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FEMS Microbiology Letters 225 (2003) 59–67

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

The *Streptomyces coelicolor ssgB* gene is required for early stages of sporulation

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Received 13 March 2003; received in revised form 20 May 2003; accepted 10 June 2003

First published online 4 July 2003

Abstract

ssgB was identified as a novel early sporulation gene in *Streptomyces coelicolor*. An *ssgB* deletion mutant failed to sporulate, overproduced actinorhodin, and its colonies were significantly larger than those of the parental strain, suggesting an important role for the *ssgB* gene product in the process of growth cessation prior to sporulation-specific cell division. This places *ssgB* temporally before the paralogous sporulation gene *ssgA*. Analysis of *ssgB* mutant hyphae by electron microscopy and by confocal fluorescence microscopy showed that it was defective in the initiation of sporulation, as no sporulation septa could be identified, and DNA segregation had not yet been initiated in the mutant.

Keywords: *Streptomyces*; Cell division; Development; SsgA; Gene disruption; Sporulation

1. Introduction

Streptomycetes are Gram-positive soil bacteria with a complex mycelial life cycle that is directed towards the production of exospores [1]. On transition from vegetative to aerial growth, secondary metabolites are produced, many of which have antimicrobial properties. Similar to filamentous fungi, streptomycetes display two types of cell division [2]. During vegetative growth, infrequent cell division results in the formation of cross-walls, which dissect the hyphae into large multinucleoid compartments. In the aerial hyphae, synchronous cell division results in the formation of physically separated spores. Our research focuses on the nature of the differences between these two apparently distinct events.

In most microorganisms, cell division is essential for growth and viability [3], but not in streptomycetes, where *ftsZ* mutants of *Streptomyces coelicolor* are viable [4], although both vegetative and developmental cell division were absent, resulting in long and aseptate hyphae. FtsZ itself plays an important role in the discrimination between the two cell division events. An exciting discovery was made recently by Grantcharova and coworkers [5], who showed that a mutation in FtsZ itself could lead to specific inhibition of sporulation-specific cell division, without obvious effects on vegetative cell division, presumably by interfering with the formation of protofilaments during sporulation. Disruption of the developmental *ftsZ* promoter strongly reduced transcription of *ftsZ* in the aerial hyphae, resulting in a *whi* (non-sporulating) phenotype [6].

An important, but still poorly understood regulator of cell division is *ssgA*, which was identified as a multi-copy suppressor of a hyper-sporulating mutant of *Streptomyces griseus* [7]. The gene plays a key role in the regulation of sporulation-specific cell division, and is essential for correct sporulation of *S. coelicolor* and *S. griseus* on solid media [8,9]. In *S. griseus*, the developmental control of *ssgA* is mediated by A-factor, as its transcription depends on the A-factor-dependent protein AdpA [10]. Increased expression of SsgA results in mycelial fragmentation and even submerged sporulation of *S. coelicolor* [8]. From a

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0378-1097/03/$22.00
doi:10.1016/S0378-1097(03)00481-6

biotechnological perspective, SsgA-induced fragmentation is possibly of great importance as it may improve the notoriously difficult fermentation behavior of streptomycetes. While *ssgA* is only found in actinomycetes, close inspection of the *S. coelicolor* genome [11] revealed six possible *ssgA* paralogues in this organism, which we named *ssgB–ssgG* (summarized in Table 1). Four of these, *ssgB*, *ssgC*, *ssgD*, and *ssgG*, had already been identified as *ssgA*-like by the genome project, and the amino acid alignments have previously been shown [8].

Analysis of *ssgB* transcription in surface-grown cultures showed that transcription of *ssgB* coincides with aerial mycelium formation and depends on the developmental σ^H [12], a σ factor that also plays a role in stress responses [13]. In this study we analyze the role of *ssgB* in *S. coelicolor* development, and show that it is important for early stages of the sporulation process.

2. Materials and methods

2.1. Bacterial strains and media

Escherichia coli K-12 strains JM109 [14] and ET12567 [15] were used for propagating plasmids, and were grown and transformed by standard procedures [14]; transformants were selected in L broth containing the appropriate antibiotics. *S. coelicolor* M145 was obtained from the John Innes Centre strain collection. Preparation of growth media, protoplast preparation and transformation were performed as described in [15]. SFM medium was used to make spore suspensions. R2YE agar plates were used for regenerating protoplasts and, after addition of the appropriate antibiotic, for selecting recombinants. Phenotypic characterization of mutants was done on SFM, R2YE and MM agar plates [15]. Salt stress experiments with *S. coelicolor* M145 and GSB1 were performed on R2YE or MM agar plates, using the following NaCl concentrations: 0 mM, 100 mM, 250 mM, 500 mM, 750 mM, and 1 M. For standard cultivation of *Streptomyces*, and for DNA isolation, YEME or tryptone soy broth (Difco) containing 10% (w/v) sucrose (designated TSBS) was used.

2.2. Plasmids and constructs

Plasmid pBR-KO was created by inserting an approximately 1-kb *Bcl*I fragment harboring the thiostrepton resistance gene *tsr* [15] into the *Bam*HI site of pBR322, and subsequently replacing the *Eco*RI-*Hin*dIII fragment by the multiple cloning site of pUC18. This construct was used to generate the *ssgB* disruption construct p*ΔssgB*. Primers (30 nt each) were designed so as to generate two *ssgB*-flanking DNA fragments of exactly 1500 bp, the left part up to and including the GTG translational start codon, and the right part from and including the TGA stop codon. These two fragments were amplified by polymerase chain reaction (PCR), and cloned into the pBR322 derivative pBR-KO, as *Eco*RI-*Bam*HI and *Bam*HI-*Hin*dIII fragments, respectively. The apramycin resistance cassette was subsequently introduced in the *Bam*HI site between the two amplified fragments, resulting in p*ΔssgB*. The insert of this construct is shown in Fig. 1A.

To generate plasmids for complementation of the mutant, a 1.1-kb *Eco*RI fragment harboring the entire *ssgB* gene and flanking sequences (Fig. 1A) was cloned into the low-copy-number vector pHJL401 (less than five copies per chromosome [15]), generating pGWB2, and into the high-copy-number vector pWHM3 (around 50 copies per chromosome [15]), generating pGWB3. In another construct, designated pGWB6, *ssgB* was positioned behind the constitutive *ermE* promoter by replacing *ssgA* by *ssgB* in pGWS4. As insert of pGWB6, a 490-bp DNA fragment harboring the complete *ssgB* gene was used, generated by PCR with oligonucleotides ssgB5 (5′-ctggaattcatatgaacaccacggtcagctgcg), specifying an *Nde*I site overlapping the translational start codon (underlined), and ssgB6 (5′-ctgggatccgtgtgtgccgtatgcggttgtc), creating a *Bam*HI site approximately 70 nt downstream of the gene.

Plasmids pGWS4 and pGWS7 were used for expression of *ssgA*. pGWS4 is an integrative plasmid that expresses *ssgA* from the constitutive *ermE* promoter, giving high and development-independent expression [8], while pGWS7 is a pHJL401 derivative harboring the complete *ssgA* gene and flanking sequences (van Wezel, unpublished).

Table 1
ssgA-like genes on the *S. coelicolor* genome

Gene (*ssgA*-like)	*S. coelicolor* database number	Cosmid ORF	Amino acid identity/similarity[a] of gene product to	
			SsgA	SsgB
ssgA	SCO3926	Q11.09	100/100	42/52
ssgB	SCO1541	L2.31	42/52	100/100
ssgC	SCO7289	5H1.03c	27/41	33/47
ssgD	SCO6722	5F2A.05c	29/41	37/53
ssgE	SCO3158	E87.09c	23/32	27/35
ssgF	SCO7175	8A11.03	26/41	32/41
ssgG	SCO2924	E19A.24	42/55	56/70

Location (cosmid, ORF number) and database accession numbers are given. Annotation of the *ssgA*-like genes *ssgC–ssgG* proposed in this paper.
[a]Identities/similarities (in %) correspond to end-to-end alignment.

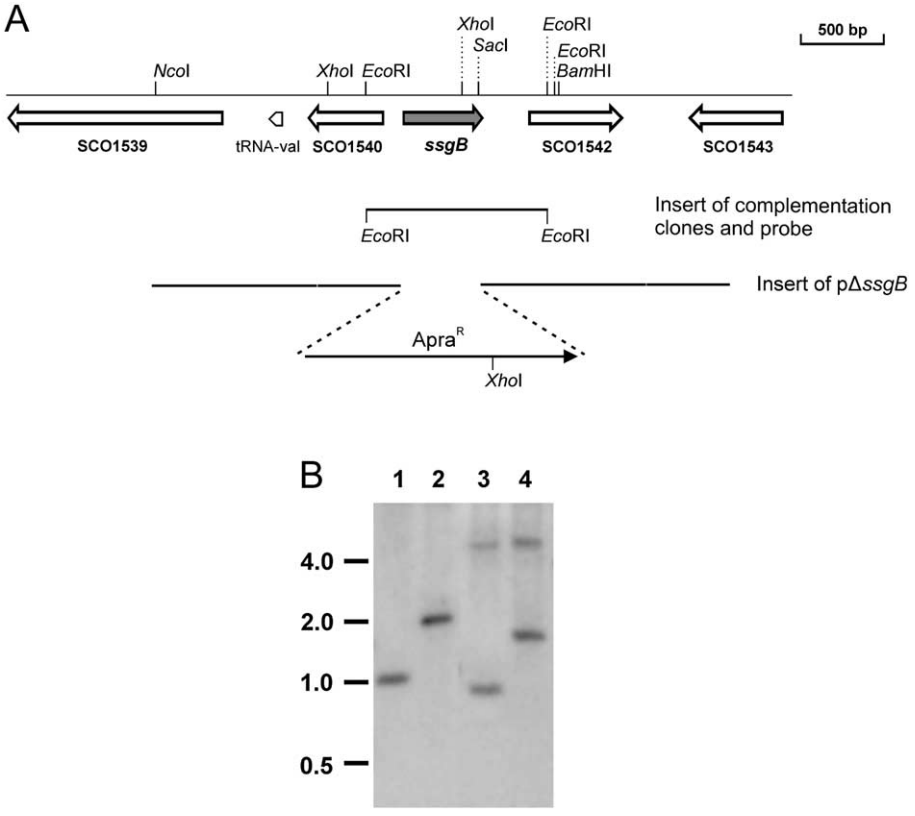

Fig. 1. Map of the genetic environment of *ssgB* and Southern analysis of the *ssgB* mutant. A: Restriction map of *ssgB* and neighboring genes. Shown below the map are the insert of the construct pΔ*ssgB* used for the replacement of *ssgB* by *aacC4* (conferring apramycin resistance), and the 1.1-kb *Eco*RI fragment that was used to complement the *ssgB* mutant in *trans*. This fragment was also used as probe for Southern hybridization (B). Genes: *SCO1539*, encodes putative extracellular sugar transporter sugar binding lipoprotein; *SCO1540*, encodes a possible membrane protein; *SCO1541*, *ssgB*; *SCO1542*, hypothetical ORF; *SCO1543*, encodes a putative membrane protein; $tRNA_{Val}$, specifies a tRNA with a CAC anticodon. B: Southern hybridization of the constructed *ssgB* null mutant. Genomic DNA isolated from the *S. coelicolor ssgB* mutant (lanes 2, 4) and its parental strain M145 (lanes 1, 3) was digested with *Eco*RI (lanes 1, 2) or *Xho*I (lanes 3, 4), separated by agarose gel electrophoresis, and blotted and hybridized according to standard procedures [14]. For probe see A. The wild-type *ssgB*-containing bands had the expected increase in size due to the replacement by the larger apramycin resistance cassette. The large 4.6-kb fragment (lanes 3, 4) corresponds to an *Xho*I fragment recognized by the 3′ end of the probe.

2.3. Southern hybridization

Chromosomal DNA samples from *S. coelicolor* M145 and its *ssgB* mutant GSB1 were digested with *Eco*RI or *Xho*I. DNAs were size-fractionated by gel electrophoresis on a 1% (w/v) agarose gel in 1×TAE and transferred to Hybond-N (Amersham) membrane. Hybridization was carried out at 42°C, in a mixture containing 50% (v/v) formamide, 2×SSC, 5×Denhardt solution, 0.2 mg ml^{-1} denatured salmon sperm DNA and 0.1% (w/v) sodium dodecyl sulfate (SDS). For high-stringency washing we used a solution of 0.1×SSC+0.1% (w/v) SDS at 65°C for 30 min. A 1.1-kb *Eco*RI fragment harboring the entire *ssgB* gene was used as the probe (Fig. 1A). The DNA was radiolabeled using the Megaprime Kit (Amersham) incorporating [α-^{32}P]dCTP.

2.4. PCR conditions

PCRs were performed in a minicycler (MJ Research, Watertown, MA, USA), using *Pfu* polymerase (Stratagene, La Jolla, CA, USA), according to the supplier's recommendations. PCR program (30 cycles): 45 s 94°C, 1 min 58°C, and 2 min 72°C. The reaction was completed by 10 min at 72°C.

2.5. Electron microscopy

For the morphological study of surface-grown mycelium of *S. coelicolor* M145 and its *ssgB* mutant by cryo-scanning electron microscopy (SEM), specimens were quickly frozen in liquid nitrogen slush and transferred directly to the cryo-transfer attachment of the microscope. Subsequently specimens were sputter-coated with a layer of 2 nm gold and examined in a JEOL JSM6700F scanning electron microscope.

Transmission electron microscopy (TEM) for the analysis of cross-sections of hyphae and spores was performed as described earlier [8].

2.6. Confocal fluorescence microscopy

Mycelium was grown for 6 days on SFM plates, and samples prepared by staining with propidium iodide as

described earlier [16]. Samples were analyzed with a Leica TCS-SP2 confocal fluorescence microscope, equipped with an oil-immersed 100× Planapo objective and a CCD camera. Digital images were assembled using ADOBE Photoshop software.

3. Results and discussion

3.1. Construction of the ssgB knock-out mutant

The gene organization around *ssgB* (*S. coelicolor* database number SCO1541; accession number NP_625820), which encodes a 138-aa SsgA-like protein, is shown in Fig. 1A. As summarized in Table 1, of the seven *ssgA*-like paralogues, *ssgG* shows by far the highest similarity to *ssgB*, with their predicted gene products sharing 56% end-to-end amino acid identity (70% similarity), the next closest homologue being *ssgA* (42% aa identity for the gene products, 52% similarity). Upstream and divergently transcribed from *ssgB* lies an open reading frame (ORF) (SCO1540) encoding a putative 150-aa membrane protein, while the downstream-located ORF (SCO1542), encoding a 205-aa hypothetical protein, is transcribed in the same direction as *ssgB*. There is no obvious similarity between the flanking regions of *ssgB* and those of any of the other *ssgA*-like genes.

To study the possible role of *ssgB* in the regulation of sporulation-specific cell division of *S. coelicolor*, a deletion mutant of *ssgB* was created by replacing the complete gene by the apramycin resistance cassette *aacC4*, using a method for gene disruption similar to that described previously for the *ssgA* knock-out mutant ([8] and references therein). The non-replicating disruption construct p*ΔssgB* was introduced into *S. coelicolor* by transformation. Transformants were selected for apramycin resistance and screened for thiostrepton sensitivity, indicative of loss of the plasmid. Six independent double recombinants were initially streaked for single colonies, and were phenotypically identical; one was selected and designated GSB1. The resulting constructed null mutant is shown schematically in Fig. 1A. Southern hybridization showed that the *ssgB* gene indeed carried the expected insertion of *aacC4* (Fig. 1B). This is shown by the predicted increase in length (by approximately 1 kb) of the wild-type 1-kb *Eco*RI and 0.9-kb *Xho*I restriction fragments, due to the replacement of *ssgB* by *aacC4*. The larger band observed in the lanes containing *Xho*I-digested DNA corresponds to the right-hand *Xho*I fragment that harbors the end of *ssgB* (Fig. 1A).

3.2. ssgB is a novel whi gene

ssgB deletion mutants had a white phenotype on all media tested, including R2YE, SFM, and MM. The phenotype on SFM plates is shown in Fig. 2A. To analyze if sporulation-specific cell division occurred in the *ssgB* mutant, we analyzed its aerial hyphae by combined light microscopy and confocal fluorescence microscopy (Fig. 2C, left and right panel, respectively), after staining the nucleoids with propidium iodide. No sporulation or DNA segregation, such as clearly seen in the parental strain M145 (see inset in Fig. 2C, top right), was observed in the *ssgB* mutant hyphae in any of the samples that were analyzed. These results strongly suggest that the process of sporulation-specific cell division was not yet initiated in the *ssgB* mutant, a conclusion supported by the lack of FtsZ assembly into so-called Z-rings, which marks the start of cell division (Noens, Koerten and van Wezel, unpublished results).

The failure of the *ssgB* mutant to initiate sporulation was further confirmed by electron microscopy (see below). Thus, we propose that *ssgB* is a novel early *whi* gene. Additionally, GSB1 mutants produced increased levels of actinorhodin on the same media, an effect also observed for the *ftsZ* mutant [4], and for transformants with enhanced expression of *ftsZ* [17]. This indicates that the regulation of actinorhodin production, which is initiated during aerial mycelium formation, is disturbed in the mutant.

Surprisingly, GSB1 colonies were significantly larger than those of the parental strain M145, sometimes reaching diameters larger than 10 mm in a relatively short time span (7–10 days) (Fig. 2A). Typically, the average colony size of the *ssgB* mutant was over twice the average size of parental colonies after 5 days. Also, while colony growth of *S. coelicolor* M145 slowed markedly prior to the onset of sporulation, *ssgB* mutant colonies continued to expand. Apparently, mechanisms of growth cessation are inhibited in the *ssgB* mutant.

3.3. The ssgB mutant is fully complemented by ssgB, but not by ssgA

To ascertain that the phenotype of the *ssgB* mutant GSB1 was solely due to the deletion of *ssgB*, and not to a possible second-site mutation, complementation construct pGWB2, harboring the intact *ssgB* gene, or pGWB6, with *ssgB* positioned behind the strong and constitutive *ermE* promoter, was introduced into GSB1. Transformants harboring either construct showed normal sporulation as compared to the parental strain M145, on MM, SFM and R2YE (Fig. 2B and phase contrast microscopy). The spores produced by the complemented *ssgB* mutant strain were indistinguishable from those of M145.

Sporulation of the *ssgB* mutant was not restored after introduction of the same *ssgB*-containing DNA fragment on the high-copy-number vector pGWB3 (Fig. 2B). A likely explanation is the titration of σ^H (and perhaps also other transcription factors) by multiple copies of the *ssgB* promoter, as similar developmental inhibition was

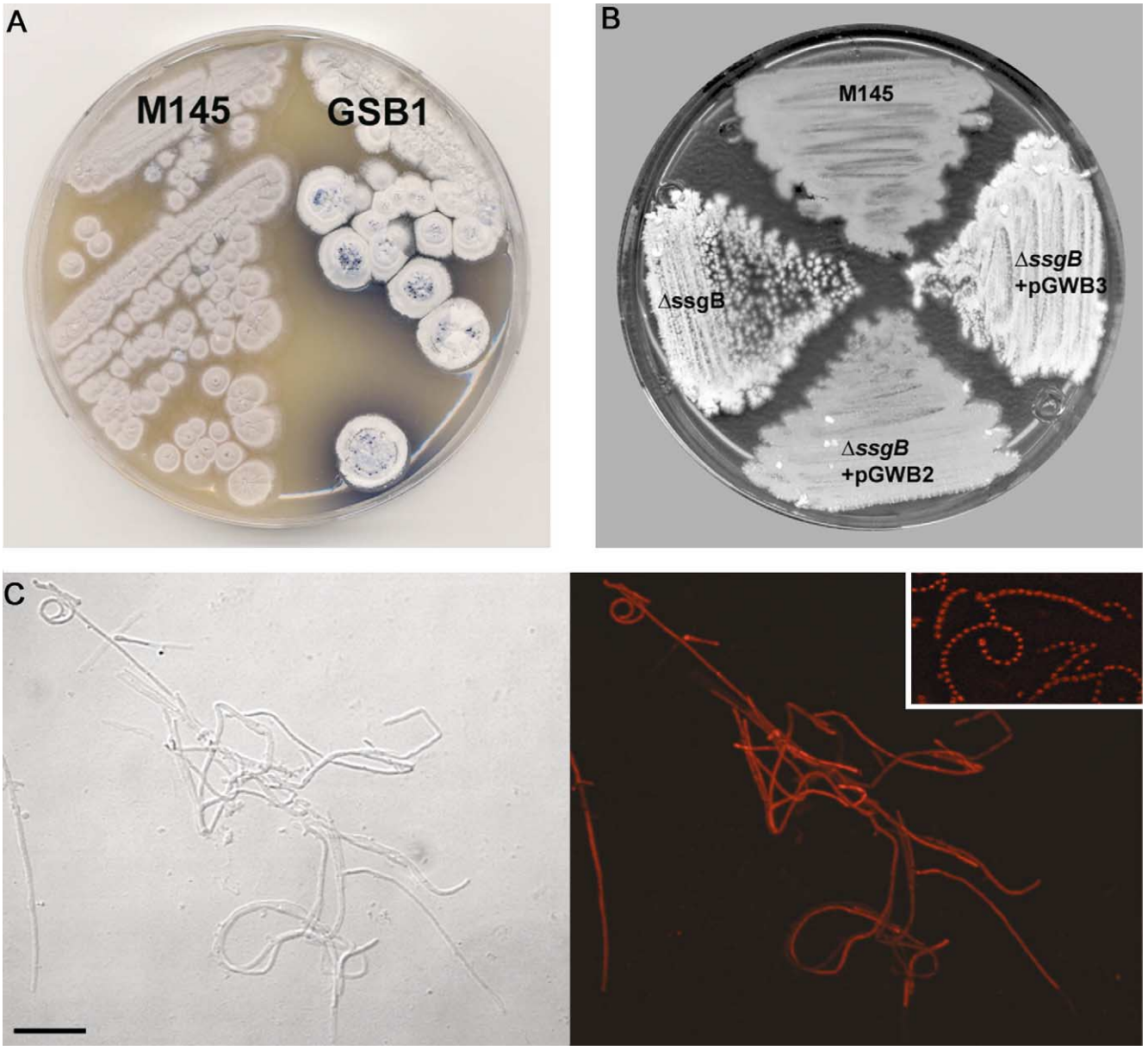

Fig. 2. Effects of deletion of the *ssgB* gene on sporulation of *S. coelicolor*. A: Phenotype of the *ssgB* null mutant GSB1 and its congenic parent *S. coelicolor* M145. Strains were grown on SFM plates for 6 days. While *S. coelicolor* M145 produces a gray aerial mycelium, GSB1 displays a white phenotype. Confocal fluorescence microscopy and light microscopy (C) and electron microscopic analysis (SEM and TEM; Figs. 3 and 4) revealed that the white appearance of GSB1 was due to its inability to produce spores. The colonies of the *ssgB* mutants were unusually large in comparison with those of the wild-type strain, and produced increased levels of actinorhodin. B: Complementation of the *ssgB* mutant. The introduction of *ssgB* on a low-copy-number vector (pGWB2), but not on a high-copy-number vector (pGWB3), restores sporulation of GSB1 to wild-type levels. Introduction of pGWB2 also restored normal colony size to GSB1 (not shown). C: Distribution of DNA in aerial hyphae of the *ssgB* mutant. Mycelium was grown for 6 days on SFM agar. A typical example of aerial hyphae of GSB1 is shown. Left panel: light micrograph, showing lack of sporulation. Note that coiling occurs in the aerial hyphae of GSB1. Right panel: the same mycelium stained for DNA with propidium iodide, clearly showing well-segregated DNA in M145 (inset picture, top right), and lack of DNA segregation in the *ssgB* mutant hyphae. Bar, 5 μm.

observed for *S. coelicolor* transformants with a multi-copy plasmid harboring solely the *ssgB* promoter region, but not with construct pGWB6, which gives high-level expression of *ssgB* from the *ermE* promoter (see above). Interestingly, pGWB6 transformants showed altered mycelial morphology in submerged cultures, with significantly smaller mycelial clumps than for wild-type cultures (not illustrated).

To establish if the *whi* phenotype of the *ssgB* mutant could be compensated for by enhanced and/or deregulated expression of *ssgA*, we introduced the expression constructs pGWS4 and pGWS7 into GSB1. Introduction of

neither pGWS4, which expresses *ssgA* from the constitutive *ermE* promoter, nor pGWS7, a low-copy plasmid harboring the complete *ssgA* gene and flanking sequences, could restore sporulation to the *ssgB* mutant, strongly suggesting that its phenotype is not due to reduced expression of *ssgA*.

3.4. *Analysis of the* ssgB *mutant hyphae by electron microscopy*

The morphology of surface-grown colonies of *S. coelicolor* GSB1 and its parental strain M145 were analyzed by cryo-SEM. While M145 produced long and regular spore chains (Fig. 3A), GSB1 formed very smooth and non-coiling aerial hyphae, which failed to sporulate (Fig. 3B). The mutant appears to be blocked in an early stage of aerial growth. Precious few aerial hyphae showed irregularly shaped globular compartments, while often tiny side branches were observed (indicated in Fig. 3C by 'S' and 'B', respectively). Branching is a phenomenon typical of vegetative hyphae, but rarely seen in hyphae of the aerial mycelium. Furthermore, aerial hyphae of the *ssgB* mutant showed a significant number of collapsed hyphae (indicated by the symbol 'C' in Fig. 3C), a phenomenon not observed for the congenic parental strain M145, though occasionally observed in other *whi* mutants [18]. Wild-type and mutant were analyzed several times, and hyphal collapse was found to be specific for the *ssgB* mutant hyphae, and not found at any time in the parental strain.

The aerial hyphae were also scrutinized for the production of septa by TEM. Chains of immature spores (Fig. 4A) and of fully developed spores (Fig. 4B) were readily observed in the parental strain M145. In the *ssgB* mutant aerial hyphae, we failed to observe compartments separated by septa, but occasionally found aerial hyphae showing indentations (Fig. 4C), perhaps corresponding to those observed by SEM (Fig. 3C). The significance of these indentations is unclear, but may reflect places of cell wall weakening. A significant change in the structure of the cell walls could not be observed. The vegetative hyphae compared well to the parental hyphae, with occasional cross-walls and no obvious cell wall anomalies (not illustrated).

3.5. *The* ssgB *mutant is sensitive to extreme salt concentrations*

Considering the dependence of the *ssgB* promoter on σ^H, which is implicated in salt stress resistance, we tested if the mutant GSB1 showed any change in salt resistance. For this, wild-type and *ssgB* mutant colonies were streaked on MM or R2YE plates containing different concentrations of sodium chloride. These experiments showed an increased sensitivity to very high salt concentrations, as the mutant failed to grow at concentrations of 750 mM NaCl and higher, while the parental M145 grew well on MM or R2YE with 1 M NaCl (not shown). No significant difference in growth was observed on media containing salt concentrations below 750 mM. While such a concentration may be physiologically irrelevant, these data

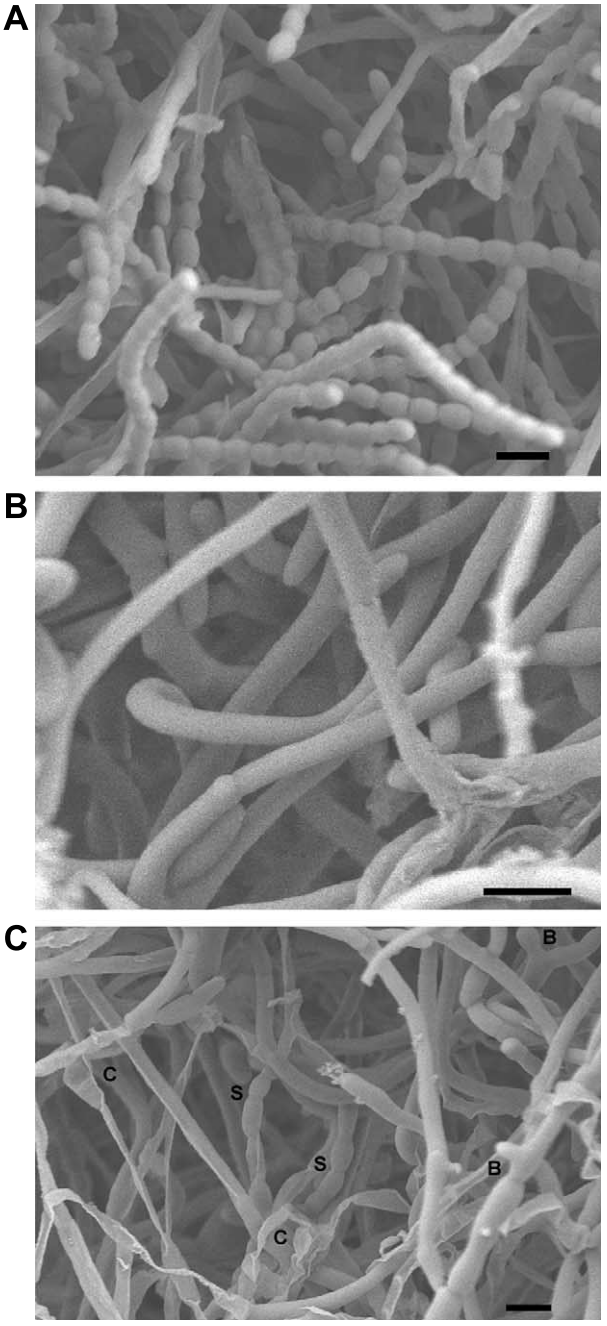

Fig. 3. Characterization of the *S. coelicolor ssgB* mutant by cryo-SEM. Samples were taken from 6-day-old cultures that were grown on SFM plates. A: Scanning electron micrograph of the surface of sporulating aerial hyphae of *S. coelicolor* M145 (phenotypic wild-type). Bar, 2 μm. B: Scanning electron micrograph of the surface of an *ssgB* mutant colony, showing smooth and non-sporulating aerial hyphae. Frequently, collapsed hyphae were observed. Bar, 5 μm. C: On rare occasions, the *ssgB* mutant produced aerial hyphae that showed signs of septation into globular, spore-sized compartments (indicated by S). The aerial hyphae also produced small side branches (indicated by B). Occasionally occurring collapsed hyphae are indicated by C. Bar, 2 μm.

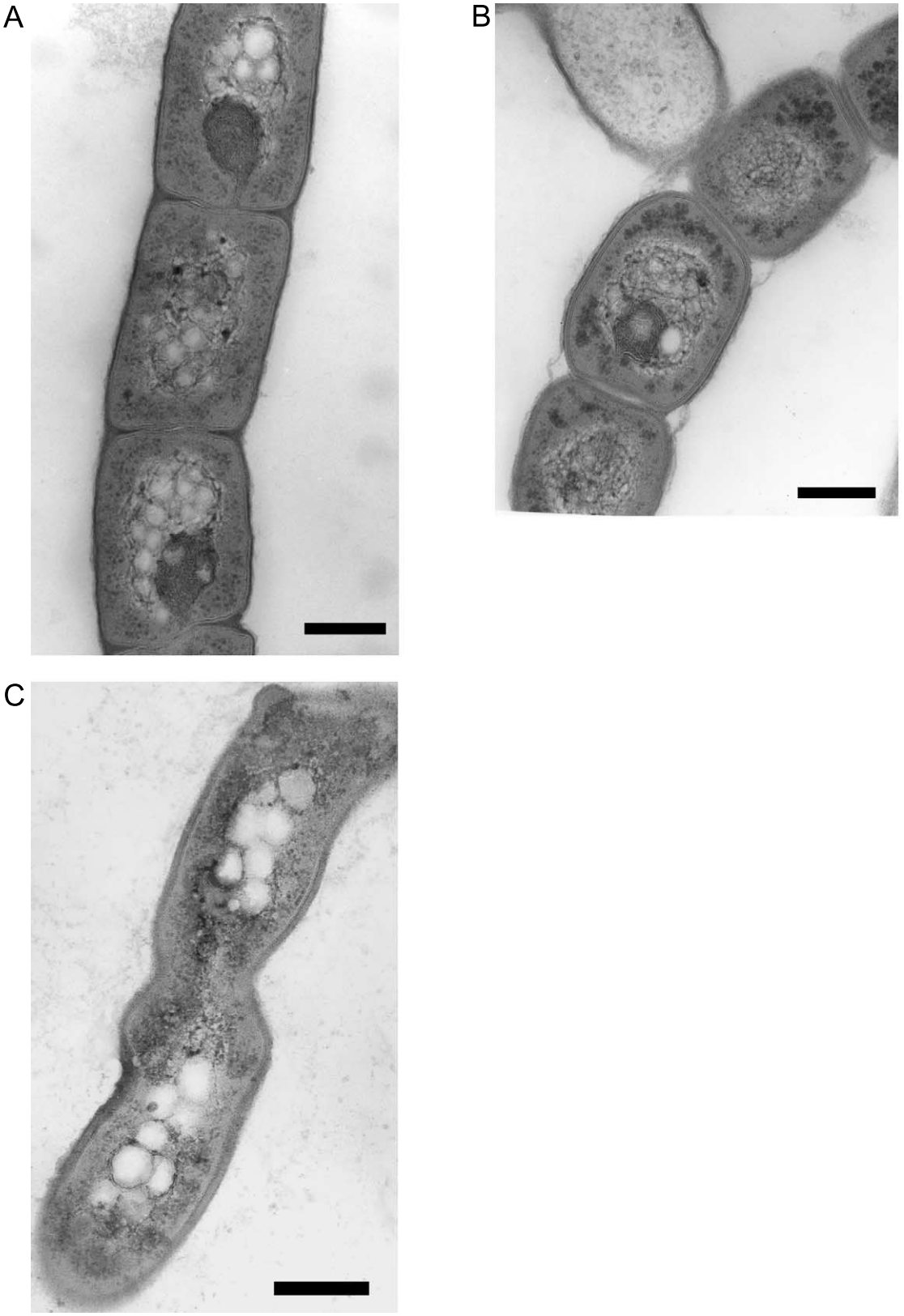

Fig. 4. Study of compartment formation in aerial hyphae by TEM. A,B: Transmission electron micrographs of immature (A) and mature (B) spore chains of *S. coelicolor* M145. C: Indentations in aerial hyphae of the *ssgB* mutant of *S. coelicolor*. On this occasion, two indentations were observed, but no septa; compare Fig. 3C (sections indicated by small arrows). Some white (electron-lucent) material accumulates in the hyphae, similar to that observed in the immature spores. Bar, 300 nm.

underline that small but significant changes may have occurred in the stability of the hyphae.

3.6. Where does ssgB fit in the developmental program?

The phenotype of the mutant, with long undifferentiated hyphae, occasional spore-like compartments and some hyphal collapse, strongly resembles that of the *whiM* mutant R432 [18]. The *whiM* locus was mapped to *S. coelicolor* cosmid I51 – far away from the *ssgB* locus or any of the other *ssgA* paralogues – but the mutation has not been pinpointed to a specific gene. Other early white mutants that are arrested in approximately the same developmental stage as *ssgB* and *whiM* are *whiJ* and *whiK* R655 [18]. *whiK* mutant R655 was found to suffer from a base change in the ribosome binding site of a gene encoding σ^{BldN}, which belongs to the family of ECF factors. *whiK* was later renamed *bldN*, as disruption of the corresponding gene resulted in a bald phenotype [19]. Apparently, *bldN*/*whiK* is active in both vegetative and aerial hyphae. An apparent feature of the *ssgB* mutant aerial hyphae is the presence of small side branches, as indicated by the small buds at the cell surface, a feature also observed for *whiK* (*bldN*) mutant R655. However, it is unclear if σ^{BldN} plays a role in the transcriptional control of *ssgB*.

There seems to be no direct correlation between the roles of *ssgB* and *ssgA*. In contrast to *ssgB* mutants, *ssgA* mutants produce significant amounts of viable spores on some media, although spore chains have reduced lengths [8]. Therefore, it is likely that SsgA is involved in the stimulation of sporulation-specific septa. Indeed, overproduction of SsgA in *S. coelicolor* triggers submerged sporulation. Conversely, *ssgB* mutants produce no sporulation septa, and the formation of large colonies suggests that mechanisms of growth cessation that are initiated prior to sporulation are not yet in place. These data strongly suggest that SsgB fulfills its regulatory role in an earlier developmental stage than SsgA.

Since *ssgB* transcription depends at least partially on σ^H [12], similarity between the phenotypes of the *sigH* and *ssgB* mutants was anticipated. However, *sigH* mutants are stalled in a later stage of development, producing viable spores, unlike the *ssgB* mutant. Apparently, the level of *ssgB* transcription observed in *sigH* mutants is sufficient to allow the formation of sporulation septa, otherwise the phenotype would resemble that of the *ssgB* null mutant. This suggests that in earlier stages of development, *ssgB* is transcribed by at least one other σ factor. Considering the high degree of similarity between the promoter sequences recognized by σ^H and σ^F, a σ factor involved in spore maturation in *S. coelicolor* [20], we anticipate that perhaps one of the σ^F-like σ factors is responsible for at least part of the transcription of *ssgB*. It is unlikely that *ssgB* should be controlled by σ^F itself, as this σ factor is expressed only at late stages of sporulation, and is important for spore maturation, while *ssgB* is expressed much earlier.

In summary, *ssgB* is a novel early *whi* gene that also plays a role in determining colony size. Analysis of the *ssgB* mutant colonies by electron microscopy suggests a position in the developmental cascade close to that of WhiM. The phenotypes of *ssgA* and *ssgB* mutants are different, and the respective mutants stalled at distinctly different points during development; this suggests that the various members of the family of SsgA-like proteins perform important roles in diverse parts of the *Streptomyces* life cycle, controlling processes related to growth and sporulation-specific cell division.

Acknowledgements

We thank J. van der Meulen for advice on TEM, J. van der Meer for help with SEM, and J. Slats for advice on fluorescence microscopy. Prof. K.F. Chater and Dr. E. Vijgenboom are thanked for discussions. This work was supported by grants from the Netherlands Royal Academy of Sciences (KNAW) to G.V.W. and from the Netherlands Applied Research Council (CW-STW) to B.J.F.K.

References

[1] Chater, K.F. (2001) Regulation of sporulation in *Streptomyces coelicolor* A3(2): a checkpoint multiplex? Curr. Opin. Microbiol. 4, 667–673.
[2] Wildermuth, H. and Hopwood, D.A. (1970) Septation during sporulation in *Streptomyces coelicolor*. J. Gen. Microbiol. 60, 51–59.
[3] Lutkenhaus, J. and Addinall, S.G. (1997) Bacterial cell division and the Z ring. Annu. Rev. Biochem. 66, 93–116.
[4] McCormick, J.R., Su, E.P., Driks, A. and Losick, R. (1994) Growth and viability of *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. Mol. Microbiol. 14, 243–254.
[5] Grantcharova, N., Ubhayasekera, W., Mowbray, S.L., McCormick, J.R. and Flårdh, K. (2003) A missense mutation in *ftsZ* differentially affects vegetative and developmentally controlled cell division in *Streptomyces coelicolor* A3(2). Mol. Microbiol. 47, 645–656.
[6] Flårdh, K., Leibovitz, E., Buttner, M.J. and Chater, K.F. (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.
[7] Kawamoto, S., Watanabe, H., Hesketh, A., Ensign, J.C. and Ochi, K. (1997) Expression analysis of the *ssgA* gene product, associated with sporulation and cell division in *Streptomyces griseus*. Microbiology 143, 1077–1086.
[8] van Wezel, G.P., van der Meulen, J., Kawamoto, S., Luiten, R.G., Koerten, H.K. and Kraal, B. (2000) *ssgA* is essential for sporulation of *Streptomyces coelicolor* A3(2) and affects hyphal development by stimulating septum formation. J. Bacteriol. 182, 5653–5662.
[9] Jiang, H. and Kendrick, K.E. (2000) Characterization of *ssfR* and *ssgA*, two genes involved in sporulation of *Streptomyces griseus*. J. Bacteriol. 182, 5521–5529.
[10] Yamazaki, H., Ohnishi, Y. and Horinouchi, S. (2003) Transcriptional switch on of *ssgA* by A-factor, which is essential for spore septum formation in *Streptomyces griseus*. J. Bacteriol. 185, 1273–1283.
[11] Bentley, S.D., Chater, K.F., Cerdeno-Tarraga, A.M. and Challis, G.L. et al. (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417, 141–147.
[12] Kormanec, J. and Sevcikova, B. (2002) The stress-response sigma

factor sigma(H) controls the expression of *ssgB*, a homologue of the sporulation-specific cell division gene *ssgA*, in *Streptomyces coelicolor* A3(2). Mol. Genet. Genomics 267, 536–543.

[13] Kelemen, G.H., Viollier, P.H., Tenor, J., Marri, L., Buttner, M.J. and Thompson, C.J. (2001) A connection between stress and development in the multicellular prokaryote *Streptomyces coelicolor* A3(2). Mol. Microbiol. 40, 804–814.

[14] Sambrook, J., Fritsch, E.F. and Maniatis T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[15] Kieser, T., Bibb, M.J., Buttner, M.J. and Chater, K.F. (2001) Practical *Streptomyces* Genetics. John Innes Foundation, Norwich.

[16] van Wezel, G.P., van der Meulen, J., Taal, E., Koerten, H.K. and Kraal, B. (2000) Effects of increased and deregulated expression of cell division genes on the morphology and on antibiotic production of streptomycetes. Antonie van Leeuwenhoek 78, 269–276.

[17] Schwedock, J., Mc Cormick, J.R., Angert, E.R., Nodwell, J.R. and Losick, R. (1997) Assembly of the cell division protein FtsZ into ladder-like structures in the aerial hyphae of *Streptomyces coelicolor*. Mol. Microbiol. 25, 847–858.

[18] Ryding, N.J., Bibb, M.J., Molle, V., Findlay, K.C., Chater, K.F. and Buttner, M.J. (1999) New sporulation loci in *Streptomyces coelicolor* A3(2). J. Bacteriol. 181, 5419–5425.

[19] Bibb, M.J., Molle, V. and Buttner, M.J. (2000) Sigma(BldN), an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2). J. Bacteriol. 182, 4606–4616.

[20] Potuckova, L., Kelemen, G.H., Findlay, K.C., Lonetto, M.A., Buttner, M.J. and Kormanec, J. (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.