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Genetics

Cell growth and λ phage development controlled by the same essential *Escherichia coli* gene, *ftsH/hflB*

(lysogenization/protease/chaperone/iron regulation)

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ABSTRACT **The λ phage choice between lysis and lysogeny is influenced by certain host functions in *Escherichia coli*. We found that the frequency of λ lysogenization is markedly increased in the *ftsH1* temperature-sensitive mutant. The *ftsH* gene, previously shown to code for an essential inner membrane protein with putative ATPase activity, is identical to *hflB*, a gene involved in the stability of the phage cII activator protein. The lysogenic decision controlled by FtsH/HflB is independent of that controlled by the protease HflA. Overproduction of FtsH/HflB suppresses the high frequency of lysogenization in an *hflA* null mutant. The FtsH/HflB protein, which stimulates cII degradation, may be a component of an HflA-independent proteolytic pathway, or it may act as a chaperone, maintaining cII in a conformation subject to proteolysis via such a pathway. Suppressor mutations of *ftsH1* temperature-sensitive lethality, located in the *fur* gene (coding for the ferric uptake regulator), did not restore FtsH/HflB activity with respect to λ lysogenization.**

When temperate phage infect a host cell, two productive outcomes are possible, lysis and lysogeny (for reviews see refs. 1 and 2). Many factors influence this decision, including the genotype and the physiological state of the infected cell, the phage genotype, and the multiplicity of infection. For phage λ, *Escherichia coli* mutants are known which favor the lytic response (*lon, cya*) or the lysogenic response (*hflA, hflB*) (for review see ref. 3). Phage λ itself has complex regulation, changes in which can tip the balance one way or the other. Perhaps the most important single regulator is the phage cII protein (4). This protein is a transcriptional activator of the c*I* gene (coding for the phage repressor, which maintains the lysogenic state once established) and of the *int* gene (coding for integrase, required for integration of the phage DNA into the bacterial chromosome, a prerequisite for inheritance of the lysogenic state). Thus, a high cII concentration shortly after infection tends to favor lysogeny, whereas a low cII concentration favors lysis (5, 6).

The cII concentration seems to be the critical factor in the lysis–lysogeny decision, and the regulation of this concentration is intricate. Transcription of the c*II* gene is repressed by the phage repressors cI and Cro and enhanced by the N protein through antitermination. cII translation is enhanced by the host protein complex IHF. Once synthesized, cII protein is rapidly degraded. Its stability is affected positively by the phage cIII protein and negatively by the host *hflA* and *hflB* gene products, and the HflA protein complex has been shown to cleave cII *in vitro* (5–7).

It is remarkable that under exponential growth conditions some infected cells of a bacterial population follow the lytic pathway and others the lysogenic. The basis for this difference is unclear. One possibility is that cell age can influence the decision between lysis and lysogeny. Better knowledge of host products that affect the decision may help shed light on the basis for differences among individual cells.

We demonstrate here that the *ftsH1* mutation markedly alters the balance between lysis and lysogeny. The original *ftsH* strain, Y16, makes filaments at 42°C and is thermosensitive for growth (8). It was found to contain two mutated genes, *ftsI* and *ftsH* (9). Filamentation is due to the alteration of the *ftsI* gene, encoding a septum-forming enzyme, penicillin-binding protein 3 (PBP3), whereas thermosensitivity (and strain-dependent filamentation) is due to the *ftsH1* mutation (9). The *ftsH* gene is located at 69 min on the *E. coli* genetic map (8) and encodes a 70.7-kDa protein (10, 11). The deduced protein sequence has striking homologies (38–48% identity in a stretch of ≈200 amino acids) with a eukaryotic protein family having putative ATPase activity, including Sec18p, Cdc48p, Pas1p, VCP, NSF, SUG, TBP-1, MSS1p, and BCS1p (11, 12). In contrast to its eukaryotic homologues (except BCS1), FtsH is a membrane protein, anchored in the inner membrane at the NH_2 terminus, with most of the protein facing into the cytoplasm (13). It has been suggested that FtsH is required for normal PBP3 activity since the amount of PBP3 in the membrane decreases in the *ftsH1* mutant (refs. 9, 10, and 14; T.T. and T.O., unpublished observation).

We show here that the *ftsH* gene is identical to *hflB*, which is involved in λ phage development. The *hflB* gene (for high frequency of lysogenization) was identified through a bacterial mutant which enhances the lysogenic response (6, 15). The *hflB29* mutation results in increased stability of the phage cII protein; it has been suggested that the HflB protein codes for a protease active on cII (6). In the absence of phage, the mutant does not exhibit any particular physiological phenotype (6).

The discovery that FtsH and HflB are identical and the collective data on these two proteins lead us to propose that FtsH/HflB governs an essential ATP-dependent protease activity. It may be a membrane component of this protease. Alternatively, it may be a chaperone-like protein which maintains potential protease substrates in an accessible configuration. In either case, it seems to be involved in proteolysis by a specific protease. Suppressors of *ftsH1* lethality have been located in the *fur* gene, encoding the ferric uptake regulator. One explanation of this observation is that the Fur protein (or a Fur-controlled protein) is a cellular target of the FtsH/HflB-controlled protease. Alternatively, FtsH/HflB activity could be related to the cell's iron metabolism; by analogy with an FtsH eukaryotic homologue, we suggest that it might participate in the assembly of iron–sulfur proteins.

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MATERIALS AND METHODS

Bacterial Strains, Phages, and Plasmids. The strains used in this work are all *E. coli* K-12 derivatives. We constructed two isogenic series, one in strain 594, genotype *lac-3350* (*lacZ lacI*$^+$) *galK2 galT22 rpsL179* (16), and one in strain CSH50, genotype Δ(*pro–lac*) *thi araD139 rpsL* (17). The *ftsH1* and *hflB29* alleles were introduced by cotransduction with a *zgj-203*::Tn*10* insertion (which is also present in the *ftsH*$^+$ strains) and the *fur*::Tn*5* and *hflA*::Tn*5* alleles by direct transduction. The sources of mutations were Y16 for *ftsH1* (8), X9393 for *hflB29* (6), X9392 for *hflA*::Tn*5* (6), QC1470 for *fur*::Tn*5* (18), and CAG12152 for *zgj-203*::Tn*10* (19). The λ phages used were λ c$^+$ (20), λ cIII67 (4), λ c17 (21), λ c17 cII (22), λ c17 *int* (laboratory collection), λ cI857(Ts) Cam105 (23), and λ c$^+$ Cam105 (see below). Phage P1 vir was used for transduction as described (17). Plasmid pAR145 carries the intact *ftsH* gene cloned downstream of the *lac* promoter of a low copy number chloramphenicol-resistant pSC101 derivative, pHSG575 (13). In the absence of induction, FtsH is expressed, presumably from its own promoter. Plasmid pULB6234 carries an *Eco*RI–*Pst* I fragment with the intact *ftsH* gene inserted at the *Eco*RI and *Pst* I sites of low copy number vector pRK7813 (24). Since pULB6234 confers tetracycline resistance, it was used for lysogeny frequency tests with λ c$^+$ Cam105.

The phage λ c$^+$ Cam105 was constructed by crossing λ cI857 Cam105 (25) and λ c$^+$ (multiplicity of infection 5 each). Strain 594 was infected with the lysate at a multiplicity of infection of 10^{-4}, and chloramphenicol-resistant lysogens were selected at 42°C. A culture of one such lysogen was irradiated with UV light to produce a phage lysate. To confirm that the lysate contained the recombinant phage λ c$^+$ Cam105, isolated plaques were shown to contain temperature-resistant, chloramphenicol-resistant lysogens.

Media and Growth Conditions. *E. coli* strains were grown in LB broth (17). For λ growth, TA or TB medium (26) was used. Top agar contained 0.7% agar and solid media contained 1.2% or 1.5% agar. Antibiotics, when required, were added at the following concentrations: chloramphenicol (Cm) 20 μg/ml, kanamycin (Km) 40 μg/ml, and tetracycline (Tc) 10 μg/ml.

Test of the Hfl$^-$ Phenotype. The Hfl$^-$ phenotype was determined by analysis of plaque morphologies as described (6). A lawn of the bacterial strain to be tested was prepared by adding 2.5 ml of TA top agar to 0.2 ml of a saturated culture in TA supplemented with 0.4% maltose and plating on a TA plate. Suspensions of λ c$^+$, λ c17, λ c17 cII, and λ cIII at titers of 10^6 to 10^7 phage per ml were spotted on the lawns and the plates were incubated overnight at 30°C, 37°C, or 42°C.

Measurement of Lysogenization Frequencies. Bacterial cultures were grown to an OD_{600} of 0.6 at 30°C in TB supplemented with 10 mM $MgSO_4$ and 0.4% maltose. Cells were pelleted and resuspended in TA containing phage (multiplicity of infection 10^{-1}). After 30 min of adsorption at 0°C, free phage were removed by centrifugation and washing with cold 10 mM $MgSO_4$. The infected bacteria were resuspended in TB medium and incubated for 30 min at 30°C, 37°C, or 42°C. Appropriate dilutions were then plated at 30°C on a bacterial lawn to calculate the number of infectious centers and on TA plates supplemented with chloramphenicol to calculate the number of lysogens. Cells which are lysogenized nevertheless form infectious centers because of spontaneous induction during colony formation; with a *himA* strain as indicator bacteria, all lysogens make visible plaques, even when the lysogenization frequency is near 100%. The frequency of lysogenization is the ratio of lysogens to infectious centers. With this procedure, phage adsorption occurs at 0°C, whereas the lysis–lysogeny decision is made while the cells are growing at 30°C, 37°C, or 42°C.

Cloning and Sequencing of the *ftsH* Gene of the *hflB29* Mutant. Chromosomal DNA of the *hflB29* strain X9393 was prepared and digested with restriction endonucleases *Pst* I and *Sal* I. After electrophoresis, fragments of ≈3 kilobase pairs were recovered and cloned in the vector pHSG575 (27) digested with the same enzymes. Thirty plasmids were screened by restriction analysis. One, carrying the *ftsH* gene, was named pAR244. Double-stranded plasmid DNA was sequenced by using a BcaBest kit (Takara Shuzo, Kyoto). Synthetic primers (17- and 18-mers) corresponding to regions upstream of or within the *ftsH* gene were used.

Southern blot analysis was carried out according to Sambrook *et al.* (28).

RESULTS

Hfl$^-$ Phenotype of the *ftsH1* Mutant. In a given bacterial population, the statistical decision between lysis and lysogeny by phage λ can be estimated by the plaque morphology of λ c$^+$. Total lysis gives clear plaques, whereas total lysogeny generates no visible plaque; intermediate situations produce a range of plaque turbidity. Phage λ c$^+$ makes turbid plaques on wild-type bacteria due to the growth of immune lysogens within the plaques; on a bacterial mutant enhancing the lytic pathway the plaques are clearer, while on a mutant enhancing lysogeny the plaques become very turbid. Phage λ c17 (21) constitutively synthesizes the cII protein (positive regulator of the c*I* and *int* genes) and the O and P proteins (required for replication initiation), all in the "right" operon of λ. After infection with this mutant, cells which have chosen the lysogenic pathway nevertheless die. On bacterial mutants which strongly favor lysogeny, λ c17 does not produce plaques. λ c17 cI90 and λ c17 cII are able to form plaques on these mutants, so lytic growth is clearly possible. The failure of λ c17 to grow on bacterial mutants favoring lysogeny may be because it integrates efficiently and subsequently replicates *in situ* due to the constitutive expression of O and P, thereby killing the lysogen. In support of this explanation, we observed that λ c17 *int* (deficient for integrase) makes plaques on the *hflA*::Tn*5* strain, showing that integration of the phage is necessary for the growth inhibition (data not shown).

On the *ftsH1*(Ts) mutant at 38°C λ c$^+$ made very turbid plaques and λ c17 made no plaques at all, whereas λ c17 cII made clear plaques. This result indicates that *ftsH1* increases the frequency of lysogenization. No such effect was observed with other *fts* mutants (*ftsA13*, *ftsE*, *ftsI23*, Δ*ftsJ*, *ftsQ1*, *ftsS*, *ftsT*, and *ftsZ84*) tested at 38°C.

The *ftsH* and *hflB* Genes Are Identical. The *ftsH* gene is located near *dacB* at 69 min on the *E. coli* genetic map. The *hflB* gene, 85% cotransducible with *argG* (6), lies in the same region. We considered the hypothesis that the *hflB* gene, coding for or controlling a protease degrading the phage λ cII activator (6), is identical to *ftsH*.

Using isogenic strains, we verified that *ftsH* and *hflB* had the same phenotype (Tables 1 and 2). Derivatives of *hflB29* and *ftsH1*, constructed in two different genetic backgrounds (CSH50 and 594), were infected with various λ mutants at different temperatures and examined for plaque-forming ability (Table 1). In addition to λ c$^+$ and λ c17 described above, we used λ cIII and λ c17 cII. The λ cIII protein enhances lysogenization in the wild-type host by stabilizing cII, presumably by acting as an antiprotease or by direct protection of cII (5, 6); the λ cIII mutant thus has a lower lysogenization frequency than λ c$^+$. The double mutant λ c17 cII, isogenic with λ c17 except that cII is inactivated, was used as a control; when the cII protein is inactivated, growth of λ c17 on the *hfl* mutants is restored, suggesting interactions between cII and the Hfl proteins. Plaque-forming ability on *ftsH1* was tested at 30°C and 37°C (*ftsH1* is thermosensitive

Table 1. Effect of bacterial mutations on λ plaque morphology

Strain*	Temperature, °C	Plaque morphology†			
		λ c+	λ c17	λ cIII	λ c17 cII
Wild type	30, 37, 42	T	C	C	C
hflB29	30, 37, 42	vT	–	T	C
ftsH1	30	T	C	C	C
ftsH1	37	vT	–	T	C
Wild type/p*ftsH*+‡	30, 37, 42	C	C	C	C
ftsH1/p*ftsH*+‡	30, 37, 42	T	C	C	C
hflB29/p*ftsH*+‡	30, 37, 42	T	C	C	C
hflA::Tn*5*	30, 37, 42	vT	–	T	C
hflA::Tn*5 hflB29*	30, 37, 42	–	–	vT	C
hflA::Tn*5 ftsH1*	30	vT	–	T	C
hflA::Tn*5 ftsH1*	37	vT	–	vT	C
hflA::Tn*5*/p*ftsH*+	30, 37, 42	T	C	C	C
ftsH1 fur::Tn*5*	30	T	C	C	C
ftsH1 fur::Tn*5*	37, 42	vT	–	T	C

*All experiments were done in isogenic derivatives of CSH50 and of 594; identical results were obtained in the two series of strains. The *ftsH*+ plasmid was pAR145.
†C, clear plaques; T, turbid plaques; vT, very turbid plaques; –, no visible plaques.
‡Same results were obtained in 594 derivatives containing pULB6234.

for growth at 42°C) and compared to the plaque-forming ability on wild-type and *hflB29* strains at 30°C, 37°C, and 42°C.

The results of these spot tests (Table 1) confirmed the Hfl⁻ phenotype of the *hflB29* mutant: very turbid plaques with λ c+, no plaques with λ c17, and clear plaques with λ c17 cII, at all temperatures. The *ftsH1* mutant had the same Hfl⁻ phenotype as *hflB29* at 37°C and a wild-type phenotype at 30°C.

The frequency of lysogenization was quantified by using a λ phage conferring chloramphenicol resistance, λ Cam105. This phage carries a gene coding for chloramphenicol acetyltransferase in a nonessential region but in all other respects is identical to the wild-type λ c+. Lysogenization frequency was calculated as the ratio of chloramphenicol-resistant clones to infected cells. In the parental strain, the frequency of lysogeny was 0.1% (Table 2), whereas in the *hflB29* mutant, the frequency was 10% at all temperatures tested (30°C, 37°C, and 42°C). In the thermosensitive mutant *ftsH1*, the frequency of lysogeny was 0.6% at 30°C, increasing to 6% at 42°C. This result confirms the thermosensitive Hfl⁻ phenotype of *ftsH1* and also reveals a slight Hfl⁻ phenotype at 30°C.

Introduction of plasmid pAR145, carrying the wild-type *ftsH*+ gene, into *hflB29* derivatives suppressed the Hfl⁻ phenotype, as assayed by spot tests with λ c+, λ cIII, λ c17, and λ c17 cII (Table 1) or by direct measurement of the lysogenization frequency (Table 2). λ c+ spot tests in *hflB29* or *ftsH1* strains containing pAR145 gave turbid plaques, while a wild-type strain containing pAR145 gave clear plaques (Table 1). This difference may be explained by low expression of FtsH from pAR145 and/or a partial dominance of the FtsH altered protein in *hflB29* or *ftsH1* (Table 2).

Table 2. Frequency of lysogenization by λ Cam105

Strain*	Lysogenization frequency, %†		
	30°C	37°C‡	42°C‡
Wild type	0.1	0.1	0.1
hflB29	10	10	10
ftsH1	0.6	4	6
hflB29/p*ftsH*+	1	1	0.8
Wild type/p*ftsH*+	0.02	0.02	0.03
hflA::Tn*5*	75	33	17
hflA::Tn*5 hflB29*	100	100	100
hflA::Tn*5 ftsH1*	80	45	100
hflA::Tn*5*/p*ftsH*+	1	0.8	0.6
fur::Tn*5*	0.1	0.1	0.3
fur::Tn*5 ftsH1*	0.6	4	9

*Strains were isogenic derivatives of 594. The *ftsH*+ plasmid was pULB6234.
†Chloramphenicol-resistant clones per 100 λ c+ Cam105-infected cells at the indicated temperature.
‡Infected cells were incubated at the indicated temperature for 30 min, then plated on TA chloramphenicol plates at 30°C.

To confirm that the *ftsH* gene in the *hflB29* mutant was altered, the *ftsH* gene of strain X9393 (*hflB29*) was cloned and sequenced. The X9393 *ftsH* gene sequence agreed with the published sequence (13) with the exception of a single base substitution, A-2473 to G, changing His-536 to Arg. The mutation should inactivate an *Nde* I DNA restriction site of an *hflB29* strain; this was verified by Southern analysis of X9393 whole cell DNA cut with *Nde* I, using pAR145 fragments as probe (data not shown). The *ftsH1* mutant has two mutations, changing Glu-462 to Lys and Pro-587 to Ala (13), in the same region as the *hflB29* mutation.

The Hfl⁻ phenotype of *ftsH1*, the suppression of *hflB29* by a plasmid carrying the wild-type *ftsH* gene, the genetic location of *ftsH* and *hflB* at 69 min, and the presence of a mutation in the *ftsH* gene of the *hflB29* mutant all strongly suggest that the *ftsH* gene is identical to *hflB*.

HflA and HflB Degrade the cII Protein Through Different Pathways. The isolation of bacterial mutants for high frequency of lysogeny of phage λ permitted the isolation of *hflA* and *hflB* mutants (15). The *hflA* locus, at 95 min, has been well characterized. It codes for three polypeptides, HflX, HflK, and HflC (29, 30). HflK and HflC, which copurify as a membrane-bound complex called HflA (7, 31), are inner-membrane proteins with most of their protein facing into the cytoplasm (30). HflKC degrades cII protein *in vitro* (7) and has a proteolytic activity which has recently been proposed to be regulated by a putative GTPase activity of HflX (30).

Since the *hflB* and *hflA* mutations both increase cII half life, one might expect HflA and HflB to act through the same degradation pathway. However, the *hflB* mutation increases the frequency of lysogenization even in an *hflA* null background (6), suggesting that HflB is involved in a second pathway of cII degradation. To confirm this, we constructed an *ftsH1* mutant in which the proteolytic activity of HflA is completely inactivated by the insertion of a Tn*5* transposon into the *hflK* gene (first characterized as *hflA*::Tn*5*), which has a polar effect on expression of the *hflC* gene (6, 31). On this *ftsH1 hflA*::Tn*5* double mutant, λ c+ could not make plaques at 37°C, although it made turbid plaques on both

single mutants (Table 1). λ cIII, which makes clear plaques on a wild-type strain and turbid plaques on *hflA*::Tn*5*, *hflB29*, and *ftsH1* mutants, made very turbid plaques on the *ftsH1 hflA*::Tn*5* double mutant. These differences revealed a more pronounced Hfl$^-$ phenotype of the double mutant than the single mutants, *hflA*::Tn*5* and *ftsH1*. As expected, the extreme Hfl$^-$ phenotype was also observed with the *hflA*::Tn*5 hflB29* double mutant at all temperatures tested (30°C, 37°C, and 42°C). These results were confirmed by measurements of lysogenization frequency: all λ Cam105 infecting the double mutants *hflA hflB* (at 30°C, 37°C, and 42°C) and *hflA ftsH1* (at 42°C) gave rise to lysogens (Table 2), indicating strong stabilization of cII. The ability of the *ftsH1* and *hflB29* mutations to increase the Hfl$^-$ phenotype when the protease HflA is completely inactivated clearly shows that there are at least two pathways of cII inactivation.

The frequency of lysogenization in the *hflA* single mutant decreased from 75% at 30°C to 17% at 42°C. The frequency in the *hflA ftsH1* double mutant also decreased from 80% at 30°C to 45% at 37°C. The temperature effect is not understood, but it suggests stronger stabilization of cII at low temperatures. Very strong stabilization of the cII protein is known to prevent λ v1 v3 growth (6). Using this test, we found that λ v1 v3 made plaques on the *hflA* single mutant at 37°C and 42°C but not at 30°C. Similarly, λ v1 v3 grew on the *hflB29* and *ftsH1* single mutants (30°C, 37°C, and, for *hflB*, 42°C) but not on the double mutants *hflA hflB* or *hflA ftsH1* (30°C, 37°C, or 42°C).

Phage λ c$^+$ makes clear plaques on a wild-type strain carrying a plasmid overproducing FtsH (Table 1), showing that an excess of FtsH markedly decreases the lysogenization frequency; presumably by increasing cII degradation. Since we suspected similar anti-cII activity for FtsH/HflB and HflA, we tested whether FtsH overproduction could replace HflA activity. A plasmid overproducing FtsH, introduced into the *hflA*::Tn*5* strain, compensated for the Hfl$^-$ phenotype (Tables 1 and 2).

Excess FtsH/HflB thus seems able to replace HflA for cII degradation, although FtsH/HflB controls a proteolytic pathway different from HflA. Experiments in which we quantified the lysogenization frequency after brief exposure to 42°C (cf. *Materials and Methods*) revealed that the thermosensitive effect of *ftsH1* is perceived within 5 min (data not shown). This result suggests that FtsH/HflB action is probably not mediated through transcription or translation, but rather that FtsH either is itself a protease degrading cII protein or directly affects the action of such a protease—e.g., by interacting with the substrate.

Effect of fur Mutations on *ftsH1*. The *ftsH1* mutant cannot grow at 42°C. Extragenic suppressors of this thermosensitivity have been shown to carry a mutation in the *fur* gene, coding for the ferric uptake regulator (ref. 10; T.T. and T.O., unpublished observation). Inactivation of the *fur* gene results in the overexpression of a set of genes mostly involved in iron uptake (32). Complete inactivation of Fur by a known *fur*::Tn*5* insertion also results in *ftsH1* thermoresistance (T.T. and T.O., unpublished observation). Suppression of *ftsH1* by Fur inactivation could be due to restoration of FtsH function. We tested the Hfl phenotype in the *fur*::Tn*5 ftsH1* double mutant. Like the *ftsH1* single mutant, the double mutant had an Hfl$^+$ phenotype at 30°C but was Hfl$^-$ at 37°C and 42°C (Tables 1 and 2). Thus the suppression of thermosensitivity by *fur* does not restore FtsH$^+$-like activity with respect to cII degradation.

Since the inactivation of Fur suppresses *ftsH1* thermosensitivity at 42°C, one can speculate that the FtsH/HflB-governed protease must process an essential protein of the Fur regulon (or Fur itself). Alternatively, it is possible that the deregulation of iron uptake, resulting in increased intracellular iron in *fur* mutants, could partially compensate for the *ftsH1* deficiency (cf. *Discussion*).

Since genes regulated by Fur are strongly repressed by anaerobiosis, we tested *ftsH1* for its ability to grow anaerobically at 42°C. We found that the *ftsH1* mutant was able to grow in an anaerobic incubator at 42°C, regardless of the presence of the *fur* suppressor (C.H. and P.B., unpublished observations). This could be explained either by a reduction in the requirement for Fur-controlled products in anaerobiosis or by the change in oxidation state, resulting in an increase in ferrous iron.

DISCUSSION

Individual *E. coli* cells behave differently with respect to the lysis–lysogeny decision of infecting phage λ, even in cultures in balanced, unrestricted growth, during which cells differ only in age. The factors determining these cell-to-cell differences are not understood, although several host functions—HflA, HflB, the cAMP–cAMP-binding protein complex, and Lon—are known to influence the decision. We have searched for new host genes regulating λ lysogeny. Among nine cell division (*fts*) mutants, *ftsH1* showed an altered lysogenization frequency. Further analysis established that the *ftsH* gene is identical to *hflB*: both genes are located at 69 min, the *ftsH1* mutant has an Hfl$^-$ phenotype, the *hflB29* mutant has a point mutation in its *ftsH* gene, and an *ftsH*$^+$ plasmid suppresses the Hfl$^-$ phenotype of *hflB29*.

This identity allows us to pool genetic and molecular information on FtsH and HflB. Studies of the *ftsH* gene have shown that it codes for an inner membrane protein of 70.7 kDa (10, 11, 13). The deduced protein sequence, with a potential ATP-binding site, has significant homology with a eukaryotic family of putative ATPases, suggesting that FtsH/HflB is an ATPase (11, 12). The *hflB29* mutation increases the lysogenization frequency of phage λ by stabilizing the λ cII protein (which in turn activates transcription of cI, repressor of the lytic functions). This stabilization shows that FtsH/HflB is involved, directly or indirectly, in the degradation of cII (6).

It has been demonstrated *in vitro* that the HflA complex has anti-cII protease activity (7). This complex is dispensable for bacterial growth and, in *hflA* null mutants, cII is still somewhat unstable, indicating the existence of a second anti-cII protease. Although the *hflB29* mutant has the same phenotype as *hflA*, it has not been shown that HflB is itself a protease. Like HflA (30, 31), FtsH/HflB is a membrane protein, but in contrast to it, it is essential. A strain in which the *ftsH* gene has been deleted is viable only if it expresses a wild-type *ftsH* gene on a plasmid (T.T., T.O., Y.A., and K.I., unpublished observations).

Introduction of the *hflB29* (6) or *ftsH1* allele into an *hflA*::Tn*5* mutant exacerbated the Hfl$^-$ phenotype, showing cumulative and independent effects of HflA and HflB on the cII protein. Nevertheless, overproduction of FtsH/HflB suppresses the *hflA*::Tn*5* mutation, showing that excess FtsH/HflB can substitute for deficiency of the HflA protease. These results indicate that FtsH/HflB is involved in a proteolytic pathway, although its precise role remains to be determined. It could be a component of the protease itself. A cII-specific degradation activity, independent of HflA and still associated with the membrane (7), may well be FtsH/HflB. An ATP-stimulated endoproteolytic activity observed in membrane extracts (33) could also be attributed to FtsH/HflB, which has putative ATPase activity. *N*-Ethylmaleimide (NEM), which oxidizes accessible —SH groups, protects cII from degradation, even in an *hflA* mutant (5), suggesting that NEM acts on the FtsH/HflB-governed protease activity. Interestingly, one of the FtsH/HflB eukaryote homologues is NSF, an NEM-sensitive fusion protein. Further-

more, one of the eukaryotic FtsH homologues is the ATPase subunit for an energy-dependent protease which has weak homologies with the ATP-binding subunit, ClpA, of the *E. coli* Clp protease (34, 35). A search for weak sequence homology in an *E. coli* data bank showed that FtsH belongs to the ClpA superfamily, including several proteases or protease regulatory subunits (E. Koonin and K. Rudd, personal communication). FtsH/HflB could be the ATP-dependent regulatory subunit of another proteolytic complex.

FtsH/HflB has an essential function clearly involved, directly or indirectly, in a proteolytic pathway. Cell lethality in the absence of FtsH function may be due to an excess of one or more target proteins of this protease. Inactivation of the ferric iron uptake regulator, Fur, restores growth of the *ftsH1* mutant at 42°C (10), although the *ftsH1 fur*::Tn*5* double mutant retains a temperature-dependent Hfl$^-$ phenotype. One interpretation of this result, as argued above, is that Fur is itself a substrate of the FtsH/HflB-governed protease and, when protease activity is low, the excess of Fur overrepresses a gene whose product is essential for aerobic growth. An alternative interpretation depends on the fact that *fur* inactivation, like anaerobiosis, results in an increase in intracellular ferrous iron. One can thus speculate that a high iron pool overcomes *ftsH1* lethality, either by stabilizing the mutant FtsH1 protein or by acting on its target.

The *BCS1* gene of *Saccharomyces cerevisiae* is the only known *ftsH* eukaryote homologue found in membranes (36). Interestingly, *bcs1* mutants are respiration deficient and present a gross reduction in the Rieske iron–sulfur protein. The BCS1 protein may carry out a chaperone-like function (36). Although *E. coli* and *S. cerevisiae* are only distantly related, it is tempting to assign a similar role to FtsH and BCS1. Such a role would be compatible with the finding that FtsH may have chaperone-like activity involved in membrane protein assembly (T.T., T.O., Y.A. and K.I., unpublished observations). The striking suppression of *ftsH1* thermosensitivity by *fur* inactivation or in anaerobiosis and the analogy with BCS1 suggest the following model. FtsH is a chaperone-like protein involved in the assembly of iron–sulfur proteins and in the proteolytic degradation of λ cII protein, where it could be required for the presentation of cII to an as yet unidentified protease. For the first role, but not for the second, *ftsH1* deficiency can be compensated for by increased intracellular ferrous iron.

The predicted amino acid sequence of FtsH is similar to the sequences of a family of eukaryotic proteins with putative ATPase activity (11, 12) involved in processes such as membrane fusion (37, 38), cell division (39), protein assembly (36), and regulation of gene expression (40). On the basis of our results, we propose that all these proteins may be energy-dependent chaperones involved in proteolytic pathways.

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1. Herskowitz, I. & Hagen, D. (1980) *Annu. Rev. Genet.* **14,** 399–445.
2. Echols, H. (1986) *Trends Genet.* **2,** 26–30.
3. Friedman, D. I., Obson, E. R., Corven, D. & Gellert, M. (1984) *Microbiol. Rev.* **48,** 299–325.
4. Kaiser, A. D. (1957) *Virology* **3,** 42–61.
5. Hoyt, M. A., Knight, D. M., Das, A., Miller, H. I. & Echols, H. (1982) *Cell* **31,** 565–573.
6. Banuett, F., Hoyt, M. A., McFarlane, L., Echols, H. & Herskowitz, I. (1986) *J. Mol. Biol.* **187,** 213–224.
7. Cheng, H. H., Muhlrad, P. J., Hoyt, M. A. & Echols, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7882–7886.
8. Santos, D. & de Almeida, D. F. (1975) *J. Bacteriol.* **124,** 1502–1507.
9. Begg, K. J., Tomoyasu, T., Donachie, W. D., Khattar, M., Niki, H., Yamanaka, K., Hiraga, S. & Ogura, T. (1992) *J. Bacteriol.* **174,** 2416–2417.
10. Ogura, T., Tomoyasu, T., Yuki, T., Morimura, S., Begg, K. J., Donachie, W. D., Mori, H., Niki, H. & Hiraga, S. (1991) *Res. Microbiol.* **142,** 279–282.
11. Tomoyasu, T., Yuki, T., Morimura, S., Mori, H., Yamanaka, K., Niki, H., Hiraga, S. & Ogura, T. (1993) *J. Bacteriol.* **175,** 1344–1351.
12. Erdmann, R., Wiebel, F. F., Flessau, A., Rytka, J., Beyer, A., Fröhlich, K.-U. & Kunau, W.-H. (1991) *Cell* **64,** 499–510.
13. Tomoyasu, T., Yamanaka, K., Murata, K., Suzaki, T., Bouloc, P., Kato, A., Niki, H., Hiraga, S. & Ogura, T. (1993) *J. Bacteriol.* **175,** 1352–1357.
14. de Souza Ferreira, L. C., Keck, W., Betzner, A. & Schwarz, U. (1987) *J. Bacteriol.* **169,** 5776–5781.
15. Gautsch, J. W. & Wulff, D. L. (1974) *Genetics* **77,** 435–448.
16. Campbell, A. (1961) *Virology* **14,** 22–52.
17. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
18. Tardat, B. & Touati, D. (1991) *Mol. Microbiol.* **5,** 455–465.
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. Thomas, R. (1966) *J. Mol. Biol.* **22,** 79–85.
21. Pereira da Silva, L. H. & Jacob, F. (1968) *Ann. Inst. Pasteur* **115,** 145–158.
22. Lecocq, J. P. (1975) Thesis (Université Libre de Bruxelles, Brussels).
23. Munter, C. J., MacHattier, L. A. & Shapiro, J. A. (1983) *J. Bacteriol.* **153,** 976–990.
24. Jones, J. D. G. & Gutterson, N. (1987) *Gene* **61,** 299–306.
25. Jones, M. O. & Herskowitz, I. (1975) *Virology* **88,** 199–212.
26. Arber, W., Enquist, L., Hohn, B., Murray, N. E. & Murray, K. (1983) in *Lambda II,* eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 433–466.
27. Takeshita, S., Sato, M., Toba, M., Masahashi, W. & Hashimoto-Gotoh, T. (1987) *Gene* **61,** 63–74.
28. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
29. Banuett, F. & Herskowitz, I. (1987) *J. Bacteriol.* **169,** 4076–4085.
30. Noble, J. A., Innis, M. A., Koonin, E. V., Rudd, K. E., Banuett, F. & Herskowitz, I. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10866–10870.
31. Zorick, T. S. & Echols, H. (1991) *J. Bacteriol.* **173,** 6307–6310.
32. Braun, V. & Hantke, K. (1991) in *Handbook of Microbial Iron Chelates,* ed. Winkelman, G. (CRC, Boca Raton, FL), pp. 107–138.
33. Voellmy, R. W. & Goldberg, A. L. (1982) *Nature (London)* **290,** 419–421.
34. Dubiel, W., Ferrell, K., Pratt, G. & Rechsteiner, M. (1992) *J. Biol. Chem.* **267,** 22699–22702.
35. Rechsteiner, M., Hoffman, L. & Dubiel, W. (1993) *J. Biol. Chem.* **268,** 6065–6068.
36. Nobrega, F. G., Nobrega, M. P. & Tzagoloff, A. (1992) *EMBO J.* **11,** 3821–3829.
37. Wilson, D. W., Wilcox, C. A., Flynn, G. C., Chen, E., Kuang, W.-J., Henzel, W. J., Block, M. R., Ullrich, A. & Rothman, J. E. (1989) *Nature (London)* **339,** 355–359.
38. Söllner, T., Whiteheart, W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. & Rothman, J. E. (1993) *Nature (London)* **362,** 318–323.
39. Fröhlish, K.-U., Fries, H.-W., Rüdiger, M., Ederman, R., Botstein, D. & Mecke, D. (1991) *J. Cell Biol.* **114,** 443–453.
40. Nelbock, P., Dillon, P. J., Perkins, A. & Rosen, C. A. (1990) *Science* **248,** 1650–1653.