*JB Minireview—Quality Control of the Cellular Protein Systems*

*J. Biochem.* 2009;146(4)449–454
doi:10.1093/jb/mvp071

# Quality Control of Cytoplasmic Membrane Proteins in *Escherichia coli*

**Yoshinori Akiyama***
*Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan*

Received March 31, 2009; accepted April 11, 2009; published online May 19, 2009

**In *Escherichia coli*, like in any organism, the cytoplasmic (inner or plasma) membrane proteins play essential roles in transport of small and macro-molecules as well as in transmission of environmental signals across the membrane. Their quality control is critically important for growth and survival of the cell. However, our knowledge about the players and mechanisms of the system is still limited. This review focuses on proteolytic quality control of membrane proteins, in which two membrane-integrated proteases, FtsH and HtpX, with different modes of action, play central roles. The prohibitin family membrane protein complexes (HflKC and QmcA) contribute to the quality control system as a regulatory factor of FtsH and also as a possible membrane-chaperone. Failure of the quality control system to function normally leads to accumulation of malfolded cytoplasmic membrane proteins, which in turn activate the stress response pathways previously believed to be specialized for sensing protein abnormalities outside the cytoplasmic membrane. In fact, many of the cytoplasmic membrane quality control factors are stress induced. Further characterization of them as well as of the stress-sensing mechanisms would prove useful to obtain an integrated picture of the membrane protein quality control system.**

**Key words: *E. coli*, stress responses, membrane proteases, membrane chaperones, protein degradation.**

Cells of a Gram-negative bacterium *Escherichia coli* are surrounded by two layers of membranes, outer and cytoplasmic (inner). The cytoplasmic membrane has a more complex protein profile than the outer membrane; it was estimated that about 20% of *E. coli* ORFs encode proteins destined to this location (*1*). They include transporters, channels, receptors and enzymes involved in the synthesis and metabolism of membrane constituents, accounting for a large part of the biological activities of the membrane. These membrane proteins should be properly synthesized, inserted into the membrane and folded into an active conformation, which may further assemble into a functional complex. Defects in such processes could occur under normal and stressed conditions. Moreover, hazardous environmental changes could induce denaturation and inactivation of pre-formed membrane proteins. The resulting accumulation of abnormal membrane proteins would disturb the membrane structure and function, eventually compromising cellular integrity and viability. Therefore, it is important for cells to monitor protein folding states and to quickly eliminate and/or repair the abnormal proteins. Despite the physiological importance, protein quality control at the cytoplasmic membrane has only poorly been investigated as compared to analogous processes at other cellular compartments. We have been characterizing factors involved in protein quality control in the cytoplasmic membrane (Fig. 1).

## FtsH

While most cytoplasmic membrane proteins are assumed to be stable under normal growth conditions, their abnormal states can be subject to rapid degradation. An ATP-dependent and membrane-bound zinc metalloprotease FtsH is the first enzyme that was shown to be responsible for membrane protein degradation (*2*). FtsH degrades SecY, the central membrane subunit of the protein translocation machinery, and Fo*a*, a subunit of the Fo sector of proton ATPase, when they have failed to assemble into the respective complex (*3*, *4*). YccA, a stress-inducible membrane protein receives slow FtsH-dependent degradation when overexpressed (*5*).

FtsH also degrades several short-lived cytoplasmic proteins such as $\sigma^{32}$, LpxC, λCII and λxis (*2*). $\sigma^{32}$ is dedicated to transcription of the cytoplasmic heat-shock genes. FtsH, which is itself a $\sigma^{32}$-controlled heat shock protein, is thus involved in regulation of its own expression (*6*). LpxC is an enzyme that catalyses a committed step for synthesis of the lipid part of lipopolysaccharide (LPS). Stabilization of LpxC in cells defective in FtsH leads to the lethal unbalance in the synthesis of phospholipids and that of LPS (*7*). An allele (*sfhC21*) of *fabZ* involved in fatty acid biosynthesis restores both the phospholipids/LPS balance and the growth of the *ftsH* null mutant (*7*). KdtA required for the synthesis of the sugar moiety of LPS is also a substrate of FtsH (*8*). Therefore, the FtsH

*To whom correspondence should be addressed.
Tel: +81-75-751-4040, Fax: +81-75-771-5699;
E-mail: yakiyama@virus.kyoto-u.ac.jp

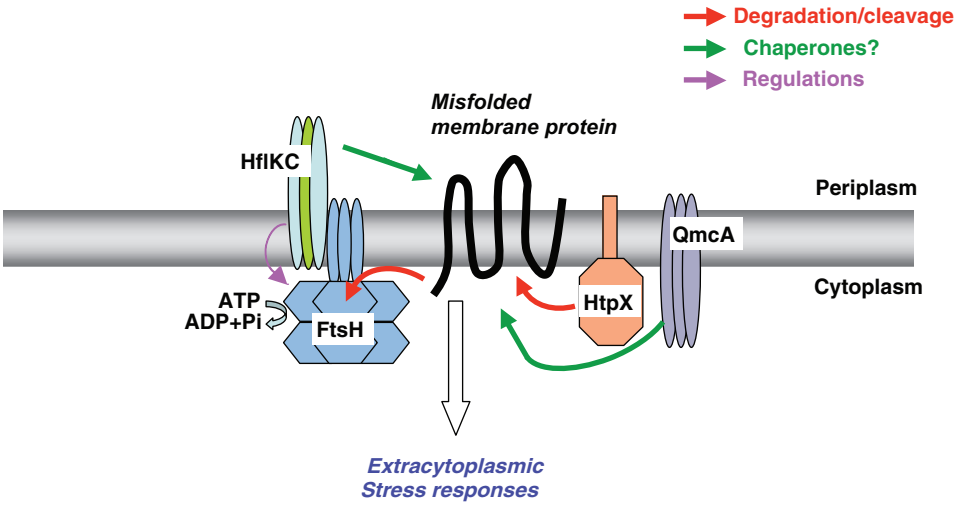


Fig. 1. **Quality control factors and stress response mechanisms that care cytoplasmic membrane proteins in *E. coli*.** FtsH dislocate abnormal membrane proteins out of the membrane for their energy-dependent and processive degradation, to which HtpX may contribute by introducing endoproteolytic cleavages into cytoplasmic loops of the substrates. PHD proteins, HflKC and QmcA function as a regulator of FtsH and/or a membrane chaperone. Abnormal membrane proteins elicit 'extracytoplasmic' stress responses.

activity is required for maintenance of both the protein and lipid compositions of the membranes.

FtsH is anchored in the membrane by the N-terminal two transmembrane segments, which is followed by the cytoplasmic $AAA^+$ ATPase domain and the metalloprotease domain (*2*). The $AAA^+$ protein modules are ubiquitously found in biological kingdoms and known to be involved in a wide variety of cellular processes (*9*). The membrane-anchoring domain of FtsH is required for its oligomerization, which is essential for the protease activity (*10*). The orthologues of FtsH in mitochondria and chloroplasts also play crucial roles in the biology of these organelles (*11*, *12*). FtsH is an ATP-hydrolysis-dependent and processive protease, although it can degrade small peptides in the presence of a non-hydrolysable ATP analogue (*13*).

Crystal structures of the $AAA^+$ ATPase domain and the whole cytoplasmic region of FtsH showed that it forms a hexameric ring-like assembly (*14*, *15*). In the ADP-bound structure of the cytoplasmic region determined by Suno *et al*. (*14*), the AAA domains adopt a 3-fold symmetry, in which each subunit alternately assumes an 'open' and a 'closed' conformation, while the protease domains have a 6-fold symmetric hexameirc structure. Although the details of how the substrate polypeptide is delivered and presented to the internally located protease active site are obscure, it was proposed that repeated cycles of a substrate binding to a conserved aromatic residue on the edge of the central pore and open–close conformational changes of the AAA domains are coupled with ATP hydrolysis to drive movement of the substrate polypeptide from the central pore of the AAA domain through a tunnel to the internal protease active sites. In addition to ATP hydrolysis, proton motive force (PMF) has been shown to stimulate proteolytic activity of membrane-bound FtsH (*16*). PMF appears to simulate FtsH by somehow affecting the membrane-anchoring region.

Insights into features of substrates required for FtsH-dependent proteolysis were originally gained form the studies of YccA (*5*, *17*), spanning the cytoplasmic membrane seven times with its N-terminus exposed to the cytoplasm (*18*). The *yccA11* mutation, an internal deletion of eight amino acid residues from the N-terminal cytoplasmic tail, was found to render YccA refractory to proteolysis by FtsH (*5*). Systematic deletion and mutational analyses of YccA and other substrate membrane proteins showed that N-terminal cytoplasmic tails with diverse sequences can confer FtsH-susceptibility when their lengths are longer than 20 amino acid resides, which presumably provide an initiation site for the processive proteolysis (*17*). FtsH can also initiate degradation from the C-terminal cytoplasmic tails of some membrane proteins (*19*). The SsrA tag sequence (*20*) that is attached to the C-terminus of certain soluble protein can be recognized by FtsH for degradation, at least *in vitro*, suggesting that FtsH might undertake degradation of SsrA-tagged membrane proteins in the trans-translation quality control mechanism. Molecular basis for the apparently bi-directional initiation ability of FtsH is an interesting question. Moreover, FtsH can initiate *in vitro* degradation of apo-flavodoxin from its internal sites (*21*). Thus, FtsH could initiate degradation of membrane proteins from either terminus as well as from cytoplasmic loops.

Membrane-embedded and periplasmic regions of membrane proteins are sequestered from the cytosolically disposed catalytic domains of FtsH. However, FtsH-mediated degradation is generally not accompanied by accumulation of discrete degradation products containing a membrane-embedded or an periplasmically exposed domain of substrates. *In vivo* analyses of degradation of a model membrane protein carrying an immunologically recognizable periplasmic domain, whose folding state can be manipulated, demonstrated that, when the domain was unfolded, it was FtsH-dependently degraded

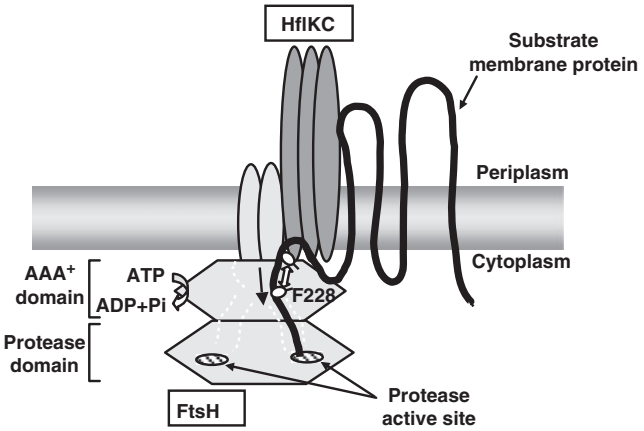


Fig. 2. **A model of FtsH action in membrane protein degradation.** HflKC might control the substrate access to FtsH within the membrane or on the periplasmic side. Upon successful encounter, a cytoplasmic tail of a substrate membrane protein is recognized by FtsH and delivered through a tunnel to the internal protease active site by cycles of movement of the central pore-aromatic residue (F228) in a manner coupled with ATP hydrplysis-dependent open–close conformational changes of the ATPase domain. Substrate dislocation and proteolysis then continues processively.

together with the region C-terminal to it. When the periplasmic domain was folded tightly, the degradation stopped in front of it. The inference that the degradation depended on the initiation event on the cytosolic side led to the 'substrate dislocation model' of FtsH-mediated membrane protein degradation (*18*) (Fig. 2).

FtsH catalysis of membrane protein dislocation and degradation was reproduced *in vitro* using proteoliposomes reconstituted from purified FtsH and a substrate membrane protein (*22*), suggesting that FtsH can dislocate a substrate from the membrane without any aid from other cellular proteins. FtsH-mediated extraction and degradation of membrane proteins is reminiscent of ER-associated degradation (ERAD) of eukaryotic membrane proteins, in which another AAA ATPase, p97, plays pivotal roles (*23*).

A putative polypeptide binding site of FtsH was mapped onto the surface of the $AAA^+$ domain (*24*). It may recognize a cytoplasmic tail of substrate membrane proteins to capture the substrate to dislocate it out of the membrane and deliver it to the protease active site. FtsH must be membrane-associated to degrade membrane proteins (*10*). Membrane tethering should be required for proper positioning of FtsH to interact with the substrate and also to generate the force for its dislocation. Substrate binding by the central pore region and conformational changes coupled with ATP binding/hydrolysis mentioned above could provide the force to extract a substrate from the membrane.

Earlier studies suggested some non-proteolytic functions of FtsH. Its mutations or overproduction can modulate topogenesis of model membrane proteins without affecting their stability (*25*), and some of such phenotypes were suppressed by overexpression of cytoplasmic molecular chaperones (*26*). In addition, the $AAA^+$ domain of a yeast FtsH homologue, Yme1, exhibits a chaperone-like activity *in vitro* (*27*). Although FtsH could possess a chaperone activity, its protein unfolding activity is much lower than that of other ATP-dependent proteases (*28*). It remains to be elucidated how generally cells utilize the protein remodelling ability of FtsH, as exemplified by its membrane protein dislocation function.

## HtpX

HtpX was first identified as a $\sigma^{32}$-controlled heat-shock protein, whose overexpression affects lysogenziation of phage λ. The primary sequence suggested its localization to the membrane as well its being a zinc-metalloprotease. Involvement of HtpX in membrane protein quality control was suggested from its rediscovery as a gene product controlled by the Cpx extracytoplasmic stress response regulator as well as from a synthetic phenotype of its deletion when combined with the *ftsH* disruption (*29*). Whereas single disruption of *htpX* is silent, its combination with Δ*ftsH* (in the presence of the *sfhC21* suppressor) results in temperature-sensitive growth failure. It was thus suggested that FtsH and HtpX have overlapping or complementary cellular functions (*29*).

HtpX is integrated into the cytoplasmic membrane with overall topology similar to that of FtsH (*29*). As expected from its metalloprotease active-site sequence motif, purified HtpX was shown to degrade casein and SecY *in vitro* (*30*). The proteolytic action of HtpX was ATP-independent and endoproteolytic, by which it introduced several cuts into cytoplasmic loops of SecY (*30*). These features are in sharp contrast to the energy dependence and the processivity characteristically observed with the FtsH action. It is conceivable that HtpX collaborates with FtsH in proteolytic quality control of membrane proteins by introducing endoproteolytic cleavages into cytoplasmic regions of the substrates. This will generate cytoplasmic tails that are subsequently recognized by FtsH to initiate rapid proteolysis of the rest of the polypeptide chain. Although HtpX-dependent cleavage was reproduced *in vivo* for overproduced SecY (*30*), 'natural substrates' of HtpX have not been identified. Recently, disruption of *htpX* was shown to repress swarming motility without impairing swimming of individual cells (*31*). HtpX might participate in the phenomenon of swarming through degradation of some envelope proteins that affect swarming-related cell-to-cell interactions. *Escherichia coli* has three uncharacterized HtpX homologues, presumably one in the cytoplasmic membrane and the other two in the outer membrane (*30*).

Oma1 is a yeast protein having sequence homology with HtpX and physiological functions that overlap those of the m-AAA protease, a mitochondrial FtsH homologue (*32*). It was reported that Oma1 cleaves loop regions of a substrate membrane protein (Oxa1) at both sides of the membrane, but it is unknown how Oma1, a possible inner membrane protease, can achieve it.

## PROHIBITIN HOMOLOGUES

Three *E. coli* membrane proteins, HflK, HflC and QmcA, are known to contain a PHB (Prohibitin homologous)

domain (also known as an SPFH domain). Eukaryotic PHB proteins possess protein- and/or lipid-binding properties and many of them are associated with the lipid raft of membranes in various cellular compartments (*33*). They have been implicated to be involved in many different cellular processes, including apoptosis, cell proliferation, senescence and regulation of mitochondrial functions, but their exact roles are poorly understood. Our studies suggest that the *E. coli* PHB proteins participate in the protein quality control of the cytoplasmic membrane.

*HflK and HflC*—HflK and HflC are cytoplasmic membrane proteins with a type II ($N_{IN}$-$C_{OUT}$) orientation. Each of them has a large periplasmic domain and a very short or negligible length of cytoplasmic tail (*18*). They form a heteo-multimeric complex (HflKC), which interacts further with the FtsH hexamer to form a higher order complex of about 1 MDa, named 'FtsH holoenzyme'. The proposed composition of the holoenzyme is $(FtsH)_6(HflKC)_6$ (*34*). Electron microscopic analyses revealed that *Saccharomyces cerevisiae* prohibtins (Phb1 and Phb2) (*35*) and *E. coli* HflKC (Saikawa, N., unpublished data) assume asymmetric ring-like structures.

Several lines of evidence suggest that HflKC negatively regulate the proteolytic function of FtsH against membrane protein substrates (*5*, *36*, *37*). Whereas some missense mutations in *hflK* and *hflC* (*hflK13* and *hflC9*) interfere with degradation of uncomplexed SecY molecules, deletion of *hflK-hflC* accelerates it. That HflKC is a negative modulator of FtsH was supported by the *in vitro* ability of purified HflKC to inhibit FtsH-catalysed proteolysis of SecY and other proteins. In contrast, degradation of soluble substrates is not markedly affected either by the *hflK13*/*hflC9* mutation or the *hflK-hflC* deletion. Thus, it appears that HflKC differentially modulates the proteolytic function of FtsH depending on the class of its substrates, membrane-integrated *vs*. soluble. Similar differential effects were observed with the YccA11 mutant protein, which is no longer degraded by FtsH but remains associated with it (*5*). Interestingly, the YccA11 inhibitory effect depends on HflKC. This mutant protein does not affect the FtsH-dependent rapid degradation of CII or RpoH. Collectively, it is suggested that membrane-bound and soluble substrates are presented to FtsH *via* different pathways and that HflKC is a regulatory factor of substrate selection.

Recent co-purification and cross-linking studies revealed that YidC, a membrane protein with essential roles in membrane protein biogenesis (insertion into membrane and folding), interacts with FtsH/HflKC, both of which are also in the proximity of a nascent membrane protein (*38*). It is a tempting possibility that the proteolytic quality control machine is ready to work at very early stages of the biosynthesis of membrane proteins.

As discussed above, the proteolytic activity of FtsH is modulated by HflK/HflC, a prohibitin homologue complex. YccA could also have regulatory roles for the FtsH activity against membrane protein substrate. Recently, Silhavy and colleagues showed that FtsH degrade the SecYEG translocon complex when it is 'jammed' with a folded domain of a preprotein. They propose that YccA is a regulator of this clearance reaction (van Stelten, J., Silva, F., Belin, D., Silhavy, T.J., personal communication). There might be another class of regulators that interact with a substrate of FtsH. A membrane-spanning and small hydrophobic peptide, MgtR, directly binds to MgtC, a multi-spanning membrane protein required for intra-macrophage survival of *Salmolella enterica*, and promotes its degradation by FtsH (*39*). MgtR seems to be a substrate-specific regulator and conceptually similar to adaptor proteins of other $AAA^+$ proteases (*40*). Thus, multiple layers of regulatory devices ensure the diverse physiological roles of FtsH.

*QmcA*—QmcA (or YbbK) is another *E. coli* PHB-domain-containing protein that also belongs to a group of prokaryotic proteins called p-stomatins (*41*). It was identified as a multi-copy suppressor against the growth defect of the *ftsH-htpX* double disruption mutant (see above). Interestingly, QmcA assumes the type I orientation and has a large cytoplasmic domain, opposite to the features of HflK and HflC. It presumably forms a homo-oligomer. Pull-down assays showed that QmcA interacts with FtsH albeit weakly. Overexpression of HflKC also partially rescues the *ftsH-htpX* growth defect. Additional knockouts of both *qmcA* and *hflKC*, but not a single knockout of either of them, exacerbate the growth defect of the Δ*ftsH-htpX* strain, suggesting further that QmcA and HflKC have related or similar functions in quality control of membrane proteins. As the *hflKC* mutational and overexpression effects can be observed in the absence of FtsH, HflKC must have a function other than a simple modulator of the FtsH activity. Eukaryotic prohibits have been implicated to possess a membrane protein-specific chaperone activity (*42*). It is an intriguing possibility that QmcA and HflKC serve as a membrane chaperone on each side of the membrane. Such an organization of membrane chaperones would be beneficial for the cell to care a wide range of membrane proteins with diverse topologies of membrane integration.

## MEMBRANE–PROTEIN STRESS

Protein abnormalities are sensed by stress response mechanisms, leading to increased expression of chaperones, folding catalysts and proteases. Such mechanisms are specialized to deal with stresses imposed to different cellular locations. In *E. coli*, cytoplasmic stresses are dealt with by activation of $\sigma^{32}$ and cell envelope stresses are mainly dealt with either by activation of another alternative sigma factor, $\sigma^E$, or a two-component system, Cpx. Cytoplasmic membrane proteins can generate both classes of stresses. When targeting of nascent membrane proteins to the cytoplasmic membrane is impaired [for instance, by dysfunction of the signal recognition particle (SRP)], the $\sigma^{32}$ stress response is upregulated, in which increased levels of cytosolic energy-dependent proteases Lon and HslUV(ClpQY) become growth-essential (*43*). The membrane-bound quality control factors FtsH, HflK/C and HtpX are also regulated by $\sigma^{32}$, suggesting that they also contribute

to elimination of unintegrated membrane protein precursors.

Although the $\sigma^E$ and the Cpx envelope stress responses (ESR) had been believed to solely respond to accumulation of abnormal proteins in the periplasm and the outer membrane (*44*), we have shown that these systems also sense abnormal proteins in the cytoplasmic membrane. Cells devoid of the FtsH quality control protease contained increased levels of Cpx- and $\sigma^E$-dependent transcription, which was further upregulated upon overproduction of an FtsH substrate membrane protein (*22*). ESR upregulation was also observed in cells depleted for YidC, which is known to be required for correct folding of some membrane proteins (*45*, *46*). A class of SecY alterations was shown not only to generate mal-folded states of lactose permease and presumably of other membrane proteins but also to elicit the ESR (*29*, *46*). Lack of poshpatidylethanolamine, a lipid required for membrane protein topogenesis, also induces the Cpx stress response (*47*). Thus, the ESR systems sense membrane-integrated but abnormal states of proteins in the cytoplasmic membrane.

That the FtsH-HtpX protease combination is essential for cell viability together with the fact that HtpX is Cpx-regulated (*29*) suggests that these membrane proteases combat the 'membrane stresses' by eliminating misfolded membrane proteins. Recent analyses of the lethal effects of aminoglycoside abtibiotics indicate that accumulation of mistranslated membrane proteins is a lethal element, in which HflKC has some role. Intriguingly, the Cpx system enhances the antibiotic-induced cell death (*48*), posing a question of why ESR, which protects cells under physiological stress, should function otherwise in response to a non-physiological category of membrane/envelope perturbations. The mechanism of stress-sensing in the cytoplasmic membrane is an important unsolved question. It should also be asked whether abnormal membrane proteins can indeed be repaired. To address these questions we probably need to identify many more additional quality control components that could exist in the membrane.

## ACKNOWLEDGEMENTS

I express special thanks to K. Ito for directing my attention to some of the recent publications as well as for critically reading and editing the manuscript.

## REFERENCES

1. Boyd, D., Schierle, C., and Beckwith, J. (1998) How many membrane proteins are there? *Protein Sci.* **7**, 201–205
2. Ito, K. and Akiyama, Y. (2005) Cellular functions, mechanism of action, and regulation of FtsH protease. *Annu. Rev. Microbiol.* **59**, 211–231
3. Kihara, A., Akiyama, Y., and Ito, K. (1995) FtsH is required for proteolytic elimination of uncomplexed forms of SecY, an essential protein translocase subunit. *Proc. Natl Acad. Sci. USA* **92**, 4532–4536
4. Akiyama, Y., Kihara, A., and Ito, K. (1996) Subunit *a* of proton ATPase $F_0$ sector is a substrate of the FtsH protease in *Escherichia coli*. *FEBS Lett.* **399**, 26–28
5. Kihara, A., Akiyama, Y., and Ito, K. (1998) Different pathways for protein degradation by the FtsH/HflKC membrane-embedded protease complex: an implication from the interference by a mutant form of a new substrate protein, YccA. *J. Mol. Biol.* **279**, 175–188
6. Herman, C., Thévenet, D., D'Ari, R., and Bouloc, P. (1995) FtsH/HflB controls the heat shock response in *Escherichia coli* via $\sigma^{32}$ proteolysis. *Proc. Natl Acad. Sci. USA* **92**, 3516–3520
7. Ogura, T., Inoue, K., Tatsuta, T., Suzaki, T., Karata, K., Young, K., Su, L.-H., Fierke, C.A., Jackman, J.E., Raetz, C.R. H., Coleman, J., Tomoyasu, T., and Matsuzawa, H. (1999) Balanced biosynthesis of major membrane components through regulated degradation of the committed enzyme of lipid A biosynthesis by the AAA protease FtsH (HflB) in *Escherichia coli*. *Mol. Microbiol.* **31**, 833–844
8. Katz, C. and Ron, E.Z. (2008) Dual role of FtsH in regulating lipopolysaccharide biosynthesis in *Escherichia coli*. *J. Bacteriol.* **190**, 7117–7122
9. White, S.R. and Lauring, B. (2007) $AAA^+$ ATPases: achieving diversity of function with conserved machinery. *Traffic* **8**, 1657–1667
10. Akiyama, Y. and Ito, K. (2000) Roles of multimerization and membrane association in the proteolytic functions of FtsH (HflB). *EMBO J.* **19**, 3888–3895
11. Sakamoto, W. (2006) Protein degradation machineries in plastids. *Ann. Rev. Plant Biol.* **57**, 599–621
12. Koppen, M. and Langer, T. (2007) Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases. *Crit. Rev. Biochem. Mol. Biol.* **42**, 221–242
13. Yamada-Inagawa, T., Okuno, T., Karata, K., Yamanaka, K., and Ogura, T. (2003) Conserved pore residues in the AAA protease FtsH are important for proteolysis and its coupling to ATP hydrolysis. *J. Biol. Chem.* **278**, 50182–50187
14. Suno, R., Niwa, H., Tsuchiya, D., Zhang, X., Yoshida, M., and Morikawa, K. (2006) Structure of the whole cytosolic region of ATP-dependent protease FtsH. *Mol. Cell* **22**, 575–585
15. Bieniossek, C., Schalch, T., Bumann, M., Meister, M., Meier, R., and Baumann, U. (2006) The molecular architecture of the metalloprotease FtsH. *Proc. Natl Acad. Sci. USA* **103**, 3066–3071
16. Akiyama, Y. (2002) Membrane potential stimulates the proteolytic activity of FtsH, a membrane-bound ATP-dependent protease in *E. coli*. *Proc. Natl Acad. Sci. USA* **99**, 8066–8071
17. Chiba, S., Akiyama, Y., Mori, H., Matsuo, E., and Ito, K. (2000) Length recognition at the N-terminal tail for the initiation of FtsH-mediated proteolysis. *EMBO Rep.* **1**, 47–52
18. Kihara, A., Akiyama, Y., and Ito, K. (1999) Dislocation of membrane proteins in FtsH-mediated proteolysis. *EMBO J.* **18**, 2970–2981
19. Chiba, S., Akiyama, Y., and Ito, K. (2002) Membrane protein degradation by FtsH can be initiated from either end. *J. Bacteriol.* **184**, 4775–4782
20. Herman, C., Thévenet, D., Bouloc, P., Walker, G.C., and D'Ari, R. (1998) Degradation of carboxy-terminal-tagged cytoplasmic protein by the *Escherichia coli* protease HflB (FtsH). *Genes Dev.* **12**, 1348–1355
21. Okuno, T., Yamanaka, K., and Ogura, T. (2006) An AAA protease FtsH can initiate proteolysis from internal sites of a model substrate, apo-flavodoxin. *Genes Cells* **11**, 261–268
22. Akiyama, Y. and Ito, K. (2003) Reconstitution of membrane proteolysis by FtsH. *J. Biol. Chem.* **278**, 18146–18153
23. Nakatsukasa, K. and Brodsky, J.L. (2008) The recognition and retrotranslocation of misfolded proteins from the endoplasmic reticulum. *Traffic* **9**, 861–870
24. Niwa, H., Tsuchiya, D., Makyio, H., Yoshida, M., and Morikawa, K. (2002) Hexameric ring structure of the ATPase domain of the membrane-integrated

metalloprotease FtsH from *Thermus thermophilus* HB8. *Structure* **10**, 1415–1423
25. Akiyama, Y., Ogura, T., and Ito, K. (1994) Involvement of FtsH in protein assembly into and through the membrane. I. Mutations that reduce retention efficiency of a cytoplasmic reporter. *J. Biol. Chem.* **269**, 5218–5224
26. Shirai, Y., Akiyama, Y., and Ito, K. (1996) Suppression of *ftsH* mutant phenotypes by overproduction of molecular chaperones. *J. Bacteriol.* **178**, 1141–1145
27. Leonhard, K., Stiegler, A., Neupert, W., and Langer, T. (1999) Chaperone-like activity of the AAA domain of the yeast Yme1 AAA protease. *Nature* **398**, 348–351
28. Herman, C., Prakash, S., Lu, C.Z., Matouschek, A., and Gross, C.A. (2003) Lack of a robust unfoldase activity confers a unique level of substrate specificity to the universal AAA protease FtsH. *Mol. Cell* **11**, 659–669
29. Shimohata, N., Chiba, S., Saikawa, N., Ito, K., and Akiyama, Y. (2002) The Cpx stress response system of *Escherichia coli* senses plasma membrane proteins and controls HtpX, a membrane protease with a cytosolic active site. *Genes Cells* **7**, 653–662
30. Sakoh, M., Ito, K., and Akiyama, Y. (2005) Proteolytic activity of HtpX, a membrane-bound and stress-controlled protease from *Escherichia coli*. *J. Biol. Chem.* **280**, 33305–33310
31. Inoue, T., Shingaki, R., Hirose, S., Waki, K., Mori, H., and Fukui, K. (2007) Genome-wide screening of genes required for swarming motility in *Escherichia coli* K-12. *J. Bacteriol.* **189**, 950–957
32. Kaser, M., Kambacheld, M., Kisters-Woike, B., and Langer, T. (2003) Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease. *J. Biol. Chem.* **278**, 46414–46423
33. Browman, D.T., Hoegg, M.B., and Robbins, S.M. (2007) The SPFH domain-containing proteins: more than lipid raft markers. *Trends Cell Biol.* **17**, 394–402
34. Saikawa, N., Akiyama, Y., and Ito, K. (2004) FtsH exists as an exceptionally large complex containing HflKC in the plasma membrane of *Escherichia coli*. *J. Struct. Biol.* **146**, 123–129
35. Tatsuta, T., Model, K., and Langer, T. (2005) Formation of membrane-bound ring complexes by prohibitins in mitochondria. *Mol. Biol. Cell* **16**, 248–259
36. Kihara, A., Akiyama, Y., and Ito, K. (1996) A protease complex in the Escherichia coli plasma membrane: HflKC (HflA) forms a complex with FtsH (HflB), regulating its proteolytic activity against SecY. *EMBO J.* **15**, 6122–6131
37. Kihara, A., Akiyama, Y., and Ito, K. (1997) Host regulation of lysogenic decision in bacteriophage λ: transmembrane modulation of FtsH (HflB), the cII degrading protease, by HflKC (HflA). *Proc. Natl Acad. Sci. USA* **94**, 5544–5549
38. van Bloois, E., Dekker, H.L., Froderberg, L., Houben, E.N., Urbanus, M.L., de Koster, C.G., de Gier, J.W., and Luirink, J. (2008) Detection of cross-links between FtsH, YidC, HflK/C suggests a linked role for these proteins in quality control upon insertion of bacterial inner membrane proteins. *FEBS Lett.* **582**, 1419–1424
39. Alix, E. and Blanc-Potard, A.B. (2008) Peptide-assisted degradation of the Salmonella MgtC virulence factor. *EMBO J.* **27**, 546–557
40. Baker, T.A. and Sauer, R.T. (2006) ATP-dependent proteases of bacteria: recognition logic and operating principles. *Trends Biochem. Sci.* **31**, 647–653
41. Chiba, S., Ito, K., and Akiyama, Y. (2006) The *Escherichia coli* plasma membrane contains two PHB (prohibitin homology) domain protein complexes of opposite orientations. *Mol. Microbiol.* **60**, 448–457
42. Nijtmans, L.G.J., deJong, L., Sanz, M.A., Coates, P.J., Berden, J.A., Beck, J.W., Muijsers, A.O., van der Spek, H., and Grivell, L.A. (2000) Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. *EMBO J.* **19**, 2444–2451
43. Bernstein, H.D. and Hyndman, J.B. (2001) Physiological basis for conservation of the signal recognition particle targeting pathway in *Escherichia coli*. *J. Bacteriol.* **183**, 2187–2197
44. Rowley, G., Spector, M., Kormanec, J., and Roberts, M. (2006) Pushing the envelope: extracytoplasmic stress responses in bacterial pathogens. *Nature Rev.* **4**, 383–394
45. Nagamori, S., Smirnova, I.N., and Kaback, H.R. (2004) Role of YidC in folding of polytopic membrane proteins. *J. Cell Biol.* **165**, 53–62
46. Shimohata, N., Nagamori, S., Akiyama, Y., Kaback, H.R., and Ito, K. (2007) SecY alterations that impair membrane protein folding and generate a membrane stress. *J. Cell Biol.* **176**, 307–317
47. Mileykovskaya, E. and Dowhan, W. (1997) The Cpx two-component signal transduction pathway is activated in *Escherichia coli* mutant strains lacking phosphatidylethanolamine. *J. Bacteriol.* **179**, 1029–1034
48. Kohanski, M.A., Dwyer, D.J., Wierzbowski, J., Cottarel, G., and Collins, J.J. (2008) Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* **135**, 679–690