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Partial Purification of Factors for Differential Transcription of the *rrnD* Promoters for Ribosomal RNA Synthesis in *Streptomyces coelicolor*

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The *Streptomyces coelicolor* A3(2) genome contains six operons (*rrnA* to *F*) for ribosomal RNA synthesis. Transcription from *rrnD* occurs from four promoters (p1 to p4). We found that transcripts from the p1 and p3 promoters were most abundant *in vivo* in the early exponential phase. However, at later phases of exponential and stationary growth, transcripts from the p1 promoter decreased drastically, with the p3 and p4 transcripts constituting the major forms. Partially purified RNA polymerase supported transcription from the p3 and p4 promoters, whereas pure reconstituted RNA polymerase with core enzyme (E) and the major vegetative sigma factor σ^{HrdB} ($E{\cdot}\sigma^{HrdB}$) did not. In order to assess any potential requirement for additional factor(s) that allow transcription from the p3 and p4 promoters, we fractionated a partially purified RNA polymerase preparation by denaturing gel filtration chromatography. We found that transcription from the p3 and p4 promoters required factor(s) of about 30-35 kDa in addition to RNAP holoenzyme ($E{\cdot}\sigma^{HrdB}$). Therefore, transcription from the p3 and p4 promoters, which contain a consensus -10 region but no -35 for σ^{HrdB} recognition, are likely to be regulated by transcription factor(s) that modulate RNA polymerase holoenzyme activity in *S. coelicolor*.

Keywords: ribosomal RNA, RNA polymerase, transcription, reconstitution

Streptomyces coelicolor is a Gram-positive soil bacterium that shows a complex differentiation process that includes filamentous vegetative growth, aerial hyphae formation, sporulation, and production of antibiotics through secondary metabolic pathways. It possesses linear chromosomal DNA of about 8.7 Mb, with a high GC content (72.1%), and six operons for ribosomal RNA (*rrn*) synthesis (Bentley *et al.*, 2002). The *rrn* operons are distributed in the central core region of the chromosome, extending from around 1.5 Mb to 6.4 Mb. All of these operons have the typical bacterial organization of 16S-23S-5S rRNA, with no tRNA genes found in the 16S-23S rRNA spaces (Baylis and Bibb, 1988a). Rapidly dividing *Escherichia coli* contains seven *rrn* operons (Kiss *et al.*, 1977), of which four operons, *rrnA*, *rrnB*, *rrnC*, and *rrnE*, are located adjacent to *oriC* (Condon *et al.*, 1992). Several different tRNA genes are found in the spacer region between the 16S and 23S rRNAs in *E. coli*. *Mycobacterium tuberculosis*, a slow-growing pathogen, has a single *rrn* operon (Kempsell *et al.*, 1992; Cole *et al.*, 1998), which is oddly located about 1.5 Mb from the putative *oriC* (Cole *et al.*, 1998). The single *rrn* operon and its location may be related to the slow growth of *M. tuberculosis* (Cole and Saint Girons, 1994).

The control of rRNA synthesis is a complex process that is fine-tuned to the cellular requirement for ribosomes and the balanced synthesis of ribosomal components (Nomura *et al.*, 1984; Lindahl and Zengel, 1986). Most of this control occurs at the level of the initiation of transcription. The regulation of rRNA transcription has been characterized best in *E. coli* (Kiss *et al.*, 1977), where each of seven rRNA operons have two promoters, P1 and P2 (Cole and Saint Girons, 1994; Condon *et al.*, 1995). These promoters show a high level of similarity to the σ^{70}-dependent promoter consensus sequence in the -10 and -35 hexamers, which are separated by 16 bp. Stringent, growth rate-dependent controls act at the P1 promoters, while the P2 promoters appear to be transcribed constitutively at a low level (Sarmientos and Cashel, 1983; Gourse *et al.*, 1996). The P1 promoters contain another RNAP recognition element, positioned at -57 to -41 with respect to the transcription start site. The element is referred to as the UP element, which interacts with two α subunits of RNAP and stimulates transcription of *rrn* operons 20- to 50-fold *in vivo*. In addition, the FIS transcription factor increases transcription 3- to 8-fold by binding to sites upstream of the UP element (Ross *et al.*, 1990; Hirvonen *et al.*, 2001). Thus, the unusual strength of *rrn* P1 promoters in *E. coli* results from the presence of UP elements and FIS. In contrast, several protein factors are known to act as negative regulators of rRNA transcription. The H-NS DNA-binding protein cooperatively binds to the upstream sequences of *rrn* P1 and antagonizes FIS-mediated activation (Afflerbach *et al.*, 1998; Afflerbach *et al.*, 1999). DksA, GreA, and GreB bind to the secondary channel of RNAP and decrease the half-life of the RNAP- promoter complex (Potrykus *et al.*, 2006; Rutherford *et al.*, 2007). In *S. coelicolor*, the regulation of rRNA transcription is rela-

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tively less understood. The *rrnA* and *rrnD* operons in *S. coelicolor* contain four tandem promoters upstream of the 16S rRNA genes (Baylis and Bibb, 1988b; Van Wezel *et al.*, 1994). In contrast to *E. coli,* all four promoters of the *rrnD* operon appear to be subject to stringent control (Strauch *et al.*, 1991).

In this work, we compared the *in vivo* transcription pattern of *rrnD* promoters with that *in vitro*. The stationary phase-specific transcripts from the p3 and p4 promoters observed *in vivo* were synthesized *in vitro* only in the presence of additional factor(s) other than the RNA polymerase holoenzyme containing housekeeping sigma factor σ^{HrdB}. In the search for such factor(s), the partially purified RNA polymerase complex was denatured with 6 M urea, and the denatured proteins were fractionated according to molecular weights. When the fractionated and renatured proteins were added to holo RNAP containing σ^{HrdB}, certain fractions enabled transcription from the p3 and p4 promoters. We propose that, among the proteins co-purified with RNA polymerase, with a molecular mass in the range of about 30 to 35 kDa, a factor or factors exist that allow transcription from the p3 and p4 promoters *in vivo*.

Materials and Methods

Bacterial strains and culture conditions

Streptomyces coelicolor A3(2) strain M145 was grown in YEME medium (Hopwood *et al.*, 1985) containing 5 mM $MgCl_2$ and 10% sucrose. To isolate RNA polymerase, a freshly grown seed culture (200 ml) was inoculated in 4 liters of YEME broth in a 5 L fermenter, aerated at 0.5 volume air/volume media/min, and agitated at 250 rpm at 30°C. The mycelium was harvested from the fermenter at late exponential phase and stored at 70°C until use. *Escherichia coli* DH5α was used for all initial transformation of plasmids and propagation of plasmids. *E. coli* cells were grown in LB or TB supplemented with appropriate antibiotics.

S1 nuclease protection analysis of rrnD transcripts

RNA was isolated from *S. coelicolor* cells grown in YEME as described previously (Hopwood *et al.*, 1985). Cells were harvested at 12, 18, 36, and 60 h, and isolated RNA was quantified by measuring the O.D. at 260 and 280 nm. The probe for S1 mapping of the *rrnD* was prepared by PCR using the rrnD5 primer; 5'-CTGGCCTACGTCTACGTTCT-3' and rrnD3 primer; 5'-CGATCAGGTCGGGGTATCAA-3' from pIJ2820 containing a 1 kb *Nsp*HI fragment (Baylis and Bibb, 1988b). The *rrnD* plasmid was provided by Dr. M. Bibb of the John Innes Center. The PCR product was labeled with [γ-^{32}P] ATP and T4 polynucleotide kinase. The 532 bp probe was generated by digesting the labeled fragment with *Acc*I. The S1 nuclease protection assay was performed as described previously (Smith and Owen, 1991). The S1 signals were analyzed by autoradiography after running on a 7 M urea - 5% (w/v) polyacrylamide gel.

Purification of RNA polymerase from S. coelicolor

RNA polymerase was purified from cell pellets according to the procedures developed for the purification of *S. coelicolor* RNAP (Kang *et al.*, 1997; Hahn *et al.*, 2003). In brief, RNA polymerase was purified from about 20 g of *S. coelicolor* mycelium by Polymin P precipitation, salt extraction, and ammonium sulfate precipitation. The pellet of fractionated RNAP was dissolved in TE (10 mM Tris·HCl and 0.1 mM EDTA) and applied to a HeparinSepharose column. Further purification was performed by a Superdex 200 HR 10/30 column (Pharmacia) and a Mono-Q HR 5/5 FPLC anion exchange column (Pharmacia). The trailing fractions (eluates at ~0.50 M NaCl) of the RNAP peak eluted from the Mono-Q column were used as core RNAP.

Preparation of the rrnD DNA template

The PCR product that had been amplified by rrnD5 and rrnD3 primers was cloned into the *Sma*I site of pUC18 (Kang *et al.*, 1997). The 437 bp DNA fragment containing *rrnD* promoters was generated by digestion of the plasmid with *Acc*I and *Ava*I. The DNA fragment was eluted in DNA elution buffer (500 mM ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA; pH 8.0, 0.1% SDS) after separation on 5% polyacrylamide gel.

In vitro transcription assay

The run-off transcription assay was performed *in vitro* as described previously (Kang *et al.*, 1997). In brief, 1.5 pmol of RNAP was incubated at 30°C for 5 min with 0.15 pmol of *rrnD* template DNA in transcription buffer. RNA synthesis was initiated by the addition of NTP mix containing 2 μCi [α-^{32}P] CTP (400 Ci/mmole) and heparin. Transcripts were analyzed by autoradiography after separation on 5% polyacrylamide gel containing 7 M urea.

Fractionation and reconstitution of RNAP holoenzyme complex through denaturing gel chromatography

The RNAP complex that had been partially purified by Superdex HR 200 chromatography was incubated in a denaturation buffer [50 mM Tris·HCl (pH 8.0 at 4°C), 1 mM EDTA, 10 mM DTT, 0.2 M KCl, 10 mM $MgCl_2$, 6 M Urea] at 37°C for 30 min and re-applied to a Superdex HR 200 column. Proteins were eluted by TG_5ED [10 mM Tris·HCl (pH 7.9 at 4°C), 0.1 mM EDTA, 0.1 mM DTT, 10% glycerol] buffer containing 0.3 M NaCl and 4.5 M urea. The eluted proteins containing urea were allowed to renature through the dialysis of urea against renaturation buffer [20 mM Tris·HCl (pH 7.8 at 4°C), 10 mM $MgCl_2$, 10 mM KCl, 0.1 mM EDTA, 50% (v/v) glycerol] for 12-16 h with one change of the buffer. Seven microliter of the renatured protein sample was then added to 1 pmol of core or reconstituted holo RNAP with σ^{HrdB}, and the mixtures were incubated on ice for 10 min. After the addition of 0.2 pmol of *rrnD* template DNA, the mixtures were incubated at 30°C for 30 min and subjected to an *in vitro* transcription assay as described above.

Results and Discussion

Growth phase-dependent expression of the rrnD promoters

The *Streptomyces coelicolor rrnD* operon is one of the six *rrn* operons that encode ribosomal RNAs arranged in the order of 16S-23S-5S; it is located about 1 Mb from *oriC* in the chromosome. It is transcribed from four promoters, located within an approximately 240 bp intergenic region between

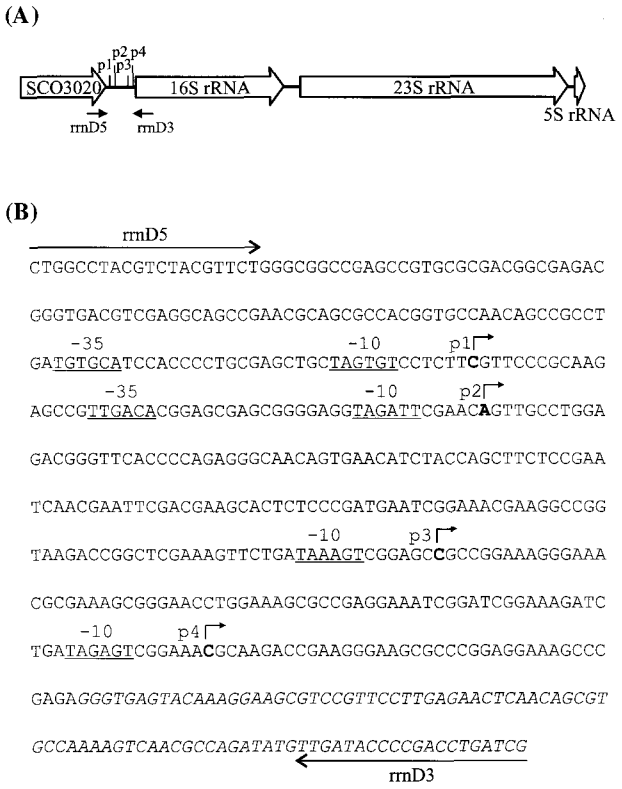

Fig. 1. The structure of the *rrnD* operon in *S. coelicolor*. (A) Genetic organization of the *rrnD* operon. Four transcription start sites of the *rrnD* operon are shown by bars. The positions of primers used for PCR amplification of the *rrnD* upstream region are indicated by arrows. (B) Nucleotide sequence of the *rrnD* upstream region. Transcriptional start sites are indicated by bent arrows (Baylis and Bibb, 1988b). Consensus-like -35 and -10 sequences that can be recognized by σ^{HrdB} are underlined. The sequence of 16S rRNA is indicated in italics. PCR primer sequences, rrnD5 and rrnD3, are indicated by arrows.

SCO3020, encoding a putative integral membrane protein, and the 16S rRNA gene (Fig. 1A; Baylis and Bibb, 1988b). The nucleotide sequence of the *rrnD* promoter region is shown in Fig. 1B. S1 mapping analysis was carried out to investigate the growth phase-dependent transcript profile of the *rrnD* promoters *in vivo*. *S. coelicolor* M145 cells were harvested at various phases of growth in YEME medium: 12 h (early exponential), 18 h (late exponential), 36 h (stationary), and 60 h (late stationary phase) (Fig. 2A). In the early exponential phase, p1 transcripts were most abundant, with slightly lower amounts of p3 transcripts. From the late exponential to stationary phases, the level of p1 transcript was very low, while that of the p3 transcript was relatively unchanged (Fig. 2B). Transcripts from the p4 promoter were barely detected at early exponential phase, and appeared at the late exponential through stationary phases. Our observation is consistent with previous studies (Strauch *et al.*, 1991), which indicated that p3 and p4 are stronger promoters than p1 and p2 in exponential phase. We found that these promoters also persist as the major promoters in the stationary phase.

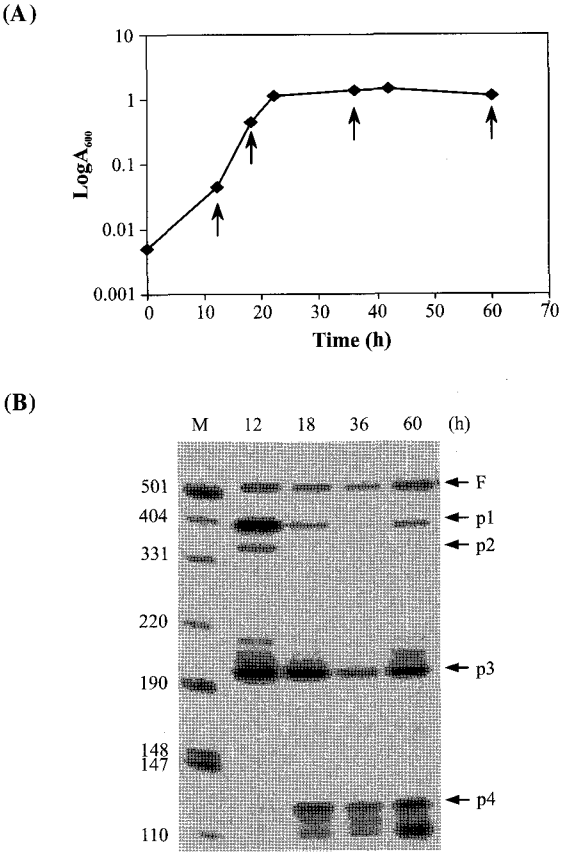

Fig. 2. Transcript profile from *rrnD* promoters *in vivo* during the growth of *S. coelicolor*. (A) *S. coelicolor* cells were grown in liquid YEME media containing 10.3% sucrose, and growth was monitored by measurement of the O.D. at 600 nm. Cells were harvested for RNA preparation at 12, 18, 36, and 60 h, as indicated by arrows. (B) S1 mapping analysis of *rrnD* transcripts at various growth phases of *S. coelicolor*. RNAs were isolated as described in Materials and Methods. The PCR product generated by rrnD5 and rrnD3 primers was labeled with [γ-^{32}P]ATP uniquely at the 5' end and used as a probe for the S1 mapping of the *rrnD* gene. F represents full-length protection of the probe. Promoter-specific transcripts are indicated by arrows.

rrnD p1 TGA**TGTGCA**TCCACCCCTGCGAGCTGC**TAGTGT**CCTCTT***C***
rrnD p2 GCCG**TTGACA**CGGAGCGAGCGGGGAGG**TAGATT**CGAAC***A***G
rrnD P3 CCGGTAAGACCGGCTCGAAAGTTCTGA**TAAAGT**CGGAGC***C***
rrnD p4 CGAGGAAATCCGGATCGGAAAGATCTGA**TAGAGT**CGGAAA***C***
σ^{HrdB} consensus **TTGaCA** (N17-18) **TAgaaT**

Fig. 3. Comparison of the *rrnD* promoter sequences. Putative -35 and -10 sequences are shown in bold letters, and transcription start sites are noted in bold italic letters. Identical sequences between the *rrnD* p3 and p4 promoters are underlined. The consensus promoter sequences for σ^{HrdB} recognition are presented in bold letters.

Sequence comparison of the rrnD promoters

The nucleotide sequences of the four *rrnD* promoters were compared with the consensus promoter sequence recognized by σ^{HrdB}, the principal sigma factor in *S. coelicolor* (Fig. 3). All of the *rrnD* promoters contain the consensus-like -10

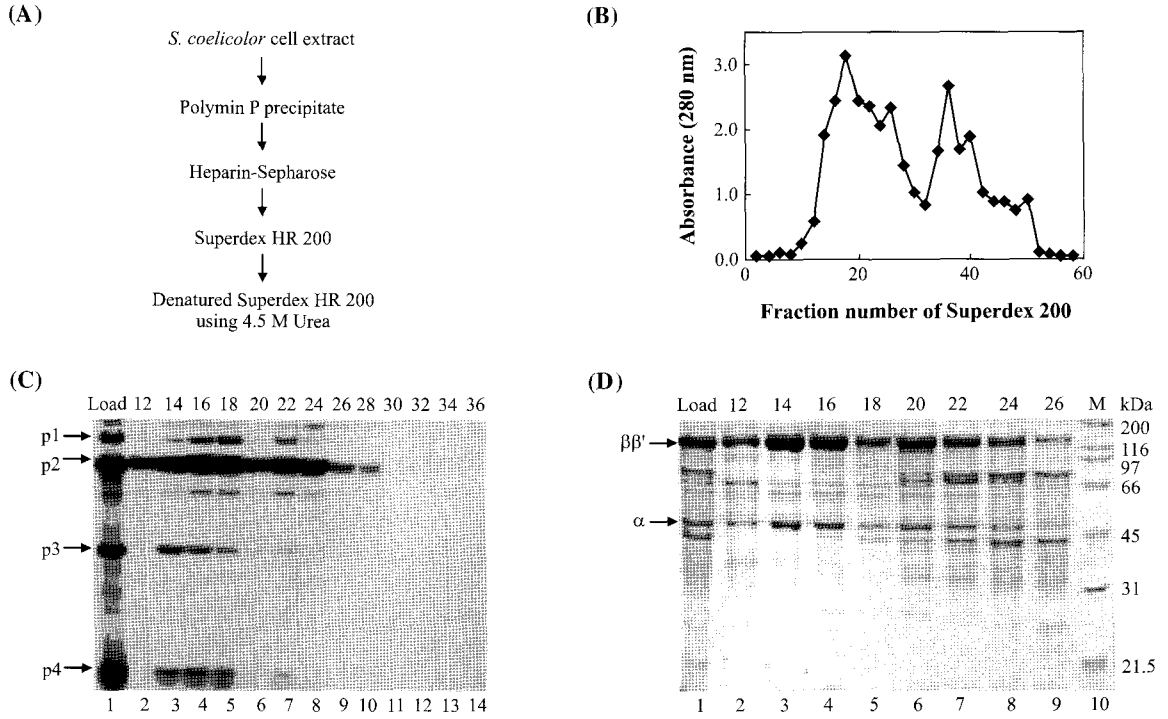

Fig. 4. Transcription of *rrnD* promoters *in vitro*. (A) Schematic procedure for the isolation of factors necessary for transcribing *rrnD* p3 and p4 promoters. (B) Elution profile from the Superdex 200 column. RNAP fractions partially purified by heparin-Sepharose column were loaded on the Superdex 200 and the eluates after an elution volume of 5 ml was collected. (C) *In vitro* transcription assay with fractions purified from the Superdex 200. Transcripts from the p1 to p4 promoters are indicated by arrows. The transcription reaction contained 2 μl aliquots of eluted fractions 12-36 (lanes 2 to 14) from the Superdex 200 column, and 2 μl of crude RNAP sample (0.3 mg/ml) obtained from the heparin-Sepharose column prior to loading on the Superdex 200 column (lane 1, load). (D) Protein profile of RNAP fractions that exhibit transcriptional activity in (C). Ten μg proteins in the loaded sample (lane 1) and Superdex-eluted fractions (12 to 26; lanes 2-9) were analyzed on a 0.1% SDS-10% polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. The positions for RNAP subunits (β, β', and α) are indicated.

sequences, but show some difference in the nucleotide sequences of their -35 regions. The p2 promoter contains the highest matching consensus sequence of the -35 hexamer with a spacing of 17 bp, whereas the p1 promoter shows relatively lower homology in the -35 region with a spacing of 18 bp. In the p3 and p4 promoters, no sequences matching the consensus -35 hexamer are found upstream of the TA-rich -10 box. Close examination of the p3 and p4 promoter sequences revealed that they are quite similar. These facts suggest that the p1 and p2 promoters could be transcribed by σ^{HrdB}, while the p3 and p4 promoters might be recognized by an alternate sigma factor other than σ^{HrdB} or an additional transcription factor in the presence of σ^{HrdB}.

In vitro transcription of the rrnD promoters

To isolate factors conferring differential transcription of *rrnD* promoters, the RNA polymerase complex was purified from *S. coelicolor* M145 cells that were grown to late exponential phase, as summarized in Fig. 4A. When an *in vitro* transcription assay was performed using RNAP that was partially purified using a heparin-Sepharose column (Fig. 4C, lane load), the transcription pattern of the *rrnD* promoters was different from the result of S1 mapping of *in vivo* transcripts (Fig. 2B). Compared with the *in vivo* pattern, the p2 transcripts were the most abundant, and the transcription from p1 appeared to be relatively weak *in vitro* with partially purified RNAP. We repeated the transcription assay using various RNAP prepared from different growth phases, but the results did not change (data not shown). Using a supercoiled *rrnD* template instead of the linear form did not change the transcript pattern either (data not shown). One possible explanation for our observation of different transcription of p1 and p2 promoters could be that the *in vivo* presence of an additional factor, which is lost during purification steps, allows efficient transcription from p1, and hence, suppresses transcription from the closely located downstream p2 promoter. In the absence of this factor, the p2 promoter with a highly matching consensus sequence for σ^{HrdB} will be actively engaged in transcription *in vitro*. Another explanation is that *in vivo* DNA conformation of *rrnD* p1 and p2 promoter upstream regions could be different from that occurring *in vitro*, and may block access of RNAP. In *E. coli*, all upstream rRNA fragments are known to exhibit intrinsic curvature and have different affinities for FIS and H-NS (Hillebrand *et al.*, 2005). A regulatory protein LRP co-purified with H-NS was recently shown to constrain supercoils by binding to rRNA p1 promoter upstream regions and inhibit the transcription of rRNA (Pul *et al.*, 2005; Pul *et al.*, 2007).

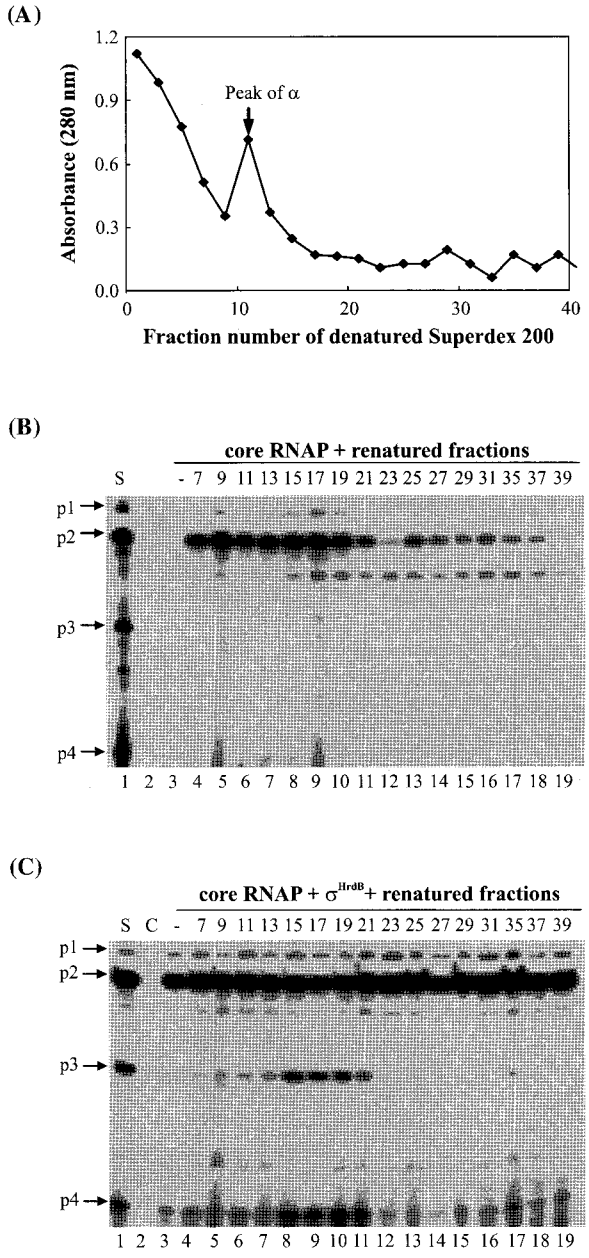

Fig. 5. Reconstitution of components that transcribe the p3 and p4 promoters *in vitro*. (A) Elution profile of p3/p4 transcribing fractions from the Superdex 200 column under denaturing conditions. The fractions (14-18) that supported transcription from the p3 and p4 promoters, as shown in Fig. 4, were incubated in a denaturation buffer containing 6 M urea and fractionated by a Superdex 200 column using 0.3 M NaCl TG_5ED buffer containing 4.5 M urea. (B) Transcription assay with reconstituted components of purified core RNAP plus renatured protein fractions. The denatured proteins that were fractionated through Superdex 200 were renatured and incubated with Mono-Q-purified core RNAP (lanes 4-19). Before loading on the denaturing gel, the p3/p4-active fractions were examined for transcribing activity (lane 1; S), along with core RNAP only (lane 3; -). (C) Transcription assay with reconstituted components of purified core RNAP, σ^{HrdB}, and renatured protein fractions. The pre-loaded RNAP preparation (S; lane 1), core RNAP (C; lane 2), and $E{\cdot}\sigma^{HrdB}$ holoenzyme alone without added fractions (-; lane 3) were examined in parallel.

Reconstitution of transcription from the rrnD p3 and p4 promoters

We observed that further purification through a Mono-Q column following heparin-Sepharose chromatography no longer allowed transcription from p3 and p4 promoters to occur *in vitro*. This finding made us suspect that there is some factor or factors necessary for p3 and p4 transcription, and that these potential factors are easily dissociated from the RNAP preparation through an anionic exchange column. Therefore, Superdex 200 gel permeation chromatography was carried out instead of purification using a Mono-Q column. The partially purified RNAP fractions from the heparin-Sepharose column were pooled and loaded on Superdex 200 column, the elution profile of which is shown in Fig. 4B. When each fraction was assayed for transcription activity for *rrnD* promoters *in vitro*, a differential pattern was observed (Fig. 4C, lanes 2-14). While the transcriptional activity of the p2 promoter stayed relatively strong from fractions 12 to 24, the p3 and p4 transcriptional activity was confined to fractions 14 to 18. The p3 transcription activity appeared to decrease gradually from fraction 14 to fraction 18. The protein profile in a 10 μg aliquot of each fraction that exhibits transcription activity was examined on SDS-polyacrylamide gel with Coomassie staining (Fig. 4D). We compared the protein profiles of fractions 14-18, which were responsible for the transcribing activities of p3 and p4, with those of fractions 20-26. All fractions contained RNA polymerase core subunits (ββ'α) and additional proteins. The additional protein band pattern was quite different. Although we were not able to identify individual proteins, it is quite likely that differences in the protein profile of each RNA polymerase fraction are responsible for the differential patterns observed in transcription from *rrnD* promoters.

We previously attempted to elute protein factors from the denaturing gel and renature them in the presence of GroEL (Brown *et al.*, 1992). The renatured protein sample did not allow transcription from p3 and p4, whereas the p1 and p2 promoters were actively transcribed, most likely due to successful renaturation of σ^{HrdB}. Therefore, we then attempted to recover the p3 and p4 transcribing activity from the Superose fractions using milder denaturation methods. Fractions 14 to 18, shown in Fig. 4D, were pooled and dissolved in a denaturation buffer containing 6 M urea. The denatured proteins were again fractionated through Superdex 200 with 4.5 M urea buffer and eluted as shown in Fig. 5A. The peak position of the denatured α subunit is indicated. Each fraction was renatured by the dialysis of urea against renaturation buffer. The renatured fraction was incubated with core RNA polymerase that was purified from a Mono-Q column. The reconstituted RNA polymerase sample was examined for its transcribing activity for *rrnD* promoters. As shown in Fig. 5B, only the p2 transcripts were abundantly synthesized over the entire fractions, with strongest activity from fractions 7 to 19. Weak transcription activity for p1 was also detected in fractions 9-17, while no activity was detected for p3 or p4. From this result, it can be inferred that the denatured σ^{HrdB} was eluted broadly, overlapping with a peak of the RNAP α subunit, and enabled transcription of the p2 promoter. On the other hand, factors required for p3 and p4 could have been lost or may not have been

refolded into an active form. Yet another possibility is that the p3 and p4 promoters may require an additional protein factor, in addition to a sigma factor, for their transcription.

Since p3 and p4 contain a -10 region that resembles a σ^{HrdB}-dependent consensus sequence, we tested the possible requirement of additional protein factors in addition to holo RNA polymerase containing σ^{HrdB} ($E{\cdot}\sigma^{HrdB}$). Results in Fig. 5C demonstrate that the p3 and p4 transcripts are not efficiently synthesized with $E{\cdot}\sigma^{HrdB}$ holo RNAP in the absence of any additional proteins (Fig. 5C, lane 3). The addition of renatured proteins in fractions 15 to 21 allowed efficient transcription from the p3 and p4 promoters. These fractions were eluted following the α subunit (37 kDa) peak, and estimated to be in the size range of 30-35 kDa. Therefore, our results suggest that a specific protein factor with a molecular mass of 30-35 kDa is required for transcription of the p3 and p4 promoters in the presence of $E{\cdot}\sigma^{HrdB}$ holo RNA polymerase. The identity of such an activating factor needs be determined by analyzing the proteome of fractions 15 to 21. Despite the different transcriptional patterns of p3 and p4 promoters *in vivo* (Fig. 2B), the *in vitro* reconstitution assay indicated that both p3 and p4 could be regulated by a common transcription factor. This may be supported by the fact that upstream sequences of p3 and p4 promoters show 55% similarity.

In the regions containing the p3 and p4 promoters, a sequence motif of g/cAAAg/c is directly repeated ten times (Baylis and Bibb, 1988b). The A-rich region is also found close to the p3 and p4 promoters of the *rrnA* operon and, thus, it has been proposed to play an important role in regulating *rrn* p3 and p4 promoters. In *E. coli*, an upstream (A+T)-rich region referred to as a UP element increases the transcription of *rrnB* P1 by about 30-fold through interaction with the C-terminal domain of the RNA polymerase α subunit (Ross *et al.*, 1990). In *E. coli*, another positive regulation of P1 promoters occurs through the FIS protein. FIS forms a homodimer with 11.2 kDa subunits (Kostrewa *et al.*, 1991) and recognizes a 15 bp degenerate consensus sequence upstream of the UP element. In the *S. coelicolor* genome, however, no FIS homologue with significant sequence similarity was found. The role of the g/cAAAg/c repeat sequence has not yet been clearly identified. A probable hypothesis could involve the binding of an activating protein to these repeat sequences. However, more detailed analysis is required.

Conclusions

In this study, we found that the *rrnD* p3 and p4 promoters, which are major promoters in the mid-to-late exponential and stationary phases in *S. coelicolor in vivo*, are transcribed by crude RNA polymerase preparation *in vitro*, but not by purified reconstituted holoenzyme containing σ^{HrdB} alone. Transcription of *rrnD* p3 and p4 promoters required an additional factor that was loosely associated with RNA polymerase components. The size of this specific factor is slightly smaller than the α subunit, with a mass of about 30-35 kDa, as estimated by Superdex 200 gel filtration in the presence denaturant (4.5 M urea). This observation is consistent with the fact that the p3 and p4 promoters contain a good -10 consensus sequence for σ^{HrdB} recognition, but lack consensus -35 hexamers. Our work provides the basis for the identification of activating factors for *rrn* p3 and p4 promoters, which will reveal an interesting aspect of ribosomal RNA synthesis and regulation in this organism.

Acknowledgements

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