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The HflB Protease of *Escherichia coli* Degrades Its Inhibitor λcIII

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The cIII protein of bacteriophage λ is known to protect two regulatory proteins from degradation by the essential *Escherichia coli* protease HflB (also known as FtsH), viz., the λcII protein and the host heat shock sigma factor σ^{32}. λcIII, itself an unstable protein, is partially stabilized when the HflB concentration is decreased, and its half-life is decreased when HflB is overproduced, strongly suggesting that it is degraded by HflB in vivo. The in vivo degradation of λcIII (unlike that of σ^{32}) does not require the molecular chaperone DnaK. Furthermore, the half-life of λcIII is not affected by depletion of the endogenous ATP pool, suggesting that λcIII degradation is ATP independent (unlike that of λcII and σ^{32}). The λcIII protein, which is predicted to contain a 22-amino-acid amphipathic helix, is associated with the membrane, and nonlethal overproduction of λcIII makes cells hypersensitive to the detergent sodium dodecyl sulfate. This could reflect a direct λcIII-membrane interaction or an indirect association via the membrane-bound HflB protein, which is known to be involved in the assembly of certain periplasmic and outer membrane proteins.

Infection of a bacterial cell by a temperate bacteriophage can result in cell lysis and virion production or in the establishment of the lysogenic state, in which all but a small number of phage genes are repressed by phage-specified repressors (37). In the case of phage λ and its host *Escherichia coli*, most of the regulatory elements determining the choice between lysis and lysogeny have been identified (13). It was early shown (24) that maintenance of the lysogenic state requires the λcI repressor and that establishment of repression requires, in addition, the λcII and λcIII proteins, which are not expressed in established lysogens. λcII activates the transcription of the CI gene, and λcIII partially stabilizes the highly unstable λcII protein (12).

Many lambdoid phages have been described, all with similar gene disposition and regulation. In general, the regulatory proteins are not interchangeable. A striking exception is cIII, which is not specific to a given phage (26). The cI and cII proteins are known to act at specific sites on the phage DNA. The interchangeability of cIII suggests that its target is a host protein or site (26).

The λcIII protein has 54 amino acids (10). The domain from residues 16 to 37 can form an amphipathic helix and seems to be sufficient for λcIII activity (26). Other cIII homologs appear to have a similar amphipathic helix, and mutations in this region can result in loss of at least some cIII activities (26). The λcIII protein, in addition to stabilizing λcII, also stabilizes the host sigma factor σ^{32}, responsible for transcribing genes of the heat shock regulon (4). Both λcII and σ^{32} are substrates of the essential host protease HflB, also known as FtsH (5, 18, 19, 43). Overexpression of λcIII prevents cell growth (26), and this inhibition is relieved by simultaneous overexpression of HflB (19, 43), which strongly suggests that λcIII inhibits this protease and is consistent with the idea that the cIII target is host specified.

HflB is an essential 70-kDa protein anchored in the inner membrane by its amino terminus, with its active domain in the cytoplasm (2, 44). Its sequence includes a conserved 200-amino-acid module characteristic of the so-called AAA family of proteins (stands for ATPase associated with various activities) (9, 28, 45), and it also has an HEXXH motif characteristic of zinc proteases. Purified HflB can hydrolyze σ^{32} in vitro; the reaction requires ATP and is stimulated by certain metal ions (17, 43). This demonstrates that HflB has protease activity and makes it the first essential protease identified in *E. coli.*

λcIII is itself unstable (27), implying the existence of a proteolytic system capable of degrading it. The known properties of λcIII—stabilization of λcII and σ^{32} and growth inhibition of the host cell—could be explained if λcIII inhibits or competes efficiently for HflB. In the present report, we present data showing that λcIII is indeed a substrate of HflB and suggesting that it affects the inner membrane.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. The strains used in this work are all *E. coli* K-12 derivatives (Table 1). There are isogenic strains derived from JM105 (46), which carries a *lacI*^{q} allele and overproduces the Lac repressor.

P1 *vir*-mediated transduction was carried out as previously described (33). Plasmid preparations, transformation, cloning, and ligation were carried out as described by Sambrook et al. (38). The Δ*hflB3*::*kan* (Δ*ftsH3*::*kan*) allele was introduced by transduction to kanamycin resistance in the presence of 70 μM L-arabinose for strains carrying plasmid pBHB1.

Plasmid pAR145 (45) carries the intact *hflB* gene cloned downstream of the *lac* promoter in a low-copy-number, chloramphenicol-resistant pSC101 derivative, pHSG575. Plasmid pSTD1-1 (3) 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. Plasmid pSTD61 (3) carries the ATPase-negative *hflB* gene cloned downstream of the *lac* promoter in pHSG575. Plasmid p*tac*cIII (27) carries the λCIII gene under control of the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible *tac* promoter of the ampicillin-resistant vector p*tac* 12H. Plasmid pCG179 (42) carries the *rpoH* gene under control of the *tac* promoter.

Plasmid pBHB1 carries the intact *hflB* gene cloned downstream of the *ara* promoter in a low-copy-number, chloramphenicol-resistant pACYC184 derivative, pBAD33 (16). This plasmid was constructed by ligating the *Eco*RI-*Hin*dIII fragment of pSTD1-1 with the intact *hflB* gene inserted at the polylinker *Eco*RI-*Hin*dIII sites of pBAD33.

Plasmid pTar carries the cII*cy42* mutant gene cloned downstream of the p^{tac} promoter on a pBR322 derivative, pKK226-3. This plasmid was constructed by ligating the blunted *Nsi*I-*Bgl*II fragment of phage 434 cII*cy42* into the *Hin*cII site of pUC18, to give pULB6227. The *Eco*RI-*Hin*dIII fragment of pULB6227 containing cII*cy42* was inserted into the *Eco*RI-*Hin*dIII polylinker of pKK223-3, to give pTar.

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TABLE 1. Bacterial strains

Strain	Genotype	Origin or construction
JM105	*supE endA sbcB15 hsdR4 rpsL thi* Δ(*lac-proAB*)/F′ (*traD36 pro*$^+$ *lacI*^{q} *lacZ*ΔM15)	46
WM1389	*leuB6 thr-1*::Tn*10 supE44 lacY1 dnaK756*(Ts)	32
GW8306	*araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rpsR fibB301* Δ*dnaK52 sidB1*/pJM41/pJM100	30
SL93	*lacZ3350 galK2 galT22 rpsL179 zgj-203*::Tn*10 hflB1*(Ts)	18
SL320	JM105/p*tac*cIII	19
SL353	*lacZ3350 galK2 galT22 rpsL179 zgj-25*::Tn*10* Δ*hflB3::kan*/pAR171	17
SL430	JM105/p*tac*cIII/pAR145	19
SL438	JM105/pCG179	19
SL453	JM105/p*tac*12H	19
SL471	JM105 *dnaK756 thr-1*::Tn*10*	JM105 + P1/WM1389
SL475	JM105/p*tac*cIII/pHSG575	SL320 + pHSG575
SL552	JM105 *dnaK756 thr-1*::Tn*10*/p*tac*cIII	SL471 + p*tac*cIII
SL584	JM105 *zgj-203*::Tn*10 hflB1*(Ts)	JM105 + P1/SL93
SL586	JM105 *zgj-203*::Tn*10 hflB1*(Ts)/p*tac*cIII	SL320 + P1/SL93
SL711	JM105 Δ*dnaK52*/p*tac*cIII	SL320 + P1/GW8306
SL745	JM105/p*tac*cIII/pSTD61	SL320 + pSTD61
SL765	JM105/pBHB1	JM105 + pBHB1
SL785	JM105/pBHB1/p*tac*cIII	SL765 + p*tac*cIII
SL790	JM105 Δ*hflB3*::*kan*/pBHB1/p*tac*cIII	SL785 + P1/SL353

Culture conditions. Cells were grown in M63 medium supplemented with 0.4% glycerol and 1 μg of thiamine per ml or in Luria-Bertani (LB) broth (33). For HflB depletion, cells were grown in M63 glucose medium plus 19 amino acids (lacking proline). 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.

Measurement of cIII stability. All strains carried a *lacI*^{q} allele and plasmid p*tac*cIII, in which the CIII gene is under control of the *tac* promoter. Cells were grown at 30°C in glycerol minimal medium supplemented with antibiotics and then shifted to 37 or 42°C ($t = -36$ min), induced with 1 mM IPTG ($t = -16$ min), pulse-labeled ($t = -1$ min) with [^{14}C]proline (9.32 GBq/mmol, 1.85 MBq/ml; ICN Biomedicals, Inc.), and then chased ($t = 0$) with 100 μg of cold proline per ml. In experiments with pBHB1, cultures were shifted to 37°C at the time arabinose was removed. Samples were electrophoresed on 14% sodium dodecyl sulfate (SDS)-polyacrylamide Tris-tricine gels. The λcIII band was clearly visible on the gels at an apparent molecular weight of 6,000; radioactivity in this band was revealed with a Molecular Dynamics PhosphorImager and quantified with the ImageQuant 1.33 program (Molecular Dynamics).

ATP depletion. ATP depletion was essentially as described by Olden and Goldberg (35). Cells were grown in M63 minimal glycerol medium to mid-log phase; the culture was then divided in two parts, one of which was depleted of ATP by addition of 1 mM sodium cyanide, 10 mM sodium arsenate, and 20 mM α-methylglucoside. Pulse-chase experiments were identical to those at 37°C described above, except that the inhibitors were added 2 min after the pulse and the first sample was taken 6 min after the chase, since the intracellular level of ATP decreases by 95% within 10 min (35).

Membrane isolation. A culture of strain SL320, growing exponentially at 37°C in minimal glycerol medium supplemented with antibiotics, was induced for 15 min with 10^{-4} M IPTG, pulsed for 4 min with [^{14}C]proline (9.32 GBq/mmol, 1.85 MBq/ml; ICN Biomedicals, Inc.), and put on ice. All subsequent steps were carried out at 0°C. Labeled cells were collected by centrifugation of 10 ml (5 min at 2,000 × *g*). The pellet was resuspended in 10 ml of HEPES buffer (10^{-2} M, pH 7.4) containing 10^{-3} M proline and centrifuged again. The washed cells were resuspended in 1 ml of the same buffer. The cell suspension was then disrupted by sonication (1 min, Soniprep 150, MSE); unbroken cells were removed by centrifugation (5 min at 2,000 × *g*). The supernatant fraction (1 ml) was layered on a two-step gradient consisting of 0.8 ml of 60% sucrose and 2.5 ml of 25% sucrose and centrifuged for 90 min in a Beckman SW50.1 rotor at 43,000 rpm. Two adjacent bands were visible near the 25-to-60% sucrose interface. The bands were removed together by puncturing the side of the tube with a no. 20 needle attached to a syringe. The resulting suspension (crude membrane, 0.5 ml) was precipitated with 5% cold trichloroacetic acid. The upper part of the tube (cytoplasm, 1.6 ml) was precipitated under the same conditions. The pellets were solubilized in SDS sample buffer and electrophoresed on SDS–14% polyacrylamide Tris-tricine gels. Radioactivity in the λcIII band was revealed with a Molecular Dynamics PhosphorImager and quantified with the ImageQuant 1.33 program (Molecular Dynamics).

Density gradient analysis. The membrane fraction, isolated as described above, was adjusted to 30% sucrose (wt/wt), measured by refractometry, and deposited on a flotation gradient according to the method described by Ishidate et al. (23). After centrifugation in a Beckman SW41 rotor at 36,000 rpm for 16 h, the inner and outer membranes are found in the density range 1.22 to 1.27; inclusions, which are denser, would be at the bottom of the tube.

RESULTS

Proteolytic degradation of λcIII depends on HflB. The λcIII protein stabilizes λcII and σ^{32}, and overproduction inhibits cell growth. We previously presented evidence suggesting that these phenotypes result from inhibition of HflB activity by λcIII (19). The λcIII protein is itself unstable (27), although the proteolytic pathway degrading it has not been identified. Since the inhibition of HflB by λcIII might involve a direct interaction between the two proteins, we tested the influence of HflB on λcIII stability.

We first measured the effect on λcIII stability of reduced HflB activity. For this purpose, we constructed plasmid pBHB1, placing the *hflB* gene under control of the arabinose-inducible promoter p^{araBAD} (16). A pBHB1-bearing strain was then transduced to Δ*hflB*::*kan*, deleting the chromosomal copy of *hflB*. This strain absolutely required arabinose to grow; its plating efficiency on LB plates lacking arabinose was 10^{-3}. The λcIII protein was provided by plasmid p*tac*cIII, in which the λcIII gene is cloned under control of the IPTG-inducible *tac* promoter (27). Strain SL790, of genotype Δ*hflB*::*kan*/pBHB1/p*tac*cIII, was grown in minimal glycerol medium containing 19 amino acids and 30 μM arabinose at 37°C. The culture was centrifuged, washed, and resuspended in the same medium lacking arabinose but containing glucose, to prevent expression of the p^{araBAD} promoter. After 5 h of incubation at 37°C, the half-life of λcIII, determined by pulse-chase, was 26 min (Fig. 1), compared to 10 min in the presence of arabinose (not shown) and 7 min in the wild-type strain (Fig. 1).

The *hflB1*(Ts) mutation strongly reduces the proteolysis of λcII (35a) and σ^{32} at 42°C (19, 43). However, the half-life of λcIII in an *hflB1*/p*tac*cIII strain after 30 min at 42°C was only slightly increased (Fig. 2). This is reminiscent of the substrate specificity exhibited by the *hflB29* mutant, in which λcII is stabilized (5, 18) but σ^{32} is not (19).

Since a decrease in HflB partially stabilizes λcIII, increasing the HflB concentration might be expected to destabilize it, as is the case for σ^{32} (19, 43). This would account for our earlier result that an excess of HflB counteracts the lethality of λcIII overproduction (19, 43). Strain SL430 carries plasmid pAR145, in which the expression of *hflB* is under control of the p^{lac} promoter (45). The half-life of λcIII was 2.7 min when HflB synthesis was induced (Fig. 2), compared to 9.9 min in the

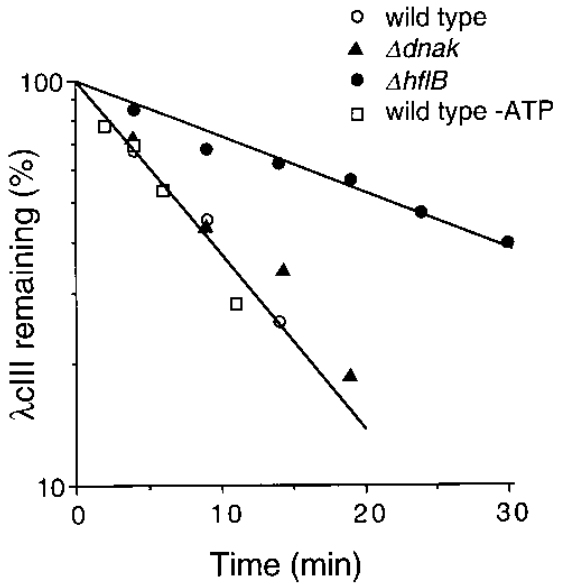

FIG. 1. Kinetics of λcIII degradation. Pulse-chase experiments were carried out at 37°C as described in Materials and Methods. Strains were SL320 (open circles), SL711 (closed triangles), SL790 (closed circles), and SL320 with a depletion of ATP (open squares). All experiments were repeated two to four times, with at least four points for each chase; there was no significant variation in the results.

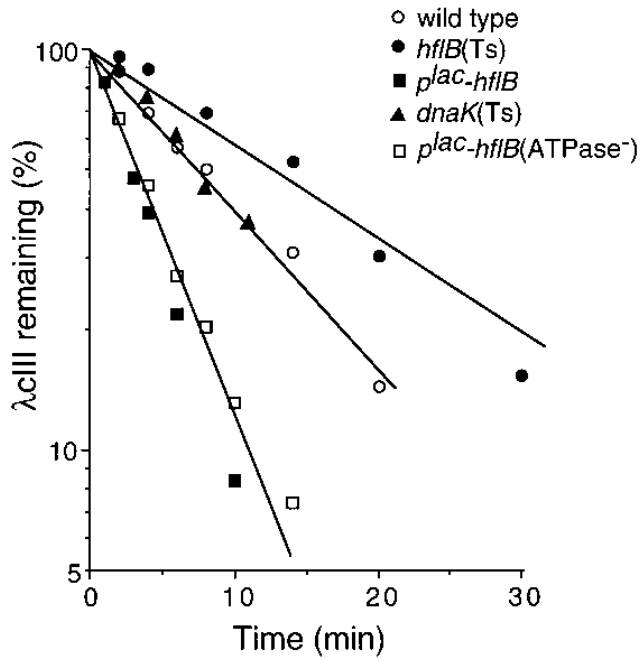

FIG. 2. Kinetics of λcIII degradation. Pulse-chase experiments were carried out at 42°C as described in Materials and Methods. Strains were SL320 (open circles), SL586 (closed circles), SL430 (closed squares), SL552 (closed triangles), and SL745 (open squares).

control strain, carrying only the vector (data not shown). Thus, there is a correlation between the quantity of HflB protease and the instability of λcIII.

λcIII degradation is DnaK independent. The molecular chaperones DnaK, DnaJ, and GrpE are required for degradation of σ^{32} (14, 29, 40, 41), probably to present it to the protease HflB. A peptide composed of residues 14 to 37 of λcIII has strong affinity for DnaK in vitro (7). Since λcIII, like σ^{32}, is degraded via the HflB pathway, we looked for a possible role of this chaperone pathway in λcIII action and degradation.

An excess of λcIII blocks bacterial growth (4, 26), probably by inhibiting HflB (19, 43). If λcIII must be presented to HflB by DnaK, λcIII lethality might be relieved in the absence of DnaK. We constructed strain SL711, with a deletion of *dnaK* and carrying the plasmid p*tac*cIII. This strain is viable (in the absence of IPTG) at temperatures between 30 and 37°C (36). Its plating efficiency was 2×10^{-4} when λcIII synthesis was induced on glucose-plus-IPTG plates at 37°C, compared to 3×10^{-4} in the control (*dnaK*$^+$) strain.

We next measured λcIII stability in the absence of DnaK. In strain SL711, the half-life of λcIII was not affected by the absence of DnaK (Fig. 1). It was also unaffected in a *dnaK756*(Ts) mutant at 42°C (Fig. 2).

These observations show that λcIII-induced lethality and λcIII degradation are DnaK independent.

ATP depletion does not prevent λcIII proteolysis. ATP depletion has been shown to stabilize most abnormal proteins in vivo (25, 35). In vitro work, however, has suggested that ATP hydrolysis may not be required for the degradation of small oligopeptide substrates by Lon and Clp (15). HflB has ATPase activity, and the degradation of σ^{32} by purified HflB in vitro absolutely requires ATP hydrolysis (43). λcIII, with a molecular mass of 6.0 kDa, is a rather small protein. We therefore examined the ATP dependence of its proteolysis in vivo.

Inactivation of the ATP-binding motif of HflB by site-directed mutagenesis has been shown to have a dominant negative lethal effect (3). Plasmid pSTD61 carries an ATPase-negative *hflB* allele under control of the *lac* promoter (3), and this mutant protein has recently been shown to lack ATPase activity in vitro (1). Nevertheless, induction of this dominant mutant HflB protein destabilized λcIII as much as wild-type HflB (Fig. 2).

It has been shown that the addition to an *E. coli* culture of a mixture of inhibitors of ATP synthesis can cause a 20-fold drop in the endogenous ATP pool (35). We added this mixture to an exponentially growing culture of a wild-type strain carrying the plasmid p*tac*cIII (see Materials and Methods). The collapse of the ATP pool did not slow down the proteolysis of λcIII (Fig. 1).

In a control experiment, we used a plasmid coding for the unstable phage regulator, λcII. (We could not use σ^{32}, since its hydrolysis requires DnaK, which in turn requires ATP.) The λcII protein is a DnaK-independent substrate of HflB (35a). The half-life of λcII, 3 min without inhibitors, became 10 min in the absence of ATP, validating the use of these inhibitors (Fig. 3).

The observations presented above strongly suggest that λcIII degradation by HflB is ATP independent.

λcIII is associated with the membrane. λcIII has a domain capable of forming an amphipathic helix, which could have affinity for membranes (31). Furthermore, HflB is anchored in the inner membrane, meaning that λcIII must approach the membrane to be degraded. We therefore looked to see whether the λcIII protein is membrane associated.

We fractionated crude extracts of cells overproducing λcIII by ultracentrifugation. The λcIII protein was indeed predominantly in the membrane fraction (Fig. 4). No inclusion bodies or aggregates of λcIII were formed during this short induction period, as shown in a parallel experiment in which the membrane fraction was recentrifuged in a sucrose density gradient

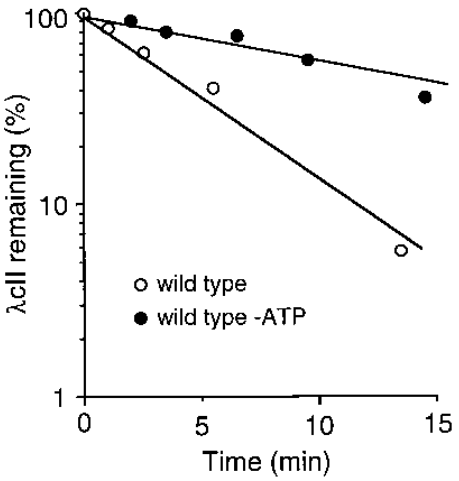

FIG. 3. Kinetics of λcII degradation. Pulse-chase experiments were carried out at 37°C as described in Materials and Methods. Strains were JM105/pTar (open circles) and JM105/pTar with a depletion of ATP (closed circles).

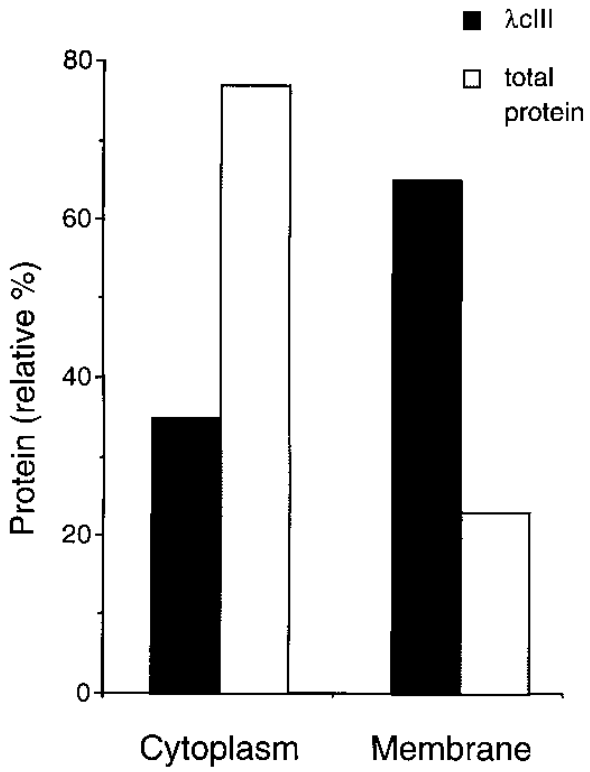

FIG. 4. λcIII is membrane associated. Crude extracts were fractionated as described in Materials and Methods. The percentages of total protein and of λcIII in the cytoplasmic and membrane fractions are shown.

(see Materials and Methods); the λcIII protein remained with the membrane material.

Cells with alterations in their membrane structure or its protein content often exhibit sensitivity to bile salts or detergents such as SDS. We speculated that the association of λcIII with the membrane could result in such an alteration. The expression of λcIII in strain SL320 was induced by means of p*tac*cIII and 10^{-4} M IPTG. This level of λcIII expression did not significantly affect cell viability on LB plates at 37°C; however, it dramatically lowered survival in the presence of 0.2% SDS (Table 2).

λcIII expression stabilizes σ^{32}, resulting in an increased concentration, and excess σ^{32} can, in some conditions, prevent colony formation. To see whether the SDS sensitivity observed by λcIII overproduction was caused simply by the higher σ^{32} concentration, we repeated the experiment with strain SL438, which carries a plasmid with the σ^{32} structural gene *rpoH* under control of the *tac* promoter. The plating efficiency of strain SL438 on LB plates containing 0.2% SDS was not significantly affected by σ^{32} overproduction (Table 2).

It is possible that excess λcIII reduces HflB activity to produce the phenotype called Std^- (stands for stop transfer defective). Std^- alleles of *hflB* affect the assembly of certain periplasmic and outer membrane proteins (2), although the precise role of HflB in this process is not yet clear. However, a known *hflB*(Std) allele, transduced into strain JM105 or 594, did not cause SDS sensitivity.

The results presented above show that overproduction of λcIII, but not of σ^{32}, increases the cell's sensitivity to SDS, consistent with the observation that λcIII is membrane associated.

DISCUSSION

Lysogenization by phage λ requires a number of phage and host factors involved in establishing repression and in integrating the phage DNA within the bacterial genome. The unstable λcII protein is a key regulator of the lysis-lysogeny decision. At high concentrations, it activates the transcription of repressor and integrase genes, favoring lysogenization, whereas at low concentrations, the lytic pathway is chosen (12). The λcIII protein, by stabilizing λcII, plays a major role in the establishment of lysogeny. λcIII is a small protein of only 54 amino acids. From its primary structure, residues 16 to 37 are predicted to form an amphipathic helix. Typically, the hydrophobic face of such a structure is shielded from the surrounding medium by other domains of the protein. In the case of λcIII, however, there is not enough protein left to shield the helix. λcIII might form dimers, as suggested by Kornitzer et al. (26), or else interact with some other protein or with the membrane.

In vitro, the amphipathic domain of λcIII interacts strongly with the molecular chaperone DnaK (14). This is perhaps not unexpected, since chaperones are basically designed to recognize hydrophobic domains of proteins. Such regions are generally found in unfolded or denatured proteins, which the chaperones then fold properly or conduct to a proteolytic system, with the whole process requiring ATP hydrolysis. ATP-dependent proteases also tend to recognize hydrophobic domains of proteins as substrates, and indeed the two functions, those of chaperone and protease, overlap somewhat, at least at the level of substrate recognition.

The λcIII protein is unstable (27). In the present work, we show that its half-life in vivo increases when there is less HflB and decreases when there is excess HflB. This strongly suggests that λcIII is degraded by the essential host protease HflB, implying a direct λcIII-HflB interaction. These results can explain why an excess of HflB suppresses the lethality of λcIII. In agreement with this conclusion, recent in vitro data indicate that purified HflB protein degrades λcIII (35a).

The λcIII protein stabilizes the phage regulator λcII and the host regulator σ^{32}, both of which are substrates of HflB. Stabilization of these proteins may result simply from the fact that λcIII is an alternative substrate, competing for the active site of HflB. It is also possible that λcIII inhibits the ATPase activity of HflB, analogous to the action of the T4-encoded PinA

TABLE 2. λcIII-induced sensitivity to SDS

Growth condition	Efficiency of plating[a] in strain:		
	SL320 (p*tac*cIII)	SL438 [pCG179 (σ^{32})]	SL453 (control)
10^{-4} M IPTG	0.4	0.9	1.1
0.2% SDS	0.1	0.7	0.3
10^{-4} M IPTG + 0.2% SDS	$<2 \times 10^{-4}$	0.2	0.4

[a] Relative to titer on LB plates.

protein on Lon protease (19a, 39). A third possibility is that λcIII interacts directly with the substrates, preventing their degradation.

We also show that the in vivo degradation of λcIII does not require the chaperone DnaK. Thus the induction of the heat shock response by λcIII may reflect the λcIII-HflB interaction and not involve a λcIII-DnaK interaction, as suggested by Tomoyasu et al. (43). The in vivo degradation of λcII by HflB has also been shown to be DnaK independent (35a). This is in contrast to the hydrolysis of σ^{32} by HflB, which is DnaK dependent in vivo (although not in vitro [43]).

Furthermore, the hydrolysis of λcIII is not inhibited when the ATP pool is depleted or when a dominant ATPase-negative HflB protein is overproduced in the cell, suggesting that the reaction is ATP independent in vivo. This is again in contrast to the hydrolysis of σ^{32}, which requires ATP even in the absence of DnaK (43). It has been suggested that the role of ATP hydrolysis is to confer substrate specificity and accessibility (15). ATP-dependent proteases often possess associated chaperone activity (47), by which ATP hydrolysis may serve to uncover recognition signals. It is possible that the recognition signals of the small λcIII protein are readily found by HflB with less energy expenditure.

λcIII is found preferentially in the membrane fraction after ultracentrifugation of crude lysates (Fig. 4). This could reflect a direct interaction of the hydrophobic face of λcIII with the inner membrane or an indirect association via the interaction of λcIII with HflB, itself an integral membrane protein (31). Nonlethal overproduction of λcIII makes the cell sensitive to the detergent SDS. The original *hflB1* strain Y16 was also reported to be altered in envelope integrity (11), although the strain carried an *ftsI* mutation as well (6). Typically, SDS sensitivity is associated with alterations of the outer membrane, presumably letting more detergent reach the periplasmic space and inner membrane (34).

The λcII protein has been shown to be degraded in vivo and in vitro by the HflA proteolytic pathway (8, 20), which is thought to be independent of HflB (5, 18). This protease, dispensable for *E. coli* growth, does not have other known substrates at present. Genetic inactivation of *hflA* does not stabilize σ^{32} (19). It has been suggested that λcIII inhibits HflA-dependent degradation of λcII (20). However, it has not so far been possible to set up an experimental system entirely devoid of HflB, whether by mutation or by dilution of preexisting protein. Interference by residual HflB activity makes it difficult to evaluate possible effects of λcIII on HflA activity.

Unstable proteins are often regulators which the cell, under certain conditions, may wish to get rid of quickly. An example is the SulA protein, induced when DNA is damaged (21) and eliminated by Lon protease when the damage is repaired (22). The primary role of λcIII after infection is to encourage the lysogeny pathway, the end point of which is a viable lysogenic bacterium. Since λcIII seems to inhibit the essential HflB protein, it is clearly necessary to eliminate excess λcIII and restore HflB activity once lysogeny has been established.

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