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AAA+ Proteases: ATP-Fueled Machines of Protein Destruction

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disassembly, proteasome, protein degradation, protein translocation, protein unfolding

Abstract

AAA+ family proteolytic machines (ClpXP, ClpAP, ClpCP, HslUV, Lon, FtsH, PAN/20S, and the 26S proteasome) perform protein quality control and are used in regulatory circuits in all cells. These machines contain a compartmental protease, with active sites sequestered in an interior chamber, and a hexameric ring of AAA+ ATPases. Substrate proteins are tethered to the ring, either directly or via adaptor proteins. An unstructured region of the substrate is engaged in the axial pore of the AAA+ ring, and cycles of ATP binding/hydrolysis drive conformational changes that create pulses of pulling that denature the substrate and translocate the unfolded polypeptide through the pore and into the degradation chamber. Here, we review our current understanding of the molecular mechanisms of substrate recognition, adaptor function, and ATP-fueled unfolding and translocation. The unfolding activities of these and related AAA+ machines can also be used to disassemble or remodel macromolecular complexes and to resolubilize aggregates.

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Contents

OVERVIEW

AAA+: ATPases associated with diverse cellular activities

Chaperones: proteins that interact with other proteins to antagonize aggregation and/or promote proper folding. Many are enzymes that utilize ATP

The proteome of a cell, like an urban landscape, is in constant flux. Decayed or damaged structures in a city are demolished for public safety, and functional buildings are frequently razed to free space for new developments or initiatives. In a similar fashion, the repertoire of cellular proteins undergoes constant modification to meet changing developmental and/or environmental challenges. Damaged or misfolded proteins are proteolytically degraded, helping to avoid potentially catastrophic aggregation in the densely packed intracellular milieu while recycling amino acids. In addition, degradation is also used to remove perfectly functional proteins for regulatory purposes or because they are no longer needed. It is important to note that degradation is irreversible, and thus highly specific recognition is required to avoid wasteful destruction. AAA+ proteases and disassembly chaperones are the protein-wrecking machines and molecular bulldozers of the cell. In this review, we summarize our current understanding of the energy-dependent mechanisms that these powerful enzymes use to dismantle, unfold, and degrade target proteins with exquisite specificity.

All AAA+ proteases contain at least one protein that belongs to a superfamily of ATPases associated with diverse cellular activities (1). These ATP-fueled enzymes typically function as engines or motors in intracellular processes that require mechanical work (2). In the clade associated with AAA+ proteases, these ATPases assemble into a hexameric ring with a central axial pore. Cycles of ATP binding and hydrolysis in the AAA+ ring drive conformational changes that unfold protein substrates and translocate the denatured polypeptide through the pore and into a sequestered degradation chamber of a compartmental protease, where proteolysis occurs. **Figure 1** illustrates these basic reactions, which are discussed in greater detail in subsequent sections. For a history of the discovery of AAA+ proteases and additional discussion, we refer readers to the reviews in References 3–15.

Several architectural features contribute to the specificity of AAA+ proteases either negatively or positively. For example, the barrel-like chamber in which the proteolytic active sites reside can only be accessed through entry portals that are too narrow to allow passage of even the smallest folded proteins. The AAA+ ring, by contrast, plays a positive role by recognizing specific native substrates, unfolding them, and spooling the denatured polypeptide into the proteolytic chamber. Indeed, degradation of both folded and unfolded substrates by AAA+ proteases requires active

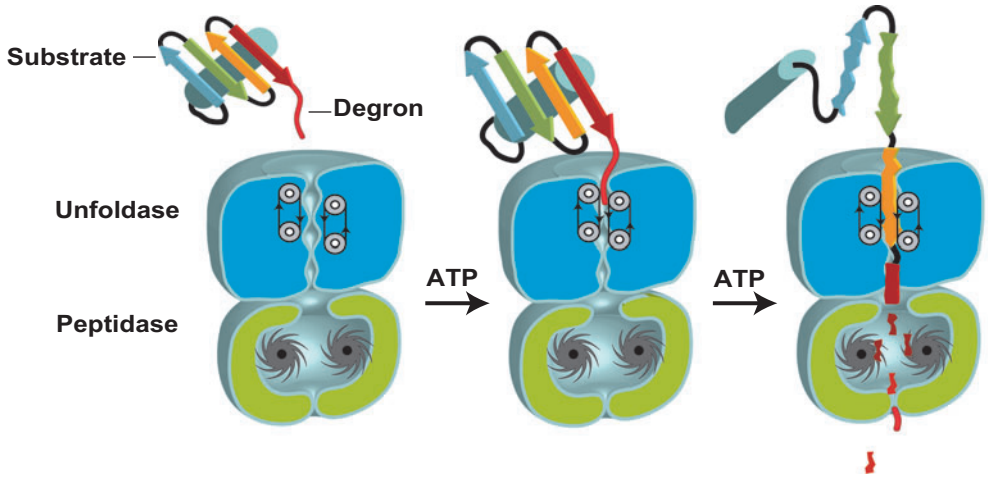

Figure 1

Basic mechanism of a AAA+ protease. A degron or degradation tag in a native substrate is initially recognized by a hexameric AAA+ unfoldase. Repetitive cycles of ATP hydrolysis then power unfolding of the substrates and translocation through the enzyme's central pore into the degradation chamber of the associated compartmental peptidase.

collaboration between the hexameric AAA+ ring and the compartmental protease.

How do AAA+ rings recognize the proper protein substrates and unfold them? One step involves the binding of an exposed peptide segment of the substrate in the axial pore of the AAA+ ring. Conformational changes in the ring, powered by ATP binding and hydrolysis, subsequently translocate this peptide and create an unfolding force when the attached protein is pulled against the pore entrance. In addition, other portions of the substrate are frequently tethered to auxiliary domains on the AAA+ ring, either directly or by specific adaptor proteins. The substrate sequences that mediate tethering or binding to the pore are called degradation tags or degrons.

AAA+ proteases are present in all kingdoms of life and can be divided into distinct families based upon the sequences of their ATPase, protease, and auxiliary domains (1, 16). Even the simplest AAA+ proteases function as large oligomers. In the Lon and FtsH families, hexamers consist of six identical subunits, each containing a AAA+ domain, a protease domain, and an additional domain. In the ClpXP, ClpAP, and HslUV families, the ClpX, ClpA, or HslU proteins form a hexameric AAA+ ring with six identical multidomain subunits, whereas the ClpP or HslV compartmental proteases are built from multiple copies of a distinct protein, which form double-ring structures with 14 and 12 subunits, respectively. In the PAN/20S protease family, the AAA+ PAN ring is again composed of six identical multidomain subunits, but the 20S protease has two kinds of subunits arranged in an $\alpha_7\beta_7\beta_7\alpha_7$ structure. The eukaryotic 26S proteasome, which contains more than 30 distinct types of subunits, is the most complicated AAA+ protease. Nevertheless, the basic architectural features found in the simpler enzymes, including a AAA+ ring and $\alpha_7\beta_7\beta_7\alpha_7$ compartmental protease, are still observed.

In the sections that follow, we provide a more detailed view of the domain organization and structures of different families of AAA+ proteases and then describe the processes of substrate recognition, translocation, unfolding, and degradation in greater depth. Afterward, we discuss how the AAA+ rings of these and related enzymes can function as disassembly chaperones and list major unanswered questions and future challenges in this field.

Degron: a protein sequence element responsible for proteolytic recognition. Some degrons function directly as recognition or degradation tags

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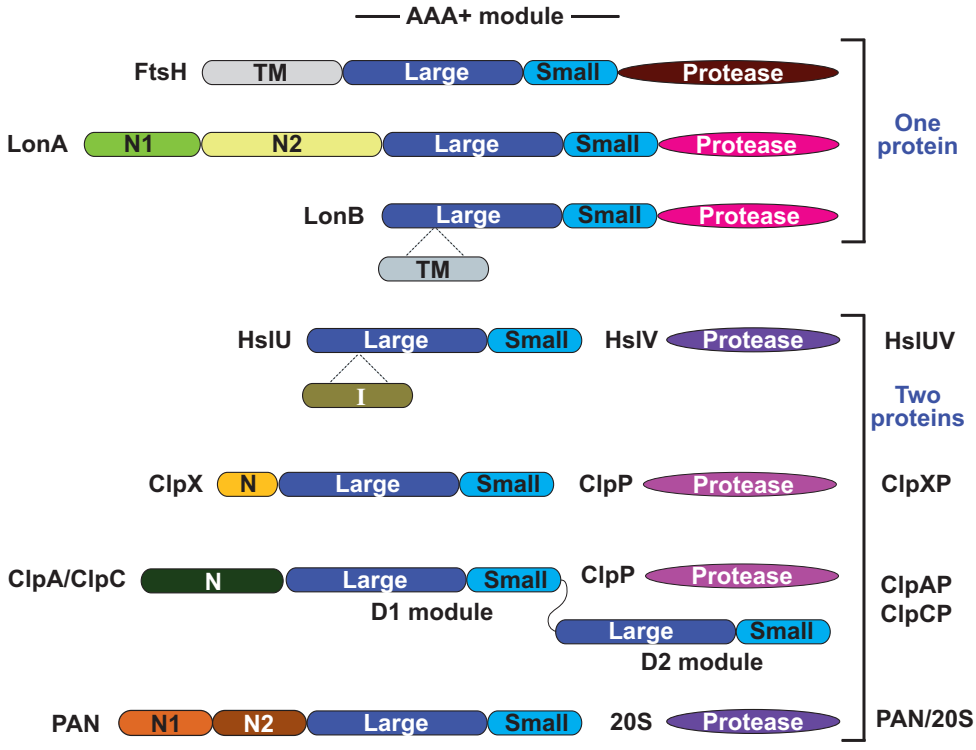

Figure 2

Domain structures of AAA+ proteases. Each protease contains one or two AAA+ modules, each consisting of a large and small domain, and additional family-specific domains. The protease domain and AAA+ module are present on the same polypeptide for FtsH, LonA, and LonB. The protease and AAA+ modules are distinct polypeptides in HslUV, ClpXP, ClpAP, ClpCP, and PAN/20S.

FAMILIES AND PHYLOGENY

Figure 2 depicts domain structures for the subunits that form the FtsH, Lon (LonA and LonB subfamilies), HslUV, ClpXP, ClpAP, ClpCP, and PAN/20S protease families. Each family contains at least one AAA+ module (~250 residues), which consists of a large and small AAA+ domain. ClpA and ClpC are unique among this group of proteolytic enzymes in having two AAA+ modules. The protease domains are linked to the AAA+ modules in Lon and FtsH but exist as distinct oligomeric complexes for the remaining families. Each group of AAA+ proteases contains at least one family-specific auxiliary domain, which often serves as a docking site for substrates or adaptors. In FtsH and LonB, these "extra" domains anchor the protease to cellular membranes (11, 13, 15).

AAA+ Ring Hexamers

Crystal structures of hexameric AAA+ rings are known for HslU, FtsH, LonB, and ClpX, and structures of the AAA+ modules in single subunits are known for ClpA, LonA, and PAN (17–30). These structures establish that the conformations of the large and small AAA+ domains are highly conserved and that ATP or ADP bind in a cleft between the large and small AAA+ domains of one subunit and the large AAA+ domain of a neighboring subunit (**Figure 3**), as has been observed in other AAA+ enzymes (31). Moreover, nucleotide binding and identity alters the rotation between the large and small AAA+ domains of a subunit, providing a mechanism to link the ATPase cycle to conformational changes in the hexameric ring.

The relationship between subunits in proteolytic AAA+ hexamers varies widely. For example, HslU and FtsH can crystallize with C_6, C_3, or C_2 symmetry, corresponding to a sixfold symmetric hexamer, a dimer of trimers, or a trimer of dimers. The subunits in ClpX hexamers assume an approximate dimer-of-trimers conformation. These structural/subunit packing variations arise because of differences in rotation between the large and small AAA+ domains of individual subunits (sometimes but not always correlated with nucleotide binding) and differences in the ways in which domains of neighboring subunits pack together in the ring (**Figure 3*b*,*c***).

Nucleotide Binding

There are six potential ATP/ADP binding sites in a hexameric ring consisting of a single AAA+ module (**Figure 3*b***), but solution studies of ClpX, HslU, and PAN show that a maximum of four are occupied at nucleotide saturation (32–34). Moreover, studies with covalently linked hexamers of ClpX indicate that nucleotide hydrolysis in a single subunit is sufficient to drive the conformational changes required for protein unfolding, translocation, and degradation (35). These results suggest that the ATPase cycle begins with an enzyme with four ATPs bound, proceeds following hydrolysis to one with three ATPs and one ADP, and then returns following ADP dissociation and ATP binding to the initial state. Some crystal structures of HslU and ClpX (**Figure 3*c***) show occupancy of just three or four subunits, as would be expected in this model. However, structures of HslU (**Figure 3*b***) and FtsH, with six bound ADPs or AMPPNPs are also observed,[1] as are structures of ClpX and FtsH hexamers with no bound nucleotide. We suspect that such structures do not represent states populated during the normal ATPase cycle, but this conjecture remains to be established.

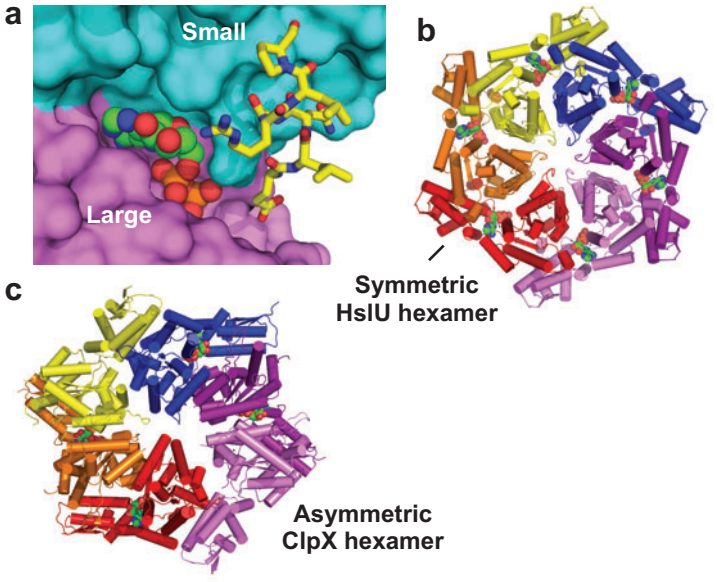

Figure 3

Nucleotide binding. (*a*) The pocket for ATP/ADP binding is located between the large (*magenta*) and small (*cyan*) AAA+ domains of a subunit. Parts of these domains are shown in surface representation for *Escherichia coli* HslU (PDB code 1HQY; chain E); ADP is shown in space-filling representation. The binding pocket consists largely of amino acids from box II (residues 18–22) and the Walker A motif (residues 57–66) in the large domain as well as from the first loop/helix (residues 335/343) and sensor II motif (residues 390–393) of the small domain. Additional nucleotide contacts from the box VII/Arg finger motif of the large domain of a neighboring subunit (residues 321–327; *stick representation*) help coordinate communication between subunits. (*b*) Sixfold symmetric hexameric ring of *Haemophilus influenzae* HslU (PDB code 1IM2) with six nucleotides bound (*space filling representation*). The I domain in this structure is not shown. (*c*) Asymmetric hexamer of *E. coli* ClpX (PDB code 3HWS) with four nucleotides bound (*space filling representation*). In the nucleotide-free subunits (*yellow* and *magenta*), a large rotation between domains occludes binding. The N domain was not present in this structure. In panels *b* and *c*, the small AAA+ domains are on the periphery of the hexameric rings, and the large AAA+ domains are closest to the ring axis.

What prevents nucleotide binding to some subunits? In the structure of the ClpX hexamer (**Figure 3*c***), a very large rotation between the large and small AAA+ domains in two subunits destroys the nucleotide-binding site by placing a helix where ATP/ADP would normally bind (27). Such extreme conformational heterogeneity has not been observed in HslU or FtsH hexamers, however, and the extent to which the ATPase cycles of these enzymes differ remains to be determined.

[1] Many of these "fully" bound structures appear to lack Mg^{2+}, which could account for the differences in the species observed crystallographically and in solution.

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Heat shock protein (Hsp): proteins whose synthesis is upregulated at high temperatures. Many chaperones, proteases, and disassembly/remodeling machines are heat shock proteins

Unfoldase: an enzyme that catalyzes the unfolding of a native substrate to generate a denatured protein

Ubiquitin: a 76-residue protein covalently attached to target proteins in eukaryotes. Four ubiquitin-length chains tether proteins to the proteasome

Double AAA+ Rings

As noted above, ClpA and ClpC contain two AAA+ modules (designated D1 and D2). Electron microscopy and crystallography show that the D1 and D2 modules form distinct stacked rings (36, 37). This double-lobed arrangement is also a hallmark of the ClpB/Hsp100 family chaperones, which are discussed below. Although the reason for having two AAA+ modules in these enzymes is unclear, biochemical experiments suggest that the two ATPase rings can fire and function independently as protein unfoldases, with the D2 ring being a stronger unfoldase (38).

Auxiliary Domains

All AAA+ proteases contain family-specific domains that connect in some fashion to the hexameric AAA+ ring (**Figure 2**). Structures are known for the intermediate (I) domain of HslU [which interrupts the AAA module (17, 19)], the N-terminal (N) domain of ClpA [which has pseudo C_2 symmetry (22)], the N domain of ClpX [a dimer stabilized by coordination of Zn^{2+} (39, 40)], the N1 and N2 domains of Lon [which form multiple oligomeric interactions in crystals (29, 41)], and the N2 domain of PAN [which forms a stable ring hexamer (28, 42)]. The auxiliary domains of different families share no structural homology. In most cases, these domains are flexibly tethered to the AAA+ ring, and the ones that form stable solution oligomers probably contribute to the stability of the attached AAA+ ring by an effective-concentration mechanism (see below). However, these family-specific domains are not required for basic AAA+ protease function, as variants of HslU, ClpA, ClpX, and FtsH lacking these domains are still active in degradation of some substrates (18, 26, 43).

RECOGNITION

Recognition of the appropriate protein substrates at the proper time and in the right context is critical given the destructive nature of degradation. As summarized below, we currently understand how some substrates are targeted to specific AAA+ proteases, which provides insight into common strategies for recognition and regulation.

Degrons

Most substrates contain sequence determinants (called recognition tags, degradation tags, or degrons) that are responsible for their recognition by a AAA+ protease. Degrons can be functionally identified by mutations that prevent degradation of known substrates or as sequences that confer susceptibility to a AAA+ protease when appended to a protein that is not normally degraded. For bacterial and archaeal AAA+ proteases, degrons are typically short unstructured peptide sequences. For example, ClpXP degrades any protein with an accessible Ala-Ala at the C terminus (44). Substrate targeting to the eukaryotic proteasome typically involves one degron that promotes addition of polyubiquitin and a second unstructured sequence that allows substrate engagement by the proteasomal unfolding machinery, although ubiquitin-independent recognition is also possible (45, 46).

Degrons mediate protease recognition in four general ways: (*a*) binding to the pore of the AAA+ ring hexamer; (*b*) binding to an auxiliary site on the protease; (*c*) binding to an adaptor protein, which binds the protease; and (*d*) mediating reactions, including covalent modification, that allow protease recognition. Many substrates contain multiple degrons. For example, the *Escherichia coli* ClpXP protease degrades proteins bearing the ssrA tag, which has the sequence AANDENYALAA-COOH. The C-terminal dipeptide and α-carboxylate of this degron are recognized by the ClpX pore, whereas the N-terminal residues are bound by SspB, an adaptor protein that also binds ClpX (47–50). Thus, degron-pore, degron-adaptor, and adaptor-protease contacts all contribute to ClpXP recognition and degradation of ssrA-tagged substrates. The latter interactions involve protein-peptide recognition (**Figure 4*a*,*b***). Several other ClpXP substrates

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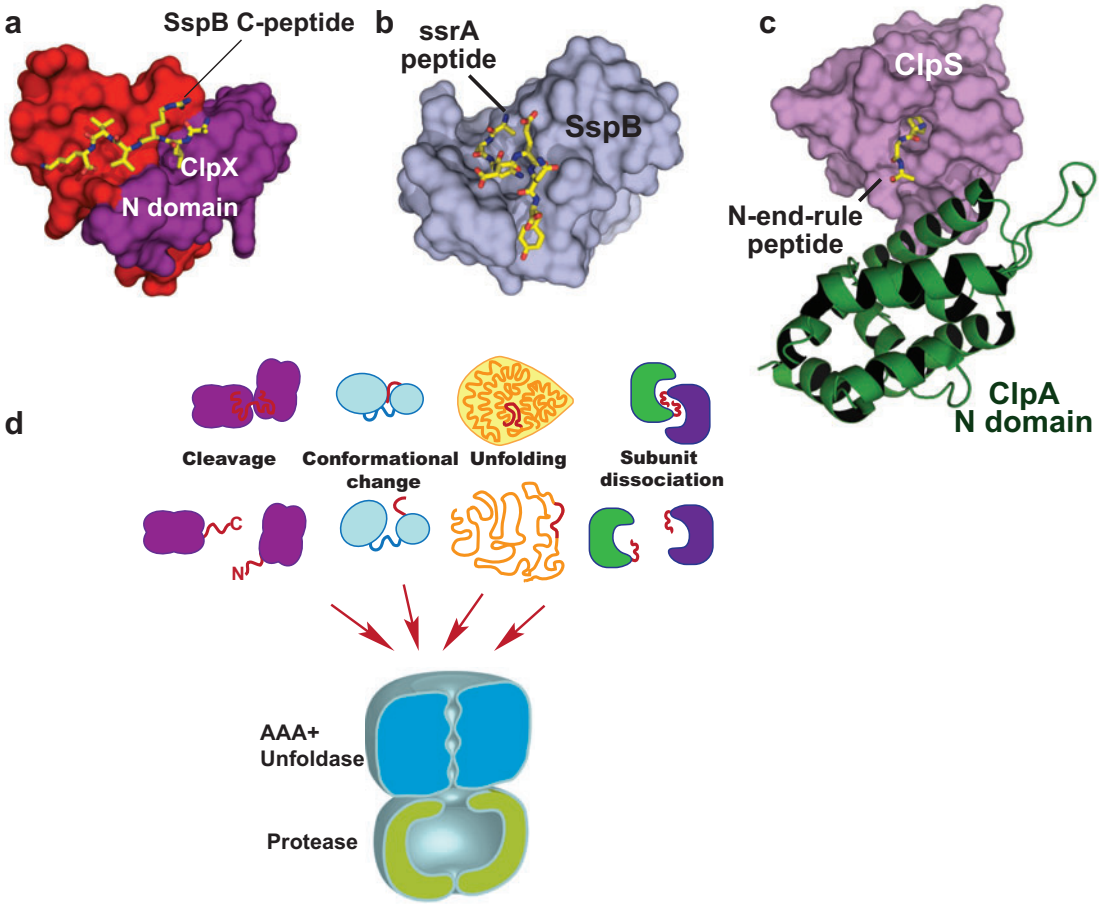

Figure 4

Recognition via peptide-protein and protein-protein interactions. (*a*) Structure (PDB code 2DS8) showing the C-terminal peptide of the SspB adaptor (*stick representation*) bound to the N-domain dimer of ClpX (*surface representation*). (*b*) Structure (PDB code 1OU8) showing a portion of the ssrA degradation tag (*stick representation*) bound to the SspB adaptor (*surface representation*). (*c*) Structures (PDB codes 3DNJ and 1MBU) showing binding of an N-end-rule peptide (*stick representation*) to the ClpS adaptor (*surface representation*) and binding of ClpS to the N domain of ClpA (*cartoon representation*). (*d*) Proteolysis is frequently regulated by controlling degron accessibility. Four common strategies are diagrammed.

also contain multiple degrons, including one sequence that interacts with the ClpX pore and distinct sequences that interact with other parts of enzyme.

Control of Degron Accessibility

Modulating the accessibility of degrons, which are buried in a native protein structure or protein-protein interface, allows degradation to be regulated (**Figure 4*d***). Fnr, for example, is a transcriptional repressor that is only degraded under oxidizing conditions, which destroy a [4Fe-4S] cluster that stabilizes Fnr dimers. Indeed, oxidation promotes dimer dissociation and reveals degradation signals in the Fnr monomer that lead to degradation by ClpXP (51). This strategy of exposing degrons in free subunits but not in complexes appears to be common. For example, intact ribosomes are highly resistant to degradation, whereas many free ribosomal subunits are good substrates for AAA+ proteases (44, 52).

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Protein unfolding is another method of revealing cryptic degrons (**Figure 4*d***). Heat shock and other global environmental stresses cause denaturation of numerous cellular proteins, whereas specific modification reactions or loss of stabilizing cofactors could result in unfolding of individual proteins. Certain AAA+ proteases play major roles in degradation of unfolded proteins. *E. coli* Lon, for example, is responsible for ~50% of the protein degradation caused by premature translational termination or the incorporation of toxic amino acid analogs, indicating that it recognizes most cellular proteins that cannot fold properly (53). Indeed, Lon is ideally suited to degrade unfolded proteins, as it recognizes degrons rich in aromatic and hydrophobic residues that would typically be buried in the hydrophobic cores of native proteins (54, 55).

Degron accessibility can also be controlled by endoproteolytic processing (**Figure 4*d***). Cleavage of two bacterial stress-response proteins, LexA and RseA, reveals degrons that target the cleavage fragments for degradation by AAA+ proteases (44, 56, 57). In such cases, the initial cleavage event can reveal degrons that were inaccessible in the uncleaved protein and/or create new α-carboxyl and α-amino groups that are recognized as part of the degron. For example, N-end-rule recognition requires a free α-amino group (58–60), whereas certain ClpX degrons require a free α-carboxylate group (48).

Addition of Polypeptide Sequences to Proteins Is a Commonly Used Marker for Destruction

Covalent addition of peptide sequences or small proteins to otherwise stable proteins is a frequently used strategy to mark specific proteins for degradation. Addition of the degradation-marker sequence can occur either posttranslationally or cotranslationally. An example of cotranslational peptide addition occurs when normal translation stalls in bacteria. This stalling triggers recruitment of the tmRNA tagging and ribosome rescue system, which mediates cotranslational addition of the ssrA tag to the C terminus of the nascent polypeptide (61). The sequence and length (9–36 residues) of the ssrA tag varies depending on the bacterial species. SsrA-tagged proteins are typically degraded by the ClpXP protease, sometimes with assistance from the SspB adaptor. In *Mycoplasmas*, which have very small genomes and encode just two AAA+ proteases, Lon degrades ssrA-tagged proteins (62, 63). Thus, irrespective of the protease employed, the tmRNA system ensures that the incomplete products of failed translation are targeted for degradation.

In eukaryotes, covalent posttranslational addition of ubiquitin is the principal method of targeting proteins for degradation by the 26S proteasome (64). Ubiquitin, a protein of 76 residues, is enzymatically cross-linked to target proteins, which require a minimum of four attached ubiquitin units to bind efficiently to the proteasome and to be degraded. Enzymes (E1, E2, and E3) bind and activate ubiquitin, recognize the substrate, and catalyze formation of an isopeptide linkage between a substrate lysine and one ubiquitin, followed by attachment of additional units to form polyubiquitin chains. Regulation of E3, which adds ubiquitin to specific substrates, is the central control point in degradation control in this system. For example, there are more than 600 different E3 enzymes in humans, and regulation occurs by numerous mechanisms, including inhibition by substrate mimics lacking modifiable lysines (65). Covalently bound ubiquitin is not degraded with the substrate because it is removed by deubiquitinating enzymes present in the proteasome.

The use of small protein, covalently added "degradation markers" has recently been shown to be more widespread in nature than previously appreciated. For example, ubiquitin-like targeting systems have been identified in some bacteria and archaea. Certain species of *Mycobacterium* and *Streptomyces* use modification by Pup (prokaryotic ubiquitin-like protein) to target substrates to a AAA+ protease, resembling the archaeal PAN/20S enzyme (66–71).

Pup, which appears to be an intrinsically disordered protein, is, like ubiquitin, conjugated to substrate lysines, but the chemistry of activation and conjugation is completely different from the reactions used to attach ubiquitin. Finaly, in archaea, ubiquitin-like small modifiers (SAMPs) have recently been discovered covalently attached to target proteins (72).

N-End-Rule Recognition

In bacteria, plants, and animals, proteins with certain N-terminal amino acids are targeted for degradation (73). These "N-degrons" are among the simplist degradation signals known, and recent advances provide insights into the molecular mechanisms of substrate recognition. Different sets of N-terminal amino acids are recognized as destablizing in different organisms; examples of these N-degrons are shown in **Table 1**.

In *E. coli*, proteins beginning with Phe, Leu, Trp, or Tyr are recognized by the well-defined N-degron binding pocket on the ClpS adaptor, which delivers them to ClpAP for degradation (58). Moreover, an N-terminal Leu or Phe can be enzymatically added to proteins beginning with Lys and Arg, allowing subsequent ClpS

Table 1 N-end-rule degron classes

Eukaryotes			
Tertiary	**Secondary**	**Primary**	**Recognized by**
N[a] (modified by deamidation)	D (modified by Arg-ylation)	RD	Class I or UBR box
Q (modified by deamidation)	E (modified by Arg-ylation)	RE	
C (modified by oxidation)	C*[b] (modified by Arg-ylation)	RC*	
		R	
		K	
		H	
		L	Class II or ClpS like
		F	
		Y	
		W	
		I	
Bacteria			
Tertiary	**Secondary**	**Primary**	**Recognized by**
None	R (modified by Leu or Phe-ylation)	F/LR	ClpS (class II)
	K (modified by Leu or Phe-ylation)	F/LK	
	(M)[c] (modified by Leu-ylation)	FM	
		L	
		F	
		Y	
		W	

[a]Typical N-end-rule residues, divided in classes, represented in the one letter amino acid code.
[b]C* denotes oxidized cysteine.
[c]To date, only one substrate with this modification has been reported (74).

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recognition and degradation. On the basis of a recent analysis of one bacterial N-end-rule substrate, it appears that this addition occasionally occurs on proteins initiating with Met (74). However, this reaction must not be general, as many proteins in bacteria retain their N-terminal Met, but are not modified and degraded.

In animals, proteins beginning with Phe, Leu, Ile, Trp, Tyr, Lys, Arg, and His are ubiquitinated by a specific E3 enzyme, targeting them to the proteasome (73, 75). The E3 binding regions that recognize these N-degrons are of two classes: the type 1 or UBR box class and the type 2 or ClpS-like class. The UBR box region on the E3 enzymes is responsible for recognizing the Lys, Arg, and His N-degrons, whereas an E3 binding region homologous to ClpS recognizes the hydrophobic side chains in this group (60, 76, 77). The structures of the UBR boxes of two E3 enzymes bound to N-degron peptides have recently been solved (78–80). This small (~70-amino acid) domain recognizes its substrates by making specific hydrogen bonds with the peptide α-amino group as well as by specific contacts with the first two amino acid side chains and the first peptide bond. Thus, we now have a view of the molecular interactions responsible for specific recognition of both class 1 and class 2 N-degrons, based on this UBR box work and the structural studies of ClpS (80).

As summarized in **Table 1**, a large variety of N-terminal residues can be destablizing, either directly (a primary destablizing residue) or after futher modification (a secondary or tertiary destablizing residue). For example, in eukaryotes, proteins beginning with Asp, Glu, Asn, Gln, or Cys can be modified by enzymatic addition of an N-terminal Arg, either directly or following Asn/Gln deamination or Cys oxidation. The rich landscape on N-degrons is even larger as it was recently determined that certain acetylated N-terminal residues can also act as N-end-rule signals in some organisms (81).

Importantly, normal posttranslational processing of proteins by methionine aminopeptidases does not usually generate N-end-rule substrates, and thus additional proteolytic or conjugation reactions are likely to be involved in their biogenesis. For example, chromosome separation during meiosis requires endoproteolytic cleavage of cohesin by separase (82). One of the resulting cohesin fragments begins with an N-end-rule residue, ensuring its complete degradation. In numerous cases, however, the modification reactions that generate N-end-rule substrates have not been well characterized.

Adaptors

Numerous proteins, commonly called adaptors, modulate the activity of AAA+ proteases. Although adaptors were first characterized as proteins that helped in delivery of specific substrates, they can have additional activities. In this section, we focus on three well-characterized adaptors, which highlight many of the features of these regulatory molecules.

SspB is a dimeric adaptor that aids in delivery of certain substrates, including ssrA-tagged proteins and N-RseA, to ClpXP. The structured domain of SspB mediates dimer formation and contains a groove that binds to the AANDENY portion of the ssrA tag (**Figure 4*b***) or a EAQPAPHQWQKMPFW sequence in N-RseA (83). These nonhomologous sequences bind competitively but in opposite orientations in the SspB groove. An unstructured region of SspB terminates with a C-terminal sequence that binds the ClpX N domain (**Figure 4*a***), thereby tethering bound substrates to ClpXP (50, 84). Tethering, in turn, increases the effective concentration of the substrate relative to the ClpX pore and decreases K_M for degradation (**Figure 5**) (85, 86). As a result, SspB-associated substrates are bound and degraded by ClpXP at lower concentrations than the corresponding free substrates. Interestingly, ClpAP also degrades free ssrA-tagged substrates but does not degrade these molecules when SspB is bound (47, 87). In the latter case, SspB blocks ClpA recognition of the substrate at the same time that it enhances recognition by ClpX.

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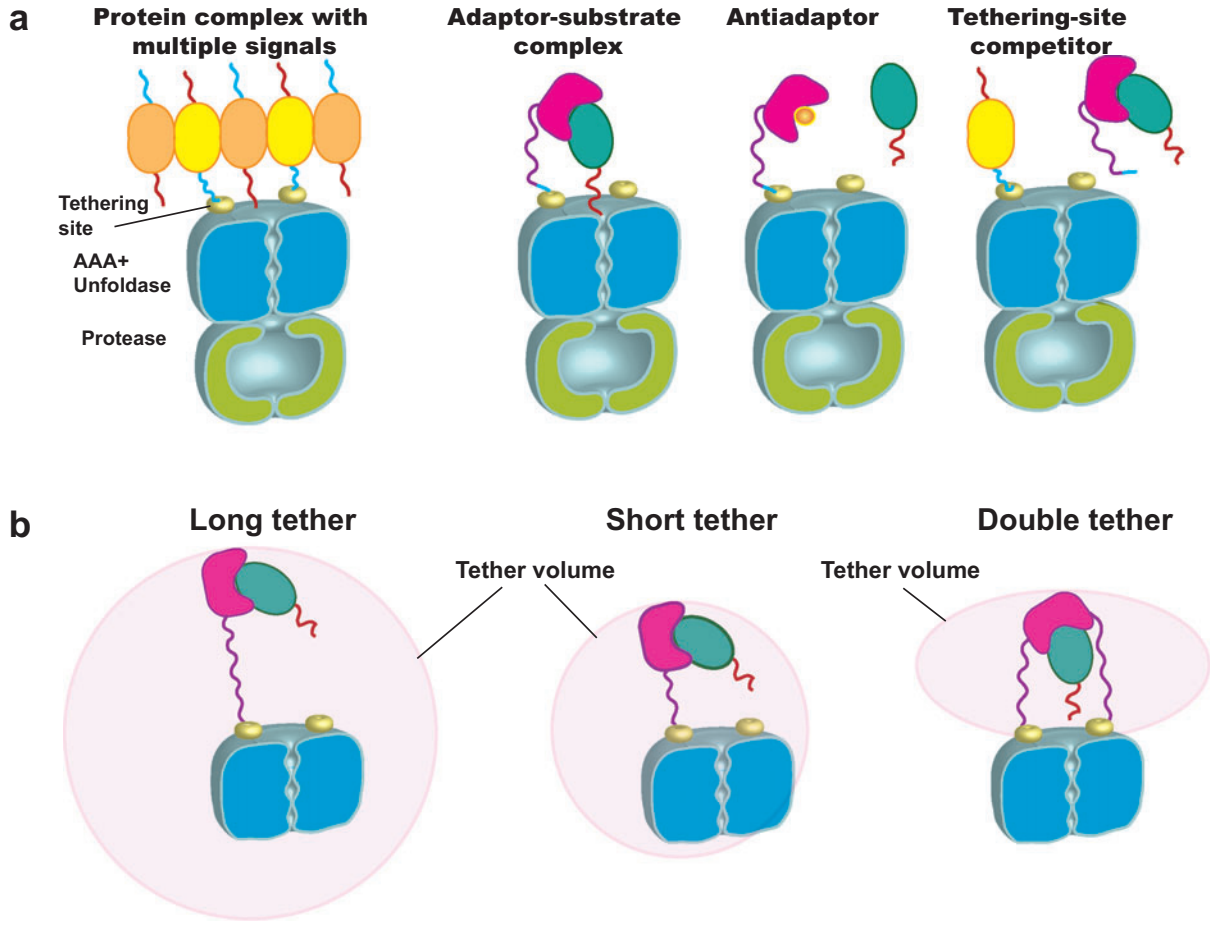

Figure 5

Multivalent interactions enhance substrate recognition and provide additional means for regulation. (*a*) A large multimeric protein and an adaptor-substrate complex are shown making multiple contacts with the AAA+ enzyme ring (tethering sites on the enzyme are shown in *yellow*). Competition by an antiadaptor and by a second protein that binds to a common tethering site are also shown. (*b*) The geometric consequences of different types of tethering on the volume the substrate can search or access while tethered but not yet engaged.

ClpS is a monomeric adaptor that contains a binding pocket for specific N-end-rule residues and also binds to the N domain of ClpA (**Figure 4*c***) (37, 60, 88, 89). However, ClpS-mediated tethering, by itself, is insufficient for ClpAP degradation. Indeed, mutations in an unstructured portion of this adaptor preserve tethering but eliminate degradation (90). Although the details are still being elucidated, an active hand-off mechanism appears to be required to transfer the ClpS-bound N-end-rule substrate and allow its engagement by the ClpA pore. Even in the absence of N-end-rule substrates, ClpS binding modifies the intrinsic substrate specificity of ClpAP. For example, unlike ClpAP, ClpAPS cannot degrade ssrA-tagged substrates, does not display autodegradation of ClpA, and degrades certain aggregated substrates (88).

MecA is a monomeric adaptor for the ClpCP protease and appears to be required for stable assembly of active ClpC hexamers in many bacterial strains (91, 92). For example, *Bacillus subtilis* ClpC only forms stable

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hexamers in the presence of MecA in vitro. The C-terminal domain of MecA binds ClpC, whereas the N-terminal domain binds the ComK protein and delivers it for ClpCP degradation. Interestingly, MecA is degraded together with ComK in this reaction. By contrast, adaptors like SspB and ClpS are not degraded during substrate delivery.

Because adaptors bind both to a substrate and to a AAA+ protease, there are ample opportunities for regulation via competition (**Figure 5*a***). For example, the ComS antiadaptor binds the MecA adaptor in a reaction that prevents binding of the ComK substrate and thus spares it from ClpCP degradation (93). Similarly, peptide sequences in the SspB adaptor and the UmuD/UmuD′ adaptor/substrate complex compete with each other for binding to the ClpX (94). Likewise, several different antiadaptors, each expressed under a different environmental condition, are key to regulating degradation of the σS substrate by ClpXP (95–97). Thus, degradation is a function not only of specific recognition but also of the entire intracellular repertoire of substrates, adaptors, and antiadaptors.

Geometric Control of Multivalent Recognition

AAA+ proteases often recognize multiple signals on a substrate and/or an associated adaptor (**Figure 5**). Each signal by itself may be too weak to allow efficient recognition/degradation, but coupling between these weak interactions can create much stronger binding. The magnitude of the energetic coupling between weak signals is determined by geometric factors (**Figure 5*b***). For example, if binding of the first signal to the protease positions the second signal very close to its receptor site, then the effective concentration will be high, and binding will be much stronger than to either individual signal. By contrast, if a long flexible linker separates the two signals, then coupled binding may be only marginally tighter than binding to the strongest signal. Geometric effects of this type have been observed using model substrates of ClpXP (98). Such geometric factors provide additional opportunities for regulation. For example, chemical modification or binding of another molecule could alter the flexibility or relative orientation of the element that connects two signals, making binding stronger or weaker.

Because weakly recognized degrons may comprise only a few amino acids, they should be relatively easy to evolve, with subsequent coupling occurring through a single polypeptide or by an assembly of complexes. Moreover, controlling the accessibility of just one of the weak degradation signals then provides a mechanism to regulate substrate recognition.

TRANSLOCATION AND UNFOLDING

AAA+ proteases can be viewed as polypeptide-translocation machines. This ATP-dependent activity is obviously essential for spooling denatured substrates through the axial pore of the AAA+ ring and into the degradation chamber of an associated compartmental protease. Translocation of a peptide tag attached to a folded protein also provides a way to apply an unfolding force by trying to pull a large object through a narrow channel. We refer readers to previous reviews for more extensive discussions of support for the translocation-coupled unfolding model (10, 12).

Translocation Machinery and Determinants

Cycles of ATP binding and hydrolysis appear to cause rigid-body changes in the conformation of the AAA+ ring, and these movements are transmitted to the substrate, at least in part, by loops that project into the axial pore and contain a sequence motif consisting of an aromatic side chain followed by a hydrophobic side chain (**Figure 6**). Replacing the aromatic residue in this sequence with a small side chain does not prevent ATP hydrolysis but effectively eliminates ATP-dependent degradation in numerous AAA+ proteases (18, 99–104).

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However, these pore loops also play important roles in substrate recognition, as discussed above, and establishing specific defects in translocation/unfolding was crucial in establishing their function. For ClpX, such defects were observed after mutating the aromatic side chain in just two of six subunits in a covalently linked hexamer (105). At saturating substrate concentrations, rates of translocation and unfolding were found to be slower for the mutant when the pore mutation was in a subunit that could hydrolyze ATP. Importantly, the cost in ATP hydrolysis of translocating an unfolded polypeptide increased ~threefold for the mutant relative to wild type, whereas the cost of unfolding a native protein was ~18-fold higher. These results suggest that many power strokes in the mutant enzyme fail to move substrate or allow it to slip following a translocation step. Moreover, when this aromatic residue was replaced with a cysteine in one subunit of a ClpX hexamer, ATP-dependent formation of a specific disulfide cross-link with a translocating polypeptide was observed, establishing that the pore loop directly contacts a spooling substrate (105). The exact way in which ATP-powered movements of pore loops drive substrate translocation has not been established and will probably require a combination of crystal structures with translocating substrates bound in the pore and single-molecule studies in which the step size of individual translocation events can be assayed.

AAA+ proteases can degrade substrates starting from either end or from internal sites (44, 55, 106–115), indicating that translocation can proceed in an N-terminal to C-terminal direction and vice versa. What parts of a polypeptide are recognized to allow the pulling events that result in translocation and unfolding? The pore loops of AAA+ proteases could, in principle, bind to peptide bonds or interact with specific side chains. Indeed, it was suggested that the critical aromatic side chain in the pore loop might contact basic or aromatic residues in the substrate via π-cation or π–π interactions (102). However, side chain recognition seems unlikely, at least for ClpXP,

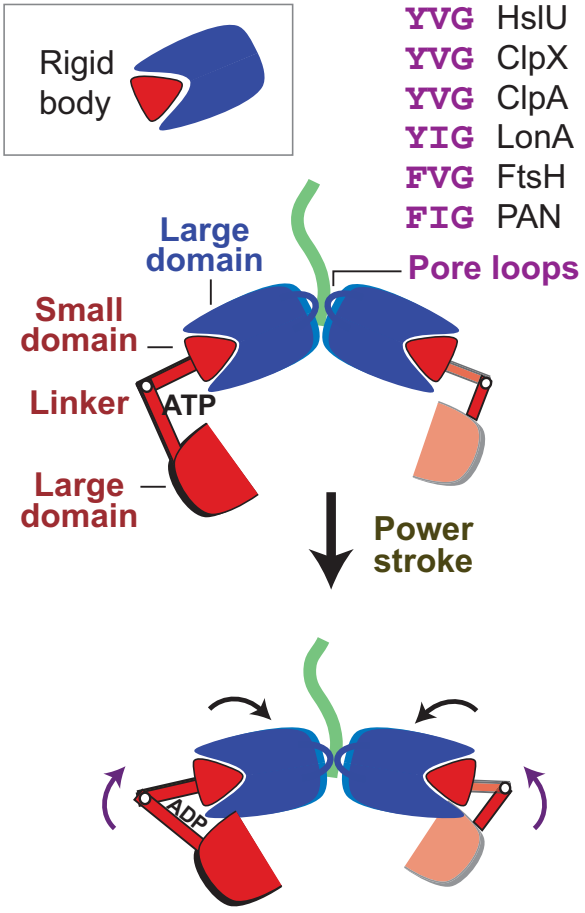

Figure 6

Highly conserved loops in the axial pore of the unfoldase contact the translocating substrate. Homologous YVG, YIG, FVG, or FIG sequences are found in the pore loops of different AAA+ unfoldases (HslU, ClpX, ClpA, LonA, FtsH, and PAN). The power stroke appears to be caused by nucleotide-dependent changes in the rotation between the large and small AAA+ domains of one subunit, which drive rigid-body movements of the entire AAA+ ring and translocate the polypeptide chain.

as this enzyme translocates peptide substrates with 10-residue stretches of proline, glycine, hydrophobic amino acids, positively and negatively charged residues, aromatic residues, and D-amino acids (116). Moreover, ClpXP recognition of peptide bonds seems unlikely because proline lacks a peptide-NH group and because substrates with nonnatural amino acids containing as many as 11 CH_2 groups between successive peptide bonds were also translocated efficiently (116). Impressively, AAA+ proteases

can also degrade disulfide-bonded substrates, which require concurrent translocation of three polypeptide chains through the axial pore (85, 112, 117, 118). How can AAA+ enzymes pull tightly on so many different sequences in single or multiple chains without recognizing specific chemical groups? A likely possibility is that the pore has an elastic character that allows it to expand to grip multiple chains or substrates with larger side chains and to contract to grip skeletal substrates like polyglycine (27, 116). According to this model, simple van der Waals contacts between the pore loops and a substrate might be sufficient to pull a polypeptide downward through the pore as the AAA+ domains and attached pore loops move during the ATPase cycle.

Limits and Costs of Forced Unfolding

The ability of AAA+ proteases to unfold native proteins is remarkable (48, 108, 115, 119, 120). For example, green fluorescent protein unfolds in solution with a half-life of years, and yet some AAA+ proteases unfold this protein in seconds. Similarly, mechanical denaturation of the I27 domain of human titin requires pulling with hundreds of piconewtons of force, but many AAA+ proteases unfold and degrade appropriately tagged titin-I27 variants. Nevertheless, some AAA+ proteases fail to unfold and thus degrade certain proteins even when these proteins bear accessible recognition tags, suggesting that some AAA+ unfoldases are more powerful than others (115, 121). However, the unfolding strength of a specific enzyme can depend upon the substrate assayed. For example, ClpXP unfolds green fluorescent protein substrates much faster than does Lon, whereas Lon degrades titin substrates much faster than ClpXP (48, 55, 120, 122). As discussed below, some of these differences may be related to the ability of the enzyme to rapidly translocate a portion of the substrate after an initial unfolding event.

After a peptide segment of a substrate is engaged by the axial pore of a AAA+ unfoldase, a few cycles of translocation are thought to pull the protein tightly against the hexameric ring and pore entry (**Figure 1**). At this point, the next translocation step will apply an unfolding force to the structural element attached to the tag. Multiple outcomes are possible: (*a*) The structure unfolds cooperatively, allowing translocation and degradation to proceed; (*b*) the protein resists unfolding and dissociates; or (*c*) the substrate resists unfolding, the translocation machinery slips, and the substrate remains bound.

The probability of outcome *a* versus *b*/*c* can be experimentally assessed by determining the amount of ATP hydrolysis required to degrade a native substrate compared to an unfolded substrate with essentially the same sequence (120). For example, ClpXP hydrolyzes an average of ~600 ATPs during the time required to degrade one molecule of native titin-I27 but only ~100 ATPs to degrade an unfolded variant of the same protein. Thus, an average of ~500 ATPase cycles are required to unfold titin-I27, although this value can be much lower for destabilized mutants. Why do some denaturation attempts succeed and others fail? At least part of the answer is that stabilities of individual molecules vary in a population of otherwise identical proteins because thermal energy is randomly distributed. Thus, the chance of denaturation per pulling event increases if the substrate happens to be transiently less stable. The rates at which AAA+ proteases unfold/degrade different substrates correlate poorly with the average global thermodynamic or kinetic stabilities of these molecules and seem instead to depend on the local mechanical stability of the structural element attached to the peptide sequence being pulled (108, 110).

Substrates that resist an unfolding attempt can dissociate completely from the AAA+ protease, but it is unclear whether dissociation or remaining bound is more frequent. The best evidence for dissociation comes from studies of substrates carrying several independently folded domains (108, 111, 115). If the first domain to be degraded contains the only recognition tag and the second domain resists unfolding, then partially degraded substrates are

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often released from the enzyme and accumulate in a form resistant to further degradation. Indeed, biological "processing" reactions of this type are carried out by the 26S proteasome and require both a domain that resists unfolding and an adjacent low-complexity sequence that the translocation pore is unable to grip tightly (123).

If an intact substrate dissociates upon failed unfolding, it can simply rebind the AAA+ protease for another attempt, in principle, ad infinitum. This mechanism may seem wasteful in terms of excessive ATP hydrolysis, but it ensures preferential degradation of the most easily unfolded proteins in a mixture of substrates (111). Moreover, AAA+ proteases must unfold an assortment of proteins, with a wide range of structures and stabilities, which probably precluded evolutionary optimization of the enzyme for unfolding any single protein. Why not simply evolve a more powerful unfoldase? One possibility is that such enzymes would degrade any transiently bound protein, including adaptors and other molecules not intended for degradation. In this regard, it is interesting that the 26S proteasome appears to have a far more powerful unfoldase activity then its simpler relatives and also uses a far more intricate mechanism of substrate selection (115). Selective pressure for the evolution of substrates that can be efficiently degraded should also be operative. In this regard, we note that most of the "high-cost" degradation reactions that have been studied biochemically use hyperstable model substrates, specifically chosen to test the limits of AAA+ unfolding and proteolysis. Thus, we suspect that natural substrates may be degraded at substantially lower energetic costs.

The rate of unfolding/degradation of some model substrates by a AAA+ protease is linearly correlated with the rate of ATP hydrolysis (124). For example, if the ATPase rate is reduced to 10% by using ATP concentrations well below K_M, then the unfolding/degradation rate is also reduced to 10%. This behavior indicates that hydrolysis of a single ATP is involved in the rate-limiting step in unfolding and is consistent with unfolding induced by a single power stroke. Strikingly, however, degradation of certain substrates requires a minimum rate or threshold of ATP hydrolysis, suggesting that multiple coordinated hydrolysis events are needed before global unfolding occurs (124). In such cases, it is possible that one power stroke unfolds only a portion of the substrate,[2] requiring rapid translocation and subsequent pulling on the remaining structure for global unfolding. The rates at which different AAA+ proteases hydrolyze ATP during substrate unfolding vary considerably (34, 115, 120, 122), and this variation may explain how one enzyme can appear to be more powerful than another when assayed using one substrate but not other substrates. For Lon, the identity of the degradation tag can also alter the rate at which a native substrate is unfolded (122), further compounding the difficultly of comparing the unfolding activities of different AAA+ enzymes. It has been proposed that different degrons bias the distribution of Lon enzyme conformations toward one that specializes in proteolysis or another that may serve a chaperone function and thus set the maximal rate of degradation (122).

Nucleotide Transactions

As noted above, only some subunits of the hexameric AAA+ rings of ClpX, HslU, and PAN bind ATP at any given time. An important unresolved question is whether binding subunits convert to nonbinding subunits and vice versa as ATP hydrolysis progresses. Whether other proteolytic AAA+ hexamers contain binding and nonbinding subunits is also unresolved. Multiple results demonstrate communication between AAA+ subunits, including positive cooperativity in ATP binding and hydrolysis, differential activities depending upon the extent of nucleotide saturation, and influences of nucleotide occupancy on hydrolysis in neighboring subunits (34, 35, 105, 125, 126).

[2] The majority of protein domains unfold cooperatively, and thus partial unfolding is probably the exception rather than the rule.

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Experiments using single-chain ClpX variants with different combinations of active and inactive subunits indicate that cycles of ATP hydrolysis in a single subunit can drive protein unfolding and translocation and show that the order in which different subunits hydrolyze ATP need not occur in a strict pattern or sequence (35). It is possible that a more regular pattern of hydrolysis would be observed in the wild-type enzyme. Alternatively, subunits positioned to make "good" contacts with the translocating substrate may have a higher probability of firing, resulting in a somewhat stochastic pattern.

DEGRADATION

Different compartmental proteases employ a variety of active sites for peptide-bond cleavage. ClpP uses a His-Asp-Ser catalytic triad, Lon uses a Lys-Ser dyad, FtsH is a Zn^{2+}-dependent peptidase, and HslV and the archaeal 20S peptidase use an N-terminal Thr as the active-site nucleophile (17, 19, 24, 26, 127–129). Indeed the β-subunits of the 20S enzyme have the same basic fold as an HslV subunit. The compartmental peptidases of PAN/20S and the 26S proteasome share a common $\alpha_7\beta_7\beta_7\alpha_7$ structure. However, each ring in the proteasome is composed of seven different α- or β-proteins, only some β-subunits are catalytically active, and these subunits have distinct preferences for cleavage after different types side chains (130, 131). The concentration of active sites in the lumen of all compartmental proteins is very high, and substrates are typically cleaved at many sites to produce peptide products with an average size of ~10 amino acids.

All compartmental proteases are constructed from rings with six subunits (HslV, Lon, FtsH) or seven subunits (ClpP and 20S enzymes), but their AAA+ ring partners invariably consist of six subunits, creating a symmetry mismatch for ClpXP, ClpAP, ClpCP, PAN/20S, and the 26S proteasome. An early proposal suggested that this mismatch might promote ATP-dependent rotation of the AAA+ ring relative to the proteolytic ring (132), but no support has emerged for this idea. Moreover, HslUV functions without a mismatch, and the AAA+ and proteolytic rings of Lon and FtsH are fused and therefore could not rotate. Some of the interactions between the AAA+ ring and compartmental protease appear to be static, whereas others are dynamic. In ClpXP, for example, static interactions are mediated by high-affinity docking of flexible loops that emerge from the AAA+ ring into clefts on the periphery of a ClpP ring, whereas dynamic interactions are mediated by relatively weak contacts between axial loops in both rings (133, 134). The latter interactions vary with the nucleotide state of individual ClpX subunits, control the rate of ATP hydrolysis during translocation, and facilitate more efficient protein unfolding by the AAA+ ring.

Unfolded polypeptides could, in principle, be degraded by self-compartmentalized proteases in the absence of a AAA+ partner, but two mechanisms limit such proteolysis. The active-site residues of HslV adopt an inactive conformation and fail to react with covalent inhibitors or cleave small peptides if an HslU ring is not bound to the HslV ring (23, 135–137). A different mechanism, gating of substrate access, controls the activities of PAN/20S, the 26S proteasome, ClpAP, and ClpXP (138–142). For example, substrate access to the chamber of the isolated 20S protease is severely limited by residues, which sterically block the entry portal, but this blockade is relieved by conformational rearrangements that accompany the binding of PAN, the regulatory complex of the 26S proteasome, or non-AAA+ regulators. The importance of restricting the activity of isolated compartmental proteases is highlighted by antibiotics that kill bacteria by binding to ClpP, blocking ClpX/ClpA binding, and activating degradation of nascent chains and other unfolded polypeptides by mimicking the gate-opening activities of ClpX/ClpA (143–145).

Compartmental proteases have been viewed as relatively static structures, but there are hints that substantial conformational changes may be functionally important in these barrel-shaped enzymes. For example, ClpP crystallizes in distinct extended and compact structures

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(146, 147). Moreover, ClpP dynamics have been proposed to open side pores transiently near the ring-ring equatorial interface, which could provide a route for peptide egress (148).

DISASSEMBLY MACHINES

AAA+ unfoldases also function in disassembly reactions uncoupled from proteolysis. For instance, ClpX alone disassembles hyperstable complexes of the MuA-transposase tetramer bound to recombined DNA by selectively unfolding one subunit of the protein complex (149, 150). In fact, some AAA+ enzymes specialize in disassembly, disaggregation, or remodeling and have no partner protease. ClpB/Hsp104 family AAA+ enzymes, for example, work in concert with refolding chaperones to resurrect aggregated proteins. Other more specialized AAA+ enzymes remodel the σ54 transcription factor, sever microtubules, and load the β- and PCNA ring-shaped clamps onto the DNA of a replication fork.

ClpB/Hsp104 Disaggregases

The bacterial ClpB enzyme and fungal/plant Hsp104 enzyme are homologous disaggregating machines that function as ring hexamers in protein quality control (151, 152). Both disaggregases have an N domain and D1 and D2 AAA+ modules that are very similar to those of ClpA/ClpC but lack the sequence motif that allows binding to the ClpP protease. In addition, ClpB and Hsp104 contain a substantial coiled-coil region, called the M or middle domain, inserted between the D1 and D2 modules (153, 154).[3]

Cells lacking ClpB/Hsp104 do not survive at high temperatures, presumably because they function to protect proteins and to antagonize the accumulation of heat-damaged proteins in deleterious aggregates. The function of ClpB/Hsp104 is intimately intertwined with the activities of the Hsp70/Hsp40 refolding chaperones (155–157). In bacteria, this system includes DnaK (Hsp70), DnaJ (Hsp40), and GrpE (a nucleotide-exchange factor). The collaboration between ClpB/Hsp104 and these chaperones is multifaceted and is not yet completely understood. An early step in disrupting protein aggregates probably involves Hsp70/KJE binding to exposed peptide sequences in the aggregate. In a subsequent recruitment step, interactions between the Hsp70/KJE proteins and the M domain of ClpB/Hsp104 tethers the aggregated protein near the AAA+ enzyme's processing center (156).

In principle, ClpB/Hsp104 could remove proteins from an aggregate by employing the protein-translocation mechanism described above for the proteolytic AAA+ ATPases. This possibility was elegantly demonstrated by engineering ClpB and Hsp104 to allow them to bind the ClpP protease and then showing that these complexes functioned as degradation machines (158, 159). However, some experiments suggest that ClpB/Hsp104 lack the robust unfoldase activity observed with AAA+ enzymes that normally function in proteolysis (160). This result has been interpreted to suggest that the action of ClpB/Hsp104 is restricted to more loosely structured regions of aggregates. As proteins are removed from an aggregate by ClpB/Hsp104, they may be passed back to an Hsp70·40/KJE partner to help the released protein fold to its native state. Some proteins, however, do not appear to require or benefit from a second Hsp70·40/KJE interaction and presumably fold efficiently on their own once they are released from ClpB/Hsp104.

Interestingly, Hsp104 also controls the activity of prion-like factors, which are inherited as amyloid fibrils (152, 161, 162). Overproduction of Hsp104 can reverse the prion state, apparently by solubilizing the fibril in a reaction analogous to disaggregation. However, the absence of Hsp104 can also prevent prior inheritance. The role of Hsp104 in prion inheritance is thought to involve a reaction in which the fibril is severed by extraction of

[3] ClpC also contains a short coiled-coil M domain.

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a subunit from an interior position, thereby producing two smaller fibrils, which can subsequently grow by addition of new protomers. The presence of smaller fibrils increases the probability of a daughter cell receiving a fibril and thus maintaining the "prion" state.

Client-Specific AAA+ Remodeling Enzymes

Other nonproteolytic AAA+ enzymes disassemble or remodel specific protein complexes. This class includes Katanin (which severs microtubules); Vps4 (which disassembles proteins polymerized on cellular membranes to promote vesicle trafficking or viral budding); the NtrC, PspF, and NifA enzymes (which remodel a closed complex of RNA polymerase, σ54, and promoter DNA to allow transcription initiation); and the clamp loaders (which assemble the PCNA and β-processivity "clamps" at DNA replication forks). Many of these enzymes share the hexameric structure and conserved protein-processing pore of the AAA+ ATPases described above, but typically they work on just one or a few client proteins. Importantly, some of these client-specific remodelers do not appear to act by translocation-mediated unfolding. For example, although the σ54-activating enzymes have a highly conserved pore motif, which is important for σ54 binding, this client is not extensively unfolded during ATP-dependent activation (163, 164). Furthermore, although the replication clamp loaders catalyze the opening of a subunit-subunit interface in the topologically closed clamp to allow it to load around a DNA duplex, this reaction does not require unfolding (165). Thus, protein remodeling by AAA+ enzymes can be achieved using a variety of mechanisms that harness the energy of ATP-binding and hydrolysis to perform work on a target protein molecule.

FUTURE DIRECTIONS AND CHALLENGES

Over the past decade, there has been an explosion of biochemical, structural, and functional information about AAA+ proteases and their substrates and adaptors. Nevertheless, many important questions remain unanswered, some concerning issues of detailed mechanism. For example, structures of AAA+ hexamers with degradation tags or translocating polypeptides bound in the axial pore are needed to help understand substrate recognition and the process of translocation at the molecular level. Similarly, the precise way in which subunits coordinate ATP binding/hydrolysis to drive the conformational changes of the AAA+ ring that perform mechanical work needs to be established. It is also important to determine if conformational transitions within compartmental proteases play roles in degradation or peptide-product egress. For many substrates, the rules that govern targeting to specific AAA+ proteases are poorly understand and need to be elucidated. Similarly, the biological roles and mechanisms of novel adaptors and antiadaptors await discovery. It is also unclear how degradation is coordinated with other parts of the protein's quality control network, whether subcellular localization of specific AAA+ proteases is functionally important, and if Lon and possibly other AAA+ proteases can function both as degradation and refolding machines. We anticipate exciting progress on many of these fronts in the near future.

SUMMARY POINTS

1. AAA+ family proteases contain a ring-shaped hexamer that uses the energy of multiple cycles of ATP binding and hydrolysis to unfold target proteins by translocating them through a central axial pore.

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2. The AAA+ hexamer translocates denatured substrates into the degradation chamber of a compartmental peptidase, whose proteolytic active sites are inaccessible to proteins not delivered in this fashion.
3. AAA+ proteases contain family-specific domains that are involved in binding substrates or adaptor proteins.
4. Substrate sequences (called degrons) facilitate recognition by binding to the pore of the AAA+ ring hexamer, by binding to auxiliary AAA+ domains or adaptor proteins that bind the protease, or by facilitating modification reactions that add a degron or reveal a previously hidden degron.
5. Substrates and adaptors often contain sequences that are only weakly bound by AAA+ proteases. Recognition of several weak degrons creates much stronger binding and allows regulation of degradation via assembly/disassembly of protein complexes.
6. The degradation activity of compartmental proteases can be controlled by the AAA+ hexamer by mechanisms involving substrate gating or allosteric remodeling of proteolytic active sites.
7. The unfolding/translocation power stroke can be driven by ATP hydrolysis in a single subunit of the AAA+ ring. The nucleotide-binding site is located between the large and small AAA+ domains, and ATP binding/hydrolysis can alter the rotation between these domains, causing rigid-body motions that propagate around the ring. These movements are transmitted to the substrate, at least in part, by conserved loops that project into the central pore.
8. Some AAA+ enzymes use translocation-mediated unfolding to solubilize aggregated proteins and disassemble macromolecular complexes.

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FUTRE ISSUES

1. What are the detailed molecular mechanisms of substrate recognition, translocation, and unfolding by AAA+ proteases?
2. How do the subunits of the AAA+ ring coordinate ATP binding/hydrolysis to drive the conformational changes needed to perform mechanical work?
3. Do conformational transitions within compartmental proteases play roles in degradation or peptide-product egress?
4. What rules govern targeting of orphan substrates to AAA+ proteases?
5. Are novel adaptors and antiadaptors involved in regulation of substrate degradation?
6. How is degradation coordinated with other parts of the protein quality control network?
7. Can some AAA+ proteases function either as degradation or refolding machines?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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