Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4902–4906, May 1995
Biochemistry

Rapid RNA polymerase genetics: One-day, no-column preparation of reconstituted recombinant *Escherichia coli* RNA polymerase

(hexahistidine tag/metal ion-affinity chromatography/transcription/transcription activation/α subunit C-terminal deletion mutant)

HONG TANG*, KONSTANTIN SEVERINOV†‡, ALEX GOLDFARB†, AND RICHARD H. EBRIGHT*§

*Department of Chemistry and Waksman Institute, Rutgers University, New Brunswick, NJ 08855; and †Public Health Research Institute, 455 First Avenue, New York, NY 10016

Communicated by Sankar Adhya, National Institutes of Health, Bethesda, MD, February 21, 1995 (received for review December 14, 1994)

ABSTRACT **We present a simple, rapid procedure for reconstitution of *Escherichia coli* RNA polymerase holoenzyme (RNAP) from individual recombinant α, β, β′, and σ^{70} subunits. Hexahistidine-tagged recombinant α subunit purified by batch-mode metal-ion-affinity chromatography is incubated with crude recombinant β, β′, and σ^{70} subunits from inclusion bodies, and the resulting reconstituted recombinant RNAP is purified by batch-mode metal-ion-affinity chromatography. RNAP prepared by this procedure is indistinguishable from RNAP prepared by conventional methods with respect to subunit stoichiometry, α–DNA interaction, catabolite gene activator protein (CAP)-independent transcription, and CAP-dependent transcription. Experiments with α(1–235), an α subunit C-terminal deletion mutant, establish that the procedure is suitable for biochemical screening of subunit lethal mutants.**

Escherichia coli RNA polymerase holoenzyme (RNAP) has subunit composition $(\alpha)_2\beta\beta'\sigma$ (1, 2). The α subunit contains determinants for protein–DNA interaction with promoter upstream elements and for protein–protein interaction with transcription activator proteins (3, 4), the σ subunit contains determinants for protein–DNA interaction with promoter −35 and −10 elements and for protein–protein interaction with transcription activator proteins (3, 5), and the β and β′ subunits contain the catalytic site (6). RNAP assembly follows the pathway $\alpha \rightarrow (\alpha)_2 \rightarrow (\alpha)_2\beta \rightarrow (\alpha)_2\beta\beta' \rightarrow (\alpha)_2\beta\beta'\sigma$ (7).

E. coli RNAP can be reconstituted in fully functional form starting from individual subunits (8–11). Reconstitution from individual recombinant subunits, produced by cloning and high-level overexpression of individual subunit genes, permits construction of mutant RNAP derivatives (12–20), including lethal mutant RNAP derivatives (13–17). Recombinant reconstitution is an extremely powerful tool for genetic dissection of RNAP structure and function. In the most noteworthy example, recombinant reconstitution of [α(1–235)]RNAP, a lethal mutant RNAP derivative lacking the C-terminal region of α subunit, led to the finding that the C-terminal region of α subunit is involved in promoter recognition and transcription activation (14, 21).

The conventional procedures for recombinant reconstitution of RNAP suffer from two limitations. (*i*) The conventional procedures are time- and labor-intensive, requiring pre-reconstitution purification of α, β, β′, and σ, and post-reconstitution purification of RNAP (four to eight columns; refs. 12, 14, and 22). (*ii*) In experiments with mutants of α, the conventional procedures yield mutant-α RNAP preparations containing substantial amounts of contaminating wild-type α (5–30%) (ref. 20; K.S., A.G., H.T., R.H.E., W. Ross, and R. Gourse, unpublished data). Kashlev *et al.* (23) recently have reported an improvement on the conventional procedures. Kashlev *et al.* use a hexahistidine-tagged β′ derivative, permitting pre-reconstitution purification of β′ and post-reconstitution purification of RNAP by metal-ion-affinity chromatography (see refs. 24 and 25). However, due to technical difficulties in purification of hexahistidine-tagged β′, the procedure of Kashlev *et al.* requires conventional pre-reconstitution purification of α and σ (four columns), requires post-reconstitution conventional further purification of RNAP (one column), and has low yields (0.4 mg of RNAP per liter of bacterial cultures producing α, β, β′, and σ). In addition, the procedure of Kashlev *et al.* does not address the problem of contamination of mutant-α RNAP derivatives with wild-type α.

In this work, we show that use of a hexahistidine-tagged α derivative permits remarkably efficient recombinant reconstitution of RNAP. The procedure of this report is rapid (1 day; no columns), has high yields (20 mg of RNAP per liter of bacterial cultures producing α, β, β′, and σ), and produces mutant-α RNAP derivatives free from contaminating wild-type α. We show further that RNAP and [α(1–235)]RNAP prepared by the procedure of this report are indistinguishable from RNAP and [α(1–235)]RNAP prepared by conventional methods with respect to subunit stoichiometries, α–DNA interaction, catabolite gene activator protein (CAP)-independent transcription, and CAP-dependent transcription.

Availability of the clones and procedure of this report should facilitate genetic analysis of RNAP structure, transcription initiation, transcription elongation, transcription termination, and transcription regulation.

MATERIALS AND METHODS

Plasmids. A list of plasmids used in this work is presented in Table 1. Plasmid pHTT7f1-NHα encodes α with an N-terminal hexahistidine tag under control of the bacteriophage T7 gene 10 promoter. Plasmid pHTT7f1-NHα was constructed by replacement of the *Xba* I–*Bam*HI segment of plasmid pET21a by the *Xba* I–*Bam*HI *rpoA* segment of plasmid pREIIα, followed by site-directed mutagenesis (29) to insert a hexahistidine coding sequence between *rpoA* codons 1 and 2 and to eliminate the *Eco*RI and *Hin*dIII sites from the pET21a multiple cloning site. Plasmid pHTT7f1-CHα encodes α with a C-terminal hexahistidine tag under control of the bacteriophage T7 gene 10 promoter. Plasmid pHTT7f1-CHα was constructed by replacement of the *Xba* I–*Bam*HI segment of plasmid pET21a by the *Xba* I–*Bam*HI *rpoA* segment of plasmid pREIIα, followed by site-directed mutagenesis (29) to delete the *rpoA* stop codon and pET21a multiple cloning site, resulting in fusion of the *rpoA* gene and the pET21a hexahistidine coding sequence and stop codon, followed by site-directed

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

Abbreviations: RNAP, RNA polymerase holoenzyme; CAP, catabolite gene activator protein.

†Present address: The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399.
§To whom reprint requests should be addressed.

Table 1. Plasmids

Plasmid	Relevant characteristics	Source or ref.
pHTT7f1-NHα	Ap^R; ori-pBR322; ori-f1; *ϕ10P-rpoA(H6,N)*	This work
pHTT7f1-CHα	Ap^R; ori-pBR322; ori-f1; *ϕ10P-rpoA(H6,C)*	This work
pHTT7f1-NHα(1–235)	Ap^R; ori-pBR322; ori-f1; *ϕ10P-rpoA(H6,N)(1–235)*	This work
pHTT7f1-σ	Ap^R; ori-pBR322; ori-f1; *ϕ10P-rpoD*	This work
pET21a	Ap^R; ori-pBR322; ori-f1; *ϕ10P*	Novagen
pREIIα	Ap^R; ori-pBR322; *lppP-'lacPUV5-rpoA*	26
pT7α	Ap^R; ori-pBR322; *ϕ10P-rpoA*	12
pMKSe2	Ap^R; ori-pBR322; *lacP-rpoB*	19
pT7β′	Ap^R; ori-pBR322; *ϕ10P-rpoC*	12
pMRG8	Ap^R; ori-pBR322; $\lambda P_L O_L$-*rpoD*	27
pMB9-203-L8UV5	Ap^R; ori-pBR322; *lacPL8-UV5*	28

Ap^R, ampicillin resistance.

mutagenesis to introduce a *Bam*HI site after the fusion gene. Plasmid pHTT7f1-NHα(1–235) encodes α(1–235) with an N-terminal hexahistidine tag under control of the bacteriophage T7 gene 10 promoter. Plasmid pHTT7f1-NHα(1–235) was constructed from plasmid pHTT7f1-NHα by site-directed mutagenesis (29) to convert *rpoA* codon 236 to a TAA stop codon. Plasmid pHTT7f1-σ encodes σ^{70} under control of the bacteriophage T7 gene 10 promoter. Plasmid pHTT7f1-σ was constructed by replacement of the *Xba* I–*Hin*dIII segment of plasmid pET21a by an *Xba* I–*Hin*dIII *rpoD* DNA fragment produced by add-on PCR.

Each of the plasmids encoding hexahistidine-tagged α or α derivative has a unique *Xba* I site preceding the *rpoA* Shine–Delgarno sequence, a unique *Eco*RI site at codon 168, a unique *Hin*dIII site at codon 229, and a unique *Bam*HI site immediately following the *rpoA* stop codon. The plasmid encoding σ has a unique *Xba* I site preceding the *rpoD* Shine–Delgarno sequence, a unique *Bam*HI site at codon 434, and a unique *Hin*dIII site immediately following the *rpoD* stop codon.

Plasmids were constructed and maintained in *E. coli* strain XL1-Blue (Stratagene). For each plasmid, the DNA nucleotide sequence of the entire *rpoA* or *rpoD* gene was verified.

Preparation of α, Nondenaturing. *E. coli* strain BL21(DE3) (Novagen; ref. 30) transformed with pHTT7f1-NHα, pHTT7f1-CHα, or pHTT7f1-NHα(1–235) was shaken at 37°C in 5 ml of LB broth (31) plus 200 μg of ampicillin per ml until OD_{600} = 0.7, induced by addition of isopropyl β-D-thiogalactoside to 1 mM, and shaken an additional 3 hr at 37°C. The culture was harvested by centrifugation (16,000 × *g*; 2 min at 4°C), the cell pellet was resuspended in 0.4 ml of buffer A (20 mM Tris·HCl, pH 7.9/500 mM NaCl/5 mM imidazole), cells were lysed by sonication, and the lysate was cleared by centrifugation (16,000 × *g*; 15 min at 4°C). Hexahistidine-tagged α or α derivative was purified by batch-mode metal-ion-affinity chromatography. The sample was adsorbed onto 0.2 ml of Ni^{2+}-NTA agarose (Novagen) in buffer A, washed twice with 1 ml of buffer A, washed once with 1.2 ml of buffer A plus 60 mM imidazole, and eluted with 0.6 ml of buffer A plus 500 mM imidazole. Adsorption, washes, and elution were performed in a siliconized 1.7-ml microcentrifuge tube with incubations of 1 min at 4°C with gentle mixing and with removal of supernatants after centrifugation (1500 × *g*; 1 min at 22°C). The yield was 400–500 μg, and the purity was >90% [protein estimated by Bradford assay (32); purity estimated by SDS/PAGE]. Samples were stored in aliquots at −80°C.

Preparation of α, Denaturing. Denaturing preparation was performed as described in the preceding section with two modifications: (*i*) prior to metal-ion-affinity chromatography, the sample volume was adjusted to 1 ml with buffer A, and hexahistidine-tagged α or α derivative was precipitated by addition of $(NH_4)_2SO_4$ to 60%, collected by centrifugation (16,000 × *g*; 20 min at 4°C), and redissolved in buffer A plus 6 M guanidine hydrochloride; and (*ii*) all solutions for metal-ion-affinity chromatography contained 6 M guanidine hydrochloride. The yield was 100–300 μg, and the purity was >95% [protein estimated by Bradford assay (32); purity estimated by SDS/PAGE].

Preparation of Crude β, β′, and σ^{70}. Inclusion bodies containing crude β, β′, and σ^{70} were isolated and washed from induced cultures of transformants of plasmid pMKSe2, plasmid pT7β, and plasmid pHTT7f1-σ, respectively (method of ref. 22). Inclusion bodies were solubilized in buffer B (6 M guanidine hydrochloride/50 mM Tris·HCl, pH 7.9/10 mM $MgCl_2$/10 μM $ZnCl_2$/1 mM EDTA/10 mM dithiothreitol/10% glycerol), and protein concentrations were adjusted to ≈10 mg/ml [protein concentrations estimated by Bradford assay (32)].

Preparation of RNAP. Two reconstitution mixtures were prepared. The first reconstitution mixture (2 ml) contained 80 μg (2 nmol) of hexahistidine-tagged α or α derivative (prepared under denaturing conditions), 300 μg (2 nmol) of crude β, and 600 μg (4 nmol) of crude β′ in buffer B. The second reconstitution mixture (0.2 ml) contained 300 μg (4 nmol) of crude σ^{70} in buffer B. (Efficient reconstitution requires use of purified hexahistidine-tagged α or α derivative and use of freshly prepared crude β, β′, and σ^{70}.) The reconstitution mixtures were dialyzed separately 16 hr at 4°C against two 500-ml changes of buffer C (50 mM Tris·HCl, pH 7.9/200 mM KCl/10 mM $MgCl_2$/10 μM $ZnCl_2$/1 mM EDTA/5 mM 2-mercaptoethanol/20% glycerol). Following dialysis, the reconstitution mixtures were combined, incubated 45 min at 30°C, and cleared by centrifugation (16,000 × *g*; 10 min at 4°C). RNAP or RNAP derivative was purified by batch-mode metal-ion-affinity chromatography. Samples were adsorbed onto 0.1 ml Ni^{2+}-NTA agarose (Qiagen, Chatsworth, CA) in buffer D (50 mM Tris·HCl, pH 7.9/0.5 mM EDTA/5% glycerol), washed three times with 1.5 ml of buffer D plus 5 mM imidazole, and eluted in 0.25 ml of buffer D plus 150 mM imidazole. Adsorption, washes, and elution were performed in a siliconized 2.0-ml microcentrifuge tube with incubations of 45 min at 4°C with gentle mixing (15 s for washes) and with removal of supernatants after centrifugation (16,000 × *g*; 2 min at 4°C). The yield was ≈400 μg, and the purity was >99%. Samples were concentrated to 50 μl by centrifugal ultrafiltration [Centricon-100 filter units (Amicon); 1000 × *g*; 30 min at 4°C], mixed with 50 μl of glycerol, and stored at −20°C.

In parallel experiments, RNAP was reconstituted from purified α, β, β′, and σ^{70} according to conventional methods (22).

DNA Binding Experiments. Electrophoretic mobility shift DNA binding experiments were performed as described in ref. 20. Experiments were performed using a 19-bp DNA fragment containing a specific DNA site for α (positions −57 to −47 of the *rrnB P*1 promoter upstream element; 5′-TCAGAAAAT-TATTTTCGGG-3′/5′-CCCGAAAATAATTTTCTGA-3′). Reaction mixtures contained (20 μl) 0–20 μM α or α derivative [prepared under nondenaturing conditions; desalted into assay

buffer by chromatography on NAP-5 (Pharmacia)], 125 nM ^{32}P-labeled DNA fragment (10 Bq/pmol; prepared as described in ref. 33), 10 mM Mops-NaOH (pH 7.0), 50 mM NaCl, 10 mM $MgCl_2$, and 5% glycerol. Reaction mixtures were incubated 1 hr at 30°C. Reaction products were analyzed by electrophoresis through 5% polyacrylamide/2.7% glycerol slab gels in 45 mM Tris-borate, pH 8.0/5 mM $MgCl_2$/0.1 mM EDTA (20 V/cm; 20 min at 4°C). Following electrophoresis, gels were dried, and radioactivity was quantified by PhosphorImager analysis (Molecular Dynamics, model 425E). Equilibrium binding constants were calculated as described in ref. 34.

For each preparation of α or α derivative, the fraction of molecules active in sequence-specific DNA binding (0.4–0.7) was determined by titration of the DNA fragment under stoichiometric binding conditions (100 μM DNA fragment; 50–400 μM α or derivative); all data are reported in terms of molar concentrations of active dimers.

***In Vitro* Transcription Experiments.** Abortive initiation *in vitro* transcription experiments were performed as described in ref. 35. Experiments were performed using as templates a 203-bp *Eco*RI–*Eco*RI DNA fragment of plasmid pMB9-203-L8UV5 and a 314-bp *Ava* II–*Eco*RI DNA fragment of bacteriophage M13mp2-ICAP (35). Reaction mixtures contained (25 μl) 0.5 nM DNA fragment, 60 nM *E. coli* RNAP or RNAP derivative, 200 nM σ^{70} (prepared as described in ref. 22), 0 or 20 nM CAP (prepared as described in ref. 36), 0.2 mM cAMP, 0.5 mM ApA (ICN Biomedicals), 50 nM [α^{32}P]UTP (30 Bq/fmol), 40 mM Tris·HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 5% glycerol. Reaction components except RNAP or RNAP derivative, ApA, and [α^{32}P]UTP were preequilibrated for 10 min at 37°C. Reactions were initiated by addition of RNAP or RNAP derivative, followed after 10 min at 37°C by addition of ApA and [α^{32}P]UTP. Reactions were terminated after a further 15 min at 37°C by addition of 5 μl of 0.5 M EDTA. The reaction product [^{32}P]ApApUpU was resolved by paper chromatography in water/saturated ammonium sulfate/isopropyl alcohol (18:80:2, vol/vol; R_f = 0.05) and was quantified by PhosphorImager analysis.

Titration experiments with the *lacPL8-UV5* promoter and RNAP concentrations of 20, 40, 60, 80, and 160 nM establish that, for each RNAP derivative, 60 nM is subsaturating.

The data in this report are from experiments with a 4-fold molar excess of σ^{70} (cf. ref. 14). Essentially identical data are obtained in experiments with no excess σ^{70}.

RESULTS

Preparation of α and α(1–235). Plasmids encoding α subunit with an N-terminal or C-terminal hexahistidine tag under control of the bacteriophage T7 gene 10 promoter were constructed. Using these plasmids, α subunit with an N-terminal or C-terminal hexahistidine tag was overproduced in *E. coli* to the level of 20–40% of total soluble protein and was purified to near-homogeneity after cell lysis and a single step of batch-mode metal-ion-affinity chromatography (Fig. 1*A*). Purification was essentially equally successful under nondenaturing conditions (producing material suitable for studies of α structure and function) and under denaturing conditions (producing material suitable for recombinant reconstitution of RNAP) (Fig. 1*A*). Similar results were obtained with α subunit C-terminal-deletion mutant α(1–235) (Fig. 1*B*).

Preparation of RNAP and [α(1–235)]RNAP. Denatured recombinant α subunit with an N-terminal or C-terminal hexahistidine tag purified by batch-mode metal-ion-affinity chromatography and crude recombinant β and β' from inclusion bodies were mixed in the molar ratio 1:1:2, and denaturant was removed by dialysis over 16 hr. Crude recombinant σ^{70} from inclusion bodies was added to the $\alpha/\beta/\beta'$ reconstitution mix in 4-fold molar excess, and the reaction mixture was incubated 45 min at 30°C. The resulting reconstituted recombinant RNAP was purified to homogeneity in a single step of batch-mode metal-ion-affinity chromatography. The efficiency of reconstitution and post-reconstitution purification was fully 90% (i.e., fully 90% of α added to the reconstitution mixture was recovered in RNAP). The overall yield was 20 mg of RNAP per liter of bacterial cultures producing α, β, β', and σ^{70}. RNAP prepared by this procedure was indistinguishable from RNAP reconstituted by conventional methods with respect to subunit stoichiometry, exhibiting an α:β:β' subunit stoichiometry of 2:1:1 and a σ^{70} saturation of 100% (Fig. 2*A*).

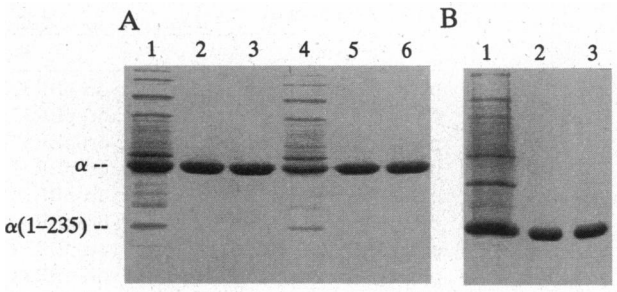

FIG. 1. Preparation of α and α(1–235). (*A*) α with an N-terminal (lanes 1–3) or C-terminal (lanes 4–6) hexahistidine tag. Lanes: 1 and 4, induced culture (total soluble protein); 2 and 5, product of nondenaturing batch-mode metal-ion-affinity chromatography; 3 and 6, product of denaturing batch-mode metal-ion-affinity chromatography. (*B*) α(1–235) with an N-terminal hexahistidine tag. Lanes as in *A*.

Similar results were obtained in reconstitutions carried out with α subunit C-terminal-deletion mutant α(1–235) (Fig. 2*B*). [α(1–235)]RNAP prepared by this procedure contained no detectable contaminating wild-type α (<<1%; Fig. 2*B*).

DNA Binding. The α subunit of RNAP participates in promoter recognition through direct, sequence-specific protein–DNA interaction (21). The α subunit recognizes an (A+T)-rich DNA site present immediately upstream of the −35 hexamer in certain strong promoters, notably the *rrnB P1* promoter (the "upstream element" or "UP"; ref. 21). The determinants for α-DNA interaction lie within the C-terminal 85 amino acids of α (amino acids 245–329) (21, 26). Therefore, whereas wild-type α is competent for α–DNA interaction, α(1–235) is not.

To assess DNA binding by hexahistidine-tagged α and hexahistidine-tagged α(1–235), we performed electrophoretic mobility shift DNA binding experiments using a 19-bp DNA

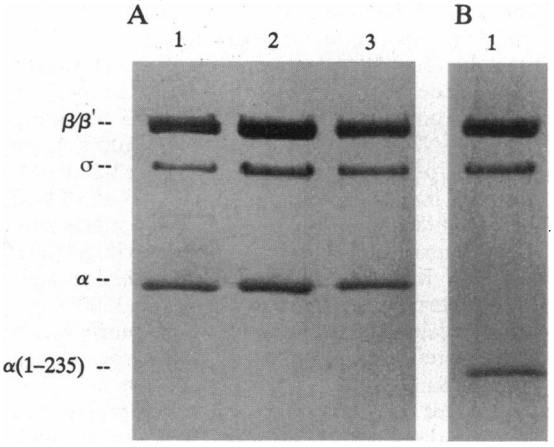

FIG. 2. Preparation of RNAP and [α(1–235)]RNAP. (*A*) RNAP. Lanes: 1, conventional procedure (22); 2, procedure of this report using N-terminally hexahistidine-tagged α; 3, procedure of this report using C-terminally hexahistidine-tagged α. (*B*) [α(1–235)]RNAP. Lane 1: procedure of this report using N-terminally hexahistidine-tagged α(1–235).

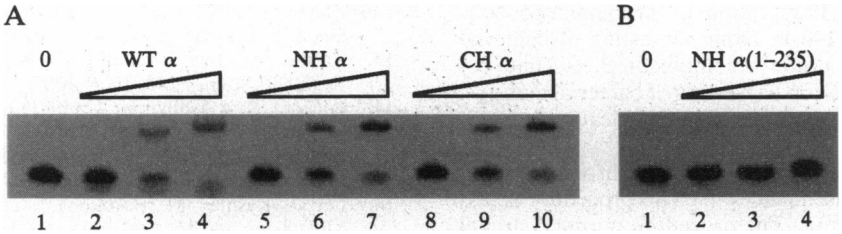

FIG. 3. DNA binding. (*A*) DNA binding experiments with α. Lanes: 1, no α; 2–4, 1.25, 2.5, and 5 μM wild-type α; 5–7, 1.25, 2.5, and 5 μM N-terminally hexahistidine-tagged α; 8–10, 1.25, 2.5, and 5 μM C-terminally hexahistidine-tagged α. (*B*) DNA binding experiments with α(1–235). Lanes: 1, no α; 2–4, 1.25, 2.5, and 5 μM N-terminally hexahistidine-tagged α(1–235).

fragment containing positions −57 to −47 of the *rrnB P1* promoter upstream element. The results are presented in Fig. 3 and Table 2. Within experimental error, wild-type α, N-terminally hexahistidine-tagged α, and C-terminally hexahistidine-tagged α exhibited identical DNA binding affinities. N-terminally hexahistidine-tagged α(1–235) exhibited no detectable DNA binding affinity.

We conclude that the presence of an N-terminal or C-terminal hexahistidine tag does not impair α-DNA interaction. Consistent with this conclusion, RNAP containing N-terminally or C-terminally hexahistidine-tagged α is essentially indistinguishable from RNAP containing wild-type α with respect to upstream-element-dependent transcription at the *rrnB P1* promoter (H.T., K.S., R.H.E., W. Ross, T. Gaal, and R. Gourse, unpublished results).

Transcription. The α subunit of RNAP is the target for transcription activation by CAP at the *lac* promoter (14, 18, 20, 37, 38). The determinants for α-CAP interaction are contained within the C-terminal region of α (amino acids 258–265). Therefore, whereas wild-type RNAP is competent for CAP-dependent transcription, [α(1–235)]RNAP is not.

To assess transcription by RNAP containing hexahistidine-tagged α and by [α(1–235)]RNAP containing N-terminally hexahistidine-tagged α(1–235), we performed abortive initiation *in vitro* transcription experiments. We analyzed two promoters: (*i*) the CAP-independent *lacPL8-UV5* promoter, and (*ii*) the CAP-dependent *lacP(ICAP)* promoter (a derivative of the *lac* promoter having a consensus DNA site for CAP; see refs. 20, 35, and 38). The results for CAP-independent transcription at *lacPL8-UV5* are presented in Fig. 4*A*. Within experimental error, RNAP containing wild-type α, N-terminally hexahistidine-tagged α, C-terminally hexahistidine-tagged α, and N-terminally hexahistidine-tagged α(1–235) exhibited identical, high CAP-independent transcription. The results for CAP-dependent transcription at *lacP(ICAP)* are presented in Fig. 4*B*. Within experimental error, RNAP containing wild-type α, N-terminally hexahistidine-tagged α, and C-terminally hexahistidine-tagged α exhibited identical, high CAP-dependent transcription; RNAP containing N-terminally hexahistidine-tagged α(1–235) exhibited almost no CAP-dependent transcription (<1% wild-type).

We conclude that reconstitution according to the procedure of this report is successful and that the presence of an N-terminal or C-terminal hexahistidine tag impairs neither CAP-independent nor CAP-dependent transcription.

Table 2. α–DNA interaction *in vitro*

Protein	K_{app}, M^{-1}
Wild-type α	2.0 (±0.7) × 10^5
N-terminally hexahistidine-tagged α	2.1 (±0.7) × 10^5
C-terminally hexahistidine-tagged α	2.2 (±0.5) × 10^5

Values are expressed as mean ± 1 SD. Data are from Fig. 3 (processed as in ref. 34).

DISCUSSION

Even with recent technical advances (22, 23), recombinant reconstitution of RNAP has been prohibitively time- and labor-intensive. Here, we report a procedure that overcomes these limitations. The procedure of this report permits recombinant reconstitution of RNAP and RNAP derivatives within 21–24 hr starting with cell pellets of bacterial cultures produc-

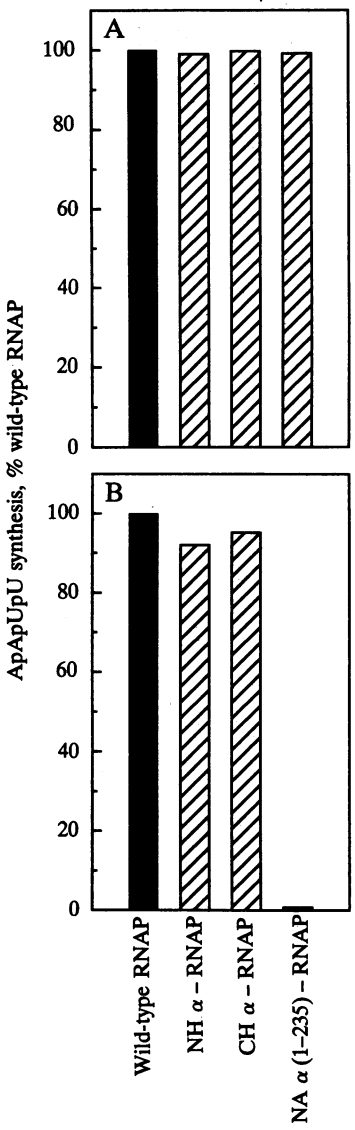

FIG. 4. Transcription. (*A*) CAP-independent transcription (*lacPL8-UV5* promoter; experiments carried out in the absence of CAP; 100% = 1.3 × 10^5 units). (*B*) CAP-dependent transcription [*lacP(ICAP)* promoter; experiments carried out in the presence of saturating CAP; 100% = 2.0 × 10^5 units].

ing α, β, β', and σ^{70}. (Preparation of near-homogeneous recombinant α requires 1–2 hr from harvesting of bacterial cultures. Preparation of crude recombinant β, β', and σ^{70} requires a total of 1–2 hr from harvesting of bacterial cultures. Reconstitution and post-reconstitution purification require 19–20 hr.)

The procedure of this report has no column chromatography steps. This has three consequences. (*i*) The procedure is less time- and labor-intensive. (*ii*) The procedure permits parallel processing of multiple samples (e.g., multiple mutants)—a critical advantage for biochemical screening of RNAP subunit mutants. (*iii*) The procedure readily can be scaled up for production of multimilligram or gram quantities of reconstituted recombinant RNAP and RNAP derivatives—a critical advantage for structural studies of RNAP (K.S., R.H.E., and Seth Darst, unpublished data).

In terms of RNAP structure and function, our results with N-terminally and C-terminally hexahistidine-tagged α subunits indicate that the N and C termini of α subunit are nonessential for CAP-independent transcription at the *lacPL8-UV5* promoter and CAP-dependent transcription at the *lac* promoter and, most likely, are exposed on the surface of RNAP.

We note that eukaryotic RNAP II contains a subunit homologous in sequence, stoichiometry, and assembly function to *E. coli* RNAP α subunit—i.e., RPB3 (reviewed in refs. 39–42). It may be possible to facilitate purification of RNAP II, and even to reconstitute RNAP II, by a strategy analogous to that used here.

We thank Drs. W. Ross and R. Gourse for discussion and for communicating results prior to publication. This work was supported by National Institutes of Health Grants GM41376 and GM51527 to R.H.E. and GM30717 to A.G. and by a Jane Coffin Childs postdoctoral fellowship to K.S.

1. Chamberlin, M. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 17–67.
2. Burgess, R. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 69–100.
3. Busby, S. & Ebright, R. (1994) *Cell* **79,** 743–746.
4. Ebright, R. & Busby, S. (1995) *Curr. Opin. Genet. Dev.*, in press.
5. Gross, C., Lonetto, M. & Losick, R. (1992) in *Transcriptional Regulation,* eds. McKnight, S. & Yamamoto, K. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 129–176.
6. Krakow, J., Rhodes, G. & Jovin, T. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 126–157.
7. Zillig, W., Palm, P. & Heil, A. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 101–125.
8. Ishihama, A., Fukuda, R. & Ito, K. (1973) *J. Mol. Biol.* **79,** 127–136.
9. Fukuda, R. & Ishihama, A. (1974) *J. Mol. Biol.* **87,** 523–540.
10. Saitoh, T. & Ishihama, A. (1975) *J. Mol. Biol.* **104,** 621–635.
11. Palm, P., Heil, A., Boyd, D., Grampp, B. & Zillig, W. (1975) *Eur. J. Biochem.* **53,** 283–291.
12. Zalenskaya, K., Lee, J., Chandrasekhar, N. G., Shin, Y. K., Slutsky, M. & Goldfarb, A. (1990) *Gene* **89,** 7–12.
13. Kashlev, M., Lee, J., Zalenskaya, K., Nikiforov, V. & Goldfarb, A. (1990) *Science* **248,** 1006–1009.
14. Igarashi, K. & Ishihama, A. (1991) *Cell* **65,** 1015–1022.
15. Mustaev, A., Kashlev, M., Lee, J., Polyakov, A., Lebedev, A., Zalenskaya, K., Grachev, M., Goldfarb, A. & Nikiforov, V. (1991) *J. Biol. Chem.* **266,** 23927–23931.
16. Lee, J., Kashlev, M., Borokhov, S. & Goldfarb, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6018–6022.
17. Martin, E., Sagitov, V., Burova, E., Nikiforov, V. & Goldfarb, A. (1992) *J. Biol. Chem.* **267,** 20175–20180.
18. Zou, C., Fujita, N., Igarashi, K. & Ishihama, A. (1992) *Mol. Microbiol.* **6,** 2599–2605.
19. Severinov, K., Soushko, M., Goldfarb, A. & Nikiforov, V. (1993) *J. Biol. Chem.* **268,** 14820–14825.
20. Tang, H., Severinov, K., Goldfarb, A., Fenyo, D., Chait, B. & Ebright, R. (1994) *Genes Dev.* **8,** 3058–3067.
21. Ross, W., Gosink, K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K. & Gourse, R. (1993) *Science* **262,** 1407–1413.
22. Borukhov, S. & Goldfarb, A. (1993) *Protein Expression Purif.* **4,** 503–511.
23. Kashlev, M., Martin, E., Polyakov, A., Severinov, K., Nikiforov, V. & Goldfarb, A. (1993) *Gene* **130,** 9–14.
24. Hochuli, E., Dobeli, H. & Schacher, A. (1987) *J. Chromatogr.* **411,** 177–184.
25. Hochuli, E., Bannwarth, W., Dobeli, H., Gentz, R. & Stuber, D. (1988) *Bio/Technology* **6,** 1321–1325.
26. Blatter, E., Ross, W., Tang, H., Gourse, R. & Ebright, R. (1994) *Cell* **78,** 889–896.
27. Gribskov, M. & Burgess, R. (1983) *Gene* **26,** 109–118.
28. Malan, T. P., Kolb, A., Buc, H. & McClure, W. (1984) *J. Mol. Biol.* **180,** 881–909.
29. Kunkel, T., Bebenek, K. & McClary, J. (1991) *Methods Enzymol.* **204,** 125–138.
30. Studier, F. W., Rosenberg, A., Dunn, J. & Dubendorff, J. (1990) *Methods Enzymol.* **185,** 60–89.
31. Miller, J. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
32. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
33. Ebright, R., Ebright, Y. & Gunasekera, A. (1989) *Nucleic Acids Res.* **17,** 10295–10305.
34. Gunasekera, A., Ebright, Y. & Ebright, R. (1992) *J. Biol. Chem.* **267,** 14713–14720.
35. Zhang, X., Zhou, Y., Ebright, Y. & Ebright, R. (1992) *J. Biol. Chem.* **267,** 8136–8139.
36. Zhang, X., Gunasekera, A., Ebright, Y. & Ebright, R. (1991) *J. Biomol. Struct. Dyn.* **9,** 463–473.
37. Kolb, A., Kolb, A., Igarashi, K., Ishihama, A., Lavigne, M., Buckle, M. & Buc, H. (1993) *Nucleic Acids Res.* **21,** 319–326.
38. Chen, Y., Ebright, Y. & Ebright, R. (1994) *Science* **265,** 90–92.
39. Woychik, N. & Young, R. (1990) *Trends Biochem. Sci.* **15,** 347–351.
40. Young, R. (1991) *Annu. Rev. Biochem.* **60,** 689–715.
41. Sentenac, A., Riva, M., Thuriaux, P., Buhler, J.-M., Treich, I., Carles, C., Werner, M., Ruet, A., Huet, J., Mann, C., Chiannilkulchai, N., Stettler, S. & Mariotte, S. (1992) in *Transcriptional Regulation*, eds. McKnight, S. & Yamamoto, K. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 27–54.
42. Archambault, J. & Friesen, J. (1993) *Microbiol. Rev.* **57,** 703–724.