

## Condensation of the Forespore Nucleoid Early in Sporulation of *Bacillus* Species

BARBARA SETLOW, NANCY MAGILL, PETER FEBBRORIELLO, LINA NAKHIMOVSKY,  
DENNIS E. KOPPEL, AND PETER SETLOW\*

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032

Received 9 April 1991/Accepted 25 July 1991

**Fluorescence microscopic examination coupled with digital videoimage analysis of 4',6-diamidino-2-phenylindole-stained sporulating cells of *Bacillus megaterium* or *Bacillus subtilis* revealed a striking condensation of the forespore nucleoid. While both mother cell and forespore compartments had equal amounts of DNA, the forespore nucleoid became >2-fold more condensed than the mother cell nucleoid. The condensation of the forespore nucleoid began after only the first hour of sporulation, 2 to 3 h before expression of most forespore-specific genes including those for small, acid-soluble spore proteins, and was abolished in *spo0* mutants but not in *spoil* or *spoilII* mutants. It is possible that this striking condensation of forespore DNA plays some role in modulating gene expression during sporulation.**

Sporulation in *Bacillus* species is characterized by the appearance of a number of new gene products at defined times during this differentiation process. This temporal pattern of gene expression is regulated in large part by ordered changes in the specificity of the cell's RNA polymerase through alterations in the pattern of associated sigma factors (10, 12). In addition to temporal control of gene expression, sporulation is characterized by spatial control of gene expression. A key event early in sporulation is the appearance of an asymmetric septum dividing the cell into large and small compartments, with the small cell destined to become the forespore which will be engulfed by the larger mother cell. As would be expected, given the morphological difference between mother cell and forespore compartments, there are different patterns of gene expression in the two compartments (20, 23). The differential gene expression in these compartments is also due, at least in part, to differences in RNA polymerase sigma factors, with one forespore-specific sigma factor ( $\sigma^G$ ) and one mother cell-specific sigma factor ( $\sigma^K$ ) identified to date (12, 23, 25). While this difference in sigma factors may explain much of the differential gene expression in the two compartments after the third hour ( $t_3$ ) of sporulation, it is not clear how this compartment-specific difference in sigma factors is established, i.e., how compartment-specific gene expression is initiated. One formal possibility is that there is some difference in the structure of the genomes in the forespore and mother cell. Surprisingly, this possibility has not been considered in recent years, despite early evidence, primarily from light microscopy, that the forespore nucleoid becomes significantly condensed during sporulation (15, 16, 28, 29). While forespore nucleoid condensation was clearly an early event in sporulation, this early work unfortunately did not precisely place the change within the time framework of the overall differentiation process, in particular with respect to synthesis of various sporulation-specific gene products. Consequently, we undertook an analysis of gross nucleoid structure during sporulation by using fluorescence microscopy of 4',6-diamidino-2-phenylindole (DAPI)-stained cells and have compared the times of changes in nucleoid struc-

ture with those for synthesis of sporulation-specific proteins such as alkaline phosphatase, glucose dehydrogenase, and small, acid-soluble spore proteins (SASP). Comparison of the kinetics of changes in forespore nucleoid structure and SASP synthesis was of particular interest, because SASP are extremely abundant, forespore-specific, DNA binding proteins which are known to, among other things, have striking effects on forespore DNA topology (13, 19). As a further aid in placing forespore nucleoid condensation within the overall framework of sporulation, we have analyzed the effects of a number of *spo* mutations on forespore nucleoid structure. As a result of all of these analyses, we conclude that a dramatic condensation of the forespore nucleoid takes place very early in sporulation, at or slightly before the asymmetric septation dividing mother cell and forespore, and that this nucleoid condensation is independent of SASP synthesis.

### MATERIALS AND METHODS

**Cell growth, enzyme assays, and DNA staining.** The strains used in this work were *Bacillus megaterium* QM B1551 and *Bacillus subtilis* 168 as well as strains of the latter species carrying asporogenous mutations, deletions of *ssp* genes, or an *sspA-lacZ* fusion. The sources of these strains were the *Bacillus* Genetic Stock Center or have been described previously (7, 11, 24). Sporulation was induced by nutrient exhaustion during growth either in supplemented nutrient broth (21) at 30°C (*B. megaterium*) or in 2× SG medium (5) at 37°C (*B. subtilis*). In some experiments with *B. subtilis*, sporulation was induced by the resuspension method (22). At various times, samples (1 ml) were centrifuged in a microcentrifuge, rinsed with 1 ml of saline-phosphate (0.15 M NaCl, 25 mM NaPO<sub>4</sub> [pH 7.0]), and suspended in saline-phosphate at an optical density at 600 nm of 0.2; 150 μl of electron microscopy-grade glutaraldehyde (25%) was added to 1 ml of cell suspension, and cells were allowed to fix overnight at room temperature. In some cases, multiple cell samples were harvested by centrifugation and extra samples were frozen for later extraction and assay of alkaline phosphatase, β-galactosidase (from an *sspA-lacZ* fusion), or glucose dehydrogenase as previously described (3, 11). Fixed cells were diluted 1/1 with the DNA stain DAPI (0.2 μg/ml in water) (2), and 1 ml was placed on a coverslip,

\* Corresponding author.

which had previously been coated with 0.01% polylysine, for 30 min and then air dried. After 30 min at room temperature, the 1 ml of liquid was removed, and the coverslip was rinsed by immersion in distilled water and allowed to air dry. A drop of saline-phosphate was placed on the dry coverslip, which was then placed on a microscope slide, and the edges of the coverslip were sealed with nail polish. In some experiments, cells were harvested, rinsed as described above, stained briefly (10 min) with DAPI without fixation, and examined immediately by fluorescence microscopy with untreated coverslips. In other experiments, 2  $\mu$ l of DAPI (1  $\mu$ g/ml) was mixed with 10  $\mu$ l of growing cells, and this mix was added to 90  $\mu$ l of supplemented nutrient broth in 0.65% low-gel-temperature agarose at 37°C. Small aliquots (~5  $\mu$ l) were placed on a prewarmed (37°C) slide, an untreated coverslip was added, and the slide was allowed to cool to room temperature prior to examination. In one experiment, DAPI (3  $\mu$ g/ml) was present throughout growth and sporulation in supplemented nutrient broth, and cells were examined directly by fluorescence microscopy. This concentration of DAPI had no detectable effect on growth or sporulation of *B. megaterium*. Microscope slides were viewed and photographed on a Nikon Diaphot-TMD fluorescence microscope equipped for epifluorescence. Excitation was in the UV range with an EX365 filter and a BA400 barrier filter. The fluorescence observed was blue, indicating that DNA was being stained (2).

In one experiment, nucleoids were isolated from sporulating cells of *B. subtilis* 168 by the procedure described by Guillen et al. (6). These nucleoids were centrifuged on a 10-ml sucrose gradient (20 to 60% in 0.2 M Tris-HCl [pH 8.2]) for 30 min at 17,000 rpm in an SW41 rotor. Fractions of 0.5 ml were collected, nucleic acid was detected by its  $A_{260}$ , and 50- $\mu$ l aliquots of nucleoid bands (in the middle of the gradient) were mixed with an equal volume of 0.5 M Tris-HCl (pH 6.8) and incubated at 12°C for 45 min with or without deoxyribonuclease II (85 U) (porcine spleen; Sigma Chemical Co.); samples were stained with DAPI and examined by fluorescence microscopy.

**Digital video image analysis.** Samples were mounted on the stage of an Ortholux II microscope (Wild-Leitz) and illuminated through the fluorescence-incident-light illuminator with a 100-W mercury lamp and a 360-DF40 interference filter (Omega Optical). Illumination periods were delimited with a computer-controlled electronic shutter. Fluorescence was collected with a 100 $\times$  immersion (numerical aperture, 1.32) objective, imaged onto the plane of a secondary field diaphragm mounted above the microscope, and reimaged onto the faceplate of a Dage-MTI, Inc., series 66 silicon-intensified-target camera equipped with the manual-gain and high-voltage control options. The field diaphragm above the microscope formed a border around the fluorescence images to provide an absolute black-level standard for the automatic black-level camera circuitry. The digitization, processing, and analysis of the video signal were performed with an RTI Station II imaging system (Recognition Technology, Inc.), with an IBM AT as the host computer.

Special-purpose software was written to automatically record the time series of uncorrected fluorescence images [ $F_u(r, t_n)$ ] in order to correct these images for background fluorescence, nonuniform illumination, and camera response. Each fluorescence image is a real-time average of 2<sup>8</sup> video frames. The computer-controlled shutter is closed during the time required to store each image on a disk, limiting sample illumination to the time of image integration. Only data within defined rectangular areas of interest are

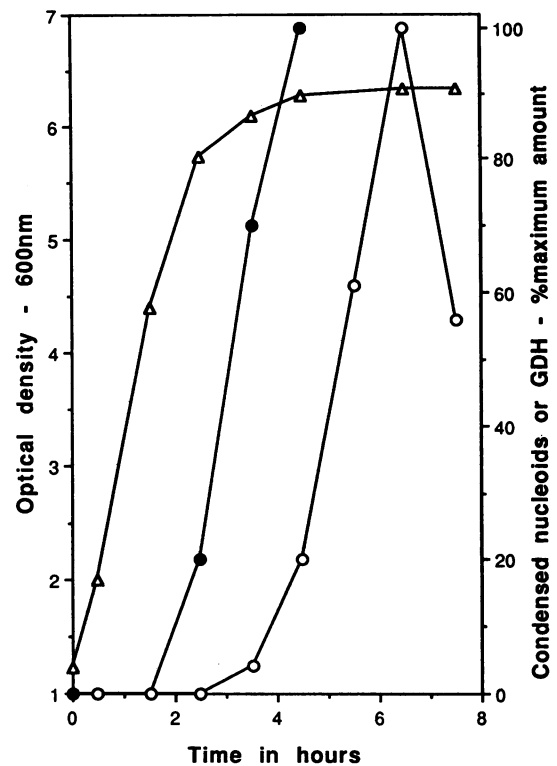


FIG. 1. Appearance of glucose dehydrogenase (GDH) and condensed nucleoids during growth and sporulation of *B. megaterium*. Cells were grown, harvested, and assayed for glucose dehydrogenase or fixed and stained as described in Materials and Methods. For determination of the percentage of cells with condensed nucleoids, fluorescence micrographs (Fig. 2) were examined and ~200 sporulating cells were scored for the presence of full or partially condensed nucleoids; this value reached a maximum of ~90%. Symbols:  $\Delta$ , optical density at 600 nm;  $\bullet$ , condensed nucleoids; and  $\circ$ , glucose dehydrogenase.

stored for subsequent analysis, making optimal use of disk storage space.

For correction purposes, three auxiliary images were recorded: a background image of a cell-free area on the same slide [ $F_b(r)$ ], an image of fluorescence from a thin uniform layer of fluorescent dye solution [ $F_d(r)$ ], and a black-level image taken without illumination [ $F_o(r)$ ]. The corrected fluorescence images were calculated as  $F(r, t_n) = (A [F_u(r, t_n) - F_b(r)] / [F_d(r) - F_o(r)] + B$ , where  $B$  is a constant offset (typically set to 25) added to eliminate possible negative numbers in background areas, which otherwise would show up on the video monitor as large positive values. Parameter  $A$  is a multiplicative constant set to make use of the full dynamic range of the digital frame stores. All subsequent quantitative analysis of the corrected images took constants  $A$  and  $B$  into account.

## RESULTS

Examination of DAPI-stained *B. megaterium* by fluorescence microscopy showed that early in sporulation, significantly condensed DNA appeared (Fig. 1 and 2c to f). This DNA condensation took place 2 to 2 1/2 h before the appearance of glucose dehydrogenase, a biochemical marker for stage III of sporulation (Fig. 1). In growing cells, the nucleoid was distributed throughout the cell, with a lobular

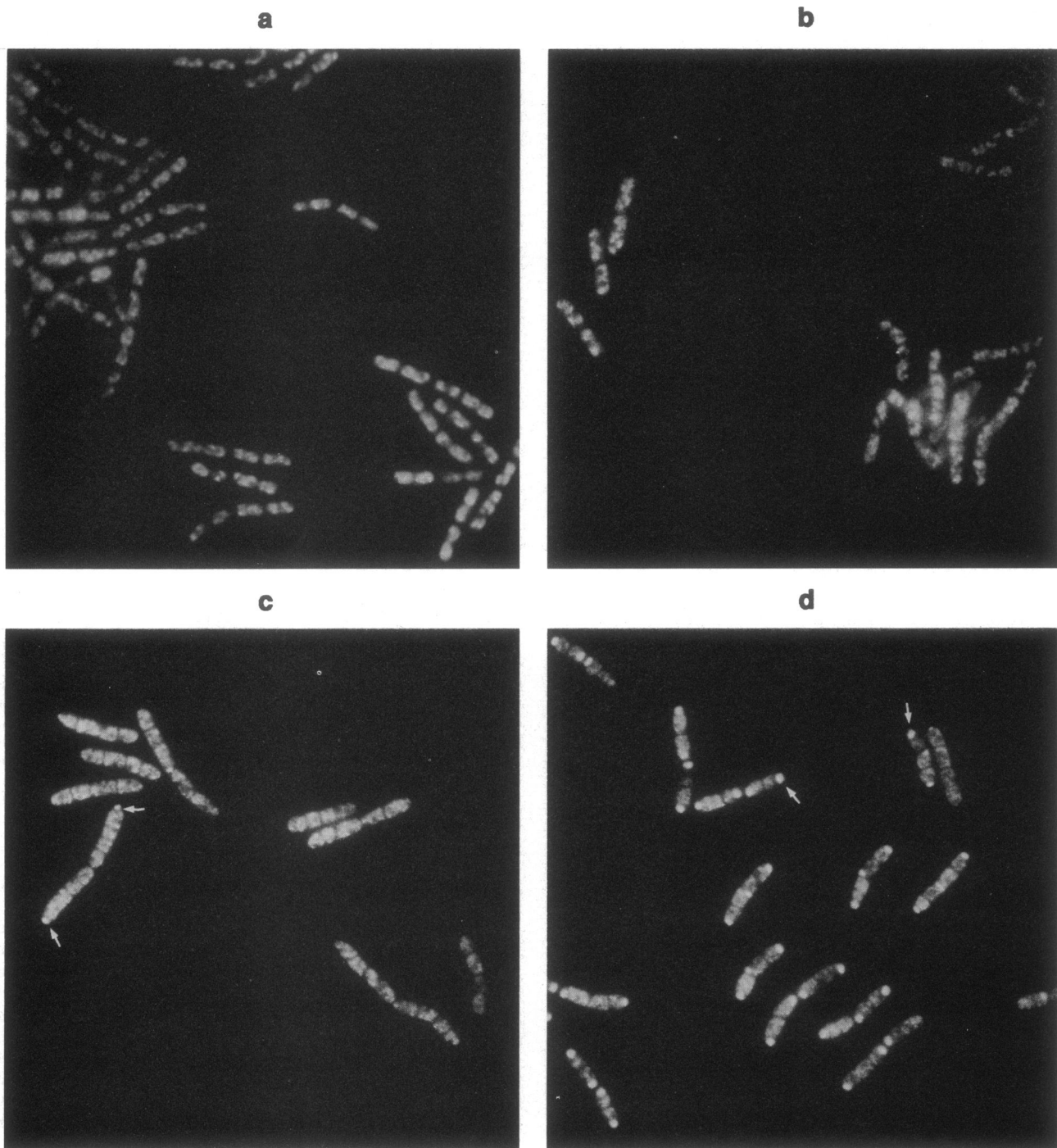


FIG. 2. Fluorescence micrographs of *B. megaterium* cells during growth and sporulation. Cells harvested at various times in sporulation were fixed, stained, and photographed as described in Materials and Methods. The magnification is  $1.8 \cdot 10^3$ , and the letters a to h above individual frames denote the times of harvest of cells grown as for Fig. 1. These times are as follows: a, 0.5 h; b, 1.5 h; c, 2.5 h; d, 3.5 h; e, 4.5 h; f, 5.5 h; g, 6.5 h; h, 7.5 h. The arrows in panels c and d denote the beginning of nucleoid condensation and condensed forespore nucleoids, respectively, as discussed in the text.

appearance similar to that seen in other bacteria (Fig. 2a and b) (8, 26). The DNA condensation began as small bright dots (arrows, Fig. 2c) which increased significantly in size to larger dots (arrows, Fig. 2d; Fig. 2e and f). Eventually, the

located became refractory to DAPI staining, presumably because the stain no longer penetrated into the maturing spore (Fig. 2f to h) (see below). As noted above, the condensed DNA was almost always seen at the outermost poles of cell pairs or at the outermost and innermost poles of

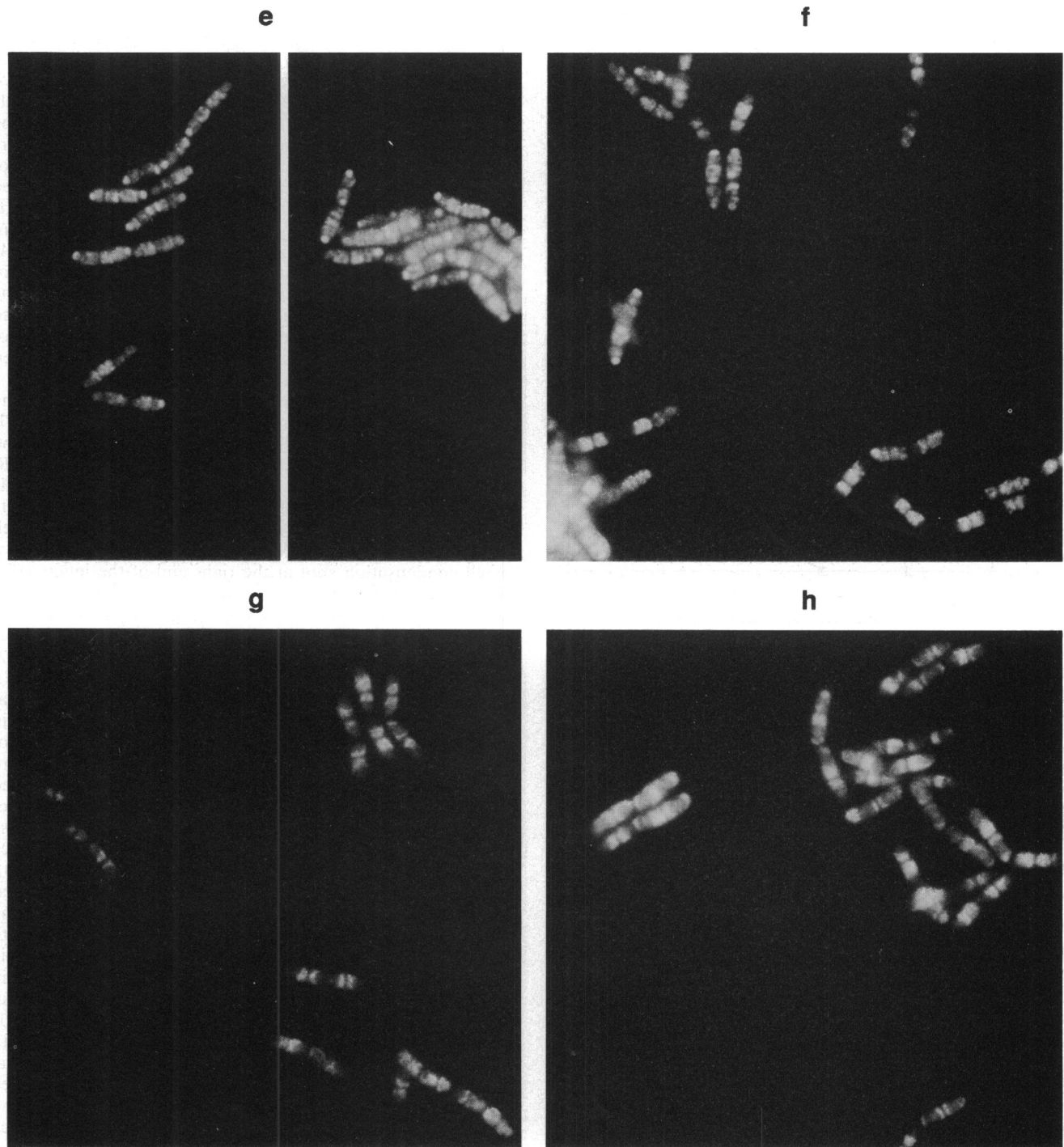


FIG. 2—Continued.

four cell chains. This spatial pattern is identical to that seen previously for the positioning of the forespore compartment (4). Indeed, examination of these slides by light microscopy revealed that the condensed DNA was always over the forespore compartment (data not shown) and presumably represents the forespore nucleoid. This condensed forespore nucleoid was also seen early in sporulation when unfixed cells were stained with DAPI just before fluorescence micro-

scopic examination or when cells were sporulated with DAPI and then examined without other treatment. Thus, the condensed DNA does not appear to be a fixation artifact.

A condensed forespore nucleoid also appeared during sporulation of *B. subtilis* by either nutrient exhaustion (data not shown) or the resuspension method (Fig. 3 and 4a to d); again, this condensed nucleoid disappeared as forespores matured and became impermeable (data not shown). As

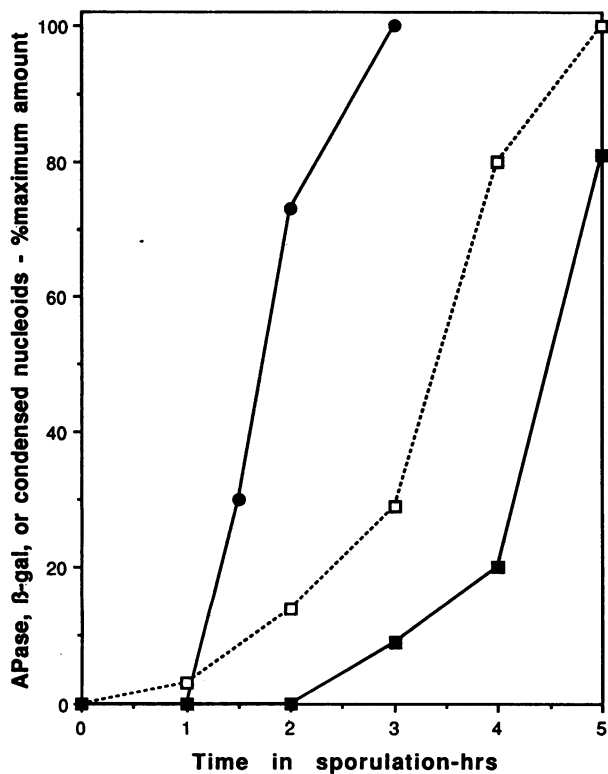


FIG. 3. Appearance of alkaline phosphatase (APase),  $\beta$ -galactosidase ( $\beta$ -gal), and condensed nucleoids during sporulation of *B. subtilis*, *B. subtilis* PS346 ( $Spo^+$ , *sspA-lacZ*) (11) was sporulated by the resuspension method, with  $t_0$  being the time of resuspension. At various times, samples were taken and assayed for alkaline phosphatase and  $\beta$ -galactosidase, fixed, and stained as described in Materials and Methods. For determination of the percentage of cells with condensed nucleoids, fluorescence micrographs (Fig. 4) were examined and  $\sim 200$  sporulating cells were scored for the presence of fully or partially condensed nucleoids. The value for the latter reached a maximum of 70%. Symbols:  $\bullet$ , condensed nucleoids;  $\square$ , alkaline phosphatase;  $\blacksquare$ ,  $\beta$ -galactosidase.

found with *B. megaterium*, the condensed DNA appeared early in *B. subtilis* sporulation, 1 to 2 h before alkaline phosphatase and 2 1/2 to 3 h before the appearance of  $\beta$ -galactosidase from an *sspA-lacZ* fusion (Fig. 3). The latter enzyme is known to increase in parallel with glucose dehydrogenase (11). Consequently, during sporulation of both *B. megaterium* and *B. subtilis*, forespore nucleoid condensation precedes glucose dehydrogenase synthesis by  $\sim 2$  1/2 h. Isolation of condensed nucleoids from  $t_3$  cells of *B. subtilis* allowed us to demonstrate that the condensed DAPI staining material was destroyed by DNase treatment (see Materials and Methods) (data not shown). As was the case with *B. megaterium*, condensed forespore nucleoids were also seen in unfixed DAPI-stained sporulating cells of *B. subtilis*.

We also took advantage of the large number of *B. subtilis* mutants blocked at specific points in sporulation to determine the *spo* gene dependence of forespore nucleoid condensation. Cells were grown in  $2\times$  SG medium, harvested, fixed, stained, and examined as described in Materials and Methods. Cells harvested at hourly intervals from  $t_1$  to  $t_6$  were examined. (See Fig. 4e to i for examples.) Condensation was blocked in all six *spo0* mutants (*spo0A12*, *spo0E11*, *spo0F221*, *spo0H75*, *spo0J93*, and *spo0K141*) tested but not

in any *spoII* or *spoIII* mutants (*spoIIAC1*, *spoIIIB131*, *spoIID66*, *spoIIE64*, *spoIIG55*, *spoIIIB2*, *spoIIIC295*, *spoIIE36*, and *spoIIIG::pPS1110*) (Fig. 4e to i). Strikingly, in several *spoII* mutants (*spoIIAC1*, *spoIIE64*, and *spoIIG55*) (Fig. 4g and h, arrows labeled d), many sporulating cells contained two condensed nucleoids at opposite poles of the cell, with little intervening staining material. This appears directly related to the formation in these *spo* mutants of asymmetric septa at both ends of the sporulating cell (termed an abortively disporic phenotype), with both small compartments containing DNA and the central larger compartment devoid of DNA (14, 27). The deletion of the *ssp* genes (*sspA*, *sspB*, and *sspE*) which code for the three abundant SASP of *B. subtilis* spores, two of which are major DNA binding proteins and affect forespore DNA topology (13, 19), also had no effect on forespore nucleoid condensation (data not shown). This is not surprising, since *ssp* genes are expressed in parallel with glucose dehydrogenase (11).

In order to obtain more quantitative information on the forespore nucleoid condensation, we also subjected DAPI-stained *B. megaterium* cells at various stages of growth and sporulation to digital video image analysis (Fig. 5). *B. megaterium* rather than *B. subtilis* was chosen for this analysis because the smaller size of the latter precluded its video image analysis with our system. Examination of a cell at the beginning stage of nucleoid condensation showed a small condensation spot at the right end of the image (Fig. 5A1) which is evidenced by a small peak in the two dimensional distribution of fluorescence (Fig. 5A2) and the topographical map of this distribution (Fig. 5A3). In cells in later stages of sporulation, the intensity of the condensed DNA was higher and the size was much larger (Fig. 5B and C). Examination of a cell even later in sporulation revealed that there was partial decondensation of the forespore nucleoid in some of the cells (Fig. 5D, right cell), although the amount of total fluorescence in this partially decondensed forespore nucleoid was the same as that in the fully condensed nucleoid (Fig. 5D) (data not shown). In some of the plots of the two-dimensional distribution of fluorescence from these partially decondensed forespore nucleoids, the fluorescence was lowest in the middle (Fig. 5D2 and data not shown), suggesting that the nucleoid may have a ringlike structure at this stage. While this conclusion stretches the limits of the technique used, conversion of the forespore chromosome to a ringlike structure has been seen by other microscopic analyses (4, 15, 28, 29). Analyses of the total fluorescence in the mother cell and forespore from cells in which full nucleoid condensation had taken place are given in Table 1. Note that both live and fixed cells were examined, with similar results. For all the cells examined, the average total fluorescence in the forespore ( $\langle\langle F_f \rangle\rangle$ ) and the mother cell ( $\langle\langle F_m \rangle\rangle$ ) and the ratio of these values were calculated. As a result of partial overlapping of the out-of-focus fluorescence in the forespore and mother cell compartments, the fluorescence of the forespore is underestimated and that of the mother cell is overestimated. This leads to a systematic  $\sim 10\%$  error (underestimation) in the ratio  $\langle\langle F_f \rangle\rangle / \langle\langle F_m \rangle\rangle$ ; hence, this ratio is very close to unity, implying that the number of chromosomes in the forespore and in the mother cell is the same. This has been implied earlier on the basis of other evidence (4, 15, 16).

The average image area (in pixels) of the forespore and mother cell nucleoids from which the fluorescence was measured is also given in Table 1, along with the ratio of the fluorescence densities in the forespore and mother cell (average total fluorescence per pixel). The systematic 10%

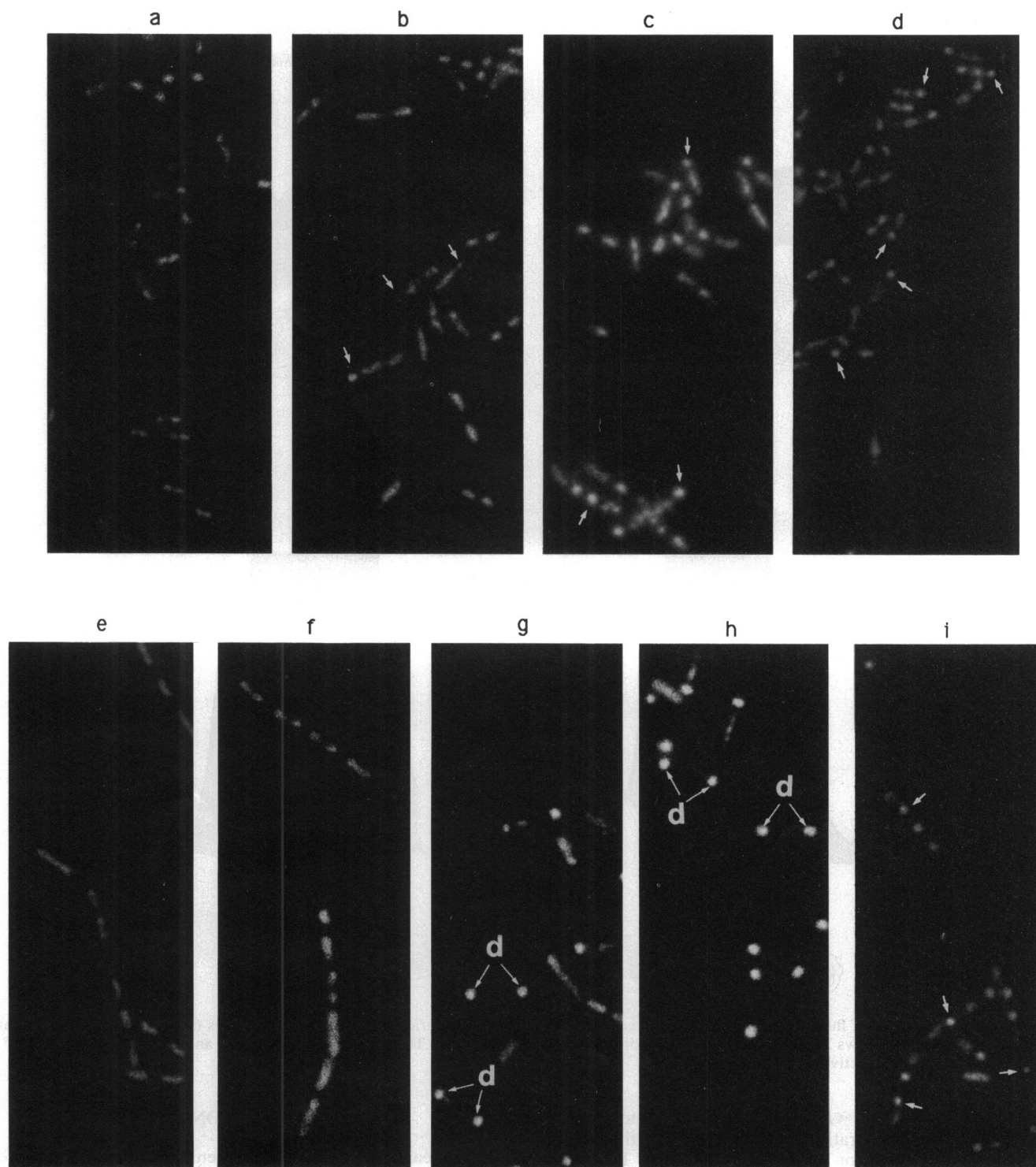


FIG. 4. Fluorescence micrographs of *B. subtilis* Spo<sup>+</sup> and Spo<sup>-</sup> strains during sporulation. Cells of strain PS346 (Spo<sup>+</sup>) (a to d) were harvested at  $t_0$  (a),  $t_{1.5}$  (b),  $t_2$  (c), and  $t_3$  (d) of sporulation as described in the legend to Fig. 3. Cells of asporogenous strains (e to i) were harvested at  $\sim t_4$  of sporulation by the nutrient exhaustion method; the strains in each panel are as follows: e, PS70 (*pheA1 spo0A trpC2*); f, PS67 (*pheA1 spo0H75 trpC2*); g, PS546 (*spoIIE64 trpC2*); h, PS1175 (*spoIIAC1 trpC2*); i, PS1129 (*spoIIIG::pPS1110*). Cells were harvested, fixed, stained, and photographed as described in Materials and Methods. The magnification is  $3.9 \cdot 10^3$  in panels a, b, c, d, e, f, and i; in panels g and h, it is  $4.5 \cdot 10^3$ . The arrows in panel b denote either condensed forespore nucleoids or the beginning of forespore nucleoid condensation. The arrows in panels c, d, and i denote condensed forespore nucleoids. The arrows in panels g and h labeled d point to two condensed forespore nucleoids at opposite ends of a single sporulating cell, with little if any intervening DNA, i.e., an abortively disporic cell.

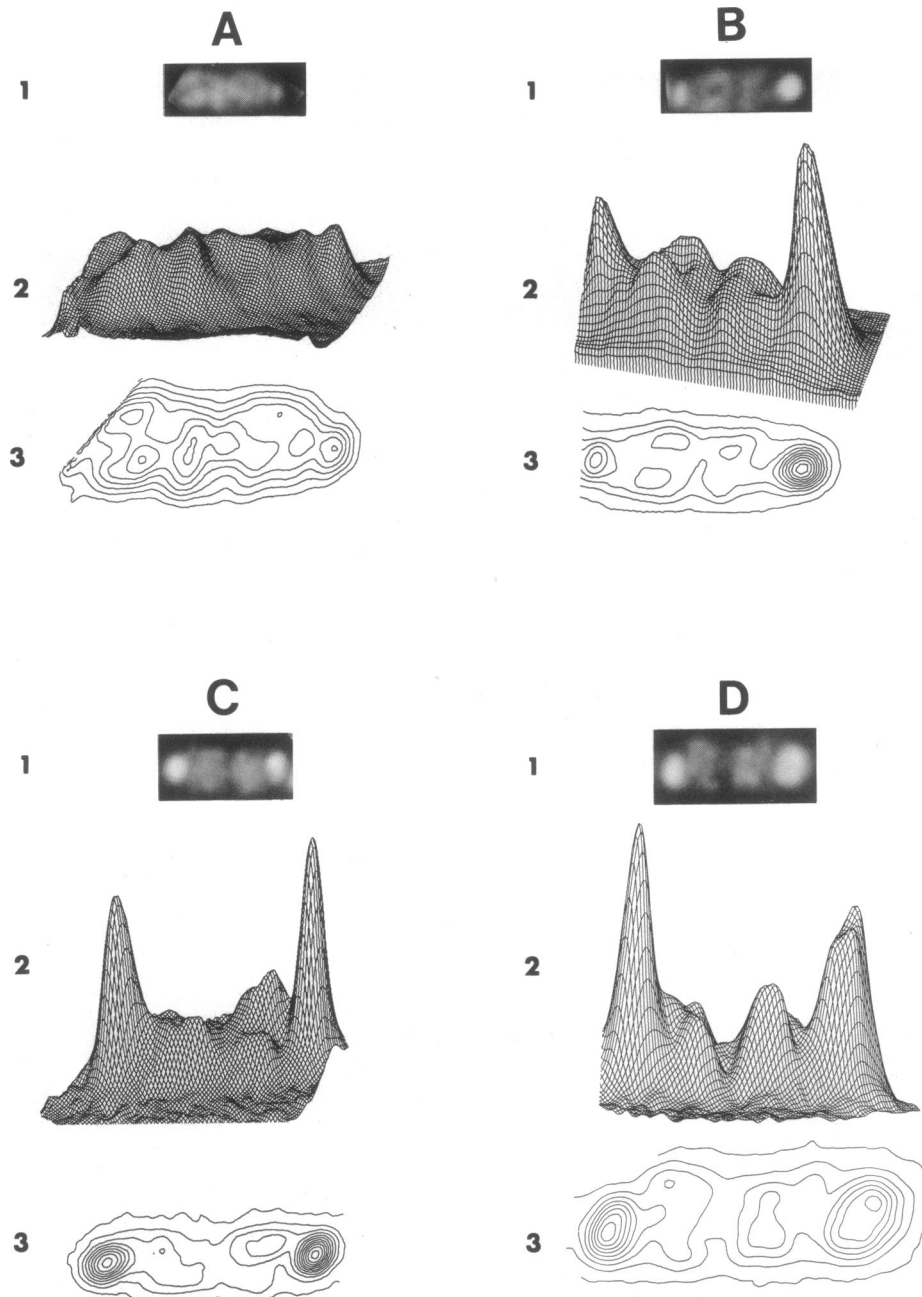


FIG. 5. Distribution of fluorescence intensity of fixed DAPI-stained *B. megaterium* cells. Individual images (rows 1) were analyzed for their two-dimensional (rows 2) or topological (rows 3) distribution of fluorescence. The cells in panels A, B, C, and D were harvested at 2.5, 3.5, 4.5, and 5.5 h, respectively, as shown in Fig. 1.

underestimation in the  $\langle F_f \rangle / \langle F_m \rangle$  ratio leads to a similar underestimation in the ratio of fluorescence densities; hence, a more correct value for the latter is about 1.6. This result shows that the amount of DNA per unit of volume of the nucleoid is on average 1.6 times higher in the forespore than in the mother cell. However, this value is only the average ratio. Examination of the topographical distributions of fluorescence (Fig. 5B and C) indicates that the maximum levels of fluorescence in the center of the nucleoid can be  $>2$  times higher in the forespore than in the mother cell, although later in sporulation, the maximum value approaches the average value of 1.6 (Fig. 5D).

#### DISCUSSION

It appears likely that the condensation of the forespore nucleoid early in sporulation may have major implications for gene expression in the forespore compartment. Consequently, it is crucial to definitively establish that our observation of this phenomenon in DAPI-stained cells is not an artifact. The first question that can be asked is whether the condensed DAPI staining material is indeed DNA. We believe it is DNA for at least six reasons. First, while DAPI can stain other cellular materials, in almost all cells which have been examined, DNA accounts for by far the majority

TABLE 1. DAPI fluorescence in the forespore and mother cell compartments of *B. megaterium*<sup>a</sup>

Compartment from:	Mean total fluorescence ( $\ll F \gg$ ) <sup>b</sup>	Mean ratio ( $\ll F/F_m \gg$ )	Mean nucleoid image area (A) (pixels)	Mean ratio ( $\ll A/A_m \gg$ )	Mean ratio of total fluorescence per pixel ( $\ll F_f \cdot A_m / F_m \cdot A_f \gg$ )
Mother cell	8,786 $\pm$ 1,186	0.88 $\pm$ 0.14 <sup>c</sup>	130 $\pm$ 26	0.62 $\pm$ 0.18 <sup>d</sup>	1.54 <sup>e</sup>
Forespore	7,673 $\pm$ 1,025		77 $\pm$ 14		

<sup>a</sup> Cells were grown, harvested at a time corresponding to 3.5 h in Fig. 1, stained, and analyzed as described in Materials and Methods. Values presented are the averages of data from 38 different cells, 10 of which were stained without prior fixation in low-temperature agarose and the remainder of which were stained after fixation.

<sup>b</sup> Arbitrary units.

<sup>c</sup> The value for unfixed cells was 0.85  $\pm$  0.13, and for fixed cells it was 0.90  $\pm$  0.14.

<sup>d</sup> The value for unfixed cells was 0.51  $\pm$  0.12, and for fixed cells it was 0.66  $\pm$  0.18.

<sup>e</sup> The value for unfixed cells was 1.76, and for fixed cells it was 1.46.

of the DAPI fluorescence (2). Second, as noted in Materials and Methods, the DAPI fluorescence from stained sporulating cells was blue, while the fluorescence from other molecules stained by DAPI is yellow to orange (2). Third, the general appearance of the putative vegetative cell nucleoid upon DAPI staining is very similar to that seen by other procedures, including confocal scanning light microscopy (8, 26). Fourth, we found equal amounts of DAPI fluorescence from both mother cell and forespore compartments at  $\sim t_2$  to  $t_3$  of sporulation. If DAPI stains both nucleoids equally (which is of course an assumption), then this indicates that both compartments have equal amounts of DAPI staining material, i.e., DNA, and other direct analyses have indicated that forespores and mother cells have similar amounts of DNA (4). Fifth, the condensed forespore DAPI staining material (as well as the less-condensed mother cell material) was destroyed by DNase. Finally, the presence of a highly condensed forespore chromosome has been seen previously (15, 16, 28, 29) when fixed sporulating cells of *Bacillus cereus* and *B. subtilis* were stained with one of several other cytological DNA stains. Furthermore, the presence in abortively disporic *spoII* mutants of two condensed forespore nucleoids at opposite ends of the cells with little intervening DNA, which was seen with DAPI staining in this work, has also been seen by using a different fixation and staining regimen for *B. cereus* (27).

A second question that should be asked is whether the condensed forespore nucleoid is an artifact of the fixation procedures used prior to staining. While cell fixation can yield artifactual morphological features, we do not believe that this is the case with the condensed forespore nucleoid for at least three reasons. First, the condensed forespore nucleoid has been observed by using a variety of different fixation procedures (15, 16, 27, 28, and this work). Second, we have observed the same condensed forespore nucleoid in *B. megaterium* and *B. subtilis* by DAPI staining of unfixed cells as well as in *B. megaterium* by growth and sporulation of cells with a low DAPI concentration and immediate fluorescence microscopic examination of these living cells. Finally, there is a strong a priori argument that forespore nucleoid condensation must take place at or just before completion of the asymmetric septum (see below).

As noted previously, the septum dividing the mother cell and forespore compartments is positioned asymmetrically. Indeed, analysis of electron micrographs of cells in which this septum is just or almost complete (4, 16) indicates that the forespore compartment initially encompasses only 15 to 20% of the volume of the sporulating cell. As noted above, both mother cell and forespore compartments have similar amounts of DNA, as is indicated by previous work as well as our data. Therefore, if the nucleoid in *B. subtilis* vegetative

cells and cells at  $t_0$  occupies a major fraction of the cell's volume (as is the case in *Escherichia coli* [8, 26] and appears to be the case from our DAPI fluorescence micrographs of *B. megaterium*), then the forespore nucleoid must be condensed relative to the mother cell nucleoid in order to be enclosed in the initial forespore compartment. This is in fact what is observed, and the slight decondensation of the forespore nucleoid as sporulation proceeds is consistent with the increasing forespore volume during this period (4, 16, 28).

If condensation of the forespore nucleoid is indeed a real event, two obvious questions are (i) what drives this process, and (ii) what is its possible significance? We do not know what drives forespore nucleoid condensation, but the process appears intimately associated with formation of the asymmetric septum, as all *spo* mutants which make the septum (*spoII* and *spoIII* mutants [14]) exhibit condensed nucleoids, while *spo0* mutants, which do not make the septum, do not. Interestingly, some *spoII* mutants, in particular *spoIIA*, *spoIIE*, and *spoIIG* mutants, often make two asymmetric septa at opposite ends of a single cell (14), and both small compartments have condensed nucleoids. Obviously, it would be most desirable to have a *spo* mutant which cannot form the asymmetric septum yet still can form condensed nucleoids. However, since it is likely that many of the genes required for these processes may also be needed for cell growth and division, such mutants may either be leaky, or if not leaky, may be lethal. A second part of the question of the mechanism of the forespore nucleoid condensation is how exactly the nucleoid is condensed. This process seems likely to involve proteins. While a number of putative chromosomal proteins in *B. subtilis* have been identified (1, 9, 17, 18), most have not been proven to be on the cell nucleoid in vivo, and essentially nothing is known about their function.

We also know nothing about the possible significance of forespore nucleoid condensation. However, it seems likely that this condensation has drastic effects on gene expression from the forespore chromosome, given the dramatic effects on gene expression associated with chromosome condensation and decondensation in higher-order organisms. One possibility is that forespore nucleoid condensation is a means of inactivating much of the forespore genome from  $t_1$  to  $t_3$  of sporulation (analogous to X chromosome inactivation in higher-order organisms), with subsequent nucleoid decondensation allowing selective activation of appropriate regions of the forespore chromosome. While this suggestion is only speculative at present, it adds an additional factor which may be involved in regulating forespore gene expression and certainly a factor worth further analysis.



## ACKNOWLEDGMENTS

This work was supported by a grant from the Army Research Office to P.S. and a grant from the National Institutes of Health (GM23585) to D.E.K.

We are grateful to Lori Tausta and Larry Klobutcher for assistance with the fluorescence microscopy.

## REFERENCES

- Brehm, S. P., F. LeHegar, and J. A. Hoch. 1974. Developmental modulations of deoxyribonucleic acid-binding proteins of *Bacillus subtilis* during sporulation stages. *J. Bacteriol.* **120**:1443-1450.
- Coleman, A. W., M. J. Maguire, and J. R. Coleman. 1981. Mithramycin- and 4',6-diamidino-2-phenylindole (DAPI)-DNA staining for fluorescence microspectrophotometric measurement of DNA in nuclei, plasmids, and virus particles. *J. Histochem. Cytochem.* **29**:959-968.
- Errington, J., and J. Mandelstam. 1983. Variety of sporulation phenotypes resulting from mutations in a single regulatory locus, *spoIIA*, in *Bacillus subtilis*. *J. Gen. Microbiol.* **129**:2091-2101.
- Freese, E. 1972. Sporulation of bacilli, a model of cellular differentiation. *Curr. Top. Dev. Biol.* **7**:85-124.
- Goldrick, S., and P. Setlow. 1983. Expression of a *Bacillus megaterium* sporulation-specific gene during sporulation of *Bacillus subtilis*. *J. Bacteriol.* **155**:1459-1462.
- Guillen, N., F. LeHegar, A.-M. Fleury, and L. Hirschbein. 1978. Folded chromosomes of vegetative *Bacillus subtilis*: composition and properties. *Nucleic Acids Res.* **5**:475-489.
- Hackett, R. H., and P. Setlow. 1988. Properties of spores of *Bacillus subtilis* strains which lack the major small, acid-soluble protein. *J. Bacteriol.* **170**:1403-1404.
- Hobot, J. A., M.-A. Bjornsti, and E. Kellenberger. 1987. Use of on-section immunolabeling and cryosubstitution for studies of bacterial DNA distribution. *J. Bacteriol.* **169**:2055-2062.
- Kimura, M., and K. S. Wilson. 1983. On the DNA binding protein II from *Bacillus stearothermophilus*. II. The amino acid sequence and its relationship to those of homologous proteins from other prokaryotes. *J. Biol. Chem.* **258**:4007-4011.
- Losick, R., and L. Kroos. 1989. Dependence pathways for expression of genes involved in endospore formation in *Bacillus subtilis*, p. 223-242. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
- Mason, J. M., R. H. Hackett, and P. Setlow. 1988. Regulation of expression of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* gene fusions. *J. Bacteriol.* **170**:239-244.
- Moran, C. P., Jr. 1989. Sigma factors and the regulation of transcription, p. 167-184. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
- Nicholson, W. L., and P. Setlow. 1989. Dramatic increase in the negative superhelicity of plasmid DNA in the forespore compartment of sporulating cells of *Bacillus subtilis*. *J. Bacteriol.* **172**:7-14.
- Piggot, P. J., and J. G. Coote. 1976. Genetic aspects of bacterial endospore formation. *Bacteriol. Rev.* **40**:908-962.
- Robinow, C. F. 1960. Morphology of bacterial spores, their development and germination, p. 207-248. *In* I. C. Gunsalus and R. Y. Stanier (ed.), *The bacteria*, vol. I. Academic Press, Inc., New York.
- Ryter, A. 1965. Etude morphologique de la sporulation de *Bacillus subtilis*. *Ann. Inst. Pasteur (Paris)* **108**:41-60.
- Salti, V., F. LeHegar, Y. Fontaine, and L. Hirschbein. 1989. Purification and properties of the DNA-binding protein HPB12 from the *B. subtilis* nucleoid. *Biochim. Biophys. Acta* **1009**:161-167.
- Salti, V., F. LeHegar, and L. Hirschbein. 1985. Isolation and characterization of small, heat-stable DNA-binding proteins from *Bacillus subtilis* nucleoids. *J. Gen. Microbiol.* **131**:581-590.
- Setlow, P. 1988. Small, acid-soluble spore proteins of *Bacillus* species: structure, synthesis, genetics, function, and degradation. *Annu. Rev. Microbiol.* **42**:319-338.
- Setlow, P. 1989. Forespore-specific genes of *Bacillus subtilis*: function and regulation of expression, p. 211-222. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
- Setlow, P., and A. Kornberg. 1969. Biochemical studies of bacterial sporulation and germination. XVII. Sulfhydryl and disulfide levels in dormancy and germination. *J. Bacteriol.* **100**:1155-1160.
- Sterlini, J. M., and J. Mandelstam. 1969. Commitment to sporulation in *Bacillus subtilis* and its relationship to development of actinomycin resistance. *Biochem. J.* **113**:29-37.
- Stragier, P. 1989. Temporal and spatial control of gene expression during sporulation: from facts to speculations, p. 243-254. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
- Sun, D., R. M. Cabrera-Martinez, and P. Setlow. 1991. Control of transcription of the *Bacillus subtilis* *spoIIIG* gene, which codes for the forespore-specific transcription factor  $\sigma^G$ . *J. Bacteriol.* **173**:2977-2984.
- Sun, D., P. Stragier, and P. Setlow. 1989. Identification of a new  $\sigma$ -factor involved in compartmentalized gene expression during sporulation of *Bacillus subtilis*. *Genes Dev.* **3**:141-149.
- Valkenburg, J. A. C., C. L. Woldringh, G. J. Brakenhoff, H. T. M. van der Voort, and N. Nanninga. 1985. Confocal scanning light microscopy of the *Escherichia coli* nucleoid: comparison with phase-contrast and electron microscopic images. *J. Bacteriol.* **161**:478-483.
- Young, I. E. 1964. Characteristics of an abortively disporic variant of *Bacillus cereus*. *J. Bacteriol.* **88**:242-254.
- Young, I. E., and P. C. Fitz-James. 1959. Chemical and morphological studies of bacterial spore formation. I. The formation of spores in *Bacillus cereus*. *J. Biophys. Biochem. Cytol.* **6**:467-482.
- Young, I. E., and P. C. Fitz-James. 1989. Chemical and morphological studies of bacterial spore formation. II. Spore and parasporal protein formation in *Bacillus cereus* var. *Alesti*. *J. Biophys. Biochem. Cytol.* **6**:483-506.