The *glpP* and *glpF* genes of the glycerol regulon in *Bacillus subtilis*

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The *Bacillus subtilis* *glpP*FKD region contains genes essential for growth on glycerol or glycerol 3-phosphate (G3P). The nucleotide sequence of *glpP* encoding a regulatory protein and the previously unidentified *glpF* encoding the glycerol uptake facilitator was determined. *glpF* is located immediately upstream of *glpK* and the two genes were shown to constitute one operon which is transcribed separately from *glpP*. A σA-type promoter and the transcriptional start point for *glpFK* were identified. In the 5' untranslated leader sequence (UTL) of *glpFK* mRNA a conserved inverted repeat is found. The repeat is believed to be involved in the control of expression of *glpFK* by termination/antitermination of transcription, a control mechanism previously suggested for the regulation of *glpD* encoding G3P dehydrogenase. Expression of *glpFK* and *glpD* requires the inducer G3P and the *glpP* gene product. A 2.9 kb *B. subtilis* chromosomal DNA fragment containing the *glpP* open reading frame was cloned to give plasmid pLUM7. pLUM7 contains a functional *glpP* open reading frame as shown by its ability to complement various *glpP* mutants. Immediately upstream of *glpP* an open reading frame is found (ORF1). Disrupting ORF1 by plasmid integration in the *B. subtilis* chromosome does not affect the ability to grow on glycerol as sole carbon and energy source. With the present report all *B. subtilis* *glp* genes located at 75° on the chromosomal map have been identified.

## Introduction

The *Bacillus subtilis* *glp* regulon contains genes specific for growth on glycerol or glycerol 3-phosphate (G3P) as sole carbon and energy source. Four *glp* genes have been identified. These genes are *glpT* encoding a G3P permease (Lindgren, 1978; R.-P. Nilsson and others, unpublished), *glpK* encoding glycerol kinase, *glpD* encoding G3P dehydrogenase and *glpP* encoding a regulatory protein (Lindgren & Rutberg, 1974, 1976). The *glpT* gene maps at 15° on the *B. subtilis* chromosomal map whereas the other three *glp* genes map at 75°. The *glpK*, *glpD* and most likely also the *glpT* gene are induced by glycerol or G3P, G3P being the true inducer. Induction of these genes also requires the *glpP* gene product and induction is repressed by glucose (Lindgren & Rutberg, 1974). The mechanism of induction of *glpD* has been studied in some detail. The *glpD* gene constitutes a monocistronic operon which is transcribed from a constitutive, glucose-insensitive promoter, but a full length *glpD* transcript is only found in induced cells. An inverted repeat is located in the 5' untranslated leader sequence (UTL) of *glpD* mRNA and this repeat is suggested to act as a transcriptional stop signal. Production of a full length *glpD* mRNA is thought to be controlled by termination/antitermination of a transcript initiated at the constitutive *glpD* promoter. In the presence of G3P, the *glpP* gene product is thought to enhance transcription across and beyond the inverted repeat (Holmberg & Rutberg, 1991, 1992).

We have previously reported the cloning of a 7 kb *EcoRI-PstI* *B. subtilis* chromosomal DNA fragment which carries wild type alleles of *glpP*, *glpK* and *glpD* mutations, and the nucleotide sequences of *glpK* and *glpD* have been presented (Holmberg et al., 1990). The plasmid carrying the 7 kb fragment is a derivative of plasmid pPHP13 and is called pLUM30. Here we report the nucleotide sequences of the two additional *glp* genes contained in pLUM30, *glpP* and the previously unidentified *glpF*. The latter gene is proposed to encode a membrane protein which facilitates diffusion of glycerol.

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**Abbreviations:** Ap, ampicillin; Cm, chloramphenicol; C23O, catechol-2,3-dioxygenase; Em, erythromycin; G3P, glycerol 3-phosphate; Pm, phleomycin.

The nucleotide sequence data reported in this paper have been submitted to GenBank and assigned the accession number M99611.
across the cell membrane. Transcriptional analysis has revealed that glpP and glpFK represent two separate operons. The presence of a conserved inverted repeat in the UTL of glpFK mRNA indicates that expression of these genes is controlled by a mechanism similar to that proposed for glpD. With the present report all B. subtilis glp genes located at 75° on the chromosomal map have been identified.

Methods

Strains and plasmids. The bacterial strains and plasmids used in this work are listed in Table 1. In LGU0402 a chromosomal DNA fragment starting at position 88 and ending at position 1234 (Fig. 2) is deleted and replaced with a DNA fragment from pUB110 containing the ble gene, using methods described by Friden et al. (1987).

Media. Bacterial strains were kept on tryptose blood agar base (TBAB) plates. For preparation of plasmid DNA the bacteria were grown in Luria broth. Minimal salts solution was that of Anagnostopoulo & Spizizen (1961). Glucose or glycerol was added to a concentration of 5 g 1⁻¹ (30 mM and 50 mM, respectively). G3P was added to a concentration of 40 mM. Casamino acids were added to a concentration of 5 g 1⁻¹. Antibiotics were added to the following concentrations: ampicillin (Ap) 50 mg 1⁻¹, chloramphenicol (Cm) 5 mg 1⁻¹ (B. subtilis) or 12.5 mg 1⁻¹ (Escherichia coli), erythromycin (Em) 5 mg 1⁻¹ (B. subtilis) or 150 mg 1⁻¹ (E. coli) and phleomycin (Pm) 0.15 mg 1⁻¹.

Transformation. Competent B. subtilis cells were prepared as described by Arwert & Venema (1973). E. coli cells were made competent as described by Mandel & Higa (1970).

DNA techniques. Chromosomal DNA was prepared by standard techniques. Plasmid DNA was prepared by the alkaline lysis method of Ish-Horowicz & Burke (1981). When preparing plasmid DNA from B. subtilis, the cells were treated with lysozyme (5 g 1⁻¹) prior to lysis with NaOH/SDS. Plasmid DNA from E. coli XL1-Blue was prepared by the boiling method of Ausubel et al. (1987). DNA fragments separated on agarose gels were purified using Gene Clean (Bio101).

DNA sequence analysis. Nucleotide sequences were determined by the dideoxy chain-termination method of Sanger et al. (1977), using Sequenase (USB) and [α-³²P]dATP. Templates used were DNA fragments cloned into pHP13 or pBluescript II KS(−). Primers used were universal or specific to the B. subtilis chromosomal DNA being sequenced. Analysis of nucleotide sequences was done using the GCG sequence software package (Devereux et al., 1984).

Growth of bacteria, preparation of cell free extracts and enzyme assays. Cell cultures were grown in minimal salts medium supplemented with Casamino acids and required amino acids. When appropriate glucose, Cm or Em was added. The cultures were grown to early exponential phase (OD₆₀₀ = 0.5) and then divided into two parts. To one part G3P was added and to the other part no addition was made. The cultures were then incubated at 37 °C for 2 h. Cell free extracts were prepared as described by Lindgren & Rutberg (1974). G3P dehydrogenase activity was measured as described by Lin et al. (1962). Catechol-2,3-dioxygenase (C23O) activity was measured as described by Sala-Trepat & Evans (1971). The extinction coefficient of 2-hydroxyxymuconic semialdehyde at pH 8.0 (33.9 nm⁻¹ cm⁻¹) was estimated from the data reported by Bayly et al. (1966). The amount of protein was determined by the Lowry method.

RNA preparation. Cell cultures were grown at 37 °C on a rotary shaker in minimal salts solution supplemented with Casamino acids. When appropriate Cm was added. The cultures were grown to an OD₆₀₀ of 9-3 and then divided into two parts. To one part glycerol was added and to the other part no addition was made. The cultures were then incubated at 37 °C for 1 h. Total RNA was extracted as described by Resnekov et al. (1990).

Primer extension. Primer extension was performed as described by Ayer & Dynan (1988). For each reaction 40 μg total RNA, 0.2 pmol ³²P-end-labelled primer and 1 U avian myeloblastosis virus reverse transcriptase (Pharmacia) were used. RNA and primer were annealed by incubation at 80 °C for 5 min followed by incubation on ice for 5 min. The primer was end-labelled as described by Sambrook et al. (1989) using T4 polynucleotide kinase (Boehringer Mannheim) and [²⁻³²P]ATP (> 5000 Ci mmol⁻¹, Amersham) (1 Ci = 37 GBq). Primer extension products were separated on polyacrylamide (6%, w/v, acrylamide, 0.3%, w/v, bisacrylamide) gels containing 7 M urea.

Northern analysis. Northern blot and hybridization analysis was performed according to the protocols of Amersham. RNA was blotted onto Hybond-N filters (Amersham). ³²P-labelled probes were synthesized using a random-primed DNA labelling kit (Boehringer Mannheim) and [α-³²P]dTCTP (> 3000 Ci mmol⁻¹, Amersham). RNA molecular mass markers (BRL) were used to determine the size of transcripts.

Results and Discussion

Nucleotide sequences of glpP and glpF

A schematic representation of the glpP, glpF, glpK and glpD region of the B. subtilis chromosome is shown in Fig. 1. The nucleotide sequences of glpP, glpF and adjacent regions are shown in Fig. 2.

An open reading frame, designated ORF1, ends at position 300 which is 27 bp upstream of the proposed start codon of glpP (see below). Sequencing of most of both strands of ORF1 (which is fully contained within the cloned EcoRI–PstI fragment) shows that it can encode a protein of 50 kDa. The predicted amino acid sequence of the ORF1 protein shows 33% identity with that of the B. sphaericus BioA protein and has several conserved motifs characteristic for other aminotransferases (Fig. 3). It should be pointed out, however, that the B. subtilis bioA gene has been mapped at 268° (Pigott et al., 1990) whereas ORF1 is located at 75° on the B. subtilis chromosomal map. To test if ORF1 encodes a protein essential for glycerol catabolism, a gene disruption experiment was performed. An ORF1–internal 470 bp SphI fragment was cloned in the B. subtilis integration plasmid pHV32 (Niaudet et al., 1982) to give plasmid pLUM316 (Fig. 1). pLUM316 can only replicate in E. coli but carries a cat gene which is expressed in both E. coli and B. subtilis. B. subtilis BR95 was transformed with pLUM316 and transformants resistant to Cm were selected. These transformants have
Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Genotype/phenotype</th>
<th>Source/reference</th>
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</thead>
<tbody>
<tr>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR95</td>
<td>ilvCI pheA1 trpC2</td>
<td>Our collection</td>
</tr>
<tr>
<td>LUG2506</td>
<td>ilvCI trpC2 glpP6</td>
<td>Our collection</td>
</tr>
<tr>
<td>LUG2509</td>
<td>ilvCI trpC2 glpP9</td>
<td>Our collection</td>
</tr>
<tr>
<td>LUG2512</td>
<td>ilvCI trpC2 glpP12</td>
<td>Our collection</td>
</tr>
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<td>Holmberg al. (1990)</td>
</tr>
<tr>
<td>LUG0402</td>
<td>ilvCI pheA1 trpC2</td>
<td>This work</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JM83</td>
<td>ara Δ(lac-proAB)</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>JM109</td>
<td>endA1 recA1 hsdR17 supE44 gyrA96 recA1 thiA(lac-proAB) F &lt;traD36 proAB+ lacF lacZAM15 Tn10(TcR)&gt;</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>XL1-Blue</td>
<td>recA1 endA1 gyrA96 hsdR17 supE44 recA1 thi lac F &lt;proAB+ lacF lacZAM15 Tn10(TcR)&gt;</td>
<td>Stratagene</td>
</tr>
<tr>
<td><strong>Plasmid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHP13</td>
<td>CmR EmR</td>
<td>Haima et al. (1987)</td>
</tr>
<tr>
<td>pUC18</td>
<td>ApR</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>pHV32</td>
<td>ApR CmR TeR</td>
<td>Niaudet et al. (1982)</td>
</tr>
<tr>
<td>pAMB22</td>
<td>CmR TeR XylE-</td>
<td>Zukowski &amp; Miller (1986)</td>
</tr>
<tr>
<td>pUB110</td>
<td>KmR PeR</td>
<td>Gryczan et al. (1978)</td>
</tr>
<tr>
<td>pBluescript I1 KS(−)</td>
<td>ApR</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pLUM30</td>
<td>CmR EmR</td>
<td>Holmberg et al. (1990)</td>
</tr>
<tr>
<td>pLUM304</td>
<td>CmR EmR</td>
<td>This work</td>
</tr>
<tr>
<td>pLUM309</td>
<td>CmR</td>
<td>Holmberg et al. (1990)</td>
</tr>
<tr>
<td>pLUM316</td>
<td>ApR CmR TeR</td>
<td>This work</td>
</tr>
<tr>
<td>pLUM47</td>
<td>EmR</td>
<td>This work</td>
</tr>
<tr>
<td>pLUM52</td>
<td>CmR TeR XylE+</td>
<td>Holmberg &amp; Rutberg (1991)</td>
</tr>
<tr>
<td>pLUM610</td>
<td>CmR TeR XylE+</td>
<td>This work</td>
</tr>
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</table>

no additional nutritional requirements and they grow
with glycerol as sole carbon and energy source. To
confirm that pLUM316 had integrated close to the glp
region, chromosomal DNA from one of the trans-
formants was used to transform a glpP mutant,
LUG2506, to CmR. Of 180 CmR transformants tested,
177 were also Glp+ confirming that pLUM316 had
integrated close to the glp genes at 75°. This was also
verified by Southern blot analysis (data not shown).

At 27 bp downstream of the stop codon of ORF1 a
second open reading frame (ORF2) starts with an ATG
at position 328 and ends with a TGA stop codon at
position 906 (Fig. 2). ORF2 can encode a protein of 192
amino acids with a molecular mass of 22 kDa. ORF2 is
preceded by a typical ribosome binding site (rbs;
AAAGGAG, AG = −56 kJ). Upstream of the proposed
ATG start codon there is a canonical σA −10
sequence separated by 16 bp from a possible −35
sequence (Fig. 2). Overlapping this possible promoter
region from positions 266 to 279 is a sequence where
13/14 bp conform to the ‘glucose repression sequence’,
−T-G-A/T-N-A-N-C-G-N-T-N-A/T-C-A-, proposed by
Weickert & Chambliss (1990). ORF2 is contained within
a 4 kb EcoRI–ClaI fragment, (Fig. 1). This fragment,
which contains wild type alleles of several glpP muta-

Fig. 1. Schematic representation of the glpP, glpF, glpK and glpD
region of the B. subtilis chromosome. Inverted repeats are indicated
with hairpin symbols. Arrows indicate direction of transcription. The
chromosomal DNA fragment present in each pLUM plasmid is shown.
pLUM304, pLUM316, pLUM309 and pLUM610 are derivatives of
pLUM30. C, ClaI; D, DraI, E, EcoRI; EV, EcoRV; H, HindIII; N,
NcoI; P, PstI; S, SphI. Restriction endonuclease sites shown are not
necessarily unique.
tions, was subcloned from pLUM30 in plasmid pHPl3 to give plasmid pLUM304. The gZpP gene product is known to act in trans (Holmberg & Rutberg, 1991). To test for glpP complementing activity by pLUM304 the plasmid was introduced into strains LUG0401/pLUM52 and LUG0402. In LUG0401 the whole glp region from
Fig. 2. Nucleotide sequence of part of ORF1, glpP, glpF and the beginning of glpK of *B. subtilis*. Both strands were sequenced and all restriction sites fully overlapped. Potential rbs and promoter sequences are underlined. Nucleotides conforming to the ‘glucose repression sequence’ are marked with A. The C to T transition occurring in pLUM304 is indicated. ▽ shows the deletion startpoint in the two deleted plasmid derivatives of pLUM309. The inverted repeat preceding glpFK is indicated with horizontal arrows. The transcriptional startpoint of glpFK is indicated with a vertical arrow. The 17-mer used in primer extension experiments is indicated. Left-margin numbers refer to the nucleotide sequence, right-margin to amino acid sequences. Nucleotide position 1230 in the sequence published here corresponds to position 1 in the nucleotide sequence published by Holmberg et al. (1990).
Table 2. Complementation of B. subtilis GlpP mutants by pLUM7 and pLUM304

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid(s)</th>
<th>Addition</th>
<th>G3P-DH*</th>
<th>C230†</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUG0401</td>
<td>pLUM52</td>
<td>-</td>
<td>-</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>LUG0401</td>
<td>pLUM52 + pLUM7</td>
<td>G3P</td>
<td>-</td>
<td>400</td>
</tr>
<tr>
<td>LUG0401</td>
<td>pLUM52 + pLUM304</td>
<td>G3P</td>
<td>-</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>LUG0402</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LUG0402</td>
<td>pLUM7</td>
<td>G3P</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>LUG0402</td>
<td>pLUM304</td>
<td>G3P</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>BR95</td>
<td>-</td>
<td>G3P</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

* G3P dehydrogenase activity is expressed as nmol substrate (G3P) converted min⁻¹ (mg protein)⁻¹.
† C230 activity is expressed as nmol product (2-hydroxymuconic semialdehyde) formed min⁻¹ (mg protein)⁻¹.

The table shows the complementation of B. subtilis GlpP mutants by plasmids pLUM52, pLUM7, pLUM304, and BR95. The activities of G3P and C230 dehydrogenases are measured and compared for different strains and plasmid combinations.

The glpP to glpD is deleted; pLUM52 is a derivative of pAMB22, where the glpD promoter region has been placed in front of the reporter gene xylE. In LUG0402 glpD is deleted, but the strain has an intact glpD gene with its promoter region. Introduction of pLUM304 into LUG0401/pLUM52 and LUG0402 did not restore G3P inducibility of either C230 or G3P dehydrogenase activity, indicating that the glpP gene supposedly carried by pLUM304 is not functional. We therefore re cloned glpP from BR95 on a 2.9 kbp EcoR1–NcoI fragment (Fig. 1) in pHPl3 to give plasmid pLUM7. When pLUM7 was introduced into LUG0401/pLUM52 and LUG0402, increased activities of C230 and G3P dehydrogenase could be induced by G3P (Table 2). Thus, pLUM7 carries a functional glpP gene. A comparison of the sequence of the glpP region in pLUM304 and pLUM7 revealed that in pLUM304 a C to T transition has occurred at position 469 (Fig. 2) which generates a TAA stop codon in the proposed glpP open reading frame. We do not understand by what mechanism this mutation has occurred. However, the fact that introduction of a stop codon in ORF2 leads to loss of glpP function strongly supports the suggestion that ORF2 represents glpP.

A third open reading frame, ORF3, starts with an ATG codon at position 1085 and ends with a TAA stop codon at position 1909.
glpK is located 18 bp downstream of this stop codon. ORF3 can encode a protein of 274 amino acids and with a molecular mass of 29 kDa. A hydropathy plot (Kyte & Doolittle, 1982) of the predicted amino acid sequence of the ORF3 protein reveals several hydrophobic stretches and the protein has the potential to form 6–7 membrane spanning segments. The ORF3 protein shows 35% identity with the E. coli GlpF protein (Fig. 4). The latter protein is a membrane protein involved in facilitated diffusion of glycerol across the cell membrane. The E. coli GlpF protein belongs to the so called MIP family of integral membrane proteins which includes transport proteins from bacteria, plants, insects and animals (Pao et al., 1991; Bairoch, 1991). Amino acid residues that are conserved throughout the MIP family are also conserved in the ORF3 protein (Fig. 4). In E. coli, glpF is located immediately upstream of glpK and the two genes form one operon (Sweet et al., 1990). This is also true for B. subtilis ORF3 and glpK (see below). We are confident that ORF3 encodes the GlpF protein of B. subtilis.

Transcription of glpF and glpP

As mentioned above a σA-type glpF promoter is suggested from the nucleotide sequence. To test for promoter activity the following experiment was done. A 606 bp EcoRV–DraI fragment containing the proposed glpF promoter (positions 558 to 1164, Fig. 2) was cloned by blunt end ligation in the SmaI site of pUC18 in E. coli JM109. The identity and orientation of the insert was verified by DNA sequencing. Plasmid DNA was then cleaved with EcoRI and HincII, sites for which flank the insert. The insert was then ligated to EcoRI-SmaI cleaved pAMB22 DNA which places it upstream of the xylE reporter gene. The ligation mix was used to transform BR95 to CmR and the CmR transformants were tested for C230 activity. Plasmid DNA was extracted from one C230-positive transformant and the identity of the plasmid, pLUM610 (Fig. 1), was verified by restriction enzyme analysis and by DNA sequencing of the insert. C230 activity was then measured in BR95/pLUM610 grown in the presence or absence of G3P and glucose. The results of these experiments (Table 3) show that the EcoRV–DraI fragment has promoter activity in B. subtilis. The C230 activity increased two-to threefold in cells grown with G3P and decreased when glucose was also present.

To determine the start point of the transcript initiated from the promoter in the EcoRV–DraI fragment the following experiment was done. BR95 and BR95/pLUM610 were grown with and without glycerol, total RNA was extracted and used in primer extension experiments. The primer used was a 17-mer corresponding to positions 973 to 989 (Fig. 2). The results of these experiments (Fig. 6) place the start of the transcripts at an A residue at position 938 (Fig. 2). This residue is located 10 nucleotides downstream of the centre of the proposed σA − 10 sequence. The conserved inverted
The inverted repeats are presented as stem–loop structures. Highly conserved regions are boxed. The −10 regions and transcriptional start points are indicated.

Table 3. Activity of C230 in B. subtilis BR95 carrying different plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Addition(s)</th>
<th>C230 activity *</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAMB22</td>
<td></td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>G3P</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>pLUM610</td>
<td>G3P</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G3P + glucose</td>
<td>8</td>
</tr>
</tbody>
</table>

* C230 activity is expressed as nmol product (2-hydroxy-muconic semialdehyde) formed min⁻¹ (mg protein)⁻¹.

repeat is located downstream of the transcriptional start point in the UTL of the glpF transcript. Probably the inverted repeat serves as a conditional stop signal for transcription as has been suggested previously for the inverted repeat in the UTL of glpD mRNA (Holmberg & Rutberg, 1991, 1992). The stop codon of glpF and the start codon of glpK are separated by 18 bp suggesting that these two genes may constitute an operon. To test this suggestion a Northern blot analysis was done. BR95 was grown with and without glycerol, total RNA was extracted and probed with an 1100 bp NcoI–ClaI fragment (Fig. 1). RNA from LUG0401 grown in the presence of glycerol was used as a control. In uninduced BR95 a transcript of about 2400 nucleotides (nt) was detected which corresponds in size to that expected for a glpFK mRNA (Fig. 7). The relative amount of this transcript increased about fivefold when BR95 was grown with glycerol. A transcript of about 4400 nt was also found in glycerol-grown cells, which is thought to represent a glpFKD transcript. A transcript of similar size is also found in induced cells when using a probe specific for glpD (Holmberg & Rutberg, 1992). As expected, no transcript hybridizing with the probe was
found in LUG0401. From the above results we conclude that glpF and glpK constitute one operon.

The xylE reporter gene in pAMB22 was used to search for promoters upstream of glpP. However, no significant promoter activity was found within 240 bp upstream of the proposed start codon for glpP. Also no transcript initiated in the intergenic region between ORF1 and glpP has been observed in primer extension experiments. Northern blots were then done to search for a glpP transcript. The probes used were a 275 bp HindIII-EcoRV fragment which extends from 45 bp upstream of the start of glpP and into this gene, and a glpP-internal 210 bp EcoRV-Hind fragment (Fig. 2). Using either probe a weak signal (compared to, e.g. uninduced glpFK transcript) corresponding to a transcript(s) of 1800 to 2100 nt was found (Fig. 8). This corresponds in size to a transcript initiated upstream of ORF1 and extending to the start of glpF. The fact that disrupting ORF1 by plasmid integration does not lead to a Glp-negative phenotype could be explained by glpP being transcribed from a new promoter located in the plasmid inserted into ORF1. It seems clear from the above results, however, that glpP is not cotranscribed with glpFK.

Conclusions

The present results together with previously published experiments have identified all B. subtilis glp genes located at 75° on the chromosomal map. These four genes represent three separate transcription units, namely, glpP, glpFK and glpD. The activities of glpFK and glpD are subject to several negative and positive controls involving, e.g. GlpP, the phosphoenolpyruvate:sugar phosphotransferase system (PTS; Reizer et al., 1988) and glucose repression. Further work is now directed towards understanding the molecular nature of these controls.

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Fig. 8. Northern analysis of glpP-specific transcripts. Lanes as described in the legend to Fig. 7. The 32P-labelled probes used were synthesized from (a) the HindIII–EcoRV DNA fragment, and (b) the EcoRV–Hinfl DNA fragment, which is internal for glpP. The positions of the RNA size markers, visualized by staining with methylene blue, are indicated on the left.

References


