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News and Commentary

IAPs – the ubiquitin connection

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One way or another, almost all proteins containing baculoviral IAP repeat domains (BIRs) have been associated with ubiquitin (the only exception being NAIP). The RING-bearing IAPs, XIAP, ML-IAP, cIAP1, cIAP2, DIAP1, and DIAP2 act as ubiquitin E3 ligases. The giant BIR containing protein BRUCE bears a ubiquitin conjugation (UBC) E2 domain, and Survivin is degraded by ubiquitin-targeted proteolysis at the end of mitosis. Current studies of BIR containing proteins illustrate the fundamental importance of the ubiquitin system in the regulation of protein function and abundance during cell death and cell division.

The finding that the zinc-binding RING domain of proteins such as *c-cbl* allows them to function as ubiquitin ligases[1] prompted other groups to see if the same would be true for RING-bearing IAPs, namely mammalian XIAP, cIAP1, cIAP2, ML-IAP/livin/KIAP and hILP2, and *Drosophila* IAPs DIAP1 and DIAP2. Using *in vitro* assays, Yang *et al*.[2] found that cIAP1 and XIAP could auto-ubiquitylate, and for this they required an intact RING domain. Focusing on cIAP2, Huang *et al*.[3] found that both full-length cIAP2, or its RING alone, could act as an E3 ligase *in vitro* to promote ubiquitylation of itself, as well as mono-ubiquitylation of caspases 3 and 7. Subsequently, all other proteins that bind to IAPs, such as caspases, IAP antagonists, and TRAFs have been tested to see if they could be ubiquitylated by IAPs (Figure 1a).

Other caspases were tested to see if they could also be substrates of IAP-mediated ubiquitylation. The *Drosophila* IAP DIAP1 was found to mediate ubiquitylation of the caspase 9-like protease Dronc[4,5] and, more recently, XIAP has been reported to ubiquitylate caspase 9 itself.[6]

IAPs can be regulated by proapoptotic proteins, such as insect Reaper (Rpr), Grim, and HID, and mammalian Smac/DIABLO and HtrA2/Omi, that bind to the IAPs' BIR domains via amino-terminal IAP binding motifs (IBMs).[7,8] XIAP was found to mediate ubiquitylation of Smac/DIABLO *in vitro,*[9] and DIAP1 was reported to cause ubiquitylation of the *Drosophila* IAP antagonists Grim, HID, and Rpr.[10]

cIAP1 and cIAP2 were initially identified as proteins that bound to TRAF1 and TRAF2 in a complex associated with the cytoplasmic domain of TNF Receptor 2.[11,12] Wu *et al*. reported that TNF signalling promotes formation of a complex of the E2 ligase Ubc6 together with TRAF2 and cIAP1 in the endoplasmic reticulum. In this complex cIAP1 caused ubquitylation of TRAF2, targeting it for degradation.[13] RIP also participates in TNFR signalling, and is required for activation of NFκB.[14] Park *et al*. found that cIAP1 and cIAP2 (but not TRAF1 or TRAF2) could poly-ubiquitinate RIP, to target it for destruction.[15]

While these experiments have demonstrated that IAPs can ubiquitylate many other interacting proteins, many of the assays were conducted using *in vitro* systems, so it will be important to confirm them in intact cells and *in vivo*. From experiments studying IAPs *in vivo*, in both *Drosophila* and mice, it appears that one of the most important targets of IAP-mediated ubiquitylation are IAPs themselves. For example, levels of cIAP2 are raised in cells from cIAP1 gene deleted mice, and this is not due to increase in transcription of the cIAP2 gene, but appears to be due to increased stability of cIAP2 protein. Consistent with this observation, transfection studies showed that cIAP1 can directly regulate abundance of cIAP2 by ubiquitylating it, and targeting it for degradation.[16]

Two important sites on which XIAP can be ubiquitylated are on BIR3, namely lysine 322 and lysine 328.[17] Mutation of these sites significantly reduced ubiquitylation of XIAP, but did not reduce its antiapoptotic activity when overexpressed. This could indicate that ubiquitylation of XIAP is not an important factor determining its abundance or activity, but it is also possible be that overexpression of XIAP by powerful promoters in 293HEK cells is able to swamp the system that normally controls its abundance.

Deletion of the *Drosophila* IAP DIAP1 results in massive apoptosis and embryonic lethality.[18] DIAP1 promotes the ubiquitination of itself as well as the caspase Dronc.[4,19] Furthermore, following its cleavage by downstream caspases Dcp-1 or Drice, DIAP1 itself is ubiquitylated and degraded by the 'N-end rule' pathway.[20,21] It is possible that this pathway, or a variant of it, is also responsible for degrading proteins with IBMs.[22]

The N-end rule, which was initially deciphered in yeast,[23] but also operates in higher eukaryotes, describes how the half-life of proteins can be determined by their amino-terminal residues.[24] A specific E3 ligase, Ubr1p, recognizes proteins with certain amino-terminal sequences, and targets them for ubiquitylation and destruction. For example, yeast proteins with an amino-terminal phenylalanine are bound and ubiquitylated by Ubr1p and consequently have a half-life of only 3 min, whereas proteins with an amino-terminal valine that are not recognized by Ubr1p have a half-life of more than 20 h.[25] Since the IBM is a neo-amino terminus that can be generated by amino-terminal methionine peptidase[26] acting on Grim, Rpr or HID, by mitochondrial import proteases acting on the precursors for mitochondrial proteins such as Smac/DIABLO,[27] or by caspases, acting on substrates including procaspases, the RING-mediated ubiquitylation and degradation of IBM-bearing proteins might be a special case of the N-end rule, that operates when IAPs and their substrates are in the same compartment. Indeed, according to rules determined by

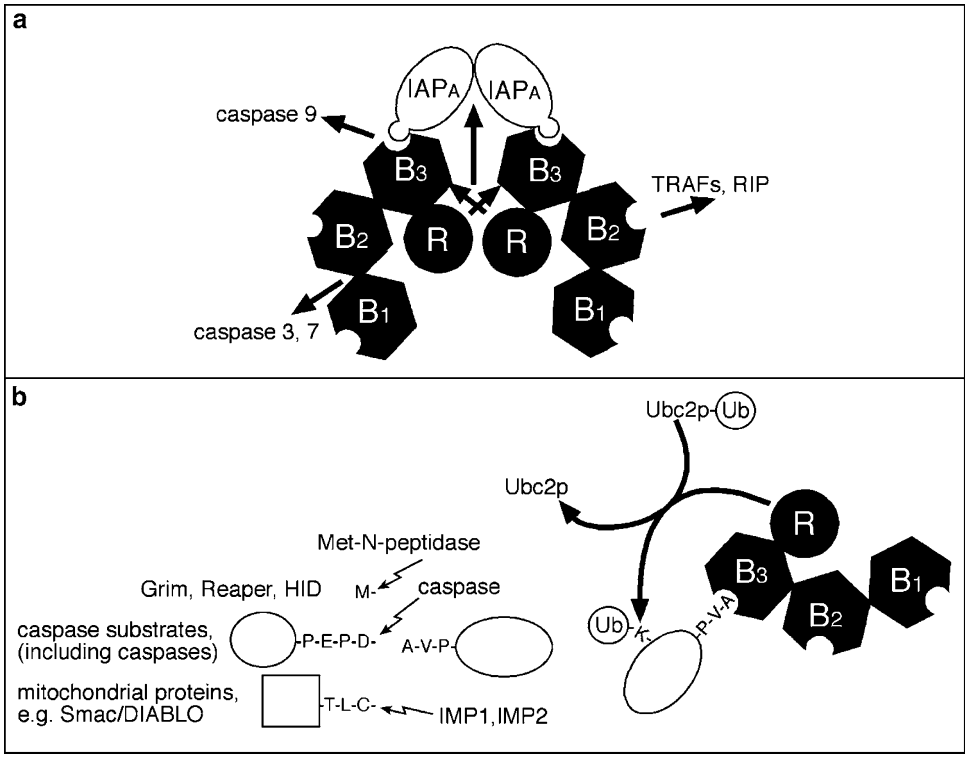

Figure 1 (**a**) Using their RING domains (R), IAPs (black) can act as E3 ubiquitin ligases, catalyzing ubiquitylation (indicated by arrows) of many of the proteins they interact with, such as caspase 9, that interacts with BIR3, and caspases 3 and 7, that bind to the region flanking BIR2. cIAP1 and cIAP2 have been reported to ubiquitylate TRAF2 and RIP. Via their BIR2 and BIR3 domains, IAPs can bind to IAP antagonists (IAPA), such as mammalian Smac/DIABLO and insect Rpr, Grim, and HID. Auto-ubiquitylation of IAPs can also occur. Activation of IAPs' E3 ligase activity might be promoted by IAP antagonists, or by dimerization, and possibly the IAP antagonists promote IAP dimerization. (**b**) IBMs can be generated by methionine amino peptidase, caspases, or the mitochondrial import proteases Imp1 and Imp2.[27] If they are in the same compartment, proteins with IBMs can bind to the BIRs of IAPs, and may be ubiquitylated. Using Varshavsky's terminology,[24] the IAPs would be acting in this model as E3 N-recognins for a type-3 primary destabilizing Ala residues at the amino-termini of proteins with IBMs

Varshavsky, amino-terminal A, S or T are primary (type-3) destabilizing residues[24] (Figure 1b).

The *Drosophila* IAP antagonists Rpr, Grim, HID and Sickle can bind to DIAP1 via their amino-terminal IBM, which has the residues Ala–Val–Pro (or something similar).[8] By binding to the BIRs, they can compete with processed effector caspases, which also bind to the BIRs via IBMs created when the caspase precursors are processed.[28] However, in addition to merely competing for binding sites, the *Drosophila* IAP antagonists also destabilize DIAP1, by promoting its degradation by the proteasome,[4,29,30] although the details of how each does so remains controversial. For example, Ryoo *et al*.[29] showed that Rpr caused degradation of DIAP1 in a RING dependent manner, presumably, by promoting DIAP1 auto-ubiquitylation and destruction, whereas Yoo *et al*.[3] found that HID stimulated DIAP1 RING-dependent poly-ubiquitylation and degradation, while Rpr and Grim down-regulated DIAP1 through mechanisms that did not require ubiquitin ligase function of DIAP1.

How Grim, Rpr, and HID activate the E3 activity of DIAP1 is not known, but one hypothesis is that they promote DIAP1 dimerization. When heterologously expressed in mammalian cells, Grim can activate the ubiquitin ligase activity of XIAP, causing it to auto-ubiquitylate and be degraded by the proteasome.[31] In contrast, although XIAP has been reported to catalyze ubiquitylation of Smac/DIABLO *in vivo* as well as *in vitro*,[6,32] Smac/DIABLO does not promote the ubiquitin ligase activity of IAPs the way that insect IAP binding proteins do, and may even antagonize it.[31,33]

The giant protein BRUCE was identified as a BIR-bearing protein that has a UBC-like domain, but not a RING domain.[34] The exact role of BRUCE, and its *Drosophila* homologue dBRUCE remains uncertain but gene deletion studies in the mouse and fly have begun to reveal some of its secrets. Expression of dBRUCE seemed to reduce cell death in *Drosophila*, and for it to do so its UBC-like E2 domain was required in addition to the BIR domain. However, the mechanism by which dBRUCE acted was obscure, as it did not promote degradation of Grim or HID directly, or inhibit Dronc or the *Drosophila* Bcl-2 family member Debcl.[35]

Several groups have reported deleting BRUCE in mice.[36–38] While all agree that deletion of BRUCE results in embryonic lethality, its exact role remains controversial, with suggested activities including preventing p53 activation,[38] directly binding to both unprocessed and mature Smac/DIABLO and both pro- and processed caspase 9, and targeting them for degradation.[36,39,40]

Survivin is a BIR containing protein that acts together with INCENP and Aurora kinase B in a complex required for chromosome segregation and cytokinesis during mitosis.[41–43] The abundance of Survivin is coupled to the cell cycle. Small amounts of Survivin exist during interphase, but it

accumulates on the centromeres as its genes are transactivated during prophase. At telophase, Survivin localizes to the midbody, and it is degraded by the ubiquitin-proteasome pathway.[44]

Just as a full understanding of the biological role of the ubiquitin-proteasome system remains a long way off, analysis of its role in IAP function is still in its infancy. Clearly, RING-bearing IAPs are not simply passive caspase inhibitors, but can also act as E3 ligases to determine the abundance of components of the apoptosis mechanism, as well as themselves. But their roles may extend beyond this, to perform other functions such as modulating TNFR signalling,[13] or tidying up wayward mitochondrial proteins.[45]

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