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Intracellular trafficking of yeast telomerase components

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Telomerase uses an internal RNA moiety as template for the synthesis of telomere repeats. In *Saccharomyces cerevisiae*, the telomerase holoenzyme contains the telomerase reverse transcriptase subunit Est2p, the telomerase RNA moiety TLC1, the telomerase associated proteins Est1p and Est3p, and Sm proteins. Here we assess telomerase assembly by determining the localization of telomerase components. We found that Est1p, Est2p and TLC1 can migrate independently of each other to the nucleus. With limiting amounts of TLC1, overexpressed Est1p and Est2p accumulated in the nucleolus, whereas enzymatically active Est2p–TLC1 complexes are distributed over the entire nucleus. The distribution to the nucleoplasm depended on the specific interaction between Est2p and TLC1 but was independent of Est1p and Est3p. Altogether, our results suggest a role of the nucleolus in telomerase biogenesis. We also describe experiments that support a transient cytoplasmic localization of *TLC1* RNA.

INTRODUCTION

Telomerase is a ribonucleoprotein (RNP) polymerase that uses an internal RNA moiety as template for the synthesis of telomere repeats (Greider and Blackburn, 1989; Lingner *et al.*, 1997b). In *Saccharomyces cerevisiae*, telomerase assembly involves association of three Ever Shorter Telomeres (*EST1–3*) gene products and presumably seven Sm proteins with the telomerase RNA moiety TLC1 (Lundblad and Szostak, 1989; Singer and Gottschling, 1994; Lendvay *et al.*, 1996; Seto *et al.*, 1999; Hughes *et al.*, 2000). It has remained unclear which cellular compartments are involved in the assembly and if it occurs in an ordered manner. In budding yeast, *TLC1* is transcribed by RNA polymerase II and polyadenylated (Chapon *et al.*, 1997). Whether the RNA moiety of telomerase is exported from the nucleus to the cytoplasm as other polyadenylated RNAs remained unclear (Huang and Carmichael, 1996). The TLC1 transcript has a trimethyl G cap and is associated with Sm proteins, which also bind to snRNAs essential for intron splicing (Seto *et al.*, 1999). These properties of the TLC1 transcript suggest that it shares the biogenesis pathway with other snRNAs. Most vertebrate snRNAs undergo several steps of maturation in the cytoplasmic compartment (including association with Sm proteins and hypermethylation to a trimethylguanosine cap) before being re-imported into the nucleus as mature RNPs (Mattaj, 1986; Izaurralde *et al.*, 1995; Huber *et al.*, 1998; Ohno *et al.*, 2000; Will and Luhrmann, 2001). In yeast, hypermethylation of the cap structure may occur in the nucleolus (Mouaikel *et al.*, 2002) and it is uncertain whether maturation of snRNPs involves the cytoplasm.

Here, we report on the subcellular localization of overexpressed telomerase components. We found that Est2p and Est1p localize independently from each other to the nucleolus but that the assembled Est2p–TLC1 complex is mainly nucleoplasmic. We also analyzed the TLC1 distribution in heterokaryons and found that TLC1 is able to migrate from one nucleus to another. In addition, an open reading frame (ORF) was translated when provided as a fusion transcript with TLC1. These results support the notion that TLC1 is exported from the nucleus to the cytoplasm as part of the telomerase biogenesis pathway. We conclude that yeast telomerase biogenesis involves several cellular compartments and nuclear sub-compartments.

RESULTS AND DISCUSSION

Localization of telomerase components

In order to determine the localization of telomerase, we replaced the endogenous copy of *EST2* with a GFP–*EST2* fusion gene whose expression was controlled with the *GAL1* promoter to obtain the strain YKF15. Cells carrying the fusion construct

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did not senesce or show considerable telomere shortening when grown both on glucose (data not shown) or galactose (see below). This shows that the chimeric protein is functional and that low expression due to leakage of *GAL1* promoter in glucose is sufficient to prevent the cells from senescing. Nonetheless, detection of GFP–Est2p by fluorescence microscopy was only possible upon induction in galactose. It revealed an enrichment of the protein in a crescent-shaped zone at the nuclear periphery (Figure 1). This zone was identified as the nucleolus by co-staining with the nucleolar protein Nop1p (Schimmang *et al.*, 1989). A small amount of GFP–Est2p was detected in the nucleoplasm but not enriched at telomeres that cluster at the nuclear periphery (Figure 1A, telomeres labeled by immunostaining against Rap1p; Gotta *et al.*, 1996). Deletion of the telomerase RNA gene *TLC1* or the telomerase protein subunit encoding genes *EST1* and *EST3* did not affect nuclear import or nucleolar enrichment of Est2p (Figure 2A and B), indicating that this localization is inherent to Est2p itself.

Nuclear localization of an HA-tagged Est1 was described previously (Zhou *et al.*, 2000), but nuclear versus nucleolar localization was not distinguished. To determine the subcellular localization of Est1p we used the same strategy as for Est2p and found that a functional fusion protein between GFP and Est1p expressed from the *GAL1* promoter was also present in the nucleoplasm and enriched in the nucleolus (Figure 1C). To conclude, both Est2p and Est1p were concentrated in the nucleolus when expressed from the *GAL1* promoter. Several hypotheses could account for this observation. First, Est2p and Est1p may have accumulated in the nucleolus because their overexpression resulted in an accumulation at an intermediate step of telomerase biogenesis. Secondly, the nucleolus may serve to stockpile mature telomerase and thus participate in the control of the access of telomerase to the telomere. Finally, our finding could simply reflect a general affinity of Est1p and Est2p for a subcellular site containing a high concentration of RNA. The results below indicate that the nucleolus does not stockpile mature telomerase.

In order to detect the RNA subunit of telomerase, we performed *in situ* hybridization using a DNA-based probe containing Alexa 546-derivatized dUTP. We found that overexpressed telomerase RNA was distributed over the entire nucleus with no preferential accumulation in the nucleolus (Figure 1D). The localization of TLC1 did not change upon deletion of *EST1*, *EST2* or *EST3* (data not shown).

Active telomerase is localized in the nucleoplasm

Telomerase activity requires Est2p and TLC1, which together make up the catalytic core (Lingner *et al.*, 1997b). We reasoned that overexpression of both moieties would be necessary to see the assembled complex. We therefore transformed the P_{GAL1}-GFP–*EST2* fusion gene harboring strain YKF15 with a plasmid encoding TLC1 under the control of the *GAL1* promoter and, as a control, with the empty plasmid pRS314. We observed no difference in growth when both strains were grown in galactose, showing that induction of both GFP–Est2p and TLC1 was not toxic. Direct observation of GFP–Est2p showed that the catalytic subunit was redistributed over the entire nucleus upon TLC1 overexpression (Figure 2A, compare panels 1 and 2). Mutants of TLC1 impaired for binding to Est2p (Livengood *et al.*, 2002) did not mediate the relocalization of Est2p (Figure 2B, panels 1 and 2). Therefore, the redistribution was dependent on the specific interaction between Est2p and TLC1. TLC1 RNA localization remained unchanged under these conditions (data not shown). This suggested that both subunits co-localize in the nucleoplasm when assembled. To test this hypothesis, we determined *in vitro* telomerase activity as a measure for assembled telomerase, from strains expressing endogenous levels of telomerase and from strains overexpressing GFP–Est2p, TLC1 or both. Telomerase activity was strongly increased in extracts from the strain overexpressing both Est2p and TLC1 (Figure 2C, lane 4), whereas it was similar to wild type when only one of the components was overexpressed (Figure 2C, lanes 2 and 3). When extract derived from the TLC1 overexpressing strain was mixed with extract from the Est2p overexpressing strain, no increase in telomerase activity was detected, ruling out a productive association of the two subunits *in vitro* (Figure 2C, compare lanes 2 and 3 with 6). Together, our results indicate that assembled and active Est2p–TLC1 complexes colocalize with each other in the nucleoplasm. However, the increased *in vitro* telomerase activity was not sufficient to increase telomere length (Figure 2D, compare lane 4 with 1–3). Telomere lengthening may have been limited for example by telomerase recruitment factors such as Est1p or Cdc13p (Evans and Lundblad, 1999), or by components of the telomeric chromatin.

The results above indicated that the Est2p–TLC1 telomerase core complex is mainly nucleoplasmic, whereas unassembled Est2p accumulates in the nucleolus. To examine the role of Est1p or Est3p in Est2p–TLC1 assembly or its relocalization, *EST1* and *EST3* were deleted separately in the strain overexpressing both Est2p and TLC1 and in the strain overexpressing only Est2p. All resultant strains showed a senescence phenotype, yet direct observation under the microscope revealed that redistribution of GFP–Est2p from nucleolus to nucleoplasm upon TLC1 overexpression did not depend on the presence of Est1p (Figure 2A, panels 3 and 4) or Est3p (Figure 2A, panels 5 and 6). Furthermore, deletion of the Est1p binding site in TLC1 did not affect the TLC1-mediated relocalization of Est2p to the nucleoplasm (Figure 2B, panels 3 and 4). Thus, Est1p and Est3p do not influence the steady-state localization of Est2p and TLC1 or their association, which is consistent with previous studies showing that Est1p and Est3p are not required for telomerase activity *in vitro* (Cohn and Blackburn, 1995; Lingner *et al.*, 1997a).

Nuclear export and re-import of TLC1

In order to test whether yeast telomerase RNA is exported from the nucleus to the cytoplasm, we analyzed TLC1 RNA redistribution in yeast heterokaryons containing nuclei that overexpress *TLC1* and nuclei that express TLC1 at endogenous levels. In yeast, heterokaryons can be obtained by mating two strains of appropriate genotype and by preventing nuclear fusion in the resulting zygote with the dominant *kar1-Δ15* allele (Vallen *et al.*, 1992; Flach *et al.*, 1994). We therefore mated one strain carrying a plasmid encoding *TLC1* under the control of the *GAL1* promoter with another strain carrying the *kar1-Δ15* allele and the GFP–*NOP1* marker (Figure 3A). The population obtained contained up to 25% of zygotes (determined cytologically). It was subsequently incubated for 12 h in selective medium containing galactose. This prevented growth of the parental

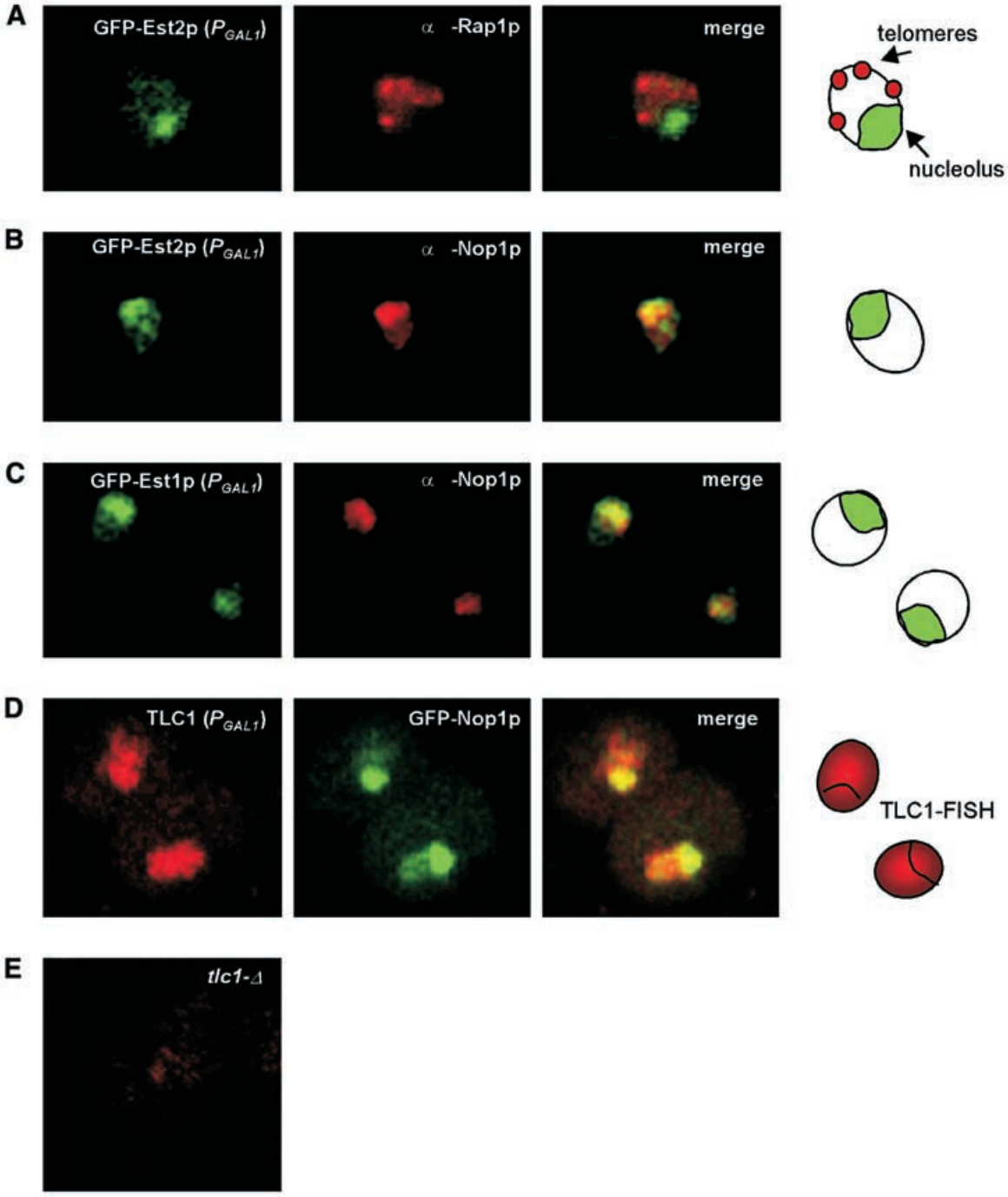

Fig. 1. Localization of telomerase components. Confocal images of strains YKF5 (**A**), YKF15 (**B**) and YT39 (**C**) grown in galactose-containing medium at 25°C and prepared for indirect immunofluorescence using an antibody against Rap1p (A) or Nop1p (B and C). (**D**) Confocal images of FYC2-6A/BL2 transformed with both pRS314-P_{GAL1}-*TLC1* and pUN100-GFP–*NOP1*, grown in galactose-selective medium and prepared for *in situ* hybridization using the PCR Alexa 546-coupled probe. (**E**) Signal obtained with YT1 (*tlc1*-Δ) under the same conditions as (D) is shown as a control.

strains and allowed expression of TLC1 from the *GAL1* promoter. In accordance with previous results (Conde and Fink, 1976; Vallen *et al.*, 1992), we obtained a final population with ~15% of multinucleated cells. Only 2–3% of diploids arose due to leakage of the *kar1-Δ15* allele. The population was fixed and prepared to allow TLC1 detection by *in situ* hybridization. Multinucleated cells derived from zygotes were unambiguously identified by containing at least one nucleus that stained positive for TLC1 and GFP–Nop1p. In Figure 3B, two representative heterokaryons are shown. The top row shows a heterokaryon (representing 10–60% of identified heterokaryons, depending on the experiment) in which TLC1 RNA was present in all the nuclei

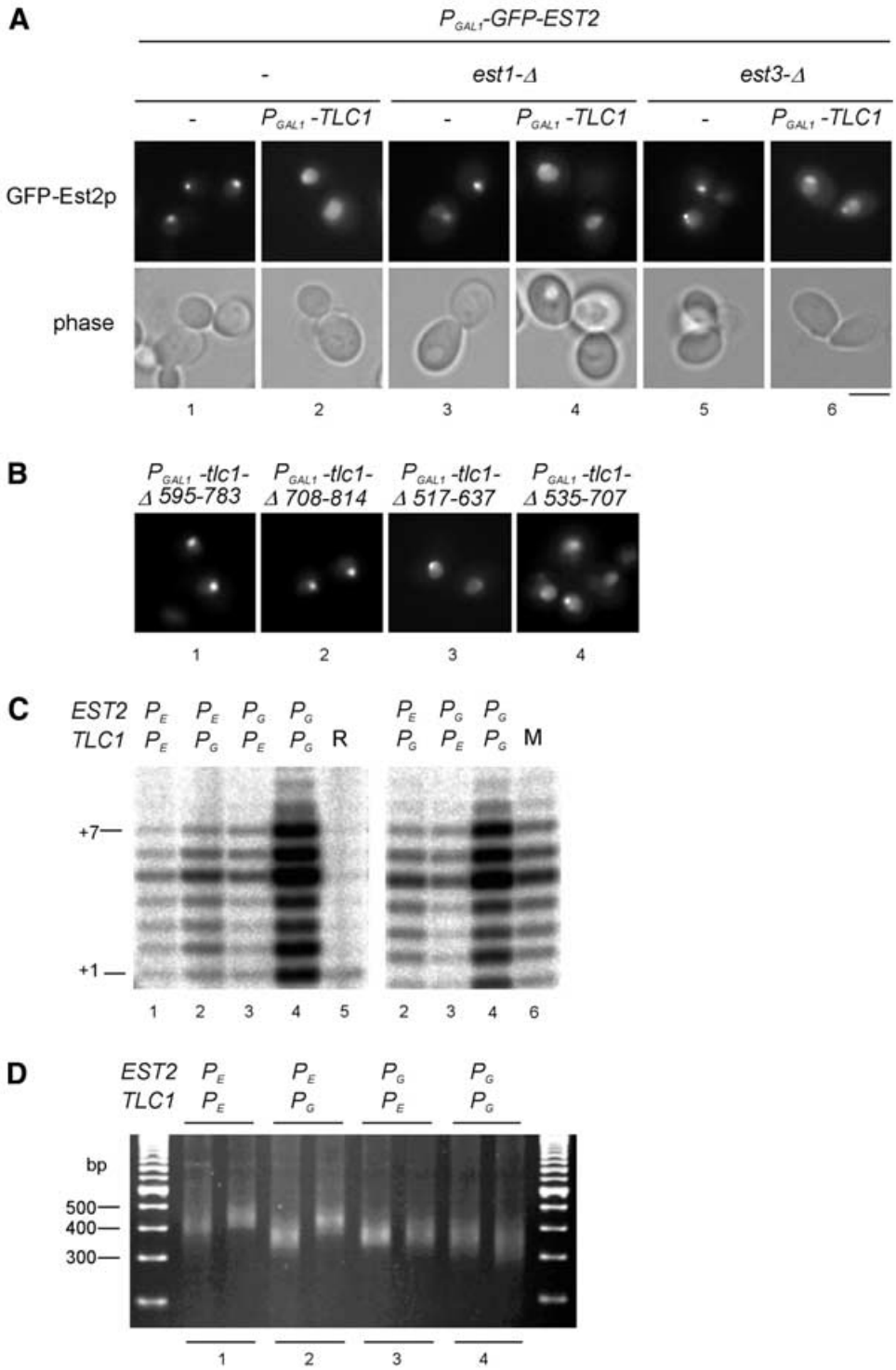

Fig. 2. Est2p is redistributed to the nucleoplasm upon TLC1 overexpression. (**A**) YKF15 (1 and 2), YT21 (3 and 4) and YT23 (5 and 6) were transformed either with the empty plasmid pRS314 (1, 3 and 5) or with pRS314-P_{GAL1}-*TLC1* (2, 4 and 6) and grown in selective medium containing galactose at 25°C. Cells were visualized with a Zeiss Axiovert 100. Bar: 6 µm. (**B**) Redistribution of Est2p from the nucleolus to the nucleoplasm upon TLC1 overexpression is dependent on the Est2p binding site in TLC1. YKF15 was transformed with pRS314-P_{GAL1}-*TLC1*-derived plasmids expressing deletion alleles of TLC1 as indicated, which impair the binding to Est2p (2), Est1p (3 and 4) or both (1) (Livengood *et al*., 2002). Cells were analyzed as in (A). (**C**) Telomerase assays performed as described (Forstemann and Lingner, 2001) using extracts from FYBL1-4D (lanes 1 and 2) and YKF15 (lanes 3 and 4) transformed either with pRS314 (lanes 1 and 3) or pRS314-P_{GAL1}-*TLC1* (lanes 2 and 4). Lane 5, extract that was pre-treated with RNase A. Lane 6, mixture of extracts derived from the strain overexpressing Est2p (lane 3) or TLC1 (lane 2). Cells were grown in galactose-containing selective medium. (**D**) Telomere length determined by telomere-PCR as described previously (Forstemann *et al*., 2000) using an oligonucleotide (o286, see Supplementary data) that specifically amplified the telomere of chromosome IL. Two independent clones were analyzed for each strain. Lanes 1–4 as in (B).

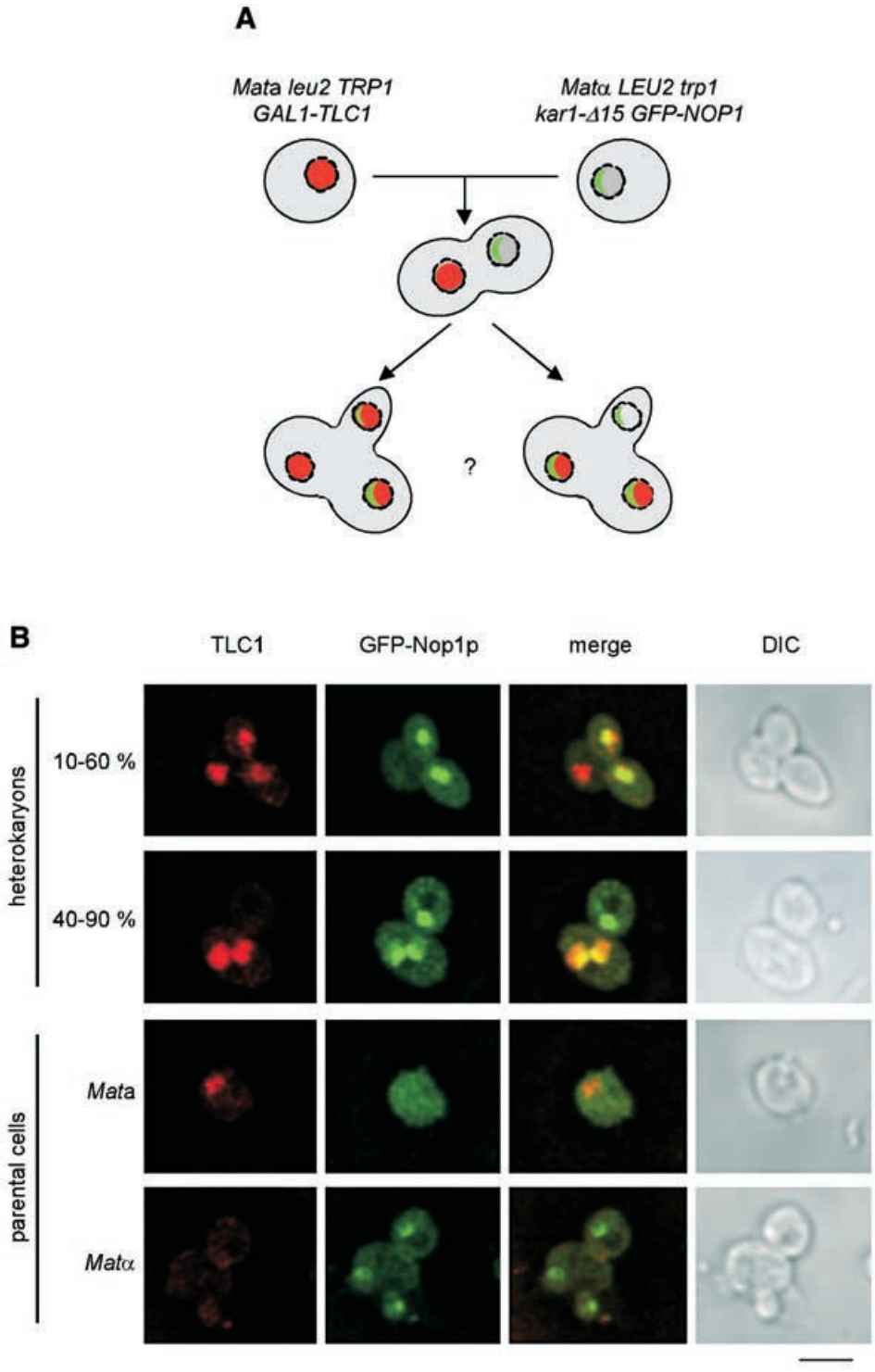

Fig. 3. Heterokaryon analysis. (**A**) Scheme of the analysis. Heterokaryons were created by mating a *Mat***a** strain overexpressing *TLC1* (FYBL1-4D transformed with pRS314-P_{GAL1}-TLC1) with a *Mat*α strain carrying the *kar1-Δ15* allele and the GFP–*NOP1* marker [MS113 (Vallen *et al.*, 1992) transformed with pUN100-GFP–*NOP1*]. Upon mating and incubation, we tested whether the TLC1 signal was detected in all the nuclei of heterokaryons indicating that there was export (heterokaryon in the left) or whether TLC1 was restricted to the nucleus where it was overexpressed, indicating nuclear retention (heterokaryon on the right). (**B**) The first two rows correspond to confocal images of representative heterokaryons obtained. Percentages report to the total of heterokaryons as defined in the text. The two lower rows show parental cells treated under the same conditions. Bar: 6 μm.

of the heterokaryon. Thus, overexpressed TLC1 RNA was able to migrate from one nucleus to another. Furthermore, our results show that both export and import pathways for TLC1 RNA exist. The re-import of TLC1 into the nucleus argues that trafficking

Table I. Yeast strains

Strain	Relevant genotype
GA426[a]	*Mat***a** *ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 DIA5-1*
FYBL1-4D[b]	*Mat***a** *ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-Δ202*
FYC2-6A/BL2[c]	*Matα ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200*
FYBL2[c]	*Mat***a***/Matα ura3-Δ851/+ trp1-Δ63/+ leu2-Δ1/+ his3-Δ200/+ lys2-Δ202/+*
YKF5[d]	*Mat***a** *ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 DIA5-1 His3MX6:*P_{GAL1}*-GFP–EST2*
YKF15[d]	*Mat***a** *ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-Δ202 His3MX6:*P_{GAL1}*-GFP–EST2*
YT39[d]	*Mat***a***/Matα ura3-Δ851/+ trp1-Δ63/+ leu2-Δ1/+ his3-Δ200/+ lys2-Δ202/+ kanMX6:*P_{GAL1}*-GFP–EST1/+*
YT1[d]	*Matα ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 tlc1-Δ::kanMX6*
YT21[d]	*ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 est1-Δ::KANMX6 His3MX6:*P_{GAL1}*-GFP–EST2*
YT23[d]	*ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 est3-Δ::HIS3MX6 His3MX6:*P_{GAL1}*-GFP–EST2*
MS113[e]	*Matα ura3-52 trp1-Δ1 leu2-3,112 kar1-Δ15*
YT7[d]	*Matα ura3-Δ851 trp1-Δ63 leu2-Δ1 his3-Δ200 cox17-Δ::His3MX6*

[a]Gasser strain collection.
[b]Obtained from B. Dujon.
[c]Fairhead *et al*. (1996).
[d]This work.
[e]Vallen *et al*. (1992).

may also occur at physiological levels and that the export of overexpressed TLC1 was not solely due to the saturation of a putative nuclear retention signal.

Our inability to detect TLC1 RNA at endogenous levels precludes performing this experiment without overexpression of *TLC1*. Therefore, we undertook an independent approach to determine whether TLC1 RNA is exported to the cytoplasm. We reasoned that a transcript present transiently in the cytoplasmic compartment would be translatable by ribosomes. In this case, an ORF provided as a fusion transcript with the TLC1 RNA would allow production of a functional protein. Our results presented as Supplementary data available at *EMBO reports* Online show that a chimeric transcript containing an ORF embedded in TLC1 is translatable. This suggests that endogenous TLC1 may also be exported from the nucleus to the cytoplasm as part of its life cycle. However, we cannot rule out the possibility that the ORF itself recruited the mRNA export machinery (Ohno *et al*., 2002) or that translation occurred in the nucleus, although to date translation in the nucleus has only been demonstrated for very small peptides (Iborra *et al*., 2001). Taken together with the heterokaryon analysis, our results support the notion that *TLC1* RNA maturation involves a step in the cytoplasm. However, more sensitive detection methods to localize endogenous TLC1 are required to clarify the issue.

Speculation

In this report, we provide evidence that telomerase biogenesis in *S. cerevisiae* involves several distinct subcellular compartments: i.e. the nucleolus, the nucleoplasm and the cytoplasm. Intracellular trafficking supports a multistep character of telomerase enzyme assembly. The RNA subunit is subject to several maturation and assembly steps involving polyadenylation (Chapon *et al*., 1997), hypermethylation at the 5′ end, association with the Sm proteins (Seto *et al*., 1999) and association with Est proteins. Our results suggest that some of these maturation steps may occur in the cytoplasm. In addition, assembly steps that occur in the nucleolus are more clearly emerging. The association of the telomerase RNA and the catalytic moiety may take place in the nucleolus, as supported by the nucleolar accumulation of overexpressed Est2p and Est1p. The assembly of telomerases from other species is a chaperone-assisted process (Holt *et al*., 1999; Licht and Collins, 1999). Since the nucleolus is considered to be a site with a high concentration of *trans*-acting factors required for the assembly of ribosomes and other RNPs (Pederson, 1998; Sleeman and Lamond, 1999; Venema and Tollervey, 1999), it may provide factors that assist in the assembly of telomerase. One of these factors could be the recently identified methyltransferase responsible for the cap hypermethylation of snRNAs and snoRNAs (Mouaikel *et al*., 2002). In support of this, it was shown that vertebrate telomerase RNA contains an H/ACA motif that targets the RNA to nucleoli where it was hypothesized to associate with the catalytic subunit of telomerase (Mitchell *et al*., 1999; Lukowiak *et al*., 2001). We found that the assembled Est2p–TLC1 telomerase core is localized in the nucleoplasm. Thus, association of Est2p with TLC1 may trigger its relocation to or its retention in the nucleoplasm. This localization does not depend on the telomerase associated proteins Est1p or Est3p. Thus it is possible that both of these proteins modulate telomerase activity downstream of the relocation of the assembled complex. It is already known that Est1p is required for telomerase recruitment to telomeres (Evans and Lundblad, 1999). In the case of Est3p, it is at present unclear how it affects the action of telomerase at telomeres *in vivo*.

METHODS

Yeast strains and plasmids. See Table I and Supplementary data.
Immunostaining and TLC1 detection. Rap1p and Nop1p immunostaining was performed as described previously (Gotta *et al*.,

1996; Teixeira *et al.*, 1997). The probe for *in situ* hybridization was created by incorporating Alexa Fluor 546-dUTP (Molecular Probes) in a PCR using pRS314-*TLC1* as a template and oligonucleotides o1 and o2 as primers. The product was purified from unincorporated nucleotides and digested with *Rsa*I and *Mbo*I. Fixation and spheroplasting of cells was performed as described previously (Teixeira *et al.*, 1997). Slides were treated for *in situ* hybridization as described in Gotta *et al.* (1999) without the RNase treatment. Images were acquired on Zeiss LSM410 or LSM510 confocal microscopes.

Heterokaryon assays. Mating experiments were performed essentially as described previously (Flach *et al.*, 1994). *Mat***a** parental cells (5×10^6), grown in raffinose tryptophan-dropout medium, were mixed in equal amounts with the *Mat*α cells and concentrated on 0.8 µm pore size filters. The filters were placed on galactose-containing media and incubated at 30°C for 5.5 h. The cells were recovered by vortexing the filter in 10 ml of synthetic medium containing galactose and lacking leucine and tryptophan. The suspension was then incubated for 12 h at 30°C with agitation before *in situ* hybridization.

Supplementary data. Supplementary data are available at *EMBO reports* Online.

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REFERENCES

Chapon, C., Cech, T.R. and Zaug, A.J. (1997) Polyadenylation of telomerase RNA in budding yeast. *RNA*, **3**, 1337–1351.

Cohn, M. and Blackburn, E.H. (1995) Telomerase in yeast. *Science*, **269**, 396–400.

Conde, J. and Fink, G.R. (1976) A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc. Natl Acad. Sci. USA*, **73**, 3651–3655.

Evans, S.K. and Lundblad, V. (1999) Est1 and Cdc13 as comediators of telomerase access. *Science*, **286**, 117–120.

Fairhead, C., Llorente, B., Denis, F., Soler, M. and Dujon, B. (1996) New vectors for combinatorial deletions in yeast chromosomes and for gap-repair cloning using 'split-marker' recombination. *Yeast*, **12**, 1439–1457.

Flach, J., Bossie, M., Vogel, J., Corbett, A., Jinks, T., Willins, D.A. and Silver, P.A. (1994) A yeast RNA-binding protein shuttles between the nucleus and the cytoplasm. *Mol. Cell. Biol.*, **14**, 8399–8407.

Forstemann, K. and Lingner, J. (2001) Molecular basis for telomere repeat divergence in budding yeast. *Mol. Cell. Biol.*, **21**, 7277–7286.

Forstemann, K., Hoss, M. and Lingner, J. (2000) Telomerase-dependent repeat divergence at the 3′ ends of yeast telomeres. *Nucleic Acids Res.*, **28**, 2690–2694.

Gotta, M., Laroche, T., Formenton, A., Maillet, L., Scherthan, H. and Gasser, S.M. (1996) The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. *J. Cell Biol.*, **134**, 1349–1363.

Gotta, M., Laroche, T. and Gasser, S.M. (1999) Analysis of nuclear organization in *Saccharomyces cerevisiae*. *Methods Enzymol.*, **304**, 663–672.

Greider, C.W. and Blackburn, E.H. (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature*, **337**, 331–337.

Holt, S.E. *et al.* (1999) Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes Dev.*, **13**, 817–826.

Huang, Y.Q. and Carmichael, G.G. (1996) Role of polyadenylation in nucleocytoplasmic transport of mRNA. *Mol. Cell. Biol.*, **16**, 1534–1542.

Huber, J., Cronshagen, U., Kadokura, M., Marshallsay, C., Wada, T., Sekine, M. and Luhrmann, R. (1998) Snurportin1, an m3G-cap-specific nuclear import receptor with a novel domain structure. *EMBO J.*, **17**, 4114–4126.

Hughes, T.R., Evans, S.K., Weilbaecher, R.G. and Lundblad, V. (2000) The Est3 protein is a subunit of yeast telomerase. *Curr. Biol.*, **10**, 809–812.

Iborra, F.J., Jackson, D.A. and Cook, P.R. (2001) Coupled transcription and translation within nuclei of mammalian cells. *Science*, **293**, 1139–1142.

Izaurralde, E., Lewis, J., Gamberi, C., Jarmolowski, A., McGuigan, C. and Mattaj, I.W. (1995) A cap-binding protein complex mediating U snRNA export. *Nature*, **376**, 709–712.

Lendvay, T.S., Morris, D.K., Sah, J., Balasubramanian, B. and Lundblad, V. (1996) Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional EST genes. *Genetics*, **144**, 1399–1412.

Licht, J.D. and Collins, K. (1999) Telomerase RNA function in recombinant Tetrahymena telomerase. *Genes Dev.*, **13**, 1116–1125.

Lingner, J., Cech, T.R., Hughes, T.R. and Lundblad, V. (1997a) Three ever shorter telomere (Est) genes are dispensable for *in vitro* yeast telomerase activity. *Proc. Natl Acad. Sci. USA*, **94**, 11190–11195.

Lingner, J., Hughes, T.R., Shevchenko, A., Mann, M., Lundblad, V. and Cech, T.R. (1997b) Reverse transcriptase motifs in the catalytic subunit of telomerase. *Science*, **276**, 561–567.

Livengood, A.J., Zaug, A.J. and Cech, T.R. (2002) Essential regions of *Saccharomyces cerevisiae* telomerase RNA: separate elements for Est1p and Est2p interaction. *Mol. Cell. Biol.*, **22**, 2366–2374.

Lukowiak, A.A., Narayanan, A., Li, Z.H., Terns, R.M. and Terns, M.P. (2001) The snoRNA domain of vertebrate telomerase RNA functions to localize the RNA within the nucleus. *RNA*, **7**, 1833–1844.

Lundblad, V. and Szostak, J.W. (1989) A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell*, **57**, 633–643.

Mattaj, I.W. (1986) Cap trimethylation of U snRNA is cytoplasmic and dependent on U snRNP protein binding. *Cell*, **46**, 905–911.

Mitchell, J.R., Wood, E. and Collins, K. (1999) A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*, **402**, 551–555.

Mouaikel, J., Verheggen, C., Bertrand, E., Tazi, J. and Bordonne, R. (2002) Hypermethylation of the cap structure of both yeast snRNAs and snoRNAs requires a conserved methyltransferase that is localized to the nucleolus. *Mol. Cell*, **9**, 891–901.

Ohno, M., Segref, A., Bachi, A., Wilm, M. and Mattaj, I.W. (2000) PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. *Cell*, **101**, 187–198.

Ohno, M., Segref, A., Kuersten, S. and Mattaj, I.W. (2002) Identity elements used in export of mRNAs. *Mol. Cell*, **9**, 659–671.

Pederson, T. (1998) The plurifunctional nucleolus. *Nucleic Acids Res.*, **26**, 3871–3876.

Schimmang, T., Tollervey, D., Kern, H., Frank, R. and Hurt, E.C. (1989) A yeast nucleolar protein related to mammalian fibrillarin is associated with small nucleolar RNA and is essential for viability. *EMBO J.*, **8**, 4015–4024.

Seto, A.G., Zaug, A.J., Sobel, S.G., Wolin, S.L. and Cech, T.R. (1999) *Saccharomyces cerevisiae* telomerase is an Sm small nuclear ribonucleoprotein particle. *Nature*, **401**, 177–180.

Singer, M.S. and Gottschling, D.E. (1994) TLC1: template RNA component of *Saccharomyces cerevisiae* telomerase. *Science*, **266**, 404–409.

Sleeman, J.E. and Lamond, A.I. (1999) Newly assembled snRNPs associate with coiled bodies before speckles, suggesting a nuclear snRNP maturation pathway. *Curr. Biol.*, **9**, 1065–1074.

Teixeira, M.T., Siniossoglou, S., Podtelejnikov, S., Benichou, J.C., Mann, M., Dujon, B., Hurt, E. and Fabre, E. (1997) Two functionally distinct domains generated by *in vivo* cleavage of Nup145p: a novel biogenesis pathway for nucleoporins. *EMBO J.*, **16**, 5086–5097.

Vallen, E.A., Hiller, M.A., Scherson, T.Y. and Rose, M.D. (1992) Separate domains of *KAR1* mediate distinct functions in mitosis and nuclear fusion. *J. Cell Biol.*, **117**, 1277–1287.

Venema, J. and Tollervey, D. (1999) Ribosome synthesis in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.*, **33**, 261–311.

Will, C.L. and Luhrmann, R.U.R. (2001) Spliceosomal UsnRNP biogenesis, structure and function. *Curr. Opin. Cell Biol.*, **13**, 290–301.

Zhou, J., Hidaka, K. and Futcher, B. (2000) The Est1 subunit of yeast telomerase binds the Tlc1 telomerase RNA. *Mol. Cell. Biol.*, **20**, 1947–1955.

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