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Cross Talk between tRNA and rRNA Synthesis in *Saccharomyces cerevisiae*

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Temperature-sensitive RNA polymerase III (*rpc160-112* and *rpc160-270*) mutants were analyzed for the synthesis of tRNAs and rRNAs in vivo, using a double-isotopic-labeling technique in which cells are pulse-labeled with [^{33}P]orthophosphate and coextracted with [^{3}H]uracil-labeled wild-type cells. Individual RNA species were monitored by Northern blot hybridization or amplified by reverse transcription. These mutants impaired the synthesis of RNA polymerase III transcripts with little or no influence on mRNA synthesis but also largely turned off the formation of the 25S, 18S, and 5.8S mature rRNA species derived from the common 35S transcript produced by RNA polymerase I. In the *rpc160-270* mutant, this parallel inhibition of tRNA and rRNA synthesis also occurred at the permissive temperature (25°C) and correlated with an accumulation of 20S pre-rRNA. In the *rpc160-112* mutant, inhibition of rRNA synthesis and the accumulation of 20S pre-rRNA were found only at 37°C. The steady-state rRNA/tRNA ratio of these mutants reflected their tRNA and rRNA synthesis pattern: the *rpc160-112* mutant had the threefold shortage in tRNA expected from its preferential defect in tRNA synthesis at 25°C, whereas *rpc160-270* cells completely adjusted their rRNA/tRNA ratio down to a wild-type level, consistent with the tight coupling of tRNA and rRNA synthesis in vivo. Finally, an RNA polymerase I (*rpa190-2*) mutant grown at the permissive temperature had an enhanced level of pre-tRNA, suggesting the existence of a physiological coupling between rRNA synthesis and pre-tRNA processing.

The existence of three nuclear transcription systems is documented for all eukaryotes investigated so far. RNA polymerase I synthesizes the three largest rRNAs, RNA polymerase II produces mRNAs and many noncoding RNAs, and RNA polymerase III makes tRNAs and 5S rRNA, as well as a few small noncoding RNAs. Exceptions to this transcriptional specialization are rare and mostly concern noncoding RNA species that can be produced by either RNA polymerase II or III, depending on the phylum considered (reference 39 and references therein). Given its universality, the triplication of the transcriptional apparatus must provide a major selective advantage to the eukaryotic cell, probably by facilitating the separate control of mRNA, rRNA, and tRNA synthesis in response to changes in the environment or in the cell growth rate. On the other hand, RNA polymerases I and III deliver matching amounts of tRNAs and rRNAs to the protein synthesis machinery and may thus need to operate in a closely coordinated way (references 21, 38, and 39 and references therein). In fact, the extent to which the three nuclear RNA polymerases are coordinated relative to each other remains largely undetermined, although this is presumably a key aspect of the transcriptional regulation of growth.

Yeast (*Saccharomyces cerevisiae*) is a particularly convenient model organism to study transcription and its regulation. Its three nuclear RNA polymerases are biochemically and genetically well characterized and contain 14, 12, and 17 subunits. Five of the subunits are common to the three enzymes and two others are shared by RNA polymerases I and III, thus providing a potential target for common regulatory controls (references 3, 5, and 42 and references therein). These common subunits are structurally conserved among eukaryotes, and the corresponding polypeptides are interchangeable in vivo between budding yeast (*S. cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), and humans (20, 27, 28, 29). Yeast is also the only eukaryote from which temperature-sensitive mutants are available for each of the the three transcription enzymes (12, 22, 40). These mutants provide a unique opportunity to assess the interdependency of the three RNA polymerases by using such mutants to separately turn off each RNA polymerase in vivo and to examine how this affects the physiological activity of the other two transcription systems.

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MATERIALS AND METHODS

Yeast strains and growth conditions. Yeast strains are listed in Table 1, and their growth patterns on YPD (1% yeast extract, 2% Bacto Peptone, and 2% glucose) are shown in Fig. 1. In vivo labeling with $^{33}P_i$ and [^{3}H]uracil was done in low-phosphate medium (YPD*) and in casein hydrolysate medium lacking uracil (25, 28), respectively. Growth was monitored with a Hach Ratio/XR turbidimeter. One turbidimetry unit corresponds to about 2×10^8 haploid cells per ml (for the wild-type strain W303-1b) and to an absorbance of 7.2 at 600 nm. Wild-type cells grown on YPD and YPD* had a doubling time of 110 min at 30°C, but growth was slightly slower under the conditions of ^{3}H labeling (doubling time, 130 min).

The *rpc160-112* and *rpc160-270* mutants are well-characterized RNA polymerase III mutants that inhibit transcription in vitro (9, 34). The *rpb1-1* (strain RY260) mutant is a tight conditional mutant of RNA polymerase II that rapidly stops mRNA synthesis when shifted to 37°C (22). Our data (see Fig. 2) indicate a slower decrease in mRNA accumulation than in the original report. The presence of an extragenic suppressor of *rpb1-1* in our RY260 isolate was ruled out by appropriate genetic crosses, but the possibility of a mild intragenic suppressor cannot be excluded. The *rpb1-1* mutant also rapidly inhibits rRNA and tRNA synthesis at 37°C (22) (data not shown). The *rpa190-2* mutant is an RNA polymerase I mutant with a strong temperature-sensitive growth defect (40) (Fig. 1). Yet our in vivo labeling data show that its RNA polymerase I defect, already quite strong at 25°C, is not much increased at 37°C (see Fig. 3).

In vivo labeling. A total of 250 μCi of $^{33}P_i$ (at 10 μCi/μl) was added to 5-ml log-phase cultures that were further grown for 10 min before the addition of 20

TABLE 1. Yeast strains used in this study

Strain	Genotype	Origin or reference
RY260	*MAT* **a** *ura3-52 rpb1-1*	22
YPH52	*MAT* **a** *ura3-52 his3-Δ200 trp1Δ1 lys2-801a ade2-1*	30
W303-1b	*MATα CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2–1*	24
NOY264	*MATα rpa190-2 trp1-Δ1 his4-Δ401 leu2-3,112 ura3-52 CAN1-100*	40
MW657	*MATα ade2-1 lys2-801 ura3-52 trp1-Δ1 his3-Δ200 rpc160-Δ1::HIS3*/pC160–112 (*TRP1 CEN4 rpc160-112*)	9
MW1029	*MAT***a** *ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpc160-τ1::HIS3*/pC160–270 (*TRP1 CEN4 rpc160-270*)	34
CJY058	*MATα ura3-52 his3-Δ200 gea21-τ::HIS3 leu2-3,112 pep4-Δ::LEU2 lys2-801*	Anne Peyroche, unpublished data
OG27GF	*MATα ura3 his3-D200 gea2-Δ::HIS3 leu2 pep4-Δ::LEU2 top1-Δ::LEU2 met15-Δ trp1 lys2-801*/pGEN-GFP3 (2μm *TRP1 GFP*)/pFL44-L (2 μm *URA3*)	Offspring of OG23-1D × CJYO58, transformed by pFL44 and pGEN-GFP3
OG23-1D	*MAT***a** *his3-Δ200 leu2 ura3 trp1 met15-Δ1 lys2–801*	D156-1 × JS209
JS209	*MATα his3-Δ200 leu2-Δ1 ura3-167 trp1-Δ63 met15-Δ1*	31
D156-1c	*MAT***a** *rpa34-Δ::HIS3 ura3-52 ade2-1 llys2-801 his3-Δ200 trp1 leu2*	11

ml of ice-cold water. The culture was spiked with a 100-μl aliquot of wild-type cells labeled with [^{3}H]uracil (see below). Labeling data were expressed by measuring the ratio between $^{33}P_i$- and ^{3}H-labeling signals. The external ^{3}H control notably improves the quantification of the $^{33}P_i$ pulse-labeling assay, since it bypasses experimental variations in the efficiency of RNA extraction or recovery, assuming that the cells behave similarly during the extraction and RNA purification procedures. It also minimizes experimental artifacts related to gel loading and electrophoresis. Cells were harvested by centrifugation, washed twice with ice-cold water, frozen in an ice-ethanol bath, and stored at −80°C. $^{33}P_i$ uptake, measured by counting the radioactivity left in the culture supernatants, ranged between 30 and 60% of the exogenous $^{33}P_i$. The reference sample of [^{3}H]uracil-labeled wild-type cells (strain OG27GF) (Table 1) was prepared by adding 5 mCi of [^{3}H]uracil (at 1 mCi/ml) to a 100-ml culture of cells grown exponentially at 30°C, at an optical density of 0.24 at 600 nm. Cells were further grown for 1.5 h, leading to a 95% incorporation of [^{3}H]uracil, harvested by centrifugation, washed twice with ice-cold water, and resuspended in 20 ml of water. They were dispatched in 100-μl samples frozen in ethanol-dry ice and stored at −80°C.

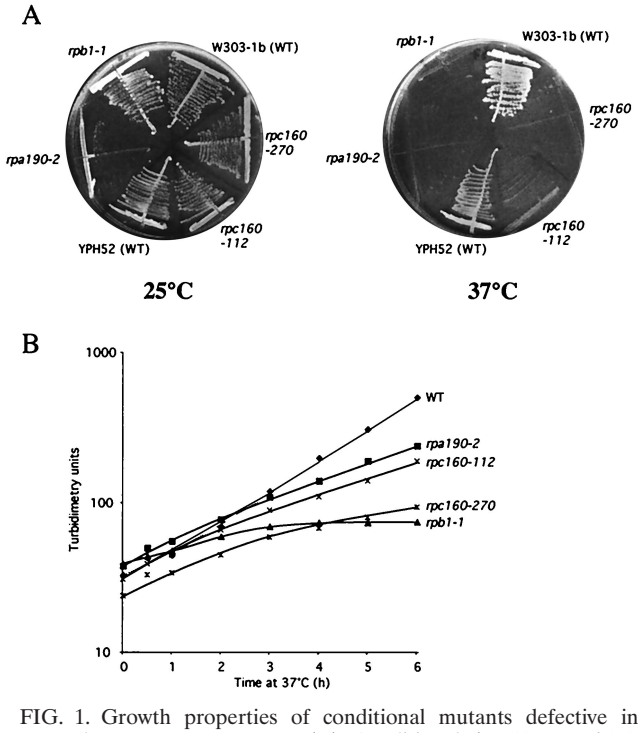

FIG. 1. Growth properties of conditional mutants defective in RNA polymerase I, II, or III. (A) Conditional (*rpa190-2*, *rpb1-1*, *rpc160-112*, and *rpc160-270*) mutants and two wild-type (WT) strains (W303-1b and YPH52) were streaked onto YPD plates and incubated for 4 days at 25 and 37°C. Doubling times in liquid medium at 25°C were 140 min (wild-type and *rpb1-1* cells), 180 min (*rpc160-112* cells), 200 min (*rpa190-2* cells), and 250 min (*rpc160-270* cells). The pedigrees and genotypes of the corresponding strains are given in Table 1. (B) Growth responses of the *rpb1-1*, *rpa190-2*, *rpc160-112*, and *rpc160-270* mutant strains and of the wild-type strain W303-1b in YPD liquid cultures grown exponentially at 25°C and shifted to 37°C for 6 h. Growth was monitored by turbidimetry (see Materials and Methods).

RNA extraction. Cell cultures (5 ml) were pelleted by centrifugation, suspended in 0.5 ml of 50 mM sodium acetate buffer (pH 5.3) with 10 mM EDTA and 1% sodium dodecyl sulfate, and mixed with an equal volume of buffered phenol prewarmed at 65°C. Cells were broken in an Eaton press with a homemade device or by extraction with 200 μl of glass beads. Comparable yields of RNA were obtained by either method. For the glass bead extraction, cells went through five consecutive cycles of vigorous vortexing at room temperature (2 min) and transfer to a 65°C bath (1 min) before being frozen in liquid nitrogen and thawed at 65°C. The whole procedure was done twice. After centrifugation (15,000 × *g*) for 20 min at 4°C and extraction in 0.5 ml of phenol-dichloromethane-isoamyl alcohol (25:24:1), nucleic acids were precipitated in 2.5 volumes of ethanol and 0.2 volume of 10 M LiCl, rinsed with 70% ethanol, and dissolved in 50 μl of RNase-free water treated with diethyl pyrocarbonate. This was treated for 2 h at 37°C in the presence of alkaline phosphatase (5 U/μg of RNA). After phenol extraction and ethanol precipitation, RNA was dissolved in 50 μl of RNase-free water and stored at −20°C. RNA radioactivity was measured by precipitating 1 μg of RNA with 4 μg of carrier tRNA in 1 ml of 5% trichloroacetic acid. After 2 h on ice, the RNA precipitate was filtered through a 0.45-μm-pore-size membrane (Millipore) and counted in biodegradable counting scintillant (Amersham Pharmacia Biotech). A specific radioactivity of about 10^{-2} μCi/μg of RNA was obtained in all cases.

RNA quantification. RNA (10 μg) was separated by electrophoresis on 6% polyacrylamide–8 M urea gels and autoradiographed with Kodak Biomax MR film. In the case of the two large rRNA species, 1 μg of RNA was loaded onto 1% agarose denaturing formaldehyde gels. The gels and their autoradiograms were superimposed to locate the major stable RNA species (tRNAs and 5S, 5.8S, 18S, and 25S rRNA). This was facilitated by using ^{33}P rather than ^{32}P labeling, due to the superior resolution of the autoradiographic signals. The corresponding gel positions were punched with an awl, generating 2.3-mm-diameter spots, as illustrated in Fig. 3. This material was rehydrated for 30 min in 25 μl of water and left overnight at room temperature in 0.5 ml of NCS-II tissue solubilizer (Amersham Pharmacia Biotech). Samples were equilibrated for 1 week in the dark in 1 ml of BCS-NA scintillant, and $^{33}P_i$ and ^{3}H activity was counted. Spots located between the autoradiographic signals were collected and measured in the same way, providing a measure of the background level of radioactivity. The signal-to-noise ratio was always higher than 10-fold (and usually close to 100-fold) for all experimental data presented here.

RNA levels were determined by Northern blot analysis (except for *PEP4* mRNA; see below), using standard conditions. Briefly, 10 μg of RNA was separated on a 1% agarose denaturing formaldehyde gel, blotted onto a nylon membrane, and hybridized overnight to radiolabeled oligonucleotide probes in

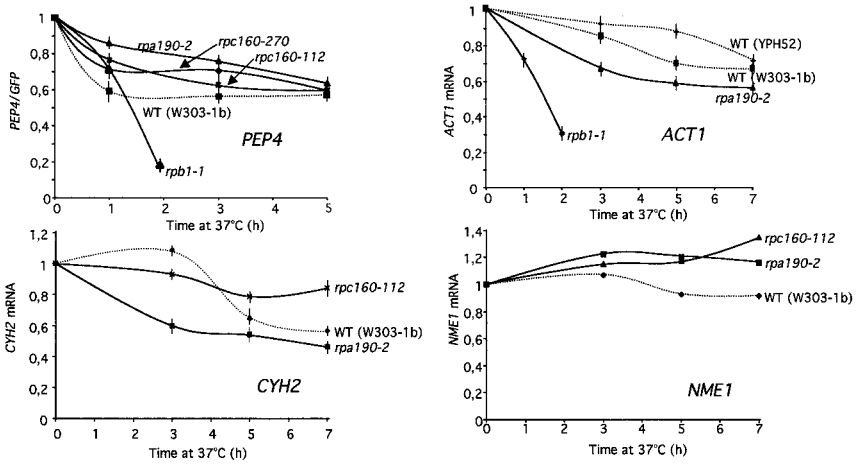

FIG. 2. mRNA synthesis in RNA polymerase I, II, and III mutants shifted to 37°C. Steady-state levels of *PEP4*, *ACT1*, *NME1*, and *CYH2* RNAs in RNA polymerase I (*rpa190-2*), II (*rpb1-1*), and III (*rpc160-112* and *rpc160-270*) mutants were compared to those in the W303-1b and YPH52 wild-type (WT) strains. *ACT1*, *CYH2*, and *NME1* RNA levels were determined by Northern hybridization. *PEP4* levels were determined by RT-PCR of mutant or wild-type cultures spiked with an aliquot of *pep4*-Δ wild-type cells (strain OG27GF) (Table 1) expressing a plasmid-borne copy of the *GFP* gene. The *GFP* mRNA served as an RNA recovery marker of *PEP4* mRNA (see Materials and Methods). Error bars correspond to experimental values obtained in at least two entirely independent RT-PCR or Northern blot experiments, except for *NME1* hybridization data (one experiment only). Experimental values were normalized to the wild-type control, arbitrarily taken to have a level of 1.

0.5 M sodium phosphate buffer (pH 7.2) with 10 mM EDTA and 7% sodium dodecyl sulfate. *ACT1*, *CYH2*, and *NME1* RNAs were hybridized to 5′-TGAA GAAGATTGAGCAGCGGTTTG-3′, 5′-CATGTTAATTCTGTGGTGATGT TGAC-3′, and 5′-CGTCATAACTATGGTTTAG-3′ probes, and *PEP4* mRNA was quantified by reverse transcription-PCR (RT-PCR) of RNA from mutant or wild-type cultures spiked with a small aliquot of wild-type cells (strain OG27GF), as described above for the in vivo double-labeling assay. OG27GF has a deletion of the *PEP4* gene and harbors the *GFP* gene (Table 1). *GFP* mRNA, amplified from the 5′-GTAACAAGACTGGACCATCACC-3′ and 5′-GGTGAAGGTGA TGCTACTTACGG-3′ primers, served as an RNA recovery marker of the *PEP4* mRNA, which was amplified from the 5′-GACCGGTCCAACCCTTCTTGG-3′ and 5′-GGTTCCTTGGCTTGTTTCC-3′ primers. One microgram of total RNA was reverse transcribed for 1 h at 42°C with 100 pmol of appropriate oligonucleotide primers. The RT-PCR amplification signals were directly proportional to the amount of RNA, over a range of 0.1 to 10 μg. RT was stopped by adding 180 μl of water to the 20-μl reaction volume. Ten-microliter samples were amplified by PCR (15 cycles) in the presence of 25 μCi of [α-^{32}P]dCTP, using 10 pmol of the corresponding oligonucleotide primers. A sample of 5 μl of each reaction product was loaded on a 6% polyacrylamide–8 M urea gel, dried, and analyzed with a Molecular Dynamics PhosphorImager.

RESULTS

mRNA synthesis is uncoupled from rRNA or tRNA synthesis in RNA polymerase I and III mutants. Unlike the RNA polymerase II *rpb1-1* mutant, RNA polymerase I (*rpa190-2*) and III (*rpc160-112* and *rpc160-270*) mutants continue to grow for at least 6 h after the temperature shift (Fig. 1) and thus have little effect on the synthesis of essential mRNAs. This was confirmed by measuring the levels of individual RNA polymerase II transcripts such as the *PEP4*, *ACT1*, and *CYH2* mRNAs and the RNA of RNase MRP encoded by *NME1* (Fig. 2). Likewise, cells that are deprived of the largest subunit of RNA polymerase I (by controlling its transcription with the galactose-repressible *GAL1* promoter) have little effect on the synthesis of several ribosomal proteins (41). Thus, the level of RNA polymerase II-dependent transcription in vivo is largely uncoupled from the activity of the other two transcription enzymes.

RNA polymerase III mutants coordinately block rRNA and tRNA synthesis at 37°C. RNA polymerase III (*rpc160-112* and *rpc160-270*) mutants distinctly impair tRNA synthesis at 25°C (consistent with the detectable growth defect at this temperature) and completely prevent it at 37°C, as shown by in vivo labeling data (Fig. 3). Furthermore, Northern hybridization (Fig. 4) shows that the *rpc160-112* mutant strongly reduces the steady-state level of pre-tRNA^{Leu3} relative to the mature tRNA^{Leu3}. They also reveal a marked depletion in the *SCR1* RNA component of the signal recognition particle. This RNA is predicted to be an RNA polymerase III transcript because of the presence of a typical RNA polymerase III terminator at its 3′ end (10). Previous experiments based on [^{3}H]uracil pulse-labeling (32) indicated that RNA polymerase III mutants hardly affect 5S rRNA synthesis in vivo, despite overwhelming evidence that the latter is made by RNA polymerase III in vitro (26, 35). The more quantitative double-label technique used here shows that *rpc160-112* and *rpc160-270* cells distinctly affect 5S rRNA synthesis, albeit less than tRNA synthesis, thus reconciling the in vitro and in vivo data (Fig. 3 and 5A).

Beyond their effect on RNA polymerase III transcripts, *rpc160-112* and *rpc160-270* cells also strongly reduce the synthesis of the 5.8S, 18S, and 25S rRNAs, which derive from a common single transcript made by RNA polymerase I. In the case of the *rpc160-112* mutant, a shift to the restrictive temperature (37°C) leads to a tight adjustment of the de novo synthesis of large rRNAs in response to the temperature-sensitive RNA polymerase III defect. As seen in Fig. 5B, the relative rates of tRNA and rRNA synthesis were down to the wild-type level within 3 h after the shift, i.e., well before growth arrest (Fig. 1). In the case of the *rpc160-270* mutant, this pleiotropic effect on rRNA synthesis also occurs at 25°C.

Since a 10-min pulse with $^{33}P_i$ is close to the time needed to process pre-rRNA in vivo (36), our labeling data do not distinguish between transcriptional and posttranscriptional effects

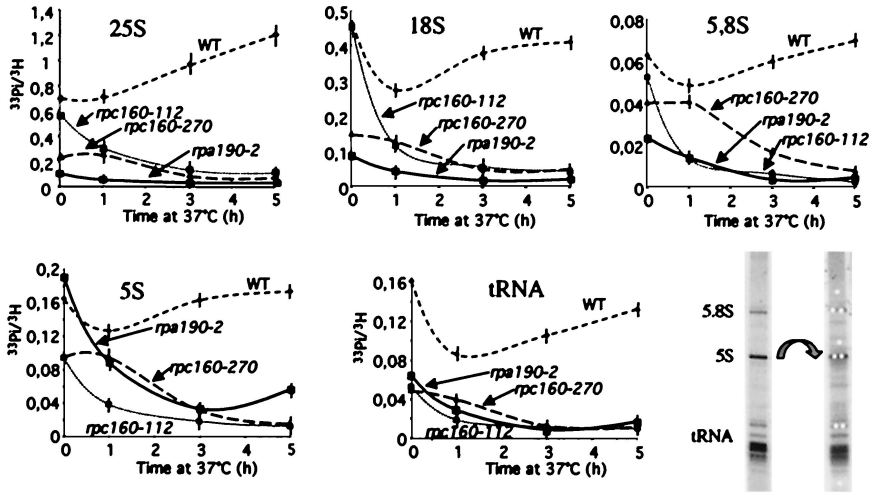

FIG. 3. tRNA and rRNA synthesis in RNA polymerase I and III mutants. Mutant (*rpc160-112*, *rpc160-270*, and *rpa190-2*) and wild-type (WT) (W303-1b) strains were shifted from 25 to 37°C in low-phosphate medium (YPD*). Cells were labeled in vivo for 10 min with $^{33}P_i$ and coextracted with a small amount of tritiated wild-type cells (strain OG27GF grown at 30°C) to provide an interval RNA recovery standard. tRNAs and rRNAs were separated by gel electrophoresis and assayed for ^{33}P and ^{3}H radioactivity. An example of gel separation is provided (the holes correspond to the recovery of RNA by awl punching, as described in Materials and Methods). Error bars correspond to experimental values obtained in at least two entirely independent in vivo labeling experiments.

on rRNA biogenesis. Previous work from this laboratory suggests that RNA polymerase III defects may impair pre-rRNA processing in vivo. Thus, the *rpc160-112* mutant and another conditional (*rpc160-41*) mutant (12) have a mild effect on the maturation of 5.8S rRNA at the semipermissive temperature of 30°C (13). Moreover, yeast mutants specifically defective in the biogenesis of 5S rRNA accumulate the 27S pre-rRNA precursor of 25S rRNA, with no effect on 20S, the precursor of 18S rRNA (7) (Fig. 4). *rpc160-112* and *rpc160-270* cells had a symmetrical effect on rRNA processing and distinctly enhanced the level of 20S pre-rRNA. In the *rpc160-112* mutant, this occurred only at 37°C, while the *rpc160-270* mutant had this effect at both temperatures (Fig. 4). Hence, the effect of these two mutants on 18S rRNA synthesis (as measured by pulse-labeling) correlates with, and at least partly results from, an rRNA processing defect.

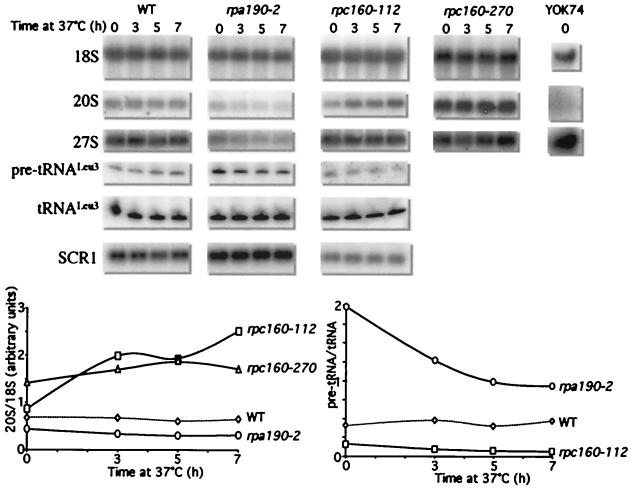

FIG. 4. Northern hybridization of RNA polymerase I or III mutants. Northern blot hybridizations with 1 μg of total RNA extracted from wild-type (WT) and mutant cells (same strains as in the previous figures) exponentially grown at 25°C and then shifted to the restrictive temperature (37°C) for 3, 5, and 7 h are shown. The localization data of the pre-rRNA cleavage sites were taken from reference 36). The oligonucleotide probes used specifically hybridized to the 20S pre-rRNA (5′-GCACAGAAATCTCTCACCGT-3′, located between cleavage sites D and A2), 27S pre-rRNA (5′-GCCTAGACGCTCTC TTCTTA-3′, located between cleavage sites C2 and C1 and recognizing all 27S species), 25S rRNA (5′-CCGTGAAATGTTTCTTGGCG TGAG-3′), 18S rRNA (5′-GCCGACGACCGTGGTCTGAAC-3′, internal to the mature 18S sequence), pre-tRNA^{Leu3} (5′-CCAAACA ACCACTTATTTGTTGA-3′, corresponding to the 5′ leader sequence 19), and mature tRNA^{Leu3} (5′-GAACTCTTGCATCTTACGATAC-3′), and *SCR1* (5′-CCATCACGGGTCACCT-3′).

Our Northern hybridization data also show a good match between the steady-state levels of mature rRNAs and tRNAs and their rates of synthesis as predicted by in vivo labeling data (Fig. 5C). The *rpc160-112* mutant has an almost threefold deficit in tRNAs when grown at 25°C, consistent with its limited effect on rRNA synthesis under these conditions. *rpc160-270* cells accumulate tRNAs and rRNAs in the same ratio as wild-type cells, as expected from their coordinated effects on tRNA and rRNA synthesis, even when grown at 25°C. The allele-specific difference consistently observed between the *rpc160-112* and *rpc160-270* mutants is puzzling, given that they were constructed in the same isogenic background and have similar growth patterns (Fig. 1). Other RNA polymerase III mutants behave like the *rpc160-112* mutant in the sense that they have a deficit in tRNA at the permissive temperature (12, 32) (data not shown), and there may thus be something special about the *rpc160-270* mutant. A cryptic suppressor mutant seems unlikely, given the perfectly regular 2:2 segregation of its temperature-sensitive growth defect in meiotic crosses (data not shown). We note, however, that the *rpc160-112* mutant has a direct catalytic defect (9) with a rapid transcriptional arrest at 37°C (Fig. 3), whereas the elongational defect of the *rpc160-270* mutant correlates with a high level of the cleaving RNase

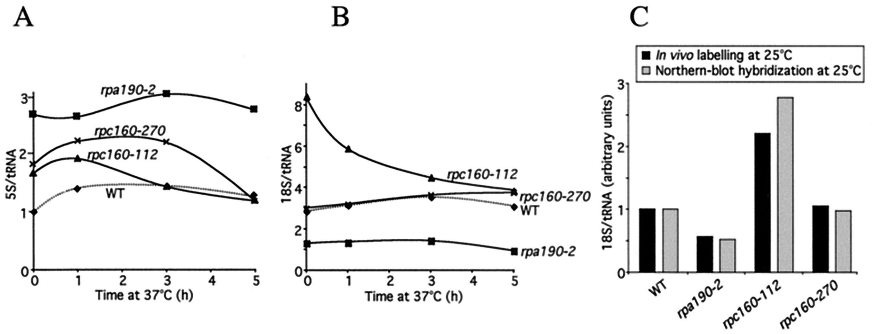

FIG. 5. Comparison of pulse-labeling and steady-state levels of tRNA and rRNAs. (A) relative rates of 5S rRNA and tRNA synthesis in RNA polymerase I (*rpa190–2*) and III (*rpc160–112* and *rpc160–270*) mutants compared to the W303-1b wild type (WT). The data were replotted from Fig. 3. (B) Relative rates of 18S rRNA and tRNA synthesis in RNA polymerase I (*rpa190–2*) and III (*rpc160–112* and *rpc160–270*) mutants compared to the W303-1b wild type. The data were replotted from Fig. 3. (C) Steady-state levels of 18S rRNA and tRNA synthesis in RNA polymerase I (*rpa190–2*) and III *rpc160–112* and *rpc160–270*) mutants compared to the wild type (W303-1b). RNA levels were determined by Northern blotting using a 5′-GCCGACGACCGTGGTCTGAAC-3′ internal 18S rRNA probe and a 5′-GAACTCTTGCATCTTACGATAC-3′ internal $tRNA^{Leu3}$ probe.

activity of RNA polymerase III (34) and a somewhat delayed transcriptional arrest in vivo.

An RNA polymerase I-defective mutant interferes with tRNA processing at the permissive temperature. Given that RNA polymerase III mutants affect the overall rate of rRNA synthesis and also interfere with pre-rRNA processing, we wondered if RNA polymerase I mutants might have a reciprocal effect on tRNAs. In vivo labeling data suggest that this may be the case (compare the rates of tRNA and rRNA synthesis in *rpa190-2* cells in Fig. 3) but are inconclusive, because the transcriptional defect of the *rpa190-2* mutant, already quite strong at 25°C, is hardly aggravated at 37°C (a similar situation was observed for the *rpa190-1* mutant [data not shown]). Yet, *rpa190-1* and *rpa190-2* cells are strongly temperature sensitive in terms of growth, suggesting that rRNA synthesis may be especially growth limiting at 37°C. Northern hybridization data, however, show that *rpa190-2* cells grown at 25°C have a high level of pre-$tRNA^{Leu3}$ (Fig. 4) and thus interfere with pre-tRNA processing, perhaps in relation to the nucleolar localization of tRNA processing enzymes (1). We cannot rule out the possibility that there is, in addition, some effect on the transcriptional synthesis of tRNAs, as is indeed suggested by the partial drop in the pre-tRNA/tRNA ratio when *rpa190-2* cells are shifted to 37°C. However, the high content of 7SL RNA (*SCR1*) found in *rpa190-2* cells at both temperatures argues against a general and massive effect on RNA polymerase III-dependent transcription.

DISCUSSION

Yeasts are fast-growing cells that invest a substantial amount of metabolic energy in ribosome biogenesis and may therefore need to precisely adjust the synthesis of rRNAs, tRNAs, and ribosomal proteins as a function of the growth rate. This regulation is fairly well understood as far as ribosomal proteins are concerned (38), but comparatively little is known of the control of tRNA and rRNA synthesis. In particular, the extent to which yeast RNA polymerases I and III are coregulated relative to each other and to the transcriptional synthesis of ribosomal proteins is still a moot point. In fact, the main evidence for coordinated control of the transcriptional synthesis of rRNA, ribosomal protein mRNAs, and tRNAs is that blocking protein secretion inhibits these three processes in a way that requires protein kinase C (19). On the other hand, rRNA and tRNA synthesis can be uncoupled under physiological conditions, such as amino acid starvation (6, 23). Even under balanced growth conditions, the cellular levels of tRNAs and rRNAs are roughly but not strictly constant, since rRNAs are more strongly affected than tRNAs in slow-growing cells (15, 32, 37). Finally, conditional mutants of RNA polymerase I or III have no effect on the transcription of ribosomal protein genes by RNA polymerase II (reference 41 and this study), showing that there is no obligatory link between the transcriptional synthesis of rRNA and of ribosomal protein mRNAs.

We show here that RNA polymerase III mutants turn off the formation of the three large rRNA species (25S, 18S, and 5.8S) in parallel to the reduced rate of tRNA synthesis, thereby adapting the flux of newly synthesized rRNA to the low level of tRNA synthesis and keeping the rRNA/tRNA steady-state ratio at the wild-type level. An obvious concern is that this could somehow be the indirect result of a common dependency on growth rate. The fast response of *rpc160-112* cells when shifted to 37°C argues against this interpretation, since they reach a low rate of rRNA synthesis within one doubling time, well before growth arrest is observed. This coordinated synthesis of tRNAs and rRNAs could partly involve transcriptional effects, as in secretion-defective cells (19), and may perhaps also reflect changes in RNA turnover. However, our data strongly suggest an additional effect on pre-RNA processing, as shown by the increase of 20S pre-rRNA observed in the *rpc160-112* and *rpc160-270* mutants. This is consistent with previous data showing that RNA polymerase III mutants grown at 30°C have minor but distinct effects on pre-rRNA processing (13). Conversely, we also observed that RNA polymerase I mutant cells accumulate a high level of pre-$tRNA^{Leu3}$ and thus probably interfere with tRNA processing.

The mechanism by which RNA polymerase III may control pre-rRNA processing is unknown. One possibility is that a hypothetical RNA polymerase III holoenzyme (5) may contain or contact nucleolar proteins participating in pre-rRNA pro-

cessing. This could arguably account for the allele-specific differences in the rRNA processing defects of the *rpc160-112* and *rpc160-270* mutants at 25°C, as these mutants are thought to have a different effect on the conformation of the elongating RNA polymerase III complex (34). Alternatively, RNA polymerase III transcripts could directly participate in pre-rRNA processing. This is the case for U3 snRNA in plants (16) or RNase MRP RNA in mammals (43), but the yeast counterparts are made by RNA polymerase II (reference 14 and this work). RNase P RNA is another candidate, as it affects 5.8S rRNA maturation in vivo (4) and is an RNA polymerase III transcript in organisms ranging from yeasts (17) to humans (2). Moreover, its high dosage partly suppresses a mutant defective in the RNA polymerase III initiation factor TFIIIC (18). Finally, yeast 5S rRNA mutants interfere with pre-rRNA processing, providing another link to RNA polymerase III (7). Native 5S rRNA is short-lived (probably reflecting its lack of nucleotide modification) (33) unless it is complexed by yeast ribosomal protein L1 (8). It could therefore operate as a sensor, stimulating pre-rRNA processing in response to RNA polymerase III activity. Unlike RNA polymerase III mutants, however, 5S rRNA mutants mainly interfere with 25S rRNA maturation, with little effect on 18S rRNA (7) (Fig. 4).

In human cells, transcriptional controls over tRNA and rRNA synthesis are probably critical to the (de)regulation of differentiated cell growth upon viral infection or tumorigenesis, as shown by the inhibitory effect of the retinoblastoma and p53 tumor-suppressing factors on RNA polymerases I and III (reference 39 and references therein). Our observation that yeast cells adjust pre-rRNA processing as a function of RNA polymerase III activity extends the repertoire of homeostatic controls of ribosome synthesis (21, 38). It would be interesting to know if a similar situation exists in human cells. Moreover, U6 snRNA and the signal recognition particle RNA are made by RNA polymerase III in organisms ranging from yeasts to humans, thus relating RNA polymerase III activity to mRNA splicing and cotranslational protein secretion. Taken together, these data underscore the highly pleiotropic role of RNA polymerase III in modulating the main steps of RNA and protein synthesis.

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