COMMENTARY

IAP proteins: sticking it to Smac

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Dogma has it that suppression of the programmed cell death pathway by the IAP (inhibitor of apoptosis) proteins is achieved by their direct enzymic inhibition of the chief executioners of the apoptotic process, the caspases. In turn, the IAPs themselves can be neutralized by Smac/DIABLO (second mitochondrial activator of caspases/direct IAP binding protein with low pI), a protein which in healthy cells is thought to be sequestered in the mitochondria, but which, in response to apoptotic stimuli, is released from the mitochondria into the cytosol where it can bind to IAPs, displacing caspases and thus perpetuating the apoptotic signal. While this is an elegant and attractive model, recent studies have suggested that IAPs can also suppress apoptotic cell death independently of their ability to inhibit caspases, and two reports in this issue

of the *Biochemical Journal* reach the interesting conclusion that the cytoprotective IAPs, ML-IAP (melanoma IAP) and ILP-2 (IAP-like protein 2), exert their effects not through direct caspase inhibition, but through the neutralization of Smac/DIABLO. The predicted outcome of these studies is a delicately controlled equilibrium between the activities of IAPs and Smac/DIABLO, leading to a dynamic regulation of the apoptotic threshold.

Key words: apoptosis, caspase, inhibitor of apoptosis (IAP) protein, melanoma inhibitor of apoptosis (ML-IAP), Smac/DIABLO (second mitochondrial activator of caspases/direct IAP binding protein with low pI), X-linked IAP (XIAP).

A feature common to all successful metazoans is the ability to control cell number. This property is essential for multicellular organisms in developmental and adult phases, and is achieved by modulating both cellular birth and death [1]. The latter event is largely effected through the activation of a delicately orchestrated series of events that lead to deconstruction, death and ultimately removal of the cell in a process known as apoptosis, in certain situations referred to as programmed cell death. The biochemistry and molecular genetics of apoptosis have been the subject of much investigation, and many components of the pathway have been uncovered. The central effectors of this mechanism are the caspases – a family of cysteine proteases with a preference for cleaving adjacent to aspartate residues, and these have been highly conserved throughout metazoan evolution [2]. In a healthy cell, caspases are generally thought to be held in an inactive form, either by enforced monomerization or by retention in an inactive zymogenic state [3]. The initiation of a pro-apoptotic stimulus can trigger the activation of a hierarchical cascade of caspases in a manner often compared with the blood coagulation pathway, but in this case leading to the proteolytic cleavage of essential cellular components that results in the demise of the cell.

Caspase activation and its prevention are subject to very tight control by many cellular factors. For example, members of the Bcl-2 family of both positive and negative regulators of apoptosis play a critical role in the control of the apoptotic threshold [4]. This family of proteins are not thought to directly associate with caspases, but appear to function upstream to integrate the cellular signals that trigger the activation of the caspases. On the other hand, certain members of the IAP (inhibitor of apoptosis) protein family, which were first identified a decade ago in baculoviruses, have been shown to bind directly to specific caspases, and through this binding are thought to directly suppress caspase activity. This class of IAP is exemplified by XIAP (X-linked IAP), which can inhibit apoptosis when ectopically expressed and has been shown *in vitro* to function as a potent inhibitor of caspases 3, 7 and 9. However, its role *in vivo* is somewhat less clear, since *xiap*-de-

ficient and wild-type mice show no drastic differences in caspase activity [5], although a recent study has suggested a neurological phenotype in these mice [6].

In underscoring a central role for IAPs such as XIAP in regulating caspase activity, a number of factors have subsequently been discovered that can suppress the activity of the IAPs themselves. Most pertinent here is Smac [second mitochondrial activator of caspases; known as DIABLO (direct IAP binding protein with low pI) in mouse], a nuclear encoded, mitochondrially localized protein which is released into the cytosol in response to apoptotic stimuli that disrupt the integrity of the mitochondria [7,8]. Smac/DIABLO negatively regulates the caspase inhibitory properties of XIAP by binding into the same pockets in XIAP which are used to bind caspases; when XIAP binds Smac/DIABLO, the caspases are displaced and primed to effect the execution phase of apoptosis [9].

Recently, some interesting twists have emerged to suggest that this scheme may not be as simple as originally thought. One of the earliest hints of this came from a study by Silke et al. [10], in which point mutants of XIAP defective in caspase inhibition, but retaining Smac-binding properties, were still cytoprotective. The implications of the resulting model are puzzling: if the binding of Smac/DIABLO to XIAP neutralizes the cytoprotective effects of XIAP, how could binding of XIAP to Smac/DIABLO block the death-promoting function of Smac/DIABLO? Which of these two opposing directions is more important? One way to attempt to answer this might be to examine XIAP- or Smac/DIABLO-deficient cells, to determine whether XIAP is still cytoprotective in a Smac/DIABLO-null cell, or whether Smac/DIABLO can potentiate death in an XIAP-deficient cell. However, this is fraught with technical problems, since Smac/DIABLO binds not only to XIAP but to other cellular IAPs, and additional proteins have been identified that can function in an analogous fashion to Smac/DIABLO.

In this issue of the *Biochemical Journal* two reports [11,12], as well as a recent study from our laboratory [13], support the overall

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conclusion of the study by Silke et al. [10], but approach the question through the analysis not of XIAP, but of three distinct protective IAPs. The study by Vucic et al. [11] is an elegant structural characterization of ML-IAP (melanoma IAP [14]), and the study by Shin et al. [12] examines the structural and functional properties of human ILP-2 (IAP-like protein 2; also known as BIRC8 [15,16]) and our group has examined the protective properties of a baculoviral IAP (Op-IAP [17]). The first study shows that, despite its strongly protective properties, ML-IAP is significantly inferior to XIAP in terms of caspase inhibition, but has a very high affinity for Smac. The study goes on to define the structural reasons for this disparity, and shows that substitution of just three residues can enhance the caspase 9 inhibitory activity of ML-IAP to levels similar to those observed with XIAP.

The second study [12] focuses on ILP-2, the product of a human testis-specific mRNA that is very closely related to the C-terminal region of XIAP. This report [12] shows that, similar to ML-IAP, ILP-2 is a weak caspase 9 inhibitor, and additionally that, in expression studies, ILP-2 is a highly unstable molecule. A stabilized form of ILP-2 containing nine additional N-terminal residues was crystallized in complex with Smac/DIABLO, and a strong association between the two molecules was revealed.

As an aside from the central theme of this discussion, these papers [11,12] raise the controversial issue of whether *ILP-2* is truly a functional gene or a testis-specific expressed pseudogene. *ILP-2* is encoded by an intronless transcript derived by retrotransposition from XIAP. We have reported that the *ILP-2* gene is found only in great apes [15], and that where it is found the open reading frame is conserved, but definitive proof of the existence of *ILP-2* has been hampered by its extremely close similarity to XIAP, and to the existence of a widely expressed proteolytic fragment of XIAP which is virtually indistinguishable from *ILP-2* [18]. The stabilization of *ILP-2* by the addition of nine unrelated residues at the N-terminus is reminiscent of the use, in the two papers describing the functional properties of this factor, of synthetic N-terminal epitope tags [15,16]. Diplomatically, the authors of the present studies [12] suggest that *ILP-2* may require a (currently unidentified) testis-specific stabilizing protein or chaperone to be fully functional, although the alternative possibility must be considered, namely that *ILP-2* might simply be the product of an expressed pseudogene.

The study from our group [13] examined the cytoprotective properties of Op-IAP in human cells, and concluded that while expression of Op-IAP in human cells blocked the activation of caspase 3, this was not due to direct binding of Op-IAP to the caspase but to an upstream property. This study [13] also found a strong association of Op-IAP with, and an ability to induce the ubiquitinylation of, Smac/DIABLO.

Taken together, these very different studies draw a common conclusion: IAPs can suppress cell death not only through the inhibition of caspases, but through the direct binding (and presumably inhibition of) Smac/DIABLO. This finding raises several interesting questions: did molecules such as Smac/DIABLO (and other analogous factors such as Omi/HtrA2) exist before IAPs, and did IAPs evolve subsequently to suppress the activity of Smac-like factors? Do proteins such as ML-IAP provide protection only in a cell expressing other caspase-inhibiting IAPs, such as XIAP,

thus serving to 'soak up' excess Smac/DIABLO and protect these IAPs from Smac/DIABLO? Again, these questions may only be resolved if we can determine definitively whether the apoptosis-promoting effects of Smac/DIABLO require IAPs. This is an issue that will not be solved overnight, and given the intense current research into the therapeutic potential of Smac/DIABLO mimetics, the studies [11,12] presented in this issue of the *Biochemical Journal* will provoke considerable discussion.

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