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The Putative σ Factor KatF Has a Central Role in Development of Starvation-Mediated General Resistance in *Escherichia coli*

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KatF is required for the expression of some 32 carbon starvation proteins in *Escherichia coli* including 6 previously identified as Pex. Mutants with the *katF* gene survive carbon and nitrogen starvation poorly. Many of the KatF-regulated starvation proteins are common to those induced by other stresses, and the mutant failed to develop starvation-mediated cross protection to osmotic, oxidative, and heat stresses. Furthermore, thermal resistance was not induced in the mutant by heat preadaptation, and it exhibited an altered pattern of protein synthesis at elevated temperature. Thus, KatF is a major switch that controls the starvation-mediated resistant state in *E. coli*.

During the 2 to 4 h after the onset of carbon starvation, *Escherichia coli* undergoes a temporally programmed expression of special genes, which results in cells possessing enhanced resistance to a variety of stresses, viz., starvation, hyperosmosis, oxidation, heat, and low pH (2, 4, 5, 9, 10). We have identified two major classes of carbon starvation genes: the cyclic AMP-dependent *cst* genes, and the *pex* genes, whose induction upon starvation is independent of cyclic AMP (21). Several *pex* genes are also induced by starvation for other individual nutrients, as well as by cell exposure to a variety of other stresses (2, 4, 5). There is strong suggestive evidence that it is the induction of this class of genes that confers the starvation-induced general resistance on *E. coli* (4, 5, 9, 10, 21).

We have recently presented evidence that a minor σ factor, namely, σ^{32} (15), is a starvation protein and regulates the induction of some of the *pex* genes during starvation (3) and that a strain with mutation in the *rpoH* gene (the gene that encodes σ^{32}) survives starvation poorly. Loewen and coworkers (7, 12, 13) have identified another putative σ factor in *E. coli* which has a role in the induction of two genes in the stationary phase, *katE* and *xthA*. These genes encode hydroperoxidase II and exonuclease III, respectively, which are believed to be involved in resistance to H_2O_2 (18).

Our primary interest in the studies presented in this report was to determine whether, by analogy to σ^{32}, KatF had a role in the induction of Pex proteins during starvation. We found that several such proteins, many of which are induced by a variety of other stresses, were indeed regulated by this putative σ factor. This in turn led us to undertake a detailed investigation of the effect of a *katF* mutation on the ability of *E. coli* to develop starvation-mediated cross protection to other stresses, as well as its capacity to adapt to specific individual stresses. The results show that KatF is a major stress response switch in *E. coli*.

MATERIALS AND METHODS

Bacterial strains and growth media. The bacterial strains used in this study are described in Table 1. The *E. coli* K-12 strain used is a Stanford strain (λ^- F^-) used in our previous work. Transductions were performed with P1 *vir* as described previously (22). M9 minimal (11), morpholinepropanesulfonic acid (MOPS) minimal (14), and LB (11) media were prepared as described previously. When specified, media were supplemented with tetracycline (25 μg/ml).

Pulse-labeling of proteins and two-dimensional gel electrophoresis. Proteins were pulse-labeled and separated on two-dimensional gels as previously described (2). Culture samples were removed at appropriate times and pulse-labeled with 10^{-8} M L-[^{35}S]methionine (12 μCi/ml; 1,072 to 1,097 Ci/mmol) for 3 min at the specified temperature. The cells were then chased for 1 min with unlabeled methionine (10^{-5} M), and proteins were precipitated with 10% trichloroacetic acid at 4°C and separated by the two-dimensional electrophoretic technique of O'Farrell (16). Equivalent amounts of radioactivity (ca. 750,000 cpm) were loaded on each gel. Labeled proteins were visualized by autoradiography on XAR-5 film (Eastman Kodak Co.).

Starvation protocol. Carbon starvation was attained by subculturing overnight cultures grown in M9 medium into prewarmed M9 medium supplemented with 0.025% (wt/vol) glucose. The onset of starvation was determined by the cessation of growth as measured by A_{660} determination (2, 21). Nitrogen starvation was attained by subculturing overnight cultures grown in MOPS medium into prewarmed

TABLE 1. *E. coli* and phage strains used in this study

Strain or phage	Genotype	Reference or source[a]
E. coli		
K-12	Wild type (λ^- F^-)	Our laboratory strain
UM120	*thi-1* HfrH *katE*::Tn*10*	20
UM122	*thi-1* HfrH *katF*::Tn*10*	20
UM202	*thi-1* HfrH *katG*::Tn*10*	20
AMS150	Like K-12, but *katF*::Tn*10*	P1 (UM122) × K-12 → Tc^r
AMS151	Like K-12, but *katE*::Tn*10*	P1 (UM120) × K-12 → Tc^r
AMS152	Like K-12, but *katG*::Tn*10*	P1 (UM202) × K-12 → Tc^r
Phage P1 *vir*		Our laboratory strain

[a] Tc^r, tetracycline resistance.

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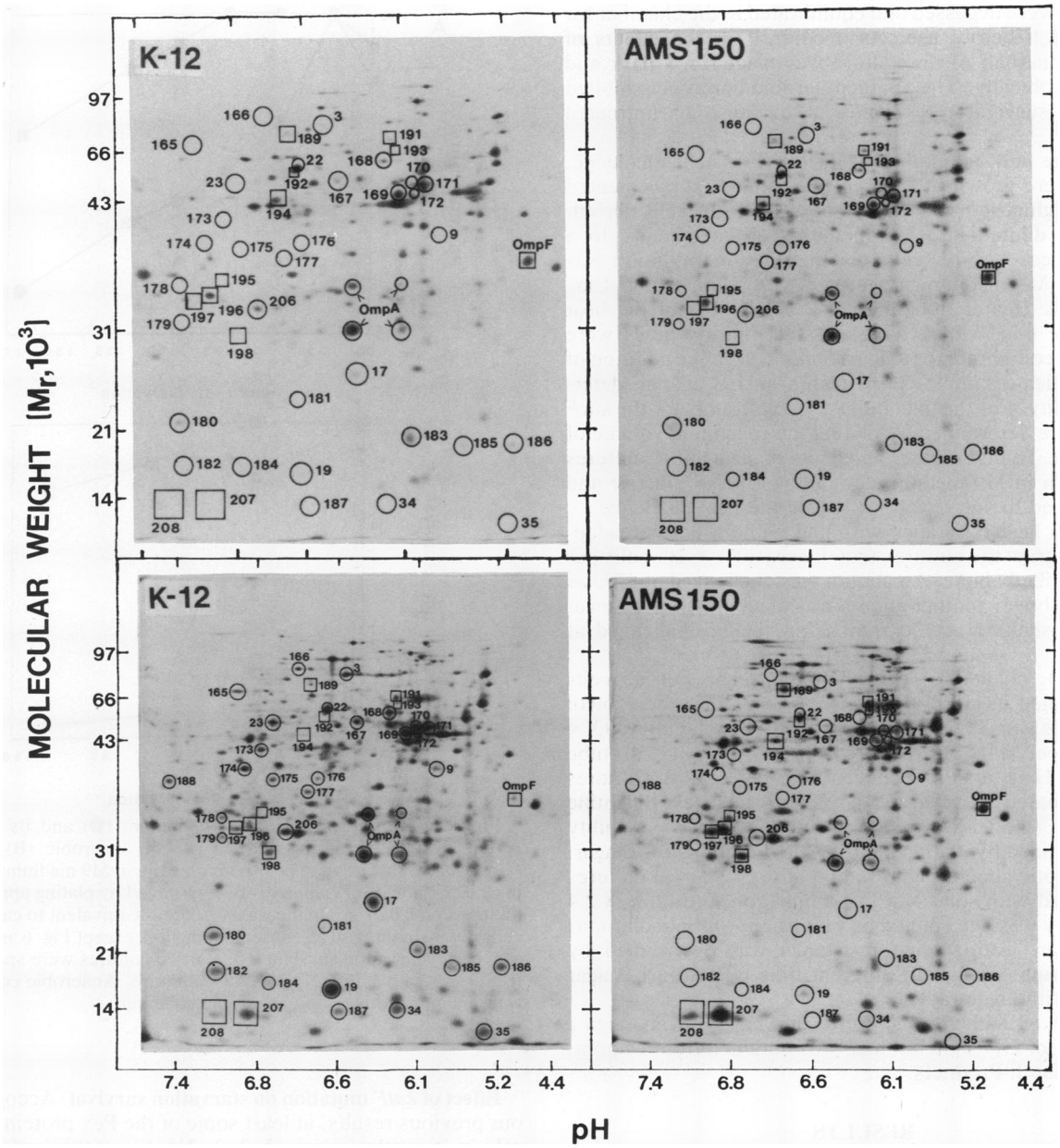

FIG. 1. Two-dimensional autoradiographs of polypeptides synthesized by *E. coli* K-12 or the *katF*::Tn*10* strain AMS150 during logarithmic growth (top panels) or at 120 min of carbon starvation (bottom panels). Circles indicate proteins which were not synthesized or which were synthesized at reduced levels in AMS150 during starvation. Squares indicate proteins which were hyperinduced in the mutant during starvation. Numbers below 165 correspond to those previously published (2, 9, 21). Spots numbered 165 and higher represent newly designated proteins. Spots whose migration corresponds to the positions of OmpA and OmpF (17) are designated. Spot 188 is not visible in the gels shown in the upper panels. Note the shift in the isoelectric focusing axis of the gel of K-12 during exponential growth (upper left panel).

MOPS medium supplemented with 0.57 mM NH_4Cl. Cultures were grown until NH_4^+ was exhausted and growth ceased. They were incubated aerobically with shaking at 150 rpm in a New Brunswick G76 Gyratory Water Bath Shaker at either 29 or 37°C, as noted. Cultures attained a density of approximately 3×10^8 cells per ml (A_{660} of 0.3) at the onset of starvation. Viability was determined by plating appropriate dilutions on LB agar, after correcting for loss of H_2O due to evaporation. Except where noted, tetracycline was omitted from the media.

Anaerobic starvation protocol. Cultures were grown as described above. At the onset of starvation, equal halves were put into serum bottles placed on ice. Sterile resazurin (1 μg/ml) was added, and the cultures were sparged with a 79% N_2–21% CO_2 mixture for 1 h; as judged by resazurin color, they were anaerobic at this time. They were then transferred to a Coy anaerobic chamber containing an atmosphere of 80% N_2, 10% CO_2, and 10% H_2 and mixed on a Tech Stir 20 (Scientific Products). Viability was determined by plating appropriate dilutions on LB agar. All materials

and media were degassed and equilibrated in the chamber for at least 12 h before use. As a control for the effects of sparging, one-half of the culture was placed in a flask and shaken aerobically. The sparged aerobic controls exhibited starvation survival rates identical to those of untreated cultures.

Challenge and adaptation protocols. For heat challenge, cultures were grown at 29°C in M9 medium supplemented with 0.4% glucose. At a density of ca. 3×10^8 cells per ml, they were diluted into M9 medium without glucose to a density of ca. 3×10^3 cells per ml. The final dilution was made into M9 medium prewarmed to 57°C in a Lab Line Multi-Block Heater (model 2097-4). The wells of the heat block were filled with water, and the dilution tubes were allowed to equilibrate for at least 5 min before the addition of cells. Duplicate samples were plated on LB agar to determine viability. For the final points, viable counts for the *katF* mutant were determined by plating the remaining volume of the culture. To investigate the effects of starvation, cultures were grown in M9 medium containing 0.025% glucose and were allowed to starve for 4 h before the heat challenge.

To study heat resistance with and without heat preadaptation, we grew the cultures to a density of 3×10^8 cells per ml as described above. An aliquot was challenged at 57°C as described above; another aliquot was incubated for 30 min at 42°C with shaking at 150 rpm before being challenged at 57°C.

For H_2O_2 challenge, logarithmic-phase or starved cells were obtained as described above except that the growth temperature was 37°C. Three milliliters of each culture (3×10^8 cells per ml) was transferred to a 15-cm test tube containing H_2O_2 at a final concentration of 15 mM. Cultures were incubated at 37°C on a New Brunswick Scientific Rollerdrum (model TC-7) run at maximum speed; viability was determined by plating appropriate dilutions on LB agar.

For osmotic shock, logarithmic-phase or starved cultures were treated with solid NaCl to a final concentration of 2.4 M. Viability was determined by plating appropriate dilutions on LB agar. Tetracycline resistance was determined by replica plating colonies at different time points on LB agar with and without tetracycline.

Materials. Biochemicals were purchased from Sigma Chemical Co. L-[^{35}S]methionine was obtained from Dupont, NEN Research Products.

RESULTS

Two-dimensional gel analysis of carbon starvation proteins affected by KatF. The *katF*::Tn*10* mutant (AMS150) and its isogenic parent strain K-12 were pulse-labeled with [^{35}S]methionine at various times before and after the onset of carbon starvation. Bulk protein synthesis rates of the mutant and wild type, as measured by incorporation of [^{35}S]methionine, were similar during growth and starvation (data not shown). As determined by visual comparison of the autoradiograms, at least 32 polypeptides were absent or synthesized at reduced levels in the *katF* mutant compared with the wild type during carbon starvation (Fig. 1). Of these, six have been previously identified as Pex proteins (polypeptides 3, 17, 19, 22, 23, and 34), some of which are also induced by other stresses (see references 4 and 5) (Table 2). The starved mutant also exhibited reduced expression of a set of polypeptides that migrated at the position corresponding to OmpA (17). The synthesis of several polypeptides was elevated in the starved mutant (Fig. 1). One of these could be OmpF, as judged by its position on the autoradiograph (17).

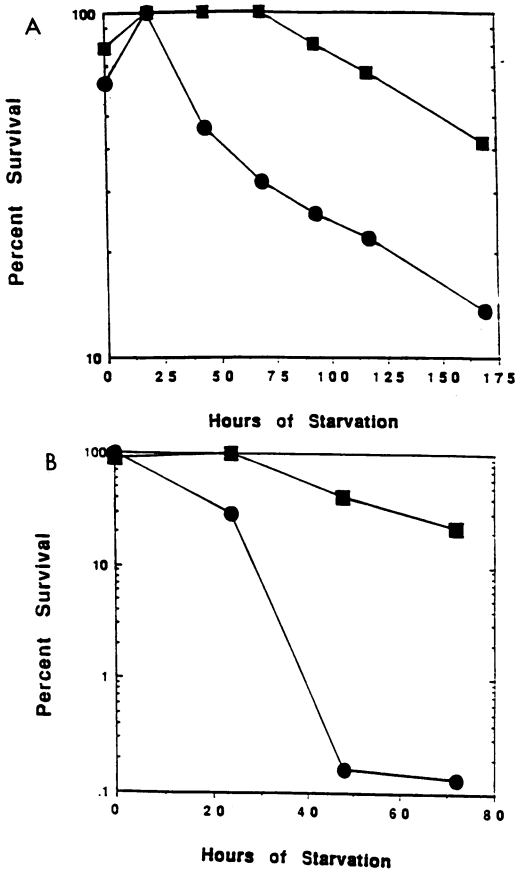

FIG. 2. Viability of a *katF*::Tn*10* mutant (●) and its isogenic parent, K-12 (■), during aerobic (A) or anaerobic (B) glucose starvation. Cultures were grown aerobically in M9 medium containing 0.025% glucose. Viability was determined by plating appropriate dilutions on LB agar. On the *y* axis, 100% is equivalent to ca. 3×10^8 cells per ml in this and all subsequent figures except Fig. 6 and 7. For anaerobic starvation survival (B), starved cultures were sparged on ice for 1 h with a 79% N_2–21% CO_2 mixture. Anaerobic conditions were maintained in a Coy anaerobic chamber.

Effect of *katF* mutation on starvation survival. According to our previous results, at least some of the Pex proteins play a role in starvation survival (2, 3, 21). Since the *katF* mutant failed to induce some of these, we investigated its survival during glucose starvation; it was significantly impaired (Fig. 2A). While these studies were in progress, Mulvey et al. (13) published their findings that the *E. coli katF* mutant survives carbon starvation poorly.

Two of the genes known to be regulated by KatF encode proteins that protect the cell from oxidative stress, either by decomposing the oxidizing species or by repairing its damage. Since these proteins are not synthesized at appreciable levels by the mutant (18), the increased sensitivity of the mutant to starvation could merely have been another manifestation of its enhanced sensitivity to the oxidative stress. However, the mutant also exhibited greater sensitivity to starvation than the wild type under anaerobic conditions (Fig. 2B).

The mutant also exhibited enhanced sensitivity to nitrogen starvation (Fig. 3). This agrees with our working hypothesis (9) that Pex protein induction also has a role in survival during starvation for other nutrients (2, 10, 21).

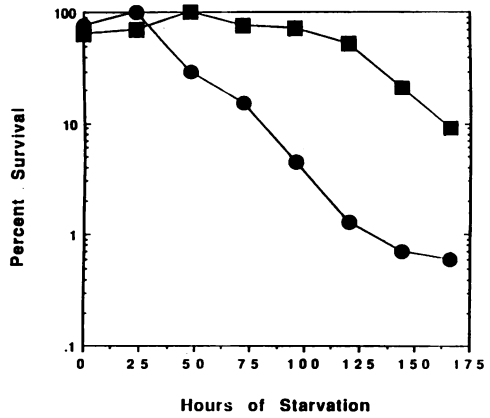

FIG. 3. Viability of a *katF*::Tn*10* mutant (●) and its isogenic parent, K-12 (■), during nitrogen starvation. Cultures were grown in MOPS medium containing 0.57 mM NH_4Cl. Viability was determined by plating dilutions on LB agar.

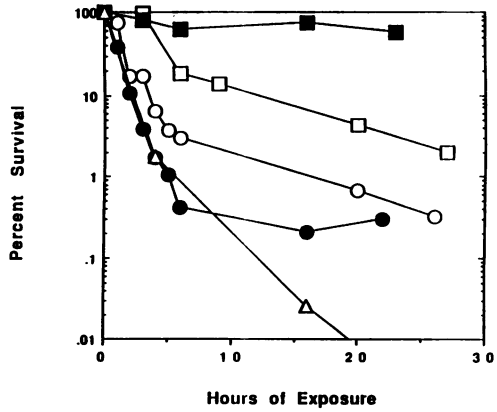

FIG. 4. Osmotic resistance of a *katF*::Tn*10* mutant (○, ●) and its isogenic parent, K-12 (□, ■), during growth and after starvation. Cultures were grown in M9 medium at 37°C and were challenged with 2.4 M NaCl during exponential growth (open symbols) or after 4 h of glucose starvation (closed symbols). The resistance of a starved culture of the *katF* mutant in the presence of tetracycline (△) was also determined. Viability was determined by plating dilutions on LB agar.

Effect of *katF* mutation on starvation-mediated resistance to osmotic shock. As stated above, starved cells possess enhanced resistance to several stresses which is attributable to Pex protein synthesis (4, 5, 9). Synthesis of nine proteins induced commonly by osmotic shock and starvation (4) was impaired in the starved *katF* mutant (Table 2 and spots 169, 182, and 183 in Fig. 1). As predicted by these results, the mutant failed to develop starvation-mediated resistance to hyperosmosis; in contrast, the 4-h-starved wild-type strain developed marked resistance (Fig. 4).

The rapid decrease in viability observed in the starved *katF* culture during osmotic shock stopped after 6 h of exposure. The culture retained approximately 0.3% of its initial viability over the next 16 h. If tetracycline was included in the medium during growth and starvation, the viability of the starved *katF* mutant continued to decrease past this point (Fig. 4). Replica plating of survivors from the culture without tetracycline on tetracycline-supplemented medium showed that at 22 h of osmotic shock, virtually all were sensitive to this drug (data not shown). Thus, excision of the Tn*10* and reversion to wild type could have occurred in the survivors; instances of precise or nearly precise excision of Tn*10* during stress have been reported (24).

TABLE 2. Selected polypeptides positively regulated by KatF during carbon starvation

Spot no.[a]	Independent of cyclic AMP[b]	Induced by heat[c]	Induced by H_2O_2[c]	Induced by hyperosmosis[d]
3	Yes	No	No	No
9	No	No	No	No
17	Yes	Yes	Yes	Yes
19	Yes	No	Yes	Yes
22	Yes	Yes	No	No
23	Yes	No	Yes	No
34	Yes	No	Yes	Yes
35	No	No	No	Yes
169	ND[e]	No	No	Yes

[a] Spot numbers below 165 correspond to those listed by Matin (9). Numbers above 165 are newly designated.
[b] Dependence on cyclic AMP for induction during carbon starvation was determined by visual examination of previously published gels (21).
[c] Induction by heat and H_2O_2 was determined by visual examination of previously published gels (5).
[d] Induction by hyperosmosis was determined by visual examination of previously published gels (4).
[e] ND, not determined.

Effect of mutations in *katE*, *katF*, and *katG* on starvation-mediated H_2O_2 resistance. Another stress against which starvation cross protects is oxidation (4). Several Pex proteins common to oxidative stress were not induced in the starved *katF* mutant. Furthermore, among the proteins that KatF is known to regulate in the stationary phase are those concerned with oxidative stress. Thus, it was of interest to determine whether the *katF* mutation affects starvation-mediated resistance to H_2O_2. The *katF* mutation made the cells extremely sensitive to H_2O_2 during both growth and starvation (Fig. 5A). After 15 min, the exponential-phase mutant was virtually nonviable; in contrast, after 30 min of exposure, some of the exponential-phase wild-type cells were still viable. A 4-h-starved wild-type culture retained nearly full viability for up to 60 min of exposure to H_2O_2; the starved mutant, however, although less sensitive than its exponential counterpart, was virtually nonviable by 30 min. Thus, KatF is necessary for starvation-induced cross protection from oxidation.

The *katF* mutation affects the expression of a large number of genes. To determine whether the effect of the *katF* mutation on H_2O_2 cross protection was attributable specifically to its regulation of hydroperoxidase II (encoded by *katE*), we transduced *katE*::Tn*10* into K-12 (strain AMS151). For purposes of comparison, a K-12 strain containing a *katG*::Tn*10* mutation was also constructed (strain AMS152). *katG* encodes hydroperoxidase I catalase (8), whose expression is regulated by OxyR and not by KatF (20). The ability of these strains to develop starvation-induced H_2O_2 resistance was examined.

The resistance of both the *katE* and the *katG* mutants to H_2O_2 was lower than that of the wild type during both growth and starvation (Fig. 5B) but was significantly greater than that of the *katF* mutant (Fig. 5A). The H_2O_2 resistances of the *katE* and *katG* mutants were nearly identical during starvation and growth, suggesting that they are equally effective in protecting the cells from oxidative stress.

Effect of *katF* mutation on starvation-mediated thermal

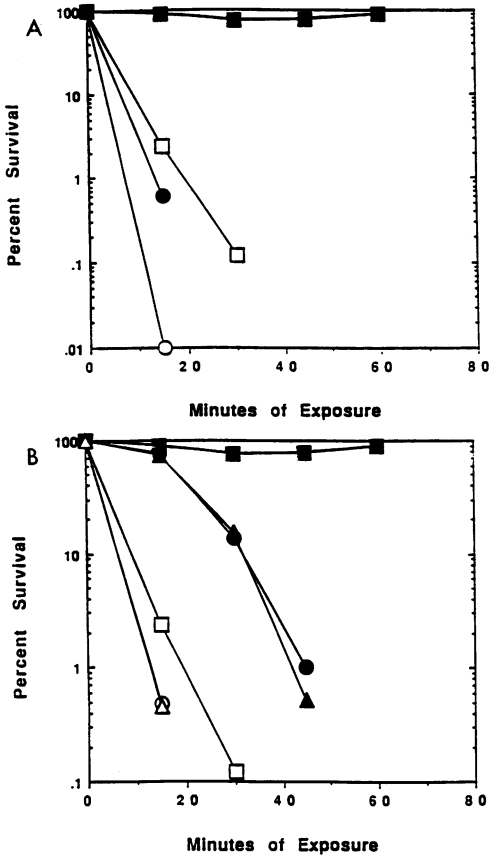

FIG. 5. Induction of H_2O_2 resistance by starvation. (A) H_2O_2 resistance of a *katF*::Tn*10* mutant (○, ●) and its isogenic parent, K-12 (□, ■). (B) H_2O_2 resistance of *katE*::Tn*10* (○, ●), *katG*::Tn*10* (△, ▲), and their isogenic parent, K-12 (□, ■). Cultures were challenged with 15 mM H_2O_2 during exponential growth (open symbols) or after 4 h of glucose starvation (closed symbols), and their viabilities were determined by plating dilutions on LB agar. All cultures were kept at 37°C throughout the experiment.

resistance. Exposure to 57°C is another stress against which starvation cross protects (5). Two of the Pex proteins which were not synthesized during starvation in the *katF* mutant, polypeptides 17 and 22 (Fig. 1), are also induced by heat shock in the wild type (5). We therefore determined the thermal resistance of the mutant. Logarithmic-phase cells and cells starved for carbon for 4 h were challenged by a rapid shift from 29 to 57°C. In both cases, the mutant was markedly more sensitive to heat than was the wild type (Fig. 6). For example, after a 2-min challenge, the logarithmic-phase wild type retained 83% viability, while the log-phase culture of the *katF* mutant was only 3.4% viable. Corresponding figures for the starved cultures were 100 and 0%, respectively.

Heat-induced thermal resistance and protein synthesis in the *katF* mutant. We have previously shown that although the *rpoH* mutant is impaired in developing starvation-induced thermal resistance, it can develop thermal resistance by a prior exposure to 42°C (3). To determine whether this was also true of the *katF* mutant, we shifted logarithmically growing cultures of the mutant and wild type from 29 to 42°C for 30 min before challenge at 57°C. The mutant was highly sensitive to heat, and preadaptation to the lower temperature failed to confer thermal protection. In contrast, the control

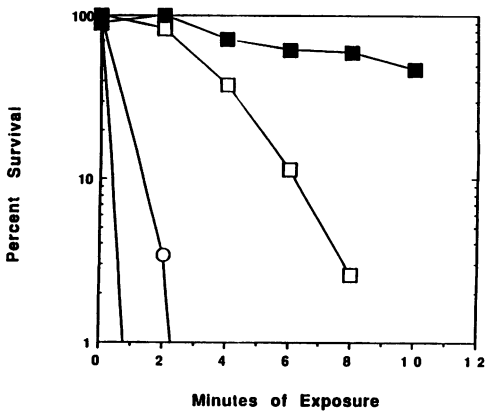

FIG. 6. Starvation-induced thermal resistance of wild-type *E. coli* K-12 (□, ■) and its *katF*::Tn*10* derivative (○, ●). Cultures were grown at 29°C in M9 medium containing either 0.025% (for starved cultures) or 0.4% (for exponentially growing cultures) glucose. Aliquots of cultures during exponential growth (open symbols) or after 4 h of glucose starvation (closed symbols) were challenged at 57°C, and viability was determined by plating on LB agar. On the *y* axis, 100% is equivalent to ca. 3×10^3 cells per ml. The 2-min point for the starved AMS150 culture is zero, so the symbol (●) does not appear on the graph.

wild type exhibited a markedly enhanced survival at 57°C upon prior exposure to 42°C (Fig. 7).

Since the *katF* mutant did not exhibit a heat adaptation response, its protein synthesis pattern was examined to determine whether it failed to synthesize some of the heat shock proteins. Cultures were pulse-labeled at 29°C and at 5 and 10 min after a shift up to 42°C and analyzed by two-dimensional gel electrophoresis (Fig. 8). At 29°C, the mutant also exhibited a different pattern of protein synthesis than the wild type. At 5 min of exposure to 42°C, the mutant was deficient in the synthesis of eight proteins compared with the wild type (polypeptides 3, 19, 174, 176, 182, 183, 200, and 203); furthermore, some proteins (5, 204, and 205) were synthesized at lower levels. The heat shock polypeptides, 17 and 22, which were missing in the mutant during

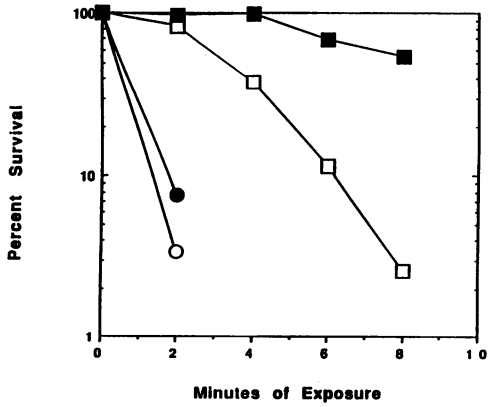

FIG. 7. Heat-induced thermal resistance of a *katF*::Tn*10* mutant (○, ●) and its isogenic parent, K-12 (□, ■). Cultures were grown at 29°C in M9 medium containing 0.4% glucose. They were either untreated (open symbols) or shifted to 42°C for 30 min (closed symbols) before being challenged at 57°C and tested for viability. On the *y* axis, 100% is equivalent to ca. 3×10^3 cells per ml.

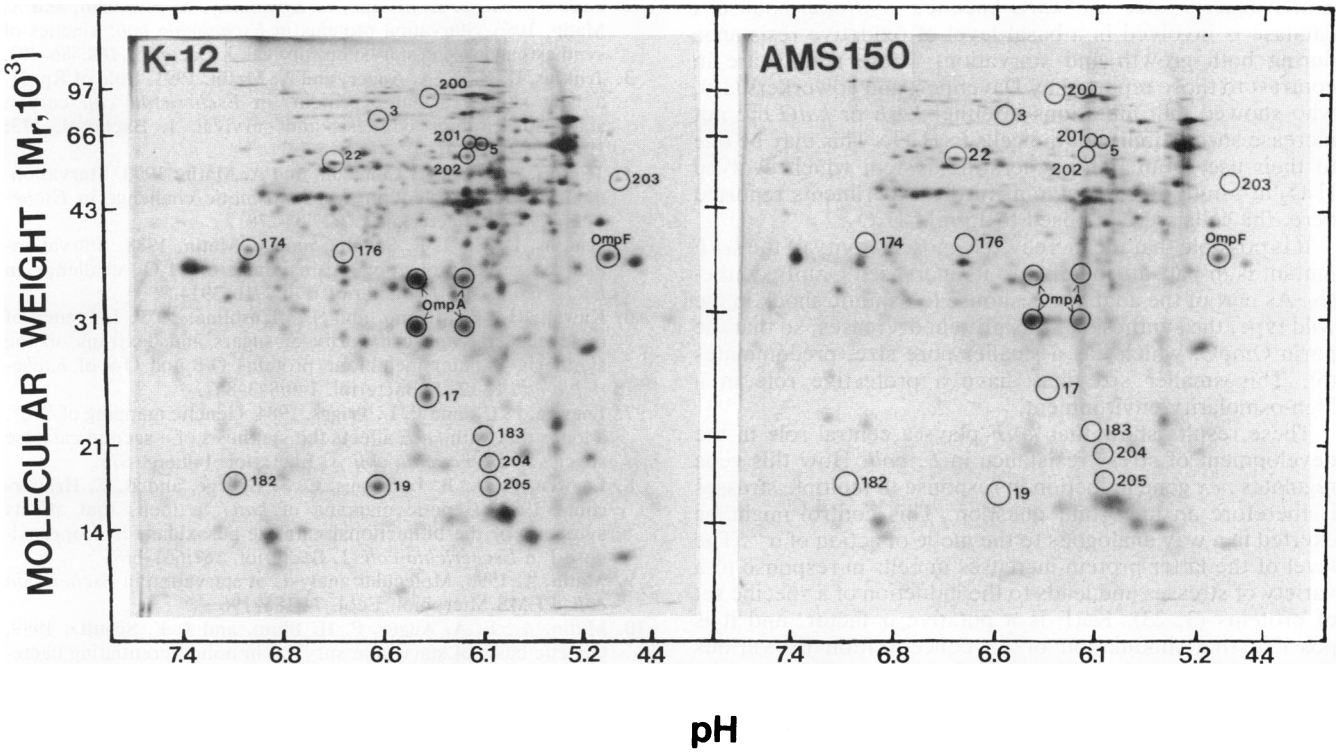

FIG. 8. Two-dimensional autoradiographs of proteins synthesized in *E. coli* K-12 or in the *katF*::Tn*10* derivative strain AMS150 at 10 min after a temperature shift from 29 to 42°C. Circles indicate proteins which were not synthesized or whose synthesis was altered at 5 or 10 min in the mutant. Spots whose migration corresponds to OmpA and OmpF (17) are designated.

starvation were induced during heat shock, although polypeptide 17 was synthesized at lower levels than in the wild type. At 10 min of exposure to 42°C, some 14 polypeptides induced in the wild type were missing from the mutant. In addition to those absent at 5 min, polypeptides 5, 17, 201, 202, 204, and 205 were not synthesized. Also, four polypeptides which migrated at positions corresponding to OmpA (17) were synthesized at lower levels in the mutant. The synthesis of the σ^{32}-regulated heat shock proteins DnaK, GroEL, and HtpG appeared to be identical in the mutant and the wild type.

DISCUSSION

We show here that the *katF* gene product controls the synthesis of at least 32 proteins that are induced upon carbon starvation in *E. coli*, since the synthesis of these proteins in the *katF* mutant was either absent or greatly reduced under starvation conditions. These include six proteins previously identified as Pex (21), as well as at least two Cst proteins. The six Pex proteins belong to the core set that is synthesized by *E. coli* in response to starvation for several individual nutrients. We have previously postulated that the core set of proteins is likely to be particularly important in the starvation survival of *E. coli* (2, 9, 10, 21). This postulation is supported by the finding that the *katF* mutant survives starvation for either carbon or nitrogen poorly, the former also under anaerobic conditions.

Many of the Pex proteins not induced in the starved *katF* mutant are among those that *E. coli* synthesizes upon exposure to a variety of individual stresses and have been previously suggested by us to play a role in starvation-mediated cross protection of *E. coli* against other stresses. This postulation is also strongly supported by the findings reported here, namely, that the *katF* mutant fails to develop starvation-induced cross protection against osmotic, oxidative, and heat stresses. For example, the *katF* mutant failed to induce the synthesis of the osmotic stress Pex proteins 17, 19, and 34 during starvation and remained highly susceptible to the effects of osmotic shock. It is also clear that the role of KatF in stress management in *E. coli* extends beyond resistances induced by starvation, since the *katF* mutant also failed to develop thermal resistance in response to an adaptive dose of heat that induces resistance in the wild type.

Although the Pex class of proteins appears to be involved in starvation-mediated cross protection as well as adaptive protection to individual stresses, the data do not permit conclusions concerning the importance of specific polypeptides in resistance to a specific stress. Thus, the heat shock Pex polypeptides 17 and 22, which are not induced in the *katF* mutant by starvation, are induced upon adaptive heat treatment, although both pretreatments failed to induce thermal resistance. This may be due to the reduced synthesis of polypeptide 17 or because other proteins act in concert with these KatF-regulated proteins to confer thermal resistance.

The role of some other proteins is more clear. It is evident that the *katE*-encoded hydroperoxidase II catalase has a direct role in starvation-mediated resistance to oxidation. Thus, the *katE* mutant was less able than the wild type to develop oxidative protection in response to starvation. The *katG* mutation also impaired the development of starvation-mediated cross protection to oxidative stress. *katG* is under the control of OxyR and is not induced by starvation (20).

This suggests that the *katG*-encoded hydroperoxidase I catalase is involved in a basal level of oxidative resistance during both growth and starvation. These results are in contrast to those reported by Davenport and coworkers (19), who showed that mutations in either *katE* or *katG* did not increase susceptibility of the cells to H_2O_2. This may be due to their use of an H_2O_2-generating system which evolved H_2O_2 at a rate of 1 mmol/min. In the experiments reported here, the cells were exposed to 15 mM H_2O_2.

It is possible that the increased osmosensitivity of the *katF* mutant is in part attributable to its increased OmpF synthesis. As part of the adaptive response to osmotic shock in the wild type, the synthesis of this protein decreases, so that the porin OmpC, which has a smaller pore size, predominates (6). This smaller size may have a protective role in a high-osmolarity environment.

These results show that *katF* plays a central role in the development of stress resistance in *E. coli*. How this gene regulates *pex* gene induction in response to multiple stresses is therefore an important question. This control might be exerted in a way analogous to the mode of action of σ^{32}. The level of the latter protein increases in cells in response to a variety of stresses and leads to the induction of a specific set of proteins (3, 23). KatF is a putative σ factor, and it is possible that modulation of its concentration by various stresses plays a role in the induction of proteins under its control. There is at present, however, no direct evidence that KatF is a σ factor or that its levels are modulated in stressed cells. It is noteworthy that KatF appears to be involved in the synthesis of at least two Cst proteins in response to starvation. To the extent that the regulation of this class has been examined, it appears to be controlled by a σ^{70} RNA polymerase holoenzyme (1). Thus, either KatF acts in concert with σ^{70} RNA polymerase as a positive transcriptional activator or these *cst* genes may possess multiple promoters. Furthermore, the differential regulation of KatF-regulated proteins under a variety of stresses suggests the involvement of additional transcriptional activators unique to each condition. These and other questions concerning KatF-mediated gene regulation, as well as the biochemical role of KatF-regulated proteins, would be of central focus in future investigations of the stress physiology of *E. coli*.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

After this paper was submitted for publication, R. Lange and R. Hengge-Aronis (Mol. Microbiol. 5:49–59, 1991) reported the effect of a regulatory mutation (subsequently identified in the *kal-F* gene) on stationary-phase genes. Some of their findings are similar to those reported here.

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