Expression of the *Streptomyces aureofaciens* glyceraldehyde-3-phosphate dehydrogenase gene (*gap*) is developmentally regulated and induced by glucose

J. Kormanec, A. Lempel'ová, R. Nováková, B. Řežuchová and D. Homérová

Author for correspondence: J. Kormanec. Tel: +42 1 7 378 2432. Fax: +42 1 7 372 316.
e-mail: umbijkor@savba.sk

Institute of Molecular
Biology, Slovak Academy
of Sciences, 842 51
Bratislava, Slovak Republic

In previous experiments, the *Streptomyces aureofaciens gap* gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was identified. To investigate expression of the gene, S1 nuclease mapping and Northern blot hybridization were performed using RNA prepared from *S. aureofaciens* cultivated under various conditions. These studies suggested monocistronic organization and developmental regulation of the gene. A single promoter, *gap-P*, was identified upstream of the *gap* coding region. In cultures grown on solid medium in the absence of glucose, its transcription was induced at the time of aerial mycelium formation. In addition, *gap* transcription was also induced in substrate mycelium by glucose. A promoter-bearing DNA fragment was inserted into two promoter-probe vectors, to give expression patterns consistent with the results of direct RNA analysis.

Keywords: *Streptomyces aureofaciens*, differentiation, transcription, promoter, glyceraldehyde-3-phosphate dehydrogenase

INTRODUCTION

Most work on mycelial Gram-positive *Streptomyces* species has been concerned with molecular studies on the genes for secondary metabolism. Molecular studies of primary metabolism have received comparatively little attention in these organisms. Studies with several examples of primary metabolic genes have indicated different regulatory mechanisms from those of *Escherichia coli* (Angell *et al.*, 1992; Smith & Chater, 1988).

Recently, we have identified and partially characterized a gene, *gap*, encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in *Streptomyces aureofaciens* (Kormanec *et al.*, 1995). GAPDH, a key enzyme of glycolysis, reversibly catalyses the oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate using NAD⁺ as coenzyme (Harris & Waters, 1976). GAPDH-encoding genes have been identified in various bacteria and transcriptional studies have been reported. In *Zymomonas mobilis*, cultivated in the

presence of glucose, at least two tandem promoters were identified upstream of the *gap* gene, but other carbon sources were not investigated (Conway *et al.*, 1987). Based on the GAPDH activities in the presence and absence of glucose, constitutive expression of the *gap* gene has been suggested in *Corynebacterium glutamicum* (Eikmanns, 1992). The only detailed study of transcriptional regulation has been done in *E. coli* by Charpentier & Branlant (1994). Their analysis of *gapA* transcription revealed a complex regulation comprising four tandemly organized promoters recognized by two different RNA polymerase holoenzymes.

To elucidate a possible function for the *gap* gene in *S. aureofaciens*, we have analysed its expression under various conditions, including different stages of development and the presence of different carbon sources. The data obtained show temporally different expression of the *gap* gene during *S. aureofaciens* differentiation and induction of expression by glucose.

METHODS

Bacterial strains, plasmids and culture conditions. *S. aureofaciens* CCM3239 wild-type (ATCC 10762) was from the Czechoslovak Collection of Micro-organisms, Brno, Czech Republic. *S. lividans* TK24 (Hopwood *et al.*, 1985) was a kind

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; tsp, transcription start point.

The GenBank/EMBL/DBJ accession number for the sequence reported in this paper is U21191.

Fig. 1. Restriction map of the *gap* gene and strategy for transcript mapping. Filled boxes indicate ORFs. The angled arrow represents the apparent direction of transcription from the *gap*-P promoter. The line below the map represents a DNA fragment (5'-labelled at the end marked with an asterisk) that was used as probe in S1 nuclease mapping. The black bar below the maps represents the probe used for Northern hybridization analysis. Relevant restriction sites used in S1 mapping and cloning are indicated.

gift from D. A. Hopwood, John Innes Institute, Norwich, UK. Plasmid pARC1 (Horinouchi & Beppu, 1985) was a kind gift from S. Horinouchi, University of Tokyo, and pIJ4083 (Clayton & Bibb, 1990) from Mervyn Bibb, John Innes Institute, Norwich, UK. *E. coli* SURE (Stratagene) was used as host and plasmid pBluescriptII SK+ (Stratagene) was used for *E. coli* cloning experiments. Growth, transformation of *S. lividans* and plasmid isolation were carried out as described in Hopwood *et al.* (1985). Thiostrepton was used for selection of pIJ4083- and pARC1-based plasmids at a final concentration of $10 \mu\text{g ml}^{-1}$, low enough to avoid inhibition of development but high enough to maintain selection. For RNA isolation, *S. aureofaciens* was cultured to late exponential phase (20 h) in liquid NMP medium (Hopwood *et al.*, 1985) containing different carbon sources at 1% final concentration. For RNA isolation from surface culture, 10^8 spores of *S. aureofaciens* wild-type strain were spread on sterile cellophane membranes placed on Bennet medium (Horinouchi *et al.*, 1983) and grown for 13 h (substrate mycelium), 19 h (beginning of aerial mycelium) and 36 h (late aerial mycelium stage). Conditions for *E. coli* growth and transformation were as described in Ausubel *et al.* (1987).

DNA manipulations. DNA manipulations in *E. coli* were done as described in Ausubel *et al.* (1987) and those in *Streptomyces* were as described by Hopwood *et al.* (1985). DNA fragments were isolated from agarose gel using GeneClean (Bio101). Nucleotide sequencing was performed by the chemical method (Maxam & Gilbert, 1980).

RNA isolation. Total RNA was prepared from surface cultures of *S. aureofaciens* as described previously (Kormanec & Farkašovský, 1994). The integrity of RNA was indicated by sharp rRNA bands after electrophoresis in agarose containing 2.2 M formaldehyde (Ausubel *et al.*, 1987) and staining with ethidium bromide.

S1 nuclease mapping. High-resolution S1 nuclease mapping was performed as described previously (Kormanec & Farkašovský, 1993). Samples (40 μg) of RNA (estimated spectrophotometrically using a Beckman DU-20 spectrophotometer) were hybridized to approximately 0.1 pmol suitable DNA probe labelled at one 5' end with [γ - ^{32}P]ATP [approx. 10^6 c.p.m. (pmol probe) $^{-1}$]. The probe used (Fig. 1) was an 800 bp *SalI*-*BstEII* fragment uniquely labelled at the 5' end at the *BstEII* site. The *hrdB*-P2 promoter probe has been

described by Kormanec & Farkašovský (1993). The protected DNA fragments were analysed on DNA sequencing gels together with G+A and T+C sequencing ladders derived from the end-labelled fragments (Maxam & Gilbert, 1980). Before assigning the transcription start point (tsp), 1.5 nt was subtracted from the length of the protected fragment to account for the difference in the 3' ends resulting from S1 nuclease digestion and the chemical sequencing reactions.

Northern blot hybridization. Samples of RNA (10 μg) were dissolved in formamide and separated on 1.2% (w/v) agarose gels in the presence of 2.2 M formaldehyde as described in Ausubel *et al.* (1987). After electrophoresis, RNA was blotted onto a Hybond-N membrane (Amersham) and hybridized at 44 °C for 16 h in hybridization solution [50% (v/v) formamide, 5 $\times$ SSPE (1 $\times$ SSPE is 0.18 M NaCl/10 mM NaH₂PO₄/1 mM EDTA, pH 7.4), 5 $\times$ Denhardt's solution (1 $\times$ Denhardt's solution is 0.02% Ficoll/0.02% BSA/0.02% polyvinylpyrrolidone), 0.5% SDS, 20 μg fresh denatured sonicated herring sperm DNA ml $^{-1}$] with a random-primed-labelled (Boehringer) 1170 bp *BamHI*-*PstI* DNA fragment (Fig. 1). After hybridization, the filter was washed successively for 15 min at room temperature in 2 $\times$ SSC/0.1% SDS, 0.5 $\times$ SSC/0.1% SDS, 0.1 $\times$ SSC/0.1% SDS, and twice for 30 min at 65 °C in 0.1 $\times$ SSC/0.1% SDS and autoradiographed (1 $\times$ SSC is 0.3 M NaCl/0.03 M sodium citrate, pH 7).

Detection of promoter activity *in vivo*. The activity of promoters cloned in promoter-probe plasmid pIJ4083 was detected on plates using the *xylE* reporter gene. Plates containing patches of *S. lividans* TK24 carrying plasmid pXYL-GAP1 or pIJ4083 at various developmental stages were sprayed with an aqueous solution of 0.5 M catechol. A yellow colour, associated with production of 2-hydroxymuconic semialdehyde, was visible after about 10 min. For quantitative assays, 10^8 spores of *S. lividans* containing the corresponding plasmid were spread on sterile cellophane membranes placed on MM or Bennet medium (with 1% carbon source and 10 μg thiostrepton ml $^{-1}$) and grown for 24, 30, 50, 70, 120 and 170 h. Samples were collected in duplicate and cell extracts were prepared as described in Homerová *et al.* (1996). The activity of catechol 2,3-dioxygenase activity was determined as described by Ingram *et al.* (1989). The protein concentration was determined by the method of Bradford (1976). Activity of promoters after cloning in promoter-probe plasmid pARC1 was detected during differentiation of *S. lividans* TK24 grown on Bennet medium with 10 μg thiostrepton ml $^{-1}$ and different carbon sources (1% final concentration) as described by Horinouchi & Beppu (1985).

RESULTS

The 5' upstream region of the *gap* gene has promoter activity

Upstream of the *S. aureofaciens gap* gene there is a 185 bp intergenic region that suggests the presence of a promoter directing *gap* expression (Kormanec *et al.*, 1995). To prove this, the 800 bp *SalI*-*BstEII* fragment containing this region (Fig. 1) was cloned in high-copy-number promoter-probe plasmid pIJ4083 (Clayton & Bibb, 1990). Since this plasmid is unable to replicate in *S. aureofaciens* (Kormanec *et al.*, 1993), the corresponding recombinant pXYL-GAP1 was used to transform the heterologous host *S. lividans* TK24. The activity of catechol 2,3-dioxygenase encoded by the *xylE* reporter gene was checked during differentiation of colonies on

Fig. 2. Time course of catechol 2,3-dioxygenase activity in *S. lividans* containing pXYL-GAP1 grown on solid medium. Spores (10^8) were spread on sterile cellophane membranes placed on MM or Bennet medium (with 1% carbon source and $10 \mu\text{g}$ thiostrepton ml^{-1}) and grown for 24, 30, 50, 70, 120 and 170 h. Cell extracts were obtained and activity measured as described in Methods. The mean specific activity of catechol 2,3-dioxygenase in *S. lividans* containing pIJ4083 was 2 mU mg^{-1} . This value was subtracted from the results. The graphs represent mean data from duplicate sets of samples isolated from the same plates.

solid medium growing on different carbon sources (glucose, maltose, galactose and mannitol) by spraying the plates with a 0.5 M solution of catechol. This colourless compound is converted by catechol 2,3-dioxygenase to the intensely yellow 2-hydroxymuconic semialdehyde (Ingram *et al.*, 1989). Two types of media were used; minimal medium MM (Hopwood *et al.*, 1985) and rich Bennet medium (Horinouchi *et al.*, 1983). Results indicated clear promoter activity of the cloned fragment that was both carbon-source-dependent and developmentally regulated. For all carbon sources other than glucose, yellow colour (indicating promoter activity) was detected only at the time of aerial mycelium formation. When grown in the presence of glucose, the promoter was active from the beginning of growth. As a negative control, *S. lividans*/pIJ4083 (on the same plate) was white after spraying with catechol during all stages of differentiation. To quantitatively estimate induction of *xylE* reporter expression by glucose, the specific activity of catechol 2,3-dioxygenase was determined in a cell extract of *S. lividans* containing pXYL-GAP1 or pIJ4083 grown on solid medium with several representative carbon sources for 24, 30, 50, 70, 120 and 170 h (Fig. 2). The results suggested that the putative *gap* promoter was induced by glucose in substrate mycelium, and in the absence of glucose the promoter was induced at the time of aerial mycelium formation. In all cases, the activity of the promoter decreased in the late stages of differentiation.

To investigate whether the high copy number of the pIJ4083-based plasmid influenced the induction of *gap*-

P promoter, the same promoter-bearing fragment as above was cloned in low-copy-number promoter-probe plasmid pARC1 (Horinouchi & Beppu, 1985), resulting in plasmid pARC-GAP1. Strong production of brown marker pigment occurred in substrate mycelium of *S. lividans* containing pARC-GAP1 grown on solid Bennet medium in the presence of glucose. When maltose, mannitol or galactose were used as carbon source, the marker brown pigment was almost invisible in substrate mycelium. However, the expression of the pigment dramatically increased at the time of aerial mycelium formation and remained until the late developmental stages. The results were thus similar to the *xylE* reporter system. Together, these results suggest a rather complicated regulation of the *gap* gene in which expression is regulated in two different ways. In substrate mycelium, *gap* expression is induced by glucose; during differentiation, the expression is induced by an unknown signal at the time of aerial mycelium formation.

The *gap* gene is transcribed from a single promoter

Since in previous experiments heterologous strain *S. lividans* was used to detect promoter activity, high-resolution S1 nuclease mapping was used to confirm these results using the RNA from *S. aureofaciens*. RNA was extracted from *S. aureofaciens* grown in liquid NMP medium in the presence of different carbon sources (glucose, mannitol and maltose), and from *S. aureofaciens* grown on solid Bennet medium with maltose during differentiation. This is the best medium for

Fig. 3. High-resolution S1 nuclease mapping of apparent tsps for the *S. aureofaciens gap* gene. A 5'-labelled DNA fragment (Fig. 1) was hybridized with 40 µg RNA and treated with 100 U S1 nuclease as described in Methods. Lanes on right: 1, RNA from *S. aureofaciens* grown in minimal liquid NMP medium with glucose to the end of exponential phase (20 h); 2, RNA from *S. aureofaciens* grown in minimal liquid NMP medium with mannitol to the end of exponential phase (20 h); 3, RNA from *S. aureofaciens* grown in minimal liquid NMP medium with maltose to the end of exponential phase (20 h); 4, RNA from *S. aureofaciens* grown on solid Bennet medium with maltose for 13 h (substrate mycelium); 5, RNA from *S. aureofaciens* grown on solid Bennet medium with maltose for 19 h (beginning of aerial mycelium formation); 6, RNA from *S. aureofaciens* grown on solid Bennet medium with maltose for 36 h (aerial mycelium approximately 5 h before pigment production characteristic of sporulation); 7, *E. coli* tRNA as control. A control S1 nuclease mapping experiment with the same RNA samples was done using a DNA probe for the *hrdB-P2* promoter (lanes on left). Thin horizontal arrows indicate the position of RNA-protected fragments and bold angled arrows indicate the nucleotides corresponding to apparent tsps after subtraction of 1.5 nt. Tracks A and T are G+A and T+C sequencing ladder fragments, respectively (Maxam & Gilbert, 1980). All of the S1 nuclease mapping experiments were performed three times with independent sets of RNA samples. In every experiment, the same RNA preparations were hybridized in parallel with all of the probes.

sporulation of *S. aureofaciens*. Surface-grown cells of *S. aureofaciens* were harvested 13, 19 and 36 h after inoculation of spores. These times correspond to the substrate mycelium growth (13 h), as well as early (19 h) and late (36 h) aerial mycelium formation. RNA was hybridized to the 5'-labelled probe indicated in Fig. 1. After S1 treatment, a single RNA-protected fragment was identified (Fig. 3). The fragment corresponded to an

Fig. 4. Northern blot hybridization analysis of the *gap* transcript. Total RNA (10 µg) was separated on a 1.2% agarose gel in the presence of 2.2 M formaldehyde, blotted to Hybond-N membrane (Amersham) and hybridized with random-primed-labelled DNA probe (Fig. 1) as described in Methods. Lanes: 1, RNA from *S. aureofaciens* grown in minimal liquid NMP medium with mannitol to the end of exponential phase (20 h); 2, RNA from *S. aureofaciens* grown in minimal liquid NMP medium with glucose to the end of exponential phase (20 h); 3, RNA from *S. aureofaciens* grown on solid Bennet medium with maltose for 13 h (substrate mycelium); 4, RNA from *S. aureofaciens* grown on solid Bennet medium with maltose for 19 h (beginning of aerial mycelium formation); 5, RNA from *S. aureofaciens* grown on solid Bennet medium with maltose for 36 h (aerial mycelium approximately 5 h before pigment production characteristic of sporulation). Positions of the internal rRNAs shown. The size of the *gap* transcript was calculated using the rRNAs as internal standards, assuming that their sizes are as in *S. ambofaciens*: 23S rRNA = 3120 nt; 16S rRNA = 1528 nt (Pernodet et al., 1989).

apparent promoter termed *gap-P* with a tsp at a G residue 23 bp upstream from the most likely translation initiation codon (see Fig. 5). Substantially more RNA-protected fragment was identified with RNA isolated from *S. aureofaciens* grown in liquid minimal medium glucose-NMP (Fig. 3, lane 1), compared to cultures grown in mannitol- or maltose-NMP (Fig. 3, lanes 2 and 3). The same RNA-protected fragment was identified using RNA prepared from surface-grown *S. aureofaciens*. The intensity of the RNA-protected fragment was much higher using RNA prepared from the beginning of aerial mycelium formation (Fig. 3, lane 5) than with RNA isolated from substrate mycelium (Fig. 3, lane 4). The intensity of the protected fragment decreased substantially using RNA from a late stage of differentiation (Fig. 3, lane 6). A very weak fragment was visible only after long overexposure of the autoradiogram (data not shown). No RNA-protected fragment was identified with tRNA as a control (Fig. 3, lane 7).

ORF1 (AraC-like)

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I L G T T P S E Y R R T F T R G E *
CATCTCGGCACCAACGCCGAGCGAGTACCGGCGCACCTTCACCGGGCGAATAGCCGGG 420
CGCGCCCGCCCGCTGGCGGATCCTTGGCGACCGTGGCAATCCCGCGCTGTACAGGCC 480
GCGACCAACCGGGGAACTTGGTGGCGAACCCTGAAAGGACACGCTCATGACTCGCATCGC 540
CATCAACGGATTCCGCGCATCGGACGCAACGTGCTGCGGCGCTGCTCGAACGCGACAG 600
I N G F G R I G R N V L R A L L E R D S

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Angled arrows indicate the likely position of the tsp and the direction of transcription. The G residue of the tsp is bold. A presumptive RBS is underlined. The one-letter amino acid code is used under the second position of each codon. A stop codon is marked by an asterisk. Inverted repeats are designated by thin arrows. A tandem repeat is designated by bold arrows.

Fig. 5. The nucleotide sequence of the *S. aureofaciens* *gap*-P promoter region. The angled arrow indicates the likely position of the tsp and the direction of transcription. The G residue of the tsp is bold. A presumptive RBS is underlined. The one-letter amino acid code is used under the second position of each codon. A stop codon is marked by an asterisk. Inverted repeats are designated by thin arrows. A tandem repeat is designated by bold arrows. The numbers correspond to the nucleotide positions in the published sequence of the gene (Kormanec *et al.*, 1995).

As a comparative control for normalization, S1 mapping was performed with the same RNA samples using a probe fragment specific for *S. aureofaciens* *hrdB*-P2 promoter, which is expressed fairly constantly during differentiation (Kormanec & Farkašovsky, 1993). As shown in Fig. 3, protected fragments of similar intensities corresponding to the *hrdB*-P2 promoter were identified with all RNA samples.

The *gap* transcript is monocistronic

Results of previous studies (Kormanec *et al.*, 1995) suggested that the identified *gap* gene was not organized in a multicistronic operon. To verify this, Northern blot hybridization was performed with RNA isolated from *S. aureofaciens* cultured under similar conditions as for S1 mapping experiments, using a DNA fragment comprising the whole *gap* gene as a probe (Fig. 1). The results revealed a unique signal of about 1060 nt (Fig. 4). This length closely corresponded to the predicted size of *gap* transcript (about 1070 nt), supposing that transcription was initiated at the *gap*-P promoter and terminated at the inverted repeat downstream of the *gap* gene (Kormanec *et al.*, 1995). The *gap* transcript was induced by glucose in substrate mycelium (Fig. 4, lane 2) and at the time of aerial mycelium formation using RNA isolated from *S. aureofaciens* grown on solid medium (Fig. 4, lane 4), similar to the results of S1 mapping. The results of Northern blot hybridization proved the proposed monocistronic organization of *gap* in *S. aureofaciens*.

Characterization of the *gap*-P promoter

The sequence of the *gap*-P promoter and flanking regions is shown in Fig. 5. An inspection of the DNA sequence upstream of the apparent tsp of the *gap*-P promoter revealed several inverted and direct repeats that might be implicated in regulation of its activity. The

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gap-P      -35      -10      +1
CAATCCCG  CTGTACGGCCGCGACCAAGCGGGGGAACCTGGTGG
hrdA-P2    -35      -10      +1
CGTCCGAT  CATCGG-GG-TTCCGCGCGGGGGTAACCATCCGGTCA

gap-P      -35      -10      +1
CAATCCCG  CTGTACGGCCGCGACCAAGCGGGGGAACCTGGTGG
rpoZ-P2    -35      -10      +1
TCCGGGCG  TGC-GCCCCGCGCCATCCCGTACTCTTCGCGCA

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Fig. 6. Comparison of the putative *S. aureofaciens* *gap*-P promoter with promoters having the greatest similarity in the -10 and -35 regions; *hrdA*-P2 directs expression of the putative σ factor gene *hrdA* in *S. aureofaciens* (Kormanec & Farkašovsky, 1993) and *rpoZ*-P2 directs the expression of the putative σ factor gene *rpoZ*, crucial in initiating sporulation in *S. aureofaciens* (Kormanec *et al.*, 1996). Apparent tsp, -10 and -35 positions are in bold. The putative -10 and -35 regions are underlined. The conserved CCGC region is highlighted in black.

putative -10 (GAACCT) and -35 (CTGTCA) regions of the *gap*-P promoter (Fig. 6) show limited similarity to the -10 (TAgPuPuT) and -35 (TTGACPu) consensus sequences for $E\sigma^{70}$ -like promoters (Strohl, 1992).

Comparison of the putative *gap*-P promoter with 139 compiled promoter regions from *Streptomyces* spp. (Strohl, 1992) revealed some similarity to two *S. aureofaciens* promoters: *hrdA*-P2 (mostly around the -10 region) and *rpoZ*-P2 (highest similarity around the -35 region) (Fig. 6). The *hrdA*-P2 promoter directs expression of a putative σ factor gene (*hrdA*) homologous to the principal σ factor in *S. aureofaciens* (Kormanec & Farkašovsky, 1993), and the *rpoZ*-P2 promoter is upstream of the putative σ factor gene that is crucial in initiating sporulation in *S. aureofaciens* (*rpoZ*) (Kormanec *et al.*, 1994, 1996). It is interesting that, similar to *gap*-P, activity of both promoters is induced at the beginning of aerial mycelium formation (Kormanec & Farkašovsky, 1993; Kormanec *et al.*, 1996). Thus, all these promoters might be regulated by a common mechanism at the beginning of sporulation. It is worth mentioning that the conserved region CCGC is present in all of these promoters around the -35 region (Fig. 6).

DISCUSSION

Our data reported here demonstrate a different regulation of *gap* expression compared to other bacteria. Based on *in vitro* transcription studies, the *S. aureofaciens* *gap* gene is transcribed as a monocistronic mRNA from a single promoter. Interestingly, its transcription is strongly induced by the presence of glucose in the culture medium. To our knowledge, this is the first report of induction of bacterial glycolytic genes by glucose. In the filamentous fungus *Aspergillus oryzae*, transcription of another glycolytic gene, encoding enolase, is also induced in the presence of glucose (Machida *et al.*, 1996).

When *gap* expression was investigated in the absence of

glucose during differentiation on solid medium, strong induction of the same promoter was identified at the time of aerial mycelium formation. This dual type of regulation of a single promoter was surprising. Whereas *Streptomyces* genes are regulated by tandem promoters that are differentially activated, in *S. aureofaciens*, *gap* is under the control of a single promoter with a dual regulatory role. In the presence of glucose, substrate mycelium expression is induced by glucose, but in its absence, expression is induced by a signal(s) at the time of aerial mycelium formation. In this respect, it is interesting that the *gap*-P promoter is most similar to two *S. aureofaciens* promoters, *hrdA*-P2 and *rpoZ*-P2, which are similarly induced at the time of aerial mycelium formation. Glucose induction of the *S. aureofaciens gap* gene is intriguing since most hexose sugars are catabolized by the glycolytic pathway and catabolism of each sugar requires GAPDH. This may be the role of the second *gap* gene suggested in *S. aureofaciens* (Kormanec *et al.*, 1995). The cloning of this gene is in progress. On the other hand, the induction at the time of aerial mycelium formation in the absence of glucose might be connected with phase I glycogen degradation that occurs during *Streptomyces* differentiation in substrate hyphae that undergo aerial mycelium formation (Homerová *et al.*, 1996; Plaskitt & Chater, 1995). Based on this assumption, the expression of *gap* might be induced not only by external glucose, but also by some glucose metabolite that is generated after phase I glycogen degradation at the beginning of aerial mycelium formation. Based on the S1 mapping data, the expression of the *gap* gene substantially decreased later in sporulation when secondary (phase II) accumulation and degradation of glycogen occurs. This might be a possible role of the proposed second *gap* gene also in glycogen consumption.

A putative gene homologous to transcriptional activators of the AraC family has been identified upstream of the *S. aureofaciens gap* gene (Kormanec *et al.*, 1995). Usually transcriptional activators of this family are located upstream of the gene (operon) they regulate (Gallegos *et al.*, 1993). Thus, it is possible that glucose induction of the *S. aureofaciens gap* gene is mediated by a protein encoded by this gene. Experiments to determine this are in progress. However, some preliminary experiments have already indicated the binding of this protein on the DNA fragment containing the promoter region of *gap* (J. Kormanec, unpublished results). The results from transcriptional fusions of *gap* in *S. lividans* show similar temporal expression as shown by S1 mapping data in *S. aureofaciens*. This suggests that *S. lividans* might contain a *gap* gene under similar regulation to the *gap* gene in *S. aureofaciens*, or is able to supply this putative activator *in trans*.

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