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Stable expression in yeast of the mature form of human telomerase RNA depends on its association with the box H/ACA small nucleolar RNP proteins Cbf5p, Nhp2p and Nop10p

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ABSTRACT

Telomerase is a ribonucleoprotein (RNP) particle required for the replication of telomeres. The RNA component, termed hTR, of human telomerase contains a domain structurally and functionally related to box H/ACA small nucleolar RNAs (snoRNAs). Furthermore, hTR is known to be associated with two core components of H/ACA snoRNPs, hGar1p and Dyskerin (the human counterpart of yeast Cbf5p). To assess the functional importance of the association of hTR with H/ACA snoRNP core proteins, we have attempted to express hTR in a genetically tractable system, *Saccharomyces cerevisiae*. Both mature non-polyadenylated and polyadenylated forms of hTR accumulate in yeast. The former is associated with all yeast H/ACA snoRNP core proteins, unlike TLC1 RNA, the endogenous RNA component of yeast telomerase. We show that the presence of the H/ACA snoRNP proteins Cbf5p, Nhp2p and Nop10p, but not Gar1p, is required for the accumulation of mature non-polyadenylated hTR in yeast, while accumulation of TLC1 RNA is not affected by the absence of any of these proteins. Our results demonstrate that yeast telomerase is unrelated to H/ACA snoRNPs. In addition, they show that the accumulation in yeast of the mature RNA component of human telomerase depends on its association with three of the four core H/ACA snoRNP proteins. It is likely that this is the case in human cells as well.

INTRODUCTION

Telomerase is a ribonucleoprotein (RNP) particle found from yeast to human, that is required for the complete replication of telomeres (1). In all organisms, the RNA component of telomerase contains a small region that is used as a template by the reverse transcriptase subunit of the particle to synthesize telomeric repeats. Until recently, the structure and function of the rest of the RNA component [designated hTR (2)] of human telomerase remained ill-defined. Work by Mitchell *et al.* (3) and Chen *et al.* (4) have provided insight into these issues. The former noticed that the 3′ half of the RNA component of mouse and human telomerases can be folded into a structure reminiscent of that adopted by box H/ACA small nucleolar RNAs (snoRNAs) (3). These RNAs are involved in chemical modification and cleavage of the pre-rRNA (5–7). Comparison of the sequences of the RNA component of telomerases from 32 different vertebrate species then led Chen *et al.* (4) to propose a phylogenetically supported secondary structure model for vertebrate telomerase RNA. This model does include a domain akin to box H/ACA snoRNAs at the 3′-end of the RNA (4). The structure of box H/ACA snoRNAs is characterized by the succession of two irregular stem–loops containing an internal bulge. These stem–loop structures are separated by a single-stranded hinge region containing the conserved H box (consensus 5′-ANANNA-3′) and followed by a single-stranded tail containing the 5′-ACA-3′ motif situated 3 nt upstream from the mature 3′-end of the snoRNA (8,9). Mutations of conserved nucleotides of the H or ACA box impair accumulation and function of box H/ACA snoRNAs (8–10). Moreover, such mutations prevent the nucleolar accumulation of box H/ACA snoRNAs injected into *Xenopus* oocytes (11). The functional similarities between the H and ACA boxes of hTR and those of the box H/ACA snoRNAs were confirmed by mutational analyses. Mutations that modify the putative H or ACA box of hTR, so that they no longer conform to the consensus, drastically lower the hTR levels in HeLa cells (3). In contrast, mutations that result in an H box that does not deviate from the consensus have no effect on hTR accumulation (3). Similarities between box H/ACA snoRNAs and hTR extend also to their sub-nuclear localization. A small but significant proportion of hTR is found within nucleoli (3). Moreover, hTR injected into *Xenopus* oocytes accumulates within the fibrillar regions of nucleoli (11). Strikingly, mutating the ACA box of hTR prevents the accumulation of this RNA within nucleoli, as is observed for box H/ACA snoRNAs (11).

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Whether the presence of an H/ACA-like domain in telomerase RNA is a feature specific to vertebrates is unclear. However, the phylogenetically supported secondary structure model proposed for the telomerase RNA of ciliates does not contain a domain obviously related to box H/ACA snoRNAs (12–14). No convincing secondary structure model for *Saccharomyces cerevisiae* telomerase RNA [TLC1 (15)] is yet available but the presence of a match to the consensus binding site of Sm proteins near the 3′-end of TLC1 RNA was noticed by Seto *et al.* (16). Remarkably, mutations within this putative Sm-binding site drastically lower the levels of the TLC1 RNA (16).

The discovery that hTR RNA contains an H/ACA-like domain crucial for its *in vivo* accumulation, 3′-end processing and intra-nuclear trafficking prompted the suggestion that hTR does associate with H/ACA snoRNP proteins. Four core H/ACA snoRNP proteins, all essential for viability, have been identified in yeast: Cbf5p (17–20), Gar1p (8,9,21), Nhp2p (20,22) and Nop10p (22). In the absence of Cbf5p, Nhp2p or Nop10p, H/ACA snoRNPs cannot accumulate in yeast cells (19,20,22). Both Gar1p (23) and Nhp2p (A.Henras, C.Dez, J.Noaillac-Depeyre, Y.Henry and M.Caizergues-Ferrer, manuscript submitted) have been proposed to directly contact box H/ACA snoRNAs. The human homologs of yeast Cbf5p, termed Dyskerin (24), and yeast Gar1p, termed hGar1p (25), have been identified. Immunoprecipitation experiments reveal that both Dyskerin and hGar1p are in fact associated with hTR (25,26).

To assess the functional importance of the association of hTR with H/ACA snoRNP proteins, we decided to express hTR in a genetically tractable system, *S.cerevisiae*. We demonstrate that the mature form of hTR is associated with all four H/ACA snoRNP proteins in yeast and that absence of Cbf5p, Nhp2p and Nop10p prevents the accumulation of the mature form of hTR. In contrast, we find that TLC1 is not associated with H/ACA snoRNP proteins and that depletion of these proteins has no effect on TLC1 levels.

MATERIALS AND METHODS

Strains, media and plasmids

Escherichia coli DH5α strain [*F′*, *endA1*, *hsdr17* (r_k^- m_k^+), *supE44*, *thi-1*, *recA1*, *gyrA* (*Nal*^{r}), *relA1*, Δ(*lacIZYA-argF*)*U169*, *deoR*, (ϕ*80dlac*Δ(*lacZ*)*M15*)] grown on LB (1% bacto-tryptone, 0.5% bacto-yeast extract, 1% NaCl) liquid or solid media was used for all cloning procedures.

Saccharomyces cerevisiae strains were grown in YNB medium [0.17% yeast nitrogen base, 0.5% $(NH_4)_2SO_4$] supplemented with 2% glucose or 2% galactose and the required amino acids.

Plasmids allowing expression of hTR in yeast were produced as follows. The entire coding sequence of hTR was released from phTR1 (25) by *Sfc*I and *Fsp*I digestions. The 5′-overhangs produced by the *Sfc*I digestion were filled-in by treatment with the Klenow fragment of *E.coli* DNA polymerase I. The resulting blunt-ended fragment was inserted in plasmid pFL45esno (10), previously digested by *Kpn*I and treated with T4 DNA polymerase to remove the 3′-overhangs left by the *Kpn*I digestion. In the resulting plasmid, $phTR_{TRP1}$, the sequence encoding hTR is inserted in-between the promoter and terminator regions of *SNR5*. $phTR_{TRP1}$ contains the *TRP1* selection marker. For transformation into certain strains expressing epitope-tagged proteins (see below), it was necessary to exchange the *TRP1* marker in $phTR_{TRP1}$ for either the *LEU2* or *URA3* markers. *LEU2* and *URA3* gene cassettes obtained from pFL46S (27) or pFL38 (27) by *Bgl*II digestion were used to replace the *TRP1*-containing *Bgl*II fragment of $phTR_{TRP1}$, creating plasmids $phTR_{LEU2}$ and $phTR_{URA3}$, respectively. To avoid unnecessary details in the main text, the distinction between the different forms of phTR was not made in the Results section.

A strain expressing hTR and Cbf5pZZ was obtained by transforming strain YDL524-18 (19) with $phTR_{TRP1}$. A strain expressing hTR and Gar1pZZ was obtained by transforming strain YO79 with plasmids pMCGZZ1 (22) and $phTR_{LEU2}$. YO79 was derived from the *GAL::gar1* strain YO24 (21) by disruption of the *LEU2* locus by the *LYS2* marker gene. A strain expressing hTR and Nhp2pZZ was obtained by transforming strain YO346 (A.Henras, C. Dez, J.Noaillac-Depeyre, Y.Henry and M.Caizergues-Ferrer, manuscript submitted) with plasmid $phTR_{URA3}$. A strain expressing hTR and Nop10pZZ was obtained by transforming strain YO249 [a *GAL*::*nop10ZZ* strain described previously (22)] with plasmids $phTR_{TRP1}$ and pFH35, a centromeric plasmid containing the *LEU2* marker gene and the *NOP10ZZ* gene under the control of the *NOP10* promoter. A strain expressing hTR and ZZNop1p was obtained by transforming strain JG540 (22) with plasmids $phTR_{TRP1}$ and pUN100Nt (a gift from Dr T. Bergès, Université de Poitiers). Strains that contain the gene encoding hTR and that conditionally transcribe the *CBF5*, *GAR1*, *NHP2* and *NOP10* genes were obtained by transforming the *GAL*::*cbf5* strain YDL521-1 (19), the *GAL*::*gar1* strain YO24 (21), the *GAL*::*nhp2* strain Y0253 (22) and the *GAL*::*nop10* strain YO247 (22) with plasmid $phTR_{TRP1}$.

RNA extractions, northern hybridizations and purification of polyA$^+$ RNAs

RNA extractions were performed as described previously (28). Polyadenylated RNAs were purified using the 'PolyA Tract mRNA Isolation System IV' (Promega) as instructed by the supplier. Electrophoresis and transfer of RNAs to nylon membranes was performed as described previously (22). In most cases, 10 µg of total RNA was analyzed. In experiments presented in Figures 1 and 4, 7% of the polyadenylated and non-polyadenylated RNA fractions obtained from 300 µg of total RNA were used. Specific RNAs were detected by hybridization of membranes with kinased oligonucleotide probes. The following antisense oligonucleotides were used: anti-TLC1, 5′-GATCAATCCGAAATCCGACACTATCTCTTCACCAT-CGAG-3′; anti-hTR, 5′-CTGGGCAGGCGACCCGCCGCAG-GTCCCCGGGAGGGGCGAACGGGCCAGCAGCT-3′; anti-yU3, 5′-ATGGGGCTCATCAACCAAGTTGG-3′; anti-snR37, 5′-GATAGTATTAACCACTACTG-3′. The sequence of the antisense oligonucleotide used to detect snR42 has been reported previously (22). Hybridization and washing conditions used with antisense oligonucleotide probes allowing detection of snoRNAs have been detailed previously (22). The anti-TLC1 oligonucleotide was hybridized to northern membranes overnight at 37°C in 6× SSC, 5× Denhardt's, 0.5% SDS, 120 µg/ml heat-denatured salmon sperm DNA. Membranes were then washed twice in 2× SSC, 0.1% SDS at 37°C for 15 min and twice in 1× SSC, 0.1% SDS at 50°C for 15 min. The anti-hTR

oligonucleotide was hybridized to northern membranes overnight at 37°C in 50% formamide, 5× SSC, 5× Denhardt's, 1% SDS, 120 µg/ml heat-denatured salmon sperm DNA. Membranes were then washed once in 2× SSC, 0.1% SDS at 37°C for 15 min, once in 1× SSC, 0.1% SDS at 42°C for 15 min and finally twice in 0.1× SSC, 0.1% SDS at 48°C for 15 min each time.

Immunoprecipitations

Immunoprecipitations of ZZ-tagged proteins from yeast extracts were performed as described previously (9).

RESULTS

Both mature non-polyadenylated hTR and a heterogeneous population of polyadenylated hTR species can accumulate in yeast cells

In order to assess the functional importance of the association of H/ACA snoRNP proteins with hTR, we wished to express this RNA in a genetically tractable system. Because it had been previously demonstrated that both human C/D-type and H/ACA-type snoRNAs could be expressed in yeast and were functional in this organism (10,29), we chose *S.cerevisiae* as our model system. The DNA fragment encoding the whole of hTR was cloned in the yeast expression vector pFL45esno (10), producing a plasmid we termed phTR. In such a construct, hTR expression is controlled by the promoter and terminator sequences of the yeast *SNR5* gene that encodes the snR5 H/ACA snoRNA (30). pFL45esno and phTR were transformed in yeast and RNAs extracted from the resulting transformed strains were analyzed by northern hybridization. Blots were incubated with an antisense oligonucleotide probe designed to hybridize to nucleotides 181–233 of hTR. Using this probe, an RNA of the expected size for mature hTR (451 nt) as well as a heterogeneous population of extended species can be detected in the RNA sample extracted from the strain containing phTR (Fig. 1, lane 2). These species are absent in the RNA sample extracted from the strain transformed with pFL45esno (Fig. 1, lane 1). A fraction of certain snoRNAs (31) and yeast telomerase RNA (32) is polyadenylated in *S.cerevisiae*. Therefore, we tested whether the bulk of the extended species just described consists of polyadenylated hTR RNAs. A sample of total RNAs extracted from the yeast strain containing phTR was incubated with biotinylated oligo(dT) and polyadenylated RNAs bound to these oligonucleotides were precipitated using streptavidin-coated magnetic beads. RNAs recovered from the supernatant (lacking the polyadenylated species) or the pellet (containing the polyadenylated species) were analyzed by northern hybridization. The bulk of the extended species is recovered from the pellet (Fig. 1, lane 4), indicating that these species are polyadenylated. We conclude that in a yeast strain containing phTR, both mature non-polyadenylated hTR and polyadenylated forms of hTR accumulate.

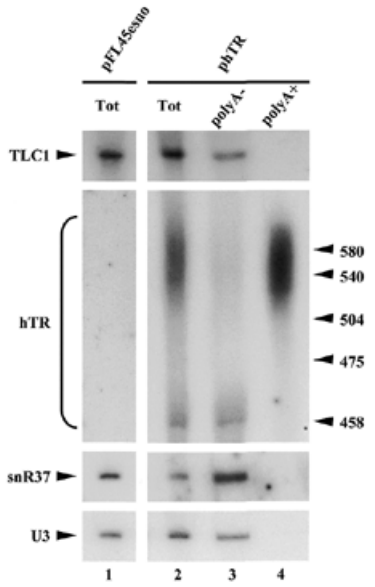

Figure 1. Both mature polyA⁻ hTR RNA and polyA⁺ forms of this RNA can accumulate in *S.cerevisiae*. Total RNAs (Tot) were extracted from yeast strains transformed either with pFL45esno expression vector lacking insert (lane 1) or with the phTR plasmid, derived from pFL45esno, that carries the DNA fragment encoding hTR (lane 2). A sample of the total RNA preparation obtained from the strain containing phTR was incubated with biotinylated oligo(dT) and polyadenylated RNAs bound to oligo(dT) were precipitated using streptavidin-coated magnetic beads. RNAs were recovered from the supernatant (polyA⁻, lane 3) or the pellet (polyA⁺, lane 4). All RNAs were separated on a 6% polyacrylamide gel, transferred to a nylon membrane and hybridized with antisense oligonucleotide probes detecting the TLC1, hTR, snR37 or U3 RNA. Positions of molecular weight markers are indicated on the right.

Mature non-polyadenylated hTR is associated in yeast with all four core H/ACA snoRNP proteins, unlike the RNA component of yeast telomerase

To assess whether hTR species expressed in yeast are associated with all four H/ACA snoRNP proteins, immunoprecipitation experiments were performed. For that purpose, phTR was transformed in yeast strains expressing Cbf5p, Gar1p, Nhp2p or Nop10p proteins tagged at their C-terminus with two IgG-binding domains (ZZ domains) derived from *Staphylococcus aureus* protein A. We also transformed phTR in a strain expressing Nop1p tagged at its N-terminus with the two ZZ domains. Total cellular extracts were prepared from the resulting strains and immunoprecipitation experiments were carried out using IgG–Sepharose to which the ZZ moieties of the tagged proteins avidly bind. Northern analysis of RNAs extracted from the pellet fractions (Fig. 2) reveals that the mature non-polyadenylated form of hTR is co-immunoprecipitated along with the Cbf5pZZ, Gar1pZZ, Nhp2pZZ and Nop10pZZ proteins as efficiently as the endogenous yeast box H/ACA snoRNAs tested (Fig. 2, lanes 1–8). In contrast, only a minor fraction of the extended hTR species is precipitated. No co-precipitation of hTR RNAs with ZZ–Nop1p is observed (Fig. 2, lanes 9 and 10). We were also unable to detect an interaction between TLC1 and any of the core H/ACA snoRNP proteins (Fig. 2).

Cbf5p, Nhp2p and Nop10p are required for the accumulation of mature non-polyadenylated hTR in yeast

We have previously shown that H/ACA snoRNPs cannot accumulate if cells are deprived of Cbf5p (19), Nhp2p (22) or Nop10p (22). In contrast, H/ACA particles can assemble in the absence of Gar1p, although these remain non-functional (33). To assess the consequences of the depletion of any of the four core H/ACA snoRNP proteins on hTR accumulation, plasmid

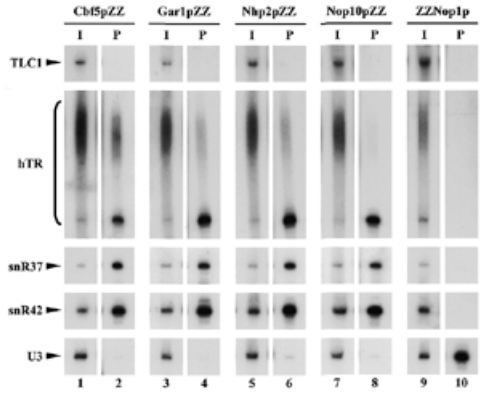

Figure 2. Mature polyA⁻ hTR expressed in yeast interacts with all four core proteins of H/ACA snoRNPs. Immunoprecipitation experiments were carried out using IgG–Sepharose and extracts from strains expressing hTR and either Cbf5pZZ (lanes 1 and 2), Gar1pZZ (lanes 3 and 4), Nhp2pZZ (lanes 5 and 6), Nop10pZZ (lanes 7 and 8) or ZZNop1p (lanes 9 and 10). RNAs extracted from one-tenth of the input extracts (I) or from the pellets following immunoprecipitation (P) were separated on a 6% polyacrylamide gel and transferred to a nylon membrane. RNAs indicated on the left were detected by hybridization with antisense oligonucleotide probes.

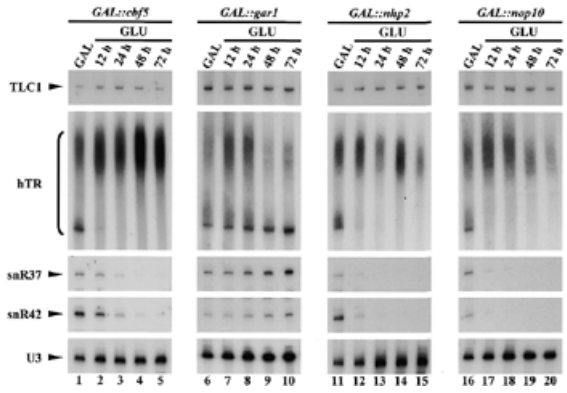

Figure 3. Accumulation in *S.cerevisiae* of mature polyA⁻ hTR requires the presence of Cbf5p, Nhp2p and Nop10p. *GAL::cbf5* (lanes 1–5), *GAL::gar1* (lanes 6–10), *GAL::nhp2* (lanes 11–15) and *GAL::nop10* (lanes 16–20) strains transformed with phTR were grown in galactose-containing medium (lanes 1, 6, 11 and 16) and were then transferred to glucose-containing medium for 12, 24, 48 or 72 h. At each time-point, culture samples were collected from which total RNAs were extracted. These were separated on 6% polyacrylamide gels and transferred to nylon membranes. RNAs indicated on the left were detected by hybridization with specific oligonucleotide probes.

phTR was transformed into strains *GAL*::*cbf5* (19), *GAL*::*gar1* (21), *GAL*::*nhp2* (22) and *GAL*::*nop10* (22). In these strains, the endogenous promoters of the *CBF5*, *GAR1*, *NHP2* or *NOP10* genes have been replaced by a *GAL* promoter, to ensure conditional transcription of these genes: on galactose-containing medium, their transcription is induced whereas it is repressed on glucose-containing medium. Strains *GAL*::*cbf5*, *GAL*::*gar1*, *GAL*::*nhp2* and *GAL*::*nop10* transformed by phTR were propagated on galactose-containing minimal media and were then shifted to glucose-containing minimal media. Samples from galactose-containing cultures and from cultures grown on glucose-containing media for 12, 24, 48 and 72 h were collected. Total proteins and RNAs were extracted from these samples for western (data not shown) and northern analyses (Fig. 3). Western analyses reveal that the steady-state levels of all four H/ACA snoRNP proteins are strongly reduced after 12 h of growth in glucose-containing media and that these proteins are almost undetectable after 24 h of growth in such media (data not shown). Depletion of Cbf5p, Nhp2p and Nop10p is correlated with a drastic decrease in the steady-state levels of the mature non-polyadenylated form of hTR (Fig. 3). In fact, levels of hTR seem to decrease even faster during the depletion time-courses than those of endogenous H/ACA snoRNAs snR37 or snR42. In contrast, the absence of Gar1p does not reduce the levels of the mature non-polyadenylated form of hTR. Interestingly, the steady-state levels of the heterogeneous extended hTR species, that seem to only weakly associate with H/ACA snoRNP proteins (Fig. 2), do not appear to be significantly affected by the depletion of any one of these proteins. Moreover, the steady-state levels of the RNA component of yeast telomerase remain unchanged.

It has been reported that in wild-type yeast cells, a minor fraction (~5%) of total TLC1 is polyadenylated and that this form is the precursor to mature non-polyadenylated TLC1 (32). The possibility that depletion of H/ACA snoRNP proteins results in over-accumulation of the polyadenylated

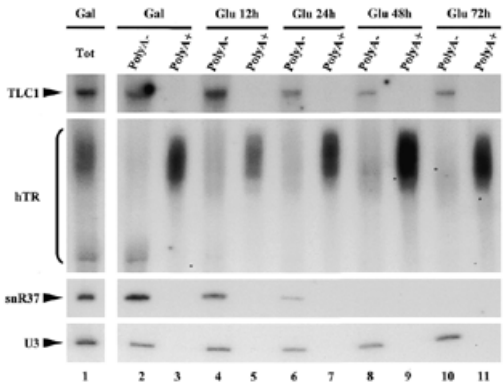

Figure 4. Accumulation in *S.cerevisiae* of polyadenylated forms of hTR and of polyA⁻ mature TLC1 does not require the presence of Nhp2p. The *GAL::nhp2* strain transformed with phTR was grown in galactose-containing medium (lanes 1–3) and was then transferred to glucose-containing medium for 12, 24, 48 and 72 h. Culture samples were collected from which total RNAs were extracted (Tot). Samples of the total RNA preparations were incubated with biotinylated oligo(dT) and polyadenylated RNAs were precipitated using streptavidin-coated magnetic beads. RNAs were recovered from the supernatants (polyA⁻) or the pellets (polyA⁺). All RNAs were separated on a 6% polyacrylamide gel, transferred to a nylon membrane and hybridized with antisense oligonucleotide probes detecting TLC1, hTR, snR37 or U3 RNA.

form of TLC1 that would mask a decrease in the levels of mature non-polyadenylated TLC1 could not be excluded (the polyA⁺ and polyA⁻ TLC1 species differ only by ~80 nt and are difficult to distinguish). To rule out this possibility, polyA⁺ RNAs were purified from RNA samples extracted from the *GAL::nhp2* strain containing phTR grown in glucose-containing medium for different times. As shown in Figure 4, the TLC1 species that are detected in RNA samples extracted from Nhp2p depleted cells clearly correspond to deadenylated TLC1. In contrast, the bulk of the extended heterogeneous hTR

species that accumulate in Nhp2p depleted cells is polyadenylated.

DISCUSSION

The discovery that hTR RNA contains a domain related to box H/ACA snoRNAs prompted the suggestion that human telomerase contains at least a subset of the H/ACA snoRNP proteins. This was confirmed by the recent findings that hTR RNA is associated with two of these proteins, Dyskerin and hGar1p (25,26). What could be the role(s) of these proteins regarding telomerase assembly and activity? The two most likely hypotheses are that H/ACA snoRNP proteins are involved (i) in the intranuclear trafficking of the telomerase complex and (ii) in the 3′-end processing of the telomerase RNA component and hence are required for its normal accumulation. These two processes are in fact likely to be interconnected. To investigate the second hypothesis, we decided to express hTR in *S.cerevisiae*, in which the production of the four known H/ACA snoRNP proteins can be easily turned off. We find that the mature form of hTR can accumulate in yeast, an observation also reported by Autexier and coworkers (34,35) while our work was in progress. We provide evidence that mature hTR is associated in yeast with the four known H/ACA snoRNP proteins, including Nhp2p and Nop10p. It is therefore likely that the human counterparts of the yeast Nhp2p and Nop10p proteins are also associated with hTR in human cells.

In addition to the mature form of hTR, a heterogeneous population of extended forms of hTR accumulate in yeast. Most of these species are polyadenylated. Polyadenylation of RNA species other than mRNAs has already been documented in yeast. In particular, 5–10% of TLC1, the RNA component of *S.cerevisiae* telomerase, is polyadenylated at steady-state (32). It is envisaged, but not proven, that the polyadenylated form of TLC1 is the precursor to the mature form of TLC1 that lacks a polyA tail (32). Interestingly, a small proportion of the yeast snR33 H/ACA snoRNA is also polyadenylated in wild-type cells (31). This polyadenylated form of snR33 may also be the precursor to the mature polyA$^-$ snR33 snoRNA. We do not know at this stage whether the polyadenylated forms of hTR that accumulate in yeast correspond to dead-end products or whether they constitute precursors to mature non-polyadenylated hTR. Polyadenylated forms of hTR have not yet been reported in human cells (36), but these forms may represent a very minor fraction of the steady-state hTR population and could therefore have gone undetected (32).

Whatever the nature of the precursor(s) to mature non-polyadenylated hTR in yeast cells, it is clear from our data that accumulation of this RNA species in *S.cerevisiae* depends on its association with the core H/ACA snoRNP proteins Cbf5p, Nhp2p and Nop10p. This is most likely the case in human cells as well. Indeed, the importance of Dyskerin (the human homolog of Cbf5p) for the accumulation of hTR in human cells is underscored by the following observation. Levels of hTR are found to be lower in primary fibroblasts from patients suffering from the X-linked form of the disease dyskeratosis congenita, that express a mutated form of Dyskerin, than in corresponding cells from healthy individuals (26).

Depletion of Gar1p, the fourth known core component of H/ACA snoRNPs, has no effect on the accumulation of mature hTR in yeast. Remarkably, accumulation of box H/ACA snoRNAs is dependent on Cbf5p (19), Nhp2p (22) and Nop10p (22) but is also unaffected by depletion of Gar1p (21; this work). This strongly suggests that the four core H/ACA snoRNP proteins assemble with the H/ACA domain of hTR and with box H/ACA snoRNAs in a similar fashion.

No secondary structure model supported by phylogenetic comparison is yet available for the very large TLC1 RNA (1.3 kb). Given the scarcity of conserved nucleotides in box H/ACA snoRNAs, it is difficult to rule out the possibility, by mere inspection of the primary structure of TLC1, that this RNA contains an H/ACA-like domain. Our data strongly suggest that mature TLC1 is not associated with any of the four known H/ACA snoRNP proteins, and show that depletion of any of these proteins has no effect on the accumulation of mature TLC1. From these results, we conclude that no functional H/ACA-like motif is present in TLC1. In contrast, convincing evidence has been provided that TLC1 contains a binding site for the Sm proteins, which are common to several UsnRNPs (16). Both the integrity of the Sm site on the TLC1 RNA and the presence of at least two of the Sm proteins are needed for accumulation of the mature form of TLC1 (16). Thus it seems that Sm protein binding to TLC1 RNA is required for its accumulation, just as assembly of H/ACA snoRNP proteins on the H/ACA domain of hTR is required for the accumulation of this RNA.

The following model can be put forward to explain how H/ACA snoRNP proteins participate in hTR 3′-end formation and promote hTR accumulation. The H/ACA snoRNP protein(s) bound to the ACA box (situated just 3 nt upstream from the 3′-end of mature hTR) could stop the advance of 3′→5′ exonucleases, for example components of the human exosome complex (37), during maturation of a 3′-extended hTR precursor. In this model, box H/ACA proteins provide a barrier to further degradation, thus preventing the turnover of hTR and defining the 3′-end of the mature form of this RNA. In addition, the possibility that H/ACA snoRNP proteins also actively promote the digestion of the 3′-extension of hTR precursors by exonucleases cannot be excluded (38). It is less easy to propose a model explaining how Sm proteins promote accumulation of TLC1. It will be interesting to assess whether the Sm protein binding site of TLC1 can be substituted for the H/ACA domain in hTR and vice versa.

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