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News and Commentary

Caspases: opening the boxes and interpreting the arrows

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The year 2002 sees the 10th anniversary of the caspases. During the early 1990s groups at Immunex and Merck had been following the protease responsible for the post-translational maturation of the interleukin-1β precursor, and simultaneously published the identity and characteristics of the enzyme, ICE (caspase 1).[1,2] Shortly afterwards the key *C. elegans* death gene Ced3 turned out to be related to ICE,[3] and paradigms for a role of specific ICE-like proteolysis in mammalian apoptosis were elucidated.[4] The caspases received their official name about 5 years ago[5] as the number of caspases and the number of publications in the area began to heat up. Thus ended the first wave of discovery.

Today, the consensus view of caspases places them in two main camps. First are the cytokine activators related to caspase 1, probably including mouse caspases 11 and 12 their close homologs caspases 4 and 5 in humans. Their main role is to respond to bacterial infection by rapidly converting active cytokines (IL-1β, IL-18) from intracellular stores. Confirmation of the important roles of the caspases in the inflammatory cytokine response comes from gene ablation experiments in mice. Animals ablated in caspase 1 or 11 are deficient in cytokine processing,[6,7] but without any overt apoptotic phenotype. The second camp constitutes the apoptotic caspases that transduce and execute death signals. The phenotypes of these knockouts are very gross, evidently anti-apoptotic, and vary from early embryonic lethality (caspase 8) to perinatal lethality (caspases 3 and 9),[8–10] to relatively mild with defects in the process of normal oocyte ablation.[11] Currently, caspase 14 may be the odd one out, being involved in keratinocyte differentiation.[12,13] On the basis of a lot of biochemistry and cell biology it has been possible to place the apoptotic caspases in a pathway, such that some are activated by others (Figure 1). This core pathway probably represents a minimal apoptotic program, and certainly its simplicity is complicated by cell-specific additions that help to fine tune individual cell fates. Nevertheless, the basic order and at least some of the essential functions and, importantly, endogenous regulators of the caspases are known, and thus ends the second wave of discovery.

But there is something a little dissatisfying about boxes, ovals and arrows. Why stop here when structural tools are available to see how caspases work, how they are activated, and how they are regulated? In principle this should have a major impact on the design of reagents and pharmaceuticals to probe the core of the apoptotic pathway. So the third wave of discovery is under way to look inside the boxes and interpret the arrows.

Early successes in this area to define the structure-activity-function algorithm came with 3-D elucidation of active caspases.[14–20] They showed a conserved picture, with differences mainly in the all-important specificity-defining clefts that bind substrate. Each active caspase is a dimer of identical catalytic domains, and each domain consists of a large and a small subunit. More recently the structures of the regulators: p35, CrmA and the BIR domains of XIAP became available.[21–24] So, now that the boxes were open, it became time to unravel the arrows. In pathway diagrams an arrow signifies a process that converts one signal to another, or prevents that signal from being transmitted. In the apoptotic pathway these processes are zymogen activation, and protease inhibition.

To allow the latent apoptotic program to respond to death signals, caspases are restrained in an inactive form – the zymogen. The dogma for pro-caspase activation says that they are activated by proteolysis between the large and small subunits. Therefore, a perplexing question posed by the dogma is how are the initiators caspase 8 and 9 activated if there are no proteases 'above' them? Indeed, this is a common problem for many proteolytic pathways (coagulation and fibrinolysis for example), not just apoptosis. To the initial rescue came the induced proximity hypothesis.[25–28] The hypothesis said that initiator caspases are recruited to protein complexes (Figure 1) whose assembly forces a locally high concentration of caspase zymogens. This clustering of zymogens possessing a small amount of intrinsic enzymatic activity would allow for processing in *trans*, and activation of the first protease in the cascade. This seemed like a good hypothesis, and still may be true for Ced3 and caspase 8. However, recent data on the 3-D structure of caspase 9 offer another explanation for the conversion of its zymogen to the active form.[29]

Pro-caspase 9 at the concentrations found *in vivo* contains the equivalent of a single domain, but this domain is in an inactive conformation. The substrate binding cleft is dislocated and the catalytic residues are distorted. Induced proximity forced within the Apaf-1 apoptosome may lead to dimerization, resulting in an ordering of the active site with development of catalytic competence. This model offers a slightly different explanation of the induced proximity hypothesis, since no proteolytic processing of pro-caspase 9 would be required to drive activation. Moreover, the model provides some interesting targets from a pharmaceutical perspective. Can pharmaceutical reagents be found to lock the monomeric form into the active conformation? This could be useful when neoplastic cells must be killed. Can others be found to block the dimerization interface and thereby prevent inappropriate activation of the intrinsic pathway in acute apoptotic episodes?

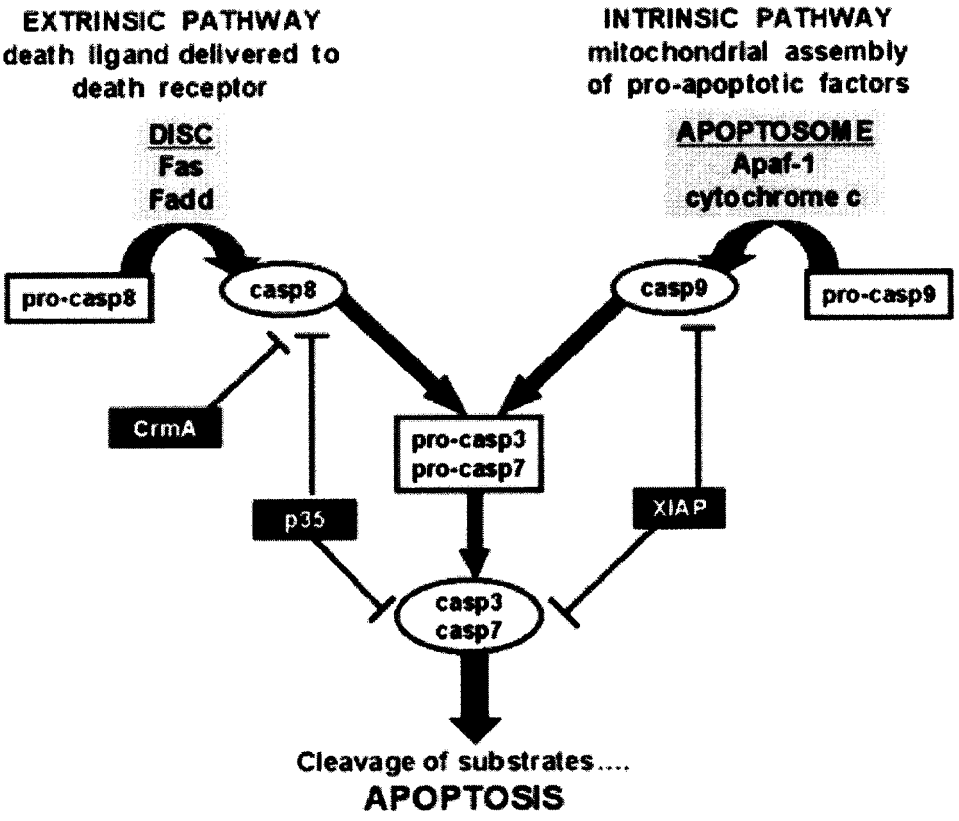

Figure 1 Two initiation pathways, triggered by separate events, converge at a common point to execute apoptosis in mammals. Natural inhibitors (black boxes) effect different points on the pathways. Cowpoxvirus CrmA is specific for caspase 8 (and also caspase 1), thereby attenuating the host inflammatory and apoptotic response to infection. Baculovirus p35 is a very broad range inhibitor, inhibiting all known caspases, with the possible exception of caspase 9.[36] XIAP is an endogenous caspase inhibitor that targets caspases 3, 7 and 9.[37] The extrinsic apoptosis pathway is triggered through the extracellular ligation of death receptors by their cognate ligands—resulting in receptor clustering, adapter recruitment, and activation of the apical protease caspase 8 (active forms of caspases in ovals). Thus, death receptors (exemplified here by Fas) act as a conduit for the transmission of extracellular death signals into the cell's interior. The intrinsic pathway responds primarily to cellular stress (ionizing radiation, cytotoxic drugs, etc.) as well as some neurodevelopmental cues, with the mitochondrion acting as an important integrator. Activation of the apical protease caspase 9 occurs when it is driven into a catalytic conformation by its co-factor Apaf-1, which itself requires prior binding to cytochrome *c*. Both pathways activate the executioner proteases caspases 3 and 7

How about the other arrows – the inhibitory ones? Whereas the mechanism of CrmA is still speculative, the recent 3-D structure of the complex of caspase 8 with p35[30] discloses a mechanism uniquely adapted by baculoviruses to ablate caspase activity. Caspases attack a substrate-like sequence in a surface loop that extends far out from the p35 framework. A movement of the N-terminus pinches the active site, knocking the catalytic residues out of kilter, and traps the enzyme half way through its normal substrate pathway. The caspase is frozen as a thiol ester, and the reaction is essentially irreversible. The flexibility of the pincer interaction between the inhibitor and the protease implies that the only requirement for inhibition is that cleavage occurs within the substrate-like loop of p35. Moreover, this loop in p35 is extremely large and flexible[21] and allows the inhibitor to adapt to multiple caspases. The broad reactivity, combined with its ability to trap caspases once they have cleaved the loop, makes p35 an ideal tool for the virus to evade the apoptotic response of infected host cells.

In contrast to the broad specificity of p35, the endogenous mammalian caspase inhibitor XIAP contains one domain (BIR2) that only inhibits caspases 3 and its very close paralog caspase 7, and another domain (BIR3) that only inhibits caspase 9. The structural basis of caspase 9 inhibition by BIR3 remains to be determined, but the mode of inhibition of caspases 3 and 7 by BIR2, recently elucidated by three groups,[31–33] reveals probably the strangest solution of how to specifically inhibit proteases ever seen. A segment of about 10 residues N-terminal to the BIR2 domain binds onto the surface of the caspase and blocks substrate access. This is conventional, but convention is violated because the segment binds backwards with respect to the normal substrate binding orientation. Not surprisingly, the normal substrate binding pockets are for the most part unoccupied by the inhibitory peptide. In fact, the peptide side chains have explored new pockets and surfaces that are only found in caspases 3 and 7. Hence the exquisite specificity of the BIR2 domain. Medicinal chemists may well be tempted to explore these newly identified pockets on caspases 3 and 7, since their occupancy by small molecules should prevent inhibition of caspases 3 and 7 by XIAP, yet still allow the normal catalytic function of the caspases.

Some of the boxes are open, and some of the arrows are interpreted. But much awaits us in this third wave of

discovery. How are the executioner caspases maintained as zymogens, and what is the structural/molecular explanation of their activation by proteolysis? Steps to interpret this process suggest unique stabilization by conserved elements in the inter-domain linker of the executioners.[34] What is the structural/molecular basis for the inhibition of caspase 9 by the BIR3 domain of XIAP? Steps to interpret this process suggest a completely different mechanism to BIR2.[35] Sometime in the near future these questions will be answered, and most of the boxes will be open. But don't worry, currently, there are just over six PubMed entries *per day* with the word 'caspase' in them, and the rate of publications is still increasing. There is always another wave to ride, and only Doug Green can predict what it's going to be.

1. Cerretti DP *et al.* (1992) Science 256: 97 – 100
2. Thornberry NA *et al.* (1992) Nature 356: 768 – 774
3. Yuan J *et al.* (1993) Cell 75: 641 – 652
4. Lazebnik YA *et al.* (1994) Nature 371: 346 – 347
5. Alnemri ES *et al.* (1996) Cell 87: p171
6. Kuida K *et al.* (1995) Science 267: 2000 – 2003
7. Wang S *et al.* (1998) Cell 92: 501 – 509
8. Kuida K *et al.* (1998) Cell 94: 325 – 337
9. Kuida K *et al.* (1996) Nature 384: 368 – 372
10. Varfolomeev EE *et al.* (1998) Immunity 9: 267 – 276
11. Morita Y *et al.* (2001) Cell Death Differ. 8: 614 – 620
12. Lippens S *et al.* (2000) Cell Death Differ. 7: 1218 – 1224
13. Eckhart L *et al.* (2000) J. Invest. Dermatol. 115: 1148 – 1151
14. Blanchard H *et al.* (1999) Structure 27: 1125 – 1133
15. Mittl PR *et al.* (1997) J. Biol. Chem. 272: 6539 – 6547
16. Rotonda J *et al.* (1996) Nature Struct. Biol. 3: 619 – 625
17. Walker NPC *et al.* (1994) Cell 78: 343 – 352
18. Watt W *et al.* (1999) Structure 27: 1135 – 1143
19. Wei Y *et al.* (2000) Chem. Biol. 7: 423 – 432
20. Wilson KP *et al.* (1994) Nature 370: 270 – 275
21. Fisher AJ *et al.* (1999) EMBO J. 18: 2031 – 2039
22. Renatus M *et al.* (2000) Structure Fold Des. 8: 789 – 797
23. Sun C *et al.* (1999) Nature 401: 818 – 822
24. Sun C *et al.* (2000) J. Biol. Chem. 275: 33777 – 33781
25. Muzio M *et al.* (1998) J. Biol. Chem. 273: 2926 – 2930
26. Martin DA *et al.* (1998) J. Biol. Chem. 273: 4345 – 4349
27. Yang X *et al.* (1998) Mol. Cell 1: 319 – 325
28. Srinivasula SM *et al.* (1998) Mol. Cell 1: 949 – 957
29. Renatus M *et al.* (2001) Proc. Natl. Acad. Sci. USA in press
30. Xu G *et al.* (2001) Nature 410: 494 – 497
31. Riedl SJ *et al.* (2001) Cell 104: 791 – 800
32. Huang Y *et al.* (2001) Cell 104: 781 – 790
33. Chai J *et al.* (2001) Cell 104: 769 – 780
34. Roy S *et al.* (2001) Proc. Natl. Acad. Sci. USA 98: 6132 – 6137
35. Srinivasula SM *et al.* (2001) Nature 410: 112 – 116
36. Vier J *et al.* (2000) Biochem. Biophys. Res. Commun. 276: 855 – 861
37. Deveraux QL *et al.* (1999) Genes Dev. 13: 239 – 252