

Universidad de Chile



Regulation of Apoptosis Signaling

Claudio Hetz, PhD

*Biomedical Neuroscience Institute, Faculty of Medicine
University of Chile, Santiago, Chile*

APOPTOSIS

- **Definition and characteristic features**
- **Regulatory mechanisms: Intrinsic and extrinsic pathways**
- **Mitochondrial-mediated apoptosis**
- **The BCL-2 Protein Family**
- **Apoptosis an disease conditions: Cancer and Neurodegeneration**

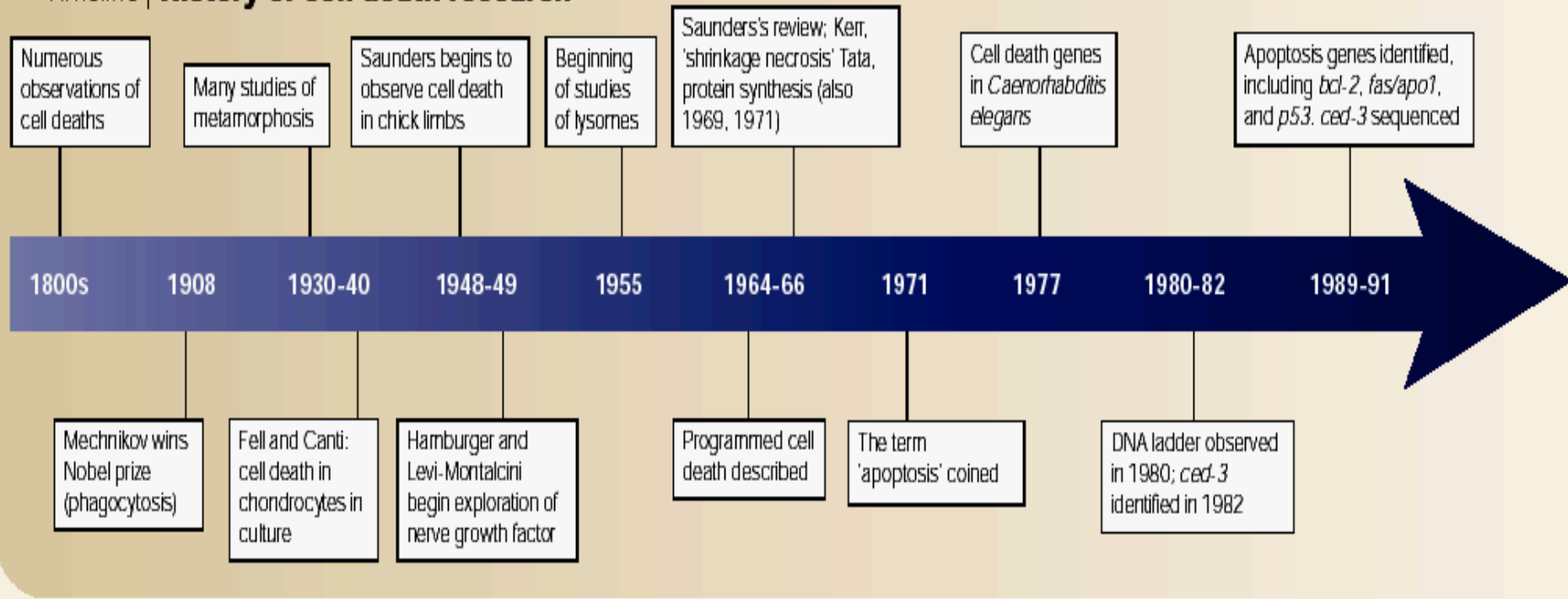
Apoptosis

- Apoptosis (1972)
 - Greek word “falling off”
- Built-in (programmed) mechanism
- or self-destruction-suicide
- Type of programmed cell death based upon morphological features



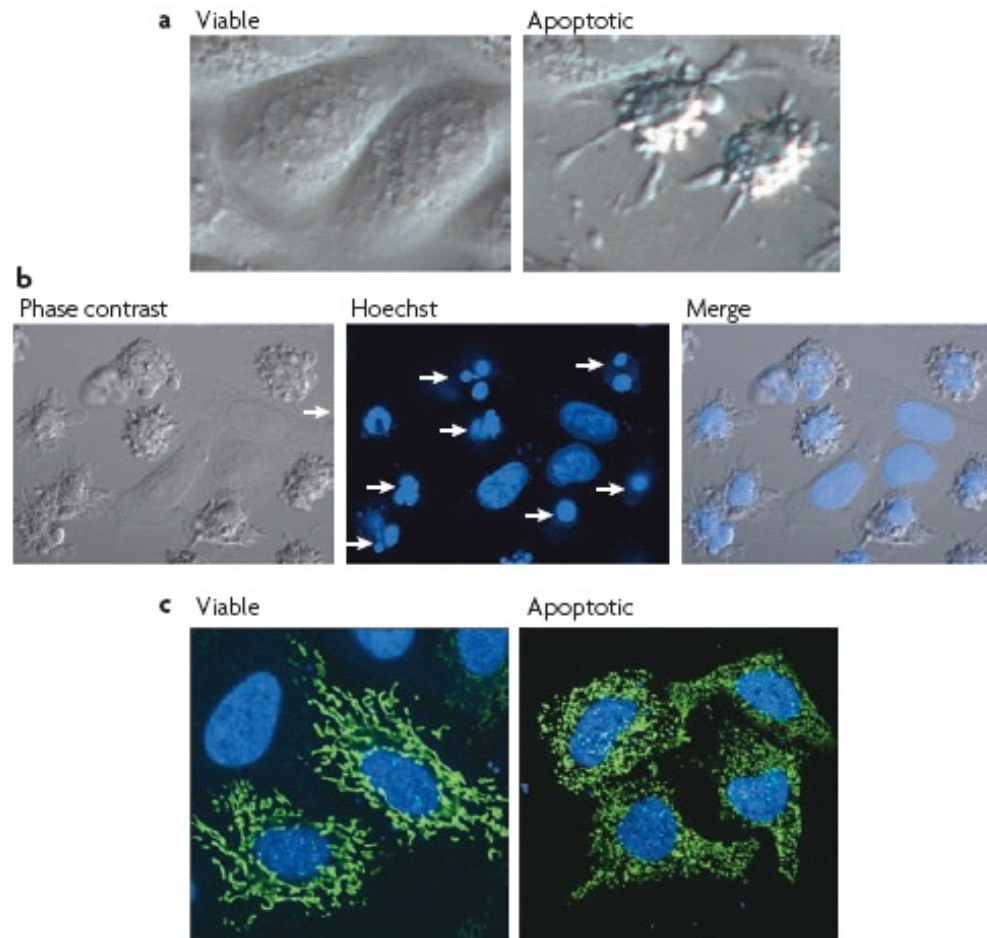
The History of Cell Death Research

Timeline | History of cell death research



Properties of Apoptotic cells

- Activation of caspases.
- Accumulation of phosphatidylserine in the outer plasma membrane leaflet.
- Fragmentation of DNA, cuts between nucleosomes.
- Fragmentation of membranes, nucleus and finally the entire cell.
- Phagocytosis of cell fragments by macrophages.



Apoptosis: Intrinsic and extrinsic pathways

EXTRINSIC

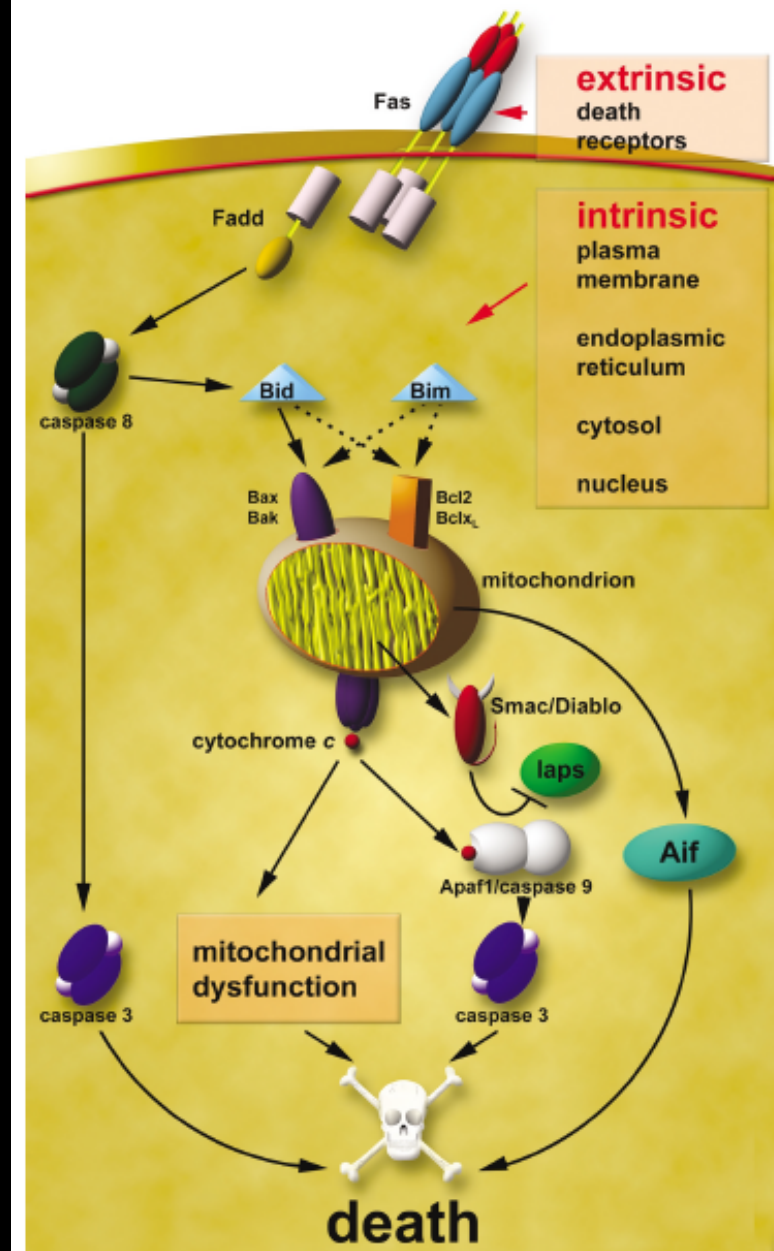
- Death receptor pathway. Activated by the engagement of receptors on cell surface

INTRINSIC

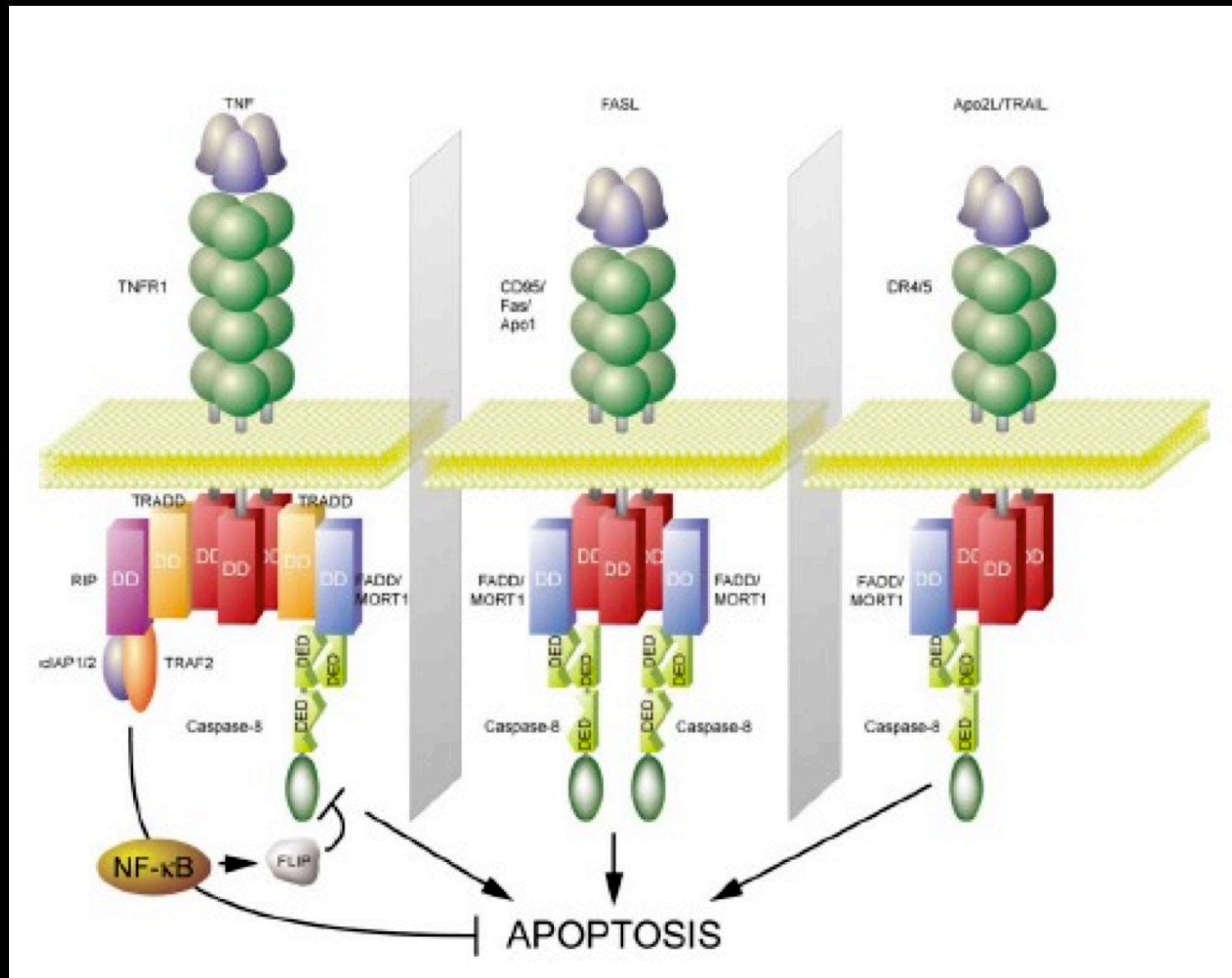
- Mitochondrial pathway. Involves release of cytochrome c and other proteins from mitochondria

OUTCOME

- Activation of downstream common signaling players: the caspase family



Extrinsic pathways: The death receptors



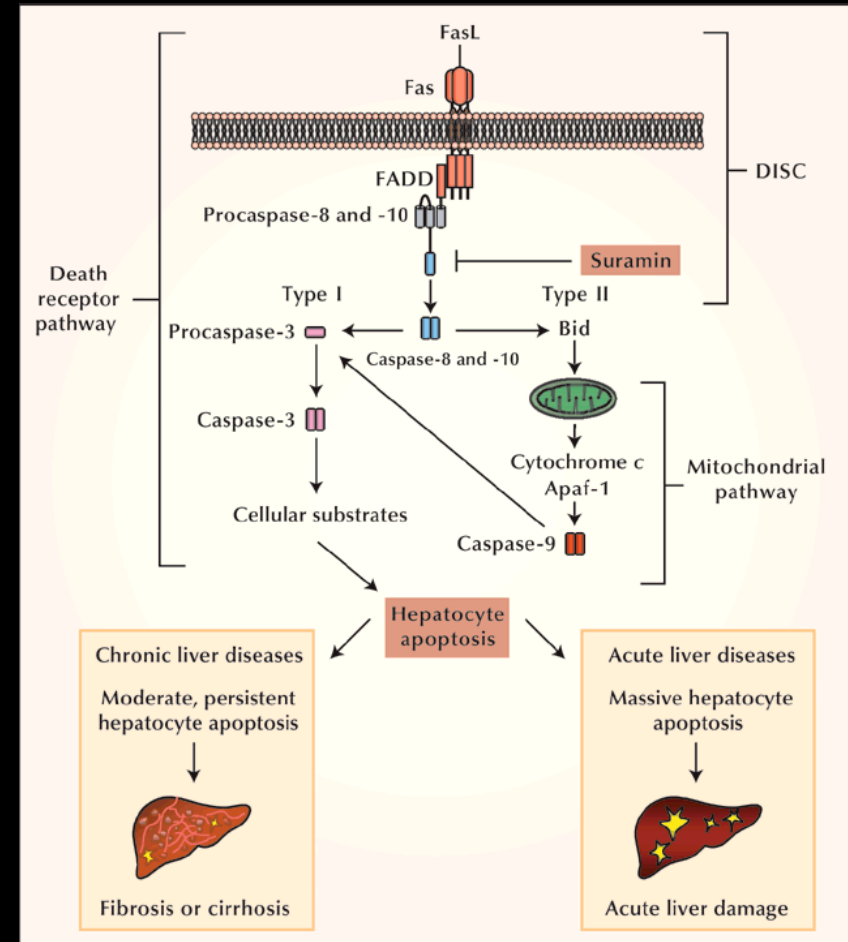
Danial and Korsmeyer. (2004) *Cell*

CD95/FAS: The prototypic death receptor

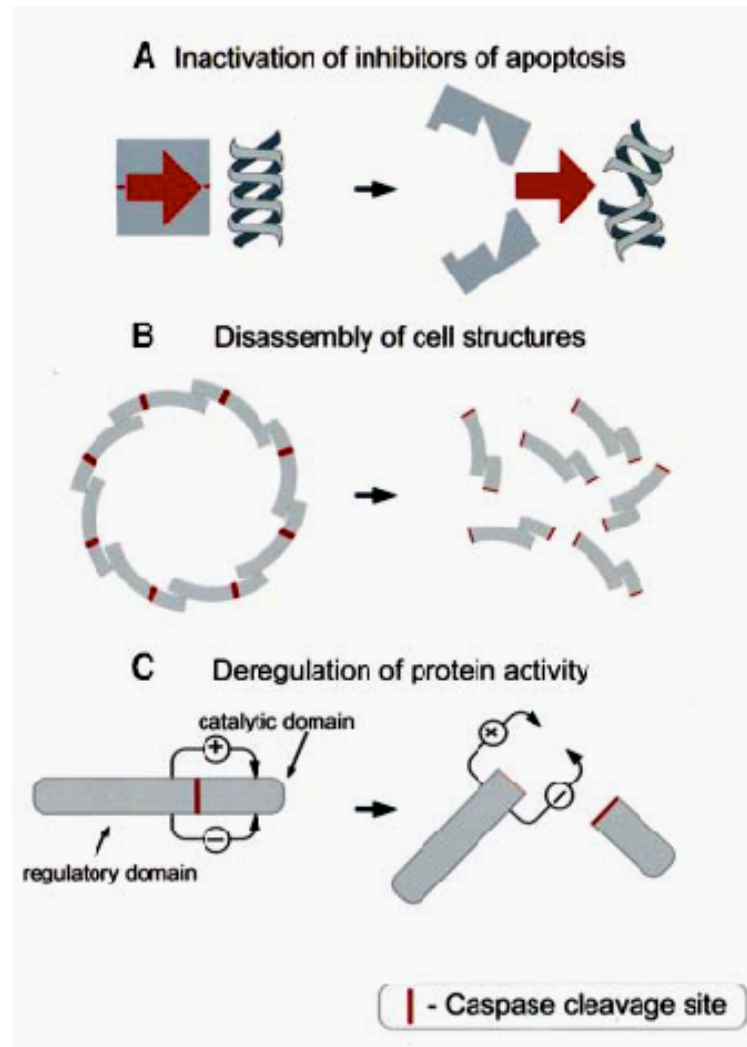
- Mice injected with the CD95 monoclonal antibody exhibited massive apoptosis of liver cells.
- Spontaneous development of autoimmune disease in three CD95/CD95L mutant mouse strains.

Autoimmunity characterized by massive accumulation of lymphocytes (primarily CD4⁺/CD8⁺/B220⁺ T cells), production of high titer autoreactive antibodies, and various related pathologies.

Humans: Autoimmune lympho-proliferative syndrome (ALPS).



How Caspases Disassemble a cell?



Caspase substrates

1) Apoptotic and inflammatory regulators

<i>Proapoptotic</i>	<i>Antiapoptotic</i>		<i>Inflammatory molecules</i>
Bid	Bcl-2	RIP	IL-1 β
Caspases	Bcl-xL	p65/RelA	IL-18
DFF45	FLIP _L	IKK β	IL-16
p28 Bap31	XIAP	cIAP-1	

2) Protein kinases and other signal transduction regulators

DNA-PK	MEKK1	FAK	Akt
Raf-1	Mst1	Mst2	SLK
PITSLRE p110	PITSLRE p170	PKN	PRK2
Fyn	Wee1	SRPK1	SRPK2
CaMK IV	CaMK II α	CaMKK	ROCK 1
IKK β	RIP	PKC θ	PKC δ
PKC μ	PKC ζ	SPAK	PP2A
RasGAP	Cbl	Cbl-b	ZAP-70

3) Cytosolic and nuclear structural proteins

α -fodrin	actin	Gas-2	gelsolin
Beta II-spectrin	β -Catenin	Cytokeratin 18	NuMA
Lamin A	Lamin B	Lamin C	DNA pol. I
HnRNP proteins C1 and C2		70-kDa protein of U1 snRNP	
topoisomerases I and II		UBF	

4) Repair factors

PARP	DNA-PK	Rad51	ATM
Rad9			

5) Cell cycle regulators

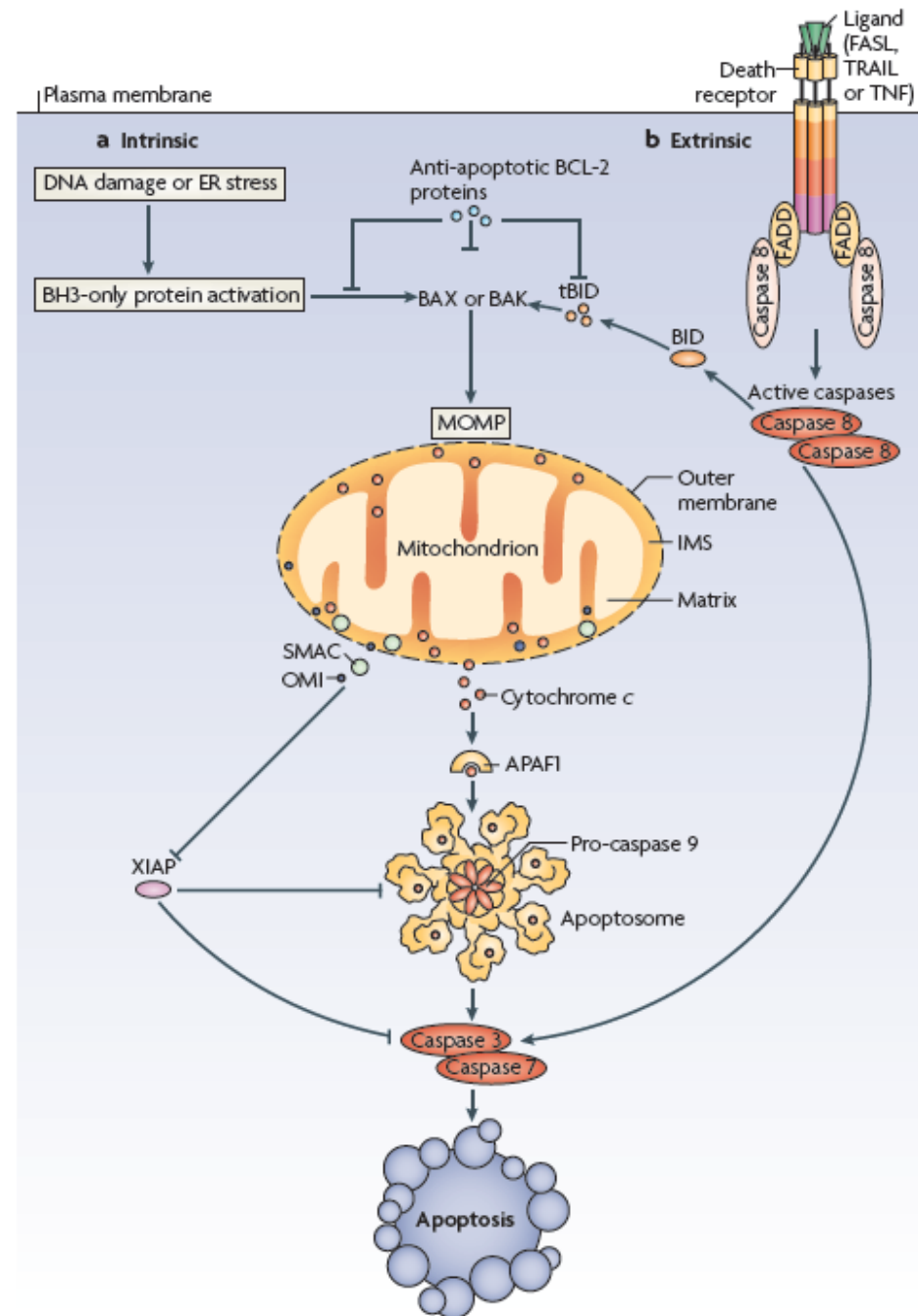
p27	p21	Rb	Mdm2
Cdc27	Wee1		

6) Disease related factors

huntingtin	ataxin-3	androgen receptor	atropin-1
APP			

Intrinsic and extrinsic pathways:
Not a simple definition.

“The BCL-2 protein family and the Gateway of death”



Apoptosis

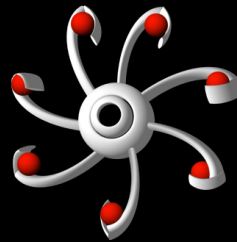
DEATH SIGNAL

Cyto c
release

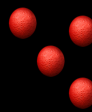
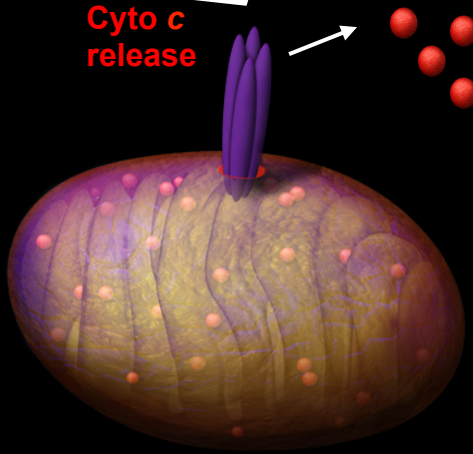
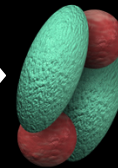
Apoptosome

Effector
Caspases

APOPTOSIS

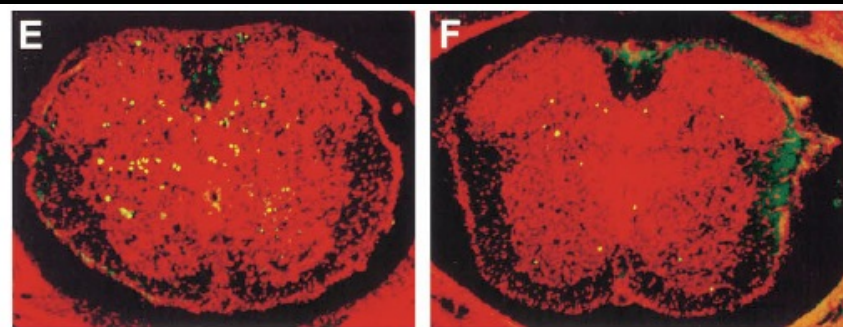
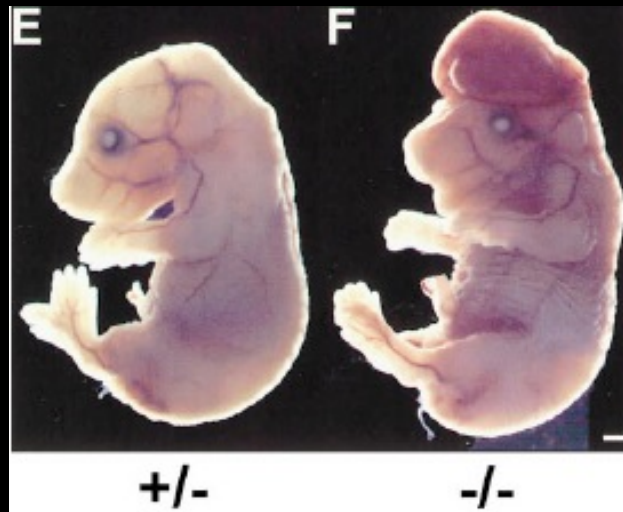


(Apaf-1,
Caspase-9,
Cytochrome c, *ATP*)

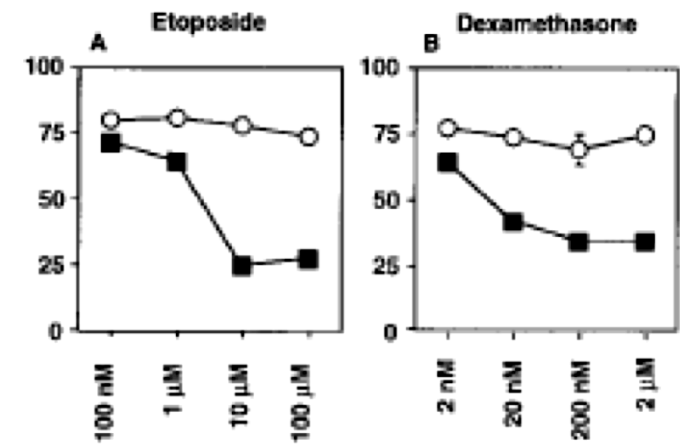


Reduced Apoptosis and Cytochrome c-Mediated Caspase Activation in Mice Lacking Caspase 9

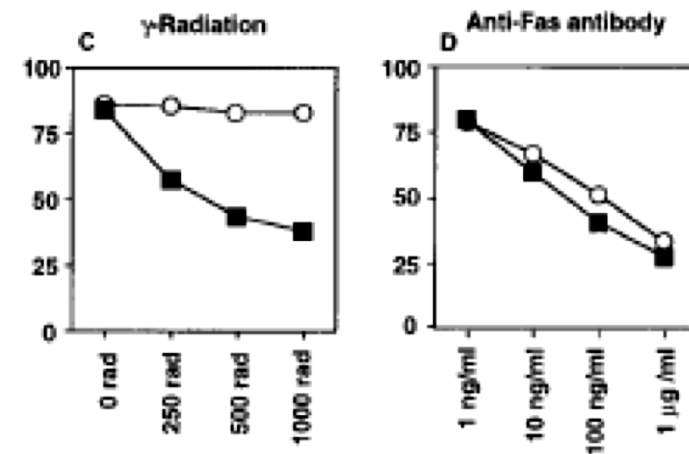
Kuida et al (1998) *Cell*



Survival (%)

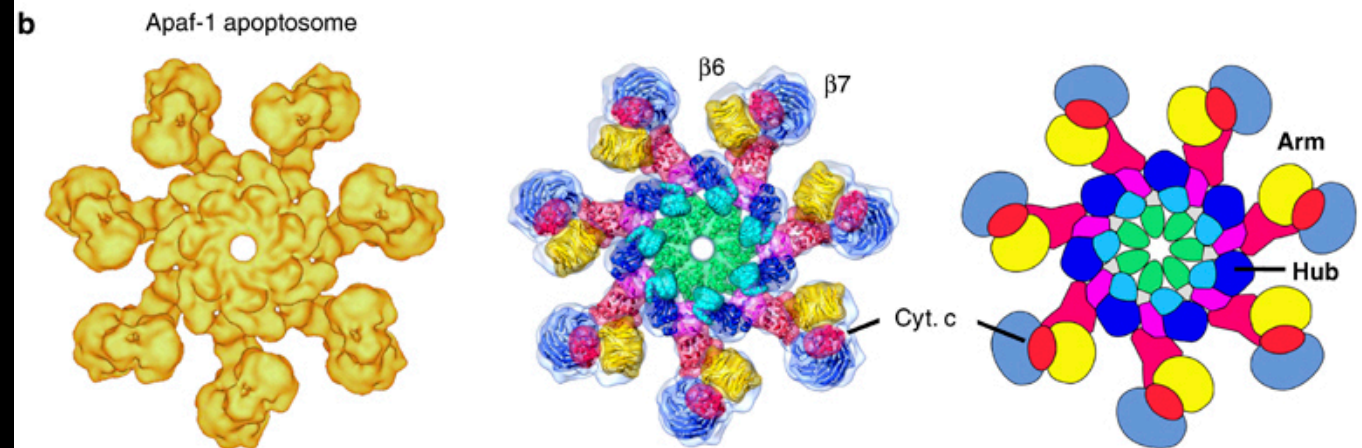
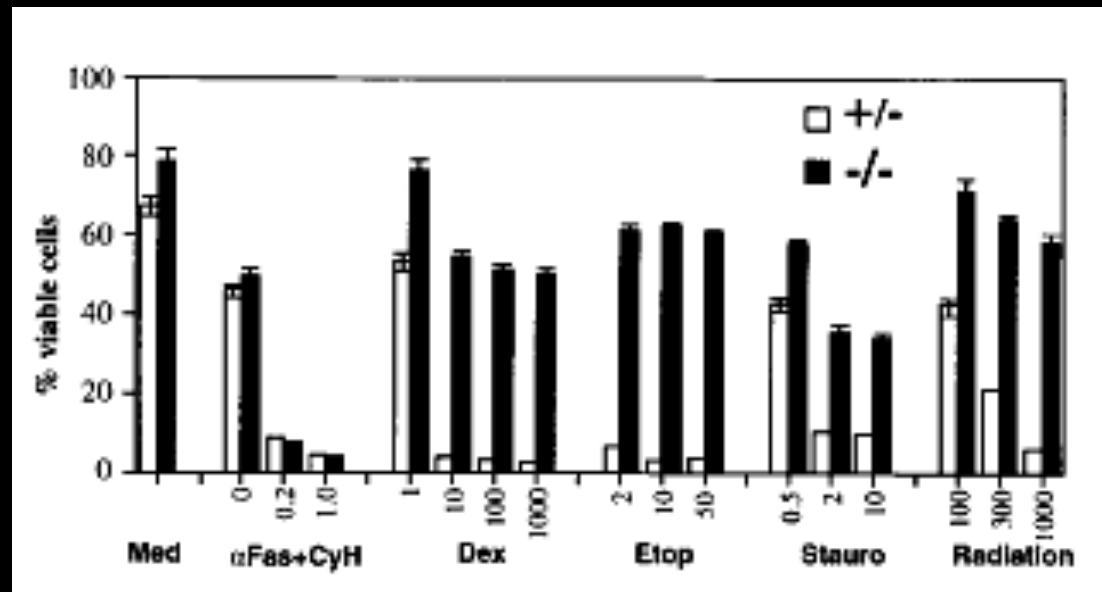
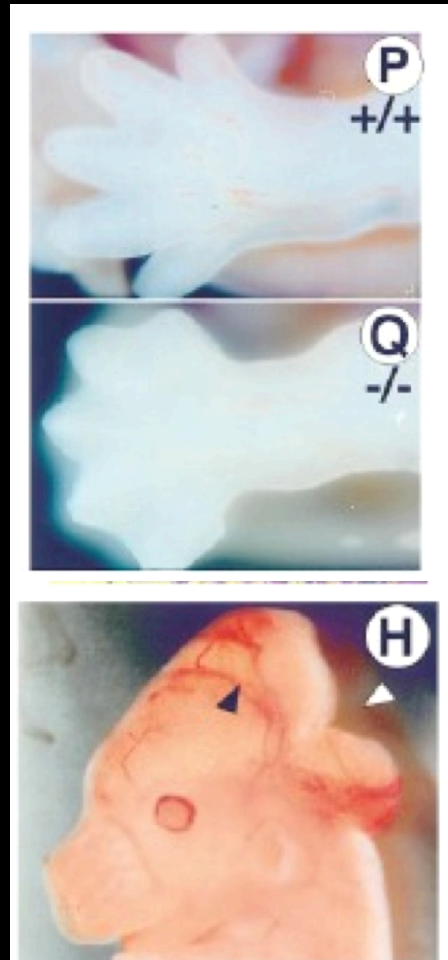


Survival (%)

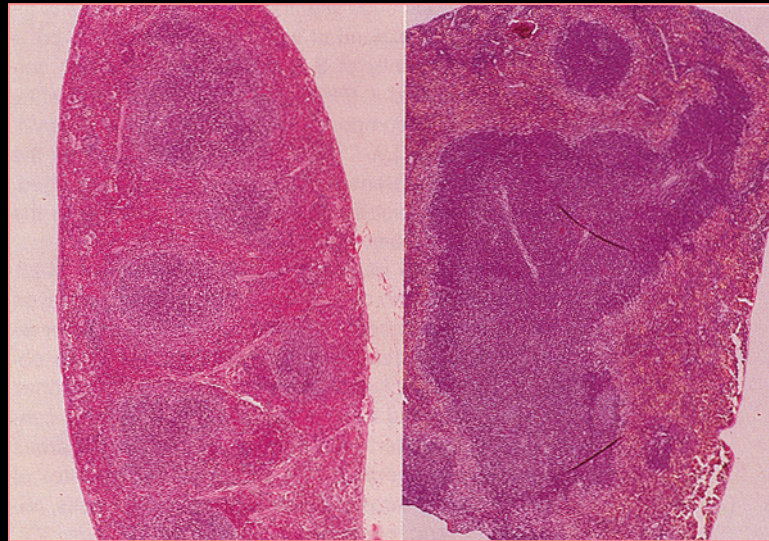
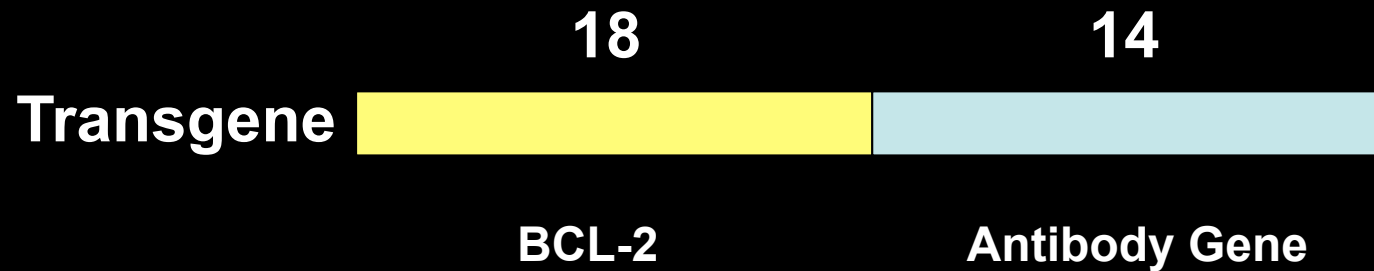


Apaf1 Is Required for Mitochondrial Pathways of Apoptosis and Brain Development

Yoshida et al (2002) *Cell*

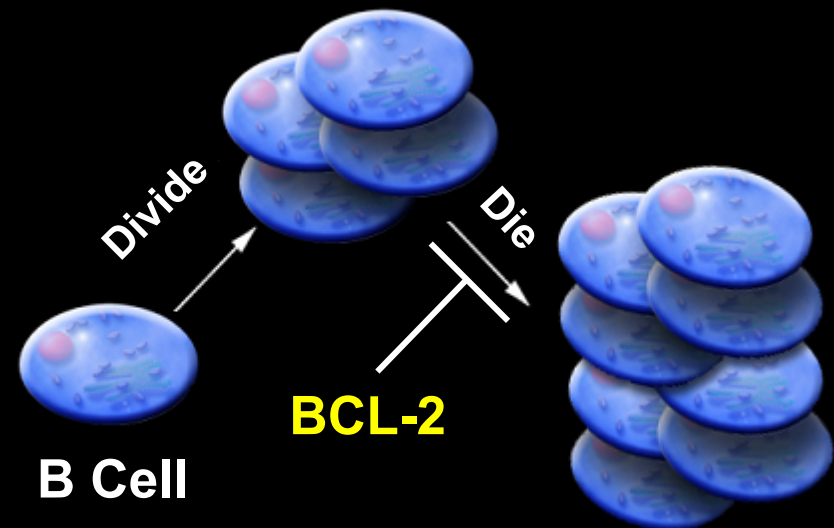


Model of Human Lymphoma



Normal
Mouse

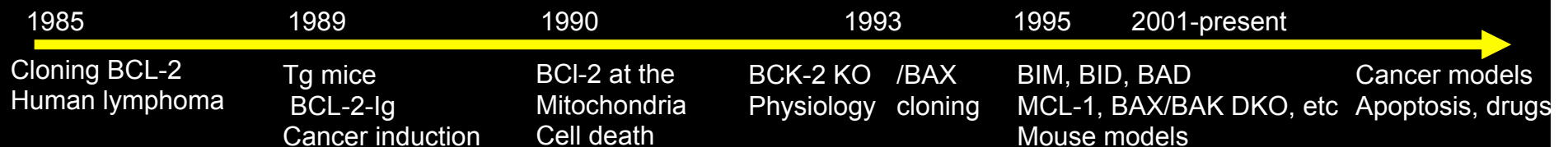
Transgenic
Mouse



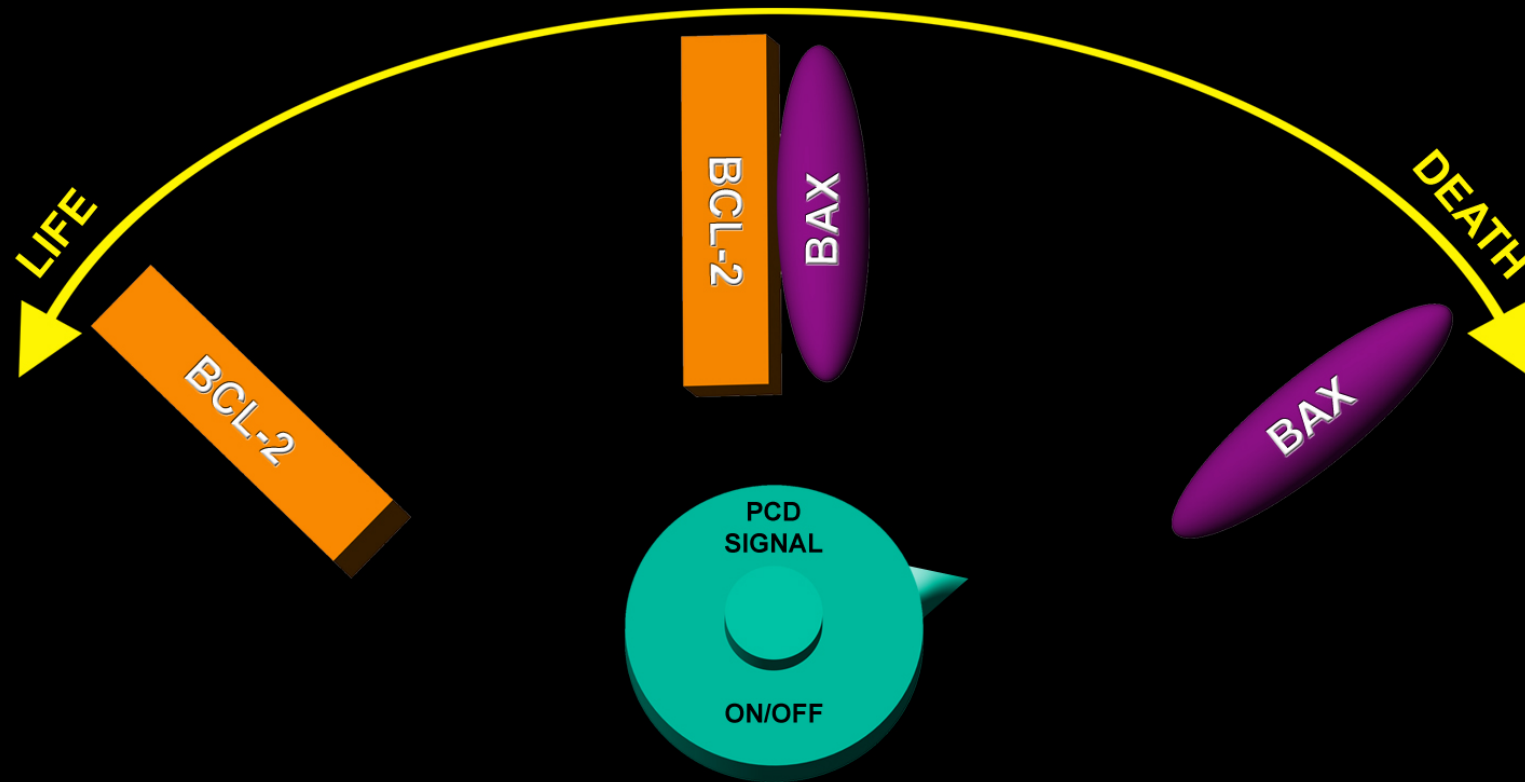
BCL-2

- **Cardinal Member of Mammalian Cell Death Pathway**
- **New Category of Oncogenes: Regulators of Apoptosis**
- **Localized to Mitochondria**

Stanley Korsmeyer



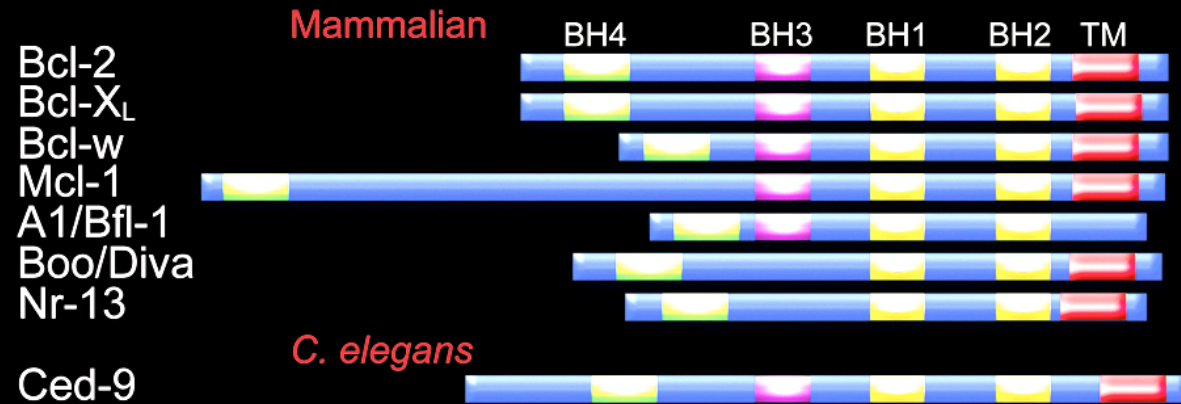
An Evil Twin: BAX



Life/Death Rheostat

BCL-2 Family

Anti-apoptotic

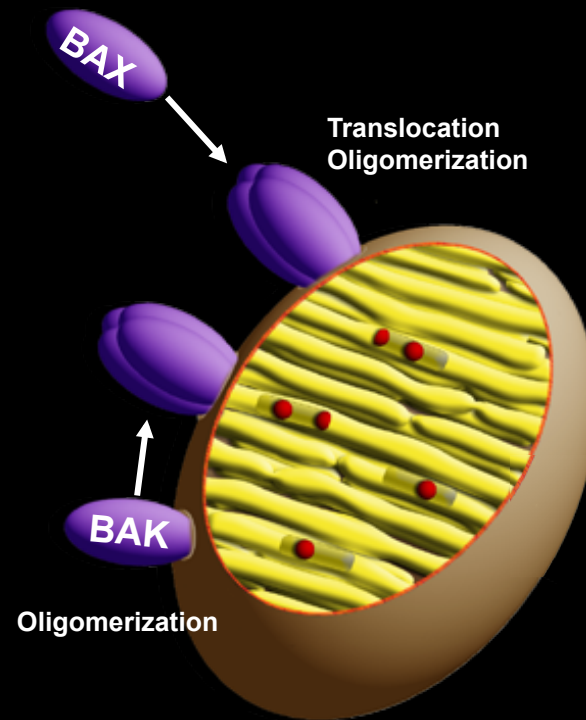


Pro-apoptotic



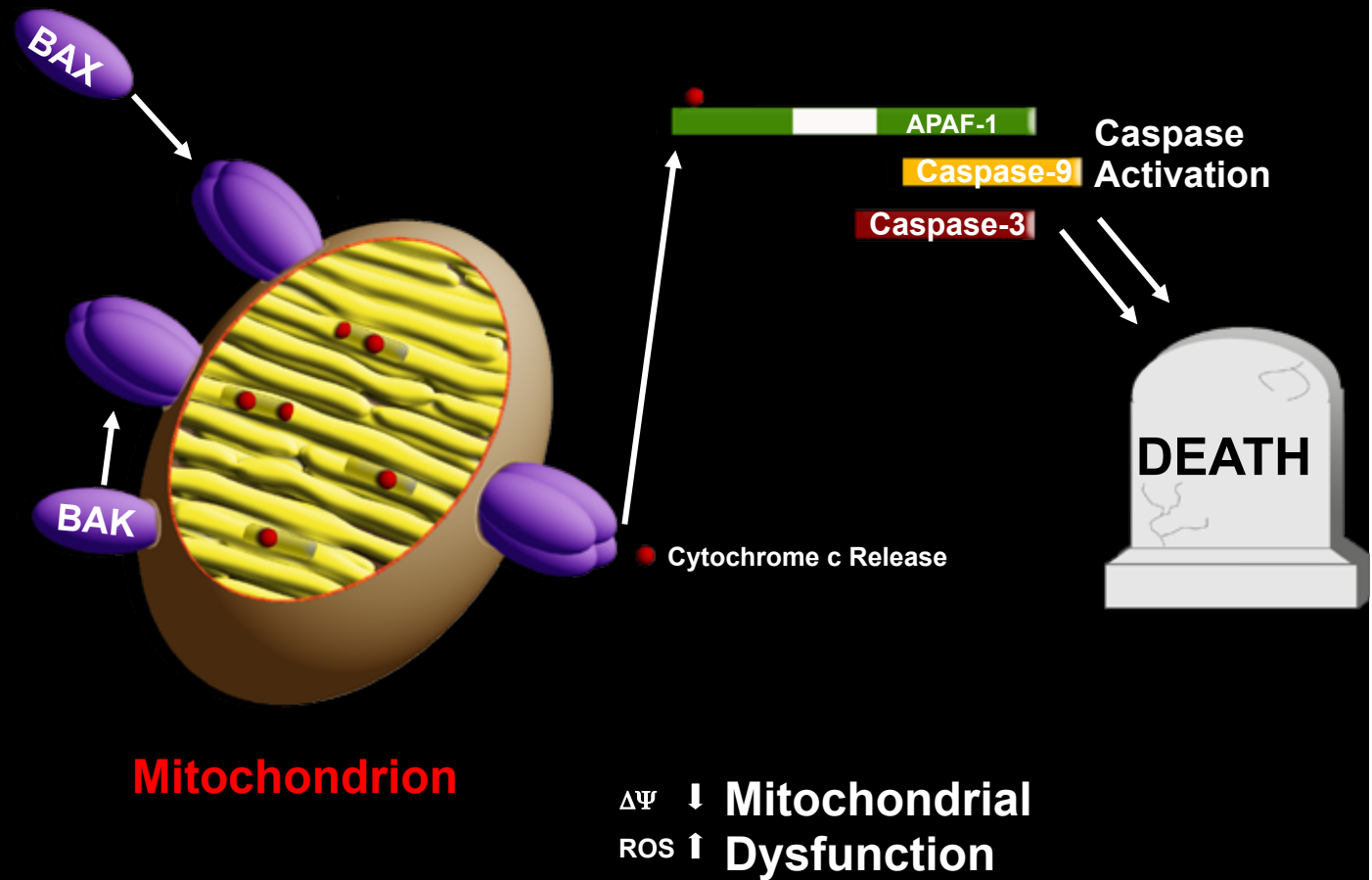
Activation of BAX/BAK: The Mitochondrial Gateway to Apoptosis

Oltzvai et al., (1993) *Cell*
Yin et al., (1994) *Nature*
Wei et al., (2001) *Science*

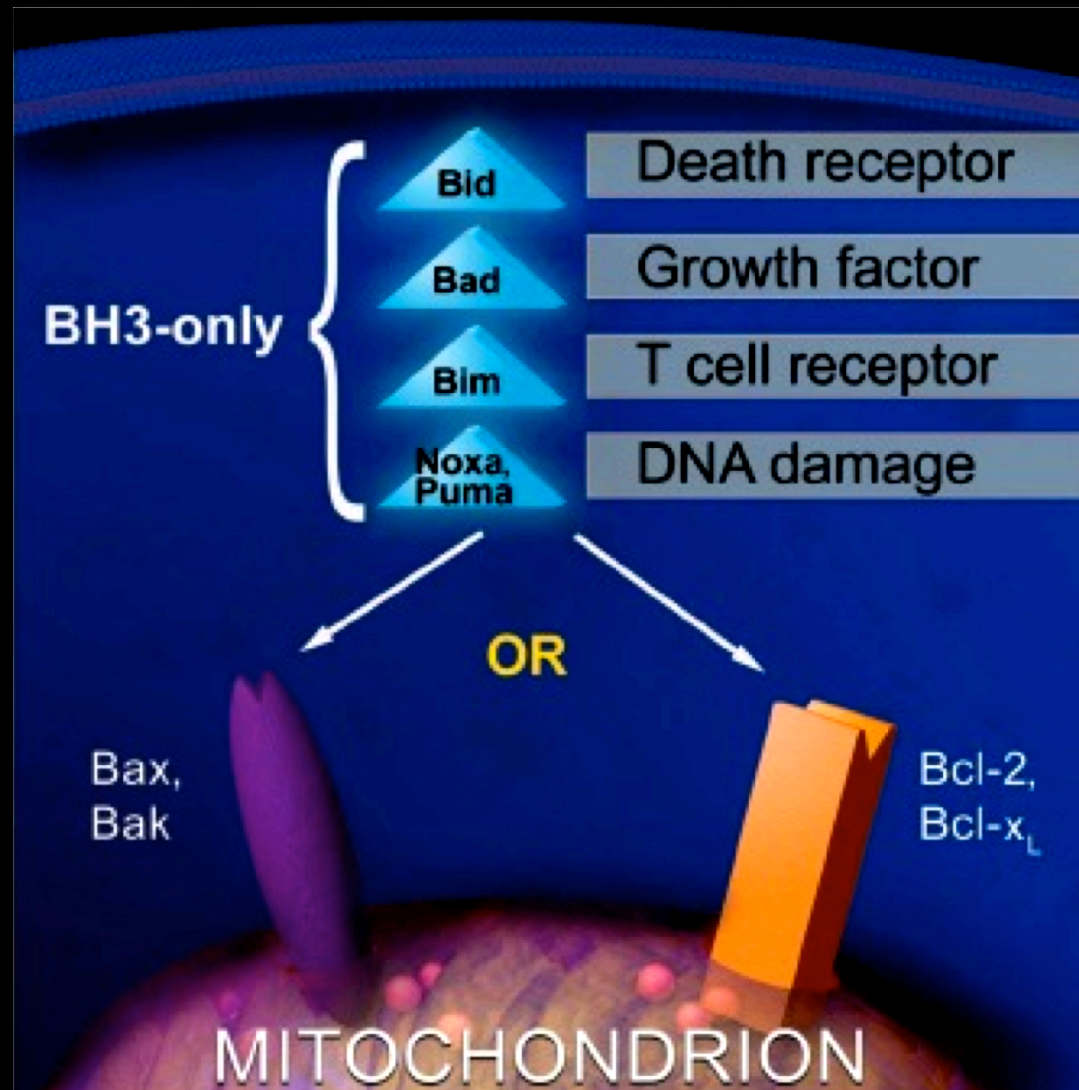


Activation of BAX/BAK: The Mitochondrial Gateway to Apoptosis

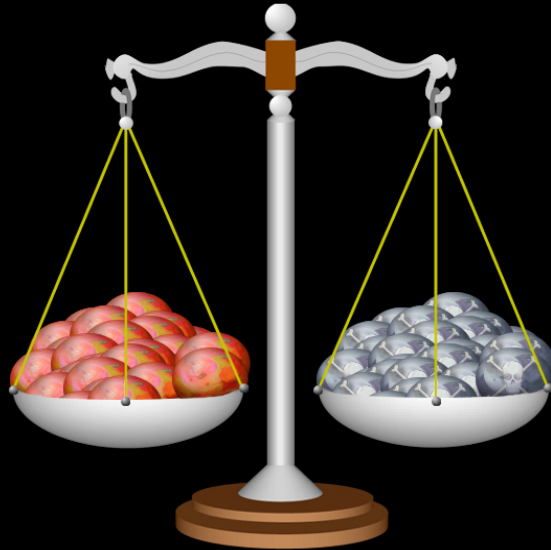
Oltzvai et al., (1993) *Cell*
Yin et al., (1994) *Nature*
Wei et al., (2001) *Science*



The BH3-only proteins: Specialized cell death sentinels



Normal Tissue

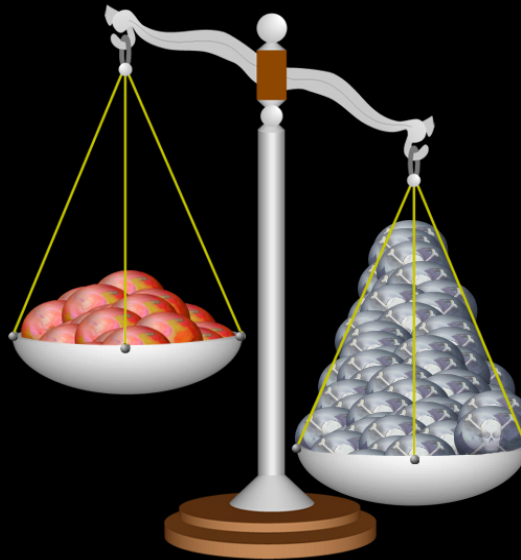


New Cells

Cell Death

Homeostasis

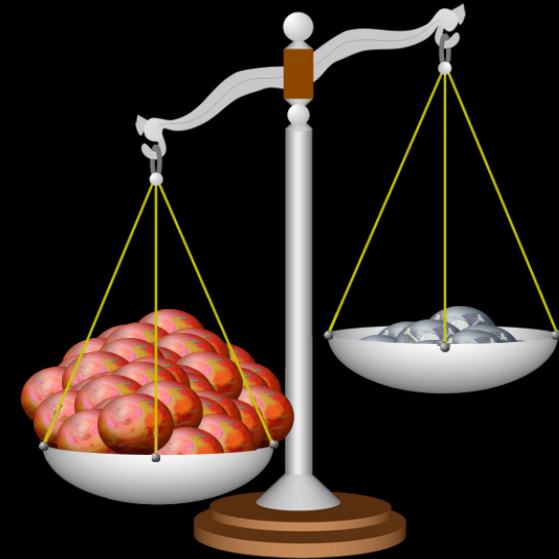
Diseases of Disordered Cell Death



New Cells

Cell Death

Neurodegeneration
Immunodeficiency

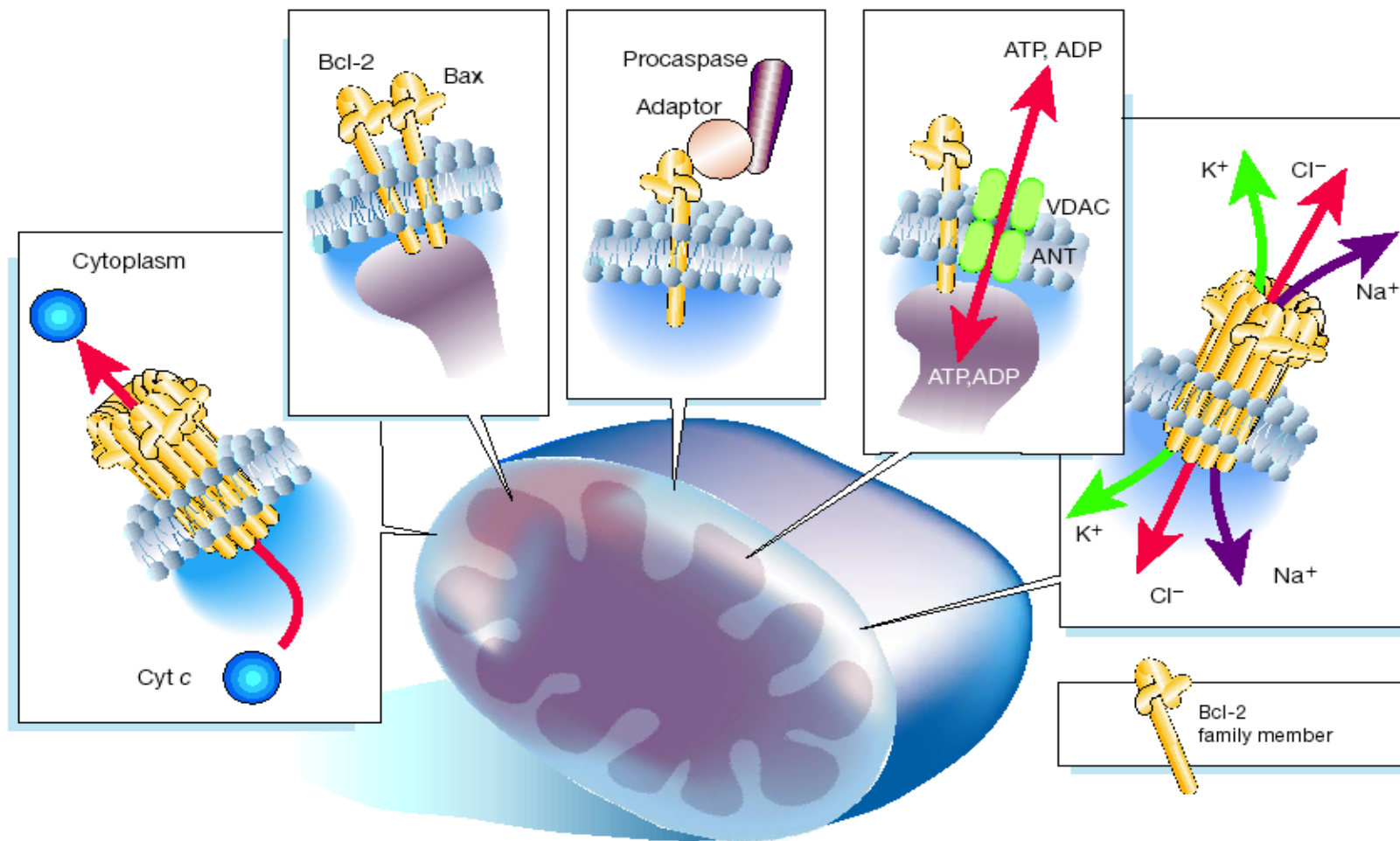


New Cells

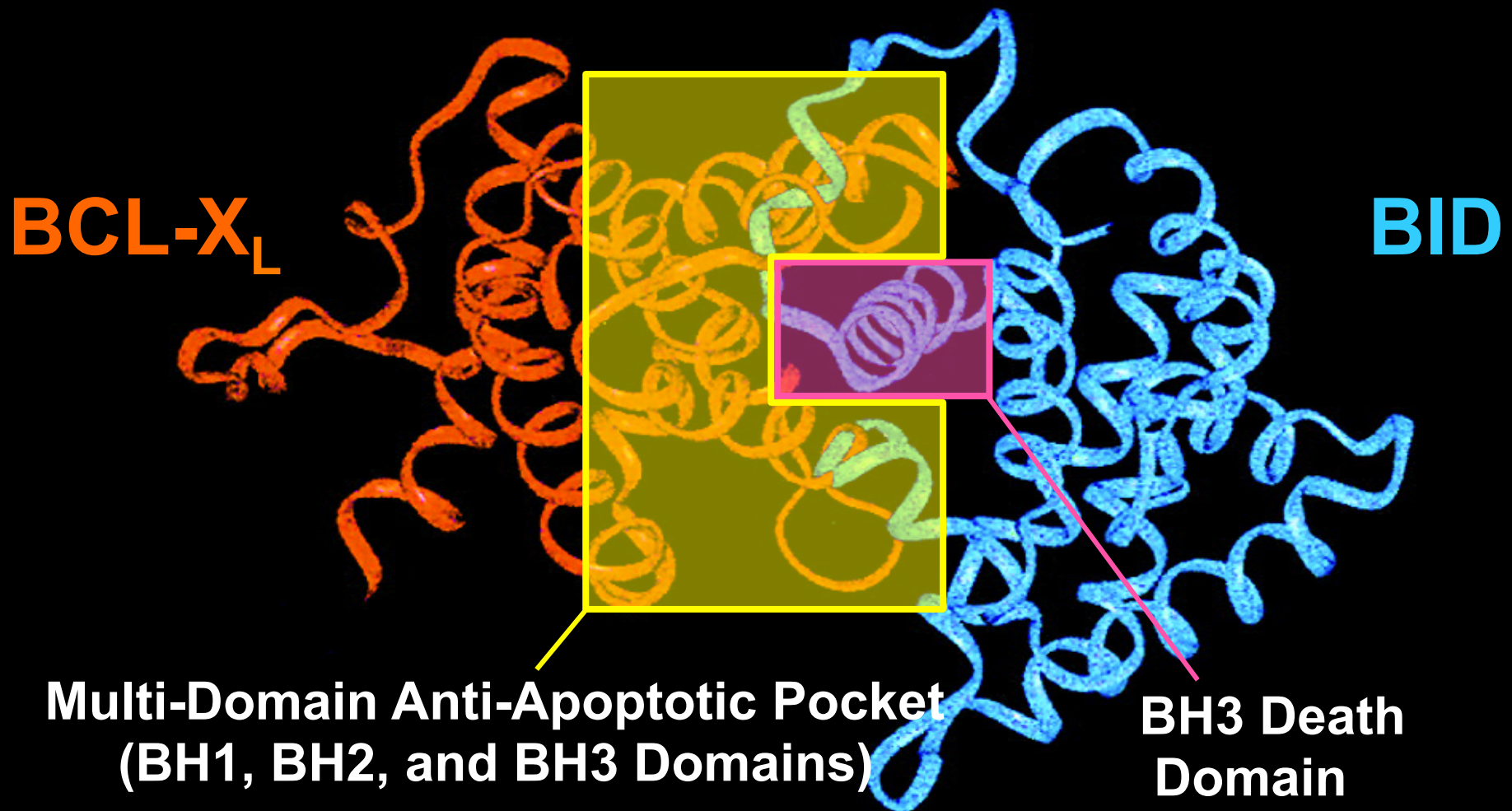
Cell Death

Cancer
Autoimmunity

Possible Mechanisms of action of Bcl-2 Family Members

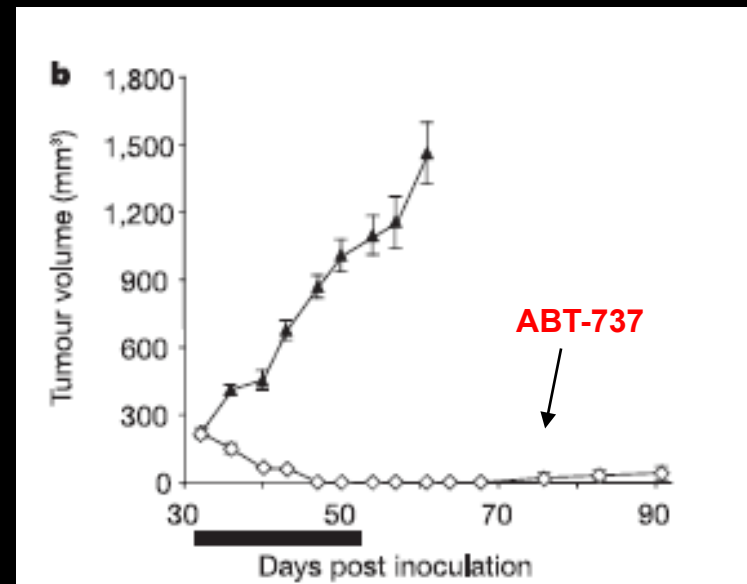
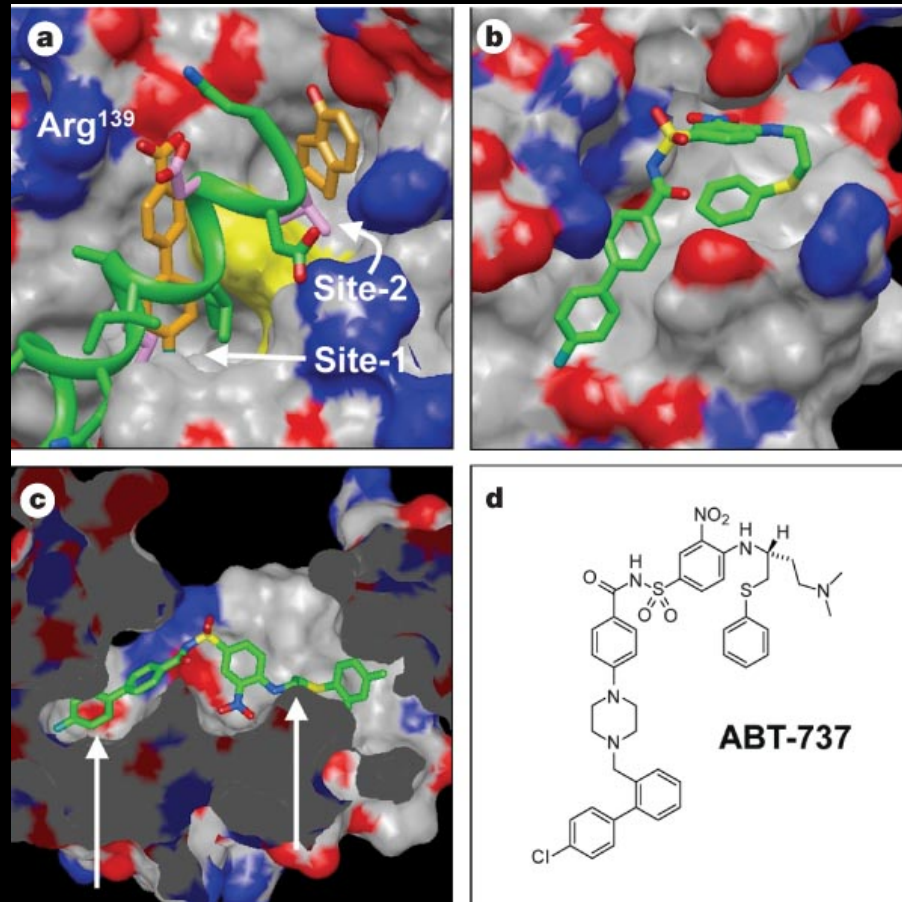


CANCER: Resistance to Apoptosis
Excess Anti-Apoptotics Sequester BH3 Domains



BH3 Mimetics: Prototype Cancer Therapeutics to Restore Apoptosis

Small Molecules: (ABT-737). Generated using NMR-based screening, parallel synthesis and structure based design. Idum Pharmaceuticals.



Olterdorf *et al.* (2005) *Nature*

Apoptosis in the treatment of cancer

An important goal of cancer drug development should be to facilitate apoptosis in neoplastic cells. Drugs that restore a p o p t o s i s m i g h t selectively kill cancer cells that have triggered a death signal and have become d e p e n d e n t o n the deregulation of apoptosis pathways.

Table 1 | **Status of apoptosis-inducing agents**

Compound	Company	Status
Anti-TRAILR1 agonistic antibody	Human Genome Sciences/ Cambridge Antibody Technology/ Takeda	Phase I and II trials initiated for solid tumours
Anti-TRAILR2 agonistic antibody	Human Genome Sciences/ Cambridge Antibody Technology	Phase I studies initiated in the United Kingdom and the United States
TRAIL	Genentech/Amgen	Phase I studies initiated in solid tumours
Oblimersen/Genasense (antisense oligonucleotide targeting <i>BCL2</i>)	Genta Inc.	Failed to meet primary end-point in phase III trial in malignant melanoma and multiple myeloma, phase III clinical trial and pre-registration in CLL
SPC-2996 (antisense oligonucleotide targeting <i>BCL2</i>)	Santaris Pharma	Phase I/II in CLL in Europe
AT 101((-)-Gossypol)	Ascenta Therapeutics Inc.	Phase I in CLL
Small-molecule BCL2-family inhibitor	Gemin X Biotech	Phase I in CLL
ABT-737 (small-molecule BCL2-family inhibitor)	Abbott Laboratories/Fizer (Idun)	Preclinical
IPI-983L/IPI-194 (small-molecule BCL2-family inhibitors)	Infinity Pharmaceuticals	Preclinical
XIAP-BIR2 inhibitor	Burnham Institute	Preclinical
XIAP-BIR3 inhibitor	UT Southwestern	Preclinical
XIAP-BIR3 inhibitor	Abbott Laboratories	Preclinical
Nutlins (MDM2 inhibitors)	Wyeth	Preclinical

BIR, baculovirus IAP repeat; CLL, chronic lymphocytic leukaemia; TNF, tumour-necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; XIAP, X-linked inhibitor of apoptosis protein.

Fesik *Nat Rev Cancer* (2005)

APOPTOSIS

- **Definition and characteristic features**
- **Regulatory mechanisms: Intrinsic and extrinsic pathways**
- **Mitochondrial-mediated apoptosis**
- **The BCL-2 Protein Family**
- **Apoptosis an disease conditions: Cancer and Neurodegeneration**