A cryptic proline permease in *Salmonella typhimurium*

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Wild-type *Salmonella typhimurium* expresses three proline transport systems: a high-affinity proline transport system encoded by the *putP* gene, and two glycine betaine transport systems with a lower affinity for proline encoded by the *prop* and *proU* genes. Although proline uptake by the ProP and ProU transport systems is sufficient to supplement a proline auxotroph, it is not efficient enough to allow proline utilization as a sole source of carbon or nitrogen. Thus, the PutP transport system is required for utilization of proline as a carbon or nitrogen source. In this study, an overexpression suppressor, designated *proY*, which allows proline utilization in a *putP* genetic background and does not require the function of any of the known proline transport systems, was cloned and characterized. The suppressor gene, designated *proY*, maps at 8 min on the *S. typhimurium* linkage map, distant from any of the other characterized proline transport genes. The DNA sequence of the *proY* gene predicts that it encodes a hydrophobic integral membrane protein, with strong similarity to a family of amino acid transporters. The suppressor phenotype requires either a multicopy clone of the *pmY*+ gene or both a single copy of the *prop* gene and a *pmZ* mutation. These results indicate that the *proY* gene is the structural gene for a cryptic proline transporter that is silent unless overexpressed on a multicopy plasmid or activated by a *proZ* mutation.

**Keywords**: proline transport, cryptic gene, multicopy suppression, *Salmonella typhimurium*

**INTRODUCTION**

Transport of nutrients across the cytoplasmic membrane is an essential and often rate-limiting step in bacterial growth. Thus, it is not surprising that there are multiple transport systems for many nutrients. Such 'redundant' transport systems often have different transport kinetics, allowing efficient uptake of a particular substrate under a variety of environmental conditions. Furthermore, when faced with the inability to transport an essential substrate, there is strong selective pressure for suppressors that overcome this problem. Such mutants may arise by activation of a cryptic transport system (e.g., Giel et al., 1996; Hall et al., 1983; Wang & Roth, 1988) or by changing the substrate specificity of a functional transport system (e.g., Begley et al., 1996; Fsihi et al., 1993; King & Wilson, 1990).

Proline transport can be mediated by three different transport systems in *Salmonella typhimurium* and *Escherichia coli* (Wood, 1988; Wood & Zadworny, 1979). The *putP* gene encodes proline permease, a sodium/proline symport system. Proline uptake by the *putP* gene product is sufficient to allow the cells to use proline as sole carbon and nitrogen source (Maloy & Roth, 1983; Ratzkin & Roth, 1978; Wood & Zadworny, 1979). The *prop* gene encodes an ion-driven transport system with a low affinity for proline and glycine betaine (Milner et al., 1988). The *proU* locus encodes a binding-protein-dependent transport system with a low affinity for proline and a high affinity for glycine betaine (Csonka & Epstein, 1996; Higgins et al., 1987). Both the *prop* and *proU* permeases are activated by osmotic stress. However, the rate of proline transport by these...
two systems is too low to allow growth on proline as the sole carbon or nitrogen source (Csonka, 1982; Grothe et al., 1986; Menzel & Roth, 1980; Stalmach et al., 1983).

In a previous study, we isolated pseudorevertants which suppressed the P5N phenotype of putP insertion mutants. The growth properties of the resulting pseudorevertants indicate that they can use proline more efficiently than the putP parental strain (Ekena et al., 1990). The mutation maps at 76.5 min on the S. typhimurium linkage map. None of the known proline transport mutants maps near this region, and the suppressor phenotype does not require any of the known transport genes. These results suggest that a new proline transport system was activated in the pseudorevertants, thus the locus was designated proZ. In this study, we show that the proline transport activity in the proZ mutants is not directly mediated by the proZ gene product, but is due to activation of another gene, designated proY. The DNA sequence of the proY gene shows strong similarity to a family of transport proteins.

METHODS

Strains and growth conditions. The genotypes of the strains and plasmids used in this study are shown in Table 1. The rich medium used was 0.8% nutrient broth (Difco) with 5% NaCl. Three kinds of minimal medium were used: E medium with 0.2% glucose was used to characterize auxotrophs (Vogel & Bonner, 1956); NCE medium which lacks citrate was used to characterize growth on alternative carbon sources (Maloy & Roth, 1983); and NCN medium which lacks both carbon and nitrogen sources was used to characterize growth on alternative nitrogen sources (Ratzkin & Roth, 1978). NCN medium with 0.2% succinate as a carbon source, 0.2% L-proline as a nitrogen source and 0.2% triphenyltetrazolium chloride as a growth indicator was used to check the ability of cells to utilize proline as the sole nitrogen source. The following supplements were added to minimal medium when required: t-arginine (0.6 μg ml⁻¹), t-methionine (0.3 μg ml⁻¹), L-tryptophan (0.1 μg ml⁻¹), L-isoleucine (10 μg ml⁻¹), l-valine (20 μg ml⁻¹), glycyll-l-isoleucine (20 μg ml⁻¹), glycyll-l-valine (40 μg ml⁻¹), glycyll-l-leucine (20 μg ml⁻¹) and calcium pantothenate (1 μg ml⁻¹). To select for transposons or plasmids, ampicillin was added to rich medium at 20 μg ml⁻¹, and kanamycin was added to rich medium at 50 μg ml⁻¹ to 250 μg ml⁻¹. High neomycin/ampicillin/Tris plates were prepared by adding 250 μg neomycin ml⁻¹, 200 μg ampicillin ml⁻¹ and 10 mM Tris/HCl (pH 7.4) to rich medium.

The livA mutants affect a branched-chain amino acid transport system: livA strains can use glycyll-valine and isoleucine as a source of branched-chain amino acids, but livA mutants are unable to grow on minimal medium with glycyll-valine and isoleucine (Onishi et al., 1988).

Genetic techniques. Preparation of phage lysates and transductions was done as previously described (Maloy, 1989). Transductions were done with P22 Htt05/1 int-210, a high-frequency generalized transducing phage that cannot form stable lysogens. Green plates (Davis et al., 1980) and EBU plates (Bochner, 1984) were used to screen for phage-free transductants. Sensitivity of strains to phage was verified by cross-streaking against a P22 c2 mutant.

MudJ insertion mutations were isolated by transitory cis-complementation as described by Hughes & Roth (1988). When KanT transductants were selected, phage and bacteria were mixed, incubated for 45 min at 37 °C to allow phenotypic expression, then spread onto selective plates. MudJ insertions in multicopy plasmids were selected on high neomycin/ampicillin/Tris plates.

Tandem duplications of the chromosomal DNA between cysG at 75.5 min and metE at 86 min were constructed as shown in Fig. 3 (Hughes & Roth, 1985). To confirm the presence of tandem duplications, presumptive merodiploids were grown overnight at 37 °C in nonselective medium to allow segregation of chromosomal duplications. Dilutions of these cultures were plated on nutrient agar, incubated overnight at 37 °C, then the resulting colonies were replica-plated onto selective media to test the phenotypes.

DNA cloning and sequencing. Complementing clones were isolated from a random library of 8–12 kb fragments from a Sau3A partial digest of S. typhimurium chromosomal DNA cloned into the BamHI site of pBR328 (Hmiel et al., 1986). Plasmids were purified by a modified alkaline lysis/LiCl method (Maloy et al., 1996). Restriction digests, ligation and other molecular techniques were done as recommended by the enzyme suppliers. Nested deletions of the cloned gene constructed with the Erase-a-Base kit (Promega) were used for DNA sequencing. The 2.27 kb HindIII-EcoRI fragment of pPC84 was cloned into pBluescript II SK(+) and pTZ18U. Two sets of unidirectional exonuclease III deletions were constructed from these plasmids yielding nested deletions differing in size by approximately 200 bp. Electrocompetent recipients were prepared and electroporated with plasmid DNA (Maloy et al., 1996) using a Gene Pulser apparatus (Biorad). Double-stranded plasmids were sequenced using Sequenase (US Biochemical). DNA fragments subcloned into pTZ or pBluescript II SK(+) were sequenced by using the reverse primer and universal primer. The DNA sequence was confirmed using complementary internal primers obtained from Operon Technologies. A primer (5'-TTCTGCTATCTC-AAAGTGAAT-3') complementary to the left end of Mu was used to determine the DNA sequences adjacent to MudJ insertions.

Computer analysis. The DNA sequence and the predicted amino acid sequence of the open reading frame were analysed using the BLAST algorithm (Altschul et al., 1990), the BLITZ server for the MPsrch program (Strurrock & Collins, 1993), and the Wisconsin Package version 8.1 (Genetics Computer Group, Madison, WI, USA). The hydropathic profile of the predicted open reading frame protein was determined by the Kyte & Doolittle method using the DNA Strider software with a window of 11 residues (Claros & von Heijne, 1994; Kyte & Doolittle, 1982; von Heijne, 1987).

RESULTS

Multicopy suppression of putP

In wild-type cells, the PutP protein is required for transport of sufficient proline to allow growth on proline as a sole carbon or nitrogen source. Therefore, putP mutants are unable to grow on medium with proline as a sole nitrogen source. We previously isolated mutants in a gene designated proZ which suppress this phenotype, allowing putP mutants to grow on proline as sole nitrogen source (Ekena et al., 1990). To further characterize the proZ mutant, we wanted to clone the...
Table 1. Strains and plasmids used

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Genotype*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. typhimurium†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KA261</td>
<td>ilvC livA1 brnQ3</td>
<td>Matsubara et al. (1987)</td>
</tr>
<tr>
<td>MST1209</td>
<td>putP1024::MudJ</td>
<td>Myers et al. (1991)</td>
</tr>
<tr>
<td>MST1644</td>
<td>putP1166::Tn10dCam</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>MST2049</td>
<td>putP1024::MudJ proZ2031 gzi-2460::Tn10 (30% linked to proZ)</td>
<td>Ekena et al. (1990)</td>
</tr>
<tr>
<td>MST2530</td>
<td>ilvC livA1 brnQ3/pPC80</td>
<td>This study</td>
</tr>
<tr>
<td>MST2532</td>
<td>ilvC livA1 brnQ3/pPC82</td>
<td>This study</td>
</tr>
<tr>
<td>MST2533</td>
<td>ilvC livA1 brnQ3/pPC83</td>
<td>This study</td>
</tr>
<tr>
<td>MST3205</td>
<td>ilvC livA1 brnQ3 zai-2469::Tn10dTet (22% linked to brnQ*)</td>
<td>This study</td>
</tr>
<tr>
<td>MST2409</td>
<td>putP1024::MudJ proZ2031 gzi-2460::Tn10 (10% linked to proZ)</td>
<td>This study</td>
</tr>
<tr>
<td>MST3209</td>
<td>putP1166::Tn10dCam proZ2031 gzi-2460::Tn10</td>
<td>This study</td>
</tr>
<tr>
<td>MST3210</td>
<td>ilvC livA1 brnQ3 putP1024::MudJ</td>
<td>This study</td>
</tr>
<tr>
<td>MST3234</td>
<td>trpC2 argG1828::Tn10 (Fels2)~</td>
<td>This study</td>
</tr>
<tr>
<td>MST3235</td>
<td>putP1166::Tn10dCam proZ2031 argG1828::Tn10</td>
<td>This study</td>
</tr>
<tr>
<td>MST3237</td>
<td>putP1166::Tn10dCam proZ2031</td>
<td>This study</td>
</tr>
<tr>
<td>MST3245</td>
<td>putP1166::Tn10dCam proZ2031 gzi-2460::Tn10 proY::MudJ</td>
<td>This study</td>
</tr>
<tr>
<td>MST3251</td>
<td>putP1166::Tn10dCam proY::MudJ</td>
<td>This study</td>
</tr>
<tr>
<td>TN3552</td>
<td>leuB Cad85 recD::Tn10</td>
<td>C. Miller†</td>
</tr>
<tr>
<td>TT10289</td>
<td>hisD9953::MudJ hisA9949::Mud1</td>
<td>Hughes &amp; Roth (1985)</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XL-1 Blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F′ Tn10 proAB lacI lacZ(M15)]</td>
<td>Stratagene</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pBR322</td>
<td></td>
<td>Covarrubias et al. (1981)</td>
</tr>
<tr>
<td>pBR328</td>
<td></td>
<td>Covarrubias et al. (1981)</td>
</tr>
<tr>
<td>pTZ18U</td>
<td></td>
<td>Stratagene</td>
</tr>
<tr>
<td>pBluescript II SK(+)</td>
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<td></td>
</tr>
<tr>
<td>pPC80</td>
<td>14.3 kb Sau3A fragment from S. typhimurium containing brnQ and proY in the BamHI site of pBR328</td>
<td>This study</td>
</tr>
<tr>
<td>pPC82</td>
<td>13.7 kb Sau3A fragment from S. typhimurium containing brnQ and proY in the BamHI site of pBR328</td>
<td>This study</td>
</tr>
<tr>
<td>pPC83</td>
<td>12.5 kb Sau3A fragment from S. typhimurium containing brnQ and proY in the BamHI site of pBR328</td>
<td>This study</td>
</tr>
<tr>
<td>pPC84</td>
<td>8.5 kb EcoRI fragment from pPC80 (GVI+ PSN+)</td>
<td>This study</td>
</tr>
<tr>
<td>pPC86</td>
<td>8.5 kb EcoRI fragment from pPC83 (GVI+ PSN+)</td>
<td>This study</td>
</tr>
<tr>
<td>pPC87</td>
<td>8.5 kb HindIII fragment from pPC80 (GVI+ PSN+)</td>
<td>This study</td>
</tr>
<tr>
<td>pPC88</td>
<td>11.9 kb EcoRI fragment from pPC82 (GVI+ PSN+)</td>
<td>This study</td>
</tr>
</tbody>
</table>

*The genetic nomenclature is described in Sanderson et al. (1996). MudJ is an abbreviation for Mu d1734, a Kan' mini-Mud that forms lac operon fusions (Beatriz et al., 1984). The designation proY was assigned after consultation with Ken Sanderson at the Salmonella Genetic Stock Center (University of Calgary, Canada). Tn10dCam is derived from Tn10dTet but with Cam' substituted for Tet'. (Elliott & Roth, 1988).

† All the S. typhimurium strains used were derived from LT2.
‡ Obtained from Charles Miller, Department of Microbiology, University of Illinois, Urbana, IL, USA.

**proZ**+ gene. Because the proZ+ gene does not have a known phenotype, there was no direct selection for the desired clones. Therefore, we used an indirect strategy: the livA gene and the proZ gene are very closely linked, so it seemed likely that clones which carry the livA+ gene might carry the proZ+ gene as well. However, rather than cloning the proZ+ gene, this strategy yielded clones of a different gene that functions as a multicopy suppressor of the PSN- phenotype.

Strain KA261( **ilvC livA brnQ3** ) is auxotrophic for isoleucine and valine due to a mutation in the **ilvC** biosynthetic gene. Furthermore, KA261 cannot directly use isoleucine and valine due to mutations in the two major branched-chain transport systems, encoded by **livA** and **brnQ**. Therefore, the auxotrophic requirement is supplemented with glycy-leucine and glycy-valine, which are transported by dipeptide transport systems and then cleaved to the corresponding amino acids once
inside the cell. Clones that complement the branched-chain amino acid transport defect were isolated from a random library of *S. typhimurium* chromosomal DNA cloned into pBR328. Plasmids from this library were introduced into KA261 by selecting for Amp<sup>+</sup> transformants, and the resulting colonies were screened for growth on minimal glucose plates supplemented with glycyl-valine and isoleucine (Matsubara et al., 1987).

Four complementing clones were isolated. One of the clones allowed KA261 to grow on minimal glucose medium without branched-chain amino acids, indicating that the clone complemented the *ilvC* mutation in KA261. The other three clones (pPC80, pPC82 and pPC83) allowed KA261 to grow on minimal glucose medium supplemented with glycyl-valine and isoleucine (GVI) but not on unsupplemented minimal glucose medium, indicating that these clones complemented the branched-chain amino acid transport defect (Fig. 1). When MST1644 (*putP<sup>1166::Tnl</sup>*::*Tn10dCam*) was transformed with each of these plasmids, the PSN<sup>+</sup> phenotype conferred by the *putP* mutation was also complemented.

Restriction mapping indicated that the three clones carried an overlapping region of chromosomal DNA. To locate the gene(s) responsible for the GVI<sup>+</sup> and PSN<sup>+</sup> phenotypes, subclones of these plasmids were constructed (Fig. 1). Based upon the phenotypes of the subclones, pPC84 contains the gene or genes responsible for the PSN<sup>+</sup> phenotype and the GVI<sup>+</sup> phenotype, while pPC87 only contains the gene which confers the GVI<sup>+</sup> phenotype. These results suggest that two separate genes are responsible for complementation of the two different phenotypes.

**The multicopy suppression is conferred by a single cloned gene**

To further localize the regions that encode the PSN<sup>+</sup> and GVI<sup>+</sup> phenotypes, MudJ insertion mutations were isolated in the plasmid clones. More than 60 MudJ insertion mutants were isolated on pPC80 and the locations of the MudJ insertions were determined by restriction mapping. Of the 60 independent MudJ insertions, 24 insertions were within the complementing *S. typhimurium* DNA fragment.

Plasmids with the MudJ insertions were purified then introduced into the two bacterial strains KA261 (*ilvC livA brnQ<sup>+</sup>*) and MST1209 (*putP*). If a single gene was responsible for both the GVI<sup>+</sup> and PSN<sup>+</sup> phenotypes, MudJ insertions in the complementing DNA fragment would be expected to either abolish both phenotypes or maintain both phenotypes. Likewise, if two separate genes were responsible for the two phenotypes but they were located in an operon, insertion mutations in the upstream gene would abolish both phenotypes. In contrast, if two independent genes were responsible for the GVI<sup>+</sup> and PSN<sup>+</sup> phenotypes, the MudJ insertions would abolish one phenotype without affecting the other. The results for several of the insertion mutations are shown in Fig. 1. In each case, the insertions which abolish the GVI<sup>+</sup> phenotype have no effect on the PSN<sup>+</sup> phenotype, and the insertions which abolish the PSN<sup>+</sup> phenotype have no effect on the GVI<sup>+</sup> phenotype, indicating that the two phenotypes are probably due to two adjacent but independent genes.

To confirm that these results were not due to multicopy
Cryptic proline permease

(a) Hydropathy plot of the predicted ProY protein. A window size of 11 residues was used. Positive scores correspond to hydrophobic regions.

(b) Predicted membrane topology of the ProY protein in the cytoplasmic membrane. LL, loop length; KR, number of lysine and arginine residues in the respective loop.

Table 2. Some proteins with substantial amino acid similarity to ProY

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Phenotype</th>
<th>Similarity (%)</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>pheP</td>
<td>Phe-specific transporter</td>
<td>66</td>
<td>43</td>
</tr>
<tr>
<td>E. coli</td>
<td>aroP</td>
<td>Aromatic amino acid transporter</td>
<td>65</td>
<td>43</td>
</tr>
<tr>
<td>E. coli</td>
<td>lysP</td>
<td>Lys-specific permease</td>
<td>58</td>
<td>36</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>PUT4</td>
<td>Pro-specific permease</td>
<td>55</td>
<td>30</td>
</tr>
<tr>
<td>Emericella nidulans</td>
<td>putX</td>
<td>Pro transporter</td>
<td>53</td>
<td>27</td>
</tr>
</tbody>
</table>

* The accession numbers of genes listed in this table are: pheP, sp/P24207; putX, sp/P18696; aroP, sp/P15993; lysP, sp/P25737; and PUT4, sp/P15380.

† Identity or similarity is shown as the percentage of identical or similar amino acids between the proY gene and the genes indicated as determined by using the Wisconsin Package version 8.1.

Effects of the MudJ insertions on pPC84 were moved onto the chromosome in single copy. Each plasmid was linearized with the restriction enzyme BclI, then the linear DNA fragments were introduced into a recD::Tn10 strain by electroporation, selecting for the Kan' phenotype of MudJ. The recD mutation inactivates exonuclease V, allowing homologous recombination between the linear DNA fragments and the corresponding gene(s) on the chromosome (Biek & Cohen, 1986; Miesel & Roth, 1994; Russell et al., 1989). Phage lysates grown on the resulting strains were then used to transduce the chromosomal MudJ insertions into strains MST3205 (ilvC ilvA brnQ) and MST3237 (putP proZ). The phenotypes of the chromosomal MudJ mutations were identical to that observed with the multicopy plasmids, confirming that the PSN+ phenotype and the GVI+ phenotype are encoded by two independent genes.

The multicopy suppressor encodes the structural gene for a new transport protein

The DNA sequence of the fragment including the region required for the PSN+ phenotype was determined. The sequence of the initial 500 bp of the region responsible for the GVI+ phenotype was identical to the brnQ+ gene (Ohnishi et al., 1988). The region responsible for the PSN+ phenotype included a 1371 bp open reading frame. The translated open reading frame would encode a
Table 3. Phenotypes of proY and proZ mutants

<table>
<thead>
<tr>
<th>Chromosomal alleles*</th>
<th>Plasmid alleles</th>
<th>Growth on PSN†</th>
<th>Sensitivity to proline analogues‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DHP</td>
</tr>
<tr>
<td>putP⁺ proZ⁺ proY⁺</td>
<td>—</td>
<td>+</td>
<td>s</td>
</tr>
<tr>
<td>putP proZ⁺ proY⁺</td>
<td>—</td>
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<td>+</td>
<td>s</td>
</tr>
<tr>
<td>putP proZ proY⁺</td>
<td>—</td>
<td>—</td>
<td>r</td>
</tr>
</tbody>
</table>

* The putP mutation used was putP1166::Tn10dCam. Each of the proY::MudJ mutations gave identical results.

†PSN indicates utilization of proline as a sole nitrogen source. Growth on PSN is a sensitive indicator of proline transport in these strains.

‡Sensitivity to the toxic proline analogues dehydroproline (DHP) and azetidine-2-carboxylic acid (AZT) was tested by determining the zone of inhibition as described by Dila & Maloy (1987). Substrate specificity was evaluated by measuring the zone of inhibition on plates spread with other amino acids.

![Fig. 3. Construction of proZ tandem duplications with a MudJ join-point. Recombination of two transducing fragments carrying metE::MudJ and cysG::MudJ with the recipient chromosome in the replication fork. The duplication was constructed by selecting for recombination between two transducing fragments with different auxotrophic MudJ insertions. Since both MudJ insertions are oriented in the same direction, recombination between the two MudJ insertions results in prototrophic Kan' colonies. Transduction of the tandem duplication allows replacement of one of the two proZ alleles. The triangle represents the linked Tn10 insertion.](image)

protein of 456 amino acids with a molecular mass of 50014 Da. The hydropathy profile and secondary structure predictions of the translated amino acid sequence predict that it is an integral membrane protein with 12 potential transmembrane domains (Fig. 2). When the predicted amino acid sequence of the open reading frame was compared with the GenBank database, a family of amino acid permeases showed significant similarity to the open reading frame (Table 2). These results suggest that this sequence encodes the structural
gene for a cryptic proline permease. This gene has been designated proY. An almost identical gene is located at the corresponding position on the E. coli chromosome (GenBank accession no. U82664).

The proZ suppressor phenotype requires the proY gene

Suppression of the PSN− phenotype of putP mutants required either (i) a multicopy clone of the proY+ gene or (ii) a single, chromosomal copy of the proY+ gene and a proZ mutation (Table 3). In either case, mutations which disrupt the proY gene eliminate the suppressor phenotype, indicating that the proY phenotype is epistatic to proZ. Furthermore, proline transport by strains with proZ and proY suppressors is virtually identical: neither the proZ nor proY suppressor requires the putP, proP or proU gene products, and the substrate specificity of transport by proY is essentially identical to that determined for proZ mutants (Table 3). These results imply that both ProZ and ProY affect the same cryptic proline transport system.

The proZ suppressor mutation is dominant

To determine if the proZ mutation is dominant or recessive, we constructed chromosomal duplications of the proZ gene as shown in Fig. 3. Merodiploids were obtained by transduction of the chromosomal duplication with a Tn10 insertion approximately 30% linked to a proZ mutation. If the wild-type proZ+ allele was dominant, both the proZ/proZ+ and proZ+/proZ+ merodiploids would have a PSN− phenotype. Hence, all the resulting recombinants would be PSN−. In contrast, if the proZ allele was dominant, the proZ/proZ+ merodiploids would have a PSN+ phenotype but the proZ+/proZ+ merodiploids would have a PSN− phenotype. Hence the ratio of PSN+ to PSN− recombinants would be determined by the linkage of proZ to the Tn10 insertion. The results indicated that the proZ mutation is dominant: 35/100 of the transductants were PSN+, like the proZ parent strain. Furthermore, haploid KanR segregants of the proZ/proZ+ merodiploids yielded approximately equal numbers of colonies with the PSN+ (proZ) and PSN− (proZ+) phenotype. Similar results were obtained with merodiploids that carry the corresponding region of DNA from E. coli on F’140 (66–81 min) (Holloway & Low, 1996). These results imply that the proZ mutation is not a simple loss of function mutation and that suppression of the PSN− phenotype in haploid cells requires the activation of proY by the proZ gene product.

DISCUSSION

Multicopy suppressors of proline transport mutants encode a cryptic proline permease

We previously characterized mutations in the proZ locus which seem to activate a new proline transport system. Based on genetic mapping results, we showed that the proZ mutation is located at 76.5 min on the S. typhimurium linkage map, close to the liuA gene (Ekena et al., 1990). To further characterize the suppressor phenotype, we attempted to clone the wild-type proZ gene. There is no direct selection for proZ+ clones, but we expected clones that complement the closely linked liuA mutation to also carry the proZ+ gene. Therefore, we screened a random library of S. typhimurium DNA cloned into pBR328 for clones that complement the branched-chain amino acid transport defect in strain KA261 (liuC livA brnQ). Instead of isolating clones that carry the branched-chain amino acid transport system encoded by liuA, we obtained clones that carry the branched-chain amino acid transport system encoded by brnQ. Serendipitously, a gene adjacent to the brnQ gene on these clones allowed putP mutants to transport proline. We designated this gene proY. The predicted amino acid sequence of the proY gene shows strong similarity to a large family of amino acid permeases, including proline permeases from other organisms (Table 2). These results suggest that proY is the structural gene for an amino acid permease.

What is the relationship between the branched-chain amino acid and proline transport systems?

In S. typhimurium, transport of branched-chain amino acids is mediated by three different systems: a high-affinity transport system (LIV-I), and two low-affinity transport systems (LIV-II and LIV-III) (Kiritani & Ohnishi, 1987). liuA is a structural gene of the LIV-I system, located at 76-5 min on the linkage map. brnQ is a structural gene of the LIV-II system, located at 8 min on the linkage map (Sanderson et al., 1996). Based on the analysis of mutants and transport kinetics, the LIV-II system appears to be the major branched-chain amino acid transport system (Ohnishi et al., 1988). Thus, it is not surprising that we isolated clones that carry the brnQ+ gene when screening for complementation of the branched-chain amino acid transport defect.

The proY gene was located adjacent to the brnQ gene. Polar insertion mutations in brnQ cannot transport branched-chain amino acids but retain proline transport, and polar insertion mutations in proY cannot transport proline but retain branched-chain amino acid transport (Fig. 1). Therefore, although adjacent, the proY and brnQ genes encode proteins with discrete functions. Furthermore, the lack of polarity indicates that the brnQ and proY genes are not in a single operon. These results suggest that the location of the proY and proZ genes adjacent to branched-chain amino acid transport genes may simply be an evolutionary coincidence.

What is the relationship between the proZ and proY genes?

Insertion mutations in proY abolished the PSN+ phenotype in the proZ mutant background, indicating that proY mutations are epistatic to the proZ mutations (Fig.
1. Taken together, these results and the DNA sequence analysis suggest that the proY gene encodes a cryptic permease that is directly responsible for the PSN+ phenotype. There are at least two possible models that could explain the role of proZ: ProZ may regulate ProY expression or ProZ may affect the enzyme activity of ProY. In either case, since the proZ mutation enhances proline transport, the ProZ phenotype could be due to either the loss of a negative effector normally made in proZ+ cells or the gain of a positive effector not normally made in proZ+ cells. If the wild-type ProZ is a negative effector of ProY, the proZ mutation would be expected to be recessive, but if the mutant ProZ is a positive effector for ProY, the proZ mutation would be expected to be dominant. The phenotype of proZ/proZ+ merodiploids indicates that the proZ mutation is dominant, suggesting that the ProZ phenotype is due to a gain of function mutation which ‘turns on’ the proY function.

How do such ‘new’ transport systems arise?

In addition to direct experimental evidence that there are many redundant transport systems in bacteria, genome sequencing studies suggest that transport proteins comprise a substantial proportion of the genomic coding potential. Many transport systems are encoded by closely related families of genes. Irrespective of the substrate of the transport system, families of transport proteins show striking sequence similarity. The sequence comparisons suggest that differences in substrate specificity only require minor changes in amino acid residues at the active site of the protein. Thus, mutants with new transport functions may evolve by minor modifications of other functional permeases. Alternatively, cryptic transport systems may be maintained as silent genes that are activated by a mutation which turns on gene expression by providing a promoter or altering a regulatory protein (Hall et al., 1983). The ability to readily alter the repertoire of substrates transported may provide an evolutionary advantage by allowing bacteria to take advantage of new sources of nutrients.

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