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Regulation of Transcription of the *Escherichia coli* Phosphoenolpyruvate Carboxykinase Locus: Studies with *pck-lacZ* Operon Fusions

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Mutants of *Escherichia coli* containing genetic fusions of *lacZ* to the *pck* (phosphoenolpyruvate carboxykinase) locus were isolated by using Mu d(*lacZ* Amp^r) bacteriophage. Synthesis of β-galactosidase in these strains is regulated by cyclic AMP and glucose (catabolite repression). Synthesis of β-galactosidase by *pck-lacZ* fusions was induced in log-phase cells growing on gluconeogenic media, was repressed by glucose, and was also induced up to 100-fold at the onset of stationary phase in LB medium. This stationary-phase induction required cyclic AMP and some other unknown regulatory signal.

Phosphoenolpyruvate (PEP) carboxykinase (EC 4.1.1.49) is regulated by catabolite repression, and maximum levels of this enzyme are also induced at the onset of the stationary phase of growth (4). Catabolite repression probably serves the purpose of inhibiting gluconeogenesis when glucose and other carbohydrate carbon sources are available. Stationary-phase induction of PEP carboxykinase may be required for the synthesis of carbohydrate storage reserves in the stationary phase. It has been observed that glycogen synthesis (14), proteolysis (15), and certain Krebs cycle enzymes (3) are also induced in the stationary phase (4).

Previous studies of PEP carboxykinase induction have been limited by the insensitivity of assays for this enzyme in crude extracts or toluene-treated cells. It appears, however, that stationary-phase induction requires a regulatory signal in addition to cyclic AMP (cAMP), since induction does not occur in log-phase cells grown on LB medium in the presence of 5 mM cAMP. The mechanism of stationary-phase induction has not been determined, and fluctuations in intracellular cAMP levels have not been ruled out.

In this work, fusions of the transcriptional control sites of the PEP carboxykinase gene (*pck*) to the β-galactosidase structural gene (*lacZ*) have been isolated and characterized to assess whether stationary-phase induction is exerted at the transcriptional level and to develop a sensitive and convenient assay for *pck* expression. Mu d(*lacZ* Amp^r) bacteriophage was used to generate one-step operon fusions (2) at the *pck* locus. Induction of β-galactosidase in *pck-lacZ* strains grown on different media has been examined. These experiments showed that the *pck-lacZ* fusions resembled the known variation of PEP carboxykinase enzyme levels, implying that PEP carboxykinase is regulated at the transcriptional level. The magnitude of the stationary-phase induction was shown to be up to 100-fold in *pck-lacZ* fusions. Induction data were obtained for glycerol and succinate carbon sources, and intracellular cAMP levels were correlated with stationary-phase induction for LB medium.

MATERIALS AND METHODS

Growth conditions and media. The growth conditions and media used have been described previously (4). All strains containing Mu dl bacteriophage were grown at 32°C from a 1 to 5% inoculum of fresh overnight culture grown on the same medium and shaken at 300 rpm on an orbital shaker. The carbon sources indicated below were added to medium A at 0.4%. LB medium contained 1% tryptone (Difco), 0.5% yeast extract (Difco), and 1% NaCl, adjusted to pH 7.2. LB plus glucose contained, in addition, 0.4% glucose. LBCM was LB medium plus 10 mM $CaCl_2$ and 5 mM $MgCl_2$. Tetracycline, kanamycin, and ampicillin were added to media at 20, 25, and 30 μg/ml, respectively. Indicator plates contained 5-bromo-4-chloro-3-indoyl-β-D-galactoside at a concentration of 40 μg/ml (8).

Bacterial strains. Bacterial strains used were all derivatives of *Escherichia coli* K-12 and are listed in Table 1. Strains with fusions of *lacZ* to the *pck* promoter were isolated with Mu d(*lacZ* Amp^r *c*ts62) phage (2), which is referred to below as Mu dl. The doubly lysogenic strain HG90 (Mu dl; Mu *hpl* Kan^r *c*ts62) was used to prepare lysates of Mu dl (6). HG90 was unstable and was constructed freshly as required from singly lysogenic strains (6).

Strain DF1651 (*pps lac*; Table 1) was used for the isolation of *pck-lacZ* fusions since the *pps* mutation in combination with a *pck* mutation produces a Succinate$^-$ (Suc$^-$) phenotype (inability to grow on medium A with 0.4% succinate as carbon source) (4). DF1651 was grown to a density of 10^8 cells per ml in LBCM at 32°C and infected with a lysate derived from strain HG90. The multiplicity of infection was adjusted to give a predominance of single lysogens (Amp^r Kan^s) compared with double lysogens (Amp^r Kan^r).

After infection with Mu dl, cells were grown overnight on medium A plus 0.4% glucose and subjected to cycloserine selection three times (6), selecting for mutants able to grow on glucose but not succinate as a carbon source. Cells were spread on LB plates containing ampicillin. Small colonies were picked onto succinate and glucose plates, since *pck pps* strains produce small colonies on LB medium without glucose (unpublished data). Suc$^-$ isolates were screened for PEP carboxykinase activity, and their mutations were mapped on the *E. coli* chromosome as described below.

Strain HG120 (*pps* Δ*lacU169*) was constructed in four steps. The *his*::Tn*10* insertion from strain DB6989 was transferred into strain YMC9 by transduction with P1 *vir* (8), selecting for resistance to tetracycline. The *argG*::Tn*5* insertion from strain DB6924 was then transduced into the resulting strain (selecting for kanamycin resistance). The resulting His$^-$ Tet^r Arg$^-$ Kan^r strain was mated for 20 min

with strain AT2572-1 (Hfr *pps*) and plated on minimal medium A plates lacking histidine and containing kanamycin. The resulting His$^+$ Tet^s Arg$^-$ Kan^r colonies were screened for the *pps* mutation (growth on pyruvate as a carbon source) (4) and the *lac* deletion of strain YMC9 (Table 1). A *pps* Δ*lacU169 argG*::Tn*5* isolate was transduced to Arg$^+$ (Kan^s) to produce strain HG120 (*pps lacU169*), using P1 *vir* grown on strain YMC9.

Strain HG154 (*pck-13*::Mu dl *pps* Δ*lacU169* Δ*cya zie-290*::Tn*10*) was constructed in two steps. The *zie*::Tn*10* insertion, which maps near *cya* (1), was transduced into strain CA8445 (Δ*cya* Δ*crp*), selecting for tetracycline resistance. P1 *vir* lysates were made from 12 Tet^r transductants and used to transduce strain HG137 (*pck-13*::Mu dl *pps* Δ*lacU169*) to Tet^r on tetrazolium plates containing 1% arabinose and 1% galactose (and tetracycline). Tet^r Ara$^-$ Gal$^-$ colonies were obtained from 3 of the 12 lysates at a frequency of about 20%, indicating that the Δ*cya* deletion had been transferred. The other nine lysates produced only Tet^r Ara$^+$ Gal$^+$ colonies.

Biochemical procedures. Assays of β-galactosidase activity in cells treated with hexadecyltrimethylammonium bromide were as described by Rothstein et al. (12). Assays of PEP carboxykinase activity in toluene-treated cells and French-pressed extracts were described previously (4).

Samples of cultures for the determination of intracellular and extracellular cAMP were prepared as described by Peterkofsky and Gazdar (11), except the volume of culture sampled was 1 ml instead of 10 ml, the dried cell samples were added to 50 mM sodium acetate buffer at pH 6.2 instead of pH 4, and extracellular fluid was also adjusted to pH 6.2. cAMP was assayed by ^{125}I radioimmunoassay (13) with the kit available from New England Nuclear Corp. [^{3}H]cAMP (3.64 nCi, equivalent to ca. 0.1 pmol) was added to the cells after filtration to determine the recovery of cAMP during extraction. The assay was verified by measuring the amount of immunologically active cAMP before and after treatment of samples with 0.2 U of cyclic nucleotide phosphodiesterase (enzyme from beef heart; Sigma Chemical Co.). Intracellular cAMP concentrations were calculated by using a value of 2.9 $\times$ 10^{-9} mg of protein per cell (from Lowry protein determinations and plate counts) and a cell volume of 10^{-12} cm^3 (11).

RESULTS

Isolation of *pck-lacZ* fusions. A typical lysate prepared by thermal induction of strain HG90 (Mu dl; Mu *hpl* Kan^r *cts62*) had titers of 2 $\times$ 10^{10} PFU/ml, 10^4 Kan^r transductants per ml, and 2 $\times$ 10^5 Amp^r transductants per ml when used to infect strain DF1651. Similar titers have been obtained with other *E. coli* strains (6). When 200 Amp^r colonies were picked on LB plates containing both ampicillin and kanamycin, 9 colonies were Amp^r Kan^r, indicating that ca. 5% of the Mu dl lysogens also contained Mu *hpl* Kan^r *cts62* helper phage.

A total of 138 Suc$^-$ Amp^r isolates were obtained by infection of DF1651 (*pps lac*) with Mu dl and cycloserine selection as described above. From these, 11 independent isolates were obtained which had undetectable levels of PEP carboxykinase activity (<0.5 nmol min^{-1} mg^{-1}) when assayed in French-pressed extracts and were Amp^r and Kan^s. To determine whether these 11 isolates had Mu dl insertions in the *pck* gene, P1 lysates of strains HG49 (*pps asd*), HG53 (*pps glpD3*), and HG47 (*pps aroB351*) were used to transduce each isolate to Suc$^+$. Of the Suc$^+$ transductants, 99% were Amp^s (at least 200 colonies each) and showed linkages with the *asd* gene (18 to 30%), the *glpD* gene (32 to 59%), and the *aroB* gene (18 to 26%) similar to those reported for the *pck* structural gene (4).

TABLE 1. Bacterial strains

Strain	Genotype	Source
HG90	Mu dl; Mu Kan^r *hpl* cts62	Reference 6
DF1651	F$^-$ *thi pps lac pyrD edd-1 his tyrA rpsL*	Institut Pasteur
YMC9	Δ*lacU169 endA1 thi-1 hsdR17 supE44*	B. Magasanik
HG120	*pps* Δ*lacU169* derivative of YMC9	This work
HG49	HfrH *pps asd*	Reference 4
HG47	*pps aroB351 mal-354 tsx-352*	Reference 4
HG53	*pps glpD3 pit-1 pst-2 leu*	*pck*$^+$ derivative of HG54, reference 4
SK2226	F$^-$ *zie-290*::Tn*10* Δ(*gpt-proA*)*62 trp-3 hisG4 argH1*	CGSC[a]
CA8445	HfrH Δ*cya* Δ*crp*	J. Beckwith
DB6989	*his*::Tn*10 pro endA1 hsdR17 supE44*	D. Botstein
DB6924	*argG*::Tn*5 pro endA1 hsdR17 supE44*	D. Botstein
AT2572-1	Hfr *pps*	CGSC[a]

[a] *E. coli* Genetic Stock Center.

Revertants, obtained from the *pck-lacZ* fusion strains on succinate plates at a frequency of about 10^{-9}, had also regained the ability to grow on medium A plates with 0.4% pyruvate as a carbon source and still had undetectable levels of PEP carboxykinase. These were therefore *pps*$^+$ revertants (4), and *pck*$^+$ revertants were not detected.

Five of the *pck*::Mu dl insertion strains produced blue colonies on 5-bromo-4-chloro-3-indoyl-β-D-galactoside plates after 40 h, whereas six strains formed white colonies. This was probably caused by random insertion of Mu dl, causing about one-half of the isolates to be oriented in the direction required for transcription from the *pck* promoter (2, 6, 12).

All of the *pck*::Mu dl insertion strains isolated from strain

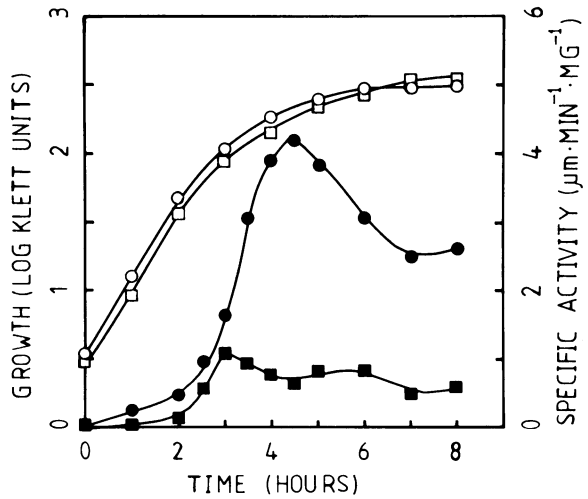

FIG. 1. Growth (optical density in Klett units) and β-galactosidase specific activity were plotted for strain HG137 (*pck-13*::Mu dl). Symbols: ○, growth on LB medium; ●, β-galactosidase levels for LB medium; □, growth on LB plus 0.4% glucose; ■, β-galactosidase levels for LB plus glucose.

DF1651 (*pps lac*) segregated a few colonies (0.5 to 2%) which were darker or lighter colored on 5-bromo-4-chloro-3-indoyl-β-D-galactoside plates. This may have been due to recombination between the *lac* genes on Mu d1 and the *lac* operon from strain DF1651. To stabilize the *pck*::Mu d1 insertions they were transduced into a *pps* strain with a deletion of the entire *lac* operon. Strain HG120 (*pps lacU169*) was constructed, as described above, and transformed with plasmid pGW600 which contains the Mu c^+ gene coding for the Mu repressor (7). Each *pck*::Mu d1 insertion was transduced into HG120(pGW600), selecting for Amp^r. Amp^r isolates were then streaked on LB plates, and Tet^s colonies were isolated which were cured of the plasmid. The genetic linkages of the *pck*::Mu d1 insertions to *glpD* and *aroB* were then rechecked as described above in the *pps lac* deletion strain. The Lac phenotypes of the *pck*::Mu d1 insertions in the *lacU169* deletion strain resembled the original phenotypes in strain DF1651, but were stable.

Stationary-phase induction of *pck-lacZ* fusions. All five of the strains expressing β-galactosidase activity showed a marked increase in specific activity just before the onset of the stationary phase of growth on LB medium. Typical results are shown for strain HG137 in Fig. 1. Addition of glucose to the LB medium resulted in a three- to fivefold depression of β-galactosidase levels with negligible effects on cell growth. The other Lac^+ *pck-lacZ* fusion strains had similar growth and induction curves in the presence or absense of glucose (data not shown), except there was variation in the maximum levels of β-galactosidase produced. Strain HG132 had levels similar to those of strain HG137, whereas levels in strains HG138, HG134, and HG139 were lower (Table 2). In all cases, levels were depressed by the addition of glucose (Table 2). Induction of β-galactosidase in stationary phase and reduction of β-galactosidase levels by glucose support the view that the *lacZ* gene is fused to the *pck* promoter (4, 5).

TABLE 2. Enzyme levels in *pck-lacZ* fusion strains

Strain	Genotype	β-Galactosidase (nmol min^{-1} mg^{-1})[a]		PEP carboxykinase (nmol min^{-1} mg^{-1}) in LB[b]
		LB	LB + glucose	
DF1651	*pps lac*	<0.5	ND[c]	32
HG120	*pps ΔlacU169*	<0.5	ND	28
HG137	*pck-13*::Mu d1 *pps ΔlacU169*	4,800	1,100	<0.5
HG132	*pck-8*::Mu d1 *pps ΔlacU169*	4,700	1,200	<0.5
HG138	*pck-14*::Mu d1 *pps ΔlacU169*	1,700	430	<0.5
HG134	*pck-10*::Mu d1 *pps ΔlacU169*	1,300	430	<0.5
HG139	*pck-15*::Mu d1 *pps ΔlacU169*	76	24	<0.5
HG133[d]	*pck-9*::Mu d1 *pps ΔlacU169*	<0.5	<0.5	<0.5
HG149	*pck-13*::Mu d1 pps^+ *ΔlacU169*	4,600	1,500	<0.5
HG154	*pck-13*::Mu d1 *Δcya pps ΔlacU169*	490	ND	ND

[a] Maximum levels of β-galactosidase were determined from induction curves, similar to that in Fig. 1, on LB medium in the presence or absence of 0.4% glucose.

[b] PEP carboxykinase levels were assayed in cells grown to early stationary phase (ca. 250 Klett units) in 500 ml of LB medium and disrupted with a French pressure cell.

[c] ND, Not done.

[d] Five other Lac^- *pck*::Mu d1 insertion strains had enzyme levels similar to those of HG133 (data not shown).

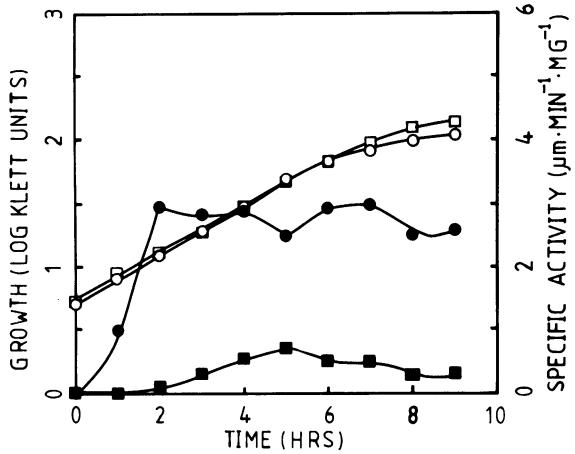

FIG. 2. Growth on minimal medium (Klett units) and β-galactosidase specific activity were plotted for strain HG137 (*pck-13*::Mu d1). Symbols: ○, growth on medium A plus glycerol; ●, β-galactosidase for medium A plus glycerol; □, growth on minimal medium plus glucose; ■, β-galactosidase for medium A plus glucose.

Strain HG137 was also grown on LB plus 5 mM cAMP and LB plus glucose plus 5 mM cAMP. Growth curves on LB medium were not affected significantly by cAMP, and induction curves (not shown) were similar to those for HG137 grown on LB medium without additions (Fig. 1).

Levels of β-galactosidase increased up to 100-fold in *pck-lacZ* strains grown on LB medium (Fig. 1) just before the onset of stationary phase. This contrasts with the five- to sixfold induction of PEP carboxykinase activity previously observed in pck^+ strains (4). The reason for this difference may be the much higher sensitivity of the β-galactosidase assay which permits more accurate measurements of low levels of enzyme activity. The previous induction studies with small volumes of cells treated with toluene probably underestimated the induction of PEP carboxykinase. Also, levels reported for cells growing logarithmically were often based on cells harvested at ca. 100 Klett units (4), which was probably too high a density to allow minimum levels of PEP carboxykinase activity to be observed. Careful measurement of PEP carboxykinase levels in 500-ml cultures of DF1651 grown on LB medium to log phase (50 Klett units) and early stationary phase (250 Klett units) revealed that log-phase levels were undetectable (<0.5 nmol min^{-1} mg^{-1}) and early stationary phase levels were 25 to 35 nmol min^{-1} mg^{-1}. This indicated that PEP carboxykinase levels were induced at least 50-fold during the stationary phase of growth.

Expression of *pck-lacZ* fusions during growth on minimal media. Figure 2 shows the growth and β-galactosidase levels for strain HG137 grown on medium A plus glycerol and medium A plus glucose. It is apparent that β-galactosidase induction occurred in the log phase on minimal medium plus glycerol and that levels were lower when glucose was the carbon source than when glycerol was the carbon source. Similar results were obtained with strain HG132 (data not shown).

To determine whether the *pps* mutation has any effect on a *pck-lacZ* fusion, a P1 *vir* lysate of strain YMC9 (pps^+) was used to transduce *pck-lacZ* fusions to pps^+ on pyruvate plates. All such $Pyruvate^+$ isolates were Suc^+ as expected for pps^+

recombinants (4). β-galactosidase levels in strain HG149 (*pps*$^+$ *pck-13*::Mu dl) are shown in Table 2. Induction curves from this strain (not shown) were very similar to those of HG137 when cultures were grown on LB, LB plus glucose, medium A plus glycerol, and medium A plus glucose. When this strain was grown on medium A plus succinate, growth was quite slow (doubling time of 190 min) and β-galactosidase levels were quite high (ca. 5,000 nmol min^{-1} mg^{-1}) from start to finish of the growth (data not shown).

Effects of cAMP levels on *pck* transcription. In light of the marked effects of glucose and cAMP on the expression of *pck-lacZ* fusions, it is of interest to determine whether cAMP is essential for transcription of the *pck* gene. Strain HG154 (*pck-13*::Mu dl Δ*cya*) was therefore constructed as described above. This strain had very low maximum levels of β-galactosidase expression (almost 10-fold lower than the *cya*$^+$ fusion strain) when grown on LB medium (Fig. 3). When HG154 was grown on LB plus 5 mM cAMP, however, β-galactosidase levels were as high as those of the wild type (Fig. 3).

The levels of cAMP in strains HG137 (*cya*$^+$ *pck-13*::Mu dl) and HG154 (Δ*cya pck-13*::Mu dl) grown on LB medium are shown in Fig. 4. These data are from experiments shown in Fig. 1 and 3. Intracellular cAMP levels were corrected for extracellular cAMP trapped on the filter and cells (10). It is evident that the levels of cAMP in the *cya*$^+$ *pck-lacZ* strain were high and fairly constant during growth on LB medium (see reference 10). No increase in cAMP level was observed in connection with the induction of β-galactosidase synthesis at the onset of the stationary phase, although such increases have been reported during the stationary phase for other growth media (10). The Δ*cya pck-lacZ* strain had fairly constant, low levels of cAMP. The low level of antigen measured in the Δ*cya* strain was probably cAMP, since the levels were within the range of the immunoassay and were reduced to undetectable levels by treatment with cyclic nucleotide phophodiesterase. The extracellular cAMP level was 10 to 18 nM for the Δ*cya* strain, but this was probably due to the presence of 30 nM cAMP in fresh LB medium (which includes yeast extract). Also, the sensitivity of the assay to ATP, ADP, AMP, cCMP, and cGMP was less than 0.01% of the sensitivity to cAMP at the midpoint of the standard curve (data not shown).

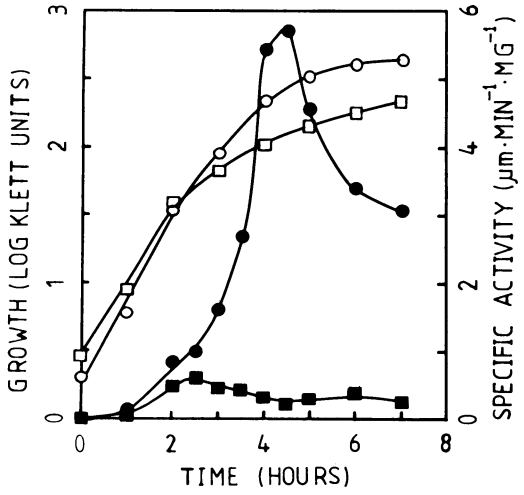

FIG. 3. Growth (Klett units) and β-galactosidase specific activity for strain HG154 (Δ*cya pck-13*::Mu dl) grown on LB medium in the presence or absence of 5 mM exogenous cAMP. Symbols: □, growth on LB medium; ■, β-galactosidase levels on LB medium; ○, growth on LB plus 5 mM cAMP; ●, β-galactosidase levels for LB plus 5 mM cAMP.

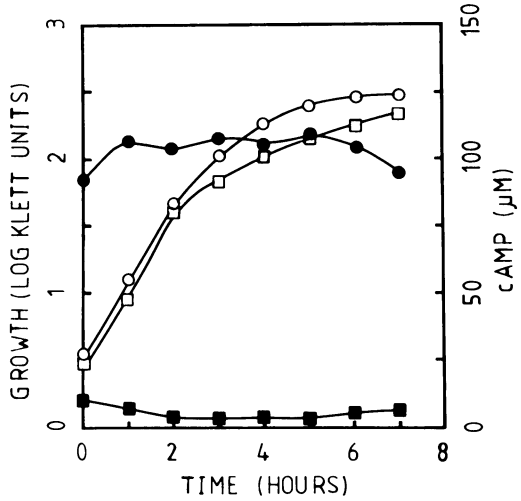

FIG. 4. Growth (Klett units) and cAMP levels for strains HG137 (*pck-13*::Mu dl) and HG154 (Δ*cya pck-13*::Mu dl) grown on LB medium. Symbols: ○, growth of strain HG137; ●, cAMP levels for HG137; □, growth of strain HG154; ■, cAMP levels for HG154.

DISCUSSION

The *pck-lacZ* fusions were induced up to 100-fold at the onset of the stationary phase in cells grown on LB medium (Fig. 1), and the levels of β-galactosidase were lowered by the addition of glucose to the medium (Fig. 1, Table 2). These data support the idea that the *lacZ* structural gene is fused to the *pck* promoter. A previous study, with the less-sensitive $^{14}CO_2$ exchange assay for PEP carboxykinase in toluene-treated cells, underestimated induction of the *pck* gene (4), since the present study demonstrated stationary-phase induction of up to 100-fold with *pck-lacZ* fusions. This was confirmed here by more careful measurement of PEP carboxykinase levels in larger cultures disrupted with a French pressure cell.

Five different *pck-lacZ* fusions expressed a range of maximum levels in β-galactosidase expression (Table 2). This phenomenon has been observed in other fusion studies (e.g., see references 2 and 6) and may involve polar effects of different fusion joints on β-galactosidase translation. Variation in expression could be caused by terminator sites or multiple promoters in the leader region of a gene or by adjacent deletions to a gene whose regulation resembled that of *pck*. All five *pck-lacZ* fusions were induced in the stationary phase and repressed by glucose (Table 2), had the specific growth phenotype of *pck* mutations (4), and represented unique Mu dl insertions at or near the *pck* locus based on transduction data. It is therefore likely that they were caused by insertions in the *pck* locus or operon.

The *pck-lacZ* fusions were induced during the log phase when cells were grown on minimal medium plus glycerol (Fig. 2). This observation was not made previously (4). It is likely that high cAMP levels in glycerol-grown cells (9) contribute to this induction, although induction did not occur until the stationary phase with LB medium (when cAMP levels were also high). Levels of β-galactosidase were lower when *pck-lacZ* fusion strains were grown on minimal medium plus glucose, in which cAMP levels are known to be

lower (9, 10). When the *pps*$^+$ *pck-lacZ* fusion strain was grown on medium A plus succinate, β-galactosidase levels were 60 to 70% higher than on medium A plus glycerol and constantly elevated throughout the growth (data not shown). This could be due to higher cAMP levels in cells grown on succinate (9) or due to induction by succinate or other Krebs cycle intermediates. Induction by Krebs cycle intermediates would have a function in the cells, since succinate is a precursor of the substrate of PEP carboxykinase, oxaloacetic acid. Decline of β-galactosidase specific activity after maximum induction (Fig. 1) could be attributed to cessation of enzyme synthesis just before cessation of growth as indicated by differential plots of the data (data not shown).

A deletion of the *cya* (adenyl cyclase) gene lowered the expression of a *pck-lacZ* fusion more drastically than the addition of glucose to the medium (Fig. 1 and 3), but cAMP reversed the effects of the *cya* deletion or glucose. The low residual levels of β-galactosidase in the Δ*cya pck-lacZ* strain may be due to cAMP in the LB medium (as discussed previously). Unfortunately, this strain grew extremely slowly on minimal medium plus glycerol or succinate, and induction curves could not be generated. It is evident from the experiments with the Δ*cya pck-lacZ* strain that cAMP is necessary for induction of PEP carboxykinase synthesis.

On LB medium, β-galactosidase synthesis was not induced in *pck-lacZ* fusions until the onset of the stationary phase, although, cAMP levels were high throughout growth (Fig. 1 and 4). Also, the *pck-lacZ* fusions were not induced during log-phase growth on LB medium plus 5 mM cAMP (data not shown). It is likely that another regulatory signal in addition to cAMP is involved in the induction of PEP carboxykinase synthesis in the stationary phase.

It has been reported that the enzymes of glycogen biosynthesis (14) and proteolysis (15) and certain Krebs cycle enzymes (3) are induced during the stationary phase. These inductions may serve the purpose of providing storage reserves in stationary-phase cells. Gluconeogenesis, and therefore PEP carboxykinase activity, would be required for glycogen synthesis in the stationary phase. In any case, it would be of interest to determine the mechanism by which PEP carboxykinase synthesis is induced in stationary phase and whether it is related mechanistically to other stationary-phase inductions. One possibility is that a type of catabolite repression which can not be reversed by cAMP (16) lowers PEP carboxykinase levels in the log phase on LB medium and enzyme synthesis is derepressed by depletion of catabolites in the stationary phase.

This work has shown that PEP carboxykinase synthesis is genetically regulated by cAMP and glucose (catabolite repression). PEP carboxykinase synthesis is induced during logarithic growth on gluconeogenic substrates (glycerol and succinate). Also, *pck* transcription is activated ca. 100-fold during the stationary phase on LB medium, which may serve to facilitate glycogen synthesis in the stationary phase. Stationary-phase induction depends upon the presence of cAMP and some additional, as yet unknown, regulatory signal.

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