Two Dissociable Subunits of Yeast RNA Polymerase II Stimulate the Initiation of Transcription at a Promoter in Vitro*

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RNA polymerase II lacking the fourth and seventh largest subunits (pol II Δ4/7) was purified from *Saccharomyces cerevisiae* strain rpb-4, in which the gene for the fourth largest subunit is deleted. pol II Δ4/7 was indistinguishable from wild-type pol II (holoenzyme) in promoter-independent initiation/chain elongation activity (400–800 nmol of nucleotide incorporated/10 min/mg of protein at 22 °C), in rate of chain elongation (20–25 nucleotides/s), and in the recognition of pause sites in the DNA template. In contrast to pol II holoenzyme, pol II Δ4/7 was inactive in promoter-directed initiation of transcription in vitro. The addition of an equimolar complex of the fourth and seventh largest subunits, purified from pol II holoenzyme by ion-exchange chromatography in the presence of urea, restored promoter-directed initiation activity to pol II Δ4/7. The transcriptional activator protein Gal4-VP16 could also elicit promoter-directed initiation by pol II Δ4/7 from a promoter with a Gal4 binding site. Complementation was observed between extracts of strain rpb-4, lacking the fourth largest subunit, and strain Y260-1, with a defect in the largest subunit. These extracts were individually inactive, but a mixture would support promoter-directed initiation. The fourth and seventh largest subunits may, therefore, shuttle between polymerase molecules.

The two largest subunits of RNA polymerase, which account for about two-thirds of the mass of the molecule, have been conserved in amino acid sequence from prokaryotes to eukaryotes (1). These subunits of the *Escherichia coli* enzyme contain binding sites for the DNA template, nucleotides, and RNA product, so the conservation of amino acid sequences probably reflects conservation of the basic mechanism of RNA synthesis (2). By contrast, both the number and sequences of the smaller subunits vary widely, presumably because these subunits are involved in promoter specificity and regulation. For example, the σ subunit of *E. coli* RNA polymerase is responsible for promoter selection and is released from the core enzyme following the initiation phase of the transcription reaction (2). In the eukaryotic enzymes, the role of the σ subunit appears to be divided among multiple subunits and accessory factors. In the case of RNA polymerase II (pol II),† responsible for the synthesis of mRNA in eukaryotes, a factor termed TFIID, BTF1, or r recognizes the "TATA" sequence associated with most (pol II) promoters (3). At least three more accessory factors appear to be required in addition to TFIID for initiation at pol II promoters (3, 4). Here we report that a complex of two small subunits of pol II also plays an important role in the initiation of transcription.

Pol II is made up of 10 subunits, ranging in size from about 6 to 190 kDa (5). In addition to the two largest subunits (denoted 1 and 2), conserved across species, subunits 5, 6, and 8 are common to the three types of eukaryotic cellular RNA polymerases (I, II, and III) (6) and so are presumed to play fundamental roles in RNA synthesis. Both biochemical and genetic evidence have been obtained bearing on the functions of subunits 4 and 7. A form of pol II that lacks these two subunits (here denoted pol II Δ4/7) was first isolated by anion-exchange chromatography of the 10-subunit enzyme (holoenzyme) in the presence of urea (7, 8). Only slight differences were detected between pol II Δ4/7 and holoenzyme in the capacity to synthesize RNA on a deaerated DNA template, so there is evidently no requirement for subunits 4 and 7 in promoter-independent (nonspecific) initiation and RNA chain elongation (8).

Two yeast mutant strains are thought to contain predominantly the pol II Δ4/7 enzyme. One strain, rpo BI, contains a mutation in the largest subunit (8), whereas the other strain, rpb-4, lacks the gene for subunit 4 (9). pol II purified from these strains lacks both subunits 4 and 7, suggesting that subunit 7 must interact with subunit 4 to associate with the polymerase. Although rpb-4 is viable, it grows slowly, is sensitive to heat and cold, and is auxotrophic for inositol. Because pol II Δ4/7 is apparently fully functional in RNA chain elongation, the phenotype of rpb-4 presumably reflects the involvement of subunits 4 and 7 in initiation or in some other aspect of polymerase assembly, maintenance, or activity.

A system was recently described for the promoter-directed (specific) initiation of transcription by yeast pol II in vitro (10, 11). With the use of this system, we have now characterized pol II Δ4/7 isolated from strain rpb-4. Differences in specific initiation activity between pol II Δ4/7 and holoenzyme were found that might account for the phenotype of the mutant strain.

**EXPERIMENTAL PROCEDURES**

*Materials—* Yeast strain BJ256 was kindly provided by Dr. E. Jones (Carnegie-Mellon University, Pittsburgh, PA). Strains Y260-1 and rpb-4 have been described (9, 11, 12). [α-32P]UTP, 650 Ci/mmol, was purchased from ICN Biomedicals (Costa Mesa, CA), and [α-32P]CTP,

*The abbreviations used are: pol II, polymerase II; HPLC, high-performance liquid chromatography.*
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>400 Ci/mmol, from Amersham Corp.

**Protein Purification**—Wild-type yeast pol II (holoenzyme) was purified from strain *B. j. 926* as described (11). pol II Δ4/7 was purified by the same procedure from strain *rpb-4*, grown at 24 °C, and harvested at an *A*₂₆₀ value of 6–8. For the isolation of subunits 4 and 7, holoenzyme (200 μg) was incubated for 3 h in 4.0 mL of 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 mM potassium acetate, 0.1 mM dithiothreitol, 5% glycerol, and 2 mL urea (buffer A). The solution was then applied to a Bio-Gel SEC DEAE-5 PW column (7.5 × 72 cm) equilibrated in the same buffer. The column was developed with a 20-ml linear gradient of 0.05–1 M potassium acetate in buffer A. Fractions containing subunits 4 and 7 were dialyzed against 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 30 mM ammonium sulfate, 50% glycerol, and were frozen in liquid nitrogen and stored at −70 °C. Gal4-VP16 fusion protein was enriched to 50% purity as described (13).

**Transcription Assays**—Non-specific initiation/chain elongation with poly(rC) as template was measured as described (14). Promoter-specific initiation assays were as described (15) in a reaction volume of 30 μL containing 4–6 μL of nuclear extract (12–20 μg of protein/ml of nuclear extract) and 20 μCi of [α-³²P]UTP (about 1 μM UTP). The template (125 ng except where noted) was pGal4CG- (15) with a Gal4 protein binding site upstream of the yeast CYC1 promoter fused to a 377-base pair sequence lacking guanosine residues in the coding strand. Promoter-specific transcripts were quantified with the use of an AMBIS radioanalytic imaging system (Ambis Systems, San Diego, CA).

Templates with single-stranded extensions on the 3′ ends were presynthesized as described (16). In particular, template pCyTK243B, containing human histone H3.3 intron terminators (denoted Tla, Tlb, and TII) (17), was produced by cleavage of plasmid pTK243B* at a unique Smal site, followed by the addition of oligo(dC) extensions with terminal transferase and treatment with SsI and MluI. On this template, RNA polymerase II synthesizes transcripts of 326 nucleotides (run-off), 197 nucleotides (Tla), 182 nucleotides (Tlb), and 138 nucleotides (TII). For preparation of template pCyTK202, used for measurement of elongation rates, plasmid pTK202* was linearized with Smal, oligo(dC) extensions were added, and the DNA was treated with SacI. This template contained no strong termination tides (run-off), 197 nucleotides (Tla), 182 nucleotides (Tlb), and 138 nucleotides (TII).

**Elongation and Termination** by pol II Holoenzyme and pol II Δ4/7—Purified RNA polymerase can initiate transcription at the ends of templates with 3′-terminal extensions (16). Such templates were used for the identification and characterization of a variety of blocks to transcription elongation (17, 20–23). Yeast pol II holoenzyme and pol II Δ4/7 were analyzed by this approach to determine their elongation

FIG. 1. pol II holoenzyme, pol II Δ4/7, and subunit 4/7 complex. Purified yeast pol II holoenzyme was fractionated on a DEAE-5PW HPLC column in 2 μM urea as described. Part of the elution profile is shown. Inset, starting pol II holoenzyme (10 μg, denoted pol II), pol II Δ4/7 purified from strain *rpb-4* as described (10 μg, denoted Δ4/7), and subunit 4/7 complex eluted from the HPLC column (0.2 μg, denoted 4/7) were analyzed by electrophoresis in a sodium dodecyl sulfate-15% polyacrylamide gel and stained with Coomassie Brilliant Blue (24). The *M* values of subunits 1–10, from comparison with molecular weight markers, are 205, 150, 45, 34, 30, 26, 21, 18, 16, and 12.5 kDa.

2 Kerppola, T. K., and Kane, C. M., EMBO. J., submitted for publication.

3 G. Rice, personal communication.
rates and termination properties.

The elongation rates of the two enzymes were indistinguishable, 20–25 nucleotides/s (data not shown). Both enzymes stopped at the same triplet of intrinsic termination sites in the human histone H3.3 gene (Fig. 3), and the efficiencies of transcription were almost equally effective in this assay, raising the possibility that subunits 4 and 7 might shuttle from the inactive to the active state (13). We were surprised to find that activity can also be restored to such an extract by an activator at a promoter with an activator binding site.

Possible Exchange of Subunits 4 and 7 between Polymerase Molecules—Nuclear extracts from strain Y260-1 were previously used to assay promoter-directed initiation of transcription by purified pol II holoenzyme (11). This strain is temperature-sensitive because of a mutation in the largest subunit of the enzyme (12), and warming an extract briefly prevents initiation. Activity is restored by holoenzyme in proportion to the amount added (Fig. 6, panel A). We were surprised to find that activity can also be restored to such an extract by an activator at a promoter with an activator binding site. The converse possibility that the largest subunit can exchange from the pol II Δ4/7 to the heat-inactivated polymerase is unlikely; the dissociation of this subunit from the polymerase under transcription conditions has never been observed nor has the dissociation of the homologous subunit from the E. coli enzyme.
transcription specifically. The subunit 4/7 complex has some characteristics normally associated with a σ factor. First, as already mentioned, the subunit 4/7 complex is required for initiation at a minimal promoter in vitro, but not for chain elongation or termination. Second, there are similarities in the amino acid sequences of subunit 4 and σ factors (9). There are, of course, limits to the analogy between the subunit 4/7 complex and σ. The subunit 4/7 complex is not sufficient for promoter recognition and initiation. Rather, a set of factors is required for these functions in eukaryotes, and the subunit 4/7 complex is a member of this set. Like other initiation factors, the subunit 4/7 complex may associate reversibly with the enzyme in the course of the initiation-elongation cycle. Our observation of in vitro complementation between defective holoenzyme and pol II Δ4/7 would be consistent with such reversible association.

*rpb-4* represents the second of two yeast pol II mutants we have examined in which the measurement of promoter-directed initiation gives very different results from the traditional assay of nonspecific initiation and chain elongation. Extracts of strain Y260-1 contain virtually no detectable activity in the nonspecific assay (12), but are as effective as wild-type extracts in promoter-directed initiation (11). Conversely, extracts of *rpb-4* possess full activity in the nonspecific assay but fail to support promoter-directed initiation. The use of both types of assay is, therefore, warranted in future characterizations of pol II mutants and preparations.

Preparations of yeast pol II holoenzyme contain a mixture of holoenzyme and pol II Δ4/7. The less than stoichiometric amounts of subunits 4 and 7 relative to the other subunits may not be artifactual because of dissociation and loss during purification, but rather may be functionally significant if, as mentioned above, the subunit 4/7 complex serves as an initiation factor, interacting reversibly with the enzyme. It will be of interest to learn whether mammalian pol II exhibits similar subunit dissociation and whether there are functional consequences.

Although the subunit 4/7 complex is required for specific initiation at a minimal promoter in vitro, strain *rpb-4*, which lacks subunit 4, is viable. This apparent contradiction may be explained by our results with the transcriptional activator Gal4-VP16. An *rpb-4* extract supports initiation at a promoter with a Gal4 binding site in the presence of Gal4-VP16. We suggest that transcription occurs at a low level in the absence of subunit 4 and is raised above the threshold for detection by Gal4-VP16. The low level of transcription in the absence of subunit 4 might be sufficient for viability. Alternatively, it may be argued that all yeast promoters contain activator binding sites, and the stimulatory effect of activators in the absence of subunit 4 results in sufficient transcription for cell growth. In either case, the level of transcription is lower than in the presence of both an activator and subunit 4, accounting for the slow growth of strain *rpb-4*.

Our results also bear on the question of what component of the pol II transcription apparatus is the target of activator proteins. The finding that Gal4-VP16 stimulates transcription in the absence of subunits 4 and 7 indicates that these two subunits cannot be the sole targets of the VP16 activation domain.

**DISCUSSION**

An analogy may be drawn between pol II Δ4/7 and the core RNA polymerase from bacteria, which lacks the σ subunit. Both enzymes possess RNA chain elongation and termination activities, but neither can recognize a promoter or initiate transcription specifically. The subunit 4/7 complex has some characteristics normally associated with a σ factor. First, as already mentioned, the subunit 4/7 complex is required for initiation at a minimal promoter in vitro, but not for chain elongation or termination. Second, there are similarities in the amino acid sequences of subunit 4 and σ factors (9). There are, of course, limits to the analogy between the subunit 4/7 complex and σ. The subunit 4/7 complex is not sufficient for promoter recognition and initiation. Rather, a set of factors is required for these functions in eukaryotes, and the subunit 4/7 complex is a member of this set. Like other initiation factors, the subunit 4/7 complex may associate reversibly with the enzyme in the course of the initiation-elongation cycle. Our observation of in vitro complementation between defective holoenzyme and pol II Δ4/7 would be consistent with such reversible association.

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**Acknowledgments**—We gratefully acknowledge Nancy Thompson and Richard Burgess for the gift of the immunoaffinity column.

**Note Added in Proof**—Recently, the gene for the polypeptide that

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4 A. M. Edwards, unpublished observation.

5 A. M. Edwards and S. A. Darst, unpublished observation.
associates with yeast RNA polymerase II (pol II) and migrates ahead of subunit 9 on denaturing gel electrophoresis (see Fig. 1 and Ref. 11) has been cloned and sequenced. This gene, rpb-11, is not related to any of the known yeast pol II genes and thus may constitute an additional subunit of yeast pol II. This raises the total number of subunits in yeast pol II to 11.

REFERENCES

1. Woychik, N. A., and Young, R. A. (1990) Trends Biochem. Sci. 15, 347–351
2. Krakow, J. S., Rhodes, G., and Jovin, T. M. (1976) in RNA Polymerase (Losick, R., and Chamberlin, M., eds), pp. 127–157, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Saltzman, A. G., and Weinmann, R. (1989) FASEB J. 3, 1723–1733
4. Conaway, J. W., Reines, D., and Conaway, R. C. (1990) J. Biol. Chem. 265, 7552–7558
5. Sentenac, A., and Hall, B. (1982) in The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 561–606, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
6. Woychik, N. A., Liao, S.-M., Kolodziej, P. A., and Young, R. A. (1990) Genes & Dev. 4, 513–523
7. Dedieu, S., Wyers, F., Sentenac, A., and Fromageot, P. (1976) Eur. J. Biochem. 65, 543–552
8. Ruet, A., Sentenac, A., Fromageot, P., Winsor, B., and Lacroute, F. (1980) J. Biol. Chem. 255, 6450–6455
9. Woychik, N. A., and Young, R. A. (1989) Mol. Cell. Biol. 9, 2854–2859
10. Lue, N. F., and Kornberg, R. D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 486–490
11. Edwards, A. M., Darst, S. A., Feaver, W. J., Thompson, N. E., Burgess, R. R., and Kornberg, R. D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2122–2126
12. Nonet, N., Scafe, C., Sexton, J., and Young, R. A. (1987) Mol. Cell. Biol. 7, 1602–1611
13. Chasman, D., Leatherwood, J., Carey, M., Ptashne, M., and Kornberg, R. D. (1989) Mol. Cell. Biol. 11, 4746–4749
14. Ruet, A., Sentenac, A., and Fromageot, P. (1978) Eur. J. Biochem. 90, 325–330
15. Lue, N. F., Planagan, P., Sugimoto, K., and Kornberg, R. D. (1989) Science 248, 661–664
16. Kadesch, T. R., and Chamberlin, M. J. (1982) J. Biol. Chem. 257, 5286–5295
17. Reines, D., Wells, D., Chamberlin, M. J., and Kane, C. M. (1987) J. Mol. Biol. 196, 299–319
18. Thompson, N., Aronson, D. B., and Burgess, R. R. (1990) J. Biol. Chem. 265, 7069–7077
19. Kolodziej, P. A., Woychik, N., Liao, S.-M., and Young, R. A. (1990) Mol. Cell. Biol. 10, 1915–1920
20. Dedrick, R. L., and Chamberlin, M. J. (1985) Biochemistry 24, 2245–2253
21. Dedrick, R. L., Kane, C. M., and Chamberlin, M. J. (1987) J. Biol. Chem. 262, 9098–9108
22. Kerppola, T. K., and Kane, C. M. (1989) Mol. Cell. Biol. 8, 4389–4394
23. Kerppola, T. K., and Kane, C. M. (1990) Biochemistry 29, 269–278
24. Laemmli, U. K. (1970) Nature 227, 680–685

N. Woychik and R. A. Young, manuscript in preparation.