Two Transcribing Activities Are Involved in Expression of the *Streptomyces* Galactose Operon

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The *Streptomyces* galactose operon is transcribed from two independently regulated promoters: *galP1*, located at the 5' end of the operon and responsible for galactose-dependent transcription of the operon, and *galP2*, an internal constitutive promoter. We identified and partially separated two distinct transcribing activities involved in expression of this operon. Using RNA polymerase from *Streptomyces lividans* and *Streptomyces coelicolor* partially purified by chromatography on heparin-agarose and DNA-cellulose, we detected activities capable of initiating transcription in vitro specifically from either *galP1* or *galP2*. Circumstantial evidence suggests that the activity for *galP2* transcription is a holoenzyme species associated with the previously described σ^{28} protein (referred to here as σ^C). The *galP1*-transcribing activity is more difficult to evaluate. This activity may correspond to a holoenzyme species associated with σ^A (formerly σ^{35}), although other possibilities are discussed. This would be the second reported example of a catabolite-controlled gene in *Streptomyces* species expressed from multiple promoters recognized by different holoenzyme forms. This may indicate that the involvement of RNA polymerase heterogeneity in gene expression in *Streptomyces* species is a more general strategy for regulation than the specialized gene expression seen in *Escherichia coli*.

Information gained from gene regulation studies in *Escherichia coli* has led to the formulation of some basic concepts that may apply to all bacteria. Nevertheless, as such studies have been extended to other organisms, it has become clear that very different strategies may be used in bacteria distantly related to *E. coli* to impose similar regulatory controls. Sometimes these different strategies involve novel molecular mechanisms and thus expand our appreciation for the range of ways in which bacteria can regulate gene expression. Regulation of tryptophan biosynthesis genes in *E. coli* and *Bacillus subtilis* is a remarkable example. In *E. coli*, the *trp* operon is regulated in part by an attenuation mechanism that involves ribosome stalling in a Trp codon-rich leader transcript (23). In the gram-positive species *B. subtilis*, the *trp* operon is also regulated by a form of transcriptional attenuation, but through a completely different mechanism that involves a *trans*-acting protein for which tryptophan itself appears to be an allosteric effector (35).

Catabolite control is an example of an even more fundamental regulatory phenomenon that appears to be mediated in gram-positive bacteria by a very different mechanism than in *E. coli*. In *E. coli*, an important component of catabolite control is mediated by cyclic AMP (cAMP) and the cAMP receptor protein (32). Current evidence suggests that gram-positive bacteria respond to repressing carbon sources by a mechanism that is independent of cAMP. For example, in *Streptomyces coelicolor*, cAMP is present, but intracellular levels of cAMP do not change with the addition of a repressing carbon source (17). Instead, glucose repression in *S. coelicolor* is perhaps regulated, at least in part, by glucose kinase (17). *B. subtilis*, another gram-positive species, lacks adenylate cyclase (and presumably, cAMP) entirely (34).

Beyond these simple observations, virtually nothing is known about the mechanism of catabolite repression in *Streptomyces* spp., despite the involvement of catabolite control in regulating some aspects of development (7, 8) and

secondary metabolism in these morphologically complex and commercially important bacteria. For example, *Streptomyces* spp. produce the great majority of antibiotics of medical or agricultural importance (2), as well as a large number of extracellular hydrolytic enzymes (39), and the synthesis of many of these compounds appears to be directly or indirectly influenced by catabolite repression (27, 33). Among the catabolite-controlled *Streptomyces* genes that have been investigated, perhaps the best characterized in terms of its transcriptional organization is the *S. coelicolor* *dagA* gene, whose product is a hydrolytic exoenzyme that degrades agar (18). Buttner et al. (6) have recently shown that at least three forms of RNA polymerase, each associated with a different sigma species, are involved in the transcription of four different and apparently independent promoters for *dagA*. The reason for this unexpected complexity is unknown, and nothing is understood about the mechanism by which glucose levels influence *dagA* expression.

In this report, we describe the initial characterization of two transcribing activities involved in the expression of the *gal* operon promoters of *Streptomyces* spp. (The work we describe here involves a study of *S. lividans* and *S. coelicolor*, and we assume that what is true for these organisms will apply to other streptomycetes. We emphasize, however, that while this may be true in general, there may well be exceptions.) Transcription of the *gal* operon is subject to induction by galactose and repression by glucose (1, 12). As with *dagA*, these and previous studies have revealed unexpected complexity at the level of both promoter organization and RNA polymerase heterogeneity. In *Streptomyces* spp., as in *E. coli*, galactose utilization genes are organized within a polycistronic operon and transcription of the operon in vivo is directed by two different promoters. However, in *Streptomyces* spp., only one promoter, *galP1*, is located at the 5' end of the operon and produces a transcript which encodes all three of the *gal* gene products (*galT*, *galE*, and *galK*), while the second promoter, *galP2*, is located between

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FIG. 1. Nucleotide sequence of the *galP1* and *galP2* promoter regions. The apparent transcription start sites are underlined and indicated as +1.

galT and *galE* and produces a transcript which encodes only *galE* and *galK* (see Fig. 2). Only *galP1* is influenced by levels of galactose and glucose in the growth medium; *galP2* is a low-level constitutive promoter. As with *dagA*, nothing is understood about the way in which carbon utilization affects expression of the *gal* operon. In this work, we partially purified two distinct forms of RNA polymerase that recognize *galP1* and *galP2*. This is the first step in dissecting the transcriptional machinery involved in induction and repression of the *galP1* promoter.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Streptomyces* strains used were *S. coelicolor* A3(2) (19) and *S. lividans* 1326 (4). *E. coli* TB1 (Focus 6:7, 1984) was used as the host for the plasmids described below. *E. coli* plasmids used in this study were pMS500 (30), pMI340 (38), pUC9 (40), and pUC18 (41). Plasmid pUC9-P1 is a pUC9 derivative containing a 210-base-pair (bp) *Sau3A*-*AluI* fragment that includes the *galP1* promoter. This fragment was cloned into pUC9 with the *Bam*HI and *Sma*I polylinker sites. Plasmid pUC9-P2:3 is a pUC9 derivative containing a 355-bp *Ban*I fragment that includes the *galP2* promoter. This *Ban*I fragment was treated with reverse transcriptase to produce flush ends (26) before insertion into the *Sma*I site of pUC9. pUC18-TT1 is a pUC18 derivative containing a synthetic DNA sequence inserted at the *Bam*HI site. Insertion of this oligonucleotide created unique *Bgl*II and *Bcl*I sites while retaining the original unique *Bam*HI site. Plasmid 3BglTT1, used for S1 nuclease protection analysis, is a pUC18-TT1 derivative containing a 410-bp *Sau3A* fragment that includes *galP1*. The *Sau3A* site immediately downstream from *galP1* is a partial *Bgl*II sequence. 3BglTT1 contains the *Sau3A* fragment oriented within pUC18-TT1 such that a *Bgl*II site was created at the downstream *Sau3A* site. Plasmid DNA was prepared by alkaline lysis (5) followed by equilibrium centrifugation in cesium chloride-ethidium bromide gradients (10). Plasmid DNA was transformed into *CaCl*₂-treated *E. coli* cells as described by Cohen et al. (11). TB1 transformants were grown in LB broth or LB agar (29) containing 50 µg of ampicillin per ml for plasmid selection.

RNA polymerase preparation. *S. lividans* 1326 or *S. coelicolor* A3(2) cultures were grown at 28°C in YEME medium (4) plus 34% sucrose to the late log phase, collected by centrifugation, washed with buffer A (0.01 M Tris hydrochloride [pH 8], 1 mM EDTA, 0.3 M dithiothreitol, 0.3 mg of phenylmethylsulfonyl fluoride per ml, 10% glycerol) containing 0.1 M KCl, fast frozen on dry ice, and stored at -70°C. A 100-g sample of cells that had been suspended in buffer A was disrupted by passage through a French pressure cell at 15,000 lb/in², and RNA polymerase was partially purified by phase partitioning as previously described (36), except that after dialysis the RNA polymerase-containing fraction was precipitated by the addition of 0.163 g of ammonium sulfate per ml (approximately 30% of saturation) and centrifuged for 10 min at 15,000 × g. The supernatant was applied to a 10-ml

heparin-agarose column (2 by 5.5 cm), and bound material was eluted with a 0.25 to 0.70 M linear KCl gradient in 50 ml of buffer A. Fractions containing RNA polymerase activity were identified in runoff transcription assays (38). When further purification was required, fractions containing certain portions of the RNA polymerase activity were pooled, dialyzed against buffer A, and applied to a 5-ml DNA-cellulose column (1.3 by 5 cm). Bound material was eluted with a 0.35 to 0.65 M KCl gradient in 50 ml of buffer A.

RNA synthesis: DNA templates and assay conditions. DNA templates containing the *B. subtilis* *veg* or *ctc* promoters were prepared by *Bam*HI digestion of plasmid pMS500 or pMI340, respectively (30, 38). A 210-bp fragment containing *galP1* was obtained by digestion of pUC9-P1 with *Bam*HI, and a 340-bp fragment containing *galP2* was obtained by digestion of pUC9-P2:3 with *Eco*RI. Promoter-containing fragments were separated from digested plasmid DNAs by polyacrylamide gel electrophoresis and recovered from gel slices by electroelution. Enzyme fractions were assayed for activity by incubation of 2 µl of the column fractions for 10 min at 37°C in 40-µl reaction mixtures containing 1 µg of the relevant purified restriction fragment of 2 µg of linearized plasmid DNA, 0.5 mM each ATP, GTP, and UTP, and 0.5 µM [³²P]CTP (10 µCi). RNA synthesis was initiated by the addition of RNA polymerase. After 1 min at 37°C, 6 µg of heparin was added to prevent reinitiation; after 5 min, 1.5 mM unlabeled CTP was added; after a further 5 min, the reaction was stopped on ice. Radioactive RNA was concentrated from the reaction mixtures by ethanol precipitation and fractionated by electrophoresis in a 6% polyacrylamide gel containing 7 M urea (28).

S1 nuclease mapping. RNA was isolated from *S. lividans* or *S. coelicolor* strains that had been grown in 50 ml of YM medium containing either 1% glucose or 1% galactose as previously described (12). The entire culture volume was quick cooled by the addition of 50 ml of 20 mM NaN₃ in 95% ethanol that had been cooled to -70°C. RNA was isolated by phenol extraction and further purified by centrifugation through a cushion of CsCl (9). The *galP1*-directed transcript was detected with a 420-bp *Bam*HI-*Bgl*II fragment from 3BglTT1 that had been end labeled at the *Bgl*II site with T4 polynucleotide kinase (26). RNA (50 µg) was hybridized to 0.5 µg of 5'-end-labeled DNA fragment in 50 µl of hybridization buffer (3). After 3 h at 59°C, the hybridization reaction was terminated by the addition of 12.5 µl of the reaction mixture to 0.1 ml of S1 buffer (12) containing 100 U of S1 nuclease. S1 digestion was for 30 min at 37°C. The protected fragments were separated on a 7% polyacrylamide gel containing 7 M urea.

RESULTS

Initial transcription studies. The DNA sequences and apparent transcription start sites for the *S. lividans* *galP1* and *galP2* promoters were determined previously (12) and are shown in Fig. 1. Assuming that the start sites determined

FIG. 2. Restriction maps of the *S. lividans* and *S. coelicolor* *gal* operons. The location and orientation of the structural genes are indicated by arrows. The position and direction of transcription of the *galP1* and *galP2* promoters are indicated by P1 and P2, respectively.

by S1 nuclease protection experiments represent the actual transcription initiation sites, the absence of homology between *galP1* and *galP2* at positions where RNA polymerase would be expected to make specific contacts (+1 to -50) raised the possibility that different forms of RNA polymerase recognized these two promoters. Indeed, as described below, RNA polymerase partially purified from *S. coelicolor* contained *galP1*- and *galP2*-transcribing activities that were separated during gradient elution from heparin-agarose.

To determine whether similar transcribing activities could be resolved in *S. lividans* extracts, we used purification protocols developed for *S. coelicolor* (38) to prepare RNA polymerase from *S. lividans*. In addition to using runoff templates containing the *galP1* and *galP2* promoters, we included templates containing the *B. subtilis* promoters for *veg* and *ctc* (31, 38). Previous studies had revealed transcribing activities in *S. coelicolor* extracts for both of these *B. subtilis* promoters (38). Unexpectedly, in RNA polymerase partially purified from *S. lividans*, only *galP2*-transcribing activity was detected with the *Streptomyces* templates. Moreover, although *ctc*-transcribing activity was readily demonstrated, *veg*-transcribing activity was completely absent.

Given the high degree of relatedness between *S. lividans* and *S. coelicolor*, we considered it very unlikely that *S. lividans* was actually devoid of *galP1*- and *veg*-transcribing activities, particularly since *galP1* was known to be active *in vivo* in *S. lividans*. We suspected instead that the *galP1*- and *veg*-transcribing activities were present in *S. lividans* extracts but were being masked by an "inhibitor," such as a protease or a nuclease, that coeluted with the *galP1*- and *veg*-transcribing activities in early-eluting fractions from heparin-agarose. To test this hypothesis, we added early-eluting fractions from *S. lividans* extracts to early-eluting fractions from *S. coelicolor* that were active in transcribing from *galP1*- and *veg*-containing templates. As predicted, this abolished the transcribing activities present in the *S. coelicolor* extracts (data not shown). Efforts to purify *galP1*- or *veg*-transcribing activities away from this inhibitory factor(s) have so far been unsuccessful.

In part because of these technical problems, we decided to carry out subsequent *in vitro* transcription studies with *S. coelicolor* RNA polymerase fractions, providing we could demonstrate that transcription of the *S. coelicolor gal* operon was directed by promoters functionally similar to *galP1* and *galP2* of *S. lividans*. We also anticipated that working with *S. coelicolor* extracts would make it easier to carry out a combination of biochemical and genetic approaches in future work.

Comparative analysis of *S. lividans* and *S. coelicolor gal* operons. Kendall and colleagues (21) have recently cloned from *S. coelicolor* a restriction fragment that contains its *galK* gene. A comparison of the restriction map of this fragment with a map derived from DNA containing the *S. lividans gal* operon is shown in Fig. 2. This comparison revealed a perfect correspondence between the two maps over the entire region of overlap, which extends for more than 7 kilobases, consistent with other studies which have indicated a very high degree of overall genetic similarity between *S. lividans* and *S. coelicolor*. Previously published work also suggested that the *S. coelicolor gal* operon was repressed by glucose and induced by galactose in a way that might closely resemble the behavior of the *S. lividans* operon (17).

To investigate with higher resolution the physical and functional similarity of promoters controlling expression of the *S. lividans* and *S. coelicolor gal* operons, we did a series of S1 protection experiments with RNA isolated from cultures grown in the presence of either glucose or galactose. The end-labeled probe for these experiments consisted of a 410-bp *Sau3A* fragment from *S. lividans* that included *galP1* (Fig. 3). RNA isolated from *S. lividans* and *S. coelicolor* bacteria grown in the presence of galactose and the absence of glucose was hybridized with the probe, treated with S1 endonuclease, and analyzed for comparison on the same polyacrylamide gel (Fig. 3). A single primary protection product of 310 bases was observed in both cases. No detectable quantity of probe was protected by RNA isolated from bacteria grown in the presence of glucose and the absence of galactose. From this we concluded that *galP1* has

FIG. 3. S1 protection analysis of the *galP1* galactose-induced transcript. (A) Molecular weight standards (shown on the left, in base pairs) consisted of ^{32}P -end-labeled, *Hinf*-digested pBR322 DNA (lane 1). An apparent 5' terminus for the galactose-induced transcript was identified with the hybridization probe shown in panel B. This probe was hybridized to yeast tRNA (lane 2), RNA isolated from *S. lividans* grown in the presence of glucose (lane 3) or galactose (lane 4), and RNA isolated from *S. coelicolor* grown in the presence of glucose (lane 5) or galactose (lane 6). The arrow indicates the position of the protected probe. (B) Partial restriction map of the hybridization probe. The position of the *galT* gene within the *Bam*HI-*Bgl*II fragment is indicated by the bold bar. *, Position of the 5' end label.

a functionally identical and probably physically identical counterpart in *S. coelicolor*.

Partial separation of *galP1*- and *galP2*-transcribing activities. To examine transcription initiation from *galP1* and *galP2* in vitro, we tested promoter-containing DNA fragments with partially purified RNA polymerase from *S. coelicolor* in runoff transcription assays. RNA polymerase that had been purified by phase partitioning was applied to a heparin-agarose column and eluted with a linear gradient of KCl. The DNA templates used were gel-purified fragments containing either *galP1* or *galP2*. With transcription initiating at positions identical to the start sites determined by S1 protection mapping, the predicted sizes for the runoff transcripts were 95 and 120 bases, respectively. The previously characterized *veg* and *ctc* promoters of *B. subtilis* were used as controls to monitor separation of holoenzyme forms (data not shown). Transcribing activities for *veg*, *ctc*, *galP1*, and *galP2* were readily detected, with *veg*- and *galP1*-transcribing activities eluting at lower salt concentrations and *ctc*- and *galP2*-transcribing activities eluting at higher concentrations (data not shown). The sizes of runoff RNAs

generated in these transcription reactions were precisely as predicted from in vivo S1 protection experiments.

As indicated by the results in Fig. 4, gradient elution of *S. coelicolor* RNA polymerase from heparin-agarose resulted in partial but definite separation of *galP1*- and *galP2*-transcribing activities, with fractions containing the peak of *galP1* activity eluting at a lower salt concentration than fractions containing the peak of *galP2* activity. This demonstrated that two distinct transcribing activities recognize *galP1* and *galP2* in vitro and is consistent with the possibility that these transcribing activities represent holoenzyme forms associated with different σ species. Indeed, when proteins present in column eluates were fractionated by electrophoresis and visualized by silver staining, σ candidates were identified for both *galP1*- and *galP2*-transcribing activities as polypeptides which coeluted with the profile of the transcribing activity (data not shown). However, preliminary efforts to recover these proteins from gel slices by electroelution and to reconstitute transcribing activities in the presence of polymerase core have so far been unsuccessful.

Further characterization of *galP2*-transcribing activity. In previous work (38), *veg*-transcribing activity was shown to be due to a holoenzyme form associated with a 35-kilodalton σ species, referred to here as σ^A , and *ctc*-transcribing activity was shown to be due to a 49-kilodalton σ species, referred to here as σ^B . The inability to detect *veg*-transcribing activity in RNA polymerase fractions isolated from *S. lividans* ruled out the involvement of σ^A -associated holoenzyme in transcription from the *galP2* promoter. To investigate whether *galP2* activity might be due to σ^B -associated holoenzyme, heparin-agarose fractions of *S. lividans* RNA polymerase that contained *galP2*-transcribing activity were pooled and applied to a DNA-cellulose column. A shallow gradient elution of RNA polymerase activity adhering to this column resulted in definite separation of *galP2*- and *ctc*-transcribing activities (Fig. 5). No transcription was detected from *veg* or *galP1* templates (data not shown). From this we inferred that *galP2*-transcribing activity was not due to σ^B -associated RNA polymerase. In addition, these data provide further evidence that *galP1*- and *galP2*-transcribing activities are separate.

DISCUSSION

We report here the existence of two distinct transcribing activities involved in expression of the *Streptomyces gal* operon. One of these activities elutes from heparin-agarose at a lower salt concentration and is capable of initiating transcription in vitro specifically from *galP1*, a glucose-repressed and galactose-induced promoter located at the 5' end of the *gal* operon. The other activity elutes at a higher salt concentration and initiates transcription specifically from *galP2*, a low-level constitutive promoter internal to the operon. Both transcribing activities initiated transcription in vitro from the same start sites demonstrated to be used in vivo by S1 nuclease protection experiments. We consider it likely that these activities represent distinct RNA polymerase forms associated with different sigma subunits, although we have so far been unable to establish this conclusively by reconstituting the activities from gel-purified proteins added back to polymerase core.

The *galP1*-transcribing activity is difficult to evaluate. First, no unique σ candidate could be identified in silver-stained sodium dodecyl sulfate-polyacrylamide gels of column fractions containing the activity peak. Second, the

FIG. 4. Partial separation of *galP1*- and *galP2*-transcribing activities. Runoff RNAs were generated with *galP1*- or *galP2*-containing templates and *S. coelicolor* RNA polymerase partially purified by gradient elution from heparin-agarose. (A) Partial restriction map of the DNA fragments used as templates in the runoff transcription assays. The expected size of each runoff transcript is indicated below the restriction map. (B) RNA generated from the *galP1*-containing template. (C) RNA generated from the *galP2*-containing template. Every other fraction eluted from the column was assayed as indicated by the numbers on top of each autoradiogram. After separation on a 7% polyacrylamide gel containing 7 M urea, the ^{32}P -labeled runoff transcripts were visualized by autoradiography. The position of the runoff transcript is indicated by an arrow. Shown to the left of each autoradiogram are the positions of the molecular weight standards which consisted of end-labeled, *Hinf*-digested pBR322 DNA.

FIG. 5. Partial separation of the *ctc*- and *galP2*-transcribing activities. Runoff RNAs were generated with *ctc*- or *galP2*-containing templates and *S. lividans* RNA polymerase partially purified by gradient elution from DNA-cellulose. (A) Partial restriction map of the *galP2* template. The expected size of the *galP2* runoff transcript is indicated below the restriction map. (B) RNA generated from the *ctc*-containing template. (C) RNA generated from the *galP2*-containing template. After separation on a 7% polyacrylamide gel containing 7 M urea, the ^{32}P -labeled runoff transcripts were visualized by autoradiography. The numbers on top of each autoradiogram indicate the DNA-cellulose column fraction used in the runoff transcription assay. The position of the runoff transcript is indicated by an arrow. Shown to the left of each autoradiogram is the position of the molecular weight standards (in base pairs) which consisted of end-labeled, *Hinf*-digested pBR322 DNA.

promoter sequence (Fig. 1) contains complexities that are difficult to interpret, perhaps reflecting the presence of overlapping regulatory signals. At present, there is no evidence to indicate which base pairs within *galP1* are involved in RNA polymerase binding. However, centered around -10 are two hexamers (TGTTAT and TATGTT) which match reasonably well the -10 sequence (TACAAT) of the *veg* promoter which is transcribed in vitro by σ^A -associated holoenzyme. An inspection of the sequence 17 bp upstream of these hexamers revealed no similarity to the *veg* -35 sequence (TTGACA). Centered around -39 , however, is a hexamer (GTGACA) with an almost perfect match to this sequence spaced 21 and 24 bp upstream from the tentative -10 regions. In *E. coli*, such similarities would be dismissed because of this unusual spacing (25, 31, 37). However, the spacing of the *galP1* tentative -10 and -35 sequences is reminiscent of the tightly regulated, temporally controlled *spoII*E and *spoII*G promoters of *B. subtilis* (14, 22). These promoters contain hexamers which have nearly perfect homology to the sequences TTGACA and TATAAT, but like *galP1*, these regions have the uncharacteristic spacing of 21 bp for *spoII*E (14) and 22 bp for *spoII*G (22). In view of this precedent, we cannot dismiss the possibility that σ^A -

galP2 CCTGGAACTTTTCACTTCCGCGTACGTCGGCAAGCTGAAGTTCCTCG
 * * * * *
dagP2 GCGCTTCGGAACTTTTTGCACGACGCGAGCTCTCGAATTTT

FIG. 6. DNA sequences of the regions upstream of the apparent transcription start sites (underlined) of the *galP2* and *dagP2* promoters. Asterisks show identical bases. Note the 9-base sequence common to both promoters (underlined).

associated RNA polymerase can be made to recognize promoters with such inappropriately spaced consensus sequences by regulatory proteins that may interact either with the promoter or with the polymerase itself. Recent work of Takahashi and colleagues (H. Takahashi, K. Tanaka, and T. Shiina, Abstr. 7th Int. Symp. Biol. Actinomycetes 1988, p. 9) raises another possibility, however. In contrast with *E. coli* and *B. subtilis*, which both contain a single abundant σ species (σ^{70} in *E. coli* and σ^A in *B. subtilis*) responsible for recognizing promoters that conform to the prototypical eubacterial consensus sequence, Takahashi et al. have found that *S. coelicolor* contains a family of four distinct but closely related σ proteins, one of which presumably corresponds with the σ^A species that transcribes *veg*-like promoters. Perhaps one or more of the other *Streptomyces* σ^A -like proteins recognizes similar consensus sequences, but separated by a spacing of 21 or 24 bp (like those of *galP1*).

For *galP2*-transcribing activity, we speculate (in the absence of successful reconstitution experiments, and we emphasize that at this time there is no evidence to support this speculation) that this activity may be due to the previously described σ^{28} -associated (referred to here as σ^C) holoenzyme activity (6). There is striking similarity between the sequences of *galP2* and *dagP2* in regions where RNA polymerase would be expected to make specific contacts (Fig. 6).

The *Streptomyces* galactose operon is only one example of a set of genes in which expression is controlled by more than one promoter. Multiple promoters are frequently clustered at the 5' end of an operon or gene. One striking example is the catabolite-controlled *dagA* gene of *Streptomyces* species; transcription is directed by four independent promoters transcribed by at least three different forms of RNA polymerase (6). It is less common, however, for a promoter to be contained within an operon. One example is the *rpoD* gene of *E. coli*, but in this case the internal promoter is active only during the heat shock response and is not "normally" used for transcription (13).

Like multiple promoters, multiple forms of RNA polymerase provide additional flexibility in the modulation of gene expression. Although RNA polymerase heterogeneity is emerging as a common, perhaps universal, feature of the gene expression machinery in both gram-positive and gram-negative bacteria (13, 24), the norm in most species appears to be the use of a single, abundant holoenzyme form for the expression of most genes that function via ordinary primary metabolic pathways. Secondary holoenzyme forms, usually associated with low-abundance σ species, are used in the expression of specialized sets of genes involved in sporulation (24), chemotaxis (15), heat shock (13), nitrogen utilization (16, 20), or other special activities. The recent work of Takahashi and colleagues (Abstr. 7th Int. Symp. Biol. Actinomycetes 1988) suggests that *Streptomyces* species have evolved an extra layer of complexity involving the use of multiple polymerase forms even for the expression of genes, such as those of the *gal* operon, whose products participate in primary carbon utilization pathways. The transcriptional complexity of *dagA*, a transcription unit with four promoters

recognized by at least three different holoenzymes, is even more puzzling. Whether these complexities represent the norm for *Streptomyces* genes, perhaps reflecting the unusual morphological and metabolic complexity of the organism itself, may be clarified as additional genes and operons are subjected to similar analysis.

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