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Expression of AbrB, a Transition State Regulator from *Bacillus subtilis*, Is Growth Phase Dependent in a Manner Resembling That of Fis, the Nucleoid Binding Protein from *Escherichia coli*

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The transition state regulator AbrB functions as an activator, a repressor, and a preventer of gene expression in *Bacillus subtilis*. In this paper, we show that expression of *abrB* is growth phase dependent. Accumulation of *abrB* transcript is restricted to a short period spanning the transition between the lag and exponential phases of the growth cycle. The level of *abrB* transcript then falls sharply, and transcript cannot be detected at the mid-exponential period of the growth cycle. The level of AbrB protein is also maximal during early exponential growth but decreases gradually throughout the remainder of the growth cycle. The abrupt reduction of *abrB* transcript level during the early period of the growth cycle is effected by the phosphorylated form of the response regulator Spo0A and to a lesser extent by negative autoregulation. The growth cycle-dependent expression of *abrB* is very similar to that observed for *fis* in *Escherichia coli* and in *Salmonella typhimurium*. Although AbrB and Fis are not homologous proteins, they display extensive similarity in terms of size, DNA binding characteristics, growth cycle-dependent patterns of expression, and their control over the expression of a varied group of operons. We hypothesize therefore that AbrB, like Fis, is a nucleoid binding protein.

Under conditions of nutrient excess, bacterial cell number increases exponentially with the cellular metabolism being directed towards production of the structural components required for cell growth and division. When the supply of nutrients is poor, cell growth and division are reduced to a very low level and the cellular metabolism is directed towards cell survival. The survival strategy adopted by each bacterial species depends on the environmental niche it occupies. In the case of *Bacillus subtilis*, a soil-dwelling bacterium, the cells (i) secrete a variety of enzymes, such as proteases, amylases, and glucanases; (ii) produce a range of antibiotics; (iii) become motile; and (iv) develop the ability to take up DNA from the medium (for a review of these topics, see reference 40). These processes are characteristic of stationary-phase cells and are not observed in cells growing exponentially. It is evident, therefore, that the transition between these distinct physiological states must involve very significant changes in gene expression.

A number of regulatory proteins (e.g., AbrB, Hpr, Sin Sen, Ten, and Pai) which control gene expression in a growth cycle-dependent manner have been identified in *B. subtilis*. They are called transition state regulators because of their role in regulating gene expression during the transition between the exponential and stationary phases of the growth cycle (33, 47; for a review, see reference 46). The cellular levels of AbrB (antibiotic resistance), Hpr (hyperproduction), and Sin (sporulation inhibition) are responsive to the prevailing environmental and nutritional conditions through Spo0A-mediated control of *abrB* expression. Spo0A is a response regulator protein whose activity is controlled by phosphorylation (18, 19, 41). Spo0A~P negatively regulates expression of *abrB*, while AbrB both negatively autoregulates its expression and positively regulates expression of *hpr* (31, 47, 49). In addition, Spo0A~P, AbrB, and Hpr regulate expression of *sin* (19, 39). Therefore, the activity of each transition state regulator is responsive both to the prevailing nutritional and environmental conditions and to the levels of the other transition state regulators. Through interaction between the transition state regulators, a complex web of regulatory activity is established which controls gene expression in the physiological states characteristic of the exponential and stationary phases of the growth cycle and also regulates the transition between these states.

The number and variety of genes and cellular processes controlled by transition state regulators are an interesting feature of this group of genes. A compilation of the cellular processes in which transition state regulators participate is presented by Strauch and Hoch (46) and includes (i) production of proteases, phosphatases, amylases, nitrogen-utilizing enzymes, transport systems, autolysins, and antibiotics and (ii) processes such as oxidative stress, competence development, catabolite repression, motility, and sporulation. Three modes of regulation have been established for AbrB (46). Expression of *tycA* (tyrocidine synthetase), *spo0E* (a Spo0A phosphatase), and *spo0H* (a sigma factor) is constitutive in *abrB* null mutants, indicating that AbrB functions as a simple repressor of these genes (12, 26, 32, 53). AbrB is a positive regulator of *hpr*, *hut*, *rbs*, and *argC-F* expression (10, 29, 31, 43) and of some genes for competence development (17). A third mode of AbrB regulation is that of preventer (42, 46). This mode of regulation can be demonstrated genetically: (i) in the presence of high AbrB levels (in *spo0A* mutants), gene expression is repressed during the stationary phase of the growth cycle, and this phenotype can be suppressed by mutation of the *abrB* gene; (ii) in the absence of AbrB (in *abrB* null mutants), a normal pattern of gene expression is observed. Expression of *spoVG* (a sporulation gene), *aprE* (a protease), and *dpp* (a dipeptide transport system) is controlled by AbrB in this manner (7, 38, 57). Expression of these genes therefore is subject to other forms of regulation which are epistatic to the control exerted by AbrB. The preventer role of AbrB is viewed as a regulatory mechanism to safeguard exponentially growing cells from expressing genes, and manifesting cellular processes, which are characteristic of the stationary-phase physiological state.

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TABLE 1. Strains and plasmids

Strain or plasmid	Genotype or description	Source or reference
Strains		
E. coli tg1	K-12 Δ(*lac pro*) *supE thi hsdR* F′ *traD36 proAB lacI lacZ*ΔM15	Amersham
B. subtilis		
JH642	*trpC2 pheA1*	BGSC[a]
JH649	*trpC2 pheA1 spo0F221*	BGSC
JH703	*trpC2 pheA1 spo0A*Δ204	J. A. Hoch
JH13311		J. A. Hoch
KD913	*trpC2 pheA1 abrB*Δ205	This work
KD914	*trpC2 pheA1 spo0A*Δ204 *abrB*Δ205	This work
Plasmids		
pGEM-7Zf(+)	ColE1-derived cloning vector.	Promega, Madison, Wis.
pG$^+$host4	Temperature-sensitive derivative of pWV01; Em^r	Biswas et al. (4)
pABRB2	pGEM-7Zf(+) with the 5′ region of *abrB* cloned into the *Sma*I site	This work
pABRB4	pABRB2 with the 3′ region of *abrB* cloned into the *Cla*I-*Bam*HI site	This work
pG$^+$hostΔ205	The juxtaposed *abrB* fragments cloned in pABRB4 were excised on an *Eco*RI-*Cla*I fragment and cloned into pG+host4	This work

[a] BGSC, *Bacillus* Genetic Stock Center, Ohio State University, Columbus.

It has been established that AbrB, Hpr, and Sin are DNA binding proteins (14, 21, 48). AbrB is hexameric in solution and contains a single helix-turn-helix motif located in the C terminus of the protein (48). Gel retardation experiments suggest that AbrB binds cooperatively to DNA and probably only to one face of the helix (13, 48). Footprinting experiments show that AbrB can protect a larger region of DNA than is found for conventional DNA binding proteins. The binding of AbrB to the promoter of the *abrB* gene, for example, protects a region of approximately 105 bases (48). The promoters to which AbrB binds are AT rich, but only some have an intrinsic bend in the DNA (44, 45). A degenerate consensus WAWWT TTWCAAAAAAW (W = A/T) has been derived from 20 binding sites located within the promoter regions of 14 genes (44). In contrast, the optimal AbrB binding site has multiple TGGNA sequences (N = any base) with spacings of 4 or 5 bp (55). It is very interesting, therefore, that none of the 20 in vivo sites identified within promoter regions are optimal for binding of AbrB.

It is clear that AbrB regulates expression of a wide variety of genes which perform very diverse functions within the cell. The role played by AbrB will depend both on *abrB* expression levels and on the levels of protein which persist throughout the growth cycle. In this study, we have examined the control of *abrB* expression and estimated the accumulated levels of *abrB* mRNA and protein throughout the growth cycle. Our results show that *abrB* mRNA accumulates maximally during the lag and very early log stages of the growth cycle and declines sharply to undetectable levels before the mid-exponential phase of the growth cycle. The maximal level of AbrB protein is approximately coincident with the maximal transcript level and then declines gradually throughout the growth cycle. This expression pattern is very similar to that of *fis* in *Escherichia coli* and *Salmonella typhimurium*. Although they are not homologous proteins, AbrB and Fis display many similarities, and we hypothesize that AbrB is, like Fis, a nucleoid binding protein.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Strains KD913 and KD914, carrying the partially deleted *abrB* gene, were constructed as follows. Primer MOR20 (5′GCTGTTATTTCGGTAGTTTCC3′) and primer MOR19 (5′CACGTCCTAATTCATCAAC3′) were used to amplify a fragment of 231 bp which contains the promoter region of *abrB* and extends 52 bases (the first 17 codons) into the structural gene. This was cloned into the *Sma*I site of pGEM7 to give the plasmid pABRB2. Primers MOR22 [5′(ATCGAT)CGAAATCCAAAACCAGC3′] and MOR23 [5′(GGATCC)AAGCGTGAAGTGTACG3′] were used to amplify a 133-bp fragment containing the last 10 codons of the gene and the terminator (flanked by *Cla*I and *Bam*HI sites) which was directionally cloned into pABRB2 to give plasmid pABRB4. The juxtaposed 5′ and 3′ ends of the *abrB* gene were excised from pABRB4 on an *Eco*RI-*Bam*HI fragment and cloned into pG$^+$host4 to give pG$^+$hostΔ205. Strains carrying a deleted *abrB* gene were constructed by using this plasmid by the method described by Biswas et al. (4). Plasmid pG$^+$hostΔ205 was transformed into strains JH703 and JH642, and integrants were selected by growth on erythromycin (5 $\mu g \cdot ml^{-1}$) at 37°C. Plasmid excision was stimulated by growth of the integrants at 28°C and screening for erythromycin sensitivity. Erythromycin-sensitive strains were screened by using PCR to detect excision events which resulted in partial deletion of the *abrB* gene. Therefore, the *abrB* gene in strains KD913 and KD914 has 69 codons missing (between nucleotides 52 and 257) from the middle of the structural gene. *B. subtilis* strains were grown in SM liquid medium (37). Solid medium was made with SM containing 1.5% agar (Difco). To investigate expression of *abrB* during the transition between the lag and exponential phases of the growth cycle, overnight cell cultures were diluted 100-fold and growth was monitored subsequently by measuring CFU.

Primer extension. RNA preparation and primer extension analysis were performed as described by O'Reilly et al. (29). The autoradiograms were scanned by using a Bio-Rad model GS-670 imaging densitometer and molecular analyst software.

Bacterial transformation. *B. subtilis* transformation was performed according to the method described by Anagnostopoulos and Spizizen (1). *E. coli* transformations were carried out as described by Sambrook et al. (36).

Western blot (immunoblot) analysis. Extracts were prepared from bacterial cells harvested at the designated periods of the growth cycle. Cells were boiled for 10 min in 1× sample buffer containing 0.1% sodium dodecyl sulfate (SDS) and 10 mM dithiothreitol. Cell extracts were resolved on SDS–15% polyacrylamide gels and transferred to an Optitran reinforced nitrocellulose (Schleicher and Schuell, Dassel, Germany) membrane as detailed by Sambrook et al. (36). Western analysis was carried out as described by Sambrook et al. (36). The membrane was incubated in 10 ml of blocking buffer to which 5 μl of polyclonal rabbit antiserum raised against purified AbrB protein was added (a kind gift from Mark Strauch, Scripps Clinic and Research Foundation). After being washed, the filter was incubated with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.). The AbrB protein was then visualized by incubation with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (BCIP) tablets (SIGMA*FAST*; Sigma Chemical Co.).

RESULTS

Initiation of *abrB* transcription. Expression of *abrB* was examined by primer extension analysis. One reverse transcript which initiates at the T residue indicated in Fig. 1 was ob-

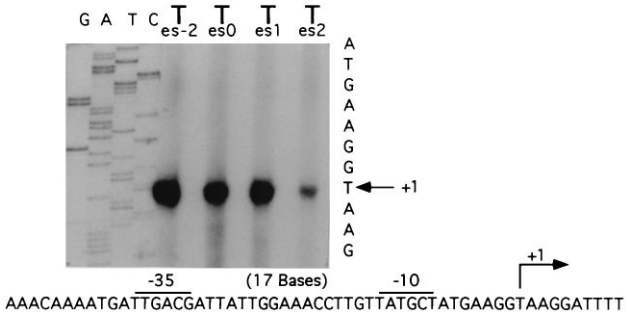

FIG. 1. Primer extension analysis of *abrB* transcripts in JH703 during the growth cycle. The single reverse transcript is that obtained with 12.5 μg of total RNA prepared from cells harvested at T_{es-2}, T_{es0}, T_{es1}, and T_{es2} of the growth cycle. Time is expressed relative to T_{es0} (the point of transition between the exponential and stationary phases of the growth cycle). The sequencing ladder was generated by using the same primer as that used for primer extension, and the individual reactions are labelled G, A, T, and C. Part of the sequence upstream of *abrB* is shown beside the autoradiogram, and the initiation point of transcription (+1) is indicated (arrow). The full SigA promoter sequence (33) is shown below the autoradiogram; the putative −35 and −10 regions are overlined, and the spacing between them is shown in parentheses.

served. This is preceded by a sequence which conforms to a consensus SigA promoter. This promoter and initiation point of transcription correspond to the P2 promoter identified by Perego et al. (33). Transcription of *abrB* initiates at this nucleotide at all stages of the growth cycle and in all strains examined. Therefore, it is concluded that under the present growth conditions, *abrB* is transcribed from a single SigA-type promoter.

Expression of *abrB* during the exponential and stationary phases of the growth cycle. Expression of *abrB* was examined by primer extension analysis during the exponential and stationary phases of the growth cycle (Fig. 2A). Time is measured relative to T_{es0}, the time of transition between the exponential and stationary phases of the growth cycle. In the wild-type strain JH642, the level of accumulated *abrB* mRNA is high during the early exponential phase (T_{es-3}) of the growth cycle (Fig. 2A). However, the level decreases sharply between the early and mid-exponential phases (T_{es-3} and $T_{es-1.5}$, respectively) and remains at this low level for the remainder of the growth cycle. It has been established previously that expression of *abrB* is negatively regulated both by AbrB and by Spo0A (47, 49). To assess the contribution of each regulator to the control of *abrB* expression during the growth cycle, the level of *abrB* transcript was established in strains KD913 (*abrB*Δ205), JH703 (*spo0A*Δ204), and KD914 (*spo0A*Δ204 *abrB*Δ205). There is a high and uniform level of accumulated *abrB* mRNA in strain KD914 at all stages of the growth cycle (Fig. 2A). It is noteworthy that the level of *abrB* transcript in strain KD914 at T_{es-3} is higher than that observed in the wild-type strain at a similar stage of the growth cycle. The level of accumulated transcript in strain KD913 at T_{es-3} is approximately the same as that observed in the wild-type strain at this time. However, the level in KD913 at $T_{es-1.5}$ is substantially higher than that observed in the wild-type strain at a similar stage of the growth cycle. No *abrB* transcript could be detected in strain KD913 between T_{es0} and T_{es2} of the growth cycle, similar to the result found for the wild-type (JH642) strain (Fig. 2A). The level of accumulated *abrB* mRNA in JH703 at T_{es-3} is high and similar to that observed in strain KD914 at this time of the growth cycle (Fig. 2A). While a decrease in the level of *abrB* transcript

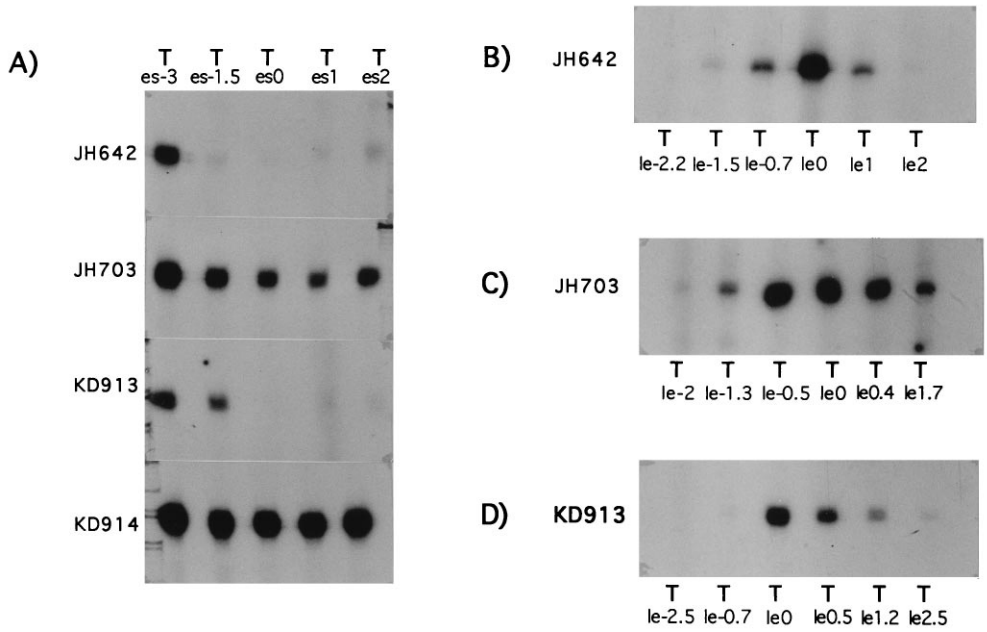

FIG. 2. Measurement of *abrB* transcript accumulation during the growth cycle of *B. subtilis*. (A) Levels of *abrB* transcript observed in strains JH642 (wild type), JH703 (*spo0A*Δ204), KD913 (*abrB*Δ205), and KD914 (*spo0A*Δ204 *abrB*Δ205) during the exponential and stationary phases of the growth cycle. The amount of reverse transcript is that obtained from 12.5 μg of total RNA isolated from cells at various times of the growth cycle. Time is measured relative to T_{es0} of the growth cycle: T_{es-3}, 3 h pretransition/early exponential phase; $T_{es-1.5}$, 1.5 h pretransition/mid-exponential phase; T_{es0}, transition between exponential and stationary phases; T_{es1}, 1 h posttransition; and T_{es2}, 2 h posttransition. (B to D) Levels of *abrB* transcript observed during the lag and early exponential stages of the growth cycle for strains JH642, JH703, and KD913, respectively. For these periods of the growth cycle, the amount of reverse *abrB* transcript is that obtained by using the amount of total RNA isolated from 5×10^7 cells at each time point. Time is measured relative to the point of transition between the lag and exponential phases (T_{le}) of the growth cycle. The first sample of each time course was taken at the point of dilution of a stationary-phase culture into fresh medium.

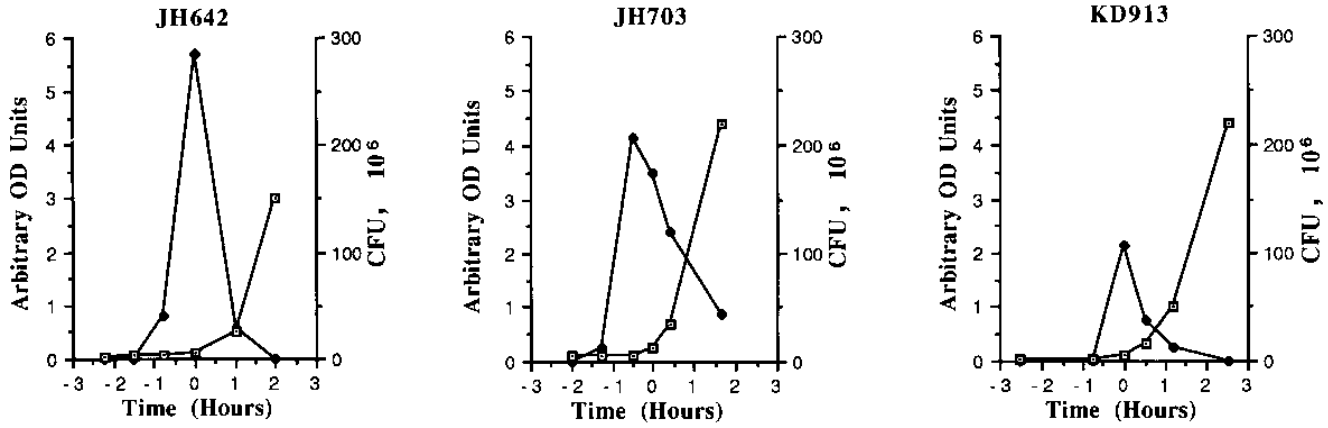

FIG. 3. Quantitation of the level of *abrB* transcript during the lag and early exponential phases of the growth cycle. The autoradiograms shown in Fig. 2B to D were quantitated as described in Materials and Methods, and the level of *abrB* transcript was expressed in arbitrary optical density (OD) units. Cell growth was monitored by measuring the number of CFU. Time was measured relative to $T_{le}0$, which we define as the time at which an increase in CFU is first observed. ◆, level of *abrB* transcript; ⊡, CFU.

can be discerned in JH703 cells during the course of the growth cycle, the level remains very substantially higher than those observed in strains JH642 and KD913. However, they are not as high as those found in KD914 cells during the latter stages of the growth cycle. These data demonstrate that the level of *abrB* transcript is maximal during the early exponential phase of the growth cycle and that the growth cycle-dependent pattern of *abrB* transcription is accomplished through negative regulation both by Spo0A and by AbrB. Spo0A regulates *abrB* expression at most stages of the growth cycle examined, whereas autoregulation is observed only during the mid-exponential stage of the growth cycle.

Expression of *abrB* during the lag and exponential stages of the growth cycle. Two intriguing features of the pattern of *abrB* expression are the high level of transcript in cells during the early exponential phase of the growth cycle and the abrupt decrease in transcript level which occurs between the early and mid-exponential stages of the growth cycle. To further investigate these features, the pattern of accumulated *abrB* transcript was determined from the time of inoculation of cells into fresh medium up to the early exponential phase of the growth cycle in strains JH642, JH703, and KD913. Five milliliters of an overnight culture was diluted 100-fold in fresh medium, and cell growth was monitored by determining CFU. The amount of total RNA per cell varies between the lag and exponential stages of the growth cycle (reference 3 and unpublished results). Therefore, the level of *abrB* transcript was determined in a constant number of cells (5×10^7) harvested from the lag and early exponential stages of the growth cycle. Time is measured relative to T_{le0} (the point of transition between the lag and exponential phases of the growth cycle), which we define as the time at which an increase in CFU is first observed. The autoradiographs obtained from these experiments are shown in Fig. 2B to D. A quantitation of the data from Fig. 2B, C, and D is shown in Fig. 3. Accumulation of *abrB* transcript in JH642 cells is confined to a short period spanning the transition between the lag and exponential phases of the growth cycle (Fig. 2B and 3). No *abrB* transcript was detected at $T_{le-2.2}$, while the level at $T_{le-1.5}$ was barely detectable. The level of transcript increases significantly at $T_{le-0.7}$, and the maximal level attained was observed at T_{le0}. The level of transcript at $T_{le1.0}$ is approximately the same as that observed at $T_{le-0.7}$, and a further decrease to an undetectable level is evident at $T_{le2.0}$ (at this point, the cells have reached an optical density at 550 nm of 1.0, the mid-exponential phase of the growth cycle). In strain JH703, transcript is first detectable at $T_{-1.3}$ and has increased to the maximal level at $T_{le-0.5}$ (Fig. 2C and 3). Although a reduction in the level of transcript was observed both at T_{le0} and $T_{le0.4}$, a very significant level of transcript is still present at $T_{le1.7}$ (the mid-exponential point of the growth cycle). In strain KD913, *abrB* transcript is first observed at T_{le0}, at which time the maximal level of message is attained (Fig. 2D and 3). There is a gradual decrease in transcript level between T_{le0} and $T_{le1.2}$, and a barely detectable level of transcript is observed at $T_{le2.5}$ (Fig. 2D and 3). These data demonstrate that accumulation of *abrB* transcript is confined to a short period spanning the transition between the lag and exponential phases of the growth cycle. The sharp reduction in the level of transcript which occurs between the lag phase-exponential phase transition (T_{le0}) and the mid-exponential phase of the growth cycle is mediated by the response regulator Spo0A and to a lesser extent by AbrB.

Expression of *abrB* in strains with mutations in the genes which encode the constituent proteins of the phosphorelay. The regulation of *abrB* expression by Spo0A is thought to be effected by the phosphorylated form of the response regulator protein Spo0A. Spo0A is phosphorylated through a phosphorelay comprising Spo0F and Spo0B (6). To assess the role of Spo0A~P in controlling *abrB* expression, *abrB* transcript levels during the growth cycle were determined in strains with mutations in components of the phosphorelay. The strains examined were JH649 (*spo0F221*) and JH13311 (a nonphosphorylatable form of Spo0A), and the results are shown in Fig. 4. The levels of *abrB* transcript accumulation in wild-type (JH642) and *spo0A* mutant (JH703) strains are presented for comparison. The profile of *abrB* transcript accumulation in these strains is similar to that observed in a separate experiment whose results are shown in Fig. 2. During exponential growth (T_{es-3} and $T_{es-1.5}$), the level of *abrB* transcript in strain JH649 (*spo0F*) is high and similar to that of JH642 at the early exponential period of the growth cycle. There is a significantly higher level of transcript at $T_{-1.5}$ in strain JH649 compared to that of the wild-type strain at the corresponding period of the growth cycle. A sharp decrease in *abrB* transcript level was observed in strain JH649 between the mid-exponential and transition phases of the growth cycle. Although the level of accumulated *abrB* mRNA in strain JH649 is very low from T_{es0} to T_{es2}, it is higher than that observed in the wild-type strain at

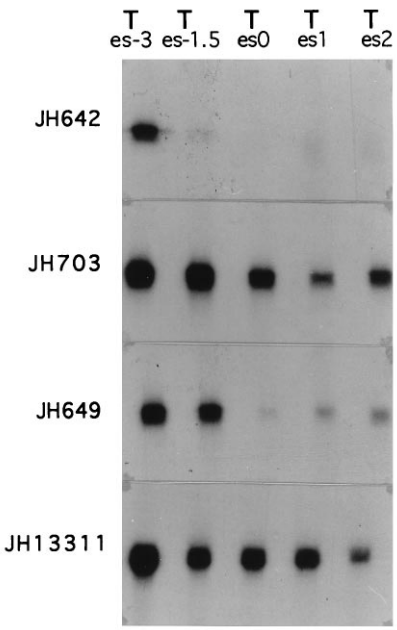

FIG. 4. Pattern of *abrB* transcript accumulation in strains with mutations in components of the phosphorelay during the growth cycle. Transcript is detected by primer extension analysis. The strains are JH642 (wild type), JH703 (*spo0A*Δ204), JH649 (*spo0F221*), and JH13311 (nonphosphorylatable Spo0A). Time is measured relative to the point of transition (T_{es0}) between the exponential and stationary phases of the growth cycle.

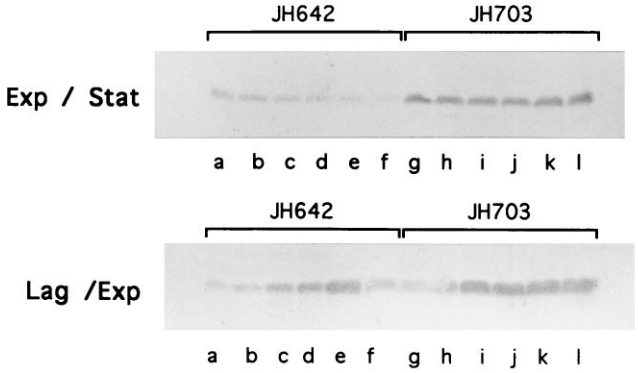

FIG. 5. Accumulation of AbrB in JH642 and JH703 during the growth cycle. Cell extracts were prepared from JH642 (lanes a to f) and JH703 (lanes g to l) cells during the exponential-stationary growth phases (Exp/Stat) and during the lag and early exponential phases (Lag/Exp) of two independent growth cycles. In the Exp/Stat samples, approximately 10 μg of total cell protein was loaded onto each lane, and in the Lag/Exp samples, 8×10^6 cell equivalents were loaded onto each lane. Times for Exp/Stat, JH642: lane a, T_{es-3}; lane b, $T_{es-1.5}$; lane c, T_{es-1}; lane d, T_{es0}; lane e, T_{es1}; lane f, T_{es2}. Times for Exp/Stat, JH703: lane g, $T_{es-2.3}$; lane h, T_{es-1}; lane i, $T_{es-0.8}$; lane j, T_{es0}; lane k, T_{es1}; lane l, T_{es2}. Times for Lag/Exp, JH642: lane a, $T_{le-2.2}$; lane b, $T_{le-1.5}$; lane c, $T_{le-0.7}$; lane d, T_{le0}; lane e, T_{le1}; lane f, T_{le2}. Times for Lag/Exp, JH703: lane g, T_{le-2}; lane h, $T_{le-1.3}$; lane i, $T_{le-0.5}$; lane j, T_{le0}; lane k, $T_{le1.2}$; lane l, $T_{le2.5}$.

similar stages of the growth cycle. These data demonstrate that the level of *abrB* transcript in JH649 cells is lower than that observed in strain JH703 (Δ*spo0A*) at corresponding points of the growth cycle (Fig. 4). This suggests either that the unphosphorylated form of Spo0A can regulate expression of *abrB* or that Spo0A can be phosphorylated independently of the Spo0F component of the phosphorelay. To distinguish between these possibilities, the level of *abrB* transcript in strain JH13311 was determined. Spo0A cannot be phosphorylated in this strain, since all the aspartate residues have been converted to asparagine (49). The pattern and level of *abrB* expression in strain JH13311 are very similar to those observed in strain JH703 at corresponding stages of the growth cycle. Therefore, the nonphosphorylated (but also mutated) form of Spo0A is not significantly active in the regulation of *abrB* expression. These data suggest that phosphorylation of Spo0A through the Spo0F component of the phosphorelay occurs during exponential growth and is important for regulating expression of *abrB*. Our data also suggest that Spo0A can be phosphorylated independently of Spo0F during the later stages of the growth cycle to an extent which is sufficient to suppress *abrB* transcript accumulation.

Accumulation of AbrB protein during the growth cycle. To investigate the accumulation of AbrB protein during the growth cycle, total cell extracts were resolved by SDS-polyacrylamide gel electrophoresis and transferred to membrane electrophoretically and AbrB protein was visualized as described in Materials and Methods. The levels of AbrB in extracts prepared from JH642 and JH703 cells at the indicated times of the growth cycle are shown in Fig. 5 (Exp/Stat, lanes a to f and g to l, respectively). In this experiment, equivalent amounts of total cell protein were loaded in each lane. In strain JH642, it is clear that the level of AbrB is highest during the early exponential period (T_{es-3}, lane a) of the growth cycle. Thereafter, a gradual decrease in the level of protein is observed until T_{es2} (Fig. 5, Exp/Stat, lane f), at which stage only very low levels of protein are observed. The level of AbrB protein in cells of strain JH703 is significantly higher than the maximal level observed in wild-type cells. In addition, it is evident that this high level of AbrB in JH703 cells does not vary significantly during the growth cycle (Fig. 5, Exp/Stat, lanes g to l). The accumulation of AbrB protein in JH642 cells (lanes a to f) and JH703 cells (lanes g to l) during the lag and early exponential phases of the growth cycle was then examined (Fig. 5, Lag/Exp). These protein extracts and the RNA preparations used for primer extension analysis (shown in Fig. 2B to D) were made from cells of the same growth experiment. In this experiment, extracts from equivalent numbers of cells were loaded onto each lane. A low level of protein is evident in JH642 cells at $T_{le-2.2}$ and $T_{le-1.5}$ (Fig. 5, Lag/Exp, lanes a and b, respectively). This level has increased at $T_{le-0.7}$ and T_{le0} (lanes c and d, respectively), and the maximal level is observed at T_{le1} (lane e). However, there is a dramatic decrease in the level of AbrB protein per cell between T_{le1} and T_{le2} (Fig. 5, Lag/Exp, lanes e and f, respectively). This coincides with an exponential increase in cell number at this time. The level of AbrB protein per cell in JH703 is low at $T_{le-2.0}$ (lane g) but rises significantly between T_{le-2} and $T_{le-1.3}$ (lanes g and h, respectively). In JH703 cells, the maximal level of AbrB protein is observed at $T_{le-0.5}$ (Fig. 5, Lag/Exp, lane i), and the level remains high and approximately invariant for the remainder of growth during the experiment. It is evident, therefore, that the level of AbrB protein first increases during the lag phase of the growth cycle, is maximal approximately 1 h after the transition from lag phase to exponential phase, and falls thereafter for the remainder of the growth cycle. In contrast, the level of AbrB in JH703 cells rises rapidly during the lag phase of the growth cycle but remains high during the remaining stages of the growth cycle examined. This profile of AbrB protein levels in JH703 is consistent with the accumulation of *abrB* transcript in this strain.

DISCUSSION

Growth cycle-dependent expression of AbrB. The transition state regulator AbrB plays a very important role in controlling gene expression in *B. subtilis* (42, 46). This role is varied, since AbrB can function as an activator, a repressor, and a preventer of gene expression. In the preventer mode of regulation, it is proposed that high AbrB levels function to prevent expression of stationary-phase-specific genes during exponential growth, while low levels are a requirement, but not a sufficient condition, for post-exponential-phase gene expression (42, 46). This suggests that the level of AbrB may vary under certain growth and/or environmental conditions. In this paper, the pattern and control of *abrB* expression throughout the growth cycle are presented. Our data demonstrate that *abrB* expression is highly growth phase dependent. Accumulation of *abrB* transcript is confined to a short period spanning the transition between the lag and exponential phases of the growth cycle. Transcription of *abrB* can be detected up to 1.5 h before cell growth (measured in CFU) is observed. The highest level of transcript is observed at the point of transition between the lag and exponential phases of the growth cycle. The level decreases during early exponential growth (T_{le+1}), and transcript cannot be detected at the mid-exponential phase or at subsequent stages of the growth cycle. The pattern of AbrB protein accumulation is similar to that of transcript accumulation. Initially during the lag phase, the level of AbrB protein is low. Levels increase and attain a peak during the period when transcript levels are high, i.e., from $T_{le-0.7}$ to T_{le1}. It is interesting, however, that the maximal *abrB* transcript level was observed at T_{le0}, whereas the maximal AbrB protein level was observed at T_{le1}. There is an abrupt decrease in the level of AbrB per cell between T_{le1} and T_{le2}, the point at which transcript levels also fall to undetectable levels. There is a gradual decrease in AbrB protein level between the early exponential and stationary phases of the growth cycle (our results) (13). Therefore, both the *abrB* transcript and the protein levels decrease between the exponential and stationary phases of the growth cycle, although the decay of transcript appears faster than the decay of AbrB protein. These data show that the highest levels of *abrB* transcript and protein are found during the transition from lag-phase to exponential growth (T_{le0}), i.e., in cells which are either preparing, or have just begun, to grow exponentially. Why are AbrB levels at their highest during the transition between lag and exponential growth phases? The observations that levels of AbrB fall throughout exponential growth and that cells with mutations in *abrB* can grow exponentially with a doubling time similar to that of wild-type cells show that high AbrB levels are not required for exponential growth. This suggests that the high level of AbrB present at T_{le0} may function to rouse cells from their quiescent state and to promote exponential growth. Once this is achieved, AbrB levels fall, thereby permitting expression of stationary-phase-specific genes should the prevailing growth and/or environmental conditions require it. The preventer role of AbrB in regulating post-exponential-phase gene expression can be explained by the growth cycle dependency of *abrB* expression. Throughout the growth cycle, cells with mutations in *spo0A* (which contain constitutively high levels of AbrB protein) may be in a physiological state characteristic of the lag phase-exponential phase (T_{le}) transition and so are incapable of expressing stationary-phase functions. The suppression of this phenotype in *spo0A abrB* double-mutant cells mimics the natural fall in AbrB level observed in wild-type cells during the growth cycle and therefore permits post-exponential-phase-specific gene expression. The designation of AbrB as a transition state regulator is therefore particularly appropriate, since it appears to play a very important role in both transitions (T_{le} and T_{es}) of the growth cycle.

Similarities between AbrB and Fis. Has the unusual growth cycle-dependent pattern of *abrB* expression been observed for other genes? The pattern of *fis* expression established both in *E. coli* and in *S. typhimurium* is very similar to that of *abrB* in *B. subtilis*. The level of *fis* transcript present in stationary-phase cells is very low, and there are only 100 molecules of Fis protein per cell (3, 30). Upon inoculation of fresh medium with stationary-phase cells, the level of *fis* mRNA increases dramatically even before the cells enter exponential growth. This increase, which peaks at approximately the point of transition between the lag and exponential growth phases, is transient, and mRNA levels have again fallen to a very low level before the mid-exponential phase of the growth cycle (3, 30). During the period of mRNA accumulation, the level of Fis protein increases to approximately 40,000 to 50,000 molecules per cell (3, 30). During the remainder of the growth cycle, the level of Fis protein per cell declines, consistent with a dilution of the protein in exponentially growing cells in the absence of new protein synthesis (3). It is therefore evident that the growth phase-dependent expression of *fis* both in *E. coli* and in *S. typhimurium* is very similar to that of *abrB* in *B. subtilis*. In fact, the similarities between Fis and AbrB are very striking (for a review of Fis, see reference 9; for a review of AbrB, see references 42 and 46). Both Fis and AbrB are small proteins (98 and 96 amino acids, respectively) which have a high proportion of positively charged amino acids (13 and 15, respectively). Both are DNA binding proteins, and each contains one helix-turn-helix motif located in the C terminus of the protein (23, 56). Both Fis and AbrB bind to highly degenerate consensus sequences with a bias for A and T residues (9, 44). The available evidence supports the view that the binding of these proteins generates a bend in the DNA (15, 23, 45, 56). In addition to the similarity in growth cycle-dependent expression of *fis* and *abrB* already outlined, both genes are subject to negative autoregulation which is manifested during the early period of the growth cycle (3, 28, 30, 47; this work). A characteristic feature of both Fis and AbrB is that they regulate expression of a very wide variety of genes. Fis stimulates site-specific DNA inversion catalyzed by the Hin, Gin, and Cin family of invertases (20, 22). It also (i) is involved in the integration and excision of lambda phage (2, 50), (ii) enhances transcription of rRNA and tRNA operons (27, 35), (iii) stimulates *oriC*-dependent DNA replication (8, 15), and (iv) is involved in the regulation of expression of 14 operons encoding proteins of very diverse functions (54). Notwithstanding the wide variety of operons whose expression is affected by Fis and AbrB, so far there is little overlap evident in their regulatory activity in their respective hosts. However, it has been established that expression of the ribose transport operons of *E. coli* and *B. subtilis* is regulated by Fis and AbrB, respectively (16, 43). There is little homology evident between the proteins at the amino acid level. Therefore, AbrB and Fis are not homologous proteins. Are there homologs of AbrB and Fis in other organisms? The Fis proteins present in *E. coli* and *S. typhimurium* are identical at the amino acid level, and they diverge by only 1.7% at the DNA sequence level (30). There is a Fis homolog in *Haemophilus influenzae* which is 81% identical at the amino acid level to the *E. coli* and *S. typhimurium* genes (11). However, a Fis homolog in gram-positive bacteria has not been found so far. There are two proteins in *B. subtilis* with homology to AbrB: ORF1 (accession no. D37799), which is located upstream of *kinC* and is 78% identical to AbrB, and *yabL* (accession no. D26185), which is 61% identical to AbrB within the amino-terminal 47 amino acids. No representative of the AbrB

gene family has been found so far in gram-negative bacteria. It is possible, therefore, that different proteins have evolved in gram-positive and gram-negative bacteria to perform the functions of AbrB and Fis. Despite these considerations, the similarities (particularly the growth cycle-dependent expression pattern) between Fis and AbrB are striking, and we hypothesize that AbrB is, like Fis, a nucleoid binding protein.

Spo0A controls growth cycle-dependent *abrB* expression. Expression of *fis* and *abrB* is restricted to a short period which spans the transition between the lag and exponential phases of the growth cycle of their respective hosts. However, *fis* and *abrB* transcripts are not detectable at the mid-exponential phase of the growth cycle (3, 28, 30; this work). The shutoff of *fis* transcription in *E. coli* is partially effected by negative autoregulation (3, 30). In *fis* mutants, transcription of *fis* is still turned off during exponential growth but at a slightly later time than is observed in wild-type cells, indicating that there must be another regulator involved (3, 28). In this paper, we show that, like *fis*, transcription of *abrB* is also negatively autoregulated during the early exponential growth phase. In *abrB* mutants, the period during which *abrB* transcript can be observed is extended to the mid-exponential phase of the growth cycle. The level of transcript then falls to undetectable levels between the mid-exponential and transition (T_{es}) phases of the growth cycle. Therefore, negative autoregulation is partly responsible for the fall in *abrB* transcript levels similar to that found for *fis* in *E. coli*. However, there must be a second regulator involved in the growth cycle-dependent restriction of *abrB* expression. Our data demonstrate that Spo0A is the primary regulator responsible for the dramatic reduction in *abrB* transcript level during the early period of the growth cycle. In a strain with a mutation in *spo0A*, the level of *abrB* transcript is very high throughout the growth cycle, although a decrease is discernible during the later stages. This decrease is probably due to negative autoregulation caused by the very high levels of AbrB present. However, in a *spo0A abrB* double-mutant strain, the level of *abrB* transcript is high and invariant throughout the growth cycle. Therefore, the growth cycle-dependent repression of *abrB* observed in wild-type cells during exponential growth is primarily effected by the transcriptional regulator Spo0A and to a lesser extent by negative autoregulation. Autoregulation appears to be operative only during the early period of the growth cycle, when levels of AbrB are highest. Spo0A is phosphorylated through a phosphorelay involving a number of kinases (KinA, KinB, and KinC), Spo0F, and Spo0B (6, 24, 25, 34, 51, 52). Is it the phosphorylated form of Spo0A which represses *abrB* expression during the early exponential growth phase? The fact that the level of *abrB* transcript in a strain containing a *spo0A* allele which cannot be phosphorylated is very similar to the profile observed in a strain carrying a partially deleted form of *spo0A* suggests that Spo0A~P is the active regulator of *abrB* expression. An important caveat to this conclusion is that the *spo0A* allele is multiply mutated and therefore may not be (fully) active. What role does the phosphorelay play in the phosphorylation of Spo0A during the growth cycle? The pattern of *abrB* transcription was investigated in a strain carrying a mutation in *spo0F*, the first component of the phosphorelay. In this strain, the cessation of *abrB* transcription is delayed until a point between the mid-exponential and the transition (T_{es}) phases of the growth cycle. Therefore, Spo0A appears to be phosphorylated via the phosphorelay during the early stages of the growth cycle. Phosphorylation of Spo0A, however, appears to occur independently of Spo0F during the later stages of the growth cycle. This alternative pathway of Spo0A phosphorylation, which may be mediated by KinC (24, 25), is not as effective as the phosphorelay since, in contrast to the case for wild-type cells, a low level of *abrB* transcript is consistently observed in cells with mutations in *spo0F* at the later stages of the growth cycle. It remains to be established whether the alternate pathway of Spo0A phosphorylation involves the Spo0B component of the phosphorelay or whether Spo0A can be phosphorylated directly.

In conclusion, we show that, although they are not homologous proteins, AbrB from *B. subtilis* and Fis, the nucleoid binding protein from *E. coli* and *S. typhimurium*, display striking similarities. We hypothesize that AbrB is also a nucleoid binding protein. It is possible that AbrB and Fis play similar roles in their respective hosts although they may not be strict functional equivalents. The growth cycle-dependent termination of *abrB* expression in *B. subtilis* is carried out primarily by the phosphorylated form of the response regulator Spo0A and, to a lesser extent, by negative autoregulation. This mode of regulation makes expression of *abrB* and of the genes it regulates responsive to the prevailing growth, nutritional, and/or environmental conditions. Since Spo0A homologs have been observed in only gram-positive bacteria (5), it will be interesting to discover the regulator responsible for growth cycle-dependent control of *fis* expression. In particular, it will be interesting to determine whether it is a member of a two-component system and, if so, to establish whether the stimuli to which it responds are similar to those to which Spo0A is responsive.

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