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

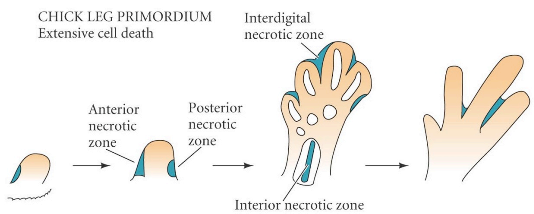
Programmed Cell Death / Apoptosis

Cell Death in Development

Cell death plays an important role in morphogenesis.

Example: Interdigital death in limb bud.

Cell Death in the chick limb bud



~~necrotic~~ -- **apoptotic**

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

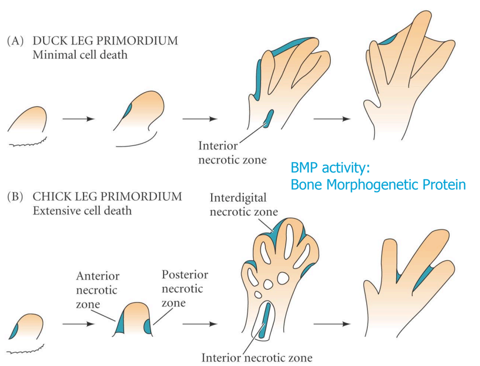
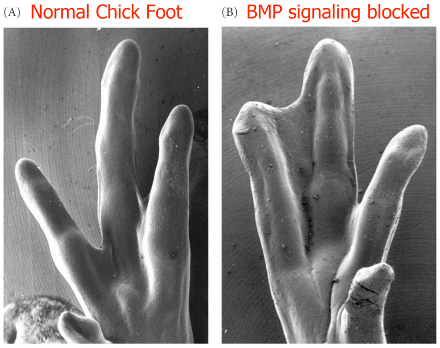


Figure 16.24 Inhibition of Cell Death leaves webbing

(A) Normal Chick Foot (B) BMP signaling blocked



Cell death is (in the vertebrates) prominent in

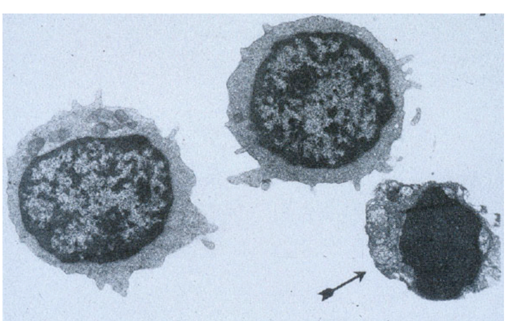
- developing nervous system
- developing and mature immune system
  1. Immune cells recognizing 'self' die during immune system development.
  2. Immune challenge results in proliferation of cells; when these cells are no longer needed, they die.

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

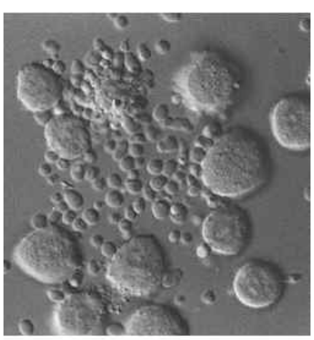
- shows 'ends' of chromosomes
  - -- few ends in normal cells
  - -- many in apoptotic cells undergoing fragmentation

Two types of cell death:

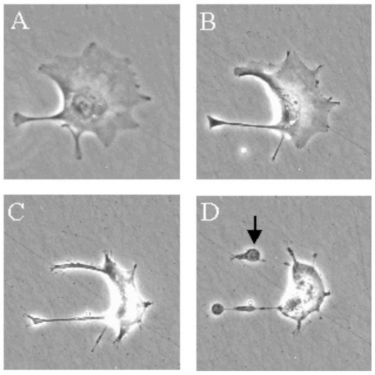
Necrosis - caused by acute injury, involves cell lysis
- undesirable because cell contents are released

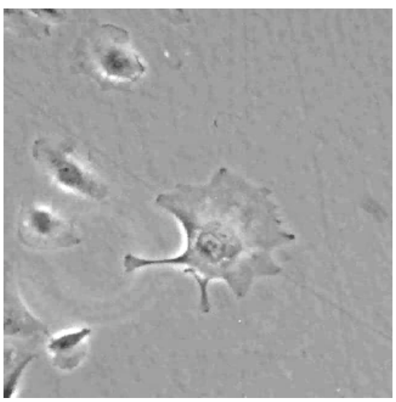
Apoptosis / Programmed Cell Death

- stereotyped pattern of events including
nuclear condensation
chromosome fragmentation
cell membrane blebbing



Sf21 (lepidopteran insect) cells undergoing apoptosis following infection with a mutant baculovirus lacking the anti-apoptotic p35 gene





Two types of cell death:

<u>Necrosis</u> - caused by acute injury, involves cell lysis
- undesirable because cell contents are released

<u>Apoptosis / Programmed Cell Death</u>

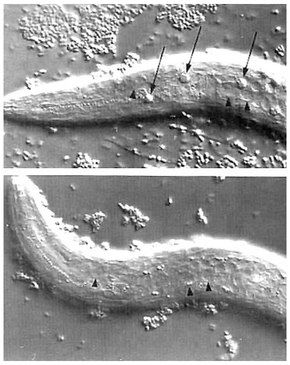
- stereotyped pattern of events including
nuclear condensation
chromosome fragmentation
cell membrane blebbing
phagocytosis by nearby cells

- active suicide program, often requiring gene activation (transcription & translation)

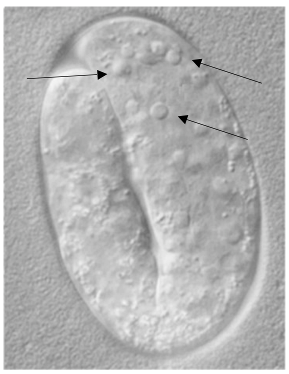
Programmed cell death genetic basis first studied in the nematode *C. elegans*

Wild type

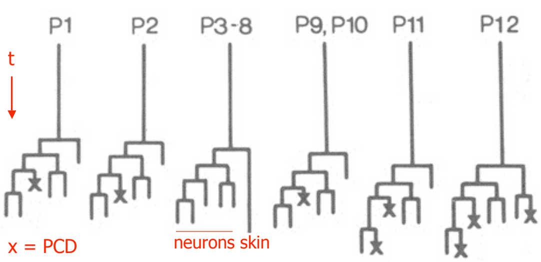
ced mutant



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*

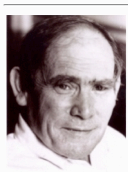
Digestion of DNA: *nuc-1*

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

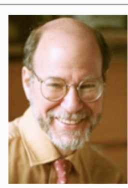
Nobel Prize for work in the nematode *C. elegans* (including cell death genetics)

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



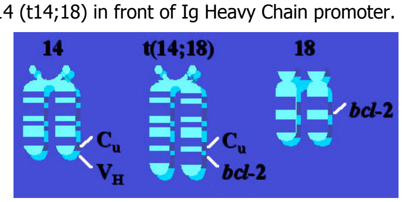
Bob Horvitz

John Sulston

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.

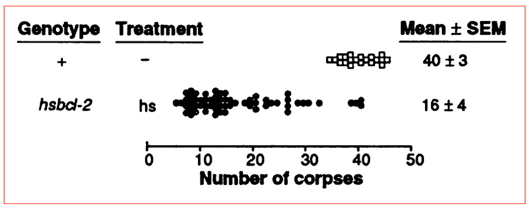
Mammalian Apoptosis Genes

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.

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

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



Cells that normally die rescued by human Bcl-2 protein

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

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.

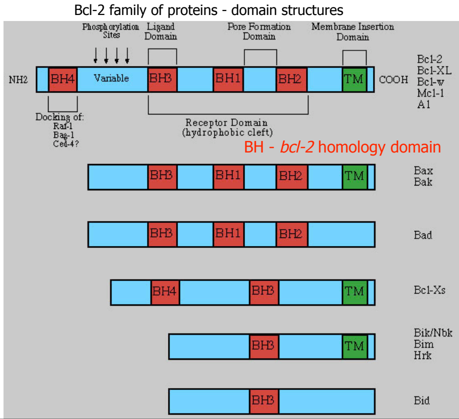
Three subfamilies:

1) Bcl-2 subfamily (e.g., bcl-2, Bcl-XL) promotes cell survival.

2) Bax subfamily (e.g., Bax, Bix) is pro-apoptotic.

3) BH3 subfamily (e.g., Bad, Bik) is pro-apoptotic.

Bcl-2 family of proteins - domain structures



Mammalian Apoptosis Genes

Other worm *ced* genes also have mammalian homologs.

egl-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).

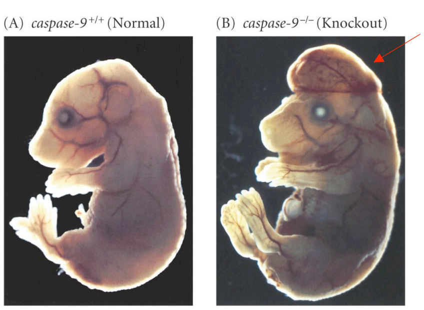
Mammals have 10 Caspases functioning in apoptosis:

Caspase-1: ICE protease

Caspase-9: closest *ced-3* homolog

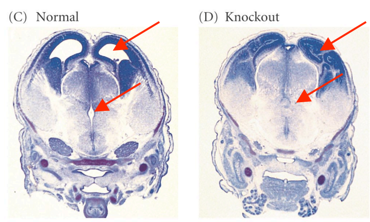
Caspase-9 (-) mutant in mice is PCD-deficient, especially in the CNS.

Figure 6.28(1) ***Disruption of Normal Brain Development by Blocking Apoptosis***



Decrease in cell death causes severe nervous system abnormalities.

Figure 6.28 ***Disruption of Normal Brain Development by Blocking Apoptosis***



Note lack of spaces (ventricles) found in normal brain.

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.

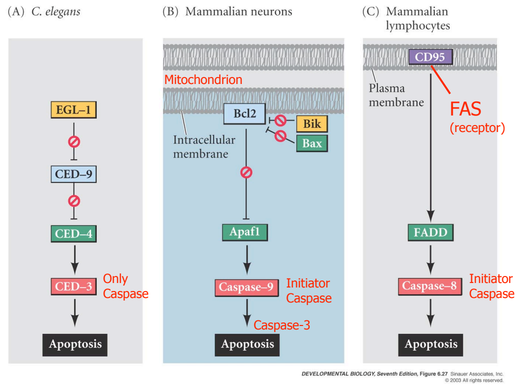
Apaf-1 binds to Pro-caspase-9, promoting its auto-cleavage to active caspase form.

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.

Figure 6.27 ***Apoptosis Pathways in Nematodes and Mammals***



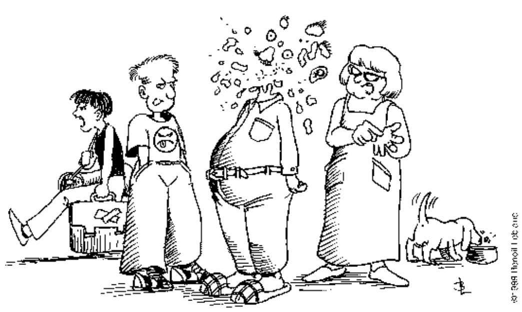
"Death Receptors"

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.