recipient. Four studies were published which were heterogeneous in their findings (Glaser et al., 2002; Laflamme et al., 2002; Muller et al., 2002; Quaini et al., 2002). Two studies found ample evidence of Y chromosome-bearing cardiac myocytes in the transplanted hearts. A second study found this to be a very rare finding and questioned its significance. A third report found no evidence of Y chromosome-bearing cardiac myocytes. Multiple editorials then appeared, with much finger pointing about these discrepancies and the various parties each claiming that their own techniques were superior. The source of this exceptional furor appears to lie in the potential for medical therapy that these studies hold. The source of the cells from the various recipients remains unknown.

In addition to circulating stem cells, there has been considerable interest in the existence of resident stem cells in tissues other than the bone marrow. The article by Beltrami et al. in this issue of *Cell* provides convincing evidence that there is a stem cell population that resides in the adult heart. The stem cells can be isolated and expanded in culture indefinitely. The cells were identified and then isolated from the hearts of older adult rats (20–23 months of age). They are characterized based on the following pattern of cell surface markers: Lin−, c-Kit+, CD45−, CD34−. These cells were able to differentiate into cardiac myocytes, smooth muscle cells, and endothelial cells in culture. However, in culture, the "differentiated" cells had an immature phenotype. To test whether these cells could achieve full mature differentiation in vivo, they were injected into the myocardium of rats subjected to a myocardial infarction. These cells formed new myocardium and the hearts exhibited functional improvement. In fact, the average infarct size was greater in the treated animals than the controls, most likely because untreated animals did not survive with infarcts as large as those in the treated group. The cells were also able to form endothelial and smooth muscle structures. One potential caveat for these findings would be if the stem cells had fused with existing host cells—this might appear to be differentiation when, in fact, it would be hybrid cells giving the appearance of differentiation. However, the Beltrami et al. study ruled this out by a number of criteria, including showing that the number of new myocytes is orders of magnitude higher than the injected cells and stating that the DNA content of the new cells is diploid and not tetraploid. The cells purified from the adult rat heart satisfy all of the properties of cardiac stem cells. They are clonogenic, self-renewing, and able to give rise to at least three different cell types. Finally, they participate in the formation of new, functional myocardium.

Despite the convincing nature of this study, there are numerous interesting questions to be pursued. Multipotent adult progenitor cells, or MAPCs, which have also been shown to have cardiogenic potential, are characterized as being negative for the marker (c-Kit) used to define the cells in the Beltrami et al. study. In addition, the fact that the adult stem cells in this study can form endothelium, smooth muscle, and cardiac muscle is confounding, since these three cell types arise from three different cell lineages. Finally, if these cells exist and lie dormant in the heart, why do they not mobilize and divide in response to an injury? The answers to these questions will certainly make for some interesting biology and perhaps future therapies.

Leslie A. Leinwand
Department of Molecular, Cellular, and Developmental Biology
University of Colorado
Boulder, Colorado 80309

Selected Reading

Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., et al. (2003). Cell *114*, this issue, 763–776.

Glaser, R., Lu, M.M., Narula, N., and Epstein, J.A. (2002). Circulation *106*, 17–19.

Jackson, K.A., Majka, S.M., Wang, H., Pocius, J., Hartley, C.J., Majesky, M.W., Entman, M.L., Michael, L.H., Hirschi, K.K., and Goodell, M.A. (2001). J. Clin. Invest. *107*, 1395–1402.

Krause, D.S., Theise, N.D., Collector, M.I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., and Sharkis, S.J. (2001). Cell *105*, 369–377.

Laflamme, M.A., Myerson, D., Saffitz, J.E., and Murry, C.E. (2002). Circ. Res. *90*, 634–640.

Leferovich, J.M., Bedelbaeva, K., Samulewicz, S., Zhang, X.M., Zwas, D., Lankford, E.B., and Heber-Katz, E. (2001). Proc. Natl. Acad. Sci. USA *98*, 9830–9835.

Muller, P., Pfeiffer, P., Koglin, J., Schafers, H.J., Seeland, U., Janzen, I., Urbschat, S., and Bohm, M. (2002). Circulation *106*, 31–35.

Mummery, C., Ward-van Oostwaard, D., Doevendans, P., Spijker, R., van den Brink, S., Hassink, R., van der Heyden, M., Opthof, T., Pera, M., de la Riviere, A.B., et al. (2003). Circulation *107*, 2733–2740.

Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D.M., Leri, A., and Anversa, P. (2001a). Proc. Natl. Acad. Sci. USA *98*, 10344–10349.

Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S.M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D.M., et al. (2001b). Nature *410*, 701–705.

Quaini, F., Urbanek, K., Beltrami, A.P., Finato, N., Beltrami, C.A., Nadal-Ginard, B., Kajstura, J., Leri, A., and Anversa, P. (2002). N. Engl. J. Med. *346*, 5–15.

Apoptosis: A Process with a (β)NAC for Complexity

Most programmed cell deaths in the nematode *C. elegans* require *ced-3* caspase activity. In a recent paper, Bloss et al. (2003) reveal a new *C. elegans* death inhibitor, *icd-1*, whose loss can promote apoptosis independently of *ced-3*.

Apoptosis (or programmed cell death), a ubiquitous metazoan cell death process, is crucial for proper organismal development, maintenance of cell number homeostasis, and elimination of diseased or otherwise harmful cells. In many instances, organismal life is impossible in the absence of the machinery for cell death. Genetic studies in model organisms have proven a fertile ground for the discovery and subsequent study of genes that regulate apoptosis. Pioneering work by Horvitz and colleagues in the 1980s identified three genes essential for

proper control of virtually all programmed cell deaths in the nematode *C. elegans*. These genes, *ced-3*, *ced-4*, and *ced-9*, were shown to act in a genetic pathway, such that *ced-9* inhibits *ced-4*, *ced-4* activates *ced-3*, and *ced-3* promotes cell death (Figure 1; Metzstein et al., 1998). Thus, *ced-4* and *ced-3* are proapoptotic genes, whereas *ced-9* is antiapoptotic. An avalanche of studies in the 1990s confirmed that the *C. elegans* apoptosis genes and aspects of their interactions were conserved in vertebrates and in the fruit fly. *bcl-2* and related genes were found to be similar to *ced-9*, the Apaf-1 adaptor gene resembled *ced-4*, and *ced-3* was shown to encode a caspase protease. These studies suggested the existence of a core pathway regulating apoptosis in all metazoans.

Despite the molecular similarities of death genes across the animal kingdom, differences in the apparent regulation of the death process were also quickly recognized. For example, in the fruit fly, a cascade of protein interactions and degradations involving the reaper/hid/grim and IAP proteins was shown to be at least as important in regulating caspase activity as the core apoptotic pathway (Ryoo et al., 2002). In vertebrates, a set of mitochondrial factors, including cytochrome C, were shown to regulate cell death (Wang, 2001). Neither of these modes of regulation was recognized in *C. elegans*, raising the possibility that independent mechanisms for regulating cell death may have evolved in different organisms.

But was apoptosis understood enough to draw such a conclusion, or were researchers poking at the same process but from different angles (much like the story of the blind men and the elephant)? Several studies suggested that the *C. elegans* pathway was incomplete. For example, localization studies of the proteins encoded by *ced-9* and *ced-4* demonstrated that both were localized to mitochondria in living cells, and that the CED-4 protein translocated to nuclear membranes in dying cells (Chen et al., 2000). These observations suggested that mitochondrial regulation of cell death may be important in *C. elegans* after all. Even more puzzling were observations indicating that cell death in *C. elegans* could proceed in the absence of functional *ced-3* caspase. Ellis and Horvitz (1986) showed that death of the *C. elegans* male linker cell could occur as scheduled even in animals harboring a mutant *ced-3* gene. Another study showed that cell death induced by overexpression of *ced-4* could only be partially suppressed in a *ced-3* mutant background (Shaham and Horvitz, 1996). And yet another study revealed that a small but consistent number of programmed cell deaths still occurred in embryos lacking *ced-3* caspase activity (Shaham et al., 1999).

The inability to reconcile these observations with the notion of a single core pathway that regulates apoptosis suggests that our understanding of cell death regulatory pathways may be inadequate to argue that the control of the process evolved separately in different organisms. A recent paper by Bloss et al. (2003) not only confirms that cell death regulation in nematodes is complex, but also introduces a new player into this complexity.

Bloss et al. discovered that inactivation of a *C. elegans* gene they named *icd-1* (*i*nhibitor of *c*ell *d*eath-*1*) using the method of RNA interference (RNAi) could result in one of two phenotypes: early embryonic arrest with no evidence of programmed cell death, or accumulation of large numbers of dying cells in animals that developed past the early embryo stage. The authors showed that at least some of the extra dying cells they observed in *icd-1*(RNAi) embryos should have normally survived. Thus, similarly to *ced-9*, *icd-1* is an antiapoptotic gene in *C. elegans*. The authors support this conclusion by demonstrating that global overexpression of *icd-1* can prevent normally occurring developmental cell deaths.

The early embryonic lethality of *icd-1*(RNAi) mutant animals could not be prevented by eliminating *ced-3* or *ced-4* functions in these embryos, suggesting that *icd-1* may have other essential, nonapoptotic activities. However, the excessive number of dying cells in *icd-1*(RNAi) embryos that progressed beyond early development could be clearly prevented by eliminating *ced-4* function, suggesting that *icd-1* may normally engage the core apoptotic machinery to prevent the deaths of cells that should normally live. Surprisingly, however, eliminating *ced-3* activity prevented excessive cell death in *icd-1*(RNAi) embryos just beginning to undergo morphogenesis, but did little to prevent excessive cell death in more mature embryos and larvae.

icd-1 encodes the *C. elegans* version of the β subunit of the nascent-polypeptide associated complex (NAC). The function of this complex is not very well understood; however, there is evidence that it is involved in targeting of actively translated proteins to appropriate cellular locations. Specifically, targeting of some proteins to mitochondria may be regulated by the NAC (Rospert et al., 2002). Consistent with this, Bloss et al. show that ICD-1 protein is normally localized to mitochondria.

The studies of *icd-1* add to observations suggesting that alternatives to the core pathway of apoptosis may exist for regulating cell demise in *C. elegans* (Figure 1).

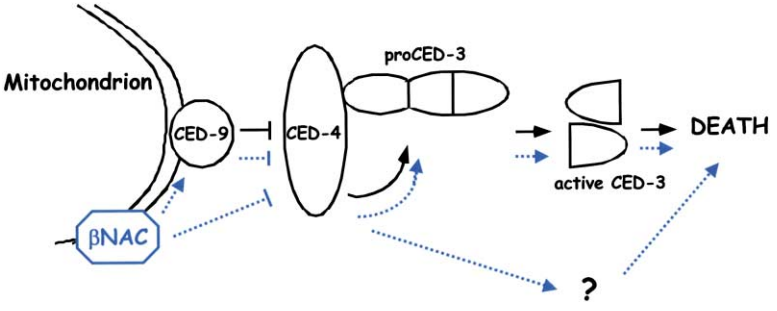

Figure 1. Possible Interactions of the *C. elegans* βNAC with the Core Apoptosis Pathway

Black text and shapes indicate the established core pathway. Blue text and shapes indicate possible interactions of βNAC with this pathway.

The studies also refocus attention on the possible role of mitochondria in *C. elegans* apoptosis. As with clues revealed in the midst of a good mystery novel, the paper by Bloss et al. raises many questions. For example, how does *icd-1* function to inhibit apoptosis? Mutants in the *ced-9* gene have a phenotypic spectrum similar to that of *icd-1* mutants, including early embryonic arrest and excess cell death in embryos that develop further (Hengartner et al., 1992). Thus, one plausible hypothesis is that ICD-1 protein shuttles nascent CED-9 protein on ribosomes to mitochondria. This idea is easily tested by examining CED-9 localization in *icd-1*(RNAi) animals. Another question raised by the *icd-1* studies is how cell death can proceed independently of *ced-3*? Bloss et al. suggest that another of the *C. elegans* caspases (perhaps CSP-1B) may be activated by loss of ICD-1. Alternatively, a novel caspase-independent pathway may exist for killing cells in *C. elegans*. Expression of a pan-caspase inhibitor protein such as the baculovirus p35 protein in *icd-1*(RNAi) mutants might distinguish between these possibilities.

The 2002 Nobel Prize awarded to Sydney Brenner, John Sulston, and Bob Horvitz for their seminal work on the genetic basis of apoptosis in *C. elegans* recognized the efforts of these individuals in opening a new area of study in Biology. Recent work, including the paper by Bloss et al., suggests that we may have only begun to scratch the surface of this important and interesting process.

Shai Shaham
Laboratory of Developmental Genetics
The Rockefeller University
New York, NY 10021

Selected Reading

Bloss, T.A., Witze, E.S., and Rothman, J.H. (2003). Nature *424*, 1066–1071.

Chen, F., Hersh, B.M., Conradt, B., Zhou, Z., Riemer, D., Gruenbaum, Y., and Horvitz, H.R. (2000). Science *287*, 1485–1489.

Ellis, H.M., and Horvitz, H.R. (1986). Cell *44*, 817–829.

Hengartner, M.O., Ellis, R.E., and Horvitz, H.R. (1992). Nature *356*, 494–499.

Metzstein, M.M., Stanfield, G.M., and Horvitz, H.R. (1998). Trends Genet. *14*, 410–416.

Rospert, S., Dubaquie, Y., and Gautschi, M. (2002). Cell. Mol. Life Sci. *59*, 1632–1639.

Ryoo, H.D., Bergmann, A., Gonen, H., Ciechanover, A., and Steller, H. (2002). Nat. Cell Biol. *4*, 432–438.

Shaham, S., and Horvitz, H.R. (1996). Genes Dev. *10*, 578–591.

Shaham, S., Reddien, P.W., Davies, B., and Horvitz, H.R. (1999). Genetics *153*, 1655–1671.

Wang, X. (2001). Genes Dev. *15*, 2922–2933.