Where to start and when to stop

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The activity of a handful of transcription factors, such as mammalian NF-κB, *Drosophila melanogaster* Cubitus interruptus and yeast Spt23 and Mga2, are regulated through partial protein degradation by the proteasome. New data now show that the proteasome activates membrane-bound Spt23 and Mga2 by initiating their proteolysis at an internal site and then degrading the proteins bidirectionally toward both ends of the polypeptide chain, modifying our ideas on how the proteasome degrades targeted substrates.

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The ubiquitin-proteasome system controls the concentrations of hundreds of proteins and influences almost every aspect of cellular regulation, including cell cycle, cell proliferation, gene expression and signal transduction[1]. The number of proteins known to be modified with ubiquitin is growing rapidly[2]. With so many proteins involved in such a wide range of processes regulated by the proteasome, it is not surprising that failure in this system contributes to the pathogenesis of many diseases, including cancer and neurological disorders[1]. The proteasome is a barrel-shaped particle with its proteolytic active sites buried deep inside its structure, only accessible through a narrow channel that is too small to allow passage of folded proteins[1]. The proteasome recognizes most of its substrates by a ubiquitin modification on the substrate, then engages them at an initiation site and unfolds and degrades them by sequentially running along their polypeptide chain in either direction, from N to C terminus or from C to N terminus[3]. The proteasome is highly processive and degrades substrates completely into small peptides of about 8 amino acid residues in length[1]. This complete degradation of proteins avoids creating protein fragments with undesirable activities, such as a catalytic domain without the control of a regulatory domain.

Besides this classical role in complete protein degradation, the proteasome is involved in less widely appreciated functions, particularly in the regulation of gene expression. For example, transcription can be stimulated by a nonproteolytic function of the proteasome, possibly in the remodeling of transcription factor complexes and chromatin[4]. In other cases, the function of transcriptional activators can be increased by the destruction of these activators[5]. The proteasome can also control the activities of a few transcription factors by degrading them only partially. The best-established examples are mammalian NF-κB[6], *Drosophila* Cubitus interruptus (Ci)[7] and its vertebrate homologs Gli2 and Gli3 (ref. 8), as well as the homologous yeast proteins Spt23 and Mga2 (ref. 9) (**Fig. 1**). In these cases, the partial protein digestion by the proteasome results in smaller protein fragments with new biological functions. For NF-κB, one of the subunits is synthesized as a larger precursor protein, p105, which remains inactive in the cytosol because of a masked nuclear localization signal next to the N-terminal DNA-binding domain. p105 also inhibits transcription by trapping other subunits of the NF-κB family in the cytosol. Stress or inflammatory signals induce the ubiquitination of p105, and the proteasome then degrades the C-terminal part of the protein to generate the N-terminal protein fragment p50. This processing unmasks the nuclear localization signal, allowing movement of p50 into the nucleus, where it regulates gene transcription with other members of its protein family. The yeast transcription factors Spt23 and Mga2 help regulate membrane fluidity by controlling levels of unsaturated fatty acids, which is essential to preserve the integrity of cell membranes. The Spt23 and Mga2 C termini are anchored in the endoplasmic reticulum (ER) membrane and thus remain inactive. After ubiquitination, the C-terminal part of the protein, including the transmembrane anchor, is degraded completely by the proteasome[9]. The N-terminal region is spared and released into the cytosol as p90, and it is free to migrate into the nucleus to drive gene expression. Partial degradation of a transcription factor can also shut off signaling pathways. In the cases of Ci and the Gli proteins, the proteasome transforms a full-length transcriptional activator into a fragment that acts as a competitive repressor of the full-length form in response to changes in Hedgehog signaling during development[7,8]. Thus, the processing reaction allows the direct switch of a signaling pathway from an activated state to a repressed state, increasing the dynamic range that can be achieved through one signaling molecule.

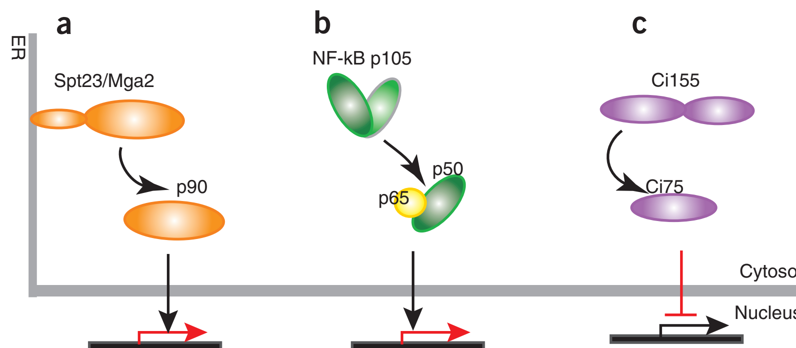

Figure 1 Examples of proteasomal processing in the cell. (**a**–**c**) The proteasome degrades transcription factors Spt23 and Mga2 (**a**), NF-κB subunit p105 (**b**) and Ci (**c**) only partially to generate biologically functional protein fragments. Processing can activate a transcription factor (**a**), transform a molecule from an inhibitor to an activator of transcription (**b**) or convert an activator into a competitive repressor (**c**).

But how is only partial degradation achieved? In the cases of Spt23 and Mga2, the initial, and maybe biggest, question was how degradation would begin in the first place. In the simplest view of proteasome degradation, proteolysis starts at either of the free termini of the substrate's polypeptide chain. There are three main reasons for this view: first, it is the simplest mechanism; second, the degradation channel through which the substrate has to travel is narrow, so that although two strands

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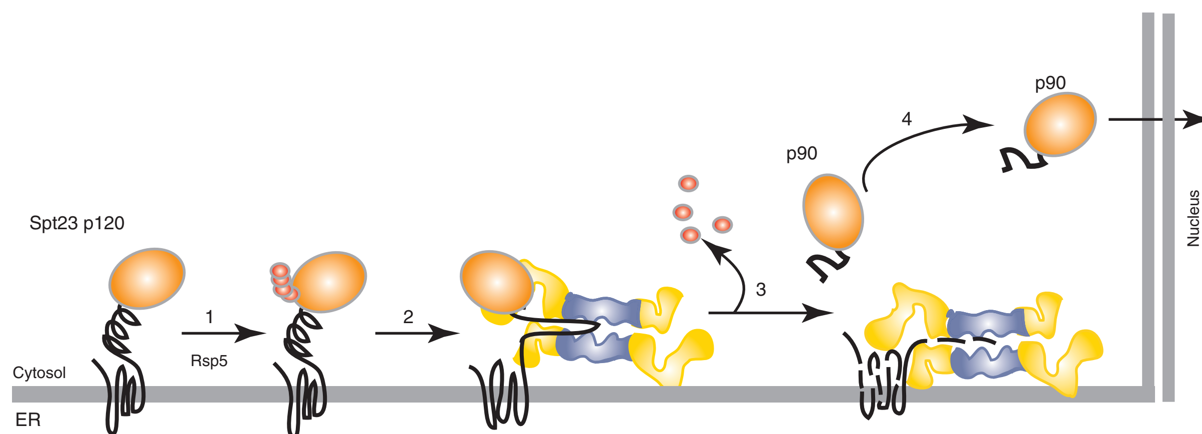

Figure 2 Model for processing of the membrane-bound yeast transcription factor Spt23[14]. Inactive precursor Spt23 is anchored in the ER membrane and becomes ubiquitinated by the Rsp5 ubiquitin ligase (1). The ubiquitination sites are not known. After ubiquitination, the proteasome initiates degradation at an internal site and proteolyses the polypeptide chains toward both the N and C termini (2). The C-terminal part of Spt23 is degraded completely, but the N-terminal part of the protein is protected by an IPT domain, shown in orange (3). The N-terminal fragment is released as p90 and translocates into the nucleus to activate transcription of the *OLE1* gene (4).

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of a polypeptide chain could fit through it at the same time[10], this seems unfavorable; and third, prokaryotic ATP-dependent proteases target most proteins via N- or C-terminal targeting sequences and degrade them from these same sequences, suggesting that the eukaryotic proteasome may function in a similar way. However, this precise mechanism is not possible for Spt23 and Mga2, because the N-terminal half of the proteins survives degradation intact and the C terminus is anchored in the membrane. To resolve this problem, the Jentsch group proposed the loop model of degradation, in which the proteasome engages the substrate by feeding an internal loop into its degradation channel[11]. Two pieces of evidence suggested that this mode of degradation might be feasible. First, a protein containing one or two disulfide bridges can be degraded without reduction of the cross-links, which indicates that the several peptide strands can reach the proteolytic sites simultaneously[12]. Second, experiments with model proteins showed that the proteasome can degrade a circular peptide[13] or be forced to initiate degradation between two folded domains[3,13]. However, a limitation to these studies was that both were done under fairly artificial conditions.

On page 691 of this issue, an elegant and exceptionally clear-cut study by Piwko and Jenstch[14] demonstrates convincingly that the proteasome can and does initiate the degradation of yeast Spt23 and Mga2 at an internal site under physiological conditions (**Fig. 2**). This discovery provides a crucial insight into the mechanism of proteasome action. The authors used two experimental tricks to establish that degradation initiates internally. They first blocked the C terminus of Mga2 by fusing a dihydrofolate reductase (DHFR) domain to it. DHFR can be stabilized against unfolding by the substrate analog methotrexate, which protects it from proteasomal degradation[15]. Thus, their observation that the Mga2-DHFR fusion protein is processed by the proteasome demonstrates that degradation must begin internally, with an endoproteolytic cut. To confirm the mechanism, the authors monitored the fate of both N-terminal and C-terminal fragments of Spt23 that are generated after the internal cut. Under most experimental conditions, C-terminal degradation intermediates are not observed, presumably because they are degraded too rapidly. The authors were able to trap these intermediates using the second trick, which takes advantage of yeast strains in which the function of the proteasome is mildly impaired owing to mutations in either a proteolytic or an ATPase subunit. Both mutations slow degradation rates without abolishing proteasome function and lead to the accumulation of N-terminal and C-terminal fragments of Spt23. This result suggests that, after the initial cut, proteolysis continues bidirectionally toward both the N and C termini of the protein. Undoubtedly, the finding that the proteasome can initiate degradation internally will not be limited to Spt23 and Mga2. For example, it has been suggested that processing of p105 to p50 occurs by an endoproteolytic cut of a nascent polypeptide chain[16], and the observations of Piwko and Jentsch could provide a mechanism for this model. Countless other examples will probably be found.

This leaves the question of why the proteasome does not degrade both halves of the protein completely but instead spares the N-terminal parts of Spt23 and Mga2. The authors found that the Ig-like/plexins/transcription factors (IPT) domain in the N-terminal part of Spt23 is important in this decision, as deletion of the IPT domain prevents accumulation of the p90 fragment (**Fig. 2**). The importance of folded domains in the mechanism of partial degradation has been described for the processing of the NF-κB precursor p105 and the transcription factor Ci. Destabilization of the IPT domain in p50 (ref. 17) and a zinc finger in Ci[18] abolishes fragment formation, and replacing these domains with DHFR restores it[17,18]. Processing can also be caused by an ornithine decarboxylase domain[19,20]. These domains are not related to each other, suggesting that it is the unfolding behavior and not another property of these proteins that is responsible for the processing. For p50 and Ci, a second sequence is necessary to allow processing. In p50 this element is a stretch of about 30 glycine residues[18,21,22] and in Ci it is a sequence of 20 residues rich in asparagine and glutamine[18]. These simple sequence stretches may allow the proteasome, once its progress has been stalled by a folded domain, to dissociate from its substrate, in effect weakening the unfolding activity of the proteasome[18,20]. Both Spt23 and Mga2 contain short simple sequences rich in asparagine, aspartate and glutamine residues adjacent to their IPT domains, but it is not known whether these simple sequences are required for processing. Alternatively, either the Spt23 and Mga2 IPT

domains might be too difficult to unfold or the yeast proteasome might have a very weak unfolding activity. Of course, it is also possible that the processing of Spt23 and Mga2 in yeast occurs by a different mechanism than processing in flies and vertebrates.

It will be interesting to further investigate the differences in processing determinants used by different biological systems. In addition, the number of known examples of proteasomal processing is small but growing. Whether this type of processing is a regulatory mechanism restricted to a handful of transcription factors or is instead a more general cellular function of the proteasome will be an important question for future work.

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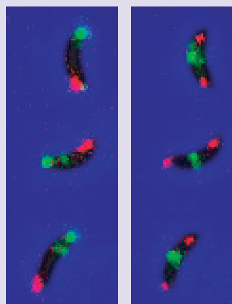
A tale of two halves

The ability of cells to position the site of the division plane so that two daughter cells with full genomes are faithfully generated from generation to generation is an intriguing aspect of cell biology. In bacteria, the site where the membrane pinches in during cell division (the midzone) is defined by the location of a ring-like structure formed by a tubulin-like protein, FtsZ. FtsZ localization was thought to be directed by two mechanisms. The first uses the membrane-tethered MinCD complex to inhibit FtsZ polymerization at the poles, thereby directing FtsZ filaments to the midzone. In the second, FtsZ assembly is prevented near the bacterial nucleoid by nonspecific chromosome-binding factors. As the nucleoid resides at the midcell until replication forces the two chromosomes to segregate to opposite poles, this second mechanism prevents FtsZ assembly from occurring before segregation but leaves the midzone free of inhibitor after segregation. However, neither of the two systems seems to operate in *Caulobacter crescentus*.



In a recent study, Martin Thanbichler and Lucy Shapiro have defined a new mechanism by which the FtsZ ring is properly positioned (*Cell* **126**, 147–162, 2006). In a screen for cell cycle-regulated genes, they isolated an essential gene encoding an ATPase of unknown function, termed MipZ. When MipZ function was repressed, cells became elongated, with the division site occurring unequally. When MipZ was overexpressed, cells again became elongated but there was little cell division, and the division that did occur was focused at the extreme ends of the cell.

Caulobacter exists in two phases: a mobile swarmer cell with a polar flagellum and an immobile stalk cell with a stalk replacing the flagellum. When a new flagellum forms opposite the stalk, the cell divides asymmetrically to yield swarmer and stalk cells. The authors observed that MipZ localizes to the flagellar pole in swarmer cells and to both poles in stalk cells before cell division, after which MipZ is found at the stalk pole. This localization pattern is reminiscent of that of the replication origin, and indeed, MipZ colocalizes with the origin, although the signals do not entirely overlap. The slight discontinuity in signals suggested that MipZ might actually associate with a cluster of sites (*parS*) for ParB, a DNA-partitioning protein that is located several kilobases from the origin. This was confirmed by colocalization and reconstitution of the ParB-MipZ interaction in *Escherichia coli*. It is important to note that although MipZ forms a focus at *parS*, mediated by its interaction with ParB, it forms a gradient toward the midcell.

When MipZ's ATPase motif is mutated, the protein becomes evenly distributed through the cell rather than focused at the origin, and the cells have a filamentous appearance similar to what occurs with MipZ overexpression. FtsZ localizes where MipZ is not present (that is, at the pole opposite the flagellum in swarmer cells and in the midcell during S phase). Therefore, to test whether MipZ has a direct effect on FtsZ assembly, MipZ expression was induced after formation of the FtsZ ring. Immediately, the ring dissolved and cell division was inhibited. By contrast, when MipZ was depleted, FtsZ formed a ring but also other foci, and the division plane was displaced. These results show that MipZ is necessary for the formation of one correctly positioned FtsZ ring.

At the start of S phase, ParB is located on an origin near the stalked pole, and FtsZ is at the opposite pole (left panel; ParB is red and FtsZ is green). MipZ is produced, and the origin, with ParB and MipZ, moves toward the opposite pole. When this happens, FtsZ is rapidly displaced from the pole and appears at the midcell, where polymerization can occur (right panel). The data suggest that FtsZ always localizes to the area containing the lowest concentration of MipZ (which maximizes its distance from ParB and the origin).

But is there a direct effect of MipZ on FtsZ assembly? The addition of GTP to FtsZ promotes its assembly *in vitro*. When MipZ is included, less FtsZ polymer is formed, the polymers are shorter and curved, and they are associated with MipZ. This result resembles what is seen when depolymerizing tubulin is bound by GDP at the ends of microtubules; in agreement with this analogy, MipZ was found to increase the GTPase rate of FtsZ, thereby affecting its assembly.

This study reveals another way in which chromosome movement can be coupled to cell division. MipZ can both interact with ParB, similarly to ParA DNA-partitioning proteins, and inhibit FtsZ ring assembly, similarly to MinC (although by a different mechanism).

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