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When, how and why? Regulated proteolysis by the essential FtsH protease in *Escherichia coli*

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Abstract: Cellular proteomes are dynamic and adjusted to permanently changing conditions by ATP-fueled proteolytic machineries. Among the five AAA⁺ proteases in *Escherichia coli* FtsH is the only essential and membrane-anchored metalloprotease. FtsH is a homohexamer that uses its ATPase domain to unfold and translocate substrates that are subsequently degraded without the need of ATP in the proteolytic chamber of the protease domain. FtsH eliminates misfolded proteins in the context of general quality control and properly folded proteins for regulatory reasons. Recent trapping approaches have revealed a number of novel FtsH substrates. This review summarizes the substrate diversity of FtsH and presents details on the surprisingly diverse recognition principles of three well-characterized substrates: LpxC, the key enzyme of lipopolysaccharide biosynthesis; RpoH, the alternative heat-shock sigma factor and YfgM, a bifunctional membrane protein implicated in periplasmic chaperone functions and cytoplasmic stress adaptation.

Keywords: AAA protease; FtsH; heat-shock response; lipopolysaccharide biosynthesis; regulated proteolysis; substrate trapping.

Introduction

All living cells rapidly adapt their proteome in response to changing environmental conditions by a number of transcriptional and translational control mechanisms. Proteolysis acts at the post-translational level and is typically catalyzed by ATP-dependent proteases. The Gram-negative model organism *Escherichia coli* harbors five different AAA⁺ (ATPases associated with a variety of cellular activities) proteases, namely ClpXP, ClpAP, HslUV, Lon and FtsH (Baker and Sauer, 2006; Sauer and Baker, 2011; Nyquist and Martin, 2014). All AAA⁺ proteases have in common that they form oligomeric structures with a central pore through which a substrate is threaded in order to reach the proteolytic chamber. AAA⁺ proteases can be divided into two functional domains, the ATPase and the protease domain. While the ATPase domain catalyzes ATP-dependent unfolding and translocation of the substrate into the proteolytic chamber, the protease domain degrades the unfolded substrate into small peptide fragments of five to 25 amino acids in length (Sauer et al., 2004; Baker and Sauer, 2006; Sauer and Baker, 2011; Bittner et al., 2016).

FtsH is the only membrane-anchored and essential protease in *E. coli*. It assembles with the membrane-anchored HflK/HflC complex, which influences the stability of some substrates like SecY or the bacteriophage lambda protein CII (Kihara et al., 1996, 2001; Saikawa et al., 2004), whereas turnover of other FtsH substrates like RpoH is not altered by HflK/C (Kihara et al., 1998). The most important function of FtsH is the regulation of the optimal ratio between phospholipids (PL) and lipopolysaccharides (LPS) in the outer membrane by degrading LpxC, the key enzyme of LPS biosynthesis (for details see the following section) (Tomoyasu et al., 1993, 1995; Ogura et al., 1999). As proteolysis is energy-demanding and irreversible, degradation of natively folded and active proteins is strictly regulated. Access of substrates to the protease requires recognition motifs, so-called degrons that can be localized at either terminus or at internal sites of a protein. A sequestered degron gets exposed when the protein is subjected to degradation. For many protease substrates, the aid of dedicated adaptor proteins is required for conditional degradation (Sauer et al., 2004; Sauer and Baker, 2011; Gur et al., 2013).

This review focusses on FtsH, a universal protease. Proteobacteria code for one copy of FtsH, whereas cyanobacteria like *Synechocystis* possess four and *Arabidopsis* chloroplasts even nine orthologs with crucial functions in...
photosynthesis (Nishimura et al., 2016). Several proteases of the FtsH type are also present in mitochondria of yeast, mammals and plants (Tatsuta and Langer, 2009; Janska et al., 2010, 2013). The ATPase and protease domains of each subunit of the homohexamer are encoded on a single gene, which led to its classification as single-chain chaperonin (Schumann, 1999). The ATPase domain of FtsH contains typical elements of ATPase function like the Walker A and Walker B motifs, an arginine finger and a sensor 1 residue (Karata et al., 2001; Ito and Akiyama, 2005; Langklotz et al., 2012; Bittner et al., 2016). FtsH is unique among the AAA+ proteases in E. coli as it contains a second region of homology (SRH), which categorizes FtsH into the AAA subfamily (Karata et al., 1999). Moreover, FtsH is the only metalloprotease among the AAA+ proteases. A conserved HEXXH-motif (X = any amino acid) in the protease domain is essential for coordinating the catalytic Zn2+ ion (Bieniossek et al., 2006).

FtsH degrades proteins for two reasons: (i) quality control of aberrant proteins and (ii) regulated proteolysis of intact proteins under specific conditions. Because the number of known FtsH substrates was relatively low, trapping approaches with an ATPase-proficient but proteolytically inactive FtsH variant (FtsHtrap) have been established. FtsHtrap is still capable of unfolding and translocating substrates into the inactivated proteolytic chamber, which contains an amino acid exchange of the first histidine of the HEXXH-motif against tyrosine. Proteins captured with FtsHtrap can then be purified and analyzed via mass-spectrometry (Westphal et al., 2012; Arends et al., 2016; Bittner et al., 2016). Putative substrates are validated by in vivo degradation experiments determining the half-lives of mildly overexpressed recombinant His-tagged proteins after inhibition of protein biosynthesis followed by Western blot analysis. Two independent trapping approaches revealed nine novel FtsH substrates, extending the number of known FtsH substrates in E. coli to 21 (Table 1). The substrate repertoire demonstrates that FtsH is involved in a remarkable variety of cellular processes like the quality control of membrane-anchored (SecY, YccA, Foa and PspC) (Kihara et al., 1995, 1996, 1999; Akiyama et al., 1996a,b; Chiba et al., 2000, 2002; Singh and Darwin, 2011) and cytosolic proteins (SsrA-tagged proteins) (Herman et al., 1998), the lysis/lysogeny decision of phage λ (Herman et al., 1997; Kihara et al., 1997, 2001; Shotland et al., 1997; Leffers and

Table 1: FtsH substrates in E. coli and their mechanism of recognition.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Recognition mechanism/modulators/localization of degrons</th>
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<tr>
<td><strong>Cytoplasmic substrates</strong></td>
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<tr>
<td>SsrA</td>
<td>C terminus</td>
<td>(Herman et al., 1998)</td>
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<td>LpxC</td>
<td>C terminus, adaptor (?)</td>
<td>(Ogura et al., 1999; Führer et al., 2006, 2007)</td>
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<td>λCII</td>
<td>HflD, HflK/C; C terminus</td>
<td>(Kihara et al., 1997, 2001; Shotland et al., 1997; Kobiler et al., 2002)</td>
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<td>λCIII</td>
<td>Internal; terminal</td>
<td>(Herman et al., 1997)</td>
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<td>λXis</td>
<td>ND</td>
<td>(Leffers and Gottesman, 1998)</td>
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<tr>
<td>RpoH</td>
<td>DnaK/J, GroEL/ES; internal</td>
<td>(Herman et al., 1995; Horikoshi et al., 2004; Obrist and Narberhaus, 2005; Obrist et al., 2007, 2009)</td>
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<td>SoxS</td>
<td>N terminus</td>
<td>(Griffith et al., 2004)</td>
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<td>IscS</td>
<td>ND</td>
<td>(Arends et al., 2016)</td>
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<td>YhbT</td>
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<td><strong>Membrane-anchored substrates</strong></td>
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<tr>
<td>SecY</td>
<td>Unassembled with Sec translocon; HflK/C</td>
<td>(Akiyama et al., 1996b; Kihara et al., 1996)</td>
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<tr>
<td>YccA</td>
<td>N terminus min. length: 20 amino acids</td>
<td>(Chiba et al., 2000; Chiba et al., 2002)</td>
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<tr>
<td>PspC</td>
<td>Unassembled with Psp system</td>
<td>(Singh and Darwin, 2011)</td>
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<tr>
<td>Foa</td>
<td>Unassembled with H+ ATPase</td>
<td>(Akiyama et al., 1996a)</td>
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<td>KdtA</td>
<td>ND</td>
<td>(Katz and Ron, 2008)</td>
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<td>DadA</td>
<td>ND</td>
<td>(Westphal et al., 2012)</td>
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<td>FdoH</td>
<td>ND</td>
<td>(Westphal et al., 2012)</td>
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<tr>
<td>YfgM</td>
<td>N terminus; adaptor (?)</td>
<td>(Westphal et al., 2012; Bittner et al., 2015)</td>
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<td>PpiD</td>
<td>ND</td>
<td>(Bittner et al., 2015)</td>
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<tr>
<td>SecD</td>
<td>ND</td>
<td>(Arends et al., 2016)</td>
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<td>ExbD</td>
<td>ND</td>
<td>(Arends et al., 2016)</td>
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<td>YlaC</td>
<td>ND</td>
<td>(Arends et al., 2016)</td>
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Localization of degrons, involved adaptor proteins and recognition mechanism are listed. Selected references are given for details. ND, Not determined; (?), possible mechanism.
Gottesman, 1998; Kobiler et al., 2002) and various stress responses.

For most of these FtsH substrates, the mechanistic details of recognition and degradation are not yet understood. In this review, we will summarize findings on the three best-studied substrates (LpxC, RpoH and YfgM) and describe when, how and why they are degraded.

Conditional proteolysis of selected FtsH substrates

LpxC, the gate keeper of LPS biosynthesis

Physiologically the most important FtsH substrate in *E. coli* and other enterobacteria is LpxC, the key enzyme in lipopolysaccharide (LPS) biosynthesis. The outer membrane of Gram-negative bacteria is composed of LPS and phospholipids (PL) and serves as permeability barrier against environmental stresses (Nikaido and Vaara, 1985; Delcour, 2009). The amount of LPS is directly correlated to the level of LpxC, the UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylgulcosamine deacetylase catalyzing the first committed step in LPS biosynthesis (Anderson et al., 1993; Ogura et al., 1999). LPS and PL biosynthesis are synchronized because they share the same precursor molecule R-3-hydroxymyristoyl-acyl carrier protein (ACP). Both depletion and overproduction of LPS is toxic, which renders LpxC, the enzyme that represents the bottleneck in LPS biosynthesis, and FtsH, the protease that controls its intracellular concentration, essential in *E. coli* and many other Gram-negative bacteria (Ogura et al., 1999; Langklotz et al., 2011). An *E. coli ftsH* deletion strain is only viable due to a suppressor mutation within the *fabZ* gene, whose gene product recruits R-3-hydroxymyristoyl-ACP into the PL biosynthesis pathway (Figure 1B).

The critical importance of LpxC in controlling the flux towards LPS production raised the question whether proteolysis of the enzyme is constitutive or adjusted to the ambient conditions. Consistent with the cellular demand, degradation of LpxC negatively correlates with the growth rate, i.e. the enzyme is stable during fast growth and rapidly degraded when cells grow slowly (Figure 1B) (Schäkermann et al., 2013). A factor that is involved in growth-rate dependent turn-over of LpxC is the alarmone (p)ppGpp (Schäkermann et al., 2013). (p)ppGpp, best known for its role in the stringent response, is involved in regulating many processes in response to various stress and starvation signals (Potrykus and Cashel, 2008; Gaca et al., 2015). In the absence of the signaling molecule, proteolysis of LpxC is mis-regulated. In stark contrast to the situation in wild-type cells, degradation is fast when the (p)ppGpp-negative mutant grows rapidly and is slow when it grows poorly (Schäkermann et al., 2013). The mechanism of alarmone-controlled LpxC degradation is under investigation. Controlled turnover of LpxC seems to be a complex multi-factorial process. Experimental and biocomputational analysis suggests that lipid A disaccharide, an intermediate of the LPS biosynthesis pathway downstream of LpxC, is the feedback source that stimulates FtsH-dependent degradation of LpxC (Emiola et al., 2014, 2016). KdtA (WaaA), an enzyme in LPS biosynthesis acting downstream of LpxC, was reported to be another FtsH substrate suggesting a backup security system in the same pathway (Katz and Ron, 2008).

Degradation of LpxC requires its C terminus in a sequence- and length-dependent manner. The degron at the very C terminus contains the sequence LAXXXXXVLA (X: any amino acid), and the unstructured C-terminal tail must at least be 20 amino acids long (Figure 2B) (Führer et al., 2006, 2007). Interestingly, this motif is necessary but not sufficient to mediate FtsH-specific degradation. Fusion of the C-terminus to the stable glutathione-S-transferase (GST) from *Schistosoma japonicum* resulted in FtsH-independent degradation (Führer et al., 2007). This is presumably due to the similarity of the LpxC degron to the SsrA-tag (AANDENYALAA), which acts as a general recognition motif for AAA+ proteases (Figure 3A) (Keller et al., 1996; Gottesman et al., 1998; Herman et al., 1998; Choy et al., 2007; Moore and Sauer, 2007; Lies and Maurizi, 2008). The SsrA-tag is directly bound by the pore residues of the ATPase domain of ClpX (Levchenko et al., 2003; Martin et al., 2008a,b). Therefore, it is likely that the aromatic FtsH pore directly binds the unstructured C-terminal LpxC motif (Führer et al., 2007). However, routing of the GST protein carrying the LpxC terminus to various proteases suggests that an internal region in LpxC or an additional factor is required for FtsH-specific targeting. A potential candidate as adapter protein is LapB (formerly known as YciM). It is an essential inner membrane protein believed to couple LPS biosynthesis and transport across the membrane (Klein et al., 2014; Mahalakshmi et al., 2014). Interestingly, LapB acts on LPS biosynthesis by altering the LpxC level in dependence of FtsH. Absence or overexpression of LapB lead to increased or decreased LpxC amounts, respectively (Mahalakshmi et al., 2014). Recently, it was proposed that another not yet defined protease contributes to LpxC degradation (Emiola et al., 2016).
Figure 1: Degradation pathways of three conditional substrates of the membrane-anchored FtsH protease. (A) Growth phase-dependent degradation of YfgM. YfgM, a single transmembrane protein with a short cytoplasmic N terminus, acts as a negative regulator of RcsB, the response regulator of the Rcs phosphorelay system. This system further consists of the sensor protein RcsF, the sensor kinase RcsC and the phosphotransferase RcsD and is responsible for adaptation to various stress conditions. YfgM stability is adjusted to the growth phase. YfgM is stabilized in exponential growth phase and rapidly degraded in the stationary phase. Original data obtained from (Bittner et al., 2015). (B) Growth rate-dependent degradation of LpxC. LpxC is the key enzyme in lipopolysaccharide (LPS) biosynthesis. It competes with FabZ of the phospholipid biosynthesis pathway for the common precursor R-3-hydroxymyristoyl-ACP. The amount of LpxC is adjusted to the cellular needs by FtsH-dependent degradation. When cells grow slowly, LpxC is rapidly degraded as less LPS is needed. When cells grow fast, LpxC is stable. Original data obtained from (Schäkermann et al., 2013). (C) Temperature-dependent degradation of RpoH. During normal temperature conditions, the chaperones DnaK/DnaJ direct RpoH to FtsH-mediated degradation. Upon heat shock, the chaperones are titrated away from RpoH by denaturized/misfolded proteins resulting in RpoH stabilization. Free RpoH forms a complex with the RNA polymerase leading to expression of the heat-shock regulon. Original data obtained from (Obrist and Narberhaus, 2005). The Western blots in the insets show the respective protein stability over time after blocking translation by the addition of spectinomycin. OM: outer membrane; IM: inner membrane; ACP: acyl carrier protein; RNAP: RNA polymerase; HS: heat shock. Protein models are based on the following structures: FtsH (PDB: 3KDS; 4V0B), LpxC (4MDT), RpoH was modeled on the basis of RpoS (5IPL) using swiss-model (Arnold et al., 2006; Bordoli et al., 2009; Biasini et al., 2014), periplasmic domain of YfgM was modeled using I-Tasser (Zhang, 2008; Roy et al., 2010).

Figure 2: Degron diversity of selected FtsH substrates. Amino acids important for degradation of YfgM (A), LpxC (B) and RpoH (C) are highlighted. Residues marked in red are critical for degradation and substitutions against other amino acids lead to stabilization. Y4 and E5 in YfgM (marked in green) are critical for stabilization during early growth phases. Amino acids marked in gray seem are not involved in recognition. The amino acids of RpoH correspond to positions L47, A50, I54 (region 2.1) and A131, K134 (region C). For more detailed information, see main text.
A C-terminal degron
LpxC EEKFKAPGSAVLLA
SsrA EKIENDENYALAN

B N-terminal degron
YfgM METYMNENAYL-----
ExbD MAMHGVNLDFNCM-----
YlaC MTYQRLLTSVATIS-----

Figure 3: Comparison of N- and C-terminal FtsH degrons.
(A) The conserved degron of the SsrA-tag and LpxC mainly consists of non-polar amino acids (highlighted in gray). (B) Sequence alignment of the N-terminal degron of YfgM and the N-termini of the membrane-anchored FtsH substrates ExbD and YlaC. Adopted from Arends et al. (2016). Conserved amino acid properties of important residues for degradation are colored from light gray to dark gray (polar, non-polar and acidic).

The essential function of LpxC in Gram-negative bacteria makes it an attractive drug target (Onishi et al., 1996; Vaara, 1996; Mdluli et al., 2006) and various LpxC inhibitors have been reported (Kalinin and Holl, 2016). Since FtsH is essential in balancing LPS and PL biosynthesis in E. coli, it might be another interesting target for antimicrobial agents. It is important to note, however, that not all Gram-negative bacteria seem to control LPS production via FtsH-mediated LpxC degradation. Alpha-proteobacteria are likely to use the Lon protease for this purpose and LpxC of Pseudomonas aeruginosa does not seem to be a protease substrate at all (Langklotz et al., 2011).

RpoH, the master regulator of the heat-shock response

Exposure of all living cells to heat-stress conditions induces a rapid emergency reaction called the heat-shock response. In E. coli, the sigma factor RpoH (σ32) is responsible for transcription of the heat-shock regulon, which encodes most of the heat-shock genes like chaperones and proteases (Yura and Nakahigashi, 1999). Expression of the rpoH gene is primarily regulated at translational and post-translational level. Translation efficiency is controlled via a secondary structure, a so-called RNA thermometer (RNAT) reaching from the 5'-untranslated into the coding region of the rpoH mRNA. At physiological temperatures, the RNAT is closed and ribosome binding is prevented. Melting of the RNAT structure with rising temperatures permits ribosome access and translation initiation (Nagai et al., 1991; Yuzawa et al., 1993; Morita et al., 1999a,b).

A major role in the heat-shock response plays the post-translational regulation through proteolysis of RpoH by FtsH and to a minor extent by other AAA+ proteases (Herman et al., 1995; Tomoyasu et al., 1995; Kanemori et al., 1997; Xu et al., 2015). Here, not the temperature itself is perceived. Instead, the consequences of a temperature upshift are monitored. In concert with the cellular demand, degradation of RpoH is temperature-dependent. The amount of the sigma factor is low (~ 50 molecules per cell) at physiological temperatures and increases during heat shock (42°C) about 20-fold to ~ 1000 molecules per cell (Straus et al., 1987). RpoH degradation is assisted by the chaperone systems DnaK/DnaJ/GrpE and GroEL/GroES (Straus et al., 1990; Tomoyasu et al., 1998; Guisbert et al., 2004). At physiological temperatures, these chaperones bind to RpoH and direct it to FtsH-mediated degradation resulting in RpoH half-lives of about 1 to 2 min (Figure 1C). The high burden of misfolded proteins after heat shock occupies the chaperone systems titrating them away from RpoH and leaving it free and ready to associate with the core RNA polymerase (RNAP) (Gamer et al., 1992, 1996; Horikoshi et al., 2004). The products of the induced heat-shock genes, among them chaperones and proteases, help to overcome the heat-inflicted damage. As a consequence, the heat-shock response is shut-off when the level of available DnaK/DnaJ is sufficient to sequester RpoH again (Straus et al., 1987, 1990; Blaszczak et al., 1995; Gamer et al., 1996).

The recognition of RpoH follows its own rules and has nothing in common with recognition of other substrates. Because the N- or C-terminal ends of RpoH are not required for degradation, two genetic screens were employed. A bacterial one-hybrid-system aimed at the identification of FtsH-resistant RpoH variants. The system is based on the two functional domains (T18 and T25) of the adenylate cyclase (AC) from Bordetella pertussis, which produces the second messenger cyclic adenosine monophosphate (cAMP). A fusion protein comprised of a stable linker between T18 and T25 is able to complement the lacZ strain. cAMP production can then be measured in the lacZ strain, e.g. by β-galactosidase (β-galactosidase) assays (Ladant and Ullmann, 1999; Dautin et al., 2000). A fusion of RpoH between T18 and T25 resulted in low β-galactosidase activity suggesting that the fusion protein is a substrate of FtsH. Generation of random mutations in the RpoH-linker by error prone PCR and screening for elevated β-galactosidase activity identified amino acids crucial for degradation. Exchanges in amino acids L47, A50, and F4 resulted in RpoH stabilization. Interestingly, these residues line up on the face of an exposed α-helix in region 2.1 (Obrist and Narberhaus, 2005) (Figure 2C). Sigma factors are divided into distinct regions based on sequence alignments and numbered from the N to the C terminus (Lonetto et al., 1992; Wösten, 1998). The
identified residues in region 2.1 of the N-terminal part of RpoH are known for core-RNAP binding. The same region was identified in a mutant screen for RpoH variants with increased activity based on the assumption that stabilized variants might exhibit higher transcriptional activity (Horikoshi et al., 2004). This internal and structured degron does not seem to be sufficient for FtsH-dependent degradation of RpoH as point mutations in this region did not stabilize RpoH completely. Moreover, introduction of RpoH-region 2.1 into the sigma factor RpoS, a ClpXP substrate, did not convert RpoS into an FtsH substrate (Obritz et al., 2007). A second turnover element for FtsH-dependent RpoH degradation lies in region C, a region located in the center of the sigma factor (Obritz et al., 2009), which is needed for binding of the RNAP (Nakahigashi et al., 1995; Joo et al., 1998; Arsène et al., 1999). Here, amino acids A^{131} and K^{134} contribute to RpoH degradation (Obritz et al., 2009) (Figure 2C). Combination of amino acid exchanges in region 2.1 and region C drastically stabilized RpoH. Most importantly, an RpoH fragment (residues 37–147) comprised of region 2.1 and region C is degraded by FtsH suggesting that these two regions are sufficient for specific recognition (Obritz and Narberhaus, 2005; Obritz et al., 2007, 2009). DnaJ interacts directly with region 2.1 (Rodriguez et al., 2008; Suzuki et al., 2012; Noguchi et al., 2014; Miyazaki et al., 2016) and it is assumed that DnaJ-induced conformational changes promote DnaK-binding to region 3.2 of RpoH (Rodriguez et al., 2008). The mechanism of how exactly the concerted binding of the DnaKJ system to two different segments in the native sigma factor targets it to FtsH-mediated proteolysis is not yet fully understood.

Recently, the model of RpoH degradation was further extended by the finding that the signal recognition particle (SRP) composed of the Ffh protein in complex with the 4.5S RNA, and its membrane-anchored receptor FtsY are involved in regulation of RpoH degradation (Lim et al., 2013; Miyazaki et al., 2016). Like dnaK/dnaJ/grpE, groEL/groES and ftsH, the ffh gene is part of the g^{12} regulon (Nonaka et al., 2006). Ffh binds via its signal peptide-binding site to the homeostatic control region 2.1 of RpoH in vivo and in vitro (Lim et al., 2013; Miyazaki et al., 2016). This interaction does not require the involvement of DnaK/DnaJ (Miyazaki et al., 2016). Intriguingly, RpoH does not contain a typical signal peptide or transmembrane segment which is usually recognized by Ffh. Region 2.1 seems to form an amphipathic helix of which the hydrophobic part could be bound by Ffh (Miyazaki et al., 2016). Based on these novel findings it is believed that Ffh and the chaperones bind to RpoH in a sequential manner. RpoH is first recruited to the inner membrane via the SRP/FtsY-dependent pathway followed by the transfer to the chaperone-dependent system to induce degradation by FtsH (Lim et al., 2013; Miyazaki et al., 2016).

### YfgM, a mediator of the cytoplasmic and extracytoplasmic stress responses

YfgM was identified as FtsH substrate by a trapping approach with an inactive FtsH variant (Westphal et al., 2012). It is a transmembrane protein with a total of 206 amino acids. A short unstructured N-terminus of 19 amino acids faces the cytoplasm. The majority of the protein is alpha helical and the C-terminus is located in the periplasm (Maddalo et al., 2011). YfgM has interaction partners in various compartments. It interacts with the membrane-anchored periplasmic chaperone PpiD and is an ancillary subunit of the Sec translocon (Maddalo et al., 2011; Götzke et al., 2014). YfgM also interacts with the cytoplasmic response regulator RcsB of the Rcs signal transduction system (Lasserre et al., 2006; Bittner et al., 2015). The Rcs system is a multi-component phosphorelay that coordinates the adaptation to various stresses. Briefly, a stress signal is sensed by the sensor lipoprotein RcsF in the outer membrane. The signal is then passed on to the sensor kinase RcsC in the inner membrane. RcsC autophosphorylates and transfers the phosphate group to the phosphotransferase RcsD in the inner membrane, which then phosphorylates the response regulator RcsB (Figure 1A). RcsB forms homodimers or heterodimers with auxiliary proteins. These dimers activate the expression of adaptation genes involved in acid or osmotic tolerance, in cell division and in adaptation to stationary phase (Carballés et al., 1999; Davalos-Garcia et al., 2001; Majdalani et al., 2002; Majdalani and Gottesman, 2005; Peterson et al., 2006; Clarke, 2010; Johnson et al., 2011). YfgM is bifunctional as it serves, on the one hand, as periplasmic chaperone for proteins, which are translocated via the Sec pathway (Götzke et al., 2014, 2015) and, on the other hand, as negative regulator of RcsB (Bittner et al., 2015). To relieve the inhibitory effect, YfgM is degraded by FtsH under conditions requiring RcsB-dependent gene expression. This is for instance the case under osmotic shock conditions or during stress conditions related to stationary growth phase (Figure 1A) (Westphal et al., 2012; Bittner et al., 2015).

Degradation of YfgM by FtsH requires its cytosolic N terminus. N-terminally truncated YfgM variants were stable in every growth phase and a detailed point-mutation survey revealed that the degron of YfgM comprises the first 14 N-terminal residues (MEIYENDQVEAV-) (Figure 2A). The importance of individual amino acids for degradation varies. For instance, E^2 and P are essential for
degradation, Y<sup>e</sup> and E<sup>5</sup> are important for stabilization of YfgM during early growth phases and the region from the sixth to the 14th amino acid contributes to the characteristic degradation profile of YfgM (Bittner et al., 2015). This sequence-dependent degron is contrasted by the recognition principle used for misfolded membrane-anchored proteins subjected to FtsH-mediated quality control. They are recognized via a minimal length of about 20 amino acids of their exposed N or C termini and the exact amino acid composition is irrelevant for recognition (Kihara et al., 1999; Chiba et al., 2000, 2002). The YfgM degron contains acidic amino acids that are not expected to directly interact with the aromatic/hydrophobic FtsH pore motif and that do not occur in other degrons like the LpxC recognition motif or the SsrA-tag. Interestingly, the C-terminal degron of the phage λ CII protein contains acidic glutamates like the N-terminal YfgM degron (Kobiler et al., 2002; Datta et al., 2005). Unexpectedly, degradation of YfgM was not altered when its interaction partners PpiD or RcsB are missing (Bittner et al., 2015). This finding further distinguishes YfgM degradation from degradation of other membrane-anchored proteins as they are predominantly degraded when unassembled from their interaction partners (Kihara et al., 1995; Akiyama et al., 1996a,b; Singh and Darwin, 2011).

Provided that a fusion protein has the same topology as YfgM, its degron is able to confer the typical YfgM degradation profile (Figure 1A). This was shown for PpiD. When its N-terminus was exchanged against the N terminus of YfgM, PpiD was stable in exponential phase but degraded in stationary phase. The YfgM degron did not convert the soluble protein GST into an FtsH substrate indicating that both the exact amino acid sequence and membrane anchoring are critical for degradation (Bittner et al., 2015).

Factors responsible for the conditional proteolysis of YfgM are not yet known. The absence of the alarmone (p)ppGpp slightly accelerated YfgM degradation (Bittner et al., 2015) but did by far not have the massive effect that was observed for LpxC (Schäkermann et al., 2013). Most likely, YfgM proteolysis is regulated via an adaptor protein that is expressed in response to the growth phase and during acidic or osmotic stress conditions when YfgM is degraded.

### Three FtsH substrates – three degradation pathways

The <i>E. coli</i> FtsH protease modulates a diverse array of cellular processes ranging from the essential LPS biosynthesis to various stress responses (Table 1). In contrast to misfolded substrates prone to protein quality control, the recognition of fully functional substrates under conditions when they are not required poses a substantial challenge and serious threat to the cell. Complex regulatory networks have been established to strictly control the turnover of the LpxC enzyme and the stress response factors RpoH and YfgM. One emerging theme is that factors involved in the FtsH-controlled pathway (chaperones in the heat-shock response or precursors of LPS biosynthesis) play a role in monitoring the cellular status and signaling it to the proteolytic machinery. An inspection of the currently known FtsH degrons suggests at least some commonalities. It has already been reported that the C terminus of LpxC resembles the SsrA tag (Figure 3A) (Führer et al., 2006, 2007). The peculiar YfgM degron might have analogs in the FtsH substrates YlaC and ExbD that are topologically similar to YfgM. Like YfgM, the biopolymer transport protein ExbD has a single transmembrane domain and a short cytosolic N terminus of 22 amino acids (Kampfenkel and Braun, 1992). YlaC, a protein of unknown function, is predicted to have two transmembrane domains and both termini are suggested to face the cytoplasm (Daley et al., 2005). Comparison of the N-terminal YfgM degron with the N termini of ExbD and YlaC indicates some conservation of residues critical for YfgM degradation (Figure 3B) (Arends et al., 2016). Further experimental studies are needed to clarify if these conserved residues are crucial for FtsH-dependent degradation. Time will tell whether all the newly identified FtsH substrates follow their individual degradation schemes or whether some common principles do exist for this unique protease.

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### References


expression by controlling the synthesis and stability of $\sigma^{32}$. Genes Dev. 4, 2202–2209.


