Reciprocal Regulation between SigK and Differentiation Programs in *Streptomyces coelicolor*\(^\dagger\)\(^\S\)

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Here we reported that deletion of SigK (SCO6520), a sigma factor in *Streptomyces coelicolor*, caused an earlier switch from vegetative mycelia to aerial mycelia and higher expression of *chlP* and *chpH* than that in the wild type. Loss of SigK also resulted in accelerated and enhanced production of antibiotics, actinorhodin, and methylenomycin (15, 21). The completed genome sequencing project reveals that *S. coelicolor* has 65 sigma factors in total, the highest number in bacteria so far identified, which was presumably consistent with its elaborate transcriptional regulations on morphogenesis, secondary metabolism, and stress responses (2, 16). Of them, 10 group 3 sigma factors of the \(\sigma^{70}\) family were revealed, including SigB (SCO0600), SigL (SCO7278), SigI (SCO3068), SigN (SCO4034), SigF (SCO4035), SigH (SCO5243), SigK (SCO0620), SigM (SCO7314), SigG (SCO7341), and \(\alpha^{WhiG}\) (SCO5621), according to the order similarity to *Bacillus subtilis* SigB, a central regulator of stress responses (24). The typical group 3 sigma factors of the \(\sigma^{70}\) family have three independent domains, \(\sigma2\), \(\sigma3\), and \(\sigma4\), which are essential for the promoter recognition and RNA polymerase (RNP) binding (16, 29).

In *S. coelicolor*, most of these group 3 sigma factors positively modulate the morphological differentiations at various developmental stages. SigB was proposed as a master pluripotent sigma factor, since *sigB* mutant is bald, shows sensitivity to high levels of osmotic stress, and produces a higher level of Act but a lower level of Red (24). Interestingly, the mycothiol, which plays a significant role in the detoxification of thiol-reactive substances (28), was synthesized at a lower level in the *sigB* mutant after osmotic stress, thus leading to more oxidated (carboxylated) proteins (24). Microarray analysis suggests the transcriptional control of more than 280 genes by SigB after osmotic induction and a hierarchically transcriptional and posttranscriptional control order from SigB to SigL and SigM. Consistent with hierarchical regulation, SigB positively controls the aerial hyphal development and SigL is required for the sporulation, while SigM is involved in the full formation of spores (11, 24). \(\alpha^{WhiG}\) is required for the trigger of sporulation onset (10), and SigF is proposed to control proper spore formation and integrity (30), while SigH is essential for efficient septation of aerial hyphae into spores (32). Recently, delayed aerial mycelium formation and sporulation in the *sigN* mutant was observed on glucose-containing medium, and higher expression of the *sigN* promoter is restricted to the “subapical stem,” also suggesting the possible involvement of SigN in proper morphogenesis (12).

Meanwhile, these alternative sigma factors are regulated by developmental programs at both transcriptional and posttranslational levels. *sigBp1* expression is silenced at the vegetative mycelium phase but persistently increases when morphological differentiation progresses, consistent with the requirement of SigB for development into aerial hyphae and spores (11, 24). The *sigN* promoter is also positively regulated by the developmental programs (12). *sigF* expression is limited only to spores, accordant to its indispensability in proper spore formation (20, 30). However, four promoters of *sigH* are differentially induced during development, since both *sigHp1* and *sigHp2* are constitutively active, while *sigHp3* activity is increased but *sigHp4* is decreasingly expressed (23). Interestingly, three isoforms of SigH, SigH-\(\sigma^{32}\), SigH-\(\sigma^{31}\), and SigH-\(\sigma^{32}\), are observed as the

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primary translational products synthesized from three distinct translation initiation sites at the early developmental stages, but SigH-$\sigma^7$ is accumulated while SigH-$\sigma^{52}$ and SigH-$\sigma^{51}$ are eliminated and proteolytically truncated into SigH-$\sigma^7$ and SigH-$\sigma^{51}$, respectively, concurrent with the morphological transitions and suggesting a function in development (36).

Here we characterized the sigma factor SigK (SCO6520) as a negative regulator controlling the vegetative-aerial mycelium switch and secondary metabolism, and we also provide evidence that SigK expression was regulated positively at the transcriptional level and that SigK protein degraded during the development into aerial mycelia.

### MATERIALS AND METHODS

#### Bacterial strains and growth media.

The *S. coelicolor* strains used in this study are listed in Table 1. *Escherichia coli* strain ET10257 (26) containing helper plasmid pUZ8002 was used for routine plasmid transfer into *S. coelicolor* by conjugation, as described previously (21). *E. coli* strain TGI was used for the usual transformation in plasmid construction. *E. coli* strains were cultured at 37°C in solid or liquid Lennox broth medium. *S. coelicolor* strains were grown at 30°C on MS or R2YE for morphological and secondary metabolism analysis or in 3% tryptic soya broth liquid medium for genomic DNA preparation (21).

#### Plasmid construction

All primer sequences are provided (see Table S1 in the supplemental material). Plasmid pLM12 was transformed into the strains by PCR analysis (data not shown) and Southern blot analysis (see Fig. S1). About 30 g of total RNA was subjected to reverse transcription (RT)-PCR analysis with primers 15 and 16, primers 17 and 18, primers 19 and 20, and primers 21 and 22, and sequenced to pTA2 after one dA addition. All the PCRs were conducted with KOD-Plus polymerase (Toyobo), and the cloned fragments were verified by DNA sequencing.

#### sigK knockout and complementation.

sigK was knocked out in the wild-type strain M145 genome by an in-frame deletion strategy via double-crossover homologous recombination (21). Knockout plasmid pLM14 was introduced into M145 by conjugation, and the transformants were subinoculated on apramycin-containing MS medium at 37°C for integration of pLM14 at the sigk locus, as verified by Southern blot analysis (data not shown). After three rounds of relaxed growth at 37°C on MS without apramycin, the recombinants were streaked on single colonies and the sigk mutant was screened out from apramycin-sensitive strains by PCR analysis (data not shown) and Southern blot analysis (see Fig. S1). Plasmid pLM12 was transformed into the sigk mutant for complementation.

#### RNA preparation

About 50 mg of mycelia collected from MS or R2YE plates overlayed with cellophane was resuspended in 500 μl of ice-cold buffer (1% sodium dodecyl sulfate, 4% β-mercaptoethanol, 5 mM EDTA in diethyl pyrocarbonate [DEPC]-treated H₂O and homogenized by ultrasonication. The lysate was immediately extracted thoroughly with 500 μl of phenol-chloroform–isoamyl alcohol (pH 5.3) twice and once with 500 μl of chloroform. After centrifugation at 13,000 × g for 10 min at 4°C, the nucleic acids were precipitated with 500 μl of isopropanol in the presence of 50 μl of Na-actetyl (pH 5.3) and washed with 70% ethanol. The genomic DNA was removed after treatment with RNase-free DNase I (Takara). Total RNA was precipitated after extraction with phenol-chloroform–isoamyl alcohol (pH 5.3) and resuspended in DEPC-treated H₂O. The concentration of RNA was determined by spectrometry.

#### Low-resolution S1 nuclease protection assay

About 30 μg of total RNA was subjected to S1 nuclease mapping as described previously (31), with some modifications. Briefly, RNA was hybridized to about 10 ng of ssDNA probe(s) in annealing buffer (0.4 M NaCl, 60 mM piperazine-N,N'-bis(2-ethanesulfonic acid) [PIPES], pH 6.4, 1 mM EDTA) at 37°C for 16 h after denaturation at 90°C for 10 min. The single-stranded nucleic acids were removed with 100 U of S1 nuclease at 37°C for 1 h, and the hybrids were precipitated and subjected to Southern blot analysis by neutral transfer to nylon membrane (31). The biotin-labeled probes for Southern blot hybridization were prepared by PCR with

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**TABLE 1. *S. coelicolor* strains used in this study**

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<tr>
<th>Strain</th>
<th>Description</th>
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<tr>
<td>LM1</td>
<td>In-frame deletion of sigK in M145</td>
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</tr>
<tr>
<td>LM2</td>
<td>M1/pLM12</td>
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<td>LM4</td>
<td>M145/pLM6</td>
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**TABLE 2. Plasmids used in this study**

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universal primers 26 and 27 from pTA2 vector with cloned fragments in the presence of biotin-11-dUTP (Fermentas) (25).

The ssDNA probes for RNA hybridization were prepared with λ exonuclease (Fermentas). The 5’-phosphorylated primer 25 and unphosphorylated primer 27 were used to amplify the double-stranded DNA from pTA2 vector with cloned fragments. The PCR products were purified and digested with λ exonuclease to remove the 5’ phosphorylated sense strand DNA. The antisense ssDNA was extracted once with phenol-chloroform–isoamyl alcohol (pH 8.0), precipitated with isopropanol, and resuspended in DEPC-treated H2O.

Protein analysis. For assays of sigK promoter activity and SigK protein regulation, spores were spread on cellophanes overlaid on MS plates (21). After incubation for the indicated time, cells were collected, resuspended in lysis buffer (100 mM NaH2PO4, 10 mM Tris, pH 8.0, 1 mM phenylmethylsulfonyl fluoride), and destroyed by ultrasonication. Total protein concentration was determined by the Bradford method (Invitrogen) (7). About 20 μg of total protein was loaded for Western blot analysis (24) with anti-green fluorescent protein (GFP) anti-body (Proteintech Group) or anti-FLAG M2 antibody (Sigma) and Coomassie brilliant blue R250 staining of total protein for loading control (Beyotime, Haimen, China).

Antibiotic assay. Antibiotic assays for cell-associated Act and Red were performed as described previously (8, 21, 35, 39). Briefly, about 10 mg of mycelia cultured on R2YE covered with cellophanes at various developmental stages was formed as described previously (8, 21, 35, 39). Briefly, about 10 mg of mycelia (100 mM NaH2PO4, 10 mM Tris, pH 8.0, 1 mM phenylmethylsulfonyl fluoride), incubation for the indicated time, cells were collected, resuspended in lysis buffer (100 mM NaH2PO4, 10 mM Tris, pH 8.0, 1 mM phenylmethylsulfonyl fluoride), and destroyed by ultrasonication. Total protein concentration was determined by the Bradford method (Invitrogen) (7). About 20 μg of total protein was loaded for Western blot analysis (24) with anti-green fluorescent protein (GFP) antibody (Proteintech Group) or anti-FLAG M2 antibody (Sigma) and Coomassie brilliant blue R250 staining of total protein for loading control (Beyotime, Haimen, China).

Loss of sigK results in the earlier appearance of aerial mycelia and spores. The genome sequencing project has revealed that SCO6520 encoded a putative sigma factor, SigK (2). Domain anatomy by BLAST indicated that SigK contained three independent domains, α2, α3, and α4 (data not shown). Moreover, SigK could interact with the RNAP core enzyme in vivo and initiate transcription from the Bacillus subtilis ctc promoter in vitro (data not shown), suggesting that SigK was also an alternative group 3 sigma factor of the σ70 family (16, 29).

In S. coelicolor, all group 3 sigma factors showed high levels of similarity to Bacillus subtilis SigB, which is the central regulator for various stress responses and expression of more than 200 genes (24). However, SigK exhibited 46% identity and 63% similarity to S. coelicolor SigB. BLAST analysis (http://www.genedb.org/gedenb/scoelicolor/blast.jsp) also revealed that SigK was more related to SigG, with 71% identity and 84% similarity, and second-most related to SigM, with 69% identity and 80% similarity (24). However, the sigG mutant has phenotypes indistinguishable from those of the wild type (22), while SigM is required for efficient sporulation (24), suggesting the diversity of potential functions of SigK-related sigma factors.

To examine the functions of SigK in S. coelicolor, the sigK gene was knocked out by the in-frame deletion method (21), which left 39 bp and 123 bp intact at 5’ and 3’ of the sigK open reading frame, respectively, thus eliminating the coding regions for σ2, σ3, and σ4 domains. Successful deletion of sigK in the genome of strain M145 was verified by PCR analysis (data not shown) and Southern blotting (see Fig. S1 in the supplemental material).

Previous global microarray measurement has revealed decreased or increased expression of sigK after a high-temperature shift or a high osmotic shift, respectively, and sigJ, sigL, and/or sigB, sigK, sigM, sigH, and sigL were expressed in a sequential order upon the sucrose upshift (19). This implied additional possible roles of SigK on heat shock adaptation and osmoprotection by elaborate cooperative regulation with other sigma factors. However, we did not observe any significant growth difference between the wild type and the sigK mutant after temperature elevation and osmotic increase with KCl or sucrose (data not shown).

On R2YE medium for 40 h, the sigK mutant showed precocious aerial mycelium formation, as demonstrated in Fig. 1A, where the mutant is covered with aerial hyphae while the wild-type strain M145 has just begun developing into the aerial mycelium stage (Fig. 1A). After incubation for 4 days, the gray appearance of sigK mutant colonies indicated matured spores with pigment deposition, but the wild-type strain still stayed at the aerial mycelium stage (Fig. 1A). After incubation for 4 days, the gray appearance of sigK mutant colonies indicated matured spores with pigment deposition, but the wild-type strain still stayed at the aerial mycelium stage (Fig. 1A). After incubation for 4 days, the gray appearance of sigK mutant colonies indicated matured spores with pigment deposition, but the wild-type strain still stayed at the aerial mycelium stage (Fig. 1A).
The emergence of aerial hyphae requires the hydrophobic surfactants, such as chaplins and SapB protein, which facilitate the vegetative mycelium to overcome the surface tension of the mycelium-air interface (9, 13). When the expression levels of two of the chaplin genes, chpE and chpH (13), were examined, both the sigK mutant and the wild-type strain showed relatively low and similar expression levels of chpE and chpH at the vegetative hypha stage. After entering the aerial mycelium phase, the sigK mutant exhibited higher expression levels of chpE and chpH than the wild type (Fig. 1D), which might account for the accelerated aerial hypha formation after deletion of sigK. These data suggested that SigK had a repressive role for morphological development.

Similar phenotypes were also observed on MS medium (data not shown). All these data suggested that loss of SigK accelerated the developmental processes by quickening the transition into aerial mycelia and spores and that SigK was a repressive regulator of the developmental program of S. coelicolor.

**sigK deletion accelerates production of antibiotics.** The secondary metabolism profiles were also checked qualitatively and quantitatively, in both the sigK mutant and the wild type. On the R2YE plate after 24 h, the sigK null mutant appeared redder than the wild type and the complemented strain, suggesting that sigK also inhibits secondary metabolism (Fig. 2A). After a further 1 day of incubation, the sigK mutant lawn was already deep purple, indicating the production of blue antibiotic Act, while the other two strains had just begun to turn purple (Fig. 2A). The sigK mutant was much purpler after further incubation, indicating the higher production of Act (data not shown). Consistent with the observation on plates, quantitative measurement of the Act and Red amounts, respectively, also showed that the sigK mutant had earlier and higher production levels of antibiotics than the wild type on R2YE medium (Fig. 2B and C). In particular, Act production was more than 10 times higher in the sigK mutant after 6 days (Fig. 2C). The overproduction of Red and Act in the sigK mutant coincided with higher expression of redD and actII-orf4 in the sigK mutant (Fig. 2D), which encode two positive transcription activators of gene clusters for Red and Act production, respectively (14, 34). These phenomena suggested that SigK also had a repressive role in secondary metabolism.

**sigK expression increases before transition into aerial mycelia.** Our results suggested that SigK was a negative regulator of morphogenesis and secondary metabolism of S. coelicolor. However, when we checked the expression profile of sigK by the S1 nuclease protection assay during morphological development, sigK expression gradually increased on MS plates before 22 h when the cells were at the vegetative mycelium stage but were consistent after development into aerial mycelia and spores (Fig. 3A). The sigK expression pattern was further validated with a promoter-probing plasmid, in which GFP was under the control of the sigK promoter (sigKp). After incubation on MS plates for 14 h, the sigKp activity was comparatively low, but it increased after a further 8-h incubation when the cells were still in the vegetative mycelium phase (Fig. 3B). At the aerial mycelium (24 and 29 h) (Fig. 3B) and spore (41 and 48 h) (Fig. 4) stages, the sigKp activity was consistent with that at the 22-h stage. As a control, the band was not observed in the strain bearing the plasmid without a promoter, indicating the specificity of GFP expression driven from sigKp activity (data not shown). Since the GFP protein is stable in bacteria (1), the increase of GFP protein driven from sigKp might result from the accumulation of GFP at vegetative hypha stage. To exclude this possibility, GFP was then expressed under the control of a constitutive promoter ermEp* and found to exhibit a steady protein level during the vegetative mycelium development (Fig. 3C), even after development into aerial hyphae and spores (Fig. 5A). This excluded the interference of GFP sta-
bility with the monitoring of promoter activity with GFP as a reporter and also suggested the specific augmentation of sigKp activity at the vegetative hypha stage. All of the data described above suggested that sigK expression or the sigKp activity was positively regulated earlier before development into the aerial mycelium stage but was almost invariable at the late stage of vegetative mycelia and throughout the aerial mycelium and spore phases.

**sigK expression is partially dependent on SigK during the vegetative mycelium phase.** Some SigB-like sigma factors in *S. coelicolor* can regulate transcription on its own promoter, such as SigB on sigBp (11), SigH on sigHpz (32), and SigN on sigNp (12). We then checked whether sigK expression could also be autoregulated. With GFP as a reporter protein, about five-times-lower sigKp activity was observed in the sigK mutant than in the wild type at the 16-h incubation time point, when the cells were developing into vegetative hyphae. But wild-type cells showed only about 1.5-times-higher yields of GFP protein than the sigK mutant at the 22-h point, when the wild type was still at the vegetative mycelium stage, while aerial mycelia had begun to erect in the sigK mutant (Fig. 4). These results suggested partial positive autoregulation of sigKp activity from SigK and decreased dependence of sigK expression on SigK during the development of vegetative mycelia. However, no apparent difference in sigKp activity was observed after 24 h between the sigK mutant and the wild type (Fig. 4), suggesting independence of sigKp activity on SigK after development into aerial mycelia.

**Posttranscriptional regulation of SigK.** If SigK somehow inhibits the developmental stages of the normal *S. coelicolor* life cycle, how is this repression relieved given that sigK expression is persistent throughout the developmental growth? To address this question, we further investigated the stability of the SigK protein along with the developmental progression. SigK was C-terminally tagged with GFP and was expressed under *ermEp*+. As a control, GFP was also expressed under *ermEp*+, and consistent expression of GFP protein during the developmental courses was observed (Fig. 5A), suggesting that the *ermEp*+ activity and posttranscription of GFP were independent of morphological transitions. Interestingly, the SigK-GFP fusion protein, with an estimated molecular mass of about 58 kDa, was detected to be at about 70 kDa after 22 h of incubation, when the cells were developing into vegetative mycelia, but vanished after an incubation of 29 h or longer, when cells were developing into aerial mycelia and spores (Fig. 5A). The SigK-GFP protein could restore the sigK mutant phenotypes (data not shown), suggesting that this fusion protein was stable and functional. Moreover, two small fragments, one about 28 kDa (Fig. 5A, arrow b) and the other about 30 kDa (Fig. 5A, arrow a) could also be detected at the vegetative mycelium stage but disappeared after further morphological development. But all of these three bands were not detected in the GFP control sample (Fig. 5A), suggesting that the two smaller fragments were derived from SigK-GFP. However, another smaller band, with molecular mass similar to that of GFP, was constitutively detected throughout the developmental phases even with the disappearance of SigK-GFP and its derivatives (Fig. 5A). If a truncated isoform of SigK, which
The signal peptide of SigK contained a stop codon at the C terminus of SigK, was expressed at the same condition, this GFP band was still constitutively observed (data not shown), suggesting another translation initiation for GFP, which had the start codon ATG within the NdeI site.

We then checked whether the two smaller bands (Fig. 5A, arrows a and b) were specific for SigK-GFP. AtpD, the ATP synthase β subunit of S. coelicolor in catalysis of ATP production (37), was expressed with fusion to GFP in the same way as SigK-GFP, and the two bands, smaller than AtpD-GFP but slightly larger than GFP, were repeatedly observed (Fig. 5B). This suggested that these were nonspecific fragments arising only in the vegetative hypha stage. Moreover, though the AtpD-GFP protein level decreased gradually, it was detected throughout all the developmental stages (Fig. 5B), which was consistent with its biological activity as the housekeeping protein for ATP production (17, 38), and also suggested the specific disappearance of SigK-GFP in aerial hyphae and spores.

**Developmental phase dependence of SigK degradation.** The two smaller derivatives (Fig. 5A, arrows a and b) of SigK-GFP could have resulted from the posttranslational modification of SigK-GFP by two independent cleavages or two alternative translation initiations, as proposed for GFP in Fig. 5A. To discriminate these two possibilities, SigK-GFP was further tagged at its N terminus with 3FLAG. Examination of the 3FLAG-SigK-GFP protein (apparently about 72 kDa) dynamics by immunoblot analysis with anti-GFP with narrower time intervals showed that the SigK fusion protein level dropped sharply at the vegetative hypha stage and was very faint at 24 h but undetectable after 26 h, when cells were developing into aerial hyphae (Fig. 6A). Meanwhile, immunoblot analysis with anti-FLAG antibody also showed that 3FLAG-SigK-GFP protein decreased sharply in the vegetative hypha phase, but this fusion protein was detected at the 26-h time point and was absent after further incubation (Fig. 6A). These results suggested that the SigK protein was comparatively abundant at the vegetative stage, although it was in continuing decline and disappeared by the aerial stage.

FIG. 5. Posttranscriptional regulation of SigK. (A) Wild-type strain M145 containing plasmid pLM1 or pLM6 for expression of GFP or SigK-GFP, respectively, was harvested after being cultured on MS plates covered with cellophane at 30°C at different time points for vegetative mycelia (V), aerial hyphae (A), and spores (S) and treated as described in legend to Fig. 3B. Two small fragments are indicated by arrows a and b. Protein molecular mass markers are shown to the left as kilodaltons. (B) Wild-type strain M145 containing plasmid pLM8 expressing AtpD-GFP was inoculated on MS plates covered with cellophane at 30°C, and mycelia were gathered at 22, 29, 41, 48, 72, and 144 h after incubation for immunoblot analysis and Coomassie brilliant blue staining as described in legend to Fig. 3B. Different morphological phases of vegetative mycelia (V), aerial hyphae (A), and spores (S) are indicated.

FIG. 6. Developmental phase dependence of degradation of SigK. (A) Mycelia of strain LM9 (M145-ermEp-3flag-sigK-egfp) were collected from MS plates with cellophane at 30°C after incubation for different hours and treated as described in legend to Fig. 3B but with an additional anti-FLAG antibody for immunoblot analysis. Arrows a, b, a’, and b’ indicate the proteolytically cleaved fragments when detected with anti-GFP and anti-FLAG antibodies, respectively. Protein molecular mass markers are shown on the left as kilodaltons. The presence of vegetative mycelia (V), aerial hyphae (A), and spores (S) are shown. (B) Mycelia of strain LM5 (M145+ermEp-3flag-sigK) were treated in the same way as described for strain LM9 in panel A, but the immunoblot experiment was demonstrated only with the anti-FLAG antibody.
of vegetative mycelia) and was almost undetectable after 26 h of incubation, the amount of 3FLAG-SigK driven from sigKp was much lower than 3FLAG-SigK-GFP expressed from ermEp*. Also, the 3FLAG-SigK protein level did not decrease as rapidly as SigK fusion proteins under ermEp* (Fig. 6a and 7), which was consistent with observations that ermEp* was one of the strong constitutive promoters and that sigKp activity increased during vegetative mycelium growth. These data suggested that the endogenous SigK protein level fell concomitantly with vegetative hypha development and faded away during the vegetative-aerial mycelium transition.

**DISCUSSION**

**SigK, a repressive sigma factor in the group 3 subfamily**. SigK (SCO6520) was previously supposed to be one of 10 SigB-like sigma factors as determined by genome mining (2). Of the other nine sigma factors in the group 3 subfamily characterized to date, seven (SigB, SigL, SigN, SigF, SigH, SigM, and SigW) were positive regulators in morphological development (10, 12, 24, 30, 32). Meanwhile, the sigL mutant showed a complete absence of Act (24), and the sigB mutant showed enhanced Act but decreased Red production (11, 24). We reported the dual repressive roles of SigK on morphological development and secondary metabolism, suggesting that this alternative sigma factor (SigK) had unique functions in the group 3 σ70 subfamily. It was also suggested that the functional diversity and hierarchy of these sigma factors in this group might play a vital role in the precise transcription controlling for the proper subsistence of *S. coelicolor* under various environmental stresses.

How does SigK control the developmental processes? We presumed that SigK, as a negative regulator for development, antagonistically binds to the RNAP core enzyme against other sigma factors as positive regulators, such as seven group 3 members or one of the extracellular function sigma factors, BldN, as mentioned above. The gradual removal of SigK during morphological development causes the andante occupancy of other sigma factors on the promoters of genes for the proper development. Another possibility is that although SigK is a transcriptional activator, it positively controls the expression of some genes encoding the repressor proteins. Loss of SigK in the sigK mutant results in the advanced shutdown of these gene expressions, thus leading to the precocious maturation of *Streptomyces* cells.

**Developmental phase dependence of SigK protein degrada-**

SigK protein, under the control of *ermEp*, showed a sharp decline during morphological development. It was relatively abundant and decreased rapidly in the vegetative mycelium phase, was faint during the vegetative-aerial mycelium transition, and was absent at the early phase of aerial hyphae. Moreover, two independent cleavages at the linker sequence between SigK and GFP were proposed, and the proteolytical products vanished rapidly before developing into aerial mycelia. Further expression of 3FLAG-SigK under sigKp showed endogenous dynamics of SigK during morphological development. 3FLAG-SigK displayed a dynamics pattern similar to that of SigK fusion proteins under *ermEp*, except that they decreased much more gradually during vegetative hypha growth. This set of dynamics of SigK was consistent with the

![Fig. 7. Endogenous dynamics of SigK. Immunoblot analysis of about 20 μg total protein from LM9 (M145+ermEp-3flag-sigK-egfp) (lane 1) and LM10 (M145+sigKp-3flag-sigK) (lanes 2 to 7) grown on MS medium at 30°C for the indicated hours with the anti-FLAG antibody. Arrows a' and b' indicate the proteolytical cleaved fragments, as described in legend to Fig. 6a. Protein molecular mass marker are shown to the left as kilodaltons. Vegetative mycelia (V), aerial hyphae (A), and spores (S), indicating different morphological phases, are shown. Coomassie brilliant blue staining of total protein is shown for the loading control.](http://jb.asm.org/)

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repressive roles of SigK on morphological development and secondary metabolism. Since SigK disappeared when cells were developing into aerial mycelia, the removal of SigK as a block might account for the initiation of aerial hypha formation, and the accelerated sporulation in the sigk mutant might result from advanced aerial hypha formation, not from sigk deletion.

Several sigma factors have been reported proteolytically processed in S. coelicolor, for example, SigH as introduced above and one of the extracellular function sigma factors, BldN. BldN is required for the formation of aerial mycelia (4), but BldN is translated into a pro-σBmut and subsequently proteolytically processed into mature BldN by removal of about 86 residues at its N terminus when aerial mycelia emerge (3). Here we initially reported the specific degradation of another sigma factor, SigK, concomitant with the morphological developmental phases.

Possible correlations between sigk expression and level of SigK protein. One of the interesting observations was that sigk expression gradually enhanced before the aerial mycelium development. SigK protein was most probably because of the increased level change of SigK expressed under sigKp, which excluded the possible transcriptional effects on ermEp*, resulting from fast degradation of SigK to an appropriate processing from the sigKp promoter after development into aerial mycelia, thus causing the advanced disappearance of SigK and accelerated aerial hyphal formation as in the sigk mutant. Nevertheless, further efforts would be required to explain the noneconomical expression from the sigk promoter after development into aerial hyphae, when the cells lacked the SigK protein because of its rapid degradation.

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