an excellent phenocopy of both of the individual knockout strains, showing pronounced lipopolysaccharide resistance, TNF resistance, defective scab formation, and *Listeria* sensitivity. Once more, however, no developmental functions were revealed by any of these experiments.

Therefore, the results of De Togni and co-workers showing a developmental defect in the LT-α knockout mouse are at first surprising. Deletion of the LT-α gene results in a distinctive phenotype, characterized by the absence of lymph nodes and by disordered splenic architecture. Because the two "classical" (55 and 75 kD) TNF receptors cannot be implicated as essential participants in the ontogeny of lymph nodes and spleen, either by gene knockout or by inhibitor gene transduction studies, suspicion immediately centers on the heteromeric ligand (LT-α–LT-β) and its receptor, TNFRrp.

TNFRrp was, before the new findings of Crowe and co-workers, known only as a complementary DNA clone (*24*). Recognized as a member of the TNF receptor family on the basis of homology, it was a receptor in search of a ligand. Now known to be an essential part of the ligand for TNFRrp, LT-β was originally identified as a "tether" for LT-α subunits. This finding explained why a proportion of LT-α is in a cell-bound state—an observation that originally could not easily be reconciled with the fact that the protein, as initially translated, lacks the transmembrane domain that characterizes all other members of the ligand family (*25*).

It now seems likely that TNFRrp serves an essential organogenetic function. But what is the nature of this function? Because mice lacking the LT-α–LT-β heteromer have normal numbers of peripheral blood monocytes, T cells, and B cells, mutation of TNFRrp does not forbid the differentiation of these cells or their survival. Rather, it prohibits their organization to form lymph nodes.

Lymph nodes are structures of mesodermal origin. Lymphocytes are recirculated through the lymph nodes by way of endolymphatic recirculation, a process that depends on homing receptors located on the high venular endothelium of vessels supplying lymph nodes and Peyer's patches (*26*). It is tempting to consider that the LT-α–LT-β heteromer and its receptor may somehow be involved in homing, but much more work remains to establish this. Further, next to nothing is known of the forces that initiate the aggregation of lymphocytes, macrophages, and other cells that form lymph nodes.

The studies of De Togni and co-workers at once underscore the limitations and the power of gene knockout procedures as an analytical technique, particularly when applied in the study of multigene families. Deletion of a single gene may affect the function of more than one protein. As such, the deletion of the LT-α gene has led to the ablation of both the LT-α homotrimer and the LT-α–LT-β heteromer. In contrast, single gene deletion may be insufficient to reveal the function of a protein, given the existence of functionally redundant molecules. Thus, LT-α gene knockout cannot be expected to fully ablate signal transduction through the 55- and 75-kD receptors, which can be triggered by the TNF homotrimer as well as the LT-α homotrimer. One prediction offered by the results of Crowe and De Togni is that deletion of the LT-β gene or the TNFRrp gene, or the use of inhibitor molecules capable of engaging the LT-α–LT-β heteromer would also prevent the formation of lymph nodes.

Is the developmental function of the LT-α–LT-β heteromer and its receptor mediated by the triggering of a signal transduction pathway in the receptor-containing cell, upon a simple mechanical interaction, or upon a combination of the two? Mutational analysis of the TNFRrp cytoplasmic domain might well provide an answer.

References

1. J. T. Browning *et al.*, *Cell* **72**, 847 (1993).
2. P. D. Crowe *et al.*, *Science* **264**, 707 (1994).
3. P. De Togni *et al.*, *ibid.*, p. 703.
4. S. A. Marsters, A. D. Frutkin, N. J. Simpson, B. M. Fendly, A. Ahkenazi, *J. Biol. Chem.* **267**, 5747 (1992).
5. D. W. Banner *et al.*, *Cell* **73**, 431 (1993).
6. H. Engelmann *et al.*, *J. Biol. Chem.* **265**, 14497 (1990).
7. B. C. Trauth *et al.*, *Science* **245**, 301 (1989).
8. N. Itoh *et al.*, *Cell* **66**, 233 (1991).
9. R. A. Heller, K. Song, N. Fan, *Cell* **73**, 216 (1993).
10. L. A. Tartaglia *et al.*, *J. Immunol.* **151**, 4637 (1993).
11. R. Watanabe-Fukunaga, C. I. Brannan, N. G. Copeland, N. A. Jenkins, S. Nagata, *Nature* **356**, 314 (1992).
12. T. Suda, T. Takahashi, P. Golstein, S. Nagata, *Cell* **75**, 1169 (1993).
13. T. Takahashi *et al.*, *ibid.* **76**, 969 (1994).
14. R. C. Allen *et al.*, *Science* **259**, 990 (1993).
15. U. Korthuer *et al.*, *Nature* **361**, 539 (1993).
16. R. Fuleihan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2170 (1993).
17. J. P. DiSanto, J. Y. Bonnefoy, J. F. Gauchat, A. Fischer, G. de Saint Basile, *Nature* **361**, 541 (1993).
18. K.-F. Lee *et al.*, *Cell* **69**, 737 (1992).
19. K. Pfeffer *et al.*, *ibid.* **73**, 457 (1993).
20. J. Rothe *et al.*, *Nature* **364**, 798 (1993).
21. M. Moore and D. Goeddel, personal communication.
22. J. Kolls, K. Peppel, M. Silva, B. Beutler, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 215 (1994).
23. K. Peppel, A. Poltorak, I. Melhado, F. Jirik, B. Beutler, *J. Immunol.* **151**, 5699 (1993).
24. M. Baens *et al.* **16**, 214 (1993); GenBank accession number LO4270.
25. P. W. Gray *et al. Nature* **312**, 721 (1984).
26. J. Michl, Q. Y. Qui, H. M. Kuerer, *Curr. Opin. Immunol.* **3**, 373 (1991).
27. M. J. Eck and S. R. Sprang, *J. Biol. Chem.* **264**, 17595 (1989).
28. M. J. Eck, M. Ultsch, E. Rinderknecht, A. M. de Vos, S. R. Sprang, *ibid.* **257**, 2119 (1992).
29. N. Q. McDonald *et al.*, *Nature* **354**, 411 (1991).

Cell Death Genes: *Drosophila* Enters the Field

Martin C. Raff

Large numbers of apparently healthy cells die during the lifetime of an animal (*1*). These normal cell deaths have a distinctive morphology, called apoptosis: The cell and its nucleus condense and often fragment, and the cell or fragments are rapidly phagocytosed either by neighboring cells or macrophages (*2*). The deaths are thought to be suicides in which the cell activates an intrinsic death program and kills itself; for this reason, the process is often called programmed cell death (PCD) (*3*). PCD is regulated by cell-cell interactions so that the organism can eliminate unwanted cells (*4*). Remarkably, although the death program is a fundamental property of animal cells, it is still not known how the cells die. A significant step toward solving this puzzle is reported in this week's issue of *Science*, in which Steller and his colleagues describe the first gene in the fruit fly *Drosophila* that seems to be specifically involved in PCD (*5*).

The strongest evidence for an intrinsic death program in animal cells originally came from genetic studies in the nematode *Caenorhabditis elegans*. Two genes, *ced-3* and *ced-4*, were shown to be required for the 131 normal cell deaths that occur during the rapid development of the mature hermaphrodite worm, which contains only about 1000 somatic cells (*3*). Both genes must act in the dying cells or their close ancestors, and if either gene is inactivated by mutation, none of the 131 cell deaths occurs. *ced-4* encodes a novel protein (*6*), whereas *ced-3* encodes a protein that is homologous to the mammalian cysteine protease interleukin-1β (IL-1β) converting enzyme (ICE) (*7*), which in macrophages cleaves the IL-1β peptide from a larger pre-

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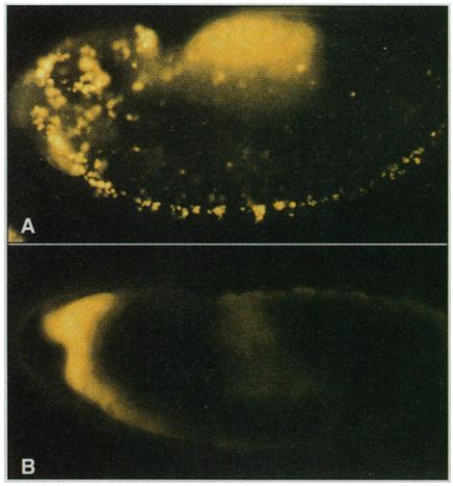
cursor (8). Interestingly, if ICE is overexpressed in rat fibroblasts, it induces them to undergo PCD (9), suggesting that a protease similar to CED-3 and ICE may be involved in PCD in vertebrate cells. This suggestion is supported by the recent report that a specific ICE inhibitor can suppress PCD in chick sensory neurons deprived of nerve growth factor (*10*). It is not clear, however, how either CED-3 (or ICE) or CED-4 contributes to PCD; it is uncertain, for example, whether they are activators or effectors of the death program.

A third C. *elegans* gene, *ced-9*, encodes a protein that inhibits the death program; if it is inactivated by mutation, most of the cells that would normally live now die and the animal dies early in development, but only if both *ced-3* and *ced-4* are functionally intact (*11*). It seems that the only reason that some cells normally survive in the developing worm is that CED-9 keeps the *ced-3*/*ced-4*–dependent death program suppressed in these cells. CED-9 is both structurally (*12*) and functionally (*12, 13*) homologous to the mammalian protein encoded by the proto-oncogene *bcl-2*, which inhibits PCD in many, but not all, mammalian cells (*14*) and, if given the opportunity, can even inhibit PCD in C. *elegans* (*12, 13*). These important findings suggest that both the cell death program and some of the proteins that regulate it have been conserved in evolution from worms to humans.

Until now, such classical genetic approaches to studying the molecular mechanism or mechanisms of PCD have been confined to C. *elegans*. Inexplicably, although PCD is known to be an important feature of normal *Drosophila* development (*15, 16*) and to be increased in many *Drosophila* mutants (*16, 17*), no one had used the power of *Drosophila* genetics to search for genes specifically involved in PCD. Now, as reported on page 677 of this issue of *Science*, Steller and his colleagues have succeeded in doing so, with dramatic results (*5*). They initially examined the pattern of cell death in 129 strains of *Drosophila* embryos homozygous for chromosomal deletions and found three overlapping deletions on the third chromosome that abolish all normal cell deaths (see figure), even though the normal deaths involve multiple cell types and show considerable morphological diversity. They subsequently found another mutation in the same chromosomal region, but without a cytologically obvious deletion, that had the same phenotype. This mutant, called *H99*, dies late in embryogenesis, before hatching, with many extra cells in its nervous system (and presumably elsewhere). Mosaic analysis in the developing eye suggests that the mutation does not impair cell survival, proliferation, or differentiation, suggesting that the lethal effect on the embryo is probably due to the lack of PCD and the consequent interference with development.



Evading the grim reaper. Normal cell death does not occur in the *H99* mutant of *Drosophila*. Normal (**A**) and mutant (**B**) embryos were stained with acridine orange, which specifically labels dying cells.

Steller and his colleagues then cloned the DNA in the region deleted in the *H99* mutant and identified a gene, which they call *reaper* (*rpr*), that is at least partially responsible for the PCD-deficient phenotype; *reaper* complementary DNA, which encodes a novel small protein of 65 amino acids, partially restores PCD in transgenic *H99* embryos. In normal embryogenesis, *reaper* mRNA appears to be specifically expressed in all of the cells destined to die, starting at about an hour or two before they display the morphological features of PCD.

The *H99* mutants are not only deficient in normal cell death. The extra apoptotic cell deaths that are seen in normal embryos after irradiation with low doses (~500 rads) of x-rays or that occur in various developmental mutations in *Drosophila* do not occur in *H99* embryos, indicating that multiple PCD-activating pathways normally converge on a *reaper*-dependent mechanism to induce cell death. Is the Reaper protein, then, part of the effector machinery of the cell death program? A crucial experiment suggests that it is not; if *H99* embryos are blasted with very high doses (4000 rads) of x-rays, small numbers of cells die that have the typical features of apoptosis, suggesting that the effector machinery of the death program is intact in the mutants but that its activation is impaired.

The genetic studies of PCD in C. *elegans* have had an enormous impact on the cell death field. The new studies in *Drosophila* have now substantially extended the findings in the worm. Whereas PCD-deficient mutant worms can live a normal life-span of several weeks (*3*), PCD-deficient mutant flies die before the embryos hatch, suggesting that normal cell death may be required for the construction of a viable animal as complex as a fly (although it is still theoretically possible that the *H99* deletion inactivates a crucial function in addition to PCD and thereby kills the embryo). Moreover, it has so far not been possible to visualize the expression of *ced-3* or *ced-4* in individual cells in developing worms in the way that the expression of *reaper* has been visualized in individual cells in fly embryos; and whereas *reaper* has been placed upstream of the effector machinery of the death program, it is still unclear whether *ced-3* and *ced-4* operate upstream, although this seems likely. It will be important to determine whether homologs of the C. *elegans* cell death genes exist in *Drosophila* and vice versa, and if they do, how they are ordered in the death pathway. The search for vertebrate homologs of *reaper* is no doubt already under way.

Despite the breathtaking pace of PCD research in the past year or two, the effector mechanism or mechanisms that kill the cell have remained elusive; it is not known what proteins lie downstream of Reaper, CED-3, and ICE. Nor is it clear where or how CED-9, BCL-2, and related proteins act to regulate the death pathway. Now that *Drosophila* has entered the field (along with a rapidly increasing proportion of the biomedical research community), progress in understanding how the death program is executed and regulated is bound to accelerate.

References and Notes

1. A. Glucksmann, *Biol. Rev. Camb. Philos. Soc.* **26**, 59 (1951); J. W. Saunders Jr., *Science* **154**, 604 (1966).
2. A. Wyllie, J. F. R. Kerr, A. R. Currie, *Int. Rev. Cytol.* **68**, 251 (1980).
3. R. E. Ellis, J. Yuan, H. R. Horvitz, *Annu. Rev. Cell Biol.* **7**, 663 (1991).
4. M. C. Raff, *Nature* **356**, 397 (1992).
5. K. White *et al.*, *Science* **264**, 677 (1994).
6. J. Yuan and H. R. Horvitz, *Development* **116**, 309 (1992).
7. J. Yuan *et al.*, *Cell* **75**, 641 (1993).
8. D. P. Cerretti *et al.*, *Science* **256**, 97 (1992); N. A. Thornberry *et al.*, *Nature* **356**, 768 (1992).
9. M. Miura, H. Zhu, R. Rotello, E. A. Hartwieg, J. Yuan, *Cell* **75**, 653 (1993).
10. V. Gagliardini *et al.*, *Science* **263**, 826 (1994).
11. M. O. Hengartner, R. E. Ellis, H. R. Horvitz, *Nature* **356**, 494 (1992).
12. M. O. Hengartner and H. R. Horvitz, *Cell* **76**, 665 (1994).
13. D. L. Vaux, I. L. Weissman, S. K. Kim, *Science* **258**, 1955 (1992).
14. D. L. Vaux, S. Cory, J. M. Adams, *Nature* **335**, 440 (1988); T. J. McDonnell *et al.*, *Cell* **67**, 879 (1992); A. Strasser, A. W. Harris, S. Cory, *ibid.*, p. 889; I. Garcia, I. Martinou, Y. Tsujimoto, J.-C. Martinou, *Science* **258**, 302 (1992); J. C. Reed, *J. Cell Biol.* **124**, 1 (1994).
15. F. Giorgi and P. Deri, *J. Embryol. Exp. Morphol.* **35**, 521 (1976).
16. J. M. Abrams, K. White, L. I. Fessler, H. Steller, *Development* **117**, 29 (1993).
17. See references 18 to 21 in (*5*).
18. Supported by a program grant from the Medical Research Council.