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Developmental Control of a *parAB* Promoter Leads to Formation of Sporulation-Associated ParB Complexes in *Streptomyces coelicolor*

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The *Streptomyces coelicolor* partitioning protein ParB binds to numerous *parS* sites in the *oriC*-proximal part of the linear chromosome. ParB binding results in the formation of large complexes, which behave differentially during the complex life cycle (D. Jakimowicz, B. Gust, J. Zakrzewska-Czerwinska, and K. F. Chater, J. Bacteriol. 187:3572–3580, 2005). Here we have analyzed the transcriptional regulation that underpins this developmentally specific behavior. Analysis of promoter mutations showed that the irregularly spaced complexes present in vegetative hyphae are dependent on the constitutive $parABp_1$ promoter, while sporulation-specific induction of the promoter $parABp_2$ is required for the assembly of arrays of ParB complexes in aerial hyphae and thus is necessary for efficient chromosome segregation. Expression from $parABp_2$ depended absolutely on two sporulation regulatory genes, *whiA* and *whiB*, and partially on two others, *whiH* and *whiI*, all four of which are needed for sporulation septation. Because of this pattern of dependence, we investigated the transcription of these four *whi* genes in *whiA* and *whiB* mutants, revealing significant regulatory interplay between *whiA* and *whiB*. A strain in which sporulation septation (but not vegetative septation) was blocked by mutation of a sporulation-specific promoter of *ftsZ* showed close to wild-type induction of $parABp_2$ and formed fairly regular ParB-enhanced green fluorescent protein foci in aerial hyphae, ruling out strong morphological coupling or checkpoint regulation between septation and DNA partitioning during sporulation. A model for developmental regulation of $parABp_2$ expression is presented.

Streptomycetes are gram-positive mycelial soil bacteria with unusual cell division features. In particular, their large linear chromosomes do not show clear-cut partitioning during most of their morphologically complex life cycle (10, 11, 14, 21). The elongated and often branched compartments of vegetative hyphae contain several copies of unsegregated chromosomes. During further development of the *Streptomyces coelicolor* colony growing on an agar surface (but not in submerged culture), new branches grow into the air for many tens of microns, forming a layer of white aerial mycelium. After cessation of growth, the long tip compartments of aerial hyphae differentiate into chains of exospores. This process starts with the assembly of a regular ladder of FtsZ rings, which are precursors of sporulation septa (46). Formation of sporulation septa is accompanied by chromosome condensation (which is somewhat impaired in *ftsZ* mutants [16]) and chromosome segregation into unigenomic prespore compartments. During maturation of the spores, the compartments round up and the spore walls thicken and acquire color through synthesis of a polyketide pigment, gray in the case of *S. coelicolor* and therefore giving rise to gray colonies (9).

White colony (*whi*) mutants cannot undergo maturation of aerial hyphae. Several *whi* genes (including *whiA*, *whiB*, *whiH*, *whiI*, and *whiG*) are regulators of the early stages of sporulation (1, 2, 8, 11, 13, 15, 44). Mutants of these five genes are defective in sporulation septation (15). *whiA* encodes a protein of unknown function with orthologues in most other gram-positive bacteria (2). *whiB* belongs to group of genes found only in actinomycetes, encoding small putative transcription factors containing an Fe-S cluster (13, 25). *whiH* encodes a member of the GntR family of transcription factors (44), and *whiI* encodes an atypical member of the response regulator family of proteins but is not adjacent to a potential sensor kinase gene (1). Both *whiH* and *whiI* are dependent on the sigma factor encoded by *whiG* (12).

Both *whiA* and *whiB* have two promoters, one low-level constitutive and another strongly transcribed at the time of aerial mycelium growth (2, 49). *whiA* and *whiB* deletion mutants have abnormally long coiled aerial hyphae, implying that they are defective in signals for growth cessation (15). In these mutants, chromosomes remain in an uncondensed state with continuous distribution along aerial hyphae. It has been proposed that WhiA/WhiB-dependent growth cessation of aerial hyphae generates signals that are recognized by, and change the behavior of, WhiH and WhiI (11). *whiI* and *whiH* transcription is also highly induced at the time of sporulation (1). *whiI* and *whiH* mutants both have loosely coiled aerial hyphae, of more or less wild-type length, differing from each other in the extent of chromosome condensation (1, 15). *whiI* mutants show the same lack of condensation as *whiA* and *whiB* mutants, while in the *whiH* mutant DNA becomes partially condensed, forming irregular patches (1, 15).

Segregation of bacterial chromosomes is most extensively studied in single-celled bacteria that divide by binary fission. It is an active process closely coupled to replication (for recent reviews, see references 3, 18, 30, 42, 48, and 52). ParAB homologues were among the earliest identified proteins involved

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TABLE 1. Strains used in this study

Strain	Relevant genotype	Source or reference
E. coli strains		
DH5α	*supE44* Δ*lacU169*(ϕ80*lacZ*Δ*M15*)*hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Lab stock
BW25113/pIJ790	K-12 derivative: Δ*araBAD* Δ*rhaBAD*/λ-Red(*gam bet exo*) *cat araC rep101*(Ts)	20
ET12567/pUZ8002	*dam-13*::Tn*9 dcm cat tet hsd zjj-201*::Tn*10*/*tra neo* RP4	41
S. coelicolor strains		
M145	$SCP1^-$ $SCP2^-$	5
J2538	M145 *parB*::*apra*	29
J2401	M145 *whiA*::*hyg*	15
J2402	M145 *whiB*::*hyg*	15
C70	A3(2) *whiB70*	8
J2450	M145 *whiI*::*hyg*	1
J2210	M145 *whiH*::*hyg*	15
J2418	M145 Δ*ftsZ*::*aphI attB*C31::pKF33[*ftsZ*Δp_2]	16
J3310	M145 *parB-egfp*	24
J3311	M145 *whiA*::*hyg parB-egfp-apra*	This study
J3312	M145 *whiB*::*hyg parB-egfp-apra*	This study
J3313	M145 *whiI*::*hyg parB-egfp-apra*	This study
J3314	M145 *whiH*::*hyg parB-egfp-apra*	This study
J3315	M145 Δ*ftsZ*::*aphI attB*C31::pKF33[*ftsZ*Δp_2] *parB-egf-apra*	This study
J3325	M145 *parAB*Δp_1 *parB-egfp*	This study
J3326	M145 *parAB*Δp_2 *parB-egfp*	This study

in chromosome segregation (reviewed in references 6 and 17) and have been studied particularly in *Bacillus subtilis* (22, 39), *Caulobacter crescentus* (38), *Pseudomonas putida* (32), and *Pseudomonas aeruginosa* (4). Their exact functions in chromosome segregation are still not clear. In *C. crescentus* both genes are essential (37), while in *B. subtilis* neither is essential, though *spo0J* (encoding the ParB homologue) is required for formation of endospores and for proper chromosome partitioning during vegetative growth (22, 47). ParB homologues are DNA binding proteins interacting with 14- to 16-bp partitioning sites (*parS*) (6, 17). In *B. subtilis* Spo0J binds to eight *parS* sites in the 20% of the chromosome around the replication origin (31, 33, 34), and Spo0J/ParB colocalizes with the *oriC*-proximal part of the *B. subtilis* and *C. crescentus* chromosomes (19, 34, 38, 51).

Due to the large size and linearity of the *Streptomyces* chromosome (8.7 Mb for *S. coelicolor*, 9 Mb for *S. avermitilis*) (5, 40) and the complexity of growth and morphological development of these organisms, *Streptomyces* chromosome segregation is expected to be more complex than that of rod-shaped bacteria dividing by binary fission. As in *B. subtilis*, the *Streptomyces parAB* genes are arranged in a two-gene operon (29). Disruption of the *S. coelicolor parAB* operon does not visibly affect colony growth, but chromosome partitioning aberrations are observed in about 13% of spores (29). The *S. coelicolor* chromosome contains 24 *parS* sites clustered within a 400-kb region around *oriC* (5% of the chromosome). Interaction studies, both in vitro and in vivo, indicated that most of the *S. coelicolor parS* sites are involved in the formation of large nucleoprotein complexes, which also seem to include the segments between *parS* sites (23). Construction of a strain expressing a ParB-enhanced green fluorescent protein (EGFP) fusion revealed ParB complexes, seen as fluorescent foci, that behaved differently during vegetative growth and in sporulating aerial hyphae (24). In vegetative hyphae, foci formed only transiently during the chromosome replication cycle and were small and irregularly spaced except close to hyphal tips, where complexes appeared to be larger and longer-lived. In contrast, regularly spaced large foci formed shortly before sporulation septation in aerial hyphae, and they disappeared after septation had been completed. Arrays of ParB foci in aerial hyphae were necessary for efficient DNA segregation into spores. Consistent with a role of ParB during sporulation, one of two *parAB* promoters is strongly expressed at the time immediately preceding sporulation (29). Here, we analyze transcriptional regulation of the *parAB* operon, its dependence on sporulation signals, and its effect on the formation of the ParB complexes.

MATERIALS AND METHODS

DNA manipulation and bacterial growth conditions. DNA manipulations were carried out by standard protocols (45). Enzymes were supplied by Roche or New England BioLabs, isotopes were from Amersham-Pharmacia Biotech, and oligonucleotides were from Invitrogen. The *S. coelicolor* and *Escherichia coli* strains are listed in Table 1. Culture conditions, antibiotic concentrations, and transformation and conjugation methods followed general procedures for *E. coli* (45) and *Streptomyces* (28). *S. coelicolor* strains were cultivated in tryptone soy broth–yeast extract-malt extract (1:1) complex liquid medium or on mannitol soy flour (MS) agar plates unless otherwise stated.

Construction of strains carrying EGFP fusion proteins. *S. coelicolor* mutants expressing ParB-EGFP in different genetic backgrounds were constructed by introducing *parB-egfp* into the *parB* chromosomal locus of different strains. The previously described cosmid H24 *parB-egfp-apra* (24) was used to transform ET12567/pUZ8002, from which it was mobilized into *whi* mutant derivatives of M145 by conjugation. Cosmid H24 *parB-egfp kan*::*vio-oriT* (24) was used to construct *parAB* promoter mutants. To obtain promoter mutants, first the *parAB* promoter region was replaced by the *apra* cassette amplified with oligonucleotides $p_{prom\text{-}apra\text{-}fw}$ and $p_{prom\text{-}apra\text{-}rv}$ flanked by unique SwaI restriction sites. Subsequently, H24 *parABp*::*apra parB-egfp kan*::*vio-oriT* was linearized with SwaI and used for coelectroporation of arabinose-induced BW25113/pIJ790 with PCR products encompassing the promoter region containing the desired mutations (obtained using the oligonucleotides for mutation sites, $p_{\Delta 1p\text{-}fw}$/$p_{\Delta 1p\text{-}rv}$ and $p_{\Delta 2p\text{-}fw}$/$p_{\Delta 2p\text{-}rv}$, and outside primers $p_{prom\text{-}fw}$/$p_{prom\text{-}rv}$). $Apra^s$ transformants were screened for the promoter mutations by restriction digestion of the PCR product, and clones verified by sequencing were used for conjugation into *S. coelicolor* strain J2538. Chromosomal DNA of all strains constructed was checked by PCR and sequencing. Cell extracts were checked by phosphorimager scanning after sodium dodecyl sulfate-

TABLE 2. Oligonucleotides used in this study

Name[a]	Sequence[b]	Application
$p_{prom\text{-}apra\text{-}fw}$	CACGCATGCCGGAGTGTCGCGGCAGTTCGGCATCAGCGGC*ATTTAAAT* GGAACTTCATGAGCTCAGCC	Insert SwaI restriction site in *parAB* promoter
$p_{prom\text{-}apra\text{-}rv}$	CAGCACGACCGATGCGCGTGTCGTCCATCGGAGGCGGTGT*ATTTAAAT* AGCTCCATCAGCAAAAGGGG	
$p_{\Delta 1p\text{-}fw}$	CCAGAGGCATGGGAGG**GGCCG**GCCCCTGCGAGCCTGAAGTCG	Mutation of $parABp_1$
$p_{\Delta 1p\text{-}rv}$	CGACTTCAGGCTCGCA**GGGGCCGG**CCCCTCCCATGCCTCTGG	
$p_{\Delta 2p\text{-}fw}$	GTTCGGCATCAGCGGC**GGCCGGCCCC**GTTTCACGTGAAACGTCGC	Mutation of $parABp_2$
$p_{\Delta 2p\text{-}rv}$	GCGACGTTTCACGTGAAAC**GGGGCCGG**CCGCCGCTGATGCCGAAC	
$p_{prom\text{-}fw}$	CGCAAGCTTTCCACACAAGCTGCCCTGCT	Amplification of *parAB* promoter
$p_{prom\text{-}rv}$	CCGGATCCGACCCGGGTCTGCTCGGGTCGC	
$p_{parABS1}$*	CATCGGAGGCGGTGTTTCACG	
p_{hrdB1}*	GCCATGACAGAGACGGACTCGGCG	Amplification of *hrdB* probe
p_{hrdB2}	CGGCCGCAAGGTACGAGTTGATGA	
p_{FP180}	AATACCGCATCAGGCGCCATTCG	Amplification of *whiA* probe (on pIJ6412 [2] template)
p_{OWA7}*	GCCAGCAGCTCCGGGTCGTG	
p_{whiB2}	ATGGGCTTGGTTCCGCA	Amplification of *whiB* probe
p_{whiB4}*	CGAGTTCCTCGTCCGCGTCGTCG	
p_{whiH7}*	ACGGGTAGCGGTCGAGTTCGCCCGGGT	Amplification of *whiH* probe
p_{whiH2}	GTCGTCGTACCGCTCGTACAG	
p_{OWI7}	GGGTCCGCACGTCCGGAGGA	Amplification of *whiI* probe
p_{OWI8}*	GACGGTGGAACGGACGCGCG	

[a] *, radiolabeled for S1 nuclease protection assay.
[b] Boldface indicates mutated nucleotides, and italics indicate restriction sites.

polyacrylamide gel electrophoresis and by Western blotting using polyclonal antibodies against ParB protein, as described previously (24). Promoter mutations were verified by transcriptional analysis of the obtained strains.

Microscopy. Strains for microscopic observations were inoculated in the acute-angled junction of coverslips inserted at 45° in MM agar containing 1% mannitol (28). Staining procedures were as described previously (46). Briefly, mycelium was fixed for 10 min with paraformaldehyde-glutaraldehyde mixture, digested for 2 min with 1 mg/ml lysozyme, and incubated for 1 h with 10 μg ml^{-1} wheat germ agglutinin-tetramethylrhodamine (WGA) conjugate (Molecular Probes) for cell wall visualization. After five washes with phosphate-buffered saline, coverslips were mounted in Slow-Fade (Molecular Probes) antifade reagent. Confocal laser scanning microscopy was carried out using a Leica SP2 microscope, equipped with a 63× objective and 488- and 543-nm lasers. Images in TIFF format were analyzed using Leica-Lite software (version 2.0; Heidelberg Microsystems).

RNA preparation and S1 nuclease protection assays. For total RNA preparation, cultures were grown on cellophane membranes on MM agar containing 1% mannitol and were harvested at different time points as described previously (1). S1 nuclease protection assays were performed using 30 μg of RNA as described by Kelemen et al. (26). Probes were generated by amplification of the promoter region with the pairs of oligonucleotides listed in Table 2.

RESULTS

Spatial separation of $parABp_1$ and $parABp_2$ promoter expression. Previous work (29) showed that the two promoters of *parAB* have distinct temporal patterns of activity. To investigate if the promoters were also expressed in a spatially specific manner, we introduced mutations expected to interrupt the −10 region of $parABp_1$ or $parABp_2$ into strain J3310, previously engineered to produce the ParB-EGFP fusion. The strains obtained, J3325 ($parAB\Delta p_1$) and J3326 ($parAB\Delta p_2$), were subjected to microscopic analysis.

In J3326, ParB-EGFP formed foci in vegetative mycelium with the same distribution and intensity as in the parental J3310 strain (Fig. 1). On the other hand, only faint, diffused fluorescence was visible in the aerial hyphae of J3326, and arrays of bright foci were never observed. In addition, we analyzed sporulation-associated chromosome segregation in J3326. DNA staining of its spore chains showed that 13% of spores were anucleate, just as observed previously for the *parB* deletion mutant, confirming that the production of elevated levels of ParB-EGFP is vital for proper chromosome partitioning in aerial hyphae.

J3325 had an exactly complementary phenotype (Fig. 1). Inactivation of $parABp_1$ entirely abolished ParB-EGFP complex formation in vegetative mycelium but did not influence either the fluorescence intensity or the spacing of sporulation-associated complex formation in aerial hyphae (1.3 μm for 91 foci measured). Moreover, J3325 was no more defective in DNA segregation into spores (4% anucleate) than its parental strain, J3310. Thus, our results demonstrated that $parABp_1$ and $parABp_2$ promoter activities are distinct both temporally and spatially.

Sporulation-associated ParB-EGFP foci are absent or markedly reduced in the aerial mycelium of early-sporulation (*whi*) mutants. Since the formation of ParB complexes in aerial hyphae coincides with sporulation-associated DNA condensation and septation, we investigated the assembly of such complexes in nonsporulating mutants. An *egfp-apra* cassette was introduced downstream of *parB* in four early *whi* mutant strains, to give J3311 (*whiA* disruption), J3312 (*whiB* disruption), J3313 (*whiI* disruption), and J3314 (*whiH* disruption). Comparing ParB-EGFP fluorescence in these strains to that in wild-type strain J3310, we found wild-type complexes in the vegetative mycelium of the mutants but not in the aerial mycelium (Fig. 2). Fluorescence was very weak and diffuse in aerial hyphae of J3311 (*whiA* disruption) and J3312 (*whiB* disruption). The aerial hyphae of J3313 (Δ*whiI*) displayed tiny, abundant fluorescent dots, but they were not regularly spaced and their signal intensity was about half of the intensity of the J3310 foci. Similarly, some irregularly spaced and rather weak foci (about three times weaker than the J3310 signals) could be distinguished in aerial mycelium of J3314 (*whiH* disruption), and in

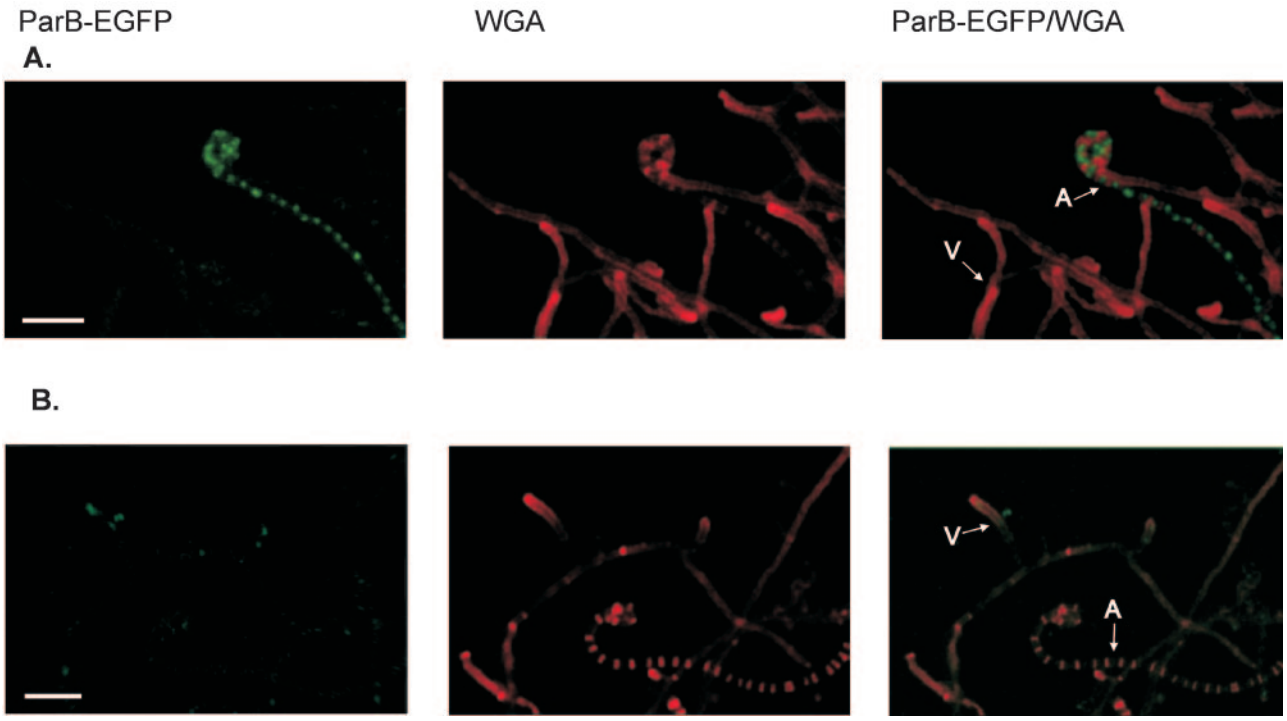


FIG. 1. ParB-EGFP complex formation in *parAB* promoter mutant strains (J3325 and J3326). Sample images show typical distributions of ParB-EGFP foci in vegetative and aerial mycelium of the $parABp_1$ (A) and $parABp_2$ (B) mutants, respectively. Images show ParB-EGFP fluorescence, cell walls stained with WGA conjugate, and an overlay of the two fluorescence signals. V, examples of vegetative hyphae; A, examples of aerial hyphae. Scale bars, 5 μm.

older cultures (more than 48 h) some hyphae could be found with brighter and more regular foci.

Efficient activation of the developmentally specific $parABp_2$ promoter is dependent on *whi* gene products. Clearly the efficient formation of sporulation-associated ParB-EGFP foci depended on all four *whi* genes. Two explanations might account for this: the *whi* genes might be necessary either for adequate levels of ParB or for the correct assembly of the complexes. We therefore investigated the activity of the *parAB* promoters in *whiA*, *whiB*, *whiI*, and *whiH* deletion mutants by using S1 nuclease protection analysis of RNA samples isolated at different time points from growing and differentiating surface cultures (Fig. 3). In all of these experiments, control S1 digestions were carried out with a probe for *hrdB*, whose expression is approximately constitutive (7) and which is often used as a combined semiquantitative standard and control of RNA quality.

The constitutive $parABp_1$ promoter was not affected in the nonsporulating strains. However, $parABp_2$ transcription, which in the wild-type M145 strain was strongly upregulated at the time of sporulation septation, was abolished in *whiA* and *whiB* deletion strains; in *whiI* and *whiH* mutants, its activity was detectable even at the earliest time point but stayed at a low and fairly unvarying level and, unlike the wild-type situation, was not switched off at later time points.

Thus, either the *whi* gene products themselves or a developmental signal(s) absent from the *whi* mutants is necessary for efficient activation of $parABp_2$, and the consequent reduced level of ParB may be insufficient for the formation of the large number of complexes usually occurring during sporulation septation. Moreover, the eventual downregulation of $parABp_2$ associated with spore maturation also appeared to be dependent on *whiH* and *whiI*.

Formation of regularly spaced ParB-EGFP complexes in aerial hyphae does not require sporulation septation. Since all four *whi* mutants tested had severe defects in sporulation septation, the reduced *parB* expression and inefficient ParB-EGFP complex formation in their aerial hyphae might involve some kind of morphological coupling of *parAB* transcription to the initiation of sporulation septation. To investigate this further, we used a strain, J2418, that is deficient in sporulation-associated *ftsZ* expression. In *S. coelicolor*, $ftsZp_2$ (one of three promoters) is upregulated before sporulation septation to provide enough protein for the efficient and synchronous formation of multiple Z rings (16). Strain J2418 contains an $ftsZp_2$ promoter mutation that abolishes the effective sporulation-specific increase of *ftsZ* expression. The strain is therefore largely defective in sporulation septation but is expected to be unaffected in expression of *whiA*, *whiB*, *whiH*, and *whiI*.

An S1 protection assay of *parAB* in J2418 showed that it was induced at the time corresponding to aerial hyphal maturation but that upregulation was less efficient than in the wild type (Fig. 4A). Moreover, a low-level signal was detected even at early time points, and there was not a complete shutdown in the later time points. We interpret the latter observation as indicating that sporulation septation itself participates in the signal cascade that results in the shutdown of $parABp_2$. The early expression, which was also seen with *whiI* and *whiH* mutants, may be a consequence of inevitable differences in the

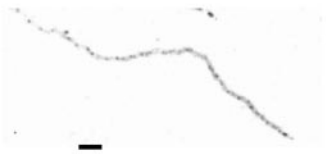
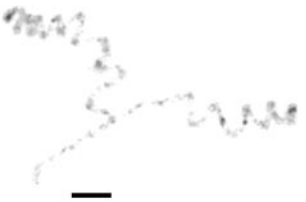
strain	vegetative mycelium ParB complexes	aerial mycelium	
		DNA condensation	ParB complexes
J3310 wt	irregular foci, foci associated with tips	separated nucleoids ++	arrays of regular , bright foci 
J3311 *whiA*	as in J3310	chromosomes continuous along hyphae -	weak diffuse fluorescence 
J3312 *whiB*	as in J3310	chromosomes continuous along hyphae -	weak diffuse fluorescence 
J3313 *whiI*	as in J3310	chromosomes continuous along hyphae -	tiny abundant dots 
J3314 *whiH*	as in J3310	chromosomes partially separated and partially condensed +/-	weak irregular foci, stronger and more regular in older hyphae 

FIG. 2. Comparison of DNA condensation and assembly of ParB complexes in the wild type (wt) and in *whi* mutants. Images show examples of EGFP fluorescence in aerial hyphae. Scale bars, 5 μm.

inoculum consisting of aerial hyphal fragments from the inoculum of spores used for the wild type. Many previous analyses of expression of different genes in these mutants have shown similar early expression (e.g., in reference 1). We also used the *ftsZp*$_2$ mutant to investigate whether sporulation septation played any role in the positioning of ParB foci. We constructed J3315, a J2418 (*ftsZ*Δ*p*$_2$) derivative expressing the ParB-EGFP fusion protein, and compared ParB-EGFP fluorescence in J3315 and in the wild type, J3310. Bright ParB-EGFP foci were seen in some aerial hyphae of strain J3315 (Fig. 4B), with spacing (1.3 ± 0.4 μm) similar to that in the wild-type strain J3310 (1.3 ± 0.3 μm), although somewhat less regular (Fig. 4C). Notably, aerial hyphae containing ParB foci were much less frequently seen than in J3310. This may have been due either to their increased transience or to a more asynchronous appearance. Summarizing our microscopic and transcriptional analysis, ParB complex formation is not tightly dependent on septation. Circumstantial evidence indicates that septation is not tightly coupled to ParB complex formation either, since mutants disrupted in *parB* form abundant spore chains. How-

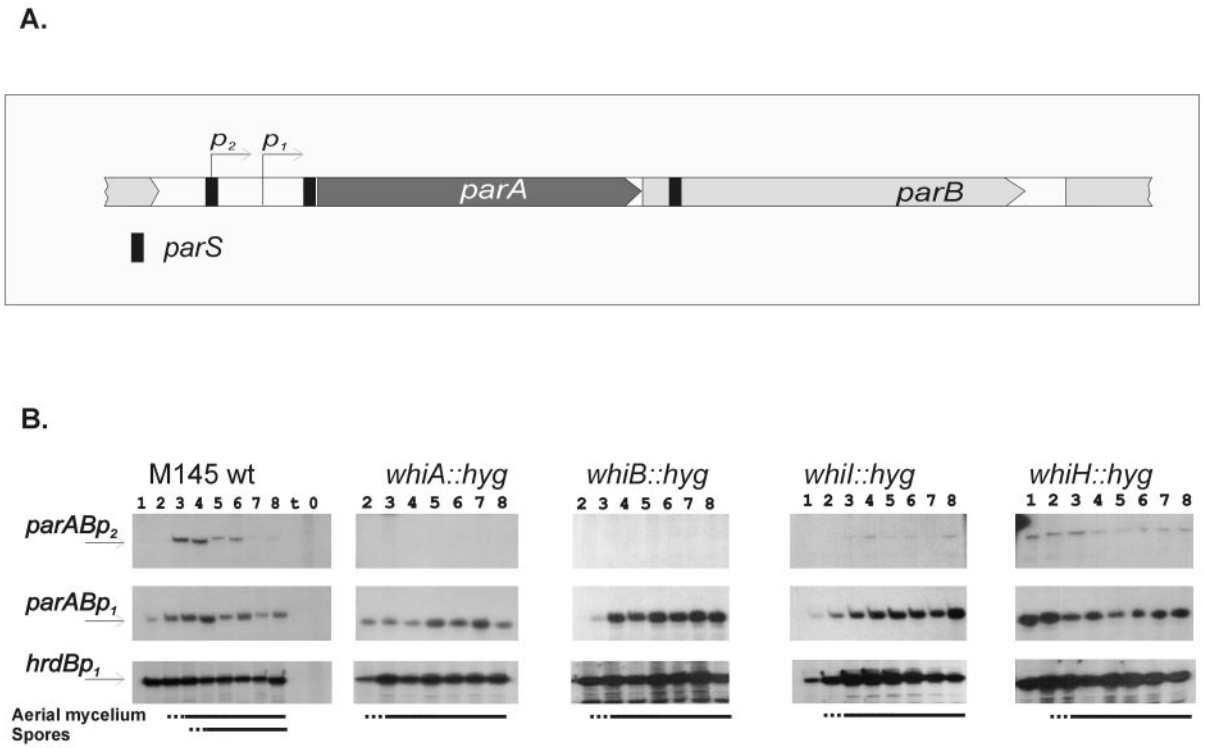


FIG. 3. Transcriptional activity of the *parAB* promoter in *whi* mutants. (A) *parAB* promoter region. (B) S1 nuclease protection analysis of *parAB* transcripts in the wild-type strain and in *whi* gene disruption mutants. Total RNA was extracted from cultures growing on MS agar at the corresponding time points: 1, 18 h; 2, 24 h; 3, 36 h; 4, 48 h; 5, 60 h; 6, 72 h; 7, 84 h; 8, 96 h. A control reaction with yeast tRNA was included in the lane labeled "t." *hrdB* is the control for a constitutively expressed gene. The lines at the bottom indicate time points at which aerial mycelium and spore chains were detected. Transcripts from $parABp_1$ and $parABp_2$ were detected with the same end-labeled probe and are therefore comparable.

ever, the shutdown of $parABp_2$ activity that usually accompanies spore maturation does appear to depend on a sporulation septation checkpoint.

Regulatory preamble to the activation of $parABp_2$. To investigate further the nature of the strong dependence of $parABp_2$ on the *whiA* and *whiB* genes, we carried out surveys of the transcriptional interdependence of these two genes on each other and of their interplay with the *whiH* and *whiI* genes (areas that have not been investigated in previous studies of *whi* gene expression). The cultures studied were M145 and its *whiA* and *whiB* disruption mutants (15), together with a nonisogenic *whiB* point mutant (*whiB70*) (8). The latter strain was used in order to extend the results with the *whiB*::*hyg* mutant, in which the absence of the entire *whiB* gene made it impossible to evaluate the activity of the developmentally regulated *whiB* promoter.

In a first experiment, RNA samples from the M145 control strain were hybridized to mixed probes for the developmentally regulated promoters of all four *whi* genes, in order to facilitate comparisons of their time courses of expression. In each case, there was a weak signal at the earliest time points, sharply increasing in strength at the time point at which spore formation became obvious (though a slight increase in all four, and particularly *whiH*, was also detected in the preceding sample, corresponding to the first emergence of aerial mycelium) (Fig. 5).

In the *whiA* mutant, expression of the sporulation-associated $whiAp_2$ promoter was reduced to a constitutive low level, as if the WhiA protein were needed in a positive feedback circuit for the upshift associated with sporulation. On the other hand, sporulation-associated $whiBp_2$ transcription was somewhat increased, although still developmentally regulated, suggesting that WhiA is involved in negative regulation of *whiB*. In both *whiB* mutants, $whiAp_2$ expression was low and constitutive, indicating that WhiB contributes in some way to *whiA* autoinduction. Judging by the use of *whiB70* RNA, *whiB* seems to be slightly overexpressed in a *whiB* mutant, as if WhiB contributed to its own repression (note that overexpression of *whiB* in the *whiB70* mutant and in a *whiA* point mutant was also reported in an early study, but only one time point was examined in that work [46]). Interestingly, both *whiB* and *whiA* were needed for the sporulation-associated upshift in *whiI* expression but not for that of *whiH*.

DISCUSSION

Previously it was shown that *parB* is required for proper chromosome segregation during sporulation, a finding reinforced by strong induction of the $parABp_2$ promoter at the time of sporulation (29). The use of a ParB-EGFP fusion revealed that ParB forms nucleoprotein complexes over the *oriC* region of the chromosome that behave differently in *S.*

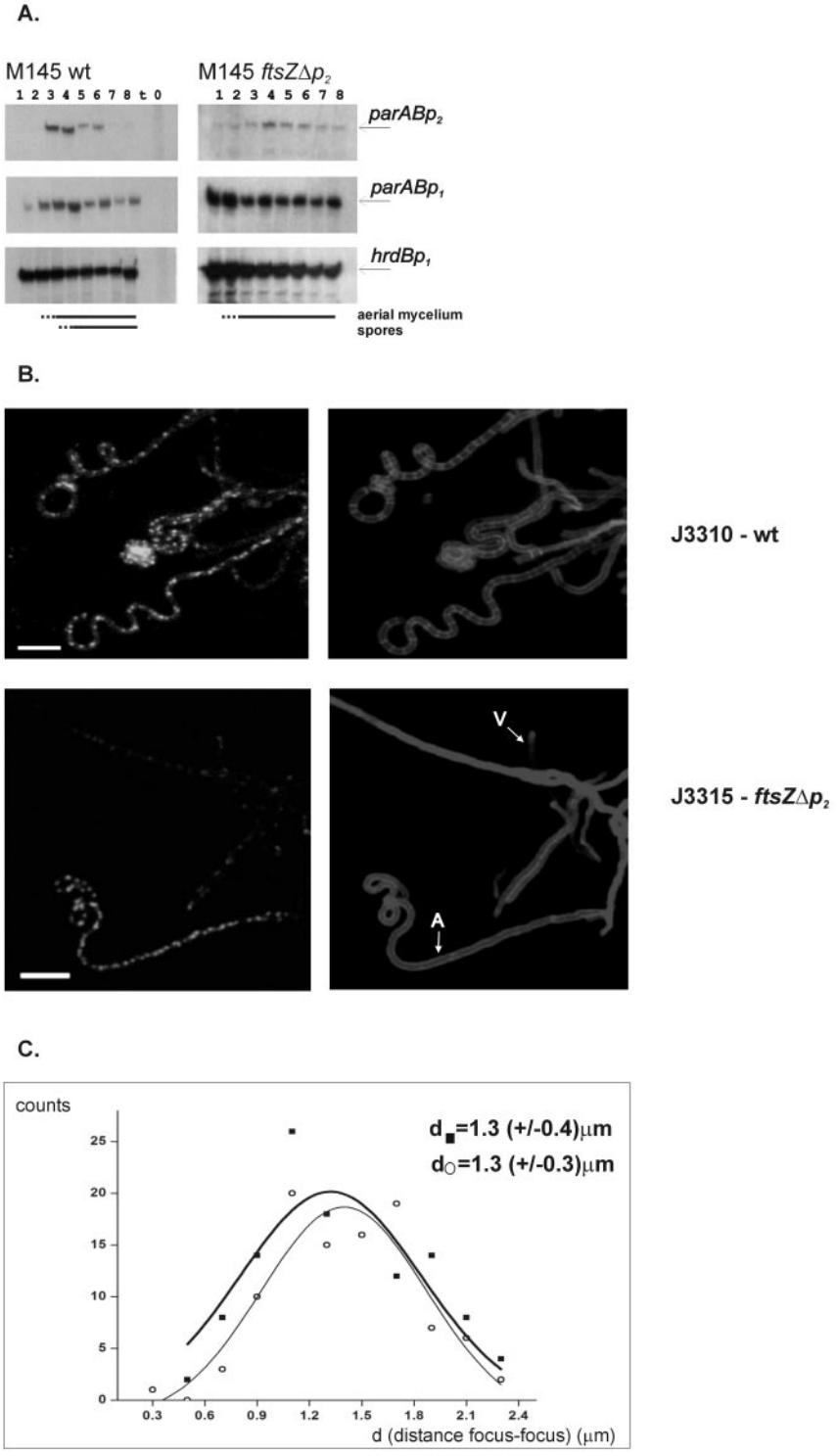


FIG. 4. Lack of dependence of ParB complex formation on sporulation septation. (A) S1 nuclease protection analysis of *parAB* transcripts in J2418. (B) Distribution of ParB-EGFP foci in aerial mycelium of the wild-type strain J3310 and an *ftsZp*$_2$ deletion mutant (J3315) deficient in septum formation. Images show ParB-EGFP fluorescence (left) and cell walls stained with WGA conjugate (right). V, example of vegetative hyphae; A, example of aerial hyphae. Scale bar, 5 μm. (C) Gaussian distribution of distances measured between ParB-EGFP complexes in arrays of ParB-EGFP foci in the aerial hyphae of strains J3310 (wild type) (○) and J3315 (■).

coelicolor vegetative and aerial hyphae (24). In aerial hyphae, the complexes assist DNA partitioning at the time of sporulation. Here, we have related formation of the ParB complexes to transcriptional activity of the two *parAB* promoters in the wild type and in different developmental mutants.

Formation of ParB complexes during vegetative growth and sporulation depends on the differential activities of two *parAB*

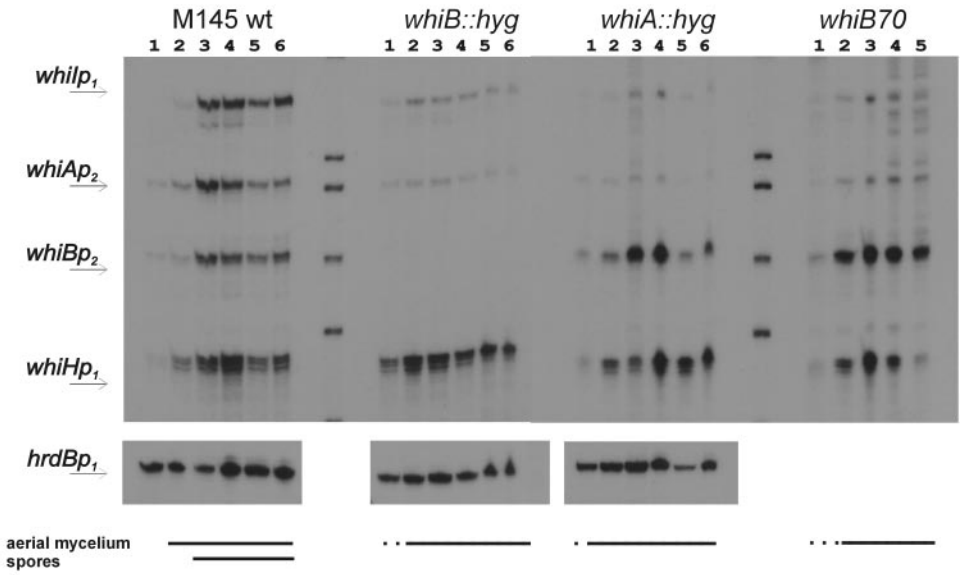


FIG. 5. Transcriptional analysis of *whi* genes in *whiA* and *whiB* mutants. Shown are S1 nuclease protection analyses of *whi* transcripts in the wild-type strain and in *whi* gene disruption mutants. Total RNA was extracted from cultures growing on MS agar at the corresponding time points: 1, 24 h; 2, 36 h; 3, 48 h; 4, 72 h; 5, 96 h; 6, 120 h. The lines at the bottom indicate time points at which aerial mycelium and spore chains were detected.

promoters. The provision of ParB at levels appropriate for the assembly of the complexes observed in different hyphal types is associated temporally with the differentially expressed activities of two promoters for the *parAB* operon. The natural constitutive level of $parABp_1$ transcription is required and sufficient for normal complex formation in vegetative mycelium, while the induction of $parABp_2$ transcription is necessary and sufficient both for presporulation complex formation in aerial hyphae and for proper partitioning of chromosomes into prespore compartments. Although $parABp_1$ is not needed for complex formation during sporulation and therefore is dispensable for DNA segregation into spores, we cannot exclude that it may be active in aerial hyphae. Several other *Streptomyces* promoters have been shown to be subject to temporal and spatial regulation during colony development. For example, *sigF* (43) and $sigHp_2$ (27) activities are restricted to sporulating aerial hyphae, and *redD* is active only in substrate hyphae (50). Circumstantial evidence points to more such cases, but the case of the promoter region of *ftsZ* is particularly similar to that of *parAB* (16). In in vitro transcription experiments with RNA polymerase purified from *S. coelicolor*, the transcript from $parABp_2$ was absent while the transcript from $parABp_1$ was present (L. Servin-Gonzalez and D. Jakimowicz, unpublished data), suggesting the requirement for an activator to transcribe the $parABp_2$ promoter. However, it is still possible that signals necessary for $parABp_2$ induction in aerial hyphae of sporulating strains may operate by relieving repression rather than by direct transcriptional activation.

***parABp*$_2$ transcription is controlled by the developmental regulatory network that coordinates sporulation.** $parABp_1$ is constitutively expressed, and its activity was not changed in four nonsporulating mutants (*whiA*, *whiB*, *whiH*, and *whiI*). Consistent with this, the formation of ParB-EGFP foci in vegetative hyphae of all these mutants was no different from that of the wild-type strain. In contrast, $parABp_2$ showed clear evidence of developmental control, which correlated well with the degree of impairment in the formation of sporulation-associated ParB-EGFP foci in the *whi* mutants and with the sporulation-associated chromosome partitioning defect of a $parABp_2$ mutant.

The complete absence of p_2 expression and the presumably resultant absence of presporulation ParB foci in the *whiA* and *whiB* mutants is particularly striking and strongly suggests that WhiA and/or WhiB may be directly implicated in controlling $parABp_2$ transcription. Low levels of WhiA and WhiB are probably present in nondifferentiating mycelium, in view of the activity of additional, apparently constitutive promoters for the corresponding genes (2, 49); however, either because WhiA and WhiB are present at insufficient levels or because additional regulatory factors are involved, $parABp_2$ is not activated.

The ability of WhiA and WhiB to bring about the shutdown of aerial growth before sporulation septation (15) may be mediated partly via an effect of ParB complexes on preventing the initiation of further rounds of replication, but this cannot be the whole story, since sporulation was not significantly impaired in a *parB* mutant (apart from the reduced regularity of DNA partitioning).

As the aerial mycelium matures, there are much higher levels of expression of *whiA* and *whiB*, while other possible accessory developmental regulators of $parABp_2$, such as WhiH and WhiI, are also more abundant, permitting strong $parABp_2$ expression. We found that mutations in *whiH* or *whiI* diminish $parABp_2$ activity, keeping it at a fairly unvarying low level, possibly reflecting some kind of modulating role for WhiH and WhiI. This could involve a direct effect of WhiI and WhiH on $parABp_2$ promoter activity or be more indirect, perhaps involving morphological or physiological checkpoints. Such check-

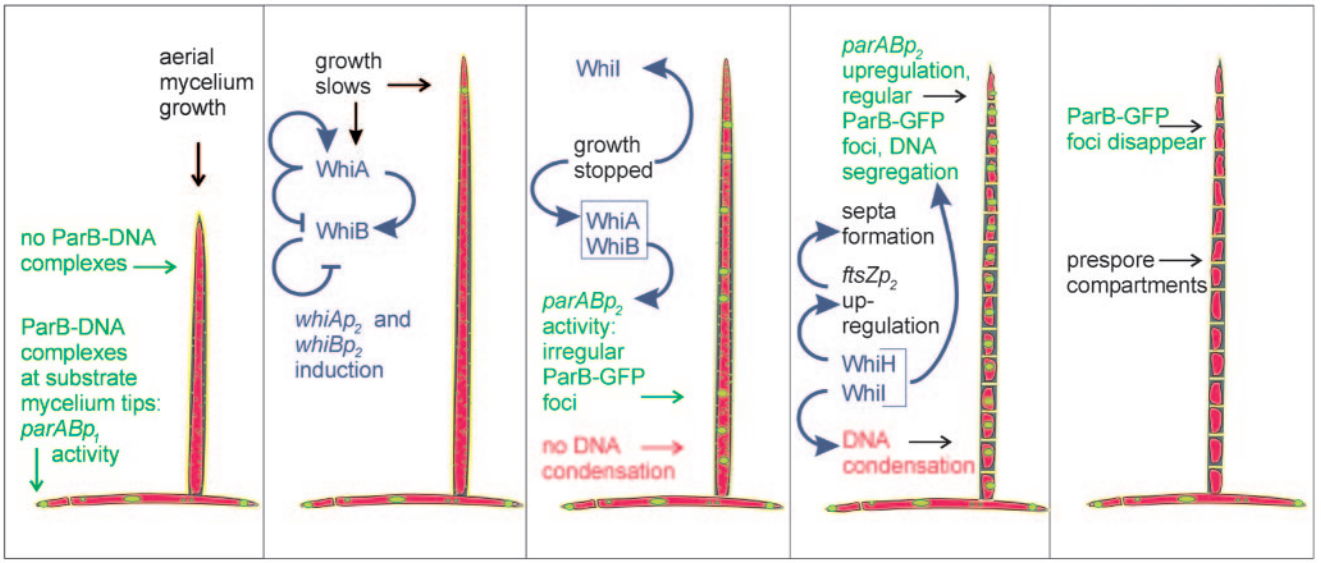


FIG. 6. Model of *parAB* promoter expression during *S. coelicolor* development in relation to the regulatory network of *whi* genes, assembly of ParB complexes, DNA partitioning, and sporulation. Blue arrows indicate *whi* gene autoregulation and their regulation of sporulation processes. Color coding is as follows: red, DNA; green, ParB; yellow, cell wall.

points would have to precede chromosome condensation, since ParB foci form before DNA condensation takes place (24).

***parABp*$_2$ transcription and ParB complex formation are not tightly coupled with sporulation septation.** Sporulation septation is probably not the key route through which *parABp*$_2$ is activated, since promoter activity was only slightly impaired, if at all, in a mutant largely lacking the sporulation septa because of a mutation in the sporulation-specific *ftsZp*$_2$ promoter. *parABp*$_2$ activity in the *ftsZp*$_2$-deficient mutant was high enough to provide sufficient protein for the formation of ParB complexes in aerial hyphae. This contrasts with a report that in *B. subtilis* multinucleate filamentous cells depleted of FtsZ, Spo0J complexes were scattered (35). Thus, *S. coelicolor* is exceptional not only in its ability to survive deletion of *ftsZ* (36) but also in its loose coupling of DNA segregation to sporulation septation. A dependence of positions of ParB foci on the positioning of FtsZ rings was not entirely ruled out, but the spacing of foci was only marginally less regular in the sporulation septation-deficient *ftsZp*$_2$ promoter mutant. It remains to be examined whether there is any dependence of the positioning of FtsZ rings on the ParB-*oriC* complex, but we note that the size of prespore compartments seemed to be more variable in *parB* mutants, either null (29) or defective in the putative DNA binding region of ParB (24). What is unambiguous is that during sporulation, neither ParB focus formation nor FtsZ ring formation per se depends on the other process.

Regulatory network controlling *parABp*$_2$. The finding that sporulation-specific *parAB* expression is completely dependent on *whiA* and *whiB*, with some degree of dependence on *whiI*, led us to analyze the effects of *whiA* and *whiB* mutations on sporulation regulatory genes. In the time courses examined, even though we evaluated several genes in a single reaction tube, we could not clearly distinguish any differences in the time of onset of transcription. However, the time intervals between samples were large (12 h) in relation to the overall time for aerial growth and sporulation to be accomplished (probably less than 20 h), so it remains possible that there are real differences in expression kinetics between the genes.

The developmentally associated increased expression of *whiA*, *whiB*, and *whiI*—and therefore, presumably, the increased abundance of WhiA, WhiB, and WhiI—appears to result from the regulatory interactions of these proteins and the corresponding genes. We propose the following working model, which extends earlier models (e.g., reference 11) (Fig. 6). WhiB, now shown to be a redox-sensitive protein containing a 4Fe-4S cluster (25), is an autorepressor in its reduced state and an activator of *whiA*, while WhiA is an autoactivator and a repressor of *whiB* (conceivably, WhiA and WhiB may actually interact at both promoters). Thus, *whiA* and *whiB* mutants overtranscribe *whiBp*$_2$ and undertranscribe *whiAp*$_2$. When an aerial hypha stops extending, a transient redox shock associated with the sudden change in physiology oxidizes WhiB, eliminating its autorepressing activity and increasing WhiB levels significantly. The increased WhiB levels contribute to the activation of *whiAp*$_2$ and thus initiate the accumulation of WhiA. The increasing amount of WhiA further stimulates *whiAp*$_2$ expression, and WhiA builds up to levels high enough to influence the regulation of other genes, including *parB* (*parABp*$_2$ promoter).

It was also noticeable that *whiI* expression was reduced in *whiA* and *whiB* mutants. Since WhiI is a response regulator-like protein (albeit somewhat atypical) (1), it is likely to change its activity during development in response to a signal. We postulate that this signal may not be sufficiently strong in *whiA* and *whiB* mutants, so any effects of WhiI that depend on the signal may not be manifested. One of these effects, it appears, is relief from autorepression; thus, the low-level expression of *whiI* in *whiA* and *whiB* mutants indicated by our present data would be a prediction of this model. Low-level expression of *whiI* might itself be expected to have developmental conse-

quences, including effects on sporulation-associated DNA condensation (1).

Conclusions. In summary, we have shown that formation of two different types of ParB complexes in vegetative and aerial hyphae depends on the differential activity of two *parAB* promoters, one of which is dependent on several sporulation regulatory genes. Further studies are necessary to find the signals that regulate ParB complex formation. In addition to control at the level of gene expression, other factors/checkpoints may influence the assembly of the complex, perhaps associated with proper growth cessation. It is also plausible that interaction of ParB with some cellular component present only in aerial hyphae is a prerequisite for proper complex assembly and/or localization. Such a component(s) could itself depend on *whi* gene products.

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REFERENCES

1. **Ainsa, J. A., H. D. Parry, and K. F. Chater.** 1999. A response regulator-like protein that functions at an intermediate stage of sporulation in *Streptomyces coelicolor* A3(2). Mol. Microbiol. **34:**607–619.
2. **Ainsa, J. A., N. J. Ryding, N. Hartley, K. C. Findlay, C. J. Bruton, and K. F. Chater.** 2000. WhiA, a protein of unknown function conserved among gram-positive bacteria, is essential for sporulation in *Streptomyces coelicolor* A3(2). J. Bacteriol. **182:**5470–5478.
3. **Bartosik, A. A., and G. Jagura-Burdzy.** 2005. Bacterial chromosome segregation. Acta Biochim. Pol. **52:**1–34.
4. **Bartosik, A. A., K. Lasocki, J. Mierzejewska, C. M. Thomas, and G. Jagura-Burdzy.** 2004. ParB of *Pseudomonas aeruginosa*: interactions with its partner ParA and its target *parS* and specific effects on bacterial growth. J. Bacteriol. **186:**6983–6998.
5. **Bentley, S. D., K. F. Chater, A. M. Cerdeno-Tarraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C. H. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabbinowitsch, M. A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B. G. Barrell, J. Parkhill, and D. A. Hopwood.** 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature **417:**141–147.
6. **Bignell, C., and C. M. Thomas.** 2001. The bacterial ParA-ParB partitioning proteins. J. Biotechnol. **91:**1–34.
7. **Buttner, M. J., K. F. Chater, and M. J. Bibb.** 1990. Cloning, disruption, and transcriptional analysis of three RNA polymerase sigma factor genes of *Streptomyces coelicolor* A3(2). J. Bacteriol. **172:**3367–3378.
8. **Chater, K. F.** 1972. A morphological and genetic mapping study of white colony mutants of *Streptomyces coelicolor*. J. Gen. Microbiol. **72:**9–28.
9. **Chater, K. F.** 1993. Genetics of differentiation in Streptomyces. Annu. Rev. Microbiol. **47:**685–713.
10. **Chater, K. F.** 1998. Taking a genetic scalpel to the *Streptomyces* colony. Microbiology **144:**1465–1478.
11. **Chater, K. F.** 2001. Regulation of sporulation in *Streptomyces coelicolor* A3(2): a checkpoint multiplex? Curr. Opin. Microbiol. **4:**667–673.
12. **Chater, K. F., C. J. Bruton, K. A. Plaskitt, M. J. Buttner, C. Mendez, and J. D. Helmann.** 1989. The developmental fate of *S. coelicolor* hyphae depends upon a gene product homologous with the motility sigma factor of *B. subtilis*. Cell **59:**133–143.
13. **Davis, N. K., and K. F. Chater.** 1992. The *Streptomyces coelicolor whiB* gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. Mol. Gen. Genet. **232:**351–358.
14. **Flardh, K.** 2003. Growth polarity and cell division in *Streptomyces*. Curr. Opin. Microbiol. **6:**564–571.
15. **Flardh, K., K. C. Findlay, and K. F. Chater.** 1999. Association of early sporulation genes with suggested developmental decision points in *Streptomyces coelicolor* A3(2). Microbiology **145:**2229–2243.
16. **Flardh, K., E. Leibovitz, M. J. Buttner, and K. F. Chater.** 2000. Generation of a non-sporulating strain of *Streptomyces coelicolor* A3(2) by the manipulation of a developmentally controlled *ftsZ* promoter. Mol. Microbiol. **38:**737–749.
17. **Gerdes, K., J. Moller-Jensen, and R. Bugge Jensen.** 2000. Plasmid and chromosome partitioning: surprises from phylogeny. Mol. Microbiol. **37:**455–466.
18. **Gerdes, K., J. Moller-Jensen, G. Ebersbach, T. Kruse, and K. Nordstrom.** 2004. Bacterial mitotic machineries. Cell **116:**359–366.
19. **Glaser, P., M. E. Sharpe, B. Raether, M. Perego, K. Ohlsen, and J. Errington.** 1997. Dynamic, mitotic-like behavior of a bacterial protein required for accurate chromosome partitioning. Genes Dev. **11:**1160–1168.
20. **Gust, B., G. L. Challis, K. Fowler, T. Kieser, and K. F. Chater.** 2003. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc. Natl. Acad. Sci. USA **100:**1541–1546.
21. **Hopwood, D. A., K. F. Chater, and M. J. Bibb.** 1995. Genetics of antibiotic production in *Streptomyces coelicolor* A3(2), a model streptomycete. Biotechnology **28:**65–102.
22. **Ireton, K., N. W. Gunther, and A. D. Grossman.** 1994. *spo0J* is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. J. Bacteriol. **176:**5320–5329.
23. **Jakimowicz, D., K. Chater, and J. Zakrzewska-Czerwinska.** 2002. The ParB protein of *Streptomyces coelicolor* A3(2) recognizes a cluster of *parS* sequences within the origin-proximal region of the linear chromosome. Mol. Microbiol. **45:**1365–1377.
24. **Jakimowicz, D., B. Gust, J. Zakrzewska-Czerwinska, and K. F. Chater.** 2005. Developmental-stage-specific assembly of ParB complexes in *Streptomyces coelicolor* hyphae. J. Bacteriol. **187:**3572–3580.
25. **Jakimowicz, P., M. R. Cheesman, W. R. Bishai, K. F. Chater, A. J. Thomson, and M. J. Buttner.** 2005. Evidence that the *Streptomyces* developmental protein WhiD, a member of the WhiB family, binds a [4Fe-4S] cluster. J. Biol. Chem. **280:**8309–8315.
26. **Kelemen, G. H., G. L. Brown, J. Kormanec, L. Potuckova, K. F. Chater, and M. J. Buttner.** 1996. The positions of the sigma-factor genes, *whiG* and *sigF*, in the hierarchy controlling the development of spore chains in the aerial hyphae of *Streptomyces coelicolor* A3(2). Mol. Microbiol. **21:**593–603.
27. **Kelemen, G. H., P. H. Viollier, J. Tenor, L. Marri, M. J. Buttner, and C. J. Thompson.** 2001. A connection between stress and development in the multicellular prokaryote *Streptomyces coelicolor* A3(2). Mol. Microbiol. **40:**804–814.
28. **Kieser, T., M. J. Bibb, M. J. Buttner, K. F. Chater, and D. A. Hopwood.** 2000. Practical Streptomyces genetics. The John Innes Foundation, Norwich, Conn.
29. **Kim, H. J., M. J. Calcutt, F. J. Schmidt, and K. F. Chater.** 2000. Partitioning of the linear chromosome during sporulation of *Streptomyces coelicolor* A3(2) involves an *oriC*-linked *parAB* locus. J. Bacteriol. **182:**1313–1320.
30. **Leonard, T. A., J. Moller-Jensen, and J. Lowe.** 2005. Towards understanding the molecular basis of bacterial DNA segregation. Philos. Trans. R. Soc. Lond. B **360:**523–535.
31. **Lewis, P. J., and J. Errington.** 1997. Direct evidence for active segregation of *oriC* regions of the *Bacillus subtilis* chromosome and co-localization with the SpoOJ partitioning protein. Mol. Microbiol. **25:**945–954.
32. **Lewis, R. A., C. R. Bignell, W. Zeng, A. C. Jones, and C. M. Thomas.** 2002. Chromosome loss from par mutants of *Pseudomonas putida* depends on growth medium and phase of growth. Microbiology **148:**537–548.
33. **Lin, D. C., and A. D. Grossman.** 1998. Identification and characterization of a bacterial chromosome partitioning site. Cell **92:**675–685.
34. **Lin, D. C., P. A. Levin, and A. D. Grossman.** 1997. Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **94:**4721–4726.
35. **Marston, A. L., and J. Errington.** 1999. Dynamic movement of the ParA-like Soj protein of *B. subtilis* and its dual role in nucleoid organization and developmental regulation. Mol. Cell **4:**673–682.
36. **McCormick, J. R., E. P. Su, A. Driks, and R. Losick.** 1994. Growth and viability of *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. Mol. Microbiol. **14:**243–254.
37. **Mohl, D. A., J. Easter, Jr., and J. W. Gober.** 2001. The chromosome partitioning protein, ParB, is required for cytokinesis in *Caulobacter crescentus*. Mol. Microbiol. **42:**741–755.
38. **Mohl, D. A., and J. W. Gober.** 1997. Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. Cell **88:**675–684.
39. **Ogasawara, N., and H. Yoshikawa.** 1992. Genes and their organization in the replication origin region of the bacterial chromosome. Mol. Microbiol. **6:**629–634.
40. **Omura, S., H. Ikeda, J. Ishikawa, A. Hanamoto, C. Takahashi, M. Shinose, Y. Takahashi, H. Horikawa, H. Nakazawa, T. Osonoe, H. Kikuchi, T. Shiba, Y. Sakaki, and M. Hattori.** 2001. Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. Proc. Natl. Acad. Sci. USA **98:**12215–12220.
41. **Paget, M. S., L. Chamberlin, A. Atrih, S. J. Foster, and M. J. Buttner.** 1999. Evidence that the extracytoplasmic function sigma factor σ^E is required for

normal cell wall structure in *Streptomyces coelicolor* A3(2). J. Bacteriol. **181:**204–211.
42. **Pogliano, K., J. Pogliano, and E. Becker.** 2003. Chromosome segregation in Eubacteria. Curr. Opin. Microbiol. **6:**586–593.
43. **Potuckova, L., G. H. Kelemen, K. C. Findlay, M. A. Lonetto, M. J. Buttner, and J. Kormanec.** 1995. A new RNA polymerase sigma factor, sigma F, is required for the late stages of morphological differentiation in *Streptomyces* spp. Mol. Microbiol. **17:**37–48.
44. **Ryding, N. J., G. H. Kelemen, C. A. Whatling, K. Flardh, M. J. Buttner, and K. F. Chater.** 1998. A developmentally regulated gene encoding a repressor-like protein is essential for sporulation in *Streptomyces coelicolor* A3(2). Mol. Microbiol. **29:**343–357.
45. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
46. **Schwedock, J., J. R. McCormick, E. R. Angert, J. R. Nodwell, and R. Losick.** 1997. Assembly of the cell division protein FtsZ into ladder-like structures in the aerial hyphae of *Streptomyces coelicolor*. Mol. Microbiol. **25:**847–858.
47. **Sharpe, M. E., and J. Errington.** 1996. The *Bacillus subtilis soj-spo0J* locus is required for a centromere-like function involved in prespore chromosome partitioning. Mol. Microbiol. **21:**501–509.
48. **Sherratt, D. J.** 2003. Bacterial chromosome dynamics. Science **301:**780–785.
49. **Soliveri, J., K. L. Brown, M. J. Buttner, and K. F. Chater.** 1992. Two promoters for the *whiB* sporulation gene of *Streptomyces coelicolor* A3(2) and their activities in relation to development. J. Bacteriol. **174:**6215–6220.
50. **Sun, J., G. H. Kelemen, J. M. Fernandez-Abalos, and M. J. Bibb.** 1999. Green fluorescent protein as a reporter for spatial and temporal gene expression in Streptomyces coelicolor A3(2). Microbiology **145:**2221–2227.
51. **Webb, C. D., A. Teleman, S. Gordon, A. Straight, A. Belmont, D. C. Lin, A. D. Grossman, A. Wright, and R. Losick.** 1997. Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. Cell **88:**667–674.
52. **Wu, L. J.** 2004. Structure and segregation of the bacterial nucleoid. Curr. Opin. Genet. Dev. **14:**126–132.