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Telomerase from Yeast *Saccharomyces cerevisiae* Is Active *in vitro* as a Monomer

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Abstract—A system for isolation of yeast telomerase *via* RNA affinity tag in TLC1 RNA was developed. Streptavidin aptamer was inserted at two different positions in TLC1 RNA. Telomerase with TLC1 RNA with one of these inserts is functional *in vivo* and can be isolated by affinity chromatography *in vitro*. A telomerase preparation isolated using this technique from a strain producing two distinguishable TLC1 RNA molecules (with and without aptameric insertion) resulted in isolation of active telomerase containing only TLC1 RNA with the aptamer. Our results indicate that yeast telomerase is active *in vitro* as a monomer.

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Telomerase is a ribonucleoprotein complex that elongates 3′-overhangs at the end of telomeres. *Saccharomyces cerevisiae* telomerase contains RNA (TLC1) and at least three protein subunits crucial for telomere length maintenance, namely, Est1p, Est2p, and Est3p [1, 2]. Est2p is a catalytic reverse transcriptase subunit that in the complex with TLC1 can elongate a specific DNA primer *in vitro* [3]. Together Est2p and TLC1 form telomerase core enzyme [3]. Est1p and Est3p have been proposed to play essential regulatory roles *in vivo* [4, 5]. TLC1 contains the template for telomere repeat synthesis and binding sites for a number of proteins and can serve as a platform for the assembly of telomerase complex [6].

In spite of intensive study of telomerase, its stoichiometric composition remains unknown. Data concerning the possibility for yeast telomerase to form a dimer are contradictory in the literature. Results obtained in [7, 8] supported the idea that yeast telomerase can form a dimer. Prescott and Blackburn [7] have shown that inactive telomerase with mutated template region became functional in the presence of wild type TLC1 that resulted in the incorporation of both mutant and wild type repeat sequences into telomeres. Moreover, both oligonucleotides complementary either to wild type or mutated template were shown to be associated with each other *in vitro* in active telomerase complex isolated by affinity chromatography from a strain containing both mutant and wild type TLC1 [8]. Gipson and colleagues described the TLC1 region 1204-1209 as being responsible for telomerase dimerization [9]. However, it was shown that this region is missing in mature TLC1 [6].

Data against telomerase dimerization have also been reported [10, 11]. TLC1 with a deletion that prevents its binding to the Est2p was not found to be co-immunoprecipitated with Est2p from a strain expressing both wild type TLC1 and TLC1 with deletion, and similarly mutant TLC1 lacking Est1p binding domain was not found to be co-immunoprecipitated with Est1p from the strain that contained both mutant and wild type TLC1 as it should be if telomerase were a dimer [10]. It should be mentioned that Est1p and Est2p binding sites in TLC1 do not overlap. Co-immunoprecipitation of fragments of TLC1 with Est1p and Est2p could be detected in the presence of wild type TLC1 RNA because of indirect interaction if telomerase were a dimer. Another set of data in favor of monomer form of telomerase was obtained when a point mutation was introduced into Est2p (Est2p-ala1) that prevented its binding to Est3p but did not influence its binding to TLC1 [11]. Such telomerase was active *in vitro*, but led to senescence *in vivo*. Est3p was not co-immunoprecipitated with Est2p when both mutant and wild type

Abbreviations: RNP, ribonucleoprotein; TLC1 RNA, yeast telomerase RNA.

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protein were expressed in the cell [11] as one would expect in the case of telomerase dimerization.

Solving the problem of whether active yeast telomerase is a dimer or a monomer could lead us to understanding the mechanism of enzyme action. To answer the question whether yeast telomerase is active in dimeric or monomeric form, we have introduced a specific affinity tag (aptamer to streptavidin [12]) into telomerase RNA to a position that does not affect the telomerase function *in vivo*. Such insertion allows purification of telomerase complex *via* TLC1 and examination of the presence of two different TLC1 RNA in telomerase complex. The advantage of this approach is the possibility of isolating telomerase complex directly *via* a component of the core enzyme.

MATERIALS AND METHODS

Plasmids and strains. Strain DBY-746α (*ura3-52*, *leu2-3*,*112*, *trp1-289*, *his3-Δ1*) was obtained from Dr. M. Ter-Avanesyan (Moscow, Russia). Strain (*tlc1Δ::KAN*) was constructed from strain DBY-746 by using plasmid pFA6a-kanMX6 [13]. To insert the aptamer tag in the TLC1 RNA molecule, the *TLC1* gene was cloned to M13mp19 vector [14] from genomic DNA. By means of Kunkel mutagenesis [15] two restriction sites (*Xma*I and *Kpn*I) were inserted in regions 1005-1019 (insertion 1) and 1056-1068 (insertion 2). The DNA fragment coding the aptamer tag [12] (obtained by annealing of two complementary oligodeoxyribonucleotides OVID1 (5′-CCGGCTGGGCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGCCC-3′) and OVID2 (5′-GGCCGGGCCGGCCCGCGACTATCTTACGCACTTGCATGATTCTGGTCGGCCCAGCCGGGTAC-3′) was inserted using the *Xma*I and *Kpn*I restriction sites. Plasmids pTLCins1 and pTLCins2 were obtained from a plasmid derivative of M13mp19 containing the *TLC1* gene with insert and a plasmid pSD107 [16] (contains wild type *TLC1* gene with its endogenous promoter and terminator in vector pRS314 [17]) that was a gift from Dr. D. Gottschling (USA). Plasmid pTLC_URA was obtained by cloning a fragment of plasmid pSD107 coding for the wild type *TLC1* gene with its promoter and terminator to vector pRS316 with *URA3* marker [17] using *Not*I and *Xho*I restriction sites. Plasmid shuffling (pTLC_URA exchange for pTLCins1 or pTLCins2) was done by a classical approach [18]. As a result, we obtained the strains (*tlc1Δ::KAN*, pTLCins1) and (*tlc1Δ::KAN*, pTLCins2) expressing only TLC1 with the corresponding insertion, (*tlc1Δ::KAN*, pSD107) expressing wild type TLC1 and (*tlc1Δ::KAN*, pRS314) without TLC1, and (*tlc1Δ::KAN*, pTLCins1 + pTLC_URA) expressing wild type TLC1 and TLC1 with the insertion at the same time.

RT-PCR analysis. Whole cell RNA for RT-PCR was obtained using the RNeasy Mini Kit (Qiagen, USA) or the hot phenol extraction method [19]. Other RNA samples for RT-PCR analysis were obtained by phenol extraction, chloroform–isoamyl alcohol (24 : 1) extraction, and ethanol precipitation in the presence with 1/10 volume 3 M NaOAc and tRNA from *Escherichia coli* as a carrier (5 μg per probe). Samples containing RNA bound on streptavidin-Sepharose resin were treated with proteinase K. A 3-μl aliquot of proteinase K (20 mg/ml) and 200 μl of "stop" buffer (20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.2% SDS) were added to 50 μl of resin suspension and incubated for 1 h at 30°C. All samples were treated with DNase I (1 U/μg nucleic acids, 1 h at 37°C). After that they were extracted with phenol, chloroform–isoamyl alcohol (24 : 1), and precipitated with ethanol in the presence with 1/10 volume 3 M NaOAc. All samples were diluted in an equal amount of water, and RNA concentration was measured spectrophotometrically at 260 nm. The volume of initial extract containing 0.1-0.5 μg RNA was used for RT-PCR analysis. An equal volume of unbound fraction and corresponding volume of bound fraction corrected for TLC1 concentration in binding from extract were taken for RT-PCR analysis. RT-PCR reactions were done using the OneStep RT-PCR Kit (Qiagen). Gene-specific primers for TLC1 were P2 (5′-GTTTATTCTAGTTTTTTCCG-3′) and T8 (5′-CGAAGGCATTAGGAGAAG-3′). Gene specific primers for scR1 RNA were scr1-for (5′-GGTGGGATGGATACGTTGAG-3′) and scr1-rev (5′-TAGCCGGGACACTTCAGAAC-3′). RT-PCR products were analyzed by electrophoresis in 2% agarose gel with TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3).

Yeast phenotype analysis by dilution assay on plates. For phenotype analysis, a series of plates with increasing number of cell generations from rescue of plasmid pTLC_URA coding for wild type *TLC1* gene was obtained. A colony of yeast cells were grown in 5 ml of synthetic complete medium (SC) without tryptophan with glucose (SC – Trp) to saturation. The culture was diluted with fresh medium to A_{600} = 0.02 and continued being grown. An aliquot of diluted culture was serially diluted fivefold with fresh medium and put on SC – Trp plates. This manipulation was repeated 10 times every 24 h. Equal numbers of cells from diluted cultures were used to compare phenotype of different strains.

Affinity isolation of telomerase with streptavidin-Sepharose. Yeast cells were grown to A_{600} = 1 in 1.6-3.2 liters of SC – Trp medium with glucose. Cells were pelleted by centrifugation (5 min, 5000 rpm, 4°C, JA-10 rotor; Beckman, USA) and washed with ice-cold sterile water four times and finally with ice-cold buffer "str" for isolation (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100). The cells were disrupted mechanically with mortar and pestle in the presence of zirconium beads (0.5 μm) in liquid nitrogen and then thawed with ice-cold "str" buffer

(10 ml) with protease inhibitor Complete (Roche, Switzerland), phenylmethylsulfonyl fluoride (0.5 mM), and RNasin (40 U/ml of extract) (Helicon, Russia). Cell debris was removed by centrifugation once at 5000*g* for 5 min and twice at 15,000 rpm for 15 min at 4°C (rotor JA-20; Beckman). An aliquot of the extract was removed for following analysis and total protein concentration measurement with a Compat-Able Protein Assay Kit and BCA Protein Assay Reagent (Pierce Biotechnology, USA). Then avidin (5 μg per mg total protein) was added, and the sample was incubated for 10 min at 4°C to prevent binding of biotinylated proteins from the extract [12]. Then yeast extract (10 mg/ml total protein) was added to streptavidin-Sepharose resin (200 μl of resin from GE Healthcare (USA) for 10 ml of extract) that had been preliminarily washed with buffer "str". The mixture was incubated on a rotary shaker at 4°C for 1.5 h. After binding, the resin was washed six times with buffer "str". The resin obtained after binding and all other fractions were frozen in liquid nitrogen and stored at −80°C for further analysis.

Isolation of telomerase using ion-exchange chromatography on DEAE-cellulose. Yeast telomerase was prepared generally according to [20, 21] with only slight modification—telomerase fraction was obtained by gradient elution (sodium acetate concentration from 100 mM to 1 M) from a DEAE-cellulose column.

Telomerase assay *in vitro*. Telomerase activity was analyzed by adding 20 μl of reaction mix to 20 μl of resin suspension obtained after isolation or DEAE fraction. The final solution contained 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 1 mM DTT, 1 mM spermidine, 0.05 mM EDTA (contributed by telomerase fraction), 5% (v/v) glycerol (contributed by telomerase fraction), 100 μM dTTP, 20 μCi [α-^{32}P]dGTP (3000 Ci/mmol), and 5 μM oligodeoxyribonucleotide TEL11 (5′-TGGTGTGTGGG-3′). Control reactions were pretreated with RNase A (1 μl, 10 mg/ml) added to 20 μl of telomerase fraction and incubated at 30°C for 30 min. The telomerase reaction was carried for 1 h at 30°C followed by addition of 200 μl of "stop" buffer (20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.2% SDS) and 3 μl proteinase K (20 mg/ml). After incubation at 30°C for 1 h, reaction products were extracted twice with equal volumes of phenol, once with an equal volume of chloroform–isoamyl alcohol (24 : 1), and precipitated with 3 volumes of ethanol in the presence of 1/10 volume of 3 M NaOAc and 5 μg of tRNA from *E. coli* as a carrier. The resulting pellet was washed twice with 80% ethanol, dried, and dissolved in formamide loading buffer (80% of deionized formamide, 1× TBE buffer, 0.1% xylene cyanole, and 0.1% bromophenol blue). Reaction products along with 5′-^{32}P-phosphorylated oligodeoxyribonucleotide TEL11 as a length marker were electrophoretically separated in a 15% TBE denaturing polyacrylamide gel and analyzed using the PhosphorImager system (Molecular Dynamics, USA).

RESULTS

Construction of yeast strains expressing TLC1 with streptavidin aptamer. We selected streptavidin aptamer [12] (Fig. 1a) from the spectrum of RNA affinity tags because affinity chromatography using this aptamer was successfully applied in our laboratory for efficient isolation of ribonucleoprotein complexes under mild conditions preventing dissociation and degradation of components [22, 23].

Streptavidin aptamer should be inserted in TLC1 without changing its functional properties, the structure of the aptamer should be preserved, and it should be located at the surface of ribonucleoprotein (RNP) complex to allow affinity isolation. Substitution of the loop-end of the stable helix, which is not important functionally with the aptamer, is known to have higher probability for insertion in RNP complex to keep its structure and to be located at the surface of the RNP complex [24]. On the basis of the analysis of TLC1 secondary structure models developed using phylogenetic approaches [6, 25] and partially confirmed biochemically [25], we selected regions 1005-1019 (insertion 1) and 1056-1068 (insertion 2) in TLC1 RNA. These regions are indicated on the secondary structure model of TLC1 [6] in Fig. 1. These insertions are located outside of known binding sites for Est2p [10, 26], Est1p [27], Ku70/Ku80 [28], or Sm proteins [29].

First, a DNA fragment coding for aptamer was inserted into the corresponding regions of the *TLC1* gene. Then, based on obtained gene plasmids pTLCins1 or pTLCins2 were obtained.

In the next stage, yeast strains were obtained with TLC1 with one of the insertions as the only source of telomerase RNA in order to test the influence of the insertion on the functional properties of telomerase RNA. For that purpose, we used the strain in which *TLC1* gene was substituted for *KAN* on the chromosome and the cell growth was supported by the expression of telomerase RNA under a control of native promoter and terminator from a plasmid pTLC_URA with *Ura3* marker. This plasmid was substituted for pTLCins1 or pTLCins2 and as a result strains (*tlc1Δ::KAN*, pTLCins1) and (*tlc1Δ::KAN*, pTLCins2) expressing only TLC1 with the corresponding insertion were obtained. In control experiments strains (*tlc1Δ::KAN*, pSD107) expressing wild type TLC1 and (*tlc1Δ::KAN*, pRS314) without TLC1 were used.

Substitution of *TLC1* for *KAN* on the chromosome was verified by PCR from genomic DNA with a number of primers according to the scheme shown in Fig. 2a. The results of analysis of different PCR products are shown in Fig. 2b. Only predicted PCR products are seen in the corresponding lanes.

The expression of corresponding TLC1 (wild type or with the insertion) and the absence of TLC1 were confirmed by RT-PCR analysis with the primers complementary to the regions on both sides of the insertions. The RT-PCR product from TLC1 with the insertion is 50 bp

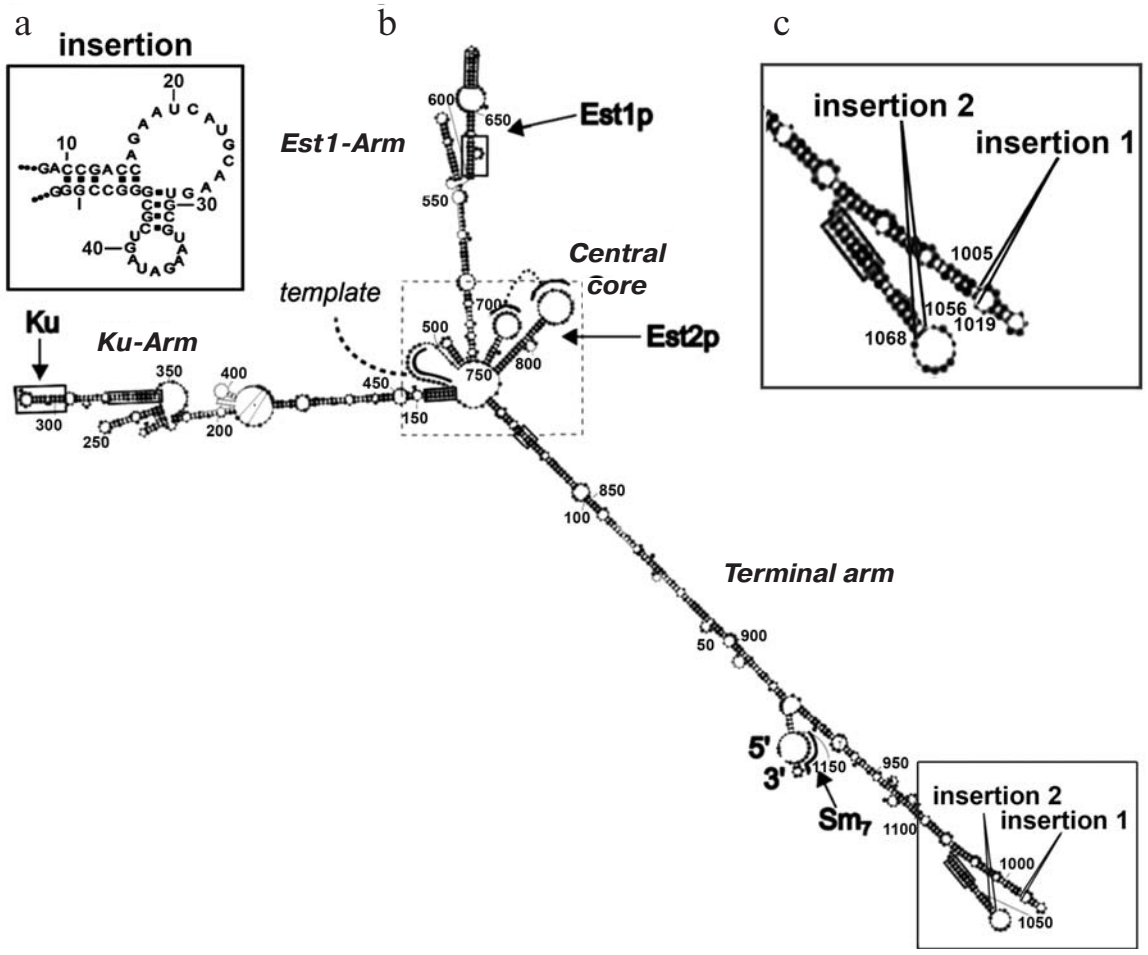

Fig. 1. Aptamer insertions 1 and 2 in the secondary structure of TLC1 RNA. a) Secondary structure of streptavidin aptamer tag [12]. b) Location of insertion 1 and insertion 2 in a secondary structure of TLC1 RNA proposed by Zappulla and Cech [6]. c) Detailed description of the location of insertion 1 and insertion 2 in the terminal arm of TLC1 RNA.

longer than that one from wild type TLC1, thus the products can be separated by agarose gel electrophoresis. The results of this analysis are shown in Fig. 2c. It is clear that in the case of the strain with only wild type TLC1 expressed the expected product is seen (Fig. 2c, lane *2*). This product is absent in the ΔTLC1 strain (Fig. 2c, lane *4*), and a larger product appears when TLC1 with one of the insertions is expressed (Fig. 2c, lanes *6* and *8*). In the control experiment (Fig. 2d) expression level of scR1 RNA (the component of signal recognition particle (SRP)), which is not related to telomerase, was measured as an internal control. Control reactions without reverse transcription (RT(–)) were performed for all samples (Figs. 2c and 2d). No product indicates there is no DNA in the probe that could produce a signal in RT-PCR.

Testing functional activity of TLC1 with the insertion *in vivo*. The inactivation of TLC1 leads to cell senescence after about 50 generations from loss of plasmid pTLC_URA coding for TLC1 [4, 30]. We analyzed cell growth in a series of generations according to a standard procedure [31] for the strains (*tlc1Δ::KAN*, pSD107) expressing wild type TLC1, (*tlc1Δ::KAN*, pRS314) with the deletion of TLC1, and for strains (*tlc1Δ::KAN*, pTLCins1) and (*tlc1Δ::KAN*, pTLCins2) expressing TLC1 with the corresponding insertion. The data are shown in Fig. 3. A plate with cells that were preliminarily grown in liquid culture for 30 generations from rescue of a plasmid pTLC_URA coding for wild type TLC1 is shown in Fig. 3a. The cell growth of all strains in serial dilutions is similar for all strains. A plate with cells that were preliminarily grown in liquid culture for 70 generations from rescue of plasmid pTLC_URA is shown in Fig. 3b. The cell growth of the strains expressing wild type TLC1 and TLC1 with the insertions is similar and differs from the growth of the strain with TLC1 deletion. Thus, we conclude that the insertions do not significantly influence the functional properties of TLC1 *in vivo*.

Affinity isolation of telomerase *via* aptamer insertion in TLC1. For telomerase isolation, cell extracts were incubated with streptavidin-Sepharose, which was then washed several times and used for further analysis. Isolated RNA was analyzed by RT-PCR as described ear-

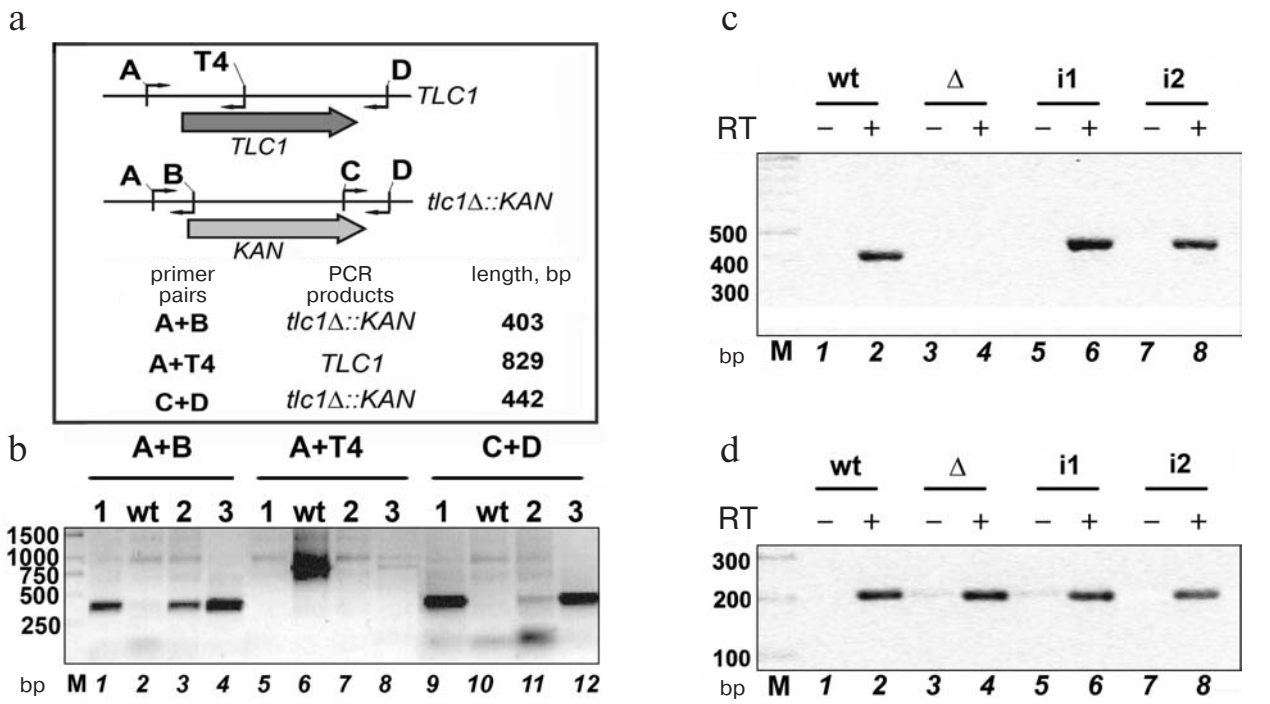

Fig. 2. Characteristic of strains expressing TLC1 RNA with insertion. a) Schematic representation of PCR-based method of verification that the *TLC1* gene was replaced with the *KAN* gene on the chromosome. Lengths of expected PCR-products are shown. b) PCR analysis of yeast DNA isolated from strains (*tlc1Δ::KAN*) (wt, wild type strain; 1-3 are clones). Lanes: M, molecular weight marker; *1-4*) PCR analysis of genomic DNA with primer pair A+B for strains 1, wt, 2, and 3, accordingly; *5-8*) the same with primer pair A+T4; *9-12*) the same with primer pair C+D. c) RT-PCR analysis with gene-specific primers to TLC1 RNA of whole cell RNA from strains containing TLC1 RNA with insertion, wild type, and not containing TLC1 RNA. RT(–) and RT(+), RT-PCR analysis without and with reverse transcription reaction, correspondingly. Lanes: M, molecular weight marker; *1*, *2*) RT-PCR analysis for strain (*tlc1Δ::KAN*, pSD107) expressing wild type TLC1 (wt); *3*, *4*) the same for strain (*tlc1Δ::KAN*, pRS314) without TLC1 ("Δ"); *5*, *6*) the same for strain (*tlc1Δ::KAN*, pTLCins1) expressing only TLC1 with the corresponding insertion 1 ("i1"); *7*, *8*) the same for strain (*tlc1Δ::KAN*, pTLCins2) expressing only TLC1 with the corresponding insertion 2 ("i2"). d) RT-PCR analysis with gene-specific primers to scR1 RNA of whole cell RNA from the described strain. Designation of lanes is as in panel (c).

lier for detection of TLC1 expression in the obtained strains. The extracts from the strain with wild type TLC1 was used as a control. The data are shown in Fig. 4a. One can see that a significant amount of TLC1 with insertion 1 was bound to the resin in contrast to wild type RNA (Fig. 4a, compare lanes *6* and *12*), and similar small amounts of scR1 RNA (specificity control) could be detected on the resin (Fig. 4b, lane *6*). Thus, we can isolate only aptamer containing products. In the next step, telomerase activity was analyzed by direct assay [20, 21]. The data are shown in Fig. 4d. The telomerase-containing fraction prepared by ion-exchange chromatography according to the classical procedure [20, 21] was used as a positive control (Fig. 4f) and RNase A treatment as a negative control. No activity was detected in the case of wild type TLC1 (Fig. 4d, lane *4*), and clear telomerase elongation pattern was observed in the case of TLC1 with the insertion (Fig. 4d, lane *6*). Isolated telomerase was able to synthesize no more than one telomeric repeat, similar to telomerase-containing fraction prepared by ion-exchange chromatography (Fig. 4f, lane *2*).

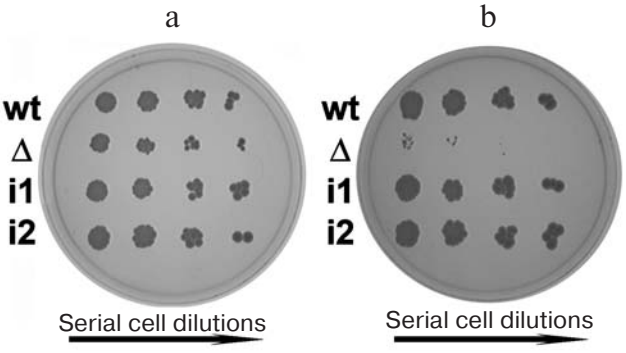

Fig. 3. Phenotype of obtained strains expressing TLC1 RNA with insertion. Equal numbers of cells from different strains were plated on SC – Trp plates with serial dilutions. a, b) Plates with yeast cells plated after, respectively, 30 and 70 generations of growth from rescue of plasmid pTLC_URA coding for wild type TLC1. Designation: wt, strain (*tlc1Δ::KAN*, pSD107) expressing wild type TLC1; Δ, strain (*tlc1Δ::KAN*, pRS314) without TLC1; i1 and i2, strains (*tlc1Δ::KAN*, pTLCins1) and (*tlc1Δ::KAN*, pTLCins2) expressing only TLC1 with the corresponding insertion, i1 and i2.

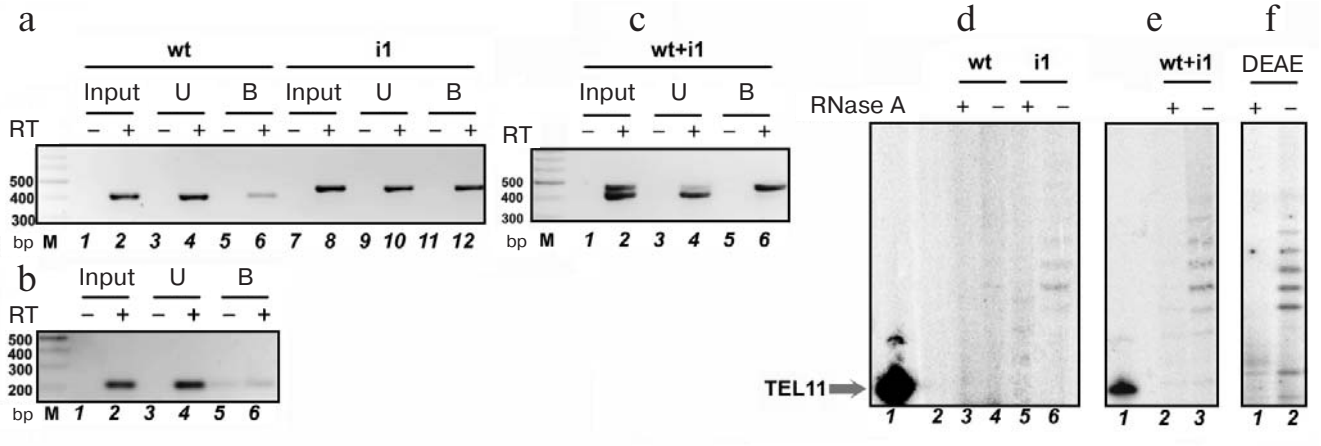

Fig. 4. Isolation of telomerase on streptavidin-Sepharose resin from the strain expressing TLC1 RNA with insertion 1 alone and from the strain expressing TLC1 RNA with insertion 1 and wild type TLC1 RNA simultaneously under the same conditions. a) RT-PCR analysis with gene-specific primers to TLC1 RNA of probes obtained in telomerase isolation experiment. RT(–) and RT(+), RT-PCR analysis without and with reverse transcription reaction, correspondingly. Panel "wt" corresponds to RT-PCR analysis of RNA from strain (*tlc1Δ::KAN*, pSD107) expressing wild type TLC1 RNA. Lanes: M, molecular weight marker; *1*, *2*) RNA probe from initial yeast extract (Input); *3*, *4*) RNA probe from unbound fraction (U); *5*, *6*) RNA probe bound on the resin (B). Panel "i1" corresponds to RT-PCR analysis of RNA from strain (*tlc1Δ::KAN*, pTLCins1) expressing TLC1 RNA with the insertion 1. Lanes *7-12* are indicated as lanes *1-6*. b) RT-PCR analysis with gene-specific primers to scR1 RNA of probes obtained in telomerase isolation experiment from strain (*tlc1Δ::KAN*, pTLCins1). Designation of lanes *1-6* is as in panel (a). c) RT-PCR analysis of TLC1 from telomerase isolation from strain (*tlc1Δ::KAN*, pTLCins1 + pTLC_URA) expressing both TLC1 RNA with insertion 1 and wild type TLC1 RNA at the same time. Lanes *1-6* are as in panel (a). d) Telomerase activity test for telomerase isolated on the resin. RNase A(+) and RNase A(–), reaction with and without pretreatment with RNase A. Lanes: *1*) 5′-^{32}P-phosphorylated oligodeoxyribonucleotide TEL11; *2*) empty; *3*, *4*) telomerase activity test for telomerase bound on the resin from strain (*tlc1Δ::KAN*, pSD107); *5*, *6*) the same for strain (*tlc1Δ::KAN*, pTLCins1). e) Telomerase activity test for telomerase isolated from a strain (*tlc1Δ::KAN*, pTLCins1 + pTLC_URA). Lanes: *1*) 5′-^{32}P-phosphorylated oligodeoxyribonucleotide TEL11; *2*, *3*) telomerase activity test for telomerase bound on the resin from strain (*tlc1Δ::KAN*, pTLCins1 + pTLC_URA) with and without pretreatment with RNase A. f) Telomerase activity test for telomerase obtained with classical approach using ion-exchange chromatography. Lanes: *1*, *2*) telomerase activity test for telomerase isolated using DEAE from initial strain DBY-746 with and without pretreatment with RNase A, respectively.

It should be mentioned that in the case of TLC1 with insertion 2 we were not able to detect specific binding of TLC1 to the affinity resin. Although RNA with this insertion was functionally active, the insertion was either hidden in the telomerase complex or did not possess the necessary structure for binding to the resin.

Analysis of telomerase dimerization. To test whether yeast telomerase forms a dimer, we expressed wild type TLC1 and TLC1 with the insertion simultaneously at the comparable expression level. For that purpose the plasmid (pTLCins1) expressing RNA with the affinity tag was introduced into the strain (*tlc1Δ::KAN*, pTLC_URA). The resulting strain (*tlc1Δ::KAN*, pTLC_URA + pTLCins1) contained two plasmids with the genes for wild type and tagged TLC1 under the control of the same promoter, which should provide similar expression level. Indeed, in the cell extracts both RNAs could be detected in comparable amounts (Fig. 4c, lane *2*). The telomerase fraction was immobilized on the resin as described above, and the presence of both variants of TLC1 was analyzed by RT-PCR. Already in the unbound fraction, one can see the difference in the amounts of the two RNAs (Fig. 4c, lane *4*). Only the signal of TLC1 with the insertion could be detected in the bound fraction (Fig. 4c, lane *6*). It should be mentioned that even using an oversensitive RT-PCR test that allows detection of a few RNA molecules, we were not able to see the signal from wild type TLC1. The activity of isolated telomerase complex was measured by direct assay, and one can see that isolated telomerase is indeed active (Fig. 4e, lane *3*).

DISCUSSION

The fact that active telomerase can be isolated by means of RNA affinity tag from cell extract with the equal amount of wild type and tagged RNA indicates that at least *in vitro* yeast telomerase can function as a monomer. One can propose that the insertion might specifically prevent telomerase dimerization. However, this does not contradict our conclusion. Our results in favor that active telomerase is not a dimer are in agreement with the finding based on co-immunoprecipitation of TLC1 fragments with Est1p and Est2p and co-immunoprecipitation of different variants of Est2p with Est3p [10, 11].

We cannot exclude the possibility that telomerase may form dimers. For example, in agreement with [9] dimers might form at a certain step of the telomerase RNA processing. It was shown that the composition of telomerase holoenzyme differs at different stages of the cell cycle [32]. We cannot exclude the possibility that telomerase might form dimers *via* protein–protein inter-

action when it is activated in the late S-phase of the cell cycle [32].

Among telomerases from different organisms, there are enzymes that function in monomeric form as well as enzymes that function in dimeric form. It was shown that *Tetrahymena thermophila* telomerase works as a monomer [33], human telomerase is reported to be a dimer [34] as well as the enzyme from *Euplotes crassus* [35], and telomerase from *Euplotes aediculatus* could be either a monomer or a dimer [36, 37]. The question whether dimeric enzymes differ functionally from monomeric enzymes is very interesting. An answer to it could lead us to a more detailed view of how this enzyme works. It was proposed earlier that telomerase dimerization is important for telomerase processivity (ability to add several telomeric repeats in one cycle of substrate elongation) or for coordinated elongation of two sister chromatid *in vivo* [8, 34]. It has been shown that processivity of telomerase is not connected with its dimerization since monomer *T. thermophila* telomerase is processive [33]. Human dimer telomerase is processive not because of dimerization as it does not switch the templates during processive synthesis [38]. Telomerase from yeast *S. cerevisiae* is not processive *in vitro*. However, it was shown that two different template sequences corresponding to different template regions of two molecules of TLC1 expressing simultaneously in a cell are built into one telomere *in vivo* [7]. That could be explained either by template switching within a dimer [7] or telomerase dissociation–association with the telomere. It could not be concluded from our data whether telomerase is a dimer or monomer *in vivo*, but our data indicate that *in vitro* dimerization is not required for yeast telomerase to be active.

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