ATP-dependent Processivity of a Telomerase Activity from Saccharomyces cerevisiae*

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Extracts of Saccharomyces cerevisiae were shown to support the elongation of oligodeoxynucleotides with telomere-like sequences. The primer sequence specificity of this elongation activity, its incorporation of dG and dT but not dA or dC from the corresponding triphosphates, and its sensitivity to RNase A and RNase H are all consistent with it being a telomerase. In contrast to the reported properties of other telomerases, the presence of ATP enhances the efficiency of initiation of the yeast enzyme and improves its processivity. Hydrolysis of ATP appears to be unnecessary for the observed effects, as the βγ-imido or the γ-thio derivative of ATP is nearly as effective.

Telomerase is a ribonucleoprotein complex that adds GT-rich repeats to oligomer or polymeric deoxynucleotide primers with telomere-like sequences at their 3′ ends (1). Because the enzyme uses an integral RNA component as the template in the synthesis of the repetitive deoxynucleotide sequences, it can be considered a specialized reverse transcriptase. Telomerase is necessary for maintaining the length and integrity of telomeres, which in turn have a variety of important physiologic functions, including the stabilization of chromosome ends against illegitimate recombination, the repression of telomere-proximal genes, and the contribution to chromosome organization inside the nucleus (2).

Telomerase has been identified in a number of organisms, including ciliated protozoa, mouse, and human (3–5). The RNA component of the enzyme complex has been cloned from several protozoa (6–9) and more recently from yeast (10), and genes encoding two polypeptide components of Tetrahymena telomerase were recently cloned (11). In addition to being characterized at the gene level, the relative abundance of the EST1 protein in their amino acid sequences. Despite the rapid accumulation of information on yeast genes that affect telomeres, study of yeast telomerase to date has been hampered by the lack of a suitable in vitro assay. We describe such an assay in this report and show that the yeast enzyme exhibits an ATP dependence that was unknown for telomerases previously characterized.

EXPERIMENTAL PROCEDURES

Yeast Strains, Buffers, and Primers—Yeast strain BCY123 (a pep4::HIS3 prb1::LEU2 bar1::HISG lys2::GAL1/10-GAL4 can1 ade2 trpl1 trp2-3 his3 leu2-3, 112 cir+ GAL1+ RAF1+ SUC+ ) was acquired from B. Cairnes, Stanford University. The following protease inhibitor mixture was used in all buffers: 1 mM phenylmethylsulfonyl fluoride, 2 mM benzamide, 2 μM pepstatin A, and 1 μg/ml leupeptin. Buffer A contains 20 mM HEPES-NaOH, pH 7.6, 1 mM magnesium acetate, 20% glycerol (v/v), 1 mM DTT. Buffer A(500) contains 20 mM HEPES-NaOH, pH 7.3, 3 mM magnesium acetate, 60% glycerol (v/v), 3 mM DTT. The following oligodeoxynucleotide primers were used as substrates or for RNase H inactivation (contiguous regions complementary to the TLC1 RNA template are underlined): TEL1, TGGGTTGTGGTGGTGGTTG; TEL2, TGGGTTGTGGTGGTGGTTG; TEL3, TGGGTTGTGGTGGTGGTTG; TEL4, TGGGTTGTGGTGGTGGTTG; TEL5, GGTTGTGGTGGTGGTG; TEL6, TGGGTTGTGGTGGTGGTG; TEL7, TGGGTTGTGGTGGTGGTG; TELCA, CCACCACCCACCCCA; TELH1, GGTTGTGGTGGTGGTG.

Yeast Extracts and Protein Fractions—Nuclear extracts were prepared as described (19). Whole cell extracts were prepared as follows. BCY123 cells were grown in YPD medium to late log phase (~5 × 10^7 cells/ml) and harvested by centrifugation. The cell pellets were washed once in ice-cold water and resuspended with 1 ml of lysis buffer per 2 g of cell paste. An equal volume of glass beads (Sigma, 400–600 μm) was added to the cell suspension, and the resulting mixture was stirred in a bead beater (Biospec). The bead beater chamber was surrounded by an icesalt mixture kept at −10 °C, and ten 30-s pulses of stirring interspersed by a 2.5-min cooling period in between were applied. Cell lysates were collected and cleared by two successive centrifugations: first in a GSA rotor (Sorval) at 9,000 rpm for 10 min and then in a Ti 60 rotor (Beckman) at 50,000 rpm for 1 h. A typical extract had a protein concentration of about 50 mg/ml.

Partial Purification of Yeast Telomerases—Telomerase activity was partially purified by passage through a POROS 50 HS (Perseptive Biosystems) column. The column was equilibrated in buffer A(100). Extracts were adjusted with 5 mM potassium acetate to a conductivity equivalent to buffer A(100), loaded onto the column, and eluted stepwise with buffer A(500) and buffer A(1500). The bulk of the telomerase activity was eluted at the buffer A(1500) step, resulting in a 300-fold enrichment of activity. All telomerase assays shown in the figures were done with about 1 μg of the partially purified telomerase fraction.

Yeast Telomerase Assays—A typical in vitro telomerase reaction was carried out in 25 μl containing the following: 50 mM HEPES-NaOH, pH 7.5, 4 mM magnesium glutamate, 30 mM potassium glutamate, 0.1 mM EDTA, 1 mM DTT, 5% glycerol (v/v), 5 mM dTTP, 20 μCi of [α-^32P]dGTP (DuPont NEN, 3000 Ci/mM), 1 mM ATP, 5 μM primer oligodeoxynucleotide, and about 1 μg of partially purified fractions. Following incubation at 22 °C for 1.5 h, the reaction was stopped with 80 μl of 10 mM Tris-HCl, 1 The abbreviations used are: DTT, dithiothreitol; AMPPNP, adenosine 5′-(β,γ-imido)triphosphate; ATPγS, adenosine 5′-O-(thiotriphosphate).

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Yeast Telomerase

RESULTS

Identification of Yeast Telomerase Activity—The approach employed for detecting yeast telomerase activity generally followed that utilized in an earlier effort aimed at detecting accurate initiation by yeast RNA polymerase II in vitro (19).

Nuclear extracts were used initially as the source of telomerase to minimize possible interference or inhibition by cytoplasmic contaminants. For the deoxynucleotide primers, GT-rich oligonucleotides complementary to the TLC1 template RNA were tried on the assumption that this might result in better recognition by the telomerase complex. ATP and an energy-regenerating system were included in initial reactions in accordance with earlier transcription assays, even though in previous studies of telomerases no ATP involvement was implicated. The inclusion of ATP proved beneficial in the detection of yeast telomerase activity (see below). Once primer elongation activity in yeast cell extracts became detectable, optimization of reaction conditions and improvements in the extract preparation were carried out (see “Experimental Procedures” for protocols currently in use).

Deoxynucleotide Dependence of the Putative Yeast Telomerase Activity—A primer extension activity was detected in yeast extracts and active chromatographic fractions of the extracts. No products were obtained in the absence of primers (see Fig. 1, lane 1). The products can be labeled by [\(\alpha^{32}\text{P}\)]dATP if cold dGTP is included or by [\(\alpha^{32}\text{P}\)]dGTP if cold dTTP is included (Fig. 1, lanes 1 and 4). Neither deoxynucleotide triphosphate alone suffices for significant extension (Fig. 1, lanes 2 and 5).

Interestingly, the extension products were shorter when [\(\alpha^{32}\text{P}\)]dTTP was used than when [\(\alpha^{32}\text{P}\)]dGTP was used. Because \(\alpha^{32}\text{P}\)-labeled nucleotides were included at low concentrations, the difference in product length probably reflects a higher \(K_{\text{m}}\) of the enzyme for dTTP. Neither [\(\alpha^{32}\text{P}\)]dCTP nor [\(\alpha^{32}\text{P}\)]dATP can be incorporated even in the presence of cold dGTP and dTTP. Thus, the primer extension products consist exclusively of dGMP and dTMP, consistent with being formed through the action of a yeast telomerase.

RNase A and RNase H Inactivation of the Yeast Telomerase Activity—Since all telomerases characterized to date are ribonucleoprotein complexes, we tested the yeast activity for sensitivity to RNase A. As shown in Fig. 2, preincubation of the telomerase fraction in the presence of RNase A abolishes subsequent primer elongation (lane 4), whereas the inclusion of an RNase inhibitor during preincubation antagonizes the effect of RNase A (lane 3). In addition, heat or proteinase K treatment abolishes primer elongation activity (data not shown), suggesting that the yeast activity requires both an RNA and a protein component(s).

To demonstrate a specific requirement of the yeast activity for TLC1 RNA, the RNA component of yeast telomerase (10), we pretreated the telomerase fraction with oligodeoxynucleotides complementary to TLC1 RNA and RNase H. The primer TEL2, which has a shorter stretch of sequence complementary to the template RNA than TEL1, was used in these assays to minimize substrate-dependent degradation of TLC1 RNA. As shown in Fig. 2, RNase H completely abolishes telomerase activity in the presence of an 18-mer antisense oligonucleotide TELH1 (lane 2), which is complementary to the template region of TLC1 but not in the presence of a noncomplementary oligodeoxynucleotide TELCA of the same length (lane 1).

Primer Specificity of Yeast Telomerase—As indicated in Fig. 3, a number of GT-rich oligonucleotides can serve as primers for extension by yeast telomerase. The length of sequence complementarity between the primer and TLC1 RNA appears to correlate with the ability of the primer to serve as substrate for extension by the activity. For example, TEL1 and TEL4 (13-nucleotide complementarity) are more proficient than TEL2 (10-nucleotide complementarity) or TEL3 and TEL5 (9-nucleotide complementarity). For oligodeoxynucleotides longer than 11-mer with the same length of nucleotides complementary to TLC1 RNA, the priming proficiency is insensitive to the total length of the primer: the 17-mer TEL4 is slightly more proficient than the 21-mer TEL1, both of which contain a 13-nucleotide stretch complementary to TLC1, and the 23-mer TEL5 is comparable with the 17-mer TEL3, both of which contain a 9-nucleotide stretch complementary to TLC1 RNA. In contrast to the Tetrahymena enzyme, the yeast telomerase can act processively on a primer as short as 8 nucleotides (TEL7),...
although the extent of the reaction is reduced by about 10-fold relative to the 17-mer TEL4.

ATP Modulation of Yeast Telomerase Activity—As shown in Fig. 4, the presence of ATP has strong effects on primer extension by the yeast activity. The average length of the extension products is much shorter in the absence of ATP (40 nucleotides compared with 250 nucleotides in the presence of ATP), and the overall number of extended chains is lower by 2-fold. The stimulatory effects of ATP can be fully substituted by the β,γ-imido analog of ATP (AMPPNP). Enhancement of activity by AMPPNP was evident at 50 μM concentration of the nucleotide, suggesting that its effects are not a result of ATP contamination. The γ-thio analog of ATP (ATPγS) was also effective. Interestingly, in the presence of dATP, the extension products were longer, but the overall reaction in terms of dGMP incorporation was inhibited by 15-fold relative to the ATP-stimulated reaction. The effectiveness of AMPPNP and ATPγS in the primer extension reaction suggests that ATP binding to the telomerase complex and/or its associated factor(s), rather than ATP hydrolysis by the assembly, is responsible for the observed effects. ATP binding appears to modulate telomerase activity in two ways, increasing both the number of productive initiation by the yeast enzyme and its processivity. A high concentration of dATP appears to inhibit the binding/initiation effect while maintaining the processivity of yeast telomerase. Other ribonucleotide triphosphates cannot function in place of ATP (data not shown).

**DISCUSSION**

We have identified and partially purified from yeast extracts an activity that fulfills several criteria for being a telomerase. In particular, the inactivation of the activity by RNase H in the presence of an antisense oligodeoxynucleotide complementary to the TLC1 template RNA indicates that primer elongation is specifically dependent on the RNA component of the yeast telomerase. Our finding therefore opens up the prospect for a combined biochemical and genetic analysis of yeast telomerase.

The yeast telomeric repeats are more irregular in sequence in comparison with repeats in many other organisms such as...
human and Tetrahymena (20). In the case of the latter two enzymes, the regularity of the repeat and the presence of preferred pulse sites within them give rise to elongation products that are integral multiples of the repeat unit (3, 5). Not surprisingly, such a pattern was not readily discernible in the yeast enzyme reaction products. Nevertheless, an interesting question with regard to the yeast enzyme is whether a unique sequence is generated in a single round of extension of a particular primer. It is evident that the yeast enzyme preferentially generates products of specific lengths. If this reflects preferential pulsing or termination by the enzyme at a particular template position, then our results are consistent with the hypothesis that a unique sequence is made. This can be confirmed by cloning and sequencing the in vitro products of the activity.

One unexpected finding in this work is the role of ATP in the modulation of yeast telomerase activity, which has not been previously observed in the characterization of other telomerases. Since ATP binding appears to suffice, the observed effects are unlikely to be due to a catalytic helicase that exposes single-stranded substrate for telomerase binding. ATP binding could, for example, trigger a conformational change in the telomerase ribonucleoprotein complex or its accessory protein(s) to lower the dissociation rate of the complex. In contrast to the yeast enzyme, human and Tetrahymena telomerases are processive in the absence of ATP (3, 5), and the mouse enzyme reportedly acts distributively even in the presence of ATP (4). It is possible that modulation of telomerase activity by ATP is unique to the yeast enzyme. However, in the case of the human enzyme, interpretation of the experimental data might have been complicated by the presence of dATP, which was needed as a substrate for the synthesis of human telomeric repeats (5). As shown in this work, dATP is an effective substitute for ATP in maintaining the processivity of the yeast enzyme and thus might act in similar ways in the human enzyme-catalyzed reaction.

Note Added in Proof—Two papers have appeared after the submission of this communication (Lin, J.-J., and Zakian, V. A. (1995) Cell 81, 1127–1135; Cohn, M., and Blackburn, E. H. (1995) Science 269, 396–400) in which the detection of telomerase activity in yeast cell extracts or partially purified fractions was reported.

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