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NOTES

The σ^E Subunit of *Bacillus subtilis* RNA Polymerase Is Present in Both Forespore and Mother Cell Compartments

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Bacillus subtilis **cells harvested 3.5 h after the onset of sporulation ($t_{3.5}$) were fractionated into extracts enriched in either mother cell or forespore components and were analyzed immunologically for σ^E and its precursor protein, P^{31}. We determined by Western blot (immunoblot) analysis that equivalent amounts of P^{31} and σ^E were present in both mother cell and forespore extracts. This result implies that, although σ^E is not synthesized until a stage in development when the cell is partitioned into progenitor forespore and mother cell compartments, it probably directs the transcription of genes that are expressed in both of these structures.**

Endospore formation by *Bacillus subtilis* is a simple developmental process that requires the activation of sporulation-specific genes in a precise temporal sequence. Although the mechanism by which sporulation genes are properly expressed is still unclear, novel RNA polymerase sigma factors are believed to be important elements of spore gene transcription (12). At least four sporulation-essential genes (*sigH*, *spoIIAC*, *sigE*, and *spoIIIG*) encode sigma-like proteins (2, 4, 6, 10, 17, 19; P. Stragier, Abstr. 10th Int. Spores Conf., abstr. 26; P. Setlow, Abstr. 10th Int. Spores Conf., abstr. 28). σ^E, the *sigE* gene product, is the best characterized of these proteins. One of the more intriguing aspects of σ^E is its complex and highly regulated mode of synthesis.

Synthesis of σ^E is controlled both at the level of transcription and by a posttranslational modification of the *sigE* gene product (9, 11). The primary translation product of *sigE* is not the sigma factor itself but rather an inactive precursor protein (P^{31}) (20). P^{31} is processed into σ^E by the removal of 29 amino acids from the P^{31} amino terminus (11). This reaction is catalyzed by a developmentally regulated proteolytic activity which is first detected in *B. subtilis* between 1 h (t_1) and 2 h (t_2) into sporulation (11, 21). The stage of development at which the processing of P^{31} occurs corresponds to that period during which the forespore septum is synthesized (1). This structure partitions the developing cell into progenitor mother cell and forespore compartments. It had previously been reported that a sporulation-specific RNA polymerase subunit (δ_1), which is probably identical to σ^E, is selectively associated with the RNA polymerase fraction that can be extracted from forespores but not from mother cells (13). If this is true, then it is possible that the coincidence of P^{31} processing with the formation of the spore septum is a consequence of a forespore-specific activity that only appears once compartmentalization occurs. Assuming that this notion is true, we would anticipate that P^{31}, whose synthesis begins before septation occurs, would be present in both mother cell and forespore compartments, while σ^E would be present only in the forespore fraction. To test this possibility, we separated sporulating *B. subtilis* into mother cell and forespore fractions and then assayed the resulting extracts for the presence of P^{31} and σ^E in a Western blot (immunoblot) assay.

B. subtilis SMY was grown in DS medium (16) and harvested 3.5 h after the end of the logarithmic growth phase. Although this is later in development than is ideal for visualizing P^{31} (20), it is the earliest stage at which forespores can be routinely isolated from mother cell extracts (3). Mother cell and forespore extracts were prepared by the protoplast disruption procedure described by Ellar and Posgate (3). The protocol was modified to employ a single centrifugation step (8,000 rpm for 15 min in a Sorvall SS-34 rotor) to separate the forespores from the mother cell extract. Forespore and whole-cell extracts were prepared by passing the resuspended forespore pellet or a whole-cell sample through a French pressure cell (20,000 lb/in^2).

As a measure of the efficiency of the fractionation protocol, the mother cell and forespore extracts were analyzed for glucose dehydrogenase and alkaline phosphatase. Glucose dehydrogenase is a forespore-specific enzyme (5), while alkaline phosphatase, a membrane-bound and secreted enzyme (7), is associated with the mother cell compartment (14). The forespore portion of our extract contained 90% of the total glucose dehydrogenase activity that was present in the preparation but only 25% of the alkaline phosphatase activity and 32% of the total protein (Table 1). On the basis of its glucose dehydrogenase content, it would appear that the fractionation procedure did in fact generate a component greatly enriched in forespore-specific materials. We view the persistence of alkaline phosphatase in the forespore extract as resulting from particulate mother cell debris copurifying with the forespore fraction. Microscopic examination of the forespore preparation prior to its disruption revealed no intact cells, only forespores and cell debris (data not shown). On the basis of these results, we conclude that our mother cell extract is depleted of forespore components and that our forespore extract, although probably contaminated with mother cell membrane and cell wall debris, is enriched for these materials.

To determine whether P^{31} or σ^E was predominantly in one or the other fraction, equivalent amounts of whole-cell, mother cell, and forespore extracts were fractionated by

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TABLE 1. Distribution of protein and enzymes in mother cell and forespore extracts[a]

Extract	Protein (%)	Glucose dehydrogenase (%)	Alkaline phosphatase (%)
Mother cell	68	9.2	75.4
Forespore	32	90.8	24.6

[a] Sporulating *B. subtilis* cells at $t_{3.5}$ were fractionated into crude mother cell and forespore extracts (3) and analyzed for their protein, glucose dehydrogenase, and alkaline phosphatase composition following dialysis against 10 mM Tris (pH 8.0)–1 mM EDTA–15 mM dithiothreitol. The content of each fraction is given as the percentage of the total protein or enzyme activity present in both extracts that is found in that fraction. Of the total protein present in the unfractionated extract, 60% was recovered in the extract fractions, as was 45% of the alkaline phosphatase activity and 160% of the glucose dehydrogenase activity. Apparently, the fractionation protocol enhanced the activity of the glucose dehydrogenase. The protein concentration was determined by using Coomassie (brilliant) blue (Bio-Rad Laboratories). Alkaline phosphatase was measured as described by Sterlini and Mandelstam (16) as modified by Nakayama et al. (14). Glucose dehydrogenase was assayed by the method of Fujita et al. (5).

sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with a monoclonal antibody that bound to both of these proteins (20). The results show that, regardless of whether equal amounts of protein (Fig. 1, lanes A to C) or equal extract fractions from the same volume of cell culture (lanes D to F) were analyzed, there was no obvious enrichment for σ^E in the forespore extract. Instead, the data reveal that the relative amount of σ^E in each extract parallels the protein concentration of the extract. P^{31} is too minor a component of the extract at $t_{3.5}$ to be readily observed in a Western blot prepared from our standard (100-μg) extract sample. Therefore, we increased the amount of protein used in our analysis fivefold to

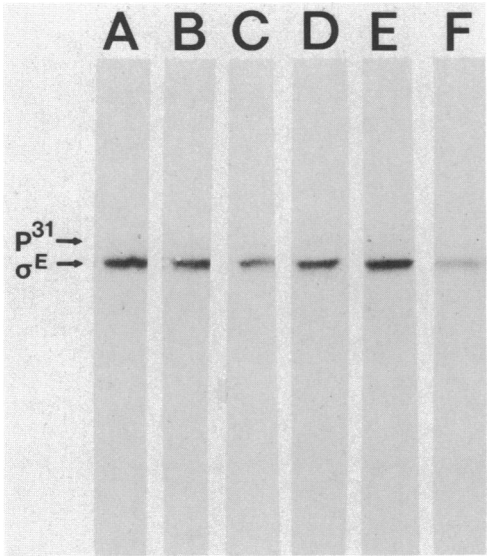

FIG. 1. Immunologic analysis of σ^E in mother cell and forespore extracts. Protein samples (100 μg) (lanes A to C) or total protein extracted from 1.5 ml of culture (lanes D to F) was electrophoresed through a sodium dodecyl sulfate-polyacrylamide gel (15% polyacrylamide), electrophoretically transferred to nitrocellulose, and probed with an anti-P^{31}/σ^E monoclonal antibody as described previously (21). Lanes: A and D, whole-cell extract; B and E, mother cell extract; C and F, forespore extract. Antigen-antibody complexes were visualized with a horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Hyclone Inc.).

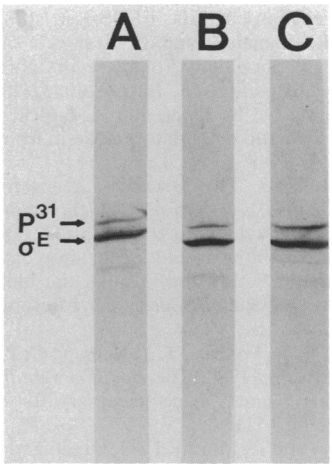

FIG. 2. Immunological analysis of P^{31} in mother cell and forespore extracts. Protein samples (500 μg) of whole cells (lane A), mother cells (lane B), or forespores (lane C) were electrophoresed and analyzed as described in the legend to Fig. 1.

visualize the P^{31}. This experiment shows that P^{31} is also present in similar amounts in each of the cell compartments (Fig. 2). Furthermore, the relative proportion of P^{31} to σ^E seems to be similar in each of the extracts that we analyzed. We conclude that P^{31} is likely to be synthesized and processed into σ^E to a similar degree in mother cell and forespore compartments.

The presence of σ^E in both the forespore and mother cell compartments reduces the probability that its role in sporulation could be limited to the activation of forespore-specific genes. It is more likely that the σ^E regulon will include genes expressed during an intermediate stage in sporulation in both mother cells and forespores. The coincidence of the time of septum formation with the appearance of the P^{31} processing, as well as the finding that several genes needed for normal septum synthesis are also essential for the processing of P^{31} (8, 11, 18, 21), has suggested to us and others that the physical presence of the septum might be a prerequisite for P^{31} processing (8, 11, 18). It has been hypothesized that the appearance of the septum acts as a developmental clock which signals the activation of σ^E by carrying within it the enzymatic machinery for P^{31} processing. If the septum does carry the processing activity, the data presented in this paper indicate that the processing machinery would be accessible from both sides of the septum membrane and that it would not be confined to one compartment.

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