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Autogenous Control of PspF, a Constitutively Active Enhancer-Binding Protein of *Escherichia coli*

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***Escherichia coli* σ^{54}-dependent phage shock protein operon (*pspA* to *-E*) transcription is under the control of PspF, a constitutively active activator. σ^{70}-dependent transcription of *pspF* is under autogenous control by wild-type PspF but not by a DNA-binding mutant, PspFΔHTH. Negative autoregulation of PspF is continual and not affected by stimuli, like f1 pIV, that induce the *pspA* to *-E* operon. PspF production is independent of PspA (the negative regulator of the *pspA* to *-E* operon) and of PspB and -C (positive regulators).**

PspF is an activator of σ^{54}-containing RNA polymerase (RNAP) required for transcription of the *psp* operon from the *pspA* to *-E* promoter (9). PspF belongs to the enhancer-binding protein (EBP) family of activators but, unlike most, does not have an N-terminal regulatory domain and is constitutively active.

The target for activation by PspF, the *pspA* to *-E* operon, is induced by expression of filamentous phage pIV protein, many of its homologs, and a variety of other stresses including extreme heat shock, hyperosmotic shock, inhibition of fatty acid biosynthesis, uncouplers, and nutrient shifts that reduce the energy charge of the cell (reviewed in reference 15). In the absence of PspA, the product of the first gene of the operon, cells are slow to translocate proteins and are less able to survive at alkaline pH in stationary phase (10, 11, 27). A postulated role for PspA is to help stabilize cells against the loss of membrane potential when under stress (10). PspA also has a regulatory function (to date not distinguishable from its potential direct physiological function); it represses transcription from the *pspA* to *-E* promoter (5, 25). Inducing stimuli overcome the negative regulation by PspA and induce the expression of the *psp* operon (25). PspB and -C, positive regulators of the *psp* response, are absolutely required for induction by filamentous phage pIV, partially required for induction by ethanol treatment and hyperosmotic shock, and not required at all for induction by extreme heat shock (25). PspA, -B, and -C are not DNA-binding proteins and do not resemble the two-component sensor and response regulator proteins used to control many bacterial responses to their environment.

As diagrammed in Fig. 1, *pspF* is close to and transcribed divergently from the *pspA* to *-E* genes (9). The *psp* intervening region contains several *cis*-acting sequences needed either for the transcription of *pspF* or *pspA* to *-E*. The *pspA* to *-E* operon is transcribed by σ^{54}-RNAP (25) and for normal expression requires PspF (9), which binds to two elements shown (Fig. 1) as upstream activating sequences (UASs) I and II (determined by gel retardation assay and DNase I footprinting to be a *psp* enhancer [7]), as well as integration host factor (IHF), bound between the UASs and the σ^{54} promoter (26). *pspF* transcription is under the control of one major (P1) and two minor (P2 and P3) σ^{70} promoters (9). From the standpoint of *pspF* transcription, IHF binds downstream of P3, and UASs I and II overlap the other two *pspF* promoters, P1 and P2. Hence, PspF binding to the UASs might be expected to inhibit transcription of *pspF*. Previous results have suggested that this hypothesis is reasonable; *pspF* is transcribed at a very low level, and the rate of transcription remains unaffected upon encounter with inducing stimuli (9).

Since binding of wild-type PspF to the *psp* enhancer facilitates activation of *pspA* to *-E* transcription, the chromosomally encoded ($pspF_{877}$) DNA-binding mutant PspFΔHTH, in which the helix-turn-helix (HTH) motif is deleted, is not capable of activating *pspA* to *-E* transcription in vivo, either under noninducing or inducing conditions (9). However, when overproduced from multicopy plasmids, both PspF and PspFΔHTH activate transcription and induce the *pspA* to *-E* operon in the absence of any other stimuli (9).

It has been shown that many EBPs are present at low concentrations in unstimulated cells and that environmental stimuli result, via positive autoregulation, in an increase in the amount of these activators, e.g., NtrC (20, 22, 23). Since the *pspF* gene is constitutively transcribed and is translated into a stable and constitutively active protein with an intrinsic ATPase activity, the intracellular concentration of PspF must be limited and well controlled in order to prevent transactivation of unrelated σ^{54}-dependent promoters in *E. coli.*

In this study we address the specifics of the putative *pspF* autoregulation and examine the roles of the UAS elements and PspF binding and the potential influence of PspA in the regulation of *pspF* transcription under normal growth conditions or with inducing stimuli. The amounts of PspF and PspFΔHTH protein in vivo correlate with activation of transcription of *pspA* to *-E* under both noninducing and inducing conditions.

Autogenous control of *pspF* transcription in vivo. A 206-bp DNA fragment containing the *pspF* promoter region was cloned upstream of the *trpA′-lacZYA* genes in fusion vector pRS415 (24) (Fig. 1). The DNA fragment was generated by PCR with primers F5 (5′-ATGGATCCATGATGAAAT-3′ [introduces a *Bam*HI site]) and A5 (5′-GGAATTCACGA TGTCGGC-3′ [introduces an *Eco*RI site]) and Pfu polymerase (Stratagene); it was ligated into *Bam*HI- and *Eco*RI-digested pRS415, creating pMJ12, which contains the *pspF-lacZ* transcriptional fusion Φ(*pspF-lacZ*). The accuracy of the pMJ12 construct was confirmed by DNA sequence analysis. *Escherichia coli* C600 (*thr leu thi*$^+$ *sulI*$^+$ T1^r 15^r ϕ80^r) (from our laboratory collection) was used as host for the λB305 (1) lysogen experiments. Strain K1758 [C600 λB305/pRS415 lysogen

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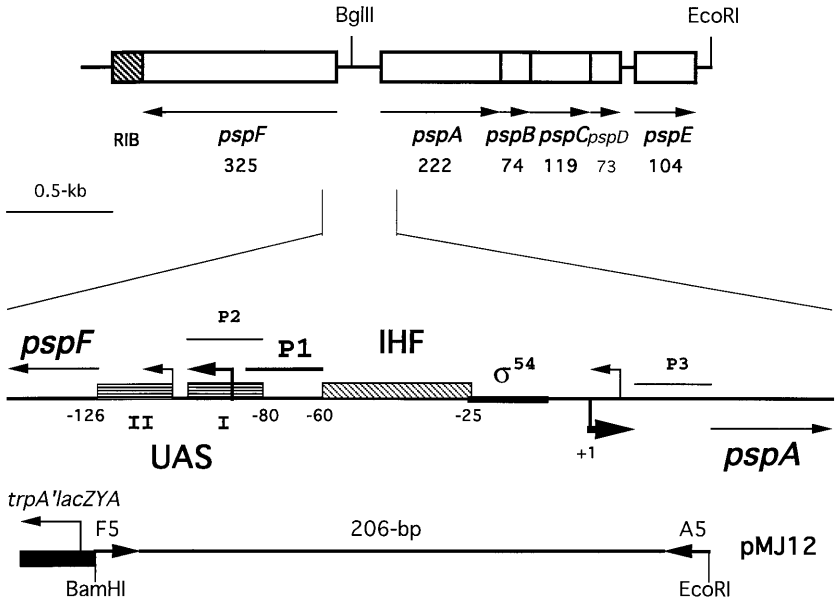

FIG. 1. Construction of the *pspF-lacZ* transcriptional fusion vector pMJ12. (Upper part) Schematic representation of *pspF* and *pspA* to *-E* genes (white blocks). The numbers indicate the lengths of the proteins encoded by the relevant genes. Hatched box, RIB element. (Middle part) Schematic representation of the *pspF-pspABCDE* promoter-regulatory region. The locations of the upstream activating sequences (UASs I and II) or the *psp* enhancer to which PspF binds, the IHF-binding site, the σ^{54} recognition sequence (σ^{54}, *pspABCDE* promoter), the *pspF* σ^{70}-dependent promoters (P1, P2, and P3), the *pspABCDE* start of transcription (bracket arrow, +1) and starts (bracket arrows) of the *pspF* transcriptional units are indicated. (Lower part) Construction of the *pspF-lacZ* transcriptional fusion. pBRPS-1 was used as a source of the *pspF-pspABCDE* promoter-regulatory region. A 206-bp DNA fragment generated by PCR was cloned into pRS415 (black block) to yield pMJ12.

(λ^-)] carries the *lacZ* and *bla* genes from the pRS415 fusion vector. K1759 [C600 λ^- Φ(*pspF-lacZ*)] was constructed by infecting with λB305/pMJ12 [Φ(*pspF-lacZ*)], picking blue plaques from X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)-containing plates, and then screening for the λB305/pMJ12 [Φ(*pspF-lacZ*)], Amp^r, λ lysogen with integrated Φ(*pspF-lacZ*). K1760 [K1759 *pspF*::mTn*10-tet* (*pspF*$_{877}$) (Tet^r)] was constructed by P1 *vir*/K1527 transduction of K1759. Transduction was performed as described previously (13). *E. coli* strains carrying the *pspF-lacZ* transcriptional fusion were assayed for β-galactosidase activity by the method of Miller (14).

A transcriptional fusion between the *pspF* promoter and a promoterless *lacZ*, when introduced as a single copy λ derivative, gave rise to a modest level of β-galactosidase expression (relative to a promoterless construct) in a *pspF*$^+$ background (Table 1). By contrast, when the strain contained a mutant *pspF*$_{877}$ gene with a deletion that removes the sequences that encode the HTH DNA-binding domain, expression of β-galactosidase was about 10 times as high as in the *pspF*$^+$ strain (Table 1). Induction of the *pspA* to *-E* operon by expression of filamentous phage pIV (pNAL200) did not appreciably change β-galactosidase levels (Table 1), indicating that *pspF* transcription is not influenced by the products of the *pspA* to *-E* operon. (For overproduction of f1 pIV [pNAL200, from our laboratory collection, contains f1 gene IV under the control of the IPTG {isopropyl-β-D-thiogalactopyranoside}-inducible *tac* promoter], cultures were induced with a final concentration of 2 mM IPTG.)

The *pspF-lacZ* transcriptional fusion was also introduced on a multicopy plasmid, pMJ12. As shown in Table 2, cells which lacked *pspF* {K1247[PK2212] [Δ*kdpABC-5 thi-1* Δ*trkE* Tet^s *rha-4 lacZ48 gal-33 malA35 zci-233*::Tn*10* Δ(*aldH-ordL-goaG-pspF-pspABCDE*) λ^r λ^-] [8]} expressed high levels of β-galactosidase, as did cells which bore *pspF*$_{877}$ {K1527[K561 *pspF*::mTn*10-tet* (*pspF*$_{877}$) (Tet^r)] [9]} which encodes a protein that cannot bind DNA. Neither induction of the *pspABCDE* operon by phage f1 nor the absence of the *pspABCDE* genes (strain J134 [K561 Δ*pspABC*::*kan*] [25]) affected β-galactosidase levels (Table 2). When the same plasmid was present in a *pspF*$^+$ strain {K561 [HfrC λ^+ *relA1 spoT1* T$_2^r$ (*ompF627 fadL701*) *lacI*^{q}] [3]}, the level of β-galactosidase activity was markedly lower whether or not the *pspABC* genes were present on the chromosome, and it was only slightly affected by filamentous phage infection (Table 2).

PspF inhibits its own transcription in vitro. PspF activates transcription from the *pspA* to *-E* promoter in vitro (4). This transcription also requires σ^{54}-containing RNA polymerase and is stimulated by IHF and by the presence of the UASs and

TABLE 1. β-Galactosidase activities of the chromosomal transcriptional fusion *pspF-lacZ* before and after induction by filamentous phage f1 pIV in *pspF*$^+$ and *pspF*$_{877}$ strains

Strain	Relevant genotype	β-Galactosidase activity (Miller Units)[a]
C600[b]	*pspF*$^+$	4 ± 1
K1758[c]	*pspF*$^+$	13 ± 3
K1759	*pspF*$^+$ Φ*pspF-lacZ*	48 ± 6
K1760	*pspF*$_{877}$ Φ*pspF-lacZ*	582 ± 12
C600(pNAL200)[d]	*pspF*$^+$	6 ± 3
K1758(pNAL200)	*pspF*$^+$	15 ± 5
K1759(pNAL200)	*pspF*$^+$ Φ*pspF-lacZ*	45 ± 8
K1760(pNAL200)	*pspF*$_{877}$ Φ*pspF-lacZ*	490 ± 15

[a] LacZ specific activities were calculated as follows: 1,000 × (A_{420} − 1.75 × A_{550})/5 min × 0.5 ml × A_{600}. Results are presented as means ± standard deviations from three independent experiments.

[b] Used as a control for residual β-galactosidase activity.

[c] λ lysogen of the pRS415 fusion vector carrying the *lacZ* reporter gene without the insert.

[d] pNAL200 produces f1 pIV upon induction by IPTG at 2 mM.

TABLE 2. β-Galactosidase activities before and after f1 infection, obtained by using transcriptional fusion *pspF-lacZ* on multicopy plasmid pMJ12 in strains K561, K1527, and J134

Strain	Relevant genotype	β-Galactosidase activity (Miller units)[a]	
		No induction	Induction by f1[b]
K1247(pMJ12)[c]	Δ(*pspF-pspABCDE*) Φ*pspF-lacZ*	6,208 ± 168	ND[d]
K1527(pMJ12)	$pspF_{877}$ Φ*pspF-lacZ*	6,300 ± 122	5,100 ± 99
K561(pMJ12)	$pspF^+$ Φ*pspF-lacZ*	1,876 ± 82	1,815 ± 66
J134(pMJ12)	$pspF^+$ Δ*pspABC*::*kan* Φ*pspF-lacZ*	1,780 ± 59	1,520 ± 110
K561(pRS415)[e]	$pspF^+$ Φ*lacZ*	128 ±21	150 ± 18

[a] LacZ specific activities were calculated as follows: $1{,}000 \times (A_{420} - 1.75 \times A_{550})/5 \text{ min} \times 0.5 \text{ ml} \times A_{600}$. Results are presented as means ± standard deviations from three independent experiments.

[b] Cells were induced by phage f1 (from our laboratory collection) infection for 30 min at 37°C.

[c] Strain K1247 [Δ(*pspF-pspABCDE*)], carrying the *pspF-lacZ* transcriptional fusion vector pMJ12, was used to obtain β-galactosidase activity in the absence of PspF.

[d] ND, not determined.

[e] β-Galactosidase background activities under noninducing or inducing conditions; pRS415 is a fusion vector carrying the *lacZ* reporter gene without the insert.

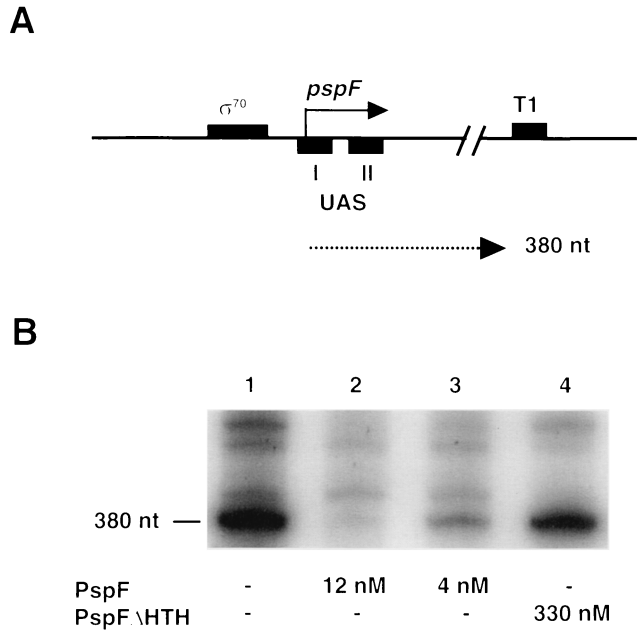

FIG. 2. Inhibition of *pspF* transcription in vitro by PspF. (A) The *pspF* P1 promoter region and structure of the in vitro transcription template. Transcription from P1 utilizes RNAP containing the σ^{70} subunit. The *rrnB* transcription terminator T1 is 380 nt downstream of the *pspF* transcription start. UASs I and II are the sites to which PspF binds. Note that the *pspF* start site lies within UAS I. (B) In vitro transcription reactions carried out on supercoiled pJD17 templates. The 380-nt transcripts were quantified with NIH Image (version 1.52) and normalized to a control transcript (generated from the chloramphenicol resistance gene carried on the plasmid; the relative values for lanes 1 through 4 are 1, 0.12, 0.38, and 1.1, respectively.

the IHF-binding site in the template. PspFΔHTH, which is unable to bind DNA, can also activate transcription but only if provided at about 300 times the concentration of wild-type PspF protein (4).

Transcription of PspF can be measured in a similar system, by using the template shown in Fig. 2A and RNAP holoenzyme (containing σ^{70}). To construct pJD17, the template for in vitro transcription assays, PCR primers JD68 (5′-GGCTGGATCC TTCGCCGATGATGAGCACC-3′) and JD69 (5′-GGCTG GTACCCTTTTTCCTGCCATTCAACC-3′) and *Taq* polymerase (Perkin-Elmer) were used to amplify the *pspF* promoter region from plasmid pPS-1 (2). The 501-bp fragment obtained contains the entire *pspF* promoter region and 110 bp of the *pspF* coding sequence. The fragment also includes the divergent σ^{54}-dependent *pspA* to *-E* promoter. This fragment was digested with *Bam*HI and *Kpn*I and cloned into the *Bam*HI and *Kpn*I sites of pGZ119EH (12), upstream of the *rrnB* T1 terminator. The *tac* promoter upstream of the *Bam*HI site was then removed by digesting with *Eco*RV and *Sac*I, blunt-ending with the Klenow fragment of DNA polymerase I (Bio-Rad Laboratories [BRL]), and ligating with T4 DNA ligase (BRL). In vitro transcription reactions were carried out in 50 mM Tris-acetate (pH 7.9), 118 mM K-acetate, 27 mM NH_4-acetate, 13.2 mM Mg-acetate, and 1 mg of bovine serum albumin per ml. All reaction mixtures also contained 0.5 mM GTP, 0.5 mM UTP, 2 mM ATP, 2 mM dithiothreitol and 0.3 U of recombinant RNasin (Promega). Supercoiled plasmid template (pJD17) was used at 5 nM, and RNAP holoenzyme (Epicentre Technologies) was used at 8 nM. PspF and PspFΔHTH were incubated with the template in the reaction buffer at 37°C for 10 min. RNAP was added and incubation was continued at 37°C for 5 min. Then 0.5 μl of [^{32}P]CTP (10 μCi/μl; New England Nuclear) was added, and after 10 min, nonradioactive CTP (1 mM) was added and the incubation was continued for 10 min. The reaction mixtures then were placed on ice, and an aliquot was extracted with phenol-chloroform and precipitated in ethanol with tRNA (50 μg/ml). The pellet was resuspended in RNase-free glass-distilled H_2O, mixed with 10 μl of formamide loading buffer, loaded on 4% polyacrylamide–7 M urea gels, and run at 225 V in 0.5× sequencing Tris-borate-EDTA buffer. The gels were subjected to autoradiography (New England Nuclear), and the results were analyzed and quantified with NIH Image (version 1.52). Transcription from the major σ^{70} P1 promoter of *pspF* and termination at *rrnB* T1 results in a transcript length of 380 nucleotides (nt).

When PspF is added to the reaction mixture at 12 nM, *pspF* transcription is almost completely abolished, and it is reduced at 4 nM (Fig. 2B). In contrast, when PspFΔHTH is added at a much higher concentration, 330 nM, *pspF* transcription is barely affected (Fig. 2B). PspF, even when present at 50 nM, binds specifically to the UASs, and no other binding of PspF is observed in the *psp* promoter-regulatory region, while PspFΔHTH at 200 nM does not bind, specifically or nonspecifically, to the DNA (7). Hence, wild-type PspF inhibits its own transcription presumably by binding to the UASs.

PspF and PspFΔHTH protein production in vivo and control of their expression in *trans*. Polyclonal antibodies raised to purified His-tagged PspF precipitate both PspF and PspFΔHTH with the same specificity and affinity (data not shown). We used Western blot analysis to estimate the production of both PspF and PspFΔHTH proteins in vivo. Cell extract or purified His_6 PspF was diluted in sodium dodecyl sulfate (SDS) sample buffer (60 mM Tris-HCl [pH 6.8], 2% [wt/vol] SDS, 10% [vol/vol] glycerol, 5% [vol/vol] β-mercaptoethanol, 0.01% bromphenol blue) and subjected to electrophoresis through a 12.5% (wt/vol) polyacrylamide gel containing SDS. Proteins were transferred to a polyvinylidene difluoride membrane (Westran; Schleicher & Schuell) with a Bio-Rad Trans-Blot apparatus at 200 V for 50 min in 1× blotting buffer

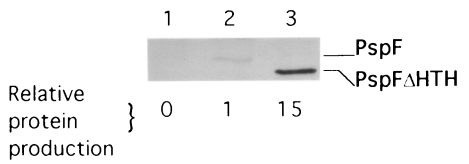

FIG. 3. Autogenous regulation of PspF production. Western blots of cell extracts from a Δ*pspF* strain (lane 1), a $pspF^+$ strain (lane 2), and a $pspF_{877}$ mutant strain (lane 3), by using anti-PspF serum, are shown. Bands were quantified by microdensitometry, and the relative amounts of protein (in arbitrary units) are listed under the lanes. (Production of PspF and PspFΔHTH were measured in five independent experiments, and the relative value for PspFΔHTH is 16±7.)

(15 mM Tris-HCl [pH 10.5], 120 mM glycine, 20% methanol). Membranes were blocked for 2 h with 5% (wt/vol) dried milk in Tris-buffered saline (TBS)-Tween 20 buffer (10 mM Tris-HCl [pH 8.0], 300 mM NaCl, 0.05% Tween 20), washed three times for 10 min with 20 ml of TBS-Tween 20, and then incubated with a 1:2,000 dilution of anti-PspF serum in 20 ml of TBS-Tween 20 for 1 h. The membranes were washed three times for 10 min with 20 ml of TBS-Tween 20, incubated with goat anti-rabbit secondary antibody-horseradish peroxidase conjugate (IgG-HRP reagent; Amersham), and washed as described above. Western blot signals were visualized by enhanced chemiluminescence (ECL) detection reagent (Amersham), used as specified by the manufacturer and quantified on a double-beam recording MK III C microdensitometer (Joyce, Loebl & Co. Ltd.).

Western blot analysis of a cell extract from a $pspF^+$ strain shows a faint band of PspF (Fig. 3); an extract from a $pspF_{877}$ strain gives a much stronger band (Fig. 3). About 15 times as much PspFΔHTH as wild-type PspF is made. With the use of serial dilutions of purified PspF as a standard, we estimate from comparable Western blots that strain K561 ($pspF^+$) harvested at 5.8×10^8 cells ml^{-1} contains about 130 copies of PspF per cell.

Figure 4 shows the amounts of PspF and PspFΔHTH made in cells with a variety of plasmid and chromosomal $pspF^+$ and $pspF_{877}$ genes. Lanes 1 and 2 confirm earlier results that 15 to 20 times as much PspFΔHTH is made in cells with the $pspF_{877}$ mutation as is PspF in a wild-type cell. The chromosomally encoded level of PspF is sufficient to confer strong inducibility on the *pspA* to *-E* operon (9). Even though PspFΔHTH was made in much larger quantities, the *pspA* to *-E* operon is not inducible. Expressing wild-type PspF from a multicopy plasmid (pMJ7 [9] carries the intact *pspF* gene) increases the amount in the cell 30 times (compare lanes 1 and 3 or 5 [Fig. 4]) and decreases the production of chromosomally encoded PspFΔHTH 10-fold (Fig. 4, lane 3); the effect of this PspF overproduction is to induce the *pspA* to *-E* operon constitutively (9). Not surprisingly, this effect is obtained whether the chromosomal *pspF* gene is the wild type or a mutant. When PspFΔHTH is expressed from a multicopy plasmid (pMJ3 [9] carries $pspF_{877}$) in the $pspF_{877}$ strain, a 140-fold larger amount of PspFΔHTH is made than PspF in a wild-type strain (Fig. 4, lane 4), and PspFΔHTH, like overproduced wild-type PspF, activates the *pspA* to *-E* operon constitutively (9). In a $pspF^+$ strain, the amount of PspFΔHTH made from the same plasmid (pMJ3) is much lower than in a $pspF_{877}$ strain (Fig. 4, lane 6) and is comparable to the amount of plasmid-encoded, wild-type protein in either a $pspF^+$ or $pspF_{877}$ background (Fig. 4, lanes 5 and 3). This level of PspFΔHTH expression is not sufficient to make expression of the *pspA* to *-E* operon constitutive (9). Expression is inducible due to the PspF encoded by the

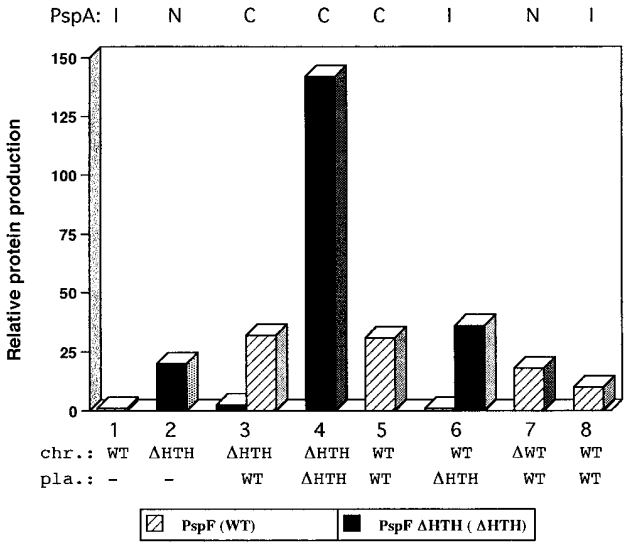

FIG. 4. PspF and PspFΔHTH production in cells containing different combinations of chromosomal (chr.) and plasmid (pla.) *pspF* and $pspF_{877}$ genes. Lane 1, K561 ($pspF^+$); lane 2, K1527 ($pspF_{877}$); lane 3, K1527(pMJ7); lane 4, K1527(pMJ3); lane 5, K561(pMJ7); lane 6, K561(pMJ3); lane 7, K1247(pMJ7) [K1247 is Δ(*pspF pspA-E*)]; lane 8, K561(pBRPS-1). pMJ7 includes promoters P1 and P2 (Fig. 1) and encodes PspF. pMJ3 is the same as pMJ7 but has a mini-Tn*10* insertion in the *pspF* gene ($pspF_{877}$) and encodes PspFΔHTH. pBRPS-1 includes the *pspABCDE* genes, the entire intergenic region, and *pspF*. Bands on Western blots were quantified by microdensitometry and are reported in arbitrary units (an average from two independent experiments) on the vertical axis relative to the amount produced by K561 ($pspF^+$) in lane 1 (pspF = 1). These strains were tested for PspA production before and after induction by either heat shock or ethanol treatment in separate experiments (shown in reference 9), and their PspA status (C, constitutive; I, inducible; N, no expression with or without stimulus) is shown at the top of the figure.

chromosomal copy of $pspF^+$, which suggests that PspFΔHTH, at least at these levels, is not dominant negative. A plasmid expressing wild-type PspF in a strain deleted for all of the *psp* genes makes a little less PspF (Fig. 4, lane 7) than in a psp^+ strain, but this may not be a significant difference. Since this strain is deleted for the *psp* operon, expression of PspA cannot be observed. Finally, placing the entire *pspA* to *-E* operon on the plasmid together with $pspF^+$ (pBRPS-1 [27]), which substantially increases the uninduced level of all the *psp* proteins, leads to a reduction in PspF production (Fig. 4, lane 8).

These data strongly support an autogenous control mechanism for *pspF* transcription that is mediated by the binding of PspF to the *psp* enhancer (UASs). The observation that the presence of the entire *pspA* to *-E* operon on the plasmid reduces expression of PspF is somewhat surprising, because this construct contains an additional promoter (P3) driving *pspF* transcription. The construct containing the remainder of the *pspA* to *-E* operon differs in two ways from those in pMJ7 and pMJ3: besides containing the *pspA* to *-E* genes, it contains the IHF-binding site in the *psp* promoter-regulatory region and the *pspA* to *-E* σ^{54} promoter. It may be that binding of either IHF or σ^{54}-RNAP to their respective sites affects PspF binding so as to tighten autogenous control.

Production of chromosomally encoded PspF is independent of PspA, PspB, and PspC and of induction by f1 pIV. To determine how PspF production is affected by PspA, the negative regulator of the *pspA* to *-E* operon, or by PspB and -C, the positive regulators, we measured the synthesis of wild-type activator from the chromosomal copy of $pspF^+$ in strains overproducing PspA [K561(pJLB24); pJLB24 (25) carries the *pspA*

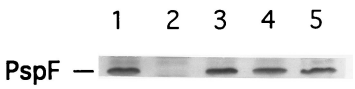

FIG. 5. PspF protein levels in strains in which proteins encoded by the *pspA* to *-E* operon are absent, overproduced, or induced. Lane 1, K561 (*pspF*$^+$ *psp ABCDE*$^+$); lane 2, K1247 [Δ(*pspF pspABCDE*)]; lane 3, J134 (*pspF*$^+$ Δ*pspABCDE*); lane 4, K561(pJLB24) (*pspF*$^+$ *pspABCDE*$^+$; PspA is overproduced from pJLB24); lane 5, K561(pNAL200) (*pspF*$^+$ *pspABCDE*$^+$; *psp* operon induced by f1 pIV from pNAL200). Bands were detected by Western blotting with PspF antiserum and quantified by microdensitometry. The gel was overexposed. The relative amounts of PspF (in arbitrary units) obtained in three replicate experiments were, as shown in lanes 1 through 5, 1, 0, 1.3±0.4, 1.1±0.3, and 1.2±0.4, respectively. The background values have been subtracted.

gene under the control of the *lac* UV5 promoter-operator], deleted for PspA to -E (J134), or overproducing the *psp* operon inducer, f1 pIV [K561(pNAL200)]. For overproduction of PspA and f1 pIV, cultures were induced with a final concentration of 2 mM IPTG. Western blot analysis showed that neither lack of PspA, -B, and -C (Fig. 5, lane 3) nor overproduction of PspA (Fig. 5, lane 4) significantly changed the level of PspF compared to its level in the wild-type control in strain K561 (Fig. 5, lane 1). The same is true for the production of PspF under f1 pIV-inducing conditions (Fig. 5, lane 5). These results show that PspF synthesis is not the target for the negative or positive control of *psp* operon expression mediated by PspA or PspB and -C, respectively.

Conclusions. The data presented here show that PspF is negatively autogenously regulated. This allows the cell to maintain a constant and low intracellular level of the activator, so that the *pspA* to *-E* promoter can be induced but is not gratuitously activated, and perhaps to prevent PspF from crossactivating heterologous σ^{54}-dependent promoters. The autogenous control of *pspF* transcription observed in the in vitro transcription assay shows that PspF does not require the presence of an auxiliary factor(s) to repress its own transcription.

There are about 130 copies of PspF in a wild-type cell, and approximately 15 times as much PspFΔHTH in a $pspF_{877}$ mutant. These protein determinations agree reasonably well with measurements of β-galactosidase activity in lysates from cells carrying a single-copy *pspF-lacZ* transcriptional fusion. The interpretation of this similarity is complicated by the fact that the deletion which removes the HTH motif from PspF also removes the RIB (reiterative IHF bacterial interspread mosaic) element 3′ to the *pspF* gene (9). In the absence of this RIB element the steady-state level of *pspF* RNA is lowered at least fivefold, presumably because the RNA is less stable (8). The lower levels of mRNA and higher levels of protein in the $pspF_{877}$ strain imply that translation of the *pspF* message is much more efficient in the absence of intact PspF and suggest that the overlap of UAS II and the *pspF* ribosome-binding site affects translation.

Purified PspFΔHTH showed approximately 0.3% of the in vitro activity of intact PspF in transcription from the *pspA* to *-E* σ^{54}-dependent promoter (4). The data obtained in this study show that when overproduced and present in a 140-fold higher concentration than chromosomally encoded PspF, the DNA-binding mutant activates *pspA* to *-E* transcription even in unstimulated cells. This implies that when produced at high levels, PspFΔHTH directly interacts with σ^{54}-RNAP bound to the *pspA* to *-E* promoter and that binding of the wild-type PspF activator to a specific UAS(s) and the presence of IHF-bent DNA must increase the effectiveness of the activator-σ^{54}-RNAP contact approximately 100 times. This is in accord with results that show that the local concentration of activator proteins bound to DNA sites in the vicinity of the promoter can be increased more than 100-fold by intrinsic or protein-induced distortion of the regular DNA conformation (22).

Negative autoregulation is a property shared by many transcription activators (19). For other activators of the EBP class, inducing stimuli can result in an increase in the concentration of the activator as well as its activation (16, 18, 21). There appears to be no comparable circuit for PspF. PspF is a bidirectional regulator that functions both as a constitutive activator and as a permanent repressor. The continual and independent autogenous control of PspF maintains the physiological intracellular concentration of the activator at a low level, which, in stimulated cells, is sufficient for specific control and induction of the *pspA* to *-E* operon by PspF bound to the *psp* enhancer. For the *pspA* to *-E* response to multiple environmental and intracellular stimuli, the negative regulation imposed by PspA seems to be the target of the PspBC-dependent and PspBC-independent signal transduction cascade. *pspA* to *-E* σ^{54} promoter activity in vivo is not regulated by the concentration of the activator but rather by the negative regulation imposed by PspA. PspA and the positive regulators PspB and -C do not act on *pspF* transcription nor on PspF production itself, presumably because they do not interfere with or change the binding of PspF to the specific UASs. Since overproduction of PspF does stimulate PspA synthesis from the *pspA* to *-E* promoter (7, 9), it is likely that PspF is a target for regulation by PspA and that overproduction of the activator bypasses this regulation. PspA may act as a negative regulator by interacting either with σ^{54}-RNAP or with PspF, thereby blocking the contact between PspF and the σ^{54}-RNAP holoenzyme. Other possible targets could be PspF self-association and the formation of a catalytically active complex. The potential determinant responsible for PspA function as a negative regulator lies in the C-terminal region of PspA (not in its coiled-coil region) (25). Alignment of PspA with PspF and the other EBPs identifies an amino acid sequence in the PspA C-terminal region which is homologous to a relatively conserved amino acid sequence block in the C3 region of the central domain of the PspF and other EBPs (6). It has been shown that this amino acid sequence contains residues important for ATP binding, ATPase activity, and interaction of NtrC with σ^{54}-RNAP (17). Hence, it is possible that this sequence in PspA mimics the homologous PspF sequence and competes for the same target on σ^{54}.

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