SigB, an alternative sigma factor of the myxobacterium Stigmatella aurantiaca, is synthesized during development and heat shock

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Alternative sigma factors have been detected in the myxobacterium Stigmatella aurantiaca during indole-induced sporulation, fruiting body formation and heat shock using an antiserum raised against sigma factor SigB. The time course of sigB gene expression was analysed by RT-PCR and by determining β-galactosidase activity during development in a merodiploid strain that harboured a sigB–lacZ fusion gene. Inactivation of the sigB gene by insertion of the neo gene resulted in the loss of one sigma factor as shown by Western analysis. Neither fruiting body formation nor sporulation, nor the production of possible SigB targets, such as DnaK, GroEL or HspA, were affected.

Keywords: myxobacteria, developmental biology, Western analysis

INTRODUCTION

Stigmatella aurantiaca belongs to the myxobacteria, which represent a group of social prokaryotes (Reichenbach & Dworkin, 1992). Upon starvation, cells move into aggregation centres and form fruiting bodies differentiated into a stalk bearing several sporangioles at its top, which in turn contain several thousands of myxospores (Lünsdorf et al., 1995; Voelz & Reichenbach, 1969). Sporulation of vegetative cells may be induced independently from fruiting body formation with indole and some of its derivatives (Gerth et al., 1993). Fruiting body development of the related myxobacterium, Myxococcus xanthus, is strictly coupled to a time-dependent synthesis of regulatory factors (Kim et al., 1992) which stimulate the expression of many genes and govern pattern formation (Julien et al., 2000). Inactivation of the genes encoding these regulatory factors would lead to a defect in fruiting.


A comparative study of various eubacterial major sigma factors revealed the presence of highly conserved domains (Helmann & Chamberlin, 1988) that may also be present in minor sigma factors as was shown for SigB of S. aurantiaca (Skladny et al., 1992). The sigB gene was extracted from an established  życ11 gene library of chromosomal S. aurantiaca DNA using degenerate oligonucleotides derived from a highly conserved domain of sigma factors. The sigB gene encodes a 296 aa polypeptide with a predicted molecular mass of about 33-3 kDa that shows extensive homology to proteins of the group of secondary sigma factors, especially to those belonging to the heat-shock sigma family (Lonetto et al., 1992). The previously identified DnaK binding domains of E. coli RpoH are conserved at positions 115–122 (LKSWSLVK instead of LRNWRIVK) and 130–137 (RKLFFSLA instead of RKLFFNLR). These domains

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have been shown to mediate chaperone-dependent control of RpoH levels and are conserved in many eu-bacterial heat-shock sigma factors (McCarty et al., 1996). There is striking homology of the S. aurantiaca SigB to the secondary sigma factors SigB, SigC, SigD and SigE of M. xanthus (Apelian & Inouye, 1990, 1993; Ueki & Inouye, 1997, 1998) particularly in subregions 2 and 4 that are involved in the recognition of the promoter region (Helmann & Chamberlin, 1988). These subregions are also conserved in the vegetative sigma factors such as SigA of S. aurantiaca (Skladny, 1994; Skladny et al., 1994). In this communication we report the characterization of the S. aurantiaca sigB gene that is developmentally regulated.

METHODS

Bacterial strains and plasmids. Strains and plasmids are listed in Table 1. For DNA sequencing, fragments were cloned into pBSII SK+ and M13mp18. For the expression of sigB gene fragments, pQE9 and pQE11 (Qiagen) were used. For heat shock, cells were grown at 30 °C and shifted to 37 °C. Escherichia coli strains Y1090−, XL-1 Blue and M15(pREP4) were grown in the media recommended by the manufacturers (Stratagene, Qiagen).

Electroporation of S. aurantiaca. Electroporation was performed as described by Stamm et al. (1999).

DNA manipulations. Standard genetic techniques for in vitro DNA manipulations and cloning were used (Sambrook et al., 1989). Genomic DNA was prepared as described by Neumann et al. (1992). Southern blot hybridization was performed under standard conditions at 68 °C (Sambrook et al., 1989). Post-hybridization washes were carried out at 65 °C. Plaque and colony hybridizations were done under the same conditions.

DNA sequencing. To determine the DNA sequence of plasmid pL15, pBSII SK+ and M13 mp18 were used for subcloning. The DNA sequence was determined by the method of Sanger et al. (1977), by using T7 polymerase on ssDNA and denatured plasmid dsDNA. Synthetic oligonucleotides were used for sequencing. Deaza-dATP, deaza-dGTP and formamide-containing sequencing gels were used to resolve compressions.

Western blot analysis. Fruiting bodies which had been scraped off starvation agar plates, vegetative cells, indole-induced spores or heat-shocked cells were suspended in a buffer containing 50 mM MOPS, pH 7.5, 10 mM MgCl₂, 10 mM dithiothreitol and 1 mM PMSF. Cells and spores were broken by sonication (Branson Sonifier, Cell Disrupter B15) with glass beads (diam. 0.1 mm) at 4 °C for 1 min in an Eppendorf tube using a cuphorn (Branson EDP 101-151-003). To remove glass

Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Characteristics</th>
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<tr>
<td>E. coli</td>
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<td>XL-1 Blue</td>
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<td>lacZAM15 Tn10 (Tc')</td>
<td>Stratagene</td>
</tr>
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<td>Y1090−</td>
<td>ΔlacU169 aln araD139 strA supF mcrAB hsdR trpC22::Tn10 (Tc')</td>
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<td></td>
<td>(pMC9 Ap' Tc')</td>
<td>stratagene</td>
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<tr>
<td>S. aurantiaca</td>
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<td>DW4/3-1</td>
<td>Wild-type (Sm')</td>
<td>Qualls et al. (1978)</td>
</tr>
<tr>
<td>BS3</td>
<td>DW4/3-1, sigB::neo (Km'Sm')</td>
<td>This study</td>
</tr>
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<td>B/Z2</td>
<td>DW4/3-1, sigB::(sigB-lacZ-neo) (merodiploid for sigB) (pBSII SK +) (Sm' Km')</td>
<td>This study</td>
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<tr>
<td>Plasmids</td>
<td></td>
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<tr>
<td>pBS9</td>
<td>blA::(lacZ-neo) in pBS SK − (Ap' Km')</td>
<td>Silakowski et al. (1996)</td>
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<td>BamHI/HindIII sigB fragment in pQE11 (Ap')</td>
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beads and cell debris, the samples were centrifuged at 15000 g at 4 °C for 10 min. Aliquots of the supernatant containing 10 µg protein were mixed with the same volume of 2× Laemmli sample buffer and incubated for 10 min at 98 °C. After electrophoresis of the samples in a 12.5% or 15% SDS-polyacrylamide gel, the protein bands were electrophoretically blotted onto a sheet of Immobilon PVDF membrane (Millipore) using a semi-dry blotting apparatus (Pegasus; Phase) as described elsewhere (Harlow & Lane, 1988; Kvhse-Andersen, 1984). Immunodetection was accomplished by using antisera raised against SigB and HspA from S. aurantiaca and DnaK and GroEL from E. coli, and goat anti-rabbit antisera conjugated with alkaline phosphatase as second antibody as described by the manufacturer (Sigma).

**Gel electrophoresis.** SDS-polyacrylamide (12.5 or 15%, w/v) gels were employed according to the procedure of Laemmli (1970) and stained with Coomassie brilliant blue R-250.

**Construction of plasmids expressing the sigB gene of S. aurantiaca.** Due to unknown reasons, the expression of the complete sigB gene of S. aurantiaca was not possible in E. coli. For the production of antigen needed for raising antisera against SigB, sigB was cloned as two fragments encoding the N-terminal part of SigB without the potential start codon was amplified by PCR and cloned into the HindIII/PstI sites of pQE9, resulting in pQE9SigB. The DNA fragment encoding the N-terminal part of SigB without the potential start codon was amplified by PCR and cloned into the HindIII/PstI sites of pQE9, resulting in pQE9sigB. The DNA fragment encoding the N-terminal part of the sigma factor was amplified by PCR and cloned into the BamHI/EcoRI sites of pQE11, resulting in plasmid pQE11sigB. Induction of cloned fragments resulted in the synthesis of fusion proteins with N-terminal His-tags. The fusion proteins were purified by immobilized metal ion affinity chromatography (IMAC) as described by the manufacturer (Qiagen). The proteins were checked for purity by analytical SDS-PAGE. The concentration of the protein was determined by the method of Bradford (1976).

**Production of antisera.** A rabbit was primed with about 100 µg of an equimolar mixture of both SigB-fusion proteins suspended in RAS-adjuvants (SEBAK) and boosted at 2-week intervals with the same amount of protein in RAS-adjuvants. The antibody titre was monitored by Western analysis with the purified antigen. The antisera reacted specifically with the purified antigen. The antiserum reacted specifically with the purified antigen. The antiserum reacted specifically with the purified antigen. The antiserum reacted specifically with the purified antigen.

**RNA isolation and RT-PCR.** For the preparation of total RNA, cells (about 2×10^8) or induced-spores were harvested by centrifugation, or fruiting bodies were scraped off agar plates, and RNA was extracted as described by Chomczynski & Sacchi (1987). RNA was further purified with the SV Total RNA Isolation Kit (Promega), according to the manufacturer’s protocol. About 200 ng of RNA were used for the RT-PCR with the Access RT-PCR System (Promega) and the primers 5′-sigBC (5′-GACAGATCCCGCCCGCCATG-3′) and 3′-sigBC (5′-GGTTTAGCATCCTGCATGGT-3′). The primer extension of the sigB transcript. Primer extension analysis was performed by using a reverse oligonucleotide (5′-GGTTTAGCATCCTGCATGGT-3′) that was end-labelled with T4 polynucleotide kinase and (β-32P) ATP. The labelled oligonucleotide was purified by gel filtration using a Sephadex G-50 column and was hybridized with 10 µg S. aurantiaca wild-type RNA at 65 °C for 90 min. Reverse transcription was performed with AMV reverse transcriptase (Stratagene) at 42 °C for 1 h. The synthesized cDNA was treated with RNase A (Serva), purified by phenol extraction and then analysed on a sequencing gel of 6% polyacrylamide containing 7 M urea.

**Construction of strain B/22 harbouring a sigB-lacZ fusion gene.** A BamHI/SalI 4.6 kb fragment containing a 3.3-kbp BamHI/HindIII promoterless lacZ fusion gene from the minitransposon TnslacZ (de Lorenzo et al., 1990) and a HindIII/SalI 1.3-kbp neo gene from pUC4KIXX were isolated from plasmid pBS9 (Silakowski et al., 1996) and cloned into BamHI/SalI-restricted pBSII SK+, resulting in pBSlacZ. An EcoRI/BglII 1.2-kbp fragment harbouring the upstream region of sigB and the ATG start codon from sigB was taken from plasmid pL15 and cloned into the EcoRI/BamHI-restricted plasmid pBSlacZ, leading to pBSsigBlacZ. pBSsigBlacZ was introduced into S. aurantiaca DW4/3-1 by electroporation. Kanamycin-resistant clones were obtained at a frequency of 2×10^8. A single recombination event led to the merodiploid strain B/22 containing the wild-type sigB gene and a SigB-lacZ fusion gene as shown by Southern analysis (data not shown).

**Construction of the sigB mutant strain BS53.** The neo gene of plasmid pUC4KIXX (Pharmacia) was isolated after BamHI restriction and inserted into the BglII site of sigB (Składny et al., 1992) of plasmid pL15. The orientation of the neo gene was divergent to that of sigB. The resulting plasmid pBS28 was linearized with ScaI and introduced into the S. aurantiaca wild-type strain DW4/3-1 by electroporation. A kanamycin-resistant mutant strain (BS53) was obtained in which the wild-type gene was replaced by the sigB gene with the neo insertion.

**Determination of β-galactosidase activity.** β-Galactosidase activity was determined by the method of Miller (1972) in cell extracts of vegetative, heat-shocked, indole-treated and starving cells of S. aurantiaca (Kroos et al., 1986). Vegetative, heat-shocked and indole-induced cells were harvested and suspended in TPM buffer (10 mM Tris/HCl, pH 7.5, 1 mM KH_2PO_4, 8 mM MgSO_4). Developmental cells were scraped off water agar plates and suspended in TPM buffer. All subsequent manipulations were performed in TPM buffer. Harvests were disrupted by sonication as described above for Western blot analysis.

**RESULTS**

**Identification of S. aurantiaca SigB protein.** For the immunological identification of the SigB polypeptide in S. aurantiaca cells, SigB was raised. As it was not possible to clone the entire sigB gene downstream of the lac promoter in high-copy-number plasmids (pQE), possibly due to a toxic effect on E. coli cells, the sigB gene was expressed as two fragments. The 5′ends of the sigB gene fragments were fused to a sequence encoding six consecutive histidine residues. This allowed easy purification of the gene products by metal ion chelate chromatography. An equimolar mixture of both peptides was used for immunization.

S. aurantiaca SigB is a member of the σ^70 protein family.
**Fig. 1.** Comparison of the sigma factor patterns of the wild-type (WT) strain DW4/3-1 and mutant strain BS53. Western blot analyses of the cell lysates after heat shock (a), starvation (b) and indole-induced sporulation (c). From each lysate 10 µg protein was separated in a 12.5% acrylamide gel, transferred to a polyvinylidene difluoride membrane and analysed with anti-SigB polyvalent antiserum (1:10000). The size of the proteins was estimated with a low range marker (Bio-Rad).

(Skladny et al., 1992). Members of this family harbour peptide domains that are highly conserved (Lonetto et al., 1992) and are the reason for cross-reactivity of sigma factor antisera. To determine which of the cross-reacting polypeptides (see Fig. 1) is encoded by sigB and if sigB is involved in fruiting body formation or sporulation, the sigB gene was inactivated. For this purpose, the neo gene isolated from pUC4KIXX was inserted into a unique BglII site near the start of translation of sigB in plasmid pL15. The resulting plasmid, pBS28, was linearized and transferred into *S. aurantiaca* cells by electroporation. Southern analysis (not shown) confirmed that the wild-type sigB gene carries the neo disruption (Fig. 2). One of the transformants, BS53, was analysed further.

Western blot analysis was performed on crude extracts from *S. aurantiaca* cells under different physiological conditions using the antiserum raised against the mixture of the SigB fusion proteins. Vegetative cells of wild-type and mutant BS53 form mainly one cross-reacting polypeptide with an apparent molecular mass of about 100 kDa that corresponds to the main sigma factor (SigA) with a calculated molecular mass of 79-9 kDa (Heidelbach et al., 1992; Skladny et al., 1994). This sigma factor is detected under all physiological conditions. Extracts of heat-shocked wild-type cells contained two further cross-reacting polypeptides with an apparent molecular mass of about 35 and 36 kDa (Fig. 1a). They were detectable between 5 and 25 min after
the increase in temperature from 30 to 37 °C, but were not present after 60 min. The intensity of staining for each of these polypeptides in immunoblots suggests a high degree of relatedness between both proteins. In mutant BS53 the 36 kDa polypeptide was absent. Only the 35 kDa and a weakly stained 37 kDa polypeptide appeared during heat treatment (Fig. 1a). During fruiting body formation, two polypeptides of about 35 and 36 kDa are observed 24 and 48 h after the beginning of starvation in the wild-type (Fig. 1b). In cell extracts of mutant BS53 only the band with the 35 kDa polypeptide was detected (Fig. 1b).

The two polypeptides of 35 and 36 kDa were also present in extracts of wild-type cells in which sporulation was induced by indole. The 36 kDa band appeared about 10 min after the addition of indole, while the 35 kDa polypeptide appeared about 30 min after adding indole (data not shown). The larger band reacted strongly with the anti-SigB antibody, while the smaller band reacted weakly. Furthermore, a cross-reacting polypeptide with a molecular mass below 30 kDa was found. The 36 kDa polypeptide was absent in extracts of mutant BS53 (Fig. 1c). Instead, the cross-reacting polypeptide of 35 kDa was abundant. In addition, the amount of a polypeptide with an apparent molecular mass of about 37 kDa increased during indole treatment in mutant BS53 cell extracts, whereas the amount of the cross-reacting polypeptide of about 30 kDa was much less.

To investigate whether inactivation of the sigB gene affects the heat-shock response of S. aurantiaca, Western analysis was performed using antisera raised against the heat-shock proteins DnaK and GroEL from E. coli and HspA from S. aurantiaca. No significant difference in DnaK, GroEL and HspA formation was observed when comparing cell extracts from the wild-type and mutant BS53 subjected to heat shock (data not shown).

**RT-PCR analyses of sigB transcription of mutant BS53 and wild-type cells**

For the determination of the size of the sigB gene transcript, Northern analysis was performed with the sigB fragment as gene probe. During indole-induced sporulation hybridizing RNA of about 1-1 kb was observed 10 to 60 min after the addition of indole (data not shown). During starvation, the hybridizing transcript was detectable between about 5 and 20 h after the beginning of starvation (Skladny, 1994) (data not shown). No signal was detected with RNA of vegetative cells. Due to the high homology between the sigma factor genes of S. aurantiaca and M. xanthus that may be induced simultaneously under various physiological conditions, the signals obtained in a Northern analysis using the sigB gene of S. aurantiaca as probe may be derived from transcripts of different sigma factor genes. Therefore, to confirm the results obtained by Western blot analysis with the SigB antiserum, RT-PCR was performed with RNA isolated from cells during vegetative growth, heat shock, fruiting body formation and indole-induced sporulation. With a sigB-specific primer (bp 12–35 of the sigB ORF; GenBank accession no. 2269).
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**Fig. 4.** Primer extension analysis (left) was used to identify the transcriptional start site for the expression of the **sigB** gene. A sequencing reaction (TCGA), performed with the same primer, is shown next to the primer extension analysis on an RNA template from cells treated with indole for 10 and 20 min. A portion of the DNA sequence is indicated on the right. +1 indicates the putative transcriptional start site as determined by the migrational position of the main primer extension product.

Z14970) and a reverse primer homologous to **S. aurantiaca** **sigB** and **sigC** (bp 528–548 of the **sigB** ORF; GenBank accession no. U27311) the 536 bp PCR fragment was not detected when analysing RNA from vegetatively growing wild-type cells or mutant BS53 cells (Fig. 3). In a time period between 10 and 30 min after the temperature upshift from 30 to 37 °C the **sigB** transcript was detectable by PCR in the wild-type but not in mutant BS53. About 14 h after the beginning of starvation the **sigB** transcript is detectable in the RNA from the wild-type cells, but not in that from the mutant cells. About 5 to 25 min after the addition of indole the **sigB** transcript was detected in RNA from the wild-type but not in the RNA from mutant BS53. **SigB** transcription ceased 60 min after the addition of indole. In addition, a second transcript that resulted in a smaller PCR fragment was detected in the wild-type 15 and 60 min after indole treatment. Using the mutant RNA a very faint signal of the same size was observed 5 and 25 min after addition of indole (Fig. 3). Both PCR fragments were cloned and sequenced. The larger is identical with part of the **sigB** gene; the smaller encodes a putative polypeptide that has significant similarity to a hypothetical protein of **Chlorobium tepidum** (GenBank accession no. AF287482).

**Determination of the of **sigB** transcription initiation site**

The transcription start site of **sigB** was determined by primer extension using a reverse primer ranging from bp -3 to +15 of the **sigB** ORF and total RNA from indole-induced cells. Under these conditions transcription initiation sites located 72 and 74 bp upstream of the translation start site were detected (Fig. 4). No typical promoter sequence was found upstream of the transcription start site.

**Fig. 5.** β-Galactosidase activity of strain B/Z2 during heat shock (a), starvation (b) and indole-induced sporulation (c). β-Galactosidase specific activity is expressed in Miller units (nmol ONPG mg⁻¹ min⁻¹).

**SigB expression**

For the determination of the temporal expression of **sigB** during heat shock, indole-induced sporulation and fruiting body formation, a promoterless **lacZ** gene was fused to the ATG start codon of the **sigB** gene. The fusion gene containing 1-2 kbp of the upstream region of **sigB** and **lacZ** was cloned into the plasmid pBSII SK+ and transferred into **S. aurantiaca** by electroporation. The 1-2 kbp upstream sequence was shown to harbour
the sigB promoter (see Fig. 4). About 700 bp upstream of the sigB translation initiation site an ORF for a putative polypeptide was found with significant homology to a hypothetical polypeptide from Pseudomonas aeruginosa. After transfer into S. aurantiaca the hybrid gene should be under the transcriptional control of the sigB promoter and the potential regulatory upstream region. The hybrid gene integrated into the wild-type genome by a single crossover recombination leading to the merodiploid mutant B/Z2. This homologous recombination event led to a tandem arrangement of the ΔsigB-lacZ gene, followed by the plasmid sequence, the 1200 bp upstream region and the wild-type sigB (Fig. 2). As the indicator gene was fused to the ATG start codon of sigB, integration could occur only into the sigB-specific upstream region and not into conserved gene sequences of other alternative sigma factors. The result of the recombinational event was determined by Southern analysis of the chromosomal DNA (data not shown). As expected, the resulting transconjugant clones, which were selected by their kanamycin resistance, formed fruiting bodies like the wild-type strain.

To analyse sigB expression in strain B/Z2, the time dependence of specific β-galactosidase activity was determined under various conditions in crude cell extracts (Fig. 5). A significant increase in β-galactosidase activity was detected during heat shock, starvation and indole-induced sporulation. A background activity was detected during vegetative growth. During the first 3 min after the beginning of heat shock a steep increase in β-galactosidase activity was observed and then during the next 55 min there was a more gradual increase (Fig. 5a). Starvation leads to a slow increase in β-galactosidase activity. The rise in specific activity continued even 50 h after the beginning of the starvation period (Fig. 5b). About 24 h after the beginning of starvation the first mature fruiting bodies appeared. Indole leads to a fast increase in β-galactosidase activity during the first 60 min after addition of the compound. Thereafter, a slow rise in activity was observed during the next 2 h (Fig. 5c). During this time period the first myxospores appeared.

**DISCUSSION**

SigB, a gene for an alternative sigma factor (SigB) of the myxobacterium S. aurantiaca has been cloned (Skladny et al., 1992) and characterized. The DNA-derived amino acid sequence of the polypeptide showed significant homology to alternative sigma factors belonging to the σ family, especially to SigB, SigC, SigD and SigE of M. xanthus (Apelian & Inouye, 1990, 1993; Ueki & Inouye, 1997, 1998).

The expression in E. coli of the complete sigB gene inserted into multicopy plasmids was not successful, possibly due to a toxic effect of the SigB protein. Therefore, the gene was split into two fragments that were expressed successfully in E. coli. An antiserum raised against a mixture of the two polypeptides was used in this study.

The antiserum cross-reacted with at least two proteins of 35 kDa and 36 kDa that were present in indole-induced spores, fruiting bodies and after heat shock (Fig. 1a–c). In extracts of vegetatively grown cells both cross-reacting polypeptides of 35 and 36 kDa were absent. This suggests the developmentally regulated formation of the alternative sigma factors. In contrast, the major sigma factor, SigA, of S. aurantiaca (Skladny, 1994) is formed during vegetative growth, heat shock and development.

To determine whether the 35 or the 36 kDa polypeptide is encoded by sigB, the sigma factor gene was inactivated by inserting the neo gene a few base pairs downstream of the start codon (Fig. 2). Comparison of the Western blots of cell extracts obtained from mutant BS53 and wild-type cells under various physiological conditions (Fig. 1a–c) revealed the 36 kDa polypeptide to be encoded by sigB, as it is absent in mutant BS53.

During indole-induced sporulation the cross-reacting 35 kDa polypeptide appears at an earlier stage of development (Skladny, 1994) (data not shown). It is suggested that this polypeptide is an additional sigma factor that resembles SigB. Similar observations for the expression of alternative sigma factors in M. xanthus have been described (Apelian & Inouye, 1990, 1993; Ueki & Inouye, 1998). The antiserum against SigB cross-reacts with some other protein species with different apparent molecular masses, possibly additional sigma factors. This may be due to the high level of homology between these polypeptides as shown for sigma factors for many different bacterial species (Lonetto et al., 1992).

Transcription of sigB was detected by RT-PCR during the early stage in indole-induced sporulation (Fig. 3) and during the middle stage in fruiting body formation. This correlates quite well with the conversion of vegetative cells into myxospores. SigB transcription was not detected during vegetative growth. Under heat-shock conditions sigB expression started 10 min after the temperature shift. During indole-induced sporulation there was an additional smaller PCR product detected using a sigB-specific forward primer to reduce a non-specific response due to the highly conserved regions of sigma factors. This PCR product was also detected in very small amounts when using RNA from the sigB mutant BS53. It encodes a putative polypeptide with significant homology to a hypothetical protein from C. tepidum. The results of the Western blot and RT-PCR analyses agree well with those obtained from the determination of β-galactosidase activity in the merodiploid mutant B/Z2. The discrepancy between sigB transcription during fruiting as determined by RT-PCR and the synthesis of β-galactosidase in the merodiploid mutant BZ/2 could be due to the fact that the expression of sigB reaches a threshold for detection by RT-PCR only after 14 h.

Though sigB expression seems to be mainly coupled to development, neither indole-induced sporulation nor fruiting body formation is impaired in the sigB mutant.
BS53. The structure of the BS53 fruiting body was investigated by light and electron microscopy. No changes in the fruiting body and spore structure were detected as compared to that of the wild-type (H. Lünsdorf, GFB Braunschweig, personal communication). Furthermore, germination of the fruiting body spores was not affected in BS53. This is in contrast to the results obtained for *M. xanthus*, where alternative sigma factors seem to be involved in myxospore maturation (Apelian & Inouye, 1990; Ueki & Inouye, 1998). Furthermore, the synthesis of the heat-shock proteins DnaK, GroEL and HspA (Heidelbach *et al.*, 1993) is not affected in mutant BS53 as shown by Western analysis (data not shown). It seems that the loss of SigB in BS53 is compensated by other sigma factors such as the 35 or the 37 kDa polypeptides detected in the Western blot shown in Fig. 1.

In addition to the results obtained by Western blot analysis a further indication of the presence of multiple genes for alternative sigma factors comes from Southern blot analyses using different oligonucleotides that encode highly conserved regions of sigma factors. Using these oligonucleotides as gene probes, up to eight cross-hybridizing DNA fragments were detected in *S. aurantiaca* DNA (Skladny, 1994). Two of the fragments correspond to the previously identified genes for SigA and SigB. This suggests that there might be a multitude of sigma factor genes of the heat-shock type present in the genome of *S. aurantiaca*. One of these cross-hybridizing fragments has recently been cloned and sequenced. It encodes another sigma factor (SigC) of the heat-shock sigma type. It has to be tested whether this gene encodes the 35 kDa cross-reacting polypeptide.

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**REFERENCES**


with chain-terminating inhibitors. Proc Natl Acad Sci USA 74, 5463–5467.


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