put and productivity, compared with sampling in a discrete mode. Furthermore, an operator needs to be present only for the 5 minutes that it takes to change the u-channel sample. Under these circumstances, it is not difficult to process 10 m of sediment a day, and it becomes feasible to undertake paleomagnetic studies that involve many tens of meters or even a few hundred meters of core.

As with other major technological advances, the revolutionary capabilities of the long-core cryogenic magnetometer are the result of a series of incremental steps involving improvements in the design and operation of cryogenic magnetometers (*1*), development of the u-channel sampling technique (*2*, *3*), and careful validation of the scientific results (*4*). The latest new magnetometers can impart different types of laboratory-induced magnetizations to a demagnetized sample. The demagnetization of the new magnetizations provides additional information about the nature of the magnetic carriers (*5*, *6*).

There are two main areas for which these capabilities are particularly useful. The first is for studies of the behavior of the geomagnetic field. Sediments contain the most continuous record of geomagnetic field behavior, but in earlier studies the time involved in making measurements compromised the number of samples and the closeness of those samples. With long-core magnetometers, one obtains a complete, continuous record of geomagnetic field behavior so that one can see the field evolve as it changes direction.

More importantly, long-core magnetometers are leading to an appreciation of the vector nature of the paleomagnetic signal. Until quite recently, the focus of most paleomagnetic studies was on the directional record of the geomagnetic field. Except for time-consuming studies that were specifically designed to determine the absolute paleomagnetic intensity, little attention was paid to the intensity of magnetization of individual samples. Recently, there have been substantial advances in methods for determining the relative paleointensity record in sedimentary sequences (*7*, *8*). The basic approach is to use measurements of laboratory-induced magnetizations to separate the variations in magnetization intensity that are due to concentration from those that are due to actual intensity variations of the original geomagnetic field. The new methods are particularly suited for studies involving u-channels, and considerable attention is currently focused on this area. Although some of the results are quite controversial, there is certainly more interest now in the vector nature of the geomagnetic field than there has been since the inception of paleomagnetism almost 50 years ago. Moreover, there is growing evidence that certain changes in the directional record of the geomagnetic field are coupled to changes in the intensity record (*9*–*11*), making it all the more important to deal with the paleomagnetic signal as a fully three-dimensional vector.

The second area of application for long-core measurements of u-channels is environmental magnetism, a new and rapidly evolving field that makes use of the influence of environmental processes on the size and nature of the magnetic carriers (*12*). The advantages of this approach are that the methods are relatively rapid, simple, nondestructive, and inexpensive and that they can be used to address problems that may be inaccessible with other techniques. The methods of environmental magnetism have been applied to studies of catchment areas, sediment transport, diagenesis, paleoseismology, paleoclimate reconstruction, and various types of pollution. Before the development of the long-core magnetometer, the limiting factor in many environmental magnetic studies was the time required to make a wide range of magnetic measurements on a large number of discrete samples. With the time constraint removed, the field is capable of reaching its full research potential.

Of course, every technological advance creates new problems and challenges. Long-core magnetometers are capable of creating enormous databases. In one study in my laboratory, we have already amassed over 1 million vector measurements. Standard methods for display and analysis of paleomagnetic data, developed for studies that might involve at most a few thousand measurements, are clearly inadequate for the new databases, and more advanced computer graphics techniques need to be developed. These methods will probably lead to insights about the use and interpretation of paleomagnetic data that, in turn, will spur additional advances in paleomagnetic technology.

References

1. W. S. Goree and M. Fuller, *Rev. Geophys. Res.* **77**, 1534 (1976).
2. L. Tauxe, J. L. LaBrecque, R. Dodson, M. Fuller, *Eos* **64**, 219 (1983).
3. R. J. Weeks *et al.*, *Geophys. J. Int.* **114**, 651 (1993).
4. E. A. Nagy and J.-P. Valet, *Geophys. Res. Lett.* **20**, 671 (1993).
5. C. Richter, A. P. Roberts, J. S. Stoner, L. D. Benning, C. T. Chi, *Proc. Ocean Drill. Program Sci. Results*, in press.
6. J. S. Stoner, C. Richter, A. P. Roberts, *ibid.*, in press.
7. J. King *et al.*, *J. Geophys. Res.* **88**, 5911 (1983).
8. L. Tauxe, *Rev. Geophys.* **31**, 319 (1993).
9. J.-P. Valet and L. Meynadier, *Nature* **366**, 234 (1993).
10. K. L. Verosub *et al.*, *Geophys. Res. Lett.* **23**, 467 (1996).
11. J. E. T. Channell and B. Lehman, *Nature* **389**, 712 (1997).
12. K. L. Verosub and A. P. Roberts, *J. Geophys. Res.* **100**, 2175 (1995).

PERSPECTIVES: APOPTOSIS

Death by Crowd Control

Michael Hengartner

Animals use apoptosis, or programmed cell death, to eliminate extraneous or dangerous cells. The muscle of this controlled cellular deconstruction is provided by the caspase family of cysteine proteases, which cleave key targets in the cell [(*1*), see page 1312]. Caspases normally exist in cells as inactive proenzymes; proteolytic processing at a few specific sites unleashes their latent enzymatic activity and triggers cell destruction.

So how is this proteolytic activation brought about? There are many caspases in mammals (13 at last count), and even more ways to kill a cell, which initially raised the specter of a vast army of caspase-activating mechanisms. Thankfully, recent work by a number of groups, including a report by Yang *et al.* in this issue of *Science* on page 1352 (*2*), suggests that the cell uses only a limited number of strategies, which are mined over and over. One of these is the use of "apoptotic chaperones," which herd together inactive proenzymes to increase their local concentration and ease them into conformations that promote their activation.

Caspases can be activated by two distinct mechanisms. Because all caspases have similar cleavage specificity, the simplest way to activate a procaspase is to expose it to a previously activated caspase molecule. This "caspase cascade" is used extensively by cells for the activation of the downstream effector caspases: caspase-3, caspase-6, and caspase-7 (*1*).

The second strategy, "induced proximity," was first observed in caspase-8, an initiator caspase that acts downstream of the Fas/CD95 death receptor [(*3*), see page 1305]. Upon ligand binding, Fas receptor molecules aggregate into a membrane-bound complex. This signaling complex recruits, via the receptor-bound adapter protein FADD, several procaspase-8 molecules, resulting in a high local concentration of procaspase-8. Under these conditions, the low protease activity inherent to procaspases is sufficient to drive intermolecular prote-

The author is at the Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA. E-mail: hengartn@cshl.org

olytic activation of the receptor-associated procaspase-8 molecules (*1*).

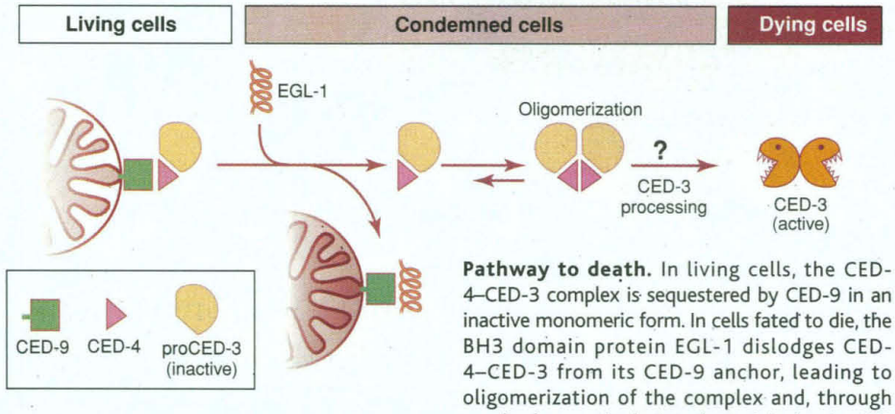
Are all initiator caspases activated by a proximity-induced mechanism? Artificial crowding, either by overexpression or forced aggregation, will also activate other procaspases—but these conditions do not necessarily reflect what goes on in the cell (*1*). However, two recent reports suggest that proximity-induced activation is also used to activate at least two other caspases: caspase-9 and the nematode caspase homolog CED-3 (*2*, *4*).

The cell death pathway in *Caenorhabditis elegans* is in many ways a miniature version of the mammalian apoptotic pathway. Genetic analysis has identified three key worm cell death proteins. Each of these has at least one functional equivalent in mammals: CED-9 is a member of the Bcl-2 family of apoptosis regulators [(*5*), see page 1322], CED-4 is homologous to Apaf-1, and CED-3 is the prototypical worm caspase (*6*). Biochemical experiments have suggested that CED-4 can interact with both CED-9 and proCED-3. Free CED-4 promotes CED-3 autoprocessing, whereas CED-9–bound CED-4 can still bind proCED-3, but not activate it. In living cells, the three proteins have been postulated to be locked into an inactive ternary complex, the apoptosome (see the figure). In cells fated to die, a pro-apoptotic stimulus, possibly mediated by the recently identified BH3 domain protein EGL-1 (*7*), induces a chain of events that likely include dissociation of CED-4–CED-3 from CED-9 and ultimately activation of CED-3.

How does CED-4 promote CED-3 processing? Yang *et al.* suggest that it does so by promoting proCED-3 aggregation (*2*). The authors made the important discovery that CED-4 interacts not only with CED-9 and proCED-3, but also with itself. Although many of the biochemical details of this interaction are still sketchy (What is the stochiometry? Is the interaction direct, or mediated by a cytosolic factor?), its functional implications are clear: By interacting at the same time with proCED-3 and with another CED-4 molecule, CED-4 brings multiple proCED-3 molecules into close apposition—the hallmark of induced proximity caspase activation. Supporting the idea that CED-4 oligomerization is biologically relevant, Yang *et al.* found that point mutations that prevent CED-4 oligomerization eliminate its pro-apoptotic activity.

For this model to function, CED-4 oligomerization must be regulated, because CED-3 should be activated only in doomed cells. Indeed, Yang *et al.* found that CED-9–bound CED-4 could not oligomerize, either because of steric hindrance or a CED-9–induced conformational change. This observation nicely explains how CED-9 inhibits CED-4 activity: By preventing CED-4 from oligomerization, CED-9 keeps proCED-3 in a safe, monomeric state (see the figure).

Are any of these wormy data relevant to mammals? Indeed, a similar story has been unfolding with Apaf-1, the mammalian homolog of CED-4. Like its worm counterpart, Apaf-1 promotes caspase activation and has been reported to interact with both Bcl-x_L and caspase-9 (homologs of CED-9 and CED-3, respectively). In a recent paper in *Molecular Cell* (*4*), Srinivasula *et al.* extend the similarity between CED-4 and Apaf-1 one step further by demonstrating that a truncated form of Apaf-1, which contains only the domain homologous to CED-4, is constitutively active and contains all the elements necessary for caspase-9 activation. Truncated Apaf-1 oligomerizes and thereby brings together multiple procaspase-9 molecules. Thus, induced proximity is likely also to participate in the activation of caspase-9.



Pathway to death. In living cells, the CED-4–CED-3 complex is sequestered by CED-9 in an inactive monomeric form. In cells fated to die, the BH3 domain protein EGL-1 dislodges CED-4–CED-3 from its CED-9 anchor, leading to oligomerization of the complex and, through poorly characterized steps that might involve ATP hydrolysis, intermolecular processing of CED-3.

Nevertheless many questions remain. First and foremost, is the function of CED-4 and Apaf-1 solely to generate a high local concentration of procaspase, or do they have a more involved role? Several observations point toward the latter. For example, several domains in CED-4 bind to CED-3, and mutations that result in the loss of any one of these interaction points prevent CED-3 activation, even though they do not affect the overall ability of CED-4 to interact with CED-3 (*8*). Similarly, Srinivasula *et al.* reported that although Apaf-1 can interact with and recruit a chimeric procaspase that contains the prodomain of caspase-9 linked to caspase-3, it cannot activate this chimera (*4*). The moral: being close might not always be enough.

The current models also fail to account for the presence of a nucleotide-binding domain in both CED-4 and Apaf-1. Surely this domain, presumed to have ATPase activity, must serve some function. Indeed, CED-4L, an anti-apoptotic alternative splice form of CED-4 that contains a 24–amino acid insertion in the nucleotide-binding domain, has an interaction pattern similar to that of normal CED-4, but it cannot activate CED-3 (*2*, *9*). Similarly, in the absence of ATP, truncated Apaf-1 can induce procaspase-9 processing, but the cleaved protease is unable to process procaspase-3, its normal substrate (*4*). These results suggest that activation of both CED-3 and caspase-9 might require an ATP hydrolysis–induced conformational change. Whether this conformational change is in the caspase (to promote processing) or in the activator (to promote release of the cleaved enzyme) is open to debate and to future experimental probing.

From this angle, the known function and proposed mechanism of action of CED-4 and Apaf-1 are strikingly similar, conceptually, to the roles of chaperones such as the heat-shock proteins. The addition of an energy-dependent step presumably decreases the probability of stochastic, inadvertent activation of caspases in normal cells, while still allowing for precise and efficient activation once the decision to die is made. Whether activation of the remaining caspases is under similar regulation remains to be determined. But in nature, a good idea is seldom used just once.

References

1. N. A. Thornberry and Y. Lazebnik, *Science* **281**, 1312 (1998).
2. X. Yang, H. Y. Chang, D. Baltimore, *ibid.*, p. 1355.
3. A. Ashkenazi and V. M. Dixit, *ibid.*, p. 1305.
4. S. M. Srinivasula, M. Ahmad, T. Fernandes-Alnemri, E. S. Alnemri, *Mol. Cell* **1**, 949 (1998).
5. J. M. Adams and S. Cory, *Science* **281**, 1322 (1998).
6. M. E. Peter, A. E. Heufelder, M. O. Hengartner, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 12736 (1997).
7. B. Conradt and H. R. Horvitz, *Cell* **93**, 519 (1998).
8. D. Chaudhary, K. O'Rourke, A. M. Chinnaiyan, V. M. Dixit, *J. Biol. Chem.* **273**, 17708 (1998).
9. A. M. Chinnaiyan, K. O'Rourke, B. R. Lane, V. M. Dixit, *Science* **275**, 1122 (1997).