Functional Reconstitution of Human Telomerase Expressed in Saccharomyces cerevisiae

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Telomerase is a ribonucleoprotein enzyme complex that adds DNA repeats at the ends of chromosomes. In an effort to establish an in vivo heterologous expression system for active human telomerase, we expressed human telomerase reverse transcriptase (hTERT) in Saccharomyces cerevisiae and affinity-purified the protein as a fusion with glutathione S-transferase (GST). Addition of the GST moiety to the N terminus of hTERT did not interfere with telomerase activity when GST-hTERT was expressed in rabbit reticulocyte lysate (RRL) in the presence of the human telomerase RNA (hTR). Active human telomerase was immunoprecipitated from yeast lysates that co-expressed GST-hTERT and hTR. In addition, telomerase activity could be reconstituted in vitro by the addition of hTR to GST-hTERT that was immunoprecipitated from either RRL or S. cerevisiae lysates. The expression and reconstitution of human telomerase activity in yeast will provide powerful biochemical and genetic tools to study the various components required for the assembly and function of this enzyme.

Eukaryotic cells possess linear chromosomes that are predicted to lose terminal sequences after every DNA replication event (1, 2). To circumvent this end replication problem, most eukaryotic cells possess a ribonucleoprotein (RNP)1 enzyme, telomerase, which uses its RNA template to synthesize DNA repeats at the ends of chromosomes (3–5). The protein and RNA components of this enzyme complex have been identified in several organisms from yeast to humans (3, 6). In humans, the catalytic subunit of telomerase (hTERT, for human telomerase reverse transcriptase) is a 127-kDa protein with distinctive motifs common to reverse transcriptases (RT) as well as a telomerase-specific (T) motif (7–10).

The first in vitro reconstitution system used to study human telomerase consisted of adding recombinantly synthesized human telomerase RNA (hTR) to partially purified, micrococcal nuclease-treated 293 cell extracts (11). More recently, human and Tetrahymena thermophila telomerase reconstitution has been accomplished by the in vitro transcription and translation of the protein catalytic component (hTERT and p133, respectively) in the presence of telomerase RNA in rabbit reticulocyte lysates (RRL) (12–14). Studies using RRL-reconstituted telomerase suggest that hTERT and hTR are the only two components necessary to reconstitute human telomerase activity in vitro. Interaction of the telomerase-associated protein (TP1) with telomerase, which is observed in mouse cell extracts, was not seen in RRL (13, 15). However, several lines of evidence suggest that proteins in the RRL are necessary for the assembly of a functional telomerase enzyme. Using the yeast two-hybrid system, Holt and co-workers (16) identified a chaperone protein, p23, that interacts with hTERT and that could be associated with a complex called “the foldosome,” important for ribonucleoprotein assembly. In addition, a recent study on the role of the T. thermophila telomerase RNA in telomerase function suggests that proteins other than the catalytic subunit are necessary for the functional assembly of this RNP in rabbit reticulocyte lysates (17).

Telomerase is active in most transformed and tumor cell lines, whereas the majority of normal diploid cells demonstrate no detectable telomerase activity (1, 18, 19). Consequently, the telomerase enzyme has become an attractive target for chemotherapy. A better understanding of the biogenesis and structure of this RNP complex will therefore be necessary to evaluate the role of telomerase in cellular immortalization and cancer. Reconstitution of the telomerase RNP by overexpression of its components in heterologous organisms will be crucial to the study of the molecular mechanisms of this enzyme. In this study, we investigated whether human telomerase activity could be reconstituted by expressing hTERT and hTR in Saccharomyces cerevisiae. We describe the expression and glutathione-Sepharose affinity purification of the human telomerase catalytic subunit as a fusion to glutathione S-transferase (GST) in yeast. In addition, we demonstrate that S. cerevisiae is capable of reconstituting a functional human telomerase enzyme when GST-hTERT and hTR are co-expressed.

MATERIALS AND METHODS

Yeast Strain—Yeast strain eLABY886 (MATa, leu2, Ura3–32, his3, pra1, prw2, prc1, cps) was used as a host strain for hTERT protein expression and also as a source of yeast cell lysate (20).

Construction of Plasmids—Clone 712562, containing bases 3–52, was used to generate a unique restriction site in the hTR cDNA (11). A new construct (phTRTDNC) was sequenced using an Applied Biosys-
tems automatic sequence to confirm the presence of the mutation.

To generate the hTERT expression plasmids, the htert gene from pGRN121 (7) was amplified by PCR with the 5′ primer 5′-TGCTCTA-GACCCCGGCGTCTCCCCGC-3′ and the 3′ primer 5′-CTTGGGTCGTGGGGTGCTGAC-3′ containing XhoI and HindIII sites, respectively, as well as with the 5′ primer 5′-CCGAACCTGGTCGGTGTCATGC-3′ containing XhoI and HindIII sites. The 5′-CCGAACCTGGTCGGTGTCATGC-3′ and the 3′ primer 5′-GAATCGGGCGCGTGTCGAC-3′ containing EcoRI and NotI sites, respectively. The XhoI-HindIII- and EcoRI-NotI-digested PCR fragments were then cloned in pEGKT (22) digested with XhoI-HindIII and in pET-28b (Novagen) digested with EcoRI-NotI, respectively. To generate the hTR DDBN yeast expression construct, a 2.5-kilobase MsiI-HindIII fragment of pEGKT-hTERT was replaced with a MluI-HindIII fragment from a derivative of pHTTNDNC containing the D868N mutation. To generate the pET-28a-GST-hTERT construct, the pEGKT-hTERT plasmid was linearized by digestion with HindIII. This linearized construct was subjected to partial digestion by the SacI restriction enzyme using a standard method (23). This resulted in a 4150-bp GST-hTERT DNA fragment, which was gel-purified and cloned into the SacI-HindIII sites of pET-28a (Novagen). To construct the hTR yeast expression vector, the hTR gene was amplified by PCR from pGRN33 (24) using the 5′ primer 5′-CGCCGATCCGGCGCGCCCCGCGC-3′ and the 3′ primer 5′-CGCGGATCCGGCGCAGCGCACCGGGTTGCGG-3′, both containing BamHI restriction sites. The BamHI-digested PCR fragment was cloned into the BamHI site of the p413-GAL1 vector (25).

Protein Expression and Affinity Purification—Yeast containing the different constructs were grown in selective medium containing 2% raffinose to an A600nm of 0.6–0.8. To induce transcription from the GAL1 promoter, galactose was added to a final concentration of 4%, and growth continued for 12–16 h. Cells were harvested, washed with sterile water, and resuspended in either radioimmune precipitation buffer (RIPA) or TMG buffer (26). Because of the inefficiency of the yeast protein extraction, 25 ml of a yeast culture was lysed by vortexing with glass beads for 6 pulses of 30 s with at least a 1-min interval (4 °C) between each pulse. After removal of the glass beads and cell debris by two centrifugations (10,000 × g) at 4 °C, the specific proteins in the lysates were affinity-purified by the addition of glutathione-Sepharose (Amersham Pharmacia Biotech) for 2 h at 4 °C, washed four times with lysis buffer (supplemented with NaCl to a final concentration of 0.5 M), and subjected to SDS-PAGE for either Coomassie Blue (Bio-Rad) staining or nitrocellulose transfer.

In Vitro Transcription and Translation—pET-28b-hTERT and pET-28a-GST-hTERT plasmids were included in coupled transcription/translation (Promega) reactions (10–15 μl) at a final concentration of 25 ng/μl for 75 μl or without 10 ng/μl of gel-purified hTERT telomerase RNA. Gel-purified human and T. thermophila telomerase RNA were generated as described previously (11, 27).

Immunoprecipitations—Immunoprecipitations were performed using yeast protein lysates (in TMG buffer) or 2–3 μl of reticulocyte lysate previously diluted into 500 μl of Buffer A (11). After a 1-h incubation at 4 °C with preimmune serum, lysates were subjected to immunoprecipitation with specific antibodies (anti-GST from Amersham Pharmacia Biotech; anti-T7 from Novagen; anti-MYC from Invitrogen) for 1 h at 4 °C. This was followed by incubation with pre-washed and lysis buffer-pre-equilibrated protein A-Sepharose (Amersham Pharmacia Biotech) for an additional 2 h. Antibody-coated beads were then washed four times with the respective lysis buffer and subjected to SDS-PAGE/Western blot analysis, TRAP assay, and RT-PCR.

Telomerase Activity Assays—Telomerase activity was assayed by a two-tube telomerase assay (modified TRAP) as described previously (11), with minor modifications; PCR reactions were performed for 25 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min 30 s, using Taq polymerase from MBI Fermentas. ACX, TSNT, and NT primers (28) were used at a final concentration of 0.4 pmol/μl, 2 × 10−3 M, and 20 fmol/μl, respectively. Amplification of TSNT by TS and NT primers generated a 36-bp PCR internal control (IC). The positive control used in TRAP assays consisted of partially purified 293 cell extracts prepared as described previously (11).

RESULTS AND DISCUSSION

Expression and Activity of a GST-hTERT Fusion in Rabbit Reticulocyte Lysates—Since the cloning of the gene encoding the catalytic subunit of the human telomerase enzyme, reconstruction of enzymatic activity has been achieved by expressing hTERT in the presence of hTR in rabbit reticulocyte lysates (12, 13). To extend the study of human telomerase, we expressed a GST-tagged hTERT in yeast (Fig. 2). To first determine whether the GST moiety fused to the N terminus of hTERT interfered with telomerase function, the entire GST-hTERT coding sequence from pEGKT-hTERT was cloned into a T7 expression vector (see “Materials and Methods”). This GST-hTERT construct was transcribed and translated in a RRL in the presence of hTR and was immunoprecipitated using a goat anti-GST antibody (Fig. 1B, lane 15). The immunoprecipitate was then assayed for telomerase activity using a modified TRAP assay (see “Materials and Methods”). The GST-hTERT/hTR complex was active in the telomerase assay (Fig. 1A, lane 15), whereas no telomerase activity was detected when a T7-tagged hTERT/hTR complex was subjected to immunoprecipitation using the same anti-GST antibody (Fig. 1A, lane 14). As expected, immunoprecipitation of hTERT or GST-hTERT proteins synthesized in the absence of hTR did not reconstitute telomerase activity (Fig. 1A, lanes 7, 8, and 13), although these proteins were expressed (Fig. 1B). Immunoprecipitates of hTERT-hTR or GST-hTERT/hTR complexes were also prepared with a monoclonal antibody recognizing a small tag (T7 tag) fused to the N terminus of both proteins (Fig. 1B, lanes 9 and 10). Both T7-tagged immunoprecipitated complexes demonstrated telomerase activity (Fig. 1A, lanes 9 and 10). Immunoprecipitates prepared with a control anti-MYC antibody did not possess detectable telomerase activity (Fig. 1A, lanes 1–5). The results shown in Fig. 1 indicate that addition of the 25-kDa GST protein to the N terminus of the human telomerase catalytic subunit does not prevent the functional reconstitution of telomerase activity by hTERT in RRL.

Expression and Affinity Purification of the Human Telomerase Catalytic Component from S. cerevisiae—The GST-hTERT

![Fig. 1. Telomerase activity of the GST-hTERT Fusion in rabbit reticulocyte lysates. A. [3H]Sulphostidine-labelled hTERT and GST-hTERT were synthesized in a rabbit reticulocyte lysate in the presence or absence of the hTR. As a control, RRL synthesis was performed with hTR RNA only (lanes 1, 6, and 11). Aliquots from each reaction were diluted with buffer (see “Materials and Methods”) and immunoprecipitated using anti-MYC (lanes 1–5), anti-T7 (lanes 6–10), or anti-GST (lanes 11–15) antibodies. After extensive washing, one-tenth of the immunoprecipitate (IP) was assayed for telomerase activity (A), and the remainder of the beads were subjected to SDS-PAGE and autoradiography (B). 293, 0.1 μg of partially purified 293 cell extract; IC, internal PCR control for the TRAP assay.](Image)
Reconstitution of Human Telomerase Activity by Co-expression of hTERT and hTR in S. cerevisiae—As the GST-hTERT fusion expressed in RRL was functional and reconstituted human telomerase activity (Fig. 1), we examined whether telomerase activity could be reconstituted in S. cerevisiae by co-expressing the catalytic and the RNA components. The gene encoding the hTR RNA was also cloned under the control of a GAL1 promoter. This construct was transformed into yeast cells expressing GST, wild-type GST-hTERT, or GST-hTERT with a point mutation at amino acid 868 that changes a conserved Asp868 residue to an asparagine in motif C of hTERT. Following selection of the double transformants, we examined the expression of hTR in yeast that were grown in medium containing galactose. Reverse transcription and polymerase chain reaction (RT-PCR) on total yeast RNA using hTR-specific primers confirmed the presence of hTR in yeast transformed with the hTR-expressing construct; this specific RNA was not detected in control yeast transformed with the vector alone (data not shown).

Once an inducible system for co-expression of hTERT and hTR in S. cerevisiae was established, cell lysates were prepared from yeast grown in selective media containing galactose or glucose. Yeast cell lysates were subjected to immunoprecipitation using an anti-GST serum, and the immunoprecipitates were analyzed for telomerase activity (Fig. 3A). Immunoprecipitates from two independent yeast clones co-expressing the wild-type GST-hTERT and hTR were positive for telomerase activity as analyzed by the TRAP assay (Fig. 3A, lanes 3 and 4). To ensure that the activity was due to hTERT rather than a co-immunoprecipitating protein, the D868N hTERT mutant was also immunoprecipitated and was shown to lack telomerase activity (Fig. 3A, lane 6). The lack of telomerase activity observed for this active site point mutant suggests that the activity observed in lanes 3 and 4 is attributable to hTERT and also confirms that the integrity of motif C is essential for activity (12, 13, 29, 30). RT-PCR analysis of the immunoprecipitates revealed that hTR was specifically associated with the reconstituted telomerase (Fig. 3C, lanes 3 and 4), as well as with the D868N GST-hTERT point mutant (lane 6). RT-PCR analysis of immunoprecipitates from control yeast that expressed GST alone did not detect hTR (Fig. 3C, lane 7).

Reconstitution of telomerase activity as well as expression of the protein and RNA components of human telomerase were only detected from yeast grown in galactose-containing medium. Growth under repressing conditions (glucose) did not induce the expression of hTERT (Fig. 3B, lane 5) or hTR (data not shown), demonstrating the specificity of the expression system. Human telomerase activity could not be detected using immunoprecipitates from yeast transformed with control vectors (Fig. 3A, lanes 1 and 7), confirming that both hTERT and hTR components were required to reconstitute human telomerase activity. The results shown in Fig. 3 demonstrate that co-expression of hTERT and hTR in S. cerevisiae specifically reconstituted human telomerase activity.

S. cerevisiae Lysate Does Not Stimulate Assembly of the GST-hTERT·hTR Complex in Vitro—Recent reports suggest that in vitro reconstitution of human and T. thermophila telomerase RNP forms requires proteins present in rabbit reticulocyte lysates (16, 17). Similarly, we asked whether the reconstitution of telomerase activity by the addition of hTR to immunoprecipitated hTERT may be dependent on proteins present in S. cerevisiae lysate. We used anti-GST serum to immunoprecipitate RRL-expressed GST-hTERT synthesized in the absence of the human telomerase RNA, as well as S. cerevisiae-expressed wild-type and D868N mutant GST-hTERT, which were ex-

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**Figure 2.** Expression and affinity purification of the human telomerase catalytic subunit from S. cerevisiae. A, GST-hTERT was expressed and purified from yeast by affinity chromatography of crude extracts using glutathione-Sepharose. Lane M, molecular mass markers (in kilodaltons); lanes 5 and 10, uninduced soluble fractions; lanes 4 and 9, induced soluble fractions; lanes 3 and 8, unbound fractions from glutathione-Sepharose; lanes 2 and 7, bound fractions from uninduced yeast lysate; lanes 1 and 6, bound fractions from galactose-induced yeast lysate. B, glutathione-Sepharose-purified and crude lysate fractions from galactose-induced GST (lanes 5 and 8, respectively), uninduced (lanes 4 and 7, respectively), and galactose-induced GST (lanes 3 and 6, respectively) GST-hTERT were loaded onto a 7.5% SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted with an affinity-purified hTERT antibody (kindly provided by Dr L. Harrington). As controls, T7-hTERT (lane 1) and hTR (lane 2) synthesized and added, respectively, in a RRL were included. The 83- and 175-kDa molecular mass markers are indicated on the right. GLU, glucose; GAL, galactose.
pressed in the absence of the hTR-expressing construct. Following extensive washing, the immunoprecipitated proteins were incubated with either human or *T. thermophila* telomerase RNA in the presence or absence of a fresh yeast protein extract. After a 45-min incubation at 30 °C, reconstituted reactions were assayed for telomerase activity. The addition of fresh yeast protein extract was neither required for nor stimulated the telomerase activity reconstituted by the assembly of hTERT and hTR (Fig. 4, lane 1 versus lane 2 and lane 7 versus lane 8, respectively). Addition of *T. thermophila* telomerase RNA did not reconstitute human telomerase activity (lanes 3 and 9), highlighting the specificity of the reconstitution reaction for human telomerase RNA. Incubation of human telomerase RNA with the *S. cerevisiae*-expressed and immunoprecipitated GST-

![Image of Figure 3](Image 63x400 to 283x729)

**Fig. 3.** Reconstitution of human telomerase activity in *S. cerevisiae* by co-expression of hTERT and hTR. A, yeast transformed with the following constructs were grown and induced with galactose (except for lane 5): pEGKT-hTERT and p413 vector (lane 1); pEGKT-hTERT and p413/antisense hTR (abTR) (lane 2); pEGKT-hTERT clone 1 (C1) and p413-hTR (lane 3); pEGKT-hTERT clone 4 (C4) and p413/ hTR (lane 4); pEGKT-hTERT clone 4 (C4) and p413/hTR grown in the presence of glucose (GLU) (lane 5); pEGKT-hTERT D868N and p413/hTR (lane 6); pEGKT vector and p413/hTR (lane 7). Yeast lysates were subjected to immunoprecipitation using a goat anti-GST serum, and the immunoprecipitates (IP) were analyzed for: A, telomerase activity; B, expression of GST-hTERT by Western blot (WB); and C, expression and association of hTR with hTERT by RT-PCR using hTR-specific primers. For panels A-C, lanes 1-7 correspond to the constructs described above. In A, telomerase activity was analyzed by the TRAP assay, and 50 ng of partially purified 293 cell extract was used as a positive control (lane 8). IC represents the internal PCR control; G-hTERT, GST-hTERT. In B, a Western blot was performed using an affinity-purified anti-hTERT antibody. IgG, the immunoglobulins used during the immunoprecipitation. The position of the protein markers are indicated on the right in kDa. In C, RT-PCR was performed in the absence (−) or presence (+) of *in vitro* synthesized hTR as controls reactions. Lane M was loaded with a 100-bp ladder.

![Image of Figure 4](Image 341x513 to 521x729)

**Fig. 4.** *S. cerevisiae* lysate does not stimulate *in vitro* reconstitution of human telomerase RNP. Rabbit reticulocyte lysate (RRL)-expressed GST-hTERT (no hTR) and *S. cerevisiae*-expressed (no hTR) GST-hTERT wild-type and D868N mutant were immunoprecipitated (IP) using anti-GST serum. Immunoprecipitated proteins were washed extensively (five times) and incubated with 200 ng of either human or *T. thermophila* telomerase RNA in the presence or absence of fresh yeast protein extract. After 45 min at 30 °C, reconstitution reactions were diluted to 40 μl and assayed for telomerase activity by TRAP analysis. IC, internal PCR control; SC, *S. cerevisiae*. As a positive control, 50 ng of partially purified 293 cell extract was used (lane 10). hTERT D868N mutant did not reconstitute activity (Fig. 4, lanes 4 and 5), confirming the requirement for a catalytically active hTERT component. In addition to using immunoprecipitated GST-tagged hTERT in reconstitution reactions, we also expressed a T7-tagged hTERT in RRL, immunoprecipitated the protein with a monoclonal anti-T7 antibody, and functionally reconstituted telomerase activity by adding only hTR (data not shown). The results presented in Fig. 4 suggest that the *in vitro* assembly of a functional hTERT-hTR telomerase RNP using immunoprecipitated hTERT does not require factors from the yeast protein lysate.

We have expressed and affinity-purified the catalytic subunit of human telomerase as a GST fusion in the yeast *S. cerevisiae*. To our knowledge, this is the first report that describes the heterologous expression of a catalytically active, full-length, recombinant hTERT in a system other than rabbit reticulocyte lysate. We demonstrated that the GST-hTERT fusion functions to reconstitute human telomerase activity in RRL when synthesized in the presence of recombinant human telomerase RNA. We also showed that co-expression of GST-hTERT and hTR in *S. cerevisiae* produced a telomerase enzyme complex that was catalytically active *in vitro*.

Quantification of the relative telomerase activity reconstituted by the addition of *in vitro* synthesized hTR to affinity-purified GST-hTERT generated from yeast or RRL indicate that comparable amounts (as determined by silver staining) of GST-hTERT immunoprecipitated from yeast or RRL yield similar levels of telomerase activity (Fig. 4 and data not shown). However, the amount of active hTERT that can be generated in yeast is limited only by the amount of yeast cultured and the efficiency of the protein extraction, making the yeast system a more abundant and practical source of active human telomerase than RRL. Preliminary attempts to reconstitute human telomerase activity using the soluble recombinant GST-hTERT fusion expressed from yeast and *in vitro* synthesized hTR were unsuccessful (data not shown). We found that the GST-
hTERT-hTR complex synthesized in RRL and precipitated with glutathione-Sepharose had significantly less telomerase activity than the same complex precipitated with an anti-GST antibody (data not shown; Fig. 1A, lane 15), suggesting that the active conformation of the GST-hTERT fusion may be altered upon binding to glutathione.

Recent in vitro studies indicate that the assembly of a functional telomerase RNP enzyme requires specific protein components (16, 17). Two chaperone proteins, p23 and Hsp90, were suggested to be important in the assembly of the human telomerase (16).

The ability to reconstitute a functional telomerase RNP in the yeast *S. cerevisiae* (Fig. 3) suggests that the cellular machinery required for the folding and/or assembly of the human telomerase catalytic subunit with its specific hTR may have been conserved through evolution. However, reconstitution of telomerase activity in the absence of yeast protein extracts using recombinantly synthesized hTR and immunoprecipitated GST-hTERT, expressed from either RRL or *S. cerevisiae* (Fig. 4), suggests that yeast proteins may not be essential for human telomerase RNP assembly and activity in vitro. It is possible that proteins important for the assembly of the telomerase RNP in vivo were co-immunoprecipitated with the GST-hTERT protein. It is also conceivable that nonphysiological in vitro levels of both hTERT and hTR can overcome an in vivo requirement for factors that assemble the human telomerase RNP. In vitro reconstitution of *T. thermophila* telomerase using immunoprecipitated p133 (catalytic subunit of *T. thermophila*) expressed in RRL and *Tetrahymena* telomerase RNA is dependent on proteins from the rabbit reticulocyte lysate, suggesting that human and *T. thermophila* telomerase may require different factors for RNP assembly (17).

We have shown that active human telomerase can be extracted from yeast co-expressing the hTERT and hTR components. Others have changed the endogenous yeast telomerase RNA template to a human telomerase RNA template to dictate the synthesis of TTAGGG repeats at yeast telomeres without growth impairment (31). Whether the functional replacement of yeast telomerase by human telomerase is possible in vivo is presently under investigation. The in vivo expression of a catalytically active recombinant hTERT in *S. cerevisiae* will be useful for future genetic and biochemical studies.

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**REFERENCES**

1. Autenrieth, C. & Greider, C. W. (1996) Trends Biochem. Sci. 21, 387–391
2. Reddel, R. R. (1998) BioEssays 20, 977–984
3. O'Reilly, M., Teichmann, S. A. & Rhodes, D. (1999) Curr. Opin. Struct. Biol. 9, 56–65
4. Porath, J.-L. & DeBaryshe, P. G. (1999) Chromosoma 108, 73–82
5. Colgin, L. I. & Reddel, R. R. (1999) Curr. Opin. Genet. Dev. 9, 97–103
6. Nugent, C. I. & Lundblad, V. (1998) Genes Dev. 12, 1073–1085
7. Nakamura, T. M., Morin, G. B., Chapman, K. B., Weinrich, S. L., Andrews, W. H., Lingner, J., Harley, C. B. & Cech, T. R. (1997) Science 277, 955–959
8. Meyerson, M., Counter, C. M., Eaton, E. N., Ellisen, L. W., Steiner, P., Dickinson, S. C., Ziaugra, L., Beijersbergen, R. L., Davidoff, M. J., Liu, Q., Barchetti, S., Haber, D. A. & Weinberg, R. A. (1997) Cell 90, 785–795
9. Harrington, L., Zhou, W., McPhail, T., Oulton, R., Yeung, D. K. S., Mar, V., Bass, M. B. & Robinson, M. O. (1997) Genes Dev. 11, 3109–3115
10. Kilian, A., Bowtell, D. D. L., Ahmad, H. E., Hine, G. R., Venter, D. J., Keese, P. K., Duncan, L. E., Reddel, R. R. & Jefferson, R. A. (1997) Hum. Mol. Genet. 6, 2011–2019
11. Autenrieth, C., Pruzan, R., Funk, W. D. & Greider, C. W. (1996) EMBO J. 15, 5928–5935
12. Weinrich, S. L., Pruzan, R., Ma, L., Ouellette, M., Tesmer, V. M., Holt, S. E., Bodnar, A. G., Lichtsteiner, S., Kim, N. W., Trager, J. B., Taylor, R. D., Carlos, R., Andrews, W. H., Wright, W. E., Shay, J. W., Harley, C. R. & Morin, G. B. (1997) Nat. Genet. 17, 498–502
13. Beattie, T. L., Zhou, W., Robinson, M. O. & Harrington, L. (1998) Curr. Biol. 8, 177–180
14. Collins, K. & Gandhi, L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8455–8459
15. Harrington, L., McPhail, T., Mar, V., Zhou, W., Oulton, R., Bass, M. B., Arruda, I. & Robinson, M. O. (1997) Science 275, 973–977
16. Holt, S. E., Aigner, D. L., Baur, J., Tesmer, V. M., Dy, M., Ouellette, M., Trager, J. B., Morin, G. M., Toft, D. O., Shay, J. W., Wright, W. E. & White, M. A. (1999) Genes Dev. 13, 817–826
17. Licht, J. D. & Collins, K. (1999) Genes Dev. 13, 1116–1125
18. Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. C. L., Civinici, G. M., Wright, W. E., Weinrich, S. L. & Shay, J. W. (1994) Science 266, 2011–2015
19. Shay, J. W. & Bacchetti, S. (1997) Eur. J. Cancer 33, 787–791
20. Dubois, N., Colina, A. R., Aumont, F., Belhumeur, P. & de Repentigny, L. (1998) Microbiology 144, 2299–3110
21. Lederberg, G. G., Aufray, C., Polymerepolous, M. & Sours, M. B. (1996) Genomics 33, 151–152
22. Mitchell, D. A., Marshall, T. K. & Deschenes, R. J. (1993) Yeast 9, 715–723
23. Ausubel, F. M., Brent, R., Kingston, R. Moore, D., Seidman, J. G., Smith, J. A. & Struhl, K. (1998) Current Protocols in Molecular Biology, Wiley and Sons, Inc., New York
24. Feng, J., Funk, W. D., Wang, S.-S., Weinrich, S. L., Avilion, A. A., Chiu, C.-P., Adams, R. R., Chang, E., Alshepp, E. C., Yu, J., Le, S. W., West, M. D., Harley, C. B., Andrews, W. H., Greider, C. W. & Villeponteau, B. (1995) Science 269, 1236–1241
25. Mumberg, D., Muller, R. & Funk, M. (1994) Nucleic Acids Res. 22, 5767–5768
26. Cohn, M. & Blackburn, E. H. (1995) Science 269, 396–400
27. Autenrieth, C. & Greider, C. W. (1994) Genes Dev. 8, 561–575
28. Kim, N. W. & Wu, F. (1997) Nucleic Acids Res. 25, 2595–2597
29. Lingner, J., Hughes, T. R., Shevchenko, A., Mann, M., Lundblad, V. & Cech, T. R. (1997) Science 276, 561–567
30. Counter, C. M., Meyerson, M., Eaton, E. N. & Weinberg, R. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9202–9207
31. Henning, K. A., Moskowitz, N., Ashlock, M. A. & Liu, P. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5667–5671