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Biochemistry

New RNA polymerase σ factor under *spo0* control in *Bacillus subtilis*

(spore formation/promoter recognition)

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ABSTRACT **In *Bacillus subtilis* transcription of *spoVG* is activated within minutes after the initiation of sporulation. Mutations in several *spo0* genes prevent the activation of *spoVG* transcription. We have found a σ-like protein that is capable of directing core RNA polymerase to use the *spoVG* promoter in an *in vitro* run-off transcription assay. This σ-like protein was not found to be associated with RNA polymerase in a *spo0A* or *spo0B* mutant but was present in a *spo0H* mutant. We suggest that one role of the *spo0A* gene product in transcription of *spoVG* is the modulation of RNA polymerase activity by this σ-like protein.**

The σ subunit of eubacterial RNA polymerases [nucleoside-triphosphate:RNA nucleotidyltransferase (DNA-directed), EC 2.7.7.6] enables the holoenzyme to recognize and bind to specific sites on DNA known as promoters. Bacteria from several genera possess multiple σ factors, which enable RNA polymerase to utilize different classes of promoters. Binding of alternative σ factors may set apart a fraction of the cells' RNA polymerase for use on promoters of one physiologically or developmentally related set of genes. In *Bacillus subtilis*, several σ factors are thought to play a role in the temporal control of transcription during differentiation of vegetative cells into endospores. For example, σ^{29}, which is essential for sporulation, appears in *B. subtilis* about 2 hr after the initiation of sporulation (1) and directs RNA polymerase to transcribe several genes that were not previously transcribed (2).

The activation of transcription at the beginning of sporulation appears to be more complex than the temporal control exerted by the appearance of σ^{29} during sporulation. Transcription of one sporulation gene, *spoVG*, has served as the paradigm for the study of gene transcription at the beginning of sporulation (3). Within minutes after the initiation of spore formation, transcription of *spoVG* is initiated from two start points separated by about 10 base pairs (4). Two forms of RNA polymerase, $E\sigma^{37}$ and $E\sigma^{32}$ (core RNA polymerase, subunits $\beta\beta'\alpha^2$, plus σ^{37} or σ^{32}, respectively) generate transcripts from these two start points *in vitro* by interaction with two overlapping promoters, P_1 and P_2, respectively (5). Although these polymerases start transcription at the same start points as are used *in vivo*, this *in vitro* reaction does not entirely mimic the *in vivo* regulation of *spoVG*. Unlike the *in vitro* reaction, the analysis of mutants indicates that transcription of *spoVG in vivo* requires the products of at least five genes known as *spo0* genes, apparently in addition to $E\sigma^{37}$ and $E\sigma^{32}$ (6). Furthermore, another promoter (*ctc*) is used more efficiently by $E\sigma^{37}$ and $E\sigma^{32}$ *in vitro* than is the *spoVG* promoter (5), whereas the *spoVG* promoter functions more efficiently *in vivo* than the *ctc* promoter during the early stages of sporulation (7). It may be that the inefficient use of the *spoVG* promoter *in vitro* is due to the absence of a factor(s) that is contributed by the *spo0* gene products *in vivo*.

It is not known how the *spo0* gene products participate in the transcription of *spoVG* or even if their role is direct or indirect. We have found a new σ factor that greatly enhances the ability of RNA polymerase to utilize the *spoVG* promoter *in vitro*. This σ factor was not found to be associated with RNA polymerase in a mutant with a defective *spo0A* gene; therefore, we suggest that one role of *spo0A* in *spoVG* expression is to modulate the function of this σ factor.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *B. subtilis* SMY, a Marburg strain, and asporogenous mutant strains JH646 *spo0A12*, JH648 *spo0B136*, JH651 *spo0H81*, and ZB369 *spo0A abrB* were provided by R. Losick. Plasmids pCB1291, which contains the *spoVG* promoter (4), and pUC31, which contains the *ctc* promoter, have been described (8).

Purification of RNA Polymerase. RNA polymerase was partially purified from 20–30 g of cells by phase-partitioning and gel filtration as described (5). After gel filtration, the RNA polymerases were fractionated by chromatography through a calf thymus DNA-cellulose column. The polymerase was eluted from the 1 × 2 cm DNA-cellulose column with a 60-ml linear gradient of KCl (0.4–1.0 M) in buffer C (5). Fractions (2 ml) were collected, and each fraction was dialyzed against storage buffer [0.01 M Tris, pH 8.0/0.01 M $MgCl_2$/0.1 mM EDTA/0.1 M KCl/0.3 mM dithiothreitol/50% (vol/vol) glycerol/2 mM phenylmethylsulfonyl fluoride].

***In Vitro* Transcription Reactions.** The protocol for the prebinding reactions has been described (8). An equal volume (2 μl) of each fraction of RNA polymerase that was eluted from the DNA-cellulose column was incubated at 37°C for 5 min with 2 μg of DNA template (1 μg of each template in the competition assay) in a 40-μl reaction volume. RNA synthesis was initiated by the addition of 0.5 mM each of ATP, GTP, and UTP and 0.5 μM (10 μCi; 1 Ci = 37 GBq) of [α-^{32}P]CTP. After 1 min, heparin (10 μg) was added to prevent reinitiation. The mixture was incubated an additional 10 min at 37°C, at which time unlabeled CTP (1.5 mM) was added. After an additional 5 min of incubation, 40 μl of stop mix (10 M urea/0.2% bromophenol blue) was added, and 20 μl of this final reaction mixture was subjected to electrophoresis into a polyacrylamide slab gel containing 7 M urea.

Purification of σ^{30} and Reconstruction of the Holoenzyme. Approximately 50 μg of $E\sigma^{30}$-containing fractions from 85 g of *B. subtilis* SMY fractionated by gradient elution similar to that of Fig. 1 was subjected to electrophoresis into a $NaDodSO_4$ gel containing 12% polyacrylamide. Slices (1.5-mm) were cut from above, below, and at the position of σ^{30}. A marker lane containing $E\sigma^{30}$ was stained and used as a

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guide. These slices were then subjected to electroelution in an Isco electroelution device and dialyzed against renaturation buffer (1). To reconstitute the holoenzymes, 0.1 μl of the eluate (0.05%–0.5% of the total eluate) were incubated with 0.08 μg of core RNA polymerase that had been purified by DNA-cellulose chromatography (9) and were assayed by electrophoresis in an $NaDodSO_4$/polyacrylamide gel. The reconstituted holoenzyme was used in a run-off transcription assay with pCB1291 DNA that had been cleaved with *Eco*RI (see Fig. 2).

RESULTS

Assay of *spoVG* Transcribing Activity. We sought to isolate a fraction of RNA polymerase with associated factor(s) that had an enhanced ability to use the *spoVG* promoter. We took advantage of the observation made by Ollington *et al.* that in an *in vitro* transcription reaction with a limiting amount of $E\sigma^{37}$, the *ctc* promoter sequestered the polymerase and prevented transcription from the *spoVG* promoter (10). Therefore, we assayed for a form of RNA polymerase that could utilize the *spoVG* promoter in the presence of the *ctc* promoter in a mixed-template competition reaction. To visualize utilization of the *spoVG* and *ctc* promoters, we used a run-off transcription assay as described. In these run-off transcription reactions, the template that contained the *spo*VG promoter had been cleaved at the *Eco*RI restriction endonuclease recognition site, which was 120 base pairs downstream from the start point of the *spoVG* P_1 promoter. Therefore, transcription from the *spoVG* promoter would result in run-off transcripts of 120 or 110 nucleotides. The reactions contained an equal molar concentration of *ctc* promoter template. This *ctc* template had been cleaved so that transcripts that were initiated from the *ctc* promoter were 95 nucleotides long and, therefore, easily distinguished from the longer transcripts, which were initiated from the *spo*VG promoter. These radiolabeled transcripts were visualized by autoradiography after electrophoresis into a polyacrylamide gel.

RNA polymerase was isolated from *B. subtilis* SMY 30 min after the cells entered stationary phase (see *Materials and Methods*) and was fractionated by elution from a DNA-cellulose column with a linear salt gradient. Each fraction was used to transcribe the mixture of *spoVG* and *ctc* templates in the competition assay (Fig. 1*B*). Two distinct activities were detected with the mixed template competition assay. An activity that primarily generated a 95-nucleotide transcript from the *ctc* promoter was eluted early (Fig. 1*B*, lane c), and an activity that generated a 120-nucleotide transcript from the *spoVG* promoter was eluted at a higher salt concentration several fractions later (Fig. 1*B*, lane e). The activity that utilized the *ctc* promoter correlated with the presence of σ^{37}, which was visualized by $NaDodSO_4$/polyacrylamide gel analysis, (Fig. 1*A*, fraction 10), as was expected because highly purified $E\sigma^{37}$ utilizes only the *ctc* promoter in this mixed template competition assay (10). The fraction from the DNA-cellulose column that contained RNA polymerase that was able to transcribe from the *spoVG* promoter, even in the presence of the *ctc* promoter (Fig. 1*A*, fraction 14), contained a large number of proteins. However, the amount of *spoVG* transcribing activity correlated with the presence of one protein with an apparent molecular mass of 30 kDa. The *spoVG* transcribing activity, which correlated with the presence of the 30-kDa protein, was also found in *B. subtilis* SMY cells that were harvested 30 min before the end of exponential growth.

Reconstruction of the *spoVG* Transcription Activity. We next asked if this 30-kDa protein were responsible for the *spoVG* transcribing activity and if it could act as an independent σ factor *in vitro*. The 30-kDa protein was purified by electroelution from an $NaDodSO_4$/polyacrylamide gel. Slices of the polyacrylamide gel from above and below the 30-kDa protein were also electroeluted. This purified 30-kDa protein (see Fig. 4*A*, lane r) was then renatured and added to *B. subtilis* core RNA polymerase to test if it could direct the polymerase to use the *spoVG* promoter in an *in vitro* run-off transcription assay. The assay contained the *spoVG* promoter template, which had been cleaved so that transcription from the *spoVG* P_1 promoter would generate a 120-nucleotide transcript. Core RNA polymerase alone did not generate the 120-nucleotide transcript (Fig. 2, lane a), although long nonspecific transcripts were observed at the top of the lane. Addition of the 30-kDa protein to the core RNA polymerase resulted in a 120-nucleotide transcript (Fig. 2, lane c), but addition of eluates from above or below the 30-kDa protein did not (Fig. 2, lanes b and d, respectively). Because this protein has the σ-like activity of directing core RNA polymerase to utilize this promoter, we suggest that it be designated σ^{30}. Evidence that this σ has not been described previously follows.

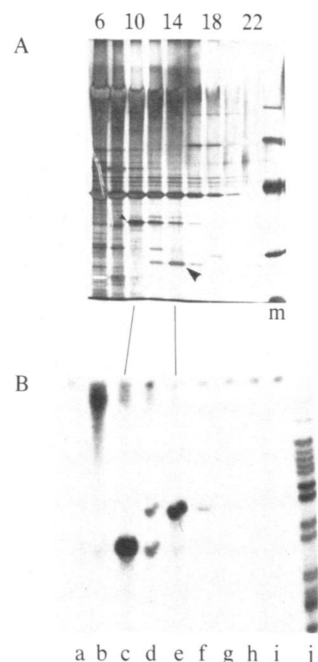

FIG. 1. Gradient elution of RNA polymerase from DNA-cellulose. Gel filtration-purified RNA polymerase from 20 g of *B. subtilis* SMY (wild type) was eluted from a DNA-cellulose column with a linear gradient of KCl as described. (*A*) The proteins in each even-numbered fraction were visualized by staining with silver (Bio-Rad reagent) after electrophoresis of 200 μl into an $NaDodSO_4$ slab gel containing 10% (wt/vol) polyacrylamide. The number above the lane indicates the fraction number. The small arrowhead indicates the position of σ^{37} in fraction 10, and the large arrowhead indicates the position of σ^{30} in fraction 14. The molecular mass markers in lane m are phosphorylase *b* 93 kDa, bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), and soybean trypsin inhibitor (22 kDa). (*B*) Each even-numbered fraction (2 μl) was used in a mixed-template transcription as described. Each reaction contained 1 μg of *spoVG* promoter template, pCB1291 that had been cut at the *Eco*RI site 120 base pairs downstream from the promoter, and 1 μg of the *ctc* promoter template, pUC31 that had been cut at the *Hin*dIII site 95 base pairs downstream from the promoter. ^{32}P-labeled run-off transcripts were visualized by autoradiography after electrophoresis into a 7 M urea slab gel containing 9% polyacrylamide. Lanes: a–i, run-off transcripts generated by RNA polymerase from fractions numbered 6–22, respectively; j, molecular mass markers from radiolabeled pBR322 that had been cut with *Hpa* II. The large arrowhead indicates the position of the 120-nucleotide run-off transcript from the *spoVG* promoter, and the small arrowhead indicates the position of the 95-nucleotide transcript that was generated from the *ctc* promoter.

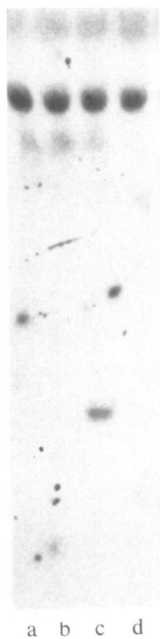

FIG. 2. Run-off transcription of *spoVG* by reconstituted $E\sigma^{30}$. Core RNA polymerase (lane a), RNA polymerase reconstituted by the addition of σ^{30} to core RNA polymerase (lane c), or core RNA polymerase to which was added protein that had been eluted from slices taken above (lane c) or below (lane d) σ^{30} on the preparative gel were used in an *in vitro* run-off transcription assay with the *spoVG* promoter template, pCB1291 that had been cut with *Eco*RI. The 120-nucleotide run-off transcript, indicated by the arrowhead, was visualized by autoradiography after electrophoresis into a 7 M urea slab gel containing 9% polyacrylamide.

σ^{30} in *spo0* Mutants. We have attempted to isolate this σ^{30} activity from several *spo0* mutants. The activity from *B. subtilis* JH651 *spo0H81* (Fig. 3*B*, lane d) correlated with the presence of the 30-kDa protein (σ^{30}) (Fig. 3*A*, fraction 18), but when we fractionated the polymerases from *B. subtilis* JH646 *spo0A12*, we did not detect the *spoVG* transcribing activity in the mixed template competition reaction (Fig. 3*D*). $NaDodSO_4$/polyacrylamide gel analysis of the proteins revealed that σ^{30} was not associated with the RNA polymerase from *B. subtilis* JH646, although σ^{37} or σ^{32} were recovered with the polymerase (Fig. 3*C*). Using this same protocol, we found that the *spoVG* transcribing activity and σ^{30} were not associated with RNA polymerase isolated from *B. subtilis* JH648 *spo0B136*.

Mutations in the *abrB* locus are able to partially suppress the effects of mutations in *spo0A* (11) and to restore transcription of *spoVG* (ref. 3; R. Losick, personal communication). Since σ^{30} was not associated with RNA polymerase in *spo0A* mutant cells, we wished to know if the *abrB* mutation would restore this protein and activity to RNA polymerase. RNA polymerase was isolated as described above from *B. subtilis* ZB369, which has a deletion of *spo0A* and a second mutation at *abrB*. The *spoVG* transcribing activity (Fig. 4*B*, lanes f, g, and h) and the 30-kDa protein were associated with RNA polymerase, although in reduced amounts (Fig. 4*A*, fraction 20). Because the activity and protein seemed to be present in reduced amounts, we assayed fraction 20 in the mixed template competition assay, using several different concentrations of RNA polymerase to be sure that the reactions were carried out with excess DNA. We concluded that the *abrB* mutation does restore the *spoVG* transcribing activity and a 30-kDa protein to the RNA polymerase.

DISCUSSION

An Additional σ Factor. We have identified a form of RNA polymerase that is capable of utilizing the *spoVG* promoter. This activity correlates with the presence of a 30-kDa protein,

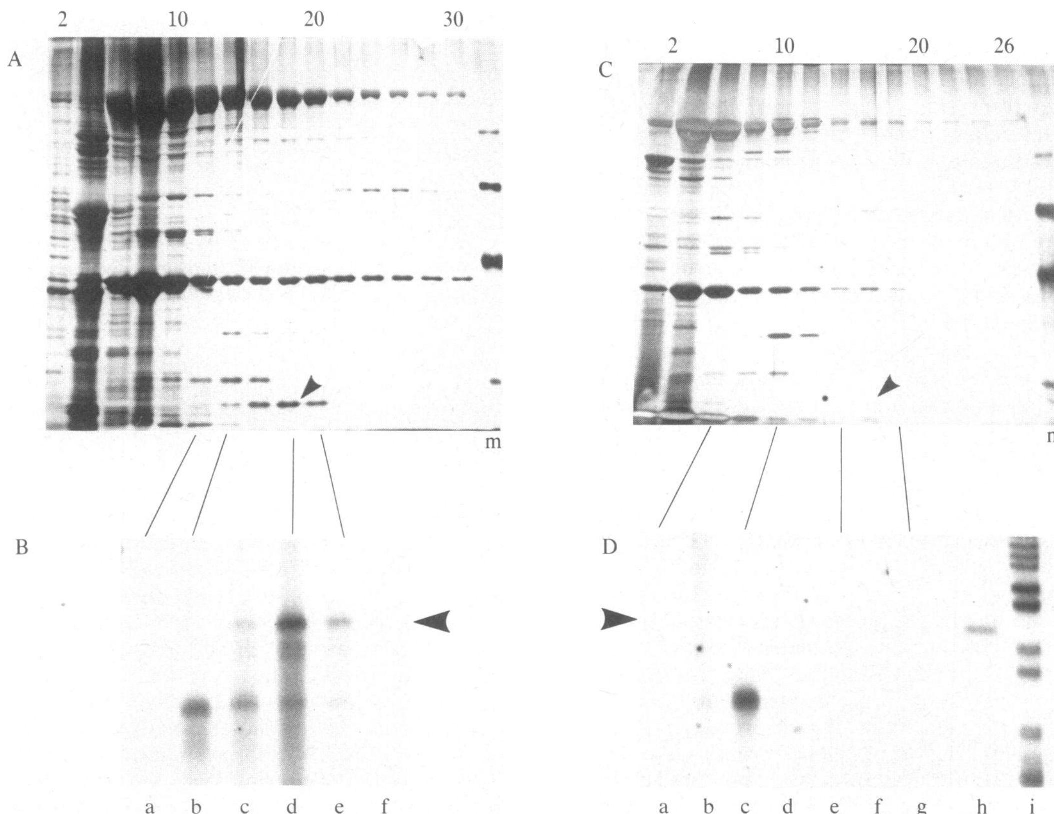

FIG. 3. $E\sigma^{30}$ in *spo0* mutants. Gel filtration-purified RNA polymerases from *B. subtilis* JH651 *spo0H81* (*A* and *B*) and JH646 *spo0A12* (*C* and *D*) were fractionated and assayed as described in Fig. 1. (*A* and *C*) The proteins in each fraction were visualized by staining with silver after electrophoresis into $NaDodSO_4$ slab gel containing 10% polyacrylamide. The large arrowheads indicate the position to which σ^{30} was expected to migrate. Molecular weight markers (same as in Fig. 1) are shown in lane m. (*B* and *D*) Run-off transcripts generated by the RNA polymerase from each even-numbered fraction from 12 to 22 in *A* (*B*) and from 6 to 18 in *C* (*D*) in the mixed-template reactions (as described in Fig. 1). The large arrowheads indicate the positions of the 120-nucleotide run-off transcript from the *spoVG* promoter. The *spoVG* transcript that was generated from the mixed templates by $E\sigma^{30}$ from *B. subtilis* SMY is shown in *D* (lane h). Molecular weight markers from ^{32}P-labeled pBR322 DNA that had been cut with *Hpa* II are shown in *D* (lane i).

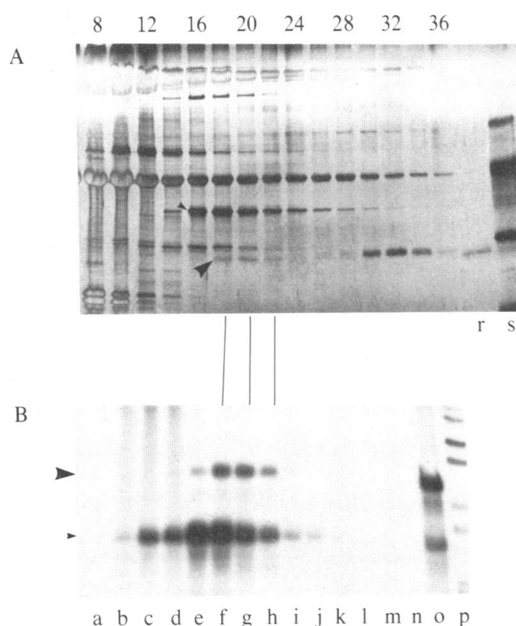

FIG. 4. Fractionated RNA polymerase from a *spo0A abrB* double mutant. Gel filtration-purified RNA polymerases from *B. subtilis* ZB369 *spo0A204 abrB703* was fractionated and assayed as described in Figs. 1 and 3 except that the polymerase was eluted from a 1 × 5 cm DNA-cellulose column with a 100-ml gradient of KCl. (*A*) Proteins in each even-numbered fraction from 8 to 36 were visualized by staining with silver after electrophoresis into a $NaDodSO_4$ slab gel containing 14% polyacrylamide. Purified σ^{30} (lane r) and the molecular weight markers described in Fig. 1 (lane s) are shown. The large arrowhead indicates the position of σ^{30}, and the small arrowhead indicates the position of σ^{37}. (*B*) Run-off transcripts generated by the RNA polymerase from each even-number fraction from 8 to 34 in *A* in the mixed-template reactions as described in Fig. 1 are shown in lanes a–n, respectively. The large arrowhead indicates the position of the 120-nucleotide run-off transcript from the *spoVG* promoter. The small arrowhead indicates the position of the 95-nucleotide runoff transcript from the *ctc* promoter. Run-off transcripts generated from the mixed templates by an $E\sigma^{30}$-containing fraction from *B. subtilis* SMY are shown in lane o. Molecular weight markers from ^{32}P-labeled pBR322 DNA that had been cut with *Hpa* II are shown in lane p.

and the reconstruction experiment (Fig. 2) demonstrated that this protein is able to direct core RNA polymerase to utilize the *spoVG* promoter in an *in vitro* run-off transcription assay. Because this 30-kDa protein was associated with RNA polymerase through several purification steps, including gel filtration, and because addition of this protein to core RNA polymerase enabled the enzyme to utilize a specific promoter, we suggest that this protein be designated a σ factor. Since σ factors have been named according to their apparent molecular weight, we suggest that this additional σ factor be referred to as σ^{30}.

This additional σ factor, σ^{30}, does not appear to be the same as the three σ factors with similar molecular weights that are known to exist in *B. subtilis*. First, it does not appear to be the σ^{29} that appears in *B. subtilis* about 2 hr after the initiation of sporulation (1) because (*i*) σ^{30} is present before the initiation of sporulation, (*ii*) $E\sigma^{29}$ does not utilize the *spoVG* promoter (12), (*iii*) the σ^{30}-associated RNA polymerase ($E\sigma^{30}$) will not utilize two promoters that are utilized by $E\sigma^{29}$ polymerase (unpublished results), and (*iv*) σ^{30} is present in *B. subtilis* JH651 *spo0H81* (Fig. 3*A*, fraction 18), a strain in which σ^{29} is not made (13). Second, σ^{30} is not the same as σ^{28} (14) because $E\sigma^{28}$ polymerase utilizes promoters with sequences that are different from that of *spoVG* (15), and $E\sigma^{30}$ will not utilize the 28-2 promoter in plasmid pMG201 (15), which is used by $E\sigma^{28}$. $E\sigma^{28}$, which was assayed by *in vitro* transcription of plasmid pMG201, was eluted from DNA-cellulose at a lower salt concentration than was $E\sigma^{30}$ (unpublished data). Furthermore, $E\sigma^{30}$ polymerase is not present in the *spo0A* mutant, whereas $E\sigma^{28}$ activity is present in the *spo0A* mutant cells (16). Third, σ^{30} is not σ^{32} because the transcript that was generated in the reconstruction experiment comigrated with the 120-nucleotide P_1 transcript, which migrated more slowly than the 110-nucleotide transcript generated by $E\sigma^{32}$ (data not shown). Furthermore, σ^{30} was not present in the *spo0A* mutant, whereas σ^{32}, which is eluted from DNA-cellulose before σ^{37}, was present (Fig. 3*C*, fractions 6, 8, and 10).

***Spo0* Control of Transcription.** Mutations in any one of five *spo0* genes prevent the efficient transcription of *spoVG* and block sporulation at the earliest stage. A mutation in *spo0H* most severely prevents transcription of *spoVG*, while mutations in *spo0A* produce significant but less severe effects (6). Mutations in *spo0F, spo0E*, or *spo0B* also decrease *spoVG* transcription, but less severely than do mutations in *spo0A* (6). A single-base-pair mutation in *spo0A* is able to suppress the effects of mutations in *spo0F, spo0E*, and *spo0B*; therefore, Hoch and colleagues have suggested that the *spo0B, spo0F*, and *spo0E* gene products may activate the *spo0A* gene product, which is present in vegetative cells (17). Since a mutation in *spo0A* prevents the association of σ^{30} with RNA polymerase (Fig. 3), we suggest that one role of *spo0A* in *spoVG* transcription is to provide σ^{30}. The modulation of the activity of σ^{30} by *spo0A* may also be essential for expression of other genes during the early stages of sporulation. It seems unlikely that *spo0A* encodes σ^{30} because we found that a mutation at *abrB* restored σ^{30} (or a protein of similar size and activity) to RNA polymerase in a strain with a deletion mutation in *spo0A*. A mutation in *spo0B* caused a decrease in the amount of σ^{30} found associated with RNA polymerase. This is consistent with the model in which *spo0B* works with *spo0A* to provide σ^{30} to RNA polymerase.

How do the products of *spo0A* and *spo0B* affect σ^{30} function? Zuber and Losick have proposed that the *abrB* gene product (AbrB) has a negative influence on *spoVG* transcription and that *spo0A* gene product (Spo0A) is an antagonist of AbrB (3). There are at least two classes of models, consistent with this proposal, that may explain the effect of Spo0A on σ^{30} function. In the first model, Spo0A positively regulates σ^{30} synthesis. In this model AbrB may repress transcription of the σ^{30} structural gene, and Spo0A may antagonize this repressor in the manner suggested by Losick. In an alternative model, Spo0A may be required for the processing of σ^{30} to an active form. In this model AbrB and Spo0A may affect expression of a processing enzyme. The processing of σ^{29} of *B. subtilis* from an inactive precursor serves as a precedent for this type of model (18).

Also unexplained are the relative roles of σ^{37} and σ^{30}. Both of these σ factors will direct RNA polymerase to use the *spoVG* P_1 promoter *in vitro*. There is no direct evidence that proves whether one or both of these σ factors interacts with the *spoVG* promoter *in vivo*, but $E\sigma^{30}$ is more active in the mixed template reaction, and the association of σ^{30} with RNA polymerase *in vivo*, like efficient transcription of *spoVG in vivo*, is dependent on the *spo0A* gene product. However, *spoVG* transcription (from both P_1 and P_2 start sites) is not completely blocked in a *spo0A* mutant (6) in which σ^{30} is evidently absent. The structural gene for σ^{37}, called *sigB*, has been cloned and mapped (19). Mutants have been isolated that have an *in vitro*-constructed insertion within *sigB*. These mutants fail to express *ctc* (19, 20). It will be interesting to see to what extent these mutations impair *spoVG* transcription. It is possible that the *spoVG* P_1 promoter is utilized *in vivo* by two different σ factors and that the absence of one does not entirely prevent transcription of this or other sporulation genes. It must be emphasized that σ^{30}, like σ^{37} and σ^{32}, is found to be associated with the RNA polymerase from

exponentially growing cells where *spoVG* is not transcribed (unpublished data). These secondary σ factors may be necessary for transcription of certain genes such as *spoVG*, but apparently they are not the regulatory factors that activate transcription at the beginning of sporulation.

Whatever the relative contribution of σ^{30} and σ^{37}, *spoVG* transcription is additionally dependent on the *spo0H* gene product. Since σ^{30} and σ^{37} are both apparently present in one *spo0H* mutant (*spo0H81*), the *spo0H* gene product may act in conjunction with one or both holoenzymes to promote transcription of *spoVG*. On the other hand, the effect on transcription of *spoVG* by *spo0H81* can be suppressed by a single base substitution in the *spoVG* P_1 promoter (3). This allele-specific interaction and our recent observation that we have been unable to isolate σ^{30} from a mutant that may have a deletion in *spo0H* (unpublished results) are consistent with, but not proof of, a model in which *spo0H* is the structural gene for σ^{30}. The identification of the structural gene that encodes σ^{30} is essential for the determination of the role of this σ factor.

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1. Haldenwang, W. G., Lang, N. & Losick, R. (1981) *Cell* **23**, 615–624.
2. Ray, L. G. & Haldenwang, W. G. (1986) *J. Bacteriol.* **166**, 472–478.
3. Losick, R., Youngman, P. & Piggot, P. J. (1987) *Annu. Rev. Genet.*, in press.
4. Moran, C. P., Jr., Lang, N., Banner, C. D. B., Haldenwang, W. G. & Losick, R. (1981) *Cell* **25**, 783–791.
5. Johnson, W. C., Moran, C. P., Jr., & Losick, R. (1983) *Nature (London)* **302**, 800–804.
6. Zuber, P. & Losick, R. (1983) *Cell* **35**, 275–283.
7. Igo, M. M. & Losick, R. (1986) *J. Mol. Biol.*, in press.
8. Tatti, K. M. & Moran, C. P., Jr. (1985) *Nature (London)* **314**, 190–192.
9. Doi, R. (1982) in *The Molecular Biology of the Bacilli*, ed. Dubnau, D. A. (Academic, New York), pp. 72–110.
10. Ollington, J. F., Haldenwang, W. G., Huynh, T. V. & Losick, R. (1981) *J. Bacteriol.* **147**, 432–442.
11. Trowsdale, J., Shiflett, M. & Hoch, J. A. (1978) *Nature (London)* **272**, 179–181.
12. Unnasch, N. E. L. (1982) Dissertation (Harvard University, Cambridge, MA).
13. Trempy, J. E., Morrison-Plummer, J. & Haldenwang, W. G. (1985) *J. Bacteriol.* **161**, 340–346.
14. Wiggs, J. L., Gilman, M. Z. & Chamberlin, M. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2762–2766.
15. Gilman, M. Z., Wiggs, J. L. & Chamberlin, M. J. (1981) *Nucleic Acids Res.* **9**, 5991–6000.
16. Wiggs, J. L. (1981) Dissertation (University of California, Berkeley).
17. Hoch, J. A., Trach, K., Kavamura, F. & Saito, H. (1985) *J. Bacteriol.* **161**, 552–555.
18. Trempy, J. E., LaBell, T. L., Ray, G. L. & Haldenwang, W. G. (1985) in *Molecular Biology of Microbial Differentiation*, eds. Hoch, J. A. & Setlow, P. (American Society for Microbiology, Washington, DC), pp. 162–169.
19. Binnie, C., Lampe, M. & Losick, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5943–5947.
20. Lampe, M., Igo, M., Schaffer, W., Binnie, C., Losick, R., Ray, C. & Moran, C. P. (1987) in *RNA Polymerase and the Regulation of Transcription*, eds. Reznikoff, W. S., Burgess, R., Dalberg, J., Gross, C., Record, T. & Whicker, M. (Elsevier Science, New York), in press.