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Programmed cell death and the control of cell survival

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SUMMARY

We draw the following tentative conclusions from our studies on programmed cell death (PCD): (i) the amount of normal cell death in mammalian development is still underestimated; (ii) most mammalian cells constitutively express the proteins required to undergo PCD; (iii) the death programme operates by default when a mammalian cell is deprived of signals from other cells; (iv) many normal cell deaths may occur because cells fail to obtain the extracellular signals they need to suppress the death programme; and (v) neither the nucleus nor mitochondrial respiration is required for PCD (or Bcl-2 protection from PCD), raising the possibility that the death programme, like mitosis, is orchestrated by a cytosolic regulator that acts on multiple organelles in parallel.

1. INTRODUCTION

PCD is a fundamental property of animal cells, allowing unwanted cells to be eliminated quickly and neatly (Wyllie *et al.* 1980; Ellis *et al.* 1991). Although the death programme is cell-intrinsic (Wyllie *et al.* 1980; Ellis *et al.* 1991), it is regulated by extracellular signals that can either activate it or suppress it (reviewed in Raff 1992). We have concentrated on signals that suppress PCD and have explored the possibility that most mammalian cells require continuous signalling from other cells to avoid PCD (Raff 1992). Dependence on survival signals would ensure that a cell only survives when and where it is needed, just as dependence on growth factors for proliferation ensures that a cell only divides when a new cell is needed. The importance of such social controls in multicellular organisms is illustrated by the devastating effects of cancer, where the controls are defective.

In this brief review, we first discuss experiments that illustrate the general importance of PCD-suppressing signals and then consider experiments that explore the nature of the death programme itself.

2. PCD IN THE OLIGODENDROCYTE LINEAGE

Oligodendrocytes make myelin in the central nervous system (CNS). Like neurons, they are postmitotic cells that develop from dividing precursors. When either oligodendrocytes or their precursor cells are isolated from the developing rat optic nerve and cultured in the absence of other cell types or exogenous signalling molecules, no matter how high the cell density, they undergo PCD within a day or so (Barres *et al.* 1992). They can be saved by factors released in culture by their normal neighbours (mainly astrocytes) isolated from the optic nerve (Barres *et al.* 1992). They can also be saved by a combination of known growth factors and cytokines. Among the factors that promote the survival of oligodendrocyte lineage cells in culture are platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), neurotrophin-3 (NT-3) and ciliary neurotrophic factor (CNTF), all of which are made by astrocytes in culture and are present in the developing optic nerve; whereas a single factor can promote short-term survival in culture, a combination of at least three of these factors is required for long-term survival (Barres *et al.* 1993*b*). Thus neither oligodendrocytes nor their precursors can survive alone in culture: they need signals from other types of cells, and their normal neighbours can provide them; without such signals, the cells kill themselves.

Many oligodendrocytes undergo PCD during normal CNS development. In the developing rat optic nerve, for example, at least half of the oligodendrocytes produced seem to die in this way (Barres *et al.* 1992). The signalling molecules that can promote the survival of oligodendrocytes *in vitro* can also do so *in vivo*: if the levels of PDGF, IGF-1, NT-3 or CNTF are experimentally increased for several days in the developing nerve, the number of dead oligodendrocytes seen in the nerve is greatly reduced and the number of oligodendrocytes is correspondingly increased (Barres *et al.* 1992, 1993*a*,*b*, 1994). These findings suggest that each of these signalling molecules is normally present in limiting amounts in the developing optic nerve and that many oligodendrocytes die because they fail to receive the signals they

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need to keep their intrinsic death programme suppressed.

What might be the function of the large-scale oligodendrocyte death in the developing optic nerve (and presumably elsewhere in the CNS)? An attractive possibility is that it helps adjust the number of oligodendrocytes to the number (and length) of axons that require myelination (Barres *et al.* 1993*a*), just as normal neuronal death is thought to help adjust the number of developing vertebrate neurons to the number of target cells they innervate (Cowan *et al.* 1984; Barde 1989; Oppenheim 1991). If so, then axons should play an important part in controlling oligodendrocyte survival, and this seems to be the case: if the postnatal optic nerve is cut just behind the eye so that all of the axons in the nerve rapidly degenerate, most of the oligodendrocytes in the nerve selectively die, suggesting that oligodendrocytes normally depend on axons for survival (Barres *et al.* 1993*a*). Although it is unclear whether axons promote oligodendrocyte survival directly or indirectly (by stimulating astrocytes to produce or release survival factors, for example), purified sensory neurons promote the survival of purified oligodendrocytes *in vitro*, suggesting that neurons can act directly, at least in culture (Barres *et al.* 1993*a*).

3. PCD IN THE DEVELOPING KIDNEY

Because cells that undergo PCD in tissues are phagocytosed and degraded quickly and do not induce inflammation, even large-scale normal cell death can be histologically inconspicuous and therefore go unrecognized (Wyllie *et al.* 1980). This was the case for oligodendrocyte death in the developing optic nerve (Barres *et al.* 1992), and it was also the case in the developing kidney. Until recently, cell death was not thought to be a feature of mammalian kidney development, but we found that the proportion of dead cells in frozen sections of developing rat kidney is more than fivefold higher than in the developing optic nerve (Coles *et al.* 1993). We estimate that the amount of normal cell death in the developing kidney may be comparable to that in the developing nervous system, supporting the view that the extent of normal cell death in mammalian development is still greatly underestimated.

If newborn rats are treated systemically with epidermal growth factor (EGF) (Coles *et al.* 1993) or IGF-1 (H. S. R. Coles, unpublished data), the number of dead cells in sections of developing kidney rapidly falls, suggesting that the normal cell death in the kidney, as in the developing nervous system, may reflect the failure of many cells to receive the signals they need to survive. It is possible that many normal cell deaths that occur in other tissues during animal development may also reflect inadequate PCD-suppressing signals.

During kidney development, metanephric mesenchymal cells are induced by invading ureteric bud cells to differentiate into epithelial cells that then form nephrons (Saxen 1987; Ekblom *et al.* 1987; Bard 1992); if, in explant cultures, the mesenchymal cells are deprived of such inducing signals, they undergo PCD, although many can be rescued if EGF is added to the culture medium (Weller *et al.* 1991; Koseki *et al.* 1992). It is therefore possible that many of the cells that die during normal kidney development are mesenchymal cells that either fail to be induced and for this reason fail to respond to new survival signals that are produced and required as development proceeds, or are induced but fail to be included in developing nephrons.

4. PCD IN LENS EPITHELIAL CELLS AND CHONDROCYTES

Although it is clear that some mammalian cells require signals from other cells to avoid PCD, it is not clear that all mammalian cells do. Blastomeres apparently do not: they can survive and cleave in the absence of signals from other cells (Biggers *et al.* 1971). It is possible, however, that once blastomeres differentiate to give rise to two distinct cell types – inner cell mass cells and trophectoderm cells – these cells and all of the nucleated cell types they give rise to become dependent on survival signals from other cells. It is not practical to test this possibility by studying each of the hundreds of mammalian cell types individually. If there are cells that can survive without signals from other cells, however, one might expect lens epithelial cells and cartilage cells (chondrocytes) to be among them, as both lens and cartilage contain only a single cell type and are not vascularized, innervated or drained by lymphatic vessels (Fawcett 1986).

Unlike oligodendrocytes or their precursors, when either lens epithelial cells (Ishizaki *et al.* 1993) or chondrocytes (Ishizaki *et al.* 1994) are cultured at high density in the absence of other cell types or exogenous signalling molecules, they can survive for many weeks, indicating that they do not require signals from other cell types to survive in culture. When cultured at low cell density, however, they undergo PCD. Culture medium from high density cultures promotes the survival of cells in low-density cultures. Thus, lens and cartilage cells seem to require signals from other cells of the same kind to avoid PCD. Such autocrine signalling among cells that reside in tissues composed of a single cell type should perhaps not be surprising.

If lens and cartilage cells need signals from other cells to avoid PCD, it seems likely that most other mammalian cells do also, at least during development and possibly in the adult as well. Chondrocytes isolated from adult rats, for example, require signals from other chondrocytes to survive in culture, just as those isolated from newborn rats do (Ishizaki *et al.* 1994).

5. THE CELL DEATH PROGRAMME

Despite its fundamental importance and apparent evolutionary conservation from nematodes to humans (Vaux *et al.* 1992; Hengartner & Horvitz, this volume), the mechanism of PCD remains unknown. Genetic studies in *C. elegans* have identified two genes, *ced-3* and *ced-4*, that are required for the 131 normal

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cell deaths that occur during the development of the hermaphrodite (Ellis *et al.* 1991; Hengartner & Horvitz, this volume). The genes have been cloned and sequenced, and *ced-3* has been shown to encode a protease that is structurally homologous to the interleukin-1β-converting enzyme (ICE) (Yuan & Horvitz 1992; Yuan *et al.* 1993), which can induce PCD when overexpressed in fibroblasts (Miura *et al.* 1993). It is still uncertain, however, how any of these proteins induce cell death; it is unclear, for example, whether they are effectors or activators of the death programme. A third gene, *ced-9*, which acts as a brake on the death programme in *C. elegans* (Hengartner *et al.* 1992), is structurally (Hengartner & Horvitz, this volume) and functionally (Vaux *et al.* 1992; Hengartner & Horvitz, this volume) homologous to the mammalian gene *bcl-2*, which suppresses PCD in many, but not all, mammalian cell types (Vaux *et al.* 1988; Korsmeyer *et al.* 1994). Bcl-2 is now known to be a member of a family of related proteins that regulate PCD in mammalian cells (Boise *et al.* 1993; Oltvai *et al.* 1993).

To test whether most mammalian cells are capable of undergoing PCD, we have used the broad-spectrum protein-kinase inhibitor staurosporine. We reasoned that, if most cells need signals from other cells to avoid PCD, inhibition of many of the protein kinases involved in the intracellular signalling pathways activated by survival signals should induce most cells to undergo PCD. We have found that high concentrations ($\geqslant 1\,\mu M$) of staurosporine induce PCD in all of the many mammalian cell types that we have tested; the only exception so far is mouse blastomeres (Jacobson *et al.* 1993; Ishizaki *et al.* 1993; Jacobson *et al.* 1994; H. S. R. Coles, K. Raff, M. C. Raff, T. J. Davies & R. L. Gardner, in preparation). Moreover, in all cases tested, protein synthesis inhibitors fail to block, and usually enhance, staurosporine-induced PCD, suggesting that most cells are not only capable of undergoing PCD but constitutively express all of the protein components required to effect the programme. In those cases where RNA or protein synthesis has been shown to be required for the induction of PCD, macromolecular synthesis seems to be required to activate or de-repress the programme, rather than effect it, as the same cells can usually be induced in other ways to undergo PCD in the absence of macromolecular synthesis (see Martin 1993).

Nuclear changes are a prominent feature of PCD, and it has often been suggested that they are the cause of death: degradation of nuclear DNA by endonucleases, for example, may kill the cell. We have found, however, in the two very different cell lines we have tested, that we can remove the nucleus from a cell and the cell will still undergo the characteristic cytoplasmic changes of apoptosis when either treated with staurosporine or deprived of survival signals; the sequence and timing of the changes are indistinguishable in the cytoplast and its nucleated parent cell (Jacobson *et al.* 1994). Moreover, if cytoplasts are prepared from cells that overexpress the Bcl-2 protein, they are protected from PCD (Jacobson *et al.* 1994). These findings suggest that the nucleus is not required for either PCD or Bcl-2 protection.

The Bcl-2 protein was initially thought to be associated with the inner mitochondrial membrane (Hockenbery *et al.* 1990), raising the possibility that the mitochondrion may be the locus of Bcl-2 action and the primary target in PCD. To explore this possibility we studied human fibroblast cell lines that, as a result of prolonged treatment with ethidium bromide, do not have mitochondrial DNA (King & Attardi 1989). These cells have nonfunctional electron transport chains and therefore cannot carry out mitochondrial respiration. Nonetheless, they are just as sensitive as their normal parent cells to staurosporine-induced PCD, or to survival-factor-deprivation-induced PCD, or to the protective effects of Bcl-2, suggesting that mitochondrial respiration is not required for either PCD or Bcl-2 protection (Jacobson *et al.* 1993).

These findings have lead us to suggest that PCD is orchestrated by a cytosolic regulator that acts on multiple organelles in parallel (Jacobson *et al.* 1994), much as the cytosolic regulator M-phase-promoting factor (MPF) orchestrates the mitotic phase of the cell cycle (Nurse 1990). In fact, PCD shares a number of features with mitosis: although the final outcome is very different, in both cases the cells round up, the plasma membrane blebs, the nuclear lamina disassembles, and the chromatin condenses. It has therefore been suggested that PCD may be an abnormal or mistimed mitosis (Ucker 1991; Rubin *et al.* 1993). It seems to us, however, that PCD is too fundamental and important to be an aberrant mitosis. We prefer the view that it is a highly specialized process that may possibly have evolved from the process of mitosis and may share some components with it.

REFERENCES

Bard, J.B.L. 1992 The development of the mouse kidney – embryogenesis writ small. *Curr. Opin. Genet. Dev.* **2**, 589–595.

Barde, Y.A. 1989 Trophic factors and neuronal survival. *Neuron* **2**, 1525–1534.

Barres, B.A., Hart, I.K., Coles, H.C., Burne, J.F., Voyvodic, J.T., Richardson, W.D. *et al.* 1992 Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* **70**, 31–46.

Barres, B.A., Jacobson, M.D., Schmid, R., Sendtner, M. & Raff, M.C. 1993*a* Does oligodendrocyte survival depend on axons? *Curr. Biol.* **3**, 489–497.

Barres, B.A., Schmid, R., Sendtner, M. & Raff, M.C. 1993*b* Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* **118**, 283–295.

Barres, B.A., Raff, M.C., Gaese, F., Bartke, I. & Barde, Y.A. 1994 A crucial role for NT-3 in oligodendrocyte development. *Nature, Lond.* **367**, 371–375.

Biggers, J.D., Whitten, W.K., Whittingham, D.G. 1971 The culture of mouse embryos in vitro. In *Methods in mammalian embryology* (ed. J. C. Daniel), pp. 86–116. San Francisco: Freeman.

Boise, L.H., González-García, M., Postema, C.E., Ding, L., Lindsten, T., Turka, L.A. *et al.* 1993 *bcl-x*, a *bcl-2*-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* **74**, 597–608.

Coles, H.S.R., Burne, J.F. & Raff, M.C. 1993 Large-scale normal cell death in the developing rat kidney and its

reduction by epidermal growth factor. *Development* **118**, 777–784.

Cowan, W.M., Fawcett, J.W., O'Leary, D.D.M. & Stanfield, B.B. 1984 Regressive events in neurogenesis. *Science, Wash.* **225**, 1258–1265.

Ekblom, P., Aufderheide, E., Klein, G., Kurz, A. & Weller, A. 1987 Cell interactions during kidney development. In *Mesenchymal-epithelial interactions in neural development* (ed. J. R. Wolff *et al.*), pp. 101–110. Heidelberg: Springer-Verlag.

Ellis, R.E., Yuan, J.Y. & Horvitz, H.R. 1991 Mechanisms and functions of cell death. *A. Rev. Cell Biol.* **7**, 663–698.

Fawcett, D.W. 1986 *Bloom and Fawcett: a textbook of histology*, 11th edn. Philadelphia: W. B. Saunders.

Hengartner, M.O., Ellis, R.E. & Horvitz, H.R. 1992 *Caenorhabditis elegans* gene ced-9 protects cells from programmed cell death. *Nature, Lond.* **356**, 494–499.

Hengartner, M.O. & Horvitz, H.R. 1994 *C. elegans* cell death gene *ced-9* encodes a functional homolog of mammalian proto-oncogene *bcl-2*. *Cell* **76**, 665–676.

Hockenbery, D.M., Nuñez, G., Milliman, C., Schreiber, R.O. & Korsmeyer, S.J. 1990 BCL-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature, Lond.* **348**, 334–336.

Ishizaki, Y., Voyvodic, J.T., Burne, J.F. & Raff, M.C. 1993 Control of lens epithelial cell survival. *J. Cell Biol.* **121**, 899–908.

Ishizaki, Y., Burne, J.F. & Raff, M.C. 1994 Autocrine dependence of chondrocyte survival in culture. *J. Cell Biol.* (In the press.)

Jacobson, M.D., Burne, J.F., King, M.P., Miyashita, T., Reed, J.C. & Raff, M.C. 1993 Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature, Lond.* **361**, 365–369.

Jacobson, M.D., Burne, J.F. & Raff, M.C. 1994 Programmed cell death and Bcl-2 protection in the absence of a nucleus. *EMBO J.* **13 13**, 1899–1910.

King, M.P. & Attardi, G. 1989 Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science, Wash.* **246**, 500–503.

Korsmeyer, S.J., Shutter, J.R., Veis, D.J., Merry, D.E. & Oltvai, Z.N. 1994 Bcl-2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death. *Semin. Cancer Biol.* (In the press.)

Koseki, C., Herzlinger, D. & Al-Awqati, Q. 1992 Apoptosis in metanephric development. *J. Cell Biol.* **119**, 1327–1333.

Martin, S.J. 1993 Apoptosis: suicide, execution or murder? *Trends Cell Biol.* **3**, 141–144.

Miura, M., Zhu, H., Rotello, R., Hartwieg, E.A. & Yuan, J. 1993 Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* **75**, 653–660.

Nurse, P. 1990 Universal control mechanism regulating onset of M phase. *Nature, Lond.* **344**, 503–508.

Oltvai, Z.N., Milliman, C.L. & Korsmeyer, S.J. 1993 Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programed cell death. *Cell* **74**, 609–619.

Oppenheim, R.W. 1991 Cell death during development of the nervous system. *A. Rev. Neurosci.* **14**, 453–501.

Raff, M.C. 1992 Social controls on cell survival and cell death. *Nature, Lond.* **356**, 397–400.

Rubin, L.L., Philpott, K.L. & Brooks, S.F. 1993 The cell cycle and cell death. *Curr. Biol.* **3**, 391–394.

Ucker, D.S. 1991 Death by suicide: one way to go in mammalian cellular development? *New Biol.* **3**, 103–109.

Vaux, D.L., Cory, S. & Adams, J.M. 1988 Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-*myc* to immortalize pre-B cells. *Nature, Lond.* **335**, 440–442.

Vaux, D.L., Weissman, I.L. & Kim, S.K. 1992 Prevention of programmed cell death in *Caenorhabditis elegans* by human *bcl-2*. *Science, Wash.* **258**, 1955–1957.

Weller, A., Sorokin, L., Illgen, E.-M. & Ekblom, P. 1991 Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factor. *Devl Biol.* **144**, 248–261.

Wyllie, A.H., Kerr, J.F.R. & Currie, A.R. 1980 Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68**, 251–306.

Yuan, J. & Horvitz, H.R. 1992 The *Caenorhabditis elegans* cell death gene *ced-4* encodes a novel protein and is expressed during the period of extensive programmed cell death. *Development* **116**, 309–320.

Yuan, J., Shaham, S., Ledoux, S., Ellis, H.M. & Horvitz, H.R. 1993 The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* **75**, 641–652.

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