Structure and function of telomeres

Elizabeth H. Blackburn

The DNA of telomeres—the terminal DNA–protein complexes of chromosomes—differs notably from other DNA sequences in both structure and function. Recent work has highlighted its remarkable mode of synthesis by the ribonucleoprotein reverse transcriptase, telomerase[1–4], as well as its ability to form unusual structures *in vitro*. Moreover, telomere synthesis by telomerase has been shown to be essential for telomere maintenance and long-term viability.

TELOMERES were originally defined functionally on the basis of early cytological and genetic studies which demonstrated that chromosomes with broken ends were unstable (reviewed in refs 1 and 2). The broken ends were able to fuse end to end, leading to dicentric, ring or other unstable chromosome forms. The contrast between this instability and the stability of normal chromosomal ends led to the concept of the telomere as being the specialized structure at the natural end of a eukaryotic chromosome, without which the chromosome is unstable. Molecular analysis has revealed how telomeres carry out another critical function: the ability to allow the end of the linear chromosomal DNA to be replicated completely without the loss of terminal bases at the 5′ end of each strand of this DNA. Such loss is predicted from the properties of the machinery of conventional semiconservative replication: its ability to work only in the 5′ to 3′ direction, and the requirement of the cellular DNA polymerases for an RNA primer (see Fig. 1).

Telomeres have proven so far to be highly conserved among all well-characterized eukaryotic nuclear chromosomes, and to be quite different from the termini of linear viral, non-nuclear plasmid or mitochrondrial DNA genomes. Hence, it is useful to define telomeres separately from the much greater variety of terminal structures found at these other DNA ends.

Telomeric sequence organization

Telomeric DNA sequences and structure are similar among otherwise widely divergent eukaryotes. The essential telomeric DNA consists of a stretch of a very simple, tandemly repeated sequence. Examples of telomeric repeat units include AGGGTT for humans as well as other vertebrates, slime moulds and trypanosomes; GGGGTT and GGGGTTTT for the ciliate protozoa *Tetrahymena* and *Euplotes* respectively; AGGGTT (C/T) for the malarial parasite *Plasmodium*, and $G_{1-3}T$ and $G_{1-8}A$ for baker's yeast and the slime mould *Dictyostelium*, respectively (reviewed in ref. 3). A terminal tract of this simple-sequence DNA, typically of a few hundred base pairs in yeast or ciliates, and thousands of base pairs in vertebrates, seems to be sufficient to maintain a stable telomere[4,5].

Although the simple telomeric repeats do not conform to a specific consensus sequence, they have a G-rich strand with an orientation specificity with respect to the end of the chromosome. At each chromosomal end, the G-rich telomeric DNA strand runs 5′ to 3′ towards the terminus and protrudes 12–16 nucleotides beyond the complementary C-rich strand, at least in the various species in which analysis has been possible[6,7] (Fig. 2).

A loosely defined group of repetitive sequences called telomere-associated sequences are commonly located adjacent and internally to the telomeric sequences. Various telomere-associated sequences are found in species ranging from yeast to humans, and include a wide variety of species-specific sequences, lengths and complexities. Although often found on several chromosomes in a species, such telomere-associated sequences are often absent altogether (reviewed in refs 1, 2 and 5). They do not seem to be essential for chromosome stability and their functional and evolutionary significance is unclear.

Telomerase

The G-rich strand of telomeres is synthesized by the ribonucleoprotein enzyme telomerase (reviewed in ref. 1). Telomerase activities have been identified *in vitro* in ciliate[8–13] and human[14] cell-free extracts. A synthetic G-rich telomeric DNA oligonucleotide[10–15] or a telomere[7,16] is elongated by polymerization, in the 5′ to 3′ direction, of deoxynucleoside triphosphate substrates into tandem repeats of the telomeric sequence of the species from which the telomerase was made. The reaction does not require ATP. The telomerase RNA and protein components both seem to be essential for activity. The telomerase RNAs of *Tetrahymena* and *Euplotes* have been identified, and contain a sequence, 5′-CAACCCCAA-3′ and 5′-CAAAACCCCAAA-3′, respectively, which is complementary to the telomeric repeats synthesized by the enzyme[8,9]. Studies *in vitro* indicated that this complementary RNA sequence acts as the template for synthesis of the G-rich telomeric DNA strand[8,9]. That the 5′-CAACCCCAA-3′ sequence in *Tetrahymena* is the template for telomere synthesis was demonstrated by site-directed mutagenesis. Expression of the mutated telomerase RNA gene in transformed *Tetrahymena* cells results in the synthesis of telomeres whose sequence corresponds to the mutated template sequence[16]. Telomerase can thus be defined as an unusual ribonucleoprotein reverse transcriptase whose RNA template is an intrinsic part of the enzyme. The current model[8,9] for the mechanism of telomere DNA synthesis by telomerase is shown in Figure 3.

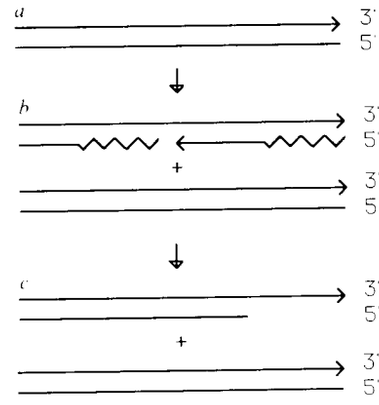

FIG. 1 The problem of complete replication of a linear DNA molecule by conventional DNA replication. *a*, DNA duplex, whose end is on the right, with 5′ and 3′ ends of each strand indicated. *b*, Parental DNA is copied by a replication fork moving from the left toward the end of the molecule. Leading strand 5′-to-3′ synthesis copies the bottom parental strand all the way to its last nucleotide. Discontinuous lagging strand synthesis, also in the 5′-to-3′ direction, copying the top parental strand, is primed by RNA primers (zig-zag lines). *c*, RNA primers are removed, and the internal gaps are filled in by extension of the discontinuous DNA and ligation. A 5′ gap in this newly synthesized strand is left because there is no primer allowing it to be filled in. Successive replication rounds will produce shortened daughter chromosomes.

In contrast to the sequence specificity of binding by the structural telomere proteins (see below), telomerase accepts as primers a variety of G-rch sequences[10,11,13-15]. A+T-rich, C-rich, or random sequence oligonucleotides are generally not used as efficiently by telomerase as primers *in vitro*. Strong evidence that telomerase recognizes various non-wild-type telomeric sequences *in vivo* as well as *in vitro* comes from experiments in which telomerase RNA genes with altered template sequences directed synthesis of several tandem GGGGTC[16] or irregular $G_{5-8}TT$ repeats (G.-L. Yu and E.H.B., unpublished work), instead of the wild-type GGGGTT repeats. These results directly demonstrate that telomerase can use a non-wild-type telomeric sequence immediately preceding the added sequence for priming *in vivo*, in agreement with the results *in vitro*.

Compelling evidence that telomerase activity is essential for long-term viability of *Tetrahymena* comes from analysis of telomerase RNA mutations *in vivo*. Overexpression of one particular mutant telomerase RNA gene in *Tetrahymena* is sufficient to cause a dominant negative phenotype characterized by telomere shortening and senescence[16]. Cell rescue has not been observed except by loss of this mutant RNA gene through recombination and/or segregating out the plasmid bearing the mutant gene[16] (J. Bradley and E.H.B., unpublished results). In yeast, loss of function of a gene essential for long-term viability, *EST1*, causes steady and continuous telomere shortening over several cell generations, and eventually senescence[17]. Cell death is preceded by increased rates of chromosome loss. The *EST1* gene encodes a reverse transcriptase-like protein[18]. This, together with the identification of the ciliate telomerases as specialized reverse transcriptases[8,9,16]and the phenotype of *est1*-deficient mutants, indicates that EST1 is a protein component of telomerase[18]. These findings imply that telomerase is essential for maintenance of telomere length and long-term viability in yeast as well as *Tetrahymena*.

Whether telomerase is used to heal broken chromosomes lacking telomeres may vary from species to species. Healing by the apparently direct addition of simple telomeric repeats to a broken end has been demonstrated in the yeasts *Saccharomyces pombe* and *Kluveromyces*, in the protozoa *Plasmodium* and *Paramecium*[19-22] and for chromosome 16 in humans[23]. Using a mutant *Tetrahymena* telomerase RNA to monitor telomerase action *in vivo*, telomerase has been shown to add the first telomeric repeats *de novo* onto the non-telomeric ends generated during the developmentally controlled chromosome fragmentation in the ciliate *Tetrahymena* (G.L. Yu and E.H.B., unpublished results). This provides a precedent for the telomerase in other eukaryotes being able to heal broken chromosomes by direct telomere addition to the broken end. In yeast, healing of broken chromosomal ends often involves recombination of large chromosomal terminal regions, including regions centromeric to the simple G_{1-3} repeat sequences, onto the broken end[24-27]. But Murray *et al.*[28] found that for a plasmid cut by a restriction enzyme so that a *Tetrahymena* telomeric sequence lay near but not at the resulting end, healing occurred by addition of G_{1-3} repeats, suggestive of telomerase-mediated healing described above for other species.

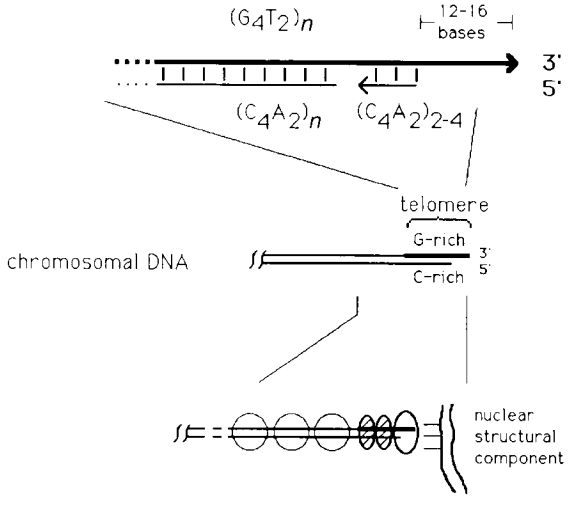

FIG. 2 Eukaryotic chromosomal telomeres. Centre, the orientation of the G-rich and C-rich telomeric DNA strands; top, details of the terminal DNA structure for the *Tetrahymena* telomere. Single-stranded breaks on the C-rich strand occur in the distal part of the duplex telomeric DNA[1], and the G-rich strand (thick line) forms a terminal DNA protrusion; bottom, telomeric proteins and their possible relation to other nuclear protein structural components. Terminally binding telomeric protein (open oval), with an unspecified association with a nuclear structure such as the nuclear envelope of lamins, may be distinct from non-terminal telomere binding protein such as RAP1 of yeast (hatched ovals). The telomeric DNA–protein complex is bordered by nucleosomes[71,72] (open circles). The drawing represents a composite of information from various species. See text for details and additional references.

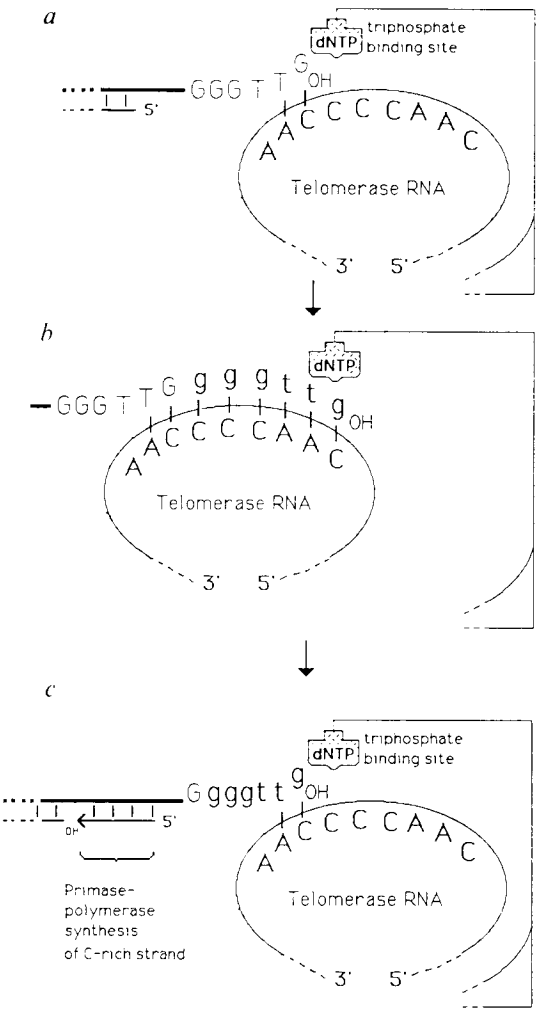

FIG. 3 Synthesis of telomeric DNA by the ribonucleoprotein enzyme telomerase from *Tetrahymena*. *a*, The 3' few nucleotides of the terminal chromosomal G-rich overhang (see text; thick line, shown arbitrarily as ending in-TTG-3') base-pair with the telomere-complementary sequence in the telomerase RNA.[1] The chromosomal end is extended by polymerization of dGTP and dTTP using the RNA as a template. The relative movement which must occur within the telomerase particle between the RNA template and the polymerization site[9] during the addition of six telomeric nucleotides is indicated schematically. *c*, The extended DNA terminus unpairs from its RNA template, becoming available for another round of elongation by telomerase and/or to primase–polymerase[43,73]. Adapted from refs 8, 9.

Illegitimate recominbation has been implicated in processes acting at the simple telomeric repeat sequences in yeast[29]. But such recombination between terminal telomeric repeats has been detected only in yeast cells transformed by linear plasmids with heterologous telomeres; specifically, its detection depends on assays *in vivo* which select for rescue or increased efficiency of function of the plasmid telomere[30,31]. Such recombination events have not been detected in yeast cells that have not been selected for these events. Furthermore, telomeric sequences are steadily and inexorably lost in otherwise wild-type *est1*$^-$ cells, and most *est1*$^-$ cells eventually die[17]. Hence recombination-mediated pathways are unlikely to be sufficient for normal telomere maintenance.

Telomeric proteins

The only biological components known to interact specifically with the extreme molecular ends of chromosomal DNA are telomerase and the telomere structural proteins. Although telomeric DNAs have been characterized from a wide variety of species, the protein structural components of telomeres have been identified with certainty in only a few species[32-35]. The strong conservation of telomeric DNA structure and function predicts that the findings made with these few known telomeric structural proteins will be generally applicable to other eukaryotic species. In the ciliate Oxytricha, a heterodimer composed of subunits with relative molecular masses 55,000 and 41,000 (55K and 41K) binds very tightly but not covalently to the overhanging G-rich protrusion, neither protecting nor requiring the adjacent duplex DNA[36]. The gene for the 55K subunit has been cloned and sequenced; it exhibits some amino-acid sequence similarity with histone H1[37]. A 50K telomeric protein in *Euplotes* binds similarly[33]. Telomere-binding proteins show sequence specificity[32,33,36,38] and their binding protects telomeric DNA against chemical modification and nucleases[32,33,36,38,39]. Such proteins are likely to be the 'cap' which protects telomeric ends *in vivo*. It has been suggested[39] that these proteins could regulate the action of telomerase *in vivo*, by binding the free DNA end and making it unavailable for further elongation. The main evidence that telomerase is distinct from these structural proteins is that it is not found tightly associated with telomeres, and telomerase activity is released in a soluble fraction from *Euplotes* nuclei lysed in hypotonic buffer (D. Shippen-Lentz and E.H.B., unpublished results), away from the telomere protein fraction[33].

Recent work indicates that the duplex portion of yeast telomeric DNA is bound *in vivo*[34,35] as well as *in vitro*[40,41] by the abundant yeast transcriptional regulator protein RAP1. The binding of RAP1 requires a consensus sequence which arises on average every 40 base pairs in the irregular telomeric $d(G_{1-3}T) \cdot d(AC_{1-3})$ repeats[34]. There is, however, no evidence that RAP1 binds the presumably single-stranded end of yeast telomeres. Instead, a separate protein analogous in function to the *Oxytricha* terminal protein could fulfil that function (Fig. 2).

Two findings *in vivo* reinforce the idea that the proteins binding to telomeric termini are distinct from those binding internal duplex regions of telomeric sequences. First, foreign telomeric sequences in internal regions of telomeres are stably and indefinitely maintained in yeast and *Tetrahymena*, with no apparent deleterious effects provided that wild-type telomeric repeats are present at the telomeric terminus[31,42-44] (G.-L. Yu and E.H.B., unpublished work). By contrast, mutated telomeric sequences at the distal ends of *Tetrahymena* macronuclear chromosomes, added continuously by the action of a mutated telomerase RNA gene, greatly impair nuclear division and lead to eventual cell death[16]. These results are consistent with a special role for a telomere terminal-binding protein like those of *Oxytricha* and *Euplotes*, which have specificity for the sequence of the G-rich strand overhang[32-34], and whose binding would be expected to be impaired by changes in this newly added end sequence. Second, in yeast placing a reporter gene within a few kilobases of various telomeric ends is sufficient to cause its transcriptional repression[45]. Strikingly, there is no repression if the same gene construct is placed the same distance from a chromosome-internal stretch of telomeric DNA sequence, despite the fact that RAP1 is expected to bind the duplex $G_{1-3}T$ repeat tracts in internal as well as telomeric locations. Again, these results argue strongly that telomeric termini are associated with protein(s) distinct from those binding duplex telomeric repeat sequences. Are these proteins associated with other nuclear components? Telomeres have long been observed cytologically to associate with the nuclear envelope (reviewed in refs 1 and 2). Binding of telomeric termini, directly or indirectly, to such a nuclear structure may be necessary for proper chromosome segregation and hence nuclear division in *Tetrahymena*[16] and possibly other species, and to produce the transcriptional repression effect in yeast.

Telomeric DNA structures

Because of their unusual sequences, telomeric DNAs can assume unusual structures in solution. Double-stranded telomeric DNA is susceptible to rapid digestion with Bal31 nuclease, despite being rich in G and C residues[5,46], and when present in supercoiled molecules, can assume unusual structures which are hypersensitive to nuclease digestion, chemical modification and strand invasion[47,48]. These properties, whose function *in vivo* is unknown, are not unique to duplex telomeric DNAs, and have been found with other duplex G-rich DNA sequences (for example, ref. 49).

The unusual structures G-rich DNA oligonucleotides can assume, and their potential biological properties, have attracted particular attention. Recent work has demonstrated that various telomeric or telomere-like DNA oligonucleotides can form a plethora of G · G base paired structures in vitro[15,39,50-55] (see ref. 56 for a recent review). I will discuss here the two kinds of structures which seem most relevant to telomere function.

First, short G-rich oligonucleotides with the same length and sequence as the 12-16-nucleotide telomeric overhang can assume intramolecular, apparently simple foldback structures, which migrate anomalously fast in non-denaturing gel electrophoresis[15,50,55]. Some of these oligonucleotides form multiple bands corresponding to discrete intramolecularly folded forms. These structures are probably stabilized by non-Watson-Crick G-G base pairing[50], but the details are not yet clear (Fig. 4*b*). Such intramolecular structures merit particular attention, because they are formed by oligonucleotides as short as the presumed substrate for activities or proteins acting on chromosomal termini. They were observed to 'melt' at or below 40–45 °C (ref. 50), well below the melting temperature of the more stable four-stranded forms described next[51].

Second, longer G-rich oligonucleotides containing at least four short runs of G residues can also fold into even more compact forms. These include a very stable, K^+- or Na^+-dependent, four-stranded intramolecular structure, a quadruple helix which has as its stabilizing element a planar array of four Hoogsteen-paired G residues[55] (Fig. 4*d*). These planar arrays are stacked upon each other, with a central K^+ or Na^+ ion between planes. G-rich telomeric DNA oligonucleotides of 12-16 nucleotides are too short to form such intramolecular four-stranded forms. But an intermolecular quadruple guanosine helix built on the same four-stranded structural principal can be formed by association between two intramolecularly-folded short (12 nucleotides) synthetic telomeric G-rich strands[54] (Fig. 4*c*).

What is the relationship of the different non-Watson-Crick structures assumed by G-rich DNA to telomere function? Ideas for how telomerase recognizes telomeric DNA primers have focused on structural rather than sequence-specific recognition[11,50]. *In vitro*, telomerase exhibits specificity for G-rich single-stranded DNA oligonucleotide primers of various sequences. Because the conditions *in vivo* are believed to

resemble those which would thermodynamically favour the stable, K^+- and Na^+-dependent quadruple helices described above, the possibility of their having a biological role has been considered. But in considering structures that could be recognized by telomerase, it should be noted that G-rich protrusions of sufficient length to form this type of intramolecular quadrupole helix have not been detected in telomeric DNA extracted from eukaryotic cells[6,7]. There is no evidence that an intra-or intermolecular quadruple helix forms *in vivo*: the patterns of protection *in vivo* from chemical methylation of the terminal nucleotides of *Oxytricha* and *Euplotes* telomeres are reconstituted *in vitro* by binding of their respective telomere binding proteins[38], and are quite different from the patterns of protection observed *in vitro* for the four-stranded form[55]. The extreme kinetic and thermodynamic stability of this four-stranded structure formed *in vitro* is difficult to reconcile with a dynamic function *in vivo*[39]. If such a four-stranded structure formed, it would be expected to persist, given its half-life for unfolding, which is of the order of many hours[39]. Evidence that the four-stranded G-rich structure may not be used *in vivo* comes from the direct demonstration that the intramolecular four-stranded structure is not bound *in vitro* by the telomere structural protein, and is disfavoured as the substrate for elongation by telomerase (ref. 39; T. Cech, personal communication; M. Lee and E.B., unpublished).

Although the existence of the highly stable four-stranded G-rich DNA forms *in vitro* is well documented, the situation is reminiscent of Z-form DNA, a distinct and defined DNA form whose function *in vivo* remains unknown. But the relationship of other, less stable G-rich structures to telomere function remains an open question. Candidates for other G · G paired structures recognized by telomerase could include the less stable simple foldback structures (see Fig. 4*b*). These have the appeal of being only moderately stable and hence able to unfold *in vivo* in response to the need to be replicated or to be bound by telomeric structural proteins.

Telomere length and loss

The model for telomere replication and maintenance best supported by available data is that the mean number of tandem simple telomeric repeats at each telomere is determined by a balance of processes that lengthen and shorten telomeres[43,57]. Many different genetic and physiological factors influence mean telomere length and, by inference, this balance in viable cells[34,58-62] Without a mechanism to counterbalance it, rounds of semiconservative DNA replication are predicted to lead to progressive shortening of the chromosome from its ends. Such shortening over many generations is indeed observed in *est1*-mutant yeast[17], and for certain cases of broken ends lacking conventional telomeric DNA sequences in *Drosophila*[63,64].

Germ-line cells in humans have an average of 10 kb of telomeric AGGGTT repeats at each chromosomal end[5,65]. Analyses of telomere length as a function of age, either in cells from people of different ages, or as a function of cell division number in primary cultures of human fibroblasts, and in certain cancerous cells, show that mean telomere length gradually decreases with increased age or cell division number[66-68]. Telomerase activity has been clearly documented in extracts of immortalized (HeLa) tissue culture cells[14]. But the data are all consistent with the idea[66-68] that telomerase activity may be low or absent in normal mammalian somatic cells. It has been suggested that the observed gradual loss of telomeric DNA could lead to chromosome instability[17] and contribute to ageing and senescence[66-69]. This is consistent with the results cited above for yeast and *Tetrahymena*. One finding inconsistent with a simple form of this idea for ageing in mammalian systems is that mouse telomeres are very long, probably 5-10 times longer than human telomeres, and are not perceptibly shorter in old compared with young mice[70].

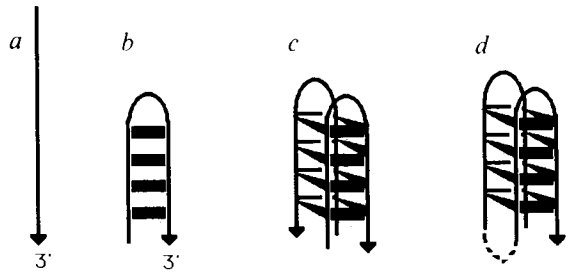

FIG. 4 Some of the structures adopted in solution by G-rich telomeric oligonucleotides containing two (*a-c*) or four (*d*) clusters of G residues. *a*, Single-stranded form. *b*, Two-stranded foldback form stabilized by non-Watson-Crick G · G base pairs (horizontal bars), present at low and high salt concentrations and visualized as a fast-migrating species in non-denaturing gel electrophoresis[50]. *c*, Monovalent cation-stabilized intermolecular quadruple helix: a dimer made up of an association of two foldback forms seen in *b* in antiparallel strand orientation, held together by additional G · G base-pairing between the two intramolecular foldbacks[54]. *d*, Monovalent cation-stabilized intramolecular quadruple helix: the same structural form as in *c* but with a continuous polynucleotide chain connecting the two halves of the molecule[55].

Drugs and telomeres

The G-rich strand of the telomere is the only essential chromosomal DNA sequence known to be synthesized by the copying of a separate RNA sequence. This unique mode of synthesis, and the special structure and behaviour of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because telomerase activity seems to be essential for protozoans or yeast, but not apparently for mammalian somatic cells, I propose that telomerase should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that binds telomerase selectively, either through its reverse-transcriptase or DNA substrate-binding properties, should selectively act against prolonged maintenance of the dividing lower eukaryote[16-18], but not impair the mammalian host over the short term, because telomerase activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would bind telomeric DNA itself. These could include drugs that selectively bind the G · G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G · G base-paired form, preventing it from adopting a structure necessary for proper function *in vivo*. Telomeres have been described as the Achilles heel of chromosomes[67,69]: perhaps it is there that drug strategies should now be aimed.

Note added in proof. The inactivity of telomerase on four-stranded DNA is described in ref. 74. □

Elizabeth H. Blackburn is in the Departments of Microbiology and Immunology and Biochemistry and Biophysics, Box 0414, San Francisco, California 94143, USA.

1. Blackburn, E. H. & Szostak, J. W. *A. Rev. Biochem.* **53,** 163-194 (1984).
2. Zakian, V. A. *A. Rev. Genet.* **23,** 579-604 (1989).
3. Blackburn, E. H. *Science* **249,** 489-490 (1990).
4. Murray, A. W. & Szostak, J. W. *Nature* **305,** 189-193 (1983).
5. Brown, W. R. A. *et al. Cell* **63,** 119-132 (1990).
6. Klobutcher, L. A., Swanton, M. T., Donini, P. & Prescott, D. M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 3015-3019 (1981).
7. Henderson, E. R. & Blackburn, E. H. *Molec. cell. Biol.* **9,** 345-348 (1989).
8. Greider, C. W. & Blackburn, E. H. *Nature* **337,** 331-337 (1989).
9. Shippen-Lentz, D. & Blackburn, E. H. *Science* **247,** 546-552 (1990).
10. Greider, C. W. & Blackburn, E. H. *Cell* **43,** 405-413 (1985).
11. Greider, C. W. & Blackburn, E. H. *Cell* **51,** 887-898 (1987).
12. Zahler, A. M. & Prescott, D. M. *Nucleic Acids Res.* **16,** 6953-6972 (1988).
13. Shippen-Lentz, D. & Blackburn, E. H. *Molec. cell. Biol.* **9,** 2761-2764 (1989).
14. Morin, G. B. *Cell* **59,** 521-529 (1989).
15. Blackburn, E. H. *et al. Genome* **31,** 553-560 (1989).
16. Yu, G.-L., Bradley, J. D., Attardi, L. D. & Blackburn, E. H. *Nature* **344,** 126-132 (1990).
17. Lundblad, V. & Szostak, J. W. *Cell* **57,** 633-643 (1989).

18. Lundblad, V. & Blackburn, E. H. *Cell* **60,** 529–530 (1990).
19. Matsumoto, T. *et al. Molec. cell. Biol.* **7,** 4424–4430 (1987).
20. Kamper, J., Meinhardt, F., Gunge, N. & Esser, K. *Molec. cell. Biol.* **9,** 3931–3937 (1989).
21. Pologe, L. G. & Ravetch, J. N. *Cell* **55,** 869–874 (1988).
22. Gilley, D., Preer, J. R., Aufterheide, K. J. & Polisky, B. *Molec. cell. Biol.* **8,** 4765–4772 (1988).
23. Wilkie, A. M., Lamb, J., Harris, P. C., Finney, R. D. & Higgs, D. R. *Nature* **346,** 868–871 (1990).
24. Dunn, B., Szauter, P., Pardue, M. L. & Szostak, J. W. *Cell* **39,** 191–201 (1984).
25. Horowitz, H. & Haber, J. E. *Molec. cell. Biol.* **5,** 2369–2380 (1985).
26. Haber, J. E. & Thorburn, P. C. *Genetics* **106,** 207–226 (1984).
27. Jger, D. & Philipsen, P. *EMBO J.* **8,** 247–254 (1989).
28. Murray, A. W., Claus, T. & Szostak, J. W. *Molec. cell. Biol.* **8,** 4642–4650 (1988).
29. Walmsley, R. W., Chan, C. S. M., Tye, B. K. & Petes, T. D. *Nature* **310,** 157–160 (1984).
30. Pluta, A. F. & Zakian, V. A. *Nature* **337,**429–433 (1989).
31. Wang, S.-S. & Zakian, V. A. *Nature* **345,** 456–458 (1990).
32. Gottschling, D. E. & Zakian, V. A. *Cell* **47,** 195–205 (1986).
33. Price, C. R. *Molec. cell. Biol.* **10,** 3421–3431 (1990).
34. Lustig, A. J., Kurtz, S. & Shore, D. **250,** 549–552 (1990).
35. Conrad, M. N., Wright, J. H., Wolf, A. J. & Zakian, V. A. *Cell* **63,** 739–750 (1990).
36. Price, C. M. & Cech, T. R. *Biochemistry* **28,** 769–774 (1989).
37. Hicke, B. J., Celander, D. W., MacDonald, G. H., Price, C. M. & Cech, T. R. *Proc. natn. Acad. Sci. U.S.A.* **87,** 1481–1485 (1990).
38. Raghuraman, M. K., Dunn, C. J., Hicke, B. J. & Cech, T. R. *Nucleic Acids Res.* **17,** 4235–4253 (1989).
39. Raghuraman, M. K. & Cech, T. R. *Nucleic Acids Res.* **18,** 4543–4552 (1990).
40. Berman, J., Tachibana, C. Y. & Tye, B.-K. *Proc. natn. Acad. Sci. U.S.A.* **83,** 3713–3717 (1986).
41. Buchman, A. R., Lue, N. F. & Kornberg, R. D. *Molec. cell. Biol.* **8,** 210–255 (1988).
42. Szostak, J. W. & Blackburn, E. H. *Cell* **29,** 245–255 (1982).
43. Shampay, J., Szostak, J. W. & Blackburn, E. H. *Nature* **310,** 154–157 (1984).
44. Riethman, H. C., Moyzis, R. K., Meyne, J., Burke, D. T. & Olson, M. V. *Proc. natn. Acad. Sci. U.S.A.* **86,** 6240–6244 (1989).
45. Gottschling, D. E., Aparicio, O. M., Billington, B. L. & Zakian, V. A. *Cell* **63,** (1990).
46. Henderson, E. *et al. Cancer Cells* **6,** 453–461 (eds Kelly, T. & Stillman, B.) (Cold Spring Harbor Laboratory, New York, 1988).
47. Budarf, M. & Blackburn, E. H. *Nucleic Acids Res.* **15,** 6273–6292 (1987).
48. Frank-Kamenetskii, M. *Nature* **342,** 737 (1989).
49. Usdin, K. & Furano, A. *Proc. natn. Acad. Sci. U.S.A.* **85,** 4416–4420 (1988).
50. Henderson, E., Hardin, C. C., Wolk, S. K., Tinoco, I. & Blackburn, E. H. *Cell* **51,** 899–908 (1987).
51. Hardin, C. C., Henderson, E. R., Watson, T. & Prosser, J. *Biochemistry* (in the press).
52. Henderson, E. R., Moore, M. & Malcolm, B. A. *Biochemistry* **29,** 732–737 (1990).
53. Sen, D. & Gilbert, W. *Nature* **344,** 410–414 (1990).
54. Sundquist, W. I. & Klug, A. *Nature* **342,** 825–829 (1989).
55. Williamson, J. R., Raghuraman, M. K. & Cech, T. R. *Cell* **59,** 871–880 (1990).
56. Sundquist, W. I. in *Nucleic Acids and Molecular Biology* (eds Lilley, D. M. J. & Eckstein, F.) Vol. 5 (Springer, in the press).
57. Shampay, J. & Blackburn, E. H. *Proc. natn. Acad. Sci. U.S.A.* **85,** 534–538 (1988).
58. Bernards, A., Michels, P. A. M., Lincke, C. R. & Borst, P. *Nature* **303,** 592–597 (1983).
59. Walmsley, R. M. & Petes, T. D. *Proc. natn. Acad. Sci. U.S.A.* **82,** 506–510 (1985).
60. Lustig, A. J. & Petes, T. D. *Proc. natn. Acad. Sci. U.S.A.* **83,** 1398–1402 (1986).
61. Larson, D. D., Spangler, E. A. & Blackburn, E. H. *Cell* **50,** 477–483 (1987).
62. Runge, K. W. & Zakian, V. A. *Molec. cell. Biol.* **9,** 1488–1497 (1989).
63. Levis, R. W. *Cell* **58,** 791–801 (1989).
64. Biessmann, H., Carter, S. B. & Mason, J. M. *Proc. natn. Acad. Sci. U.S.A.* **87,** 1758–1761 (1990).
65. Moyzis, R. K. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 6622–6626 (1988).
66. Cooke, H. J. & Smith, B. A. *Cold Spring Harb. Symp. quant. Biol.* **51,** 213–219 (1986).
67. Harley, C. B., Futcher, A. B. & Greider, C. W. *Nature* **345,** 458–460 (1990).
68. Hastie, N. D. *et al. Nature* **346,** 866–868 (1990).
69. Olovnikov, A. M. *J. theor. Biol.* **41,** 181–190 (1973).
70. Kipling, D. & Cooke, H. J. *Nature* **347,** 400–402 (1990).
71. Gottschling, D. E. & Cech, T. R. *Cell* **38,** 501–510 (1984).
72. Budarf, M. & Blackburn, E. H. *J. biol. Chem.* **261,** (1986).
73. Zahler, A. M. & Prescott, D. M. *Nucleic Acid Res.* **17,** 6299–6317 (1989).
74. Zahler, A. M., Williamson, J. R., Cech, T. R. & Prescott, D. M. *Nature* (in the press).

ACKNOWLEDGEMENTS. I thank T. Cech and my colleagues for helpful comments on the manuscript. This work was supported by the NIH.

ARTICLES

Inadequacy of effective CO_2 as a proxy in simulating the greenhouse effect of other radiatively active gases

Wei-Chyung Wang, Michael P. Dudek, Xin-Zhong Liang & J. T. Kiehl*

Atmospheric Sciences Research Center, State University of New York, Albany, New York 12005, USA
* National Center for Atmospheric Research, Boulder, Colorado 80307, USA

The use of an 'effective' CO_2 concentration to simulate the combined greenhouse effect of CO_2 and the trace gases CH_4, N_2O, CFC-11 and CFC-12 is open to question, because the radiative-forcing behaviour of CO_2 is very different from that of these other gases. Model simulations show that different radiative forcing can lead to quite different climatic effects. The thermal infrared opacity of these trace gases therefore needs to be explicitly accounted for when attempting to predict the climate response to increasing concentrations of greenhouse gases.

ATMOSPHERIC CO_2 is currently increasing at 0.5% per year, and the increase is expected to continue. Increasing CO_2 will enhance the greenhouse effect and lead to a global warming[1]. The atmosphere also contains the trace gases CH_4, N_2O, $CFCl_3$ (CFC-11) and CF_2Cl_2 (CFC-12), whose concentrations are increasing at 0.9, 0.25, 4 and 4% per year, respectively[1,2]. These trace gases have strong absorption bands at 8–20 μm[3,4] in the infrared, and their concentration increases can further augment the CO_2 greenhouse effect[5–9]. In addition, the trace gases are chemically active; their increases can perturb atmospheric O_3 with subsequent climatic effect[2,10]. But the spatial distribution of atmospheric opacity which absorbs and emits the long-wave radiation is different for CO_2 and for the trace gases; for example, $CFCl_3$ is optically thin whereas CO_2 is optically thick. This difference can lead to a different distribution of radiative forcing[6,11], which in turn will affect the dynamics with subsequent different climate responses.

Here we concentrate on two issues related to the greenhouse effects of CO_2 and trace gases: the extent to which radiatively active trace gases maintain the present climate, and how they differ from CO_2 in affecting the climate. Our model simulations indicate that trace gases provide an important radiative energy source for the present climate system on Earth. The results also indicate that the different infrared opacity of CO_2 and the trace gases can lead to different climatic effects.

Atmospheric general circulation model

Study of the climatic effects of trace gases has so far been based on the use of simple energy balance models[5,10,12]. Because of the model simplifications such as vertical lapse rate adjustment and fixed relative humidity, the differences in the temperature responses arising from differences in the radiative forcing between CO_2 and trace gases cannot be properly addressed in the energy balance models. General circulation models (GCMs), on the other hand, are based on numerical solutions of the fundamental equations governing dynamical and physical processes in the atmosphere and are more appropriate for studying the effects of radiation on atmospheric dynamics. Most GCMs neglect the trace gases[13–17], and the only GCM that explicitly includes them[18] did not study the differences in climate responses between CO_2 and these gases. Excluding trace gases will affect