Identification of a New Member of the Phage Shock Protein Response in Escherichia coli, the Phage Shock Protein G (PspG)*

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The phage shock protein operon (pspABCDE) of Escherichia coli is strongly up-regulated in response to over-expression of the filamentous phage secretin protein IV (pIV) and many other stress conditions including defects in protein export. PspA has an established role in maintenance of the proton-motive force of the cell under stress conditions. Here we present evidence for a new member of the phage shock response in E. coli. Using transcriptional profiling, we show that the synthesis of pIV in E. coli leads to a highly restricted response limited to the up-regulation of the psp operon genes and yjbO. The psp operon and yjbO are also up-regulated in response to pIV in Salmonella enterica serovar Typhimurium. yjbO is a highly conserved gene found exclusively in bacteria that contain a psp operon but is physically unlinked to the psp operon. yjbO encodes a putative inner membrane protein that is co-controlled with the psp operon genes and is predicted to be an effector of the psp response in E. coli. We present evidence that yjbO expression is driven by σ54-RNA polymerase, activated by PspF and integration host factor, and negatively regulated by PspA. PspF specifically regulates only members of the PspF regulon: pspABCDE and yjbO. We found that increased expression of YjbO results in decreased motility of bacteria. Because yjbO is co-conserved and co-regulated with the psp operon and is a member of the phage shock protein F regulon, we propose that yjbO be renamed pspG.

The phage shock protein operon (pspABCDE) was first characterized in Escherichia coli (1) and is highly conserved in many Gram-negative bacteria including several pathogens. There is good evidence that the psp genes are involved in protecting the bacterial cell during infectious processes. For example, pspC mutants of Yersinia enterocolitica are severely attenuated for virulence during infection (2) and exhibit growth defects when the type III secretion system is expressed (3).

Significantly, the psp genes are among the most highly up-regulated genes in Salmonella typhimurium during macrophage infection (4). The psp operon is also up-regulated during swarming in S. typhimurium (5) and during biofilm formation in E. coli (6).

Expression of the psp operon in E. coli is induced by protein IV (pIV),1 a secretin from filamentous phage F1 (1). pIV forms a pore in the bacterial outer membrane that is required for the assembly and export of filamentous phage (7, 8). The pIV protein is the founding member of a large family of bacterial secretins, all of which form large multimeric export channels in the outer membrane. Overexpression of several secretins, often components of the type II and type III bacterial secretion systems, has also been shown to induce expression of the psp operon (e.g. Refs. 7 and 9) establishing that the response is not restricted to a phage protein. Expression of the psp operon can also be induced following overexpression of mutant forms of the outer membrane protein PhoE that are not efficiently secreted (10). PspA synthesis is switched on under conditions that block or reduce the efficiency of the export apparatus, for example, mutants in secA, secD, and secF (10) and deletion of YidC (11, 12). Mutations in components of the twin-arginine translocation pathway also leads to PspA induction under anaerobic conditions (Ref. 13 and see also Ref. 12). Other more general stresses including extreme heat shock (50 °C), hyperosmotic shock, ethanol treatment (10%), and uncouplers of proton-motive force induce psp (reviewed in Ref. 14). The common factor that may link psp-inducing stresses is their effect in dissipating proton-motive force. Indeed, it is significant that PspA, an effector protein of the phage shock response, is known to be involved in maintaining proton-motive force under stress conditions (15). In addition to Psp protein homologues in other Gram-negative bacteria, a PspA homologue (VIPP1) has been found in Synechocystis, which is thought to be important in thylakoid formation, consistent with a role of PspA in sustaining membrane function (16).

Psp proteins mediate regulation of the psp operon (17, 18). Transcription of the psp operon is driven by the σ54-RNA polymerase (σ54-RNAP) (17), which is activated by the enhancer binding protein PspF (19) and facilitated by integration host factor (IHF) (20). The expression of PspF is negatively autogenously controlled (21). PspA negatively regulates psp transcription by binding to the activator protein PspF (22, 23). Conversely, PspB and PspC act as positive regulators of psp operon transcription by overcoming the negative regulation

1 The abbreviations used are: pIV, protein IV; IHF, integration host factor; IPTG, isopropyl 1-thio-β-D-galactopyranoside; RT, reverse transcriptase.
imposed by PspA under specific inducing conditions (e.g. pIV) (17, 24, 25). Phenotypes of cells lacking the psp operon are very subtle and include reduced survival in stationary phase at alkaline pH and changed motility (14).

Here we have used whole genome transcriptional profiling to determine the global effect of pIV synthesis in E. coli. In the highly restricted response we have identified one new gene associated with the psp system, pspG (previously yhB). pspG is physically unlinked with the psp operon but is co-conserved and co-regulated with the psp operon genes by PspA, PspF, IHF, and PspA. Several lines of evidence suggest that PspG is an effector of the phage shock system and not a regulator of psp expression.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The bacterial strains and plasmids used in this study are described in Table I. MG1655ΔpspA, MG1655ΔpspBC, and MG1655ΔpspF were constructed as described in Ref. 12. MVA29 was constructed by transducing ΔpspABC::kn from J134 (17) into MG1655. MVA19 was constructed by transducing ΔpspABC::kn from J134 and pspF::mTn10-tet from K1927 (19) into MG1655. MVA40 was constructed by transducing pspG::kn from JW5716.1 (Km+) into MG1655. MVA42 was constructed by transducing pspG::kn from JW5716.1 (Km+) into MG1655ΔpspA. Strains were grown aerobically with shaking at 37 °C. For microarray analyses, strains were grown to mid-exponential phase in N-C- minimal media were grown aerobically with shaking at 37 °C. For microarray analyses, producing JWK5716_1 (Km::pIV) MG1655. MVA40 was constructed by transducing pspG::kn into MG1655. MVA42 was constructed by transducing pspG::kn into MG1655. MVA19 was constructed by transducing pspG::kn into MG1655. MVA42 was constructed by transducing pspG::kn into MG1655. MG1655 ΔpspA was constructed by transducing ΔpspA::kn from J134 into MG1655. MVA40 was constructed by transducing pspG::kn from JW5716.1 (Km+) into MG1655ΔpspA. Strains were grown aerobically with shaking at 37 °C. For microarray analyses, strains were grown to mid-exponential phase in N-C- minimal media.

| Strain or plasmid       | Genotype or relevant characteristics                                           | Ref. or source          |
|-------------------------|--------------------------------------------------------------------------------|-------------------------|
| BW25113                 | Wild-type                                                                      | CGSC #7739              |
| BW25113 rpsN::kn        |                                                                                 | Gift from Hirotada Mori |
| JWK3169_1 (Km::pIV)     |                                                                                 | Gift from Hirotada Mori |
| JWK5716.1 (Km::pIV)     |                                                                                 | Gift from Hirotada Mori |
| MC1061                  | lac                                                                             | A gift from Michael Chandler |
| MC1068                  | MC1061 lamA::Tn10 (te')                                                       | CGSC No. 7740 (28)      |
| MG1655                 | Wild-type                                                                        | This work               |
| MG1655ΔpspA            | MG1655 ΔpspA                                                                    | This work               |
| MG1655ΔpspBC           | MG1655 ΔpspBC                                                                  | This work               |
| MVA29                  | MG1655ΔpspABC::kn                                                              | This work               |
| MVA19                  | MG1655ΔpspABC::kn pspF::mTn10-tet (pspFΔHTH)                                    | This work               |
| MVA40                  | MG1655 ΔpspG::kn                                                              | This work               |
| MVA42                  | MG1655 ΔpspA pspG::kn                                                         | This work               |
| S. typhimurium LT2      | Wild-type                                                                       | 29                      |

Plasmids

| Plasmid               | Description                                                                 | Ref. or source          |
|-----------------------|-----------------------------------------------------------------------------|-------------------------|
| pGZ119EH              | IPTG-inducible tac promoter expression vector. cm'                           | Gift from Marjorie Russel (30) |
| pPMR129               | pGZ119EH harbouring pyV. cm'                                                 | Gift from Marjorie Russel (31) |
| pMR25                 | lacZ transcriptional fusion vector. tet'                                     | 12                      |
| pS1J                  | pMR25 with pspA promoter region plus the first 21 amino acids of PspA cloned into the mcs (EcoRI-EcoRI), tet' | 12                      |
| pMC1403               | lacZ translational fusion vector. ap'                                        | 32                      |
| pL1L                  | The pspG promoter region plus the first 6 amino acids of PspG cloned in-frame into the mcs of pMC1403 (EcoRI-BamHI), ap' | This work               |
| pL2L                  | The pspG promoter region plus the first 6 amino acids of PspG cloned out of frame into the mcs of pMC1403 (EcoRI-BamHI), ap' | This work               |
| pSLE1                 | pspA promoter region (EcoRI-BamHI) cloned into the vector pTE103 (56) ap'   | 23                      |
| pJH2                  | pspG promoter region (EcoRI-BamHI) subcloned from pL1L into pTE103 ap'       | This work               |
| pBAD18-cm             | Vector cm'                                                                    | A gift from Jonathan Beckwith |
| pLL8                  | pspG (XbaI-HindIII) cloned into the mcs of pBAD18-cm. cm'                    | This work               |

| Strain or plasmid       | Genotype or relevant characteristics                                           | Ref. or source          |
|-------------------------|--------------------------------------------------------------------------------|-------------------------|
| E. coli                 | Wild-type                                                                      | CGSC #7739              |
| MG1655ΔpspA            | MG1655 ΔpspA                                                                    | This work               |
| MG1655ΔpspBC           | MG1655 ΔpspBC                                                                  | This work               |
| MG1655ΔpspF            | MG1655 ΔpspF                                                                    | This work               |
| MVA29                  | MG1655ΔpspABC::kn                                                              | This work               |
| MVA19                  | MG1655ΔpspABC::kn pspF::mTn10-tet (pspFΔHTH)                                    | This work               |
| MVA40                  | MG1655 ΔpspG::kn                                                              | This work               |
| MVA42                  | MG1655 ΔpspA pspG::kn                                                         | This work               |

microarray experiment, RNA was fluorescently labeled during reverse transcription and cDNA was hybridized to E. coli PCR product microarrays according to S. Kustu and co-workers (37). Hybridization, scanning, and normalization were carried out as described (38) and genome images were prepared (37). Experiments were performed in duplicate with a dye swap. The microarray data for the pIV experiments were generated with fluorescently labeled genomic DNA as a reference channel in each experiment using E. coli and S. typhimurium PCR product microarrays printed at IFR (39–41). Experiments were performed in duplicate, consisting of two biological replicates and two technical replicates. Microarray slides were scanned with a Genepix 4000B scanner on an Axon Instruments. Fluorescent spot and local background intensities were quantified using Genepix Pro software. For labeling, hybridization, and data analysis protocols and details of statistical filtering procedures, see the online site (ifr.bbsrc.ac.uk/Safety/Microarrays/#Protocols). Further statistical analysis was carried out using Cyber-T (visitor.ics.uci.edu/genex/cybert). RT-PCR—Qiagen® One-step RT-PCR kit was used according to the manufacturer’s instructions to amplify pspA (20 cycles) and pspG (35 cycles) from RNA samples. For amplifying pspA the primers RT-PspA(a) (5’-CTCGGATTGCGGATCAGTGTAAT-3') and RT-PspA(b) (5’-TGCCGATTGCTGCTGATCGTAT-3') were used. For amplifying pspG the primers RT-PspG(a) (5’-GCGGAACACTTTTGTGTGATTG-3') and RT-PspG(b) (5’-CGCCACGCGTCAATACGCTGATATT-3') were used. Western Blotting—Western blotting was carried out as described (23) using primary antibodies to PspA (12) and pIV (a gift from Marjorie Russell). pIV antibodies were used at a 1:10,000 dilution with donkey anti-rabbit secondary antibodies (Amersham Biosciences). β-Galactosidase Assays—β-Galactosidase assays were carried out as described (35). Bioinformatics Methods—Fuzzpro (EMBOSS programs) was used to search for consensus sequences in regions of DNA by allowing small mismatches to be introduced to the database. DNaSE I Footprinting Assays—DNase I footprinting reactions (10 μl) were carried out at 37 °C in STA buffer (25 mM Tris acetate, pH 8.0, 8 mM magnesium acetate, 10 mM KCl, 1 mM dithiothreitol, 3.5% (w/v) polyethylene glycol 8000) essentially as described (42). Briefly, 0–400
ne E. coli (reconstituted in situ with a 1:2 molar ratio of E and D) or 0–1 μM E. coli PspF was incubated with 15 nm pLL1 for 10 min and treated with 1.75 × 10^{-3} units of DNase I (Amersham Biosciences) for 2 min. The DNase I reaction was quenched by the addition of DNase I stop buffer (400 mM NaCl, 30 mM EDTA, 1% SDS) and the DNA was purified using QIAquick spin columns (Qiagen) according to the manufacturer’s instructions.

In Vitro Transcription Assay—In vitro transcription reactions (10 μl) were carried out as described (43) with a 1:5 ratio RNAP to E. coli lac

Motility Assay—Motility assays were carried out using motility agar (1% tryptone, 0.5% NaCl and 0.3% agar) plus the appropriate antibiotic. 2 μl of a fully grown LB overnight culture was pipetted into the motility agar, plates were incubated at 37 °C for 6 h, and zones of motility were measured in millimeters.

RESULTS

pIV Secretin Stress Results in the Up-regulation of pspAB-CDE and pspP—To examine the transcriptional response to pIV-induced stress in E. coli wild type MG1655 cells containing the plasmids, pPMR129 (pIV) or pGZ119EH (vector control) were grown to mid-log phase, expression from the plasmids was induced with IPTG for 1 h, and cells were harvested for RNA extraction. The synthesis of pIV reached high levels after 1 h (see Supplementary Materials and Fig. 1) indicating that it elicits a full cellular response but did not lead to reduced growth rates, or reduced yields of cells, indicating a lack of toxicity. Microarray analyses showed increased levels of psp operon transcripts in pIV-expressing cells compared with the vector control (Fig. 1A). We correlated activation of the pspA promoter with increased levels of the PspA protein using Western blotting (data not shown). Other than the psp operon genes, only a single gene, yjbO, showed a significant and sizeable up-regulation in response to pIV secretin stress (Fig. 1A; see Supplementary Materials Tables I and III). This data indicates that large transcriptional responses of E. coli to pIV are very rare and identify a new gene involved in the phage shock response, yjbO. We propose to rename this gene pspG.

To confirm that transcript levels of pspG are increased in wild type MG1655 cells expressing pIV, RT-PCR was carried out on the RNA samples used for the microarray experiments. RT-PCR clearly demonstrates that pspG transcription is up-regulated, along with pspA transcription, in pIV-expressing MG1655 cells compared with the vector control (Fig. 2A). β-Galactosidase assays using a translational reporter for PspG (pLL1) confirm that PspG is produced in response to pIV in MC1061 cells (Fig. 2B).

To determine whether the response to pIV detected in E. coli is conserved in other bacteria that contain the psp operon, a pIV expression experiment was carried out in Salmonella enterica serovar Typhimurium LT2. There is significant up-regulation of psp operon and pspG transcripts in the pIV-expressing S. typhimurium cells (Fig. 1B; see Supplementary Materials Tables I and III). The transcriptional response to pIV in S. typhimurium resembles that of E. coli in that the response to pIV secretin stress is highly restricted. Our comparative transcriptomic analysis of responses of E. coli and S. typhimurium to pIV shows that the common core of up-regulated genes are pspABCDE and pspG. Transcription of pspF does not show any change in response to pIV expression, consistent with control of PspF being exclusively at the level of activity (21).

A limited and specific response to pIV stress resembles that of E. coli lac operon. We performed a microarray experiment to show that IPTG only causes significant increased expression of lac operon genes in MG1655, no other transcriptome changes occur (see Supplementary Materials Table II). As with lac promoter activity induced by IPTG, the effect of pIV inducing stimulus under our growth conditions in E. coli appears to be close to gratuitous (44).

**pspG Transcription Is Regulated by psp-encoded Proteins—**To examine the effect of overexpression of the psp genes on the transcriptome, transcripts from cells lacking the negative regulator PspA (MG1655ΔpspA) were compared with transcripts from cells lacking the positive regulator PspF (MG1655ΔpspF). In MG1655ΔpspA, the psp operon is expressed at high levels because the negative regulator of its transcription has been removed. Conversely, in MG1655ΔpspF, expression of the psp operon is completely absent because the activator protein required for dsDNA-RNAP-driven transcription has been removed. Levels of psp expression in the MG1655ΔpspA strain are therefore close to levels in wild type cells expressing pIV, but without the production of PspA. It is clear from Fig. 3 that pspBCDE is transcribed at high levels in MG1655ΔpspA compared with MG1655ΔpspF. As with the re-
were carried out on MC1061 cells to detect transcription of regulated. The level of expression from the clear exception of the gene response to overexpression of wild type MG1655 cells to pIV stress, there is very amplified from total RNA samples from MG1655 expressing pPMR129 for 1 h; lane 3, PspG detected in MG1655 expressing pGZ119EH for 1 h; lane 4, PspG detected in MG1655 expressing pPMR129 for 1 h. PsPG is a small (−9 kDa) highly hydrophobic protein that is predicted to be an inner membrane protein (enzim.hu/hmmtop/http://www.cbs.dtu.dk/services/TMHMM/).

Because our experiments indicated that psPG transcription is regulated by the same elements that regulate psP operon transcription, we used a bioinformatic approach to search the psPG promoter region for the control elements that are present in the psP operon promoter, which are binding sites for $\sigma^{34}$, PsF, and IHF. Using the program fuzzpro (EMBOSS programs) and the consensus sequence WWWTCAA[N4]TTR for IHF binding (45) and sequences GCCACGCAATTGT for $\sigma^{34}$ binding and TAGTGTAAATGCCTAACT for PsP binding (based on the $\sigma^{34}$ and PsP binding sites in the psPG promoter) (20, 46) we found potential binding sites for $\sigma^{34}$, IHF, and PsP (Fig. 4).

Using the translational fusion for psPG (pLL1) we found that $\sigma^{34}$-RNAP and activation by PsP are required in vivo for pIV-induced PsPG expression. In the wild type strains the basal level of PsPG expression is extremely low, but is up-regulated upon induction with pIV. In mutant strains for $\sigma^{34}$ and PsP, psPG expression is abolished both before and after induction (data not shown). DNase I protection assays using purified components confirmed that the psPG upstream DNA region was bound by PsP (Fig. 5A, lane 3) and by the $\sigma^{34}$-RNAP (Fig. 5B, lane 3) at the promoter sequence predicted by bioinformatics. In vitro transcription assays established that a transcript originated from the predicted psPG promoter region, dependent upon $\sigma^{34}$, PsP, and ATP (Fig. 6A). Transcripts from the psPG promoter increased with increasing concentrations of PsP (Fig. 6B) and it appears that in the absence of IHF the psPG promoter is much more sensitive to PsP concentration than the psP promoter. Addition of IHF to the in vitro transcription assay increases the level of transcripts from the psPG promoter indicating that the predicted IHF binding site in the psPG promoter region is functional (Fig. 6C) and that the binding of IHF to the psPG promoter facilitates psPG transcription. IHF enhances psP operon transcription (20, 46) and facilitates binding of PsP to its upstream activation sequences in the psP operon regulatory region, autogenously down-regulating psPG transcription (20, 21). Consistent with this data and the increased sensitivity of the psPG promoter to PsP in comparison to the psP promoter (Fig. 6B), PsPG expression in an IHF mutant (MC1068) is increased both before and after induction by pIV in vivo (data not shown). Combined, these results provide strong evidence that psPG is tightly co-regulated with the psP operon and is a member of the PsP-dependent regulon.

PsPG Is Not a Regulator of the PsP Regulon—To test whether PsPA can be induced by pIV, extreme heat shock, or ethanol shock in cells lacking PsPG function, we carried out $\beta$-galactosidase assays using the transcriptional reporter for PsPA (pS71) in JW55716 (pPSJ1) and translation of psPG (pLL1). Lane 1, psPA detected in MG1655 expressing pGZ119EH (vector) for 1 h; lane 2, psPA detected in MG1655 expressing pPMR129 (pIV) for 1 h; lane 3, psPG detected in MG1655 expressing pGZ119EH for 1 h; lane 4, PsPG detected in MG1655 expressing pPMR129 for 1 h. PsPA and PsPG are co-conserved and co-regulated—psPG and psPFpsPABCDE are not physically linked on the chromosome, but psPG is highly conserved among bacteria in which the psP operon is conserved. Furthermore, all bacteria containing a recognizable psP operon carry a psP homologue, and psPG homologues are not present in bacteria lacking a psP operon. This shows that the psPG and psP loci are co-conserved.
units). Therefore we conclude that PspG is not involved in controlling the PspF regulon.

PspG Is an Effector of the PspF Regulon—Considering the putative membrane location of PspG, and that PspG is not involved in psp regulation per se, it is likely that this protein is an effector of the psp system. It has been shown that the psp operon is up-regulated during swarming in Salmonella (5) and psp mutants have altered motility (14) therefore we employed a motility assay to compare wild type cells to strains mutant for various psp genes to explore a possible effector function of PspG (Fig. 7). Cells lacking the negative regulator (ΔpspA and Δpsp-ABC) (therefore with increased PspG expression) show decreased motility. Note that in the presence of PspBC, motility is less decreased. As a control, strains deleted for pspBC show no change in motility. Double mutants for the activator PspF and the negative regulator PspA (therefore no PspG expression)

![Image](image1.png)

**Fig. 3.** pIV secretin stress results in a highly restricted transcriptional response. *E. coli* microarrays (from the laboratory of S. Kustu) were probed with mixtures of cDNAs from MG1655ΔpspA and MG1655ΔpspF grown on N-C- media supplemented with 0.4% glucose and 10 mM NH₄Cl to mid-log phase. Spots from fluorescence scanning of the microarrays were rearranged in genome order. The b numbers are indicated. Spots are arranged in doubles as a dye-swap experiment was carried out. Those b numbers with a red spot in the top row of the doublet and a green spot in the bottom row of the doublet are up-regulated in MG1655ΔpspA compared with MG1655ΔpspF and vice versa. For highly expressed genes, spots appear intense yellow because of image saturation. pspB, C, D, E (b1305–1308), and pspG (b4050) (highlighted) are clearly up-regulated in MG1655ΔpspA compared with MG1655ΔpspF. There is little change in gene expression across the rest of the genome.

**Fig. 4.** The pspG promoter region contains regions predicted to bind PspF, IHF, and σ²⁴. A, the pspA promoter region of *E. coli* (20, 57). B, the pspG promoter region of *E. coli*. Sites for PspF, IHF, and σ²⁴ in the pspG promoter region were predicted by bioinformatics (fuzzpro, EMBOS programs). Consensus sequence for IHF binding is WWWCTCA(N4)TTR (45) and the sequences for σ²⁴ (GGCACGCAAAT-TGT) and PspF (TAGTGTAATTCGCTAACT) binding are present in the pspA promoter.

**Fig. 5.** PspF and σ²⁴-RNA polymerase bind the pspG promoter region. A, PspF (used at 400 nM) footprint on the pspG promoter between positions −76 and −109 is shown in lane 3. Control reactions that do not contain PspF are shown in lanes 1 and 2. The reaction in lane 4 was conducted with 1000 nM PspF1–275 (PspF lacking the DNA binding domain). B, Esψ²⁴ (used at 200 nM) footprint on the pspG promoter between positions −8 and −30 is shown in lane 3. Control reactions that do not contain Esψ²⁴ are shown in lanes 1 and 2. In A and B the lanes marked A, C, G, and T contain chain terminating DNA sequencing reactions conducted with pLL1 and the chain terminating dATP, dCTP, dGTP, and dTTP, respectively. DNase I-treated (+) and -untreated (−) reactions are marked at the bottom. The DNA sequence shown on the side was predicted using bioinformatics (fuzzpro, EMBOS programs) to bind to PspF (A) and Esψ²⁴ (B). In B, the consensus promoter −12 and −24 regions of σ²⁴-dependent promoters are shown.
show slightly increased motility. Cells deleted for pspG or for
both pspA and pspG also show slightly increased motility.
Strains mutant for the activator PspF (no PspG and no Psp
operon expression) show unchanged motility. When PspG is
expressed from pLL8, motility in both wild type and ∆pspF
cells is greatly decreased (Fig. 7). Induction of PspG expression
by 0.4% arabinose decreased motility to a higher extent com-
pared with non-inducing conditions (data not shown). PspG
expression is also subject to negative regulation imposed by PspA. This is in agreement with the results of Green and Darwin (52) in Y. enterocolitica. Here we have shown that the pspG promoter is more sensitive to PspF activation than the pspA promoter implying that under stress conditions and release of PspA negative regulation, pspG responds rapidly. The transcription of pspG in vitro is
enhanced by IHF in a concentration-dependent manner. How-
ever, in vivo the basal level of pspG expression in IHF mutants
is increased. IHF works to enhance transcriptional activation
of the psp operon (20, 46) and facilitates the binding of PspF to
upstream activation sequences I and II in the psp operon regu-
laratory region. Thus IHF enhances both the activation of psp
transcription and the negative autogenous control of PspF
keeping the PspF concentration at a low level (20, 21). Strains
lacking functional IHF will therefore under normal growth
conditions have increased concentrations of the activator PspF
and decreased concentrations of the negative regulator PspA.
Hence, the increased level of pspG expression in IHF mutants
should be because of the high sensitivity of the pspG promoter
and diminished negative regulation by PspA. To summarize, our data show that pspG is a member of the PspF regulon in E. coli and Salmonella and is tightly regulated in concert with the psp operon. Because the expression of the activator PspF is constant under all growth
conditions, the key regulatory point under normal growth conditions is strong negative regulation imposed by PspA, whereas under inducing conditions this regulation is lifted leading to the co-
ordinated expression of the psp operon and pspG. In fact, PspF
specifically regulates only pspABCDEFG and pspG. pspG is phys-
ically separated from the psp operon on the chromosome, but is
conserved in all bacteria harboring the psp operon and there
are no obvious PspG homologues in bacteria that lack a recogni-
able psp operon. Therefore, the co-regulated expression of the psp operon and pspG by PspF and PspA is likely to be a
widely conserved and important feature of cellular adaptation
to secretin-induced stress. It is striking that the psp operon and
the pspG σ54 promoters, which are physically unlinked, are
both regulated by PspF, PspA, and IHF in exactly the same
fashion. Darwin and Miller (3) suggest that in Y. enterocolitica,
another genetic locus is involved in the psp response to secretin
stress because a double pspF/psp operon mutant showed a
more severe growth defect than the psp operon mutant alone.
Further evidence to support this has been reported (52). Our

DISCUSSION

Our data show that expression of pIV in both E. coli and
S. typhimurium results in the significant up-regulation of pspABCD
and pspG (yjbO) transcripts. Previously it has been shown that
the pspABCDEFG operon and pspG are among the 25
most highly up-regulated genes in S. typhimurium infecting
macrophages (4). Similarly high levels of expression of the psp
operon and pspG are also seen in S. typhimurium infecting
epithelial cells and in Shigella flexneri infecting macrophages
and epithelial cells. Therefore, the psp response linked with
pspG is observed in a range of enteric bacteria, with potentially
important roles for these genes in bacterial virulence. In con-
trast to the response of E. coli to stresses such as nitrogen
limitation (37) and specialized growth conditions (47–49),
which cause substantial changes on a transcriptional level,
the synthesis of pIV causes a very restricted change in gene tran-
scription. Restricted responses in microarray experiments have
been reported previously, for example, the limited transcriptional response to cell division inhibitors (50). It is possible that a
range of pIV-dependent changes in the cell do occur, but only
at a transalational or post-transalational level. Identifying gene
regulators through expression profiling may well prove to be a
generally challenging problem (51).

As it has been shown for the pspA promoter, both the tran-
scriptional activator, PspF, and the σ54-RNAP physically inter-
act in vitro with the pspG promoter and activation is dependent
on PspF and σ54-RNAP in vivo. pspG expression is also subject
to negative regulation imposed by PspA. This is in agreement
with the results of Green and Darwin (52) in Y. enterocolitica. Here we have shown that the pspG promoter is more sensitive
to PspF activation than the pspA promoter implying that under
stress conditions and release of PspA negative regulation, pspG
responds rapidly. The transcription of pspG in vitro is
enhanced by IHF in a concentration-dependent manner. How-
ever, in vivo the basal level of pspG expression in IHF mutants
is increased. IHF works to enhance transcriptional activation
of the psp operon (20, 46) and facilitates the binding of PspF to
upstream activation sequences I and II in the psp operon regu-
laratory region. Thus IHF enhances both the activation of psp
transcription and the negative autogenous control of PspF
keeping the PspF concentration at a low level (20, 21). Strains
lacking functional IHF will therefore under normal growth
conditions have increased concentrations of the activator PspF
and decreased concentrations of the negative regulator PspA.
Hence, the increased level of pspG expression in IHF mutants
should be because of the high sensitivity of the pspG promoter
and diminished negative regulation by PspA. To summarize, our data show that pspG is a member of the PspF regulon in E. coli and Salmonella and is tightly
regulated in concert with the psp operon. Because the expres-
sion of the activator PspF is constant under all growth
conditions, the key regulatory point under normal growth conditions is strong negative regulation imposed by PspA, whereas under inducing conditions this regulation is lifted leading to the co-
ordinated expression of the psp operon and pspG. In fact, PspF
specifically regulates only pspABCDEFG and pspG. pspG is phys-
ically separated from the psp operon on the chromosome, but is
conserved in all bacteria harboring the psp operon and there
are no obvious PspG homologues in bacteria that lack a recogni-
able psp operon. Therefore, the co-regulated expression of the psp operon and pspG by PspF and PspA is likely to be a
widely conserved and important feature of cellular adaptation
to secretin-induced stress. It is striking that the psp operon and
the pspG σ54 promoters, which are physically unlinked, are
both regulated by PspF, PspA, and IHF in exactly the same
fashion. Darwin and Miller (3) suggest that in Y. enterocolitica,
another genetic locus is involved in the psp response to secretin
stress because a double pspF/psp operon mutant showed a
more severe growth defect than the psp operon mutant alone.
Further evidence to support this has been reported (52). Our

2 L. J. Lloyd, G. Jovanovic, and M. Buck, unpublished data.

3 J. Hinton, unpublished results.
Salmonella slightly increased motility. It has been shown that swarming increased motility, whereas the lack of PspG expression causes a decrease in motility. In this study we demonstrate that increased expression of PspG results in decreased motility, judged by growth rates and yields. In this study we raise issues about previously described phenotypes attributed to the PspF regulon, and show that PspG, according to our results, is constantly in an effector state. Because PspA and the previously unknown PspF are so tightly co-regulated, our results suggest that PspG could be that PspA requires induction to switch between being a negative regulator to being an effector, whereas PspG, according to our results, is constantly in an effector state. Because PspA and the previously unknown PspF regulon member, PspA, are so tightly co-regulated, our results raise issues about previously described phenotypes attributed to PspA and PspG. The major difference between PspA and PspG could be that PspA requires induction to switch between being a negative regulator to being an effector, whereas PspG, according to our results, is constantly in an effector state.

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REFERENCES

1. Brisette, J. L., Russel, M., Weiner, L., and Model, P. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 862–866.
2. Darwin, A. J., and Miller, V. L. (1999) Mol. Microbiol. 32, 51–62.
3. Darwin, A. J., and Miller, V. L. (2001) Mol. Microbiol. 39, 429–444.
4. Eriksson, S., Luchini, S., Thompson, A., Rhen, M., and Hinton, J. C. (2003) Mol. Microbiol. 47, 103–118.
5. Wang, Q., Frye, J. G., McClellan, M., and Harshey, R. M. (2004) Mol. Microbiol. 52, 169–187.
6. Beloin, C., Vallee, J., Latour-Lambert, P., Faure, P., Krzeminiski, M., Ballestroso, D., Haagensen, J. A., Molin, S., Prensier, G., Arbeille, B., and Ghiyo, J. M. (2004) Mol. Microbiol. 51, 659–674.
7. Russel, M., and Kazmierczak, B. (1993) J. Bacteriol. 175, 3998–4007.
8. Opalka, N., Beckmann, R., Boisset, N., Simon, M. N., Russel, M., and Darst, S. A. (2003) J. Mol. Biol. 325, 461–470.
9. Posset, O., d’Enfer, C., Reys, I., and Pugsley, A. P. (1992) Mol. Microbiol. 6, 95–105.
10. Kleerebezem, M., and Tommassen, J. (1993) Mol. Microbiol. 7, 947–956.
11. van der Laan, M., Urbanus, M. L., Ten Hagen-Jongman, C. M., Nouwen, N., Oudega, B., Harms, N., Driessen, A. J., and Luitink, J. (2000) Proc. Natl. Acad. Sci. U. S. A. 100, 5801–5806.
12. Jones, S. E., Lloyd, L. J., Tan, K. K., and Buck, M. (2003) J. Bacteriol. 185, 6707–6711.
13. DeLisi, M. P., Lee, P., Palmer, T., and Georgiou, G. (2004) J. Bacteriol. 186, 366–373.
14. Model, P., Jovanovic, G., and Dworkin, J. (1997) Mol. Microbiol. 24, 255–261.
15. Kleerebezem, M., Crielaard, W., and Tommassen, J. (1996) EMBO J. 15, 162–171.
16. Westphal, S., Heins, L., Soll, J., and Vothknecht, U. C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4243–4248.
17. Weiner, L., Brissette, J. L., and Model, P. (1991) Genes Dev. 5, 1912–1923.
18. Adams, H., Teerstra, W., Koster, M., and Tommassen, J. (2002) FEBS Lett. 518, 173–176.
19. Jovanovic, G., Weiner, L., and Model, P. (1996) J. Bacteriol. 178, 1936–1945.
20. Jovanovic, G., and Model, P. (1997) Mol. Microbiol. 23, 473–481.
21. Jovanovic, G., Dworkin, J., and Model, P. (1997) J. Bacteriol. 179, 5232–5237.
22. Dworkin, J., Jovanovic, G., and Model, P. (2000) J. Bacteriol. 182, 311–319.
23. Elderkin, S., Jones, S., Schumacher, J., Studdholme, D., and Buck, M. (2002) J. Mol. Biol. 320, 33–37.
24. Brisette, J. L., Weiner, L., Ripmaster, T. L., and Model, P. (1991) J. Mol. Biol. 220, 35–48.
25. Weiner, L., Brissette, J. L., Ramani, N., and Model, P. (1995) Nucleic Acids Res. 23, 2030–2036.
26. Datsenko, K. A., and Wanner, B. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6640–6645.
27. Casadaban, M. J., and Cohen, S. N. (1980) J. Mol. Biol. 138, 179–207.
28. Blattner, F. R., Plunkett, G., 3rd, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rodie, C. K., Mayhew, G. F., Gregor, J., Davies, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mao, B., and Shao, Y. (1997) Science 277, 1453–1474.
29. McClelland, M., Sanderson, K. E., Spieth, J., Clifton, S. W., Latreille, P., Courtney, L., Purwolik, S., Ali, J., Dante, M., Du, F., Hsu, S., Layman, D., Leonard, S., Nguyen, C., Scott, K., Holmes, A., Grewal, N., Mulvany, E., Ryan, E., Sun, H., Florea, L., Miller, W., Stoneking, T., Nhan, M., Waters, R., and Wilson, R. K. (2001) Nature 413, 852–856.
30. Lessl, M., Balzer, D., Lurz, R., Waters, V. L., Guiney, D. G., and Langa, E. (1992) J. Bacteriol. 174, 2495–2500.
31. Daefler, S., Russel, M., and Model, P. (1997) J. Mol. Biol. 266, 978–992.
32. Casadaban, M. J., Chou, J., and Cohen, S. N. (1980) J. Bacteriol. 143, 971–980
33. Kustu, S., Burton, D., Garcia, E., McCarter, L., and McFarland, N. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4576–4580
34. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, 2 Ed., Cold Spring Harbor Press Laboratory Press, Cold Spring Harbor, NY
35. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
36. Lin-Chao, S., and Cohen, S. N. (1991) Cell 65, 1233–1242
37. Zimmer, D. P., Soupene, E., Lee, H. L., Wendisch, V. F., Khodursky, A. B., Peter, B. J., Bender, R. A., and Kustu, S. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14674–14679
38. Khodursky, A. B., Peter, B. J., Cozzarelli, N. R., Botstein, D., Brown, P. O., and Yanofsky, C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12170–12175
39. Anjum, M. F., Lucchini, S., Thompson, A., Hinton, J. C., and Woodward, M. J. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8784–8789
40. Thompson, A., Lucchini, S., and Hinton, J. C. (2001) Trends Microbiol. 9, 154–156
41. Burrows, P. C., Severinov, K., Ishihama, A., Buck, M., and Wigneshweraraj, S. R. (2003) J. Biol. Chem. 278, 29728–29743
42. Wigneshweraraj, S. R., Nechaev, S., Bordes, P., Jones, S., Cannon, W., Severinov, K., and Buck, M. (2003) Methods Enzymol. 370, 646–657
43. Monod, J., and Cohn, M. (1952) Adv. Enzymol. Relat. Sub. Biochem. 13, 67–119
44. Hales, L. M., Gumpert, R. I., and Gardner, J. F. (1994) J. Bacteriol. 176, 2999–3006
45. Dworkin, J., Jovanovic, G., and Model, P. (1997) J. Mol. Biol. 273, 377–388
46. Oh, M. K., Rohlin, L., Kao, K. C., and Liao, J. C. (2002) J. Biol. Chem. 277, 13175–13183
47. Salmon, K., Hung, S. P., Mekjian, K., Baldi, P., Hatfield, G. W., and Gunsalus, R. P. (2003) J. Biol. Chem. 278, 29837–29855
48. Kao, K. C., Yang, Y. L., Boscolo, R., Sabatti, C., Roychowdhury, V., and Liao, J. C. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 641–646
50. Arends, S. J., and Weiss, D. S. (2004) J. Bacteriol. 186, 880–884
51. Martinez-Antonio, A., and Collado-Vides, J. (2003) Curr. Opin. Microbiol. 6, 482–489
52. Green, R. C., and Darwin, A. J. (2004) J. Bacteriol. 186, 4910–4920
53. Berg, H. C. (2003) Annu. Rev. Microbiol. 57, 95–135
54. Fung, D. C., and Berg, H. C. (1995) Nature 375, 809–812
55. Gabel, C. V., and Berg, H. C. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 8748–8751
56. Elliott, T., and Geiduschek, E. P. (1984) Cell 36, 211–219

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32. Casadaban, M. J., Chou, J., and Cohen, S. N. (1980) J. Bacteriol. 143, 971–980
33. Kustu, S., Burton, D., Garcia, E., McCarter, L., and McFarland, N. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4576–4580
34. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, 2 Ed., Cold Spring Harbor Press Laboratory Press, Cold Spring Harbor, NY
35. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
36. Lin-Chao, S., and Cohen, S. N. (1991) Cell 65, 1233–1242
37. Zimmer, D. P., Soupene, E., Lee, H. L., Wendisch, V. F., Khodursky, A. B., Peter, B. J., Bender, R. A., and Kustu, S. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14674–14679
38. Khodursky, A. B., Peter, B. J., Cozzarelli, N. R., Botstein, D., Brown, P. O., and Yanofsky, C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12170–12175
39. Anjum, M. F., Lucchini, S., Thompson, A., Hinton, J. C., and Woodward, M. J. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8784–8789
40. Thompson, A., Lucchini, S., and Hinton, J. C. (2001) Trends Microbiol. 9, 154–156
41. Burrows, P. C., Severinov, K., Ishihama, A., Buck, M., and Wigneshweraraj, S. R. (2003) J. Biol. Chem. 278, 29728–29743
42. Wigneshweraraj, S. R., Nechaev, S., Bordes, P., Jones, S., Cannon, W., Severinov, K., and Buck, M. (2003) Methods Enzymol. 370, 646–657
43. Monod, J., and Cohn, M. (1952) Adv. Enzymol. Relat. Sub. Biochem. 13, 67–119
44. Hales, L. M., Gumpert, R. I., and Gardner, J. F. (1994) J. Bacteriol. 176, 2999–3006
45. Dworkin, J., Jovanovic, G., and Model, P. (1997) J. Mol. Biol. 273, 377–388
46. Oh, M. K., Rohlin, L., Kao, K. C., and Liao, J. C. (2002) J. Biol. Chem. 277, 13175–13183
47. Salmon, K., Hung, S. P., Mekjian, K., Baldi, P., Hatfield, G. W., and Gunsalus, R. P. (2003) J. Biol. Chem. 278, 29837–29855
48. Kao, K. C., Yang, Y. L., Boscolo, R., Sabatti, C., Roychowdhury, V., and Liao, J. C. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 641–646
50. Arends, S. J., and Weiss, D. S. (2004) J. Bacteriol. 186, 880–884
51. Martinez-Antonio, A., and Collado-Vides, J. (2003) Curr. Opin. Microbiol. 6, 482–489
52. Green, R. C., and Darwin, A. J. (2004) J. Bacteriol. 186, 4910–4920
53. Berg, H. C. (2003) Annu. Rev. Biochem. 72, 19–54
54. Fung, D. C., and Berg, H. C. (1995) Nature 375, 809–812
55. Gabel, C. V., and Berg, H. C. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 8748–8751
56. Elliott, T., and Geiduschek, E. P. (1984) Cell 36, 211–219