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The RNA Polymerase α Subunit from *Sinorhizobium meliloti* Can Assemble with RNA Polymerase Subunits from *Escherichia coli* and Function in Basal and Activated Transcription both In Vivo and In Vitro

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***Sinorhizobium meliloti*, a gram-negative soil bacterium, forms a nitrogen-fixing symbiotic relationship with members of the legume family. To facilitate our studies of transcription in *S. meliloti*, we cloned and characterized the gene for the α subunit of RNA polymerase (RNAP). *S. meliloti rpoA* encodes a 336-amino-acid, 37-kDa protein. Sequence analysis of the region surrounding *rpoA* identified six open reading frames that are found in the conserved gene order *secY* (SecY)-*adk* (Adk)-*rpsM* (S13)-*rpsK* (S11)-*rpoA* (α)-*rplQ* (L17) found in the α-proteobacteria. In vivo, S. *meliloti rpoA* expressed in *Escherichia coli* complemented a temperature sensitive mutation in *E. coli rpoA*, demonstrating that *S. meliloti* α supports RNAP assembly, sequence-specific DNA binding, and interaction with transcriptional activators in the context of *E. coli*. In vitro, we reconstituted RNAP holoenzyme from *S. meliloti* α and *E. coli* β, β′, and σ subunits. Similar to *E. coli* RNAP, the hybrid RNAP supported transcription from an *E. coli* core promoter and responded to both upstream (UP) element- and Fis-dependent transcription activation. We obtained similar results using purified RNAP from *S. meliloti*. Our results demonstrate that *S. meliloti* α functions are conserved in heterologous host *E. coli* even though the two α subunits are only 51% identical. The ability to utilize *E. coli* as a heterologous system in which to study the regulation of *S. meliloti* genes could provide an important tool for our understanding and manipulation of these processes.**

The α-proteobacterium *Sinorhizobium meliloti* is able to live either as a soil saprophyte or in a symbiotic relationship with members of the legume family, such as alfalfa. Recent studies have focused on understanding how rhizobia adapt to these unique environments, especially at the level of gene expression (6, 13, 62). For the symbiosis to occur, expression of a subset of genes, such as the *nod* and *nif* genes, must be tightly regulated (reviewed in reference 22). As is the case with other bacteria, much of the gene regulation occurs at the level of initiation of transcription (28). To facilitate our studies of transcription and its regulation in *S. meliloti,* we must understand RNA polymerase (RNAP) structure and function.

Previous work demonstrated that RNAP from *S. meliloti* displays the characteristic $\alpha_2\beta\beta'$ core subunit structure found in most bacteria (23, 45). In addition σ^{70}, σ^{54}, and σ^{72} homologs have been cloned from *S. meliloti* (47, 48, 52, 55). These results are consistent with the evidence that bacterial RNAPs display overall sequence and functional similarities, although they can exhibit some differences in individual steps during transcription such as promoter recognition and promoter escape (4). Since only a limited number of *S. meliloti* promoters have been characterized, the *cis*-acting elements are not yet as well defined as in *Escherichia coli* promoters (7, 23, 55). Nevertheless, *S. meliloti* RNAP can initiate transcription at typical *E. coli* promoters (19, 23). However, most *S. meliloti* promoters that have been characterized are not transcribed by *E. coli* RNAP in vivo or in vitro (5, 19), perhaps because the *S. meliloti* $E\sigma^{70}$ homolog recognizes these promoters slightly differently from *E. coli* $E\sigma^{70}$ or because these promoters utilize σ factors or transcription activators not found in *E. coli*.

In the past decade, based primarily on work with *E. coli*, RNAP α has emerged as a key player in both basal transcription and in transcriptional activation (reviewed in references 20 and 29). RNAP α consists of two independently folded domains connected by a flexible linker (9, 64). The amino-terminal domain (αNTD) is required for α dimerization, for RNAP assembly, and for interaction with a subset of transcription factors (32, 51); the carboxy-terminal domain (αCTD) is required for binding to the upstream (UP) element, an A+T-rich sequence found upstream of the −35 hexamer, and for interaction with a number of transcription factors (35, 53). Several screens for α mutants have identified residues required for the activation of transcription. These α-activator contacts help recruit RNAP to promoters and/or stimulate the isomerization of the RNAP-promoter complex from the closed to the open state (46). Furthermore, the αCTD may also interact with the σCTD during transcription initiation at some promoters (29).

In some cases, α-activator contacts appear be species specific, suggesting that α and activators have coevolved. For example, *Agrobacterium tumefaciens* α is required for VirG-activated transcription of the *virB* promoter in *E. coli* (39). Similarly, transcription activation from the *Bacillus subtilis*

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TABLE 1. Strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
E. coli		
DH5α	*supE44 ΔlacU169* (ϕ80*lacZΔM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	56
HN317*ts*112	F⁻ *aroE thi* Su⁻ Str *rpoA112*	36
XL1-Blue MRA	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 gyrA96 relA1 lac*	Stratagene
S. meliloti JM57	1021, pSym *nodC-lacZ*	44
Plasmids		
pBluescript SK(−)	Cloning vector, Ap^r	Stratagene
pBAD/HisB	Cloning vector, arabinose promoter, six-His tag, Ap^r	Invitrogen
pREIIα	ori-pBR322; *lppP-'lacPUV5-E. coli rpoA*, Ap^r	9
pCR2.1-TOPO	Cloning vector, Ap^r	Invitrogen
pMP1	2.1-kb *Bam*HI fragment containing *rpoA* in pBluescript SK(−)	This study
pMP2	3.3-kb *Hin*dIII fragment containing *rpoA* in pBluescript SK(−)	This study
pMP3	Full-length *rpoA* in *Eco*RI site of pBluescript SK(−)	This study
pMP16	*rpoA* cloned into pCR2.1-TOPO	This study
pMP18	*rpoA* (1–254) plus adjacent stop codon in pCR2.1-TOPO	This study
pMP19a	*rpoA* cloned into pBAD/HisB	This study
pMP21	*rpoA* (1–254) in pBAD/HisB	This study
pMP22	*E. coli rpoA* replaced by *S. meliloti rpoA* with six-His tag in pREIIα	This study
pMP25	Upstream stop codons and Shine-Dalgarno sequence, *rpoA* with six-His tag in pCR2.1-TOPO	This study
pHTT7α	ori-pBR322; ϕ10P-*E. coli rpoA*, Ap^r	9
pRLG770	Vector containing *E. coli rrnB* P1 terminator, RNA *I* promoter	54
pRLG593	pRLG770 with *lacUV5* (−59–+38)	54
pRLG862	pRLG770 with *E. coli rrnB* P1 (−88–+1)	54
pLR14	pRLG770 with *E. coli rrnB* P1 (−88–+1, SUB)	50
pRmE65	*nodD3* expressed under *trp* promoter in pTE3, IncP Tc^r	24

phage A3 promoter requires RNAP containing *B. subtilis* α and is not supported by *E. coli* RNAP (43). In both cases, the species specificity of the α-activator contact was mapped to the αCTD (40, 43). Interestingly, *Bordetella pertussis* α reconstituted into *E. coli* RNAP does not support transcription at the *E. coli* CAP-dependent *lac* promoter (58), suggesting that different activator-α specificities may exist, despite striking sequence homologies in the αCTDs of *B. pertussis* and *E. coli*.

Our ultimate goal is to understand how α interacts with transcription factors to initiate transcription at *S. meliloti* promoters. In this paper we describe the cloning and characterization of the *S. meliloti* RNAP α subunit. Furthermore, we establish that *S. meliloti* α can functionally replace *E. coli* α in vivo and that S. *meliloti* α reconstituted into *E. coli* RNAP holoenzyme can support both UP element- and Fis-dependent transcription in vitro. These results suggest that the study of transcription activation in *S. meliloti* may be facilitated by utilizing tools developed for *E. coli* RNAP.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains and plasmids used in this work are listed in Table 1. *S. meliloti* and *E. coli* were grown in Luria broth at 30 and 37°C, respectively, unless otherwise noted. Ampicillin (50 to 100 μg/ml) was used to select and maintain plasmids.

Plasmid construction. Plasmid constructions in *E. coli* were carried out as described previously (56). Full-length *rpoA* was amplified by PCR from pMP3 and cloned into pCR2.1-TOPO to create pMP16. The region of *rpoA* encoding amino acids 1 to 254 plus an added adjacent stop codon was amplified by PCR from pMP3 and cloned into pCR2.1-TOPO to create pMP18, encoding αΔ254. *Eco*RI fragments containing full-length *rpoA* from pMP16 or the coding sequence for αΔ254 from pMP18 were cloned into *Eco*RI-digested pBAD/HisB to create pMP19a and pMP21, respectively. PMP19a and pMP21 contain sequences encoding an additional 62 amino acids at the N terminus of the product of *rpoA* representing a six-His tag, an anti-Xpress epitope, an enterokinase cleavage site, and a Shine-Dalgarno-like sequence upstream of the *rpoA* product initiating methionine. *rpoA* encoding a full-length protein with a six-His tag was amplified by PCR from pMP19a and cloned into pCR2.1-TOPO to create pMP25. To create pMP22 an *Xba*I-*Eco*RV insert from pMP25 was cloned into pREIIα digested with *Bam*HI, filled in with the Klenow fragment of DNA polymerase I, and digested with *Xba*I.

DNA manipulation, sequencing, and sequence analysis. Plasmid DNA was isolated with the Miniprep kit (Qiagen, Inc.) or the Wizard DNA plasmid kit (Promega) or by CsCl banding. Genomic *S. meliloti* DNA was isolated with the Puregene DNA isolation kit (Gentra Systems).

Generation of λ-ZAP phage lysates and infection of XL1-Blue MRA DNA were performed as described previously (56). To purify phage DNA, lysed *E. coli* cultures were centrifuged for 5 min at 5,000 × *g*. Supernatants were incubated at 37°C for 1 h after addition of 1 μg each of pancreatic DNase I and RNase H (Sigma)/ml and centrifuged for 5 min at 5,000 × *g*, and then they were recentrifuged for 90 min at 100,000 × *g*. Pellets were resuspended in a solution containing 500 μl of 50 mM Tris-HCl, pH 8.0, and 500 μl of phenol and incubated (with vortexing every 5 min) for 15 min at room temperature to lyse the phage. They were extracted twice with phenol-$CHCl_3$ and once with $CHCl_3$, and the phage DNA was precipitated with ethanol, air dried, and resuspended in 200 μl of H_20. Alternatively, λ-ZAP DNA was purified with the Qiagen phage purification kit.

Restriction digestions were performed according to manufacturer's directions. DNA was gel purified with the Gene-Clean kit (Bio 101) or the Qiaex II kit (Qiagen, Inc.). Nucleotide analysis of both strands was by fluorescence sequencing with an Applied Biosystems Prism 310. The DNA sequences were assembled with the Sequencher, version 3.0, computer program (Gene Codes Corporation). Alignment of nucleotide sequences was performed with the Lasergene computer program (DNASTAR Inc.). Database searches were performed with BLAST (2).

Purification of RNAP from *S. meliloti*. *S. meliloti* RNAP was purified by the method of Burgess and Jendrisak with the following modifications (12). JM57/pRmE65 cell paste, frozen at −70°C, was the source of *S. meliloti* RNAP. Cells were broken by passage through a French press at 10,000 to 14,000 lb/in^2 in lieu of lysozyme-sodium deoxycholate lysis. A cocktail of protease inhibitors (3 μM chymotrypsin, 16 μM leupeptin, 3.6 μM pepstatin, and 130 μM phenylmethylsulfonyl fluoride [final concentrations]) was added to the crude extract. A DNA-agarose column prepared by the method of Schaller et al. (57) was used in place of DNA-cellulose. Bio-Gel A-1.5m was substituted for Bio-Gel A-5m (41). RNAP activity on a pUC derivative containing the cloned *Salmonella enterica* serovar Typhimurium *trp* promoter was assayed (18). Pooled active fractions were dialyzed against buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA, 0.1 mM

dithiothreitol) plus 50% glycerol. Proteins were analyzed by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis (SDS–12.5% PAGE) and visualized with Coomassie blue R250 (Sigma).

Isolation of *rpoA* and the surrounding region. Full-length RNAP α or peptides obtained from digestion of α with *Staphylococcus aureus* V8 protease in the presence of SDS (17) were isolated on an SDS-polyacrylamide gel, electroblotted to an Immobilon P membrane, and subjected to Edman degradation to obtain the N-terminal sequence (Protein and Nucleic Acids Facility, Stanford University). Degenerate primers based on the polypeptide sequence (rpoA 5′2, rpoA 5′3, rpoA 7, and rpoA 8) were used to obtain 400- and 800-bp PCR fragments containing part of the *S. meliloti rpoA* open reading frame (ORF). Primer and peptide sequences are available from the authors upon request. A wild-type *S. meliloti* genomic λ-library (63) was screened with the 800-bp PCR fragment to isolate three independent (nonsibling) λ-clones that contain *rpoA* and flanking genes. Southern blot analysis of the λ-clones identified a 2.2-kb *Bam*HI fragment containing *rpoA*, which was cloned into pBluescript SK(−) to create pMP1. To obtain the sequence of the *Bam*HI fragment, deletion fragments of *Eco*RV- and *Kpn*I-digested pMP1 were generated with exonuclease III as described previously (56) and sequenced. A primer (5′-S11) based on the 5′ sequence of the 2.2-kb *Bam*HI insert was used in Southern blot analysis to identify a 3.3-kb *Hin*dIII fragment, and this fragment was cloned into pBluescript SK(−) to create pMP2, which contained DNA upstream of *rpoA*. To obtain additional upstream sequence, we used chromosome walking. Library screening and Southern blot analysis were performed using digoxigenin (DIG) detection according to the manufacturer's directions (Boehringer Mannheim).

Purification of RNAP α. A 250-ml culture of DH5α containing pMP21 and a 150-ml culture of DH5α containing pMP19a were induced at an A_{600} of 0.4 with 0.002% arabinose for 3 and 4 h, respectively. Cells were harvested by centrifugation (5 min at 5,000 × *g*), resuspended in lysis buffer according to manufacturer's directions (Qiagen, Inc.), and broken by three passages through a BioNeb cell and DNA disrupter (Glas-Col). Six-His-tagged α and αΔ254 were purified on Ni^{+2}-nitrilotriacetic acid Superflow in batch under native and denaturing conditions, respectively, according to the manufacturer's directions (Qiagen, Inc.). Protein fractions were concentrated in an Ultrafree-NMWL 30K centrifugal filter (Millipore) and stored with 50% glycerol (25). *E. coli* six-His-tagged α was purified under denaturing conditions on a Ni^{+2} spin column according to the manufacturer's directions (Qiagen, Inc.). Protein concentrations were determined with the Bio-Rad protein assay. Samples were fractionated by SDS–12.5% PAGE and visualized with Coomassie blue 250 (Sigma).

Reconstitution of RNAP and in vitro transcription. Overexpression, purification, and reconstitution of RNAP holoenzymes were performed as described previously (61). *E. coli* α was replaced with purified *S. meliloti* six-His-tagged wild-type α or αΔ254. The activities of the wild-type and hybrid holoenzymes were normalized on the RNA *I* and *lacUV5* (pRLG 593) promoters. Reaction mixtures contained supercoiled plasmid DNA (20 ng); 10 mM Tris-Cl (pH 7.9); 10 mM $MgCl_2$; 1 mM dithiothreitol; 100 μg of bovine serum albumin/ml; 200 μM ATP, GTP, and CTP; 10 μM UTP; 4 μCi of [^{32}P]UTP (NEN); and 100 mM KCl. Fis-dependent transcription was measured in the same buffer but containing 170 mM KCl. Fis (200 nM final concentration) was added 15 min before addition of RNAP. Transcription was initiated by addition of RNAP and terminated by addition of stop solution (53) after 15 min at 22°C. Final RNAP concentrations in reaction mixtures were 16 (*E. coli* RNAP), 11 (*E. coli* RNAP with *S. meliloti* α), 26 (*E. coli* RNAP with *S. meliloti* α Δ254), and 7 nM (*S. meliloti* RNAP). Samples were electrophoresed on 5.5% polyacrylamide–7 M urea gels and quantified by phosphorimaging.

Nucleotide sequence accession number. The nucleotide sequence of the *rpoA* region has been deposited in the GenBank database under accession no. AF317474.

RESULTS AND DISCUSSION

Isolation of *rpoA* and surrounding macromolecular-synthesis genes. Oligonucleotides based on the N-terminal sequences of peptides generated from *S. meliloti* α were used to obtain PCR fragments containing the partial ORF of *S. meliloti rpoA*. The PCR fragments were used to screen a wild-type *S. meliloti* λ-library for clones containing *rpoA* and surrounding genes. DNA sequencing and BLAST analysis of these clones yielded ORFs that have significant homology to the genes for SecY (*secY*), adenylate kinase (*adk*), ribosomal protein S13 (*rpsM*), ribosomal protein S11 (*rpsK*), RNAP α (*rpoA*), and ribosomal protein L17 (*rplQ*) (Fig. 1). Subsequent sequence determination of the *S. meliloti* chromosome confirmed our data (14, 27).

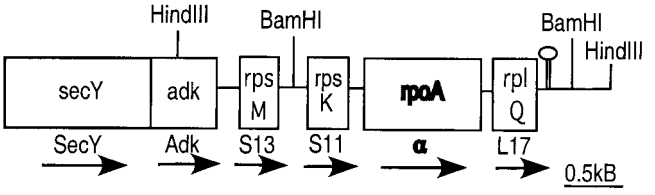

FIG. 1. Genetic organization of the *rpoA* region in *S. meliloti*. Sites for restriction enzymes mentioned in Materials and Methods are indicated. ORFs, direction of transcription, and protein names are indicated. The stem-loop structure indicates a proposed transcriptional terminator.

The genetic organization in the immediate area of *rpoA* is partially conserved among several bacterial species. *rpsD*, which encodes the S4 protein, is present immediately upstream of *rpoA* in *Bordetella pertussis*, *Pseudomonas aeruginosa*, and *E. coli* and has been implicated in translational regulation of the operon that includes *rpoA* (8, 15, 16, 59). However, *rpsD* is not part of this operon in *S. meliloti*, *B. subtilis*, *Mesorhizobium loti*, or *Rickettsia prowazekii* (3, 11, 37), indicating that S4 may not regulate this operon in these species. The second gene in this region in *S. meliloti*, *adk*, which encodes adenylate kinase, is not found in the *rpoA* region in most bacterial species for which this region has been sequenced (30, 31, 49). Based both on comparisons with the DNA sequences for *E. coli* and *B. subtilis* and on the lack of a predicted transcriptional terminator between any of the genes we sequenced, we hypothesize that at least some *S. meliloti rpoA* transcription must be initiated from a promoter upstream of *secY*, which is part of the *spc* operon (16, 60) and which is probably cotranscribed with ribosomal protein genes. Finally, we suggest that the gene coding for L17 is the last gene in the operon, because a predicted rho-independent terminator lies 23 nucleotides downstream of the L17 gene stop codon (Fig. 1). The next predicted ORF, which lies 2.4 kb downstream, encodes a putative serine protease and is not found in the *rpoA* regions of other bacteria (27).

Deduced amino acid sequence of α. Figure 2 illustrates the sequences of the α subunits from *S. meliloti* and *E. coli*. The sequence comparison indicates that the α-domain structure is conserved. RNAP αNTD residues 45, 48, and 80, which are involved in the interaction with the β subunit, and residues 86, 173, 180, and 200, which are involved in the interaction with β′, are all conserved between *E. coli* and *S. meliloti* (33). In addition, the amino acids in the αCTD essential for UP element recognition and for the interaction with transcriptional activator Fis are conserved between *E. coli* and *S. meliloti* (1, 25, 42).

Purification of α. To facilitate in vitro transcription studies, we cloned the *rpoA* ORF into a vector containing the *E. coli* arabinose promoter. This vector adds 62 amino acids including a hexahistidine tag to the NH_2 terminus of *S. meliloti* α. Previous studies have established that the N-terminal domain of *E. coli* α forms a stable polypeptide capable of assembling into functional RNAP (32, 34) but defective in recognizing the UP element and in interacting with certain transcriptional activators. Therefore, we also constructed a C-terminally truncated

```
E.c.:    1 M-QGSVTEFLKPRLVDIEQVSSTHAKVTLEPLERGFGHTLGNALRRILLSSMPGCAVTEVE 60
           M Q+   E +KP  V+      T A  + EPLERGFG TLGNALRR+LLSS +G AVT V+
S.m.:    1 MIQKNWQELIKPNKVEFASSGRTKATLVAEPLERGFGLTLGNALRRVLLSSLRGAAVTAVQ 61

E.c.:   61 IDGVLHEYSTKEGVQEDILEILLNLKGLAVRVQGKDEVILTLNKSGIGPVTAADITHDGD 120
           IDGVLHE+S+  GV+ED+ +I+LN+K +A+++ G D   + + K G G VTA DI   GD
S.m.:   62 IDGVLHEFSSIPGVREDVTDIVLNIKEIAIKMDGDDAKRMVVRKQGPGVVTAGDIQTVGD 121

E.c.:  121 VEIVKPQHVICHLTDENASISMRIKVQRGRGYVPASTRIHSEEDERPIGRLLVDACYSPV 180
           +EI+ P HVIC L DE A I M   V  G+GYVPA     + ++ PIG + VD+ YSPV
S.m.:  122 IEILNPNHVICTL-DEGAEIRMEFTVNNGKGYVPAD---RNRSEDAPIGLIPVDSLYSPV 177
                                                          ~~~~~
E.c.:  181 ERIAYNVEAARVEQRTDLDKLVIEMETNGTIDPEEAIRRAATILAEQLEAFVDLRDVRQP 240
           ++++Y VE  R  Q  D DKL + +ET+G++  E+AI  AA IL +QL  FV+  D  Q
S.m.:  178 KKVSYKVENTREGQVLDYDKLTMSIETDGSVTGEDAIAFAARILQDQLSVFVNF-DEPQK 236
           ~~~~~   ~~~
E.c.:  241 EVKEE---KPEFDPILLRPVDDLELTVRSANCLKAEAIHYIGDLVQRTEVELLKTPNLGK 297
           E +EE   +  F+P LL+ VD+LEL+VRSANCLK + I YIGDL+Q+TE E+L+TPN G+
S.m.:  237 ETEEEAVTELAFNPALLKKVDELELSVRSANCLKNDNIVYIGDLIQKTEAEMLRTPNFGR 296
                              -
E.c.:  298 KSLTEIKDVLASRGLSLGMRLENWPPASIADE          329
           KSL EIK+VLAS GL LGM + +WPP +I D
S.m.:  297 KSLNEIKEVLASMGLHLGMEVPSWPPENIEDLAKRYEDQY 336
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FIG. 2. Comparison of amino acid sequence of *S. meliloti* (S.m.) RNAP α with that from *E. coli* (E.c.) (49). Dashes, gaps introduced for alignment; ~, amino acids in the linker region; −, position of the αΔ254 deletion. Amino acid residues identical in *S. meliloti* α and *E. coli* α are indicated between the two sequences; +, conservative replacements. Amino acids essential for the αCTD interaction with the UP element are in boldface, and amino acids essential for interaction with Fis are underlined.

version of the product of *S. meliloti rpoA* in which amino acids 1 to 254 were removed (αΔ254). After induction by arabinose, six-His-tagged α and αΔ254 were purified (see Materials and Methods; Fig. 3A, lanes 1 and 2). For purposes of comparison, we also purified six-His-tagged *E. coli* α (61) (Fig. 3A, lane 3). Six-His-tagged *S. meliloti* α has an apparent molecular mass of 46 kDa, and untagged *S. meliloti* α has an apparent molecular mass of 43 kDa, both larger than the predicted molecular mass of 37.2 kDa and larger than six-His-tagged *E. coli* α (Fig. 3)

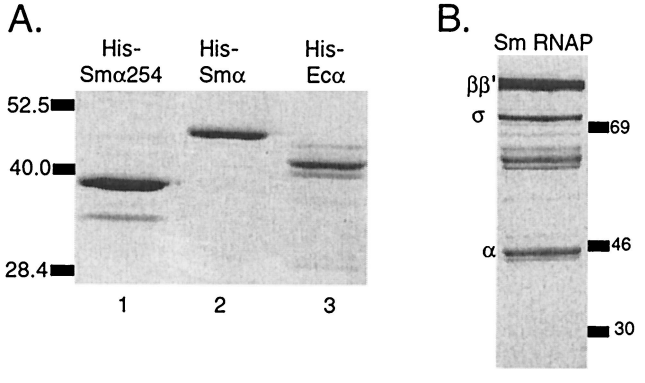

FIG. 3. Analysis of six-His-tagged purified α-subunits resolved by SDS-PAGE and stained with Coomassie blue R250. (A) *S. meliloti* (Sm) αΔ254 purified in batch on Ni^{+2} resin under native conditions from cells containing pMP21 (lane 1), six-His-tagged *S. meliloti* α purified in batch on Ni^{+2} resin under denaturing conditions from cells containing pMP19a (lane 2), and six-His-tagged *E. coli* α purified under denaturing conditions on a Ni^{+2} spin column from cells containing pHTT7α (lane 3). Six-His-tagged *S. meliloti* α has a larger molecular mass than untagged α due to the addition of 62 amino acids at the N terminus of the six-His-tagged protein (see Materials and Methods). (B) RNAP isolated from *S. meliloti*. Size markers are in kilodaltons.

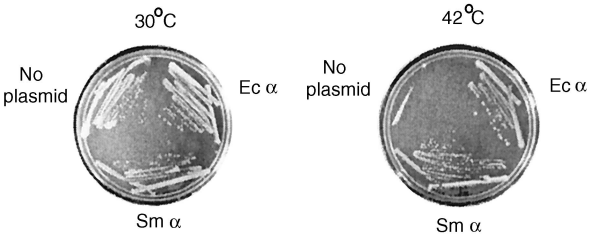

FIG. 4. Complementation of the *E. coli rpoA* mutant with *S. meliloti rpoA*. Strains carrying *rpoA112*(Ts) either alone or with plasmids coding for *E. coli* (Ec) α or *S. meliloti* (Sm) α were streaked on Luria-Bertani agar and grown for 24 h at the indicated temperatures.

(23). The purified polypeptides cross-reacted with an antibody to *E. coli* α (data not shown).

***S. meliloti* α assembles into active RNAP in *E. coli*.** To facilitate an understanding of the role of the αCTD in transcription activation of *S. meliloti* RNAP, we cloned versions of *S. meliloti rpoA* encoding full-length and C-terminally truncated (αΔ254) proteins into a broad-host-range vector for examination in *S. meliloti*. Interestingly, while the full-length clone could be introduced into *S. meliloti*, αΔ254 was deleterious to cell growth (data not shown). Therefore, we utilized *E. coli* as a heterologous system to examine transcription by *S. meliloti* RNAP in vitro. First, we showed that *S. meliloti rpoA* functions in *E. coli* by demonstrating that it complements an *E. coli rpoA* temperature sensitive mutation for growth because of a defect in RNAP assembly (38). Specifically, we transformed *E. coli rpoA112*(Ts) with a plasmid constitutively expressing six-His-tagged *S. meliloti* α or wild-type *E. coli* α (Fig. 4). We observed growth of all of the strains at the permissive temperature, 30°C. At 42°C, the nonpermissive temperature, the strains expressing *S. meliloti* or *E. coli* α were viable, even though the two proteins are only 51% identical, while the strain with only the host-encoded α was not viable. We note that while the temperature-sensitive *E. coli* mutant expressing *S. meliloti* α plated with high efficiency, forming independent single colonies on plates, it formed smaller colonies than the cells containing the plasmid encoding wild-type α. We conclude that *S. meliloti* α can assemble into a fully functional holoenzyme with the other *E. coli* RNAP subunits and is able to replace *E. coli* α for all functions essential for viability.

***S. meliloti* α assembles into functional RNAP in vitro.** We next reconstituted RNAP from *S. meliloti* α and *E. coli* β, β′, and σ in order to address whether hybrid RNAP containing only *S. meliloti* α subunits is functional and to study transcription by RNAP containing *S. meliloti* α in vitro. Recovery of the active holoenzyme was completely dependent on exogenously added purified α (data not shown), indicating that potential contamination of the other *E. coli* subunits with *E. coli* α could not account for the observed transcription. Since the amount of *S. meliloti* α required for assembly into functional RNAP was approximately the same as for *E. coli* α (see Materials and Methods), the assembly determinants in *S. meliloti* α apparently are conserved in the two species. We assayed the reconstituted enzymes (and, as a control, *S. meliloti* RNAP holoenzyme) for their abilities to initiate transcription, to respond to an UP element, and to interact with well-characterized transcriptional activator Fis. Because previous studies of *E. coli*

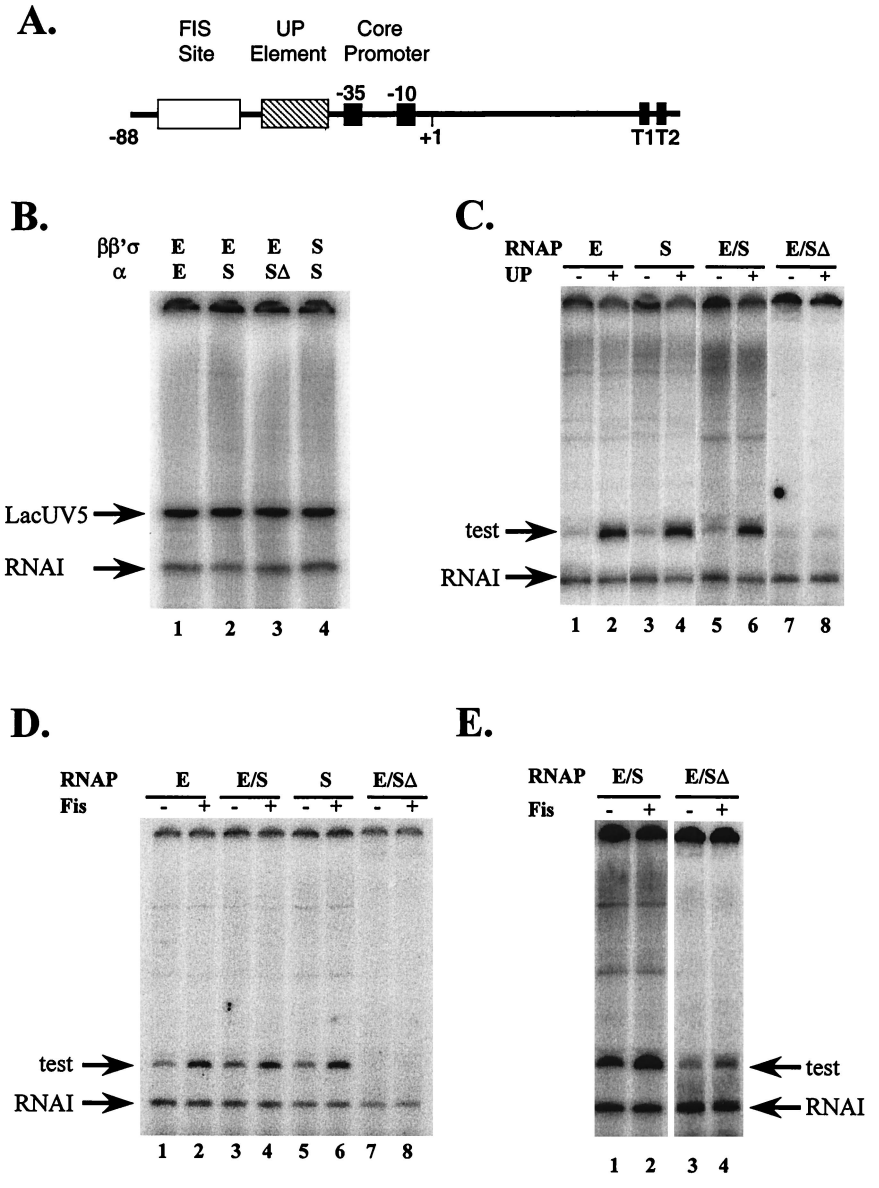

FIG. 5. In vitro transcription with reconstituted *E. coli* (E), *E. coli* containing either six-His-tagged *S. meliloti* α (E/S), or αΔ254 (E/SΔ), or *S. meliloti* RNAP (S). (A) *rrnB* P1 promoter used for in vitro transcription studies contains the Fis binding site, UP element, and the core −10 and −35 elements. Insertion of promoter fragments into the plasmid vector gives rise to a 150-nucleotide RNA fragment terminating at the *rrnB* T1 and T2 terminators. (B) Normalization of *E. coli*, *S. meliloti*, and hybrid RNAPs on the *lacUV5* and *RNA* I promoters. *RNA* I is a control promoter from the plasmid vector. (C) *E. coli*, *S. meliloti*, and hybrid RNAPs activate UP element-mediated transcription. Templates carrying *rrnB* P1 promoters with (+) and without (−) the UP element were transcribed, and the products were analyzed by denaturing gel electrophoresis. (D) *E. coli*, *S. meliloti*, and hybrid RNAPs are activated by Fis. The template containing the *rrnB* P1 UP element and Fis site was incubated with the indicated RNAP with or without Fis (200 nM). (E) Activation by Fis depends on the αCTD. Lanes 1 and 2, RNAP containing intact *S. meliloti* α (E/S); lanes 3 and 4, RNAP containing αΔ254 (E/SΔ). Reactions were performed under conditions in which basal transcription could be detected in the absence of αCTD (see text).

have demonstrated that the αCTD is essential for both UP element function and Fis-mediated transcription (10, 21, 25, 53), we also tested RNAP reconstituted with *S. meliloti* αΔ254. As a control, we tested the activity of RNAP purified from *S. meliloti* in the same assays to address whether *S. meliloti* α in native RNAP functioned similarly to *S. meliloti* α in the hybrid RNAP. We normalized the activities of the RNAP preparations on the *lacUV5* and *RNA* I promoters, two promoters that do not require the function of the αCTD (Fig. 5B, lanes 1 to 4).

To test whether *S. meliloti* α supports UP element- and/or Fis-dependent transcription, we assayed transcription at the *E. coli rrnB* P1 promoter (Fig. 5A). The presence of the UP element stimulated transcription by *E. coli* RNAP containing *S. meliloti* α 10-fold (Fig. 5C, lanes 3 and 4) under conditions where transcription by the native *E. coli* and *S. meliloti* RNAPs was stimulated 14- and 13-fold, respectively (Fig. 5C, lanes 1, 2, 5, and 6). As observed previously with *E. coli* RNAP lacking the αCTD (53), transcription from the promoter containing

the UP element was extremely weak with the *S. meliloti* αΔ254 RNAP (Fig. 5C, lanes 7 and 8). Thus, *S. meliloti* αCTD contains the determinants required for recognition of the *rrnB* P1 UP element. Since *S. meliloti rrn* promoters contain sequences homologous to *E. coli* consensus UP elements (21, 26) and since the DNA-binding determinants in *E. coli* αCTD are present in *S. meliloti* αCTD, it is likely that the UP element-α interaction is conserved in *S. meliloti*. Reconstituted *E. coli* RNAP, *S. meliloti* RNAP, and the hybrid RNAP carrying *S. meliloti* α were stimulated by Fis 5.1-, 4.8-, and 3.2-fold, respectively (Fig. 5D, lanes 1 to 6). The RNAP reconstituted with *S. meliloti* αΔ254 was less active than the other RNAP preparations under the high-salt conditions (170 mM KCl) used in this experiment (Fig. 5D, lanes 7 and 8), making it difficult to evaluate whether Fis can or cannot activate the enzyme lacking the αCTD. Therefore, the effect of Fis on the RNAP containing *S. meliloti* αΔ254 was also compared with that of the enzyme containing the full-length α under slightly more permissive conditions (100 mM KCl), where basal transcription is visible. The mutant RNAP was stimulated only 1.4-fold (Fig. 5E, lanes 3 and 4), while RNAP containing the intact *S. meliloti* α was stimulated 3.3-fold (Fig. 5E, lanes 1 and 2). Thus, we conclude that *S. meliloti* α interacts with Fis and that, as in *E. coli* (10), the interaction is mediated primarily by the αCTD.

These results demonstrate that *S. meliloti* α is competent for assembly with *E. coli* β, β′, and σ^{70}; for UP element recognition; and for recognition by a transcription factor. We showed above that *S. meliloti* α complements *E. coli* α in vivo. Our results with the hybrid enzymes, formed in vitro in the absence of *E. coli* α, support the conclusion that complementation in vivo resulted from the function of *S. meliloti* α.

Summary. We have isolated the region from *S. meliloti* encoding proteins SecY, Adk, S13, S11, α, and L17 and overexpressed and purified *S. meliloti* α and reconstituted it with the β, β′, and σ^{70} subunits from *E. coli*. In vivo assays of *E. coli* and in vitro assays using purified *S. meliloti* α demonstrate that *S. meliloti* α retains its critical functions as a hybrid enzyme with other *E. coli* RNAP subunits. The cloning and characterization of *S. meliloti* RNAP α thus provide new opportunities for studying *S. meliloti* transcription.

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