

Cell death

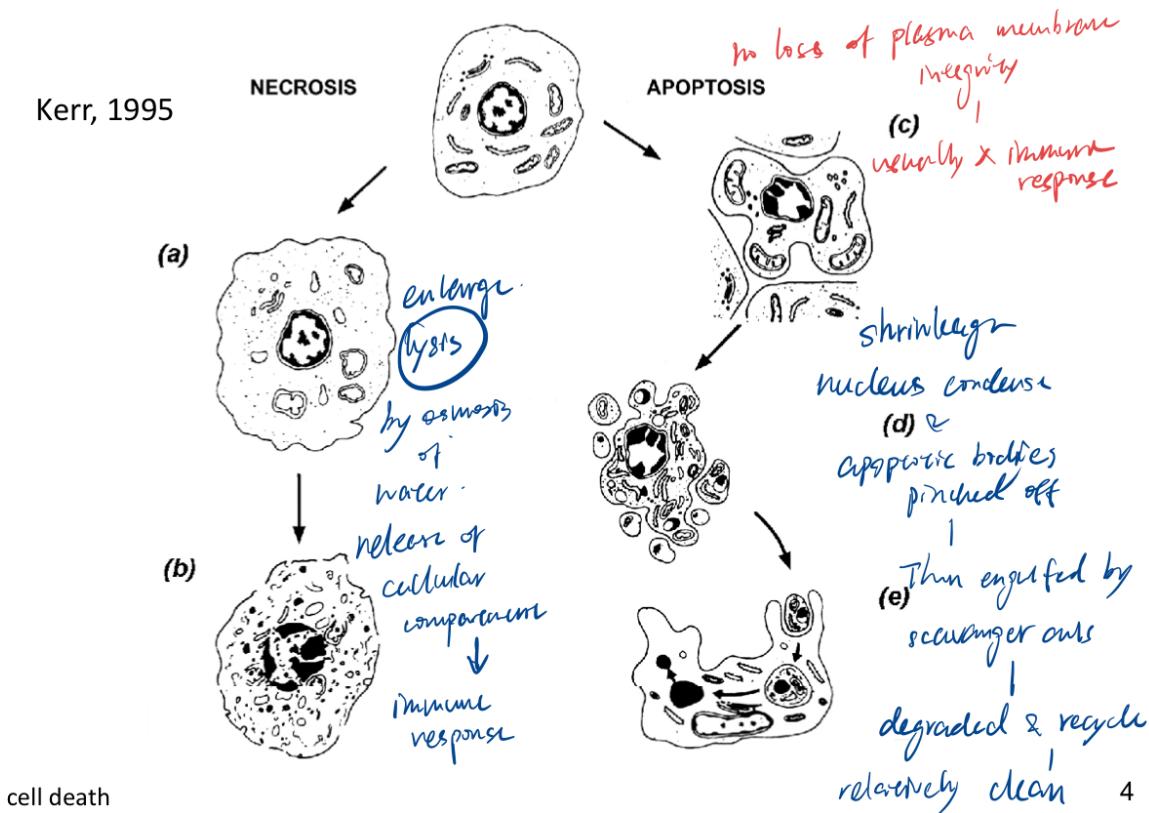
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▼ Types of cell death

- Programmed cell death
 - Physical process
 - not necessarily induces immune response
 - apoptosis; necroptosis; pyroptosis
- Necrosis
 - Induced by massive cellular damage
 - induces immune response

▼ Characteristic

'Necrosis' versus 'Apoptosis'



- Necrosis
 - Cell bloated by osmosis—lysis
 - outburst of plasma membrane
 - release cellular components → immune response
- Apoptosis
 - shrinkage
 - nucleus condenses
 - no loss of plasma membrane integrity
 - formation of apoptotic bodies & pinching off
 - The compartments are engulfed by scavenger cells for degradation & recycling

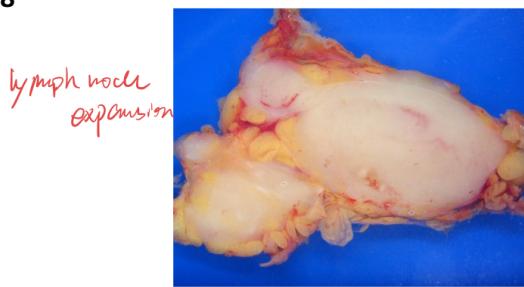
▼ Molecular characteristics of apoptosis

- smearing DNA bands on gel electrophoresis
 - The DNA fragments are actually wrapped around nucleosomes to prevent from degradation
 - Perhaps introduce nucleases to cut between nucleosomes
 - This shows that apoptosis is actually a controlled process
- Phosphatidylserine exposure “eat me” signal for scavenger cells
 - flipped from inner leaflet to outer leaflet
 - found by phagocytosis assay
 - Add liposomes containing different phospholipids
 - found that PS group significantly inhibited the phagocytosis by macrophages → confuse macrophage → fewer dead cells are engulfed by them
- Membrane blebbing

Bcl-2

1. The *bcl-2* (*bcl*, B-cell lymphoma) story

- Research area: oncology
 - Approach: cytology, genetics
 - Model: follicular lymphoma
-
- **Follicular lymphoma** is the second-most-common form of non-Hodgkin's lymphoma; defined as a lymphoma of follicle center B cells
 - In more than 60% of the cases caused by a **translocation between chromosomes 14 and 18**



<http://ookaboo.com/o/pictures/picture/13042592/>
Macroscopic_aspect_of_the_cut_surface_of

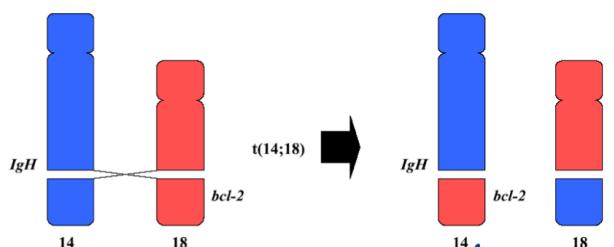
The *bcl-2* story

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The t(14; 18) (q32; q21) chromosomal translocation:

- breakage site*
14 18
for ↑ TEC Level
- fuses enhancer of immunoglobulin heavy chain (*IgH*) locus to new transcription unit on chromosome 18q21 ('*bcl-2*') *↑ expression of bcl-2 ↑*

Tsujimoto et al., 1985, Bakhshi et al., 1985, Cleary and Sklar, 1985



- causes the over-expression of a hybrid *bcl-2/IgH* transcript in B cells

Cleary et al., 1986

The *bcl-2* gene:

- Is expressed in numerous cell types
- Encodes a 239 aa protein ('Bcl-2') with no known motifs
- Bcl-2 protein is found associated with mitochondria and ER

Cleary et al., 1986

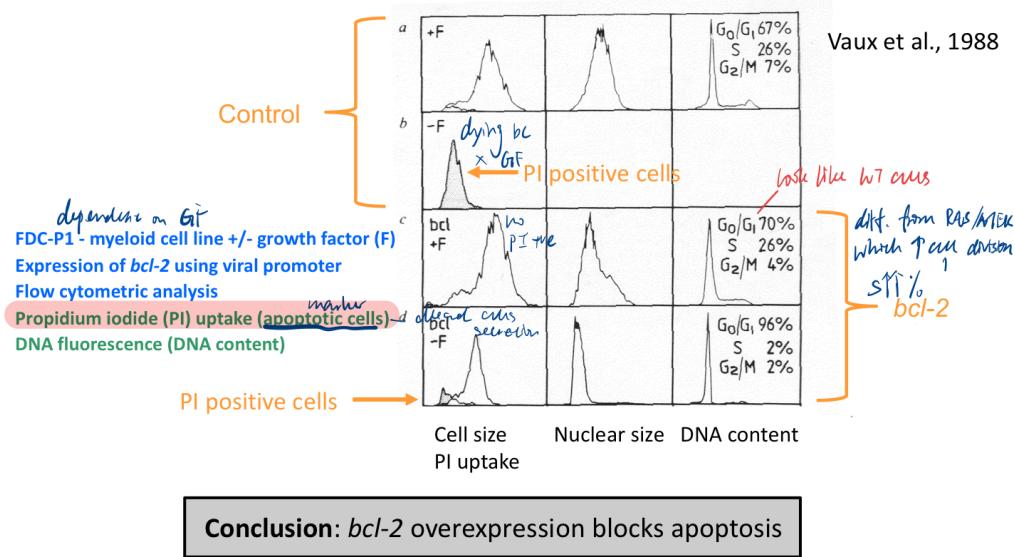
Why is over-expression of the *bcl-2* gene oncogenic?

What does the Bcl-2 protein normally do?

Approaches used to uncover *bcl-2*/Bcl-2 function:

- Over-expression phenotype? (*gain-of-function* experiment)
by adding bcl-2 to strong promoter
- Interacting proteins? *immunoprecipitation*
- Loss-of-function phenotype? (*loss-of-function* experiment, *knock-outs*)

The over-expression of the *bcl-2* gene in a myeloid cell line promotes cell survival NOT cell proliferation:



▼ Proteins interacting with Bcl-2

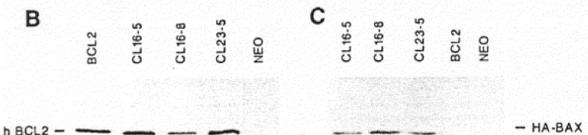
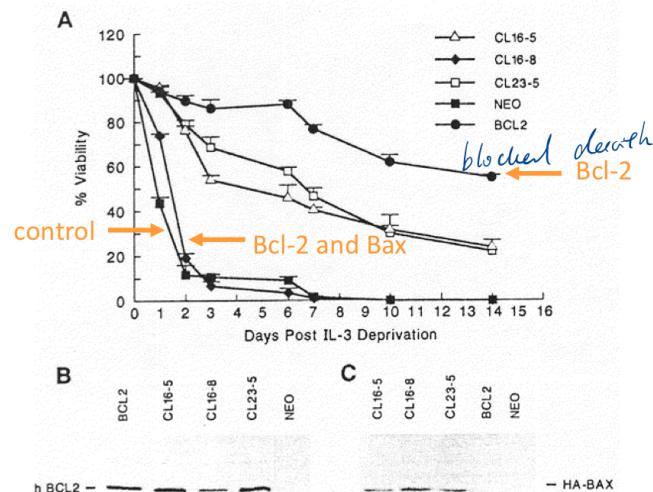
- BAX
- BAD
- Both are structurally similar to Bcl-2
 - BAX shares the BH domain
 - BAD shares the BH3 with BAX and BAD

▼ BAX can induce apoptosis & **antagonise the Bcl-2 anti-apoptotic**

BAX antagonizes the anti-apoptotic activity of Bcl-2

Oltvai et al., 1993

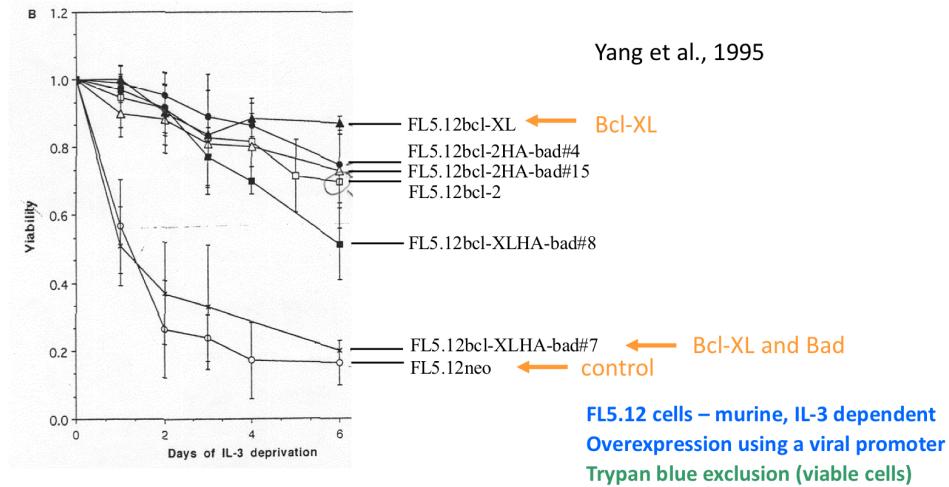
- ∴ pathology of B-lymphoma.
- bcl-2 overexpresses BAX
- ∴ apoptosis blocker?



FL5.12 cells – murine, IL-3 dependent Overexpression using a viral promoter Trypan blue exclusion (viable cells)

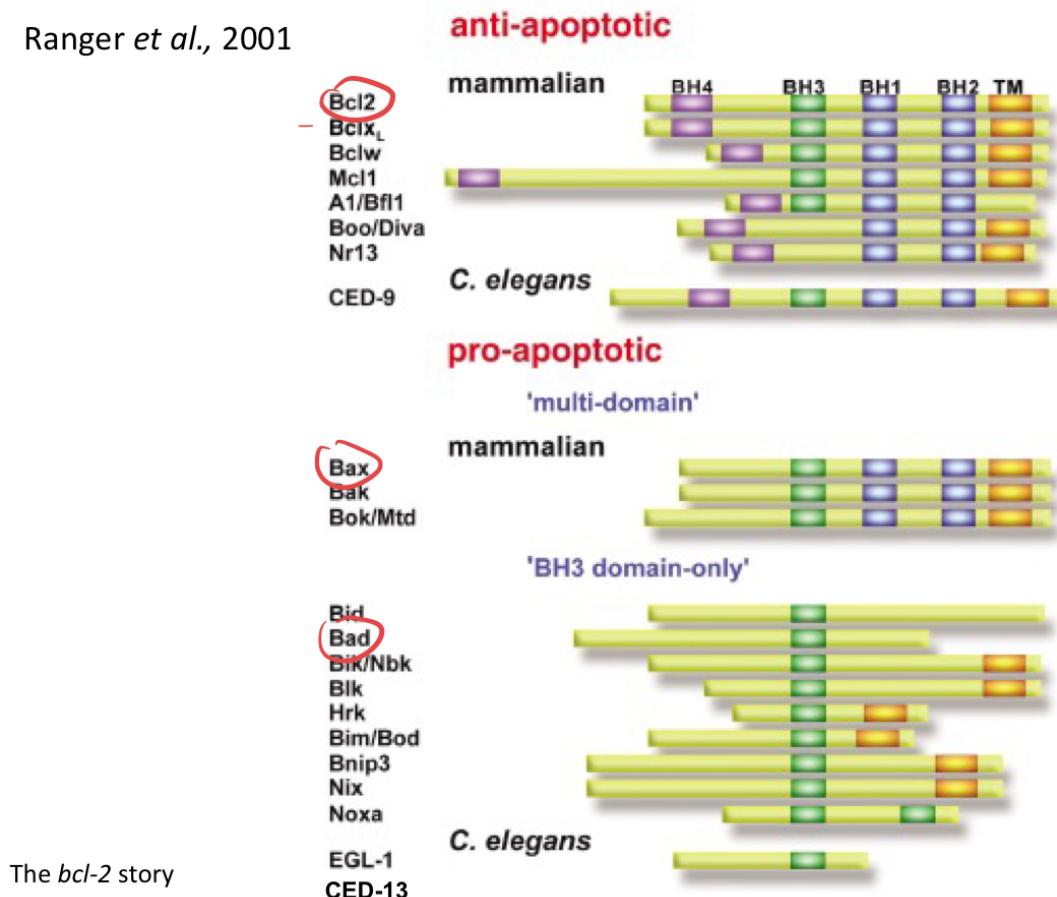
- ▼ BAD antagonises the **anti-apoptotic activity of Bcl-XL** (Bcl-2-like protein)

Bad expression antagonizes the function of Bcl-XL



The Bcl-2 superfamily of apoptosis regulators

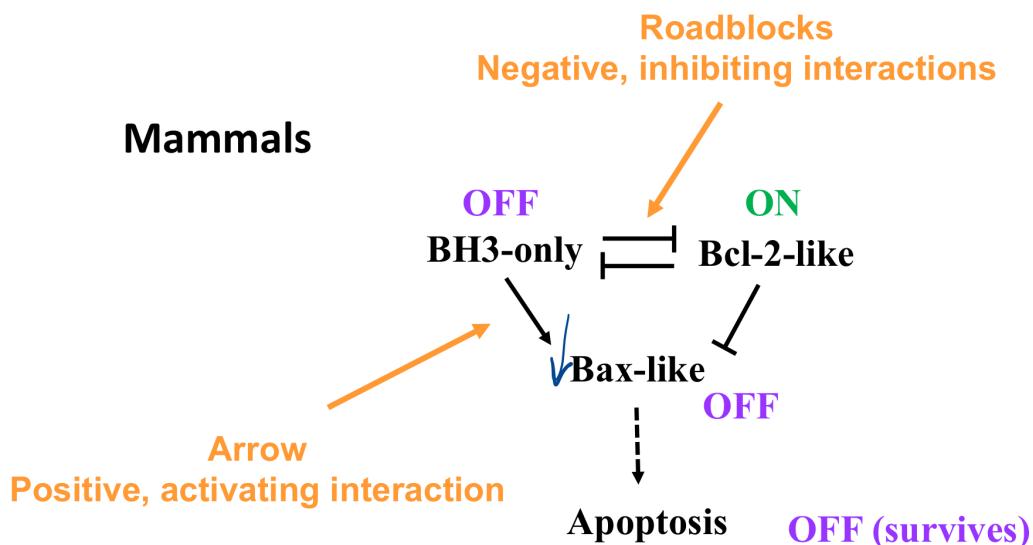
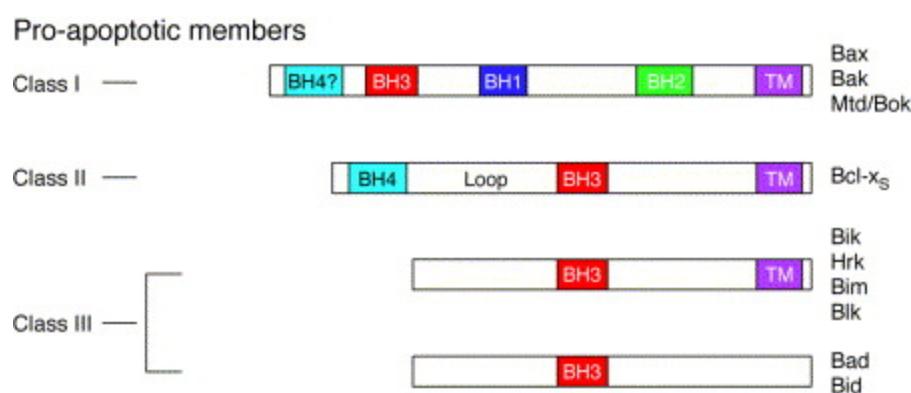
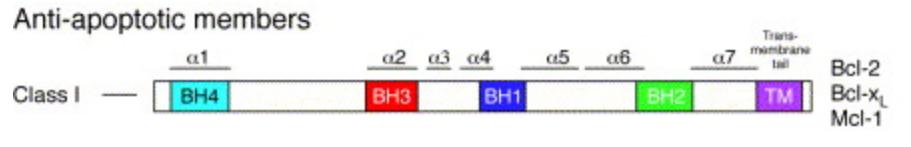
Ranger et al., 2001

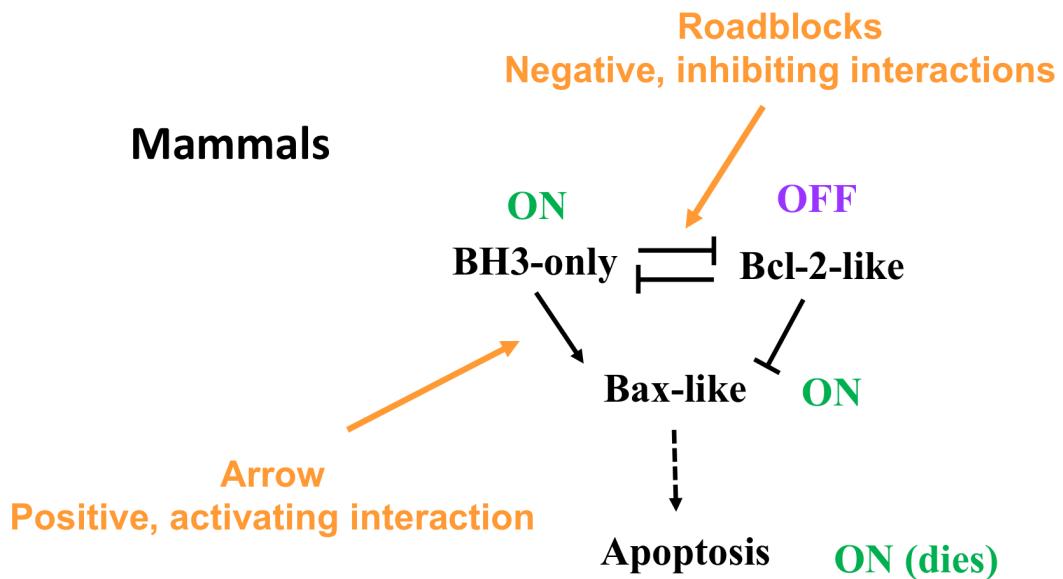


Therefore, the anti-apoptotic function is carried out by the BH4 domain!

- The fate is determined by the ratio of pro- to anti-apoptotic protein members

BH3 mimetics function by binding within the hydrophobic groove of the anti-apoptotic BCL-2 proteins, thereby **inhibiting anti-apoptotic protein activity, and lowering the threshold or apoptosis to proceed**





Caspase-mediated apoptosis

C. elegans have invariant cell lineages (every hermaphrodites have exactly 969 somatic cells)

2. The caspase story

- **Research area:** developmental biology
- **Approach:** genetics
- **Model:** *Caenorhabditis elegans*

Dr. Bailly's lectures

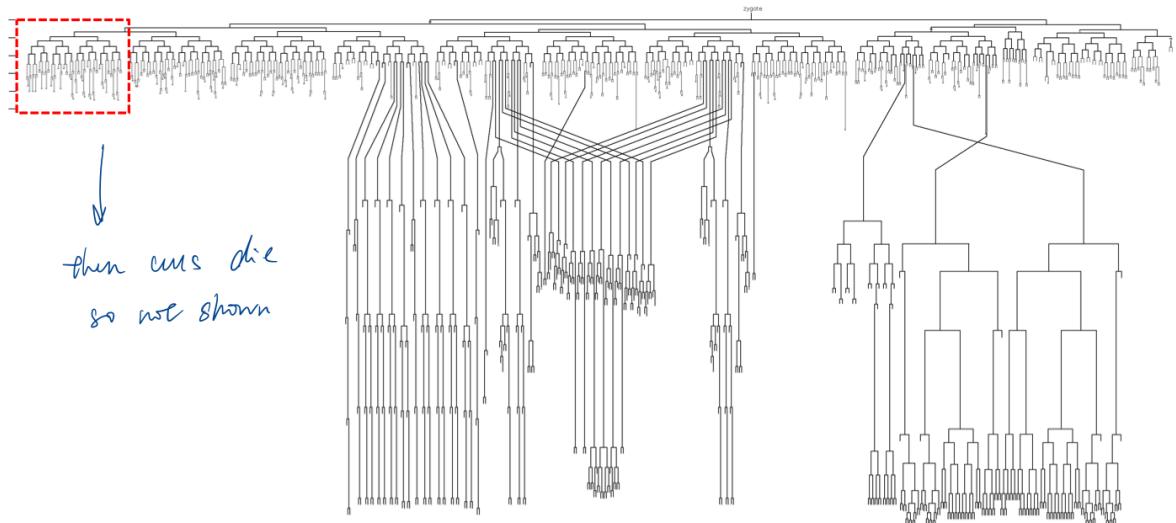
- essentially invariant cell death pattern (131 cells die, most of them through apoptosis)
- cell death can be observed in living animals using Nomarski (DIC) optics
- cell death is not essential for viability



DIC- pseudo 3D effect; no need for staining to visualise the structure; C elegans is transparent

cell line

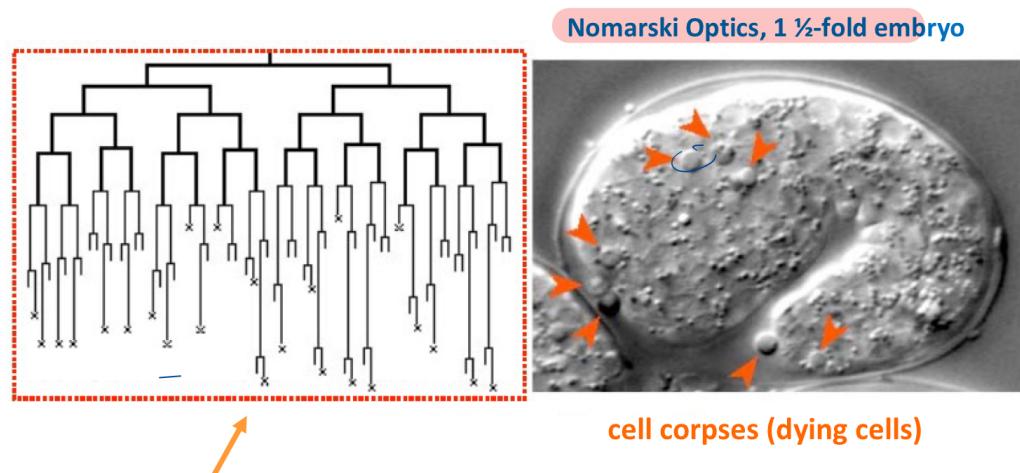
The essentially invariant *C. elegans* cell lineage



$$1090 - 131 = 959 \text{ cells alive}$$

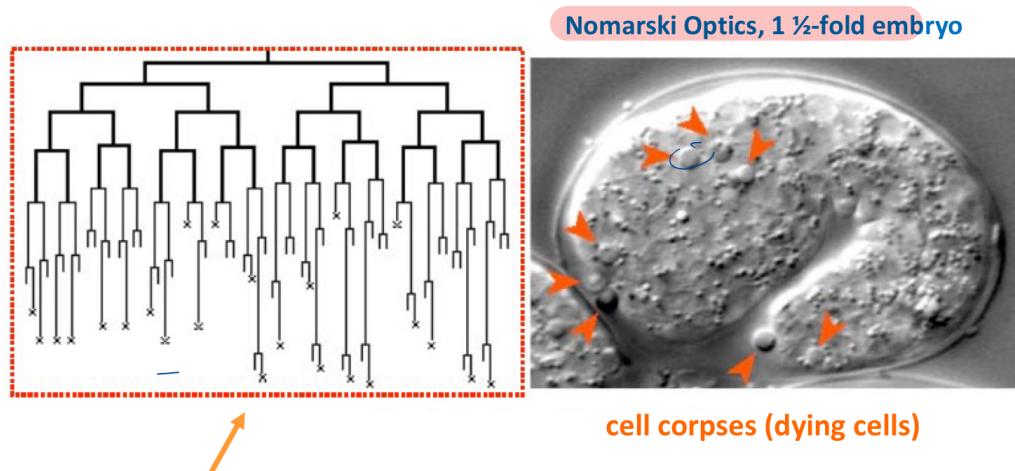
Sulston and Horvitz, 1977
Sulston et al., 1983

Analyzing genes involved in apoptosis
131 somatic cells reproducibly die during *C. elegans* development



- **Recessive** loss-of-function mutation of **ced-3 & ced-4** leads to **loss of cell death**
- **ced-3 & ced-4 regulates cell death**

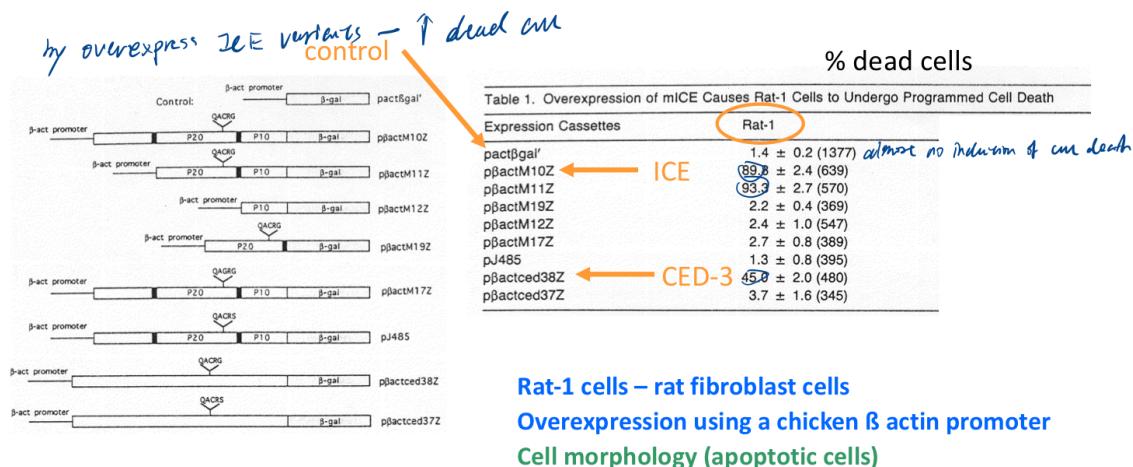
Analyzing genes involved in apoptosis
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→ *ced-3* and *ced-4* are required for apoptosis in *C. elegans* [***ced-3* encodes for caspase-1**]

- *ced-3* has similar structure to protease **ICE** (Interleukin 1-beta Converting Enzyme)
- ICE and *ced-3* function are conserved —

The expression of ICE or CED-3 in mammalian cells induces cell death



Miura et al., 1993

Conclusions: The pro-apoptotic function of CED-3/ICE has been conserved

▼ Caspase

- cysteine dependent
- inactive without stimulation
- when mature: active monomer dimerisation → **self-cleavage** → **two cleavages between prodomain and large subunit & between of large and small units** →
- cleavage site recognise tetrapeptide sequence containing **aspartate (D)**

CED-3/ICE-like proteases or 'caspases' (cysteine-dependent aspartate-specific proteases)

- Induce apoptosis when overexpressed
- Are formed as essentially inactive 'zymogens' and need to be processed in order to become fully active
- Cleavage specificity: tetrapeptide (P4-P1) – WEHD (ICE) (used as specific inhibitors and substrates)
*aspartate
nptophen*

