Phosphotransferase-mediated Regulation of Carbohydrate Utilization in *Escherichia coli* K12: the Nature of the *iex* (*crr*) and *gsr* (*tgs*) Mutations

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Mutants of *Escherichia coli* K12 defective in the gene *iex* (*crr*) no longer utilize glucose or *N*-acetylglucosamine in preference to lactose, but competition between either of these sugars and another that also enters by a phosphotransferase (PT) mechanism is not affected. In this they differ from *gsr* (*tgs*) mutants. In *gsr* mutants, glucose does not exclude any other sugar, though *N*-acetylglucosamine still does so. In *gsr* mutants that are also *ptsM* the phosphoenolpyruvate-dependent phosphorylation of glucose or methyl α -glucoside is reduced by 90%; *N*-acetylglucosamine phosphorylation is not affected. The *iex* mutation does not affect the phosphorylation of either of these compounds. The wild-type alleles *iex*⁺ and *gsr*⁺ are dominant in λ heterozygotes. Glucose inhibits the lactose permease of wild-type cells, but only when the permease is present in low amounts. The inhibition is also relieved (1) by induction of another transport system that is subject to regulation by the *iex* system or (2) by an *iex* mutation. We suggest that the *iex* gene specifies a protein that, in cells transporting certain sugars by a PT mechanism, acts to inhibit active transport systems. The protein is present in limiting concentration in the cell, sufficient only to inhibit the basal, uninduced, level of the active transport systems. In consequence the inducer (or its precursor) may be excluded from the cell and induction thus prevented.

INTRODUCTION

Certain hexoses, hexitols and amino-hexoses ('PT sugars') are taken up by *Escherichia coli* via a phosphotransferase (PT) mechanism (Kundig *et al.*, 1964). In this, the phosphate group of phosphoenolpyruvate is transferred by an enzyme (Enzyme I, specified by the gene *ptsI*) to (usually) a small histidine-containing protein (HPr, specified by *ptsH*); however, a different and larger carrier, designated Fpr, is also involved in the uptake of fructose (Waygood, 1980). The phosphorylated carriers P-HPr or P-Fpr subsequently transfer that phosphate, either directly or via further carriers loosely associated with the inner membrane and generically termed 'factors III' (Roseman, 1972) to enzymes II that are firmly embedded in this membrane. These enzymes II effect the transfer of the phosphate group to specific sugars with concomitant translocation of the sugars into the cell.

Although this overall sequence of events adequately describes the phosphoenolpyruvate-dependent uptake of relevant carbohydrates, it is not sufficient to explain two observations that imply other functions of the phosphotransferase system.

(1) Growth of *E. coli* on sugars that are taken up via 'active transport' processes ('non-PT sugars'), occurs only after prolonged lag if the organisms lack either functional enzyme I (Gershanovitch *et al.*, 1967; Wang *et al.*, 1970) or HPr (Gershanovitch *et al.*, 1977). When lactose was the substrate, this lag could be shortened by the provision of cAMP or by addition of the non-catabolizable inducer isopropyl β -D-thiogalactoside (IPTG) (Pastan & Perlman, 1969). Similarly, the addition of cAMP to cultures of mutants lacking enzyme I activity enables them to grow on glycerol (Berman *et al.*, 1970) and on many other non-PT substrates (Watts, 1979).

(2) Glucose, which is taken up via the phosphotransferase system predominantly through an enzyme II (specified by *ptsG*) that is associated with a glucose-specific 'factor III^{Glc}' (reviewed by Postma & Roseman, 1976), also exerts profound effects on the utilization of other sugars. It represses the synthesis of inducible enzymes required for the catabolism of those other sugars, and it inhibits the continued utilization of other sugars even though the transport systems and enzymes required for the utilization of those other sugars were fully active prior to the addition of glucose. The former effect, catabolite repression (Magasanik, 1961) affects the differential synthesis of cellular proteins; the latter effect, catabolite inhibition (McGinnis & Paigen, 1969, 1973), affects the activity of specific transport systems (reviewed by Kornberg *et al.*, 1980).

Two different mutations have been found that alter the expression of these regulatory functions of the phosphotransferase system. Mutants of *E. coli* altered in a function that was originally termed *crr* but, for reasons discussed below, is now termed *iex*, were selected as organisms able readily to utilize non-PT sugars despite absence of enzyme I activity (Jones-Mortimer & Kornberg, 1974). Mutants of *E. coli* altered in a function originally termed *tgs* but now termed *gsr* were selected as organisms that continued to utilize PT sugars despite the presence of the (usually toxic) glucose analogue 5-thioglucose, but that were not devoid of PtsG function (Kornberg & Watts, 1978). Both mutations map close to *ptsH* and *ptsI*, though preliminary analysis indicated that they were located on opposite sides of these genes (Kornberg & Watts, 1979; Kornberg *et al.*, 1980).

In retrospect, it is clear that the original designation of the former type of *E. coli* mutant as '*crr*' was unfortunate, and has given rise to much confusion in the literature. Jones-Mortimer & Kornberg (1974) adopted this notation because their *E. coli* mutants were apparently closely similar to, or identical with, *crr* mutants of *Salmonella typhimurium* that had been previously described by Saier & Roseman (1972). We show in this paper that the '*crr*' mutation in *E. coli* is quite different in its effects from that designated *crr* in *S. typhimurium*, and that it is the lesion originally termed *tgs* in *E. coli* that is closely similar to '*crr*' in *S. typhimurium*. We also show that the '*crr*' mutation in *E. coli* is concerned with inducer exclusion whereas '*tgs*' is specific to substrates of the PtsG system; the accompanying paper (Britton *et al.*, 1983) demonstrates that these two genes are located on opposite sites of *ptsI* on the *E. coli* genome, which emphasizes that they must be distinct genes. For these reasons, we now adopt the notation '*iex*' for the gene specifying inducer exclusion, and '*gsr*' for that specifying glucose-specific repression.

METHODS

The minimal medium was that of Ashworth & Kornberg (1966). Bacteriophage techniques are described in the accompanying paper (Britton *et al.* 1983). The *Escherichia coli* strains used are listed in Table 1. The relative utilization of carbon sources from a mixture was measured as described by Amaral & Kornberg (1975); adenylate cyclase was assayed by the method of Salomon *et al.* (1974) and the phosphotransferase activity of toluene-treated cells was measured by the procedure of Kornberg & Reeves (1972a). β -Galactoside permease was measured by determining the rate of *o*-nitrophenol formation when intact cells were incubated in the presence of *o*-nitrophenyl- β -D-galactoside (ONPG) in 50 mM-sodium phosphate buffer, pH 7.2, containing 50 μ g chloramphenicol ml⁻¹. The process of hydrolysis *in vivo* is proportional to the transport rate (Rickenberg *et al.*, 1956).

RESULTS

The phenotypes of iex and gsr mutations

Mutants affected in the genes *iex* and *gsr* differ from wild-type organisms and from each other in several important respects.

Utilization of carbon sources for growth. Since glucose is known to enter *E. coli* both via a membrane-associated uptake system specified by *ptsG* and to a lesser extent also via one specified by *ptsM* (Kornberg & Jones-Mortimer, 1975; Curtis & Epstein, 1975), and since fructose can also be taken up by the latter system (Kornberg & Jones-Mortimer, 1975) it is advisable to study the effects of the *iex* and *gsr* mutations in organisms that are *ptsM*. The rates of growth of three such strains on a variety of carbon sources are shown in Table 2. It is evident that in the presence of functioning enzyme I (i.e. at 30 °C in these *ptsI*⁺ organisms), the *iex*

Table 1. *Escherichia coli* strains usedThe λ phage are described by Britton *et al.* (1983).

Strain	Genotype						
FP6	<i>iex</i>	<i>umgC</i>	<i>ptsI</i> ^{ts}	<i>metB</i>			
FP19	<i>iex</i>	<i>umgC</i>	<i>ptsI</i> ^{ts}	<i>metB</i>	<i>ptsH</i> (λ <i>ptsH</i> ⁺ <i>iex</i> ⁺)		
FP20	<i>iex</i>	<i>umgC</i>	<i>ptsI</i> ^{ts}	<i>metB</i>	<i>ilvA</i> ::Tn5		
FP31		<i>umgC</i>	<i>ptsI</i> ^{ts}	<i>metB</i>	<i>ilvA</i> ::Tn5		
FP34		<i>umgC</i>	<i>ptsI</i> ^{ts}	<i>metB</i>	<i>ilvA</i> ::Tn5	<i>lacI</i>	
HK724		<i>umgC</i>	<i>ptsM</i>	<i>ptsI</i> ^{ts}	<i>argH</i> <i>thr</i>	<i>leu</i>	<i>cysA</i>
HK656	<i>gsr</i>	<i>umgC</i>	<i>ptsM</i>	<i>ptsI</i> ^{ts}	<i>argH</i> <i>thr</i>	<i>leu</i>	
HK727	<i>iex</i>	<i>umgC</i>	<i>ptsM</i>	<i>ptsI</i> ^{ts}	<i>argH</i> <i>thr</i>	<i>leu</i>	
JM1781	<i>gsr</i>	<i>umgC</i>	<i>thr</i>	<i>leu</i>	<i>cysA</i>		
JM1784		<i>umgC</i>	<i>thr</i>	<i>leu</i>			
JM1785	<i>gsr</i>	<i>umgC</i>	<i>thr</i>	<i>leu</i>	<i>cysA</i> (λ <i>cysA</i> ⁺ <i>gsr</i> ⁺)

Fig. 1. Effects of 5-thioglucose on the adaptation of gluconate-grown strain HK727 (*iex*) to growth on maltose (Δ , $\blacktriangle$), lactose (∇ , $\blacktriangledown$) and fructose ($\circ$, $\bullet$). The growth of cultures in the absence of 5-thioglucose is indicated by open symbols; that in its presence, by solid symbols.

mutation does not alter the wild-type character of the relevant strain and that it readily utilizes both non-PT and PT sugars. In contrast, the *gsr* mutation, introduced into the *ptsM* genetic background, greatly impairs growth on glucose; growth on other carbon sources is, however, not affected. Moreover, since *gsr ptsM* mutants grow readily on glucose-6-phosphate, which is taken up without prior hydrolysis (Fraenkel *et al.*, 1964; Winkler, 1966; Kornberg & Smith, 1969), it must be the uptake of glucose and not its subsequent catabolism that is specifically affected by the *gsr* mutation. As expected from the manner of their selection, *gsr* mutants are not inhibited by 5-thioglucose during growth on fructose; since they are also not inhibited by this (and other) analogues of glucose on any other carbon source tested, it is likely that the lesion prevents entry of the analogues into the cells.

Although both the wild-type and *iex* strains are strongly inhibited by the presence of 5-thioglucose (and other glucose analogues) in their ability to adapt to growth on PT sugars, *iex* mutants are less severely impaired by the analogue in their response to non-PT sugars than are wild-type cells (Fig. 1). This phenomenon is considered further in the last section of Results.

Competition between different sugars for uptake. It has long been established that glucose, added to wild-type cultures of *E. coli* that are utilizing either PT or non-PT sugars, inhibits the continued activity of the uptake systems for those other sugars: glucose is therefore subsequently taken up in preference (McGinnis & Paigen, 1969, 1973; Kornberg, 1972, 1973). This

Table 2. *Rates of growth of iex and gsr mutants on various carbon sources*

Inocula were grown at 30 °C on gluconate, harvested, and adapted to growth at 30 °C on hexoses at 10 mM and on disaccharides at 5 mM. Where indicated, 5-thiogluconate (TG) was added together with the carbon source, to a final concentration of 1 mM. All strains were *ptsM ptsI^{ts} umgC*.

Carbon source	Strain ...	Generation time (to nearest 5 min)		
		HK724 (+)	HK656 (<i>gsr</i>)	HK727 (<i>iex</i>)
Fructose		90	95	95
Fructose + TG		> 600	95	> 600
Glucose		90	330	90
Glucose 6-phosphate		85	90	90
Sorbitol		108	100	100
Sorbitol + TG		> 600	105	> 600
Lactose		90	95	90
Lactose + TG		> 600	100	*
Maltose		105	110	95
Maltose + TG		> 600	120	*

* The initial inhibition of growth was gradually overcome: this is shown in Fig. 1 and discussed further in the last section of Results.

Table 3. *Utilization of glucose in the presence of other sugars by iex and gsr mutants*

Cultures were grown overnight at 30 °C on the sugars listed at 10 mM. They were harvested and resuspended in duplicate flasks of growth medium containing those sugars at either 5 mM (monosaccharides) or 2.5 mM (disaccharides). When growth recommenced, unlabelled glucose to 5 mM and a trace amount of the relevant sugar, labelled with ¹⁴C, was added to one flask, and 5 mM-[¹⁴C]glucose to its duplicate. Samples were taken at intervals throughout the subsequent growth and analysed as described by Amaral & Kornberg (1975). All strains were *ptsM ptsI^{ts} umgC*.

Carbon source	Strain ...	Fraction of carbon (%) derived from glucose		
		HK724 (+)	HK656 (<i>gsr</i>)	HK727 (<i>iex</i>)
Fructose		92	30	71
Sorbitol		96	33	73
N-Acetylglucosamine		88	33	70
Lactose		94	32	38
Maltose		96	30	36

phenomenon is illustrated in Table 3. However, *gsr* strains do not show this effect: in *ptsM⁺* strains, the glucose and the carbon source that was used for growth prior to the addition of glucose are used simultaneously and to an approximately equal extent (Kornberg & Watts, 1979); in *ptsM* strains, the other sugar predominates. This applies irrespective of whether that other sugar is a PT or non-PT sugar and supports the view that the *gsr* mutation is associated with an impairment of glucose uptake.

That the *gsr* lesion affects the uptake specifically of glucose is shown in two ways. (a) Like glucose, N-acetylglucosamine strongly inhibits the continued utilization of other PT sugars, such as fructose: when this substance was added to a culture of a *gsr⁺ ptsM* organism growing on fructose, 75% of the carbon subsequently incorporated into the cells was derived from N-acetylglucosamine. This proportion was not decreased when the strain used was *gsr ptsM* although (Table 3) such a strain no longer uses glucose preferentially. (b) A second line of evidence arises from direct assay of the phosphoenolpyruvate-dependent phosphorylation of different sugars by suspensions of cells rendered permeable with toluene (Kornberg & Reeves, 1972*a, b*). The various strains used are all derived from strain PA309 (Bachmann, 1972) which

Table 4. *Phosphotransferase activities of gsr and iex mutants rendered permeable with toluene*

The overall ability of toluene-treated cultures, grown initially on 10 mM-*N*-acetylglucosamine or 10 mM-fructose, to effect the phosphoenolpyruvate-dependent phosphorylation of appropriate substrates (at 0.1 mM) was measured by the procedure of Kornberg & Reeves (1972a). All strains were *ptsM ptsI^{ts} umgC*.

Strain	Relevant genotype	Carbon source for growth	Rate of phosphorylation [nmol (mg dry mass) ⁻¹ min ⁻¹]			
			Fructose	<i>N</i> -Acetyl-glucosamine	Glucose	Methyl α -glucoside
HK724	+	<i>N</i> -Acetyl-glucosamine	10	90	95	90
		Fructose	90	<5	110	90
HK656	<i>gsr</i>	<i>N</i> -Acetyl-glucosamine	15	80	9	9
		Fructose	68	<5	10	10
HK727	<i>iex</i>	<i>N</i> -Acetyl-glucosamine	15	90	95	88
		Fructose	77	<5	121	118

Fig. 2. Relative utilization of lactose (●) and glucose (○), and fructose (▲) and glucose (○), during growth of *iex*⁺ and *iex* mutants of *E. coli*. (a, b) Experiments with strain HK724 (*iex*⁺), (c, d) experiments with strain HK727 (*iex*). All inocula were grown on fructose overnight.

expresses the activity of the principal enzyme II for the uptake of glucose and of methyl α -glucoside constitutively (*umgC*; Kornberg & Reeves, 1972a); this makes it possible to compare the activity of that component of the phosphotransferase system even though the cultures were grown on fructose or *N*-acetylglucosamine as sole carbon source. It is apparent from Table 4 that *gsr* mutants phosphorylate 0.1 mM-glucose or 0.1 mM-methyl α -glucoside much less readily than do either wild-type or *iex* strains; however, no such differences are observed in the phosphorylation of 0.1 mM-fructose or 0.1 mM-*N*-acetylglucosamine.

In contrast to these findings with *gsr* strains, Table 3 shows that, in the *iex* strain HK727,

Table 5. *Dominance of the wild-type allele gsr⁺ in the utilization of glucose and of N-acetylglucosamine preferentially to fructose, and the activity of adenylate cyclase*

Strain	Relevant genotype	Fraction of carbon (%) derived from fructose by cells growing on fructose plus:		Adenylate cyclase activity [pmol min ⁻¹ (mg protein) ⁻¹] in gluconate-grown cells in the presence of:	
		Glucose	N-Acetylglucosamine	No addition	0.1 mM-Methyl α -glucoside
JM1784	<i>gsr⁺</i>	17	25	23	2.3
JM1781	<i>gsr</i>	63	20	1.5	0.6
JM1785	<i>gsr</i> / λ <i>gsr⁺</i>	26	23	11	0.2

glucose was still used preferentially to the PT sugars fructose, sorbitol and *N*-acetylglucosamine: glucose had lost its ability to compete successfully with other sugars only when these other sugars were taken up by 'active transport', as is the case with lactose and maltose. This difference is graphically demonstrated also by Fig. 2, in which the competition between glucose and lactose, and glucose and fructose, was measured when fructose-grown cultures of strains HK 724 (*iex⁺*) and HK 727 (*iex*) were placed in media containing 5 mM-glucose and either 2.5 mM-lactose or 5 mM-fructose. The well-known exclusion of lactose by glucose (Monod, 1942) is exhibited by the *iex⁺* strain but not by its *iex* mutant; however, in both cultures, glucose still largely excludes fructose. Similarly, the *iex* mutant differs from the *gsr* mutant HK 656 in that it is not impaired in phosphotransferase activity with either glucose or methyl α -glucoside as substrate (Table 4).

Adenylate cyclase activity. The adenylate cyclase activity of all *gsr* mutants of *E. coli* that have been investigated is considerably lower than that of either wild-type strains or *iex* mutants (Kornberg & Watts, 1978, 1979; Kornberg *et al.*, 1980; Kornberg, 1981); there were no significant differences between wild-type strains and *iex* mutants in this activity. This strongly suggests that the *gsr* lesion affects a component of the phosphotransferase system that not only specifically impairs the uptake and phosphorylation of glucose and of methyl α -glucoside but that also plays a role in the activation of adenylate cyclase.

Dominance of wild-type alleles gsr⁺ and iex⁺

To obtain a better understanding of the lesions in *gsr* and *iex* mutants, heterozygous diploids were constructed by infecting the *cysA gsr* mutant JM1781 with a hybrid lambda phage (λ JM28) that carried *E. coli* DNA containing the *gsr⁺* allele; two Cys⁺ progeny strains JM1784 (*cys⁺ gsr⁺*) and JM1785 (*cys gsr*/ λ *cys⁺ gsr⁺*) were obtained. Measurement of the proportion of carbon derived from fructose when the three strains were grown on mixtures of fructose and either glucose or *N*-acetylglucosamine showed that the ability of glucose to exclude fructose was largely restored in the partial diploid (Table 5); the preference for *N*-acetylglucosamine over fructose was not affected by the nature of the *gsr* allele present. Furthermore, the activity of adenylate cyclase, which in the *gsr* mutant was only about 7% of that found in the *gsr⁺* strain, was greatly raised in the *gsr⁺/gsr* diploid. This not only shows that the wild-type allele of *gsr* is dominant but supports the view that the *gsr⁺* allele specifies one component of the phosphotransferase system involved both in the uptake of (specifically) glucose and in the activation of adenylate cyclase.

In an analogous manner, the *iex*/ λ *iex⁺* diploid strain FP19 was constructed by lysogenizing a *ptsH* mutant with the lambda phage λ JM29 that carries *E. coli* DNA including the *ptsH⁺* and *iex⁺* alleles. This diploid strain resembled the wild-type in that glucose and *N*-acetylglucosamine were used in marked preference to lactose (Table 6). However, since lactose competed effectively with either of these PT sugars in *iex* strains, the effect of the *iex⁺* allele was dominant. These results confirm those shown in Table 3, that the effect was not glucose-specific. It is also evident that the nature of the *iex* allele present exerted no significant effect on the activity of adenylate cyclase.

Table 6. Dominance of the wild-type allele *iex*⁺ in the utilization of glucose and of *N*-acetylglucosamine preferentially to lactose, and the activity of adenylate cyclase

For measurements of relative utilization of carbohydrates, cells were grown overnight on either glucose or *N*-acetylglucosamine. Cultures were resuspended in media containing these substrates (5 mM); when growth recommenced, 2.5 mM-[¹⁴C]lactose was added. For adenylate cyclase assay, cultures were grown in sorbitol: this stabilized the *ptsH/ptsH*⁺ diploid FP19.

Strain	Relevant genotype	Fraction of carbon (%) derived from lactose by cells growing on [¹⁴ C]lactose plus:		Adenylate cyclase activity [pmol min ⁻¹ (mg protein) ⁻¹] in sorbitol-grown cells in the presence of:	
		Glucose	<i>N</i> -Acetylglucosamine	No addition	0.1 mM-Methyl α -glucoside
FP31	<i>iex</i> ⁺	3	7	3.1	<0.1
FP6	<i>iex</i>	46	56	3.7	<0.1
FP19	<i>ptsH iex</i> / <i>λptsH⁺ iex⁺</i>	9	17	4.4	<0.1

The role of the *iex* gene product in inducer exclusion

The inhibitory action of glucose on the continued utilization of non-PT sugars is complex and involves at least two components. Glucose may not only serve (i) to inhibit the induction of necessary enzymes, by catabolite repression (which also involves the inhibition of adenylate cyclase and hence *gsr* gene function), but may (ii) inhibit the penetration of those first molecules of substrate that are needed to initiate the induction process: it is this latter effect of glucose that is termed 'inducer exclusion'. A further complication may be introduced when non-catabolizable analogues of glucose are used to study these processes: these analogues may accumulate, as their phosphate esters, and in consequence inhibit growth (reviewed by Ferenci & Kornberg, 1974).

Adaptation to new carbon sources. Experiments on the effects of glucose and its analogues methyl α -glucoside and 5-thioglucose on the adaptation of *iex*⁺ and *iex* mutants to new carbon sources illustrates all these features and provide an insight into the role of the *iex* gene product. These experiments may be summarized as follows. (a) The adaptation to growth on non-PT sugars, such as maltose or lactose, by gluconate-grown *iex*⁺ mutants is strongly inhibited by methyl α -glucoside or 5-thioglucose. No significant growth of strain HK 724 (*ptsI*^{ts} *ptsM*) occurred at 30 °C over 8 h, although cultures from which the glucose analogues had been omitted had grown for more than three cell doublings in that time. However, (b) when this experiment was repeated with cells that had been grown initially on maltose or lactose, the inhibitory effect of the glucose analogues on the culture now placed in medium containing the same sugar was short-lived: after a lag of 2–3 h, the cells grew almost as rapidly on maltose + 5-thioglucose, or lactose + 5-thioglucose, as they did on the non-PT sugars alone. (c) Gluconate-grown cultures of *iex* strains grew, after a short lag, on maltose or lactose even though 1 mM-5-thioglucose was present: they thus behaved like pre-induced *iex*⁺ strains (Fig. 1). (d) *iex* strains pre-induced by overnight growth on maltose or lactose continued to grow on these substrates, at rates not significantly diminished by addition of 5-thioglucose or methyl α -glucoside. In contrast, neither *iex*⁺ nor *iex* strains adapted to growth on PT sugars when the glucose analogues were present, even when the cells had been pregrown on those sugars.

Inhibition of transport. The above observations suggest that 'inducer exclusion' by glucose analogues is effective to only a limited extent and can be overcome by altering the relative proportions of induced permease and the product of the *iex*⁺ gene: this latter would, of course, be absent from *iex* strains. This view is supported by direct measurement of the effectiveness of methyl α -glucoside as an inhibitor of permease function. In these experiments, β -galactoside permease was induced by IPTG gratuitously and progressively for various lengths of time, and the activity of that permease was measured in the presence of a great excess (5 mM) of methyl α -glucoside and in its absence (Table 7). When only little permease activity had been induced, the inhibition by methyl α -glucoside was virtually complete (Fig. 3). However, methyl α -glucoside

Table 7. *Effect of methyl α -glucoside on the activity of β -galactoside permease*

Cells were grown overnight in medium containing lactate as sole carbon source. After initiation of growth in fresh lactate-containing medium, inducer (1 mM-IPTG) was added. After different times of induction, cells were harvested, washed and resuspended in 50 mM-sodium phosphate buffer, pH 7.2, containing 125 μ g chloramphenicol ml⁻¹, for determination of the β -galactoside permease activity. Permease activities are expressed as the amounts (nmol) of ONPG hydrolysed by whole cells (mg dry mass)⁻¹ min⁻¹ at 30 °C.

Strain	Relevant genotype	Time of induction (min)	Permease activity	Inhibition (%) of ONPG hydrolysis by 5 mM-methyl α -glucoside
FP31	<i>iex</i> ⁺	10	15	30
		20	36	11
		30	50	10
FP20	<i>iex</i>	10	18	< 5
		20	40	< 5
		30	57	< 5

Fig. 3. Effect of 5 mM-methyl α -glucoside on the activity of β -galactoside permease by *iex* (○) and *iex*⁺ (●) strains of *E. coli*, induced by exposure to IPTG for various lengths of time.

was only marginally effective as an inhibitor of the much greater activities of permease formed by the cells after more prolonged exposure to IPTG. This effect was not due to the metabolism of galactose, derived from the hydrolysis of ONPG used in the assay, since a *galK* mutant behaved identically. These observations confirm those of Koch (1971), who used a lactose-constitutive strain grown on glucose, of Kalachev *et al.* (1981), and of Postma (1982).

In contrast, the permease activity induced in *iex* strains in this manner was not inhibited by methyl α -glucoside at any level: this indicates that the *iex*⁺ gene product is directly involved in this effect. This is also indicated by the more detailed time-course of an experiment of this type (Fig. 3), in which the permease activity of each sample of cells after induction with IPTG, measured in the presence of methyl α -glucoside, is plotted against the activity of the same sample measured in its absence. With *iex* strains, where no inhibition is observed, the slope of the line relating these two rates is unity and passes through the origin; with *iex*⁺ strains, where inhibition is observed, the slope is identical but the intercept on the abscissa is displaced.

Two other observations suggest an interaction of the permease with a limited amount of *iex*⁺ gene product. If another transport system, such as that for maltose, which is also subject to regulation by the *iex* gene, was first induced (for example, by overnight growth of the cells on that sugar), the β -galactoside permease activity subsequently induced by IPTG was much less

Table 8. *Effect of growth substrate on the inhibition of β -galactoside permease activity by methyl α -glucoside*

Cells were grown overnight in either lactate or maltose and resuspended in fresh medium of the same type; β -galactoside permease was induced by addition of 1 mM-IPTG. The subsequent operations were as described for Table 7.

Strain	Time of induction (min)	Permease activity	Inhibition (%) of ONPG hydrolysis by 5 mM-methyl α -glucoside by cells grown on:	
			Maltose	Lactate
FP31 (<i>iex</i> ⁺)	5	8.1	29	60
	10	17	16	32

Table 9. *Factors affecting the utilization of mixtures of glucose and lactose*

Cells were grown overnight in media containing the carbon source, and inducer where appropriate, listed below. They were harvested and resuspended in duplicate flasks containing 2.5 mM-[¹⁴C]lactose and 5 mM-glucose, and 2.5 mM-lactose and 5 mM-[¹⁴C]glucose. The relative utilization of these sugars was measured over 3-4 cell doublings, as described by Amaral & Kornberg (1975).

Strain	Relevant genotype	Carbon source and inducer in overnight culture used for experiment	Fraction of carbon (%) derived from glucose by cells growing on 2.5 mM-lactose and 5 mM-glucose
FP31	<i>iex</i> ⁺	Glucose	97
		Lactose	77
		Glycerol + 1 mM-IPTG	50
FP34	<i>iex</i> ⁺ Lac ^c	Glycerol	43
FP20	<i>iex</i>	Glucose	50

sensitive to inhibition by methyl α -glucoside than it was in cells grown on e.g. lactate, whose transport does not involve *iex* gene function (Table 8). Secondly, the relative utilization of lactose in the presence of glucose, which in *iex*⁺ strains pre-grown on glucose is virtually nil (Fig. 2), could be greatly increased in two ways: (i) by prior induction of β -galactoside permease, either through growth on lactose or, more effectively, by growth in the presence of IPTG; and (ii) by mutation to constitutivity of the *lac* operon. When the β -galactoside permease was fully induced, glucose and lactose were utilized simultaneously and to an equal extent (Table 9). The same rate of uptake of these two sugars could also be obtained, even in cells pre-grown on glucose, when the strain used was *iex*.

These results can all be explained if the *iex*⁺ gene specifies an inhibitor of transport that is made in limiting amounts, so that it can be titrated out by inducing any of the permeases with which it interacts.

DISCUSSION

The recognition that *E. coli* contains two regulatory genes, designated *gsr* and *iex*, that are clearly distinguishable both by the different phenotypes of *gsr* and *iex* mutants and by their location on the *E. coli* genome (Kornberg & Watts, 1979; Britton *et al.*, 1983), appears to be in conflict with earlier work on *S. typhimurium* and with the models for the mechanisms of catabolite repression and inducer exclusion derived from them. This apparent conflict may have a simple explanation: the *gsr* and *iex* genes in *S. typhimurium* may lie in so close a proximity that some or all '*cr*' mutants of that organism may be double mutants. There are good grounds for

this belief. The *crr* mutants of *S. typhimurium* that have been described (Saier & Roseman, 1972) appear to fall into at least three classes, the properties of some of which reflect *gsr iex*⁺ and *gsr iex* mutants in *E. coli*. Detailed genetic analysis of the gene order adjacent to the markers that specify enzyme I and HPr on the *Salmonella* chromosome (Cordaro & Roseman, 1972) revealed a sequence:

crr·ptsI·ptsH·cysK

In contrast, the gene order in *E. coli*, confirmed by restriction endonuclease analysis of cloned genes (Britton *et al.*, 1983), is

cysA·gsr·ptsI·ptsH·iex·cysZ·lig

Although no direct evidence is yet available on the nature of the mutations in *S. typhimurium*, it is likely that the manner in which 'crr' mutants were selected favoured the emergence of organisms affected in more than one property. The basis of such selection lay in the observation that mutants impaired in, but not totally devoid of, enzyme I activity were still capable of inducing the permeases for non-PT sugars but that this induction was unusually sensitive to inhibition by methyl α -glucoside. Further mutants that were no longer inhibited by the glucose analogue were described as being 'carbohydrate repression resistant', the initial letters of which phrase formed the acronym used also for defining the relevant gene. One effective way in which inhibition by methyl α -glucoside can be overcome is to inactivate a component of the PT system specifically involved in the uptake of that glucose analogue and, indeed, 'crr' mutants of *S. typhimurium* have been shown to be deficient in the factor III^{Glc} (Saier & Roseman, 1976). Such mutants correspond to the *gsr* mutants of *E. coli* and, as expected, are also impaired in adenylate cyclase activity. It is possible that other 'crr' mutants of *S. typhimurium*, that are not greatly impaired in adenylate cyclase activity, may be of a type corresponding to the *iex* mutants of *E. coli* that are described in the present paper.

The recognition that, in *E. coli* there are two genes, *gsr* and *iex*, that specify distinct and separate regulatory properties makes it necessary to modify models that have been put forward for the role of the PT system in catabolite repression and inducer exclusion. One such model was proposed by Saier & Feucht (1975) and has been frequently reviewed (Saier & Stiles, 1975; Postma & Roseman, 1976; Osumi & Saier, 1982). Its central feature is that a regulatory protein RPr was postulated to exist which could control the activity of non-PT sugar permeases by binding to them at allosteric regulatory sites. The passage of phosphate from phosphoenolpyruvate to RPr, via enzyme I and HPr, would phosphorylate it: this phosphorylation to P-RPr would prevent binding to the allosteric site. On the basis of genetical evidence obtained with mutants of *S. typhimurium*, and of measurements of the binding of factor III^{Glc} (purified from *Salmonella*) to *E. coli* membranes rich in β -galactoside permease, in the presence of enzyme I, HPr and phosphoenolpyruvate, Osumi & Saier (1982) concluded that the regulatory protein RPr is identical with factor III^{Glc}. This model would not, however, explain the phenotype of *iex* mutants of *E. coli*, that are unimpaired in the uptake of methyl α -glucoside and of glucose, and in adenylate cyclase activity (which are determined by factor III^{Glc}), but in which lactose is excluded neither by glucose nor by *N*-acetylglucosamine. It would also not explain the finding that *gsr* mutants, that contain greatly diminished amounts of factor III^{Glc}, retain 'inducer exclusion' for all effective substances (like *N*-acetylglucosamine and gluconate) save glucose. We therefore postulate a modified model in which the function of the *gsr* gene product, which probably is factor III^{Glc}, is (i) to interact with the PtsG system in the membrane to effect the uptake of most of the glucose, and all of the methyl α -glucoside, that may be present in the medium, and (ii) to interact with the adenylate cyclase in the membrane in order to modulate its activity. The function of the *iex* gene product, on the other hand, is to modulate the activity of permeases for non-PT sugars, possibly in a manner analogous to that postulated for RPr.

The division of function between the *gsr* gene, which specifies glucose-specific repression, and *iex* which specifies inducer exclusion, would make it possible to resolve present confusion in the literature by an appropriate change in nomenclature. Thus, 'crr' in *S. typhimurium*, which has been termed *tgS* in *E. coli*, could become *gsr*, whereas the gene previously designated *crr* in *E. coli* could become *iex*. We shall continue to use this nomenclature henceforth.

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