Integration of Damage Signals before Commitment to Death

Programmed cell death (apoptosis) is controlled in part by the Bcl-2 family of apoptosis-promoting (Bak, Bax, Bid) and apoptosis-inhibiting (Bcl-2, Bcl-x<sub>L</sub>), proteins. Beginning on page 903, Griffiths et al. examine what may be one of the earliest events in apoptosis: the effects of damage signals on the pro-apoptotic protein Bak. Using flow cytometry, they show that although the amount of Bak does not change early on, an epitope at Bak's NH<sub>2</sub>-terminus becomes accessible after various apoptosis-inducing events, DNA strand breaks and changes in protein phosphorylation and transcription, are induced by etoposide, staurosporine, or dexamethasone. However, the epitope is not exposed by activation of the Fas-dependent apoptotic pathway.

In untreated cells, Bcl-x<sub>L</sub> and Bak coimmunoprecipitate and immunofluorescence data reveal a close association between the two proteins and mitochondria. Dissociation of Bcl-x<sub>L</sub> from Bak, perhaps due to exposure of Bak's NH<sub>2</sub>-terminus, could account for the decreased coimmunoprecipitation observed after cell damage and commitment to death. A pro-apoptotic protein in the Bcl-2 family, Bax, also undergoes a change in conformation, exposing its NH<sub>2</sub>-terminal domain, in cells undergoing apoptosis. Using HeLa cells, Desagher et al. (page 891) help answer the question: do the apoptosis inhibitors Bcl-2 and Bcl-2 inhibit the pro-apoptotic Bax, or are the anti-apoptotic influences of Bcl-2 and Bcl-2 antagonized by Bax? The data from Jean-Claude Martinou's lab suggest that Bax may be the active killer.

Others have previously described a change in Bax conformation leading to exposure of the NH<sub>2</sub>-terminal domain in a solution containing nonionic detergents. The latest results indicate that Bak's structural change is linked to the translocation of a third pro-apoptotic protein, Bid, from the cytosol to mitochondria. The conformational change is also linked to the release of cytochrome c from mitochondria. In isolated mitochondria, the Bax structural change and cytochrome c release require direct binding of Bid. Both Bcl-2 and Bcl-x<sub>L</sub>, but not caspase inhibitors, prevent the change in Bax conformation. Bid alone elicits little release of cytochrome c in mitochondria from Bax-deficient tumor cells, whereas Bax and Bid together significantly increase release. Thus, Bax appears to have an essential role in the release of cytochrome c from mitochondria induced by Bid.

Cytochrome c release need not be irreversible. Martinou et al. (page 883) indicate that cytochrome c can be released from mitochondria without any obvious damage to the outer mitochondrial membrane in primary cultured sympathetic neurons. This observation is supported by the finding that mitochondria from neurons depleted of cytochrome c can recover their cytochrome c content when NGF is added.

When NGF is eliminated, the neuronal mitochondria lose cytochrome c content. Normally this leads to cell death but this fate can be avoided by treating the cells with caspase inhibitors. The recovery of cytochrome c content after reintroduction of a trophic factor, together with the apparent integrity of the organelles indicated by morphometric electron microscopy, suggests to Martinou that the "outer mitochondrial membrane has not been damaged during the NGF deprivation." This insight contradicts the popular hypothesis that cytochrome c release reflects irreversible damage to mitochondria. It implies the existence of a control process that may involve a specific channel. One likely candidate is Bax.

Mutational Analysis

Troponin I and Myosin Heavy Chain

Kronert et al. (page 989) use genetic analysis to identify interactions between troponin I and other muscle proteins during muscle contraction. They screened Drosophila melanogaster for mutations that suppress the held-up wing position of the troponin I hdp2 mutation, which produces a degenerative muscle syndrome. This approach revealed four genetic suppressors, all of which are located on chromosome 2.

Genetic complementation tests with known myosin heavy chain alleles indicated that the four mutations are alleles of myosin heavy chain (Mhc), and cloning and sequencing of the Mhc gene from each of the mutants revealed that these mutations are all located in the head of this thick-filament molecule. Genetic studies also indicated that specific changes in Mhc structure, as opposed to changes in myosin function or levels, account for suppression of the held-up wing phenotype. The degree of genetically determined muscle dysfunction in this model can be increased or decreased by mutations in other proteins involved in muscle contraction. Such a system could provide an opportunity to identify how the interactions of genes might influence the severity of disease in other organisms.

Cell Division in Worms

Gönczy et al. (page 927) use a classical microscopy technique, video-enhanced, time-lapse Nomarski DIC microscopy, to examine cell division processes in their large scale mutational analysis of the one-cell stage of Caenorhabditis elegans.

"We have a direct way of looking at cell division processes in great detail as they are happening, without fixing the cell," says first author Pierre Gönczy. The method provides a straightforward way to screen worm mutants—in this instance, 160 maternal-effect, embryonic-lethal mutations on chromosome III—and determine their phenotypes. Among the 48 mutations at 34 loci, some cause no apparent defect in the microtubule-dependent processes that precede spindle assembly, but mutants fail to form a bipolar spindle. Others are specifically defective in cytokinesis. The loci have already been mapped by deficiencies at dis-
crete regions of chromosome III; the next step will be to identify individual genes at these loci.

Modulating Morphogenic Proteins

Bone is deposited on cartilage during endochondral ossification, so cartilage formation determines the pattern of the developing skeleton. Type II collagen is the major extracellular matrix protein in cartilage, and it may also function in induction of chondrogenesis. It is formed from one of two precursors: type IIA and type IIB. Type IIA procollagen contains an additional 69 amino acid, cysteine-rich domain at its amino terminus relative to type IIB.

Zhu et al. (page 1069) now provide evidence that the additional amino acid domain in type IIA procollagen has a significant role in chondrogenesis. Using antibodies directed against three domains of type IIA procollagen, they detect the NH₂-terminal propeptide, the fibrillar domain, and the COOH-terminal propeptides of the molecule as cartilage is deposited during bone development. Immunoelectron microscopy with antibodies to the NH₂-terminal propeptide indicated that type IIA procollagen fibrils are present in the extracellular matrix. Recombinant NH₂-terminal propeptide binds to TGF-β1 and BMP-2, which are both capable of inducing bone formation, but not to bFGF or IGF-1, which are also involved in chondrogenesis.

The findings support the suggestion that the type IIA NH₂-terminal propeptide modulates the function of morphogenetic proteins during chondrogenesis. A similar protein domain in the Xenopus protein Chordin, and the Drosophila protein Sog, binds to morphogenic factors (BMP-4 and Decapentaplegic, respectively) to establish the embryonic dorsal-ventral axis. These findings suggest a novel function for the collagen II NH₂-terminal propeptide and may establish a mechanistic paradigm for the regulation of pattern formation in the basic body plan and in the skeleton.

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