A new gene, \textit{sigG}, encoding a putative alternative sigma factor of \textit{Streptomyces coelicolor} A3(2)

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Abstract

An oligonucleotide probe encoding a peptide motif conserved in all sigma factors was used to isolate a new gene, \textit{sigG}, from a \textit{Streptomyces coelicolor} A3(2) genomic library. The deduced protein of 263 amino acids with an \( M_r \) of 29 422 showed the greatest similarity to the previously identified sporulation sigma factor (\( \sigma^F \)) of \textit{Streptomyces coelicolor}, and general stress response sigma factor (\( \sigma^B \)) of \textit{Bacillus subtilis}, mostly in domains suggested to be involved in recognition of \(-10\) and \(-35\) promoter regions. Southern-blot hybridization with DNA from several \textit{Streptomyces} spp. revealed the presence of a similar gene in all strains tested. Disruption of the \textit{S. coelicolor} \textit{sigG} gene appeared to have no obvious effect on growth, morphology, differentiation, and production of pigmented antibiotic actinorhodin and undecylprodigiosin. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

\textit{Streptomyces} are mycelial, Gram-positive bacteria which undergo a complex cycle of morphological differentiation. The differentiation is controlled at several levels, of which heterogeneity of \( \sigma \)-factors of RNA polymerase seems to play an important role [1]. The \( \sigma \)-subunits confer promoter-specific transcription initiation on RNA polymerase [2]. Biochemical and genetic analyses in \textit{Streptomyces coelicolor} A3(2) revealed at least 11 forms of RNA polymerase containing different \( \sigma \)-factors [3,4]. Two \( \sigma \)-factors have been shown to be involved in the control of the morphological differentiation. The \( \sigma \)-factor encoded by \textit{whiG} is required to trigger the onset of sporulation in \textit{S. coelicolor} [5], and the \( \sigma \)-factor encoded by \textit{sigF} is proposed to control the late stages of spore development [6].

In order to isolate new \( \sigma \)-factor genes participating in the differentiation of \textit{S. coelicolor}, we selected the strategy based on high homology among different \( \sigma \)-factors in region 2 [7]. We hybridized a \textit{S. coelicolor} A3(2) genomic library with an oligo probe encoding a peptide motif from that region. Using this approach, we have identified a new gene, \textit{sigG}, exhibiting high similarity to all conserved domains characteristic of \( \sigma \)-factors [7]. This work also reports disruption of the gene in chromosome of \textit{S. coeli- color} A3(2).
2. Materials and methods

2.1. Bacterial strains, plasmids and culture conditions

*S. coelicolor* M145 (SCP1−, SCP2−, Pgl+) [8] and J1916 (hisA1, strA1, uraA1, ΔglkA-espI19, Pgl−, SCP1−, SCP2−) [9] were kindly provided by Mark J. Buttner, John Innes Institute, Norwich, UK. *Escherichia coli* XL1Blue (Stratagene) was used as a host and plasmid *pBluescript* II SK+ (Stratagene) and pUC19 (New England Biolabs) were used for *E. coli* cloning experiments. *E. coli* ET12567 [10] was used for preparation of dam− and dcm− plasmid DNA. Growth, transformation of *S. coelicolor*, and plasmid isolation were carried out as described in [8]. SMMS [11], MM, R2, and R5 [8] solid media were used to assess morphological differentiation and antibiotic production. Liquid SMM [11] and TSB [8] media were used to check the growth of the wild-type and *sigG*-disrupted strains. Conditions for *E. coli* growth and transformation were as described [12].

2.2. DNA manipulations

DNA manipulations in *E. coli* were done as described in [12] and those in *Streptomyces* were as described in [8]. Chromosomal DNA from *S. coelicolor* strains was isolated according to [8]. One microgram of the DNA was digested with appropriate restriction endonuclease, separated by electrophoresis in 0.8% (w/v) agarose gel in TAE, and transferred on Hybond N (Amersham) as described in [12]. The membrane was hybridized with random primed [α-32P]dCTP-labeled 1021-bp *Pvu*II-*Bam*HI DNA fragment (Fig. 1a), at 45°C in 50% (v/v) formamide as described in [12]. Colony blot hybridization with random primed DNA of *S. coelicolor* was performed at 30°C in 50% (v/v) formamide as described in [18]. Two replica Hybond N filters (Amersham) were washed after hybridization in 2× SSC (0.3 M NaCl/0.03 M sodium citrate pH 7.0), 0.1% SDS at 35 and 40°C.

2.3. Disruption of *sigG*

A 2.4-kb *Pvu*II−EcoRI fragment from a pBR322 derivative plasmid pRPO5SC-10 containing *sigG* (Fig. 1a) was cloned into *pBluescript* II SK digested with EcoRI and EcoRV to create plasmid pRPO5SC-10A. A 1050 bp *Bam*HI−SacI fragment from plasmid pFK41 [13] containing the *tsr* gene of *S. azureus* was rendered blunt-ended, and inserted between *Pvu*II and *Pst*I (blunt-ended) sites of the plasmid pRPO5SC-10A, replacing 600 bp of the 3′-coding region of *sigG*, to generate plasmid pRPO5SC-10B. The 2.75-kb *Spe*I−HindIII (blunt-ended) fragment of pRPO5SC-10B containing the *tsr*-disrupted *sigG* allele was cloned into plasmid *pJU2581* [14] cut with *Sma*I and *Spe*I, replacing *tsr* gene. The resulting plasmid, pGLK−ΔsigG1 (Fig. 1a), isolated from the methylation deficient *E. coli* strain ET12567 [10], was used to transform *S. coelicolor* J1916, a *glkA* deletion derivative of *S. coelicolor* J1501 [9]. Transformants were selected by adding thiostrepton (Th) to R2YE regeneration plates [8] to give a final concentration of 10 μg ml−1. Spores from about 300 thiostrepton-resistant colonies were plated on minimal medium (MM) [8] supplemented with histidine (100 μg ml−1), uracil (15 μg ml−1), thiostrepton (50 μg ml−1), 100 mM, 2-deoxyglucose, and mannitol (0.5% w/v) as carbon source.

2.4. Electron microscopy

Transmission electron microscopy of colonies and spores was performed as described previously in [6] except that sections were examined in a Tesla T541 (Brno, Czechoslovakia) electron microscope.

Fig. 1. (a) A 4-kb *S. coelicolor* M145 *Taq*I partial chromosomal fragment containing *sigG* cloned in pRPO5SC-10, and chromosomal DNA of *S. coelicolor* J1916, *sigG*: *Tsr*1 disrupted via double cross-over. The 2750 bp *Taq*I−*Pvu*II fragment of pGLK−ΔsigG1 with flanking restriction sites from *pBluescript* SK polylinker is shown by stippled box. The black bar below the maps represents probe (1021 bp *Pvu*II−*Bam*HI fragment) used for Southern hybridization analysis. Restriction sites relevant to Southern hybridization experiment are indicated. (b) Southern hybridization analysis of chromosomal DNA from the gene replacement experiments. Lanes: 1, *Pvu*II-digested *S. coelicolor* J1916 DNA; 2, *Pvu*II-digested DNA from the *S. coelicolor* J1916 *sigG*: *Tsr*1; 3, *Sma*I-digested *S. coelicolor* J1916 DNA; 4, *Sma*I-digested DNA from the *S. coelicolor* J1916 *sigG*: *Tsr*1; 5, *Pst*I+*Cla*I-digested *S. coelicolor* J1916 DNA; 6, *Pst*I+*Cla*I-digested DNA from the *S. coelicolor* J1916 *sigG*: *Tsr*1. Lambda DNA−*Bst*EI digested was used as the molecular weight standard.
3. Results and discussion

3.1. Cloning and sequencing of the sigG gene

S. coelicolor M145 genomic library (2- to 4-kb TaqI partially digested chromosomal fragments cloned into the ClaI site of the E. coli plasmid pBR322) was hybridized with a degenerate reverse oligo probe 5'-GCSTYGATSAGSCC (where S = C or G and Y = C or T) encoding a peptide motif GLI(K,D,N,E)A, homologous to a part of the most conserved domain 2.2 of almost all c-factors [7]. Analysis of positive clones revealed eight representatives, which were sequenced. Sequence analysis of the clones revealed previously identified whiG [5] and sigF [6] genes, and in one case, plasmid pRPO5SC-10 containing a 4-kb S. coelicolor TaqI partial fragment (Fig. 1a), revealed an ORF, whose deduced product showed extensive similarity (up to 60%) to σ-factors. We named the gene sigG. Coincidence of the identified 4-kb TaqI partial fragment containing sigG in plasmid pRPO5SC-10 with S. coelicolor M145 chromosome was proved by Southern-blot hybridization (data not shown).

The 2100-bp PstI–BglII fragment (Fig. 1a) was sequenced in both strands. Analysis of the sequence by the program FRAME [15] identified the complete sigG ORF having a codon usage typical for Streptomyces with a marked preference for G or C (91.9%) in the third nucleotide of the triplet [15]. The sigG ORF encodes deduced protein of 263 aa and predicted Mr of 29 422. Analysis of the sequence upstream of sigG by program FRAME revealed a divergent ORF1 with no significant similarity with known proteins.

A ^32P-labeled DNA fragment covering the coding region of sigG (700-bp BstBI–SamI fragment, internal to sigG, Fig. 1a) was used as a probe in Southern-blot hybridization of intermediate stringency (corresponding to about 85% identity) with chromosomal DNA isolated from several Streptomyces strains (S. lividans, S. griseus, S. aureofaciens, S. anticandidus). The probe hybridized with all DNA samples used (data not shown). These results may indi-
cate universal occurrence of sigG homolog among Streptomyces spp.

3.2. Sequence similarity

Sequence comparison of the sigG-encoded deduced protein with σ-factors revealed extensive similarity, mostly in regions 2, 3, and 4, which are conserved among almost all σ-factors [7]. Although there was clear similarity with all σ-factors as aligned by Lometto et al. [7], the highest similarity was to the sigF-encoded σF of S. coelicolor (45% identity, 60% similarity), which is necessary for late stages of sporulation [6], and with σB of B. subtilis (33% identity, 53% similarity), which is involved in the control of stationary phase gene expression and the general stress response [16,17]. The comparison of these proteins (Fig. 2) has revealed similarity over the entire sequence, but the highest similarity was in regions 2.4 and 4.2, which are involved in recognition of the −10 and −35 regions of promoters. Moreover, all residues suggested to be directly involved in the −10 and −35 recognition were identical [7]. It is likely that these σ-factors may recognize similar promoter sequences.

3.3. Disruption of the sigG gene

To investigate the role of putative σG, the S. coelicolor sigG gene was disrupted in vivo employing the glkA-dependent procedure with positive selection of recombinants [14]. The procedure is based on the fact that the presence of active glucose kinase gene (glkA) results in sensitivity to the non-utilizable sugar 2-deoxyglucose. The strain S. coelicolor J1916, containing glkA mutation, is resistant to 2-deoxyglucose [14]. The tsr gene was used to replace sigG encoding regions 3 and 4, which are essential for function of σ-factors. Integration of the resulting non-replicating plasmid pGLK-AsigG1 (Fig. 1a) carrying the glkA gene by single cross-over into chromosome of S. coelicolor J1916 resulted in thiostrepton resistance and 2-deoxyglucose sensitivity. Following, spores from such transformants were screened for double cross-over event by selection to 2-deoxyglucose and thiostrepton resistance. Three such colonies were picked up and their correct integration into chromosome was confirmed by Southern-blot hybridization using a 1021-bp PvuII–BamHI fragment as a probe (Fig. 1a). In all cases, the integration occurred through double cross-over, resulting in the replacement of the wt sigG gene by the disrupted allele. Results of the genomic blot confirming correct integration of one representative, S. coelicolor J1916 sigG::Tsr1, are shown in Fig. 1b. Phenotypic analysis revealed that the sigG-disrupted strain is viable and stable. Growth in liquid TSB and SMM media was comparable with the parent J1916 strain. It was not visibly affected in colony morphology, differentiation, and production of the pigmented antibiotics undecylprodigiosin and actinorhodin, when grown on solid R2, R5 and minimal media MM and SMMS. To investigate a possible defect of the sigG mutant in the late developmental stages, we performed transmission electron microscopy of ultrathin sections of spores from S. coelicolor J1916 and sigG mutant as described in [6]. Spores from both strains have shown no obvious differences (data not shown).

Our results indicate that sigG-encoded putative σ-factor of S. coelicolor is not required for growth, differentiation, and production of antibiotics undecylprodigiosin and actinorhodin. Based on these results, the biological function of the sigG gene remains unknown. In order to examine the expression of sigG, high-resolution S1 nuclease mapping was performed using a 5′-labeled probe at single BstBI site in sigG coding region (Fig. 1a) and RNA isolated from surface-grown culture of S. coelicolor M145 during differentiation on solid minimal medium MM [8]. In repeated experiments, we did not detect any protected fragment with the probe, whereas a control probe for the hrdB promoter gave consistent expression levels using the same RNA samples (data not shown). The results of transcriptional analysis have revealed that sigG is not expressed in vegetative stage, or during differentiation. Since there is a divergent ORF1 upstream of sigG, it is unlikely that the sigG gene is transcribed as part of polycistronic message from a more upstream promoter. It might be that sigG is expressed in a special conditions. These studies are in progress. Previously, we established a method for the identification of promoters recognized by a particular Streptomyces σ-factor of RNA polymerase [18]. The identification of genes directed by RNA polymerase containing sigG-encoded σ-factor using this method...
would also elucidate its physiological function. These experiments are also in progress.

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References