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Phage shock protein, a stress protein of *Escherichia coli*

(filamentous phage/heat shock protein/σ^{32}-independent transcription/membrane protein/Psp protein)

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ABSTRACT **Filamentous phage infection induces the synthesis of large amounts of an *Escherichia coli* protein, phage shock protein (Psp), the product of a previously undescribed gene. This induction is due to the phage gene *IV* protein, pIV, an integral membrane protein. The uninduced level of Psp is undetectable, but when induced by prolonged synthesis of pIV, it can become one of the most abundant proteins in the cell. Psp is also synthesized transiently in response to several stresses (heat, ethanol, and osmotic shock). High-level synthesis occurs only after extreme treatment. Unlike the members of the heat shock regulon, Psp induction does not require the heat shock σ factor, σ^{32}; some stimuli that elicit σ^{32}-dependent heat shock proteins do not induce Psp synthesis. The level of Psp induction after extreme stress is even higher in σ^{32} mutant cells, which are unable to mount a normal heat shock response, suggesting that these parallel stress responses are interrelated.**

All viruses depend on their host for propagation. Often they use the host machinery just as the host does, but they also adapt host proteins to somewhat or completely different purposes. Viruses can also modulate host gene expression. Examination of the ways in which they alter or induce host activities has provided windows into host genes and functions that were not otherwise known to exist. For example, a set of *Escherichia coli* proteins involved in transcription antitermination were identified because phage λ requires them and specifies a protein that alters their activity (1). Infection by λ also induces the synthesis of a number of proteins, including heat shock proteins (2–4). Several heat shock proteins initially identified by their essential role in λ development (reviewed in ref. 5) are now known to be required for host survival at most temperatures (6–9).

We have found that filamentous phage infection induces the synthesis of large amounts of a previously unidentified host protein that we have called Psp (phage shock protein). Here we report some of the characteristics of the induction process. Induction is the specific result of the synthesis of the protein encoded by phage gene *IV*, pIV, an integral membrane protein (10) required for phage morphogenesis. Neither truncated forms of pIV nor pIV confined to the cytoplasm induce Psp synthesis, but some mutant forms of pIV do. Psp, like other *E. coli* stress-induced proteins, is also produced in response to a variety of environmental stimuli. Psp is strongly (but transiently) induced by extreme heat shock, by treatment with a high concentration of ethanol, and by hyperosmotic shock. Unlike the "classical" heat shock proteins, Psp synthesis does not require the heat shock σ factor, σ^{32}. Psp expression therefore parallels the heat shock response but is elicited by more extreme conditions.

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MATERIALS AND METHODS

Phage, Plasmids, and Bacterial Strains. The f1 phage were from our laboratory collection; the gene *IV* mutants are described in ref. 10, as is 4tsH2, a gene *IV* temperature-sensitive (ts) derivative of M13 isolated by Pratt *et al.* (11). Ike phage was kindly provided by Ruud Konings (University of Nijmegen, The Netherlands).

Plasmid pDG117IIA (12) is a pBR322 derivative that contains the f1 replication origin and the f1 gene (*II*) required for utilization of this origin under control of the *tac* promoter. pJLB31 contains, in addition, f1 gene *IV* under control of the *lac* promoter (*Plac*). It was constructed by cloning the *Eco*RI fragment of pJLB3 (10) that includes *Plac* and gene *IV* into one of the *Eco*RI sites of pDG117IIA. Plasmids pJLB3 and pJLB4 (pGL101 plus gene *IV*) have been described (10).

K38 [HfrC (λ) *phoA tonA22 garB10 ompF627 relA1 pit-10 spoT1 fadL701 phoM510 mcrB rrnB2*], its amber-supressing (*supD*) derivative, K37, or K91 (K38 λ^s) were used as hosts for infection by f1/M13 phage. K1037 (K38/N3) was used as host for phage Ike, which requires N pili specified by the N3 episome (13) for infection. K561 (K38 *lacI*^{q}) or K871 (K561 *recA56 srl-300*::Tn*10*) were used with plasmids that contained genes under control of *Plac*. SC122, also known as CAG440, contains a ts ochre suppressor (*supC*^{ts}) (14, 15); its derivative CAG456 (SC122 *rpoH165*^{am}; ref. 16) contains the *rpoH* amber mutation described by Cooper and Ruettinger (14). SC122 and CAG456 were kindly provided by Carol Gross (University of Wisconsin). The strains defective in potassium uptake [FRAG 5, TK2205, and TK2240 (17)] and the *trk*$^+$ *kdp*$^+$ control strain (FRAG 68) were kindly provided by Wolf Epstein (University of Chicago).

Experimental Procedures. K561/pJLB31 bacteria grown for 5 hr in DO salts (18) containing 0.4% glycerol, thiamin (5 μg/ml), 20 amino acids (1 mM each), ampicillin (100 μg/ml), and isopropyl β-D-thiogalactopyranoside (IPTG, 2 mM) were centrifuged, resuspended in 50 mM Tris Cl, pH 7.4/50 mM NaCl, sonicated, and centrifuged (100,000 × *g*, 2 hr, 4°C). The pellet was extracted with 4 M urea for 1 hr at 4°C, reextracted, and recentrifuged as above. The 4 M urea supernatants were electrophoresed in an SDS/polyacrylamide gel, and the Psp band was cut out, eluted, and used to immunize rabbits as described (10). Serum was batch-adsorbed (10).

Cells were labeled, immunoprecipitated, and fractionated as described (10). Osmotic strengths were measured on an Advanced Instruments 3W2 osmometer and are given in milliosmoles per kilogram of H_2O.

RESULTS

Filamentous Phage Infection Induces an *E. coli* Protein. Filamentous phage-infected cells contain a 25-kDa polypeptide not detected in uninfected cells. This protein is overpro-

Abbreviations: IPTG, isopropyl β-D-thiogalactopyranoside; pIV, gene *IV* protein; ts, temperature-sensitive.

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duced in cells that contain a plasmid, pJLB31, in which f1 gene *IV* is under the control of a *lac* UV5 promoter (hence IPTG-inducible) and whose copy number can be greatly amplified by the addition of IPTG. Cells containing the vector plasmid did not accumulate this protein (Fig. 1). The 25-kDa protein was isolated and used to raise antibodies in rabbits. The resulting antiserum reacted with the 25-kDa protein synthesized in the presence of IPTG in pJLB31-containing cells; it reacted with a 25-kDa protein in f1-infected cells but not in uninfected cells (Fig. 2). It did not react with any labeled protein synthesized *in vitro* with either f1 or gene *IV* plasmid DNA as template (data not shown). The N-terminal amino acid sequence (GIFSRFADIVNANINALLEKAED-PQKLVRL) of the isolated 25-kDa protein and peptide mapping confirmed that it was not derived from pIV or from any other possible f1 product. Therefore, pIV, a phage-encoded membrane protein (10), induces a bacterial protein; we have called this 25-kDa protein Psp (phage shock protein).

pIV Induction of Psp. The rate of Psp synthesis in f1-infected cells and IPTG-induced, pJLB31-containing cells is at least 50-fold the rate in uninfected cells. These high levels of Psp, compared to the low levels in uninduced pJLB31-containing cells and the barely detectable amount in uninfected cells (Fig. 2), suggest that the rate of Psp synthesis is responsive to the level of pIV in the cell. Quantitation of autoradiograms of lysates from cells pulse-labeled 30 min after f1 infection or continuously labeled from 15 to 60 min after infection indicated that pIV and Psp were synthesized at equivalent rates and to equivalent levels; the pIV concentration has been estimated to be 5–10 × 10^3 molecules per cell at 30 min after infection (10). Psp synthesis was detected by 4 min after addition of IPTG to cells containing pJLB3, a pGL101 derivative in which gene *IV* is under the control of the *lac* UV5 promoter (10). The maximal rate was reached by 10 min, well before pIV had accumulated to its steady-state level (data not shown). This rapid induction suggests that Psp synthesis is a specific response to pIV, not an indirect consequence of the membrane perturbations that often occur when secreted or membrane proteins are overproduced.

Maintenance of Psp synthesis requires continued synthesis of pIV (Fig. 3). Within a few minutes of IPTG withdrawal from pJLB4-containing cells, the rate of Psp synthesis began to decline, reaching a half-maximal rate at 15 min and the basal, uninduced rate by 40–60 min. The decline in the rate of Psp synthesis was more rapid than could be accounted for by dilution, since the doubling time of these cells was 75 min, and no decline in pulse-labeled pIV was observed after a 30-min chase (data not shown). Psp itself is also stable (unpublished data).

The ability of various filamentous phage and several mutants to induce Psp synthesis is presented in Table 1. Psp was

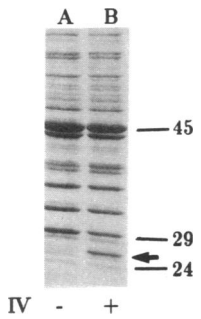

FIG. 1. Gene *IV* induces a cellular protein. Lysates from cells grown in the presence of 2 mM IPTG for 5 hr were electrophoresed in an SDS/polyacrylamide gel. Proteins were visualized with Coomassie blue. Lanes: A, K561/pDG117IIA (vector); B, K561/pJLB31 (*Plac–IV*). Molecular size standards are indicated (in kilodaltons). Arrow indicates the 25-kDa protein.

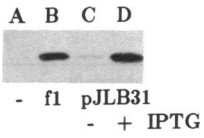

FIG. 2. Immunoprecipitation of Psp from pIV-producing cells. Uninfected (lane A) and f1-infected (lane B) K38 cells and uninduced (lane C) and IPTG-induced (lane D) K561 cells containing pJLB31 (*Plac–IV*) were labeled with [^{35}S]methionine for 1 min. Cell lysates were immunoprecipitated with Psp antiserum. Immunoprecipitates were analyzed by SDS/polyacrylamide gel electrophoresis followed by autoradiography.

synthesized in cells infected by f1$^+$ and by a related filamentous phage, Ike. The Ike and f1 pIV amino acid sequences are 46% identical (19). Psp synthesis was not induced in nonsuppressing cells infected by gene *IV* amber mutants, but it was induced in suppressor-containing cells. Cells infected by phage with mutations in gene *I*, *VIII*, or *IX*, which are also blocked in phage assembly, synthesized Psp in the absence of amber suppression.

pIV is synthesized as a precursor that is processed and secreted into the periplasm prior to its integration into the membrane (10). The mutant pIV synthesized by cells infected by an f1 mutant (R482) with a 4-codon deletion in the hydrophobic core of the gene *IV* signal sequence is not competent for phage morphogenesis (10). It is neither processed nor secreted; the unprocessed pIV is stable and accumulates in the cytoplasm in association with the cytoplasmic membrane (data not shown). Psp synthesis was not induced in R482-infected cells (Table 1) or in cells containing plasmids with two other gene *IV* signal-sequence deletions.

The mutant phage f1′ encodes a stable pIV with an altered C terminus (-Arg-Ala-Leu → -Gly-Val; ref. 20). This mutant pIV is only partially functional for phage morphogenesis (10). Nonetheless, f1′ infection induced Psp synthesis at about half the f1$^+$ rate. The mutant phage 4tsH2 (11) encodes a missense pIV (Ser-39 → Leu, mature; ref. 10). Bacteria infected by this ts mutant do not produce phage at 42°C (ref. 11 and data not shown). Nonetheless, Psp synthesis was fully induced at the nonpermissive temperature (Table 1). Thus pIV function in phage morphogenesis and its ability to induce synthesis of Psp are not inextricably linked.

Heat, Ethanol, and Osmotic Shock Induce Psp. Infection of *E. coli* with bacteriophage λ induces the synthesis of a number of proteins also transiently induced by heat shock (2–4). Because we thought f1 infection might also induce heat

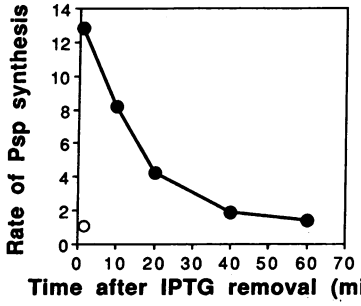

FIG. 3. Decline of the rate of Psp synthesis after IPTG removal from *Plac*–gene *IV*-containing cells. K871/pJLB4 cells were grown at 37°C in the absence of IPTG. Prior to IPTG addition (5 mM), an aliquot (○) was labeled with [^{35}S]methionine for 1 min. Forty minutes after IPTG addition, the culture was centrifuged for 15 sec in an Eppendorf microcentrifuge and washed with medium lacking IPTG, and aliquots were labeled as above at the times indicated (●). The samples were immunoprecipitated with Psp antiserum and electrophoresed, and band intensities were quantitated by microdensitometry. The rates of synthesis are relative to the uninduced rate of Psp synthesis.

Table 1. Induction of Psp synthesis by wild-type and mutant filamentous phage

Phage	Mutant gene	Psp	pIV	Morphogenesis
None		−	−	
f1$^+$		+	+	+
Ike$^+$		+	+	+
R14, R122	*I* am	+	+	−
8H1	*VIII* am	+	+	−
N113	*IX* am	+	+	−
R12, R17, R143 (K38)	*IV* am	−	−	−
R12, R17, R143 (K37)	*IV* am	+	+	+
R482 (Δ signal sequence)	*IV*	−	+*	−
f1′ (altered C terminus)	*IV*	+	+	±
M13 4tsH2 at 42°C	*IV* ts	+	+	−

K38 (nonsuppressing) or K37 (*supD*) cells were infected at 37°C or 42°C and labeled with [^{35}S]methionine after 30 min. Samples were electrophoresed in SDS/polyacrylamide gels, and Psp and pIV were detected by autoradiography of whole-cell lysates and immunoprecipitates.

*A pIV species slightly larger than pIV (normally present in the membrane) was present in the cytoplasm.

shock proteins, and thus that Psp might be a heat shock protein, the synthesis of Psp in heat-shocked, uninfected cells was examined. Psp synthesis was strongly induced after a shift from 37°C to 50°C (Fig. 4). In contrast to the classical heat shock proteins (5), which were strongly induced after temperature shift from 30°C to 42°C (data not shown), Psp synthesis was more modestly induced (≈10% the 50°C rate) by this less severe heat shock (Fig. 4). Induction was transient in both cases, as was induction of the other heat shock proteins, with the highest rates occurring 2 min after temperature shift. Although f1 infection did induce the synthesis of modest levels of the classical heat shock proteins, the effect was not due to pIV; they were not induced by expression of gene *IV* from a plasmid, and they were more strongly induced by gene *IV* (and gene *I*) amber mutant than by f1$^+$ infections (data not shown). Psp synthesis was not induced at 8, 15, or 25 min after infection by λ.

To test whether stimulation of Psp synthesis by pIV and by heat shock is additive, pJLB3-containing cells treated with IPTG to induce pIV synthesis were shifted from 37°C to 48°C. The rate of Psp synthesis in heat-shocked, pIV-synthesizing cells was substantially greater than the rate from either treatment alone (Fig. 5). If cells were heat shocked 45 min after IPTG had been removed (and Psp synthesis had returned to the basal level; see Fig. 3), the stimulation of Psp synthesis was the same as in cells that had not previously been induced for pIV synthesis (Fig. 5).

In *E. coli* as well as other organisms, the heat shock proteins can be induced by stresses other than heat, although heat is most effective (5). Among these other agents, ethanol provokes a response that most closely resembles that of heat shock. The effect of these and other treatments on the

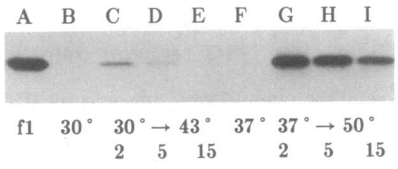

FIG. 4. Induction of Psp synthesis by heat shock. K38 bacteria were labeled with [^{35}S]methionine for 1 min before (lanes B and F) and 2 (lanes C and G), 5 (lanes D and H), and 15 (lanes E and I) min after temperature shift from 30°C to 43°C or from 37°C to 50°C, or 30 min after infection with f1 at 37°C (lane A), as indicated. The labeled samples were immunoprecipitated with Psp antiserum and electrophoresed.

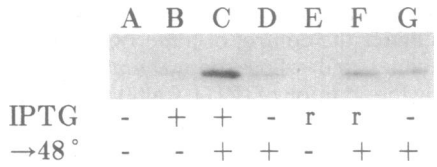

FIG. 5. Induction of Psp synthesis by pIV and heat is additive. IPTG (2 mM) was added to half of a culture of K871/pJLB3 cells growing at 37°C (t = 0 min). A portion of each half (± IPTG) was shifted to 48°C at t = 30 min. At t = 45 min, another portion of each was centrifuged and rinsed with fresh medium to remove IPTG, grown for 45 min at 37°C, then shifted to 48°C. Aliquots were pulse-labeled with [^{35}S]methionine for 1 min as indicated, immunoprecipitated with Psp antiserum, and electrophoresed. Lanes: A, − IPTG; B, + IPTG (t = 30 min); C, + IPTG, after 5 min at 48°C; D, − IPTG, after 5 min at 48°C; E, 45 min after removal of IPTG; F, 45 min after removal of IPTG and 5 min at 48°C; G, 5 min at 48°C (− IPTG). *r*, Removal of IPTG.

synthesis of Psp and of the conventional heat shock proteins is shown in Table 2. Ethanol induced the synthesis of Psp, and as was the case with heat shock, a higher concentration (10%, vol/vol) was more potent than a concentration (4%) that gives strong induction of the classical heat shock proteins (5). Psp synthesis was not induced by treatment with novobiocin, nalidixic acid, or mitomycin C, treatments that did induce the synthesis of conventional heat shock proteins.

Psp synthesis was also induced upon hyperosmotic shock produced by the addition of NaCl or sucrose (Table 2). Induction reached a maximum within 5 min after the shock and gradually declined to the basal level. Heat shock protein synthesis was also detected after osmotic shock. One of the responses to hyperosmotic shock is the intracellular accumulation of potassium, which has been shown to induce expression of several genes involved in restoration of turgor pressure (21). Although the magnitude of Psp induction by hyperosmotic shock varied from strain to strain, neither the magnitude nor the kinetics of induction in a given strain were affected by mutations causing defects in potassium uptake (data not shown). A 10-fold decrease in osmolarity did not induce Psp (Table 2).

Induction of Psp Synthesis Is Independent of the Heat Shock σ Factor, σ^{32}. Psp induction is at the level of transcription, since it is blocked by rifampicin addition (data not shown). For the heat shock regulon, which consists of at least 17 genes (5), induction is dependent upon the heat shock σ factor, σ^{32} (22), encoded by the *rpoH* gene. Unlike these classical heat shock genes, induction of Psp synthesis is independent of σ^{32}

Table 2. Induction of Psp synthesis by various treatments

Treatment	Psp	HSP
Heat shock (30°C → 42°C)	±*	+
Heat shock (37°C → 50°C)	+	+
Ethanol (4%)	±*	+
Ethanol (10%)	+	+
Novobiocin (500 μg/ml)	−	+
Nalidixic acid (40 μg/ml)	−	+
Mitomycin C (0.5, 50 μg/ml)	−	+
Hyperosmotic shock		
0.3 M NaCl (270 → 800 mosmol/kg)	±*	+
0.75 M NaCl (270 → 1600 mosmol/kg)	+	+
0.6 M sucrose (270 → 925 mosmol/kg)	±*	+
Hypoosmotic shock (800 → 80 mosmol/kg)	−	−

Cells were labeled with [^{35}S]methionine before and at several times after the indicated treatment. Total lysates and Psp immunoprecipitates were electrophoresed in SDS/polyacrylamide gels. Stimulation of the rate of synthesis of a 70-kDa and a 63-kDa protein (presumably DnaK and GroEL) was used as a measure of the classical heat shock protein (HSP) response.

*Indicates 0.1–0.2 times the amount of Psp indicated by a +.

(Fig. 6). This was shown by using a mutant strain of *E. coli* that contains an *rpoH* amber mutation and a ts suppressor (14, 16). As expected, the classical heat shock proteins were synthesized in $rpoH^+$ but not in $rpoH^{am}$ cells after temperature shift to stop further σ^{32} synthesis (Fig. 6 *Upper*) compare lanes C and E with lanes D and F) and ethanol addition (compare lanes G, I, and K with lanes H, J, and L). By contrast, Psp synthesis was induced (Fig. 6 *Lower*) in the mutant strain as well as its $rpoH^+$ parent. In fact, the rate of Psp synthesis after ethanol addition was substantially higher in the mutant than in the control cells. An unidentified ≈18-kDa protein was even more strongly induced in ethanol-treated *rpoH* mutant cells (Fig. 6 *Upper*).

Induction of Psp synthesis by pIV was also σ^{32}-independent (data not shown).

Subcellular Location of Psp. By a variation of the Osborn and Munson (23) cell fractionation procedure, Psp was recovered in both the membrane and cytoplasmic fractions but not in the periplasm (Fig. 7). When urea was added to the lysozyme/EDTA-treated cells prior to their lysis and fractionation, Psp was detected only in the cytoplasmic fraction. Upon alkali extraction of whole cells (24, 25) Psp was found in the alkali supernatant, consistent with these fractionation properties. These studies identify Psp as a cytoplasmic protein, about half of which is associated with the cytoplasmic membrane.

Cloning and Mapping the *psp* Gene. A *psp*-containing λ phage was identified by hybridization to a degenerate oligonucleotide (unpublished work). Comparison of a crude restriction map of the insert with the physical map of *E. coli* obtained by Kohara *et al.* (26) suggested that *psp* might be located between 28 and 29 min on the genetic map of *E. coli*, close to *pyrF*. This was confirmed by replacing (27) the chromosomal psp^+ gene by an interrupted, selectable *psp*::*kan* gene constructed in the plasmid-borne gene. Their high cotransduction frequency (40%) indicated that, as expected, *psp*::*kan* and *pyrF* were closely linked. Strains carrying *psp*::*kan* in place of psp^+ in the chromosome did not produce Psp in response to pIV or heat shock (data not shown). Similarly, strain PK2212, which carries an ≈14-kilobase deletion in the 28- to 29-min region of the chromosome (28) to which *psp* maps, could not be induced for Psp synthesis. Both *psp*::*kan* and Δ*psp* strains support plaque formation by f1.

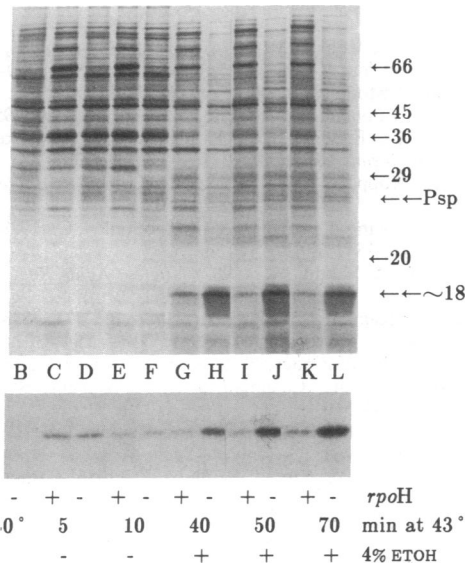

FIG. 6. Psp induction is independent of σ^{32}, the heat shock σ factor. Isogenic $supC^{ts}$ strains containing the wild-type (+, $rpoH^+$) or mutant (−, $rpoH^{am}$) σ^{32} gene were labeled for 1 min with [^{35}S]methionine before and after temperature shift (30°C → 43°C) at the indicated times. Ethanol was added to a final concentration of 4% 10 min after the shift. Samples were electrophoresed directly (*Upper*) or immunoprecipitated with Psp antiserum and electrophoresed (*Lower*).

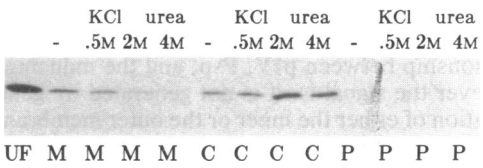

FIG. 7. Localization of Psp in f1-infected cells. K38 cells were infected with f1 at 37°C. At 30 min after infection the cells were labeled with [^{35}S]methionine for 1 min, centrifuged, and suspended in 20% sucrose/50 mM Tris Cl, pH 8.0/5 mM EDTA with lysozyme at 100 μg/ml. The sample was split into equal portions, which were fractionated in the absence (−) or presence of 0.5 M KCl, 2 M urea, or 4 M urea as described (10). Fractions were precipitated with trichloroacetic acid and immunoprecipitated. UF, unfractionated; M, membrane fraction; C, cytoplasmic fraction; P, periplasm.

DISCUSSION

Synthesis of a previously unidentified 25-kDa protein of *E. coli*, Psp (phage shock protein), is induced by filamentous phage infection and by several stresses, including shift to a lethal temperature, treatment with a lethal concentration of ethanol, and extreme osmotic shock. Psp is not GrpE (29), stringent starvation protein (30), or σ24 (31, 32), stress proteins of similar size, based on sequence comparisons and Western immunoblot analysis (J. Kaguni and J.L.B., unpublished work). Psp induction during phage infection is mediated by the phage-encoded protein pIV. It occurs when pIV is produced from a plasmid (pJLB3) at levels that have no detectable effect on cell growth. Thus Psp synthesis in this case is not a response to obvious cell damage. That Psp induction occurs very quickly after pIV synthesis begins suggests that induction by pIV is a specific process.

Induction of Psp appears to require full-length pIV; gene *IV* amber fragments and TrpE–pIV fusion proteins that contain either the N-terminal 28% or the C-terminal 72% of mature pIV did not induce Psp (data not shown). Nonetheless, induction does not require that pIV be capable of supporting phage morphogenesis, since a mutant with an amino acid substitution close to the N terminus that renders it temperature-sensitive for phage production induced Psp synthesis fully at the nonpermissive temperature. Mutant pIV proteins that contain deletions in their signal sequence failed to elicit Psp production. These proteins are restricted to the cytoplasm, suggesting that processing and correct localization of pIV are necessary for induction of Psp. Although produced in modest amounts, these proteins, which associate with the inner membrane, are lethal to the host under conditions where wild-type pIV produced by the parental plasmid is not (data not shown). Membrane perturbation, itself, does not prevent induction of Psp synthesis, since gene *I* and gene *IV* amber mutant infections exhibit similar membrane disturbances (33, 34), and Psp induction does occur in the former.

It is probable that pIV acts indirectly (by affecting the membrane or a membrane constituent) to induce Psp synthesis, not directly as a transcriptional activator, because it is a membrane protein that does not appear to have a cytoplasmic domain (10). It shares many features with outer membrane proteins and is found in both inner and outer membrane fractions (10). We have suggested that it may be part of an exit port (10).

The dependence of Psp synthesis on continued pIV synthesis could signify that one of the transient forms of pIV

(i.e., cytoplasmic pre-pIV or periplasmic pIV) generates the inducing signal. Alternatively, there could be a stoichiometric relationship between pIV, Psp, and the inducing signal.

Whatever the signal is, it is not generated by generalized perturbation of either the inner or the outer membrane. Cells infected by gene *I* or gene *IV* amber mutants accumulate virion structural proteins, suffer membrane hyperplasia, and eventually die (33, 34). Overproduction of a MalE–LacZ fusion protein (35) also causes profound inner membrane disturbances. In each of these instances, heat shock proteins are induced (our results and ref. 36), but only when pIV is produced is Psp synthesis stimulated. Another filamentous phage protein, pIII, affects the outer membrane, making cells deoxycholate-sensitive, colicin-tolerant, and unable to retain proteins in the periplasm (37). pIII does not induce Psp synthesis, nor does pIV cause the pIII-related phenotypes.

Although Psp is induced by heat shock, it is not a conventional heat shock protein. Its full induction requires more extreme treatments than induction of the classical heat shock proteins and is independent of the heat shock σ factor (σ^{32}). Indeed, the Psp response is stronger in σ^{32} mutants. Two other σ^{32}-independent heat-inducible genes have been identified; one is the σ^{32} (*rpoH*) gene itself (38), while the other, *htrA* (39) or *degP* (40), encodes a periplasmic protease (40) that is essential for viability at high temperature (39). The *htrA* and *rpoH* genes are transcribed at 50°C by RNA polymerase containing a 24-kDa σ factor (31, 32). We do not know whether *psp* transcription is also mediated by this σ factor.

Psp is also induced by hyperosmotic shock but not by hypoosmotic shock. Potassium influx, which is a consequence of hyperosmotic shock and a regulator of osmoresponsive genes (21), does not appear to mediate the induction of Psp synthesis in response to osmotic shock. At least some conventional heat shock proteins were induced by hyperosmotic shock, consistent with the observation of Sherman (41).

In no case is the nature of the actual signal(s) that induces expression of heat shock genes understood. Part of the problem in identifying the signal is the diverse nature of the inducing treatments (heat, ethanol, UV light, nalidixic acid, puromycin, osmotic shock, etc.) and their consequences. Heat and ethanol affect protein structure, synthesis, and translocation and cause DNA and membrane damage (5). In contrast to the classical heat shock genes, *psp* expression is induced by a narrower range of treatments. In particular, DNA-damaging agents are ineffective. The ability of a particular protein, pIV, to specifically induce expression of this novel "heat shock" gene may make it possible to identify the actual inducing signal.

The role of the classical heat shock proteins is only just beginning to be understood. We do not understand what role Psp may play. However, it is also induced by heat shock of *E. coli* B and C and of *S. typhimurium*. The increased Psp synthesis in σ^{32} mutants suggests that Psp and the heat shock proteins overlap in either their regulation or function. Recent results (unpublished) with the cloned *psp* gene show that it is part of an operon of five genes; the four additional products were not detected from the chromosomal genes. Synthesis of the operon mRNA is stimulated by all inducing agents. Strains lacking all or part of the *psp* operon are viable under all conditions tested so far. What is perhaps more surprising is that neither *psp* nor the other members of the operon are required for filamentous phage production.

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1. Yager, T. D. & von Hippel, P. H. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 1241–1275.
2. Drahos, D. J. & Hendrix, R. W. (1982) *J. Bacteriol.* **149,** 1050–1063.
3. Kochan, J. & Murialdo, H. (1982) *J. Bacteriol.* **149,** 1166–1170.
4. Bahl, H., Echols, H., Straus, D. B., Court, D., Crowl, R. & Georgopoulos, C. P. (1987) *Genes Dev.* **1,** 57–64.
5. Neidhardt, F. C. & VanBogelen, R. A. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 1334–1345.
6. Yura, T., Tobe, T., Ito, K. & Osawa, T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6803–6807.
7. Ang, D., Chandrasekhar, G. N., Zylicz, M. & Georgopoulos, C. (1986) *J. Bacteriol.* **167,** 25–29.
8. Fayet, O., Ziegelhoffer, T. & Georgopoulos, C. (1989) *J. Bacteriol.* **171,** 1379–1385.
9. Bukau, B. & Walker, G. C. (1989) *J. Bacteriol.* **171,** 2337–2346.
10. Brissette, J. L. & Russel, M. (1990) *J. Mol. Biol.* **211,** 565–580.
11. Pratt, D., Tzagoloff, H. & Erdahl, W. S. (1966) *Virology* **30,** 397–410.
12. Greenstein, D. & Horiuchi, K. (1987) *J. Mol. Biol.* **197,** 157–174.
13. Bradley, D. E. (1980) *Plasmid* **4,** 155–169.
14. Cooper, S. & Ruettinger, T. (1975) *Mol. Gen. Genet.* **139,** 167–176.
15. Yamamori, T. & Yura, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 860–864.
16. Baker, T. A., Grossman, A. D. & Gross, C. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6779–6783.
17. Polarek, J. W., Walderhaug, M. O. & Epstein, W. (1988) *Methods Enzymol.* **157,** 655–667.
18. Vogel, H. J. & Bonner, D. M. (1956) *J. Biol. Chem.* **218,** 97–106.
19. Peeters, B. P. H., Peters, R. M., Schoenmakers, J. G. G. & Konings, R. N. H. (1985) *J. Mol. Biol.* **181,** 27–39.
20. Ravetch, J. V., Ohsumi, M., Model, P., Vovis, G. F., Fischhoff, D. & Zinder, N. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2195–2198.
21. Csonka, L. N. (1989) *Microbiol. Rev.* **53,** 121–147.
22. Grossman, A. D., Erickson, J. W. & Gross, C. A. (1984) *Cell* **38,** 383–390.
23. Osborn, M. J. & Munson, R. (1974) *Methods Enzymol.* **31,** 642–653.
24. Steck, T. L. & Yu, J. (1973) *J. Supramol. Struct.* **1,** 220–248.
25. Russel, M. & Model, P. (1982) *Cell* **28,** 177–184.
26. Kohara, Y., Akiyama, K. & Isono, K. (1987) *Cell* **50,** 495–508.
27. Winans, S. C., Elledge, S. J., Krueger, J. H. & Walker, G. C. (1985) *J. Bacteriol.* **161,** 1219–1221.
28. Hill, T. H., Kopp, B. J. & Kuempel, P. L. (1987) *J. Bacteriol.* **170,** 662–668.
29. Lipinska, B., King, J., Ang, D. & Georgopoulos, C. (1988) *Nucleic Acids Res.* **16,** 7545–7562.
30. Seriwaza, H. & Fukuda, R. (1987) *Nucleic Acids Res.* **15,** 1153–1163.
31. Wang, Q. & Kaguni, J. M. (1989) *J. Bacteriol.* **171,** 4248–4253.
32. Erickson, J. W. & Gross, C. A. (1989) *Genes Dev.* **3,** 1462–1471.
33. Schwartz, F. M. & Zinder, N. D. (1968) *Virology* **34,** 352–355.
34. Woolford, J. L., Jr., Cashman, J. S. & Webster, R. E. (1974) *Virology* **58,** 544–560.
35. Bassford, P. J., Jr., Silhavy, T. J. & Beckwith, J. R. (1979) *J. Bacteriol.* **139,** 19–31.
36. Ito, K., Akiyama, Y., Yura, T. & Shiba, K. (1986) *J. Bacteriol.* **167,** 201–204.
37. Boeke, J. D., Model, P. & Zinder, N. D. (1982) *Mol. Gen. Genet.* **186,** 185–192.
38. Tilly, K., Erickson, J., Sharma, S. & Georgopoulos, C. (1986) *J. Bacteriol.* **168,** 1155–1158.
39. Lipinska, B., Sharma, S. & Georgopoulos, C. (1988) *Nucleic Acids Res.* **16,** 10053–10067.
40. Strauch, K. L. & Beckwith, J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1576–1580.
41. Sherman, M. Y. (1987) *Molekulyarnaya Biologiya* **21,** 189–192.