate the telomere.

Telomere length and cell division capacity – how short is too short?

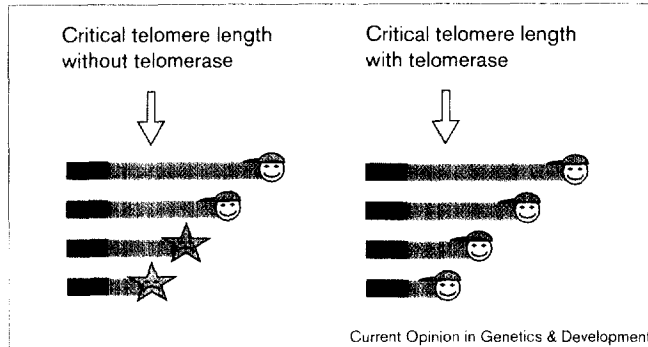
As the dust settles from the recent flurry of findings, we find that ectopic expression of telomerase activity is sufficient to overcome senescence in some, though not all, human cell types. However, when normal cells grown in culture stop dividing, they generally still contain ample tracts of telomeric DNA, suggesting that telomere shortening *per se* is not sufficient to cause cellular senescence. Notably, human cells in culture with replicative lifespans extended by oncogene transformation continue to undergo

telomere shortening until they go into crisis (Figure 1) [46–48]. Knockout of the mouse *TER* gene leads to end-to-end chromosome fusions, a hallmark of compromised telomeric function, yet the fusion junctions often contain considerable tracts of telomeric repeats [49]. Recent studies, which tested the ability of telomerase to extend the lifespan of post M1, SV40 T-Ag-transformed cells, have shed new light on these apparent paradoxes. Ectopic expression of hTERT in T-Ag-transformed HEK cells extended lifespan beyond crisis, with concomitant marked telomere lengthening [27••,30••]. Expression of hTERT (but not of catalytically inactive or carboxy-terminally HA-tagged hTERT) in T-Ag transformed human lung and foreskin fibroblasts while similarly extending lifespan, did so despite considerable telomere shortening that continued for tens of cell divisions beyond the expected crisis point [30••]. Comparably high levels of telomerase activity were induced in both studies. Thus, telomere lengthening *per se* is not required for telomerase-mediated lifespan extension. In a striking parallel, in *S. cerevisiae*, the presence of telomerase containing a mutated, though functional, TLC1 RNA subunit stabilized telomeres at significantly shorter lengths than those of senescent cells expressing a stable, but enzymatically inactive, telomerase complex [50]. Thus, the presence of enzymatically active telomerase stabilizes a telomere that would be critically short in its absence (Figure 3).

Quality of life without telomerase

Whereas the lifespan of certain cell types can be extended with hTERT expression, knockout mice lacking the essential RNA subunit of telomerase were viable and fertile for up to six generations [51]. Successive back-crossing of these telomerase-deficient mice, however, revealed a steady decrease in fertility, seen as decreased litter size, testis size, and number of primary spermatocytes [52]. At the same time, mitotic instability increased in the somatic

Figure 3



Model for telomere stabilization by telomerase. Telomeres that fall below a critical length are functionally compromised, subject to end-to-end fusions and nucleolytic degradation (bottom two telomeres on the left side). Such telomeres can be stabilized by exogenous telomerase expression (right side). Such stabilization is often, but not necessarily, accompanied by telomere lengthening. In some cases, the presence of telomerase is sufficient to stabilize telomeres independent of telomere lengthening.

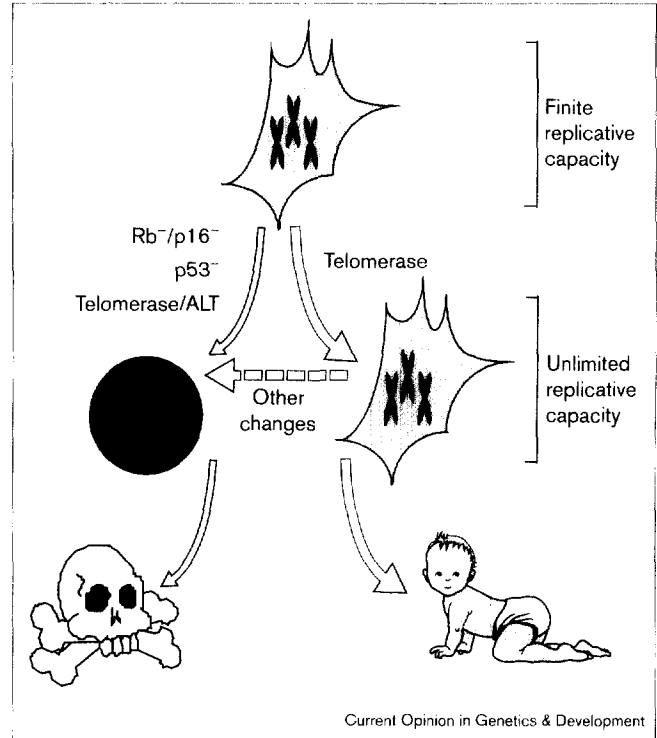
cells, as seen by increased aneuploidy and end-to-end chromosome fusions [49,52]. Similarly, mouse embryonic stem cells lacking mTER and maintained in culture showed increased levels of telomere–telomere fusions and decreased replicative lifespan in culture [53]. Finally, late-generation mTER knockout mice often died *in utero*, and neural tube closure often failed [54*]. Such failure, along with developmental defects in a number of highly proliferative tissues, was correlated with telomeric lengths shorter than in normally developing sibling embryos ([54*]; M Blasco, personal communication). Thus, significant contributions of telomerase to a normal happy life are suggested by the sterility, birth defects, and developmental defects that emerge in its absence.

Cancer and telomerase

The telomere hypothesis of cellular senescence and aging had a stunning implication: inhibition of telomerase might limit the growth of tumors without significantly affecting normal, non-proliferative tissues. The caveats of this implication have become clear through two types of recent observations. First, TER knockout mice are, at least initially, no more resistant to certain types of tumors than wild-type mice [51]. Second, an alternative (telomerase-independent) pathway for telomere maintenance, termed ALT, has been identified in human cells. ALT is probably active in the ~10% of human tumors that lack detectable telomerase activity [55]. This secondary pathway of telomere maintenance appears similar to the RAD52-dependent pathway found previously in budding yeasts — a pathway that maintains telomeres through unequal homologous recombination [38,56].

The fact remains, however, that the majority of human tumors contain elevated telomerase levels. That, coupled with the fact that the ability to diagnose cancers in early

Figure 4



Cells with a finite replicative potential can be immortalized by several methods. In some cell types, inactivation of pRb/p16 and p53, combined with telomerase maintenance, can lead to cellular immortalization accompanied by aneuploidy and the loss of growth inhibition typical of oncogenically transformed cells (in orange), whereas exogenous expression of telomerase alone can extend replicative potential apparently without causing oncogenic transformation (in green). It is possible, however, that as yet unidentified factors may later facilitate the oncogenic transformation of telomerase-immortalized cells.

stages of development often translates into increased survival rates, has hastened the use of telomerase as an indicator of cellular transformation in clinical settings. In urine analyses for the diagnosis of bladder cancers, telomerase activity appears to be a more sensitive indicator of oncogenic transformation than the more standard cytological examinations [57*]. Telomerase activity, however, may not be a reliable indicator of transformation in early stage cervical cancer lesions [58,59]. Thus the usefulness of telomerase as an early indicator for the presence of cancer is probably cell-type-dependent.

What price immortality?

Recent studies suggest that although telomerase may be able to immortalize some cell types, such immortalization does not cause oncogenic transformation, as can be judged by many criteria [31**,32**]. Furthermore, genomic instability is common in cancerous cells, yet forced telomerase expression in SV40-transformed human fibroblasts reduced the occurrence of dicentric chromosomes and hyperploidy [30**], and thus protected against at least one cause of genomic instability. Telomerase-mediated cellular

immortalization may thus have significant therapeutic usefulness in growing human tissues in culture.

Although these findings are significant, recent history warns us that it is still too soon to claim that telomerase induction will not potentiate oncogenic transformation. The obverse side of the coin is that ectopic telomerase expression also allowed pre-neoplastic, partially transformed fibroblast, HMEC, and human embryonic kidney cells to proliferate [27^{••},30^{••}]. Hence, in this context, telomerase expression exhibits a cancer-promoting property. It has been estimated that following bone-marrow transplantation, telomere shortening may cause the donated cells to 'age' by fifteen years [60]. On the one hand, could such 'aging' be obviated by ectopic expression of telomerase (Dr Jekyll) in the transplanted tissue? On the other hand, would expression of telomerase (Mr Hyde) then increase the likelihood that the transplanted tissue would itself be susceptible to oncogenic transformation?

Conclusions

We still have much to learn about both the molecular mechanisms of the telomerase reaction and its control in the proliferating cell. The recent work discussed here emphasizes the Jekyll and Hyde nature of telomerase in the context of human and mammalian biology. Data summarized here show that although ectopic expression of telomerase can immortalize cells without inducing the genomic instability associated with the cancerous state, the effects of ectopic telomerase expression are cell-type-specific. Advancing our understanding of these aspects of telomerase will be crucial to deploying it in the interest of human health. In the end, we would do well to keep in mind the words of Robert Louis Stevenson's Dr Jekyll, "I hesitated long before I put this theory to the test of practice. I knew well that I risked death; for any drug that so potently controlled and shook the very fortress of identity, might, by the least scruple of an overdose ... utterly blot out that immaterial tabernacle which I looked to it to change. But the temptation of a discovery so singular and profound at last overcame the suggestions of alarm" [61].

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