Transcription Elongation through DNA Arrest Sites

A MULTISTEP PROCESS INVOLVING BOTH RNA POLYMERASE II SUBUNIT RPB9 AND TFIIS*

(Received for publication, October 24, 1996, and in revised form, April 2, 1997)

Donald E. Awrey, Rodney G. Weilbaecher‡, Sally A. Hemming, Stephen M. Orlicky, Caroline M. Kane‡§, and Aled M. Edwards¶

From the Cancer Research Group, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario L8S 3Z5, Canada and the §Department of Molecular and Cell Biology, University of California, Berkeley, California 94720

The role of yeast RNA polymerase II (pol II) subunit RPB9 in transcript elongation was investigated by examining the biochemical properties of pol II lacking RPB9 (pol IIΔ9). The maximal rate of chain elongation was nearly identical for pol II and pol IIΔ9. By contrast, pol IIΔ9 elongated more efficiently through DNA sequences that signal the elongation complex to pause or arrest. The addition of purified recombinant RPB9 to pol IIΔ9 restored the elongation properties of the mutant polymerase to those of the wild-type enzyme. Arrested pol IIΔ9 complexes were refractory to levels of TFIIS that promoted maximal read-through with pol II. However, both pol II and pol IIΔ9 complexes stimulated with TFIIS undergo transcript cleavage, confirming that transcript cleavage and read-through activities can be uncoupled. Our observations suggest that both TFIIS and RPB9 are required to stimulate the release of RNA polymerase II from the arrested state.

In Saccharomyces cerevisiae, the genes encoding the twelve subunits of pol II have been cloned and sequenced. Five of the ten small subunits of yeast pol II (RPB5, RPB6, RPB8, RPB10, and RPB12) are common to all three nuclear RNA polymerases (4, 5). Several remaining pol II subunits have homologues in pol I and pol III (7). RPB7 is similar to the pol III subunit C25 (4, 5). Several remaining pol II subunits have homologues in pol I and pol III. RPB11 (6) is related to AC19, a subunit shared by pols I and III (7). RPB7 is similar to the pol III subunit C25 (8). RPB9 is related to the pol I subunit, A12 (9). At least six pol II small subunits from S. cerevisiae are functionally interchangeable with human subunits (RPB6, RPB7, RPB8, RPB9, RPB10, and RPB12) (10–12). Genetic analysis indicates that only two yeast pol II subunits, RPB4 and RPB9, are not essential for cell viability (13, 14).

In yeast, deletion of RPB9 results in mild temperature sensitivity and relatively normal levels of transcription in vivo. However, for most genes examined, the selectivity of the site of transcription initiation is altered, with new start sites shifted upstream relative to wild-type sites (15–17). The transcription initiation phenotype can be recapitulated in vitro, and addition of recombinant purified RPB9 (rRPB9) restores wild-type start site selection (15). RPB9 from S. cerevisiae is a 122-amino acid polypeptide that contains two zinc binding domains (14). The COOH-terminal zinc binding domain shares 25% sequence identity with that of the general transcript elongation factor TFIIS (18) and is predicted to adopt a zinc ribbon fold (17–19). This domain is required for the function of RPB9 in start site selection (20) and is required within TFIIS for elongation stimulation (20).

The homology of RPB9 to TFIIS suggested that RPB9 may play a role in transcript elongation. In addition, the altered start site selection of pol II lacking RPB9 (pol IIΔ9) suggested some alteration in positioning the catalytic center of the polymerase, and a mobile catalytic center is a prominent feature of several recent models for transcript elongation (21–24). Thus, the elongation properties of highly purified pol II and pol IIΔ9 were compared. The availability of purified rRPB9 and TFIIS was exploited to characterize the functional interactions among pol II and these factors during the transcription elongation process.

MATERIALS AND METHODS

Purification of Yeast pol IIΔ9 and pol II—RNA polymerase II lacking RPB9 (pol IIΔ9) was purified from the S. cerevisiae strain W19 (14). pol II and pol IIΔ9 were purified as described previously (25), except that 10 μM ZnCl2 was included in all buffers. The polymerase preparations were stored at −80 °C in 10 mM Tris-HCl, pH 7.9, 40 mM ammonium sulfate, 10% glycerol, 2.5 mM DTT, 100 μM EDTA, and 10 mM ZnCl2.

Expression and Purification of Recombinant RPB9—The RPB9 coding region (14) was subcloned using polymerase chain reaction amplification to generate the coding region with an NdeI restriction site at the 5′ end of the sequence and a BamHI site at the 3′ end. The amplified insert was then cloned between the NdeI and the BamHI sites of the pET15b bacterial expression vector (Novagen, Madison, WI) to generate a fusion protein between RPB9 and an N-terminal hexahistidine tag. BL21(DE3) cells containing the pET15b RPB9 plasmid were grown to an A600 of 0.8 at 37 °C. The RPB9 protein expression was induced at 30 °C by the addition of 0.5 mM isopropyl-β-D-thiogalactosidase. The cells were harvested 3 h postinduction by centrifugation, resuspended in 50 mM Heps, pH 7.5, 10% sucrose, 100 mM NaCl, 10 mM ZnCl2, 1 mM PMSF, and 1 mM benzamidine (2 ml/g of cells), frozen, thawed, and then lysed by sonication at 4 °C. All subsequent steps were performed at 4 °C. The lysate was clarified by centrifugation and then centrifuged at 55,000 g for 30 min in a Beckman SW28 rotor, and then NaCl was added to a final concentration of 500 mM. The supernatant was passed through a DE52 column (2.5 × 5 cm; Whatman, Maidstone, UK) and loaded directly onto His-

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Supported by the National Science Foundation.
‡ Supported by the Medical Research Council of Canada.
§ Supported by the National Science Foundation.
¶ Supported by the Medical Research Council.

1 The abbreviations used are: pol, polymerase; rRPB, recombinant RPB9; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; GST, glutathione S-transferase; CAPS, 3-(cyclohexylamino)propanesulfonic acid; HMK, heart myosin kinase.

2 M. Hampsey, personal communication.
Role of RNA pol II Subunit RPB9 in Elongation

bind resin (2.5 × 2.5 cm; Novagen, Madison, WI), previously charged with 200 mM NiSO4 and equilibrated in purification buffer (50 mM Hepes, pH 7.5, 10 μM ZnCl2, 10% glycerol, 1 mM PMSF, and 1 mM benzamidine) containing 5 mM imidazole. The His-bind column was washed with 5 column volumes of purification buffer containing 500 mM NaCl and 50 mM imidazole, pH 7.5. RPB9 was eluted from the column with purification buffer containing 500 mM NaCl and 300 mM imidazole, pH 7.5. The eluate was dialyzed for 8 h against purification buffer containing 100 mM NaCl and 10 mM DTT but lacking PMSF and benzamidine. The dialysate was treated with bovine thrombin (3 μg of thrombin/mg of RPB9 fusion) for 4 h at room temperature, diluted 2-fold with basic purification buffer containing 10 mM DTT, passed through a Poros S column (0.75 × 10 cm; PerSeptive Biosystems, Cambridge, MA), and loaded onto a 2-ml hydroxyapatite fast protein liquid chromatography column (Bio-Rad). The column was developed with a 5 mM to 150 mM gradient of sodium phosphate, pH 7.5 in 50 mM NaCl, 10 mM DTT, 10 μM ZnCl2, 1 mM PMSF, and 1 mM benzamidine, and RPB9 eluted at 50 mM phosphate. The purified protein was dialyzed against 5 mM Hepes, pH 7.5, 100 mM NaCl, 10 μM ZnCl2, and 10 mM DTT and stored frozen at −70 °C.

Expression and Purification of Recombinant GST-RPB9 Fusion—To generate a fusion protein of GST and RPB9, the RPB9 coding region (14) was subcloned using polymerase chain reaction amplification to generate the coding region with a BamHI restriction site at both the 5′ and 3′ ends. The amplified insert was then cloned into the BamHI site of the pGEX-2T bacterial expression vector (Pharmacia Biotech Inc.). The GST-RPB9 fusion protein was expressed as described above for recombinant RPB9 using HB101 cells. The cells were harvested and lysed as described previously, with the exception that the NaCl concentration was maintained at 100 mM. The supernatant was passed through a DE52 column (2.5 × 2.5 cm; Whatman) and loaded directly onto a glutathione-Sepharose-4B column (2.5 × 2.5 cm; Pharmacia) previously equilibrated with Buffer A (10 mM sodium phosphate, 1.8 mM potassium phosphate, 2.7 mM KCl, 140 mM NaCl 10 mM DTT, 1 mM PMSF, and 1 mM benzamidine). The glutathione column was washed with 10 column volumes of Buffer A followed by 5 column volumes of Tris buffer (50 mM Tris, pH 8.0, 10 mM DTT, 1 mM PMSF, and 1 mM benzamidine). The GST-RPB9 fusion protein was eluted from the column with Tris buffer containing 10 mM glutathione. The purified protein was dialyzed against 5 mM Hepes, pH 7.5, 100 mM NaCl, 10% glycerol, 10 μM ZnCl2, and 10 mM DTT and stored frozen at −70 °C.

Purification of Yeast TFIIIS—Yeast TFIIIS was cloned and expressed as a fusion protein containing an amino-terminal hexahistidine tag in bacterial cells (26), and the expressed protein was purified as described below. All procedures were performed at 4 °C. Bacterial cells (2 L) expressing the yeast TFIIS protein were lysed using a French pressure cell in Buffer A (20 mM Hepes, pH 7.5, 10% glycerol, 10 μM ZnCl2, 1 mM PMSF, and 1 mM benzamidine) containing 100 mM NaCl, 10 mM DTT, and 1 mM EDTA. The lysate was clarified by centrifugation at 55,000 g for 30 min in a Beckman SW28 rotor, and the supernatant was loaded directly onto a DE52 column (2.5 × 2.5 cm; Whatman) and loaded onto a 2-ml hydroxyapatite fast protein liquid chromatography column (Bio-Rad). The column was developed with a 5 mM to 150 mM gradient of sodium phosphate, pH 7.5 in 50 mM NaCl, 10 mM DTT, 10 μM ZnCl2, 1 mM PMSF, and 1 mM benzamidine. The His-bind column was washed with 10 column volumes of Buffer A containing 100 mM NaCl and 50 mM imidazole, pH 7.5. The eluate was dialyzed directly onto a Poros MC column (0.75 × 10 cm; PerSeptive Biosystems, Cambridge, MA) equilibrated with NiSO4. The column was washed with Buffer A containing 500 mM NaCl and 5 mM imidazole. Yeast TFIIIS was then eluted with Buffer A containing 500 mM NaCl and 50 mM imidazole, immediately dialyzed against Buffer A containing 50 mM NaCl and 10 mM DTT, and stored frozen at −70 °C.

Transcription Assays: Read-through and Nucleolytic Cleavage by Arrested Ternary Complexes—These procedures were carried out as described previously (26). Briefly, transcription by purified pol II was initiated from a 3′ deoxyctydylate tailed template containing a human histone H3.3 gene fragment, which contains well-characterized blocks to elongation (27). The RNA transcript was pulse-labeled at the 5′ end by incubating in the presence of 20 μCi of [α-32P]TTP (3000 Ci/mmol, DuPont NEN), 0.8 mM GTP, ATP, and UTP for 75 s, followed by elongation to the blocks to elongation in the presence of 0.1 mM unlabeled CTP for 75 s. Ternary complexes stalled at these sites were treated in two different ways. For the read-through assay, yeast TFIIIS was added to the transcription reaction was allowed to proceed for the specified intervals, and the resulting transcripts were collected by ethanol precipitation and resolved by electrophoresis on a 6% polyacrylamide (19:1 acrylamide: bisacrylamide) gel. The bands were visualized by autoradiography. The upper bands were excised and subjected to nuclease P1 digestion to estimate the number of RNA polymerase II reads through the site (28).

RESULTS

Purification of pol IIΔ9 and Recombinant RPB9—pol IIΔ9 was isolated using a combination of heparin, immunoaffinity, and anion exchange chromatography. This method yielded a purified enzyme devoid of RPB9 but contaminated by all of the remaining subunits (Fig. 1). The yield of pol IIΔ9 enzyme was similar to that of pol II, approximately 0.8 mg/100 g of packed yeast. Purified pol IIΔ9 consistently demonstrated a 2–3-fold higher specific activity than pol II in assays of promoter-independent RNA chain elongation on denatured DNA templates.
However, the maximal elongation rate was indistinguishable between the two polymerases. The enzymes were also similar in the rate and extent of pyrophosphorolysis and the temperature dependence of promoter-independent assays of RNA synthesis (data not shown).

rRPB9 was expressed and purified from *Escherichia coli*. The yield of rRPB9 was 5 mg/liter of bacterial culture. We judged rRPB9 to be functional on the basis of two observations. First, the zinc content of rRPB9 was determined by atomic absorption spectroscopy to be 2.4 ± 0.4 mol of zinc/mol of rRPB9, in keeping with the predicted stoichiometry of zinc binding by RPB9.\(^3\) Second, this RPB9 preparation restores accurate transcription start site utilization in nuclear extracts prepared from yeast cells lacking RPB9.\(^4\)

Although rRPB9 was transcriptionally active, it readily formed aggregates. Dynamic light scattering studies of rRPB9 at 3 mg/ml revealed a polydisperse preparation whose average size corresponded to a pentamer. In an attempt to reduce the propensity to aggregate, another recombinant version of RPB9 was prepared, in the form of a fusion to GST. The purified GST-RPB9 was dimeric (in keeping with the dimeric nature of native GST), monodisperse in solution, and also active in assays of promoter-dependent transcription. However, if the GST moiety was removed from the purified GST-RPB9, the RPB9 that was generated once again aggregated. We could not prepare a version of RPB9 that was unaggregated. Therefore we used RPB9 fusion proteins for reconstitution experiments. In each such experiment, the source of the RPB9 is indicated, and the corresponding state of aggregation was therefore known.

**pol IIΔ9 Elongates More Efficiently than pol II through an Intrinsic Block to Elongation**—The observation that pol II and pol IIΔ9 have the same maximal elongation rate yet pol IIΔ9 synthesizes more RNA in chain elongation assays suggested that pol IIΔ9 pauses less frequently during elongation. To test this idea, we examined the behavior of pol IIΔ9 at well characterized blocks to elongation. Purified pol II stops in *vitro* at several sites within the human histone H3.3 first intron (27). The strongest block, TIA, has been used extensively to study the elongation properties of pol II from yeast and mammalian cells (26, 28–33). We observed that pol IIΔ9 transcribes through TIA with higher efficiency than pol II; 50–65% of pol IIΔ9 elongates past TIA compared with 15–30% for pol II. The differences in elongation efficiency are moderate but reproducible; these values reflect the range of TIA read-through in more than a dozen independent experiments with at least two different preparations of each enzyme.

To exclude the possibility that the purification of pol IIΔ9 for some reason results in a more efficiently elongating enzyme and that the elongation differences were indeed a property of RPB9, we tested whether purified RPB9 could restore the wild-type elongation properties of pol IIΔ9. The addition of increasing amounts of rRPB9 or GST-RPB9 to pol IIΔ9 resulted in decreased (wild-type) levels of read-through at the TIA intrinsic block to elongation (Fig. 2A). The decrease in transcription likely results from the reconstitution of wild-type polymerase activity by RPB9 and pol IIΔ9 because addition of greater than 200-fold molar excess of rRPB9 to either pol IIΔ9 or pol II did not decrease read-through below that observed with pol II alone. Wild-type elongation levels were restored to pol IIΔ9 by a 3–5-fold molar excess of GST-RPB9 and a 10-fold molar excess of rRPB9.

The addition of purified RPB9 to pol IIΔ9 not only decreased read-through activity but also decreased the total level of transcription from the dC-tailed templates (Fig. 2B). We observed that pol IIΔ9 synthesized 3–15 times more transcripts from the dC-tailed template than did either pol IIΔ9 + RPB9 or the wild-type enzyme, even though pol IIΔ9 was only 1.5–2-fold more active than the wild-type enzyme in the normal assays for promoter-independent chain elongation. RPB9 must inhibit transcription from these templates at an early stage in the reaction because the transcripts are pulse-labeled during the formation of the first 20 or so nucleotides. Using native gel electrophoretic mobility shift assays, we have shown that the two enzymes bind the tailed template with equal affinity (data not shown). Therefore, the inhibitory effect of RPB9 takes place after binding of the polymerase and before the synthesis of the first 20 nucleotides. The mechanism of inhibition remains to be established.

**pol IIΔ9 Ternary Complexes Are Not Stimulated to Read-through Blocks to Elongation by the Elongation Factor TFIIIS**—The addition of rRPB9 to pol IIΔ9 causes more polymerase to arrest at TIA. The effect of this subunit apparently contrasts with that of the elongation factor TFIIIS, which stimulates transcription through TIA, as well as several other elongation blocks. To explore the functional interactions between RPB9 and TFIIIS, we compared read-through stimulation by TFIIIS with both pol II and pol IIΔ9-containing arrested complexes. For the wild-type enzyme, a 3-fold molar excess of TFIIIS maximally stimulates read-through of TIA by 30 min, at which time

---

\(^3\) I. Donaldson, personal communication.

\(^4\) M. W. Hull and N. A. Woychik, personal communication.
nearly two-thirds of the initially arrested complexes are reactivated (Fig. 3A). By contrast, arrested pol IIΔ9 ternary complexes were completely unresponsive to this level of TFII S during a 30-min incubation (Fig. 3B, lanes 6–10; also Fig. 3D for quantification). The apparent decrease in the levels of the TIIa transcript in Fig. 3B (lane 5) reflects a decreased level of radioactivity in the entire lane and not a TFII S-independent read-through event.

Once again, to ensure that the inability of pol IIΔ9 to respond to these levels of TFII S was caused by the lack of RPB9, we attempted to restore wild-type TFII S responsiveness by the addition of purified RPB9. A 3-fold molar excess of TFII S was added to pol IIΔ9 ternary complexes formed in the presence of RPB9. The reconstituted polymerase (pol IIΔ9 + RPB9) responded to TFII S with the same kinetics of read-through as did pol II (Fig. 3C, lanes 6–10). It was not necessary to preincubate the RPB9 with pol IIΔ9 to observe reconstitution of activity; identical results were obtained if RPB9 was added after the arrest complex formation. The resulting enzyme (pol IIΔ9 +

FIG. 3. Stimulation of elongation through arrest sites by TFII S. Ternary elongation complexes were formed using pol II (A), pol IIΔ9 (B), or pol IIΔ9 reconstituted with a 10-fold molar excess of recombinant RPB9 (C) and incubated in the presence or the absence of the indicated amounts of TFII S for 0, 1, 5, 15, and 30 min. The transcription products were resolved by gel electrophoresis and visualized by autoradiography. The relative mobilities of the TII, TIIa, and run-off (RO) transcripts are indicated on the left of each panel. D, the percentage of transcripts arrested at TIIa were determined for pol II (squares, thick line), pol IIΔ9 (triangles), and Δ9 RNA pol II reconstituted with recombinant RPB9 (circles) in the absence (open symbols) and the presence of TFII S (closed symbols). The average of three reactions is shown. wt, wild type.
RPB9) displayed an identical response to TFIIS compared with pol II (Fig. 4C, lanes 1–5). The requirement for RPB9 in read-through could be diminished by the addition of a 100-fold molar excess of TFIIS (Fig. 3). Similarly, the addition of a 100-fold molar excess of TFIIS to arrested pol II D9 complexes returned the cleavage patterns and kinetics to that seen in complexes formed with the wild-type enzyme (Fig. 4D). Therefore, in the absence of RPB9, the polymerase requires substantially higher TFIIS levels to effect cleavage and read-through.

The observed results would be explained if pol II D9 was defective in catalyzing transcript cleavage. Thus the intrinsic cleavage activities of pol II and pol II D9 were compared. We found that pol II D9 did not appear to have any intrinsic defect in catalyzing transcript cleavage. Moderate elevation in pH can stimulate intrinsic cleavage by pol II, similar to the intrinsic cleavage reaction observed with *E. coli* RNA polymerase (37). At basic pH, yeast pol II complexes halted at T1a cleave their transcripts in the absence of TFIIS. Although such intrinsic cleavage can occur at physiologic pH under some solution conditions, the conditions used to assay TFIIS-stimulated cleavage demonstrated no intrinsic cleavage over the course of 30 min (Fig. 4A and B, lanes 1 and 2). In comparing intrinsic cleavage by pol II and pol II D9 under mildly alkaline conditions, we observed no difference in the size of the cleavage products nor in the rate of transcript cleavage (Fig. 5). The intrinsic cleavage products correspond approximately to the mobilities of the TFIIS-stimulated cleavage products; however, direct determination of the cleavage increment has not been established. Extension of the intrinsic shortened transcripts at pH 9.5 can result in synthesis back to the original T1a site. Some intrinsic shortened transcripts are elongated slightly past the T1a site (see Fig. 5, Nuc lane); the mechanism of this elongation event remains to be established, and further characterization of the intrinsic cleavage activity of pol II will be presented elsewhere. In summary, the similarity of pol II and pol II D9 in the intrinsic cleavage reaction suggests that pol II D9 is deficient in some other aspect of TFIIS-mediated read-through.

**Fig. 4. Time course and titration of cleavage activity by TFIIS.** Stalled ternary complexes containing pol II (A), pol II D9 (B and D), or pol II D9 reconstituted with rRPB9 (C) were incubated with a 3-fold (A, B, and C) or a 100-fold molar excess (D) of TFIIS for 0, 1, 5, 15, and 30 min. The transcription products were resolved by gel electrophoresis and visualized by autoradiography. The relative mobilities of the T1a, T1l, and run-off (RO) transcripts and the first two cleavage products, C1 and C2, are indicated. The first two lanes in each of panels A and B demonstrate that no transcript cleavage occurs after 30 min in both pol II and pol II D9 complexes in the absence of TFIIS. The lanes designated Nuc show the products of a 30-min cleavage reaction after the addition of nucleotides for an additional 10 min.

**pol II and pol II D9 Have Equivalent Affinity for Yeast TFIIS—** A trivial explanation for our observations would be that the reduced response of pol II D9 to TFIIS arises from a reduced affinity of pol II D9 for TFIIS. Therefore, the affinity of pol II and pol II D9 for TFIIS was examined using an electrophoretic mobility shift assay that measures the association of radiolabeled TFIIS with polymerase in nondenaturing polyacrylamide gels (38). In this assay, both pol II and pol II D9 bind TFIIS with equivalent affinity (approximately 80 nM) (Fig. 6). Binding of TFIIS to either polymerase saturates at a 1:1 molar ratio and is competed by the addition of unlabeled TFIIS (data not shown). In this gel system, pol II and the pol II-TFIIS

---

5 Weilbaecher, R. G., Awrey, D. E., Edwards, A. M., and Kane, C. M., in preparation.
Role of RNA pol II Subunit RPB9 in Elongation

complex enter the gel, whereas free TFIIS migrates toward the cathode.

**DISCUSSION**

Wild-type RNA polymerase II and the enzyme lacking RPB9 have interesting differences in elongation properties, and these differences suggest that this subunit is important for transmitting signals to the elongating ternary complex. The behavior of ternary elongation complexes containing pol II (A) or pol IIΔ9 (B) were incubated at pH 9.5 for 2.5, 5, 10, and 20 min (lanes 3–6), or pH 8.0 for 0 and 20 min (lanes 1 and 2). After 20 min of incubation, an aliquot was adjusted to 800 µM rNTPs and incubated further for 5 min at 30 °C in pH 9.5 buffer (lane Nuc). The transcription products were resolved by gel electrophoresis and visualized by autoradiography.

The decreased propensity of pol IIΔ9 to arrest at intrinsic blocks to elongation might be explained if pol IIΔ9 complexes either (i) had an overall elongation rate faster then the wild-type enzyme or (ii) had reduced ability to recognize signals that lead to an intrinsic block to elongation (31). Our experiments suggest that the latter explanation is the case, namely that pol IIΔ9 complexes do not recognize intrinsic blocks to elongation or other pause sites as efficiently as the wild-type complexes. Paradoxically, although pol IIΔ9 complexes do not appear to respond to DNA sequences that trigger transcription arrest, the pol IIΔ9 complexes that do arrest appear to be more stable as judged by their reduced responsiveness to TFIIS. These results highlight the complexity of the behavior of RNA polymerases at arrest sites.

RNA polymerases likely adopt many different conformations during the elongation process (21, 39). If arrest site recognition may be mediated by only a subset of the RNA polymerase conformational changes (arrest-competent), then the efficiency of transcription arrest would be governed by the proportion of the elongating enzymes in an arrest-competent conformation. The relative amounts of each conformation might be regulated by accessory factors, substrate levels and composition, solution conditions, and polymerase subunits. The behavior of pol IIΔ9 is consistent with a role for RPB9 in increasing the amount of RNA polymerase in an arrest competent conformation. In the...
absence of RPB9, the read-through conformation might be favored, and as a result a smaller proportion of the elongating enzymes arrest. In the presence of RPB9, the arrest competent conformation would be favored, and consequently a greater proportion of wild-type complexes arrest compared with pol IIΔ9. This proposed role for RPB9 in regulating the interaction of pol II with the nucleic acid is consistent with the location of the RNA polymerase I RPB9 homologue (A12.2) (9) in the three-dimensional structure of yeast RNA polymerase I. Subunit A12.2 is located near the region thought to include the sites for DNA interaction (43) and in a region proposed to envelope the DNA template in an elongation complex. If RPB9 occupies an analogous position in RNA polymerase II, then the subunit is situated perfectly to modulate the structure of the elongation complex.

pol II and pol IIΔ9 elongation complexes also differ in the ability to respond to TFIIIS, which promotes reactivation of transcription after arrest. Arrested pol IIΔ9 complexes fail to read-through the T3a site in response to levels of TFIIIS that completely stimulate read-through by arrested wild-type complexes. We showed that the defect in pol IIΔ9 complexes is not in binding to TFIIIS or in intrinsic cleavage activity. Rather, in pol IIΔ9 complexes, TFIIIS stimulated read-through occurs much more slowly than with the wild-type enzyme. The reactivation of arrested pol IIΔ9 complexes is therefore affected at a step after TFIIIS binding and transcript cleavage. Perhaps RPB9 facilitates the conversion of an arrest-competent conformation to a read-through competent conformation, and this interconversion is an obligatory step in the read-through process. Thus, our observations define at least a three-step process for the release from the arrested state; binding to TFIIIS, induction of transcript cleavage, and then a reactivation step(s) that likely includes a conformational change. This multistep model is consistent with the observations of Ciprés-Palacín and Kane, who also showed, using mutants of TFIIIS, that transcript cleavage could be uncoupled from read-through (44).

There is a sequential appearance of transcript cleavage products in both mammalian (34) and yeast RNA polymerase II arrest complexes at the T3a site. For the mammalian enzyme, only cleavage to the first site was required for read-through (35). The cleavage to C1 can occur in pol IIΔ9 complexes in response to low levels of TFIIIS, although the kinetics are slower than with the wild-type enzyme. However, at these levels of TFIIIS, the subsequent transcript cleavage events are not detected in the absence of RPB9, nor is read-through detected even when the first cleavage event has occurred in the absence of RPB9. Our observations raise the possibility that transcript cleavage by purified pol II at the T3a site can be distinguished into two steps. The first cleavage event, to C1, is not sufficient for read-through. Rather, cleavage to the C2 site, and perhaps an accompanying conformational change, is correlated with the read-through process.

A Possible Connection between Start Site Selection and Elongation—Transcription arrest, according to the model described above, is mediated by a particular conformation(s) of the polymerase that interacts specifically with the DNA in arrest sites. Elongating polymerase is proposed to contain two DNA binding domains, one upstream and one downstream of the position of the active site (21, 45). The downstream DNA binding domain likely plays a significant role in recognition of the arrest site because in many instances the sequences that trigger arrest lie downstream of the catalytic site in regions yet to be transcribed (23). The downstream DNA binding activity of RNA polymerase may also play a role in the selection of the transcription start site. In yeast, start site selection is thought to involve specific recognition of DNA sequences ahead of the actual start site (46) because the distance from the TATA box to the start site in yeast can vary anywhere from 40 to 120 bases (47). The most frequently used start site(s) therefore cannot be chosen based on a set distance from the TATA box. Rather, the DNA sequences encompassing the start site are likely selected by pol II, perhaps via a scanning mechanism (48, 49).

In cells lacking RPB9, the selection of the start site appears less stringent than in wild-type cells. In these cells, pol IIΔ9 uses a wider range of start sites, which are located upstream of the major sites used in wild-type cells (15, 17). The pol IIΔ9 enzyme also uses these altered start sites in vitro (15). Perhaps this start site alteration is related to the altered elongation properties of the pol IIΔ9 enzyme. RNA polymerase, once recruited to the promoter, may need to recognize and bind specific sequences to position the catalytic site for accurate initiation. pol IIΔ9 may be less capable of effective recognition and binding to the start site because an increased proportion of the enzyme is in the read-through conformation. In other words, the pol IIΔ9 enzyme does not efficiently arrest to allow the correct positioning of the catalytic site at the transcription start site. RPB9 would allow pol II to adopt a conformation compatible with selective binding to the sequences that constitute the start sites of transcription.

A necessary feature of this model is that promoter sequences and arrest sites share common features recognized by RNA polymerase. Although sequence similarities are not readily apparent, promoter release and release from intrinsic arrest sites share many characteristics. First, the release of polymerase from both a promoter and intrinsic arrest sites appears to be unfavorable in that the polymerase utilizes accessory factors to facilitate each process (50–52). Second, the polymerase is catalytically active within both initiation and elongation complexes and is able to perform abortive synthesis of small transcripts while remaining in place. For example, when bound to a promoter, RNA polymerases catalyze the formation of small RNAs that are abortively terminated and ejected from the complex (53–55). Similarly in arrested ternary complexes, the enzyme can also catalyze several cycles of transcript cleavage and re-extension with ejection of the small cleaved RNAs (29, 34, 36, 56). Third, for bacterial RNA polymerase, escape from both promoter sequences and the movement through blocks to elongation is accompanied by a downstream movement of the forward edge of the enzyme, as detected by a significant increase in the size of nucleosome footprint ahead of the position of the catalytic center of the enzyme (24, 53, 57). Fourth, the biochemical analysis of yeast RNA polymerase III harboring a point mutation in the C160 subunit has also revealed functional connections between promoter clearance, arrest site recognition, and transcript cleavage (58). The C160 mutant polymerase more efficiently recognized arrest sites, less efficiently cleared the promoter, and showed increased transcript cleavage. Fifth, recent studies with E. coli RNA polymerase demonstrate that the bacterial elongation factors, GreA and GreB, also stimulate the conversion from an abortive synthesis complex at a promoter to an elongation competent ternary complex (55).

RNA polymerase lacking subunit RPB9 clearly has altered initiation and elongation properties. The ability to reconstitute wild-type pol II activity from purified pol IIΔ9 and RPB9 will facilitate the comparison of the in vivo and in vitro behavior of RPB9 mutants. Finally, the stability of the pol IIΔ9 arrested complexes, even in the presence of TFIIIS, provides a means to derive structural information about the complex of an elongating RNA polymerase bound to TFIIIS.
viding the WY9 strain and D. Reines and M. Chamberlin for helpful discussions.

REFERENCES

1. Langer, D., Hain, J., Thuriaux, P., and Zillig, W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5768–5772
2. Sentenac, A., Riva, M., Thuriaux, P., Buhler, J.-M., Treich, I., Carles, C., Werner, M., Ruet, A., Huet, J., Mann, C., Chiannilikulchai, N., Stettler, S., and Mariotte, S. (1999) Transcriptional Regulation (Conaway, R. C., and Conaway, J. W., eds) pp. 27–54, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Built, C. J., White, O., Olsen, G. J., and Venter, J. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13, 114–122
4. McKeon, K., Moore, P. A., Hall, M. W., and Woychik, N. A. (1995) Mol. Cell. Biol. 6, 759–775
5. Treich, I., Carles, C., Riva, M., and Sentenac, A. (1992) Gene Exp. 4, 313–323
6. Woychik, N., and Young, R. A. (1993) J. Biol. Chem. 268, 6895–6900
7. Hull, M. W., McKeon, K., and Woychik, N. A. (1999) Mol. Cell. Biol. 19, 265, 15030–15037
8. Sadhale, P. P., and Woychik, N. A. (1994) Mol. Cell. Biol. 14, 6164–6170
9. Nogi, Y., Yano, R., Dodd, J., Carles, C., and Nomura, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4932–4936
10. Sentenac, A., Oudet, P., and Schultz, P. (1996) EMBO J. 15, 4643–4653
11. Kaine, B. P., Mehr, I. J., and Woese, C. R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 351–356
12. Khazak, V., Sadhale, P., Woychik, N., Brent, R., and Golenis, E. (1995) Mol. Cell. Biol. 15, 799–811
13. Woychik, N., and Young, R. A. (1993) J. Biol. Chem. 268, 10799–10809
14. Hull, M. W., McKeon, K., and Woychik, N. A. (1999) Mol. Cell. Biol. 19, 265, 15030–15037
15. Hull, M. W., McKeon, K., and Woychik, N. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4112–4117
16. Woychik, N., and Young, R. A. (1993) J. Biol. Chem. 268, 19033–19035
17. McKeon, K., Moore, P. A., Hull, M. W., and Woychik, N. A. (1995) Mol. Cell. Biol. 15, 481–490
18. McKeon, K., Moore, P. A., Hull, M. W., and Woychik, N. A. (1995) Mol. Cell. Biol. 15, 6895–6900
19. Shpakovski, G., Acker, J., Wintzerith, M., Lacroux, J., Thuriaux, P., and Vigneron (1995) Cold Spring Harbor Symp. Quant. Biol. 60, 313–323
20. Wang, D., Meier, T., Chan, C., Feng, G., Lee, D., and Landick, R. (1999) Cell 81, 341–350
21. Christie, K. R., Awrey, D. E., Edwards, A. M., and Kane, C. M. (1999) J. Biol. Chem. 274, 986–989
