Antisense expression at the \textit{ptsH-ptsI} locus of \textit{Escherichia coli}

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1. SUMMARY

A Mud(Ap, \textit{lac}) prophage has been shown to be inserted into the \textit{ptsH} gene of \textit{E. coli}. The insertion is likely to have generated an operon fusion revealing antisense transcription at this locus. This suggests that the ORF previously identified, overlapping with \textit{ptsH} ORF in the opposite orientation, might be functional. A model of regulation for the \textit{ptsH-ptsI-crr} operon transcription is presented which accounts for antisense transcription.

2. INTRODUCTION

The phosphoenolpyruvate dependent carbohydrate transport system (PTS) is a central control system in most bacterial species. It is constituted of a carbohydrate specific membrane-bound permease coupled to a series of cytoplasmic phosphocarriers that permit transfer of a phosphoenolpyruvate derived phosphate group to the incoming carbon source, transported by the permease. In \textit{E. coli} and \textit{S. typhimurium} the cytoplasmic fraction of the system for glucose transport is constituted of three proteins, forming a phosphorylation cascade, HPr, Enzyme I and Enzyme III\textsubscript{Glc} [1]. The corresponding genes constitute an operon \textit{ptsH-ptsI-crr} [2]. In the course of experiments meant to identify the start and end points of the operon, we have isolated a Mud(Ap, \textit{lac}) prophage insertion at the \textit{pts} locus, that did not behave strictly as expected for a \textit{ptsH} or \textit{ptsI} defective mutant. For this reason we proposed that a new gene, tentatively named \textit{ptsJ}, and having a phenotype similar to the phenotype displayed by a \textit{ptsI} defective mutant, was present in the region [3]. In the present work, we show that the Mud insertion we have isolated is inserted at the end of the \textit{ptsH} gene, but most likely oriented in the opposite orientation with respect to \textit{ptsH} orientation. Thus, some expression could be obtained from a mRNA transcribed in the opposite orientation to that of \textit{ptsH-ptsI} transcripts. The possible significance of this observation is discussed.

3. MATERIALS AND METHODS

3.1. Bacterial strains and growth media

\textit{E. coli} K12 strains TP7220 (F\textsuperscript{−} \textit{metE gal-3 his aroB xyl}) and TP722c (F\textsuperscript{−} \textit{metE gal-3 his xyl})
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ptsI::Mud(Ap, lac) previously described by Bitoun et al. [3] were cultivated in L Broth medium [4].

3.2. DNA manipulation

Routine DNA manipulations were performed as described by Maniatis et al. [5]. DNA restriction fragments were isolated from low melting point agarose (Litex). DNA fragments used to make probes derived from plasmid pDIA3206 [2].

DNA was extracted from E. coli strains TP7220 and TP722c according to Silhavy et al. [6]. Southern blot analysis and nick translation of DNA probes were performed as described by Maniatis et al. [5].

4. RESULTS AND DISCUSSION

Bitoun et al. [3] obtained strain TP722c by insertion of the Mud(Ap, lac) prophage into the chromosome of strain TP7220 in the vicinity of the ptsH-ptsI locus. The insertion displayed a ptsI defective phenotype. However, several features of the mutation, namely the impossibility to restore a Lac+ phenotype by a simple mutational event or after deletion of the Mud insert, raised the question of the nature of the mutation.

Fig. 1 displays a restriction map of the ptsH-ptsI locus derived from De Reuse et al. [2] and Lévy and Danchin [7]. The location of the Mud insert was determined by Southern blot analysis of chromosomal DNA isolated from strains TP7220 and TP722c (data not shown). In both parental and mutant chromosomal DNA, a 1.7 kb XhoI/EcoRI (6/7) fragment hybridized with a probe consisting of a ClaI/EcoRI (4/7) fragment containing ptsH gene and most part of ptsI gene (P1 in Fig. 1). However, P1 hybridized with a 0.8 kb HindIII/XhoI (3/6) fragment in TP7220 DNA and the hybridization pattern was changed with digests of the mutant chromosomal DNA. It can be concluded from these observations that the Mud(Ap, lac) prophage is inserted upstream from the XhoI (6) restriction site.

In both parental and mutant chromosomal DNA, a 2.1 kb BamHI/PstI (2/5) DNA fragment hybridized with a probe consisting of an HindIII/XhoI (3/6) fragment containing all ptsH gene (P2 in Fig. 1). However, P2 hybridized with a 3.3 kb PstI (5/8) fragment in TP7220 DNA and this hybridization signal was not conserved with digests of the mutant chromosomal DNA. This suggests that Mud(Ap, lac) is inserted downstream from the PstI (5) restriction site. Thus, the insertion is located in the ptsH gene, between PstI (5) and XhoI (6) restriction sites, which are separated by 97 bp.

The Mud(Ap, lac) construction performed by Casadaban and Cohen [8] is not well enough defined to permit derivation of a precise restriction map of the 33 kb Mud insert. However, the size of the EcoRI fragments hybridizing with the P1 probe (Fig. 1) suggested that the Mud(Ap, lac) is inserted in an orientation opposite to that of the ptsH ORF. Consequently, the lacZ gene could be transcribed in a direction opposite to that of ptsH (Fig. 2). Other features, such as measurement of the β-galactosidase activity of strain TP722c support this contention. Firstly, the β-galactosidase rate of synthesis in strain TP722c was 150 U/mg [3] whereas expression from the ptsH-ptsI-crr operon promoter has been shown by De Reuse and Danchin [9], in strains harbouring single copies of fusions between the ptsH and the lacZ gene, to result in the synthesis of a much larger value (3000 U/mg of β-galactosidase activity). Secondly, it has been noticed that an ORF was present, superimposed on the ptsH ORF, in the opposite
Fig. 2. Restriction map of the ptsH-ptsI locus of strain TP722c chromosomal DNA. Thin lines represent chromosomal DNA, the striped box represents the Mud(Ap, lac) prophage insertion. The 33 kb Mud insert has been internally truncated on the map, as indicated. Genes are represented by boxes beneath the corresponding DNA segments. Uncertainty about the exact limits of the lig gene is indicated by the dashed part of the box.

orientation [10]. It seems therefore most likely that Mud expression corresponds to this ORF.

We propose therefore that the lacZ gene present in strain TP722c chromosome is expressed from a transcript initiated downstream from the ptsH gene and going upwards. The corresponding antisense expression would accordingly be 20 times lower than sense expression. In addition, Northern blotting analysis of the transcripts present in the region did not reveal high level mRNA expression from the direction opposite to normal ptsH-ptsI transcription [9]. Analysis of the transcripts derived from the ptsH-ptsI-crr region has revealed an unusual pattern [9]. While a transcript covers the whole operon, two major short transcripts are also present, namely a crr specific transcript, arising from a secondary promoter located inside the ptsI gene, and a ptsH specific transcript, terminating inside the ptsI ORF, in a region located upstream from the ‘anti ptsH’ ORF. It can therefore be speculated that antisense transcription (possibly coupled to translation) mediates premature transcription termination of the ptsH-ptsI-crr operon, resulting in a specific ptsH shortened transcript. It should be noted in this respect that no consensus transcription termination signal could be identified on the DNA sequence corresponding to the end of the ptsH transcript [9]. A fine tuning of this latter transcript level, as compared to the whole operon transcription efficiency, might be necessary under certain growth conditions.

Phylogenetic data, through analysis of the pts region present in other bacteria might give hints on the true significance of the observation presented above. It is known that many ORFs are prominent when DNA sequences are analysed, many of which are probably not significant. However, it is also known that important genes may be expressed at levels much lower than the level we have found in the present work (e.g. adenylate cyclase corresponds to 10 U/mg β-galactosidase, and lactose repressor corresponds to 1 U/mg β-galactosidase). This should therefore be borne in mind when expression of the ptsH-ptsI genes is analysed.

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REFERENCES


