Location and Direction of Transcription of the ptsH and ptsI Genes on the Escherichia coli K12 Genome

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Recombinant plasmids were constructed that carried various fragments of the DNA specifying the Escherichia coli genes ptsH and (part of) ptsI, the genes for the common components of the phosphoenolpyruvate:sugar phosphotransferase. Expression of plasmid-specified functions in minicells showed that ptsH and ptsI were transcribed clockwise. Most of the transcription of ptsI was from the ptsH promoter, but some was from a second site within or after ptsH.

INTRODUCTION

A number of hexoses, hexitols and amino-hexoses ('phosphotransferase sugars') are taken up by Escherichia coli via a phosphotransferase mechanism (Kundig et al., 1964). In this, the phosphate group of phosphoenolpyruvate is transferred by an enzyme (Enzyme I, M, 65000, specified by the ptsI gene) to (usually) a small histidine-containing protein (Hpr, M, 9000, specified by the ptsH gene).

We have previously reported that a plasmid, pAB65, which carried DNA derived from a specialized transducing phage integrated into the ptsI gene (Britton et al., 1983), complemented the ptsH lesion in a recA ptsH strain of E. coli, and expressed a polypeptide of M, 9000 in minicells (Lee et al., 1982; Boronat et al., 1984). The polypeptide was purified and shown to be identical to the Hpr isolated from Salmonella typhimurium (Weigel et al., 1982) by a number of criteria (Lee et al., 1982) though, at that time, none of its amino acid sequence had been determined.

Two other recombinant plasmids, pAB70 and pAB101, were constructed from pAB65 (Boronat et al., 1984), from which we tentatively mapped the ptsH gene as lying between the λH57 and H1 HindIII restriction sites. This correlated with the position of the ptsH gene on the maps of the specialized transducing phages from which the plasmids had originally been derived (Britton et al., 1983). We believe from the expression of the specialized transducing phages and the recombinant plasmids pAB65, pAB70 and pAB101 that a polypeptide of M, 49000 is a fragment of Enzyme I. The purpose of this paper is to identify the position and orientation of the ptsH and ptsI genes on the E. coli chromosome.

METHODS

The genotypes of the strains used are listed in Table 1. The chemicals used were as reported by Britton et al. (1982) and the [35S]methionine as described by Boronat et al. (1984). All restriction endonucleases and T4 DNA ligase were obtained from BRL. The minimal medium was that of Ashworth & Kornberg (1966).

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transformed using purified DNA, and minicells were prepared and plasmid DNA expressed as described by centrifugation in cesium chloride density gradients containing ethidium bromide (0.6 mg ml\(^{-1}\)) in a high speed Boronat then precipitated with ethanol and resuspended in 10 mM-Tris/HCl, 0.1 mM-EDTA pH 7.5. Cells were Alkaline phosphatase (Sigma, calf intestinal no. P552 1) treatment and its removal by phenol extraction were by plasmids instead of chloramphenicol. The plasmid DNA was purified by cesium chloride density gradient nuclease-free BSA (100 pg ml\(^{-1}\)) with saturated centrifugation in the presence of ethidium bromide.

Plasmid DNA was digested using the buffer systems of Davis et al., 1984 except that all digestions contained nuclease-free BSA (100 \(\mu\)g ml\(^{-1}\); BRL). Ligation reactions were carried out as described by Boronat et al., 1984. Alkaline phosphatase (Sigma, calf intestinal no. P5521) treatment and its removal by phenol extraction were by the procedure of Davis et al., 1980. Digested plasmid DNA was analysed in 1.0% (w/v) agarose gels [containing 0.2% polyacrylamide as described by Britton et al., 1980] except that spectinomycin (300 \(\mu\)g ml\(^{-1}\)) was used to amplify the plasmids instead of chloramphenicol. The plasmid DNA was purified by cesium chloride density gradient centrifugation in the presence of ethidium bromide.

Purification and characterization of plasmid DNA. Plasmid DNA was prepared as described by Boronat et al., 1984 except that 100 ml cultures were used and the DNA, before phenol/chloroform extraction, was subjected to centrifugation in caesium chloride density gradients containing ethidium bromide (0-6 mg ml\(^{-1}\)) in a high speed MSE 65 preparative ultracentrifuge (Davis et al., 1980). Ethidium bromide was extracted from the plasmid DNA with saturated (5 M NaCl, 10 mM-Tris/HCl, 0.1 mM-EDTA pH 7-5) butan-2-ol (Davis et al., 1980). The DNA was then precipitated with ethanol and resuspended in 10 mM-Tris/HCl, 0.1 mM-EDTA pH 7-5. Cells were purified using purified DNA, and minicells were prepared and plasmid DNA expressed as described by Boronat et al., 1984.

Plasmid DNA was digested using the buffer systems of Davis et al., 1980 except that all digestions contained nuclease-free BSA (100 \(\mu\)g ml\(^{-1}\); BRL). Ligation reactions were carried out as described by Boronat et al., 1984. Alkaline phosphatase (Sigma, calf intestinal no. P5521) treatment and its removal by phenol extraction were by the procedure of Davis et al., 1980. Digested plasmid DNA was analysed in 1-0% (w/v) agarose gels as described by Boronat et al., 1982. Small \((<1-0 kb)\) and very small \((<0-1 kb)\) fragments were analysed on 3-5-7-5% and 12-20% (w/v) polyacrylamide gradient gels [containing 0-2% polyacrylamide as described by Britton et al., 1982] in order to strengthen the gels] respectively, prepared as described by Britton et al., 1982 using the buffer systems of Maniatis et al., 1982).

Construction of \(pBR322\) derivative plasmids. These plasmids were constructed as shown in Fig. 1 using the methods described by Boronat et al., 1984.

Construction of \(pBR328\) derivative plasmids. Plasmid \(pBR328\) (Soberon et al., 1980) was prepared as described for plasmid \(pBR322\) (Boronat et al., 1984) except that spectinomycin (300 \(\mu\)g ml\(^{-1}\); Upjohn) was used to amplify the plasmids instead of chloramphenicol. The plasmid DNA was purified by cesium chloride density gradient centrifugation in the presence of ethidium bromide.

Plasmid \(pPB50\), which contained the \(PstI\) fragment \(\lambda P56-PsI\) (Fig. 2), was constructed by digesting purified \(pAB65\) DNA with \(PstI\), \(EcoRI\) and \(BamHI\) restriction endonucleases. The \(\lambda P56-PsI\) fragment was then ligated into plasmid \(pBR328\) DNA previously digested with \(PstI\). The ligated mixture was transformed into strain \(PB21\) and \(Cm^R\) \(Ap^R\) transformants were selected. The plasmids from 12 independent isolates were found to contain the \(PstI\) fragment.
Location of *E. coli* *ptsH* and *ptsI* genes

Plasmids pAB65, pAB92, pAB94, pPB63 and pPB44 were all shown to be *ptsH*+ by transformation of the *ptsH* strains FP45 or PB21 to the *PtsH*+ phenotype. All these plasmids were phenotypically ampicillin-resistant but tetracycline-sensitive.

Plasmids pPB157, pPB158, pPB164 and pPB166, which contained the *PvuII* fragment P3-P4 (Fig. 2), were constructed by digesting plasmid pAB101 with *PvuII*, *BglII* and *BamHI* restriction endonucleases. Any *PvuII* fragments were ligated into plasmid pBR328 previously digested with *PvuII* and treated with alkaline phosphatase to prevent re-ligation of the vector. The ligated mixture was transformed into strain PB21. Ampicillin-resistant colonies were selected: 12 out of 278 had the *PtsH*+ phenotype and were CmS.

Isolation of plasmids containing γ6 insertions. Guyer (1978) reported that the *F*+-mediated transfer of pBR322 resulted in the insertion of the γ6 sequence (Tn7000) into pBR322 at random sites. Sancar et al. (1979, 1981) used this technique to insert the γ6 sequence into the *E. coli* *uraA* gene cloned into pBR322. A *ptsH*+ plasmid, pPB63, was transformed into the *F*'-mediated strain PB13 to yield PB13(pPB63). Both the donor strain PB13(pPB63) and the recipient strain PB21 (*StrR recA ptsH*) were grown in Luria broth at 37 °C to a cell density of 1.8 x 10^9 cells ml^-1 (an OD₆₀₀ of 1 is equivalent to 2 x 10^8 cells ml^-1). Samples (2 x 10^8 cells) of each strain were mixed and incubated for 90 min at 37 °C. The mixture was then centrifuged in an Eppendorf 5412 micro-centrifuge for 5 min and resuspended in 700 µl Luria broth. Samples (100 µl) of the mixture were spread on nutrient agar containing ampicillin (100 µg ml^-1) and streptomycin (100 µg ml^-1) to select PB21 cells that had received insertional derivatives of pPB63. Single colonies were then tested for their ability to grow on N-acetylglucosamine as sole carbon source. Of the 152 colonies tested, 21 did not grow, indicating that the *ptsH* gene was no longer functional, perhaps because of a γ6 insertion. Cell extracts of the transformants were also assayed for the presence of Hpr as described by Lee et al. (1982).

RESULTS

Restriction mapping of the cloned *E. coli* DNA

Britton *et al.* (1983) showed that the DNA fragment JH57-B1 (Fig. 2) contains two *KpnI* sites (K1 and K2) and one *HindIII* site (H1). To elucidate the position of the *ptsH* and *ptsI* genes the DNA was digested with other restriction endonucleases. Four *PvuII* sites were found, three of which, P1, P2 and P3, were between the K1 and K2 sites. The other, P4, lies 38 bp to the right of the H1 site. Two *SmaI* sites, S1 and S2, lay between the P3 and K2 sites. A single *PslI* site, Ps1, was found just to the left of the K2 site. A single *BglII* site, Bg1, was found 0.17 kb to the left of the K1 site. These restriction sites are shown in Fig. 2. The identification of these restriction sites permitted the construction of several more recombinant plasmids.

Construction of *PtsH*+ and *PtsH*– recombinant plasmids

To locate the position of the *ptsH* and *ptsI* genes several recombinant plasmids derived from pAB65 were constructed (Fig. 1). Initially the ψ-H57-H1 fragment was subcloned from plasmid pAB65 into plasmid pBR322, resulting in two plasmids, pAB92 and pAB94, each of 7.12 kb.
The fragment was found to be orientated in opposite directions in the two plasmids. Therefore neither the \textit{ptsH} nor the \textit{ptsl} gene required the plasmid \textit{tet} gene promoter for expression. Both plasmids complemented the \textit{ptsH} lesion in FP45 and expressed polypeptides of $M$, 9000 and 49000 in minicells (Fig. 3, tracks 4 and 5).

Two new plasmids were constructed by removing the K1-K2 KpnI fragment from pre-existing plasmids; plasmid pAB65 yielded plasmid pAB95 (8.64 kb) and plasmid pAB94 yielded plasmid pPB3 (6.42 kb). Neither of these plasmids complemented the \textit{ptsH} lesion in FP45 or in strain PB21. Neither plasmid expressed polypeptides of $M$, 9000 or 49000 in minicells (Fig. 3, tracks 6 and 7). However plasmid pAB95 expressed polypeptides of $M$, 34000 and 33000 (Fig. 3, track 6), products of the \textit{cysK} gene; and of part of the \textit{ptsl} gene; its position and product are shown. The position marked A indicates the insertion site of $\psi\delta$ into the \textit{ptsH} gene in plasmids pPB169, pPB170 and pPB185. The sizes of the restriction fragments in the \textit{\lambda} DNA were derived from the \textit{\lambda} DNA map (Sanger et al., 1982). The restriction sites within \textit{\lambda} DNA are identified by numbers indicating their position on the \textit{\lambda} map. The DNA derived from \textit{E. coli} is shown as a solid line and that from bacteriophage \textit{\lambda} as an open box. E, EcoRI; Ps, PstI; P, PvuII; H, HindIII; Bg, BgfII; K, KpnI; S, SmaI; and B, BamHI. OASL, O-acetylserine (thiol)-lyase. The restriction fragment B-1-E-1 is not drawn to scale but is 6.18 kb long.

The DNA fragment \textit{\lambda}E54.3-B1 was cloned from JJM29 into plasmid pBR322 (Lee et al., 1982) and the resulting plasmid, pAB65, was mapped. The sizes (kb) of the restriction fragments, determined using either agarose or polyacrylamide gel electrophoresis as described in the text, were: \textit{\lambda}H57-Bgl, 1.00; Bgl-K1, 0.17; K1-P1, 0.095; P1-P3, 0.341; P3-S1, 0.128; S2-K2, 0.094; P3-Psl, 0.307; Psl-H1, 0.82; H1-P4, 0.038; P4-B1, 1.57. The DNA to the left of the $\lambda$ integration site has not been sub-cloned or extensively mapped (the restriction sites were determined by Britton et al., 1983). The loci of the \textit{gsr}, \textit{ptsl}, \textit{ptsl} and \textit{cysK} genes are shown. The position of the \textit{gsr} gene and the C-terminal piece of the \textit{ptsl} gene are derived from the specialized $\lambda$ transducing maps reported by Britton et al. (1983). The position of the genes and the sizes of polypeptides they specify were determined from the expression of the transducing phages (Britton et al., 1982) or from the expression of the recombinant plasmids in minicells (Boronat et al., 1984, and this paper). The recombinant plasmids carried only part of the \textit{ptsl} gene; its position and product are shown. The position marked A indicates the insertion site of $\psi\delta$ into the \textit{ptsH} gene in plasmids pPB169, pPB170 and pPB185. The sizes of the restriction fragments in the \textit{\lambda} DNA were derived from the \textit{\lambda} DNA map (Sanger et al., 1982). The restriction sites within \textit{\lambda} DNA are identified by numbers indicating their position on the \textit{\lambda} map. The DNA derived from \textit{E. coli} is shown as a solid line and that from bacteriophage \textit{\lambda} as an open box. E, EcoRI; Ps, PstI; P, PvuII; H, HindIII; Bg, BgfII; K, KpnI; S, SmaI; and B, BamHI. OASL, O-acetylserine (thiol)-lyase. The restriction fragment B-1-E-1 is not drawn to scale but is 6.18 kb long.

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Two new plasmids were constructed by removing the K1-K2 KpnI fragment from pre-existing plasmids; plasmid pAB65 yielded plasmid pAB95 (8.64 kb) and plasmid pAB94 yielded plasmid pPB3 (6.42 kb). Neither of these plasmids complemented the \textit{ptsH} lesion in FP45 or in strain PB21. Neither plasmid expressed polypeptides of $M$, 9000 or 49000 in minicells (Fig. 3, tracks 6 and 7). However plasmid pAB95 expressed polypeptides of $M$, 34000 and 33000 (Fig. 3, track 6), products of the \textit{cysK} gene; and of part of the \textit{ptsl} gene; its position and product are shown. The position marked A indicates the insertion site of $\psi\delta$ into the \textit{ptsH} gene in plasmids pPB169, pPB170 and pPB185. The sizes of the restriction fragments in the \textit{\lambda} DNA were derived from the \textit{\lambda} DNA map (Sanger et al., 1982). The restriction sites within \textit{\lambda} DNA are identified by numbers indicating their position on the \textit{\lambda} map. The DNA derived from \textit{E. coli} is shown as a solid line and that from bacteriophage \textit{\lambda} as an open box. E, EcoRI; Ps, PstI; P, PvuII; H, HindIII; Bg, BgfII; K, KpnI; S, SmaI; and B, BamHI. OASL, O-acetylserine (thiol)-lyase. The restriction fragment B-1-E-1 is not drawn to scale but is 6.18 kb long.

The sub-cloning of the DNA fragment \textit{\lambda}Ps56-Ps1 (Fig. 2) from plasmid pAB65 into plasmid pBR328 resulted in the Cm$^+$ Ap$^+$ plasmid pPB50 (7.34 kb). This plasmid was not able to complement the \textit{recA ptsH} strain PB21 nor did it express a polypeptide of $M$, 9000 in minicells. However plasmid pPB50 did express, though at a lower level than plasmids pAB65 and pAB94, a polypeptide of $M$, 49000 (Fig. 3, track 8). This indicated that the \textit{PsrI} site, Ps1, lay within the \textit{ptsH} gene and that expression of the \textit{ptsl} gene also initiated within the K1-K2 fragment from a promoter independent of the \textit{ptsH} promoter.
Location of E. coli ptsH and ptsI genes

Fig. 3. Fluorogram of the [35S]methionine-labelled polypeptides expressed from the recombinant plasmids in minicells and analysed on 12-20% gradient polyacrylamide gels at 4°C as described by Boronat et al. (1984). Track 1 shows the pattern from DS410 minicells without any plasmid DNA; track 2, ampicillin and chloramphenicol resistance gene products from plasmid pBR328; track 3, ampicillin resistance gene product from plasmid pBR322; track 4, expression pattern of plasmid pAB92; track 5, pAB94; track 6, pAB95; track 7, pPB3; track 8, pPB50; track 9, pAB65; track 10, pAB101; track 11, pPB63; track 12, pPB169; track 13, pPB170; track 14, pPB185; track 15, pPB190; track 16, pPB157; track 17, pPB158; track 18, pPB164; and track 19, pPB166. a, Enzyme I fragment; b, O-acetylserine (thiol)-lyase; c, bEa59 fragment; d, product resulting from fusion of ptsH and ptsI genes; e, β-lactamase; f, chloramphenicol-acetyltransferase; and g, Hpr.

Position of the ptsH gene by sub-cloning

The properties of plasmids pAB95, pPB3 and pPB50 indicated that the ptsH gene lay across the K2 and Ps1 restriction sites and that the promoter was to the right of Ps1. If so, one would expect to find a single PstI site followed by a single KpnI site in the DNA that encodes the protein Hpr. From the Hpr amino acid sequence established by Weigel et al. (1982) there are two potential PstI sites and one potential KpnI site (assuming appropriate codon usage):

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino acid</th>
<th>DNA sequence</th>
<th>Restriction site</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-19-20</td>
<td>Pro-Ala-Ala</td>
<td>CCT GCA GCT</td>
<td>PstI</td>
</tr>
<tr>
<td>51-52</td>
<td>Leu-Glu-Gly-Thr</td>
<td>CTG CAG GGT ACC</td>
<td>KpnI</td>
</tr>
</tbody>
</table>

Another potential PstI site exists at the last two amino acids of Hpr but the observation that removal of the K1-K2 fragment led to a hybrid polypeptide indicated that a KpnI site must lie within the ptsH structural gene. This observation combined with the mapping data, a PstI site followed by a KpnI site, rules out the possibility that the potential PstI site of the last two amino acids is the observed one. The mapping data favour a PstI site about 25 bp to the right of the K2.
site (Fig. 2). The PstI site corresponding to amino acids 51 and 52 is therefore favoured for the PstI site in the cloned DNA. The codons for amino acids 51 and 52 resulting in a PstI site and amino acids 59 and 60 resulting in a KpnI site are the most common for these amino acids in E. coli as demonstrated by Grosjean & Fiers (1982). The codons required for amino acids 18, 19 and 20 to correspond to a PstI site are not preferred. Thus it is highly probable that the codons for the amino acids at positions 51 and 52, and 59 and 60 create the PstI and KpnI restriction sites, PstI and K2, though the DNA sequence of the ptsH gene is necessary to confirm this. A SmaI site, S2 (Fig. 2), is 94 bp to the left of the K2 site but the amino acid sequence of Hpr indicates that it could not be specified by DNA containing a SmaI site. This indicates that the ptsH gene does not stretch as far as the S2 site. If the codons for amino acids 59 and 60 create the KpnI site, K2, the amino acid sequence of Hpr predicts that the ptsH gene stretches 71 bp to the left of the K2 site and ends approximately 23 bp short of the S2 SmaI site. The N-terminal sequence of the first 30 amino acids of the Hpr produced from our cloned DNA was identical to the N-terminal sequence obtained by Weigel et al. (1982) for S. typhimurium Hpr which suggests that there is a high degree of homology between the genes of the two organisms. The N-terminal amino acid of our Hpr (purified as described by Lee et al., 1982) was found to be methionine by the dansylation method described by Perham (1978). A sample (30 nmol) was used for determining the sequence of the first 30 amino acids on a liquid-phase protein sequencer. These were found to be:

Met-Phe-Gln-Gin-Glu-Val-Thr-Ile-Thr-Ala-Pro-Asn-Gly-Leu-His-Thr-Arg-Pro-Ala-Ala-Gln-Phe-Val-Lys-Glu-Ala-Lys-Gly-Phe-Thr

**Position of the ptsH gene by insertion of the γδ transposon**

In order to show that most of the ptsH gene does indeed lie to the right of the K2 site we isolated plasmids with γδ (Tn1000) insertions (Guyer, 1978) into the ptsH gene using the method of Sancar et al. (1978, 1981). The λH57-H1 fragment from plasmid pAB94 was subcloned into the HindIII site of the lacUV5 promoter-containing plasmid, pPM70. The resulting plasmid, pPB63 (6.42 kb), was shown to complement the ptsH lesion in strain PB21 and to express polypeptides of M, 9000 and 49000 in minicells (Fig. 3, track 11). This plasmid was mobilized from an F+ strain into the ptsH strain PB21 to screen for plasmids that had lost the ptsH gene function.

Extracts of 21 exconjugant strains that carried ApR plasmids and that would not grow on N-acetylglucosamine as sole carbon source, were assayed for the presence of Hpr; 13 of them were devoid of Hpr. Three putative ptsH : : γδ plasmids, pPB169, pPB170 and pPB185 and one ptsH+ plasmid, pPB190, from the same selection, were purified, mapped and expressed in minicells. Minicells containing plasmids pPB169, pPB170 and pPB185 did not express a polypeptide of M, 9000 but did express one of M, 49000 (Fig. 3, tracks 12, 13 and 14). Minicells containing plasmid pPB190 expressed polypeptides of M, 9000 and 49000 (Fig. 3, track 15). Restriction endonuclease digestion confirmed that all four plasmids contained the γδ transposon. They were all larger by 5.7 kb than plasmid pPB63 as predicted (Ohtsubo et al., 1974), and yielded the 2.7 kb and 1.3 kb BglII fragments (Iida et al., 1983), the 0.8 kb EcoRI fragment and the 2.5 kb HindIII fragments (Guyer, 1978) that are diagnostic of γδ. The absence of Hpr expression from plasmids pPB169, pPB170 and pPB185 indicated that γδ had inserted into the ptsH structural gene or its promoter region. Restriction mapping showed that all three plasmids were identical and that the γ end was approximately 0.14 kb from the K2 site, thus confirming that the insertion was in the ptsH structural gene (Fig. 2). This result also confirmed that the ptsH gene is initiated to the right of the K2 site. The low level of expression of the polypeptide of M, 49000 from these plasmids is compatible with the result obtained with plasmid pPB50: that the ptsI gene must have its own promoter (albeit less efficient than the ptsH promoter) and that most of the Enzyme I synthesis may result from transcription from the ptsH promoter.

The λH57-H1 fragment in plasmid pPB63 was re-orientated to give plasmid pPB44. In the latter plasmid the ptsH gene was contiguous with and in the opposite direction to the lacUV5 promoter. The assay of Hpr in strain PB21 either containing pPB44 or pPB63 in the presence or absence of isopropyl-β-D-thiogalactoside (a gratuitous inducer of the lactose operon) indicated
that the levels of Hpr did not alter but were fivefold higher than in the \textit{ptsH}+ haploid strain HK640: 27 \textmu mol methyl-\textalpha-glucoside-6-phosphate was produced (mg cell extract)\textsuperscript{-1} min\textsuperscript{-1} from HK640 but 135 \textmu mol from the PtsH\textsuperscript{+} plasmids in the \textit{ptsH} strain PB21.

**Position of the \textit{ptsI} gene**

To locate the right-hand end of the \textit{ptsI} gene, recombinant plasmids were constructed by ligating fragments from plasmid pAB101 incompletely digested with \textit{PvuII} into plasmid pBR328. A total of 12 independently isolated plasmids were shown to be \textit{ptsH}+; four of these, pPB157, pPB158, pPB164 and pPB166, were purified, mapped with restriction endonucleases and expressed in minicells. Plasmids pPB158 and pPB166 contained a 0·136 kb \textit{PvuII} fragment in addition to the 1·174 kb P3-P4 \textit{PvuII} fragment common to all four plasmids. The larger plasmids thus carry the P2-P4 \textit{PvuII} fragment. All four plasmids expressed a polypeptide of \textit{M}, 9000 (Hpr) but none expressed a polypeptide of \textit{M}, 49000 (Fig. 3, tracks 16, 17, 18 and 19). The larger plasmids, pPB158 and pPB166, also expressed a polypeptide of \textit{M}, 10000, presumably from the P2-P3 fragment. This polypeptide must correspond to the N-terminus of the \textit{M}, 49000 peptide expressed from pAB101. A polypeptide of \textit{M}, 49000 requires about 1·47 kb of DNA and immediately to the left of the \textit{\lambda} DNA the \textit{\lambda} attachment site contains stop codons in all three phases. The distance from the \textit{\lambda} attachment site to the P3 site is about 1·4 kb; thus the start of the \textit{ptsI} gene is near the P3 site, possibly just to the left of it.

**DISCUSSION**

We previously mapped the \textit{ptsH} and \textit{ptsI} genes between the \textit{\lambda}H57 and H1 sites of our specialized \textit{\lambda} transducing phages (Britton \textit{et al.}, 1983). We also showed that the \textit{cysK} gene mapped across the H1 site (Boronat \textit{et al.}, 1984). Though the \textit{ptsH} gene was expressed from its own promoter, the \textit{ptsI} gene might be expressed either from its own promoter or from the \textit{ptsH} promoter. The results presented here show that both the \textit{ptsH} and \textit{ptsI} genes have their own promoters and are expressed in the same direction as the \textit{cysK} gene, in a clockwise direction on the \textit{E. coli} chromosome. The level of expression of the \textit{ptsI} gene in the absence of the \textit{ptsH} promoter is however less than when the \textit{ptsH} promoter is present. This indicates that the \textit{ptsI} gene in \textit{E. coli} is transcribed from two promoters whereas in \textit{S. typhimurium} it is apparently only read from the \textit{ptsH} promoter (Cordaro \& Roseman, 1972).

Experiments using the \textit{ptsH} gene contiguous and in the opposite orientation to the lac\textit{UV5} promoter (present on plasmid pPM70), plasmids pPB63 and pPB44 respectively, indicated that there was no increase in the expression of \textit{ptsH} in the presence or absence of isopropyl \textbeta-D-thiogalactoside. This observation indicated that there was no expression from the lac\textit{UV5} promoter indicating the presence of a strong termination site after the lac\textit{UV5} promoter but before the \textit{ptsH} promoter. Mapping data would place it in the 0·58 kb of DNA lying between the end of the \textit{cysK} gene and the start of the \textit{ptsH} gene (Fig. 2). We have shown (Boronat \textit{et al.}, 1984) that repression of the \textit{cysK} gene with cysteine does not affect expression of the \textit{\lambda} \textit{ben} gene though the absence of the \textit{cysB} gene product does. This provides strong evidence for a transcription termination site after the \textit{cysK} gene which will be present on the H1-\textit{\lambda}H57 fragment.

We have mapped the position of the \textit{ptsH} gene as lying across the K2 and Ps1 restriction sites and shown that the promoter for \textit{ptsH} must lie about 150 bp to the left of the Ps1 site. The \textit{ptsI} gene initiation point lies very close to the P3 site, possibly to the left side; DNA sequencing is needed to determine this point.

The position of the genes involved in the phosphotransferase system and those adjacent to them are as shown in Fig. 2. The position of the \textit{\lambda} secondary attachment site within the \textit{ptsI} gene is derived from sizes of the restriction fragments of the plasmids from the positions of restriction sites in \textit{\lambda} deduced from the DNA sequence (Sanger \textit{et al.}, 1982). The DNA and genes to the left of the \textit{\lambda} attachment site are taken from the \textit{\lambda} restriction maps of Britton \textit{et al.} (1983).

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