RapA, a Novel RNA Polymerase-Associated Protein, Is a Bacterial Homolog of SWI2/SNF2

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We have identified a novel Escherichia coli RNA polymerase (RNAP)-associated protein, an ATPase named RapA. Almost all of this 110-kDa protein in the cell co-purifies with RNAP holoenzyme as a 1:1 complex. Purified to homogeneity, RapA also forms a stable complex with RNAP, as if it were a subunit of RNAP. The ATPase activity of RapA is stimulated by binding to RNAP, and thus, RapA and RNAP interact physically as well as functionally. Interestingly, RapA is a homolog of the SWI/SNF family of eukaryotic proteins whose members are involved in transcription activation, nucleosome remodeling, and DNA repair.

Escherichia coli RNA polymerase (RNAP)1 is the sole enzyme responsible for all transcription activity in the cell. The core RNAP, consisting of $\alpha_2\beta\beta'$ subunits, is the basic machinery capable of transcription elongation and termination at all terminators. There are many RNAP-associated proteins that modulate the activity of RNAP during different stages of transcription (1). Examples of such RNAP-associated proteins are sigma factor (1–3), NusA (4, 5), GreA, and GreB (6–10). The two largest subunits of RNAP, $\beta$ and $\beta'$, are the homologs of eukaryotic RNAP. For review, see Ref. 11 and references therein. In addition, GreA and GreB proteins are the functional homologs of SII, an eukaryotic RNA Pol II-associated protein. These homologs indicate that the basic structure and function of RNAP and RNAP-associated proteins are conserved throughout evolution.

Here we describe the identification of a novel E. coli RNAP-associated protein named RapA. RapA is found to be a homolog of the SWI/SNF family of eukaryotic proteins (12, 13). Yeast $swi$ and $snf$ genes were genetically identified to be important for transcription activation (14–17). Homologs of the SWI/SNF family have been reported in Drosophila (18–21) and humans (22–24). Purified eukaryotic SWI/SNF proteins and their homologs are able to remodel the chromatin/nucleosome structure (21, 25, 26), which could explain their role in influencing transcription activity at some genes. The human homologs of the SWI/SNF family interact with the transcription machinery, the HIV-1 integrase, and the Epstein-Barr virus EBNA2 (23, 27–29). In addition, some members of the SWI/SNF family are involved in DNA repair (30–34), and mutations in these genes are implicated in human disease (27, 34). Bacterial ORFs have been reported to be the homologs of the SWI/SNF family (35, 36), however, none of the prokaryotic homolog products had been purified. One of the unsolved questions regarding the SWI/SNF family of proteins is whether they are associated with RNAP (13). Our results have shown that RapA is an RNAP-associated protein, suggesting that association with RNAP is a conserved feature of this class of proteins.

EXPERIMENTAL PROCEDURES

Purification of RNAP—E. coli K12 cells (MG1655) were grown on 4× LB in a 10-liter fermenter. Cells were harvested at $A_{600}$ = 8 in late log growth phase (yield ~200 g of wet cell paste). About 100 g of wet cell paste was used per purification. RNAP was purified according to Hager et al. (37), but with some modifications. A single-stranded DNA-agarose (Pharmacia Biotech Inc.) column was used instead of DNA-cellulose, and the step of gel filtration on Sephacryl S-300 was omitted. The single-stranded DNA-agarose column (bed volume ~ 25 ml) was pre-equilibrated with TGED buffer (0.1 M Tris-Cl, pH 7.9, 5% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol) containing 0.2 M NaCl, and sample was loaded on the column at 1.5–2 ml/min. After loading, the column was washed with 200 ml of TGED containing 0.2 M NaCl, and the RNAP fraction was eluted with TGED containing 1.0 M NaCl at 1 ml/min. A shallow gradient (from 0.3 to 0.5 M NaCl in 400 ml of TGED buffer) was used to elute RNAP from the 10/10 preparative Mono-Q column (Pharmacia) at 0.5 ml/min. Typically, the yield for core RNAP was 16–20 mg, for holoenzyme was 10–16 mg, and for holoenzyme-RapA was 2–4 mg. FPLC System (Pharmacia) was used for the protein purification, and the purification procedures were carried out at 4 °C.

Purification of RapA—The combined RNAP-RapA fractions (peak 3 from the Mono-Q 10/10 column) were buffer exchanged with Buffer A (50 mM Hepes, 0.2 mM EDTA, 0.1 mM dithiothreitol, pH 7.5) containing 1 M (NH$_4$)$_2$SO$_4$ using Centiprep 100 concentrators (Amicon). They were then subjected to reverse linear gradient of (NH$_4$)$_2$SO$_4$ (1–0 M in 80 ml of TGED buffer). Fractions that contained the 110-kDa RapA protein were diluted with 2 volumes of TGED buffer and loaded directly on the Mono-Q 5/5 column (Pharmacia). The ATPase activity of RapA is stimulated by binding to RNAP, and thus, RapA and RNAP interact physically as well as functionally. Interestingly, RapA is a homolog of the SWI/SNF family of eukaryotic proteins whose members are involved in transcription activation, nucleosome remodeling, and DNA repair.

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1 The abbreviations used are: RNAP, RNA polymerase; ORF, open reading frame.

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contained 40 mM Tris-HCl (pH 7.4), 40 mM NaCl, 4 mM MgCl₂, 1 mM dithiothreitol, 50 μg/ml bovine serum albumin, 0.2 mM ATP and 1 μCi of [α-32P]ATP. When indicated, poly(rA) (0.25 mg), poly(dA) (0.26 mg), or poly(dA)·poly(dT) (0.26 mg) was included in the reaction mixture. The reactions were started by the addition of the purified RapA (0.2 mM) or RNAP holoenzyme (0.1 mM) or 2:1 (mol/mol) mixture of the purified RapA (0.2 mM) and RNAP holoenzyme (0.1 mM). After 30 min at 37 °C, the reactions were terminated by the addition of 1 ml of 10% SDS, lyophilized, dissolved in 5 ml of methanol and spotted onto a poly(ethyleneimine)-cellulose plate (J. T. Baker), and chromatography was carried out in 1 M LiCl, 1 M formic acid. Plates were autoradiographed and were also scanned on a PhosphorImager (Molecular Dynamics) to quantify the amount of [α-32P]ATP hydrolyzed. For determination of other (d)NTPase activity, ATP was replaced with a corresponding (d)NTP.

**Binding of Polynucleotides to Purified RapA**—The binding of polynucleotides to RapA was studied by fluorescent titrations using a Luminescence Spectrometer LS 50B (Perkin-Elmer). Protein (tryptophan) fluorescence of RapA (0.01 mg/ml in 120 mM TGED buffer containing 0.1 M NaCl and 10 mM MgCl₂) was measured in the presence of various concentrations of polynucleotides. The reductions in the fluorescence intensity of the sample (λ<sub>ex</sub> 529 nm) were calculated using the equation ΔF = F₀ - F, where F₀ is the fluorescence intensity of the sample in the absence of polynucleotides and F is the fluorescence intensity in the presence of polynucleotides.

**FIG. 1.** A 110-kDa protein copurifies with E. coli RNAP. A, schematic for the modified procedure for purification of E. coli RNAP. For detail see Experimental Procedures. B, elution profile from the Mono-Q column, demonstrating separation of the core RNAP (Peak 1), RNAP holoenzyme (Peak 2), and a 1:1 complex of RNAP holoenzyme with the 110-kDa protein, RapA (Peak 3), by a shallow NaCl gradient. C, fractions from different steps of the purification procedure were analyzed on an 8% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. The positions for RNAP and RapA are indicated.

FIG. 2. The 110-kDa protein is not related to the known subunits of RNAP. α, β/β', σ-70 subunits of RNAP and the 110-kDa protein were isolated separately from the Coomassie-stained SDS-polyacrylamide gel (by passive elution with 1% SDS, 1% Triton X-100, 6 M urea, 50 mM Tris-HCl, pH 7.4, for 2 h at 50 °C from homogenized gel slices) and loaded repeatedly on separate tracks of two identical 8% SDS-polyacrylamide gels, subjected to either silver staining (top) or immunoblotting with antibodies against both core RNAP and σ-70 (bottom). The 110-kDa protein is indicated by an arrow. Lane 1, 2 μl of the protein standards; lane 2, 1 μg of the Mono-Q-purified RNAP holoenzyme (Peak 2); lane 3, 1 μg of the Mono-Q-purified fraction containing the RNAP holoenzyme and the 110-kDa RapA protein (Peak 3); lane 4, 0.2 μg of the purified α; lane 5, 0.2 μg of the purified β/β'; lane 6, 0.1 μg of the purified σ-70 subunit of RNAP; lane 7, 0.1 μg of the purified 110-kDa RapA protein.
maximum of the emission spectrum were monitored after the additions of increasing amounts of a polynucleotide solution (for each addition, the sample was allowed to equilibrate for 10 min, and all additions were made in a total volume < 8 μl). ΔP/ΔF_max for each polynucleotide concentration was calculated, and the dissociation constants were determined from the Scatchard plots.

Other Biochemical Techniques and Reagents—RNAP concentrations were determined by UV absorbance using the extinction coefficient data of Lowe et al. (40). RNAP and RapA concentrations were also determined using the Bradford method (48) with bovine serum albumin as a standard. The purity of the RNAP and RapA preparations was estimated from overloaded SDS-polyacrylamide gels after staining with Coomassie Brilliant Blue R-250 or silver. 8% SDS-polyacrylamide stacking gels were run using a Mini-PROTEAN II Electrophoresis System (Bio-Rad). Amino acid sequencing of the N-terminal region of RapA was done by automated Edman degradation and phenylthiohydantoin-derivative analysis using Applied Biosystems model 476A protein sequencing system. For Western blot, electrophoresis was performed using Fastblot semi-dry electrophoresis blotter (Bio-Rad) and Immobilon-P membranes (Millipore). The polyclonal antibodies to highly pure core RNAP, σ-70 subunit of RNAP, or the 110-kDa RapA protein (>98% pure) were raised in rabbits by Berkeley Antibody Co., CA. The protein bands were visualized using goat anti-rabbit peroxidase-conjugated secondary antibodies (Calbiochem) and ECL Western blotting detection reagents (Amersham Life Science, Inc.).

RESULTS AND DISCUSSION

A 110-kDa Protein Copurifies with RNAP—Using a modified procedure for the purification of the E. coli DNA-dependent

![FIG. 3. The majority of the 110-kDa RapA protein in the cell co-purifies with RNAP. The fractions corresponding to different steps of the RNAP purification procedure (see Fig. 1) were immunostained with the 110-kDa RapA protein-specific polyclonal antibodies. Lane 1, 50 ng of the purified 110-kDa RapA protein; lane 2, 10 μl of the E. coli crude extract; lane 3, 10 μl of the supernatant after Polymin P precipitation; lane 4, 10 μl of the 0.5 M NaCl wash of the Polymin P precipitate; lane 5, 10 μl of the 1.0 M NaCl wash of the Polymin P precipitate; lane 6, Polymin P pellet after 1.0 M NaCl wash; lane 7, 10 μl of the single-stranded DNA agarose flow-thru; lane 8, 10 μl of the single-stranded DNA agarose 1 M NaCl eluate; lane 9, 100 ng of the purified 110-kDa RapA protein. RNAP-enriched fractions were in lanes 5 and 8 (see Fig. 1C). The band which migrated below the RapA protein (lanes 2 and 3) is of uncharacterized origin.](http://www.jbc.org/)

![FIG. 4. Purification of RapA. The detailed procedure was described under “Experimental Procedures.” A, elution profile from the phenyl Superose HR 5/5 column demonstrating separation of the 110-kDa RapA protein from the RNAP holoenzyme. B, fractions from the phenyl Superose HR 5/5 column. An aliquot corresponding to 100 μl from each fraction was loaded per track of an 8% SDS-gel stained with Coomassie Brilliant Blue R-250. Lane 1, 10 μl of the protein standards (Bio-Rad, broad range); lanes 2 and 3, combined RNAP-RapA fractions (Peak 3 from the Mono-Q 10/10 column in Fig. 1); lanes 4–10, fractions 7, 9, 11, 13, 15, 17, and 19 from the phenyl Superose HR 5/5 column. C, elution profile from the Mono-Q 5/5 column (Mono-Q-2), the final step of the RapA purification procedure. D, fractions from the Mono-Q 5/5 column (Mono-Q-2) analyzed on an 8% SDS-gel stained with Coomassie Brilliant Blue R-250. An aliquot corresponding to 80 μl from each fraction was loaded per track of an 8% SDS-gel. Lane 1, 10 μl of the protein standards (Bio-Rad, broad range); lanes 2–8, fractions 5, 7, 9, 11, 13, 15, and 17 from the Mono-Q 5/5 column (Mono Q-2).](http://www.jbc.org/)
RNA polymerase (RNAP), we identified a 110-kDa protein that consistently copurified with RNAP (Fig. 1). In the final step of Mono-Q chromatography, from which highly pure and active RNAP was obtained (37), the fraction that contained the RNAP holoenzyme and the 110-kDa protein eluted as peak 3 immediately following the core RNAP (peak 1) and holoenzyme (peak 2) in a shallow NaCl gradient (Fig. 1, B and C). A protein of about 110-kDa had been previously observed to copurify with RNAP (38–40); however, it was believed to be a proteolytic fragment of the β′ (40) or β subunit of RNAP (41). The 110-kDa protein in our preparation does not react with RNAP-specific polyclonal antibodies (Fig. 2), indicating that it is not related to the known subunits of RNAP.

The 110-kDa protein appears to be an integral component of RNAP because (i) it is associated with RNAP in nearly equimolar amounts, as estimated by quantitation of the Coomassie-stained protein bands on 8% SDS-polyacrylamide gels; (ii) there is a significant amount of the RNAP holoenzyme (20–30% in six independent protein purification procedures) associated with the 110-kDa protein; and (iii) almost all of the 110-kDa protein (>90%) from the cell is associated with RNAP, judging from the amount of this protein present during different stages of RNAP preparation, determined by immunoblotting using the 110-kDa protein-specific polyclonal antibodies (Fig. 3). Our results indicate that this 110-kDa protein is a novel RNAP-associated protein, and we name it RapA. RNAP containing the RapA protein is called RNAP-RapA hereafter.

**Purification of the 110-kDa RapA Protein**—To study the biochemical properties of this new RapA protein, we separated the 110-kDa RapA protein from RNAP and purified it to homogeneity in two steps (Figs. 1A and 4). The fractions containing RNAP holoenzyme and the 110-kDa RapA protein (Fig. 1, Mono-Q-1, Peak 3) were first subjected to hydrophobic interaction chromatography on phenyl Superose HR column (Fig. 4, A and B). The second peak eluted from the column was the 110-kDa RapA protein. Another chromatography on a Mono-Q column followed (Fig. 4, C and D), and the highly pure RapA protein was eluted as the first peak. In four repetitions of this purification procedure, about 100–250 μg of highly pure (>98%) RapA was obtained from 2–4 mg of the starting material. To obtain homogeneous RapA, the remaining contaminants (mostly RNAP) were removed by repeating the last purification step.

**Reconstitution of the RNAP-RapA Complex**—We found that a stable RNAP-RapA complex can be reconstituted with highly purified 110-kDa RapA protein and RNAP holoenzyme in vitro. When the 110-kDa RapA protein and the RNAP holoenzyme were mixed and passed through a Superose 6 HR column (Pharmacia), they coeluted as a complex (Fig. 5A). In control experiments, when these two proteins were applied separately to the column, RNAP and the 110-kDa RapA protein eluted at different fractions in gel filtration (Fig. 5, B and C). These results demonstrated that the 110-kDa RapA protein forms stable complex with RNAP, like a subunit of RNAP.

**The RapA Protein Is a Homolog of the SWI/SNF Family of Eukaryotic Proteins**—Determination of the N-terminal sequence of the 110-kDa RapA protein showed that the first 10 N-terminal amino acid residues are: Pro-Phe-Thr-Leu-Gly-Gln-Arg-Trp-Ile-Val, a sequence whose only match is the corre...
The stimulation of (d)ATPase activity of RapA by RNAP

| Enzyme | Enzyme | ATP | dATP |
|--------|--------|-----|------|
|        |        | K_{m} (mM) | k_{cat} (s^{-1}) | K_{m} (mM) | k_{cat} (s^{-1}) |
| RapA   | 0.05   | 0.06 | 0.44 | 1.8 |
| RNAP·RapA | 0.04 | 0.26 | 0.44 | 2.9 |

Table II
Hydrolysis of (d)NTPs by RapA and RNAP·RapA

| Enzyme | ATP | dATP |
|--------|-----|------|
|        | pmol of nucleotide hydrolyzed/min/μg RapA |
| RapA   | 28  | 177  |
| RNAP·RapA | 122 | 293  |

Table III
Dissociation constants of the RapA-polynucleotide complexes

| Protein | Poly(rA) (~420 bases) | Poly(rA)·Poly(dT)_{12–18} (~420 bases) | Poly(dA) (~270 bases) | Poly(dA)·Poly(dT)_{12–18} (~270 bases) |
|---------|-----------------------|---------------------------------------|-----------------------|---------------------------------------|
| RapA    | 29                    | 17                                   | 22                    | 15                                    |

RapA Is an ATPase and Its ATPase Activity Is Stimulated by RNAP—We found that RapA was capable of ATP hydrolysis (Fig. 6). The ATPase activity of RapA was not affected by the presence of single-stranded DNA (lane 3), RNA (lane 4), or double-stranded DNA (lane 5).

The ATPase activity of RapA was stimulated by the presence of RNAP (Fig. 6, lanes 10–13), which by itself did not hydrolyze ATP or did so very poorly (lanes 6–9). Thus, RNAP·RapA functions as an RNAP ATPase, a novel activity associated with RNAP. In the presence of poly(dA)·poly(dT), only RNAP·RapA exhibited RNA synthetic and ATP hydrolytic activities simultaneously (compare lane 13 to lanes 5 and 9), with the former being the predominant activity. The apparent k_{cat} of ATP hydrolysis by RNAP·RapA increased more than 4-fold, while the K_{m} for ATP changed very little (Table I). The stimulation of ATPase of other SWI/SNF members by RNAP has not been reported. The specific activities of the ATPases of RapA and RNAP·RapA were approximately 30 and 120 pmol of ADP released/min/microgram of RapA, respectively (Table II), values close to the yeast SWI/SNF ATPase (45).

We believe that the RapA protein is responsible for the ATPase activity for the following two reasons. 1) A homologous RapA protein that contained no detectable impurities by Coomassie Blue R-250 or silver stainings exhibited ATPase activity; and 2) The ATPase activity of RapA was totally eliminated when the RapA protein was depleted in the reactions by anti-RapA antibodies complexed with the protein A-agarose (data not shown). RapA or RNAP·RapA was also able to hydrolyze dATP (Table II). The k_{cat} for dATP hydrolysis was 30-fold higher than that of ATP (Table I). However, the K_{m} for dATP was more than 8-fold higher than the K_{m} for ATP, indicating that the affinity of RapA for dATP is much lower than that for ATP. The hydrolysis of dATP was enhanced by RNAP due to an increase in k_{cat} (Table I). Because the concentration of ATP in the cell is about 10-fold higher than that of dATP (46), it is likely that RNAP·RapA will use both ATP and dATP with similar efficiency. RapA or RNAP·RapA hydrolyzed other NTPs and dNTPs very poorly (Table II), a property similar to yeast RSC, another member of the SWI/SNF family (26).

RapA Is a Polynucleotide-binding Protein—RapA binds to single-stranded DNA, RNA, partial DNA-DNA, and DNA-RNA duplexes (Table III). The K_{s} values of the complexes of RapA with different nucleic acids were similar, ranging from 15 to 30 μM with a relatively better binding to partial DNA-DNA and DNA-RNA duplexes. At present, we do not know whether the polynucleotide-binding activity of RapA is altered when it forms a complex with RNAP. Nucleic acid-binding activity has been reported for other members of the SWI/SNF family (26, 47).

RapA Is Not a Helicase—Although RapA has putative helicase motifs, highly pure RapA showed no detectable helicase activity by extensive helicase assays using several different substrates. However, when RapA preparations that contained even trace amounts of RNAP (<1%) were used, an apparent helicase activity was observed. RNAP alone was capable of unwinding model partial duplex DNA substrates used in helicase assays, and this activity was not enhanced by RapA (data not shown). So far, none of the members of the SWI/SNF family have shown helicase activity.

In summary, we have identified a new RNAP-associated protein, RapA, in E. coli. This bacterial homolog of the SWI/SNF family interacts with RNAP both physically (binds to RNAP) and functionally (the ATPase of RapA is stimulated by
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RNAP). However, the effect of RapA on transcription using naked DNA templates (either linear or supercoiled) in vitro is not dramatic. Fig. 7 illustrates a representative of such experiments showing at best moderate activation in overall transcription activity by RNAP-RapA when compared with RNAP itself. This moderate activation of transcription (150 to 200%) by RapA is independent of several promoters used, and the RapA protein has no effect on the rate of elongation and on the efficiency of termination at both ρ-independent and -dependent terminators (data not shown). Possibly, some factors are missing in our in vitro transcription system. At present, we do not know the role of RapA or RNAP-RapA in the cell. Apparently, the E. coli cell that lacks a functional rapA gene is viable and able to utilize different sugars as carbon sources.3 Currently, we are studying the expression of rapA and the role of rapA under different conditions in the cell. Further study of this RapA protein and its gene is necessary to understand its function in E. coli.

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