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Protein targeting to ATP-dependent proteases

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ATP-dependent proteases control diverse cellular processes by degrading specific regulatory proteins. Recent work has shown that protein substrates are specifically transferred to ATP-dependent proteases through different routes. These routes can function in parallel or independently. In all of these targeting mechanisms, it can be useful to separate two steps: substrate binding to the protease and initiation of degradation.

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To be active, newly synthesized protein chains must fold into three-dimensional structures, but regulated unfolding is also crucially important in some biological processes, such as protein degradation by ATP-dependent proteases and protein translocation across membranes [1]. Unfolding is required during degradation because the proteolytic sites of the ATP-dependent proteases are sequestered deep inside the proteases' structures and accessible only through narrow openings. Similarly, unfolding is required during several translocation processes because the protein import channels in some organelles are not wide enough for native proteins to fit through them. The mechanisms of unfolding in both types of processes are similar to each other but different from that of unfolding induced by heat or chemical denaturants. Here we discuss how the requirement for protein unfolding during degradation affects the way ATP-dependent proteases select their substrates.

ATP-dependent proteases

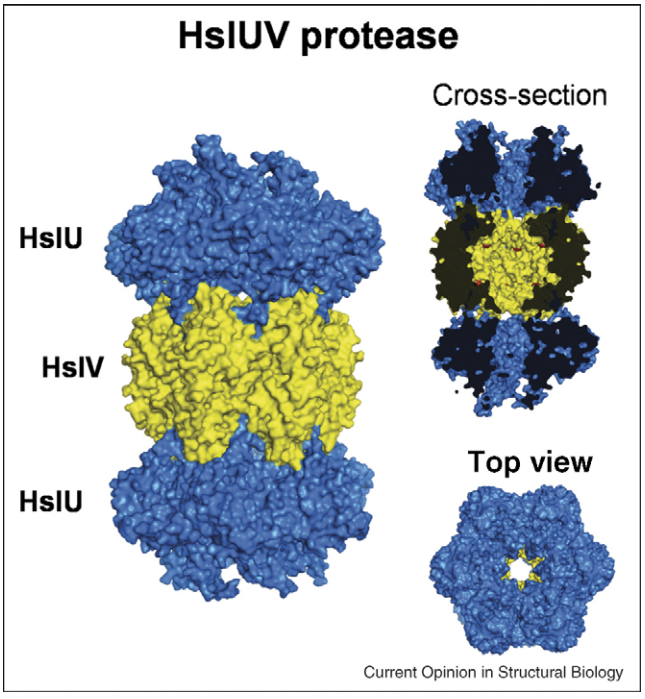
ATP-dependent proteases degrade short-lived regulatory proteins and thereby control cellular processes such as signal transduction, cell cycle, and gene transcription. The proteases also clear misfolded and aggregated proteins from the cell and produce some of the peptides to be displayed at cell surface as part of adaptive immune response. In eukaryotes, these functions are performed mainly by the proteasome. In prokaryotes and the organelles of eukaryotes, the functions are fulfilled by analogs of the proteasome, such as the ClpAP, ClpXP, HslUV, FtsH, and Lon proteases. Although ATP-dependent proteases show only relatively little sequence identity, they share a common architecture [2].

The ATP-dependent proteases all form large multisubunit particles (Figure 1). In the simplest case, FtsH protease, the particle consists six copies of a 71 kDa subunit forming a complex of approximately 425 kDa, and in the most complex case, the proteasome, the particle consists of some 40 different subunits forming a complex of 2 MDa molecular weight [3,4]. The subunits are mostly arranged in six or seven subunit rings that stack on top of each other to form cylindrical structures [2]. The proteolytic sites in all of these proteases are buried deep inside the particles and are accessible only through channels that are too narrow to allow folded proteins to pass through them [2,5]. This arrangement prevents the unintentional degradation of proteins. The ATPase subunits sit at the entrance to the proteolytic channels where they gate the channels and select and unfold substrates for degradation [2,5] (Figure 1).

Unfolding presumably occurs at the surface of the protease and the subsequent proteolysis proceeds sequentially along the substrate's polypeptide chain [6] (Figure 2). Unfolding during degradation can be much faster than spontaneous global unfolding, and the susceptibility of a protein to unfolding by the proteases is largely determined by the stability of its local structure first encountered by the protease and not the stability of the overall structure against global unfolding [6]. Proteins are more easily unraveled from surface α-helices and loops than from buried β-strands [6]. In the simplest model, the proteases catalyze unfolding by pulling at the polypeptide chain, perhaps simply as a consequence of the translocation of the polypeptide chain into the degradation channel [1]. Once the protein reaches the proteolytic sites, it is hydrolyzed into 3-30 amino acid long peptides [7,8] (Figure 2).

Besides their role in protein degradation, some ATP-dependent proteases are involved in nonproteolytic functions and most regulatory ATPase complexes show chaperone-like activity. Unfolding of a misfolded protein by ATP-dependent proteases can disrupt inappropriate

Figure 1



Structures of the bacterial ATP-dependent protease HslUV (PDB 1G3I). The protease subunits HlsV are shown in yellow, the ATPase subunits HslU are shown in blue. A side-on cross-section reveals the active site of proteolysis (red dots) in the catalytic chamber and the degradation channel that connects the active site to the exterior of the protease. End-on view shows the sixfold axis of symmetry. Structures were produced by PyMOL.

intermolecular interactions and thus assist proper protein folding if it is uncoupled from degradation. The proteasome also functions as a regulator of a variety of cellular processes including gene transcription, DNA repair, and chromatin remodeling [9]. The chaperone-like activity of the proteasome ATPase ring may also induce conformational changes in the targeted factors involved in such cellular processes.

Substrate targeting to proteases

The proteases' proteolytic sites show little intrinsic sequence preference [10] and instead substrate specificity is conferred by the regulatory complexes selecting the proteins to unfold and translocate to the degradation sites. There are three main pathways by which substrate proteins are targeted to the different proteases (Figure 3).

In eukaryotes, most substrate proteins are targeted to the proteasome by the covalent attachment of many copies of the small protein ubiquitin. Ubiquitination is carried out by a cascade of three enzymes, E1, E2, and E3, which act sequentially to attach the ubiquitin moieties to the acceptor protein. Typically, the C-terminus of ubiquitin forms an isopeptide bond with the ε-amino group of lysine residues in the substrate protein but in some rare cases ubiquitin may also be conjugated through the substrate's N-terminus or a cysteine side chain [11–13]. Yeast encodes a single E1, a few dozen E2s, and hundreds of E3 enzymes. The enzymes pass the ubiquitin from the E1 to one of the E2s and on to the substrates, which are recognized by an E3 enzyme. Once the first ubiquitin is attached to substrate, the E3 can continue to function and attach more and more ubiquitins to lysines in the first ubiquitin. However, in some cases, further extension of the polyubiquitin chain is mediated by an additional conjugating factor (E4), which binds to preformed ubiquitin chain and catalyze multiubiquitin chain assembly in conjunction with E1, E2, and E3 [14••]. The minimal proteasome targeting signal or degron consists of four ubiquitin moieties linked to each other by isopeptide bonds between carboxy termini and Lys48 [15••]. This polyubiquitin degron is recognized by the 19S regulatory particle of the proteasome through two surfaces formed by the ATPase subunits Rpn10 and Rpt5 [16,17].

Once attached, a polyubiquitin chain keeps being modified and can grow and shrink [18••]. The length of the polyubiquitin chains affects degradation [19••]. For example, the E3 anaphase-promoting complex (APC) coordinates the order of substrate degradation during the cell cycle and the timing by which substrates are degraded depends on the processivity of their ubiquitination by APC [19••]. Substrates that acquire long ubiquitination chains quickly are degraded earlier than substrates that are ubiquitinated slowly [19••].

During degradation, the polyubiquitin chain must be removed from the substrate because the proteasome cannot translocate more than two or three polypeptide chains through the degradation channel at the same time. Cells contain a large number deubiquitinating enzymes (DUBs) [20] and at least three of them, Rpn11, Uch37, and Ubp6, are located in the 19S regulatory particle and as such components of the proteasome [3,4,21,22••,23••,88••]. Rpn11 removes entire ubiquitin chains from the substrate by cleaving the isopeptide bond between the substrate and the first ubiquitin to recycle ubiquitin and to allow substrate's degradation [22••,23••]. Ubp6 trims the chain from the free end and may serve as a timer [24••]: when the ubiquitinated substrate binds to the proteasome, the proteasome will try to engage its substrate while Ubp6 shortens the ubiquitin chains from their distal end. If the ubiquitin chain has been removed before the proteasome has begun to degrade the protein, it escapes until it is ubiquitinated again and rebinds the proteasome.

The length of the ubiquitin chain appears to be regulated further and it was found recently that the E3 ligase Hul5 associates with the DUB Ubp6 on the 19S regulatory particle [4,25••]. The ubiquitin ligase activity of Hul5