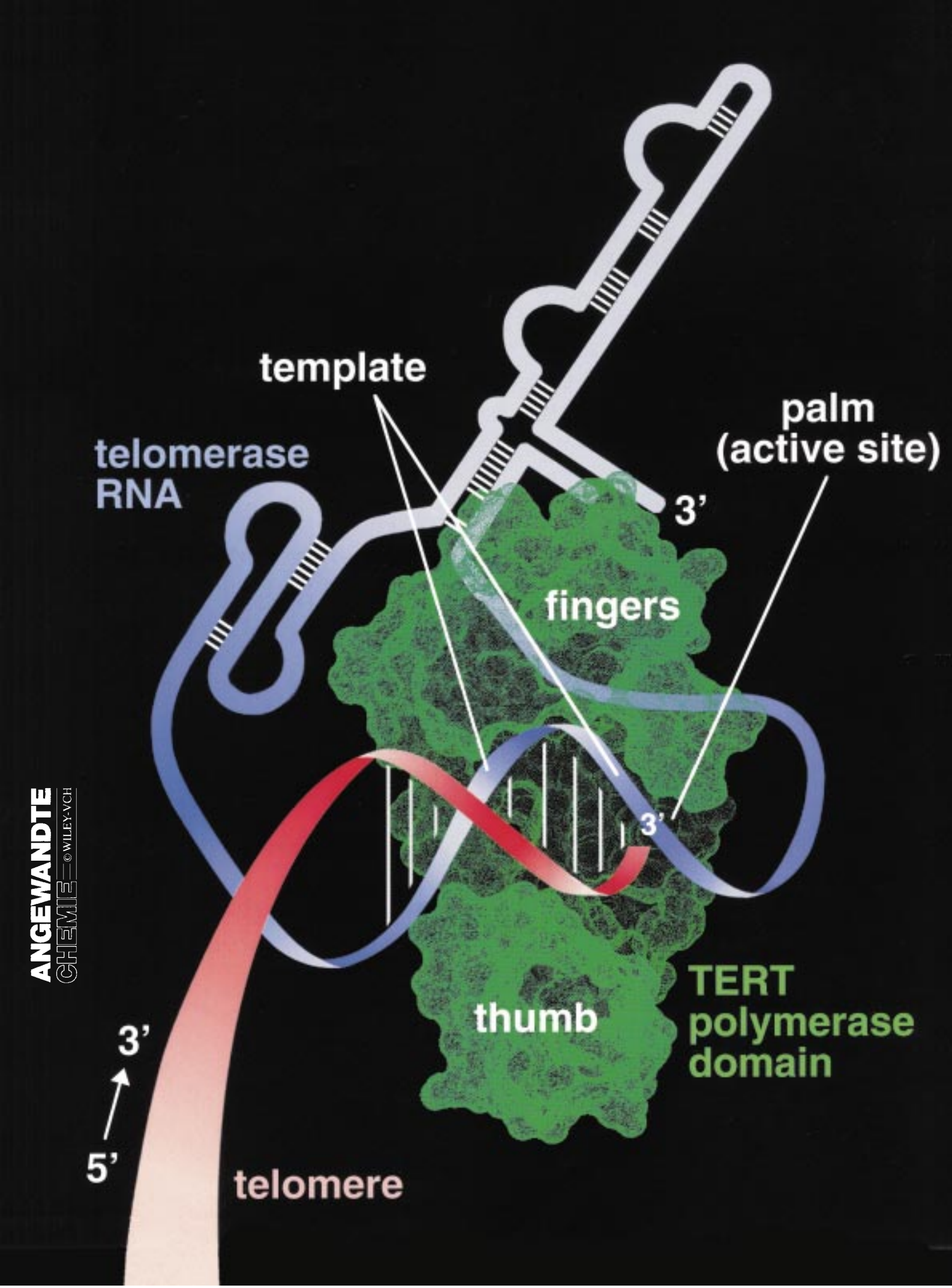

Life at the End of the Chromosome: Telomeres and Telomerase**

Thomas R. Cech*

Telomerase is the enzyme that replicates the ends of linear chromosomes, such as those found in human cells. Its absence has been implicated in cellular aging, and its reactivation promotes tumorigenesis. Telomerase is an unusual enzyme in that it contains an essential RNA subunit as well as protein components. Although the RNA component was described a decade ago, the first catalytic protein subunit of telomerase was discovered only recently through studies of a ciliated protozoan, *Euplotes aediculatus*. Computer searching and biochemical experiments then led to the identification of the genes for the corresponding telomerase subunits in yeast and humans. These proteins comprise a new branch of the polymerase family called TERT (*te*lomerase *r*everse *t*ranscriptase), distantly related to the enzyme responsible for copying the RNA of HIV (human immunodeficiency virus) and other retroviruses. The pathway by which TERT is assembled with the RNA subunit to form telomerase and the mechanism of chromosome end-replication are topics of current research. In addition, the identification of TERT provides a new focus for the development of inhibitors with promise as antitumor agents.

Keywords: enzymes · ribonucleoprotein · RNA · telomerase · telomere · tumor therapy

1. Introduction

Chemists typically begin to take notice of a biological system after the molecules involved have been identified and their biochemical function at least partially revealed. Such initial characterization provides a starting point to probe the chemical mechanism of the reaction, dissect structure–function relationships, and synthesize or identify antagonists or agonists of the process. In the case of the chromosome-end-replicating enzyme telomerase, the key biomolecular components have been identified only in the past two years. Since then, striking support has been provided for causal relationships, on the one hand between telomerase shut-off and cellular senescence, and on the other hand between telomerase reactivation and oncogenic transformation of human cells. These events now provide ample incentive for chemists to become informed about this fascinating enzyme.

Before discussing telomerase, it is useful to define its substrate, the chromosome telomere (*telos*, end; *mere*, part). Early observations of Hermann Muller, who studied fruit fly chromosomes, and of Barbara McClintock, who studied maize chromosomes, indicated that the natural ends of chromosomes are "sealed" and thereby protected from the fusion reactions characteristic of ends produced by chromosome breakage.[1] Only decades later was the molecular nature of this "cap" on the ends of the chromosome ascertained. The DNA at the chromosome ends does not consist of a complex protein-coding sequence, but instead a simple sequence such as TTGGGG in the ciliate *Tetrahymena*[2] or TTAGGG in humans is repeated dozens or even thousands of times at each chromosome end. This DNA sequence binds specific proteins, which cap the chromosome end either directly[3] or by inducing a particular DNA structure.[4]

While the bulk of telomeric DNA is double-helical, the GT-rich sequence paired with its CA-rich complement, the 3′-end of the DNA protrudes as a single-stranded extension in all eukaryotes studied (Figure 1). Complete replication of the overhanging end of such a chromosome cannot be accomplished by conventional DNA polymerases for the simple reason that an overhang has no template strand to guide its synthesis.[5] Even if the chromosome were blunt-ended, there would be a problem with complete replication of its ends.[6]

In almost all eukaryotes this chromosome end-replication problem is solved by telomerase, discovered by Carol Greider and Elizabeth Blackburn in 1985.[7] Telomerase is an unusual enzyme in that it is an RNA–protein complex (ribonucleoprotein, RNP). A portion of the RNA subunit provides the

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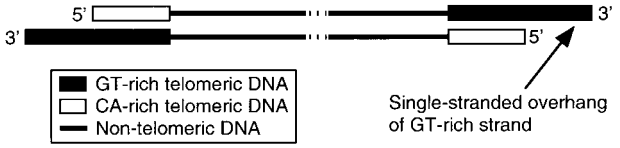

Figure 1. Telomeric DNA at the ends of eukaryotic chromosomes. Solid boxes, multiple repeats of a short sequence, typically GT-rich (TTAGGG in humans, TTTTGGGG in *Euplotes*). Open boxes, the complementary sequence, typically CA-rich (CCCTAA in humans, CCCCAAAA in *Euplotes*).

nucleic acid template that the DNA cannot provide for itself[8, 9] (Figure 2). The catalytic protein subunit (TERT)[*] catalyzes the polymerization of dNTPs onto the chromosome end (Figure 3), as described in detail in Section 4.

2. Newly Discovered Functions for Telomeres and Telomerase in Humans

2.1. Extending Cellular Lifespan

Normal human cells, when established in tissue culture, undergo only a limited number of cell divisions. They cease growing at a point called the Hayflick limit,[10a] which occurs after about 50 population doublings in the case of connective tissue cells called fibroblasts. In this state of senescence, the cells express a different set of genes and cannot be stimulated to grow by plating at lower cell density or by adding fresh medium. Although they can be forced to proliferate by infection with certain viruses or by transformation with an oncogene, such forced cell divisions lead to a state of massive cell death called crisis.

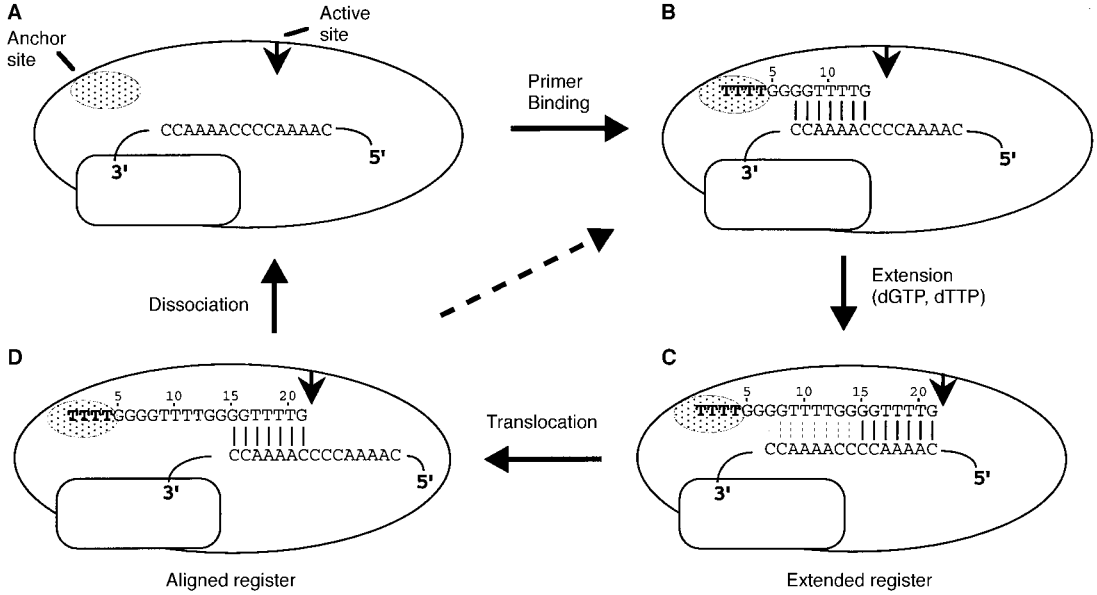

Figure 2. Telomeric DNA is synthesized by telomerase, a ribonucleoprotein enzyme. A) Empty telomerase enzyme, showing the nucleotide sequence of the RNA template region (*Euplotes* sequence). Oval represents the catalytic protein subunit (TERT); rounded rectangle represents a species-specific accessory subunit. B) DNA primer (3′-end of chromosome) binds to telomerase by base-pairing to the RNA template and by interaction with the anchor site.[61] C) Reverse transcriptase activity of TERT extends the DNA until the end of the template is reached. Enzyme maintains a more-or-less constant amount of primer-template base-pairing during elongation, preventing formation of a long duplex that would inhibit dissociation.[62] D) Following translocation, the DNA primer is in position to be extended again (dashed arrow), or it can dissociate. (Reproduced from ref. [62].)

Telomere length has been proposed to be the "meterstick" that measures the number of divisions a cell is allowed before it reaches the Hayflick limit (see ref. [10b]). Because telomer-

[*] TERT = *te*lomerase *r*everse *t*ranscriptase. This article follows the convention that the name of the gene is italicized (*TERT*, or *hTERT* for the human gene), while the protein is not italicized.

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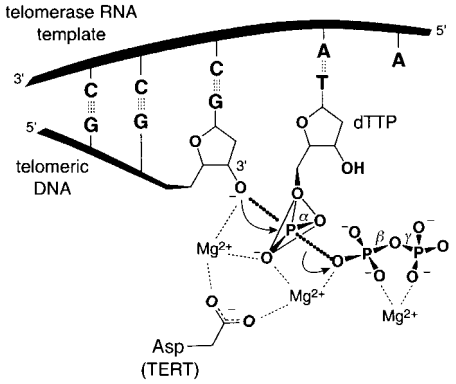

Figure 3. Possible chemical mechanism of catalysis of telomeric DNA extension by telomerase, based on a model of reverse transcriptase by Joyce and Steitz.[63] Three active-site aspartic acid side chains (only one of which is shown here) coordinate two divalent metal ions that are in turn coordinated to the nucleophile (3′-oxyanion of G), the α-phosphate of the incoming nucleotide, and the leaving group oxygen atom. Metal ion ligand coordination is indicated by dashed lines. In this hypothetical transition state diagram, the dotted lines indicate bands half-formed or half-broken.

ase is turned off in most somatic cells (not in germ-line cells) about the time of birth, the chromosome telomeres cannot be fully replicated. Thus, they shrink from their original size of about 15 kbp (bp = base pairs) at a rate of about 100 bp per cell division.[11]

It has been hypothesized that when the telomeres reach a critically short length (or when the shortest telomere in the cell reaches a critically short length), the cell enters the state of senescence. The exact signaling pathway connecting telomere length with cell cycle arrest is not known, but it may involve the release of telomere-bound proteins as their DNA binding sites are eroded away.

The correlation between the lifespan of cells in culture and telomere length did not necessarily imply any causal relationship. The discovery of hTERT (see Section 4), the catalytic subunit of human telomerase, enabled a direct test of the hypothesis. The other components required for telomerase activity are still present in telomerase-negative normal human cells, so reintroduction of hTERT is sufficient to restore telomerase activity and extend chromosomal telomeres.[12, 13] Transfection of the *hTERT* gene[*] (under control of a heterologous transcriptional promoter) also allowed two types of normal human cells—fibroblasts and retinal epithelial cells—to continue growing far beyond the Hayflick limit.[12] (These cells may in fact be immortal, but given that reviews must be written in a mortal time frame, we will settle for the description "greatly extended lifespan.") Transfection with *hTERT* did not represent merely a new way to transform human cells, as the *hTERT*-transfected cells retained a normal karyotype and were unable to grow in soft agar, a hallmark of transformed cells.

This remarkable life-extending effect is not totally generalizable, in the following sense. Other cell types (mammary epithelial cells and keratinocytes) require inactivation of the Rb/p16 growth control pathway in addition to gain of hTERT function to achieve extended lifespan.[14] (Rb is named for the retinoblastoma caused by loss or mutation of both copies of this tumor suppressor gene; p16 is another tumor suppressor acting in the same pathway as Rb.) Furthermore, some normal human cells have detectable telomerase activity in vitro but nevertheless still undergo senescence.[15]

Some have considered that the ability to extend cellular lifetime might be a key to extending human lifespan. An emphatic disclaimer is therefore in order: the relationship between the lifetime of primary cultures of human cells in vitro and the longevity of the organism is at best obscure.[16] Furthermore, as discussed in the next section, inactivation of telomerase is likely a tumor suppressor mechanism; although normal cells transfected with *hTERT* have shown no signs of transformation,[12] we now have reason to believe that they may be one step closer to transformation. This in no way detracts from the potential of using these extended-lifespan normal cells as improved subjects for cell biology research, or as "bioreactors" to produce pharmaceutically important proteins on an ongoing basis.

2.2. Telomerase Required for Carcinogenesis in Humans

This section will read very much like the previous one. What was for a long time an intriguing correlation—in this case, between reactivation of telomerase and human tumors—now appears to be a cause-and-effect relationship.

About 85 – 90 % of diverse human tumors show telomerase activity, while adjacent healthy tissue is typically telomerase-negative. Thus, telomerase, which is generally not present in somatic cells, is reactivated in tumors. The telomerase component that is missing in normal cells is the hTERT protein;[17, 18] the RNA subunit of telomerase is not only present in telomerase-negative cells, but it is efficiently recruited into an active telomerase complex upon introduction of hTERT.

Very recently, Weinberg and colleagues succeeded in solving a long-standing problem in human cancer biology: the oncogenic transformation of human cells with defined genetic components. Such transformation is easily accomplished with rodent cells by expression of two cooperating oncogenes. However, introduction of the same genes was known to be insufficient for stable transformation of human cells in culture. The missing component turned out to be hTERT.[19] Thus, one pathway to transformation of human cells is shown to require three steps: activation of proliferation (accomplished by expression of a mutant form of the *ras* oncogene), inactivation of tumor suppressors p53 and the Rb protein (accomplished by expression of the SV40 T oncoprotein) and activation of telomerase (by expression of hTERT).[**] Telomerase is therefore much more than a "marker" for neoplastic growth, it is a promising target for chemotherapeutic intervention in diverse cancers.

[*] Transfection refers to introduction of a gene into mammalian cells in a form that allows the gene to be expressed. Transformation refers to the oncogenic state characterized by unregulated growth.

[**] More accurately, the experiments[19] show that some function of hTERT is required. We assume that this function is activation of telomerase activity, but a catalytically inactive mutant of hTERT needs to be tested to further support this hypothesis.

3. RNA Component of Telomerase

3.1. Biosynthesis

The RNA components identified first were those of the ciliated protozoa. These are transcribed by RNA polymerase III, which leaves a short run of U residues at the 3′-end of the transcript.[8, 20] There was a switch in RNA polymerase usage during evolution, because the yeast RNA subunit (TLC1) is transcribed by pol II.[21] As a result, the yeast RNA is 5′-capped and (initially) 3′-polyadenylated. The human RNA is also thought to be a pol II transcript and its mature form also has a 5′-trimethylguanosine cap.[22] The 3′-half of the human RNA subunit constitutes a small nucleolar RNA (snoRNA) domain,[23] previously found only in RNAs that mediate the conversion of certain uracils to pseudouracils in stable RNAs. This snoRNA domain is required for proper 3′-end formation of the human RNA.[23]

yeast,[26] where mutation of the template sequence led to incorporation of the predicted variant sequence at chromosome ends. Mutations in the RNA do not always simply change the sequence of the DNA product, but can cause infidelity of polymerization and other problems.[27] Presumably the RNA template interacts with the active site of TERT much more intimately than the template RNAs that are copied by retroviral RTs.

Another function of the RNA subunit, at least in *S. cerevisiae*, is to provide a binding site for Sm proteins.[22] These seven proteins, previously known to be associated only with small nuclear (sn) RNAs involved in RNA processing, promote the trimethylation of the 5′-cap of snRNAs and may mediate their intracellular transport. It seems likely that other regions of telomerase RNAs will be involved in bringing yet other proteins into the telomerase complex (see Section 4.4 below), but no RNA binding site for any of these proteins has yet been specified.

3.2. RNA Structure

If the RNA subunit of telomerase were highly conserved in sequence and/or structure, one would argue that it was an attractive target for small-molecule inhibitors. However, the RNA subunit varies widely among species. Even among ciliated protozoa, very few conserved nucleotides can be ascertained, although the overall "frying-pan" like secondary structure is conserved (Figure 4).[20, 24] The human RNA subunit is much larger, 450 nt (nt = nucleotides).[25] However, when the snoRNA domain[23] is subtracted, the remaining template-containing domain of the RNA consists of 210 nt, not too different in size from its ciliate counterparts (ca. 160 – 190 nt). It will therefore be of interest to see whether the secondary structure of this 5′-domain bears any resemblance to that of the ciliate RNAs.

3.3. RNA Function

The most obvious function of the RNA subunit is to template the addition of telomeric DNA repeat sequences onto chromosome ends. All known telomerase RNAs contain about 1.5 repeats of a sequence complementary to the telomeric DNA repeat sequence of that organism. That this RNA sequence in fact acts as the template was first proven in *Tetrahymena*[9] and

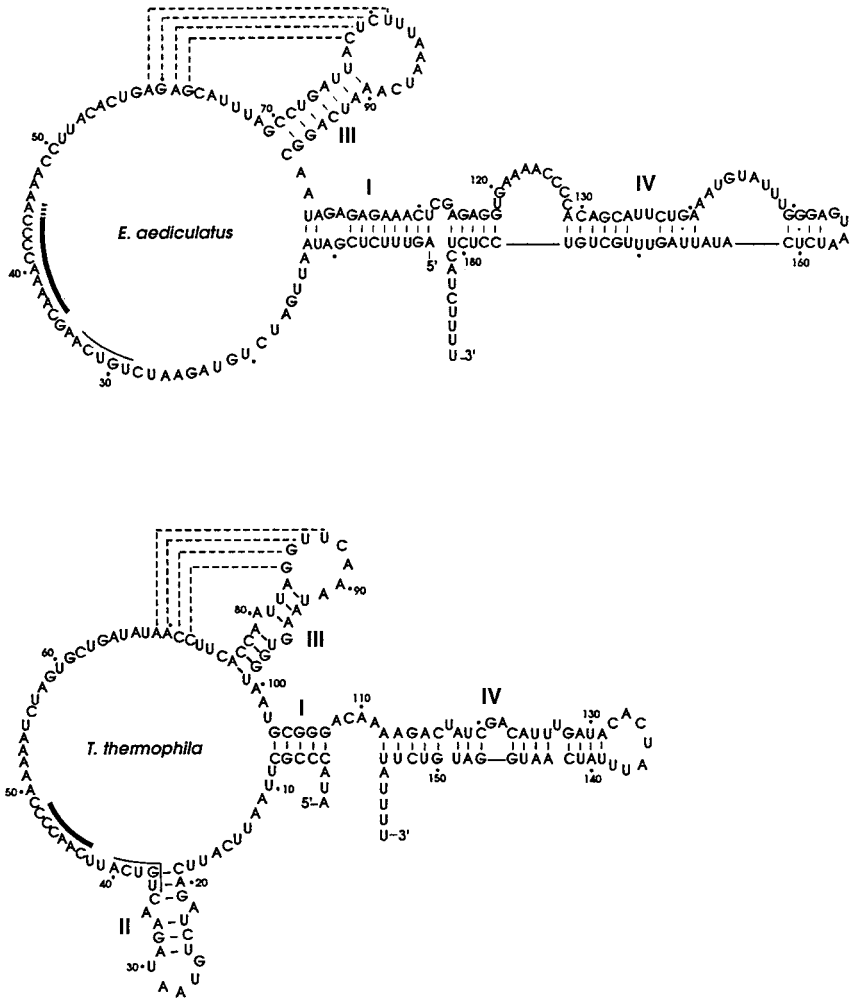

Figure 4. The secondary structure but not the nucleotide sequence of telomerase RNA is conserved among evolutionarily diverse ciliated protozoa. Dashed lines, pairing of the loop of helix III with a nearby sequence forming a pseudoknot. Bold lines highlight the template sequences. Adjacent thin lines mark a sequence conserved among ciliates. *E. aediculatus* from Lingner et al.,[20] *T. thermophila* from Romero and Blackburn.[24] (Reproduced from ref. [64].)

4. TERT and Other Protein Components

4.1. Discovery of TERT

The telomerase component that allowed both the extension of lifespan of normal human cells and the first oncogenic transformation of human cells with defined genetic components was hTERT, human telomerase reverse transcriptase. Despite intensive effort, this key human component escaped identification for many years. It was finally identified by a path that involved biochemical purification of the corresponding *Euplotes aediculatus* protein, identification and genetic analysis of its yeast homologue, and computer searching of the EST (expressed sequence tag) database for the human homologue.

Why *Euplotes*? This unicellular eukaryote carries germline, diploid micronuclei and a somatic, polyploid macronucleus within the same cell.[28] Each macronucleus has about 50000000 DNA molecules, each containing a single gene flanked by canonical telomeres (TTTTGGGG repeats ending with a 3′-overhang of two additional repeats). Thus, there are about 1000000 times more telomeres per cell in *Euplotes* than in human, and a concomitant increase in telomerase.

An affinity purification method was devised by Joachim Lingner in my laboratory to isolate *Euplotes* telomerase from a nuclear extract[29] (Figure 5). The enzyme remained fully active during purification, as judged by the ratio of enzymatic activity to the telomerase RNA content of the preparation. Two polypeptides copurified with the RNA in the active complex, one 123 kDa (p123) and one 43 kDa (p43), in amounts consistent with a 1:1:1 complex of the two proteins and the RNA.[29]

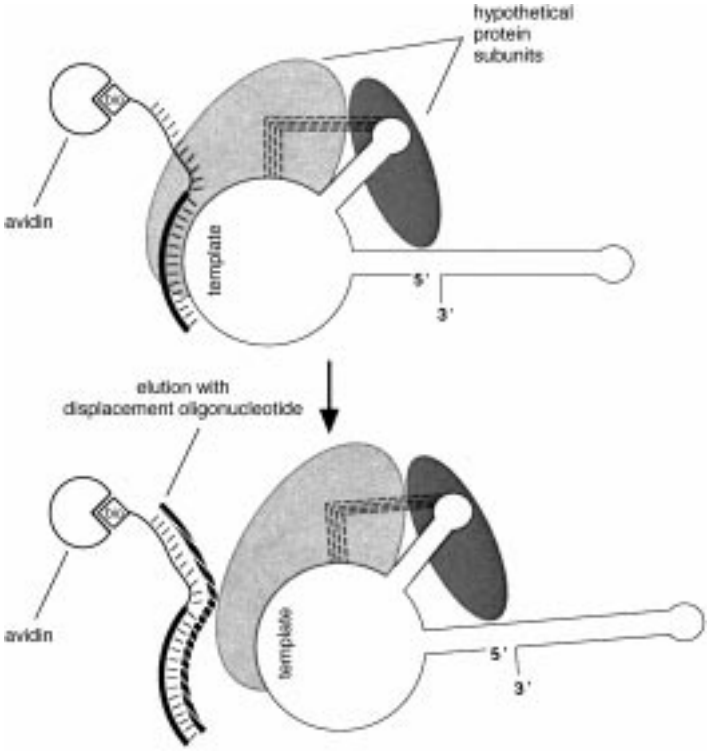

Figure 5. Active *Euplotes* telomerase was affinity-purified. Top: When a *Euplotes* extract is mixed with agarose containing a bound oligonucleotide, the telomerase is adsorbed by hybridization. Other components in the extract can then be washed away. Bottom: Telomerase is released from the column by adding a displacement oligonucleotide, which can form a more stable hybrid with the oligonucleotide on the column. (Reproduced from ref. [29].)

Electrospray mass spectroscopy in collaboration with Matthias Mann and Andrej Shevchenko (EMBL, Heidelberg) then revealed enough peptide sequences to enable cloning and sequencing of genes for both p123 and p43.[30] It was the larger of these two proteins that proved particularly interesting, because it contained reverse transcriptase motifs (short blocks of amino acid sequence characteristic of this family of proteins).[31] Because telomerase uses an RNA template to synthesize telomeric DNA, it is by definition some sort of a reverse transcriptase. The finding of RT motifs went beyond this to say that the *Euplotes* telomerase protein was related in terms of protein structure and presumably in terms of its evolutionary origin to other known RTs, such as those encoded by retrotransposons and retroviruses.[30]

This biochemical line of research then converged on a genetic approach that was ongoing, completely independently, in the laboratory of Victoria Lundblad (Baylor College of Medicine, Houston). Lundblad had set up a genetic screen to identify genes that, when mutated, gave the telomere-shortening effect expected for a telomerase mutant. Her laboratory found four such genes, which she named *EST1*, *EST2*, *EST3*, and *EST4*[32] (*EST* = *e*ver *s*horter *t*elomeres; *EST1* had been previously described by Lundblad and Szostak,[33] and *EST4* is also known as *CDC13*[34]). The best sequence match to *Euplotes* p123 in protein databases turned out to be Lundblad's Est2p.[30]

The yeast homologue was an important finding for several reasons. First, it was attractive to think that there would be a single catalytic subunit conserved among all telomerases; if so, the completely sequenced yeast genome would need to have a copy, and it did. Second, a definitive demonstration that this RT-containing protein was indeed essential for telomerase activity was much more easily carried out in yeast, where facile genetic tools are available, than in *Euplotes*. Indeed, a collaborative effort between our laboratory and the Lundblad laboratory in 1996 provided the evidence that Est2p was a reverse transcriptase and was essential for telomerase activity, both in an enzymatic assay in vitro and for telomere maintenance in vivo.[30]

The *Euplotes* p123 sequence also revealed a portion of the corresponding human sequence in the Expressed Sequence Tag database (Washington University, St. Louis). A single amino acid deletion prevented the yeast Est2p from revealing the same human sequence, but once it was identified, pairwise sequence comparisons clearly showed that it was related as well. Also by this time, Toru Nakamura in my laboratory had identified and sequenced the *Schizosaccharomyces pombe* (fission yeast) *TERT*, and this sequence also retrieved the human expressed sequence tag from the database.[17] Following this, and in collaboration with scientists from Geron Corporation we cloned and sequenced human open reading frame.[17] The same gene, now called *hTERT*, was independently cloned and sequenced by several other laboratories,[18, 35–37] all starting from the *Euplotes* p123 sequence.

Like the other TERTs, hTERT is a relatively large protein, with an overall net basic charge and reverse transcriptase motifs in its C-terminal half (Figure 6A). Subsequently, the

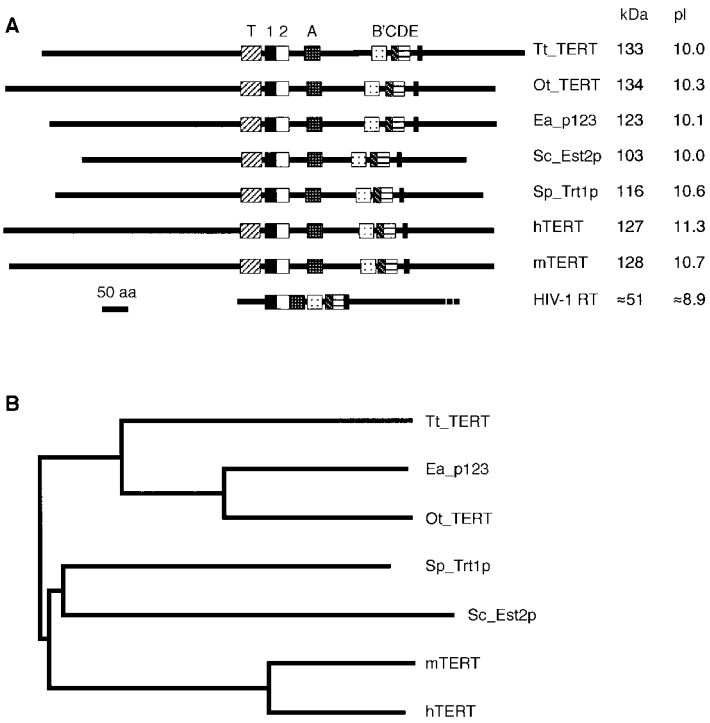

Figure 6. Relationship of the TERT family members to each other and to the HIV retroviral RT. A) Protein sequences are represented by lines. Cross-hatched box, the telomerase-specific motif T.[17] Other boxes, reverse transcriptase motifs 1, 2 and A-E.[31] HIV-1 RT, RNase H domain is omitted. B) Phylogenetic tree of TERTs calculated from sequence comparison of the motifs indicated in part A. Tt = *Tetrahymena thermophila*; Ot = *Oxytricha trifallax*; Ea = *Euplotes aediculatus*; Sc = *Saccharomyces cerevisiae* (baker's yeast); Sp = *Schizosaccharomyces pombe* (fission yeast); mTERT = mouse TERT. (Reproduced from [38].)

Tetrahymena,[38, 39] *Oxytricha*,[38], and mouse[40] TERTs were added to the collection (Figure 6A) and, very recently, that of the plant *Arabidopsis*.[41]

Expression of the *hTERT* gene was absent or barely detectable in telomerase-negative normal human cells, but easily detected in telomerase-positive immortalized human cells. This contrasted with the ubiquitous expression of the telomerase RNA component in all cells tested, and led to the idea that the presence of hTERT might be the "switch" that determined whether human cells had telomerase activity or not. This idea was subsequently verified when it was found that ectopic expression of hTERT in telomerase-negative cells was sufficient to provide chromosomal telomere lengthening in living cells as well as telomerase enzymatic activity in cell extracts.[37, 42]

4.2. A New Subclass of Polymerases

Amino acid sequence comparisons show that the TERTs represent a discrete class of reverse transcriptases, more related to each other than to other RTs.[30, 43] Features that distinguish these proteins from other RTs include the following:

1) There are some distinctive variations within the RT motifs. For example, in motif C the sequence around the two catalytic aspartic acids (D) is LxDD(F/Y)L in the TERTs compared to a consensus of (F/Y)xDDxx in other RTs, where x is a less conserved amino acid and (F/Y) means that either Phe or Tyr is found in that position.
2) A distinctive amino acid sequence called the T (Telomerase-specific) motif precedes RT motif 1.

Compared to the other RTs, the TERTs are most closely related to the RTs of certain bacterial and mitochondrial elements and non-LTR (long terminal repeat) retrotransposons. (Retrotransposons are DNA elements that move within and between genomes through an RNA intermediate, and therefore need a reverse transcriptase to regenerate a DNA copy to integrate into a chromosome. Some retrotransposons have a long sequence repeated at their termini that resembles the LTRs involved in retroviral replication; the non-LTR retrotransposons do not.) The TERTs are more distantly related to the RTs of LTR retrotransposons and viruses, and to the RNA-dependent RNA polymerases of viruses such as poliovirus.

Transposition of non-LTR retrotransposons and Group II introns occurs by target-primed reverse transcription, in which the RT extends a 3′-OH at a DNA break; the DNA break can be created either by an endonuclease or by catalytic activity intrinsic to the intron RNA. Similarly, telomerase extends the 3′-OH of the chromosomal DNA terminus. Another similarity is that certain Group II intron and mitochondrial RTs, like telomerase, are found in stable complexes with their RNA templates. Therefore, the close relationship of TERTs to the non-LTR RTs obtained by sequence comparison is supported by functional criteria as well.[43]

The phylogenetic tree of the TERTs is qualitatively similar to the tree of the organisms from which the telomerases were obtained: the two mammalian TERTs are the most closely related, the two yeast species group together, and the three ciliated protozoa form a third branch, with the two hypotrichs (*Euplotes* and *Oxytricha*) more closely related to each other than to the holotrich *Tetrahymena* (Figure 6B). Thus, the phylogenetic tree is unremarkable—it is just what one would expect for any gene that is ancient. We conclude that TERT is probably as old as the eukaryotic kingdom (see refs. [43, 44] for additional discussion).

4.3. Functions of TERT

Synthesis of telomeric DNA is the most obvious function of TERT, and as far as we know, the lengthening of chromosome telomeres it provides may be sufficient to explain the dramatic results described earlier: the extension of lifespan of normal human cells provided by ectopic expression of hTERT, and providing the "missing link" for stable transformation of human cells in culture. However, TERTs are large proteins, much larger than some of the retroviral RTs, suggesting the possibility of functions beyond their role as polymerases.

The telomerase-specific T motif of human TERT has been mutated and found to contribute to telomerase catalytic activity in vitro.[42] The N-terminal half of Est2p (the yeast TERT) has been analyzed by making a large library of randomly mutated versions and recovering those that were able to rescue senescing *est2*$^-$ yeast.[45] Four essential regions of the N-terminus were mapped, two of which are implicated in binding the RNA subunit and one of which may bring an unidentified subunit into the complex.[45] Also in yeast, Est3p is associated with the telomerase RNA in an Est2p-dependent manner,[46] which may implicate Est2p as contributing a binding site for Est3p. The specific contributions of the T motif and the rest of the N-terminal domain of TERT are only beginning to be evaluated. Thus, it is quite possible that the introduction or reactivation of TERT in human cells has consequences beyond the obvious one of lengthening telomeres.

4.4. Other Telomerase Protein Components

(Because these have been reviewed recently,[47] they will be only briefly discussed here.) Aside from TERT, the other proteins found associated with telomerase appear to be species-specific. An exception is *Tetrahymena* p80,[48] which is highly homologous to one domain of a much larger (230–240 kDa) human telomerase-associated protein now called TEP1.[49, 50] Evidence of the importance of these proteins for telomerase activity in vivo or in vitro has not yet been reported.

Two non-TERT telomerase-associated proteins that have been shown to be essential for telomerase activity in vivo in yeast are Est1p and Est3p.[32, 33] Est1p is a single-stranded telomeric DNA-binding protein, so it is plausible that its function is to help telomerase locate telomeres in the cell.[51] This would also explain why it is dispensable for in vitro telomerase enzyme assays, where the telomeric DNA substrate is presented as a protein-free oligonucleotide and is readily available to the telomerase. A function for Est3p has not been proposed.

5. Opportunities for Chemical Intervention

Now that the importance of telomerase for oncogenic transformation and for lifespan extension of human cells has been established, medicinal and pharmaceutical chemists are focussing on how they might be able to intervene in telomerase pathways. Inhibitors of telomerase are promising candidates for antitumor drugs targeted against a wide variety of cancers. Activators of telomerase, on the other hand, should be able to extend the lifespan of normal human cells in culture and might find useful medical applications in certain age-related diseases.

The most obvious anti-telomerase target is the hTERT–RNA core of the enzyme. The protein component of this complex is related to other reverse transcriptases, as described above, so nucleoside analogues comprise one obvious class of inhibitors. However, differences in key active site amino acids imply that TERTs will have structures distinct from other RTs (see Section 4.2); thus, it is not surprising that AZT (azidothymidine) and other HIV RT inhibitors are only weak inhibitors of telomerase.[52] Therefore, inhibitors must be developed specifically against hTERT. The RNA template moiety of telomerase can be targeted by oligonucleotides containing stable 2′-*O*-methylribose moieties or by peptide nucleic acids (PNAs).[53]

Activation of telomerase in normal human cells cannot be achieved by compounds directed at hTERT, since hTERT is not expressed in these cells.[17, 18] Chemical approaches to this problem may include the development of compounds that act at the level of induction of transcription of the *hTERT* gene, whose promoter is activated by the transcription factor c-Myc[54] and presumably by other proteins not yet described. Alternative mRNA splicing provides another pathway to down-regulation of telomerase in some cells, so compounds capable of redirecting the RNA splicing machinery could potentially be useful. Finally, introduction of the *hTERT* gene by gene therapy is another conceivable pathway to telomerase activation in humans, as transfection has already proven to be successful in activating telomerase in cultured cells as described in Section 2.1.

Returning to the subject of inhibition of telomerase, the recognition that it has a complex pathway of maturation and assembly[21–23] (Figure 7) suggests other possible points of intervention. In addition, Holt et al.[55] have found that the molecular chaperones p23 and hsp90 (a heat shock protein) are necessary for the assembly of telomerase components into an active enzymatic complex in vitro and appear to stay associated with the assembled complex in cell extracts. Geldanamycin (a benzoquinone), which inhibits functions of hsp90 by binding to its ATP site, blocks assembly of active telomerase in vitro and in cultured human cells.[55] A caveat regarding targeting of telomerase maturation, assembly, or chaperone components for pharmaceutical application is that such components are likely to be shared with other biochem-

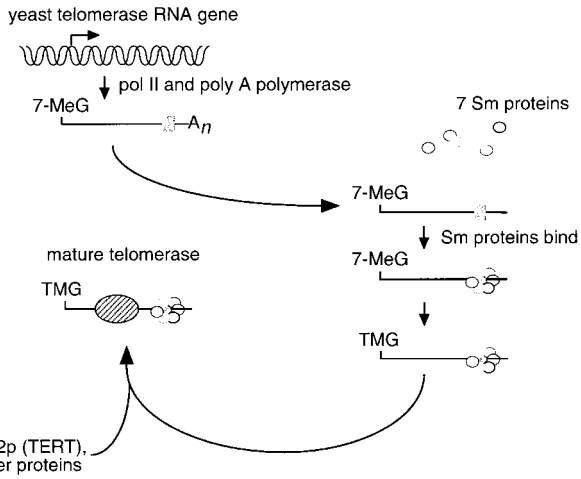

Figure 7. Telomerase is matured and assembled by a complex multi-step pathway. The model for the yeast pathway shown here incorporates data from Chapon et al.[21] and Seto et al.[22] 7-MeG = 7-methylguanosine; TMG = 2,2,7-trimethylguanosine.

ical pathways. Unless some telomerase-specific feature can be identified, inhibition of these cellular processes would presumably be toxic.

Finally, one could consider targeting the telomere (chromosome end) itself, because it is the substrate on which telomerase acts. The very ends of chromosomes have both single-stranded DNA and protein components. In many organisms including human, the single-stranded DNA contains G-rich repeats which can fold into intramolecular (or intermolecular) quadruple helix structures; each G is Hoogsteen – hydrogen-bonded to another G in a square-planar arrangement called a G-quartet.[56] This structure hides the 3′ end of the DNA from telomerase and thereby inhibits telomerase extension.[57] Hurley and co-workers[58] are developing compounds that stabilize the G-quartet form of telomeric DNA and could thereby prevent telomerase from acting. Protein components of telomeres also act as negative regulators of telomerase (see ref. [47]). Stabilizing their interaction with the DNA or inhibiting components that normally displace these proteins provides another possible route to inhibition of telomerase.

One possible challenge to successful implementation of an anti-telomerase therapeutic is suggested by the observation that a minority of human tumors use an alternative non-telomerase mechanism for maintaining telomere length.[59] This ALT (alternative lengthening of telomeres) pathway may involve extension of a telomere using another telomere as a template, facilitated by DNA recombination proteins that catalyze DNA strand invasion. Application of a telomerase inhibitor might provide a selective growth advantage for the rare cells that utilized ALT and therefore did not require telomerase. Thus, one might next be faced with developing inhibitors of the ALT pathway. On the other hand, there are always pathways for resistance to any pharmaceutical, so this potential problem should not diminish interest in development of effective anti-telomerase compounds.

6. Summary and Outlook

Telomerase is an unusual enzyme, an RNA – protein complex, that extends the repeated DNA sequences at chromosome ends and thereby enables them to be fully replicated. The last few years have witnessed major breakthroughs in our understanding of this enzyme. The catalytic protein subunit of telomerase has been identified in diverse organisms, including human, and found to represent a new branch of the reverse transcriptase family. On the biomedical front, the long-suspected roles of telomerase deactivation in cellular senescence and of telomerase activation in tumorigenesis have received decisive experimental support.

Thus, chemists now have much more incentive to develop compounds that modulate telomerase activity, and they have clearly defined molecular components to target. A most attractive target is the hTERT catalytic subunit of telomerase. In addition, one can consider developing compounds to modulate *hTERT* gene expression, to interfere with the assembly of the protein – RNA complex, and to prevent access of telomerase to its telomeric DNA substrate. Recently, dominant-negative versions of hTERT have been found to kill tumor cells by apoptosis (programmed cell death).[60] These findings provide great reason for enthusiasm that anti-telomerase pharmaceuticals will indeed have antitumor potential.

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