evolved. Provided that RNA or RNA analogues can be made to function as drugs, SELEX may offer a new and rapid approach to drug design. Even more daring is their idea that selection for binding of random proteins to a target can be used in SELEX. To do this the binding must be by nascent peptides in polysomes. The ribosome-associated mRNA coding for the selected peptide would then be isolated and amplified for use in repeated cycles of protein synthesis coupled selection. The advantages of this technique over genetic selection or large-scale screening is that far larger populations can be screened and organism viability is not an issue. Can it be that the "awesome power of genetics" has met its match?


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# Telomeres and Their Synthesis


ELIZABETH H. BLACKBURN


TELOMERES, THE SPECIALIZED DNA-PROTEIN STRUCTURES found at the ends of every eukaryotic chromosome, are required to stabilize chromosomes. Cytogenetic studies indicate that true terminal deletions of chromosomes, with loss of the telomere, are rare and that they lead to chromosome instabilities or progressive loss of terminal sequences from chromosome ends (*1–3*). Until quite recently, studies of the molecular mechanisms of telomere function and synthesis in higher eukaryotes were confounded by the low abundance of telomeres in their genomes. Such studies therefore were concentrated on certain lower eukaryotes, especially the ciliated protozoa, which possess short linear chromosomal DNA molecules. However, it is now known that the structure, function, and metabolism of telomeres are remarkably conserved among protozoans, fungi, slime molds, animals, and plants (*1–7*). As I will outline in this Perspective, this conservation appears to reflect the specialized manner in which telomeric DNA is synthesized.

Telomeric DNA, comprising the extreme molecular ends of chromosomes, consists of simple tandemly repeated sequences, characterized by clusters of G residues in one strand (Table 1). An overall strand composition asymmetry results in G-rich and complementary C-rich strands. The 3′ end of each strand of the duplex linear chromosomal DNA molecule is the G-rich telomeric strand, and it forms a 3′ terminal overhang, 12 to 16 nucleotides in length, protruding from the duplex (*3*). Each eukaryotic species has a characteristic telomeric repeat sequence. Limited sequence variations are found in some species (Table 1). However, widely divergent species can have the same telomeric repeat unit: for example, 5′-AGGGTT-3′ is the telomeric repeated sequence of acellular slime molds and humans. In human germline (sperm) nuclei, about 10 to 15 kb of this tandemly repeated sequence is found at every telomere (*7*), so that ~0.03% by weight of the total genome is telomeric DNA.

The enzyme telomerase is responsible for synthesis of the G-rich strand of telomeric DNA. Telomerase was first identified in the ciliate *Tetrahymena* (*8, 9*) and was shown to polymerize nucleotides into tandem repeats of the *Tetrahymena* telomeric DNA sequence, TTGGGG. Polymerization occurs by adding onto the 3′ end of a G-rich strand telomeric oligonucleotide primer, independent of an exogenously added nucleic acid template. The enzyme requires a DNA primer: it can use the G-rich strand telomeric sequences from all eukaryotes tested, but not random sequence DNA oligonucleotides (*8*). Similar findings were subsequently made for the telomerase activities of the ciliates *Oxytricha* and *Euplotes* (*10, 11*) and of human cells (*5*). Each telomerase synthesizes its species-specific G-rich strand sequence and has primer requirements similar to those of the *Tetrahymena* telomerase. Identification of telomerase activity in human cells suggests the generality of this enzyme in telomere synthesis in eukaryotes outside the ciliated protozoa.

The existence of telomerase can explain many properties of telomeres in vivo. Telomeres from one species can stabilize linear DNA molecules or chromosomes in another species, even though the two organisms have different telomeric DNA sequences (*3*). DNA sequencing showed that yeast telomeric DNA sequences are added to the end of a ciliate telomere in vivo (*12*). Human telomeres also function in yeast (*4*), demonstrating that this functional conservation is not limited to the lower eukaryotes. The basis for conservation of telomere function between distantly related eukaryotes may be the recognition properties of telomerases. In vitro, all telomerases require a minimum length (10 to 12 nucleotides) of G-rich strand telomeric DNA, similar to the length of the 3′ overhang of telomeric DNA, for high-affinity recognition as a primer in vitro (*5, 8, 10, 11, 13*). The ability of such G-rich synthetic DNA oligonucleotides to assume intra- and intermolecular two- and four-stranded folded structures stabilized by non–Watson-Crick base pairs (*14, 15*) correlates with the ability to be recognized as a primer by telomerase (*8, 13, 14*). However, the exact structure responsible for recognition by telomerase is not yet known.

A linear duplex DNA such as a chromosomal DNA is thought to require special means, other than normal semiconservative DNA replication, for completing the replication of its 5′ termini. The action of conventional DNA polymerases, which synthesize DNA in the 5′ to 3′ direction and usually require a nucleic acid primer, is expected to leave 5′-terminal gaps after each replication round (*1–3*). A central function of telomerase therefore appears to be to counterbalance this terminal DNA loss. Considerable variation in telomere length is common in eukaryotes, including humans (*3, 6*). If mean telomere length is determined by the balance between the addition of sequences of telomerase and their terminal loss through 5′ end attrition as described above, the variability of telomere lengths in


Department of Molecular and Cell Biology, University of California at Berkeley, Room 229 Stanley Hall, Berkeley, CA 94720.

**Table 1.** Sequences of telomeres in various species. For each species, the most common telomeric repeat unit sequences are shown 5′ to 3′.

| Group | Organisms | Telomeric repeat | Reference |
|---|---|---|---|
| Mammals | *Homo sapiens* | AGGGTT | (*3*) |
| Acellular slime molds | *Physarum polycephalum*<br>*Didymium iridis* | | |
| Filamentous fungus | *Neurospora crassus* | | |
| Kinetoplastid protozoa | *Trypanosoma*<br>*Crithidia* | | |
| Ciliated protozoa | *Tetrahymena*<br>*Glaucoma chattoni* | GGGGTT | (*3*) |
| | *Paramecium* | GGG(G/T)TT | (*3*) |
| | *Oxytricha*<br>*Stylonichia*<br>*Euplotes* | GGGGTTTT | (*3*) |
| Sporozoite protozoan | *Plasmodium* | AGGGTT(T/C) | (*3*) |
| Higher plant | *Arabidopsis* | AGGGTTT | (*3*) |
| Alga | *Chlamydomonas* | AGGGTTTT | (*21*) |
| Fission yeast | *Schizosaccharomyces pombe* | $(A)G_{2-5}TTAC$ | (*3*) |
| Budding yeast | *Saccharomyces cerevisiase* | $G_{1-3}T$ | (*3*) |
| Cellular slime mold | *Dictyostelium discoidum* | $G_{1-8}A$ | (*3*) |

vivo would arise from changing the relative rates of these processes.

Whereas telomerase only synthesizes the G-rich telomeric strand, it has been proposed that synthesis of the complementary C-rich strand is carried out by primase-polymerase–mediated discontinuous synthesis typical of semi-conservative DNA replication mechanisms (*12*). G-rich strand-templated synthesis of the C-rich strand by a primase-like activity has been found with cell-free extracts from the ciliate *Oxytricha* (*16*).

Telomerases are ribonucleoprotein enzymes, with essential RNA and protein components (*5, 8–11*). The RNA moieties of the telomerases of the ciliates *Tetrahymena* and *Euplotes* have been identified (*9, 17*). The sequence 5′-CAACCCCAA-3′ is found in the *Tetrahymena* telomerase RNA (*9*), and selective cleavage of this sequence in vitro destroys telomerase activity (*9*). This sequence could template the addition of TTGGGG repeats, and a model for the mechanism of telomerase activity incorporating this template function was proposed (*9*). An analogous sequence, 5′-CAAAACCCCAAAA-3′, is found in the telomerase RNA of *Euplotes*, and functional analyses in vitro have identified it as the template strand domain for synthesis of GGGGTTTT repeats (*17*), the telomeric sequence of *Euplotes*. Site-directed mutagenesis of the 5′-CAACCCCAA-3′ sequence in the *Tetrahymena* telomerase RNA gene, and overexpression of the mutated gene in transformed *Tetrahymena* cells, results in the synthesis of telomeres in vivo whose sequence corresponds to the mutated template sequence (*18*). These findings establish telomerase as an unusual reverse transcriptase, which carries its own internal RNA template for DNA synthesis. In yeast, deletion of the essential gene *EST1* causes telomere shortening and senescence, a phenotype predicted for telomerase mutations (*19*). Strikingly, the predicted amino acid sequence of *EST1* contains four amino acid motifs common to reverse transcriptases, suggesting that *EST1* may be a protein component of a yeast telomerase (*20*).

A telomerase RNA containing a template for DNA synthesis is intriguing in the context of the widely accepted idea of an evolutionary transition from RNA to DNA genomes. The conserved presence of a ribonucleoprotein telomerase in diverse eukaryotes suggests that this enzyme predated the separation of eukaryotes from prokaryotes. Indeed, telomerase RNA may be a relic of an RNA replicase ribozyme that became able to synthesize DNA. Perhaps as protein components took over this catalytic role they gave rise to the protein reverse transcriptases found in both eukaryotes and prokaryotes, but in the case of telomerase, a portion of the template RNA (still possibly with some catalytic role) was retained. Whereas the major function of genome replication has been taken over by DNA-dependent DNA polymerases, the specialized RNA-dependent DNA polymerase telomerase has been relegated to the role of maintenance of chromosomal ends.

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