RNA polymerase I remains intact without subunit exchange through multiple rounds of transcription in *Saccharomyces cerevisiae*

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Previous experiments using mammalian cells suggested that after each round of transcription, RNA polymerase I (Pol I) dissociates into subunits that leave and reenter the nucleolus as individual subunits, before formation of a new initiation complex. In this study, we show that the size and subunit composition of Pol I did not change significantly when Pol I was not engaged in rRNA transcription, brought about by either the absence of Pol I-specific rDNA template or specific inhibition of the transcription initiation step that requires Rrn3p. In fact, Pol I purified from cells completely lacking rDNA repeats was more active than when purified from wild-type cells in an *in vitro* transcription system designed to assay active Pol I–Rrn3p complexes. Furthermore, measurements of the exchange of A135 and A190 subunits between preexistent Pol I and newly synthesized Pol I showed that these two largest subunits of Pol I do not disassociate through many rounds of transcription *in vivo*. Thus, Pol I is not a dynamic protein complex but rather a stable enzyme.

Synthesis of the ribosome is an energetically costly and tightly regulated cellular process that involves a major fraction of cellular resources (1, 2). In eukaryotic cells, RNA polymerase I (Pol I) is responsible for the synthesis of rRNA. The components of the Pol I transcription machinery have been defined in detail (for review, see refs. 3–6). In the model organism *Saccharomyces cerevisiae*, Pol I consists of 14 subunits (for review, see refs. 5 and 7). This 14-subunit complex is referred to as intact Pol I. In addition to Pol I, several transcription factors are required for efficient transcription of rDNA. Pol I must associate with Rrn3p to be competent for transcription initiation (8–10). The formation of the Pol I–Rrn3p complex is thought to be a primary target for regulation of Pol I activity *in vivo* (9–12). Pol I–Rrn3p is recruited to the promoter with the help of core factor [CF, consisting of three subunits: Rrn6p, Rrn7p, and Rrn11p (13–15)], TATA-binding protein [TBP (16–18)], and the upstream activation factor [UAF, consisting of six subunits: Rrn5p, Rrn9p, Rrn10p, Uaf30p, and histones H3 and H4 (19–21)]. Reb1 is required for the termination of transcription (22), which is prerequisite to the release of Pol I from the rDNA. Despite the relatively clear understanding of the factors required for transcription of rDNA, the stability of intact Pol I between rounds of transcription has not been studied in detail.

Recently, Misteli and coworkers (23, 24) proposed a new kinetic model for Pol I transcription in mammalian cells. By transiently expressing GFP-tagged Pol I subunits or associated factors and taking quantitative measurements of the movements of these proteins within a nucleolus of individual cells using photobleaching microscopy, the authors could fit the data to a model with a variety of kinetic parameters. This model proposes that, at the end of each transcription cycle, Pol I dissociates into individual subunits and exits the nucleolus, and that the dissociated subunits reenter the nucleolus individually and assemble into an initiation-competent Pol I, presumably at the promoter, before the start of the next transcription cycle. Although repeating disassembly and reassembly of a relatively stable protein complex for each transcription cycle seems energetically very costly, this kinetic model, which we call the "Pol I subunit exchange model," would provide a potential for additional steps of regulation of rRNA transcription through controlling localization of the enzyme's individual subunits and efficiency of their assembly into the intact enzyme.

In this study, we examine the possible dissociation and reassociation of Pol I subunits during transcription cycles as proposed by this model using the yeast *S. cerevisiae*. We have found that the size of the Pol I enzyme does not change significantly even when the enzyme is not engaged in transcription. Furthermore, direct kinetic measurements of the exchange between Pol I subunits during cellular growth showed that the two largest Pol I subunits do not dissociate from one another between rounds of transcription. Based on these data, we suggest that Pol I is relatively stable through multiple rounds of transcription.

Materials and Methods

Media, Strains, and Plasmids. YEPD medium consists of 1% yeast extract, 2% peptone, and 2% glucose. When indicated, the medium is supplemented with 2% galactose instead of glucose (YEP-Gal). For ^{35}S labeling of proteins, cultures were grown in synthetic galactose medium (2% galactose/0.67% yeast nitrogen base) supplemented with amino acids and required bases (25), except that methionine and uracil were omitted. Unless otherwise indicated, cultures were grown at 30°C with aeration. The yeast strains and plasmids used in this study are listed in Table 1. pNOY693 was constructed by cloning a DNA fragment containing *(His)$_6$-(HA)$_3$-RPA135* downstream of the *GAL7* promoter in a pRS316 plasmid derivative.

Glycerol Gradient Centrifugation. Cells were grown in 1-liter cultures as described in the legends to Figs. 1–4, harvested by centrifugation, washed once in breakage buffer (20 mM Tris-acetate, pH 7.9/500 mM K acetate/10% glycerol/0.1% Tween 20/1 mM PMSF), suspended in a final volume of 2 ml of breakage buffer, added to an equal volume of glass beads, and disrupted in a Fast Prep bead breaker (four times for 30 sec at 4.5 setting; Thermo Savant, Woburn, MA). The lysate was cleared by centrifugation for 30 min at 16,000 × *g*, and 0.1 ml of the cleared lysate was subjected to glycerol gradient centrifugation essentially as described by Riva *et al.* (26), except that the 10–30% (vol/vol) glycerol gradients were made in the breakage buffer. Centrifugation was done at 35,000 rpm for 21 h at 4°C in a Beckman SW48 rotor. After centrifugation, 0.5-ml fractions were collected, and proteins were precipitated with 100 μl of 100% trichloroacetic acid. The precipitates were dissolved in 100 μl of 1× SDS loading dye (Bio-Rad), and 20-μl samples were analyzed by SDS/PAGE followed by Western immunoblot analysis using a polyclonal antibody against A190. Resulting data

Abbreviations: Pol I, RNA polymerase I; HA, hemagglutinin.

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Table 1. Yeast strains and plasmids used in this study

Strain or plasmid	Description
Strains	
NOY505	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100*
NOY760	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100 rpa135*Δ::*LEU2* carrying pNOY442
NOY891	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100 rdn*ΔΔ::*HIS3* carrying pNOY353
NOY908	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100 rdn*ΔΔ::*HIS3* carrying pNOY373
NOY2073	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100 rdn*ΔΔ::*HIS3* $(His)_6$-$(HA)_3$-*RPA135* carrying pNOY353
NOY2078	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100 rpa135*Δ::*LEU2* $(HA)_7$-*rrn3(S213P)* carrying pNOY442
NOY2079	*MAT*a *ade2–1 ura3–1 trp1–1 leu2–3,112 his3–11,15 can1–100* carrying pNOY693
Plasmids	
pNOY353	pTV3 derivative carrying *GAL7–35S rDNA,* 5S *rDNA, TRP1,* 2μ, *amp* (29)
pNOY373	YEp351 derivative carrying *rDNA* with promoter starting from −206 and with *XhoI-NotI* flanked enhancer, *LEU2,* 2μ, *amp* (29)
pNOY442	pRS314 (*CEN6, ARSH4,* and *TRP1*) derivative carrying $(His)_6$-$(HA)_3$-*RPA135* (27)
pNOY693	pRS316 (*CEN6, ARSH4,* and *URA3*) derivative carrying *GAL7*-$(His)_6$-$(HA)_3$-*RPA135*

were analyzed by using QUANTITY ONE software (Bio-Rad) and plotted with SIGMA PLOT 2001 (SPSS, Chicago).

Pol I Preparation and *in Vitro* Transcription. For Pol I preparation, cells were grown in 1 liter of YEP-Gal to mid-log phase ($A_{600} \approx 0.5$). Cell extracts were prepared as for glycerol gradient centrifugation described above, except that the breakage buffer also contained 20 mM imidazole. The entire cleared lysate was mixed with 0.5 ml of Chelating Sepharose (Amersham Pharmacia Biosciences) charged with $NiSO_4$ and preequilibrated with breakage buffer. The mixtures were kept for 1.5 h at 4°C with agitation and transferred to Bio-Spin disposable columns (Bio-Rad). The resin was washed with 2 ml of breakage buffer, and the proteins were eluted with 1 ml of breakage buffer containing 250 mM imidazole. This eluate was then directly mixed with 50 μl of antihemagglutinin (anti-HA) antibody conjugated to agarose beads (Sigma–Aldrich) and kept with agitation for 1.5 h at 4°C. These beads were washed four times with 1 ml of breakage buffer (no imidazole), and proteins were eluted with two subsequent treatments with 100 μl of breakage buffer containing 1 mg/ml HA peptide (sequence: YPYDVPDYA). Relative concentration of the A135 subunit of Pol I was determined by SDS/PAGE and immunoblotting by using a monoclonal antibody against the HA epitope (12CA5).

In vitro transcription assays were performed as described (27), except that the sole source of Pol I and Rrn3p in these reactions was from the Pol I preparation described above. Purified UAF, CF, and TBP were prepared as described (27, 28). The amount of Pol I preparation added to each 20-μl reaction is described in the Fig. 2 legend.

Transfer Experiment. Cells were grown in 1 liter of S-Gal–met–ura (supplemented with 7.0 mCi of a mixture of [^{35}S]methionine and [^{35}S]cysteine; EasyTag, PerkinElmer) to early log phase ($A_{600} = 0.15$). The specific activities of [^{35}S]methionine and [^{35}S]cysteine were 1,175 Ci/mmol according to the manufacturer. Then, glucose was added to 2% final concentration, and excess cold methionine and cysteine were added (final concentration of each 200 μg/ml; resulting in >200,000-fold molar excess of the labeled amino acids). This amount of cold methionine and cysteine was sufficient to prevent further incorporation of ^{35}S-labeled amino acids into newly synthesized proteins (data not shown). Samples (250 ml) were collected immediately ($t = 0$) and after 2 and 4 h of incubation (approximately one and two generations of growth, respectively, after time 0). Samples were harvested and washed in breakage buffer as described above. Before disrupting the cells, carrier unlabeled NOY505 cells were added to make the mass of the cell pellet 0.5 grams for each sample to avoid artifacts of handling/breaking very small amounts of radioactive cells. Pol I preparations were made as for *in vitro* transcription (see above), except that proteins were eluted from the anti-HA beads by boiling for 10 min in 100 μl of SDS sample dye (Bio-Rad). Samples (20 μl) were subjected to an 8–16% SDS/PAGE (Gradipore, Frenchs Forest NSW, Australia), transferred to Immobilon-P membrane (Millipore), dried, and exposed to a phosphorimager cassette. The amounts of radioactive proteins were quantified with QUANTITY ONE software, and data were plotted with SIGMA PLOT 2001.

Results

Pol I Remains Intact in *rdn*ΔΔ Strains That Do Not Use Pol I. A key proposal of the subunit exchange model for rDNA transcription in mammalian cells is that the Pol I enzyme disassembles into individual subunits upon termination of transcription and resides mostly in the nucleoplasm, when it is not engaged in transcription of rDNA template (23). We tested this prediction in *S. cerevisiae* by comparing the size of the Pol I complex extracted from wild-type cells and mutant cells where Pol I is not used for rDNA transcription.

Previously, we have demonstrated that the *RDN1* locus on chromosome XII can be deleted completely (*rdn*ΔΔ), if the strain carries a rDNA template on a multicopy plasmid (29). Two kinds of multicopy helper plasmids were shown to sustain growth of such *rdn*ΔΔ strains. One of them (pPol I) carries a single rDNA repeat to be transcribed by Pol I. The second (pPol II) carried the 35S rRNA coding region fused to the *GAL7* promoter and the 5S RNA gene and allows cell growth in galactose, but not glucose, media. In *rdn*ΔΔ strains carrying the second type of plasmid, one (or two) rounded nucleolus was observed away from the nuclear periphery, and immunofluorescence microscopy showed that the largest subunit (A190) of Pol I is present throughout the nucleoplasm without concentrating in the rounded nucleoli (30). Thus, we asked whether A190 in the nucleoplasm exists as a free subunit or as part of an intact Pol I enzyme in these cells that are using only Pol II for rDNA transcription. We also analyzed an *rdn*ΔΔ strain carrying pPol I as a control. This strain contained fragmented mininucleoli, and Pol I was primarily localized to these mininucleoli (30).

Extracts were prepared from wild-type cells, *rdn*ΔΔ cells with pPol II, and *rdn*ΔΔ cells with pPol I and subjected to glycerol gradient centrifugation. Fractions containing A190 were identified by Western blotting (Fig. 1*A*). The majority of both A190 (Fig. 1) and A135 (data not shown) migrated to the same peak fractions irrespective of the strain background, consistent with the expected position of intact Pol I. These data show that Pol I remains assembled as a large complex with the same size as the control intact Pol I even in the *rdn*ΔΔ pPol II strain, in which Pol

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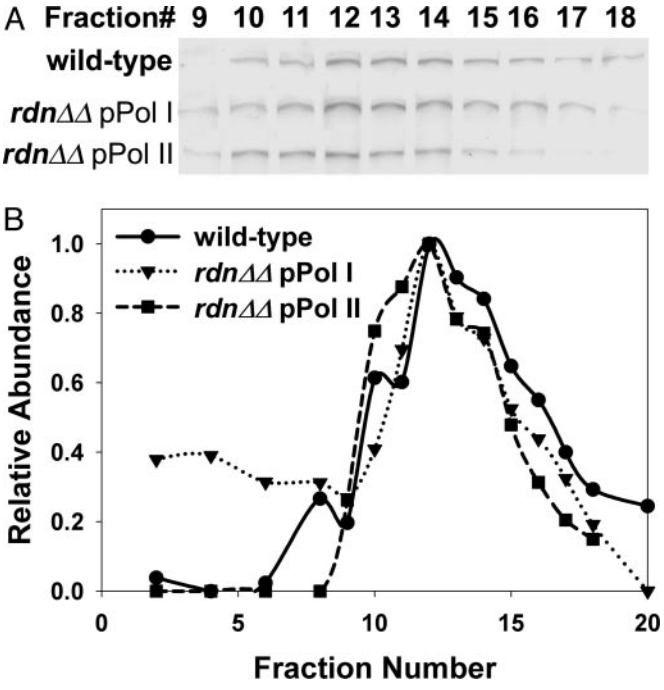

Fig. 1. Pol I remains intact when not associated with rDNA in a *rdn*ΔΔ strain synthesizing rRNA using Pol II and the *GAL7-35S rDNA* fusion gene. (*A*) Representative Western blot of Pol I peak fractions obtained after glycerol gradient centrifugation. Yeast cells were grown in YEP-Gal to mid-log phase, $A_{600} \approx 0.5$. Fractions containing Pol I were identified by SDS/PAGE followed by Western blot analysis, probing with a polyclonal antibody against A190. (*B*) Relative abundance of A190 throughout the gradients was plotted as a function of position within the gradient (fraction numbers starting from the top of the gradient) for all three strains (wild type = NOY505, *rdn*ΔΔ pPol I = NOY908, *rdn*ΔΔ pPol II = NOY891).

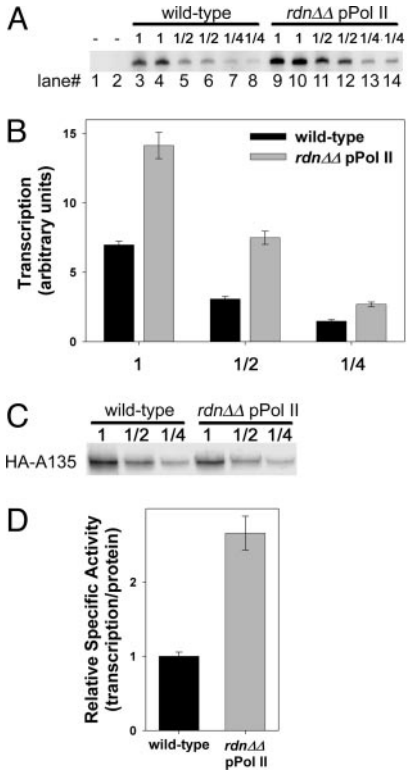

Fig. 2. Pol I is more active *in vitro* when purified from *rdn*ΔΔ pPol II (NOY2073) than when purified from wild type (NOY760). (*A*) Cultures were grown in YEP-Gal to an A_{600} of ≈0.5, and Pol I was isolated by a two-step procedure (see *Materials and Methods*). Transcription reactions were performed with all pure components (CF, TBP, UAF, and template). One microliter of dilutions of each Pol I sample (degree of dilution is indicated over each lane) was added to 19-μl transcription reactions before addition of nucleotides. Samples were diluted in breakage buffer containing HA peptide (see *Materials and Methods*), and buffer alone was added to the reactions run in lanes 1 and 2 as a negative control. Duplicate reactions were performed, and an autoradiogram of the transcripts is shown. (*B*) Quantification of transcription products shown in *A* with error between duplicate samples indicated. (*C*) Analysis of Pol I preparations used in *A* by Western blot analysis to compare relative amounts. Blots were probed with anti-HA antibody 12CA5. (*D*) Amounts of transcription products were normalized to the amounts of HA-tagged A135 in Pol I preparations and plotted relative to wild type, indicating the difference in the amount of active Pol I–Rrn3p complex in the Pol I preparations.

I is present mostly in nucleoplasm and is not actively engaged in transcription (Fig. 1*B*, *rdn*ΔΔ pPol II).

Pol I Isolated from the *rdn*ΔΔ pPol II Strain Is Active *in Vitro*. In the above experiments, Pol I appeared to remain assembled in the nucleoplasm even though it is not functioning in the *rdn*ΔΔ pPol II strain. We asked whether this Pol I complex was active for specific transcription *in vitro*.

To measure the activity of Pol I isolated from the *rdn*ΔΔ pPol II strain, we incorporated an affinity tag [$(His)_6$-$(HA)_3$] on the N terminus of A135 in this strain. Pol I was isolated from both the *rdn*ΔΔ pPol II (NOY2073) and a wild-type control strain (NOY760). Surprisingly, we found that Pol I isolated from the *rdn*ΔΔ pPol II strain was more active in specific transcription assays than that prepared from the wild-type control strain (Fig. 2 *A* and *B*). These assays were performed in the absence of both Pol I and Rrn3p, thus measuring the amount of initiation-competent Pol I–Rrn3p complex. When transcription activity was normalized to the amount of Pol I (Fig. 2*C*), we found that it was ≈2.5-fold higher for the preparation purified from the *rdn*ΔΔ pPol II strain than that purified from wild-type cells (Fig. 2*D*). Based on these data, we conclude that Pol I is not disassembled even when there are no rRNA genes with the Pol I promoter in the cell, and that Pol I does not require a Pol I specific promoter to form an initiation-competent complex with Rrn3p. Additional implications of these data are presented in *Discussion*.

Pol I Remains Intact When Rrn3p Is Inactivated. In addition to the experiments with the *rdn*ΔΔ strains, we also tested the effects of inactivation of Rrn3p on Pol I stability. We have previously shown that a mutation of a serine residue at position 213 to proline in Rrn3p resulted in temperature-sensitive growth (10). After the shift to the restrictive temperature, rRNA synthesis was reduced severely due to the inactivation of Rrn3p (>90% inhibition at 3 h after the shift), and the association of Pol I with rDNA was also largely abolished (10). Additionally, it was found that, under such conditions, nucleolar structure was disrupted, and Pol I diffused into the nucleoplasm (M. Oakes and M.N., unpublished data). Here, we tested whether the specific inhibition of transcription initiation and the resultant nucleoplasmic localization of Pol I in this mutant affected the size or composition of the Pol I complex as predicted by the Pol I subunit exchange model.

The *rrn3(S213P)* strain (NOY2078) was grown at 23°C to early log phase ($A_{600} \approx 0.1$), and the culture was divided in two. One was shifted to 37°C and incubated for 3 h, and the other was kept at 23°C. Crude extracts were made from these cells and subjected to glycerol gradient centrifugation analysis as described above. Pol I migrated to the same fractions of the gradient whether cells were grown at 23°C or 37°C (Fig. 3 *A* and *B*), indicating that the

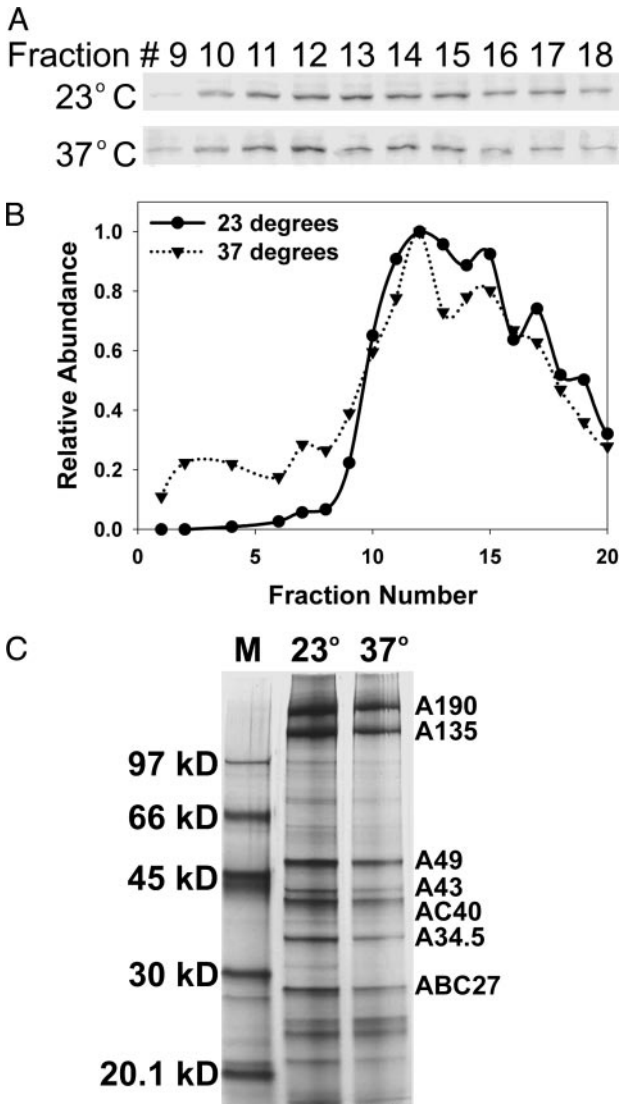

Fig. 3. Pol I remains intact when Rrn3(S213P)p is inactivated. (*A*) Representative Western blot of Pol I peak fractions obtained after glycerol gradient centrifugation. A strain containing a temperature-sensitive mutation in *RRN3* (NOY2078) was grown in YEPD at 23°C to $A_{600} \approx 0.1$, then either shifted to 37°C or maintained at 23°C for an additional 3 h. Fractions containing Pol I were identified as in Fig. 1 by using a polyclonal antibody against A190. (*B*) Relative abundance of A190 throughout the gradients was plotted as a function of position within the gradient for cells grown at 23°C and those shifted to 37°C. (*C*) $(His)_6$-$(HA)_3$-A135p was immunoprecipitated from cell extracts made from NOY2078 grown as in *A*. These immunoprecipitated samples were run on 8–16% SDS/PAGE and silver stained according to the manufacturer's protocol (Gradipore). Pol I subunit identification was based on size and abundance. Identification of Pol I subunits smaller than 27 kDa was not attempted.

size of the Pol I complex does not change significantly even when transcription initiation is prevented and nucleolar organization is disrupted by inactivation of Rrn3p (>90% inhibition; see ref. 10). Thus, the majority of Pol I remains as a large macromolecular complex after leaving the rDNA template and becoming dispersed in nucleoplasm.

Because A135 [and Rrn3p(S213P)] contained the HA epitope at their N termini in the mutant strain, we examined the subunit composition of Pol I directly by immunoprecipitating the enzyme from extracts prepared from cells that were either grown at 23°C or shifted to 37°C for 3 h. We then examined proteins in these Pol I fractions by SDS/PAGE followed by silver staining and could easily identify a Pol I subunit profile (Fig. 3*C*). It was evident that the composition of the immunoprecipitated Pol I did not change after inactivation of Rrn3p. Although we could not positively identify small Pol I subunits (<27 kDa), there was no significant change in the relative abundance of the seven largest subunits at the restrictive temperature, relative to cells growing at the permissive temperature. These data further support the conclusion that Pol I does not dissociate into subunits after leaving rDNA template and remains physically intact in nucleoplasm.

A190 and A135 Remain Associated Through Multiple Generations. Based on the data presented above, we conclude that the Pol I subunit exchange model does not apply to *S. cerevisiae*. However, this is based on experiments using mutant yeast cells. To confirm the conclusion with normally growing cells, we decided to measure the kinetics of exchange of Pol I subunits among different Pol I complexes *in vivo*, using a modified "transfer" experiment. The principle of the experiment is similar to the classic density transfer experiments of Meselson and Stahl (31); however, here we used conditional expression of an epitope-tagged protein subunit instead of density transfer to distinguish preexistent Pol I from newly synthesized Pol I.

We constructed a strain (NOY2079) containing a plasmid that conditionally expresses *(His)$_6$-(HA)$_3$-RPA135* from a galactose-inducible *GAL7* promoter, in an otherwise wild-type strain background. Therefore, the epitope-tagged *RPA135* is expressed when grown in galactose and repressed in the presence of glucose, but the strain grows irrespective of the carbon source, because the endogenous nontagged *RPA135* gene is intact. We grew this strain in minimal medium supplemented with galactose, [^{35}S]methionine, and [^{35}S]cysteine. At time 0, we added glucose and excess cold methionine and cysteine, resulting in repression of the expression of *(His)$_6$-(HA)$_3$-RPA135* and in cessation of further incorporation of ^{35}S into newly synthesized proteins. We then made extracts, isolated Pol I containing $(His)_6$-$(HA)_3$-A135, and measured the amounts of ^{35}S-labeled A190 and ^{35}S-labeled $(His)_6$-$(HA)_3$-A135 in the tagged Pol I as a function of time. The two potential extreme outcomes of this experiment can be predicted as shown in Fig. 4*A*. The Pol I subunit exchange model predicts that the ratio of ^{35}S-labeled A190 to ^{35}S-labeled $(His)_6$-$(HA)_3$-A135 in the $(His)_6$-$(HA)_3$-tagged Pol I should decrease by a factor of two for each generation, because the ratio of ^{35}S-labeled A190 to unlabeled A190 would decrease by a factor of two, and the new A190 should be able to compete equally well with ^{35}S-labeled A190 for binding to the epitope-tagged A135. On the other hand, if there were no dissociation/reassociation of Pol I subunits between rounds of transcription, the ratio of ^{35}S-labeled A190 to ^{35}S-labeled tagged A135 in the tagged Pol I would not change as a function of time (Fig. 4*A*).

In the actual experiments, we found that the intensity of the A135 band is greater than that of A190 in the tagged Pol I fractions (Fig. 4*B*). After correction for the methionine and cysteine contents of the two proteins (A135 has 52 methionine/cysteine residues, whereas A190 has 67), the molar ratio of A135 to A190 at time 0 was 2.1. Our interpretation is that we were isolating the population of the tagged A135 that was incorporated into Pol I and an excess free population of the tagged A135. Because the tagged A135 was expressed from the *GAL7* promoter in addition to the normal expression from the endogenous A135 locus, production of excess tagged A135 over other Pol I components may be expected. The data indicate that the amount of total tagged A135 decreased only slightly, if at all, after two generations of growth, suggesting that, like the tagged A135 in the Pol I complex, the presumed free tagged A135 produced in galactose medium remained largely stable. The important result is that the amount of ^{35}S-labeled A190 associated with the ^{35}S-labeled tagged A135 in the Pol I synthesized in the

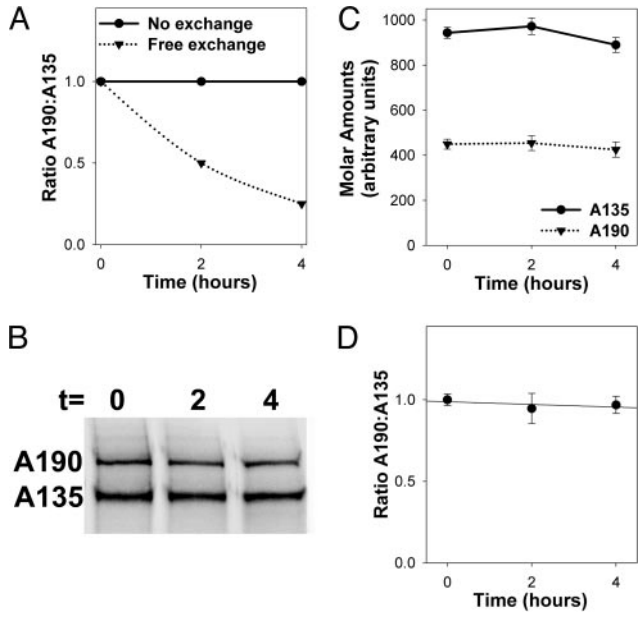

Fig. 4. A135 and A190 remain stably associated throughout many rounds of transcription. (*A*) Plot of theoretical outcomes of the transfer experiment, if A135 and A190 are exchanged freely among different Pol I complexes or stably associated in the same complex during yeast cell growth. (*B*) Representative autoradiogram of proteins in immunoprecipitated $(His)_6$-$(HA)_3$-Pol I fractions purified from NOY2079 cells taken at times indicated (in hours) (see *Materials and Methods*). Proteins were identified by size and abundance and confirmed by Western blot analysis (data not shown). (*C*) Amounts of radioactive A190 and $(His)_6$-$(HA)_3$-tagged A135 found in immunoprecipitated Pol I fractions and normalized to the methionine and cysteine contents of the two proteins. Relative values (in arbitrary units) obtained are plotted against time. (*D*) Ratios of A190 to A135 at indicated time points were calculated from the values shown in *C* and then normalized to the value at time 0. Values from three independent samples are shown with error ranges.

galactose medium remained approximately the same even after two generations of growth in the virtual absence of further synthesis of radioactive proteins (Fig. 4 *C* and *D*). These results argue against significant exchange between A135 or A190 subunits present in preexistent Pol I and those in newly synthesized Pol I in growing cells, i.e., there appear to be no dissociation and reassociation of Pol I subunits during multiple rounds of transcription *in vivo*. This conclusion probably applies to most, if not all, of the Pol I subunits; however, due to the small size and lower methionine and cysteine content of the other subunits, we could not accurately measure extents of their exchange in this set of experiments.

Discussion

Model for Pol I Transcription in Yeast. By measuring the size of Pol I and its subunit composition, we have found that Pol I does not disassemble into subunits when it is not associated with rDNA as a result of specific inhibition of transcription initiation (caused by inactivation of Rrn3p) or as a result of the absence of a Pol I-transcribed rDNA template in *S. cerevisiae*. In fact, the data presented in Fig. 2 show that the multisubunit Pol I enzyme is synthesized and is able to form a transcriptionally competent Pol I–Rrn3p complex even in the complete absence of any specific Pol I promoter. Furthermore, we used a transfer experiment to show that A135 and A190 subunits remained stably associated throughout many transcription cycles. Taken together, these data suggest that the Pol I subunit exchange model proposed by Misteli and coworkers (23) does not apply to Pol I transcription in *S. cerevisiae*, and that active Pol I can be assembled and maintained intact in the absence of a Pol I promoter in this organism.

Our data support a simpler model for the Pol I transcription cycle. UAF is bound tightly to the upstream element of the Pol I promoter regardless of whether Pol I is engaged in rRNA transcription (10, 19). After initiation of transcription, CF (and presumably TBP), but not UAF, dissociates from rDNA promoter (32), and Rrn3p is also released from the Pol I–Rrn3p complex (9, 32). Thus, these transcription factors and Pol I may exchange their partners during multiple rounds of transcription, but Pol I itself remains largely intact. Whether Pol I remains exclusively in the nucleolus or shuttles between the nucleolus and the nucleoplasm is not known, but regardless of this question, Pol I does not dissociate into individual subunits.

We note that the present studies do not exclude the possibility of dissociation/reassociation of some small protein subunits present at the "periphery" of the intact Pol I structure during yeast cell growth. For example, subunit A49 and A34.5 appear to be weakly associated with the rest of Pol I and tend to dissociate during purification using certain chromatographic procedures (33). Such proteins might also be subject to dissociation and reassociation *in vivo* under certain conditions. In addition, there might be some unidentified proteins associated with Pol I that exchange during cell growth. However, the present studies are not concerned with such loosely associated protein factors.

Technical Observations and Comments. In Figs. 1 and 3, we used glycerol gradient centrifugation to compare the size of the Pol I complex between wild-type strains and strains in which the nucleolar localization of Pol I was disrupted. Very slight changes in the mass of a large protein complex may not be detected with this method; however, any significant difference in the partition between intact Pol I complex and disassembled subunits would be evident. Therefore, if Pol I were truly disassembled after leaving rDNA template, this method would detect such a change. Furthermore, it has been shown (34) that Pol I assembly defects resulted in detectable aberrant sedimentation velocity of the enzyme in glycerol gradient centrifugation. Finally, we show here that individual A190 subunits (or smaller complexes containing A190) are detectable with this method, because we reproducibly observed the presence of small amounts of A190 in the slowly sedimenting fractions in the *rdn*ΔΔ pPol I strain (Fig. 1*B*) and in the *rrn3* mutant strain grown at 37°C (Fig. 3*B*).

We were somewhat surprised by the observation that Pol I was more active for initiation of rDNA transcription *in vitro* when isolated from the *rdn*ΔΔ pPol II strain than from wild type. The growth rate of the *rdn*ΔΔ strain is significantly slower than the wild-type control strain (≈2.5-fold), yet the amount of active Pol I–Rrn3p complex is higher than wild type. In fact, we have observed as much as a 6-fold difference in activity in other experiments (data not shown). One possible explanation for these data is that (hypothetical) feedback control of Pol I transcription is induced in an attempt to increase expression of rRNA in the *rdn*ΔΔ pPol II strain (thus a high level of Pol I–Rrn3p complex is produced). It is also possible that, because the steady-state amount of the Pol I–Rrn3p complex is mostly determined by the rate of synthesis and the rate of its consumption by the act of transcription, the Pol I–Rrn3p complex simply accumulates in these mutant cells because of the absence of Pol I transcription.

Parenthetically, we note that two possible models for the role of Rrn3p in transcription initiation were previously considered. In one model, Rrn3p is required for recruitment of Pol I to the rDNA promoter, whereas in the other model, Rrn3p activates Pol I that is already bound to the rDNA promoter. Previous chromatin immunoprecipitation experiments using the *rrn3(S213P)* mutant supported the first model and not the

second (10). Our observation of the formation of initiation competent Pol I–Rrn3p complex in the absence of any Pol I promoter (Fig. 2, *rdn*ΔΔ pPol II) is also consistent with this conclusion.

Potential Explanations for Discrepancies with Previous Data. There are at least two possible ways to explain the differences between the conclusions drawn here and those presented previously (23). Either Pol I transcription in yeast is very different from that in mammalian cells in terms of enzyme stability during transcription cycles, or the divergent conclusions are due to technical differences. Although we cannot exclude the first possibility, we favor the second, in view of the known similarity between yeast and mammals in the subunit composition of Pol I as well as the mechanism of Pol I transcription, especially that elucidated for transcription from core promoters (see reviews in refs. 4–6).

In the mammalian study, a series of photobleaching microscopy experiments were performed, and rates of movement of several Pol I subunits were estimated in a series of separate experiments, in which one of several different GFP-tagged Pol I subunits was transiently expressed in the presence of endogenously expressed untagged versions of the corresponding proteins. Thus, the possibility cannot be excluded that the movement of the GFP-tagged protein did not correctly reflect the behavior of the endogenous protein and the deviation from the native behavior varied, depending on which protein subunit was analyzed. Perhaps this technical variability led to the results supporting the Pol I subunit exchange model. However, more direct experimental tests similar to those presented in this paper must be carried out for mammalian systems before one can settle the question of Pol I subunit exchanges in mammalian cells.

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