the physiology, biochemistry, and cellular processes that allow the rapid conversion and movement of protoplasm necessary for both gametagenesis and nocturnal growth are unknown. Clifton's (*4*) investigation begins to fill this void and also documents a phenomenon of plant behavior that was previously unknown for seaweeds. This system offers interesting parallels and contrasts with other synchronized events such as mass spawning in corals, mass seed set in bamboo, and temporal release of pheromones in insects.

As a final note, although society has become increasingly aware of how ecological and environmental knowledge influences our lives, the facilities and opportunities for conducting long-term field investigations are very limited. The Smithsonian Tropical Research Institute in Panama supported Clifton's work, and it is one of the few places where in-depth field investigations can still be conducted. Such facilities and institutions are valuable resources that should be recognized and supported.

An enhanced version of this Perspective, with live links, can be seen in *Science* Online at http://www.sciencemag.org/

References

1. L. Hillis-Colinvaux, *Adv. Mar. Biol.* **17**, 1 (1980); P. J. Davies and J. F. Marshall, *Proc. 5th Int. Coral Reef Congr.* **5**, 1 (1985).
2. M. E. Hay, *Proc. 8th Int. Coral Reef Symp.*, in press, 1997.
3. V. J. Paul and W. Fenical, *Mar. Ecol. Prog. Ser.* **34**, 157 (1986); M. E. Hay, *J. Exp. Mar. Biol. Ecol.* **200**,103 (1996).
4. K. E. Clifton, *Science* **275**, 1116 (1997).
5. M. E. Hay *et al.*, *Oecologia* **75**, 233 (1988).
6. W. Boland, in *Chemical Ecology*, T. Eisner and J. Meinwald, Eds. (National Academy of Sciences, Washington, DC, 1995), pp. 87–102.
7. R. H. Richmond and C. L. Hunter, *Mar. Ecol. Prog. Ser.* **60**,185 (1990).
8. N. Lindquist and M. E. Hay, *Ecol. Monogr.* **66**, 431 (1996).

CELL BIOLOGY

Controlling Cell Death

Pierre Golstein

In many situations—both normal and pathological—cells die as a result of an orderly, stereotyped cascade of cellular events. On pages 1122, 1126, 1129, and 1132 of this issue (*8*, *9*, *12*, *13*), four reports describe the molecular basis of crucial steps in this cascade. The importance of understanding the basis of this programmed cell death was spectacularly demonstrated recently through the rescue with cell death inhibitors of mice undergoing acute liver destruction (*1*, *2*).

Programmed cell death is genetically determined, as demonstrated in the nematode *Caenorhabditis elegans*. Cell death occurring during development of this worm involves the molecules CED-3 and CED-4, which are necessary for cell death to occur, and CED-9, which protects cells from death. Genetic evidence shows that CED-9 acts as an inhibitor of CED-4 to prevent the death-inducing activity of CED-3 (*3*). In mammals, CED-3 homologs are a family of at least 10 cysteine proteases with aspartate specificity, formerly called the ICE (interleukin-1β–converting enzyme) family, now called caspases (*4*). CED-9 corresponds in mammals to a family with many members (*5*); the prototype is the Bcl-2 molecule; family members will be referred to here as Bcl-2s. The mammalian equivalents of the CED-4 molecule are not yet known.

The programmed cell death cascade can be conveniently divided into several stages (Fig. 1A). Multiple signaling pathways lead from death-triggering extracellular agents to a central control and execution stage. In this stage, the activation of CED-3/caspases occurs, which is controlled by CED-9 /Bcl-2s and presumably also involves CED-4. Bcl-2 is found mainly at the mitochondrial membrane (*6*), which, together with other arguments, suggests that mitochondria may also play a part at this stage (*7*). Downstream, caspase activation leads directly or indirectly to the characteristic "apoptotic" structural lesions accompanying cell death—cytoplasmic and chromatin condensation and DNA fragmentation. The control and execution stage thus requires at least four components, CED-3/caspases, CED-9/Bcl-2s, CED-4, and mitochondria. How do these components interact at the molecular level?

In two papers in this issue (*8*, *9*), the molecular link between Bcl-2s and caspases is shown to involve CED-4 (or its mammalian equivalent). In an impressive paper, Chinnaiyan *et al.* (*8*) initially show that overexpression of *C. elegans* CED-4 in mammalian cells leads to cell death. These transfected cells allowed further investigation on CED-4, getting around the absence of a known mammalian equivalent of CED-4 and of *C. elegans* cell lines. CED-4–induced mammalian cell death can be blocked by the overexpression of Bcl-x_L (one of the death-protective Bcl-2s) and by caspase inhibitors. These results indicate that in mammalian cells CED-4, under the control of Bcl-2s, causes death through caspase activation. Chinnaiyan *et al.* then show by coimmunoprecipitation a direct physical interaction between the proteins encoded by the genes *ced-4* and *ced-9* (or *bcl-x_L*) and the proteins encoded by *ced-4* and *ced-3* (or the mammalian caspases ICE and FLICE, which have a large prodomain, but not CPP32 or Mch-2, which have smaller prodomains). These transfection experiments made clever use of mutant molecules with the binding but not the killing properties of the wild-type molecules, to prevent induction of cell death with its resulting decrease in protein yield.

CED-4 can independently and simultaneously bind both CED-9 and CED-3, as shown by coprecipitation of CED-3 and CED-9 only when CED-4 was also cotransfected. Similar results were obtained for Bcl-x_L and ICE; however, in this case there was no requirement for CED-4, consistent with an endogenous mammalian equivalent of CED-4 that is able to link Bcl-2s and caspases. A reservation is that the techniques used do not allow detection of other molecules that might participate in, and are perhaps required for, the CED-3–CED-4–CED-9 complexes.

Where in the cells do these molecular interactions take place? Wu *et al.* (*9*) show by immunostaining plus confocal microscopy that the expression of CED-4 was diffuse in the cytoplasm when CED-4 was expressed in isolation, whereas it formed a compact granular pattern when CED-9 was coexpressed with CED-4. Similarly, in fractionation experiments CED-4 shifted upon expression of CED-9 from a cytosolic to a membrane-organelle fraction rich in mitochondria. Because Chinnaiyan *et al.* (*8*) showed that the binding of CED-9 to CED-4 did not prevent the binding of CED-4 to CED-3, when CED-9 drags CED-4 to (presumably) mitochondria, it must also drag CED-3 there. While doing this, CED-9 also modifies CED-4 to ultimately prevent CED-3/caspase activation.

These reports, added to the previous genetic, biochemical, and functional data, establish a nicely simple paradigm: CED-9/Bcl-2, at the mitochondrial membrane, binds and modulates CED-4, which in turn binds CED-3/caspases (Fig. 1B). Binding of CED-4 to CED-3/caspases may lead to caspase activation if CED-4 is not bound by CED-9/Bcl-2.

The author is at the Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France. E-mail: golstein@ciml.univ-mrs.fr

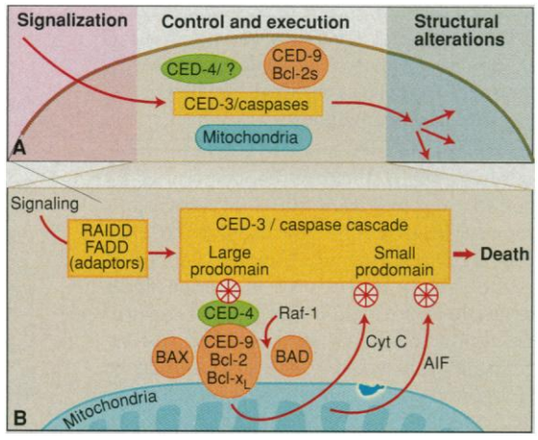
To this simple scheme, other observations add some complexity. For instance, Bcl-2 appears to drag to mitochondria also the Raf-1 kinase. BAD, yet another Bcl-2 family member, which is proapoptotic through heterodimerization with Bcl-2 or Bcl-x_L, can be phosphorylated by Raf-1 (*10*) at the mitochondrial membrane. Phosphorylated BAD cannot heterodimerize with Bcl-2, allowing Bcl-2–driven survival (*11*). Thus posttranslational modifications of some Bcl-2s, in a mitochondrial environment, can regulate cell death.

Two other reports in this issue reveal that CED-4 may not be the only intermediate between Bcl-2s and caspases. Cytochrome c (Cyt c) released from mitochondria (*12, 13*) may also be critical. In mammalian cells, there is a detectable shift of Cyt c from mitochondria to cytosol, as soon as 1 hour after the induction of cell death, together with caspase activation and cell death. Translocation of Cyt c from mitochondria to cytosol was blocked by overexpression of Bcl-2. Cyt c was released from isolated mitochondria, but much less was released from mitochondria isolated from cells overexpressing Bcl-2. The Cyt c thus released was required, together with other cytosolic factors, for subsequent caspase activation. Inhibitors of caspase activation did not block the release of Cyt c from mitochondria but blocked subsequent caspase activation and cell death.

In another cell-free system in which *Xenopus* egg extracts were used (*13*), Kluck *et al.* incubated mitochondria in cytosol, which led within 3 hours to the appearance of Cyt c in the cytosol, caspase activation, and apoptotic effects. Baculovirus-expressed Bcl-2 added to the mitochondrial fraction blocked Cyt c release, caspase activation, and the apoptotic effects. Bcl-2 had no effect when added after Cyt c release. This showed that Bcl-2–regulated Cyt c release is required for caspase activation and the apoptotic effects. The effect of added Bcl-2 was directly on the mitochondria, rather than on a cytosolic step upstream of the mitochondria. Indeed, the regulation by Bcl-2 of Cyt c release is a strong point in favor of the biological significance of this release. It would be of interest to know whether moieties other than Cyt c can be released from mitochondria under similar experimental conditions, and whether this is also under Bcl-2 control. The mechanisms by which Cyt c is released from mitochondria and by which this process is blocked by Bcl-2 are not known.

Another possible indirect connection between Bcl-2s and caspases via mitochondria has been suggested (*7*). A loss of mitochondrial membrane potential occurs during cell death. This permeability transition is under Bcl-2 control. The permeability transition leads to the release from mitochondria to the cytosol of a molecule, called AIF (apoptosis-inducing factor), apparently itself a caspase-inhibitor–sensitive protease, causing caspase activation and cell death. Interestingly, the permeability transition can be blocked by caspase inhibitors under certain circumstances, suggesting that it may require the first steps of the caspase activation cascade (*7*). However, in certain experimental systems, the permeability transition may occur more than 4 or 5 hours after the death-inducing signal (*12, 13*).

Bcl-2s thus clearly control, directly or indirectly, caspase activation, although this may not account for all possible effects of Bcl-2s on cell death. Bcl-x_L by itself is able to form ion channels in membranes (*14*), and hyperexpression of BAX can lead to cell death that does not require caspase activation (*15*). These effects of Bcl-2s may parallel those of perforin, a channel-forming molecule in granules of cytotoxic T cells. When cytotoxic T cells kill a target cell, granule exocytosis leads to the presence of perforin near the target cell. At high concentrations, perforin is able to destroy the target cell by itself, leading to necrotic rather than apoptotic cell death. At lower, sublytic concentrations, perforin seems to "help" another granule protein, the serine esterase granzyme B, to leave unidentified target cell organelles to activate caspases, which subsequently leads to death (*16, 17*). Thus, there is a teasing similarity between Bcl-2s and perforin. Both Bcl-2s and perforin are channel-formers, both can trigger by themselves cell death at high concentrations, and both at lower concentrations can lead to the release from intracellular organelles of molecules (Cyt c and granzyme B, respectively) that will lead to caspase activation and cell death. Perhaps this reflects a more general cell-death paradigm in which one molecule causes the release of another from a sequestering organelle, allowing it to execute its function.



Schematic representation of the programmed cell death cascade. (**A**) The three main stages of programmed cell death. (**B**) The control and execution stage. Members of the Bcl-2 family (for example, Bcl-2, Bcl-x_L, CED-9, BAX, and BAD), on mitochondria, interact with themselves. Bcl-2, Bcl-x_L, and CED-9 interact with CED-4. CED-4 interacts with members of the caspase family (CED-3 and caspases with large prodomains) and triggers or relays the caspase activation cascade primed through upstream signaling (via connecting molecules such as FLICE or RAIDD). A hypothesis, represented here, is that this cascade is further amplified through the effects of cytochrome c and AIF released from mitochondria, which lead to structural alterations and cell death. Starred wheels, possible sites of caspase activation.

If all of these results are reproducible and general, between Bcl-2 and caspase activation, there are at least three possible links, involving CED-4, Cyt c, or AIF. Assuming that these links are independent, they may act in parallel, adding to the apparent molecular redundancy that seems to be the rule for mammalian cell death. Alternatively, they may act in part sequentially (Fig. 1B). Each of these pathways would be sufficient to trigger caspase activation on its own under artificial circumstances such as overexpression. However, under physiological conditions each may be limiting. There might thus be a series of activating inputs on the caspase pathway, via upstream connecting molecules CED-4, Cyt c, and AIF. These inputs may act as successive reinforcements—sequential, additive activation inputs along the caspase cascade.

The data presented in this issue position key players of the control and execution stage of the cell death cascade. They pave the way to finding out how exactly these players affect one another and how they function, and to identifying the mammalian homolog of CED-4.

References

1. I. Rodriguez *et al.*, *J. Exp. Med.* **183**, 1031 (1996).
2. N. Rouquet, J. C. Pagès, T. Molina, P. Briand, V. Joulin, *Curr. Biol.* **6**, 1192 (1996).
3. S. Shaham and H. R. Horvitz, *Genes Dev.* **10**, 578 (1996).
4. E. S. Alnemri *et al.*, *Cell* **87**, 171 (1996).
5. E. White, *Genes Dev.* **10**, 1 (1996).
6. D. Hockenbery, G. Nunez, C. Milliman, R. D. Schreiber, S. J. Korsmeyer, *Nature* **348**, 334 (1990).
7. G. Kroemer, N. Zamzami, S. A. Susin, *Immunol. Today* **18**, 44 (1997).
8. A. M. Chinnaiyan, K. O'Rourke, B. R. Lane, V. M. Dixit, *Science* **275**, 1122 (1997).
9. D. Wu, H. D. Wallen, G. Nuñez, *Science* **275**, 1126 (1997).
10. H.-G. Wang, U. R. Rapp, J. C. Reed, *Cell* **87**, 629 (1996).
11. J. Zha, H. Harada, E. Yang, J. Jockel, S. J. Korsmeyer, *ibid.*, p. 619.
12. J. Yang *et al.*, *Science* **275**, 1129 (1997).
13. R. M. Kluck, E. Bossy-Wetzel, D. R. Green, D. D. Newmeyer, *Science* **275**, 1132 (1997).
14. A. J. Minn *et al.*, *Nature* **385**, 353 (1997).
15. J. L. Xiang, D. T. Chao, S. J. Korsmeyer, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 14559 (1996).
16. S. J. Martin *et al.*, *EMBO J.* **15**, 2407 (1996).
17. C. J. Froelich *et al.*, *J. Biol. Chem.* **271**, 29073 (1996).