By the time I was born, more of me had died than survived. It is no wonder I cannot remember; during that time I went through brain after brain for nine months, finally contriving the one model that could be human, equipped for language.

Lewis Thomas (1992)

Cell Death in Development

Programmed Cell Death / Apoptosis

Cell death plays an important role in morphogenesis.
Example: Interdigital death in limb bud.
Cell Death in the chick limb bud

Figure 16.24 Patterns of Cell Death in Leg Primordia of Duck and Chick Embryos

(A) DUCK LEG PRIMORDIUM
Extensive cell death

(B) CHICK LEG PRIMORDIUM
Extensive cell death

BMP activity:
Bone Morphogenetic Protein

Figure 16.24 Inhibition of Cell Death leaves webbing

(A) Normal Chick Foot

(B) BMP signaling blocked
Cell death is (in the vertebrates) prominent in
- developing nervous system
- developing and mature immune system

1. Immune cells recognizing ‘self’ die during immune system development.

2. Immune challenge results in proliferation of cells; when these cells are no longer needed, they die.

Two types of cell death:

**Necrosis** - caused by acute injury, involves cell lysis
- undesirable because cell contents are released

**Apoptosis / Programmed Cell Death**
- stereotyped pattern of events including nuclear condensation

Cell with condensed nucleus in EM
Two types of cell death:

**Necrosis** - caused by acute injury, involves cell lysis
- undesirable because cell contents are released

**Apoptosis / Programmed Cell Death**
- stereotyped pattern of events including
  - nuclear condensation
  - chromosome fragmentation

"TUNEL" - TdT-mediated dUTP Nick End Labeling
- shows 'ends' of chromosomes
  -- few ends in normal cells
  -- many in apoptotic cells undergoing fragmentation

521 (HeLa ovarian cancer) cells undergoing apoptosis
following infection with a mutant baculovirus lacking the anti-apoptotic p35 gene
Two types of cell death:

**Necrosis** - caused by acute injury, involves cell lysis
- undesirable because cell contents are released

**Apoptosis / Programmed Cell Death**
- stereotyped pattern of events including
  - nuclear condensation
  - chromosome fragmentation
  - cell membrane blebbing
  - phagocytosis by nearby cells
- active suicide program, often requiring gene activation (transcription & translation)
Programmed cell death genetic basis first studied in the nematode *C. elegans*

Wild type

*cde* mutant

PCD is particularly prominent in the *C. elegans* embryonic nervous system lineages

PCD prunes unneeded cells from the *C. elegans* nervous system

P ectodermoblast cell divisions (lineage)
Programmed cell death genetic basis first studied in the nematode *C. elegans*

Steps in cell death process identified by mutants:

- **Decision:** ced-9, egl-1
- **Execution:** ced-3, ced-4
- **Engulfment:** ced-1, ced-2
- **Digestion of DNA:** nuc-1

Vertebrates have similar proteins: *bcl-2* is homolog of *ced-9*

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2002 Nobel Prize awarded to Brenner, Horvitz & Sulston for Studies of the Genetic Regulation of *C. elegans* Development

- The Nobel Prize in Physiology or Medicine 2002 - official Nobel Committee website
- Nobel Committee Press Release

Sydney Brenner  Bob Horvitz  John Sulston

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Mammalian Apoptosis Genes

*bcl-2* was first discovered as an oncogene in B cell lymphomas. *bcl-2* protein coding sequence translocated from chromosome 18 to 14 (t14;18) in front of Ig Heavy Chain promoter.

*bcl-2* homology to *C. e. ced-9* gene gave a clue to its function.

*bcl-2* gene was permanently ON in these B cells, blocking apoptosis, immortalizing them - pre-disposing cells to cancer.
Mammalian Apoptosis Genes

*bcl-2* was shown able to block apoptosis in IL-3 deprived B cells.

*bcl-2* gene inserted and activated in *C. elegans* could rescue worm cells from programmed cell death.

Such functional conservation is the hallmark of a fundamental biochemical process shared by all animals.

Mammals have many bcl-2-like genes regulating apoptosis.

Three subfamilies:
1) Bcl-2 subfamily (e.g., bcl-2, Bcl-XL) promotes cell survival.
2) Bax subfamily (e.g., Bax, Bik) is pro-apoptotic.
3) BH3 subfamily (e.g., Bad, Bik) is pro-apoptotic.

Programmed cell death in *C. elegans* functions like that in mammals

Human *bcl-2* can function in worm to block PCD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>hs<em>bcl-2</em></td>
<td>hs</td>
<td>18 ± 4</td>
</tr>
</tbody>
</table>

Conservation of function (over great evolutionary distance) - the hallmark of a fundamental biochemical process

*Vaux et al., 1992: Prevention of Programmed Cell Death in Caenorhabditis elegans by Human bcl-2 (modified Fig. 3)*
Mammalian Apoptosis Genes

Other worm ced genes also have mammalian homologs.

*egi-1* gene first discovered as a dominant mutation causing PCD in the egg laying neuron HSN.

*Egl-1* protein is a homolog of pro-apoptotic BH3 subfamily proteins.

First mammalian *ced-3* homolog was *ICE*, a cysteine protease.

*ICE* was the first Caspase (Cysteine - Aspartate protease enzymes).

Mammals have 10 Caspases functioning in apoptosis:

- Caspase-1: *ICE* protease
- Caspase-9: closest *ced-3* homolog

Caspase-9 (-) mutant in mice is PCD-deficient, especially in the CNS.

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![Disruption of Normal Brain Development by Blocking Apoptosis](image)

*Figure 6.28(1)**

Decrease in cell death causes severe nervous system abnormalities.
Mammalian Apoptosis Genes

Other worm ced genes also have mammalian homologs. Caspases function as initiator and effector caspases. All caspases are synthesized as inactive pro-caspases. Cleavage activates caspases to be functional enzymes. Caspase-9 (Ced-3 homolog) is an initiator caspase; one of its functions is to cleave and activate the caspase-3 effector. Caspase-3 and other effectors begin digestion of cell contents.

Ced-4 homolog is the Apaf-1 protein. Apaf-1 binds to Pro-caspase-9, promoting its auto-cleavage to active caspase form.

Mammalian Apoptosis Genes

The basic molecular pathway is conserved from worms to humans. Unlike nematodes, which have a single pathway, mammals have multiple pathways to activate a caspase cleavage cascade. Some bypass the bcl-2/mitochondrial pathway.
“Death Receptors”

Fas (aka CD95 or Apo-1) is a ‘death receptor’ in the TNF receptor superfamily (TNF = Tumor Necrosis Factor)

Death receptors mediate active killing signals by (e.g.) cytotoxic T cells.

Death receptors mediate killing of auto-reactive immune cells.

Integrating signals hinting at his obsolescence, George undergoes apoptosis.