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Analysis of the Regulatory Mechanisms Controlling the Synthesis of the Hexitol Transport Systems in *Escherichia coli* K12

J. Lengeler and H. Steinberger
Institut für Biochemie, Genetik und Mikrobiologie, Lehrstuhl Biologie IX, Universität Regensburg

Summary. The synthesis of the transport systems (enzymeII-complexes) coded for in the *mtl* and in the *gut* (*srl*) operon was found to be induced by unphosphorylated D-mannitol and D-glucitol respectively. Induction from the outside however is only possible if these polyols are taken up into the cells. Induction of the D-mannitol system is immediate, resistant against catabolite repression, relatively insensitive towards transient repression and starts from a high uninduced level (5–30%). By contrast, the induction of the D-glucitol system starts at a low basal level (0.5–2.5%), does show a pronounced lag from 25 to 90 min, and is hypersensitive towards catabolite and transient repression. These differences apparently reflect primarely differences in the corresponding operator-promotor genes *mtl(P,O)* and *gut(P,O)* as well as differences in the uptake of the first, inducing hexitol molecules. For each operon additional regulatory genes exist, called *mtlR* and *gutR* respectively, in which transrecessive, temperature sensitive mutations leading to a constitutive expression of the corresponding operon can be found. The influence of these regulatory mechanisms in diauxie experiments and their importance for the differentiation of the three operons during evolution from apparently one common ancestor operon will be discussed.

Introduction

In *E.coli K12*, as shown before (Lengeler, 1975b) the hexitols D-mannitol and D-glucitol are taken up and phosphorylated by three different membrane-bound enzymeII-complexes of the PEP dependent phosphotransferase system (PTS). Both are converted in a second step by two soluble dehydrogenases to the common endproduct, D-fructose-6-P. The structural genes for the D-mannitol specific enzymes are clustered in the *mtl* operon while the D-glucitol specific enzymes are clustered in the *gut* (or *srl*) operon (Lengeler, 1975a). Despite the common endproduct, the hexitols have also been found to differ dramatically in diauxie experiments, due apparently to differences in the transport systems and enzymeII-complexes involved, the pacemaker steps of the corresponding catabolic pathways (Lengeler and Lin, 1972).

Diauxie, as suggested originally by Monod (1942) is caused primarely by the exclusion from the cell of a minor carbon source (e.g. D-glucitol) by a major one (e.g. D-mannitol or D-glucose) preferred for growth. Subsequent work revealed that the preferred substrate prevents the entry or the synthesis of the inducer (inducer exclusion) of the second catabolic pathway by interference of transport system synthesis via catabolite (permanent) and transient repression as well as by interference with transport system activity via catabolite inhibition (for reviews see Magasanik, 1970; Paigen and Williams, 1970; Pastan and Adhya, 1976). A complete analysis however of the exact role of transport systems in these complex regulation phenomena in genetically well defined, isogenic strains and for substrates and inducers taken up by biochemically well characterized transport systems is lacking thus far.

In the present paper, as the first part of such a project, we shall report on the analysis of the different regulatory mechanisms found to control the synthesis of the D-mannitol and of the D-glucitol transport system.

Material and Methods

Chemicals. Lactitol (D-galactopyranosyl-1-4-βD-glucitol) was prepared by borohydride reduction (French et al., 1953) at pH 10

For offprints contact: Dr. J. Lengeler, and H. Steinberger, Institut für Biochemie, Genetik und Mikrobiologie, Universität Regensburg, Universitätsstraße 31, D-8400 Regensburg, Federal Republik of Germany

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Table 1. Origin, genotype or phenotype of bacterial strains

Strain	Origin or reference	Markers relevant to this study
L141	Lengeler, 1975a	*mtlA gut*$^+$ *gat(P,O) lacY*
L144	Lengeler, 1975a	*mtlA gut(P,O) A51 gat*$^+$
L320	*L144*, this study	*mtlR*^{ts} *gatA*
L162	*L141*, this study	*mtl*$^+$ *gut*$^+$ *gat(P,O)*
L187	*L141*, this study	*mtlA gut*$^+$ *gatA*
L316	*L187*, this study	*mtlA gutR*^{ts} *gatA*
L188	*L162*, this study	*mtl*$^+$ *gut*$^+$ *gatA*
L148	Lengeler, 1975a	*mtlA gutR*$^+$ *(P,O)A*$^+$ *D*$^+$
L156	Lengeler, 1975a	*mtlR*$^+$ *(P,O)A*$^+$ *D*$^+$ *gutA gatA*
L194	Lengeler, 1977	*mtlA gutA52 gat(P,O)A,D lacI*$^+$ *Z*$^-$ *Y*$^+$
LS195	*L194*, this study	*gut*$^+$
LS196	*L194*, this study	*lacI*$^-$ *Z*$^+$ *Y*$^+$
L308	*LS196*, this study	*gut*$^+$
L309	*L308*, this study	*pts(H,I)*
L200	*AB313/11*, Lengeler, 1975a	*mtl*$^+$ *gut*$^+$ *gat(P,O)*

Genetic markers are according to Bachmann et al. (1976) and Lengeler (1977). The previous notation *mtl*C$^-$ or *gut*C$^-$ (C for control) has been changed to *(P,O)* for promotor/operator region (cis-dominant constitutivity mutation) while the new symbols *mtlR* or *gutR* are introduced to describe the regulatory genes, characterized by trans-recessive constitutivity mutations. The symbol ts designates a temperature sensitive phenotype

from lactose and does not contain more than 0.5% of free D-glucitol or free galactitol. D-galactopyranosyl-1-6-βD-mannitol was a gift of Dr. E.C.C. Lin (Solomon, et al., 1973).

Bacterial Strains. The origin and the genotypes of strains used in this paper are listed in Table 1. Unless stated otherwise, they are all derivatives of strain *L188*, itself a derivative of strain *L141* (Lengeler, 1975a) and usually have the following genotype in addition to the markers listed in Table 1: F$^-$ *thi his-1 argG metB rpsL* (*strA*) lacY galK xyll.

Culture Media and Growth Conditions. The media and plates used were prepared as described before (Lengeler, 1975a; Ruch et al., 1974). Induction of the D-mannitol or D-glucitol transport systems and dehydrogenases was done by adding these polyols or lactitol to cells growing exponentially on glycerol (0.2%) or casamino acids (1.0%). At the times indicated samples were taken, cooled down in the presence of 100 µg/ml chloramphenicol (carrier free), centrifuged and washed twice at 0° C. The cells were subsequently resuspended in minimal medium and used immediately to test the transport activity or alternatively, the cell pellet was kept frozen at −20° C until used to test the enzymeII-complex activities or the hexitol-P dehydrogenase activities.

Transient repression was measured by adding to inducible cells growing exponentially on glycerol at the same time D-mannitol, D-glucitol or lactitol and D-glucose (at 0.2% each) and taking samples as described for the induction studies. To measure catabolite repression the different activities were determined after growth on D-glucose vs. activities determined after growth on glycerol in strains carrying either a constitutive and cis-dominant mutation in the *mtl(P,O)* region (strain *L156*) or in the *gut(P,O)* region (strain *L148*).

Genetical Techniques. Conventional genetical techniques and the selection procedures for the different mutations affecting hexitol transport and metabolism were as described before (Lengeler 1975a; Lengeler, 1977). Mutants inducible for the *mtl* operon at 25° but constitutive at 42° C were isolated as derivatives of strain *L144 gatA*, able to grow at 42° C on D-glucitol as sole carbon source but not at 25° C. This strain with the genotype *mtlR*$^+$ *(P,O)*$^+$ *A*$^+$ *D*$^+$ *gutR*$^+$ *(P,O) A D*$^+$ *gatA* is unable to grow on D-glucitol due to the *gutA gatA* mutations unless the *mtl* coded transport system is synthesized constitutively (Lengeler, 1975a). Similarly mutants inducible for the *gut* operon at 25° but constitutive at 42° have been isolated in strain *L187* with the genotype *mtlR*$^+$ *(P,O)*$^+$ *A D*$^+$ *gutR*$^+$ *(P,O)*$^+$ *A*$^+$ *D*$^+$ *gatA*. Such strains, as shown before too (Lengeler, 1975a), are unable to grow on D-mannitol unless the *gutA* coded transport system is expressed in a constitutive way. Using D-fructose instead of D-mannitol Jones-Mortimer and Kornberg (1976) have selected in a similar way mutants inducible for the gut operon at 25° C, but constitutive at 42° C. The *lacI* derivatives were obtained by plating the transductants from a cross between a Pl.*L253 lacI* lysate and the recipient strains on lactitol plates. Lactitol is a substrate for both the enzyme β-galactosidase (E.C.3.2.1.23) and the lactose transport system but is not an inducer for the *lac* operon (unpublished data). Thus on lactitol plates only strains constitutive for the *lac* operon will grow.

Uptake and Enzyme Assays. Standard uptake assays as well as the preparation of cell extracts by lysozyme or ultrasonic treatment, the enzymeII-complex activity tests, the hexitol-P dehydrogenase assays and the protein determinations have been described (Lengeler and Lin, 1972; Lengeler, 1975a and b; Lengeler, 1977). Uptake and enzymeII-complex activities are expressed in nmoles per min. per mg of protein, dehydrogenase activities in µmoles per min. per mg of protein.

Results

1. Inducibility of the mtl and of the gut Operon: Nature of the Inducer

As shown in Figure 1a the addition of D-mannitol to cells of the wildtype strain *L188* growing exponentially on glycerol does induce a nearly immediate increase of the transport activity coded for by the gene *mtlA*. The increase in transport activity is parelleled by a strictly coordinated increase in the activity of the corresponding enzymeII-complex^{Mtl} and of the soluble enzyme D-mannitol-1-P dehydrogenase coded for by the gene *mtlD* (data not shown). Depending on the growth conditions, the uninduced basal level for all enzymes coded for in the *mtl* operon amounts from 5 to 30% of the fully induced level.

By contrast a lag lasting from 25 to 90 min elapses between the addition of D-glucitol and the earliest measurable increase of the transport activity coded for by the gene *gutA* (Fig. 1b). This unusual lag is also found for the corresponding membranebound enzymeII-complex^{Gut} (data not shown) and for the soluble enzyme D-glucitol-6-P dehydrogenase coded for by the gene *gutD* (Fig. 2). The uninduced basal level in the *gut* operon is low, varying from 0.5 to 2% of the fully induced level, depending on the growth conditions.

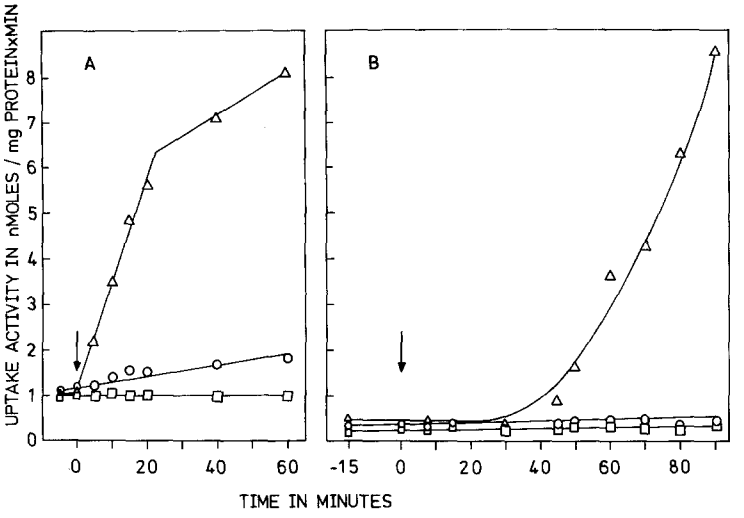

Fig. 1 A and B. Induction of the D-mannitol (*A*) and of the D-glucitol (**B**) transport system. At time 0, indicated by the arrow, 0.2% D-mannitol or D-glucitol respectively were added to cells of strain *L188* growing exponentially on glycerol and the transport activity tested in washed cells as described in materials and methods.
Additions were none (□—□), D-mannitol (**A**) or D-glucitol (**B**) (△—△), D-mannitol (**A**) or D-glucitol (**B**) + D-glucose (0.2%) (○—○)

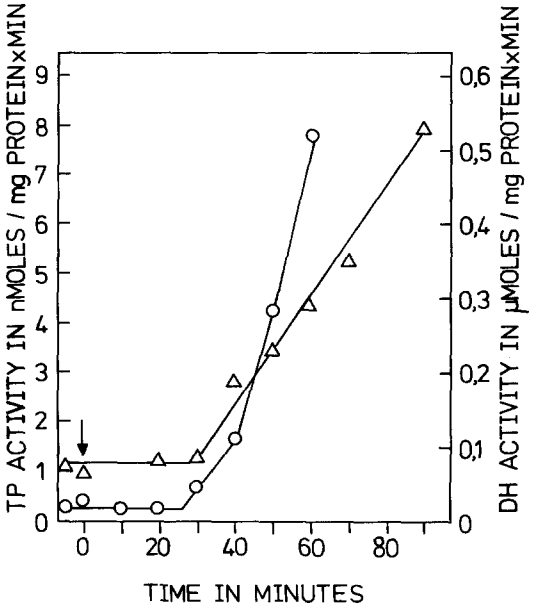

Fig. 2. Coordinated induction of the D-glucitol transport and of the D-glucitol-6-P dehydrogenase activity. At time 0, indicated by the arrow, 0.2% D-glucitol was added to cells of strain *L188* growing exponentially on glycerol. Samples of the culture were treated and tested for transport activity (○—○) and for dehydrogenase activity (△—△) as described in materials and methods

In *gat*$^+$ derivatives of strains *AB313* and *560* the specific and coordinated induction of the *mtl* operon by D-mannitol and of the *gut* operon by D-glucitol with similar induction factors as found in the *gatA* mutant *L188* has been reported before (Lengeler and Lin, 1972).

Free D-mannitol rather than D-mannitol-1-P seems to be the inducer of the *mtl* operon as indicated by its ability to induce the enzyme D-mannitol-1-P dehydrogenase in strains lacking the ability to synthesize D-mannitol-1-P, due to the absence of an active enzymeII-complex^{Mtl} (Solomon and Lin, 1972). This hypothesis is further supported by the absence of an induction lag for the *mtl* operon (Fig. 1a). By contrast the unusual lag seen in the induction of the *gut* operon might be due to the slow conversion of D-glucitol into the real inducer (e.g.D-glucitol-6-P). In order to test this hypothesis, we repeated the experiment of Solomon and Lin with D-glucitol and strains unable to synthesize D-glucitol-6-P. The strains used were *L309*, lacking the proteins HPr and enzymeI coded for in the *pts* operon ($\leqq$0.4% rest activity compared to the *pts*$^+$ wild type), and the triple mutant *LS194* (*mtlA gutA gatA*) lacking all enzymeII-complex activities specific for hexitols. Care was taken to choose the weakly polar mutation *gutA51*, in which the operator distal gene *gutD* can still be expressed (Lengeler, 1975a).

As shown in Figure 3 the addition of free D-glucitol to cells of strain *L309* growing exponentially does induce the enzyme D-glucitol-6-P dehydrogenase. Since in such strains no ATP dependent D-glucitol kinase activity and no D-glucitol-6-P is detectable (Lengeler, 1975b), the latter substance does not seem to be the inducer of the *gut* operon. Unexpectedly it was found (Fig. 3), that the transport negative mutant *LS194* cannot be induced from the outside. Thus we had to consider the possibility that only intracellular D-glucitol but not D-glucitol in the medium can induce the *gut* operon and furthermore that free D-glucitol is able to penetrate into the *pts* mutant *L309*, but not into the transport negative mutant *LS194*, carrying the *gutA51* mutation. Strains with a constitutive lactose transport system, coded for by the gene *lacY*, are able to take up the lactose analoge lactitol (D-galactopyranosyl-1,4-βD-glucitol), and to generate internally D-galactose and free, unphosphorylated D-glucitol by means of the enzyme β-galactosidase

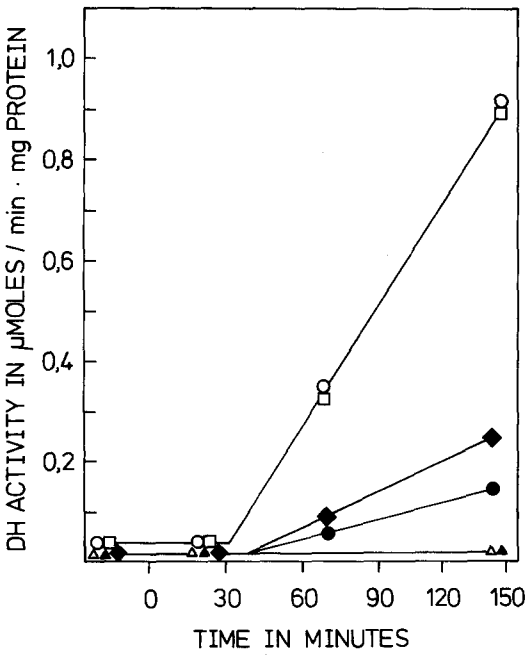

Fig. 3. Induction of the enzyme D-glucitol-6-P dehydrogenase in different transport mutants. At 0 min, 0.2% D-glucitol or lactitol were added to cells growing exponentially on glycerol (open symbols) or on casamino acids (CAA) (filled symbols). Samples were taken and dehydrogenase activity was tested as described in materials and methods for the strain *L188 mtl*$^+$ *gut*$^+$ *lacY* (○—○), *LS196 mtlA gutA51 lacI*$^-$ (△—△) or *L308 mtlA gut*$^+$ *lacI*$^-$ (□—□) in glycerol+glucitol, for the strain *LS196 mtlA gutA51 lacI*$^-$ (◆—◆) and *L194 mtlA gutA51 lacZ* (▲—▲) in CAA+lactitol, and for strain *L309 mtlA gut*$^+$ *lacI*$^-$ *pts(H,I)* (●—●) in CAA+glucitol

(Lengeler, in prep.). As shown in Figure 3, strain *LS194* (which in addition to be negative for all hexitol transport systems lacks also the *lacY* coded transport activity) is neither induced by D-glucitol nor by lactitol. By contrast the *lacI*$^-$ *Z*$^+$ *Y*$^+$ derivative *LS196*, though still not inducible by D-glucitol is induced by lactitol for the *gutD* coded dehydrogenase, supporting the hypothesis that free, but intracellular D-glucitol is the inducer of the *gut* operon.

When lactitol is added to *lacI*$^-$ *Z*$^+$ *Y*$^+$ mutants growing on glycerol, a transient growth inhibition and enzyme repression is observed during 90 to 240 min., as has been reported for the addition to such strains of lactose (v. Hofsten, 1961) and D-galactose (Lengeler, 1966). The effect is only weak for cells growing on D-glucose or on CAA, since here the lactose transport activity is partially repressed. In these media however induction of the *gut* operon by lactitol and even by D-glucitol itself is also repressed, thus explanining the low activity measured in mutants (Fig. 3) and in wildtype cells (data not shown).

In a similar experiment with D-mannitol and several of our *mtlA* mutants, after 2 h no induction of the enzyme D-mannitol-1-P dehydrogenase was detectable, in apparent contradiction to the data of Solomon and Lin (1972). After 8 h however, the time used by these authors, some induction is found in our mutants too. Again, induction of strain *L309* with D-mannitol or induction of strain *LS196* with D-galactopyranosyl-1,6-βD-mannitol is immediate (data not shown) indicating for this operon too induction by free, but unphosphorylated hexitol.

For both the *mtl* and the *gut* operon evidence for the existence of cis-dominant regulatory mutations (promotor-operator type) and called previously *mtlC* or *gutC* (C for control) has been given (Lengeler, 1975a). As long as their exact location in the genes *P* or *O* has not been established, we now prefer to give the notation (*P,O*). In addition we have isolated a new class of regulatory mutants, which seem analogous to mutants first described by Jones-Mortimer and Kornberg (1976) for the *gut* operon. As shown in Figure 4 cells of the mutant *L316* when growing at 25° C on glycerol have a low D-glucitol transport activity, which upon shift of the culture to 42° C increases without a lag to high levels. At both temperatures, the D-mannitol transport activity remains low. The mutant *L316* is normally inducible for the *gut* operon at 23° C during growth on glycerol. Its generation time on D-mannitol at 23° C is $\geqq$600 min compared to ca. 100 min at 42° C. Strain *L320* by contrast has on D-glucitol a generation time of $\geqq$660 min at 23° C and of ca. 90 min at 42° C. In this strain the D-glucitol transport activity always remains low, while the D-mannitol transport activity varies characteristically with a change in the growth temperature (data not shown). In each case the increase in transport activity is paralelled by an increase of the corresponding enzymeII-complex and dehydrogenase activities.

The temperature sensitive mutations in strains *L320* and *L316* are trans-recessive to the corresponding wildtype allel and do not map in the (*P,O*) regions. Deletion of the regulatory gene of the *gut* operon does cause a constitutive synthesis of this operon. A complete analysis of these mutations, apparently located in regulatory genes coding for *mtl* or *gut* specific repressors (negative control), and thus called *mtlR* and *gutR*, will be presented elsewhere (L. Csonka, pers. commun.; Lengeler and Auburger, in prep.).

2. Regulation by Catabolite (Permanent) Repression

Catabolite repression, some times also referred to as permanent repression, to distinguish it from transient

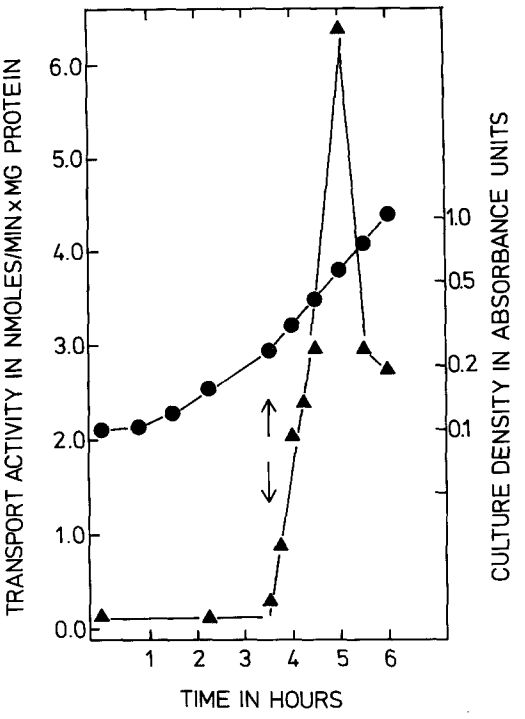

Fig. 4. Derepression of the *gut* operon by temperature shift. Cultures of strain L316 growing exponentially on glycerol at 25° C were rapidly brought to 42° C at the time indicated by the arrows. Further treatments and tests for growth (●—●) or D-glucitol transport activity (▲—▲) were as described in materials and methods

Table 2. Catabolite repression of the *mtl* and of the *gut* operon

Growth medium	Additions	Transport activities in nmoles/min mg prot.	
		in strain *L156*	in strain *L148*
glycerol	–	4.3	8.9
D-glucose	–	6.2	2.1
D-glucose	1 mM cAMP	6.1	3.7
D-glucose	5 mM cAMP	8.3	10.0

Cells of strain *L156 mtl(P,O)* for the D-mannitol transport tests and of strain *L148 gut (P,O)* for the D-glucitol transport tests were inoculated in the media indicated above, harvested and tested 3 h later as described in materials and methods

repression (see below) is known to control in a cAMP/CRP dependent reaction the transcription of catabolic operons (Pastan and Adhya, 1976). Usually it is measured in constitutive strains, thus excluding interference by inducer exclusion. We have used either strain *L156*, constitutive for the expression of the *mtl* operon, or alternatively strain *L148*, constitutive for the *gut* operon but otherwise isogenic to strains *L156* or *L188*.

As shown in Table 2, expression of the *mtl* operon is not repressed by growth on D-glucose and consequently the addition of cAMP (5 mM) only slightly increases the activity. A similar resistance is observed in inducible strains (data not shown) and seems to be characteristic for all strains of *E.coli K12* tested thus far (Monod, 1942; Lengeler and Lin, 1972). By contrast synthesis of the *gut* operon in strain *L148* and other strains of *E.coli K12* is highly sensitive towards catabolite repression caused by D-glucose (Table 2) or by D-mannitol (data not shown) and, as expected this repression is reverted completely by the addition of cAMP (5 mM). An even greater sensitivity towards catabolite repression is observed in inducible strains (Lengeler and Lin, 1972).

Unexpectedly in mutants with a tight mutation in the cya and/or in the *crp* gene not only the *gut* operon, but also the constitutive *gat* operon (Lengeler, 1977) and even the seemingly catabolite repression resistant *mtl* operon are not expressed (Yashpe and Kaplan, 1975; Lengeler, unpubl. data). A more detailed analysis of the complicated role of cAMP in the expression of PTS type transport systems and their operons will be published elsewhere.

3. Regulation by Transient Repression

As shown in Figure 1, the transient repression of the *mtl* operon triggered by the simultaneous addition of D-mannitol and D-glucose (0.2% each) to cells of the inducible strain *L188* growing exponentially on glycerol is strong, but from the very beginning, not complete. In strain *L200*, a derivative of *AB313*, (Fig. 5) or after lowering the concentration of D-glucose it is even lacking completely and only a weak catabolite repression is visible. By contrast the repression of the *gut* operon in both strains, measured under identical conditions and caused either by D-glucose or by D-mannitol is complete and lasts from 3.5 to 8 h. A simultaneous addition of cAMP (5 mM) or the use of a strain with a constitutive galactitol transport system, known to take up also D-glucitol (Lengeler, 1975b) reduces the lag to some extend, but not completely.

These differences are also reflected in the behaviour of the strains in diauxie experiments. Thus strain *L200* as shown before (Lengeler and Lin, 1972) does not show a diauxic lag when growing on a mixture of D-glucose/D-mannitol (0.03% each) while the lag in strain *L188* is ca. 5 min. This contrasts with a lag from 30 to 90 min of both strains on the mixture D-glucose or D-mannitol and D-glucitol (0.03% each). The diauxic lag with these class A substrates

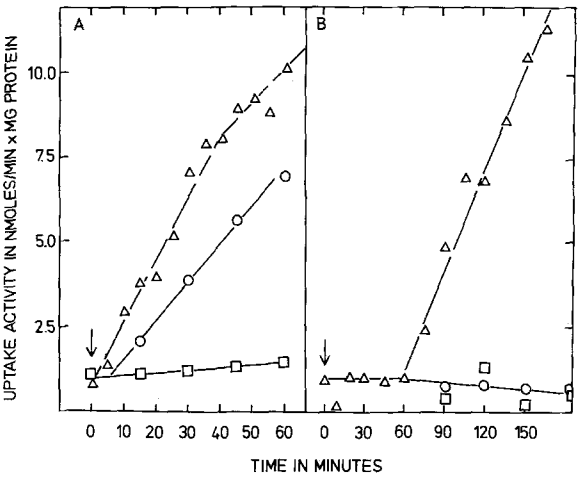

Fig. 5A and B. Test of the transient repression of the *mtl* (**A**) and of the *gut* (**B**) operon in strain *L200*. Test conditions and symbols were as described in Figure 1

is even visible when the cells have been pregrown on D-glucitol. On the other hand, no class B substrate, e.g. D-glucose-6-P, D-fructose, D-mannose, lactose, L-arabinose or glycerol does cause a diauxic growth in a mixture with D-glucitol (0.03% each) (data not shown).

Discussion

According to the data presented in this paper, coarse control of the hexitol transport system activity in *E.coli K12* first of all is by regulation of their synthesis by means of induction. The *mtl* and the *gut* operon apparently are induced only by intracellular but unphosphorylated D-mannitol or D-glucitol respectively. This, at first is suprising in view of the fact that enzymeII-complexes of the PEP dependent sugar phosphotransferase system are thought to accumulate in a vectorial reaction primarely phosphorylated substrates (Postma and Roseman, 1976). Furthermore no potent phosphatase activity specific for hexitol-phosphates is detectable in cell extracts of *E.coli K12* (Lengeler, 1975b). We have however been able to show for D-glucitol in an experiment similar to one reported by Solomon et al. (1973) for D-mannitol, that unphosphorylated D-glucitol too can be translocated through the intact cell membrane by means of the unphosphorylated enzymeII-complex^{Gut} (Lengeler, FEBS Symposium Zürich, 1976). This together with the induction experiment described in Figure 3 seems to indicate that at least the hexitol specific enzymeII-complexes might function in the non-phosphorylated state as bidirectional carriers for free hexitols, while in the phosphorylated state they catalyze a vectorial phosphorylation. Similar conclusions have been reached for other enzymeII-complexes in *E.coli* and *Salmonella typhimurium* too (Postma and Roseman, 1976; Kornberg and Jones-Mortimer, 1977).

The exceedingly long lag in the induction of the *gut* operon with its low level of uninduced enzymeII-complex^{Gut} activity compared to the short lag for the *mtl* operon with its high basal level of enzymeII-complex^{Mtl} activity thus in part might reflect a slow uptake of the inducing D-glucitol and a faster uptake of the inducing D-mannitol molecules.

Certain mutants unable efficiently to take up D-mannitol or D-glucitol cannot be induced from the outside for the corresponding hexitol-P dehydrogenase, coded for by the promotor distal gene. They thus might simulate the presence of a polar mutation in the operator proximal gene or of a pleiotropic negative mutation in the promotor/operator region or in the regulatory gene. Thus on the basis of complementation data in mutants lacking both the *gutA* and the *gutD* coded enzyme activities, a positive regulation of the *gut* operon has been suggested (McEntee, 1977). A positive regulation of this operon however is not easily compatible with the existence of temperature sensitive constitutivity mutations (Jones-Mortimer and Kornberg, 1976), which are trans-recessive to the wildtype allel, or with the observation, that deletions of these regulatory genes render the expression of the operon constitutive (L. Csonka, pers. commun.; Lengeler, unpublished data). The exact location of the different mutations obviously has to be known, before this apparent contradiction will be clarified.

Besides by induction, the synthesis of the hexitol transport systems is controled by means of catabolite repression. Both operons are regulated by this mechanism though the *mtl* operon is less affected then the *gut* operon. In fact the effect on the *mtl* operon is clearly visible only in mutants with tight mutations in the *cya* and/or the *crp* genes.

Transient repression is not well understood at present. It is known to control the transcription of catabolic operons and to influence the cAMP/CRP system (Pastan and Adhya, 1976). This repression, by contrast to the permanent catabolite repression, is strong and, as its name indicates, transient. As will be shown in a sbusequent paper (Lengeler and Steinberger, in prep.) transient repression of the hexitol operons apparently is always observed, whenever catabolite repression is combined with catabolite inhibition (Paigen and Williams, 1970).

The two classA substrates D-glucose and D-mannitol are taken up and metabolized by transport systems and catabolic pathways with a high, uninduced basal level. Their synthesis is highly resistant toward

catabolite and transient repression (Lengeler and Renner, in prep.). By contrast, all classB substrates (e.g. D-glucitol and galactitiol) have low, uninduced basal levels and their operons are (hyper)sensitive towards both repressions. The three metabolic pathways however for the dissimilation of the hexitols in *E.coli K12* and related Enterobacteriaceae are remarkably similar (Lengeler, 1977). Several enzymes of these pathways, especially the transport systems, still share the same substrates, from hexitols and their analoges up to the ring-shaped D-fructose (Jones-Mortimer and Kornberg, 1976). Furthermore, the corresponding genes have been shown to be arranged in the same sequence inside the three operons. This has prompted the speculation, that they might have evolved from a common ancestor hexitol operon (Lengeler, 1975a, 1977). Their location on the chromosome of *E.coli K12* supports such an evolution by triplication (Zipkas and Riley, 1975). Recently the hypothesis has been extended to the evolution of all enzymeII-complexes of the PEP dependent sugar phosphotransferase system from a common ancestor and an early differentiation in a hexose and in a hexitol specific subgroup is proposed (Saier, 1977). The broad substrate specificity, also reflected in the role of enzymeII-complexes as common chemoreceptors in chemotaxis (Adler and Epstein, 1974; Lengeler, 1975a), contrasts to the high specificity in the regulation of the corresponding operons. The *mtl* operon (enzymeII-complex^{Mtl}) is only induced by D-mannitol, the *gut* operon (enzymeII-complex^{Gut}) only by D-glucitol, the *ptsF* gene (*fruA*; enzymeII-complex^{Fru}) by D-fructose (Jones-Mortimer and Kornberg, 1976), the *ptsG(glcA)/crr* system (enzymeII-complex^{Glc}) in strain *L188* by D-glucose, and the *ptsM(manA)* system (enzymeII-complex^{Man}) by D-mannose (unpublished data). The *gat* operon finally (enzymeII-complex^{Gat}), constitutive in all strains of *E.coli K12* tested thus far (Lengeler, 1977), is semiconstitutive in *E.coli B* and normally inducible in *Salmonella typhimurium LT2* by galactitol, but not by D-mannitol or D-glucitol (unpublished data).

If all enzymeII-complexes have evolved from a common ancestor, then a pronounced differentiation at the substrate level occured once, to separate the hexose from the hexitol subgroup, while differentiation at the regulatory level occured for each system. The latter, accordingly, would thus play the major role in the evolution of metabolic pathways among the Enterobacteriaceae.

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