Pup grows up: *in vitro* characterization of the degradation of pupylated proteins

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By reconstituting the recently discovered prokaryotic ubiquitin-like protein (Pup)–proteasome degradation system *in vitro*, Weber-Ban and colleagues (Striebel *et al*, 2010) elucidate its mechanism and describe a surprising variation on the established principles of protease targeting. Nevertheless, their findings suggest that the bacterial and eukaryotic systems follow the same overall principles even if the details differ.

ATP-dependent proteases control the concentrations of hundreds of proteins, but they do so specifically and recognize each protein individually. These proteases show relatively little sequence conservation, but they all adopt a similar solution: their proteolytic sites are encapsulated in a degradation chamber that folded proteins cannot fit into. Proteins to be degraded are unfolded by a ring of ATP-dependent motor proteins that thread them into the degradation chamber (Baker and Sauer, 2006; Finley, 2009) (Figure 1).

The degradation signal or degron typically has two parts. The first part is a recognition element that will bind to the ATP-dependent protease. The second part is the site where degradation actually initiates. The initiation region is an unstructured sequence of amino acids that the protease can grab and pull at to unfold the substrate (Schrader *et al*, 2009).

In bacteria, substrate targeting is typically carried out through short-peptide sequences that are either part of the primary sequence of the target or are appended to mistranslated proteins stalled on the ribosome. These targeting sequences are recognized directly or through adaptors by the proteases and degradation then initiates directly at the targeting sequences (Baker and Sauer, 2006).

In eukaryotes, the targeting system is somewhat more complex. Mostly, proteins are marked for degradation by a cascade of enzymes that attaches many copies of the small protein ubiquitin to lysine side chains in the substrate. These poly-ubiquitin chains are recognized by the proteasome directly or via adaptor proteins (Finley, 2009). Next, the proteasome must engage its substrate at an unstructured region in the protein, from where the proteasome unravels the substrate and translocates it into the degradation chamber (Prakash *et al*, 2004).

Recently, an entirely new targeting system has been discovered in bacteria (Pearce *et al*, 2008), which Weber-Ban and colleagues have now elegantly dissected by reconstituting it *in vitro* (Striebel *et al*, 2010). Their fascinating findings show that the system combines aspects of both the bacterial and eukaryotic mechanisms.

Actinobacteria have acquired an encapsulated protease core particle that resembles that of the eukaryotic proteasome, apparently by horizontal gene transfer (Lupas *et al*, 1994). The core particle works together with a ring of ATPase subunits called Mpa, forming a simple cylindrical structure much like other bacterial AAA-proteases (Wang *et al*, 2009). In stark contrast, however, substrates are targeted to this protease through covalent attachment of a small protein called Pup to the side chain of lysine residues, which is

Figure 1 Comparison of different classes of ATP-dependent proteases, shown as a side-on cross-section. (A) The eukaryotic 26S proteasome, composed of a 20S core particle (blue α and β subunits) flanked by 19S regulatory subunits (magenta and orange). The 19S subunits bind to the substrate through the covalent ubiquitin modification (yellow) and unfold it by pulling on the unstructured initiation site (bright green). Ubiquitin is removed to be recycled during the degradation process. (B) The bacterial protease ClpXP, composed of rings of the protease ClpP (dark green) and the ATPase motor ClpX (red). ClpX binds to the degradation signal, in this case the *ssrA* peptide sequence (green), which also serves as the site for the initiation of degradation. (C) The actinobacterial proteasome, consisting of a 20S core particle similar to that of the 26S proteasome, and a single ring of the ATPase Mpa (purple). Mpa binds to the substrate through the covalent Pup modification (light green). Pup has an N-terminal unstructured region, which serves as the site for the initiation of degradation, leading to complete degradation of Pup.

reminiscent of the ubiquitin tags used in eukaryotes (Pearce *et al*, 2008).

The paper by Weber-Ban and colleagues shows that the Pup chain is recognized by the Mpa ATPase. Attachment of Pup to a substrate enables binding to the Mpa ATPase and leads to degradation of both Pup and the attached proteins. Interestingly, a gate-opened mutant of the proteasome is required for degradation, suggesting that additional factors may be involved *in vivo*. The N terminus of Pup is unstructured (Liao *et al*, 2009; Sutter *et al*, 2009), but is strictly required for degradation. The amino-acid sequence of the N terminus is not recognized uniquely, because test proteins are proteolysed just as well when the Pup tail is replaced with several unrelated sequences. Presumably, the tails serve as the initiation site for the actinobacterial proteasome.

The targeting mechanism of the Pup proteasome system (PPS) then combines aspects of the eukaryotic UPS and of the previously characterized bacterial systems (Figure 1). Like in the UPS, the protease recognition element is covalently

attached to target proteins post-translationally, providing the opportunity for regulated protein degradation. However, in the PPS, the Pup tag also provides the unstructured region that serves as the proteasome initiation site, just like the linear degrons recognized by bacterial ATP-dependent proteases. Thus, Pup contains the two required elements of degrons in one attachable label. Although the details differ, the bacterial and eukaryotic systems follow the same overall principles.

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Conflict of interest

The authors declare that they have no conflict of interest.

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