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News and Commentary

Till Death Do Us Part

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Even in an age where to publish or perish has become a scientific mantra, the pace of publications describing the function and discovery of the IAP antagonist, DIABLO/ Smac, has been astonishing. In the 6 months since DIABLO was described,[1,2] the structure of portions of the inhibitor of apoptosis (IAP) protein, XIAP in complex with DIABLO has been solved. As a result, a conserved IAP binding motif shared by the separate *Drosophila* IAP antagonists (Grim, Reaper and HID) and DIABLO has been identified, and a simple and elegant model of the functional interactions between XIAP, caspases and DIABLO now exists.

IAPs were first identified in baculoviruses[3] and are characterised by one or more copies of a motif known as baculoviral IAP repeat (BIR). Understanding of IAP function in *Drosophila* (recently reviewed[4]) has concentrated on the interactions between the *Drosophila* IAP antagonists and the IAPs (although not exclusively, see[5]). In contrast, the mechanism of action of mammalian IAPs has focused on their ability to directly inhibit activated caspases. This was largely because mammalian homologues of the *Drosophila* IAP antagonists were conspicuous by their absence until DIABLO was identified. DIABLO functions as an IAP antagonist. In the *Drosophila* IAP antagonists, a small N-terminal region of $\sim$14 amino acids that they share in common has long been known to be important for interaction with *Drosophila* IAPs. DIABLO has limited sequence homology, sharing identity with the *Drosophila* proteins in three critical amino acids at the N-termini.[6–8]

DIABLO was originally described in July 2000.[1,2] While Du *et al*[2] had identified a mitochondrial factor (Smac) that, in combination with APAF-1, cytochrome *c*, dATP and caspase-9 promoted activation of caspase-3, Verhagen *et al*[1] had identified a protein (DIABLO) that bound to IAPs. Together, the data suggested that DIABLO functioned to activate caspases by removing XIAP from the apoptosome.

DIABLO is normally targeted to the mitochondria by an N-terminal sequence that is subsequently removed. It is the processed form, once released from mitochondria that binds IAPs, and reverses or prevents XIAP-dependent cell survival and caspase inhibition.

The structure of the DIABLO-XIAP BIR3 complex indicates how DIABLO might separate XIAP from activated caspases. In an extremely detailed piece of work Chai *et al*,[9] showed that DIABLO exists as a dimer and that the key determinants for XIAP binding are the 4–5 N-terminal amino acids of processed DIABLO. Monomeric mutants of DIABLO were still able to bind the third BIR domain (BIR3) of XIAP, but not BIR2, most likely because the N-terminal amino acids of DIABLO bind with greater affinity to the BIR3. Dimeric DIABLO was able to bind to either the BIR2 or BIR3. Because BIR3 of XIAP bound more strongly to DIABLO than BIR2, it was the obvious candidate BIR to co-crystalise with DIABLO. DIABLO interactions with BIR3 may be what allows it to disrupt XIAP-caspase-9 interaction because it is also BIR3 of XIAP that binds caspase-9.[10,11]

The structure of a BIR3 DIABLO complex was solved independently by two groups, one determined the solution structure using a peptide consisting of the nine N-terminal amino acids of processed DIABLO,[7] and the other determined the crystal structure of BIR3 and the whole DIABLO molecule.[6] Both groups were rewarded with a beautiful structure that immediately solved the puzzle of how DIABLO prevents XIAP from inhibiting caspase-9. Simply put, the N-terminal amino acids of DIABLO not only bind to exactly the same groove of BIR3 as caspase-9, but also interact with the same residues within the groove.[12] Therefore DIABLO antagonises XIAP's inhibition of caspase-9 by physically excluding, or displacing, caspase-9 from the BIR3 of XIAP.

Why do DIABLO and caspase-9 both fight over the same part of XIAP, even down to the same residues? The answer is elegant and, in retrospect, obvious; caspase-9 and DIABLO bind to the same groove in the BIR3 because once proteolytically processed, both DIABLO and caspase-9 expose the same N-terminal IAP binding motif. In the case of DIABLO, this motif is AVPI, but it is only exposed and available for XIAP binding once DIABLO has (a) been processed in the mitochondria, and (b) been released. In the case of caspase-9, the sequence of the binding motif is ATPF (human) and AVPY (mouse), and it is made available for XIAP binding following autocatalytic cleavage of caspase-9 at the aspartate in position 315.[12]

How might this model in which DIABLO separates XIAP and caspase-9 influence our understanding of this death pathway? Caspase activation represents a feedforward loop. *In vivo*, caspase-9 processes caspase-3, and activated caspase-3 can in turn process pro-caspase-9. The first autocatalytic cleavage of caspase-9 occurs at D315, but the cleavage of caspase-9 by caspase-3 occurs at D330. Only auto-catalytically activated caspase-9 bears an N terminus that XIAP can bind. Caspase-3 activated caspase-9 cannot be inhibited by XIAP.

One could describe release of DIABLO from mitochondria as a 'ratification' of the death signal enacted by the

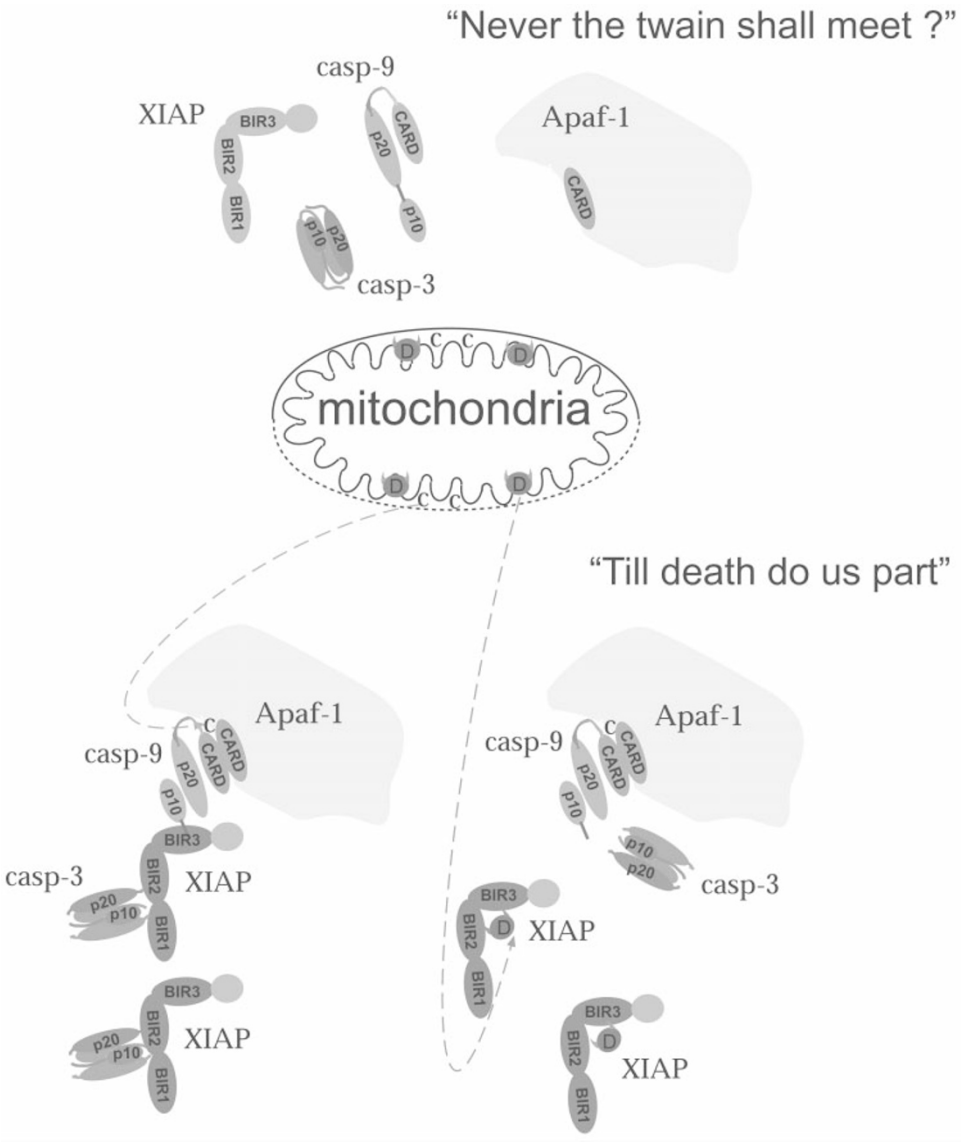

Figure 1 In the absence of a death stimulus, caspase-9 and caspase-3 are present in their zymogen form and not complexed with either APAF1 or XIAP. Cytochrome *c* (c) and DIABLO (D) are locked away in the mitochondria. Following a death stimulus, cytochrome *c* and DIABLO are released from the mitochondria. Cytochrome *c* acts as a cofactor allowing caspase-9-APAF1 binding and caspase-9 auto-processing. Caspase-3 can, in turn, be processed by caspase-9. XIAP is able to bind to both processed caspases and appears to hold these within the apoptosome.[14] DIABLO can compete for the caspase binding sites within XIAP and allows caspases to cleave their substrates. If caspase-9 becomes activated without sufficient DIABLO release then XIAP can remain bound to processed caspase-9 and prevent cell death[15]

release of cytochrome *c*. DIABLO ensures that activation of caspase-9 cannot be subverted by XIAP. Alternatively one could envisage a simple 'bio' logic circuit; caspase activation 'and' DIABLO release result in cell death, but caspase activation 'or' DIABLO release do not, because XIAP inhibits caspase activity. Another possibility is that a death stimulus needs to reach a certain threshold before it is irreversible. Enough XIAP, or insufficient DIABLO, would prevent the dire consequences of caspase activation. To what extent caspase-9 or caspase-3 can and do auto-activate without cytochrome *c* and DIABLO release, and how dangerous that might be, remains to be seen. But the feed-forward nature of caspase activation does indeed suggest that minimal activity might be deadly without IAPs to counter 'unintended' apoptosis.

Although DIABLO might be expected to be normally sufficient to compete XIAP from caspase-9, it appears that, intracellularly, no chances are taken, and caspase-3 processes caspase-9 to remove the N-terminal AVP and prevent processed caspase-9 from interacting with XIAP. Caspase-3 can then cleave XIAP itself (demonstrated only in human cell lines) thereby probably interfering with the ability of XIAP to inhibit the caspases.[10]

Feedforward loops make sense in biological process that must go to completion, after all, there's no sense in being half dead. But feedforward loops rapidly amplify small signals and are dangerous if unwanted activation takes place. XIAPs critical role might well then be to act as a brake on the feedforward loop, until DIABLO parts it from caspase-9, and death results in the ultimate separation.

Acknowledgements

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