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Role of an *Escherichia coli* stress–response operon in stationary-phase survival

(phage shock protein/σ^{54}/heat shock/starvation)

LORIN WEINER* AND PETER MODEL†

Laboratory of Genetics, The Rockefeller University, New York, NY 10021

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ABSTRACT **The phage shock protein operon (*pspABCE*) of *Escherichia coli* is strongly expressed in response to stressful environmental conditions, such as heat shock, ethanol treatment, osmotic shock, and filamentous phage infection. We show that bacteria lacking the *pspABC* genes exhibit a substantial decrease in the ability to survive prolonged incubation in stationary phase under alkaline conditions (pH 9). The *psp* mutant bacteria grow approximately as well as wild-type strains in the alkaline medium, and stationary-phase survival of the *psp* mutants improves substantially at pH values closer to the optimal growth range (pH 6–8). In late stationary-phase (1- to 2-day-old) cells, the operon can be strongly induced under certain conditions, and PspA can become one of the most highly expressed bacterial proteins. The combination of stationary-phase starvation and alkaline pH is likely to place a severe strain on the maintenance of endogenous energy sources, and, consistent with these effects, we find that *psp* expression is also induced by uncouplers of oxidative phosphorylation and other agents that interfere with energy production. The death rate of *psp* mutants in stationary phase is accelerated by the presence of wild-type bacteria in the same culture, suggesting that the *psp* operon may play a significant role in enabling *E. coli* to compete for survival under nutrient- or energy-limited conditions.**

The phage shock protein (*psp*) operon of *Escherichia coli* is strongly induced by exposure to adverse conditions such as heat shock, ethanol treatment, osmotic shock, the inhibition of protein secretion, the gene IV protein of filamentous phages (e.g., f1, M13), and homologs of the gene IV protein from various Gram-negative bacterial species (1–4). The operon consists of at least four genes, designated *pspABCE*, and is regulated primarily at the transcriptional level (5). All stress-induced transcription utilizes RNA polymerase holoenzyme containing σ^{54} (6), a σ factor not known previously to participate in the heat shock response. The *psp* genes are cooperatively activated by PspB and PspC and repressed in the absence of environmental stress by PspA (6). Most *E. coli* heat shock genes (≈17) are positively regulated by the σ factor σ^{32} (7–9), but strains containing loss of function mutations in σ^{32} exhibit a greater and prolonged induction of the *psp* operon during heat shock (1, 5). The σ^{32}-dependent heat shock proteins (Hsps) thus down-regulate *psp* expression following induction. The only *psp*-inducing agents that do not stimulate synthesis of the σ^{32}-dependent Hsps are the gene IV protein and its homologs (ref. 1; M. Russel, personal communication).

Although the operon is strongly expressed in response to harmful agents, *psp* mutant strains do not display any obvious defects in growth or viability under either standard or stressful conditions. The synthesis of substantial amounts of the phage shock proteins (Psps) during environmental challenge [≈10,000 molecules of PspA in an f1-infected cell (1)], and the existence of a multifactor network tightly regulating *psp* expression, led us to assume that the operon serves a protective or beneficial function. We have suggested that the lack of a stress-related, logarithmic-phase *psp* phenotype is due to a functional overlap of the Psps with the σ^{32}-controlled regulon (6). Both PspA and the σ^{32}-dependent heat shock system turn off *psp* expression, and this negative regulation may result from similar or overlapping activities.

We report here that the *psp* operon is required for prolonged survival in stationary phase at alkaline pH (pH 9) and that the expression of the *psp* genes can be strongly induced during stationary phase under certain conditions.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. Strains K561, J134 (K561 Δ*pspABC*), L1, and L24 (L1 Δ*pspABC*) have been described (6, 10). SG20045 (*cps-5*::Tn*10* Δ*lon-100*) (11) was kindly provided by Susan Gottesman (National Institutes of Health). SK5022 (*zci*-604::Tn*10*) was from the *E. coli* Genetic Stock Center (CGSC no. 6666). L104 (K561 *cps-5*::Tn*10*), L102 (J134 *cps-5*::Tn*10*), and L96 (J134 *psp*$^+$ *zci*-604::Tn*10*) were generated by transduction (12). The plasmid pBRPS-1, which carries the complete *psp* operon, was constructed by ligating the 4.5-kb *Eco*RI fragment of pPS-1 (5) to *Eco*RI-restricted pBR322.

Bacterial Survival in Stationary Phase. Overnight cultures of bacteria in tryptone broth (rich medium; 1% tryptone/0.1% yeast extract/0.1% glucose/0.8% NaCl) were diluted into essentially the same medium containing 100 mM 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO; pK_a of 9.0 at 25°C)/0.4% NaCl; the pH of the AMPSO buffer was adjusted with NaOH. The cells were grown to stationary phase and incubated with moderate shaking at 37°C. The cultures were titered for viable cells at various times by plating on tryptone medium (no AMPSO) with or without appropriate antibiotics.

Analysis of *psp* Expression. To analyze the rate of Psp synthesis following treatment with carbonylcyanide *m*-chlorophenylhydrazone (CCCP) or fatty acids, bacteria were grown to a density of 2×10^8 cells per ml in M9 (12) or D0 (13) salts supplemented with 0.4% glucose/5 μg of thiamine per ml/19 amino acids (0.2 mg/ml each; no methionine). Aliquots of each culture (0.2 ml) were pulse-labeled 1 min with 20 μCi of [^{35}S]methionine (New England Nuclear; 1000 Ci/mmol; 1 Ci = 37 GBq). To assay *psp* expression in

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Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; AMPSO, 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid; cfu, colony-forming unit(s); Hsp, heat shock protein; Psp, phage shock protein.

*Present address: Department of Cell Biology, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.
†To whom reprint requests should be addressed.

stationary phase, bacteria were grown in 20 mM K_2HPO_4/10 mM KCl/0.05% NH_4Cl/1 mM $MgSO_4$/50 μM $CaCl_2$/0.2% glucose/19 amino acids (0.1 mg/ml each; no methionine)/100 mM of either AMPSO (pH 9.0) or Mops (pH 7.0). The cells were labeled with [^{35}S]methionine for 1 min in logarithmic and early stationary phase; pulse periods were extended to 12 hr (with aeration) at later stationary-phase time points. Almirón *et al.* (14) found that prolonged labeling periods are necessary to detect protein synthesis in late stationary-phase cells and that the incorporation of [^{35}S]methionine is linear in K-12 strains for labeling periods of at least 24 hr. The ^{35}S-labeled proteins were precipitated with cold trichloroacetic acid (5%), resuspended in 4% SDS (25 μl), and immunoprecipitated as described (10).

RESULTS

Survival of *psp* Mutant Bacteria in Stationary Phase. The Δ*pspABC* strain J134 and its wild-type parent K561 were grown in rich medium at 37°C and incubated with aeration throughout stationary phase. For 8 days, both cultures displayed a slow, similar decline in the number of viable cells, but on the ninth day, the Δ*pspABC* strain experienced a sharp decline in viability and exhibited an ≈100-fold difference from the *psp*$^+$ strain in the number of colony-forming units (cfu)/ml (data not shown).

The pH of both cultures was found to have changed from approximately 7 to 9 over the course of the experiment, perhaps due to the release of ammonia as the bacteria metabolized amino acids. The experiment was repeated, therefore, with the addition of 100 mM AMPSO to the rich medium for a final pH of 9.0 at 37°C. Equal numbers of the *psp*$^+$ and Δ*pspABC* bacteria were diluted from overnight cultures into the alkaline medium in either separate flasks or the same flask; these cultures were then grown to stationary phase. The Δ*pspABC* strain exhibited approximately the same doubling time as the wild-type strain during logarithmic-phase growth in the alkaline medium (data not shown). After 3 days in stationary phase (in eight separate experiments), the number of cfu in the Δ*pspABC* culture (Fig. 1, ○) was 1000-fold lower than the cfu in the *psp*$^+$ culture (□). When the *psp*$^+$ and Δ*pspABC* strains were in direct competition in the same flask, the Δ*pspABC* bacteria (Fig. 1, ●) underwent an even more rapid decline in viability and after 3 days exhibited a close to 10,000-fold difference in survival from the wild-type strain. The pH of the medium was essentially constant during the experiments, decreasing only 0.1–0.2 pH unit by

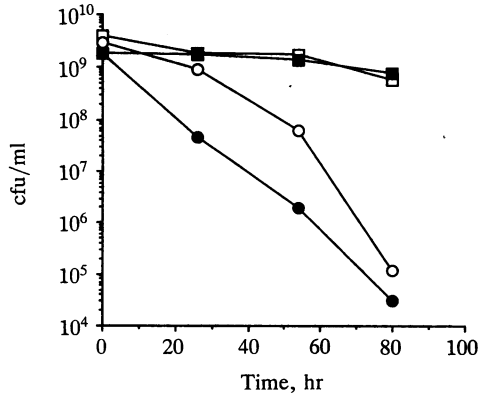

FIG. 1. Survival of *psp* mutant bacteria in stationary phase at pH 9.0. Strains K561 (*psp*$^+$) and J134 (Δ*pspABC*) were grown in tryptone broth containing 100 mM AMPSO (pH 9.0). The cultures were incubated with aeration in stationary phase and titered for cfu. K561 (□) and J134 (○) incubated in separate cultures. K561 (■) and J134 (●) maintained in the same culture.

the 72-hr time points. Strains L65 (K561 Δ*pspB*) and J136 (K561 Δ*pspC*) (6) exhibited variable survival under these conditions (the survival rates were not consistently lower than that of K561) and did not display the sensitivity of J134 (Δ*pspABC*), suggesting that the loss of PspA is necessary to produce a clear difference in viability (data not shown). Two other laboratory strains, L1 (*psp*$^+$) and L24 (Δ*pspABC*), exhibited differences in survival of 5- to 10-fold after 3 days of incubation in stationary phase at pH 9; by the fourth day of incubation, both strains underwent a large loss of viability. Thus the ability to survive under these conditions differs among strains, as L1 exhibited greater sensitivity than K561 and died sooner. The magnitude of the effect of the *psp* genes on survival is also strain-dependent, as L1 and L24 displayed differences in viability that, though significant, were smaller than those of K561 and J134.

Following 3 days of incubation in stationary phase at pH 9.0, cells began to appear in the J134 (Δ*pspABC*) culture that gave rise to very mucoid colonies. These bacteria slowly increased in number at subsequent time points (as seen in Fig. 3) and may have grown by cannibalizing the dead cells. These mucoid bacteria most likely contained suppressor mutations, and the proliferation of mutant cells with a survival advantage in late stationary phase was observed previously in studies of *surA* (15) and *rpoS* (16).

To determine whether the stationary-phase phenotype was due to the deletion of the *psp* genes, the Δ*pspABC* strain J134 was transduced to *psp*$^+$ (creating L96), and a transductant was tested for viability in stationary phase at pH 9.0. As shown in Fig. 2*A*, the ability to survive these conditions was regained through the restoration of the *psp* operon to the Δ*pspABC* bacteria; the viability of L96 was identical to that of the wild-type strain K561. The operon was also restored to strain J134 on the plasmid pBR322 (pBRPS-1). This experiment is complex because the wild-type and *psp* mutant strains did not stably maintain pBR322 in alkaline medium without tetracycline, and the presence of the plasmid and tetracycline accelerated the death rate in stationary phase. Nonetheless, the stationary-phase viability of the Δ*pspABC* strain was significantly improved by the plasmid-borne *psp* genes (Fig. 2*B*). The additional copies of the *psp* genes in bacteria carrying pBRPS-1 did not increase survival above that observed for the wild-type strain containing pBR322 and a single chromosomal copy of the operon.

The effect of the *psp* genes on stationary-phase survival was assayed as a function of pH. The viability of *psp*$^+$ and Δ*pspABC* bacteria was compared in medium containing 100 mM AMPSO titrated to either pH 9.0 or 8.2 at 37°C. The strains used in this experiment were derivatives of K561 and J134 containing null mutations in *cpsD*, a gene required for the synthesis of capsular polysaccharide. These strains were used to determine whether the inability to produce capsules would prevent the appearance of the mucoid suppressor cells in the Δ*pspABC* cultures. Following ≈3 days of stationary-phase incubation, the viability of the Δ*pspABC* strain L102 improved >1000-fold when the pH of the medium was lowered from 9.0 to 8.2 (Fig. 3). The *cpsD* mutation did not alter the survival of the wild-type or mutant strains under these conditions and also did not prevent the appearance of suppressor cells with altered colony morphology in the Δ*pspABC* pH 9.0 culture. These suppressor colonies were less mucoid than those derived from J134 but were star-shaped and whiter than the typical Δ*pspABC* colonies. These cells slowly proliferated following the rapid decrease in viability of L102 during the third day of incubation. In the cultures set to pH 8.2, a modest difference in survival of ≈5-fold emerged between the *psp*$^+$ and Δ*pspABC* strains over the course of 5 days (Fig. 3).

One possible explanation for the decreased viability of *psp*$^-$ bacteria during stationary-phase incubation at alkaline

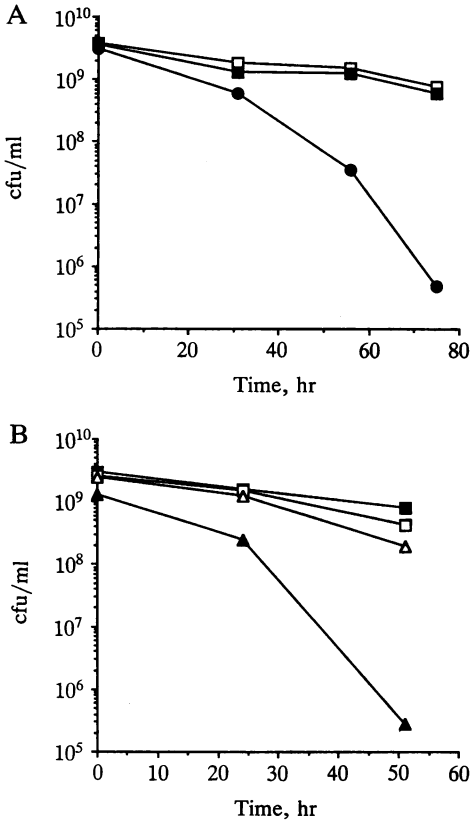

FIG. 2. Sensitivity to alkaline conditions in stationary phase results from the loss of the *psp* genes. (*A*) Strains K561 (*psp*$^+$), J134 (Δ*pspABC*), and L96 (Δ*pspABC* transduced to *psp*$^+$) were grown to stationary phase at 37°C in tryptone broth containing 100 mM AMPSO (pH 9.0). The three strains were maintained with aeration in separate cultures and assayed for cfu. □, K561; ■, L96; ●, J134. (*B*) Strains K561 and J134 containing either pBR322 or pBRPS-1 were grown to stationary phase in separate cultures as described in *A* and titered at various times. ■, K561, pBR322; □, K561, pBRPS-1; ▲, J134, pBR322; △, J134, pBRPS-1.

pH is that the *psp* mutants are more sensitive than the wild-type cells to a toxic molecule released under these conditions. To test for the presence of a toxin, K561 bacteria were incubated in rich medium at pH 9 for 3 days and then removed from the culture by centrifugation and filtration.

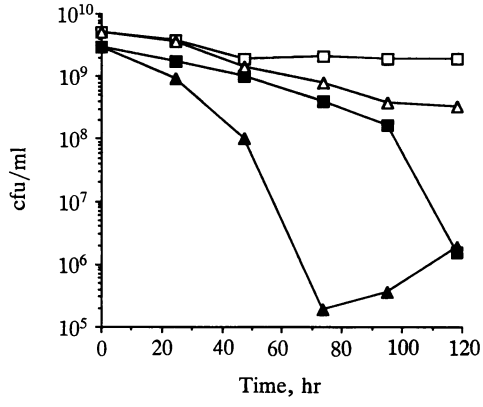

FIG. 3. Survival of *psp* mutant bacteria in stationary phase as a function of pH. Strains L104 (*psp*$^+$) and L102 (Δ*pspABC*) were grown to stationary phase at 37°C in tryptone broth containing 100 mM AMPSO titrated to either pH 9.0 or pH 8.2. The bacteria were maintained with aeration in separate cultures and titered at various times. ■, L104, pH 9.0; ▲, L102, pH 9.0; □, L104, pH 8.2; △, L102, pH 8.2.

The spent medium was added as an equal volume to separate mid-logarithmic phase, pH 9 cultures of K561 and J134. The growth of these strains was not blocked or even partially inhibited by the spent medium, strongly suggesting that a toxin does not accumulate under these conditions.

Stationary-phase bacteria are extremely resistant to thermal killing (17). To determine whether the *psp* genes play a role in the acquisition of this thermotolerance, strains K561 and L1 and their Δ*pspABC* derivatives were grown to stationary phase (37°C) in rich medium containing 100 mM Mops (pH 7.0) and shifted to 55°C. No differences in thermal resistance were observed between the *psp* mutants and their parent strains (data not shown).

Psp Expression in Stationary Phase. Strain K561 was grown in medium containing 100 mM of either AMPSO (pH 9.0) or Mops (pH 7.0) and labeled with [^{35}S]methionine at various points in logarithmic and stationary phase. Logarithmic-phase and early stationary-phase (2 hr after the cessation of growth) bacteria were pulse-labeled with [^{35}S]methionine for 1 min, but late stationary-phase cultures (1- to 2-day-old cells) require prolonged labeling periods to detect protein synthesis (14) and were incubated with the [^{35}S]methionine for 12 hr. As shown in Fig. 4, PspA was strongly expressed in late stationary-phase bacteria incubated at pH 7 (lanes 4 and 5) and was perhaps the most intensely labeled protein in 1-day-old, stationary-phase cells. In the pH 9 culture, PspA was synthesized at more modest but constant rates during logarithmic phase and early stationary phase (lanes 6–8); this level of *psp* expression was higher than that observed for the pH 7 culture at the same time points. Following 24 hr of stationary-phase incubation under alkaline conditions, very little [^{35}S]methionine was incorporated into protein, although PspA production remained detectable (lane 9); after 48 hr in stationary

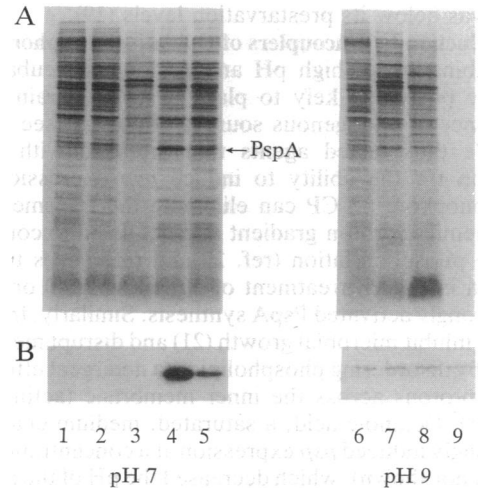

FIG. 4. PspA synthesis in stationary phase. Strain K561 was grown at 37°C in medium containing 100 mM of either Mops (pH 7.0; lanes 1–5) or AMPSO (pH 9.0; lanes 6–9) and labeled with [^{35}S]methionine at various points in logarithmic and stationary phase. The radioactivity incorporated into trichloroacetic acid-precipitable material was similar for the time points shown in lanes 1–8. ^{35}S-labeled samples normalized for total cells were electrophoresed on 15% polyacrylamide/SDS gels. (*A*) Total bacterial protein. (*B*) Immunoprecipitations using antiserum to PspA. Lanes 1 and 6, mid-logarithmic-phase cultures (5×10^8 cells per ml); lanes 2 and 7, late logarithmic-phase cultures (2×10^9 cells per ml); lanes 3 and 8, early stationary-phase cultures (2 hr after the cessation of growth; 4×10^9 cells per ml in pH 7.0 culture and 3×10^9 cells per ml in pH 9.0 culture); lanes 4 and 9, 1-day-old cultures (labeling initiated after 24 hr in stationary phase); lane 5, 2-day-old culture (labeling initiated after 48 hr in stationary phase). (*B*) Composite of two exposures of the same gel; lanes 1–5 were exposed 12 hr and lanes 6–9 were exposed 8 days.

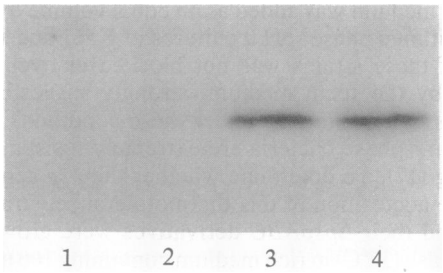

FIG. 5. *psp* activation by a proton ionophore. K561 bacteria were grown in M9 salts medium (pH 7, 37°C), treated with CCCP for 15 min, and pulse-labeled with [^{35}S]methionine. CCCP was dissolved in dimethyl sulfoxide and added in a volume that was 0.5% of the culture volume. Immunoprecipitations using anti-PspA serum were analyzed by SDS/PAGE. Lane 1, no treatment; lane 2, 0.5% dimethyl sulfoxide; lane 3, 40 μM CCCP; lane 4, 60 μM CCCP.

phase, no [^{35}S]methionine was incorporated (data not shown). This absence of protein synthesis in the pH 9 culture was correlated with a decrease in the number of surviving cells, as the cfu fell by two orders of magnitude after 36 hr of stationary-phase incubation (the number of viable cells in the pH 7 culture remained virtually the same over the course of the experiment). Similar results were obtained on immunoblots analyzing PspA expression in stationary-phase cultures grown in pH 9 tryptone broth. PspA was present in logarithmic-phase cells and did not appreciably increase in its steady-state level over 2 days of stationary-phase incubation at pH 9 (data not shown). In studies performed by others using M9 salts medium (no additional buffer), the levels of σ^{32} and the σ^{32}-dependent Hsps DnaK, GroEL, and HtpG increased at early time points (6 hr or less) during carbon starvation (17–19). The amount of σ^{32} then declined so that at 24 hr, this protein was below its prestarvation levels (19).

Psp Induction by Uncouplers of Oxidative Phosphorylation. The combination of high pH and prolonged incubation in stationary phase is likely to place a severe strain on the maintenance of endogenous sources of energy (see *Discussion*). We thus tested agents that interfere with energy production for the ability to induce *psp* expression. The proton ionophore CCCP can eliminate the transmembrane electrochemical proton gradient and act as an uncoupler of oxidative phosphorylation (ref. 20 and references therein). As shown in Fig. 5, treatment of *E. coli* with 40 or 60 μM CCCP strongly activated PspA synthesis. Similarly, free fatty acids can inhibit microbial growth (21) and disrupt membrane function by disordering phospholipids (a detergent effect) and carrying protons across the inner membrane (acting as an uncoupler). Octanoic acid, a saturated, medium-chain fatty acid, strongly induced *psp* expression at a concentration of 10 mM (data not shown), which decreased the pH of the medium by only 0.1 unit. Uncouplers are known to induce Hsp synthesis (22), and on SDS/polyacrylamide gels of total protein labeled with [^{35}S]methionine, the production of PspA and other Hsps in response to CCCP and fatty acid treatment was clearly visible (data not shown).

DISCUSSION

E. coli is a neutrophile that is able to grow in a pH range of ≈5–9, with optimal growth rates reached between pH 6 and 8 (23). At pH values of 4 or 10, the capacity for growth is lost. The bacteria maintain a constant internal pH of 7.5–7.9 and are intolerant to significant deviations from this value (23).

The Δ*pspABC* strain grows at approximately the same rate as the wild-type bacteria in the rich medium at pH 9.0, and thus the mutant does not appear to be defective in the ability to maintain pH homeostasis in logarithmic phase. Upon prolonged incubation in stationary phase under alkaline conditions, the Δ*pspABC* cells exhibit a substantial difference in survival with *psp*$^+$ bacteria, and this difference becomes even greater when the mutant and wild-type strains compete to survive in the same culture. The decreased viability of the mutant bacteria appears to be due to the loss of the *psp* genes, as restoring the operon to the mutant on a plasmid or by transduction greatly improves survival. The increased death rate of the Δ*pspABC* strain in the presence of wild-type cells suggests that the sensitivity to stationary-phase incubation at pH 9.0 does not result from simply an inability to regulate internal pH in stationary phase.

psp expression is regulated by growth phase, and following 1 day of stationary-phase incubation at pH 7, PspA is one of the most highly expressed proteins in the cell. Thus, although the absolute level of protein synthesis declines in stationary phase, the bacteria devote a significant share of their limited resources to the production of PspA. The low rate of protein synthesis presumably means that the accumulation of PspA in these cells is a slow process. During logarithmic and early stationary phase, the rate of PspA production is higher at pH 9 than at pH 7, and so PspA is present in stationary-phase cells under alkaline conditions. In late stationary-phase, pH 9 cultures, there is no clear increase in the level of PspA, apparently due to a substantial decrease in total protein synthesis. Hence, under alkaline conditions, it appears to be the accumulation of the Psps during logarithmic and early stationary phase that promotes survival at later times.

There are several possible interpretations of the effects of the *psp* genes on stationary-phase survival. One possibility involves the ability of the *psp* mutants to generate or utilize energy sources efficiently. It is generally assumed that as bacteria enter stationary phase, the cells have exhausted at least one essential nutrient, and upon prolonged incubation in spent medium, the cells may begin to starve for several nutrients. As expected for starving cells, stationary-phase *E. coli* undergo a reduction in protein synthesis and metabolic rate (24). Protein (25) and RNA (particularly rRNA; ref. 26 and references therein) degradation increases, and this self-cannibalization may provide the materials for cell maintenance and repair. The adenylate energy charge, a measure of the energy stored in adenine nucleotides, declines but appears to remain above a certain level in viable cells (27). In *Klebsiella pneumoniae*, the proton motive force was shown to decrease as well (28). As noted by Siegele and Kolter (24), many systems for nutrient uptake require ATP or an electrochemical gradient for substrate accumulation, and so the cells must maintain these energy sources to transport and exploit any newly encountered nutrients.

The proton motive force consists of two components, the membrane potential and the pH gradient. Since *E. coli* maintain a constant internal pH of 7.5–7.9, the cells increase the membrane potential as the external pH rises in order to maintain a constant proton motive force (29). At an external pH of 9.0, the pH gradient points out of the cell and opposes the proton motive force. The bacteria are thus required to generate a large compensatory membrane potential, which presumably demands energy to create and maintain.

Starving bacteria incubated at pH 9.0 for a prolonged period of time will be particularly limited in their ability to produce and maintain their endogenous energy sources. It seems possible then that the *psp* mutants lose viability more rapidly than wild-type cells under these conditions due to an inability to store, maintain, or utilize endogenous energy sources efficiently. The *psp* mutants were found to die even more rapidly when incubated with wild-type cells in the same culture, which may result from the direct competition of the mutant and wild-type strains for the limited resources remaining in the medium. The wild-type cells may take up the remaining nutrients more quickly than the *psp* mutants and

thus hasten the starvation of the mutant cells. This possibility is consistent with the properties of the agents that induce Psp synthesis. We report here that the *psp* genes are strongly expressed in response to proton ionophores and fatty acids, which can dissipate the electrochemical proton gradient across the inner membrane and potentially disrupt ATP synthesis (20, 21). In earlier work, it was found that heat shock and ethanol treatment activate *psp* expression (1), and these agents are capable of interfering with energy production as well in various organisms (20, 30–32). Given the conditions that induce *psp* expression and reduce *psp* mutant viability, Psp induction and function could be related to either energy generation directly or the inhibition of an energy-dependent process.

Kleerebezem and Tommassen (3) reported that the chromosomal deletion of *pspA* and its promoter resulted in a reduced efficiency of translocation for certain secreted proteins expressed from plasmids (in logarithmic-phase cells). Similar effects on translocation efficiency were found for σ^{32}-dependent Hsps, which most likely act as molecular chaperones and maintain precursor proteins in translocation-competent forms (33–36). Both PspA and the σ^{32}-dependent Hsps negatively regulate *psp* expression, and we have proposed that this overlap in regulatory effects may represent an overlap or similarity in function (6). PspA may interact with the translocation apparatus directly or, alternatively, the deletion of the *pspA* gene may cause the other Hsps to be titrated away from the secretion machinery in order to perform activities that compensate for the loss of PspA. Whether the effect of PspA on protein secretion is direct or indirect, a decrease in the efficiency of export is likely to cause the cell to expend energy to prevent precursor accumulation (by either the translocation or degradation of these proteins).

Spent medium from 3-day-old, wild-type cells did not affect the growth of either psp^+ or psp^- bacteria, and thus the sensitivity of *psp* mutant bacteria to stationary-phase incubation at pH 9 does not appear to result from the secretion of a toxic molecule. It remains a formal possibility that a toxin is produced but does not accumulate in stationary-phase cultures due to either its instability or reactivity.

Finally, *E. coli*, like most bacteria, are likely to spend much of their time in stationary phase in the wild, as food sources may only become available transiently. In the intestine, *E. coli* may exist close to stationary phase and exhibit a doubling time of about 20 hr in mice (37). The secretions of the duodenum are also alkaline and have a pH of 8.2–9.3. It seems possible then that in the wild, the *psp* genes could play an important role in enabling *E. coli* to compete successfully with other bacteria under conditions of nutrient or, more specifically, energy deprivation.

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