JOURNAL OF BACTERIOLOGY, May 1987, p. 2287–2290
0021-9193/87/052287-04$02.00/0

Vol. 169, No. 5

Phosphoenolpyruvate:Sugar Phosphotransferase System of *Bacillus subtilis*: Cloning of the Region Containing the *ptsH* and *ptsI* Genes and Evidence for a *crr*-Like Gene

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Received 4 November 1986/Accepted 20 January 1987

The genes *ptsI* and *ptsH*, which encode, respectively, enzyme I and Hpr, cytoplasmic proteins involved in the phosphoenolpyruvate:sugar phosphotransferase system, were cloned from *Bacillus subtilis*. A plasmid containing a 4.1-kilobase DNA fragment was shown to complement *Escherichia coli* mutations affecting the *ptsH* and *ptsI* genes. In minicells this plasmid expressed two proteins with the molecular weights expected for Hpr and enzyme I. Therefore, *ptsH* and *ptsI* are adjacent in *B. subtilis*, as in *E. coli*. In *E. coli* a third gene (*crr*), involved in glucose translocation and also in catabolite repression, is located downstream from the *ptsHI* operon. The 4.1-kilobase fragment from *B. subtilis* was shown to contain a gene that enables an *E. coli crr* mutant to use glucose. This gene, unlike the *E. coli crr* gene, was located to the left of *ptsH*.

The phosphoenolpyruvate:sugar phosphotransferase system (PTS) has been previously described for a number of bacteria, both gram positive and gram negative (for reviews, see references 12 and 16). The PTS allows the uptake and concomitant phosphorylation of a number of sugars. The PTS includes two general proteins. Enzyme I (encoded by *ptsI*) catalyzes the phosphorylation of Hpr (encoded by *ptsH*) by phosphoenolpyruvate. Phosphorylated Hpr is used as a phosphoryl donor by several sugar-specific membrane-bound enzyme IIs. For certain sugars the phosphorylating cascade is more complicated; a cytoplasmic enzyme III is necessary. In *Escherichia coli* the glucose-specific enzyme III (enzyme III^{Glc}) encoded by *crr* plays an important regulatory role in catabolite repression through modulation of adenylate cyclase activity; it also affects the function of several permeases (12).

In gram-positive bacteria the link between catabolite repression and the PTS remains unclear, but several interesting observations have been reported. In particular, it has been shown that a kinase can phosphorylate Hpr on a serine residue (14), whereas enzyme I catalyzes the phosphorylation of a histidine residue by phosphoenolpyruvate (12). The ATP-dependent serine phosphorylation of Hpr strongly reduces its ability to be phosphorylated from phosphoenolpyruvate by enzyme I (14). In *Bacillus subtilis*, glucose, sucrose, fructose, mannose, and mannitol are translocated and phosphorylated by the PTS (4). Enzyme I (4, 8), Hpr (6, 7; K. Stüber, doctoral thesis, University of Cologne, Cologne, Federal Republic of Germany, 1982), and several enzyme IIs (7, 11) have been characterized genetically or biochemically (or both). No Enzyme III has been characterized, although the regulation of glycerol uptake by the PTS might suggest the existence of an enzyme III^{Glc} similar to that described for *E. coli* (15). Furthermore, several enzyme IIIs have been characterized for the gram-positive species *Staphylococcus aureus* and *Lactobacillus casei* (16).

Cloning of the *pts* region. In *B. subtilis*, mutants affected in *ptsI* (4, 8) express levansucrase constitutively (P. Gay, doctoral thesis, University of Paris VI, Paris, France, 1979), whereas in the wild-type strain, this exocellular saccharolytic enzyme (the product of the *sacB* gene) is inducible by sucrose (5). This property was used to select a transposition into the *pts* region, as described elsewhere (S. Aymerich and M. Steinmetz, Mol. Gen. Genet., in press). We replaced the chromosomal *sacB* gene with a kanamycin resistance gene; the resulting strain (GM107) was resistant to kanamycin only in the presence of sucrose. Into GM107 we introduced a thermosensitive replication plasmid bearing Tn*917-lac*, a derivative of the transposon Tn*917* constructed by Perkins and Youngman that confers resistance to erythromycin (10). We selected mutants from GM107 that had become simultaneously resistant to erythromycin at 45°C (nonpermissive temperature for plasmid replication) and constitutively resistant to kanamycin (in the absence of sucrose). One of these mutants, GM107-4, did not grow on glucose or mannitol, suggesting that a transposition had occurred into the *pts* locus. In Tn*917-lac* there is no promoter within the transposon sequences located upstream of *lacZ*; therefore, *lacZ* expression depends on the presence of external promoters (10). In GM107-4 grown on LB medium (7a) supplemented with 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside, *lacZ* expression was observed, suggesting that the promoter of the target gene was upstream of *lacZ* (Fig. 1a). The sequences located upstream of the inserted transposon were cloned by the method of Perkins and Youngman (10), giving a plasmid (pTS1) bearing a 1.7-kilobase fragment from *B. subtilis* (Fig. 1b).

A plasmid carrying the sequences located upstream and downstream of the transposon target was constructed as described by Niaudet et al. (9). The *Eco*RI-*Bam*HI fragment from pTS1 was subcloned in pJH101 (3), and the resulting plasmid, pTS2, was used to transform a *B. subtilis* wild-type strain to chloramphenicol resistance. From a transformant containing a single copy of pTS2 integrated in the chromosome, DNA was extracted, cut with *Cla*I, ligated, and then used to transform *E. coli* to ampicillin resistance, giving a plasmid (pTS10) which bore a 5.3-kilobase fragment from *B.*

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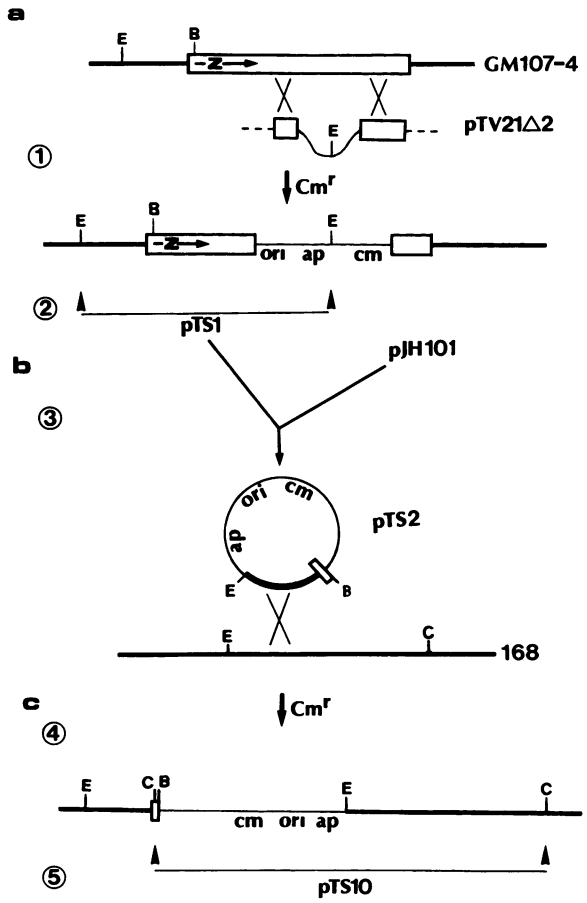

FIG. 1. Cloning of the *B. subtilis pts* locus. (a) Construction of pTS1 by the method of Perkins and Youngman (10). 1, Substitution of Tn*917-lac* material with pTV21Δ2 sequences (Cm^r selection); 2, *Eco*RI cloning of sequences located upstream from the transposon. (b) Construction of pTS2 by subcloning the short *Eco*RI-*Bam*HI fragment of pTS1 in pJH101 (3). (c) Construction of pTS10. 4, Wild-type strain 168 transformed with pTS2; 5, DNA extracted from the transformant was cut with *Cla*I, ligated, and used to transform *E. coli*, giving pTS10. ap and cm, Ampicillin and chloramphenicol resistance genes, respectively; z, *lacZ* gene; ori, pBR322 origin of replication; B, C, and E, *Bam*HI, *Cla*I and *Eco*RI sites, respectively.

subtilis (Fig. 1c). Several pTS10 derivatives were constructed by deleting one end of the insert (pTS20, pTS21, pTS22, pTS23, and pTS24) or the other (pTS101) (Fig. 2a).

Organization of the *pts* region. The pTS plasmids (and pJH101 as a control) were used to transform *E. coli* mutants (2) affected in *ptsH* (strain 1101) and *ptsI* (strain TS19). Transformants were selected on LB-ampicillin (50 mg/liter), and the phenotype of the transformants was characterized by the method of De Reuse et al. (2) on MacConkey agar-ampicillin-mannitol plates. A functional *ptsH* gene was present in pTS21, and both *ptsI* and *ptsH* were present in pTS20 (Fig. 2b). A rough localization of these genes in the insert is shown in Fig. 2c. The promoter from which *lacZ* is expressed in strain GM107-4 can be the promoter of *ptsH* or *ptsI* or of a *ptsHI* operon.

The minicell-producing *E. coli* AR1062 was transformed with several pTS plasmids, the minicells were purified, and the proteins synthesized in the presence of [^{35}S]methionine

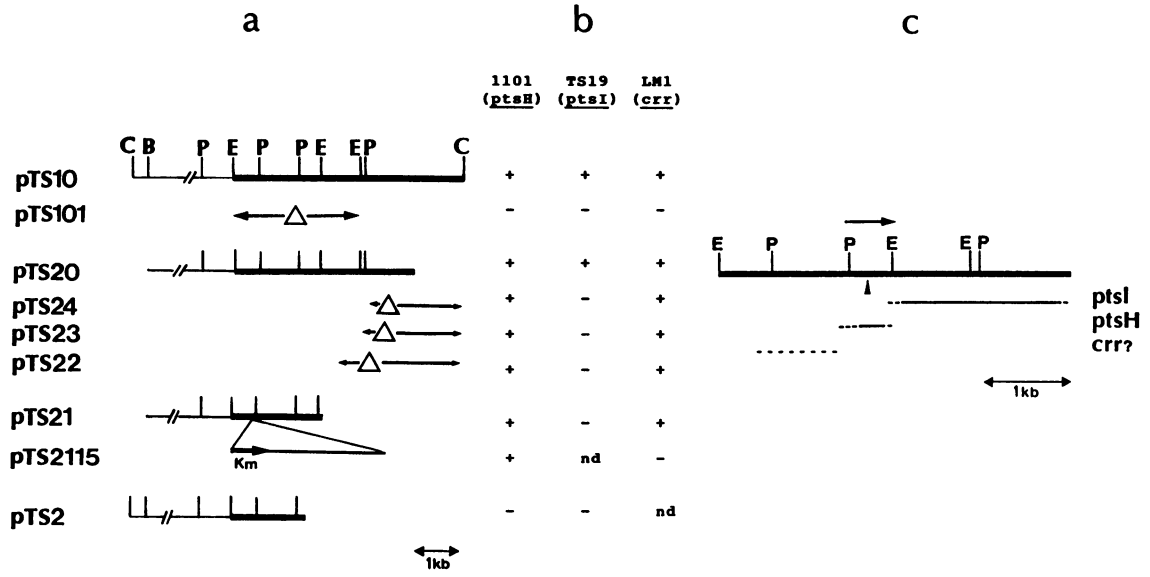

FIG. 2. Organization of the *B. subtilis pts* locus. (a) Restriction map of pTS2, pTS10, and derivatives. Restriction sites shown are *Bam*HI (B), *Cla*I (C), *Eco*RI (E), and *Pst*I (P). To obtain pTS20, pTS21, pTS22, pTS23, and pTS24, pTS10 DNA was cut with *Bam*HI, partially cut with *Sau*3A, ligated, and used to transform *E. coli*. pTS101 was obtained from pTS10 by an *Eco*RI deletion. pTS2115 was obtained by insertion, in the *B. subtilis* insert present in pTS21, of a Mu dIIPR3 prophage containing the kanamycin resistance gene (Km) oriented as indicated (→). (b) Complementation of *E. coli pts* mutants (strains 1101, TS19, and LM1) by various pTS plasmids. The relevant genotypes of the three strains are *ptsH*, *ptsI*(Ts), and *crr*, respectively. The phenotype of the transformants was determined as described in the text. +, Wild-type genotype (red colonies); −, mutant phenotype (white colonies); nd, not determined. (c) Probable positions of the *ptsI*, *ptsH*, and *crr*-like genes in the *B. subtilis* insert present in pTS20. The maximum extent of the three genes (——) and the proposed, but uncertain limits of the coding regions (-----) are indicated. The expected lengths of these coding regions are 1.9 kilobases for *ptsI* (Marquet, doctoral thesis, 1978) and 0.21 kilobases for *ptsH* (6); the coding region length for the *crr*-like gene is unknown. ↑, Target of the Tn*917-lac* transposon present in GM107-4; the promoter from which *lacZ* is expressed in this strain is located upstream of the target. →, orientation of this promoter.

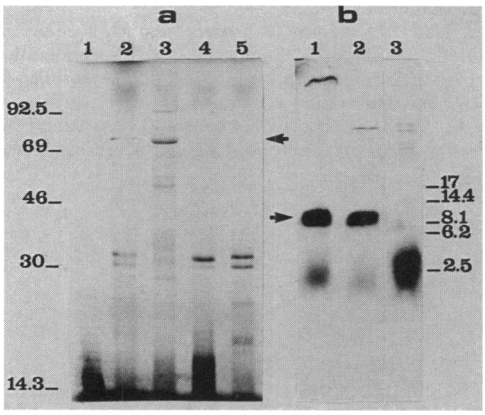

FIG. 3. Autoradiography of electropherograms of labeled proteins from minicells containing different pTS plasmids. After a 30-min incorporation of [^{35}S]methionine, the proteins synthesized were analyzed by electrophoresis (30 mA for 4 h) on 10 and 17.5% polyacrylamide gels in the presence of sodium dodecyl sulfate. The masses of the products were estimated by using the following markers. (a) 10% Acrylamide gel: phosphorylase *b* (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and lysozyme (14.3 kDa). (b) 17.5% Acrylamide gel: five polypeptides (16.9, 14.4, 8.1, 6.2, and 2.5 kDa) were derived from the cleavage of horse heart myoglobin (Sigma Chemical Co.). Lanes in panel a: 1, pTS2; 2, pTS10; 3, pTS20; 4, pTS21; 5, pTS101. Lanes in panel b: 1, pTS20; 2, pTS21; 3, pTS101.

were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1) (Fig. 3). pTS10 and pTS20 expressed a 70-kilodalton (kDa) protein, which is a good candidate for being an enzyme I (M. Marquet, doctoral thesis, University of Paris VII, Paris, France, 1978). pTS10, pTS20, and pTS21 expressed a 9.3-kDa protein, which is the mass expected for Hpr (6) (Fig. 3).

In *E. coli*, *ptsH* and *ptsI* are organized in an operon, which is transcribed from a promoter located upstream of *ptsH* (2). *ptsI* is immediately followed by the *crr* gene, which is transcribed in the same direction. To determine whether a homologous gene was present in the *B. subtilis pts* locus, several pTS plasmids (with pJH101 as a control) were used to transform an *E. coli crr* mutant, strain LM1 (2). The transformants were characterized by the method of De Reuse et al. (2) on MacConkey agar-ampicillin plates supplemented with glucose. The ability of LM1 to use glucose was restored by pTS10, pTS20, and pTS21, but not by pTS101 (Fig. 2b). pTS2115, a pTS21 derivative containing a Mu dIIPR3 prophage (13) inserted as shown in Fig. 2a, no longer allowed the use of glucose by LM1, although it still complemented *ptsH*. Thus a *crr*-like gene seems to be present in pTS21; its position is different from that of the *crr* gene observed in *E. coli*, since, as the above-described results show, it is located to the left of *ptsH* (Fig. 2c). In minicells, no 18- to 20-kDa protein (the mass of *E. coli* enzyme III^{Glc}) was observed. However, no expression of the *E. coli crr* gene was observed by De Reuse et al. (2) in a maxicell system.

These results provide preliminary evidence that the *B. subtilis pts* locus probably contains a gene homologous to the *crr* gene of *E. coli*. However, it must be kept in mind that *E. coli* has two pathways for translocating glucose, including a specific pathway through enzyme II^{Glc} and enzyme III^{Glc} and a nonspecific one through enzyme II^{Man} (*manA*) (12). Both pathways are inactivated in strain LM1 because of mutations *crr*, *manA*, and *manI* which affects the phosphomannose isomerase gene. Hence it cannot be completely excluded that our plasmids code for an analog of enzyme II^{Man} rather than for one of enzyme III^{Glc}.

This work and studies presently in progress concerning the structure and regulation of the three genes of the *pts* locus should help to clarify the links between the PTS and catabolite repression and sporulation in *B. subtilis*. In addition, these results suggest that *E. coli* mutants affected in the *pts* genes can be used to clone the homologous genes from various gram-positive bacteria.

We are very grateful to Hilde De Reuse for the kind gift of *E. coli pts* mutant strains and for helpful discussions. We thank Stéphane Aymerich for collaboration in this work, Chantal Arthaud for technical assistance, and Dick d'Ari for assistance with the English and a critical reading of the manuscript.

This work was supported in part by an Aide à la Recherche from the Mission des Biotechnologies, government of France.

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