Genetic Evidence for Modulation of the Activator by Two Regulatory Proteins Involved in the Exogenous Induction of Phosphoglycerate Transport in Salmonella typhimurium

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We previously reported the cloning of a 14.4-kilobase (kb) DNA fragment from Salmonella typhimurium (4). This fragment, which carries the genetic information for the exogenously induced phosphoglycerate transport system and its regulatory components, allowed us to identify and sequence the transporter gene, pgtp, and a regulator gene, pgta (3, 10). The nucleotide sequence of the 3.4-kb segment located between the pgtp and pgta genes has been determined and has been shown to contain two genes, pgtb and pgtc, which encode two polypeptides of 593 and 397 amino acid residues, respectively (see accompanying paper [9]). This paper presents an insertion and deletion analysis of the pgtbC genes. It is concluded that, in addition to pgta, these two regulatory genes are necessary for the inducibility of the pgtp gene expression. A model of induction by phosphoglycerates in which the pgta gene product acts as a derepressor is proposed and discussed.

MATERIALS AND METHODS

General methods and materials used in this work were as described in the accompanying paper (9).

3-PG transport assays. Strain CSR603, harboring particular plasmids, was grown at 37°C in minimal medium (medium E) (7) containing 0.5% succinate as a carbon source and supplemented with thiamine, threonine, leucine, proline, arginine, and the appropriate antibiotics. When growth reached exponential phase, cells were collected by centrifugation, washed twice with medium E, and suspended in medium E to an optical density at 660 nm of 3.0. When induction of the pgt transport system was required, 0.2% 3-phosphoglycerate (3-PG) was added to exponentially growing cells, and the cells were harvested 2 h later.

3-PG transport was measured as follows; an aliquot (25 μl) of cell suspension prepared as described above was incubated at 37°C for 2 min, when 1 μl of 250 mM glucose was added. After 15 s, 1 μl of [14C]3-PG (2.3 mM; specific activity, 55 mCi/mM) was added, and the incubation was continued for desired intervals. To terminate transport, the mixture was diluted with 2 ml of medium E. Cells were collected on cellulose acetate membranes (pore size, 0.45 μm; Schleicher & Schuell, Inc.) and washed once with 2 ml of the same medium. Membranes were dried and counted in toluene-based Omnifluor (Dupont, NEN Research Products) in a liquid scintillation counter.

RESULTS

Analysis of high-copy-number lac fusion plasmids. To determine whether the pgtb and pgtc genes were required for the expression of pgtp, lac fusion clones were constructed in vitro with the lac-bearing BamHI sequence isolated from the plasmid pMC931 of Casadaban et al. (2). The fusion clones with fusion sites located outside of the pgtp and pgta genes are shown in Fig. 1. The fusion clone pJH529 had the fusion site in the pgtc gene and was unable to confer 3-PG transport (Table 1), suggesting that the pgtc gene was required for expression of the pgtp gene. In contrast, the clones pJH525 to pJH527, pJH532, pJH536, and pJH537 with fusion sites in the pgtb gene conferred a constitutive 3-PG transport phenotype (data for only pJH525 and pJH526 are shown in Table 1). These results suggest that the pgtb gene was required for the inducibility of pgtp expression.

Analysis of high-copy-number deletion plasmids. Deletion analysis was also carried out to confirm the results from the insertion analysis described above. To this end, three plasmids with short deletions were derived from plasmid pJH6 (Fig. 2). In pSJ1 (pgtc) a NsiI-BglII deletion removed 168 base pairs (bp) from the pgtc gene, presumably creating a truncated PgtC polypeptide; in pSJ2 (pgtbC) the BglII-SnaBI deletion removed the sequence of 323 bp that in-
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latter still retained the bulk of pgtC gene, since the deletion began near the end of the pgtC gene and extended into the pgtB gene (Fig. 2). These results suggest that the activity of the pgtA gene product was affected not only by intact PgtB and PgtC proteins but also by the truncated PgtC protein of pSJ12.

Constitutive expression of pgtP (in pSJ18) required the pgtA gene product, even when the pgtB and pgtC gene products were absent. Thus, plasmid pSJ19 containing only the pgtP gene (in a 1.9-kb HindIII-AatII fragment [Fig. 4]) conferred a non-transport phenotype, whereas pSJ18, which harbored both pgtP and pgtA, conferred a constitutive phenotype (Table 2).

PgtABC polypeptides are not involved in posttranslational modulation of PgtP activity. While the results presented above strongly suggest that the three regulatory proteins, PgtABC, are most likely involved in the regulation of expression of the pgtP gene, alternatively, it is possible, although highly unlikely, that these polypeptides might be involved in posttranslational modulation of the PgtP activity, that is, PgtP might be always expressed but its activity would depend on PgtABC polypeptides. To test this possibility, a lacZ-pgtP fusion plasmid, pJHL30, was constructed as follows. A 760-bp BssHII-Ndel fragment, harboring the 5' flanking region of the pgtP gene plus the first 66 bp of the gene, was prepared from plasmid pJH585 (9), and its ends were blunted with S1 nuclease. This fragment was then ligated to lacZY-bearing plasmid pMC1403 (2) that had been cut with SmaI and dephosphorylated. By sequencing, the fusion plasmid pJHL30 (ampicillin resistant [Ap]) was found to have the 760-bp BssHII-Ndel inserted in the desired

TABLE 2. 3-PG transport conferred by low-copy-number mini-F pgt insertion and deletion plasmids*  
<table>
<thead>
<tr>
<th>Plasmid</th>
<th>3-PG transport (nmol/mg of protein/1 min)</th>
<th>Uninduced</th>
<th>Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>pJH567 (wild type)</td>
<td>0.81</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>pJH577 (pgtB)</td>
<td>0.92</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>pJH66 (wild type)</td>
<td>1.45</td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td>pSJ11 (pgtC)</td>
<td>0.44</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>pSJ12 (pgtBC)</td>
<td>1.10</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>pSJ13 (pgtB)</td>
<td>1.10</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>pSJ14 (pgtA)</td>
<td>0.49</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>pSJ18 (∆pgtBC)</td>
<td>13.8</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>pSJ19 (pgtABC)</td>
<td>0.37</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

* Transport assays were performed as described in Materials and Methods.
orientation with the NdeI end proximal to the lacZ coding region. It had 24 codons preceding codon 8 of the lacZ, 22 of which were the first 22 codons of pgtP and codons 23 and 24 being from the polylinker in pmC1403. Strain YMC9 with the lac operon deleted (1) was the recipient for transformation with plasmids. The expression of the fusions lacZ of this plasmid was examined in cells lacking the lac operon and bearing one of the following low-copy-number mini-F deletion plasmids: pJH66, pSJ11, pSJ12, pSJ13, and pSJ14 (Table 3). Mini-F-derived plasmids and pJHL30 are compatible, thus allowing complementation to be performed. It is clear from Table 3 that the effects of the deletions on the expression of lacZ are essentially identical to those observed on the expression of pgtP measured by 3-PG transport (Table 2). With plasmid pJH66 carrying intact pgtABC genes, the expressed lacZ level was very low under uninduced conditions, was slightly lower than the control without pJH66, and was increased 17.5-fold under induced conditions. With deletion plasmids pSJ11, pSJ12, pSJ13, and pSJ14, very low expression of lacZ was observed under both uninduced and induced conditions. However, with plasmid pSJ18, the lacZ expression was constitutive. These results indicate that PgtABC are involved in the regulation of expression of pgtP gene, but not in modulation of the activity of PgtP polypeptide.

**DISCUSSION**

The results presented in this paper indicate that both pgtB and pgtC are regulatory genes necessary for the induction of the pgtP gene. These two genes are flanked by pgtA and pgtP genes. Thus, altogether the pgt system of *S. typhimurium* is organized in a cluster of four genes in the order pgtP, pgtC, pgtB, and pgtA. The pgtP gene, which encodes the transporter, is transcribed from right to left, and the regulatory genes, pgtC, pgtB, and pgtA, are transcribed in the opposite direction (3, 9, 10). The three regulatory genes do not appear to form an operon, since the expression of pgtA was unaffected by deletions or insertions in pgtB and deletion in pgtBC (pSJ2). However, it is possible that pgtB and pgtC constitute an operon.

A detailed understanding of the function of the three gene products, PgtA, PgtB, and PgtC, in relation to expression of pgtP will require further investigation. However, the results presented here enable certain conclusions to be made. It seems plausible that the role of PgtA is to positively regulate the expression of the pgtP gene, since in both high-copy-number plasmids and low-copy-number mini-F plasmids, deletion of pgtA always resulted in a non-transport phenotype. PgtA may be regarded therefore as an activator or derepressor.

PgtB and PgtC are also involved in induction of pgtP, since expression of the pgtP gene was (i) inducible only when all three regulatory genes were all intact, (ii) constitutive when both pgtB and pgtC genes were totally deleted, and (iii) not expressed when either the pgtB or pgtC gene (in the low-copy-number plasmids) were deleted. It is possible that the activity of PgtA protein in controlling pgtP expression is subject to modulation by PgtB and PgtC proteins. The mechanism for this modulation is unknown. The membrane location of these three proteins (9, 10) and the fact that induction of pgtP expression responds to exogenous inducer suggests that a mechanism involving protein-protein interactions between the PgtC, PgtB, and PgtA proteins occurs within the membrane.

The data obtained with lacZ-pgtP fusion plasmid pJHL30 (Table 3) supports the conclusion that PgtB and PgtC are involved in induction, and also renders highly improbable the possibility that PgtB and PgtC might be involved in modulation of PgtP activity, since no lacZ was expressed under uninduced conditions when the PgtABC were all functional and since PgtPABC were all required for the expression of the lacZ gene under induced conditions.

A working model of induction that can form the basis for future experiments is as follows. Under noninducing conditions, the derepressor, PgtA, cannot assume the required conformation to promote DNA binding and therefore cannot facilitate pgtP gene transcription. Under inducing conditions, the derepressor can adopt an active form as a result of a conformational change that occurs when inducer binds to the receptor site on the periplasmic side of the membrane. PgtB and PgtC are presumably involved in transmission of a transmembrane signal to PgtA in response to inducer binding. PgtA can be freed from constraints imposed by PgtB and PgtC not only as a result of inducer binding but also when these proteins are removed by deletion as in plasmid pSJ18 (ΔpgtBC).

According to this model of induction, a defective receptor or defective signal transmission could explain the non-expression phenotype exhibited by plasmids pJH577, pSJ11, pSJ12, and pSJ13 that harbor insertions or deletions in the pgtB or pgtC gene. Moreover, total deletion of both pgtB and pgtC genes could account for the constitutive phenotype conferred by pSJ18. The latter finding rules out the possibility that induction might involve chemical modification of the derepressor mediated by either PgtB or PgtC protein (or both), since such a mechanism would predict a non-expression phenotype, rather than the observed constitutive phenotype of pSJ18.

There exists a discrepancy between the constitutive transport phenotype conferred by the high-copy-number pgt deletion plasmids, pSJ2 and pSJ3, and insertion plasmid pJH526 on the one hand (Table 1) and the non-transport phenotype exhibited by their low-copy-number mini-F equivalents, pSJ12, pSJ13, and pJH577 on the other (Table 2). It may be, in the context of the proposed model, that when these regulatory proteins were expressed in the high-copy-number plasmids, some derepressor molecules were free and uncomplexed, and therefore were active, but when expressed in low-copy-number plasmids, all derepressor molecules were complexed and therefore inactive. However, the reason, it is clear from the contradicting observations made here that to avoid complications that may arise with
high-copy-number plasmids, it is important to conduct regulatory studies with low-copy-number plasmids, a situation which, in terms of gene number, closely resembles a chromosomal location. Similar cautions have been made by Weston and Kadner (8) and others.

Kadner and co-workers (7) have extensively analyzed the exogenously induced uhp system involved in hexose-6-phosphate transport in *Escherichia coli*. Like pgtP expression, the expression of *uhpT* encoding the transporter requires three regulatory genes. It is not known if similar regulatory mechanisms operate for these two systems.

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LITERATURE CITED


