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Isolation and Properties of a Mutant of *Escherichia coli* with an Insertional Inactivation of the *uspA* Gene, Which Encodes a Universal Stress Protein

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Cells of *Escherichia coli* increase greatly the synthesis of a small cytoplasmic protein as soon as the cell growth rate falls below the maximal growth rate supported by the medium, regardless of the condition inhibiting growth. The gene, designated *uspA* (universal stress protein A), encoding this protein has been cloned and mapped, and its nucleotide sequence has been determined (T. Nyström and F. C. Neidhardt, Mol. Microbiol. 6:3187–3198, 1992). We now report the isolation of an *E. coli* mutant defective in UspA synthesis because of insertional inactivation of the corresponding gene. Analysis of such a mutant demonstrated that it grows at a rate indistinguishable from that of the isogenic parent but lags significantly when diluted into fresh medium, regardless of the carbon source included. In addition, the mutant exhibits a diauxic type of growth when grown on certain single substrates, such as glucose and gluconate. This growth phenotype was found to be the result of abnormal metabolism of the carbon source (e.g., glucose) accompanied by excretion into the medium of acetate. The diauxic type of growth may be attributed to the failure of cells to form acetyl coenzyme A synthetase and to form isocitrate lyase and malate synthase of the glyoxalate bypass, needed for the assimilation of the produced acetate, until glucose or gluconate has been completely exhausted. The *uspA* mutant appears to dissimilate glucose at an elevated rate that is not commensurate with its biosynthetic processes. These results suggest that the role of protein UspA may be to modulate and reorganize the flow of carbon in the central metabolic pathways of *E. coli* during growth arrest.

Bacteria generally need to express only part of their genome to become structural and functional units of an environment. Continual changes in environmental conditions can then be accommodated by complex but efficient mechanisms that alter the pattern of gene expression. While the exhaustion of a building block may require the induction of a particular operon, severe stress conditions causing growth arrest are more complex, requiring global control systems (4, 11, 33) for regulation above the operon level. Global control systems consist of multiple unlinked genes and operons coordinately controlled by a common regulatory signal or regulatory gene (17). Such global control systems are known to be activated in response to stresses such as temperature shifts (18, 19), irradiation, chemical and physical assaults, and nutrient starvation (e.g., 4, 9, 10, 12, and 13).

Growth arrest conditions are likely to activate several signals and unlinked global regulatory systems. For this reason, Smith and Neidhardt (27) proposed the use of the term stimulon to refer to the entire set of genes responding to a given environmental stimulus. The majority of the proteins encoded by a starvation or stress stimulon are, in general, uniquely induced by one specific stimulus (5, 30). However, stimulons can share member proteins (6, 7, 30, 31). We have reported that one such shared responder of the *Escherichia coli* stress stimulons is unique in that it appears to be a universal, nonspecific responder to growth arrest or to perturbations in unrestricted balanced growth (21). The induction of this protein was found to be independent of that of all the global regulators so far studied; thus, this protein could not be assigned membership in any global regulatory network (21). The gene, designated *uspA* (universal stress protein A), encoding this protein has been cloned and its DNA sequence has been determined (21). Sequence analysis revealed that the *uspA* gene is a previously unidentified gene located at the 77-min region of the *E. coli* chromosome. The increased level of protein UspA during growth inhibition appears to be primarily a result of transcriptional activation of the corresponding gene (21). The increased transcription of the gene, at least during glucose carbon starvation, is a result of the activation of a σ^{70}-dependent housekeeping promoter (21).

We now report an analysis of the consequences of insertional inactivation of the *uspA* gene. We demonstrate that the mutation in *uspA* causes abnormalities in carbon utilization, such that a significant fraction of the carbon source, which normally enters the tricarboxylic acid cycle for biosynthesis under the aerobic growth conditions used, is catabolized to acetate and released into the culture medium. The results presented indicate that the UspA protein may be involved in modulating the flow of carbon in the central metabolic pathways of *E. coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. *E. coli* K-12 strain JM105 [F′ *traD36 lacI*^{q} Δ(*lacZ*)M15 *proAB/thi rpsL endA sbcB15 hsdR4* Δ(*lac-proAB*)] was used except as otherwise noted. Strain JC7623 (*arg ara his leu pro recB21 recC22 sbcB15 thr*) was used to recombine the *uspA*::*kan* mutation into the chromosome. The mutation was transduced into *E. coli* JM105 by standard P1*vir* transduction

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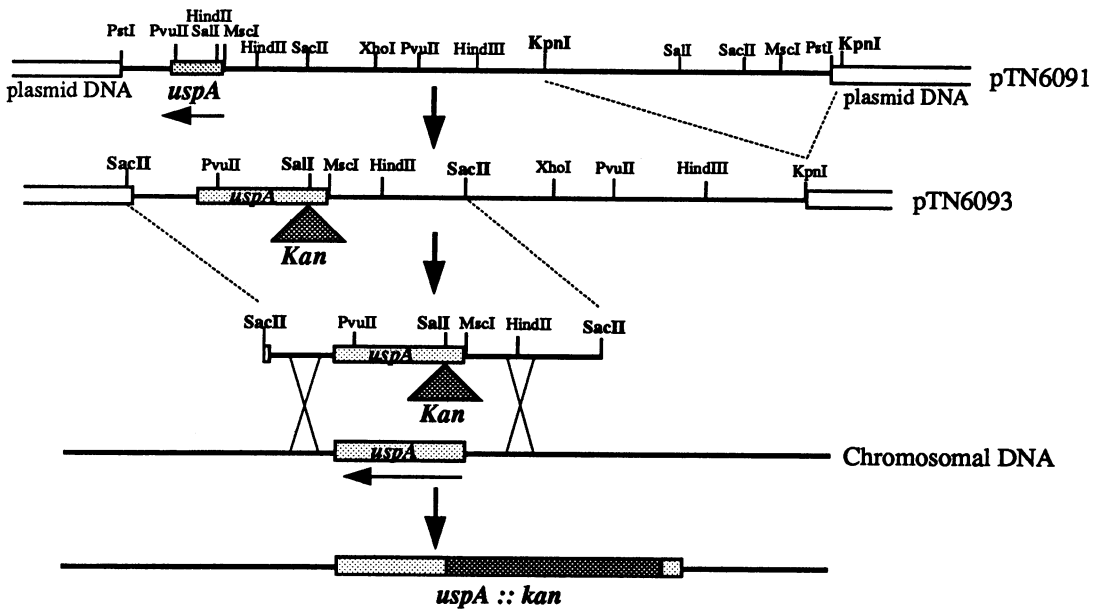

FIG. 1. Construction of a *uspA* insertion mutant by use of the kanamycin resistance gene block from Pharmacia Biochemicals. Plasmid pTN6091 was digested with *Kpn*I, and the fragment containing the *uspA* gene was subsequently religated to create plasmid pTN6093. The kanamycin resistance gene block and plasmid pTN6093 were digested with *Sal*I and ligated to create the *uspA*::*kan* recombinant. The recombinant plasmid was digested with *Sac*I, and the *uspA*::*kan* fragment was introduced into *E. coli* JC7623 by linear transformation. See Materials and Methods for detailed methodology.

procedures (16). Strain DH5αF' [*supE44 ΔlacU169* (f80*lacZ* ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] was used for transformations. Plasmid pTN6091, carrying the entire *uspA* gene (21), was used for the construction of the *uspA* insertion mutant.

Cultures were grown in liquid minimal medium consisting of MOPS (morpholinepropanesulfonic acid; 18) supplemented with glucose (0.4%) and thiamine (10 mM) in Erlenmeyer flasks placed in a rotary shaker at 37°C. When required, MOPS minimal medium was supplemented with various carbon sources as described by Wanner et al. (35). Luria broth or agar for plates was prepared as described by Sambrook et al. (25). When appropriate, the Luria broth medium was supplemented with carbenicillin (50 μg/ml) and kanamycin (50 μg/ml). For carbon starvation conditions, cells were grown aerobically in MOPS minimal medium with 5 to 8% the normal concentration of the appropriate carbon source (35). This procedure resulted in growth arrest of cells at an optical density at 420 nm (OD_{420}) of 0.5 to 0.8 (1.5×10^8 to 2.5×10^8 cells ml^{-1}). Cells grown in nonlimiting concentrations of glucose reach an OD_{420} of 10.

Bacterial cell counts and cell volume. Samples (2 ml) from cell suspensions were fixed with 250 μl of 2.5% (vol/vol) formaldehyde. Total counts and size distributions were obtained with a microcomputerized (Coulter Channelyzer 256) ZM Coulter Counter.

Mutant construction. Plasmid pTN6091 (21) was digested with *Kpn*I, and the fragment containing the *uspA* gene was subsequently religated (Fig. 1). The resulting plasmid, pTN6093, containing a unique *Sal*I site in the amino-terminal part of the structural *uspA* gene, and a kanamycin resistance gene block from Pharmacia Biochemicals, were digested with *Sal*I and ligated to create the *uspA*::*kan* recombinant (Fig. 1). Strain DH5αF' was transformed with the recombinant plasmid, which was subsequently isolated and purified by use of the protocol provided by Qiagen Inc. The plasmid carrying the kanamycin resistance gene block was digested with *Sac*I, and the *uspA*::*kan* fragment was purified by gel electrophoresis (25). This fragment was introduced into *E. coli* JC7623 by linear transformation. Kanamycin-resistant recombinants harboring the mutation integrated in the chromosome were isolated, and P1*vir* transduction (16) was used to transfer the mutation to *E. coli* JM105.

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

Bacterial metabolism of glucose. For tracing the fate of glucose carbon during the growth of cultures in glucose (0.02%)-limited MOPS medium, D-[U-^{14}C]glucose (400 nM; 8.84 GBq/mmol; 37 MBq/ml) was included in the medium, and the radioactivity of filtrates was determined at time intervals.

The filtrates were subsequently passed over Bio-Rad AG50X8 H^+ columns (5-ml bed volume) to determine the charge of the excreted compound(s), and the columns were washed with 10 ml of double-distilled water. The radioactivity of portions of eluates was counted to determine the concentrations of labeled neutral and/or acidic products in the medium. Filtrates were also subjected to analysis on a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column with isocratic elution with 8 mM H_2SO_4 (14). The eluate was monitored at 214 nm, 0.5-ml fractions were collected, and the radioactivity was measured in a Beckman LS7500 scintillation counter.

Resolution of proteins on two-dimensional polyacrylamide gels. Culture samples were processed to produce extracts for resolution on two-dimensional polyacrylamide gels by the method of O'Farrell (23) with modifications (32).

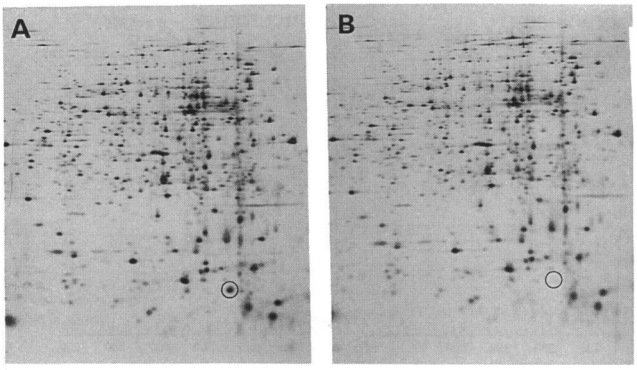

FIG. 2. Two-dimensional polyacrylamide gels of extracts of wild-type *E. coli* (A) and a kanamycin-resistant recombinant (B) glucose starved for 10 min at 37°C. The autoradiograms show the incorporation of [^{35}S]methionine for 5 min. The circled protein spot is UspA.

DNA manipulations. DNA purification, ligation, restriction analysis, and gel electrophoresis were carried out as described by Sambrook et al. (25). Restriction enzymes and T4 DNA ligase were products of New England Biolabs and Boehringer Mannheim Co.

RESULTS

Isolation of *uspA* mutants. *uspA* mutants were isolated after insertional mutagenesis of *uspA* clone pTN6093 (Fig. 1) by use of the kanamycin resistance gene block from Pharmacia Biochemicals (see Materials and Methods). The mutation was crossed into the chromosome of strain JC7623 (*arg ara his leu pro recB21 recC22 sbcB15 thr*) and subsequently introduced into strain JM105 by standard P1 transduction procedures (16). Kanamycin-resistant recombinants were then analyzed by two-dimensional gel electrophoresis to confirm their inability to synthesize protein UspA (Fig. 2). One of these mutants, TN1051, devoid of UspA expression, was further characterized.

The *uspA* mutant exhibits a diauxic type of growth when grown on glucose or gluconate. In a preliminary investigation of the nature of the physiological alterations in the *uspA* mutant, its growth characteristics were compared with those of the isogenic parent on various carbon and energy sources. Overnight cultures of the *uspA*::*kan* mutant and its isogenic parent were inoculated into MOPS minimal medium with a variety of single carbon sources, and the growth of the cells was monitored by measurement of culture turbidity. As depicted in Fig. 3, the *uspA*::*kan* mutant (TN1051) showed a significant lag when transferred to fresh prewarmed minimal medium, regardless of the carbon source in the medium. After the lag period, strain TN1051 started to grow, with a generation time indistinguishable from that of the isogenic parent (Fig. 3), but exhibited a diauxic type of growth when grown on either glucose or gluconate as the sole carbon source (Fig. 3). At a biomass of about 60% of the final yield of the wild-type culture, the growth of strain TN1051 was arrested for 60 to 120 min, after which growth resumed at a slower rate (Fig. 3). Next, the cell number and median cell volume were determined in the same experiment to confirm that the turbidity measurements accurately reflected mass increase and the diauxic growth of strain TN1051 rather than the occurrence of morphological abnormalities, such as filamentation or spheroplast formation, affecting the optical density of the culture. As illustrated in Fig. 4, growth of the wild-type culture ceased at a density of 2.5×10^8 cells per ml, while growth of the *uspA* mutant culture was arrested at a significantly lower density (1.4×10^8 cells per ml). Moreover, no significant difference in the median cell volumes of the two cultures could be detected (Fig. 4), and the biovolume (median cell volume multiplied by cell number) of the TN1051 culture was found to mimic accurately the turbidity measurements for the diauxic type of growth (data not shown). The next question to be considered, then, was whether the premature growth arrest exhibited by mutant strain TN1051 grown on glucose or gluconate resulted from a rapid exhaustion of the exogenous carbon source.

Strain TN1051 exhibits an elevated level of dissimilation of glucose. Cultures of strain JM105 and TN1051 were incubated in MOPS minimal medium initially containing 275 μg of glucose per ml, and the exhaustion of glucose during cell growth was monitored by the coupled glucose oxidase-peroxidase enzymatic method (Fig. 5). As depicted in this figure, glucose was exhausted at a lower cell density in strain TN1051, and the second phase of growth occurred in the total absence of measurable levels of exogenous glucose. This growth phenotype and the rapid dissimilation of glucose by the mutant could be complemented by a plasmid containing the structural *uspA* gene (Fig. 5B). The growth behavior of strain TN1051 resulted in the phenomenon depicted in Fig. 6. In this figure, the glucose taken up by the cells is plotted as a function of mass increase. Two different initial concentrations of glucose yielded the same results (Fig. 6). For the *uspA* mutant, growth on glucose was characterized by a rapid dissimilation of this substance at a rate exceeding that of the wild-type parent (Fig. 6). In view of the fact that strains TN1051 and JM105 grew at the same rate in glucose minimal medium, it appears that the *uspA* mutant strain dissimilates the carbon source at a rate that is not commensurate with its biosynthetic processes (Fig. 6). A simple hypothesis to account for the diauxic growth behavior of strain TN1051 would be that the elevated level of dissimilation of the carbon source by the mutant is accompanied by excretion into the medium of a metabolite(s) that can only be taken up and/or assimilated after glucose or another catabolite-repressing carbon source, such as gluconate, has been exhausted from the medium. The next experiment supported this idea.

The addition of exogenous cAMP significantly shortens the diauxic lag phase of strain TN1051. We assessed the effect of cyclic AMP (cAMP) added to the *uspA* mutant strain growing with either glucose or gluconate as the sole source of carbon in MOPS minimal medium. As depicted in Fig. 7, the mutant culture that received cAMP during growth in glucose-limited MOPS minimal medium recovered significantly faster from the diauxic lag than did the culture with no additions. The same result was obtained with gluconate-grown cells (data not shown). Nevertheless, the diauxic growth behavior of strain TN1051 could not be totally abolished by cAMP, even when its concentration or time of addition was varied.

Strain TN1051 converts a significant fraction of glucose to acetate, which is excreted into the medium. The experiments reported in Fig. 5, 6, and 7 suggest that the elevated level of dissimilation of glucose in strain TN1051 is accompanied by the conversion of this carbon source to some form that is excreted into the medium and that can be utilized for growth after catabolite repression is relieved. The following experiment confirmed this notion. Figure 8 shows the results of an experiment in which the label remaining in the medium

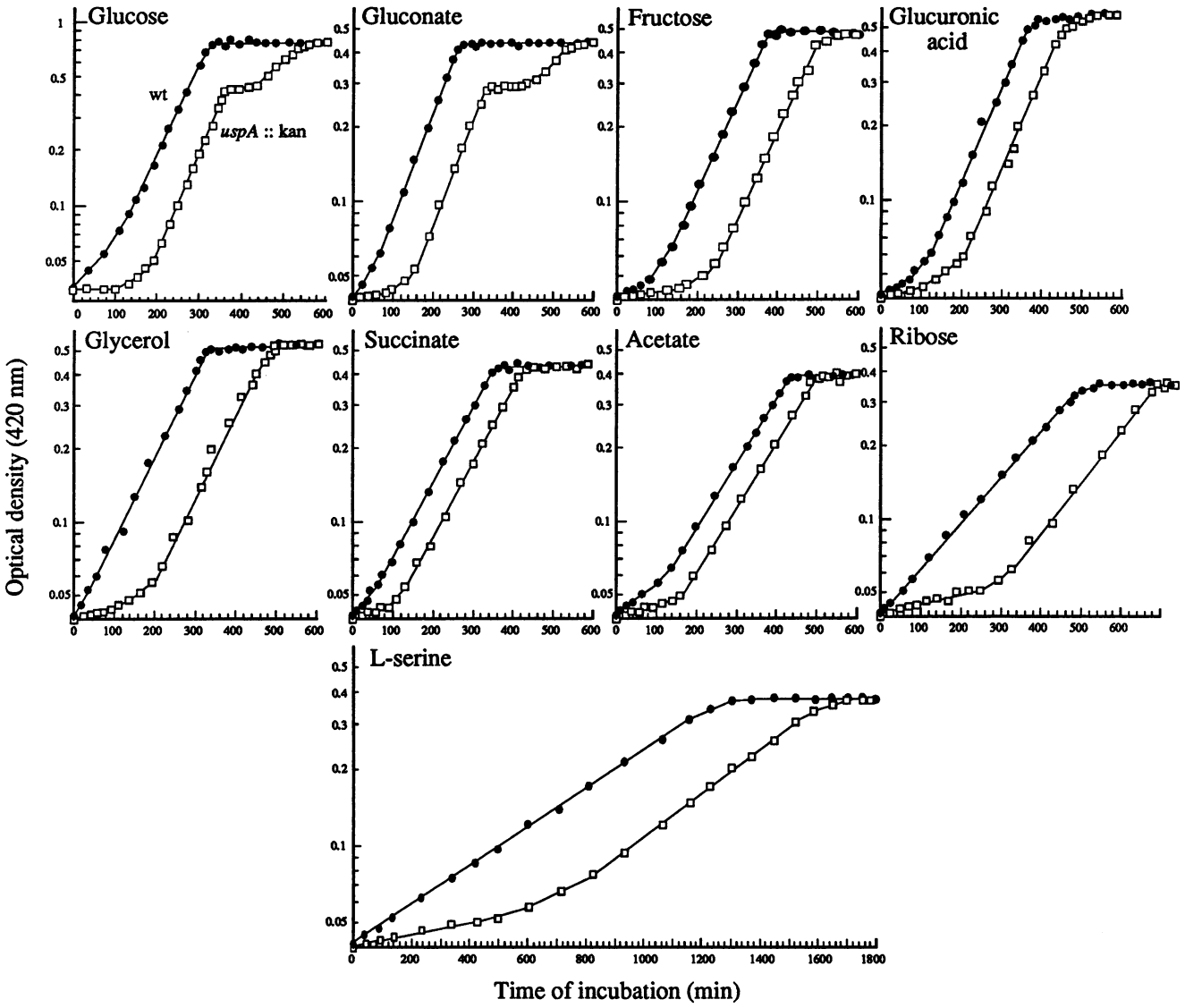

FIG. 3. Growth of the wild type (●) and *uspA* mutant TN1051 (□) at 37°C in MOPS minimal medium containing various carbon sources. The OD_{420} is plotted on a logarithmic scale as a function of time. The carbon sources were used at 0.02 to 0.032% (final concentration).

during incubation of cells with uniformly labeled exogenous glucose was analyzed. About 30 to 40% of the label could be recovered from the medium at the onset of the diauxic lag phase, when glucose was completely exhausted. Moreover, the label was subsequently taken up and exhausted from the medium in parallel with the resumption of the mass increase of strain TN1051 during the diauxic phase of growth (Fig. 8).

Labeled filtrates were isolated at intervals during the diauxic lag phase and passed over Bio-Rad AG50X8 H^+ ion-exchange columns to determine the charge of the excreted compound(s). About 95% of the label was recovered in column eluates, indicating that the compound(s) was either acidic or neutral. The eluates were subsequently subjected to analysis with a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column. About 90% of the label in the filtrate obtained from the TN1051 culture at the onset of the diauxic lag phase eluted in one peak with a retention time of 14.4 min and coeluted with acetate in the standard solution (Fig. 9A). In addition, the excreted acetate was utilized when the growth of strain TN1051 resumed and was completely depleted from the medium when the culture reached its final yield (Fig. 9B). The remaining 10% of the label eluted in several minor peaks with retention times of between 5 and 12 min (Fig. 9). These labeled peaks were also present in the filtrates of growth-arrested wild-type cultures and were not utilized by either wild-type or *uspA* mutant cultures even after prolonged incubations in the carbon-depleted medium (data not shown).

DISCUSSION

The gene *uspA* encodes a small, soluble *E. coli* protein that is synthesized in response to diverse stresses, such as nutrient starvation and exposure to heat, acid, heavy metals, oxidative stress agents, antibiotics, and uncouplers of oxidative phosphorylation (21). The induction occurs as soon as

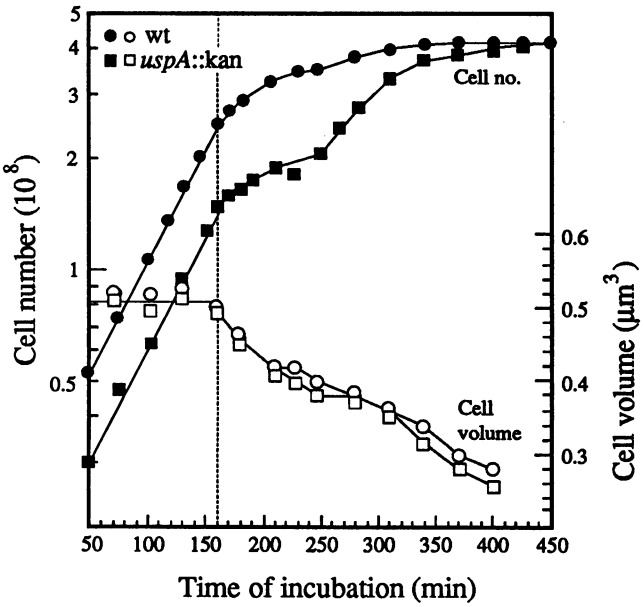

FIG. 4. Cell number and median cell volume of the wild type (wt) and *uspA* mutant TN1051 (*uspA*::kan) during growth in glucose-limited (0.02%) MOPS minimal medium at 37°C.

the growth rate falls below the maximal growth rate supported by the medium and peaks within 20 min after a change in the growth rate is observed (21). In addition, by using an experimental system in which growth and the cellular levels of ppGpp are under the control of a P_{tac}::*relA* fusion (26), we demonstrated that the cessation of growth causes the induction of UspA, even when there are no known changes in the nutritional sufficiency of the growth medium (21).

We now report the construction and growth properties of a *uspA* mutant. We found that the mutant lags significantly when diluted into fresh medium, regardless of the carbon source included, and exhibits a diauxic type of growth when grown on catabolite repressors, such as glucose and gluconate. This odd growth behavior was demonstrated to be the result of an elevated level of dissimilation of the carbon source, accompanied by the excretion and subsequent reutilization of acetate (Fig. 6, 8, and 9).

The growth of *E. coli* on acetate as the sole carbon source requires the operation of the glyoxylate shunt (8). The two unique enzymes of this shunt, isocitrate lyase and malate synthase A (Fig. 10), are normally induced only when *E. coli* is grown on acetate or fatty acids (20). In addition, the synthesis of these enzymes is subject to catabolite repression (20). Before it is metabolized, acetate must be activated to acetyl coenzyme A (acetyl-CoA) by either acetyl-CoA synthetase (1) or the acetate kinase-phosphotransacetylase pathway (20) (Fig. 10). The former of these enzymes, like the glyoxylate shunt enzymes, appears to be regulated by catabolite repression (20). Thus, the diauxic type of growth of the *uspA* mutant grown on catabolite repressors, such as glucose and gluconate (Fig. 3), may be attributed to the failure of cells to form key enzymes for the assimilation of the acetate produced until glucose or gluconate has been completely exhausted from the medium.

Our observations regarding the elevated level of dissimilation of glucose accompanied by the excretion of high concentrations of acetate for strain TN1051 suggest that protein UspA may be involved in modulating the flow of carbon in the central metabolic pathways of *E. coli*. Specifically, it appears feasible that the induction of protein UspA during growth arrest would either restrict the synthesis of acetate or alternatively increase the utilization of the acetate produced. During aerobic growth on glucose, acetate is formed from acetyl-CoA via the phosphotransacetylase

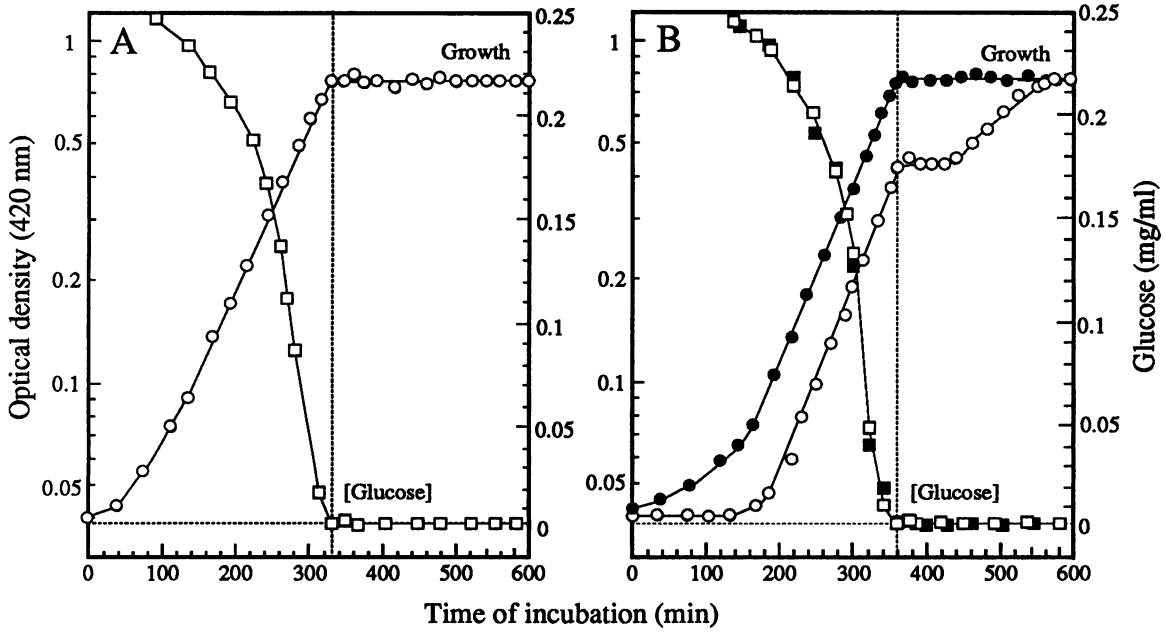

FIG. 5. Depletion of glucose by the wild type (A) and *uspA* mutant TN1051 (B) without (open symbols) and with (closed symbols) plasmid pTN6093, carrying the entire structural *uspA* gene. Cultures were grown at 37°C in MOPS minimal medium initially containing 275 μg of glucose per ml.

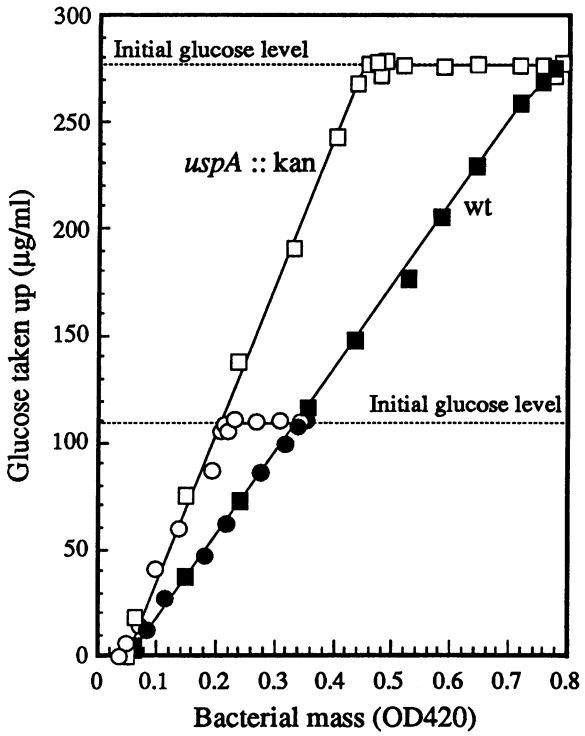

FIG. 6. Utilization of glucose during the growth of the wild type (closed symbols) and *uspA* mutant TN1051 (open symbols). Cultures were grown in MOPS minimal medium initially containing 275 μg (squares) or 110 μg (circles) of glucose per ml.

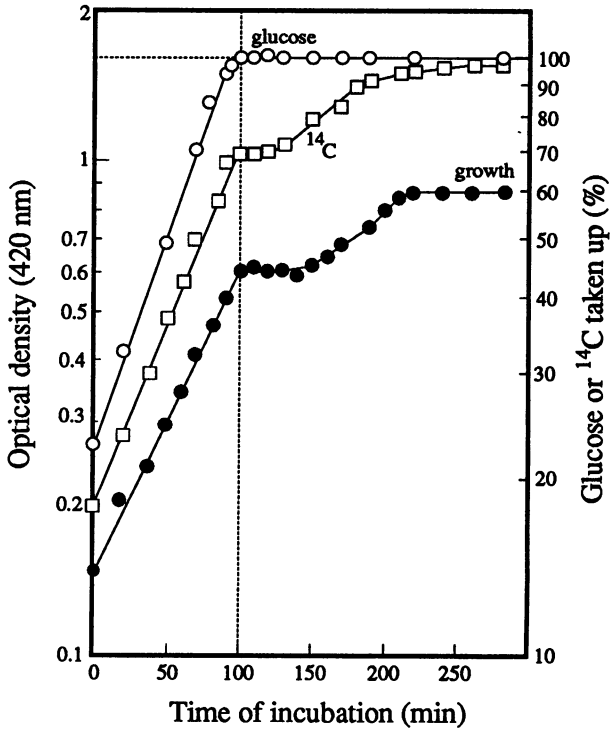

FIG. 8. Fate of [^{14}C]carbon during the metabolism of uniformly ^{14}C-labeled glucose by *uspA* mutant TN1051 grown in glucose-limited (0.02%) MOPS minimal medium. The radioactivity in filtrates (□) is expressed as the percentage of the total radioactivity of the glucose at the start of the experiment and is plotted as a function of time. Total glucose concentrations in filtrates, expressed as the percentage of glucose taken up by the cells (○), were quantitated by the coupled glucose oxidase-peroxidase enzymatic method (see Material and Methods for details).

(PTA)-acetate kinase (ACK) pathway (Fig. 10). The PTA-ACK pathway therefore constitutes a possible site for UspA control. However, we cannot rule out the possibility that the acetate production of the *uspA* mutant results from the lack of control of the flow of carbon through glycolysis rather than the PTA-ACK pathway, such that the increased production of acetate in the mutant results from elevated levels of acetyl-CoA that exceed the capacity of the enzymes of the tricarboxylic acid cycle. In addition, the increased concentrations of acetate in the medium of strain TN1051 may reflect a diminished ability of this mutant strain to recycle this compound.

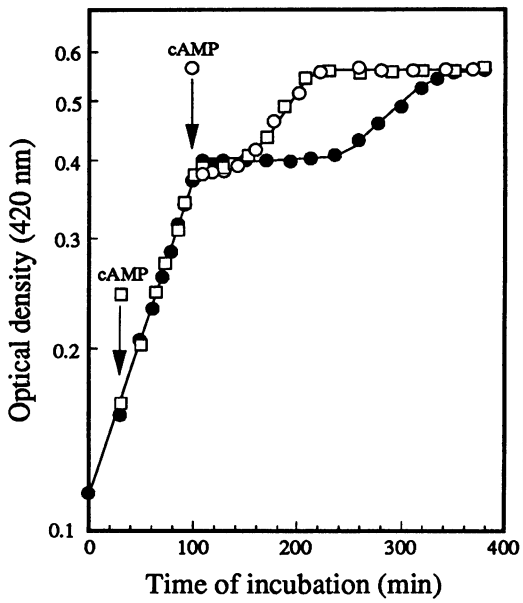

FIG. 7. Effect of cAMP (2 mM final concentration) added at different times during the growth of *uspA* mutant TN1051 in glucose-limited (0.02%) MOPS minimal medium. Symbols: ○ and □, cAMP added at the indicated time; ●, no cAMP added.

The effect of a *uspA* mutation on acetate synthesis is particularly interesting in view of the fact that acetylphosphate, an intermediate of the PTA-ACK pathway (Fig. 10), appears to be involved in P_i-independent cross-regulation of the phosphate starvation regulon (36). Conditions expected to result in increased levels of acetylphosphate lead to a P_i-independent induction of this regulon (36). In addition, acetylphosphate was recently demonstrated to cross-activate the regulator (NtrC) of the two-component regulatory system (NtrC-NtrB) controlling the nitrogen limitation regulon (3). As pointed out by Wanner (34), such cross-regulation of two-component starvation regulons may be important in the control of central pathways of energy and carbon metabolism, since C, N, P, O, and H are all assimilated via a central metabolic pathway and most, if not all, two-component regulatory systems are related to central pathways of metabolism. These observations, together with the fact that UspA, the universal stress and starvation protein of *E. coli*, appears to affect the synthesis of acetate indicate that further work should be directed at exploring how

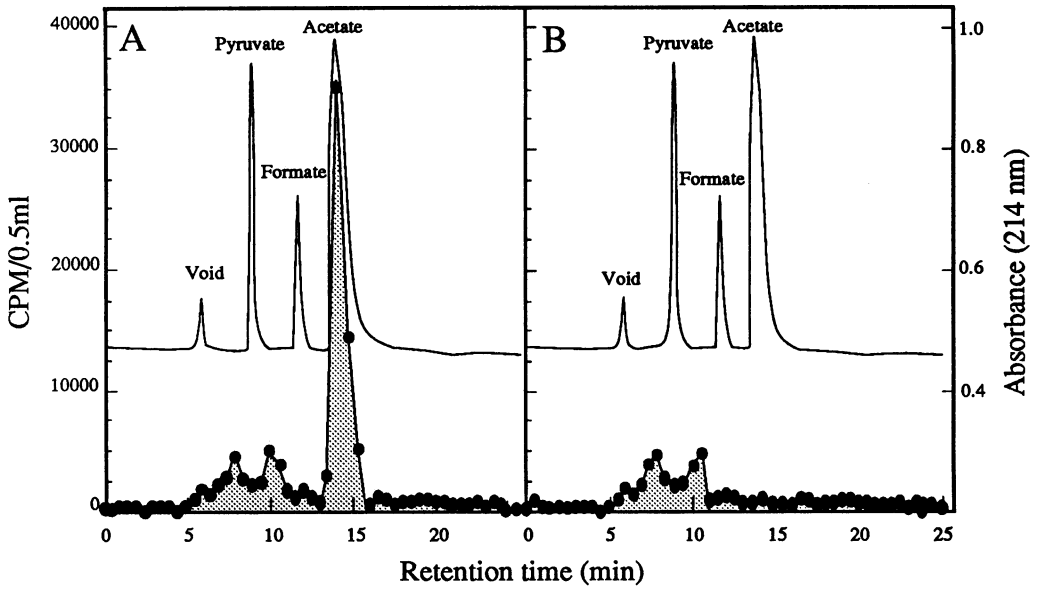

FIG. 9. Analysis of labeled filtrates obtained from a TN1051 culture at the early stage of the diauxic lag phase (A) and after the end of the diauxic growth phase (B) on a Bio-Rad Aminex HPX-87H high-pressure liquid chromatography column. The eluates were monitored at 214 nm, 0.5-ml fractions were collected, and radioactivity was measured. Labeled filtrates were coeluted with standard samples containing pyruvate, formate, and acetate. See Materials and Methods and the legend to Fig. 8 for details.

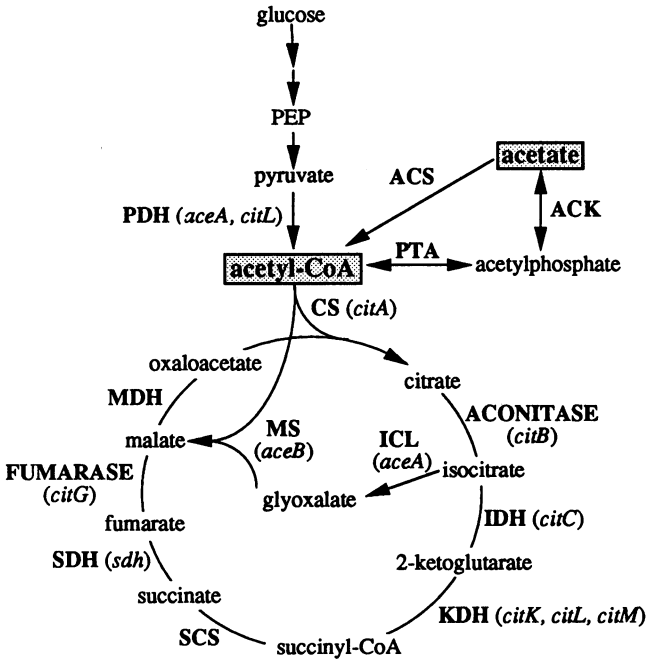

FIG. 10. Tricarboxylic acid cycle, glyoxalate shunt, and acetate metabolism pathways. The reactions are schematized, and cofactors and cosubstrates (ADP, P_i, and NAD, etc.) are not shown. Gene symbols are in parentheses. Abbreviations: ACS, acetyl-CoA synthetase; CS, citrate synthase; ICL, isocitrate lyase; IDH, isocitrate dehydrogenase; KDH, ketoglutarate dehydrogenase; MDH, malate dehydrogenase; MS, malate synthase A; PDH, pyruvate dehydrogenase complex; PEP, phosphoenolpyruvate; PTA, phosphotransacetylase; SCS, succinyl coenzyme A (succinyl-CoA) synthetase; SDH, succinate dehydrogenase.

UspA is involved in modulating the flow of carbon through the PTA-ACK pathway and whether the levels of acetylphosphate as well as acetate are subject to control by UspA.

Our results are reminiscent of the well-documented accumulation of acetyl-CoA in *Bacillus subtilis* cells subjected to nutrient depletion (C, N, or P) or decoyinine treatment, which causes a rapid decrease in the size of the pool of GTP (28). This change in the size of the pool of acetyl-CoA is the earliest known effect of a decrease in the level of GTP, which leads to sporulation of the *B. subtilis* cells (29). The mechanism responsible for the rapid accumulation of acetyl-CoA during growth arrest is not established, but it has been suggested that the activity of pyruvate dehydrogenase may respond directly to a decrease in the level of GTP (28). The alteration in the size of the pool of acetyl-CoA is followed by the induction (and/or activation) of several genes coding for different components of the tricarboxylic acid cycle. These components include citrate synthase (29), aconitase (2), fumarase (24), ketoglutarate dehydrogenase (28), and succinate dehydrogenase (15). The regulation of these genes in response to growth arrest has been suggested to be the result of a cascade of events in which the decrease in the level of GTP followed by an increase in the level of acetyl-CoA is the initial signal eventually leading to the induction of aconitase and an optimized flow of carbon to succinate for energy and to ketoglutarate for biosynthesis (28). In this scenario of economized utilization of the elevated level of acetyl-CoA, it appears to be beneficial to restrict the flow of carbon to acetate through the PTA-ACK pathway (Fig. 10). As discussed above, the role of UspA induction during growth arrest may be to restrict such a flow of carbon to acetate or, alternatively, to increase the reutilization of acetate. It would be interesting, therefore, to learn whether a UspA homolog is in fact an integral part of the response to growth arrest in *B. subtilis* and whether the synthesis of enzymes of the tricarboxylic acid cycle responds to elevated levels of acetyl-CoA (and alterations in the levels of GTP) during

growth arrest in *E. coli* as well as *B. subtilis*. We have initiated comparative studies to ascertain the presence or absence of a homologous protein in other enteric bacteria, in *B. subtilis*, and in more distant genera.

We recently observed that *E. coli* cells harboring a *uspA* insertion mutation are greatly impaired in the ability to survive prolonged periods of carbon source and energy starvation (22). We are exploring the possible link between the poor viability of strain TN1051 during carbon starvation and the abnormal utilization of the carbon source by TN1051, as reported here. This finding provides additional impetus to elucidate the mechanism by which UspA operates and the site(s) of its action in the central metabolic pathway.

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