Review

Structure and function of the bacterial AAA protease FtsH ☆

Sina Langklotz ^a, Ulrich Baumann ^{b,**}, Franz Narberhaus ^{a,*}
^a Lehrstuhl für Biologie der Mikroorganismen, Ruhr-Universität Bochum, Germany

^b Institut für Biochemie, Universität zu Köln, Germany

ARTICLE INFO

Article history:

Received 17 July 2011

Received in revised form 24 August 2011

Accepted 30 August 2011

Available online 8 September 2011

Keywords:

FtsH

Proteolysis

AAA protein

LpxC

LPS biosynthesis

Heat shock

ABSTRACT

Proteolysis of regulatory proteins or key enzymes of biosynthetic pathways is a universal mechanism to rapidly adjust the cellular proteome to particular environmental needs. Among the five energy-dependent AAA⁺ proteases in *Escherichia coli*, FtsH is the only essential protease. Moreover, FtsH is unique owing to its anchoring to the inner membrane. This review describes the structural and functional properties of FtsH. With regard to its role in cellular quality control and regulatory circuits, cytoplasmic and membrane substrates of the FtsH protease are depicted and mechanisms of FtsH-dependent proteolysis are discussed. This article is part of a Special Issue entitled: AAA ATPases: structure and function.

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1. Introduction

Multiple regulatory mechanisms have evolved to allow for a fine-tuned adaptation of the cellular proteome to the particular environmental requirements. Especially single-celled organisms like bacteria have to respond rapidly to stress conditions and changes in their environment to ensure cell survival. Regulated proteolysis is a post-translational mechanism with a direct impact on the amount of certain proteins. Many regulatory circuits or metabolic pathways are controlled by proteolysis to facilitate the immediate responses to external influences. Furthermore, proteases are crucial for the cellular quality control as they dispose of defective enzymes and enable amino acid recycling [1]. In bacteria, energy-dependent proteolysis is mediated by AAA⁺ proteases (ATPases associated with various cellular activities), which combine an ATP-driven unfolding activity with the degradation of proteins. The physiological functions of AAA⁺ proteases in Gram-negative bacteria have been studied best in *Escherichia coli*. *E. coli* is equipped with five AAA⁺ proteases: ClpXP, ClpAP, HslUV, Lon and FtsH. All these proteases serve important cellular functions, yet only FtsH is essential for viability of *E. coli* [2]. FtsH is

universally conserved in eubacteria, mitochondria and chloroplasts. Moreover, FtsH is unique as it is anchored to the inner membrane while the other proteases are cytoplasmic [3]. Active, membrane-bound variants of the Lon protease are present only in archaea lacking FtsH homologues [4, 5]. This review on bacterial FtsH will focus on recent results of structure–function analyses and on the substrate diversity and recognition logics of the protease.

2. FtsH structure and mechanism of action

AAA⁺ proteases are self-compartmentalizing proteolytic complexes. The active centers are sequestered in the interior of barrel- or ring-shaped assemblies. As access is granted only through a narrow pore, passage of even small-sized folded proteins is not permitted. A key feature of the entry pore is the occurrence of aromatic amino acids (aa) that are important for substrate translocation and recognition [19,20,23–26]. Proteins selected for disposal carry a degradation signal that is recognized either directly by these pore residues or indirectly via adaptor molecules. ATP hydrolysis triggers conformational changes in the AAA⁺ ring resulting in a pulling of the substrate towards the narrow pore, eventually resulting in unfolding and subsequent translocation events. Proteins are processively degraded into small peptides of ~6–25 aa in length.

2.1. Primary structure: domains and sequence motifs

Contrary to other AAA⁺ proteases with the exception of Lon, in FtsH both the ATPase and proteolytic activity reside on a single polypeptide that is ~650 aa in length in most eubacteria (Fig. 1). The N-terminal part of the polypeptide chain is anchored to the lipid bilayer

Abbreviations: TM, transmembrane domain; HS, heat shock; LPS, lipopolysaccharides; PL, phospholipids; PS, photosystem

☆ This article is part of a Special Issue entitled: AAA ATPases: structure and function.

* Correspondence to: F. Narberhaus, Ruhr-Universität Bochum, Lehrstuhl für Biologie der Mikroorganismen, Universitätsstr. 150, NDEF 06/783, D-44780 Bochum, Germany. Tel.: +49 234 322 3100; fax: +49 234 321 4620.

** Correspondence to: U. Baumann, Universität zu Köln, Institut für Biochemie, Zülpicher Str. 47, room 455, D-50674 Köln, Germany. Tel.: +49 221 470 7786.

E-mail addresses: ulrich.baumann@uni-koeln.de (U. Baumann),

franz.narberhaus@rub.de (F. Narberhaus).

and comprises two transmembrane (TM) helices in *E. coli* FtsH. Yet, variants containing only one TM helix (e.g. yeast mitochondrial *i*-AAA protease Yme1) or even completely lacking the TM parts appear to exist, as found by database searches for *Haemophilus influenzae* (UniProtKb entry P45219). In *E. coli*, a small periplasmic region is located between the two TM helices. The membrane-spanning part is connected to the AAA module via a glycine-rich linker of ~15–20 aa in length. The AAA moiety contains the characteristic sequence motifs of the AAA family, namely Walker A and B as well as the pore residues and the second region of homology (SRH) fingerprint. The characteristic ‘zincin’ HEXXH motif (frequently HEAGH) identifies the protease active center. At the C terminus there is a leucine-rich motif that has been implicated in co-recognition of certain substrates [6]. The C-terminal end is of variable length and is predicted to be mostly unstructured. *E. coli* FtsH has been reported to auto-catalytically clip its C terminus but the significance of this modification is unknown [7].

2.2. Tertiary structure

First pieces of three-dimensional structural information emerged from crystal structure analyses of isolated AAA domains [8, 9]. More recently, two further crystal structures of isolated FtsH AAA domains from *Helicobacter pylori* and human paraplegin have been published [10, 11]. All those single AAA domains are monomeric and void of ATPase activity. Structures of any full-length FtsH protein are not available yet, but constructs from *Thermotoga maritima* and *Thermus thermophilus* have been crystallized [12–14]. All these structures agree in monomer fold and a general hexameric assembly with dimensions of ~100 Å in diameter and 65 Å in height. While the protease domain has a virtually identical conformation in these structures and in isolation [14], large differences exist in the arrangement of the AAA domains. These soluble constructs lack the TM helices and comprise the ATPase and the protease domain (Fig. 2A). The fold of the AAA moiety is canonical with a wedge-shaped N-terminal subdomain consisting of a five-stranded parallel beta sheet (strands β 1– β 5, Fig. 1) surrounded by α helices on both sides (η 1/ α 1 to α 5/ η 3) and a C-terminal four-helix bundle (α 6– α 9). Nucleotides bind to the Walker A motif (also called P-loop) located in the ‘wedge’ domain close to the cleft between the two subdomains. The presence and type of the nucleotide influence the angle between the two subdomains, which undergo a mainly rigid-body movement with the apo-form being most open and the ADP-form most closed with the interdomain angle changing by up to 25° [15]. The AAA module is connected to the protease domain by a flexible linker of 12 aa in length featuring a strictly conserved glycine residue (G³⁹⁸, G³⁹⁹ or G⁴⁰⁴ in *E. coli*, *T. thermophilus* or *T. maritima*, respectively). The proteolytic domain is nearly all-helical, comprising 6 α -helices (α 10– α 15) and a short piece of β structure (strands β 6– β 8). There is a very faint resemblance to zincin-type proteases like Glu-zincins (e.g. thermolysin) or metzincins, mainly restricted to the relative spatial position of the HEXXH helix and the beta strands [16]. However, direction and connectivity of the secondary structure elements do not agree. Worthwhile to mention here is the dynamic nature of the so-called ‘edge strand’ β 7, which is located above the active-site helix. In other metallo-endoproteases this β strand serves for binding of the substrate backbone in an extended antiparallel fashion. In the reported FtsH crystal structures it is only observed in the apo-state, while in the ADP-bound state this segment is either α -helical or disordered. Interestingly, a crystal structure of the apo-state in complex with a peptide-derived hydroxamate inhibitor shows parallel binding [12]. The active zinc ion is coordinated by the two imidazole side-chains of the HEXXH motif on α 10 and a strictly conserved aspartic acid on α 12 (H⁴¹⁷, H⁴²¹ & D⁴⁹⁵ in *E. coli*), thus classifying FtsH as a novel Asp-zincin. The leucine-rich motif is located at the very C terminus composed of L⁵⁶⁷, L⁵⁷⁴ and L⁵⁸¹ in *E. coli*. The helix hairpin carrying this motif consists of α 14/ α 15 and has been suggested to contribute to the recognition of certain substrates,

additionally to the aromatic pore residues of the FVG motif that are directly involved in binding and translocation [6, 17–19].

2.3. Quaternary structure

The biological entity of FtsH is a homo-hexamer. The crystal packing of the monomeric AAA domain from *E. coli* FtsH above led to the claim of six-fold symmetric rings. From these and other crystallographic studies as well as from electron microscopic reconstructions the sixfold symmetry was considered for a long time as valid for all the intermediates of the ATPase cycle [20–23]. Yet, biochemical evidence hinted at the presence of non-equivalent nucleotide binding sites in at least some nucleotide states [24, 25]. Initial claims on hexamerization via AAA domain interactions together with the involvement of the TM helices proved to be at least partially wrong. While hexamerization of *E. coli* FtsH seems to depend on the TM domains [26], the crystal structures of soluble FtsH constructs from *T. maritima* and *T. thermophilus* lacking the TM helices or of the isolated protease domain from *Aquifex aeolicus* show hexameric molecules, in which a large oligomerization interface is provided by the protease alone, while rather loose interactions are observed between the AAA domains (PDB entries 2CE7, 2CEA, 2DHR, 2DI4) [12–14]. However, the stability of the hexamer may well be enhanced by the TM-helix interactions. The assembly consists of two hexameric rings, one formed by the AAA modules and the other by the protease moieties (Fig. 2B). The protease ring displays virtually perfect sixfold-symmetry in all structures. In contrast, different symmetries of the AAA rings are observed. Both ADP-bound structures from *T. maritima* and *T. thermophilus* have all six subunits loaded with ADP but their AAA rings are totally different in symmetry, which is twofold and threefold, respectively. This difference might be caused by an intentional mutation of the conserved linker glycine residue between the AAA and protease domain in the *T. thermophilus* protein. This mutation was introduced to rigidify the linker segment to improve crystal quality. However, at least under some conditions, this mutant appears as inactive and monomeric [12]. The glycine residue adopts a backbone conformation that is Ramachandran-unfavorable for other residues. The AAA ring of the apo-structure of *T. maritima* FtsH displays sixfold symmetry (Fig. 2B). The lack of bound nucleotide opens the angle between wedge and 4-helix bundle subdomains of the AAA by some 20°, resulting in a different packing of the AAA modules in comparison to the ADP-bound state. All three different quaternary structures are realized by varying interdomain angles between (stationary) protease and (mobile) AAA moieties. In the case of the twofold symmetric *T. maritima* ADP-state, opposing subunits in the sixfold ring have the same interdomain angle but differ from the others. This leads to a reduction of the symmetry from sixfold to twofold. In *T. thermophilus* mutant FtsH, alternating pairs possessing different interdomain angles within the pair form the hexameric assembly. The importance of the different conformations observed in the *T. maritima* enzyme is illustrated by the trajectories of the aromatic pore residues of the Φ VG motif (Φ : aromatic residue, in *E. coli* FtsH F²²⁸) that participate directly in substrate recognition and translocation (Fig. 2C) [17, 18, 27–30]. In the apo-structure F²²⁸ is located at the top of a flexible loop pointing outwards and is lining a circular entrance pore. In the ADP-bound state an inward-movement of these residues is observed, leading to a staggered arrangement of the pore residues. By the conformational transition from the apo- to the ADP-state the aromatic side-chains of the pore loop travel by a distance of some 40 Å. These very large rearrangements provide the mechanical force and vectorial movement for substrate unfolding and translocation into the proteolytic chamber. Recent crystal structures of Lon and ClpX have revealed complexes deviating from sixfold symmetry as well [31, 32]. Lon in the ADP-bound state is threefold symmetric and ClpX in a likely ADP-bound state has approximate twofold symmetry, although actually each of the six monomers is in a different conformation. The current notion in the AAA⁺ field is the lack of sixfold symmetry, at least in the nucleotide-bound state.

Fig. 1. Sequence alignment of FtsH homologues. Secondary structure elements are indicated above the sequence (η_1 , 3_{10} helix; T, turn). N-terminal signal peptides, the first transmembrane helices and C-terminal residues have been omitted. Key elements are indicated below the sequences in blue (TM2, transmembrane helix 2 (predicted); Walker A and B; motifs responsible for ATP binding and hydrolysis; SRH, second region of homology; Sens1, sensor-1 element; Arg-finger, arginine finger; Zn, zinc-ligands; edge, substrate-binding edge strand). FtsH homologues were abbreviated as follows and UniProtKb entries are given: *T. maritima* FtsH, FtsH-Tm (Q9WZ49); *E. coli* FtsH, FtsH-Ec (P0AAI3); *m*-AAA subunits Yta10 and Yta12 from *Saccharomyces cerevisiae*, Yta10-Sc and Yta12-Sc (P39925, P40341); *i*-AAA subunit Yme1 from *S. cerevisiae* (C7GQB4), Yme1-Sc; FtsH1 from *Arabidopsis thaliana*, FtsH1-At (Q39102). The figure was prepared using ESPript [143].

Fig. 2. Structural features of *T. maritima* FtsH. (A) Monomer structure of FtsH from *T. maritima* (PDB entries 2CE7 and 3KDS). Termini are marked by N and C, respectively. Structural features are shown as follows: AAA module, green (with the N-terminal 'wedge' in light and the C-terminal 4-helix bundle in dark color); P-loop (Walker A), pink; ADP (from 2CE7) in ball-and-stick representation. The aromatic pore residue F²³⁴ and the functionally important linker glycine are indicated. The protease active site residues H⁴²³, E⁴²⁴, H⁴²⁷ and D⁵⁰⁰ are shown as sticks and the catalytic zinc ion is drawn as cyan sphere. The edge strand is highlighted in blue. (B) Top and side view of hexameric FtsH in the ADP-bound state (left) and in the apo-form (right). In the top view, the protease rings in the back are in identical orientation. In the side view, the protease and AAA rings are located at the left and right side of each assembly, respectively. Pore phenylalanines are depicted in yellow, ADP as orange and gray sticks. (C) Different arrangement of aromatic pore residues in ADP-bound and apo-state. F²³⁴ is shown as yellow van-der-Waals spheres, ADP in blue and the zinc ligands in cyan. The polypeptide chain is represented as gray C α trace.

3. Function of FtsH in *E. coli* and other bacterial species

3.1. Role of FtsH in quality control and degradation of membrane proteins

Bacteria possess a so-called trans-translation system based on a bi-functional transfer-messenger RNA to rescue stalled ribosomes. The incompletely synthesized polypeptide is thereby tagged with a C-terminal degradation signal (—AAXXXXALAA), called "SsrA tag". Most of these proteins are degraded by ClpXP, but ClpAP, Lon and FtsH also contribute to degradation [33–36]. While FtsH probably plays a minor role in removal of aberrant cytosolic proteins, this protease is important for the quality control of membrane proteins. Approximately 21% of the open reading frames in *E. coli* encode proteins located to the cytoplasmic membrane [37]. FtsH is needed for the degradation of subunits F₀a and SecY when they are not complexed with their partner proteins to form the ATP synthase or the SecYEG translocase, respectively [38, 39]. In case of SecY, degradation also occurs when the translocase is blocked and FtsH mutants are retarded during protein trafficking [40, 41]. Recognition of membrane proteins is sequence-independent and requires exposed N or C termini of ~20 or ~10 aa in length, respectively [42, 43]. Furthermore, FtsH degrades YccA, a protein of unknown function with seven TM domains [44]. It is discussed that YccA acts as an inhibitor for the activity of FtsH against membrane proteins [40, 44]. In *Salmonella*, FtsH degrades the inner membrane protein MgtC, which has five TM domains and is needed for intramacrophage survival. Degradation of MgtC is regulated by the 30 aa peptide MgtR, although its precise contribution to proteolysis is unknown [45]. FtsH in *Synechocystis* and plant chloroplasts is required for the quality control of the photosystem (PS) [46–49]. The proteins D1 and D2 contain five TM domains and form the reaction center of PS II. Cyanobacteria like *Synechocystis* encode four FtsH proteases. FtsH encoded by *slr0228* degrades damaged or

uncomplexed D1, D2 and also CP47 and turnover of D1 requires the exposure of N-terminal residues [50, 51].

3.2. Control of the heat shock response

Upon heat shock (HS) the bacterial cell has to cope with unfolded and aggregated proteins. This is counteracted by the expression of HS genes, which in *E. coli* are dependent on the alternative sigma factor RpoH (σ^{32}) [52]. HS proteins e.g. are molecular chaperones like DnaK/J, GroEL/ES, small HSPs like IbpA/B and AAA⁺ proteases [53]. Induction of the HS response is tightly controlled by regulation of RpoH translation, activity and stability (Fig. 3A). Translation of the *rpoH* mRNA is regulated by an RNA thermometer [54–57]. Post-translational regulation of RpoH is mediated through proteolysis by FtsH and to minor extents by cytoplasmic proteases [3, 58, 59]. RpoH from the α -proteobacterium *Caulobacter crescentus* is also degraded by FtsH [60]. Yet, the degradation mechanism seems to be different. In contrast to *C. crescentus*, turnover of RpoH in *E. coli* involves chaperones like DnaK/J and GroES/EL as adapter proteins [61–63]. At physiological conditions, RpoH expression is very low and the protein is degraded with a half-life of ~1 min, resulting in a steady-state level of ~50 molecules per cell [64]. This basal level enables a rapid induction of the HS response. Upshifts in temperature are indirectly sensed by the amounts of denatured proteins, which titrate DnaK/J away from RpoH. RNAP-bound RpoH then initiates the transcription of HS genes. The transiency of the HS response is achieved as idle chaperones retarget RpoH to proteolysis when the cellular folding state improves. DnaJ binding to region 2.1 of RpoH induces a conformational change followed by association of DnaK to region 3.2 (Fig. 3B). Binding of DnaK leads to further destabilizing structural alterations that are thought to mediate delivery to FtsH [65]. Two regions of RpoH

contain residues needed for proteolysis. In region 2.1, especially amino acid substitutions at positions L⁴⁷, A⁵⁰ and I⁵⁴ influence RpoH proteolysis [66–68]. Yet, this region is not sufficient for proteolysis [69]. Two further amino acids (A¹³¹, K¹³⁴) in region C influence RpoH stability and a minimal RpoH variant composed of regions 2.1–C is degraded by FtsH [70]. The degradation motif in region 2.1 lines up to one side of an helix when modeled on solved sigma factor structures and A¹³¹ and K¹³⁴ are roughly oriented into the same direction (Fig. 3B) [67]. These domains therefore might provide a combined platform for protein–protein interactions needed for proteolysis. As substitutions in region 2.1 do not reduce sigma factor activity or chaperone binding, this region might mediate contact to FtsH.

3.3. Dual role of FtsH in lipopolysaccharide biosynthesis

Gram-negative cells are surrounded by two membranes. The outer leaflet of the outer membrane contains high amounts of lipopolysaccharides (LPS) to form a permeability barrier against external agents like detergents or antibiotics [71, 72]. LPS is composed of a polysaccharide O-antigen, a core oligosaccharide and lipid A (Fig. 4A). Lipid A is the hydrophobic anchor of LPS in the outer membrane. Unbalanced amounts of LPS disturb its protective function and tight regulation of LPS biosynthesis is crucial for cell survival. LPS is synthesized in a sequential manner. In the first step in lipid A synthesis, the acyl group of (R)-3-hydroxymyristoyl-ACP is transferred to UDP-N-acetylglucosamine (UDP-GlcN) by LpxA (Fig. 4A) [73]. (R)-3-hydroxymyristoyl-ACP is also a precursor for the synthesis of phospholipids (PL). The first committed reaction in lipid A biosynthesis is catalyzed by LpxC [74]. This step is regulated by proteolysis of LpxC by FtsH, rendering this protease essential in *E. coli* [2]. Viability of Δ *ftsH* cells is permitted by a suppressor mutation, which rebalances the LPS to PL ratio. This mutation compensates for LpxC stabilization by increased activity of FabZ, the key enzyme of PL biosynthesis [2]. FtsH serves a dual function in regulation of LPS biosynthesis as it degrades the KDO transferase KdtA, which is located to the inner membrane by one TM domain [75, 76]. The mechanisms for FtsH-dependent proteolysis of KdtA are unknown but recognition principles for LpxC have been analyzed [77–79]. Turnover of LpxC requires a sequence- and length-specific signal at the very C terminus of the protein (Fig. 4B) [77, 78]. Resembling the SsrA tag, the C terminus of LpxC is mainly composed of non-polar residues (–LAXXXXAVLA) and a critical length (~20 aa) is needed for degradation [77, 78]. C-terminal truncations and amino acid substitutions abolished degradation but had no effect on the activity of LpxC. Therefore, the flexible C terminus solely functions as a degradation signal [78, 80]. Yet, the C terminus of LpxC is a rather general degradation tag that does not confer strict specificity to FtsH when added to other polypeptides. LpxC enzymes from different species differ in their C-terminal sequences and their susceptibility to proteolysis in *E. coli* as heterologous host for degradation experiments. Proteolysis by FtsH is highly conserved in enterobacteria (*S. enterica*, *Yersinia pseudotuberculosis*, *Vibrio cholera*). In contrast *A. aeolicus* LpxC, which lacks a C-terminal extension, is stable and proteolysis of LpxC in α -proteobacteria like e.g. *Agrobacterium tumefaciens* is mediated by the Lon protease. Although its C terminus is similar to the protein from *E. coli*, LpxC from *Pseudomonas aeruginosa* is stable in both organisms and a proteolysis-independent strategy to control LpxC activity is assumed [79].

3.4. FtsH and viral infection

Another name for FtsH is HflB (high frequency of lysogenization), which reflects that FtsH plays a role in phage λ infection [81]. The *E. coli* phage λ can enter two alternative pathways. The lytic pathway leads to phage replication and lysis of the host cell. By proteolysis of λ proteins, FtsH contributes to the decision between both pathways. Degradation of CII favors the lytic pathway [82]. The C terminus of

Fig. 3. Control of the heat shock response in *E. coli* and degradation motifs in RpoH. (A) The RpoH-dependent heat shock response is regulated on several levels. Translation of RpoH is controlled by an RNA thermometer. RpoH in complex with the RNA polymerase (RNAP) initiates HS gene expression. The cellular protein folding state is monitored by the level of free DnaK/J, which target RpoH to proteolysis. Under non-HS conditions, proteolysis keeps RpoH amounts to a minimal level needed for rapid induction of the HS response upon sudden temperature upshifts. Revised from [52]. (B) The domain organization of RpoH is shown and interaction sites for DnaJ, DnaK, FtsH and RNAP as well as for binding to –10/–35 promoter regions are marked by arrows. Regions 2.1–C of RpoH are given as a structural model and residues important for degradation are indicated in the single letter amino acid code [70].

CII is a structurally flexible degradation signal enriched in non-polar residues [83, 84]. HflD acts as an adapter protein for CII and HflK/C have modulating functions in the lysis/lysogeny switch [85–87]. Turnover of CII is abolished by proteolysis of the small (54 aa), membrane-associated protein CIII, which serves as a competitive inhibitor for FtsH [88]. An internal amphipathic helix in CIII and oligomerization are important for inhibition of FtsH [89, 90]. The amphipathic helix might be used for recruiting to FtsH but the termini seem to be involved in degradation initiation [91]. Stabilized CII induces the expression of the CI repressor for entry into lysogeny. Additionally, FtsH degrades the λ DNA excisionase Xis, which is primarily controlled by Lon [92].

3.5. Hijacking of FtsH mediates colicin activity

Colicins are bactericidal proteins produced by certain strains of *E. coli* to combat competing bacteria. *E. coli* cells carrying mutations in *ftsH*, which is also termed *tolZ* (colicine tolerance), are resistant against some colicins as toxicity of colicins with nuclease activities like E2, E3 and D requires a functional FtsH protease [93, 94]. It has been suggested that uptake and activation of these colicins is mediated by FtsH-dependent processing [95, 96]. Thereby, only the C-terminal domains of e.g. D and E3 are transported into the cell to fulfill their functions.

Fig. 4. Key role of LpxC for lipid A biosynthesis and structure of LpxC from *E. coli*. (A) The first steps in lipid A biosynthesis are shown. LpxC catalyzes the second, but first committed step in this pathway. The precursor for lipid A synthesis, (R)-3-hydroxy-myristoyl-ACP is also used for phospholipid biosynthesis (indicated in gray). Lipid A forms the hydrophobic anchor for lipopolysaccharides (LPS). Core and O-antigen sugar pentose or heptose moieties of *E. coli* LPS are schematically shown as penta- or hexagons, respectively. (B) displays the solved structure of *E. coli* LpxC (PDB entry 3PS1, [144]) with the C terminus given as amino acid sequence with key residues of the degradation signal shown in bold.

3.6. Involvement of FtsH in shut-off of the superoxide stress response

The regulator SoxR (superoxide response protein) senses oxidative stress by iron-sulfur clusters and mediates transcription of *soxS* [97, 98]. SoxS activates the superoxide stress regulon and is controlled by Lon- and to a minor extent by FtsH-dependent proteolysis [99]. The short half-life (2 min) allows the rapid turn-off of this stress response and binding to DNA or RNAP protects SoxS against proteolysis by Lon [100]. The degradation signal was narrowed down to the N-terminal 17 aa but additional stabilizing amino acid substitutions around position ~40 and within residues 62–73 of SoxS were reported [101].

3.7. In vitro substrates of *E. coli* FtsH

While searching for a photometrically monitorable model substrate for proteolysis by FtsH, the flavin mononucleotide (FMN)-free apo-flavodoxin was found to be degraded in vitro [102]. FMN-containing holo-flavodoxin, which is a small electron transfer protein, is resistant to proteolysis. FMN binding is thought to stabilize the structure of flavodoxin and proteolysis is correlated to the folding state of the protein [103]. Although it remains elusive if FtsH acts on

flavodoxin in vivo, this model substrate might give insights into the directionality of both substrate translocation and degradation, as proteolysis can be initiated from internal sites of the protein [104]. Another in vitro substrate of *E. coli* FtsH is the cell division protein FtsZ. Proteolysis of FtsZ in vitro is independent of terminal sequences and is modulated by the GTP-binding state [105, 106]. Attempts to measure FtsZ degradation by FtsH in vivo were unsuccessful and FtsZ was found to be a substrate of ClpXP [107, 108].

3.8. Cytoplasmic substrates of FtsH in other bacterial species

Although FtsH is essential in *E. coli*, *Bradyrhizobium japonicum* and *H. pylori*, it is dispensable for normal growth of other bacteria but is involved in survival upon stress conditions like heat shock or osmotic stress [60, 109–116]. FtsH might ensure the quality control of membrane proteins upon these conditions or might control specific regulatory substrates. Degradation of alternative sigma factors by FtsH seems to be a common mechanism. In *C. crescentus*, FtsH degrades the extracytoplasmic function (ECF) sigma factor σ^F and the ECF sigma factor σ^W of *Bacillus subtilis* might also be an FtsH substrate [117, 118]. In *B. subtilis*, FtsH is furthermore involved in endospore development by degradation of Spo0E and SpoVM [119–121]. Spo0E regulates the phosphorelay of the master regulator Spo0A. The C terminus of Spo0E carries the degradation signal [121]. Interestingly, the 26 aa protein SpoVM is similar to λ CIII as it harbors an amphipathic helix and acts as an inhibitor of FtsH [89, 120, 122]. In *Corynebacterium glutamicum*, FtsH together with ClpCP/ClpXP degrades the nitrogen-responsive protein GlnK. GlnK is adenylated upon nitrogen starvation and binds to the global repressor AmtR for expression of nitrogen starvation genes. When nitrogen is not limited, GlnK is sequestered to the membrane to block ammonium uptake by the AmtB transporter [123]. Interestingly, FtsH might serve a quality control function for the AmtB transporter in *E. coli* [124]. In *Synechocystis*, FtsH encoded by *slr0228* regulates levels of GgpS [125]. GgpS is needed for synthesis of the osmoprotectant glucosylglycerol. *Synechocystis* is furthermore able to synthesize a CO_2 -concentrating system. FtsH is needed for induction of this system, probably by degradation of the regulator NdhR [126].

4. The FtsH interactome in the membrane

Several membrane proteins interact with FtsH (Fig. 5). The ATP-independent endoprotease HtpX was found to serve complementary or overlapping functions, as it becomes essential upon loss of FtsH and is capable of cleaving SecY in vitro [127, 128]. It was postulated that HtpX is used to alleviate quality control by pre-cleavage of membrane proteins, which would lead to exposed termini and recognition by FtsH [127, 129]. A similar mechanism found for HtpX and FtsH

Fig. 5. Topology of FtsH and its membrane protein interactome. N or C termini of inner membrane proteins connected to cellular functions of FtsH are indicated. HflK and QmcA are prohibitin-like proteins and are encoded in an operon with HflC or YbbJ, respectively. While HtpX is a zinc-dependent endoprotease, YidC is needed for the insertion of proteins into the inner membrane.

homologues in yeast mitochondria supports this assumption [130]. Two FtsH homologues are present in the inner membrane of mitochondria (see also review by Thomas Langer in this issue). While *m*-AAA is active in the matrix, *i*-AAA protrudes into the intermembrane space. The interaction of FtsH with prohibitin-like proteins is also conserved. The exact function is largely unknown, but prohibitins associate with the *m*-AAA protease in order to regulate its proteolytic activity [131, 132]. *E. coli* possesses two prohibitin-like proteins, HflK and QmcA, which interact with FtsH. HflK and QmcA have opposite orientations in the inner membrane and are encoded in operons with HflC or YbbJ, respectively [133, 134]. The complex HflK/C regulates FtsH activity against SecY, but such a role has not been reported for QmcA [133]. Interestingly, eukaryotic prohibitins are found in membrane microdomains called lipid rafts. Lipid rafts in eukaryotes compartmentalize the membrane into coordinated platforms for various cellular processes [135, 136]. Recently it was demonstrated that bacteria contain lipid rafts to orchestrate complex processes and that prohibitin-like proteins are associated to these microdomains [137]. Therefore the question arises whether FtsH is associated with lipid rafts to coordinate interaction with partner proteins or to regulate proteolysis of substrates. Furthermore, the FtsH holoenzyme comprised of FtsH₆(HflK/C)₆ interacts with YidC, which is needed for membrane protein insertion and both locate to nascent membrane proteins. This indicates a role in quality control in very early steps of membrane protein integration [138]. Both HflK/C and QmcA/YbbJ are multicopy suppressors of *E. coli* cells lacking FtsH and HtpX. Therefore they are supposed to serve membrane chaperone functions as already known for mitochondrial prohibitins [134, 139]. Although not studied in detail, both the mitochondrial *i*-AAA and FtsH are supposed to have chaperone functions [41, 124, 140–142].

5. Concluding remarks and perspectives

Owing to its membrane localization FtsH is a unique protease, which degrades various substrates in *E. coli* and other bacteria. At present, the number of identified and characterized substrates is low compared to the Clp machinery and Lon. Global studies aimed at the identification of new substrates of FtsH will broaden the understanding of FtsH-controlled cell physiology. Identification of new FtsH substrates will further help to dissect common recognition logics for this protease. So far, the emerging principles of substrate recognition by FtsH are limited to a few known substrates. The recognition logics are diverse including non-polar C-terminal sequences like in LpxC as well as internal structural motifs as found in RpoH. Substrate selectivity might be further achieved by the weak unfoldase activity of the protease [142]. Moreover, the membrane-anchoring of FtsH deserves further investigation. Membrane integration of *E. coli* FtsH is crucial for degradation of membrane proteins. Yet, the exact mechanism of the ATPase cycle and how the ATP-provided energy is transmitted into the mechanical force for translocation of proteins from the membrane has to be resolved. Certainly, the cellular function and structural functionality of FtsH is much less understood compared to its cytoplasmic counterparts. As proteases homologous to bacterial FtsH proteins are present in eukaryotes and are known to be involved in severe diseases, further research on mechanisms of proteolysis by FtsH is of major interest.

Acknowledgements

Work in the laboratory of FN was supported by a grant from the German Research Foundation (DFG, SFB 642, GTP- and ATP-dependent membrane processes). UB acknowledges the Swiss National Science Foundation grants 31-67253.01, 3100A0-108262 and 31003A-120174. We thank Kai Westphal for critical reading of this manuscript.

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