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Differential Regulation by Cyclic AMP of Starvation Protein Synthesis in *Escherichia coli*

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Of the 30 carbon starvation proteins whose induction has been previously shown to be important for starvation survival of *Escherichia coli*, two-thirds were not induced in *cya* or *crp* deletion mutants of *E. coli* at the onset of carbon starvation. The rest were induced, although not necessarily with the same temporal pattern as exhibited in the wild type. The starvation proteins that were homologous to previously identified heat shock proteins belonged to the latter class and were hyperinduced in Δ*cya* or Δ*crp* mutants during starvation. Most of the cyclic AMP-dependent proteins were synthesized in the Δ*cya* mutant if exogenous cyclic AMP was added at the onset of starvation. Furthermore, β-galactosidase induction of several carbon starvation response gene fusions occurred only in a *cya*$^+$ genetic background. Thus, two-thirds of the carbon starvation proteins of *E. coli* require cyclic AMP and its receptor protein for induction; the rest do not. The former class evidently has no role in starvation survival, since Δ*cya* or Δ*crp* mutants of either *E. coli* or *Salmonella typhimurium* survived starvation as well as their wild-type parents did. The latter class, therefore, is likely to have a direct role in starvation survival. This possibility is strengthened by the finding that nearly all of the *cya*- and *crp*-independent proteins were also induced during nitrogen starvation and, as shown previously, during phosphate starvation. Proteins whose synthesis is independent of *cya*- and *crp* control are referred to as Pex (postexponential).

Starving bacteria are of both fundamental and applied interest (14, 15, 21, 27, 28). We have shown that during the first 4 to 5 h of starvation for carbon substrates (glucose or succinate) approximately 30 proteins are induced in *Escherichia coli* K-12 (14, 15) and that these proteins are important in starvation survival (27, 28, 32). Starvation-induced proteins have different temporal patterns of synthesis—some are synthesized very transiently during starvation, whereas others have a broader peak of synthesis (15). Investigators in other laboratories have also shown that unique genes are switched on and new proteins are synthesized at the onset of starvation (10, 33, 34).

To better understand the regulation of starvation protein synthesis, we have focused on the role of the signal molecule adenosine 3′,5′-cyclic monophosphate (cAMP) and its receptor protein (CRP). Intracellular cAMP levels increase at the onset of carbon starvation (5, 8, 19, 26), and there appears to be an inverse relationship between the energetic state of the cell and cAMP levels (8, 13). This raises the possibility that cAMP acts as a signal for starvation protein synthesis. We have therefore investigated the effect of the loss of cAMP control on starvation protein synthesis in *E. coli*. Isogenic *E. coli* strains deleted in the adenylate cyclase gene (Δ*cya*) or the gene encoding CRP (Δ*crp*) were examined. The results indicate that although several starvation genes are positively regulated by cAMP, many others are not, and that the latter class of genes is also switched on during starvation for different individual nutrients.

MATERIALS AND METHODS

Bacterial strains and growth media. The bacterial strains used in this study are listed in Table 1; all were derived from *E. coli* K-12 or *Salmonella typhimurium* LT2-Z. The K-12 strain used is nonlysogenic for λ and does not contain the F plasmid (R. Dyson, J. Schultz, and A. Matin, unpublished results). Strains AMS3, AMS10, AMS11, AMS13, and AMS14 were previously designated as EZ1, JS52, JS55, JS101, and JS103, respectively, in reference 32. Transductions were performed with bacteriophage P1 *vir* for *E. coli* (20) and phage P22 HT105/1 *int* for *S. typhimurium* (31).

M9 minimal (20), morpholinepropanesulfonic acid (MOPS) minimal (22), and LB (20) media were prepared as described previously. When appropriate, the media were supplemented with the following (micrograms per milliliter): ampicillin, 50; chloramphenicol, 10; fusaric acid, 12; phosphomycin, 10; tetracycline, 12.5; streptomycin, 100; kanamycin, 50; and/or 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 40.

Construction of Δ*cya* strains of *E. coli*. *E. coli* Δ*cya* strains were constructed by P1 transduction of *metE*::Tn*10* from strain RK4349 followed by a second transduction of linked *metE*$^+$ Δ*cya-854* genes from strain CA8306. The second transduction replaced the *metE*::Tn*10* by selection for methionine prototrophy on plates containing M9 medium plus 0.4% glucose; cotransduction of Δ*cya-854* was confirmed by (i) phosphomycin resistance on plates containing MacConkey plus 1% glycerol (1), (ii) inability to grow on M9 plates containing catabolite-sensitive carbon sources (lactate, succinate, or ribose [25]), (iii) dependence of β-galactosidase induction on the presence of 15 mM exogenous cAMP (25) (applicable to strain AMS2 only), and (iv) a lower growth rate than *cya*$^+$ strains on M9 medium plus glucose (25).

Strain AMS12 was generated as a *cya*$^+$ control of strain AMS2 (Δ*cya*). The *cya*$^+$ gene from K-12 was introduced into the AMS2 chromosome by P1 transduction, and the strain was selected by the ability to grow on plates containing M9 medium plus 0.4% ribose.

Construction of Δ*crp* strains. The cotransducible Δ*crp-39 rpsL* genes of strain CA8439 were introduced into K-12 by

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TABLE 1. Bacterial strains used in this study

Strain	Genotype	Reference or source
E. coli		
K-12	Wild type (λ^- F^-)	Our laboratory strain (14, 15, 27, 28)
RK4349	F^- *ilv metB proB entA* Δ*lac strA his metE*::Tn*10*	R. Kadner
CA8306	HfrH *thi* Δ*cya-854*	7
CA8439	HfrH Δ*cya-854* Δ*crp-39 rpsL*	29
AMS2	K-12 Δ*cya-854*	This study
AMS12	AMS2 *cya*$^+$	This study
AMS10	K-12 *rpsL*	This study
AMS11	K-12 Δ*crp-39 rpsL*	This study
MC4100	F^- *araD139* Δ(*lacIPOZYA-argF*)*U169 rpsL150 relA thi ptsF25 flbB5301 deoC1*	9
AMS3	MC4100 *cst-1*::Mu dX (Ap^r Cm^r *lacZ*)[a]	15
AMS5	AMS3 Δ*cya-854* (Ap^r Cm^r *lacZ*)	This study
AMS6	K-12 Δ*lacU169*	This study
AMS8	AMS2 Δ*lacU169*	This study
AMS13	MC4100 *cst-2*::λ *plac*Mu9 (Kn^r)	This study
AMS28	AMS6 *cst-2*::λ *plac*Mu9 (Kn^r)	This study
AMS29	AMS8 *cst-2*::λ *plac*Mu9 (Kn^r)	This study
AMS14	MC4100 *cst-3*::λ *plac*Mu9 (Kn^r)	This study
AMS30	AMS6 *cst-3*::λ *plac*Mu9 (Kn^r)	This study
AMS31	AMS8 *cst-3*::λ *plac*Mu9 (Kn^r)	This study
S. typhimurium		
LT2-Z	Wild type	B. Ames
PP1037	*crp-773*::Tn*10 trpB223*	12
AMS23	LT2-Z *crp-773*::Tn*10*	This study
AMS27	AMS23 *crp* (Tet^s)	This study

[a] *cst*, cAMP-dependent carbon starvation response gene; see Discussion.

P1 transduction. *rpsL* transductants were selected by streptomycin resistance on LB plates, and cotransduction of Δ*crp-39* was confirmed as described above for the Δ*cya* strains. The addition of 15 mM exogenous cAMP did not restore β-galactosidase synthesis (25). Strain AMS10, a *crp*$^+$ *rpsL* transductant, was also isolated during this construction and was used as the *crp*$^+$ control. The starvation protein synthesis patterns of AMS10 and K-12 were virtually identical (data not shown).

The *crp*::Tn*10* of *S. typhimurium* PP1037 was transduced into strain LT2-Z by P22 transduction. Transductants were selected by tetracycline resistance and screened as described above to confirm the *crp* mutation. The resulting strain was designated AMS23.

To obtain a nonreverting *crp* mutation, strain AMS23 (*crp*::Tn*10*) was plated on the selective medium of Bochner et al. (3), and tetracycline-sensitive, fusaric acid-resistant colonies were obtained. One of these putative deletion strains, designated AMS27, was phosphomycin resistant (1) and had a reversion frequency of less than 10^{-8} on M9 medium plus 0.4% ribose.

Construction of carbon starvation response protein fusions. Strain MC4100 was infected with λ *plac*Mu9 phage by the method of Bremer et al. (6). Fusion strains were obtained by selection for kanamycin resistance and were screened on M9 medium containing X-Gal and either 0.40 or 0.02% glucose, as previously described (15). Plates were incubated at 37°C for 24 h, and colonies that were dark blue on low-glucose plates (i.e., during starvation) but white or light blue on high-glucose plates were further characterized by assaying β-galactosidase activity in liquid cultures during glucose depletion. Two strains, AMS13 and AMS14, exhibited the expected phenotype for *lac* fusion integration into a carbon starvation-inducible gene, i.e., increase in β-galactosidase activity at the onset of glucose or succinate starvation.

The λ *plac*Mu9 insertions of AMS13 or AMS14 were transduced into strains AMS6 (Δ*lac*) and AMS8 (Δ*lac* Δ*cya*); selection of *lac* fusions was by kanamycin resistance.

Starvation protocol. Carbon starvation was attained by allowing the cultures to grow in M9 medium, supplemented with glucose (0.025%, wt/vol), succinate (0.048%, wt/vol), or glycerol (0.022%, wt/vol), until the carbon source was exhausted. The onset of starvation was determined by the cessation of growth, which in control experiments was shown to be coincident with substrate exhaustion. For nitrogen starvation, cultures were grown in MOPS medium (initial concentration, 0.57 mM NH_4Cl) until NH_4^+ was exhausted. All cultures were incubated aerobically at 37°C with agitation and had attained a density of about 3×10^8 cells per ml (equivalent to an A_{660} of ca. 0.3) at the onset of starvation. Media were supplemented with kanamycin or ampicillin plus chloramphenicol to maintain selection of the λ *plac*Mu9 or Mu dX insertion strains, respectively.

Resolution of polypeptides on two-dimensional gels. Cellular polypeptides were labeled and separated on two-dimensional polyacrylamide gels as previously described (15). Briefly, samples were removed at selected times during growth or starvation and were pulse-labeled with 10^{-8} M L-[^{35}S]methionine (12 μCi/ml; 1,072 to 1,097 Ci/mmol) for 3 min at 37°C. Following a 1-min chase with unlabeled methionine (10^{-5} M), proteins were precipitated with 10% trichloroacetic acid at 4°C and separated by using the two-dimensional electrophoretic method of O'Farrell (24). Equivalent amounts of radioactivity were loaded for each sample (ca. 750,000 cpm). Labeled proteins were visualized on XAR-5 film (Eastman Kodak Co.) by either fluorography or autoradiography.

Measurements of synthesis rates of individual polypeptides. To compare the polypeptide synthesis rates of K-12 (*cya*$^+$) and AMS2 (Δ*cya*) during glucose starvation, we combined each of the ^{35}S-labeled samples described above with an internal standard of ^{3}H-labeled growth and starvation proteins before precipitation. This allowed us to correct for recovery of all growth or starvation proteins during electrophoresis and/or excision of the spots (15). The standard was prepared from the combination of two cultures of *E. coli* K-12: one culture was labeled with 0.5×10^{-6} M L-[^{3}H]leucine (20 μCi/ml; 45 Ci/mmol) during two doublings of growth on M9 medium plus 0.4% glucose. The second culture was allowed to deplete the glucose (0.025%) in the medium, at which time 7.7×10^{-6} M L-[^{3}H]leucine (25 μCi/ml; 3.25 Ci/mmol) was added. In the latter culture, the leucine was continually taken up by the starving cells for over 2 h and did not perturb the starvation state in that no growth occurred during this 2-h period.

The individual polypeptide spots were excised from dried fluorographed gels and solubilized as previously described (15). Hydrofluor scintillant (5 ml) was added to the samples, which were then quantitated in a Beckman LS9000 liquid scintillation counter. Synthesis rates were defined as the ratio of disintegrations per minute of ^{35}S to ^{3}H for the individual spot divided by the same ratio of the total labeled protein for that sample.

Quantitation of the individual polypeptides of K-12 and AMS2 (Δ*cya*) during nitrogen starvation or AMS11 (Δ*crp rpsL*) and AMS10 (*rpsL*) during glucose starvation was

performed by computer-assisted microdensitometry of autoradiograms. The abundance of each polypeptide was measured by using an interactive graphics system to integrate the counts of an individual spot (18). Strips containing known amounts of ^{14}C isotope were used to calibrate the autoradiograms. The rate of synthesis for a given polypeptide was calculated as the percent of the total counts per minute recovered on the autoradiogram.

Miscellaneous. Viability was determined by spreading serial dilutions of cultures on LB plates. As a precaution against clumping, the cultures were homogenized with a Potter-Elvehjem-type tissue grinder (Wheaton Industries) before plating; control experiments showed a 0 to 14% increase in colony counts after homogenization. β-Galactosidase activity was assayed in duplicate and corrected for light scattering, as described previously (15). Activity is expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside cleaved per minute per A_{660} unit.

Materials. Biochemicals were purchased from Sigma Chemical Co. L-[^{35}S]methionine (1,072 to 1,097 Ci/mmol; 12 mCi/ml) and L-[^{3}H]leucine (45 Ci/mmol; 1 mCi/ml) were obtained from Du Pont, NEN Research Products. Hydrofluor scintillant was purchased from National Diagnostics Inc.

RESULTS

Carbon starvation protein synthesis in *cya* and *crp* mutants. The Δ*cya* mutant (AMS2) and its K-12 *cya*$^+$ parent strain were pulse-labeled with radioactive methionine at various times before and after glucose depletion from the medium. Since starvation protein synthesis exhibits different temporal classes (15), cultures were periodically sampled and labeled during the first 4 h of starvation to ensure that all of the classes were represented. Bulk protein synthesis became negligible in both the mutant and the wild type after 4 h of starvation.

The Δ*cya* mutant induced some of the carbon starvation proteins, but not others, as illustrated by two-dimensional gel maps for two time points (Fig. 1). To obtain a more precise idea of this difference, the kinetics of synthesis during the entire 4-h period after the onset of starvation were determined for starvation proteins in the two strains. Of the 30 proteins, 19 were either not synthesized or synthesized at low levels in the Δ*cya* mutant throughout this period. Synthesis kinetics of representative proteins of this group are shown in Fig. 2, and it is evident that they do not belong to any particular kinetic class (15) of starvation proteins. The remaining 11 starvation proteins that were induced in both AMS2 and K-12 fell into three categories: those exhibiting similar induction patterns in the two strains (polypeptides 3, 17, 19, and 31); those showing temporally altered induction kinetics in AMS2 (polypeptides 2, 20, 22, 23, and 28); and those showing enhanced induction in the mutant (polypeptides 4 and 6). These last two polypeptides were heat shock proteins DnaK and GroEL, respectively, as identified in separate experiments by exposing the culture to a temperature shift from 29 to 42°C (16, 23). The synthesis pattern of one representative polypeptide of each class is shown in Fig. 3.

We also compared the kinetics of synthesis of several carbon starvation proteins in *E. coli crp*$^+$ (AMS10) or Δ*crp* (AMS11) strains during glucose depletion (32). All of the *cya*-dependent starvation proteins also required CRP. Similarly, all of the cAMP-independent proteins analyzed in the Δ*crp* mutant exhibited essentially the same kinetics as shown for the Δ*cya* mutant (Fig. 3). Thus, two-thirds of the glucose starvation proteins analyzed required both cAMP and CRP for induction; the rest did not.

Two types of control experiments were conducted to confirm this conclusion. In the first, 25 mM cAMP was exogenously supplied to a culture of AMS2 (Δ*cya*) at the time of glucose exhaustion from the medium, and the protein synthesis pattern was determined by two-dimensional gel electrophoresis as discussed above. All but three of the cAMP-dependent starvation proteins were synthesized by this culture, and the kinetics were similar to those in the wild type. The three proteins (polypeptides 7, 8, and 9) that failed to exhibit induction belonged to the temporal class that is transiently induced during glucose or succinate starvation (as represented by polypeptide 8 in Fig. 2A; see also Fig. 2A in reference 15). Their lack of induction could reflect a need for cAMP before the onset of starvation or a requirement for higher intracellular cAMP levels than could be attained under these conditions. Whatever the reason, it is clear that induction of these early proteins is not a prerequisite for the synthesis of those of subsequent temporal classes.

The second control involved transducing the *cya*$^+$ gene back into the Δ*cya* mutant AMS2 (to construct strain AMS12 [Table 1]) and determining the kinetics of starvation protein synthesis in AMS12. These were identical to the wild-type K-12 with respect to both cAMP-dependent and cAMP-independent starvation proteins throughout the 4-h period of starvation analyzed (data not shown).

Regulation by cAMP of *E. coli* carbon starvation response gene fusions. To differentiate between the expression of the genes coding for the cAMP-dependent and cAMP-independent starvation proteins, we made use of *lacZ* fusions in carbon starvation response genes (15). Several such fusions have been constructed in this laboratory by using Mu dX (15) or λ *plac*Mu9. Because the *lacZ* gene encoded by the phage is under the control of a carbon starvation response promoter in these strains, each exhibits an increase in β-galactosidase synthesis at the onset of glucose starvation. Three fusion strains (AMS3, AMS28, and AMS30 [Table 1]) were selected; they exhibited different kinetics of β-galactosidase synthesis at the onset of starvation and the gene fusions mapped at separate locations on the chromosome, indicating that different carbon starvation response genes had been affected in these strains. Transduction of the Δ*cya* gene in these strains abolished their ability to induce β-galactosidase synthesis at the onset of starvation (Fig. 4), and this ability was restored when the *cya*$^+$ gene was back-transduced into them. Thus, in all three strains these genes belonged to the class that requires cAMP for induction during starvation. Analysis of about 5,000 additional *lacZ* fusions has failed to reveal any that would induce β-galactosidase in a *cya* background (E. Auger, S. Schippa, S. Chaisson, R. Sepulveda, and A. Matin, unpublished data). Consequently, using this method we have been unable so far to demonstrate the existence of genes coding for cAMP-independent starvation proteins.

Two of the three fusion strains mentioned above that contain insertions in cAMP-dependent carbon starvation response genes (AMS28 and AMS30) were also tested for β-galactosidase synthesis during growth and starvation in M9 medium supplemented with glycerol or succinate. During exponential growth, these strains had considerably higher β-galactosidase levels in glycerol or succinate medium than in glucose medium (ca. 200 versus 15 U for AMS28, and 24 to 48 versus 14 U for AMS30). These differences in β-galactosidase activity probably correlate

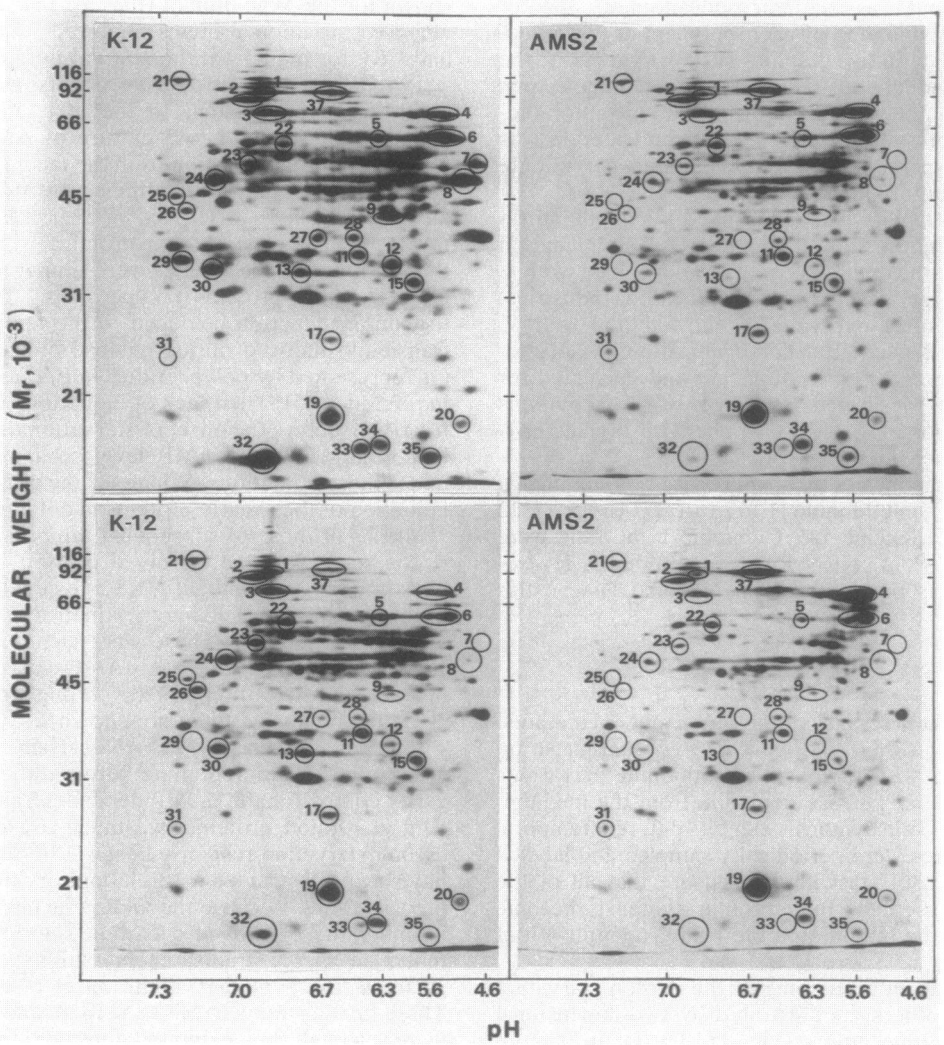

FIG. 1. Two-dimensional fluorograms of polypeptides synthesized by *E. coli* K-12 or Δ*cya* mutant AMS2 at 15 min (top panels) or 100 min (bottom panels) following glucose exhaustion from the medium. Culture samples were pulse-labeled for 3 min with [^{35}S]methionine as described in Materials and Methods. Circles indicate the polypeptides whose kinetics of synthesis were measured. To facilitate comparison with our previous work (15), the numbering system for individual starvation proteins used previously has been retained. (See Fig. 1A in reference 15 for the protein synthesis pattern during growth.) Note that the corrected pH axis (erratum to reference 15) is used in this figure.

with cAMP levels, since cAMP levels are known to be higher in *E. coli* strains grown on glycerol or succinate than on glucose (8, 13). Both the strains exhibited a marked increase in β-galactosidase levels after the exhaustion of any of the individual carbon substrates. Thus, the induction of the carbon starvation response genes in these strains is triggered by starvation for carbon sources in general and not just for glucose. Further studies have shown that the same applies to several other carbon starvation fusion strains (E. Auger and A. Matin, unpublished data). Curiously, the net increase in the β-galactosidase level tended to be higher at the onset of glycerol or succinate starvation than at the onset of glucose starvation, even though the synthesis rate of cAMP is about 20-fold higher during glucose starvation than during glycerol or succinate starvation (5). For instance, AMS28 exhibited a 420-U increase in β-galactosidase levels when starved in glycerol or succinate medium and only a 250-U increase when starved in glucose medium. Thus, it appears that even though the promoter activity of these genes correlates with cAMP synthesis rates during growth on different carbon substrates, it does not do so during starvation.

Effect of *cya* or *crp* mutations on starvation survival. As stated above, protein synthesis in *E. coli* during the initial period of starvation is necessary for starvation survival (27, 28). Since *E. coli* Δ*cya* or Δ*crp* mutants expressed some, but not all, starvation proteins, it was of interest to determine their starvation survival characteristics. In three separate experiments, the culture half-lives of glucose-starved K-12 and Δ*cya* mutant AMS2 cells were found to be the same (ca. 3.0 to 3.5 days). Likewise, the *crp*$^+$ (AMS10) and Δ*crp* (AMS11) strains exhibited very similar culture half-lives. Furthermore, an *S. typhimurium crp*::Tn*10* mutant (AMS23), a nonreverting *crp* derivative of it (AMS27), and the parent strain (LT2-Z) all had half-lives of about 3 days.

Supporting the conclusion that induction of cAMP-dependent starvation proteins is not involved in starvation survival, fusion strains AMS13 and AMS14 showed the same culture half-life during glucose starvation as did their parent,

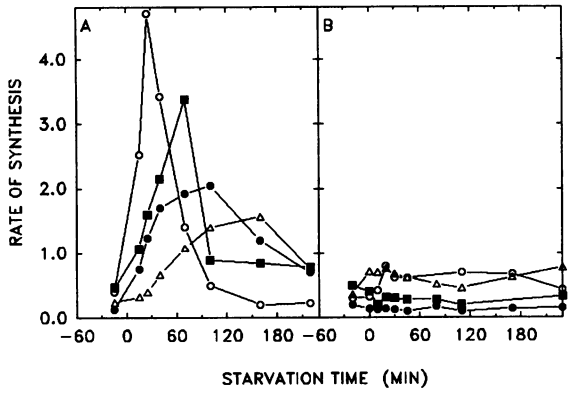

FIG. 2. Synthesis rates of representative cAMP-dependent polypeptides of *E. coli* K-12 (A) or Δ*cya* mutant AMS2 (B) at various times during glucose starvation. Zero time denotes complete exhaustion of glucose from the medium. The synthesis rate was calculated by dividing the ^{35}S to ^{3}H ratio in a given spot by the ^{35}S to ^{3}H ratio in the total protein. It is noteworthy that the induction of starvation proteins is not merely a consequence of decreased bulk protein synthesis, since [^{35}S]methionine incorporation rates were fairly constant in both strains during this period (ca. 50 to 75% of incorporation rates during growth [15]). Symbols: ○, polypeptide 8; ■, polypeptide 30; ●, polypeptide 26; △, polypeptide 11. Other cAMP-dependent polypeptides, which are not shown in the figure, are polypeptides 1, 5, 7, 9, 12, 13, 15, 21, 24, 25, 27, 29, 32, 33, and 35.

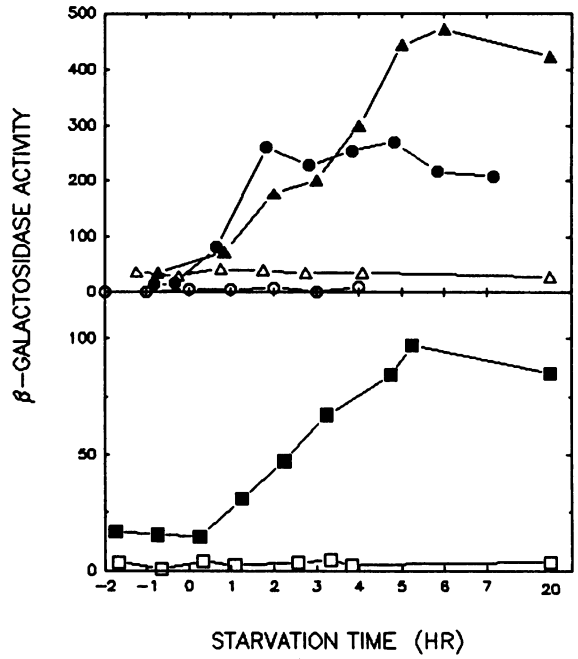

FIG. 4. β-Galactosidase activities of *E. coli cst::lacZ* fusion strains in *cya*$^{+}$ (solid symbols) or Δ*cya* (open symbols) genetic backgrounds. β-Galactosidase activity was assayed during growth and after glucose exhaustion. Symbols: ▲, AMS3; △, AMS5; ●, AMS28; ○, AMS29; ■, AMS30; □, AMS31.

MC4100. As discussed above, the carbon starvation-inducible fusions in these strains are cAMP dependent.

Protein synthesis during nitrogen starvation. It is clear that the induction of cAMP-dependent starvation proteins is not important in starvation survival, and yet our previous data (27, 28, 32) conclusively show that starvation protein induction enhances resistance to starvation. It therefore follows that it must be the cAMP-independent starvation proteins whose induction confers starvation resistance. This notion has not yet been tested directly, but other findings are consistent with it. We previously reported that 13 proteins were commonly induced regardless of whether the cells were starved for carbon, nitrogen, or phosphorus, and so suggested that these proteins were likely to be most critical in conferring resistance to starvation (15). We found that nearly all the cAMP-independent proteins that we have identified in this study belonged to this common category of starvation proteins.

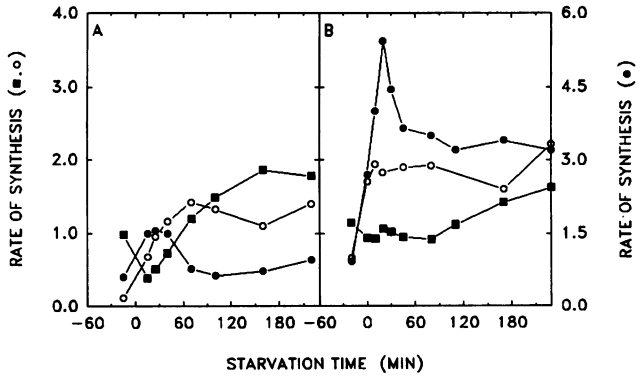

FIG. 3. Synthesis rates of representative proteins that do not require cAMP for synthesis during glucose starvation in *E. coli* K-12 (A) and Δ*cya* mutant AMS2 (B). The rates were calculated as described in the legend to Fig. 2. Symbols: ○, polypeptide 19; ■, polypeptide 22; ●, polypeptide 4.

In the previous study (15), a 30-min starvation period was used to identify starvation proteins common to glucose, nitrogen, and phosphorus starvation. Since starvation proteins fall into different temporal categories (15), it was possible that the cAMP-independent proteins were also induced at other times during starvation. We therefore determined the kinetics of cAMP-independent protein synthesis in *E. coli* K-12 throughout the first 4 h of nitrogen starvation. Of nine proteins analyzed, all except one (polypeptide 6 [GroEL], which was not induced) were induced throughout all or most of this period (Fig. 5) and exhibited kinetics that were very similar to those observed during carbon starvation. Thus, the cAMP-independent protein induction is indeed independent of the identity of the starvation nutrient.

As a control, we also determined the kinetics of cAMP-independent protein synthesis in the Δ*cya* strain (AMS2) under nitrogen starvation, and all but two proteins exhibited kinetics of synthesis similar to those of the wild type (Fig. 5). The remaining two proteins were heat shock proteins (DnaK and GroEL), and both were about fourfold hyperinduced in the Δ*cya* strain. As noted above (Fig. 3), these proteins were also hyperinduced under carbon starvation conditions in the Δ*cya* strain. Thus, the induction of these heat shock proteins by starvation appears to be negatively regulated by cAMP.

Comparison of the kinetics of cAMP-dependent starvation protein synthesis in K-12 and AMS2 (Δ*cya*) during nitrogen starvation revealed the curious fact that three of them (polypeptides 11, 24, and 35) required cAMP for induction during carbon starvation but not during nitrogen starvation (data not shown). All the rest of the proteins were not induced in either strain during nitrogen starvation.

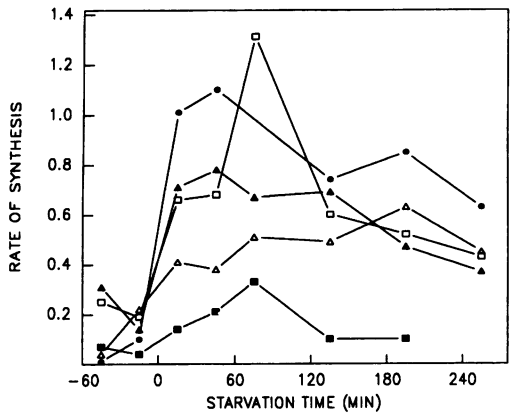

FIG. 5. Rates of synthesis of representative cAMP-independent starvation proteins in *E. coli* K-12 during nitrogen starvation. Rates of synthesis were quantitated by computer-assisted microdensitometry as described in Materials and Methods and are calculated as the percent of the total counts per minute recovered on the autoradiogram. Symbols: ●, polypeptide 3; ▲, polypeptide 34; ■, polypeptide 20; □, polypeptide 17; △, polypeptide 22. Not shown are polypeptides 19 and 23 (with kinetics similar to polypeptide 17) and polypeptide 4 (DnaK, with a maximum synthesis rate at 4 h of starvation).

DISCUSSION

The data presented in this paper show that of the 30 starvation proteins examined, 19 were not induced in a Δ*cya* or Δ*crp* strain of *E. coli*, although most of them could be induced when the Δ*cya* strain was starved in the presence of exogenous cAMP. Similarly, synthesis of β-galactosidase at the onset of starvation in a number of carbon starvation-inducible fusions occurred only in a *cya*$^+$ genetic background. Thus, about two-thirds of the starvation proteins in *E. coli* require cAMP-CRP complex for induction during starvation, but the other one-third is independent of this control. Most of the cAMP-dependent starvation proteins were induced only when the starvation regimen involved deprivation of carbon. In contrast, nearly all the cAMP-independent proteins were induced regardless of whether the starvation state was carbon, nitrogen, or phosphorus deprivation (Table 2). We propose the designation *cst* for cAMP-dependent carbon starvation response genes and *pex* for genes encoding the cAMP-independent starvation proteins. (Note that the current definition of *cst* is more restrictive than the definition used previously [15].)

The mechanism by which starvation leads to the induction of the *pex* genes remains unclear. Unique sigma factors and promoter sequences may be involved. We also do not know whether fluctuations in cAMP levels are sufficient to account for the changes in the synthesis rates of cAMP-dependent starvation proteins. We believe it to be likely that other factors also play a role, since preliminary evidence presented in this paper did not reveal strict correlation between the expected cAMP levels and the degree to which at least some of the *cst* promoters were active during starvation. Furthermore, proteins of this class encompassed all the various temporal categories of starvation proteins (15), which cannot be explained if cAMP levels were the sole determining factor. Potential candidates for factors that may be involved include specific inducer molecules generated by catabolism of endogenous carbon substrates during starvation and/or transcriptional factors uniquely synthesized during starvation that could potentiate the positive cAMP regulation. That stress conditions can induce cAMP-regulated genes has been shown for *cea*, an SOS gene that is induced by UV irradiation only in a cAMP-proficient background (2, 30). It is also clear that the cAMP-dependent starvation proteins fall into two subcategories with respect to regulation, since unlike most of the cAMP-dependent proteins, three were also induced under nitrogen starvation and did not require cAMP for induction under these conditions.

Induction of the cAMP-dependent proteins is not involved in conferring resistance to starvation. We suspect that these proteins are instead concerned primarily with preparing the cell for escape from starvation. It is known that many cAMP-controlled proteins mediate transport or catabolism of carbon substrates (1, 4, 11). Thus, induction of such proteins during starvation would be advantageous in nature, since it would enlarge the range of substrates that a bacterium can utilize without a lag, thus increasing its chances to escape starvation. An additional role for these proteins might be to facilitate bacterial adhesion to surfaces, since some starved bacteria demonstrate enhanced adherence capabilities (17).

We have previously proposed that the proteins commonly induced by different nutrients were likely to be involved in conferring resistance to starvation (15). That the induction of most of these proteins is independent of cAMP and CRP positive control is consistent with this notion, since the signal role of cAMP is confined to perturbations of the carbon and energetic state of the cell (1, 4). In contrast, considering that starving, nongrowing cells might have to face a common set of survival challenges regardless of the type of starvation, a trigger designed to adapt the cell to these problems would have to be responsive to a variety of such causes. That some cAMP-independent starvation proteins are important in starvation survival of *S. typhimurium* is also suggested by the work of Spector et al. (33).

The way in which expression of the *pex* genes is regulated and the identities and functions of the Pex proteins remain questions of central importance in understanding the nature of starvation resistance in non-sporeforming bacteria.

TABLE 2. Induction of 20 carbon starvation proteins under other starvation conditions in *E. coli* K-12

Conditions	Induction of Carbon starvation protein no.[a]:																				
	cAMP independent (Pex)									cAMP dependent (Cst)											
	3	4	6	17	19	20	22	23	34	8	11	13	24	25	26	29	30	32	33	35
Nitrogen starvation	+	+	−	+	+	+	+	+	+	−	+	−	+	−	−	−	−	−	−	+
Phosphorus starvation[b]	+	−	+	+	+	−	+	+	+	−	−	−	+	−	−	−	−	−	−	+

[a] Symbols: +, induction; −, lack of induction.
[b] Determined visually from previously published gels (15).

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