Regulation by proteolysis: developmental switches

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The energy-dependent proteases originally defined in *Escherichia coli* have proven to have particularly important roles in bacterial developmental systems, including sporulation in *Bacillus subtilis* and cell cycle in *Caulobacter*. Degradation of key regulatory proteins participates, with regulation of synthesis and activity of the regulators, to ensure tight control and, where required, irreversible commitment of the cell to specific developmental pathways.

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Introduction

Energy-dependent proteases help rid the cell of abnormal and misfolded proteins and play key roles in many regulatory cascades. In addition, the ATPase components of some of these proteases or their close relatives serve as chaperones. Our understanding of the mechanism of action of these proteases, based primarily but not exclusively on studies with the *Escherichia coli* prototypes, has continued to expand. At the same time, homologs of the *E. coli* enzymes in many other organisms have been identified and found to have critical regulatory roles, particularly in developmental pathways. This review briefly describes the energy-dependent proteases, approaches to studying their *in vivo* targets, new regulatory cascades in which they have been implicated.

The basic machinery: processive, selective proteases

In prokaryotes, on the order of six energy-dependent proteases must handle the degradation of a large variety of cellular proteins. Unlike eukaryotes, in which multiple ubiquitin tagging systems funnel multiple substrates into one cytoplasmic protease the 26S protease, substrate selection in prokaryotes must operate at the level of the protease itself. Energy-dependent proteases are generally large oligomeric assemblies. In those in which structure has been examined, the proteolytic sites are sequestered in a chamber, the entry to which is too small for most folded proteins [1,2]. Entry is believed to be mediated via the regulatory ATPase domains or subunits; it is these regulatory ATPases that also determine substrate specificity. The ATPase subunits from otherwise distinct proteases share sequence similarities that may imply similar mechanisms of action [3]. Because substrate recognition is by the regulatory ATPases, the substrate characteristics that lead to degradation are independent of any sequence requirements for peptide bond cleavage. Once a protein is recognized and bound properly by the regulatory ATPase domain, degradation of the whole polypeptide chain proceeds processively, with cleavage occurring every 5–10 amino acids.

Four ATP-dependent protease families have been recognized in *E. coli*.

Lon

Lon (also called La) is encoded by a single gene with a serine active site near the carboxyl terminus and an ATPase domain in the middle [4–10]. Recent studies of the protein from yeast mitochondria suggest that these domains can be expressed from separate polypeptides and still cooperate to restore full Lon function [11••].

ClpAP and ClpXP

ClpP, the proteolytic core of the ClpAP protease, has its serine active sites arrayed within a cavity formed from two seven-membered rings of ClpP [1]. The regulatory ATPase ring consists of six ClpA subunits [12]. ClpA can function as a chaperone *in vitro*, binding substrate in the presence of nucleotide and releasing it in an activated state when ATP is hydrolyzed; only a single round of binding and release is necessary for activation [13,14]. When ClpP is present but proteolytically inactive, substrates are translocated from the ATPase to the ClpP chamber following ATP hydrolysis [15••]. Parallel results observed *in vivo* for Lon protease (sequestration of substrates in the absence of active proteolysis) suggests that this may be a common feature of these two protease families (L van Melderen, MR Mauriz, S Gottesman, unpublished data).

ClpXP consists of an ATPase ring, ClpX, associated with the ClpP rings; the substrate specificity of ClpXP is different from that of ClpAP [16–18]. ClpX can also act as a chaperone on its own [19–21].

ClpYQ/HslUV

This protease is a hybrid, in which a ClpX-like ATPase (known as ClpY or HslU) associates with a protease subunit, ClpQ or HslV. The protease subunit has a threonine active site and sequence similarities to the eukaryotic proteasome core subunits [2,22]. Recent studies suggest that this protease at least partially overlaps Lon in substrate specificity ([23,24]; W-F Wu, YN Zhou, S Gottesman, unpublished data), emphasizing that the substrate specificity is not a characteristic of a particular protease family.

FtsH/HflB

Another single subunit protease, FtsH (also known as Hf1B), is a zinc metalloprotease with two membrane spanning regions. The active site as well as the ATPase domain, a member of the AAA family of ATPases, reside

in the cytoplasm (recently reviewed in [25]). FtsH is the only essential member of the energy-dependent proteases in *E. coli*, although in *Bacillus subtilis*, for instance, it is not essential [26,27•]. FtsH has also been observed to form rings in EM studies [28].

How are substrates recognized?

It is not yet possible to examine a protein sequence and make even an educated guess as to whether it will be unstable and, if so, what protease will degrade it. For known unstable proteins, studies of the protease recognition signals suggest that the ends of proteins are frequently implicated in protease recognition. Whether the end *per se* is a recognition motif (implying that the NH_2 or COOH are part of what is recognized) or whether recognition motifs have evolved to be near ends because the ends are more likely to be or to become accessible may vary with the particular case.

Proteins engineered to have certain abnormal amino-terminal amino acids *in vivo* are rapidly degraded (the N-end rule); degradation is dependent upon ClpAP [29–31]. It is not yet clear whether this is biologically relevant in bacteria. A carboxy-terminal tagging system leading to degradation of incomplete proteins is dependent upon a 'mobile messenger RNA' called 10Sa RNA or tmRNA, encoded by the *ssrA* gene of *E. coli*. This RNA serves as a template for the co-translational addition of 11 amino acids to the carboxyl terminus of proteins with truncated messenger RNAs [32]; the carboxy-terminal addition allows degradation by ClpAP, ClpXP, and, in some cases, FtsH [33•,34•]. Thus ClpAP recognizes both the carboxyl terminus and the amino terminus of proteins under different circumstances. The degree of redundancy among these proteases varies with particular substrates, and presumably depends upon the structure of regions further into the body of the protein. A periplasmic protease, Tsp, also recognizes the same carboxy-terminal sequence [32]. In other cases, the carboxyl terminus is also the site of recognition, although the tagging sequence is not present. Recognition of the replication protein of bacteriophage Mu, A protein, by ClpX depends upon the last eight amino acids of MuA [20]. Specificity for the MuA sequence is at least in part encoded in the carboxy-terminal region of ClpX [35]. Degradation of a number of *Caulobacter* proteins, including a component of the flagellar motor, a chemoreceptor protein and CtrA, a regulatory protein, depends upon the carboxyl terminus of these proteins [36–38].

Degradation is also significantly modulated by the interaction of the substrate with other protein components, both to stimulate degradation, and, in other cases, to sequester substrates in protected complexes. As a result, sequences identified as being involved in substrate recognition may in fact be those modulating interaction with critical proteins. RpoS (σ^S), a sigma factor degraded under certain conditions by ClpXP, must have at least some of the essential sequences for recognition in the middle of the protein, because fusions that delete the carboxyl terminus (and add on LacZ to make translational fusion proteins) are still degraded with the same pattern as the parent protein; deleting a bit further into the protein, however, leads to a stable protein [39,40]. RpoS degradation, however, requires an auxiliary protein, RssB/SprE [41,42]. Therefore, it is unclear if the sequences within RpoS are those responsible for recognition by RssB, recognition by the protease, or both. Similarly, degradation of the heat-shock sigma factor, RpoH or σ^{32}, is dependent upon FtsH but is significantly affected by DnaJ, DnaK, and GrpE [43–46]. To complicate matters further, the activity of σ^{32} is also affected by DnaK and DnaJ, in the absence of the FtsH protease [47•]. Degradation of the wild-type Mu repressor is stimulated by the presence of a mutant repressor (Mu vir) [48].

Regulatory roles for proteolysis

In many cases, proteolysis in regulatory circuits is not itself regulated. The synthesis of the substrate is presumably changed with conditions, and constant rapid degradation ensures a tight coupling between synthesis and activity. In other cases, proteolysis changes with environmental or developmental cues. As noted above, regulation of the degradation of a given protein may depend on interactions with other proteins. Degradation of UmuD′, a component of the error-prone repair mutagenesis system in *E. coli*, depends on the availability of a second component of the system, UmuC and the UmuD′ precursor, as well as the activity of the UmuD self-cleavage reaction (dependent upon RecA and DNA damage cues) [49]. MuA recognition by ClpX is affected by the interaction of MuA and MuB [20]. These protein–protein interactions are, not surprisingly, substrate specific. The requirement of RssB for RpoS degradation is also substrate specific [50•]; the regulatory step may be activation of RssB by phosphorylation [51]. It seems likely that most of the regulated proteolysis observed in both prokaryotes and eukaryotes will reflect substrate-specific protein interactions and/or modifications, rather than major changes in protease availability or activity. It is clear, however, that there are some exceptions to this. Bacteriophage T4 shuts off proteolysis by Lon and other proteases; PinA, a T4 protein, acts as an inhibitor of the ATPase of Lon [52,53]. Bacteriophage lambda RexB protein inhibits ClpP, apparently helping to save lambda lysogens from killing their hosts after certain types of stresses [54•].

Protease involvement in regulatory pathways has been recognized either as a result of observable phenotypes of protease mutants or by the recognition of specific unstable proteins and the subsequent identification of the relevant protease. The sequencing of bacterial genomes coupled with the recognition of conserved families of proteases has simplified analysis via both of these pathways. Nonetheless, we still know the full story in very few cases.

Phenotypes due to mutations in the protease

Not all phenotypes associated with mutations in the proteases reflect regulatory proteolysis. Because some of the energy-dependent proteases are involved in dealing with

abnormal proteins, and some Clp ATPases are also acting as chaperones, it is not surprising that mutations in these proteases sometime yield strains that are sensitive to stress, in particular high temperature [55–58]. In addition, a mutation in a given protease frequently will lead to stabilization of more than one substrate, complicating analysis. The *clpP* mutation in *B. subtilis* is defective in high temperature growth, sporulation, competence development, and motility. A mutation in one likely target, MecA, suppresses some but not all phenotypes, and is not fully suppressed for those, suggesting that other substrates must exist [59••].

Most instructive in defining new roles for proteolysis are mutations isolated in a search for a given phenotype, which prove to be in a protease gene. For instance, mutations in *clpP* were found in a search for mutations that increased transcription of a cell-surface protein involved in *Yersinia enterocolitica* pathogenesis [60], suggesting the involvement of an unstable transcriptional regulator in this system. A number of other processes newly found to be modulated by protease mutants are reviewed below.

Swarming and biofilms

Mutations selected in *Vibrio parahaemolyticus* for their inability to down-regulate swarmer genes, normally expressed only when the bacteria are growing on solid surfaces, during liquid growth have proven to be in the *lonS* gene, encoding a Lon homolog [61]. In *Pseudomonas fluorescens*, mutants that are defective in biofilm formation include strains with insertions in *clpP* [62].

Restriction-modification systems

Introduction of new restriction systems into *E. coli* results in delayed expression of the restriction system, while modification is expressed immediately. Mutations in *clpX* or *clpP* lead to a significant lowering in the efficiency of acquiring the new system, suggesting that degradation of a component specific to restriction but not modification may be important [63]. The fact that a *clpX* mutation has a more significant phenotype than a *clpP* mutation might suggest that some 'chaperone' activity may participate as well.

mRNA stability

Kushner and colleagues [64•,65] looked for temperature sensitive mutants with changes in mRNA decay; one mutation that had a profound effect on mRNA decay, particularly in the context of mutations in other known nucleases, has proven to be an allele of *ftsH*. In addition, the classic *hflB29* allele of *ftsH*, which is not conditionally lethal, has the mRNA decay phenotypes at all temperatures. Therefore, defects in *ftsH* lead to defects in mRNA decay, and this is independent of the essential role of FtsH [64•,65]. The best current explanation for these observations would be that some (direct or indirect) regulator of mRNA decay is subject to FtsH-dependent degradation, and lack of degradation of the protein inhibits mRNA decay.

Unstable proteins in search of a protease

In some cases, proteins that are of interest have been shown to be unstable, such as lambda cII [66]. Because we now know there are a limited number of energy-dependent proteases, it is feasible to look at the degradation of a protein of interest in representative protease mutant hosts. For lambda Xis, recognized as an unstable protein in 1971 [67], the two overlapping proteases responsible have just recently been identified as FtsH and Lon [68]. Such a search of known proteases has also proven productive in studies in *Caulobacter* (see below).

Bacterial development and proteolysis

Developmental pathways, with their requirements for timed expression of proteins at one stage but not another, are prime cases where one might expect regulated proteolysis to be particularly important. Recent studies in *B. subtilis* and *Caulobacter*, summarized below, support this idea. Although the importance of degradation of specific substrates is apparent from these studies, it is more difficult to determine if proteolysis is the prime mechanism for throwing critical switches during development or serves as a locking mechanism to prevent reversal of switches thrown by other levels of regulation.

B. subtilis

B. subtilis can grow vegetatively, develop competence under some starvation conditions, and, under more extreme conditions, sporulate. The energy-dependent proteases seem to operate in all of these pathways. Development of competence in *B. subtilis* requires a positive regulator of transcription of competence genes, ComK. ComK activates its own transcription as well as expression of downstream competence genes. If the cell is to avoid initiating the autoregulation of ComK, the level and/or activity of ComK need to be tightly regulated. Two proteins, MecA and ClpC (an ATPase with homology to ClpA), as well as ClpP, seem to participate in this process. *In vitro*, MecA and ClpC together bind ComK and may thus block its ability to autoregulate. ComS, the small protein that appears to mediate environmental signaling for competence development, overcomes this binding, possibly competing for MecA and ClpC and releasing active ComK [69]. Degradation of the bound ComK also contributes to down-regulating ComK activity; when ComS releases ComK, ComS itself becomes sensitive to degradation by the Clp protease [70•]. The probable multiple roles for ClpP and ClpC are demonstrated by the puzzling genetics of this system. *clpP* mutants do not have the phenotype of *mecA* or *clpC* mutants (constitutive ComK synthesis); in fact, they fail to make ComK [59••]. High levels of MecA accumulating in the *clpP* mutant may be part of the explanation; *clpP mecA* double mutants partially suppress the *clpP* phenotypes [59••].

Null mutations in *ftsH* in *B. subtilis*, like *clpP* mutations, block sporulation at a very early stage [27•]. A second role

late in sporulation has been inferred from the isolation of mutations in *ftsH* as suppressors of an allele of *spoVM*. *spoVM* encodes a 26 amino acid polypeptide; null mutations in the gene are defective in spore formation [71]. Because the *ftsH* alleles do not suppress null mutations in *spoVM*, it seems possible the mutant allele (which encodes a fusion protein) is unstable and degraded by FtsH, rather than that FtsH plays a normal role at this stage of sporulation. It is intriguing, however, that, *in vitro*, the SpoVM peptide but not a mutant form is degraded by FtsH and can inhibit FtsH, not unlike the inhibition of FtsH by the small lambda cIII polypeptide, a part of the cII-dependent lysogenic switch mechanism [72]. If the only function of SpoVM, however, was to inhibit FtsH, the *ftsH* mutants would be expected to suppress a null *spoVM* mutation, which they did not [71].

Caulobacter

CtrA is a central regulator of development of cell type in *Caulobacter crescentus*, and acts as a timer for cell division as well. It is unstable at a particular stage of the cell cycle, and mutations in the carboxyl terminus of the protein, which stabilize the protein, perturb cell growth [37]. This observation suggests that the degradation of CtrA is critical for *Caulobacter* growth. Jenal and coworkers [73••] found that mutations in *clpX* or *clpP* were lethal for *Caulobacter* and depleting either protein stabilizes CtrA; whether this is the only target of these proteases is not yet known. Still to be examined is the basis for the regulated proteolysis of CtrA during only one part of the growth cycle; the proteases are known to be present at all times. Lon protease also is critical for cell cycle control in this organism, degrading CcrM, an adenine DNA methyltrasferase necessary for proper timing of DNA replication [74].

A number of other proteins are subject to periodic proteolysis in *Caulobacter*, including the cell division protein FtsZ. In this case, it seems likely that regulated degradation is secondary to regulated assembly of the protein into active complexes (only the free protein is degraded) [75•].

Conclusions

The energy-dependent Lon, Clp, and FtsH families of proteases were originally defined in *E. coli*, but it has now become evident that they are widespread throughout prokaryotes and also play critical roles in eukaryotic organelles. With functions both as proteases and in some cases, as chaperones, they have been found to act as modulators in many regulatory circuits. Mutations in Clp components are defective in virulence in bacterial pathogens [60], and a Clp-like protein in humans has been implicated in an inherited torsion dystonia [76]. In the past year, the involvement of ATP-dependent proteases as direct participants in regulatory circuits, particularly for microorganisms with developmental pathways, has provided new insight into ways in which proteolysis can act in regulation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wang J, Hartling JA, Flanagan JM: **The structure of ClpP at 2.3 Å resolution suggests a model for ATP-dependent proteolysis.** *Cell* 1997, **91**:447-456.
2. Bochtler M, Ditzel L, Groll M, Huber R: **Crystal structure of heat shock locus V (HslV) from *Escherichia coli*.** *Proc Natl Acad Sci USA* 1997, **94**:6070-6074.
3. Lupas A, Flanagan JM, Tamura T, Baumeister W: **Self-compartmentalizing proteases.** *Trends Biochem Sci* 1997, **22**:399-404.
4. Fischer H, Glockshuber R: **ATP hydrolysis is not stoichiometrically linked with proteolysis in the ATP-dependent protease La from *Escherichia coli*.** *J Biol Chem* 1993, **268**:22502-22507.
5. Fischer H, Glockshuber R: **A point mutation within the ATP-binding site inactivates both catalytic functions of the ATP-dependent protease La (Lon) from *Escherichia coli*.** *FEBS Lett* 1994, **356**:101-103.
6. Starkova NN, Koroleva EP, Rumsh LD, Ginodman LM, Rotanova TV: **Mutations in the proteolytic domain of *Escherichia coli* protease Lon impair the ATPase activity of the enzyme.** *FEBS Lett* 1998, **422**:218-220.
7. Roudiak SG, Seth A, Knipfer N, Shrader TE: **The Lon protease from *Mycobacterium smegmatis*: molecular cloning, sequence analysis, functional expression, and enzymatic characterization.** *Biochemistry* 1998, **37**:377-386.
8. Yu A, Antonov VK, Gorbalenya AE, Kotova SA, Rotanova TV, Shimbarevich EV: **Site-directed mutagenesis of La protease. A catalytically active serine residue.** *FEBS Lett* 1991, **287**:211-214.
9. Rotanova TV, Kotova SA, Amerik AY, Lykov IP, Ginodman LM, Antonov VK: ***Escherichia coli* ATP-dependent protease La.** *Bioorg Khim* 1994, **20**:114-125.
10. Mel'nikov EE, Tsirul'nikov KB, Ginodman LM, Rotanova TV: ***In vitro* coupling of ATP hydrolysis to proteolysis of ATP site mutant forms of Lon-proteinase from *E. coli*.** *Bioorg Khim* 1998, **24**:293-299.
11. •• van Dijl JM, Kutejova E, Suda K, Perecko D, Schatz G, Suzuki CK: **The ATPase and proteases domains of yeast mitochondrial Lon: roles in proteolysis and respiration-dependent growth.** *Proc Natl Acad Sci USA* 1998, **95**:10584-10589.

The ATPase and protease domains of *S. cerevisiae* mitochondrial Lon can be expressed from separate plasmids, and complement *in vivo* to restore function to a *lon* mutant (which has non-functional mitochondria). Thus the organization of this protease may be functionally similar to the Clp proteases, in which ATPase and protease are in separate subunits.

12. Kessel M, Maurizi MR, Kim B, Kocsis E, Trus BL, Singh SK, Steven AC: **Homology in structural organization between *E. coli* ClpAP protease and the eukaryotic 26S proteasome.** *J Mol Biol* 1995, **250**:587-594.
13. Wickner S, Gottesman S, Skowyra D, Hoskins J, McKenney K, Maurizi MR: **A molecular chaperone, ClpA, functions like DnaK and DnaJ.** *Proc Natl Acad Sci USA* 1994, **91**:12218-12222.
14. Pak M, Wickner S: **Mechanism of protein remodeling by ClpA chaperone.** *Proc Natl Acad Sci USA* 1997, **94**:4901-4907.
15. •• Hoskins JR, Pak M, Maurizi MR, Wickner S: **The role of the ClpA chaperone in proteolysis by ClpAP.** *Proc Natl Acad Sci USA* 1998, **95**:12135-12140.

Substrates for ClpAP are normally bound by ClpA and then translocated to the proteolytic ClpP subunit for degradation. When complexes that are defective in ClpP proteolytic activity, via mutation or chemical inactivation of the active site serine, are formed, an intermediate in the degradation pathway can be isolated in which the substrate (RepA) has moved from the ATPase subunit into ClpP. This process requires ATP hydrolysis.

16. Grimaud R, Kessel M, Beuron F, Stevens AC, Maurizi MR: **Enzymatic and structural similarities between the *Escherichia coli* ATP-dependent proteases, ClpXP and ClpAP.** *J Biol Chem* 1998, **273**:12476-12481.
17. Gottesman S, Clark WP, de Crecy-Lagard V, Maurizi MR: **ClpX, an alternative subunit for the ATP-dependent Clp protease of *Escherichia coli*.** *J Biol Chem* 1993, **268**:22618-22626.

18. Wawrzynow A, Wojtkowiak D, Marzsalek J, Banecki B, Jonsen M, Graves B, Georgopoulos C, Zylicz M: **The ClpX heat-shock protein of *Escherichia coli*, the ATP-dependent substrate specificity component of the ClpP-ClpX protease, is a novel molecular chaperone.** *EMBO J* 1995, **14**:1867-1877.

19. Wawrzynow A, Banecki B, Zylicz M: **The Clp ATPases define a novel class of molecular chaperones.** *Mol Microbiol* 1996, **21**:895-899.

20. Levchenko I, Luo L, Baker TA: **Disassembly of the Mu transposase tetramer by the ClpX chaperone.** *Genes Dev* 1995, **9**:2399-2408.

21. Kruklitis R, Welty DJ, Nakai H: **ClpX protein of *Escherichia coli* activates bacteriophage Mu transposase in the strand transfer complex for initiation of Mu DNA synthesis.** *EMBO J* 1996, **15**:935-944.

22. Kessel M, Wu W-F, Gottesman S, Kocsis E, Steven AC, Maurizi MR: **Six-fold rotational symmetry of ClpQ, the *E. coli* homolog of the 20S proteasome, and its ATP-dependent activator, ClpY.** *FEBS Lett* 1996, **398**:274-278.

23. Khattar MM: **Overexpression of the hslVU operon suppresses SOS-mediated inhibition of cell division in *Escherichia coli*.** *FEBS Lett* 1997, **414**:402-404.

24. Kanemori M, Yanagi H, Yura T: **ATP-dependent HslUV/ClpQY protease participates in turnover of cell division inhibitor SulA in *Escherichia coli*.** *J Bacteriol* 1999, in press.

25. Schumann W: **FtsH- a single-chain charonin?** *FEMS Microbiol Rev* 1998, in press.

26. Herman C, Ogura T, Tomoyasu T, Hiraga S, Akiyama Y, Ito K, Thomas R, D'Ari R, Bouloc P: **Cell growth and λ phage development controlled by the same essential *Escherichia coli* gene, *ftsH/hflB*.** *Proc Natl Acad Sci USA* 1993, **90**:10861-10865.

27. • Deuerling E, Mogk A, Richter C, Purucker M, Schumann W: **The *ftsH* gene of *Bacillus subtilis* is involved in major cellular processes such as sporulation, stress adaptation and secretion.** *Mol Microbiol* 1997, **23**:921-933.

ftsH mutants, although viable in *B. subtilis* (in contrast to *E. coli*), are very heat and salt sensitive and are unable to sporulate. Many of the *ftsH* phenotypes are similar to those of a *spoOA* null mutation; *ftsH* mutants fail to accumulate SpoOA at expected levels for unknown reasons.

28. Shotland Y, Koby S, Teff D, Mansur N, Oren DA, Tatematsu K, Tomoyasu T, Kessel M, Bukau B, Ogura T, Oppenheim AB: **Proteolysis of the phage λ cII regulatory protein by FtsH (HflB) of *Escherichia coli*.** *Mol Microbiol* 1997, **24**:1303-1310.

29. Tobias JW, Shrader TE, Rocap G, Varshavsky A: **The N-end rule in bacteria.** *Science* 1991, **254**:1374-1376.

30. Varshavsky A: **The N-end rule.** *Cold Spring Harbor Symp Quant Biol* 1995, **60**:461-478.

31. Varshavsky A: **The N-end rule: functions, mysteries, uses.** *Proc Natl Acad Sci USA* 1996, **93**:12142-12149.

32. Keiler KC, Waller PRH, Sauer RT: **Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA.** *Science* 1996, **271**:990-993.

33. • Herman C, Thevenet D, Boulos P, Walker GC, D'Ari R: **Degradation of carboxy-terminal-tagged cytoplasmic proteins by the *Escherichia coli* protease HflB (FtsH).** *Genes Dev* 1998, **12**:1348-1355.

When the lambda cI amino-terminal domain carries certain carboxy-terminal extensions, the proteins become unstable. For some of these unstable proteins, FtsH is the primary protease responsible for degradation. For proteins tagged with the 11 amino acid carboxy-terminal extension encoded by SsrA (tmRNA), FtsH participates as a secondary protease, particularly at elevated temperatures.

34. • Gottesman S, Roche E, Zhou Y-N, Sauer RT: **The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system.** *Genes Dev* 1998, **12**:1338-1347.

The SsrA RNA adds an 11 amino acid extension to the carboxyl terminus of protein fragments when the mRNA ends before the end of the open reading frame; the tagged proteins are unstable *in vivo* and *in vitro*. For model substrates carrying the cI amino-terminus and the 11 amino acid SsrA extension, ClpXP and ClpAP are the primary proteases responsible for their degradation.

35. Levchenko I, Smith CK, Walsh NP, Sauer RT, Baker TA: **PDZ-like domains mediate binding specificity in the Clp/Hsp100 family of chaperones and protease regulatory subunits.** *Cell* 1997, **91**:939-947.

36. Alley MRK, Maddock JR, Shapiro L: **Requirement for the carboxyl terminus of a bacterial chemoreceptor for its targeted proteolysis.** *Science* 1993, **239**:1754-1757.

37. Domian IJ, Quon KC, Shapiro L: **Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle.** *Cell* 1997, **90**:415-424.

38. Jenal U, Shapiro L: **Cell cycle-controlled proteolysis of a flagellar motor protein that is asymmetrically distributed in the *Caulobacter* predivisional cell.** *EMBO J* 1996, **96**:2393-2406.

39. Muffler A, Traulsen DD, Lange R, Hengge-Aronis R: **Posttranscriptional osmotic regulation of the σ^S subunit of RNA polymerase in *Escherichia coli*.** *J Bacteriol* 1996, **178**:1607-1613.

40. Schweder T, Lee K-H, Lomovskaya O, Matin A: **Regulation of *Escherichia coli* starvation sigma factor (σ^s) by ClpXP protease.** *J Bacteriol* 1996, **178**:470-476.

41. Pratt LA, Silhavy TJ: **The response regulator SprE controls the stability of RpoS.** *Proc Natl Acad Sci USA* 1996, **93**:2488-2492.

42. Muffler A, Fischer D, Altuvia S, Storz G, Hengge-Aronis R: **The response regulator RssB controls stability of the σ^s subunit of RNA-polymerase in *Escherichia coli*.** *EMBO J* 1996, **15**:1333-1339.

43. Tilly K, Spence J, Georgopoulos C: **Modulation of the stability of *Escherichia coli* heat shock regulatory factor σ^{32}.** *J Bacteriol* 1989, **171**:1585-1589.

44. Straus DB, Walter WA, Gross CA: **DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32}.** *Genes Dev* 1990, **4**:2202-2209.

45. Herman C, Thévenet D, D'Ari R, Bouloc P: **Degradation of σ^{32}, the heat shock regulator in *Escherichia coli*, is governed by HflB.** *Proc Natl Acad Sci USA* 1995, **92**:3516-3520.

46. Tomoyasu T, Gamer J, Bukau B, Kanemori M, Mori H, Rutman AJ, Oppenheim AB, Yura T, Yamanaka K, Niki H *et al.*: ***Escherichia coli* FtsH is a membrane-bound, ATP-dependent protease which degrades the heat-shock transcription factor σ^{32}.** *EMBO J* 1995, **14**:2551-2560.

47. • Tatsuta T, Tomoyasu T, Bukau B, Kitagawa M, Mori H, Karata K, Ogura T: **Heat shock regulation in the *ftsH* null mutant of *Escherichia coli*: dissection of stability and activity control mechanisms of σ^{32} *in vivo*.** *Mol Microbiol* 1998, **30**:583-593.

Although FtsH is essential for *E. coli*, null mutations are viable in the presence of a second mutation in *sfhC*. This suppressor does not affect the requirement for FtsH for σ^{32} degradation, so that proper degradation of σ^{32} is not required for viability. Regulation of σ^{32} activity, dependent upon DnaK and DnaJ, however, limits the deregulation of synthesis of heat shock proteins when σ^{32} levels are abnormally high due to loss of degradation control.

48. Welty DJ, Jones JM, Nakai H: **Communication of ClpXP protease hypersensitivity to bacteriophage Mu repressor isoforms.** *J Mol Biol* 1997, **272**:31-41.

49. Frank EG, Ennis DG, Gonzalez M, Levine AS, Woodgate R: **Regulation of SOS mutagenesis by proteolysis.** *Proc Natl Acad Sci USA* 1996, **93**:10291-10296.

50. • Zhou Y-N, Gottesman S: **Regulation of proteolysis of the stationary-phase sigma factor RpoS.** *J Bacteriol* 1998, **180**:1154-1158.

RssB, a protein with homology to a response regulator, is required for the ClpXP-dependent degradation of RpoS. RssB is shown to act in a substrate-specific fashion, because it is not necessary for degradation of another ClpXP substrate, lambda O protein.

51. Bouche S, Klauck E, Fischer D, Lucassen M, Jung K, Hengge-Aronis R: **Regulation of RssB-dependent proteolysis in *Escherichia coli*: a role for acetyl phosphate in a response regulator-controlled process.** *Mol Microbiol* 1998, **27**:787-795.

52. Hilliard JJ, Simon LD, Van Melderen L, Maurizi MR: **PinA inhibits ATP hydrolysis and energy-dependent protein degradation by Lon protease.** *J Biol Chem* 1998, **273**:524-527.

53. Hilliard JJ, Maurizi MR, Simon LD: **Isolation and characterization of the phage T4 PinA protein, an inhibitor of the ATP-dependent Lon protease of *Escherichia coli*.** *J Biol Chem* 1998, **273**:518-523.

54. Engelberg-Kulka H, Reches M, Narasimhan S, Schoulaker-Schwarz R, Klemes Y, Aizenman E, Glaser G: **rexB of bacteriophage λ is an anti cell death gene.** *Proc Natl Acad Sci USA* 1998, **95**:15481-15486.
The product of the RexB protein, previously shown to inhibit the ClpXP-dependent degradation of lambda O protein, also inhibits the ClpAP-dependent degradation of MazE, a chromosomally encoded function associated with an 'addiction system'. Thus, RexB appears to affect the common ClpP subunit of these two proteases. High levels of ppGpp leads to killing by this system. The authors suggest that lambda protects lysogens from killing by certain stresses by this inhibition of Clp-dependent proteolysis.

55. Sanchez Y, Taulien J, Borkovich KA, Lindquist S: **Hsp104 is required for tolerance to many forms of stress.** *EMBO J* 1992, **11**:2357-2364.

56. Clarke AK, Schelin J, Porankiewicz J: **Inactivation of the clpP1 gene for the proteolytic subunit of the ATP-dependent Clp protease in the cyanobacterium *Synechococcus* limits growth and light acclimation.** *Plant Mol Biol* 1998, **37**:791-801.

57. Gerth U, Kruger K, Derre I, Msadek T, Hecker M: **Stress induction of the *Bacillus subtilis clpP* gene encoding a homologue of the proteolytic component of the Clp protease and the involvement of ClP and ClpX in stress tolerance.** *Mol Microbiol* 1998, **28**:787-802.

58. Porankiewicz J, Schelin J, Clarke AK: **The ATP-dependent Clp protease is essential for acclimation to UV-B and low temperature in the cyanobacterium *Synechococcus*.** *Mol Microbiol* 1998, **29**:275-283.

59. •• Msadek T, Dartois V, Kunst F, Herbaud M-L, Denizot F, Rapoport G: **ClpP of *Bacillus subtilis* is required for competence, development, motility, degradative enzyme synthesis, growth at high temperature and sporulation.** *Mol Microbiol* 1998, **27**:899-914.
clpP mutants of *B. subtilis* are defective for competence and sporulation, as well as growth at high temperature and motility. Some, but not all, of the phenotypes can be explained by high levels of MecA accumulating in the *clpP* mutant; *mecA clpP* double mutants partially suppress the *clpP* phenotypes.

60. Pederson KJ, Carlson S, Pierson DE: **The ClpP protein, a subunit of the Clp protease, modulates *ail* gene expression in *Yersinia enterocolitica*.** *Mol Microbiol* 1997, **26**:99-107.

61. Stewart BJ, Enos-Berlage JL, McCarter LL: **The *lonS* gene regulates swarmer cell differentiation of *Vibrio parahaemolyticus*.** *J Bacteriol* 1997, **179**:107-114.

62. O'Toole GA, Kolter R: **Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis.** *Mol Microbiol* 1998, **28**:449-461.

63. Makovets S, Titheradge AJB, Murray NE: **ClpX and ClpP are essential for the efficient acquisition of genes specifying type IA and IB restriction systems.** *Mol Microbiol* 1998, **28**:25-35.

64. • Granger LL, O'Hara EB, Wang R-F, Meffen FV, Armstrong K, Yancey SD, Babitzke P, Kushner SR: **The *Escherichia coli mrsC* gene is required for cell growth and mRNA decay.** *J Bacteriol* 1998, **180**:1920-1928.
Mutations in the *mrsC* gene lead to profound effects on mRNA decay when combined with mutations in multiple RNases. Surprisingly, *mrsC* is an allele of the *ftsH* gene encoding an ATP-dependent protease.

65. Wang RF, O'Hara EB, Aldea M, Bargmann CI, Gromley H, Kushner SR: ***Escherichia coli mrsC* is an allele of *hflB*, encoding a membrane-associated ATPase and protease that is required for mRNA decay.** *J Bacteriol* 1998, **180**:1929-1938.

66. Reichardt LF: **Control of bacteriophage lambda repressor synthesis after phage infection: the role of the N, cI, cII and cIII products.** *J Mol Biol* 1975, **93**:267-288.

67. Weisberg RA, Gottesman ME: **The stability of Int and Xis functions.** *In* The Bacteriophage Lambda. Edited by Hershey AD. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1971:489-500.

68. Leffers GG Jr, Gottesman S: **Lambda Xis degradation *in vivo* by Lon and FtsH.** *J Bacteriol* 1998, **180**:1573-1577.

69. Turgay K, Hamoen LW, Venema G, Dubnau D: **Biochemical characterization of a molecular switch involving the heat shock protein ClpC, which controls the activity of ComK, the competence transcription factor of *Bacillus subtilis*.** *Genes Dev* 1997, **11**:119-128.

70. • Turgay K, Hahn J, Burghoorn J, Dubnau D: **Competence in *Bacillus subtilis* is controlled by regulated proteolysis of a transcription factor.** *EMBO J* 1998, **17**:6730-6738.
ComK, a key controller of competence gene expression, is controlled by interactions with MecA and ClpC; these interactions are released by ComS. ClpP can degrade ComK, only when MecA and ClpC are also present; ComS, by releasing ComK, protects ComK from degradation and is itself degraded. Because *clpP* mutants are unable to establish competence, degradation of some components of the system, as well as the previously noted sequestration of ComK by MecA and ClpC, must be important in initiating the regulatory cascade that turns on competence genes.

71. Cutting S, Anderson M, Lysenko E, Page A, Tomoyasu T, Tatematsu K, Tatsuta T, Kroos L, Ogura T: **SpoVM, a small protein essential to development in *Bacillus subtilis*, interacts with the ATP-dependent protease FtsH.** *J Bacteriol* 1997, **179**:5534-5542.

72. Herman C, Thevenet D, D'Ari R, Bouloc P: **The HflB protease of *Escherichia coli* degrades its inhibitor λcIII.** *J Bacteriol* 1997, **179**:358-363.

73. •• Jenal U, Fuchs T: **An essential protease involved in bacterial cell cycle control.** *EMBO J* 1998, **17**:5658-5669.
clpX or *clpP* mutants of *Caulobacter* arrest in the cell cycle before initiation of cell division; a proteolytically active form of ClpXP is necessary for restoring growth. CtrA, which is known to be unstable and negatively regulates DNA replication, is stabilized *in vivo* by *clpX* or *clpP* mutants, consistent with the idea that degradation of CtrA by ClpXP allows the G1 to S transition via release of the CtrA-dependent DNA replication initiation block. How degradation is timed within the cell cycle remains to be determined.

74. Wright R, Stephens C, Zweiger C, Shapiro L, Alley MRK: ***Caulobacter* Lon protease has a critical role in cell cycle control of DNA methylation.** *Genes Dev* 1996, **10**:1532-1542.

75. • Kelly AJ, Sackett MJ, Din N, Quardokus E, Brun YV: **Cell cycle dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter*.** *Genes Dev* 1998, **12**:880-893.
The cell division protein FtsZ is subject to cell-cycle regulated transcription and degradation, and only accumulates in cells in stalked cells and not swarmer cells. FtsZ is stable when assembled into a ring at the dividing septum; proteolysis may follow disassembly of this ring and insure tight control of FtsZ availability.

76. Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, de Leon D, Brin MF, Raymond D, Corey DP *et al.*: **The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein.** *Nat Genet* 1997, **17**:40-48.