egg extracts: role of EB1 in microtubule stabilization and mechanisms of targeting to microtubules. *Mol. Biol. Cell* 13, 3614–3626
21 Kosco, K.A. *et al.* (2001) Control of microtubule dynamics by Stu2p is essential for spindle orientation and metaphase chromosome alignment in yeast. *Mol. Biol. Cell* 12, 2870–2880
22 Severin, F. *et al.* (2001) Stu2 promotes mitotic spindle elongation in anaphase. *J. Cell Biol.* 153, 435–442

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Proteasome degradation: enter the substrate

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Cells depend upon the regulated destruction of their various proteins to maintain homeostasis and change their metabolic state. A key component of this process is the proteasome – a large multisubunit protease whose catalytic sites are sequestered within a central chamber. Entry of substrates into proteasomes is regulated by activators and is generally thought to proceed sequentially, starting from one end of the substrate polypeptide. This conventional view is expanded by a recent paper, which indicates that some unfolded substrates can open the entrance to the proteolytic chamber in the absence of an activator and can enter the proteasome in a hairpin conformation to allow limited proteolysis of internal segments.

As with all chemical reagents, the activity of a protein depends upon its concentration. Cellular metabolism is therefore regulated through changes in concentration of specific proteins. This regulation occurs at many levels, including control of protein synthesis, especially at the level of transcription, and posttranslational modification, such as phosphorylation, which alters the concentration of active forms of the protein. Another widespread and effective form of posttranslational modification is proteolytic degradation, which can rapidly and irreversibly inactivate a protein by destroying it.

Proteins inside cells are typically degraded by their targeting to the membrane-enclosed lysosome or to a soluble proteolytic complex called the proteasome [1–3], which is abundant in the cytosol and nuclear compartments. Proteasomal substrates include damaged or denatured proteins and excess subunits of multisubunit complexes that need to be removed as part of basal housekeeping functions. In addition, numerous regulatory proteins, whose removal signals a change in cellular metabolism, are substrates of proteasomes. These include activators and inhibitors of the cyclin-dependent kinases that drive the eukaryotic cell cycle, transcription factors and their domains, and inhibitory proteins. The function of proteasomes is not limited to removal of their substrates. They are also essential for the processing of certain transcription factors, such as mammalian NF-κB and yeast Spt23p and Mga2p, and for the production of peptides that are displayed on the cell surface in complex with major histocompatibility complex (MHC) class I molecules.

Proteasome architecture and regulation

Given the high natural abundance of proteasomes and the ability of their catalytic sites to hydrolyze almost any sequence, it is essential that inappropriate substrates be protected from indiscriminate proteolysis. This is achieved by the architecture of the 20S proteasome, also known as the core particle, which sequesters the active sites of the proteasome inside an isolated compartment of its hollow, cylindrical structure [4] (Figure 1). To enter the 20S proteasome, substrates must pass a constriction in the outer rings of the α-subunits, known as the α-annulus, that excludes folded proteins [5,6]. Furthermore, the N-terminal residues of the α-subunits form a gate to the α-annulus by adopting precisely ordered conformations that seal the entrance [7,8]. Thus, by default, the isolated 20S proteasome is latent and spares illegitimate substrates from untimely demise.

Legitimate substrates are delivered to the interior of the 20S proteasome by an ATP-dependent activator known as PA700, also called 19S or RP, which binds to one or both rings of the α-subunits of the 20S proteasome to form a complex known as the 26S proteasome [3]. Substrates are recognized by PA700 through their posttranslational ligation to ubiquitin; substrate selection is enforced by extensive biochemical machinery that specifically attaches a polyubiquitin chain to target proteins [9–11]. PA700 binds the polyubiquitin chain, cleaves the bond connecting polyubiquitin and substrate, unfolds the substrate, opens the gate to the 20S proteasome and translocates the substrate into the catalytic chamber. A recent study by Liu *et al.* [12], however, indicates that *in vitro*, at least some substrates can enter the 20S proteasome without the assistance of an activator such as PA700 and, furthermore, can do so in a hairpin conformation rather than starting from a free polypeptide terminus.

Unfolded proteins can be degraded from either terminus

Liu *et al.* [12] addressed the question of how substrates enter the degradation chamber of the 20S proteasome by assaying the 20S and 26S proteasome-mediated degradation of the natively unfolded proteins $p21^{cip1}$ (p21) and α-synuclein, and various fusion constructs of these

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