GENETIC CONTROL OF INDUCER EXCLUSION BY *ESCHERICHIA COLI*

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1. Introduction

There are two main ways in which enteric bacteria take up carbohydrates from their external media. In 'group translocation', the uptake of a number of sugars is coupled to the transfer to them of phosphate from phosphoenolpyruvate (PEP) and the sugars thus appear inside the cells as the phosphate esters. The multi-component phosphotransferase system (PTS) that brings this about was discovered by S. Roseman et al. [1]; its properties have been extensively reviewed [2,3]. 'Active transport', on the other hand, is a term applied to the energy-linked uptake of other sugars, in which no obligatory phosphate transfer occurs and in which the sugar present externally appears, chemically unchanged, as such inside the cells. This process is intimately associated with the flow of electrons through membrane carriers and may be energized by the collapse of proton and/or electrical gradients [4,5]. Sugars that are taken up by *Escherichia coli* or *Salmonella typhimurium* via phosphotransferase-mediated 'group translocation' include glucose and fructose; they are designated 'PT-sugars' for convenience. Carbohydrates that are taken up by these bacteria via 'active transport' include lactose, maltose, pentoses, gluconate and hexose phosphates; they can be similarly grouped under the term 'non-PT-sugars' [3,6]. It would be expected from this distinction that mutants impaired in a sugar-specific component of the PEP-phosphotransferase system would not grow on the appropriate PT-sugar or, if the lesion were in a common component of that system, on any PT-sugar, but that such mutants would grow on non-PT sugars. However, many mutants of Enzyme I of the PTS (*pts I*) have been described which fail to grow both on PT-sugars and on several (though not all) non-PT carbohydrates [7–11]. In some cases, this reluctance to utilize some non-PT sugars can be overcome by the addition of adenosine 3′,5′-cyclic phosphate (cAMP) [12,13]; in other cases, cAMP appears to be ineffective but addition of the appropriate inducer, or mutation to constitutivity of the appropriate transport system, permits growth on that (but no other) substrate to occur [7–11]. These and other [14,15] findings suggest that *pts I*-mutants do not grow on certain non-PT-sugars because they are unable to accumulate enough of those sugars to induce the proteins required for the active transport and subsequent metabolism of those sugars. Since this property is also associated with an undue sensitivity of such *pts I*-mutants to repression of the synthesis of certain catabolic enzymes, this role of the phosphotransferase system has been ascribed to the activity of a gene designated *crr*$^+$ (for catabolite repression resistant): *crr*-mutants of *S. typhimurium*, that are less subject to catabolite repression, have been described [14,15] and the *crr* gene has been located on the Salmonella genome contiguous to the genes specificying the Enzyme I and HPr components of the PTS [16]. *E. coli* mutants with lower susceptibility to glucose catabolite repression have also been described, but the affected gene appears to be located at about 25 min on the genome [17,18] which is not close to that specifying the Enzyme I component of the PTS (46 min).

Bourd et al. [19] described a mutant of the Cavalli strain of *E. coli* K12 that synthesizes the Enzyme I component of the PTS normally at 27°C but ceases to do so when the temperature is raised to 37°C (*pts I*^{ts}). Through the kindness of Dr. G. I. Bourd, this mutant (*ts 19*) was made available to us and was used for the selection of further mutants. It

is the purpose of this paper to describe the properties of one such further mutant, *ts19–1* Δ, which provides evidence that, in *E. coli* as in *S. typhimurium*, non-PT sugars penetrate readily into *ptsI*-mutants only if a *crr*$^+$-gene is inactivated, and that this gene lies sufficiently close to *ptsI* on the *E. coli* genome to be covered by one deletion.

2. Materials and methods

Cultures of strain *ts 19* (Hfr-C, *met ptsI*^{ts}) were grown, and their growth was measured, as previously described [20].

The mutant *ts19–1*Δ was selected as a colony able to grow at 40°C on minimal agar plates containing 5 mM maltose as sole carbon source. The absence of PT-activity in isolated colonies was confirmed by the film technique previously described [21] and by their inability to grow on agar plates containing the PT-sugars fructose, glucose, and mannitol as carbon sources.

3. Results

Cultures of strain *ts 19* grow readily on a variety of PT-sugars at 27–30°C but rapidly cease to grow when the temperature is raised to 38°C. This property confirms the presence of a thermosensitive Enzyme I of the PTS. [17]. However, cultures of this strain that grow readily at 27–30°C on a variety of non-PT-sugars, such as maltose, lactose or galactose, initially increase in their rates of growth when the temperature is raised to 38°C but, after less than one doubling, progressively slow down. Moreover, cultures initially grown on gluconate as carbon source at 38°C (and hence devoid of PT-activity) adapt with difficulty or not at all to growth on such non-PT-sugars at that temperature, (fig. 1) although there is no impediment to continued growth on gluconate or to adaptation to growth on glucose 6-phosphate. The addition of cAMP (to 5 mM) did not overcome the inability to grow on maltose, lactose or galactose. This suggests that, in the absence of a functioning Enzyme I component of the PTS, the *ts 19*-strain of *E. coli* can neither maintain the induction of some carbohydrate transport systems already present in the cells

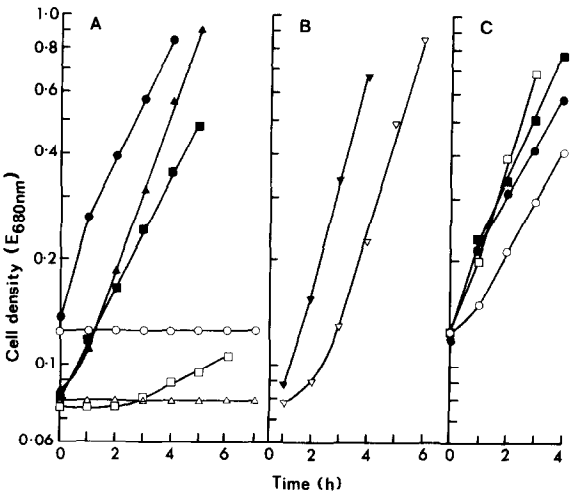

Fig. 1. Growth at 38°C of strain *ts 19* (open symbols) and its mutant *ts-1*Δ (closed symbols) on *A*: □■ galactose; △▲ glycerol; ○● maltose, *B*: ▽▼ arabinose; *C*: ○● lactose; □■ lactose + isopropylthiogalactoside. The inocula were grown on gluconate as carbon source.

nor can readily form such uptake systems.

Since strain *ts 19*, which grows readily on maltose at 30°C, does not adapt at all to growth on this sugar at 38°C, mutants derived from it that now grow on maltose must fall into one of three classes: they must have (i) mutated in the already defective Enzyme I component to restore its activity at 38°C, or (ii) mutated in a regulatory gene of the system for maltose utilization, to permit the constitutive formation of the components of that system, or (iii) mutated to *Crr*$^-$, to permit the penetration of maltose into the cell in amounts sufficient to induce the systems for maltose utilization. Mutants of type (i) and (ii) have been reported previously [11]. They can be easily recognized, since mutants of type (i) should grow on all PT-sugars at 38°C, and mutants of type (ii) should still fail to grow at 38°C on non-PT sugars other than maltose. However, the mutant *ts 19–1*Δ chosen for study did not fall into either of these categories. It no longer grew on any PT-sugar tested, even at 30°C, and failed to take up methyl-α-[^{14}C]glucoside and [^{14}C]glucose [19] at any temperature; clearly, it had lost all PT-activity. Another striking difference from its parent *ts19* was that, when grown on gluconate and transferred to

the non-PT sugars shown in fig. 1, the mutant adapted (with barely a lag) to growth not only on maltose but also on arabinose, glycerol, galactose and lactose. In further contrast to strain *ts 19*, the growth, and the adaptation to growth, of the mutant *ts 19–1Δ* on lactose were nearly as rapid in the absence as in the presence of the inducer 1 mM-isopropylthiogalactoside.

The proximity of the *crr*-gene to *ptsI* was shown further by genetic analysis of recombinants from the cross [*ts 19–1Δ*(HfrC, *met ptsI crr*) x K2.1t (*his argH thr leu str*)] which were selected for His^+ Str^R. About 60% of such recombinants were unable to utilize any PT-sugar tested (*ptsI*); two such that were examined further exhibited the ability rapidly to adapt to growth on a variety of non-PT sugars characteristic of the parent *ts19–1Δ* (fig. 1). Since the ability of such recombinants to grow on PT-sugars ($PtsI^+$) was restored with high frequency by crossing them with the Hfr-strain Kl 16 (*0-thy-ptsI-his*) for 20 min the gene(s) specifying the dysfunction of the PT-system present in the mutant *ts 19–1Δ* must lie between minutes 40 and 55 on the genome, which agrees with the location of the *ptsI* marker (minute 46). The mutation of *ts 19*, carrying a thermosensitive-*ptsI* marker, to *ts 19–1Δ*, in which PtsI-activity is absent at all temperatures and in which the Crr^+-character has also been lost, is thus a deletion that covers both these functions. The nature of the mechanism whereby the *crr*-gene exerts its activity remains to be elucidated.

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