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Carbon-starvation induction of the *ugp* operon, encoding the binding protein-dependent *sn*-glycerol-3-phosphate transport system in *Escherichia coli*

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Summary. The gene products of the *ugp* operon of *Escherichia coli* are responsible for the uptake of *sn*-glycerol-3-phosphate and certain glycerophosphodiesters. The regulation of *ugp* is mainly *phoBR*-dependent. Significant expression, however, can be observed even in the presence of high concentrations of phosphate, a condition which normally completely represses *pho* expression. *Pho*-independent *ugp* expression was found to be derepressed during the late logarithmic growth phase due to carbon starvation. Among different carbon sources tested, glucose caused the most complete repression. Addition of cAMP prevented glucose repression, indicating that a cAMP-CRP control mechanism may be directly or indirectly involved in the carbon-starvation response. This conclusion is supported by the fact that *pho*-independent *ugp* expression correlated with the presence of the *cya* and *crp* gene products.

Key words: *ugp* operon, gene regulation – Regulation

Escherichia coli possesses two transport systems for *sn*-glycerol-3-phosphate (G3P). The genes involved in the *glpT*-dependent transport system are part of the *glp* regulon, which includes the sequences coding for the enzymes required for utilization of glycerol and its derivatives as carbon and energy sources (Lin 1976; Ludtke et al. 1982). The second G3P transport system, which is also responsible for the transport and concomitant hydrolysis of glycerophosphodiesters (Brzoska and Boos 1988), is encoded by the *ugp* operon (Argast et al. 1987). This operon consists of at least five genes, *ugpB,A,E,C,* and *Q*, organized as a single transcription unit (Overduin et al. 1988). Genetic analyses have shown that *ugp* expression is co-regulated with other members of the *pho* regulon (Argast and Boos 1980; Schweizer and Boos 1985), especially *phoA*, the structural gene for alkaline phosphatase. However, a more detailed analysis revealed significant differences. Whereas *phoA* expression is completely repressed in the presence of excess phosphate (P_i) (0.64 mM), *ugp* expression is not completely repressed under such conditions (Schweizer and Boos 1984; Wanner and McSharry 1982). Wanner and McSharry (1982) demonstrated that the expression of a *ugp-lacZ* fusion gene in vivo was affected by, among other factors, carbon starvation. These results indicated that the *pho*-dependent regulation may not be the only regulatory mechanism that controls expression of the Ugp system. During our attempt to construct a phosphate-regulated expression system we found that a significant amount of P_{ugp}-directed expression of target genes could only be repressed in the presence of high concentrations of glucose, supporting the hypothesis of multiple controls of *ugp* expression (Su et al. 1990). Recently, Kasahara et al. (1991) also showed that the dual regulation occurs at two interspaced promoters. In the present study, additional physiological and genetic evidence is presented which supports the notion of dual regulation of *ugp* expression by phosphate- and carbon-starvation.

For these studies, a 174 bp *Eco*RI-*Hinf*I fragment containing the *ugp* promoter region was isolated and cloned between two transcription terminators, resulting in the P_{ugp}-based expression vector pPH3 (Su et al. 1990). Two reporter genes, *lacZ* (encoding β-galactosidase or LacZ) and *xylE* (encoding catechol 2,3-dioxygenase or XylE) (Inouye et al. 1981a), were inserted into this vector, forming plasmids pPZ3 and pPX3, respectively (Su et al. 1990). In these plasmids, expression from the *ugp* promoter can be easily monitored by measuring the respective enzyme activities.

When *ugp*-promoted XylE and LacZ expression was examined in a wild-type strain and several *pho* regulatory mutants grown under conditions of excess (TGE) and limiting (TGL) P_i, *ugp* expression from the plasmid paralleled chromosomally-derived *phoA* expression. As shown in Table 1, approximately 20% of the total β-galactosidase activity expressed from P_{ugp} was not P_i-repressible [depending on the experiment, period of pre-

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Table 1. Alkaline phosphatase and P_{ugp}-directed β-galactosidase activities in wild-type and *pho* regulatory mutants[a]

Strain (Relevant genotype)	Limiting P_i		Excess P_i		Phenotype	Strain reference
	AP	βGal	AP	βGal		
DL291 (*pho*$^+$)	636	264486	7.4	50991	repressible	Ludtke et al. (1982)
BW3921 (*Δlac pho*-510)	1617	268813	6.0	44393	repressible	B. Wanner
BW6504 (*Δlac phoU*35 *pho*-510)	2009	211636	1981.3	217850	constitutive	B. Wanner
BW6520 (*Δlac Δ(phoBphoR)* 518 *pho*-510)	17	9636	6.1	28093	uninducible	B. Wanner
BW3820 (*Δlac phoB*23)	14	8963	6.8	39439	uninducible	B. Wanner
BW3966 (*Δlac phoM*451 *phoR*68 *pho*-510)	40	13897	20.6	42925	uninducible	B. Warner

[a] Strains harboring plasmid pPZ3 [$\Phi(P_{ugp}$-*lacZ*)] were grown into mid- to late-logarithmic phase in TGE medium [TG (Echols et al. 1961) containing 10 mM KH_2PO_4, P_i] supplemented with 0.2% glucose. They were harvested, washed twice with P_i-free and glucose-free TG medium, and then inoculated into TGE (10 mM P_i, excess P_i) or TGL (0.064 mM P_i, limiting P_i) medium with 0.2% glucose. Where necessary, the TG medium was supplemented with thiamine (5 μg/ml) and ampicillin (50 μg/ml). For enzyme assays, aliquots were removed from the cultures about 6 h after entering stationary phase growth and immediately placed on ice. Chloramphenicol was added to 100 μg/ml. The ice-cold cells (0.5 ml) were pelleted, washed once with 0.1 M TRIS-HCl (pH 8.0), and resuspended in the same buffer. The cells were then permeabilized by adding 20 μl of chloroform and 10 μl of 0.1% sodium dodecyl sulfate. β-galactosidase and alkaline phosphatase were analyzed as previously described (Brickman and Beckwith 1975; Miller 1972). The molar extinction coefficients used for calculation of specific activities are $\varepsilon_{410} = 1.62 \times 10^4$ (alkaline phosphatase) and $\varepsilon_{420} = 4.5 \times 10^3$ (β-galactosidase). Specific activities are expressed in nmol of products liberated per minute per mg protein

P_i, phosphate; AP, Alkaline phosphatase; β-Gal, β-galactosidase

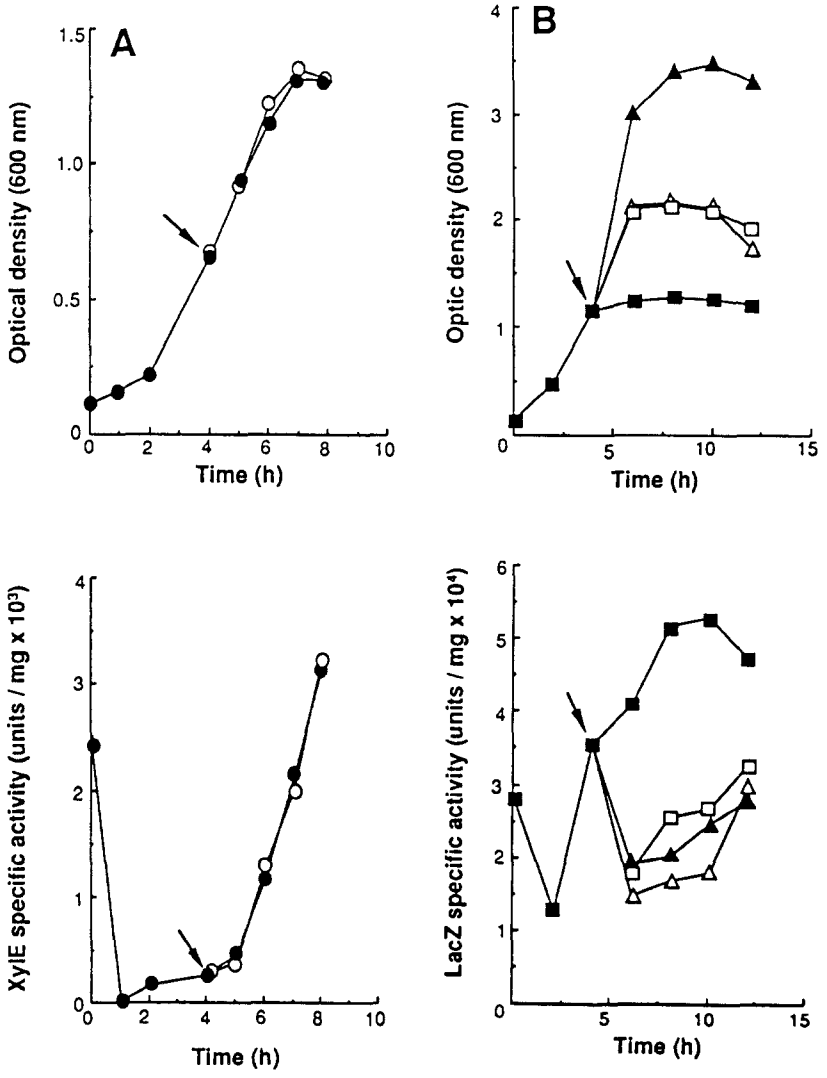

Fig. 1A, B. The *pho*-independent repression and derepression of P_{ugp}-directed catechol 2,3-dioxygenase and β-galactosidase synthesis. Strain DL219 harboring plasmid pPX3 (panel A) or pPZ3 (panel B) was grown in TGE medium and then inoculated into prewarmed TGE medium as described in Table 1. Samples were withdrawn at the indicated intervals and assayed for optical density (upper panels) and XylE activity (lower panel A) and LacZ activity (lower panel B) as described in Tables 1 and 3. At the time indicated by the *arrow*, each culture was divided. Additional P_i (10 mM; panel A, *open circles*), glucose (0.2%; panel B, *open triangles*) or TYE (Miller 1972; panel B, *closed triangles*) was added. Alternatively, the cells were harvested by centrifugation and resuspended in prewarmed fresh TGE medium (panel B, *open squares*). As a control, cultures were grown without additions or medium changes (panel A, *closed circles;* panel B, *closed squares*)

growth prior to the assay, and the indicator gene analyzed, this value varied between 5% and 20% (cf. Table 3, line 1)]. Similar levels of expression were also observed when P_i-uninducible mutants (BW6520, BW3820 and BW3966) were grown in excess P_i. Furthermore, addition of excess P_i (10 mM) to the already derepressed culture had no effect on further induction (Fig. 1 A). These results demonstrate that the observed levels of *ugp* expression were *pho*-independent under conditions of excess P_i.

Since pPH3 is a high copy-number plasmid and the strains employed in this study possess only single copies of the *pho* regulatory genes, one explanation could be that these results are due to a copy number effect as originally claimed by Schweizer and Boos (1984). However, Inouye et al. (1981 b) have shown that the regulatory proteins produced by the haploid chromosomal regulatory genes are sufficient to cause complete repression and derepression of multiple copies of *phoA*. Thus, it is very unlikely that the high gene dosage of the P_{ugp} fusion genes could account for the *pho*-independent expression. This conclusion is further supported by the following observations (data not shown): 1) the basal levels of *ugp* expression in excess P_i were not repressed when the *phoBR* genes were present on a compatible multicopy plasmid; 2) the copy number of the P_{ugp}-*lacZ* or P_{ugp}-*xylE* plasmid constructs did not significantly change during derepression, as assessed by determinations of β-lactamase specific activites; 3) when the *ugp* promoter region of the P_{ugp}-driven *lacZ* gene was replaced by the *tac* promoter (P_{tac}), uninduced β-galactosidase activity (excess P_i in the absence of IPTG) expressed from P_{ugp} was 6–7 times higher than that expressed from the P_{tac}, although the intrinsic strength of P_{ugp} is only about 80% of that of P_{tac} promoter (Su et al. 1990). The latter result also rules out the possibility that the observed levels of enzyme synthesis were due to transcriptional readthrough from a promoter within the vector.

To assess whether the observed *pho*-independent *ugp* expression was due to carbon-starvation, growth-dependent levels of *ugp*-directed XylE or LacZ synthesis were determined (Fig. 1). Upon dilution of a derepressed culture into fresh TGE medium, a significant decrease in *ugp*-directed enzyme synthesis was observed (Fig. 1 A, lower panel). During late exponential growth, a significant derepression of enzyme expression was observed. Addition of excess P_i (10 mM) had no effect on derepression, showing that it was *pho*-independent (Fig. 1 A, lower panel). Switching to fresh TGE medium, addition of TYE (Miller 1972), or addition of 0.2% glucose to the depleted growth medium during late exponential growth of the cells resulted in immediate resumption of growth, and a concomitant decrease in *ugp*-promoted LacZ synthesis (Fig. 1 B). When the cells were cultured until they reached the stationary growth phase, the specific activity of LacZ increased again (Fig. 1 B, lower panel). Fig. 1 B also displays a lack of TYE-specific induction, which was previously reported from the in vivo *lacZ* fusion studies (Wanner and McSharry 1982). Since carbon source addition exerted the same effect as did different growth media, it can be concluded that carbon-starvation is closely correlated with the *pho*-independent expression. The effects of different carbon sources on *pho*-independent *ugp* expression were further examined by carrying out experiments analogous to those described above. When the kinetics of *ugp* derepression were examined, *pho*-independent expression was repressed by all three carbon sources tested (glycerol, glucose and succinate) during log phase growth, and derepressed in late log to early stationary growth phase (Fig. 2). We observed that only glucose exerted a concentration-dependent complete repression. In the presence of high concentrations of glucose (1–2%), *pho*-independent *ugp* expression was essentially completely repressed. Addition of cAMP relieved this repression (Table 2). The

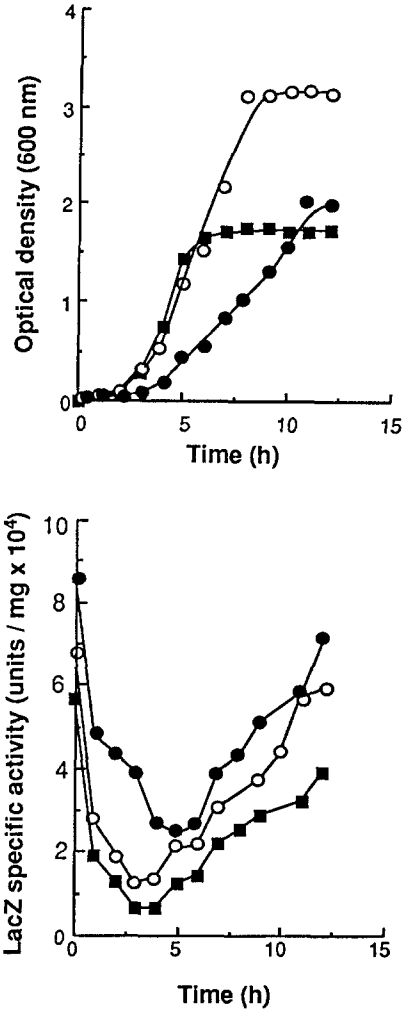

Fig. 2. Effect of carbon starvation on P_{ugp}-directed β-galactosidase expression. Strain DL291/pPZ3 was pregrown in TGE medium (glucose, *closed squares*) or in TE medium in which glucose was replaced by the indicated carbon sources (glycerol, *open circles;* succinate, *closed circles*) until complete *pho*-independent derepression was achieved. The cultures were washed twice with carbon-free TE medium. At time zero, the indicated carbon sources were added to the washed cultures to give final concentrations of 0.4%, except for glucose which was added to a final concentration of 0.2%. At the indicated time points aliquots were assayed for optical density (upper panel), and β-galactosidase (lower panel) was assayed as described in the legend to Table 1

Table 2. Effect of different carbon sources on *pho*-independent P_{ugp}-directed β-galactosidase synthesis[a]

Carbon source	Concentration (%)	cAMP[b] (mM)	β-Galactosidase (units/mg)
Succinate	0.4	0	103682
Glycerol	0.4	0	93673
Glucose	0.2	0	64738
Glucose	2.0	0	5945
Glucose	2.0	5	49579

[a] Strain DL291 harboring pPZ3 [$\Phi(P_{ugp}$-*lacZ*)] was grown in TGE medium overnight (12 h) and β-galactosidase was assayed as described in Table 1

[b] cAMP was added during late log phase ($A_{600} = 0.8$)

Table 3. P_{ugp}-directed catechol 2,3-dioxygenase expression in *crp*$^+$ *cya*$^+$ and *crp cya* strains[a]

Strain	Genotype	Excess P_i	Limiting P_i
CA8001[b]	*crp*$^+$ *cya*$^+$	2084	45047
CA8306[b]	*Δcya*-854	121	35318
JK7[b]	*Δcrp-BS*990	56	34308
CA8439[b]	*Δcrp*-39 *Δcya*-854	65	33495

[a] Strains harboring plasmid pPX3 were grown in TGE or TGL medium supplemented with 0.2% glucose and treated as described in Table 1. For strains harboring pPX3, 10 mM Fe^{3+} was required in growth media due to the high levels of XylE expression. Six hours after dilution into the indicated media, cells were suspended in 50 mM KPO_4 buffer (pH 7.5) containing 10% acetone (acetone assay buffer) and lysed by adding 10 µl of toluene plus 20 µl of 50 mM TRIS-HCl (pH 8.0), 100 mM EDTA, 100 mM DTT. The cell suspension was kept at 30 °C for 30 min and then placed on ice. The assay mixture for XylE was composed of 1 ml of acetone assay buffer, 20 µl of 0.5 mM catechol and 20 µl of lysed cells. The change in absorbance at 375 nm was recorded at room temperature. A molar extinction coefficient of $\varepsilon_{375} = 4.4 \times 10^4$ was used for calculating the specific activity. Specific activities are expressed in nmol of product liberated per minute per mg protein

[b] Strains CA8001, CA8306 and CA8439 were obtained from J. Beckwith. Strain JK7 was obtained from S. Adhya

pattern of the response of *ugp* expression to different carbon sources is reminiscent of catabolite-responsive enzyme synthesis, suggesting that the carbon-starvation induction of *ugp* may be directly or indirectly correlated with catabolite repression.

To further examine the possible involvement of the cAMP-CRP (cAMP receptor protein) control system in carbon-starvation induction, pPX3 was transformed into *crp* (JK7), *cya* (CA8306), and *cya crp* (CA8439) mutant strains and into a wild-type strain (CA8001), and levels of *ugp* expression were measured. The results shown in Table 3 indicate that the P_{ugp}-directed XylE synthesis was highly repressed in *crp* or *cya* mutants when compared to wild-type strain CA8001. These results are in agreement with the results described by Kasahara et al. (1991).

In summary, the results obtained in the present study are in agreement with previous reports that the *ugp* operon of *E. coli* is induced by both phosphate- and carbon-starvation (Wanner and McSharry 1982; Kasahara et al. 1991), although phosphate-starvation control is the major regulatory mechanism. The reasons for having a dual regulation of *ugp* expression are not clearly understood. Previous studies have shown that G3P transported via Ugp can only serve as sole source of P_i but not carbon, contrary to the conclusion reported by Kasahara et al. (1991). The reason for this so-called "Ugp paradox" is unknown, but it may be the result of a P_i imbalance caused by G3P uptake into the cell (Brzoska et al. 1987). Thus, the ability of the *ugp* promoter to be induced by phosphate- or carbon-starvation could provide the cell not only with a means to adapt to both stresses, but also with a means to fine tune the balance of carbon and phosphate.

At the present time, the mechanism(s) by which carbon starvation induction of P_{ugp} is exerted remain(s) to be elucidated. Lack of an apparent cAMP-CRP DNA binding site in the *ugp* control region (Overduin et al. 1988; Kasahara et al. 1991) indicates that the mechanism(s) that cause(s) carbon-starvation-induced *ugp* expression may themselves be cAMP-CRP-independent, and that the observed effects may be caused by cross-talk with some unidentified, globally controlled cAMP-CRP-dependent system.

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