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Genetics

Degradation of σ^{32}, the heat shock regulator in *Escherichia coli*, is governed by HflB

(protease/λcIII/λcII/FtsH/FtsJ)

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ABSTRACT **The heat shock response in *Escherichia coli* is governed by the concentration of the highly unstable σ factor σ^{32}. The essential protein HflB (FtsH), known to control proteolysis of the phage λcII protein, also governs σ^{32} degradation: an HflB-depleted strain accumulated σ^{32} and induced the heat shock response, and the half-life of σ^{32} increased by a factor up to 12 in mutants with reduced HflB function and decreased by a factor of 1.8 in a strain overexpressing HflB. The *hflB* gene is in the *ftsJ-hflB* operon, one promoter of which is positively regulated by heat shock and σ^{32}. The λcIII protein, which stabilizes σ^{32} and λcII, appears to inhibit the HflB-governed protease. The *E. coli* HflB protein controls the stability of two master regulators, λcII and σ^{32}, responsible for the lysis–lysogeny decision of phage λ and the heat shock response of the host.**

Cells protect themselves from thermal stress by induction of a set of proteins, the heat shock proteins, many of which are highly conserved from bacteria to man (1, 2). In *Escherichia coli*, this universal response is transcriptionally regulated by a special σ factor, σ^{32}, which associates with RNA polymerase to initiate transcription from promoters containing σ^{32}-specific recognition sequences (3, 4). The key determinant of heat shock regulation is the concentration of σ^{32}, a highly unstable protein (4). Its degradation involves the heat shock proteins DnaK, DnaJ, and GrpE, which are thought to chaperone σ^{32} to a proteolytic system (5–7). The protease responsible for σ^{32} degradation in *E. coli*—a central component of heat shock regulation—has not been identified.

FtsH, a 70-kDa inner membrane protein (8, 9) essential for bacterial survival (10), was recently shown to be identical to HflB (11), which is involved in proteolysis of the λcII protein (11, 12). The name HflB, which we now use, reflects the phenotype—"high frequency of lysogeny"—of *hflB* missense mutants (11, 12). λcII is the master regulator of the λ phage lysis–lysogeny decision; high λcII concentrations shortly after infection favor lysogeny, whereas low concentrations favor lysis (12, 13). Since HflB is an essential protein, one can speculate that bacterial viability depends on the degradation (or processing) of one or more bacterial proteins by the HflB-governed proteolytic pathway. However, until now, no bacterial substrates of this protease activity have been identified.

The λcIII protein stabilizes λcII (13). It also has been shown to stabilize σ^{32}, thereby inducing the heat shock response (14). It is not known whether the protection afforded by λcIII is via direct interaction with λcII and σ^{32} or via inhibition of one or more proteases involved in their degradation. Overproduction of λcIII is lethal in *E. coli* (15). HflB, an essential protein that governs an anti-λcII protease activity, seemed to be a good candidate for the target of λcIII action. If indeed λcIII inhibits the HflB-governed proteolytic pathway, then the simplest hypothesis to explain σ^{32} stabilization by λcIII is that HflB also governs σ^{32} degradation. In the present report, we test the dual hypothesis that λcIII inhibits the HflB-governed proteolytic activity and that this pathway degrades σ^{32}.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Phage. The strains used in this work are all *E. coli* K-12 derivatives. There are two isogenic series, one from strain 594, genotype *lacZ-350 galK2 galT22 rpsL179* (16), and the second from JM105 (17), which carries a $lacI^q$ allele and overproduces Lac repressor. The isogenic $rpoH^+$ and *rpoH*(Am) *supF*(Ts) strains were SC122 and K165, respectively, where Am = amber mutation and Ts = temperature sensitive (18). Heat shock induction during HflB depletion was monitored by using strain SL410 [594 Δ*ftsH3*::Km *lacIpoZ*Δ(*Mlu*) λpF13-(p*groE::lacZ*)/F′ $proAB^+$ $lacI^q$ *lacZ*Δ*M15* Tn*10*/pSTD40)], where Km = kanamycin resistant.

The *ftsH1* and *hflB29* alleles were introduced by cotransduction with a *zgj-203*::Tn*10* (19) insertion (also present in the $hflB^+$ strains). The Δ*ftsH3*::Km allele was introduced by transduction and selection for kanamycin resistance in the presence of 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG) in a strain carrying pSTD40. The sources of mutations were Y16 for *ftsH1* (20), X9393 for *hflB29* (12), AD315 for Δ*ftsH3*::Km (10), and the allele *lacIpoZ*Δ(*Mlu*) (21). P1 *vir* was used for transduction as described (22).

Plasmid pAR145 carries the intact *hflB* gene cloned downstream of the *lac* promoter in a low-copy-number chloramphenicol-resistant pSC101 derivative, pHSG575 (8). The plasmid ptacλcIII (15) carries the λcIII gene, the plasmid pcIII14–37 carries the DNA fragment of λcIII coding for amino acids 14–37 inserted in *lacZ′* (15), and plasmid pCG179 (23) carries the *rpoH* gene coding for σ^{32}; all plasmid inserts are under control of the IPTG-inducible *tac* promoter of the ampicillin-resistant vector ptac12H (23, 24). Except for SL40, the $lacI^q$ allele for 594 derivatives was provided, when necessary, by the spectinomycin-resistant plasmid pSC101lacI^q (25). Plasmid pSTD40 carries the *hflB* gene lacking its own promoter and cloned under control of the *lac* promoter in a low-copy-number chloramphenicol-resistant pSC101 derivative (10). Plasmid pULB6238 carries the 4-kb *Eco*RI-*Asp*718 fragment from pAR132 (8) cloned in pUC19 (17) digested with the same enzymes.

Culture Conditions. Cells were grown in M63 medium (22) supplemented with 0.4% glucose and 1 μg of thiamine per ml or in Luria–Bertani (LB) broth (22). When necessary, antibiotics were added at the following concentrations: ampicillin, 50 μg/ml; chloramphenicol, 15 μg/ml; kanamycin, 40 μg/ml; tetracycline, 10 μg/ml; and spectinomycin, 50 μg/ml.

Survival Against Overproduction of λcIII, LacZ′(λcIII14-37), or σ^{32}. The strains used were JM105 derivatives. Cells were grown at 37°C overnight in glucose minimal medium

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Abbreviations: IPTG, isopropyl β-D-thiogalactoside.

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containing chloramphenicol and ampicillin. They were assayed on the same solid medium at 37°C with or without 5 mM IPTG.

β-Galactosidase Assay. Heat shock induction during HflB depletion was monitored by the quantification of β-galactosidase expressed from prophage λpF13-(p*groE::lacZ*) in strain SL410, which has no other source of β-galactosidase. Cells were grown at 37°C in glucose minimal medium supplemented with tetracycline, kanamycin, and 0.1 mM IPTG. At OD_{600} = 0.2, IPTG was removed from the culture by three centrifugations and washing in medium without IPTG. At the third wash, the resuspended culture was divided in two batches, 0.1 mM IPTG was added to one, and the two cultures were incubated at 37°C. Samples were withdrawn every 30 min and assayed for β-galactosidase activity (26), and the differential rate of synthesis was determined.

Measurement of σ^{32} Stability. All strains carried a *lacI*^{q} allele and pCG179, in which the *rpoH* gene, coding for σ^{32}, is under control of the *tac* promoter (23). The protocol was essentially that described by Tilly *et al.* (23); cells were pregrown at 30°C in glucose minimal medium supplemented with spectinomycin and ampicillin, then shifted to 42°C (t = −36 min), induced with 1 mM IPTG (t = −16 min), pulse-labeled (t = −1 min) with a mixture of [^{35}S]methionine and [^{35}S]cysteine (38.3 TBq/mmol, 0.22 MBq/ml; ICN), and then chased (t = 0) with 100 μg of each amino acid per ml. Samples were electrophoresed on 10% SDS/polyacrylamide gels, and radioactivity in the σ^{32} band was revealed with a PhosphorImager (Molecular Dynamics) and quantified with the IMAGEQUANT 1.33 (Molecular Dynamics) program.

Immunoblots. Immunoblots were carried out with anti-HflB serum (9) (provided by T. Ogura, University of Kumamoto, Japan) or anti-σ^{32} serum (provided by C. Georgopoulos, Université de Genève, Switzerland and R. Burgess, University of Wisconsin). Strains JM105, JM105/ptac12H, JM105/pCG179, and SL404 (Δ*ftsH3*::Km/pSTD40) were grown in glucose minimal medium supplemented with ampicillin for ptac12H and pCG179 and with chloramphenicol for pSTD40. SC122 and K165 were grown in LB broth. Samples equivalent in mass to 1 ml at OD_{600} = 0.2 were cooled on ice, centrifuged, and resuspended in 100 μl of SDS sample buffer (17), heated at 100°C for 3 min, electrophoresed on SDS/10% polyacrylamide gels, and electrophoretically transferred to nitrocellulose (17). Blots were blocked overnight with Tris-buffered saline (137 mM NaCl/20 mM Tris, pH 7.6/0.1% Tween 20) with 5% nonfat dry milk, then probed with anti-HflB serum or anti-σ^{32} serum, and developed with an enhanced chemiluminescence (ECL) immunoblot (Western blot) detection kit (Amersham). Blots were digitalized with an Apple scanner, and relative amounts of HflB were determined with the IMAGE 1.52 (National Institutes of Health) program.

S1 Nuclease Mapping and mRNA Quantification. Total *E. coli* mRNA was isolated by phenol extraction (27) from strain 594 *zgj-203*::Tn*10* grown in modified M9 medium (28). S1 nuclease mapping was carried out as described by Sambrook *et al.* (17). Two probes were synthesized by polymerase chain reaction amplification on pULB6238 using the oligonucleotides TACTGACATCAGCACAACGGC (in *hflB*), CGCTAAAGTGTTCCTGAAGCC (in *ftsJ*) and ACAGCGTTTTACCGATGACCTG (in the upstream *greA* gene). They were end-labeled by using T4 polynucleotide kinase (Biolabs, Northbrook, IL) with [γ-^{32}P]ATP (22.2 TBq/mmol, 13 MBq/ml) and purified on a G-50 Sephadex column (Boehringer Mannheim). mRNA (20 μg) was hybridized overnight at 45°C with an excess of probes. The reaction mixtures were incubated 30 min with 120 units of S1 nuclease (Boehringer Mannheim).

DNA sequences were determined with a Sequenase DNA sequencing kit (United States Biochemical) according to the manufacturer's procedure with the oligonucleotide in *ftsJ* as primer. Samples were electrophoresed on 6% polyacrylamide gels containing 40% urea. Radioactivity was detected with Kodak XAR5 film or with a PhosphorImager (Molecular Dynamics) and quantified with the IMAGEQUANT 1.33 (Molecular Dynamics) program.

RESULTS

Growth Inhibition by Excess λcIII Is Relieved by HflB Overproduction. According to our hypothesis that HflB is the target of λcIII action, the lethality caused by λcIII overproduction would reflect titration of the essential HflB proteolytic activity. Therefore, we evaluated the effect of λcIII overproduction in the presence of excess HflB. As shown in Table 1, overproduction of HflB relieved the growth inhibition caused by excess λcIII.

A chimeric LacZ-λcIII protein [LacZ′(cIII14–37)] that includes a 24-amino acid fragment of λcIII mimics λcIII in that it enhances λ lysogenization, induces the heat shock response, and inhibits cell growth when overproduced (15). This growth inhibition was also relieved by HflB overproduction (Table 1).

These observations are consistent with the hypothesis that HflB is the target of λcIII inhibition. In fact, the λcIII protein may itself be degraded by the HflB-governed protease (preliminary observations). Thus, in excess, it may competitively inhibit the degradation of other substrates, resulting in growth inhibition.

Growth Inhibition by Excess σ^{32} Is Relieved by HflB Overproduction. Overproduction of σ^{32}, like that of λcIII, is lethal (Table 1). If, as speculated above, σ^{32} degradation is governed by HflB, this lethality may again reflect titration of HflB activity. Indeed, as with λcIII, the growth inhibition due to σ^{32} overproduction was relieved by HflB overproduction (Table 1), consistent with the idea that the HflB-governed protease degrades σ^{32}.

The HflB-Governed Protease Degrades σ^{32}. Strains in which the *hflB* gene is under *lac* promoter control are viable only in the presence of IPTG; removal of IPTG results in HflB depletion and growth inhibition after few generations (10). Protein gels from such an HflB-depleted strain revealed an increased intensity of bands that comigrate with heat shock proteins (molecular masses 70 and 60 kDa; data not shown). To see whether the heat shock response is induced in these conditions, we introduced the phage λpF13-(p*groE::lacZ*) (29) and a *lac* deletion into our strain. With this fusion, the rate of β-galactosidase synthesis is a specific assessment of transcription initiated at the heat shock promoter (29). Removal of IPTG from the medium arrested HflB synthesis; the dilution of HflB with time resulted in a 4.5-fold increase in the rate of

Table 1. HflB antagonizes the growth inhibition due to overproduction of λcIII or σ^{32}

Plasmids*	Proteins induced	+IPTG/−IPTG†
pHSG575		
+ ptac12H	—	1.0
+ ptacλcIII	λcIII	5×10^{-5}
+ pcIII14–37	LacZ′(cIII14–37)	1×10^{-5}
+ pCG179	σ^{32}	5×10^{-4}
pAR145		
+ ptac12H	HflB	8×10^{-1}
+ ptacλcIII	HflB, λcIII	4×10^{-1}
+ pcIII14–37	HflB, LacZ′(cIII14–37)	5×10^{-1}
+ pCG179	HflB, σ^{32}	5×10^{-1}

*Plasmid pHSG575 is the control into which the intact *hflB* gene was cloned downstream of the *lac* promoter to make pAR145. ptacλcIII carries the λcIII gene, pcIII14–37 carries λcIII-(14–37) gene inserted in *lacZ′*, and pCG179 carries the *rpoH* gene coding for σ^{32}. All plasmid inserts are under control of IPTG-inducible *tac* promoter of ampicillin-resistant vector ptac12H.

†Ratio of titers (no. of cells) on glucose minimal plates with or without 5 mM IPTG.

β-galactosidase synthesis, demonstrating induction of the heat shock response (Fig. 1).

To see whether heat shock induction in an HflB-depleted strain resulted from σ^{32} accumulation, the amount of HflB and σ^{32} was monitored by immunoblots at different times after IPTG removal. As the amount of HflB per mass decreased, σ^{32} increased (Fig. 2). After 5 hr, IPTG was added back to the culture. This resulted in an increase in HflB and a decrease in σ^{32} (Fig. 2).

The increase in σ^{32} in the absence of HflB could reflect increased synthesis or, more likely, decreased degradation. To evaluate the role of HflB in σ^{32} degradation, we measured the stability of σ^{32} in different *hflB* mutants. Two different alleles of the *hflB* gene—*ftsH1*(Ts) and *hflB29*—are deficient in proteolysis, since they increase the frequency of λ lysogenization by stabilizing λcII (refs. 11 and 12; data not shown). The half-life of σ^{32} was 40 sec in wild-type cells; in the *hflB29* mutant, it increased to 60 sec (Fig. 3). This marginal effect explains why the HflB system was previously reported not to be involved in σ^{32} degradation (14, 30). In contrast, in the *ftsH1*(Ts) mutant at 42°C (a nonpermissive temperature), the half-life of σ^{32} increased to 480 sec, whereas in the presence of excess HflB, it decreased to 22 sec (Fig. 3). Another unstable protein, λN, which is principally degraded by Lon protease (31), was not stabilized in the *ftsH1* mutant at 42°C (data not shown). We conclude that HflB specifically governs the degradation of λcII (11, 12) and of σ^{32}. The *hflB29* mutation specifically reduces cII proteolysis, whereas the *ftsH1* allele affects both cII and σ^{32} degradation at restrictive temperature.

Other ATP-Dependent Proteases (HflA, Lon, and ClpP) Are Not Responsible for σ^{32} Instability. λcII is also degraded by the HflA proteolytic pathway (32, 33), and analysis of two-dimensional protein gels from *hflA* and *hflB* mutants suggests that these two proteolytic pathways may have common bacterial substrates as well (34). Complete inactivation of the *hflA* locus resulted in slight stabilization of σ^{32} (half-life 144 sec). However, a multicopy plasmid carrying the three genes of the *hflA* locus did not affect σ^{32} stability (half-life 38 sec), suggesting that the stabilization is indirect. Inactivation of the Lon or ClpP protease also stabilized σ^{32} slightly (half-life, 100 sec and 83 sec, respectively), probably indirectly by increasing the accumulation of abnormal proteins (see ref. 6).

HflB Is a Heat Shock Protein. The stability of σ^{32} is decreased by the DnaK, DnaJ, and GrpE proteins (6, 23), which are thought to chaperone σ^{32} to an unknown protease. It has been suggested (7, 35) that, after a heat shock, these chaperones, drawn to other unfolded proteins, release σ^{32}, resulting in transient stabilization. These chaperones are themselves heat shock proteins under control of σ^{32}. Therefore, we looked for possible heat shock regulation of HflB. Indeed, the amount of HflB per mass was higher in cells cultivated at high temperature (Fig. 4*A*). Furthermore, the increase in HflB after a heat shock was abolished in an *rpoH* mutant in which σ^{32} synthesis is temperature sensitive (Fig. 4*A*). Finally, the amount of HflB increased when σ^{32} synthesis was artificially induced by IPTG when using a *ptac-rpoH* fusion (Fig. 4*B*). These data strongly suggest that HflB is a heat shock protein regulated by σ^{32}. In agreement with this conclusion, we previously found that, in an *hflA* null mutant in which HflB governs the principal anti-λcII activity, the λ lysogenization frequency decreases with increasing temperature (11), suggesting that λcII is more rapidly degraded by HflB after a heat shock.

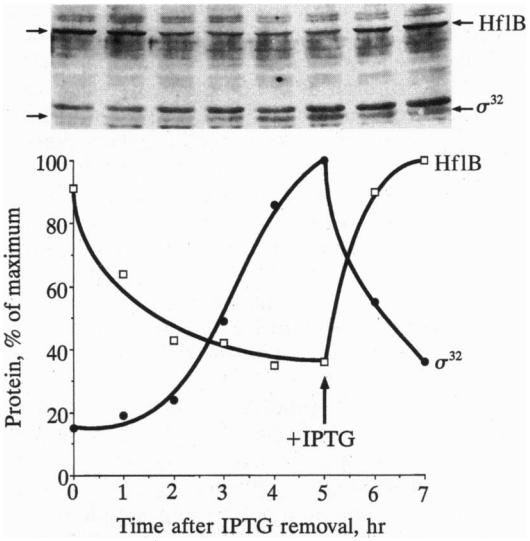

FIG. 2. Accumulation of σ^{32} during HflB depletion. Immunoblots with anti-HflB and anti-σ^{32} antisera were made with samples removed at 1-hr intervals after IPTG removal from strain SL410. After 5 hr, 100 μM IPTG was added back to the culture. The positions of HflB (upper arrow, 70 kDa) and σ^{32} (lower arrow, 32 kDa) are indicated. Below the gel is a normalized quantification of HflB (□) and σ^{32} (●).

It was recently reported (36) that λ phage 520 from the library of Kohara *et al.* (37), which carries a 19-kbp insert of *E. coli* DNA including the *ftsJ* and *hflB* genes [*ftsJ* is 102 bp upstream of *hflB* (8)], codes for 27-kDa and 70-kDa heat shock proteins regulated *in vivo* by heat shock and *in vitro* by σ^{32}. These proteins could well correspond to FtsJ [of apparent molecular mass 26 kDa (8)] and HflB (70 kDa).

By S1 nuclease mapping with a probe covering the intergenic sequence between the *greA* and *ftsJ* genes (both upstream of

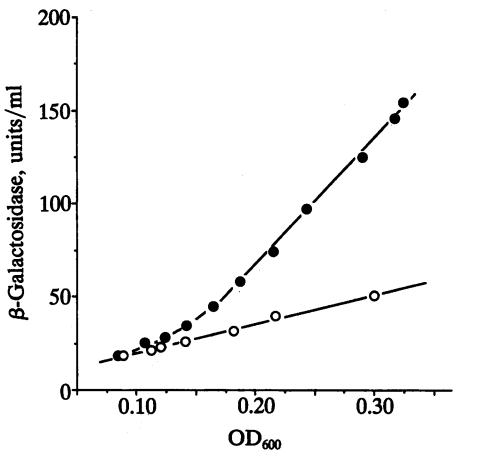

FIG. 1. Induction of the p*groE::lacZ* gene fusion during HflB depletion. ○, SL410 + IPTG; ●, SL410 − IPTG (depleted for HflB).

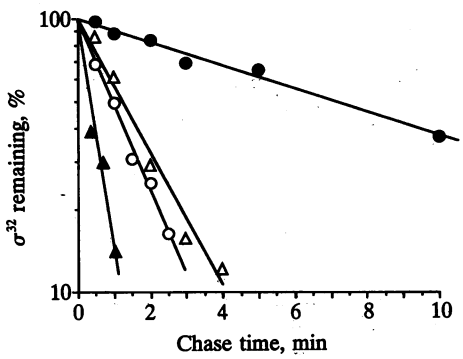

FIG. 3. HflB governs σ^{32} degradation. All strains carry *lacI*^{q} and pCG179, in which the *rpoH* gene, coding for σ^{32}, is under control of the *tac* promoter (23). ○, *hflB*$^+$; ●, *ftsH1*(Ts); △, *hflB29*; ▲, *hflB*$^+$ carrying pAR145, in which the *hflB* gene is downstream of the *lac* promoter.

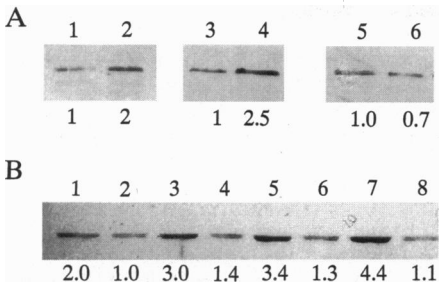

FIG. 4. HflB is a heat shock protein. An immunoblot is shown with anti-HflB antiserum and samples containing the same cell mass. (*A*) Lanes: 1 and 2, strain JM105 growing in steady state at 30°C and 42°C, respectively; 3 and 4, strain SC122 (*rpoH*$^+$) at 30°C and after 60 min at 42°C, respectively; 5 and 6, strain K165 [*rpoH*(Am) *supF*(Ts)] at 30°C and after 60 min at 42°C, respectively. (*B*) Lanes: 1, 3, 5, and 7, strain JM105 carrying plasmid pCG179 in which the expression of σ^{32} is under control of the *tac* promoter; 2, 4, 6, and 8, strain JM105 carrying the control vector ptac12H. The times of IPTG induction are 0 (lanes 1 and 2), 20 (3 and 4), 40 (5 and 6), and 60 (7 and 8) min. Below the gels is a normalized quantification of HflB.

hflB), we identified two mRNAs initiating upstream of *ftsJ* (Fig. 5*A*). With a probe covering the sequence between *greA* and *hflB*, these two mRNAs were seen to include *hflB*; a third minor transcript initiating within *ftsJ* was also detected (data not shown). Neither of the two previously proposed promoters (8) was active. The shorter mRNA (starting at promoter *P*2) increased after a heat shock (Fig. 5*B*) with kinetics similar to that of the *dnaK* heat shock promoters (data not shown), while the longer mRNA (starting at promoter *P*1) decreased in parallel (Fig. 5*B*). The extinction of *P*1 after heat shock could

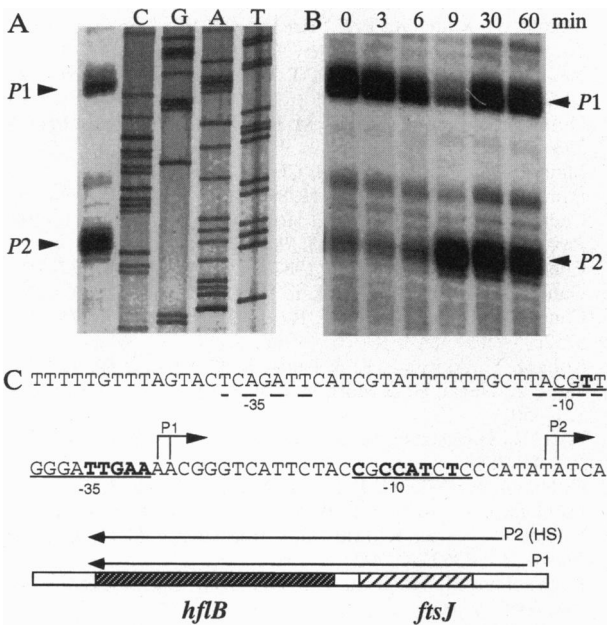

FIG. 5. The *ftsJ-hflB* operon possesses a heat shock promoter. The quantification and the promoter of the mRNAs were determined by S1 nuclease mapping with a probe covering the intergenic sequence between the *greA* and *ftsJ* genes. (*A*) Lanes: 1, S1 nuclease mapping, using a DNA probe covering the intergenic sequence *greA–ftsJ*; 2–5, DNA sequence reaction, C, G, A, and T, respectively. (*B*) Quantification of mRNA by S1 nuclease digestion after a shift from 30°C to 42°C. Lanes 1–6 correspond to samples withdrawn at 0, 3, 6, 9, 30, and 60 min, respectively, after heat shock. (*C*) DNA sequence of the promoter region of *ftsJ*. The proposed −35 and −10 regions are underlined, and bases that match the consensus heat shock sequence (38, 39) are in boldface letters. The mRNA start points deduced from the S1 mapping are indicated by arrows. The poor match of *P*1 with the σ^{70} consensus sequence suggests additional regulation.

be due to promoter occlusion since *P*1 and *P*2 are practically adjacent (Fig. 5*C*). The *P*2 promoter sequence resembles the σ^{32} recognition sequence (38).

We conclude that the *ftsJ* and *hflB* genes form an operon, with one of the two major transcripts being under heat shock regulation by σ^{32}.

DISCUSSION

From its predicted amino acid sequence (8), HflB belongs to a family of proteins (40), mostly eukaryotic, all of which have putative ATPase activity. From known or suspected activities of several of these relatives, we previously pointed out that HflB might be a chaperone or a protease (11). One of the closest HflB relatives is the S4 ATPase subunit of the 26 S proteasome (41, 42) involved in the ubiquitin-dependent proteolysis pathway (43, 44). HflB also has weak similarity with protease regulatory subunits in *E. coli*, including the ATP binding subunit ClpA (cited in ref. 11). These observations suggest that HflB is a component of a specific ATP-dependent proteolytic pathway.

Only a few ATP-dependent proteases have been identified in *E. coli* (30). The ATP-dependence of proteolytic activity has been proposed to make the protease an "intelligent machine" conferring substrate specificity (30). Except for Lon, these proteases have two subunits, one proteolytic and the other, with the ATPase activity, regulatory. HflB, which possesses putative ATPase activity, could be the regulatory subunit of the protease responsible for σ^{32} proteolysis or, like Lon, the protease itself. Recent *in vitro* data indicate that purified HflB in the presence of ATP degrades σ^{32} (T. Tomoyasu, J. Gamer, B. Bukau, M. Kanemory, H. Mori, A. J. Rutman, A. B. Oppenheim, T. Yura, K. Yamanaka, H. Niki, S. Hiraga, and T. Ogura, personal communication). This strongly suggests that HflB is the protease itself.

Although *hflB* mutants have several phenotypes (8, 10–12, 45), the only clear activity of HflB to date is to govern a specific protease activity—the first essential protease identified in *E. coli*. Cell death in the absence of HflB may reflect accumulation of lethal quantities of substrates of this protease. The one host target protein identified, σ^{32}, is indeed lethal when overproduced, providing a simple explanation for the essentiality of HflB. However, a comparison of two-dimensional protein gels from wild-type and *hflB29* strains (34) suggests that there are additional host targets that are not heat shock proteins (34) (consistent with the lack of stabilization of σ^{32} in *hflB29*) and that also may be toxic when overproduced. We suggest that other phenotypes of *hflB* mutants will ultimately be shown to result from stabilization of various host proteins.

The HflA complex, like HflB, has anti-λcII protease activity (32, 33), although it does not seem to be active on σ^{32}. No bacterial targets are known for this dispensable protease, but again comparison of two-dimensional protein gels suggests that there are such substrates, some of which, like λcII, may also be substrates for the HflB-governed protease (34).

The *E. coli* heat shock response is controlled principally by the concentration of σ^{32}, whose degradation is primarily governed by heat shock-induced proteins like DnaK, DnaJ, and GrpE. The new element of the σ^{32} degradation system, HflB, is also regulated by σ^{32}, contributing to homeostatic regulation of the heat shock response. The dispensable protein FtsJ (8) is a new heat shock protein coded for by the *ftsJ-hflB* operon; its role is unknown.

The HflB homologue from the Gram-positive bacterium *Lactococcus lactis* (46) is also induced by heat shock (P. Duwat and A. Gruss, personal communication) and seems functionally equivalent to HflB, since a cloned gene from this strain allows an *E. coli* Δ*hflB* mutant to survive at 30°C (46). Although other types of heat shock regulation have been proposed for Gram-positive species (47), these observations

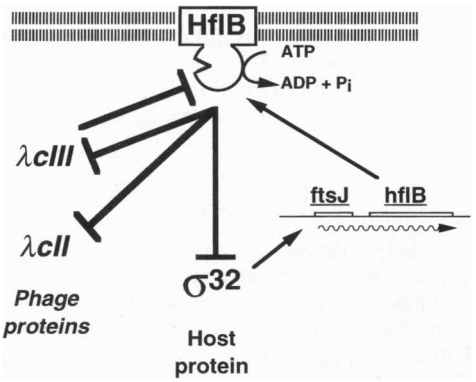

FIG. 6. The λ lysis–lysogeny decision and σ^{32} stability are controlled by the HflB-governed protease. HflB is an inner membrane protein (9) governing λcII proteolysis (11, 12). It has similarities with proteases and has putative ATPase activity (8). Inactivation of HflB stabilizes σ^{32}, whereas overproduction decreases its half-life. The *hflB* gene is positively regulated by σ^{32}, providing feedback control of σ^{32} stability. The λcIII protein stabilizes λcII and σ^{32} by inhibiting the HflB-governed protease.

suggest that the regulation of the heat shock response by HflB is highly conserved among bacteria; an equivalent to σ^{32} may exist in some Gram-positive species.

The λcIII protein, responsible for two apparently unrelated phenotypes—increase in λ lysogenization frequency and induction of the heat shock response—seems to be an inhibitor of the HflB-governed protease. HflB itself controls the degradation of at least two master regulatory proteins (Fig. 6): λcII, regulator of the lysis–lysogeny decision of phage λ (11, 12), and σ^{32}, principal determinant of the heat shock response.

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1. Morimoto, R. I., Tissières, A. & Georgopoulos, C. (1994) *Heat Shock Proteins and Molecular Chaperones* (Cold Spring Harbor Lab. Press, Plainview, NY).
2. Nover, L. (1991) *The Heat Shock Response* (CRC Press, Boca Raton, FL).
3. Grossman, A. D., Erickson, J. W. & Gross, C. A. (1984) *Cell* **38,** 383–390.
4. Straus, D. B., Walter, W. A. & Gross, C. A. (1987) *Nature (London)* **329,** 348–351.
5. Tilly, K., McKittrick, N., Zylicz, M. & Georgopoulos, C. (1983) *Cell* **34,** 641–646.
6. Straus, D., Walter, W. & Gross, C. A. (1990) *Genes Dev.* **4,** 2202–2209.
7. Liberek, K., Galistski, T. P., Zylicz, M. & Georgopoulos, C. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3516–3520.
8. Tomoyasu, T., Yuki, T., Morimura, S., Mori, H., Yamanaka, K., Niki, H., Hiraga, S. & Ogura, T. (1993) *J. Bacteriol.* **175,** 1344–1351.
9. Tomoyasu, T., Yamanaka, K., Murata, K., Suzaki, T., Bouloc, P., Kato, A., Niki, H., Hiraga, S. & Ogura, T. (1993) *J. Bacteriol.* **175,** 1352–1357.
10. Akiyama, Y., Ogura, T. & Ito, K. (1994) *J. Biol. Chem.* **269,** 5218–5224.
11. Herman, C., Ogura, T., Tomoyasu, T., Hiraga, S., Akiyama, Y., Ito, K., Thomas, R., D'Ari, R. & Bouloc, P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10861–10865.
12. Banuett, F., Hoyt, M. A., McFarlane, L., Echols, H. & Herskowitz, I. (1986) *J. Mol. Biol.* **187,** 213–224.
13. Hoyt, M. A., Knight, D. M., Das, A., Miller, H. I. & Echols, H. (1982) *Cell* **31,** 565–573.
14. Bahl, H., Echols, H., Straus, D. B., Court, D., Crowl, R. & Georgopoulos, C. P. (1987) *Genes Dev.* **1,** 57–64.
15. Kornitzer, D., Altuvia, S. & Oppenheim, A. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5217–5221.
16. Campbell, A. (1961) *Virology* **14,** 22–52.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
18. Cooper, S. & Ruettinger, T. (1975) *Mol. Gen. Genet.* **139,** 167–176.
19. Singer, M., Baker, T., Schnitzler, G., Deischel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. W. & Gross, C. A. (1989) *Microbiol. Rev.* **53,** 1–24.
20. Santos, D. & de Almeida, D. F. (1975) *J. Bacteriol.* **124,** 1502–1507.
21. Rasmussen, L. J., Møller, P. L. & Atlung, T. (1991) *J. Bacteriol.* **173,** 6390–6397.
22. Miller, J. H. (1992) *A Short Course in Bacterial Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
23. Tilly, K., Spence, J. & Georgopoulos, C. (1989) *J. Bacteriol.* **171,** 1583–1589.
24. Amann, E., Brosius, J. & Ptashne, M. (1983) *Gene* **25,** 167–178.
25. Van Melderen, L., Bernard, P. & Couturier, M. (1994) *Mol. Microbiol.* **11,** 1151–1157.
26. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
27. Barry, G., Squires, C. & Squires, C. L. (1980) *Proc. Natl. Acad. Sci. USA* **75,** 1274–1278.
28. Mecsas, J., Rouviere, P. E., Erickson, J. W., Donohue, T. J. & Gross, C. A. (1993) *Genes Dev.* **7,** 2618–2628.
29. Yano, R., Imai, M. & Yura, T. (1987) *Mol. Gen. Genet.* **207,** 24–28.
30. Gottesman, S. & Maurizi, M. R. (1992) *Microbiol. Rev.* **56,** 592–621.
31. Maurizi, M. R. (1987) *J. Biol. Chem.* **262,** 2697–2703.
32. Banuett, F. & Herskowitz, I. (1987) *J. Bacteriol.* **169,** 4076–4085.
33. Cheng, H. H., Muhlrad, P. J., Hoyt, M. A. & Echols, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7882–7886.
34. Cheng, H. H. & Echols, H. (1987) *J. Mol. Biol.* **196,** 737–740.
35. Gamer, J., Bujard, H. & Bukau, B. (1992) *Cell* **69,** 833–842.
36. Chuang, S.-E. & Blattner, F. R. (1993) *J. Bacteriol.* **175,** 5242–5252.
37. Kohara, Y., Akiyama, K. & Isono, K. (1987) *Cell* **50,** 495–508.
38. Yura, T., Nagai, H. & Mori, H. (1993) *Annu. Rev. Microbiol.* **47,** 321–350.
39. Harr, R., Haggstrom, M. & Gustafsson, P. (1983) *Nucleic Acids Res.* **11,** 2843.
40. Erdmann, R., Wiebel, F. F., Flessau, A., Rytka, J., Beyer, A., Fröhlich, K.-U. & Kunau, W.-H. (1991) *Cell* **64,** 499–510.
41. Dubiel, W., Ferrell, K., Pratt, G. & Rechsteiner, M. (1992) *J. Biol. Chem.* **267,** 22699–22702.
42. Rechsteiner, M., Hoffman, L. & Dubiel, W. (1993) *J. Biol. Chem.* **268,** 6065–6068.
43. Ghislain, M., Udvardy, A. & Mann, C. (1993) *Nature (London)* **366,** 358–361.
44. Gordon, C., McGurk, G., Dillon, P., Rosen, C. & Hastie, N. D. (1993) *Nature (London)* **366,** 355–357.
45. Begg, K. J., Tomoyasu, T., Donachie, W. D., Khattar, M., Niki, H., Yamanaka, K., Hiraga, S. & Ogura, T. (1992) *J. Bacteriol.* **174,** 2416–2417.
46. Nilsson, D., Lauridsen, A. A., Tomoyasu, T. & Ogura, T. (1994) *Microbiology* **140,** 2601–2610.
47. Zuber, U. & Shumann, W. (1994) *J. Bacteriol.* **176,** 1359–1363.