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Between Feast and Famine: Endogenous Inducer Synthesis in the Adaptation of *Escherichia coli* to Growth with Limiting Carbohydrates

ALISON DEATH AND THOMAS FERENCI*
Department of Microbiology, The University of Sydney, NSW 2006, Australia

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***Escherichia coli* adapted to growth with low carbohydrate concentrations bypassed the requirement for exogenous inducer with at least three well-studied sugar regulons. Induction of *mgl* and *gal* genes became independent of added galactose in bacteria approaching stationary phase or during continuous culture with micromolar glucose in the medium. Bacteria became independent of exogenous induction because endogenous galactose and cyclic AMP (cAMP) pools were sufficient for high expression of *mgl* and *gal* genes under glucose limitation. Limitation-stimulated induction of *mgl* was dependent on a functional *galETK* operon for synthesis of the inducer galactose. Intracellular galactose levels were maximal not during starvation (or slow steady-state growth rates approaching starvation) but at fast growth rates with micromolar glucose. The extent of *mgl/gal* induction correlated better with inducer availability than with cAMP concentrations under all conditions tested. Endogenous inducer accumulation represents an adaptation to low-nutrient environments, leading to derepression of high-affinity transport systems like Mgl essential for bacterial competitiveness at low nutrient concentrations.**

The classic model of the *lac* operon sought to explain how intracellular enzyme synthesis is upregulated when a degradable substrate is present in the medium. The requirement of extracellular sugar for intracellular induction is generally believed to hold for other well-studied sugar regulons of *Escherichia coli*. As shown below, this notion is not true for bacteria growing on low concentrations of nutrients, in which at least three sugar regulons are highly expressed in the absence of an external inducer. Here we report that internally synthesized sugar constitutes, together with elevated cyclic AMP (cAMP) pools, a hunger response in regulating *mgl* and *gal* gene expression under nutrient limitation. Endogenous inducer levels are controlled, so that galactose accumulates under conditions of scavengeable external nutrient concentrations and, to a lesser extent, under starvation or glucose-excess conditions. Hence, endogenous inducer plays a significant role in gene regulation under conditions of low-nutrient growth likely to be found in natural environments of *E. coli*; exogenous inducer is required for *gal/mgl* induction only in nutrient-rich conditions.

The idea that internally synthesized sugars permit high expression of sugar regulons is not new. But previous examples involved accumulation of sugars under nonphysiological conditions, mainly with mutants blocked in transport or metabolism. Indeed, one of the oldest reports of internal induction was of the *gal* and *mgl* operons, observed in *galK* mutants with elevated intracellular galactose levels (12). These early studies showed that an intracellular galactose pool of less than 2×10^{-4} M was sufficient for induction of Mgl and Gal functions (22). Another example of endoinduction was in the *mal* system, in which a mutation in *malQ* gives rise to elevated *mal* gene expression, because of increased levels of intracellularly synthesized maltosaccharides (7, 8).

The regulation of the *mal* and *mgl* systems by nutrient stress is particularly important for two reasons. First, the LamB glycoporin encoded in the *malKlamBmalM* operon was recently shown to have a general role in outer membrane permeability of carbohydrates under conditions of low extracellular glucose, lactose, arabinose, and glycerol concentrations (6). Second, the periplasmic glucose/galactose binding protein-dependent Mgl system, together with the LamB glycoporin, constitutes a high-affinity glucose transport system important for the competitive survival of *E. coli* under glucose limitation (5). In these studies, elevated LamB protein levels and Mgl activity were seen in the absence of maltodextrins or galactose, in the presence of glucose, conditions not previously thought to be favorable for expression of *mgl* or *mal-lamB* genes. These results prompted the current attempt to define the regulation of the *mgl* genes under glucose-limited growth conditions.

The high level of Mgl activity under glucose limitation posed the interesting question of how genes regulated by the MglD/GalS repressor as well as by cAMP receptor protein (Crp)-cAMP complex (20) respond to nutrient stress. High expression would be expected if either the repressor was downregulated or internal sugar inducer was produced. Downregulation of repressor seemed unlikely, as *galS* is actually upregulated by Crp-cAMP. Hence, we tested the possibility that limitation conditions increase the level of endogenous galactose as a means of overcoming repressor activity. In view of the finding that the GalR repressor binds the same inducers as does the GalS/MglD repressor, the level of induction of the *gal* regulon was also investigated; both regulons appeared to be upregulated by internal inducers under conditions of nutrient-limited growth.

The expression of sugar regulons, including *mgl*, is modified by Crp-cAMP function, so it was also pertinent to test levels of cAMP under conditions of high induction of the *mgl* and *gal* systems. Indeed, in confirmation of earlier findings (15), cAMP levels were much higher under carbohydrate limitation conditions and contributed to high expression of these regulons, consistent with cAMP being a global signaller of sugar limitation or starvation (14, 19). But strikingly, maximal *mgl* induc-

* Corresponding author. Phone: (612) 692-4277. Fax: (612) 692-4571.

TABLE 1. Bacterial and phage strains used in this study

Strain or phage	Genotype	Parent strain	Reference or source
MC4100	F⁻ *araD*139 Δ(*argF-lac*)U159 *rpsL150 relA1 deoC1 ptsF25rbsR25flbB*5301		4
BD21	MC4100 *mglB551 mglA*::λplacMulΦ(*mglA-lacZ*)hyb525		3
Δ4	F⁻*trpC recA rpsL sup*° Δ4(*gal-chlD-pgl-att*)		2; from P. Reeves as P3127
TP2139	F⁻*xyl argH1 ilvA lac*ΔX74 Δ*crp-39*		18
LA5731	F⁻ *ptsF lacY arg mgl-515 zee-700*::Tn*10*(P1*cml clr*1000)		W. Boos
LA5715	F⁻*lacY galK zbg*::Tn*10*		W. Boos
LA5711	F⁻*lacY zbg-710*::Tn*10*		W. Boos
BW2000	HFrG6 *mgl*::Tn*10 his*		5
BW2926	MC4100 *mgl*::Tn*10*	MC4100	This study
BW2931	MC4100 *gal*(Δ4) *zbg*::Tn*10*	MC4100	This study
BW2932	MC4100 *galK zbg*::Tn*10*	MC4100	This study

tion did not coincide with optimal cAMP concentrations inside bacteria, confirming that cAMP was not the only determinant of *mgl* expression under nutrient limitation.

We also address the question of whether the endogenous inducer synthesis in low-sugar habitats is part of the well-reviewed starvation or stationary-phase responses (10, 13, 14). Recently, these states have been most frequently studied with batch cultures entering starvation by exhausting a limiting nutrient or in agar plates where the concentration of limiting nutrient is undefined after growth but is probably also exhausted. Our results suggest that endogenous inducer synthesis is characteristic of steady-state growth but at rates limited by low (micromolar) concentrations rather than by total nutrient deprivation. Batch or plate cultures are only transiently under conditions favoring this hunger response, which is why it requires a chemostat to recognize this state.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains were derivatives of *E. coli* K-12 and are shown in Table 1. P1-mediated transduction (16) with P1 *cml clr*1000 grown on LA5731 as the donor was used to make a lysogen of BW2000. The P1 lysate from this lysogen was used to introduce *mgl*::Tn*10* into MC4100 to derive the Mgl⁻ strain BW2926. BW2931 was constructed by first preparing a lysogen derivative of LA5711 with P1 *cml clr*1000 grown on LA5731. A lysate produced from the lysogen was used to transduce Tn*10* into P3127. From one transductant, a P1 lysogen was derived and lysate was prepared which was used to transduce MC4100 to Tet^{r}Gal⁻. BW2932 was constructed by first preparing lysogen derivative of LA5715 with P1 *cml clr*1000 grown on LA5731. This strain was used to prepare the lysate used to transduce MC4100 to Tet^{r}GalK⁻.

Growth media and culture conditions. Batch-cultured bacteria were supplied with a carbon source at 0.2% (wt/vol), incubated at 37°C, and harvested during mid-log phase. For chemostat cultures, 80-ml positive-pressure chemostats and media as described in reference 6 were routinely used for continuous culture. The determination of intracellular galactose concentration needed larger culture volumes, and a commercially designed 570-ml chemostat with controls (LH Engineering, Stoke Poges, Buckinghamshire, England) was used. The inoculation and sampling of the chemostats was as previously described (5, 6). The residual glucose concentration of cultures was monitored by using glucose oxidase, also as previously described (5).

Assay of cAMP in cultures. The intracellular and extracellular concentrations of cAMP were determined with either a commercial radioimmunoassay kit (^{125}I dual range; Amersham International, Sydney, Australia) or an enzyme-linked immunoassay kit (dual range; Amersham International). Control standards revealed that the two kits gave comparable results. To determine the intracellular cAMP concentration, bacteria were grown as described above and 5 ml of culture was filtered by suction directly from the chemostat through a Supor-200 polysulfone membrane filter (pore size, 0.2 μm). The filter was immediately placed in 5 ml of ice-cold 60% ethanol solution, and the sample was stored at −20°C. Prior to the assay, the ethanol was evaporated by blowing air over the samples held on ice, and the residue was resuspended in 5 ml of cAMP assay buffer (supplied with either kit). To determine the extracellular cAMP concentration, the culture filtrate was collected as described above and was stored at −20°C prior to the assay. The enzyme-linked immunoassay procedure was in accordance with instructions provided by the manufacturer. The protocol for the radioimmunoassay was also as recommended, except that all volumes were halved.

Intracellular galactose concentration. The preparation of the cell extract was as described by Ehrmann and Boos (8), except that volumes were reduced. Bacteria from a 570-ml chemostat (0.04% carbon source in input medium) or from 500-ml batch cultures (0.2% carbon source) were harvested by centrifugation (6,000 rpm, 6,370 × *g*, 10 min). The pellet was washed in 50 ml of minimal medium A (MMA) and rewashed in 10 ml of MMA before being resuspended in water (to a final volume of 1 ml), at which point the optical density (A_{580}) was recorded. To lyse bacteria, 1 to 2 ml of boiling ethanol was added. The ethanol was immediately removed by evaporation at 85°C, before cell debris was removed by centrifugation. The supernatant was deionized by the addition of 1 g of Dowex MR3 mixed-bed ion-exchange resin before concentration by freeze-drying to a final volume of 50 μl in galactose oxidase buffer (36.3 g of Tris and 50 g of NaH_2PO_4 per 100 ml). The galactose concentration in the sample was measured by using galactose oxidase in an assay based on the assay using glucose oxidase (11). Fifty-microliter samples or standards (5×10^{-4} to 5×10^{-3} M) were added to 100 μl of reaction mixture (consisting of 2 ml of galactose oxidase buffer, 30 μg of horseradish peroxidase [Boehringer-Mannheim Australia, Castle Hill, Australia], 0.2 mg of *O*-dianisidine-di-HCl, and 30 U of galactose oxidase [Sigma Chemical Co., St. Louis, Mo.]). The mixture was vortexed for 10 s and was incubated at 30°C for 30 min. The reaction was stopped by the addition of 500 μl of 5N HCl. The end point was measured at A_{546}. To ensure that only intracellular galactose was being measured in galactose-grown cells, the supernatant from the final wash was concentrated by freeze-drying and was subjected to the galactose-oxidase assay. No galactose was detected in this wash.

Galactose transport assay. Samples (10 ml) from chemostat or batch cultures were harvested, processed, and assayed as previously described (5).

Galactokinase (GalK) assay. Fifty-milliliter samples from chemostat or batch cultures were washed twice in MMA and resuspended in 1 ml of MMA supplemented with 0.01 M mercaptoethanol and 0.001 M EDTA. Bacteria were lysed by sonication (2-min total, in 20-s pulses; Branson Cell Disruptor B15), and cell debris was removed by centrifugation. The

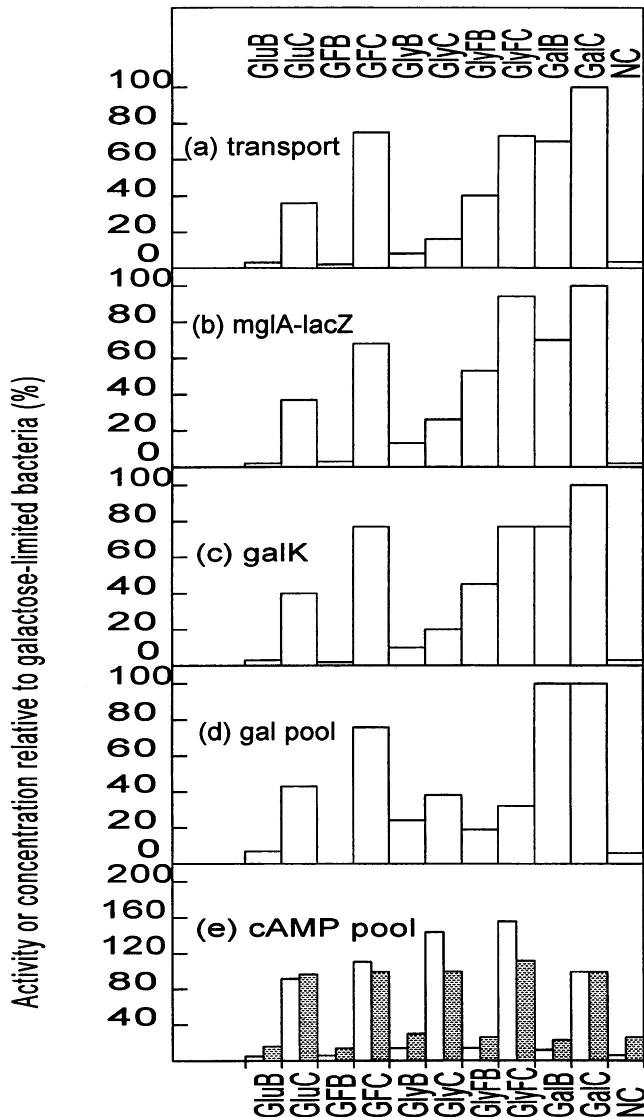

FIG. 1. Effect of carbon source and growth on Mgl and Gal induction in wild-type bacteria. Bacteria were grown in a glucose batch culture (GB); a glucose-limited chemostat (GC); a glucose plus 1 mM fucose batch (GFB); a glucose-limiting chemostat with 1 mM fucose (GFC); a glycerol batch (GlyB); a glycerol-limited chemostat (GlyC); a glycerol plus 1 mM fucose batch (GlyFB); a glycerol-limited chemostat with 1 mM fucose (GlyFC); a galactose batch (GalB); a galactose-limited chemostat (GalC); or a nitrogen-limited chemostat (NC). Batch cultures were supplemented with a 0.2% carbon source and were harvested during mid-exponential growth. For carbohydrate-limited chemostats, the input medium was supplemented with a 0.02% (wt/vol) carbon source, and the dilution rate was constant at $D = 0.3$ h^{-1}. The nitrogen-limited chemostat contained ammonium sulfate at a concentration of 0.045 g/liter and glucose at 0.4% (wt/vol). In all panels, activities or concentrations are given as percentages of the values obtained with bacteria grown under galactose limitation. Each column depicts a mean of 4 to 6 determinations. (a) Mgl transport by wild-type bacteria. Bacteria were harvested and washed in MMA, and the initial rate of uptake was measured as previously described, by using [^{14}C]galactose at a concentration of 1 μM (5). The 100% value for galactose-limited bacteria was 171 pmol of [^{14}C]galactose taken up per 10^8 bacteria per min. (b) Induction of *mgl* expression was assayed by measuring β-galactosidase activity of an *mglA-lacZ* fusion. The 100% value with galactose-limited bacteria was 608 Miller units (16). (c) GalK activity as a measure of *gal* induction. Cells were harvested, washed in MMA, and sonicated, and the rate of formation of [^{14}C]galactose-1-phosphate was determined. The 100% value with galactose-limited bacteria was 286 nmol of [^{14}C]galactose-1-phosphate formed per 10^9 bacteria per min. (d) The pool size of intracellular galactose. The extraction of intracellular galactose and the galactose oxidase assay used to determine galactose concentration are as described in Materials and Methods. The 100% value with galactose-limited bacteria was 0.38 mM intracellular galactose. (e) Accumulation of external (filled columns) and internal (open columns) cAMP in carbohydrate-rich and -limited cultures was measured by the radioimmunoassay method. The 100% value with galactose-limited bacteria was intracellular cAMP at 7×10^{-7} pmol per bacterium and extracellular cAMP at 3.6×10^{-6} pmol per bacterium.

protein concentration in the cell extract was determined by the BCA protein assay (Pierce Chemicals, Rockford, Ill.). The GalK assay used was as described by Wilson and Hogness (21).

β-Galactosidase assay. Five-milliliter samples from chemostat (0.02% carbon source) or batch cultures (0.2% carbon source, unless otherwise stated) were removed, and β-galactosidase activity was measured as described by Miller (16).

RESULTS

Derepression of the Mgl transport system during steady-state sugar-limited growth. Galactose transport, when assayed at micromolar concentrations, reflects the activity of the binding protein-dependent Mgl transport system (12). As shown recently, high levels of transport activity were seen in the absence of galactose in the medium under glucose limitation conditions in chemostats (5). As shown in Fig. 1a, the Mgl activity present in glucose-limited chemostats growing at a dilution rate of $D = 0.3$ h^{-1} was close to that found in glycerol batch cultures induced by the gratuitous inducer fucose, which is considered to be a highly induced state of Mgl. As also shown in Fig. 1a, chemostats limited by carbohydrates other than glucose, including glycerol and galactose, also had elevated levels of Mgl activity. As expected, galactose limitation gave the most favorable conditions for *mgl* expression. Glycerol limitation was less effective, but induction was still considerably higher than that found in batch cultures. Induction of Mgl activity was dependent on carbohydrate limitation rather than on starvation for any nutrient, as growth in a nitrogen-limited chemostat at identical dilution rates did not result in *mgl* derepression (Fig. 1).

Transport activity as assayed with galactose and the β-galactosidase activity of an *mglA-lacZ* transcriptional fusion gave parallel patterns of induction under chemostat conditions with different limiting nutrients, as shown in Fig. 1b. Hence the transport assay in Fig. 1a indeed reflected *mgl*-encoded activity and suggested that *mgl* expression was transcriptionally up-regulated. The presence of an *mgl*::Tn*10* mutation also abolished transport activity under all inducing conditions, indicating that the assayed transport was not a previously undefined activity (Fig. 2).

Intracellular galactose levels during glucose limitation. To explain the pattern of induction in Fig. 1a and b, we investigated the intracellular concentrations of the two signalling molecules known to influence *mgl* expression, namely, inducer (galactose) and cAMP. Intracellular sugars were extracted from bacteria as described by Ehrmann and Boos (8). Galactose was assayed by using galactose oxidase in the assay system developed for glucose oxidase (11). As shown in Fig. 1d, bacteria had high intracellular amounts of galactose under conditions leading to high Mgl activity; batch cultures had generally one sixth of chemostat levels. The concentration of

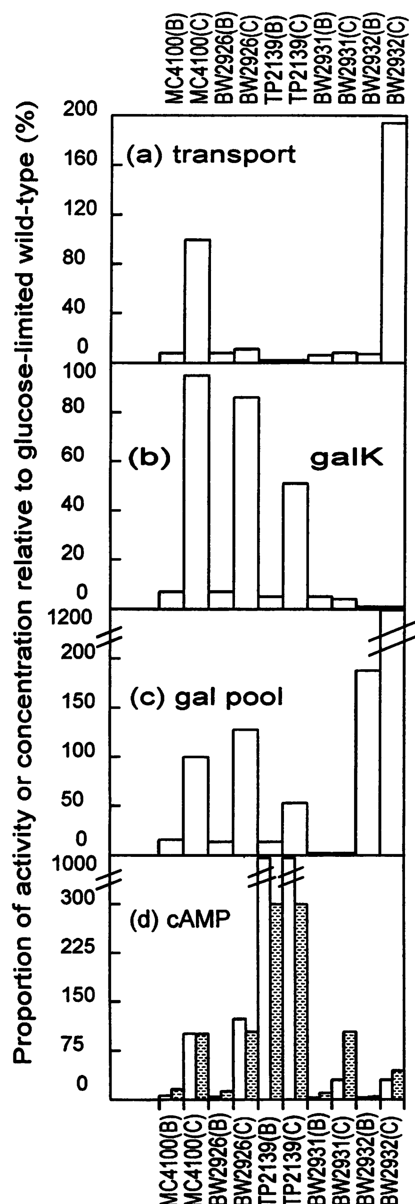

FIG. 2. Effect of *mal*, *gal*, and *crp* mutations on Mgl activity and galactose pools. The properties of wild-type (MC4100), Mgl^- (BW2926), $GalETK^-$ (BW2931), $GalK^-$ (BW2932), and Crp^- (TP2139) mutants grown in glucose-rich batch cultures (0.2%) and glucose-limited chemostat cultures (0.02%) are shown. In all panels, activities or concentrations are given as percentages of the values obtained with wild-type bacteria grown under glucose limitation. The bacteria were grown in chemostats with 0.02% glucose at a dilution rate set at between $D = 0.2$ and $D = 0.3\ h^{-1}$ for these experiments. Each column depicts a mean of 3 to 5 determinations. (a) Transport of 1 μM galactose as a measure of Mgl activity. The 100% value was 62 pmol of [^{14}C]galactose taken up per 10^8 bacteria per min. (b) GalK activity as a measure of *gal* induction. The 100% activity measured in glucose-limited wild type was 114 nmol of [^{14}C]galactose-1-phosphate formed per 10^9 bacteria per min. (c) The intracellular pool of galactose. Glucose-limited wild type contained an intracellular galactose concentration of 0.16 mM, and all other values are given as percentages of this concentration. (d) The intracellular and extracellular cAMP contents of cultures. The values are percentages of the intracellular concentration (6.8×10^{-7} pmol per bacterium) and external concentration (3.3×10^{-6} pmol per bacterium) measured in glucose-limited wild type.

galactose in glucose-limited chemostat cultures was close to the 2×10^{-4} M level previously found to derepress Mgl activity in *galK* mutants (12). Glycerol limitation resulted in the lowest internal galactose pools and the lowest Mgl activity among the nutrient-limited cultures. The strong correlation between the extent of *mgl* expression under the different growth conditions and the intracellular galactose levels found in Fig. 1d suggests that internal inducer concentration is an important determinant in limitation-induced transport. The only exception to this correlation was in cultures containing fucose, a gratuitous inducer of *mgl*.

The *gal* regulon and intracellular galactose during sugar limitation. If glucose limitation derepresses the *mgl* operon by elevating endogenous inducer levels, it would be expected that *gal* regulation would also become independent of external inducer, given that the GalR repressor is also inactivated by binding galactose (20). Hence, GalK activity was also monitored in the chemostat cultures, and these results are shown in Fig. 1c. Consistent with the result in Fig. 1d, that intracellular inducer was present, GalK activity was also significantly upregulated under sugar limitation. Both *mgl* and *gal* genes, therefore, respond to nutrient stress.

The above results raised the interesting question of how glucose limitation increases internal galactose levels. Galactose synthesis for macromolecular cell components like lipopolysaccharide uses the *pgm*, *galU*, and *galE* gene products involving the Leloir pathway (1). We therefore tested whether a *gal* deletion mutant lacking epimerase and transferase activities could produce galactose under limitation by a novel route. As shown in Fig. 2, glucose limitation with a strain containing a deletion of the *galETK* operon did not lead to induction of *mgl* or to increased galactose accumulation. Hence, the classic galactose pathway was critical for limitation-induced galactose synthesis as well as for *mgl* induction. Also consistent with this notion was the observation that the growth of the *gal* deletion mutant was greatly impaired at low glucose concentrations (removal of medium glucose was impaired and left a much higher residual level in chemostats; results not shown), indicating that the *mgl* induction pathway via *gal* gene products is important for efficient scavenging at micromolar glucose concentrations (5).

A *galK* mutation by itself did not prevent *gal* or *mgl* induction under limitation conditions and indeed had by far the highest internal galactose levels under all conditions (Fig. 2). This was consistent with earlier studies with *galK* mutants (12) and shows that GalK activity is a major route of inducer removal through phosphorylation under all conditions, including nutrient stress. However, limitation conditions permit an increase of galactose pools even in the presence of kinase activity. Regulation of GalK may be one possibility as to how galactose pools are controlled to provide an inducing steady-state concentration under limitation conditions. But further work is needed to define the metabolic control sites in more detail and of course the effectors involved.

The role of cAMP and Crp in regulation of *mgl* and *gal* genes. High cAMP levels under nutrient-limited stress conditions were expected to contribute to derepression of the Mgl system. Previous studies have indicated the catabolite-repressible nature of the Mgl system (9) and that consensus Crp-cAMP binding sites are present at the *mgl* promoter (3, 20). Continuous culture conditions with glucose limitation were shown to lead to increased cAMP levels (15), and the concentration of both intra- and extracellular cAMP in the cultures shown in Fig. 2d was consistently higher under carbon source limitation than during batch growth. However, as in Fig. 1 above, the intracellular level of cAMP did not correlate with

the extent of *mgl* expression, suggesting that the pattern of *mgl* induction is more critically dependent on the availability of endogenous galactose than on the availability of cAMP. For example, cultures growing on limiting glycerol had the highest cAMP concentrations but had some of the lower Mgl activities. Nevertheless, even under glucose limitation, the level of cAMP is high by batch culture standards and consistent with the consensus view that cAMP contributes to the response against nutrient limitation and general derepression of sugar regulons.

The extent of *mgl* derepression due to Crp-cAMP activation was also studied by testing *mgl* expression in the presence of a *crp* deletion (Fig. 2). Transport activity was poorly expressed under glucose limitation in the *crp* mutant strains. It should be noted that glucose-limited growth of *crp* bacteria was greatly impaired. Growth required far higher steady-state glucose concentrations in chemostats (results not shown), so the glucose scavenging activity of *crp* bacteria was greatly affected. This was consistent with the observations that (i) LamB expression was reduced, decreasing transport affinity for glucose (reference 6 and results not shown) and (ii) *mgl* expression was also reduced and further contributed to the impairment of glucose uptake at low concentrations (5).

Interestingly, the *crp* deletion did not totally abolish the raised galactose pools in chemostat cultures. Consistent with the higher inducer concentration, the *crp* mutant in continuous culture had increased GalK activity as well; it should be remembered that transcription of *gal* genes can take place from two promoters, one of which is Crp independent. This is in contrast to *mgl*, whose expression is minimal and whose promoter is Crp dependent (20).

Derepression of the Mgl transport system during the transition to starvation. To test whether *mgl* induction and galactose accumulation could be considered to be part of a starvation or stationary-phase response, batch cultures depleted for glucose were also investigated. As shown in Fig. 3b, galactose transport rates increased when the glucose supply in a batch culture became depleted (Fig. 3a). *mgl* induction occurred during and after the slowing of exponential growth and was highest within 3 h of growth arrest. A similar time course was seen following β-galactosidase activity of an *mglA-lacZ* transcriptional fusion (Fig. 3), confirming that *mgl* gene regulation and not just galactose transport responds to nutrient stress. Interestingly, as also shown in Fig. 3, *mgl* induction occurred later than did the peak of intracellular cAMP accumulation characteristic of transition into starvation (17) and shown in Fig. 1c. Indeed, *mgl* induction continued while intracellular cAMP levels were dropping to the steady-state starvation level.

Intracellular galactose pools also rose during starvation to about 70 μM, higher than in exponential growth but to a plateau level suboptimal for *mgl* induction; about 200 μM is necessary for high levels of *mgl* expression (12). Indeed, the extent of *mgl* induction seen in this kind of starvation response experiment is only about 10% of that seen with inducer-stimulated batch cultures (37 compared with 320 to 430 Miller units for *mglA-lacZ* fusion strains; results not shown). Also striking was the fact that Mgl activity in carbohydrate-limited chemostats in Fig. 1 is 5- to 10-fold higher than in the starvation experiment represented in Fig. 3. Hence, starvation was not optimal for *mgl* induction.

Galactose levels and Mgl induction at different nutrient-limited growth rates. To explore whether endoinduction was significant during very slow growth close to the starvation state, glucose-limited chemostats were established over a wide range of growth rates (dilution rates, D = 0.08 to D = 0.9 h^{-1}). As shown in Fig. 4, Mgl transport activity and *mglA* transcription exhibited a definite optimum, being maximal at dilution rates

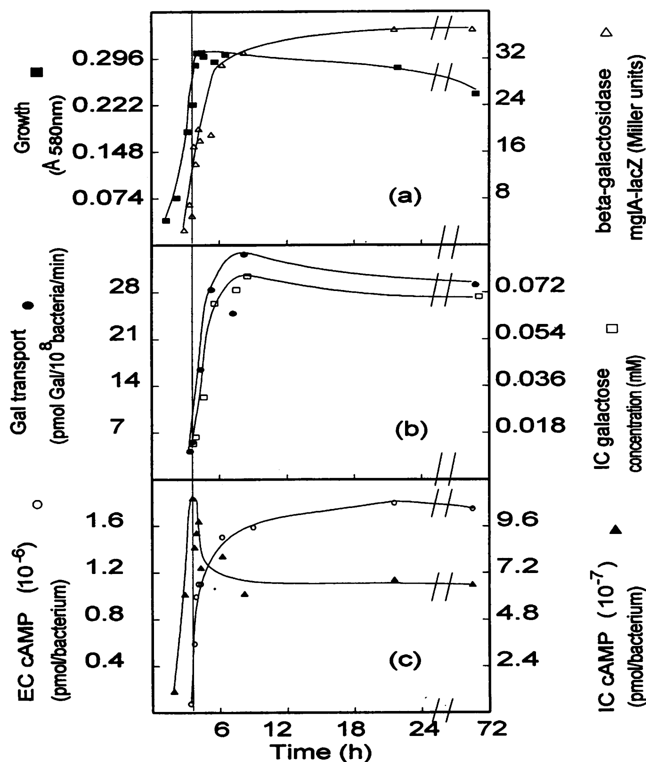

FIG. 3. Effect of glucose depletion in batch culture on the expression of the Mgl system. An overnight culture of MC4100 or BD21 (*mglA-lacZ*) was used to inoculate a 500-ml flask containing 0.02% glucose. At the points on or after the dotted line in each panel, the glucose concentration of the medium assayed with glucose oxidase became undetectable (i.e., dropped below 2 μM). (a) Bacterial growth was monitored for BD21 by determining optical density at 580 nm. Derepression of *mgl* was assayed by measuring β-galactosidase activity of the *mglA-lacZ* fusion in BD21. (b) Transport of 1 μM galactose as a measure of Mgl activity was assayed with MC4100 harvested from a 500-ml batch culture harvested at various stages of growth. The initial rate of uptake was measured as previously described, by using [^{14}C]galactose at a concentration of 1 μM (5). The rest of the culture was used to assay intracellular galactose accumulation. (c) Accumulations of intracellular and extracellular cAMP were measured for MC4100 at specified time points. A 5-ml sample was removed from the culture by suction through a membrane filter and was processed as described in Materials and Methods. The cAMP concentrations in the samples were determined by using an enzyme-linked immunoassay kit (Amersham).

of approximately 0.5 to 0.7 h^{-1}, equivalent to a doubling time of 1 to 1.5 h. Hence, high induction is not a consequence of slow growth rates in chemostats. Indeed, below a doubling time of over 3 h, the induction of Mgl activity is greatly reduced, and with doubling times of 7 h, the extent of induction of Mgl matches the batch-culture starvation level shown in Fig. 3.

There was an expected drop in *mgl* expression at near-maximal growth rates. It should be remembered that in a glucose-limited chemostat, the growth rate is governed by the steady-state glucose concentration up to washout. For the experiments represented in Fig. 4, the steady-state glucose concentration was detectable by using the glucose oxidase assay only at dilution rates above 0.72 h^{-1}. At lower growth rates, glucose concentrations were at or below 2 μM, the detection limit of the glucose oxidase assay. At D = 0.88 h^{-1}, the glucose concentration in the chemostat was 560 μM, and at

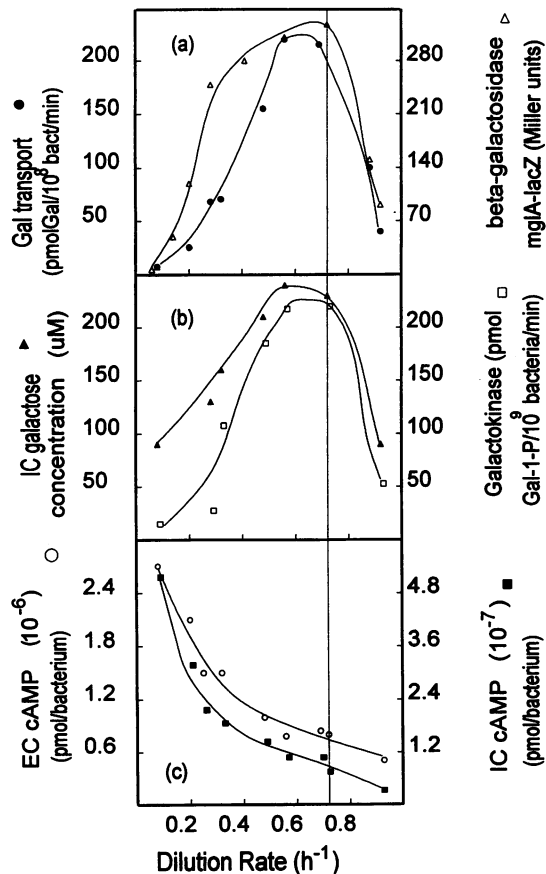

FIG. 4. Induction of *mgl* and *gal* as a function of growth rate. Continuous cultures with wild-type MC4100 or the *mglA-lacZ* fusion strain BD21 were performed in glucose-limited chemostats equilibrated at various dilution rates. (a) The degree of derepression of the *mgl* operon at different growth rates was monitored by measuring 1 μM galactose transport in wild-type bacteria and also β-galactosidase activity of the *mglA-lacZ* fusion in BD21. For both assays, bacteria were grown in 80-ml glucose-limited chemostats (0.02% carbon source). (b) The level of *gal* regulon induction was determined by assaying GalK activity. The intracellular galactose concentrations in 570-ml cultures grown at set dilution rates were from 0.04% glucose-limited chemostats. (c) Amounts of extracellular and intracellular cAMP were measured by the radioimmunoassay method. Bacteria were grown in 80-ml glucose-limited chemostats with 0.02% glucose.

$D = 0.92\ h^{-1}$, the glucose concentration was 600 μM. So, the reduction in *mgl* expression at higher growth rates is attributable to the increasingly repressive effect of glucose at medium concentrations approaching 1 mM, consistent with the continued drop in cAMP concentrations toward batch-culture values.

Most strikingly, the intracellular galactose concentrations at different dilution rates correlated closely with *mgl* expression. Low internal galactose concentrations were found at low and high growth rates (Fig. 4). At the lowest growth rate tested ($D = 0.08\ h^{-1}$), the galactose level as well as the level of induction of *mgl* matched the level found in batch starvation experiments represented in Fig. 3. So, very slow growth, with undetectable extracellular glucose concentrations in chemostats, appears to be the equivalent of starvation in batch-culture experiments for Mgl activity.

In contrast to internal galactose levels, cAMP concentrations both inside and outside bacteria were highest at $D = 0.08\ h^{-1}$. At very low growth rates, cAMP levels did not correlate with the level of *mgl* induction. Hence, from the data in Fig. 1 to 4, internal inducer has a closer influence on the extent of *mgl* induction than does cAMP, with the proviso that a certain level of Crp-cAMP needs to be available to induce at *mgl* promoters. The results with low dilution rates also suggest that inducer buildup is not a secondary effect of cAMP accumulation. It is likely that a sensor/transducer other than cAMP signals nutrient limitation to trigger endoinducer accumulation.

DISCUSSION

There is a tight correlation between the ability of *E. coli* to scavenge glucose, the state of induction of the Mgl transport system, and the pool size of galactose inside bacteria. Endogenous inducer synthesis is clearly a natural adaptation of bacteria to improve their scavenging ability in times of reduced nutrient availability. The high level of induction elicited by endogenous sugar also points to the fact that the absolute requirement for exogenous inducer in the *mgl*/*gal* regulons is restricted to high-nutrient conditions.

cAMP and its receptor protein are essential for *mgl* induction, as confirmed in this study. As well defined by others, nutrient limitation globally stimulates the conditions favorable for *mgl* induction by raising the pool size of cAMP. But high cAMP levels are by themselves not sufficient for induction of *mgl*; in the absence of the pathway for endogenous galactose synthesis, as in the *galETK* mutant, *mgl* expression is minimal even in the presence of high cAMP. Very low growth rates also result in high cAMP but low *mgl* expression. These results reinforce the conclusion that endogenous inducer, as well as cAMP, is a requirement for nutrient-limited induction of Mgl. Entry of a batch culture into starvation-induced stationary phase elicits a multitude of physiological changes (10, 14). Superficially, galactose accumulation, as shown in this study, appears to be part of a starvation response, as *mgl* induction and galactose levels rise within 3 h of the onset of starvation. However, the much more pronounced galactose pools and *mgl* expression under steady-state growth with micromolar extracellular sugar concentrations suggest that endogenous inducer synthesis is more important in a scavenging state to support nonmaximal growth rates. Hence, *mgl* induction is more a hunger response than a starvation response. Also consistent with this view was the suboptimal *mgl* induction at very low growth rates, comparable to the level seen in batch cultures of starving bacteria. In other words, our study indicates that internal inducer synthesis is optimal neither during feast nor famine but under oligotrophic conditions. These results further suggest that most previous discussions of the starvation response (10, 14) in a batch culture really considered a sum of at least two physiological states, one of which is the scavenging, hunger response. Batch cultures entering starvation transiently pass through the micromolar nutrient concentrations required to elicit the hunger response, which is therefore only poorly expressed in batch-culture experiments.

Many questions are raised by these studies. One of the most significant is whether endogenous induction is widespread in the bacterial regulation of sugar regulons. Our view is that the presence of endogenous synthesis is likely to be substantiated with inducers of other regulons, and preliminary results with extracts of glucose-limited bacteria suggest that at least eight low-molecular-weight uncharged compounds accumulate to higher levels than under nutrient-rich conditions (16a). These compounds include galactose and maltosaccharides. The latter probably account for *mal-lamB* expression under sugar limitation in the absence of exogenous inducer (6), but other

inducers and regulons remain to be investigated. Other important questions include the generality of the hunger response in bacterial physiology, the nature of the molecules recognizing and signalling carbohydrate limitation, and the signal transduction pathway ultimately affecting the metabolic steps influencing inducer pools. Much remains to be learned about the scavenging state of bacteria.

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REFERENCES

1. **Adhya, S.** 1987. The galactose operon, p. 1503–1512. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
2. **Ahmad, A.** 1984. Plasmid vectors for positive selection of cloned DNA in *Escherichia coli*. Gene **28:**37–43.
3. **Benner-Luger, D., and W. Boos.** 1988. The mglB sequence of *Salmonella typhimurium* LT2; promoter analysis by gene fusions and evidence for a divergently oriented gene coding for the mgl repressor. Mol. Gen. Genet. **214:**579–587.
4. **Casabadan, M. J.** 1976. Transposition and fusion of the lac operon to selected promoters in *E. coli* using bacteriophage Lambda and Mu. J. Mol. Biol. **104:**541–555.
5. **Death, A., and T. Ferenci.** 1993. The importance of the binding-protein-dependent Mgl system to the transport of glucose in *Escherichia coli* growing on low sugar concentrations. Res. Microbiol. **144:**529–537.
6. **Death, A., L. Notley, and T. Ferenci.** 1993. Derepression of LamB protein facilitates outer membrane permeation of carbohydrates into *Escherichia coli* under conditions of nutrient stress. J. Bacteriol. **175:**1475–1483.
7. **Decker, K., R. Peist, J. Reidl, M. Kossmann, B. Brand, and W. Boos.** 1993. Maltose and maltotriose can be formed endogenously in *Escherichia coli* from glucose and glucose-1-phosphate independently of enzymes of the maltose system. J. Bacteriol. **175:**5655–5665.
8. **Ehrmann, M., and W. Boos.** 1987. Identification of endogenous inducers of the *mal* regulon in *Escherichia coli*. J. Bacteriol. **169:**3539–3545.
9. **Henderson, P. J. F.** 1980. The inter-relationship between proton-coupled and binding-protein-dependent transport systems in bacteria. Biochem. Soc. Transact. **8:**678–679.
10. **Hengge-Aronis, R.** 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. Cell **72:**165–168.
11. **Huggett, A. S. G., and D. A. Nixon.** 1957. Glucose oxidase. Biochem. J. **66:**12P.
12. **Kalckar, H. M.** 1971. The periplasmic galactose binding protein of *Escherichia coli*. Science **174:**557–565.
13. **Kjelleberg, S., N. Albertson, K. Flaerdh, L. Holmquist, A. Jouper-Jaan, R. Marouga, J. Oestling, B. Svenblad, and D. Weichart.** 1993. How do non-differentiating bacteria adapt to starvation? Antonie Leeuwenhoek **63:**333–341.
14. **Matin, A.** 1991. The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. Mol. Microbiol. **5:**3–10.
15. **Matin, A., and M. K. Matin.** 1982. Cellular levels, excretion, and synthesis rates of cyclic AMP in *Escherichia coli* grown in continuous culture. J. Bacteriol. **149:**801–807.
16. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

16a. **Notley, L.** Unpublished results.

17. **Peterkofsky, A.** 1977. Regulation of adenylate cyclase by phosphorylation-dephosphorylation. Trends Biochem. Sci. **2:**12–14.
18. **Roy, A., P. Glaser, and A. Danchin.** 1988. Aspects of the regulation of adenylate cyclase synthesis in *Escherichia coli* K12. J. Gen. Microbiol. **134:**359–367.
19. **Schultz, J. E., G. I. Latter, and A. Matin.** 1988. Differential regulation by cyclic AMP of starvation protein synthesis in *Escherichia coli*. J. Bacteriol. **170:**3903–3909.
20. **Weickert, M. J., and S. Adhya.** 1993. The galactose regulon of *Escherichia coli*. Mol. Microbiol. **10:**245–251.
21. **Wilson, D. B., and D. S. Hogness.** 1966. Galactokinase and uridine diphosphogalactose-4-epimerase from *Escherichia coli*. Methods Enzymol. **8:**229–240.
22. **Wu, H. C. P., W. Boos, and H. M. Kalckar.** 1969. Role of the galactose transport system in the retention of intracellular galactose in *Escherichia coli*. J. Mol. Biol. **41:**109–120.