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The glucose-starvation stimulon of *Escherichia coli*: induced and repressed synthesis of enzymes of central metabolic pathways and role of acetyl phosphate in gene expression and starvation survival

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Summary

Proteins of the glucose-starvation stimulon were identified by using two-dimensional gel electrophoresis and the gene–protein database of *Escherichia coli*. Members of this stimulon included enzymes of the Embden–Meyerhof–Parnas (EMP) pathway, phosphotransacetylase (Pta) and acetate kinase (AckA) of the acetyl phosphate/acetate production pathway, and formate transacetylase. The synthesis of these enzymes was found to be induced concomitantly with the decreased synthesis of enzymes of the Krebs cycle. Thus, the modulation in the synthesis of specific proteins during aerobic glucose starvation is, in part, similar to the response of cells shifted to anaerobiosis. These modulations suggest that the glucose-starved cell increases the relative flow of carbon through the Pta–AckA pathway. Indeed, the ability to synthesize acetyl phosphate, an intermediate of the pathway, appears to be indispensable for glucose-starved cells as *pta* and *pta–ackA* double mutants were found to be impaired in their ability to survive glucose starvation. The survival characteristics of *ackA* mutants and the wild-type parent were indistinguishable. Moreover, the *pta* mutant failed to induce several proteins of the glucose-starvation stimulon.

Introduction

Cells of *Escherichia coli* respond to starvation and stress conditions by activating complex global regulatory systems for regulation above the operon level. Global control systems consist of a large number of unlinked genes and operons co-ordinately controlled by a common regulatory signal or regulatory gene. Growth arrest conditions are likely to activate several signals and unlinked regulatory systems in the cell. To avoid confusion, Smith and Neidhardt (1983b) therefore proposed the use of the term stimulon to refer to the entire set of genes/proteins responding to a given environmental stimulus. The majority of the genes/proteins of a stimulon are, in general, uniquely induced by one specific stimulus (e.g. VanBogelen *et al.*, 1990; VanBogelen and Neidhardt, 1991). However, stimulons can share member proteins (e.g. VanBogelen *et al.*, 1990; VanBogelen and Neidhardt, 1991; Nyström and Neidhardt, 1992). During the transition from vegetative growth to glucose starvation-induced growth arrest, *E. coli* increases the synthesis of a group of proteins which together make up the glucose-starvation stimulon. The proteins (Gsps; glucose starvation proteins) of this stimulon are made in a sequential, time-dependent manner and can be classified according to their kinetics of synthesis (Groat *et al.*, 1986). Only limited information exists as to the identities of the specific sensors/signals triggering the synthesis of these protein classes. It is also not clear whether the synthesis of different Gsps is independent or whether some of them represent sequential steps in an ordered cascade pathway. Although the majority of Gsps are of unknown function, several perform functions that are indispensable for the survival of carbon-starved *E. coli* (Schultz *et al.*, 1988; Lange and Hengge-Aronis, 1991; Nyström and Neidhardt, 1994), and some are members of well-characterized stress regulons (e.g. Jenkins *et al.*, 1988; VanBogelen *et al.*, 1990; Lange and Hengge-Aronis, 1991).

The aim of this work has been to identify genes/proteins of the glucose-starvation stimulon as a means of understanding (i) the physiological commitment required by *E. coli* to survive in the absence of a growth-supporting carbon/energy source, and (ii) what sensors and signals are activated in the cell in response to growth arrest. Towards this end, proteins of *E. coli*, labelled at various times during a 24 h period of starvation, were first separated by two-dimensional (2-D) gel electrophoresis in accordance with the methods used for generating the gene–protein database of *E. coli* (VanBogelen *et al.*, 1990), and classified as either glucose starvation

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inducible or repressible. Using this database, Gsps were traced to their structural gene, protein name, enzymatic function, or membership in global regulatory networks (VanBogelen *et al.*, 1990), and several Gsps whose synthesis was induced at different times during glucose starvation were tentatively identified. Gsps belonging to one kinetic class were found to be enzymes of the Embden–Meyerhof–Parnas pathway (EMP), the acetate/acetyl phosphate production pathway, and formate transacetylase. Further, the induced synthesis of these proteins was accompanied by a repressed synthesis of several enzymes of the Krebs cycle. The results indicate that the sensors/signals activated during anaerobiosis may be activated also during aerobic glucose-starvation conditions. The importance of acetyl phosphate in starvation survival and gene expression is also demonstrated.

Results

Starvation protocol

The *E. coli* strains used were grown aerobically in glucose minimal morpholinepropanesulphonic acid (MOPS), or M9 media with 0.02% glucose at 37°C. In these media, growth was abruptly arrested at an optical density (420 nm) of 0.5–0.53 when glucose was exhausted (Fig. 1). When glucose was added back to the growth-arrested culture, growth resumed at a rate typical of cells growing aerobically in this medium (Fig. 1). Thus, cells were arrested because of glucose depletion alone and not as a result of additional stresses such as the presence of excreted byproducts or oxygen exhaustion.

Several Gsps of E. coli *are enzymes of central metabolism whose synthesis is normally induced by anaerobiosis*

E. coli W3110 was pulse-labelled with [^{35}S]-methionine during exponential growth and at 2, 5, 15, and 30 min, and 1, 2, 3, 5, 7, 10, 16, and 24 h after growth ceased owing to glucose depletion. Analysis by 2-D gel electrophoresis revealed that the rates of synthesis of about 82 proteins were induced at some time during the glucose-starvation period studied. Unfortunately, a correlation between this study and the study of glucose-starvation proteins by Matin and coworkers (Groat and Matin, 1986) was impossible owing to differences in the 2-D protocols. Using the gene–protein database of *E. coli* (VanBogelen *et al.*, 1990), eight Gsps were identified as heat-shock proteins including GrpE, GroEL, DnaK, GroES, HtpH, HtpO, ClpB, and Lon. One Gsp, F050.6, belongs to the oxidation stress regulon (*oxyR*-controlled), while no protein of the *E. coli* SOS response catalogued by 2-D analysis (VanBogelen *et al.*, 1990) was identified as being a member of the glucose-starvation stimulon. Among the remaining 73 Gsps, 15 were tentatively identified as enzymes of the EMP pathway, different anapleurotic reactions, the Entner–Doudoroff pathway, and the purine salvage pathway. The induction of synthesis of these polypeptides was rather modest: 1.5- to 10-fold, and six of these enzymes and three Gsps of unknown identity have previously been reported to be induced by anaerobiosis (Fig. 2; Table 1; VanBogelen *et al.*, 1990; Smith and Neidhardt, 1983a). These proteins include, phosphofructokinase, enolase, pyruvate kinase, formate acetyltransferase, acetate kinase, phosphotransacetylase, B011.8, B015.0, and F038.0, four of which are members also of the phosphate-starvation stimulon (VanBogelen *et al.*, 1990; Table 1). Moreover, these proteins exhibited similar kinetics of synthesis during glucose starvation (Fig. 3, top, left panel). To ensure that the tentative identification of these proteins was correct, their responsiveness to anaerobiosis was also determined. As depicted in Fig. 3 (top, right panel) the synthesis of all the Gsps of this group was rapidly induced when cells were shifted to anaerobic growth conditions, and the extent of induction during anaerobiosis is very similar to that previously reported (Smith and Neidhardt, 1983a). The kinetics of synthesis, however, differed between the conditions. While the synthesis of the Gsps of this group was induced within minutes after a shift to anaerobiosis, their rates of synthesis were gradually increased during several hours of aerobic glucose starvation (Fig. 3, top panels).

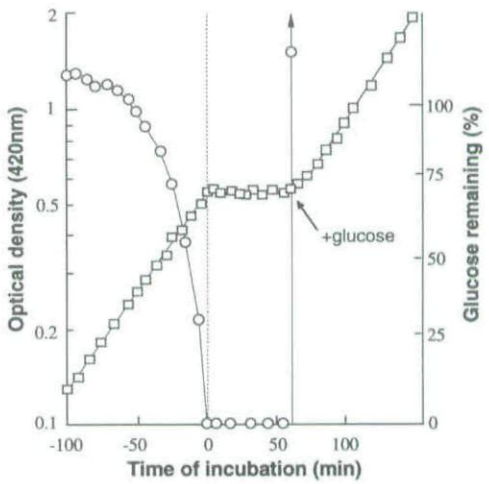

Fig. 1. Growth arrest as a result of glucose depletion by *E. coli* W3110. The culture was grown at 37°C in MOPS minimal medium initially containing 200 μg of glucose per ml. The arrow indicates the time at which 2.5 mg glucose was added to the growth-arrested culture. Optical density (squares), and glucose concentration (circles) are recorded in the graph.

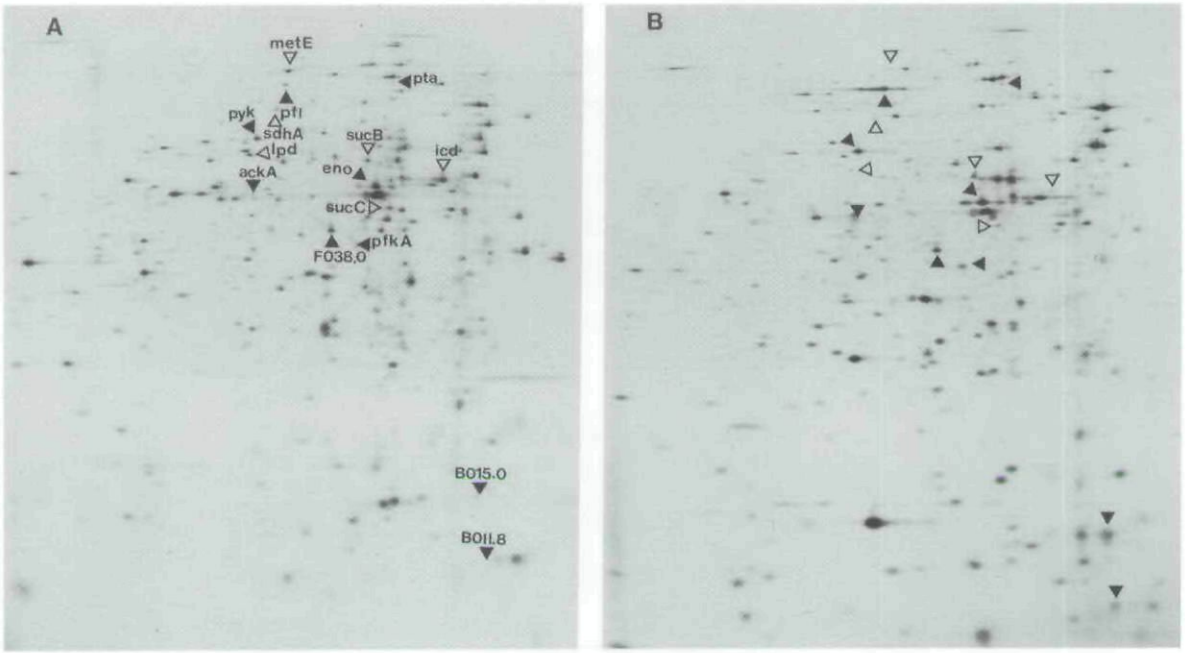

Fig. 2. Autoradiograms of 2-D polyacrylamide gels of extracts of *E. coli* W3110 growing aerobically in glucose minimal MOPS at 37°C (A), and glucose starved for 3 h (B). Cells were labelled for 5 min with [^{35}S]-methionine during exponential growth, and for 15 min after 3 h of starvation. Protein spots marked with filled triangles denote proteins whose synthesis was found to be induced by glucose starvation while open triangles denote proteins with repressed synthesis. Gene symbols are used to identify proteins (see Table 1 for full enzyme name). For pI and molecular weight designations of protein spots in the autoradiograms consult the reference gels of the 'Gene–protein database of *Escherichia coli*' (VanBogelen *et al.*, 1990).

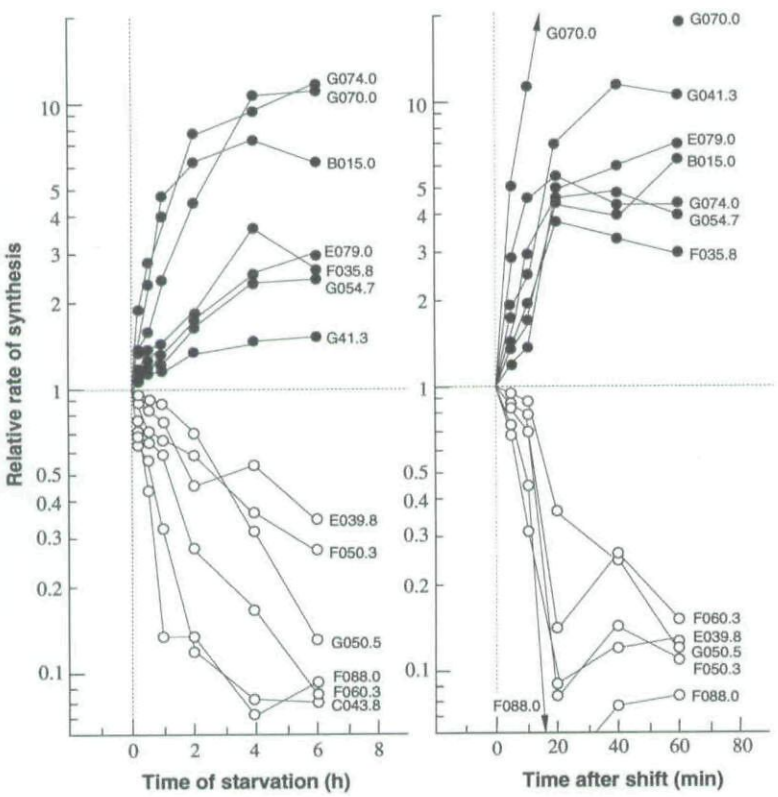

Fig. 3. Relative rates of synthesis of individual proteins at various times after glucose depletion (left panel) and after a shift from aerobic to anaerobic growth condition (right panel). All values are normalized to the measured preshift rate of synthesis. The alpha numeric designations for the proteins are used in the graph (VanBogelen *et al.*, 1990). Their corresponding enzyme names are shown in Table 1.

Table 1. Enzymes of central metabolic pathways with induced and repressed synthesis during glucose starvation.

Alpha numeric	Gene name	Protein name	Induction by other conditions
Induced by glucose starvation			
B011.8			An
B015.0			An, P
E079.0	*pta*	Phosphotransacetylase	An
F007.0	*ptsH*	Phosphohistidinoprotein-hexose phosphotransferase	
F035.8	*pfkA*	Phosphofructokinase	An, P
F038.0			An
F043.8	*eno*	Enolase	An
G041.3	*ackA*	Acetate kinase	An
G054.7	*pyk*	Pyruvate kinase	An, P
G070.0	*pfl*	Formate acetyltransferase	An
G074.0	*pfl*	Formate acetyltransferase	An, P
Repressed by glucose starvation			
C043.8	*icd*	Isocitrate dehydrogenase	Ae
E039.8	*sucC*	Succinyl-CoA synthetase, β subunit	Ae
F050.3	*sucB*	α-ketoglutarate dehydrogenase, E2	Ae
F060.3	*sdhA*	Succinate dehydrogenase, flavoprotein subunit	Ae
F088.0	*metE*	Tetrahydropteroyltriglutamate methyltransferase	Ae
G050.5	*lpd*	Lipoamide dehydrogenase, E3	Ae

Alpha numeric designations are from VanBogelen *et al.* (1990). The kinetics of synthesis during glucose starvation of proteins (alpha numerics) in boldface are shown in Fig. 2.
Abbreviations: Ae, aerobiosis; An, anaerobiosis; P, phosphate starvation.

The relative rates of synthesis of the proteins accounted for in Fig. 3 were unaffected by growth of cells on glycerol or acetate as compared to glucose and were induced by glucose starvation also in a *cya* and *crp* genetic background (data not shown) demonstrating that the induced production of these enzymes is probably not the result of elevated levels of cyclic AMP. In addition, starvation for gluconate (a non-PTS carbohydrate) elicited an induced synthesis of the enzymes shown in Fig. 3, ruling out the PTS system as the sensor for the observed response.

The syntheses of Krebs cycle enzymes normally repressed by anaerobiosis are also repressed in response to glucose starvation

Anaerobiosis-induced synthesis of the Gsps listed in Table 1 have been reported to occur concomitantly with a repressed expression of genes of the Krebs cycle, including *sucC, sucB, sdhA, lpd, icd, aceE*, and *aceF*, and *metE* (Smith and Neidhardt, 1983a; VanBogelen *et al.*, 1990). A similar reciprocal relationship between the synthesis of Gsps and Krebs cycle enzymes was observed during glucose starvation (Fig. 3, top and bottom panels). To determine whether the observed alterations in enzyme synthesis lead to an activation of fermentative pathways in glucose-starved cells, the medium of the cells was analysed with regard to excreted mixed acid fermentation products. First, the cell constituents of *E. coli* were labelled by including uniformly labelled [^{14}C]-glucose in the growth medium. Then, the fate of ^{14}C during glucose starvation was followed in culture filtrates by analysis on the Bio-Rad Aminex HPX-87H column designed for separation of mixed acid fermentation products. At the onset of glucose starvation about 5% of the label originating from glucose was recovered in filtrates and identified as acetate (Fig. 4). The accumulated acetate was subsequently

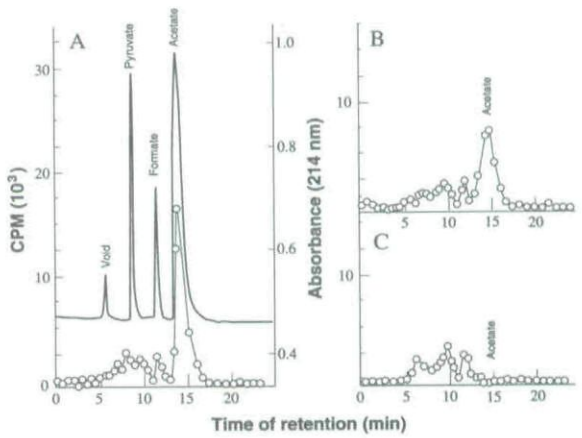

Fig. 4. Analysis of labelled filtrates obtained from an *E. coli* W3110 culture at the onset (A), after 40 min (B), and 3 h (C) of glucose starvation on a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column. The eluates were monitored at 214 nm, 0.5 ml fractions were collected, and radioactivity was measured. Labelled filtrates were coeluted with standard samples containing pyruvate, formate, and acetate. See text for details.

utilized during the first few hours of starvation (Fig. 4), and no fermentation products were found to accumulate in the medium during the starvation period studied.

The induced synthesis of Gsps and the repressed synthesis of Krebs cycle enzymes by glucose starvation is not a growth rate effect

The expression of many genes of *E. coli* has been demonstrated to be growth rate dependent (Pedersen *et al.*, 1978). The induction or repression of genes during entrance of cells into stationary phase could be due to the activation or inhibition of transcription from 'growth-rate-sensitive' promoters as the growth rate gradually decreases during the transition to growth arrest (Aldea *et al.*, 1990). To address this possibility, the levels of synthesis of the proteins shown in Fig. 3 were measured in *E. coli* growing at different rates in MOPS minimal media supplemented with different carbon sources (Fig. 5; Wanner *et al.*, 1977). No correlation between growth rate-dependent expression and expression during growth arrest was noted for the proteins of this study. In other words, the synthesis of Gsps was not inversely dependent on growth rate (Fig. 5, left panel), and synthesis of proteins repressed by glucose starvation did not show a direct growth rate dependence (Fig. 5, right panel). In fact, the levels of synthesis of most of the proteins (Krebs cycle enzymes) reported here to be repressed by glucose starvation are inversely dependent on growth rate.

Production of acetyl phosphate but not acetate/ATP is important for starvation survival

The increased synthesis of enzymes of the *pta–ackA* pathway in parallel to the repressed synthesis of the Krebs cycle enzymes may suggest that acetyl-CoA is used preferentially for acetate production, provided that no fermentative pathways are operating in the growth-arrested cells. The *pta–ackA* pathway operates primarily in the acetyl-CoA-to-acetate direction and in *E. coli* conversion of acetyl phosphate to acetate by acetate kinase provides a major source of ATP during anaerobic growth (Thauer *et al.*, 1977). The possible role of this ATP production during growth arrest was assessed by determination of the culture viability of *pta*, *ackA*, and *pta–ackA* double mutants subjected to glucose starvation. It was demonstrated that the production of acetyl phosphate rather than ATP was important for the survival of glucose-depleted cultures since *pta*, and *pta–ackA* double mutants were impaired in their ability to survive while *ackA* mutants were not (Fig. 6). The growth rates of *pta*, *pta–ackA*, and wild-type *E. coli* were indistinguishable in glucose minimal M9 at 37°C (g = 52 min), while the *ackA* mutant grew somewhat slower (g = 61 min).

Acetyl phosphate is involved in regulating the synthesis of proteins of the glucose-starvation stimulon

The pattern of protein synthesis was found to be different in the *pta* mutant as compared to the isogenic parent during both growth and glucose starvation. The differences encompass both proteins whose synthesis is induced and repressed during starvation, as well as steady-state levels of synthesis during exponential growth. The locations on 2-D gels of proteins with altered levels of synthesis in the *pta* mutant are highlighted in Fig. 7. To determine whether the observed changes were a result of the inability to synthesize acetyl phosphate rather than acetate, the pattern of protein synthesis was also analysed in the *ackA* mutant, devoid of the capacity for

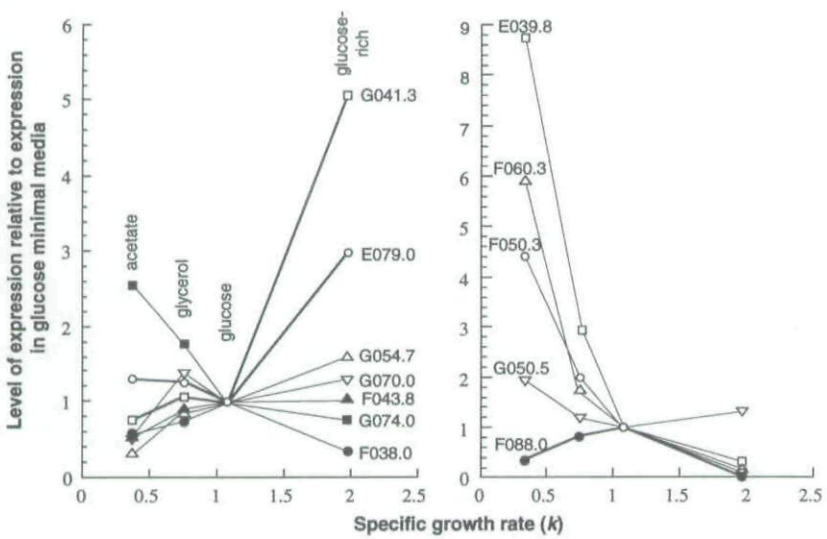

Fig. 5. Relative rates of synthesis of individual proteins at varying growth rates. Proteins with induced and repressed rates of synthesis during glucose starvation are recorded in the left and right panel, respectively. Strain W3110 was grown exponentially in minimal MOPS media supplemented with acetate, glycerol or glucose as carbon sources and glucose plus amino acids, nucleotides and vitamins for rich medium. The rates of protein synthesis are plotted relative to the rate of synthesis in glucose minimal media, which was assigned a value of 1.0. Growth rates in the different media are expressed as *k*, the first order growth-rate constant.

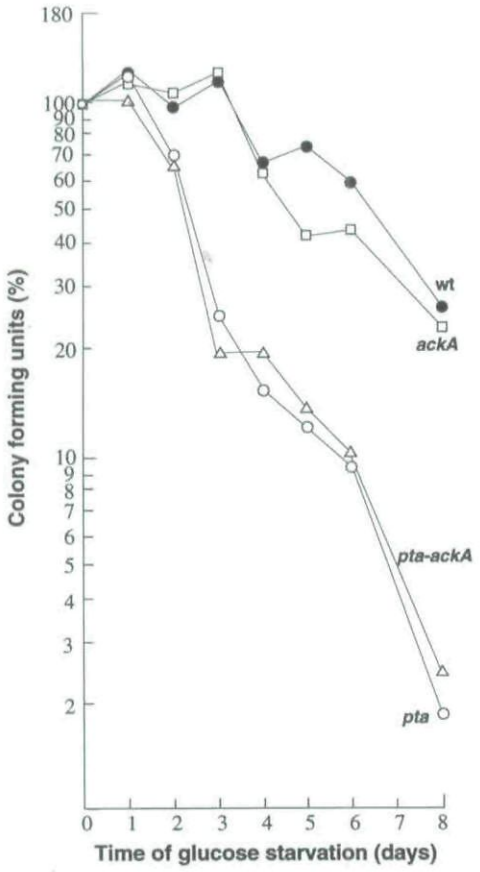

Fig. 6. Starvation survival of the wild type (BW13711), *pta* (BW16462), *ackA* (BW16545), and *pta–ackA* double (BW16463) mutant strains during glucose depletion. Strains were grown in glucose minimal M9 medium with one-twentieth the normal concentration of glucose. After growth arrest commenced, incubation was continued for 8 d under the same conditions. Viable cells were counted as colonies plated on LB plates after appropriate dilutions. 100% viability corresponds to the number of viable cells counted 1 h after growth was arrested owing to the depletion of glucose.

acetate but not acetyl phosphate production. The result of this analysis is summarized in Table 2. Proteins 1–5 are Gsps whose synthesis is induced in a *pta*-dependent, *ackA*-independent manner during glucose-starvation. Thus, these proteins of the glucose-starvation stimulon appear to be regulated, in part, by levels of acetyl phosphate. Also, proteins 12 and 16 (Table 2), whose synthesis is normally repressed during starvation, also failed to be so in the *pta* mutant. Synthesis of protein 16 also failed to be repressed in the *ackA* mutant, indicating a role for acetate rather than acetyl phosphate in the regulation of this protein.

Proteins not belonging to the glucose-starvation stimulon were also found to be subjected to control by the status of the *pta* and *ackA* genes. For example, the synthesis of proteins 6, 7, 9, 11, 13, 14, 15, 17, and 18, which was more or less unaltered during the transition of the wild-type strain to growth arrest, was significantly induced in the *pta* strain, and proteins 8 and 10 exhibited higher steady-state levels of synthesis during exponential growth in the *pta* mutant. A few of these proteins were also affected by the *ackA* mutation (Table 2).

Discussion

The analysis of the physiology of glucose-starved *E. coli* cells has been hampered, in part, by the fact that the identities or functions of most of the proteins (Gsps) being made are unknown and the pathways employed to provide endogenous energy for protein synthesis during starvation are not characterized. Moreover, the regulatory mechanisms underlying the increased synthesis of the majority of Gsps are not well understood. It has been established that some genes encoding Gsps require cAMP/CRP for induction (Schultz *et al.*, 1988), and a dozen or so are members of the *rpoS* regulon (Lange and Hengge-Aronis, 1991; McCann *et al.*, 1991). The data presented in this communication may shed some light on the physiological rearrangements that take place in response to glucose starvation and also raise several new questions.

Protein identifications of some Gsps as well as proteins whose synthesis is repressed by glucose starvation, demonstrate that the physiological response elicited in the glucose-starved cell is reminiscent of the cell's response to anaerobiosis. The identified EMP enzymes and Krebs cycle enzymes and their assignments to central metabolic pathways are depicted in Fig. 8. The similarity between the response of glucose-starved cells and cells subjected to oxygen depletion may suggest that similar changes in the routes of carbon source utilization occur during the different conditions. Specifically, depletion of oxygen, or other electron acceptors, places a greatly increased demand on the EMP pathway, minimizing the role of the Krebs cycle as a source of reducing equivalents for respiration, and diverts the flow of intermediates through glycolysis to fermentative pathways. The observed alterations in the rates of synthesis of specific proteins during glucose starvation did not result in an apparent excretion of fermentation products (Fig. 4). However, this observation does not rule out the possibility that fermentation pathways are operating because it is possible that the rate of metabolism in the growth-arrested cell is too low to allow for the detection of excreted products. Also, fermentation products may be re-utilized instantaneously by the growth-arrested population.

The ArcA repressor controls the repression of Krebs cycle genes in response to anaerobiosis although it is clear that oxygen itself is not the signal of the *arcA* system (Iuchi and Lin, 1988). Rather, it has been proposed that the signals activating the *arcA* regulon during shifts in oxygen

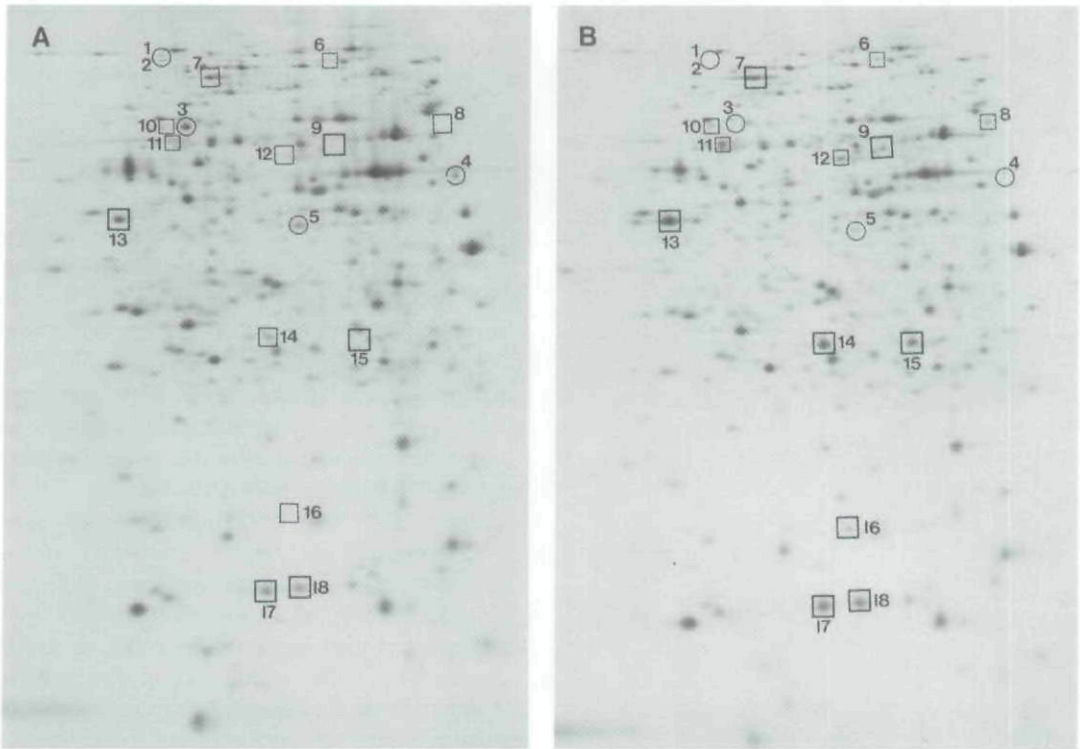

Fig. 7. Autoradiograms of 2-D polyacrylamide gels of extracts of wild-type *E. coli* BW13711 (A) and the *pta* mutant BW 16462 (B) glucose starved for 3 h. Cells were labelled for 15 min with [^{35}S]-methionine. Boxed spots denote proteins that exhibit a markedly higher rate of synthesis in BW16545 as compared to BW13711, while circled spots represent proteins that have a higher rate of production in BW13711.

Table 2. List of proteins with altered levels of synthesis in *pta* (BW16462) and *ackA* (BW16545) mutants as compared to their parent (BW13711).

			Expression					
			growth			starvation		
Alpha numeric	Protein number	x–y co-ordinate	wt	*pta*	*ackA*	wt	*pta*	*ackA*
	1	30 × 158				++		++
	2	32 × 159				++		++
	3	37 × 135.5				++		++
	4	99.5 × 126				++		++
F038.5	5	63 × 114.5				++		++
	6	69 × 156					++	++
	7	47 × 146.5				+	++	+
	8	99 × 136		++			++	
	9	72 × 129					++	
D028.3	10	32 × 134		++	++		++	++
	11	33 × 130					++	
	12	60 × 128	++	++	++		++	
	13					+	++	+
	14	55 × 90				+	++	+
	15	76 × 89.5		+			++	
	16	58 × 51	++	++	++		++	++
F013.9	17	50.5 × 33.5				+	++	+
	18	59.5 × 36				+	++	+

Expression was determined for cells growing exponentially in M9 glucose minimal medium and glucose starved for 3 h. The x–y co-ordinates are those of the standard 2-D reference gel of *E. coli* (Van Bogelen *et al.*, 1990). Three proteins are tenatitively assigned alpha numeric designations. ++ denotes markedly induced levels of synthesis; + denotes weakly induced levels of synthesis.

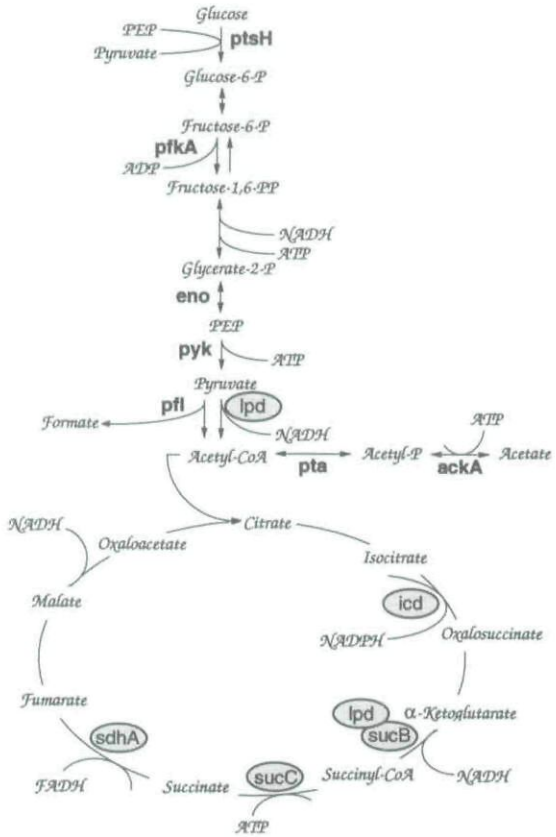

Fig. 8. Some principal routes for carbon metabolism with enzymes found to be induced or repressed by glucose starvation. Symbols in boldface denote enzymes induced by glucose starvation. Circled symbols denote enzymes repressed by glucose starvation. In general, gene symbols are used to identify enzymes (see Table 1, for complete enzyme names and alpha numeric designation). Pts has been demonstrated previously to be induced by glucose starvation (VanBogelen *et al.*, 1990) and is not included in this study. See text for details.

availability may be either (i) a graded decrease in the difference of electrical potential (or pH gradient) across the cytoplasmic membrane affecting the redox state of a sensor in the membrane (e.g. a cytochrome) or (ii) shifts in the cellular redox state influencing the ratio of oxidized to reduced forms of electron adapters in the respiratory chain (Iuchi and Lin, 1988). It is possible that these signals may also be activated during a shift from growth to stasis owing to depletion of the exogenous carbon/energy source and therefore the ArcA repressor may also be activated during carbon/energy starvation. In this context, it is interesting to note that the amount of the intermediate component menaquinone, predominantly used during anaerobic respiration, increases 4.4-fold during transition from growth to aerobic stationary phase, while ubiquinone, predominantly used during aerobic respiration, decreases 3.2-fold (Poole and Ingledew, 1987). This, together with the data presented here concerning the repressed synthesis of Krebs cycle enzymes during starvation, indicates that dehydrogenases other than NADH dehydrogenase are increasingly important for growth-arrested cells since electron transport from NADH to oxygen cannot be accomplished by menaquinone as the intermediate component (Wallace and Young, 1977). The increased synthesis of formate transacetylase (Pfl) during glucose starvation is consistent with this notion. In contrast to pyruvate dehydrogenase, Pfl provides acetyl-CoA from pyruvate without producing NADH (Fig. 8). However, Pfl is presumably inactive in the presence of oxygen (Knappe and Schmitt, 1976) making its synthesis during aerobic, glucose starvation conditions rather obscure. It should be noted that the *pfl* operon is one of only a few whose expression is regulated positively by the ArcA regulator (Sawers, 1993).

The modest induction of *pta–ackA* and genes of the EMP pathway is an integral part of the *E. coli* response to anaerobiosis (Smith and Neidhardt, 1983a). The conversion of acetyl phosphate to acetate by acetate kinase provides a major source of ATP during anaerobic growth (Thauer *et al.*, 1977). The role of this pathway during glucose exhaustion, however, appears to be connected to the synthesis of acetyl phosphate rather than ATP and acetate (Fig. 6), since *pta*, and *pta–ackA* double mutants but not *ackA* mutants are impaired in their ability to withstand glucose starvation. Acetyl phosphate is a high-energy phosphate compound and acts as a phosphoryl donor to enzyme I of the phosphoenolpyruvate:glucose phosphotransferase system (Fox *et al.*, 1986). Also, acetyl phosphate is the preferred phosphoryl donor for some binding protein-dependent transport systems (Hong *et al.*, 1979). It is possible that acetyl phosphate can substitute for ATP in other metabolic reactions and that there is a demand for such high-energy phosphate compounds in the carbon/energy-starved cell. It has been proposed that acetyl phosphate also functions as a global regulator of gene expression in *E. coli* (Wanner, 1992; Wanner and Wilmes-Riesenberg, 1992; McCleary *et al.*, 1993). Pertubations in the production of acetyl phosphate have been shown to affect the *in vivo* expression of the phosphate (Pho) and nitrogen (Ntr) regulons of *E. coli* (Wanner and Wilmes-Riesenberg, 1992; Feng *et al.*, 1992), and phosphorylation of the regulatory protein of the Ntr regulon occurs *in vitro* with acetyl phosphate as phosphate donor, bypassing the need for autophosphorylation of the sensor protein (Feng *et al.*, 1992). The data presented here concerning the global change in the pattern of protein synthesis in *pta* mutants further substantiates the suggestion that acetyl phosphate may function as a global regulator in *E. coli*. Unfortunately, most of the proteins demonstrated to be subjected to control by the state of the *pta* gene (Table 2) could not be identified using the gene–protein database

(VanBogelen and Neidhardt, 1991). One protein that responded to glucose starvation in a *pta*-dependent fashion was tentatively identified as ribosomal protein L7/L12 acetyltransferase (F038.5; Table 2) but this identification awaits confirmation by *N*-terminal sequencing. The results presented here do not exclude the possibility that the altered pattern of protein synthesis and impaired survival capacity of *pta*, *pta–ackA* mutants are due to their inability to drain acetyl-CoA levels rather than being a direct effect of acetyl phosphate.

The re-utilization of excreted acetate seen during the first few hours of starvation (Fig. 4) is probably accomplished by acetyl-CoA synthetase (Brown *et al.*, 1977), an inducible enzyme subjected to catabolite repression and responsible for acetate uptake and activation in *E. coli* (Brown *et al.*, 1977). The *pta–ackA* genes are not induced by acetate nor catabolite repressed by glucose (Brown *et al.*, 1977). In contrast, several Krebs cycle enzymes are activated by the cAMP–CRP complex (Nimmo, 1987). Levels of this complex would presumably increase during the glucose-starvation conditions employed. Yet the synthesis of Krebs cycle enzymes was found to be repressed. Also, the induced synthesis of the EMP enzymes by glucose starvation was unaffected by mutations in the *cya* and *crp* genes. Thus, regulatory mechanisms other than catabolite repression are probably responsible for the patterns of protein induction/repression reported in this communication. Moreover, the data presented in this paper (Fig. 5) exclude growth rate regulation as being the sole mechanism involved in the altered expression of Gsps and Krebs cycle enzymes during glucose starvation. It should be noted that the apparent inverse correletion between growth rate and expression of Krebs cycle enzymes may be due to the fact that the carbon sources used (acetate and glycerol) for generating the lowest growth rates are not fermentable. However, it is unlikely that this would mask a direct correlation between growth rate and expression of these enzymes, and no such correletion is observed between cells growing in glucose-minimal and glucose-rich medium (Fig. 5). The modulation in the pattern of enzyme synthesis described here was not observed in cells starved for isoleucine or nitrogen, or growth arrested by overproducing guanosine tetraphosphate (data not shown). Thus, growth arrest in general is not sufficient to elicit the response. However, starvation for glucose, gluconate or glycerol resulted in similar modulations in the synthesis of EMP and Krebs cycle enzymes (data not shown) indicating that carbon/energy starvation is the determinant for the observed response.

In conclusion, changes in the pattern of protein synthesis (this paper) as well as levels of the electron adapters, ubiquinone and menaquinone (Poole and Ingledew, 1987) indicate that the trials and tribulations of glucose/energy starvation and anaerobic existence are similar. The principal questions and directions of further research relate to the trigger mechanisms involved in the seemingly similar physiological adjustments performed by cells shifted to anaerobiosis and conditions of carbon starvation. Also, the mechanism of control of gene expression by acetyl phosphate should be addressed as well as the identities of proteins subjected to this control.

Experimental procedures

Bacterial strains, media and growth conditions

The K-12 strains of *E. coli*, W3110 (Smith and Neidhardt, 1983a), BW13711 (DE3[*lac*]X74), BW16462 (as BW13711 but *pta-200 zej-223*::Tn*10*), BW16463 (as BW13711 but Δ[*pta*, *ackA*, *hisQ*, *hisP*] *zej-223*::Tn*10*), and BW16545 (as BW13711 but *ackA200, zej-223*::Tn*10*) were used as noted. Strains BW13711, 16462, 16463, and 16545 were kindly provided by Dr Barry L. Wanner through Dr Victor deLorenzo. Cultures were grown in liquid MOPS (Neidhardt *et al.*, 1974) or M9 (Sambrook *et al.*, 1989) medium supplemented with glucose (0.4% w/v) and thiamine (10 mM) for minimal medium at 37°C, or anaerobically as described previously (Smith and Neidhardt, 1983a). For analysis of proteins induced by starvation for glucose, cells were grown aerobically in glucose minimal MOPS or M9 medium with one-twentieth the normal concentration of glucose (Nyström and Neidhardt, 1992). This procedure resulted in growth arrest of cells at an OD_{420} of 0.5–0.53 (about 1.5×10^8 cells ml^{-1}).

Resolution of proteins on two-dimensional polyacrylamide gels

Culture samples were processed to produce extracts for resolution on 2-D polyacrylamide gels by the methods of O'Farrell (1975) with modifications (VanBogelen and Neidhardt, 1990). The 2-D gel apparatuses used for the O'Farrell methodology were that of Hoefer Corporation and the Investigator 2-D Electrophoresis System of Millipore Corporation. Alphanumeric (A-N) designations and/or x–y coordinates were assigned to protein spots after matching them to the reference 2-D images of the gene–protein database of *E. coli* (VanBogelen *et al.*, 1990).

Measurement of rates of synthesis of individual proteins

At indicated times, a portion (1 ml) of a culture was removed and placed in a flask containing [^{3}H]-leucine (5 mCi $mmol^{-1}$, 100 μCi ml^{-1}). Incorporation was allowed to proceed for different lengths of time after which non-radioactive leucine (2.4 mM) was added for a chase. To this sample was added a portion of a culture of the same strain grown in [^{35}S]-methionine labelling medium as described by Nyström and Neidhardt (1992). The mixed sample was then prepared as previously described by VanBogelen and Neidhardt (1990) to produce extracts for resolution of the cellular proteins on two-dimensional gels. An autoradiogram was prepared to permit visualization of labelled proteins. Protein spots chosen for quantitative assay were sampled from the dried gel with a

syringe needle and treated as described by Pedersen *et al.* (1976) to permit measurement of their ^{3}H and ^{35}S content by scintillation counting. The differential rate of synthesis of a sampled protein was defined as the $^{3}H/^{35}S$ ratio of the sampled spot divided by the same isotope ratio of unfractionated mixed extracts.

Glucose determinations

Glucose concentrations in the culture media were determined after samples were filtered through Millex-GV (Millipore) membranes of 0.22 μm pore size. Glucose recovered in filtrates was quantified using the coupled glucose oxidase/peroxidase enzymatic method with *o*-dianisidine dihydrochloride as the chromogen (Sigma Diagnostics).

Bacterial metabolism of carbon during starvation

To trace the fate of glucose carbon during growth and subsequent starvation. Cultures were grown in glucose (0.02% w/v) limited MOPS media, with [U-^{14}C] D-glucose (400 nM; 0.24 Ci mmol^{-1}; 1 mCi ml^{-1}) and the radioactivity of filtrates were determined at time intervals.

Filtrates were then subjected to analysis on a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column (designed for the separation of mixed acid fermentation products) which was eluted isocratically with 8 mM H_2SO_4 (Matthews and Neidhardt, 1989). The eluate was monitored at 214 nm, 0.5 ml fractions were collected, and radioactivity was measured in a Beckman LS7500 scintillation counter.

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