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An Overhanging 3′ Terminus Is a Conserved Feature of Telomeres

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The reactivity of single-stranded thymidines with osmium tetraoxide was used to demonstrate the existence of a terminal overhang of the G-rich strand of telomeres from two distantly related eucaryotes, the ciliated protozoan Tetrahymena spp. and the acellular slime mold Didymium spp. Conservation of a G-strand overhang at the molecular terminus of telomeres is consistent with our suggestion that an unusual DNA structure formed by the G-strand overhang is important for telomere function (E. Henderson, C. C. Hardin, S. K. Wolk, I. Tinoco Jr., and E. H. Blackburn, Cell 51:899–908, 1987).
overhangs analogous to those previously reported for the telomeres of hypotrichous ciliates (19, 22).

The length of the overhang in both the *Tetrahymena* and *Didymium* telomeres is 12 to 16 bases. The precision of this number depends on whether or not there are single-stranded, nonthymidine nucleotides 5’ to the single-stranded thymidines. Since the osmium cleavage technique is specific for thymidine residues, a more precise number cannot be obtained by using this technique.

The G-strand overhang is a feature of *Tetrahymena* telomeres lengthened in vivo. The molecular ends of telomeres which had grown by 300 to 400 base pairs during prolonged vegetative cell divisions (2, 20) were compared with those from telomeres of standard length in *Tetrahymena* spp. The results of this comparison are shown in Fig. 2b. The ends of the short (lane 1) and long (lane 2) telomeres are indistinguishable in terms of their susceptibility to cleavage by the osmium reagent. Therefore, the 3’ overhang is maintained in telomeres actively involved in the lengthening process.

Natural *Tetrahymena* rDNA ends are substrates for telomerase in vitro. In vitro, telomerase, the enzyme most likely to be responsible for telomere elongation, utilizes telomeric G-strand primers as substrates for elongation. Duplex telomeric DNA and C-strand primers are not elongated in this assay (7, 13, 14, 17, 18, 25). We tested the ability of telomerase to recognize natural telomeres as substrates for elongation in vitro. *Tetrahymena* rDNA was incubated with telomerase as previously described (13, 14). After telomerase treatment, the rDNA was digested with *PstI* and the reactions were assayed by monitoring the incorporation of [α-32P]dGTP into a telomeric *PstI* restriction fragment approximately 1 kilobase long. Since the rDNA is a palindrome, there are two similar *PstI* restriction fragments, one at each end of the molecule. For size comparison, this telomeric fragment was labeled with Klenow fragment at discontinuities known to exist in the telomere repeats (5, 16).
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The C strand recognizes the 5' end elongation. Telomerase, presumably to telomeres, presumably to telomeres, including recombination, and recognition by telomere-specific proteins.

FIG. 3. Telomerase recognition of the natural \textit{Tetrahymena} telomere structure. Telomeres were radiolabeled at G-strand 3' discontinuities (5, 16) by Klenow fragment (lane 1) or at the molecular terminus by telomerase (lane 2). Telomerase recognized and labeled only the telomeric \textit{Prtl} restriction fragment by addition of G and, presumably, T residues, indicating that labeling was at the terminus and not at internal discontinuities. Since telomerase does not recognize the C strand as a substrate for telomerase sequence addition and there is no precedent for repetitive addition of nucleotides to the 5' end of a DNA molecule by an enzyme, we concluded that addition was to the 3' end, i.e., the G-strand overhang. An average of 90 nucleotides was added to the telomeres by telomerase.

We have shown here that an overhang of the telomeric G-rich strand is a conserved feature of the molecular terminus of telomeres. Therefore, a G-strand overhang may play a central role in telomere function. We had previously demonstrated that synthetic oligonucleotides corresponding to telomeric G-strand overhangs can assume novel higher order structures involving non-Watson-Crick base pairing interactions (15). This observation is consistent with the idea that the G-strand overhang at telomeres is not single stranded in vivo but rather assumes an unusual DNA conformation which is responsible for some of the attributes of the telomere, including its resistance to degradation and recombination, and recognition by telomere-specific proteins.

Didymium strain 2-16 rDNA was a generous gift from Margaret Silliker (Ph.D. dissertation, University of California, Berkeley, 1986). We thank Tom Cech for suggesting the use of osmium tetraoxide as a single-strand-specific probe and Carol Greider for providing telomerase.

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LITERATURE CITED