Telomere Terminal Transferase Activity from *Euplotes crassus* Adds Large Numbers of TTTTGGGG Repeats onto Telomeric Primers

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A telomere terminal transferase activity was identified in developing macronuclear extracts from *Euplotes crassus*. The activity was essentially unregulated in vitro: up to 50 tandem repeats of the *Euplotes* telomeric repeat sequence TTTTGGGG were added onto synthetic telomeric oligonucleotide primers. Both the structure of the telomere substrate and its 3′-terminal sequence were recognized. The activity was destroyed by low concentrations of RNase A.

Replication of telomeres, the essential G+C-rich structures at the ends of eucaryotic chromosomes (reviewed in references 1 and 3), is thought to involve a telomere terminal transferase (telomerase) that extends the 3′ end of the G-rich strand by de novo synthesis of telomeric repeats (4, 14). Such a telomerase activity has been identified in *Tetrahymena thermophila* extracts (4). Unregulated in vitro, the *Tetrahymena* telomerase adds more than 1,000 TTGGGG repeats onto telomeric primers (E. H. Blackburn, C. W. Greider, E. Henderson, M. Lee, J. Shampay, and D. Shippen-Lentz, Genome, in press). Further characterization of the activity showed that it is a ribonucleoprotein enzyme containing a single essential RNA species (5, 6).

The highly conserved structures of telomeres among different eucaryotes imply that the mechanism of telomere elongation may also be conserved. Besides *T. thermophila*, other potentially good sources of telomerase activity are the hypotrichous ciliates *Euplotes* and *Oxytricha* spp., which contain approximately 107 chromosomal termini per cell (reviewed in reference 7). Analysis of the telomerase enzyme in these organisms may be particularly enlightening since their telomeric repeat sequence (TTTTGGGG) is different from the *Tetrahymena* repeated sequence (TTGGGG) (2, 8). Moreover, telomere length is regulated differently in the two types of ciliates. In *Tetrahymena thermophila*, telomeres typically consist of 50 to 70 repeats; however, length fluctuations occur under certain physiological conditions (9). In contrast, telomere length in the hypotrichous ciliates is tightly regulated during vegetative growth. Macronuclear telomeres in vegetative *Euplotes crassus* consist of a 28-base-pair duplex region plus a 14-base single-stranded tail (8, 11). During *Euplotes* macronuclear development, oversized telomeres are added onto DNA molecules immediately after chromosomal fragmentation and are subsequently trimmed back to their stable vegetative lengths (13).

A telomerase activity has recently been identified in vegetative macronuclear extracts from *Oxytricha nova* (15). Unlike the *Tetrahymena* telomerase, the *Oxytricha* activity added only five to seven TTTTGGGG repeats onto telomeric primers. In this study, we analyzed telomerase activity from the developing macronuclei of another hypotrichous ciliate, *E. crassus*, to determine whether this apparent difference in telomerase activity reflects the comparatively shorter telomeres of the hypotrichs.

*E. crassus* was cultured under nonsterile conditions, using the alga *Dunalie1la salina* as the live food source (12). After 1 to 2 days of starvation, cell mating was initiated by mixing starved cultures of two mating types, cc 51(mtB mt1:III) and cc 55(mt1 mt1:1). Developing macronuclei were isolated 60 h after mating (12), the time when chromosomal fragmentation and telomere addition occur under our culture conditions (S. Tausta and L. Klobutcher, personal communication). Cells from 20 liters of mated cultures were collected on 15-μm-pore-size Nitex filters (Tetko), concentrated to 200 ml, and centrifuged at 100 × g for 3 min in an IEC clinical centrifuge. The cell pellet was suspended in 10 mM Tris (pH 7.5)-0.05% spermidine phosphate-1 mM phenylmethylsulfonyl fluoride—10 μM pepstatin—40 U of RNasin (Promega Biotech, Milwaukee, Wis.) per ml. After addition of Triton X-100 to a final concentration of 0.5%, the sample was mixed for approximately 5 s and then transferred to a sterile Dounce homogenizer. Cells were broken at room temperature with 5 to 10 strokes, and lysis was monitored by microscopy. After filtration through a 25-μm-pore-size Nitex filter to remove cell ghosts and unlysed cells, the sample was purified through a 25 to 50% Percoll (Sigma Chemical Co., St. Louis, Mo.)-sucrose step gradient. The gradient was spun at 4,000 × g for 10 min at 4°C in an HB-4 rotor of a Sorvall centrifuge (Ivan Sorvall Inc., Norwalk, Conn.). Fractions containing developing macronuclei were collected by dilution 1:1 with 5% sucrose—10 mM Tris (pH 7.5)-0.05% spermidine phosphate and centrifugation as described above. The pellet was suspended in 10 to 20 volumes of TMG buffer (10 mM Tris hydrochloride [pH 7.5], 1 mM MgCl2, 10% glycerol) plus 1 mM phenylmethylsulfonyl fluoride, 10 μM pepstatin, and 40 U of RNAsin per ml, frozen, and stored in liquid nitrogen. The final concentration of developing nuclei was approximately 107/ml.

Extracts were assayed as described previously (5) except that additional MgCl2 and spermine were omitted from the reaction cocktail. Incubation of the extract in the presence of [α-32P]dGTP, cold dTTP, and a synthetic single-stranded telomeric DNA oligonucleotide resulted in elongation of the primer by the addition of many 8-base repeats (Fig. 1). The reaction worked equally well at 22°C, the optimum temperature for *Euplotes* growth, or at 30°C, the temperature at which *Tetrahymena* telomerase was assayed (Fig. 1A). Telomerase activity was found exclusively in the nuclei and did not appear to leak out: however, hypotonic lysis of the nuclei released all of the activity into the supernatant fraction (data not shown). Although the products of the *Tetrahymena* telomerase reaction (Fig. 1A and C) appeared much
more abundant than the *Euplotes* products, the *Tetrahymena* preparation contained the equivalent of 10⁷ nuclei per ml, as opposed to only 10⁷/ml for the *Euplotes* extract. Therefore, the level of telomerase activity in mated *Euplotes* nuclear extracts was comparable to that found in whole-cell S-100 preparations from mated *Tetrahymena* cells.

To determine the total number of TTTTGGGG repeats added, the *Euplotes* telomerase products were assayed on an alkaline agarose gel (10). Products with lengths of up to 400 nucleotides (50 repeats) were detected, although the major products ranged from approximately 75 to 300 bases (Fig. 2). These data indicate that the *Euplotes* telomerase is highly regulated in vivo, since telomeres at this stage of development are approximately 80 base pairs long and are subsequently shortened and stably maintained at 28 base pairs plus a 14-base 3' protruding tail during vegetative divisions (13).

The substrate specificity for the *Euplotes* telomerase activity was similar to that of the *Tetrahymena* telomerase (5); both the structure of the telomere substrate and its 3'-terminal sequence were recognized. Primers that differed in their 3'-end sequences were tested to determine whether the permutation of the input oligonucleotide was perceived. The pattern of bands repeated with an 8-base periodicity in the elongation products was relatively shifted up or down, depending on the 3' sequence of the three input telomeric primers of identical lengths and repeat units (Fig. 1B and 3). Oligonucleotide primers ending with less than four G residues (5'-GTTGAGGTTTGGGTTGGG-3') or less than four T residues (5'-GGGGTTGGGTTGGGTTTTG-3') were elongated by the appropriate number of nucleotides to create a complete *Euplotes* telomeric repeat, GGGGTGTT, or TTTTGGGG before additional repeats were added. These results show clearly that the *Euplotes* telomerase activity, like that of *T. thermophila* (5), adds both G and T residues one nucleotide at a time to the 3' terminus of the telomeric primer rather than adding them as a single block.

The *Euplotes* activity also recognized the structure of the telomeric substrate. G-rich telomeric sequences from a variety of organisms were elongated by the addition of many 8-base repeats (Fig. 1C).

Like the *Tetrahymena* telomerase, the *Euplotes* activity contained an essential RNA component (Fig. 1D). The difference between the telomeric sequences synthesized by these two activities may be specified by the RNA moiety, since a potential templating sequence has been identified in the *Tetrahymena* telomerase RNA species (6). Preliminary experiments to identify the *Euplotes* telomerase RNA gene by cross-hybridization with *Tetrahymena* telomerase RNA indicate that the primary sequence of the telomerase RNA is not highly conserved.

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LITERATURE CITED