The sodium channels of snake neurons and skeletal muscle are implicated in TTX resistance because they are the targets of TTX and differ in their TTX sensitivity. Geffeney and colleagues now test this possibility in their new study (*2*). To quantify whole-animal resistance, these authors measured the dose of TTX required to reduce a garter snake's crawling speed by 50%. To quantify resistance of muscle to TTX, they excised snake skeletal muscle—thus adapting another preparation, "fillet of a finny snake," pioneered by the witches of *Macbeth*. Then they measured the effect of TTX on the maximum rate of rise of action potentials, a rough estimate of the sodium current. By applying both assays to individual snakes, the authors found that the sensitivity of muscle action potentials to TTX was positively associated with whole-animal sensitivity—that is, snakes with resistant muscle cells showed a major reduction in crawling speed only at high doses of TTX.

Fugu for garter snakes. Some garter snakes dine on newts and are seemingly undeterred by large quantities of the potent neurotoxin TTX in the newt's skin.

These results strongly imply that sodium channels in snake skeletal muscle are key players in the evolution of TTX resistance, although not necessarily the only players. As Geffeney and co-workers note, variations in the TTX sensitivity of neuronal sodium channels could also influence variations in whole-animal resistance, as well as account for the slow crawling speed of TTX-resistant snakes.

Variations in the TTX sensitivity of the muscle action potentials have yet to be explained. One possibility derives from the observation that mammalian muscle cells contain both TTX-sensitive and TTX-resistant sodium channels (*9*). If snakes have both types of sodium channels, then the TTX resistance of muscle and of the whole animal could be adjusted by an evolutionary alteration in the ratio of the two channel types in muscle membranes. An intriguing way of achieving this would be through a genetic alteration in the timing of the developmental program ("heterochrony") for snake skeletal muscle. During mammalian muscle development, TTX-resistant sodium channels predominate early but are mostly replaced by TTX-sensitive forms as the muscle matures and becomes innervated (*10*). Thus, variations in the TTX resistance of snake muscle could be achieved by arresting the switch between TTX-resistant and TTX-sensitive channel types at different stages of development.

Why is marked TTX resistance found only in certain populations of garter snakes, even though many other garter snake populations inhabit areas where newts also live (*2*)? This may reflect idiosyncratic (contingent) historical influences. Alternatively, perhaps the snake-newt arms race includes a third trophic level—animals that prey on the snakes themselves. Even though TTX-resistant snakes gain sole access to an untapped food resource, they may suffer an increased risk of predation because they are chronically slow (*8*, *11*). This trade-off may put evolutionary brakes on a further escalation of snake resistance to TTX (*4*, *8*). Moreover, resistance might be favored only where food for snakes is limited and where predators of snakes are rare. Testing this geographic, coevolutionary hypothesis (*12*) may prove difficult.

The work of Geffeney and colleagues demonstrates the power of integrative biology and will stimulate complementary tests of diverse neurobiological and evolutionary hypotheses. Moreover, the take-home message seems to be that getting a leg up in the predator-prey arms race requires neither arms, nor legs, nor speed.

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PERSPECTIVES: APOPTOSIS

A Cinderella Caspase Takes Center Stage

Sharad Kumar and David L. Vaux

Activation of proteolytic enzymes called caspases is a key step in the apoptotic program. Caspases exist in latent forms in almost all animal cells and become activated in response to apoptotic signals such as those induced by cell stress (for example, DNA damage and withdrawal of trophic support). The first caspases to become activated are so-called "initiator caspases." These caspases have long amino-terminal prodomains containing specific protein-protein interaction motifs. Through these domains the caspases interact with adaptor proteins that recruit them to specific "death complexes" (large multiprotein complexes that mediate caspase activation). In mammals, these death complexes include the Apaf-1/caspase-9 apoptosome and the FADD/caspase-8 death-inducing signaling complex (DISC). Once the initiator caspases are activated, they process and activate downstream effector caspases, such as caspases 3, 6, and 7 (*1*). The apoptosome and DISC are thought to account for most caspase-dependent apoptosis. The upstream signaling pathways leading to the assembly of these death complexes are often called the mitochondrial (intrinsic) and death receptor (extrinsic) pathways of apoptosis.

During stress-induced apoptosis, mitochondria release their cytochrome c, which binds to Apaf-1 and promotes apoptosome formation and caspase-9 activation (*1*). Thus, the most widely held view is that caspase-9

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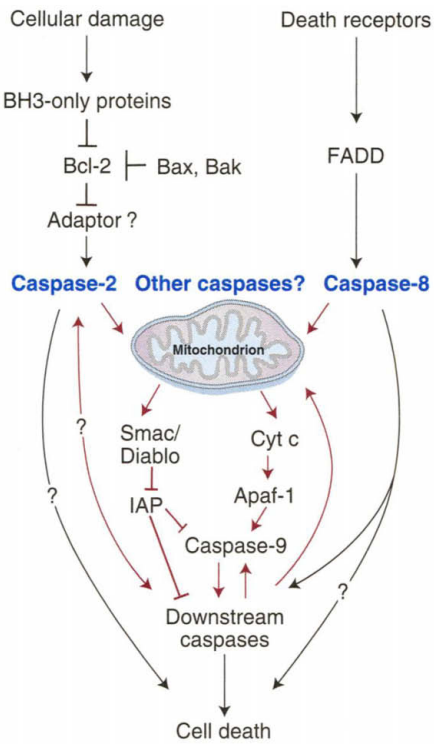
is the initiator caspase in this pathway and that mitochondrial release of cytochrome c is essential for activation of this caspase (*1, 2*). However, this view has been challenged [see (*2*) for arguments from both sides] and is now under challenge again from several reports (*3–6*), including one by Lassus *et al.* (*3*) on page 1352 of this issue. These authors show that in response to cell stress, activation of a neglected caspase, caspase-2, is required before mitochondrial permeabilization and apoptosis can take place (*3*).

Caspase-2 (Nedd2/Ich-1) was the first mammalian apoptotic caspase to be identified (*7, 8*). It closely resembles caspase-9 and CED-3 in the worm, and like them bears a caspase recruitment domain (CARD). Numerous early studies implicated caspase-2 in cell death pathways that could be rescued by Bcl-2. Caspase-2 is ubiquitously expressed, its activation occurs early in apoptosis, and antisense studies suggest that reducing the amount of caspase-2 lessens cell death in response to factor deprivation of cultured cells and sympathetic neurons (*7–11*). However, the absence of an overt phenotype in caspase-2–deficient mice (*12, 13*) led to this caspase becoming the Cinderella of the caspase field.

In their new work, Lassus and co-workers revisit caspase-2 using small interfering RNA (siRNA) to ablate its expression. They demonstrate clearly that caspase-2 is required for DNA-damage–induced apoptosis of E1A-transformed human fibroblasts and some human cancer cell lines (*3*). Expression of a caspase-2 cDNA construct that is refractory to siRNA restored the ability of cells to undergo apoptosis. Perhaps their most striking finding is that caspase-2 activity is required for translocation of the death protein Bax to the mitochondria as well as for release of the mitochondrial proteins cytochrome c and Smac/Diablo, early steps in the apoptotic program (*3*). Similar results are reported by two other groups who demonstrate that caspase-2 can induce cytochrome c, Smac, and apotosis-inducing factor (AIF) release from mitochondria directly (*4, 5*) or through cleavage of the proapoptotic protein Bid, which moves to mitochondria and facilitates cytochrome c release (*4*). Another group also reports that caspase-2 in the nucleus signals cytochrome c release from mitochondria (*6*). Using different systems and cell types, all of these studies suggest that caspase-2, not caspase-9, is an apical caspase in the proteolytic cascade initiated by stress signals. If correct, this model might help to explain why apoptosis seems to occur normally in cells other than neurons in mice lacking Apaf-1 or caspase-9 (*14*). Although caspases cannot be activated by released cytochrome c in these animals, caspase-2 or a similar caspase could still be killing cells independently of the mitochondrial pathway.

This model also gives new breath to the idea that Bcl-2 might act like its worm homolog CED-9, namely by preventing activation of a caspase and so maintaining the integrity of mitochondria indirectly (*2*). It is generally believed that Bcl-2 blocks apoptosis by preventing loss of mitochondrial membrane potential and release of mitochondrial proteins such as cytochrome c, Smac/Diablo, and AIF. These events are mediated by proapoptotic Bcl-2 family members such as Bax, Bak, and Bid. The alternative view is that Bcl-2 acts like CED-9 by preventing activation of a caspase (*2*). According to the new model (see the figure), once this caspase is activated it causes damage to cellular components, including the mitochondria. The damaged mitochondria then release cytochrome c, which promotes apoptosome-mediated activation of caspases such as caspase-9 and caspase-3, serving to amplify the caspase activation cascade.



A new model of apoptosis. Previous studies suggest that caspase-8 and caspase-9 act as initiator caspases in extrinsic and intrinsic pathways, respectively, and activation of these caspases is necessary for the initiation of caspase cascade. In the new model, intrinsic pathways, such as those initiated by cell stress, induce activation of caspase-2, which is required for permeabilization of mitochondria, release of cytochrome c, and apoptosis. Mitochondria may act as amplifiers rather than initiators of caspase activity. Current efforts to develop caspase inhibitors for treating stroke and myocardial infarction have centered on trying to block the activity of postmitochondrial caspase-9 and caspase-3. However, this approach may only inhibit the amplification loop, to delay, but not prevent, cell death.

Before this model of apoptosis is accepted, several questions must be answered. Why is the phenotype of the caspase-2 knockout mouse so subtle? Is caspase-2 the only pre-mitochondrial stress-activated caspase, or are there others that can compensate for its absence? How common is the pathway requiring caspase-2, given that some of the cell lines tested by Lassus *et al.* showed no effects on apoptosis after caspase-2 ablation? How does caspase-2 activation occur upstream of mitochondria, and what factors regulate it? Does it require a CED-4–like adaptor protein that binds to Bcl-2 and is regulated in a manner similar to CED-9–mediated inhibition of CED-3 activation?

To date, Apaf-1 remains the only convincing CED-4 homolog, and it acts downstream of mitochondria and does not bind to Bcl-2 or Bcl-x. Another puzzling observation is that in dying cells, caspase-3 mediates most of the cleavage of procaspase-2, and cells derived from Apaf-1 and caspase-9 knockout mice fail to show processing of caspase-2, which suggests that most of the caspase-2 activation may occur downstream of mitochondria (*13*). One possibility is that caspase-2 processing by caspase-3 is an amplification mechanism and that the initial caspase-2 activation upstream of mitochondria occurs without any processing.

The answers to these questions are far from academic. Any potential pharmacological use of caspase inhibitors for treating diseases such as stroke and acute myocardial infarction will require targeting caspase activation events upstream of mitochondria, rather than inhibiting caspase-9 and caspase-3. But a drug that binds to caspase-2—dare we say with the fit of a glass slipper—and allows long-term survival of cells would make a very nice conclusion to this tale.

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