To Live or Die by the Sword: The Regulation of Apoptosis by the Proteasome

The proteasome has been implicated in the control of apoptosis by modulating the levels of both pro- and antiapoptotic molecules. A recent study published in the April 9th issue of *Molecular Cell* reveals that caspase-dependent inactivation of the proteasome can amplify the activation of apoptosis.

The ubiquitin-proteasome pathway is the primary route for degrading ubiquitin-tagged proteins and some non-ubiquitinated proteins (Glickman and Ciechanover, 2002). In this pathway, the sequential action of E1, the ubiquitin-activating enzyme, E2, the ubiquitin conjugating enzyme (UBC), and E3, the ubiquitin-protein ligase, serves to attach the ubiquitin moiety (most often multiubiquitin chains) to acceptor lysine residues of protein substrates, which subsequently are targeted for degradation by the 26S proteasome. The 26S proteasome is composed of a barrel-shaped 20S core particle (CP) and two 19S regulatory particles (RP), each of which binds to opposite ends of the core particle (Glickman and Ciechanover, 2002). The 19S RP, which possesses ATPases to fuel its functions, recognizes and binds a polyubiquitinated substrate, removes its polyubiquitin chains, and then denatures and threads the substrate into the 20S proteolytic core where it is cleaved into small peptides. The ability to degrade and regulate the levels of intracellular proteins has made the proteasome an important player in regulating diverse biological processes including cell cycle progression, cell signaling, and apoptosis.

Recent studies have shown that the ubiquitin-proteasome pathway mediates the degradation of key apoptotic regulators (Jesenberger and Jentsch, 2002). Some of them are proapoptotic, like p53, caspases, and Smac/Diablo, and some are antiapoptotic, such as Mcl-1, XIAP, cIAP1, cIAP2, and DIAP1 (Bergmann et al., 2003; Nijhawan et al., 2003; Jesenberger and Jentsch, 2002). The delicate balance between these pro- and antiapoptotic factors likely determines the life versus death fate of a cell. In cells that need to live, the ubiquitin-proteasome system is employed to safeguard the inadvertent activation of proapoptotic proteins. For example, the ubiquitin-dependent degradation of p53, a tumor suppressor and apoptosis activator, is regulated by an E3 ligase, Mdm2, which targets both itself and p53 to the proteasome for degradation, preventing accumulation of the p53 protein which could inadvertently lead to apoptosis (Fang et al., 2000). Studies on the inhibitor of apoptosis (IAP) protein family indicate that IAP family proteins appear to suppress apoptosis through two distinct mechanisms. On one hand, IAP family proteins use their caspase binding BIR domains to inhibit the processing of pro-caspases or the activity of activated caspases. On the other hand, IAP family proteins can use their Ring finger domains, which contain an E3 ligase activity, to promote polyubiquitination of their substrates and themselves (Bergmann et al., 2003; Suzuki et al., 2001; Yang et al., 2000). Most of their substrates are proapoptotic proteins, such as caspases or the caspase activator, Smac/Diablo, which is released from the mitochondria upon the induction of a death signal to antagonize IAP inhibition of caspases (Du et al., 2000; Verhagen et al., 2000). Thus IAP family proteins could protect against cell death partly through degrading caspases that may be spontaneously activated or Smac that is incidentally released from mitochondria.

For cells that are induced to die due to genotoxic insults or other apoptotic stimuli, the proteasome pathway participates in the initiation of apoptosis through rapidly depleting the antiapoptotic regulators. Studies from Xiaodong Wang's group demonstrated that proteasome-mediated degradation of Mcl-1, an antiapoptotic member of the Bcl-2 family, and simultaneous inhibition of Mcl-1 protein synthesis triggered by genotoxic stresses are required for the translocation of Bax and Bcl-x_L to the mitochondria and subsequent cytochrome c release and caspase activation (Nijhawan et al., 2003). In the fruit fly, the Grim, Reaper, or Hid proteins, the *Drosophila* functional homologs of Smac/Diablo, induce apoptosis by binding to DIAP1, the *Drosophila* IAP family member, and enhancing the autoubiquitination of DIAP1, which not only releases the binding of DIAP1 to fly caspases such as Dronc or DrICE, permitting their accumulation and activation, but also promotes the rapid degradation of DIAP1, further facilitating the activation of apoptosis (Bergmann et al., 2003).

All studies conducted to date have focused on the ability of the proteasome to degrade regulatory apoptotic molecules. However, Gerald Cohen and his colleagues recently found that the proteasome itself is subjected to negative regulation during apoptosis (Sun et al., 2004). They demonstrate that the induction of apoptosis by various stimuli in multiple cell types results in the accumulation of ubiquitinated proteins at an early stage of apoptosis, which correlates with a decreased ability of apoptotic cells to degrade short-lived proteins through the proteasome pathway. The compromised ability of proteasome in apoptotic cells to degrade short-lived proteins was not due to a defect in forming de novo ubiquitinated conjugates in vitro and was observed for both the ubiquitin-dependent substrates like Smac and a well-studied ubiquitin-independent substrate, ornithine decarboxylase.

Interestingly, the decreased activity of the proteasome in apoptotic cells coincides with the caspase-dependent cleavage of three subunits of the 19S RP, S1, S5a, and S6′. Earlier work on the 19S RP suggests that the S6′ and S5a subunits recognize the polyubiquitinated substrates while S1 in conjunction with S5a and S2 hold together the lid and base of the 19S RP (Glickman and Ciechanover, 2002). The authors suggest that cleavage of S6′, S1, and S5a by caspases may result in an inability of the proteasome to recognize and degrade polyubiquitinated substrates as well as a partial dissociation of the 19S RP from the 20S core particle. As a result, the ubiquitin-dependent degradation of proapoptotic molecules such as Smac/Diablo and Omi/HtrA2 is inhibited, leading to the accumulation of these proteins and a feed-forward amplification of the apoptotic signal.

The work by Cohen and colleagues reveals an additional level of complexity to the already complicated relationship between the proteasome and apoptosis. The proteasome appears to play an important role in suppressing inadvertent activation of proapoptotic proteins in cells that need to live and participates in eliminating antiapoptotic proteins in response to apoptotic stimuli. After the initiation of apoptosis and the activation of caspases, the proteasome is then disabled to allow the build-up of proapoptotic proteins, which tilts the balance of life and death further toward the point of no return.

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Selected Reading

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