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The *Escherichia coli* FtsH Protein Is a Prokaryotic Member of a Protein Family of Putative ATPases Involved in Membrane Functions, Cell Cycle Control, and Gene Expression

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The *ftsH* gene is essential for cell viability in *Escherichia coli*. We cloned and sequenced the wild-type *ftsH* gene and the temperature-sensitive *ftsH1*(Ts) gene. It was suggested that FtsH protein was an integral membrane protein of 70.7 kDa (644 amino acid residues) with a putative ATP-binding domain. The *ftsH1*(Ts) gene was found to have two base substitutions within the coding sequence corresponding to the amino acid substitutions Glu-463 by Lys and Pro-587 by Ala. Homology search revealed that an ~200-amino-acid domain, including the putative ATP-binding sequence, is highly homologous (35 to 48% identical) to the domain found in members of a novel, eukaryotic family of putative ATPases, e.g., Sec18p, Pas1p, CDC48p, and TBP-1, which function in protein transport pathways, peroxisome assembly, cell division cycle, and gene expression, respectively. Possible implications of these observations are discussed.

Genetic approaches have identified a large number of cell division mutants in *Escherichia coli*; *fts* mutants (filamentation temperature sensitive) fail to septate when grown at high temperatures (for reviews, see references 5 and 6). Many Fts proteins and additional proteins are involved in septation; some of them constitute the machinery for septation, and the others must have regulatory functions (for reviews, see refs 4 to 6 and 20).

The *E. coli ftsH* mutant Y16 was originally isolated as a cell division mutant (27). The *ftsH* mutation responsible for the thermosensitivity in colony formation was mapped at 69 min on the chromosome (27). Results obtained from penicillin-binding assays suggest that the thermosensitive filamentation of the mutant is caused by a decrease in the amount of a septum-forming enzyme, penicillin-binding protein 3 (PBP3), in the membrane fraction (9). Recently, we have found that the Y16 mutant has mutations in two genes, one in *ftsH* and the other in *ftsI*, which encodes PBP3; both mutations decrease the amount of PBP3 (2). We previously reported that transcription and translation of *ftsI* did not decrease in *ftsH* transductants (22). This suggests that the *ftsH* gene might function in a posttranslational stage of PBP3 production. On the other hand, genetic analyses suggested that the *ftsH* mutation also affected at least one other additional protein essential for cell growth as well as PBP3 (22). This may suggest that the *ftsH* gene is not only a cell division regulator.

We also identified another gene (*ftsJ*) affecting cell division in the region upstream of the *ftsH* gene (22). A deletion mutant of *ftsJ* was constructed, and the mutant grew slowly and formed filaments, indicating that the *ftsJ* gene is not essential for growth but affects cell division (22).

In this paper, we report the nucleotide sequence of the *ftsH* gene, and some particular features of the deduced amino acid sequence of the FtsH protein. We discuss the function of the FtsH protein in conjunction with the possible significance of sequence homology between the FtsH protein and the members of a novel, eukaryotic family of putative ATPases.

MATERIALS AND METHODS

Bacterial strains, plasmids, phages, and media. The following *E. coli* K-12 strains were used in this study: Y16 [*thr-1 leu-6 thi-1 supE44 lacY1 tonA21 ftsH1*(Ts) *ftsI372*(Ts*)] (2, 27), AR1025 (*thr-1 leu-6 thi-1 supE44 lacY1 tonA21 recD1009 ΔftsJ::kan*), W3110 [IN(*rrnD-rrnE*)*1*] (14), NM539 [*supF hsdR* (P2 *cox3*)] (10), and JM103 [*supE sbcB15 hsdR4 rpsL thi* Δ(*lac-proAB*)/F' *traD36 proAB*$^+$ *lacI*^{q} *lacZΔM15*] (18). Vector plasmids pHSG439 (3), pHSG575 (30), and pHSG396 (30) were used for cloning. Vector phage EMBL4 (10) was used for preparing a library of chromosomal DNA segments of the Y16 strain. Phage M13mp11 (18) was used as a vector for sequencing. L medium (1% Bacto-Tryptone, 0.5% yeast extract, 0.5% NaCl [pH 7.4]) was used for cultivation of cells. NZCYM medium (1% NZ amine [type A; Wako Pure Chemical Industries, Ltd., Osaka, Japan], 0.5% yeast extract, 0.5% NaCl, 0.1% Casamino Acids, 0.2% $MgSO_4 \cdot 7H_2O$ [pH 7.5]) was used for propagation of EMBL4 derivative phage clones. For propagation of M13 derivative phage clones, 1× YT medium (0.8% tryptone, 0.5% yeast extract, 0.5% NaCl [pH 7.5]) and 2× YT medium (1.6% tryptone, 1% yeast extract, 0.5% NaCl [pH 7.5]) were used. Chloramphenicol was added to media, as needed, at a concentration of 20 μg/ml.

Construction of libraries and subcloning. Construction of cosmid libraries with pHSG439 and phage libraries with EMBL4 was previously described (3, 10). Recombinant phage clones were screened by plaque hybridization tech-

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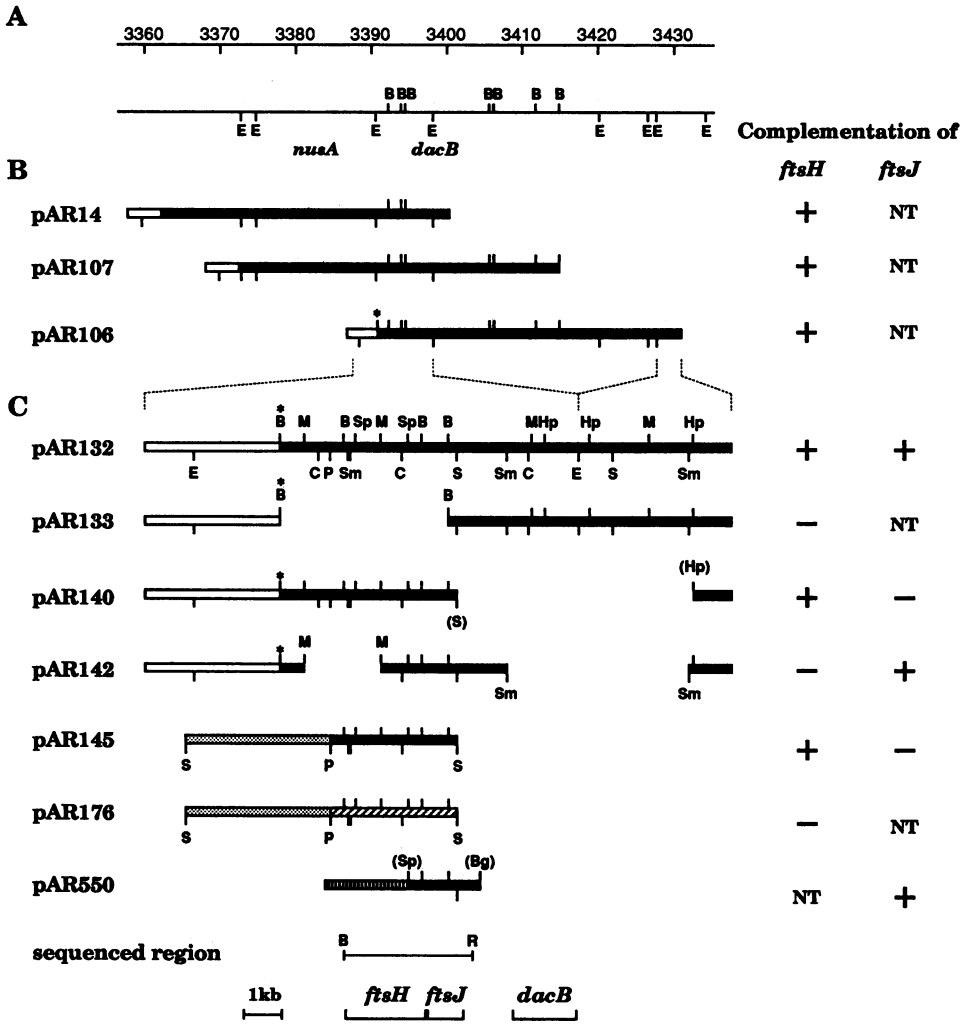


FIG. 1. Cloning of the *ftsH* gene. (A) Physical map of the 69-min region (14) and location of the known genes. (B) Chromosomal segments carried by the cosmid clones complementing the *ftsH1*(Ts) mutation. (C) Chromosomal segments carried by the plasmids. The complementation of the *ftsH1*(Ts) or Δ*ftsJ*::*kan* mutation by each plasmid is indicated to the right. Symbols: filled bar, chromosomal segment derived from the wild-type strain W3110; hatched bar, chromosomal segment derived from the *ftsH1*(Ts) Y16 mutant; open bar, pHSG439 vector DNA; dotted bar, pHSG575 vector DNA; striped bar, pHSG396 vector DNA. Abbreviations for restriction endonucleases: B, *Bam*HI; Bg, *Bgl*I; C, *Cla*I; E, *Eco*RI; Hp, *Hpa*I; M, *Mlu*I; P, *Pst*I; R, *Rsa*I; S, *Sal*I; Sm, *Sma*I; Sp, *Sph*I. The asterisked *Bam*HI site is created by the ligation of *Bam*HI-*Sau*3AI. Cleavage sites within parentheses, in pAR140 and pAR550, indicate blunt end ligation.

niques, as previously described (25), with the labeled pAR145 DNA as a probe. Subcloning, with plasmid and phage vectors, was carried out as previously described (25).

DNA sequence analysis. Subsegments of the chromosomal segments bearing the *ftsH* and *ftsJ* genes were cloned into M13mp11 and sequenced by the dideoxy method (26) to determine the nucleotide sequences of the wild-type and the mutated *ftsH* genes of W3110 and Y16, respectively. The sequencing reactions were carried out with a kit of Sequenase (U.S. Biochemicals, Cleveland, Ohio). Computer analysis of the nucleotide and the amino acid sequences was done with the SDC-GENETYX programs (Software Development Co., Tokyo, Japan) and DNA Strider (17). Homology search of amino acid sequence was done in the GenBank and the EMBL data bases.

Detection of plasmid-encoded proteins in vitro. Plasmid-encoded proteins were detected with a prokaryotic DNA-directed translation kit (Amersham) and L-[^{35}S]methionine.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been deposited in the GenBank data base under accession number M83138.

RESULTS

Cloning of the wild-type *ftsH* gene. We have previously reported that the temperature-sensitive colony formation of the *E. coli* mutant Y16 is caused by the *ftsH1*(Ts) mutation mapped at 69 min (2). We isolated cosmid clones carrying chromosomal segments derived from the wild-type strain W3110, which enables Y16 to form colonies at 42°C, to clone the wild-type *ftsH* gene. The DNA of the cosmid clones was extracted and analyzed with restriction endonucleases. All the clones were found to carry the 69-min region of the *E. coli* chromosome (Fig. 1A).

We obtained several deletion derivatives of the pAR106 cosmid and a subclone with the pHSG575 vector (Fig. 1B) to

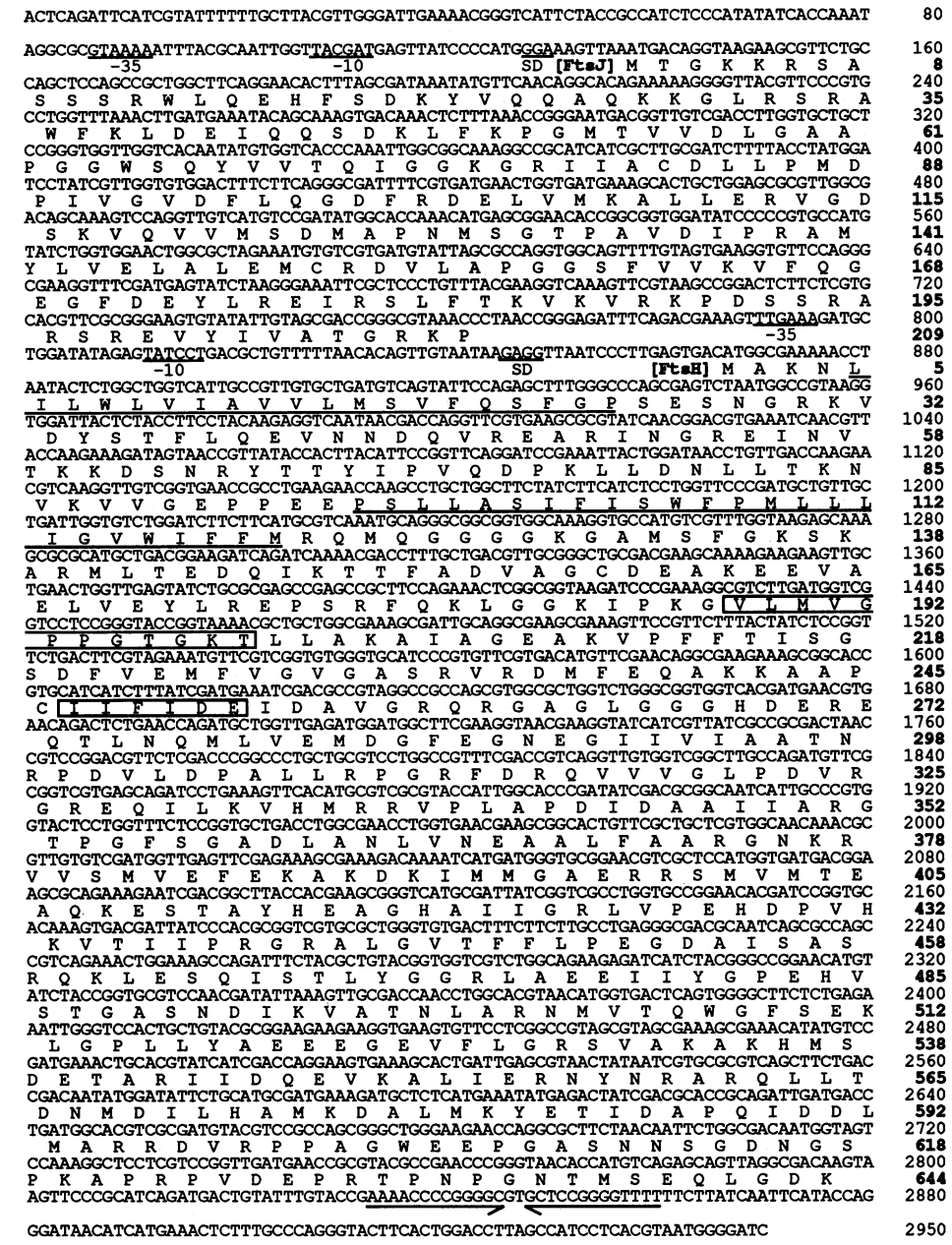


FIG. 2. Nucleotide sequence of the 2,950-bp *Rsa*I-*Bam*HI segment possessing the *ftsH* and *ftsJ* genes and the predicted amino acid sequence of the FtsH and the FtsJ proteins. The nucleotide sequence of the segment possessing the *ftsH* and *ftsJ* genes of the wild-type strain W3110 was determined. The nucleotides and the corresponding amino acids of the FtsH and FtsJ proteins are numbered on the right. The sequenced region (Fig. 1C) corresponds to 3395.5- to 3392.5-kb coordinates (counterclockwise) of the physical map of the *E. coli* chromosome (14). The boxed regions of amino acids represent the motifs A and B of a putative ATP-binding sequence. The underlined amino acids represent possible membrane-spanning regions (see Fig. 3) (31). The putative promoter regions (−35 to −10) and the ribosome binding site (SD) sequences of the *ftsH* and the *ftsJ* genes are underlined. The arrows represent inverted repeats, which is a possible signal for ρ-independent termination.

find the precise location of the *ftsH* gene. They were tested for the ability to complement the *ftsH1*(Ts) mutation. The results indicated that the 3.05-kb *Pst*I-*Sal*I segment can complement the mutation, suggesting that the segment includes the *ftsH* gene.

Cloning of the *ftsH1*(Ts) gene. We constructed an EMBL4 chromosomal DNA library of Y16 to clone the *ftsH* gene of the Y16 mutant; phage clones were screened with labeled pAR145 as a probe. Seven positive clones were found within ~1,000 screened clones. Three of them were chosen for the analysis with restriction endonucleases, and two of them were found to have the 8-kb *Eco*RI segment including the *ftsH* gene; the other one was found to have part of the *Eco*RI segment. The 3.05-kb *Pst*I-*Sal*I segment within the *Eco*RI

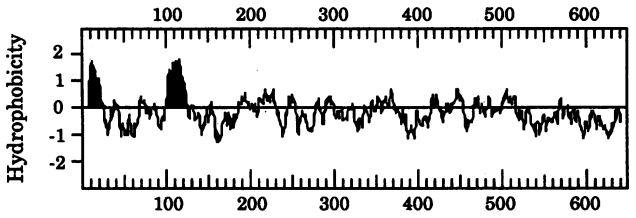


FIG. 3. Hydropathy profile of FtsH. The amino acid sequence of FtsH was analyzed by the Kyte and Doolittle method (16). The filled regions represent the hydrophobic segments.

segment was subcloned with the vector pHSG575. The resulting plasmid pAR176 (Fig. 1B) was tested for the ability to complement the *ftsH1*(Ts) mutation. As expected, pAR176 did not complement the mutation.

Nucleotide sequence of the *ftsH* gene and the deduced amino acid sequence of FtsH protein. The nucleotide sequence of the cloned *ftsH* gene and its flanking region was determined. Figure 2 shows the nucleotide sequence of the *ftsH* gene region and the deduced amino acid sequence of two open reading frames identified in this region. The open reading frame of 644 amino acid residues, with the potential to encode a protein of 70.7 kDa, was concluded to correspond to the *ftsH* gene by complementation tests described above.

The amino acid sequence of the FtsH protein was analyzed by the Kyte and Doolittle procedure (16) for distribution of hydrophobic amino acids. This protein contains two regions that are rich in hydrophobic amino acids (Fig. 3). The length of each hydrophobic segment is long enough for spanning the membrane in an α-helical structure, suggesting that FtsH traverses the membrane twice. In addition, sequence motifs characteristic of ATP-binding proteins were found within the FtsH sequence (Fig. 2).

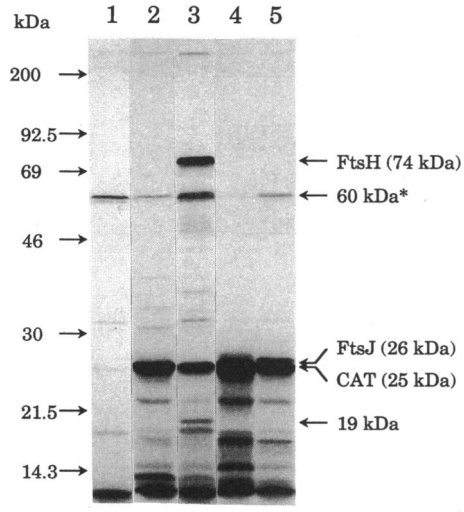


FIG. 4. Identification of FtsH and FtsJ proteins in vitro. Plasmid-encoded proteins were labeled in vitro and analyzed on sodium dodecyl sulfate-polyacrylamide gels. Lanes; 1, no DNA; 2, pHSG575 DNA; 3, pAR145 DNA; 4, pHSG396 DNA; 5, pAR550 DNA. The positions of 74-kDa FtsH protein, 26-kDa FtsJ protein, 25-kDa chloramphenicol acetyltransferase (CAT), additional 60- and 19-kDa proteins, and markers are indicated. The asterisked 60-kDa protein seen in all of the lanes may be a protein which is present in the reaction lysate and binds methionine or methionyl-tRNA as described in the product specification of the kit.

Sequencing of the mutated *ftsH1*(Ts) gene revealed two base substitutions, the 2253rd G by A and the 2625th C by G; this corresponds to the amino acid substitutions of Glu-463 by Lys and Pro-587 by Ala.

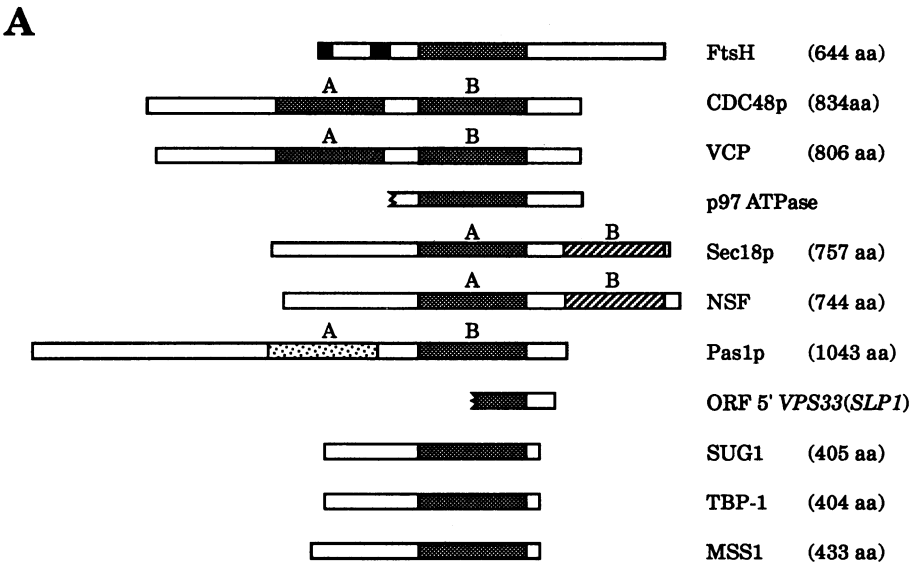
The *ftsJ* gene located in the upstream region of the *ftsH* gene. We found an open reading frame of 209 amino acid residues, with the potential to encode a protein of 23.3 kDa, in the upstream region of the *ftsH* gene (Fig. 2). Disruption of this potential gene by a kanamycin cassette caused slow growth and filamentation; the gene was designated *ftsJ* (22). The slow growth and the filamentation of the Δ*ftsJ* mutant was suppressed by a plasmid carrying the *ftsJ* gene, pAR550 (Fig. 1).

Identification of FtsH and FtsJ proteins in vitro. In vitro transcription-translation analysis was carried out to detect the FtsH and FtsJ proteins. As shown in Fig. 4, the *ftsH*$^+$ plasmid pAR145 produced 74- and 19-kDa polypeptides in addition to chloramphenicol acetyltransferase (25 kDa) encoded by the vector DNA (lane 3; compare with lane 2). The 74-kDa polypeptide should be the FtsH protein. The molecular mass is consistent with that (70.7 kDa) deduced from the sequence. In addition, this polypeptide was indeed immunoprecipitated with an antiserum, which was raised to a synthetic peptide corresponding to the amino acids 297 to 312 of FtsH (31) (data not shown). The 19-kDa polypeptide is probably a LacZα'-'FtsJ fusion protein. The in-frame *lacZα'-'ftsJ* fusion gene was constructed on pAR145 by the *Sal*I ligation (Fig. 1), and the molecular mass of LacZα'-'FtsJ should be 18 kDa.

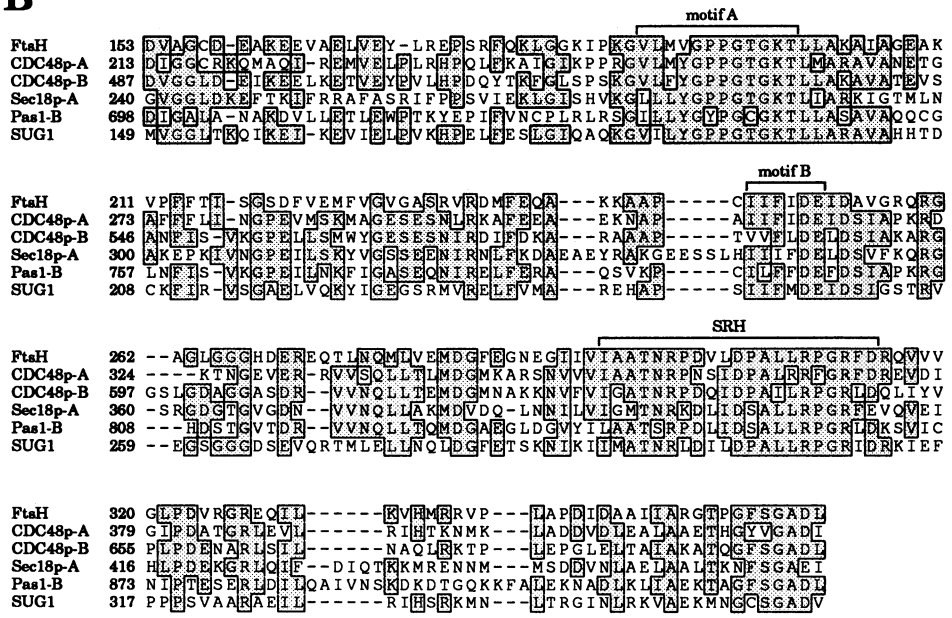
The *ftsJ*$^+$ plasmid pAR550 produced a 26-kDa polypeptide in addition to chloramphenicol acetyltransferase (lane 5). The deduced molecular mass of the FtsJ protein is 23.3 kDa, as described above.

Similarity of FtsH protein to a family of eukaryotic putative ATPases. Homology search revealed a striking homology between the FtsH protein and some eukaryotic proteins, such as *Saccharomyces cerevisiae* Sec18p (7), Pas1p (8), CDC48p (11), SUG1 (29), and an open reading frame located in the region upstream of *VPS33*(*SLP1*) (1, 33); *Xenopus laevis* p97 ATPase (23); and mammalian VCP (15), NSF (34), TBP-1 (21), and MSS1 (28). These proteins may constitute a novel family of putative ATPases (8). A diagrammatic alignment of the members of this family is presented in Fig. 5A. The region of FtsH showing strong similarity to these eukaryotic proteins corresponds to the proposed domain (~200 amino acids) common in all the members of the family, which includes a putative ATP-binding sequence and another highly conserved sequence, SRH (second region of homology) (Fig. 5B). The presence of SRH was proposed within the SUG1/TBP-1/MSS1 subfamily (29), and this subdomain is characteristic of all of the members of this family (Fig. 5B). The SRH sequence of FtsH is also well conserved. Thus, FtsH protein should also belong to this family of putative ATPases. FtsH protein, SUG1, TBP-1, and MSS1 each have only one homologous domain with a putative ATP-binding sequence, whereas the others have two domains.

The degrees of identity and similarity of the homologous domain are shown in Fig. 5C. The domain of FtsH is 35 to 48% identical (59 to 74% similar) to the other domains. This degree is almost the same within eukaryotic members. Judging from the degree of the similarity, in conjunction with the overall structural feature represented in Fig. 5A, some members should be classified into subfamilies CDC48p/VCP/p97 ATPase, Sec18p/NSF, and SUG1/TBP-1/MSS1. The domain B of Sec18p/NSF (hatched region) and the domain A



B



C

	FtsH	CDC-A	CDC-B	VCP-A	VCP-B	p97	Sec18-A	NSF-A	Pas1-B	SUG1	TBP-1	MSS1
FtsH	-	66%	72%	66%	74%	74 %	59%	61%	63%	64%	68%	68%
CDC-A	45%	-	66%	95%	65%	65 %	62%	62%	58%	65%	64%	66%
CDC-B	48%	46%	-	65%	88%	87 %	62%	61%	66%	65%	65%	65%
VCP-A	46%	86%	45%	-	64%	64 %	61%	61%	58%	62%	65%	65%
VCP-B	45%	45%	73%	44%	-	100%	63%	61%	68%	67%	68%	68%
p97	45%	44%	71%	43%	98%	-	61%	61%	68%	66%	67%	67%
Sec18-A	35%	42%	41%	38%	39%	38 %	-	83%	57%	58%	57%	54%
NSF-A	37%	41%	40%	41%	42%	42 %	71%	-	59%	60%	59%	54%
Pas1-B	36%	41%	47%	43%	47%	46 %	35%	41%	-	57%	53%	55%
SUG1	44%	43%	44%	41%	43%	42 %	34%	36%	38%	-	74%	75%
TBP-1	45%	43%	42%	43%	43%	42 %	35%	36%	35%	58%	-	73%
MSS1	46%	45%	40%	43%	41%	41 %	35%	37%	36%	59%	56%	-

Similarity

Identity

FIG. 5. Sequence comparison of FtsH with members of a novel family of eukaryotic putative ATPases. (A) Diagrammatic alignment. Shadowed regions represent regions of strongest similarity. Hatched and dotted regions represent areas displaying some resemblance to the regions of strongest similarity. Solid regions in FtsH protein represent hydrophobic sequences. The N-terminal sequences of p97 ATPase and the 5′ open reading frame (ORF) of *VPS33*(*SLP1*) are truncated because of incomplete sequencing. aa, amino acid residues. (B) Alignment of the primary sequences of the homologous domain. The position in the primary sequence of the first amino acid on every line is shown. Three or more identical amino acids in one position are boxed. Gaps are indicated by dashes. The nucleotide binding motifs (12) are marked with a bracket. (C) Quantification of the homologies in the domain. Percent identity is defined as the ratio of the number of positions containing identical amino acids between any pairwise analysis of sequences aligned as shown in panel A to the total number of amino acids. Percent similarity is defined as the ratio of the number of positions containing identical and similar amino acids to the total number of amino acids, with the following similarity rules: L = I = M = V = F = W, K = R = H, D = E = Q = N, G = A = S, T = V, A = V, and F = Y = H = W.

of Pas1p (dotted region) are rather divergent from the other domains; the identity of these domains to the other domains is only 15 to 20% (data not shown). A most striking feature of FtsH distinct from all of the other members is the presence of hydrophobic stretches long enough for membrane spanning.

Similarity of FtsJ protein to a potential protein in *S. cerevisiae*. Significant homology of amino acid sequence was observed between FtsJ protein and a putative 83-kDa protein encoded by *YCF4* in *S. cerevisiae* (Fig. 6). The entire length of FtsJ is homologous (30% identical and 53% similar) to the N-terminal part of the potential protein. The gene *YCF4* is located in the region between the *CHA1* gene coding for L-serine/L-threonine dehydratase and the *APA1*(*DTP*) gene coding for diadenosine 5′,5‴-P^1,P^4-tetraphosphate phosphorylase (13, 24). The function of the *YCF4* gene is unknown.

DISCUSSION

We cloned and sequenced the *E. coli ftsH* gene, which is essential for growth. The deduced amino acid sequence of the FtsH protein has some interesting features: (i) two hydrophobic stretches characteristic of membrane spanning, (ii) a putative ATP-binding sequence, and (iii) significant homology to a novel, eukaryotic family of putative ATPases.

We have found that the *ftsH1*(Ts) mutation causes a decrease in the amount of PBP3 at 42°C (2). The posttranslational processing of PBP3 at the C-terminal part is significantly retarded in the *ftsH1*(Ts) mutant (32). The mutation was also found to cause a marked intracellular accumulation of the precursor form of plasmid-encoded β-lactamase. Unlike the pre-β-lactamase, pre-PBP3 accumulated in the *ftsH1*(Ts) mutant was digested by proteinase K added externally to the intact spheroplasts. Evidence suggested an altered state of pre-PBP3 in the mutant. These results suggest that the FtsH function is involved in the localization processes of some envelope proteins: folding, assembly or topogenesis for PBP3 and membrane translocation for β-lactamase.

Among the eukaryotic members of the novel family of putative ATPases, which show homology with FtsH protein (Fig. 5A), yeast Sec18p and mammalian NSF have been indicated to act in the vesicle-mediated protein transport pathways (34). Pas1p is required for peroxisome assembly (8). It has been suggested that CDC48p participates in a cell cycle function related to that of Sec18p/NSF (11). Yeast *cdc48* mutants arrest as large budded cells with microtubules that protrudes from an unseparated spindle pole body and spread aberrantly throughout the cytoplasm, in the stage of nuclear division, at restrictive temperatures (11, 19). This indicates that both prokaryotes and eukaryotes have this family of ATPases, which are essential for cellular functions.

We have shown that FtsH protein is an integral cytoplasmic membrane protein spanning the membrane twice (31). In contrast to FtsH protein, none of the eukaryotic proteins described above have any hydrophobic stretches characteristic of membrane spanning (Fig. 5A). Sec18p and NSF are soluble proteins (7, 34). The p97 ATPase from *X. laevis* and its porcine homolog VCP are also diffusible components, which are distributed in both the cytoplasm and the nucleus (15, 23), although the yeast homolog CDC48p is bound loosely to components of the microsomal fraction as discussed below (11). Yeast Pas1p seems to be diffusible, too (8). Yeast SUG1 and human TBP-1 are localized in the nuclei (21, 29). So, some or maybe all of the eukaryotic members of this family of proteins are soluble components. It should be noticed, however, that many eukaryotic members may, nevertheless, be involved in membrane functions. Sec18p and NSF can be isolated in membrane-bound forms (7, 34). CDC48p is bound loosely to microsomal fractions, as described for Sec18p/NSF (11). In addition, the p97 ATPase from *X. laevis*, which is a vertebrate homolog of CDC48p, exhibits an inhibitor specificity similar to the membrane-

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FtsJ    1 M--TGKKRSASSSRWLQEHFSDKYVQQAQKKGLRSRAWFKLDETQQS-DKLFKPGMTVVD
YCF4    1 MGKTQKKN--SKGRL------DRYYYLAKEKGYRARSSFKIIQINEKYGHFLEKSKVVID

FtsJ   58 LGAAPGGWSQYVVTQIGGKGRIIACDLLPMDPIVGVDFLQGDFRDELVMKALLERVGDSK
YCF4   53 LCAAPGSWCQVASKLCPVNSLIIGVDIVPMKPMPNVITFQSDITTEDCRSKLRGYMKTWK

FtsJ   118 VQVVMSDMAPNMSGTPAVDIPRAMYLVELALEMCRDVLAPGGSFVVKVFQGEGFDEYLRE
YCF4   113 ADTVLHDGAPNVGLGWVQDAFTQSQLTLQALKLAVENLVVNGTFVTKIFRSKDYNKLIWV

FtsJ   178 IRSLFTKVKVRKPDSSRARSREVYIVATGRKP
YCF4   173 FQQLFEKVEATKPPASRNVSAEIFVVCKGFKAPKRLDPRLLDPKEVFEELPDGQQNMESK
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FIG. 6. Sequence comparison of FtsJ with a potential protein YCF4 in *S. cerevisiae*. Alignment of the primary sequences of FtsJ and YCF4 is indicated. The position in the primary sequence of the first amino acid on every line is shown. Identical amino acids are boxed, and similar amino acids are indicated by dots. Gaps are indicated by dashes.

bound vacuole-type ATPases (23). Pas1p is required for peroxisome assembly, as described above. An essential open reading frame, homologous to this family, is located adjacent to *VPS33(SLP1)*, which is involved in vacuolar morphogenesis and function (1, 33). These results suggest that this novel family of putative ATPases might act in important steps of membrane functions in both eukaryotes and prokaryotes.

As described above, FtsH and SUG1/TBP-1/MSS1 each has only one homologous domain with a putative ATP-binding sequence, whereas the other members of the family have two domains. The SUG1/TBP-1/MSS1 subfamily has been indicated to be involved in the regulation of gene expression (21, 28, 29). FtsH protein therefore may also regulate gene expression in *E. coli*. The increased expression of *ftsI* (i.e., PBP3 expression) in the *ftsH1*(Ts) mutant at 42°C (22) may be explained by the possible negative control of FtsH on *ftsI* expression. This, however, cannot explain the decrease in the amount of PBP3 in the membrane fraction of the *ftsH1*(Ts) mutant at 42°C (2). The decrease should be due to a defect at the posttranslational stage, as described above.

We cloned and sequenced another gene, *ftsJ*, which is not essential for growth but affects cell division. Although the *ftsJ* and *ftsH* genes are closely located and transcribed in the same direction, the downstream gene *ftsH* has its own potential promoter within the intergenic region (Fig. 2). The Δ*ftsJ*::*kan* mutation was fully complemented by the *ftsJ*$^+$ plasmid. Therefore, the *ftsJ* phenotypes, slow growth and filamentation, are not due to the possible polar effect by the kanamycin cassette, which may reduce *ftsH* expression. The function of the *ftsJ* gene and possible relationship between *ftsJ* and *ftsH* remain to be studied.

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ADDENDUM IN PROOF

The *YCF4* gene in *S. cerevisiae*, whose potential product shows a significant homology to FtsJ (Fig. 6), corresponds to YCL54w located on chromosome III (Oliver et al., Nature **357**:38–46, 1992).

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