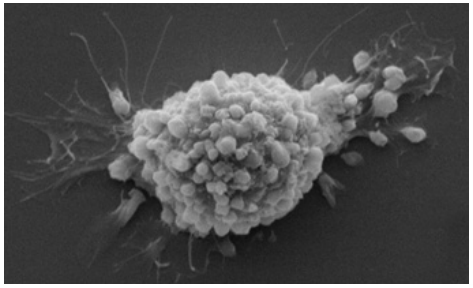
Apoptosis and Cancer

ONCOL 520. Feb. 2, 2012
Ing Swie Goping, PhD
igoping@ualberta.ca
The Biology of Cancer (Weinberg)
Chapter 9



Lecture outline

- What is apoptosis?
- What signaling pathways are activated to induce apoptosis?
- How is apoptosis regulated?
  - Bcl-2 family
  - IAP family
- How can these mechanistic insights lead to rational anti-cancer therapies?

First a refresher: p53 and apoptosis

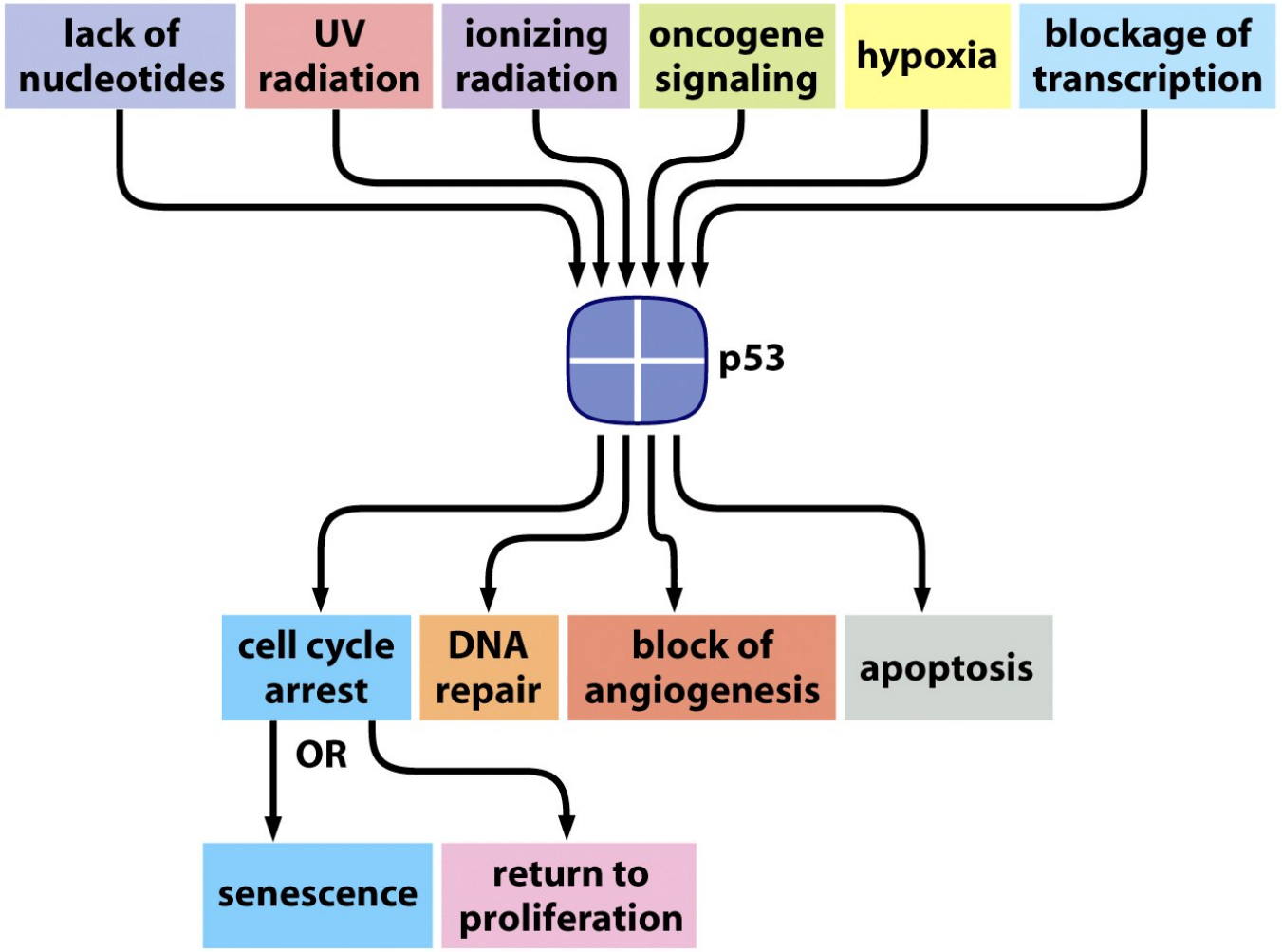


Figure 9.8 *The Biology of Cancer* (© Garland Science 2007)

refresher: p53 and apoptosis

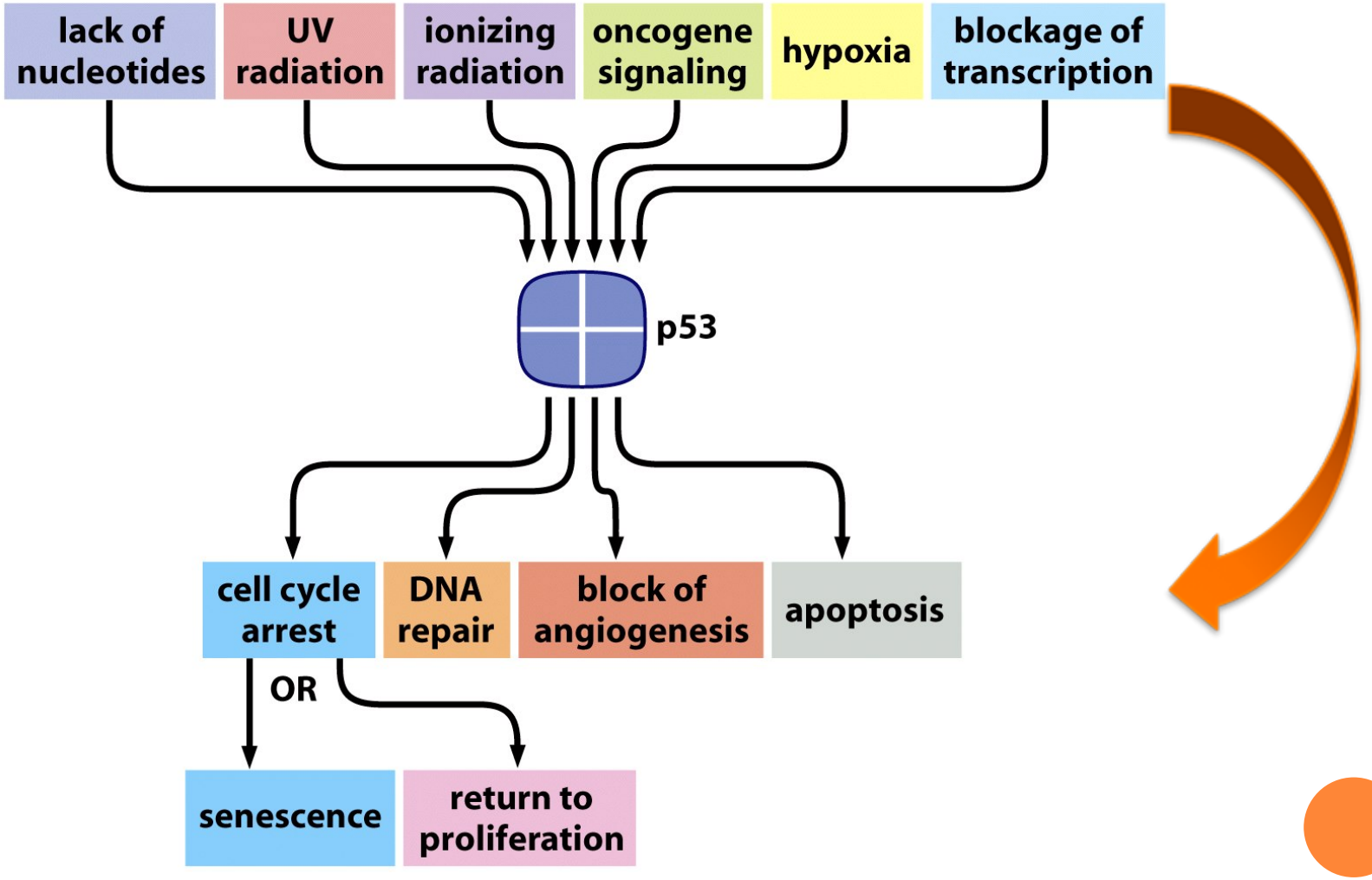
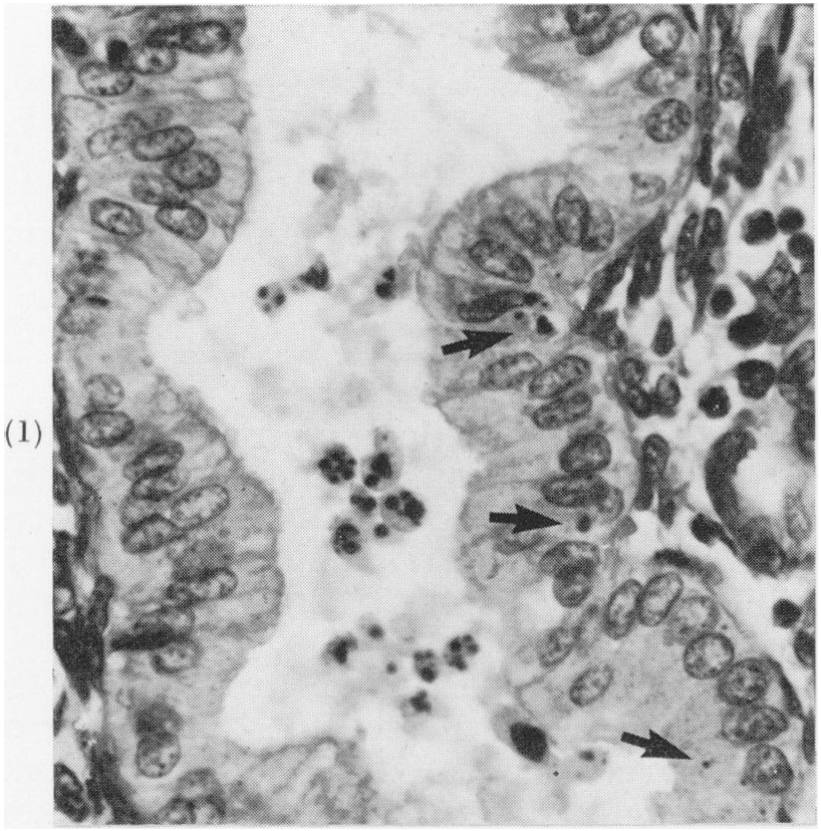


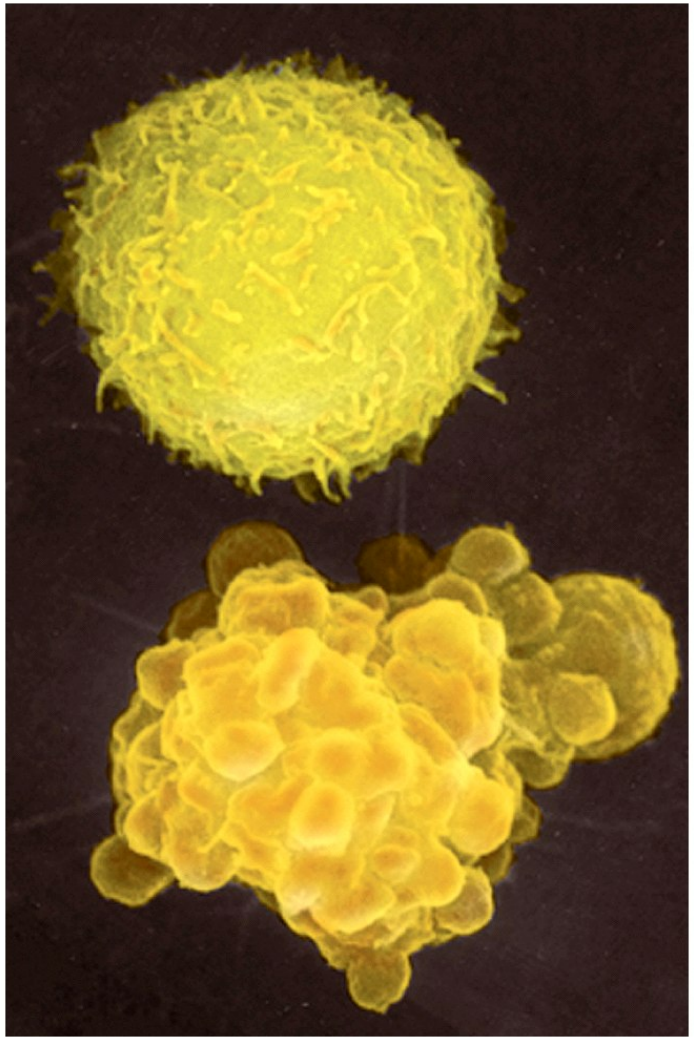
Figure 9.8 *The Biology of Cancer* (© Garland Science 2007)

Apoptosis is programmed cell death



Apoptotic bodies first described by Kerr et al., 1972, Br.J.Can 26,239

Apoptotic cells have distinct morphology



Structural changes occur in 2 stages:
1.Nuclear and cytoplasmic condensation and breaking up of cell into membrane-bound fragments (apoptotic bodies).
2.Apoptotic bodies are ingested by other cells.

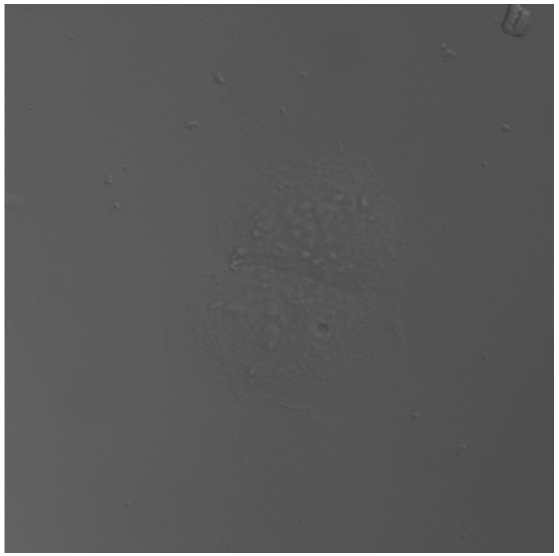


Figure 9.18a *The Biology of Cancer* (© Garland Science 2007)

Apoptosis is morphologically distinct from Necrosis

Apoptosis	Necrosis
1. Programmed cell death	1. Lethal cell injury or accidental death
2. Cell shrinkage	2. Rapid cell swelling
3. Membrane blebbing with plasma membrane integrity intact	3. Lysis and rupture of plasma membrane
4. Nuclear condensation	4. No nuclear changes
5. DNA fragmentation	5. No DNA fragmentation
6. Formation of apoptotic bodies with retention of intracellular enzymes	6. Release of intracellular enzymes into extra cellular space
7. Apoptotic bodies ingested by adjacent cells or macrophages with no inflammation	7. Influx of neutrophils and macrophages with accompanying inflammation
8. Atrophy with stromal collapse but no scarring	8. Active inflammation and scarring

Apoptosis is required for proper development and tissue homeostasis

- Development
  - E.g. nervous and immune systems
  - Digits
  - Tadpole metamorphosis
- Adult tissue—each year we turn over ~$3x10^{14}$ cells
  - Bone marrow
  - Intestine
  - Skin

Apoptosis eradicates cells that represent a threat to the integrity of the organism

- Cells with DNA damage
  - Genome damage can cause a cell to become cancerous
- Tumor cells, or cells infected with virus
  - CTL induce apoptosis of target cells
- Rationale behind anti-cancer chemotherapeutics
  - Many chemotherapeutic agents induce apoptosis in target cancer cells

Clear link between Apoptosis and Cancer

- Tissue homeostasis requires a balance between cellular proliferation and elimination
- Defective cell death mechanisms are one of the hallmarks of cancer (Hanahan and Weinberg, 2011)

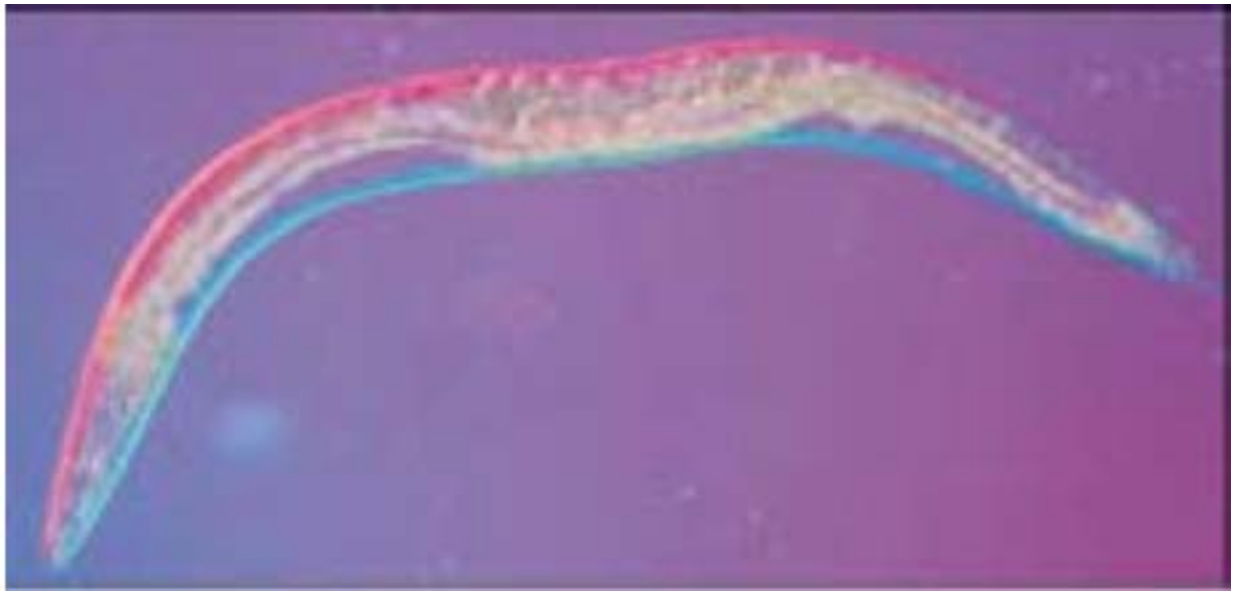
Paradox

- The apoptotic machinery is down-regulated in cancer
- Essentially all anti-cancer drugs kill cancer cells through by stimulating apoptosis (reviewed by Debatin et al., 2002; Reed, 2008)
  - Single or double-strand breaks, microtubule depolymerization or stabilization, glucocorticoid receptor activation, inhibition of estrogen and androgen receptors
- Can we develop rational targeted therapies based on an understanding of the molecular mechanisms of apoptosis?

Therefore, we need to determine…

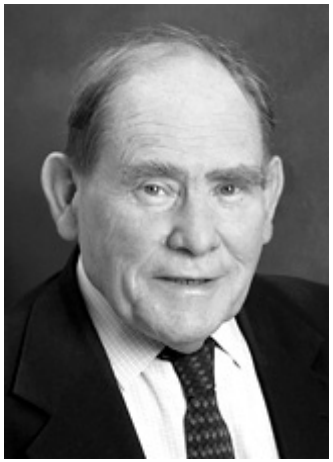
- What are the major components of the apoptotic machinery?
- How are they regulated? i.e. elucidate the signaling pathway
- Are they differentially regulated in cancer cells?
- Can they be manipulated?

Landmark studies in apoptosis research



A simple model organism to study cell lineage and cell death

Apoptosis is programmed cell death

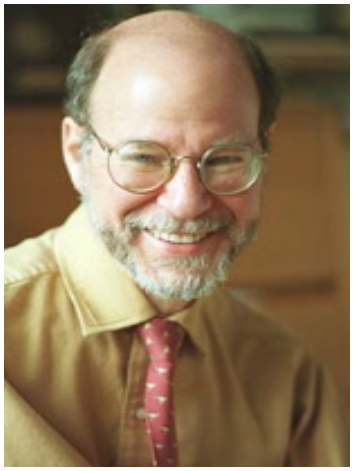


Sydney Brenner

First to use C. elegans as a model system
Berkeley, CA, USA

John E. Sulston

Mapped cell lineage and described cell death
Cambridge, UK

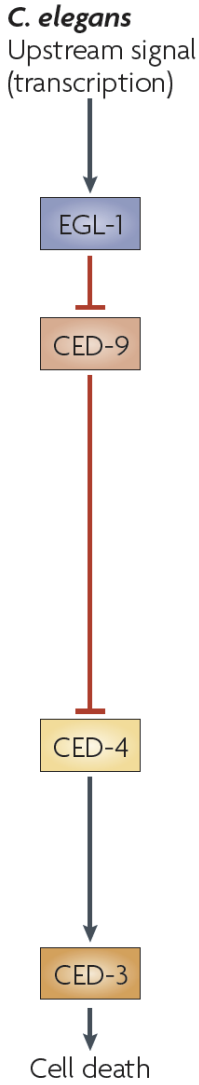


H. Robert Horvitz

Identified 'death' genes (CED-3 and CED-4) in C. elegans,
Cambridge, MA, USA

Nobel prize for physiology or medicine 2002—Cell Death

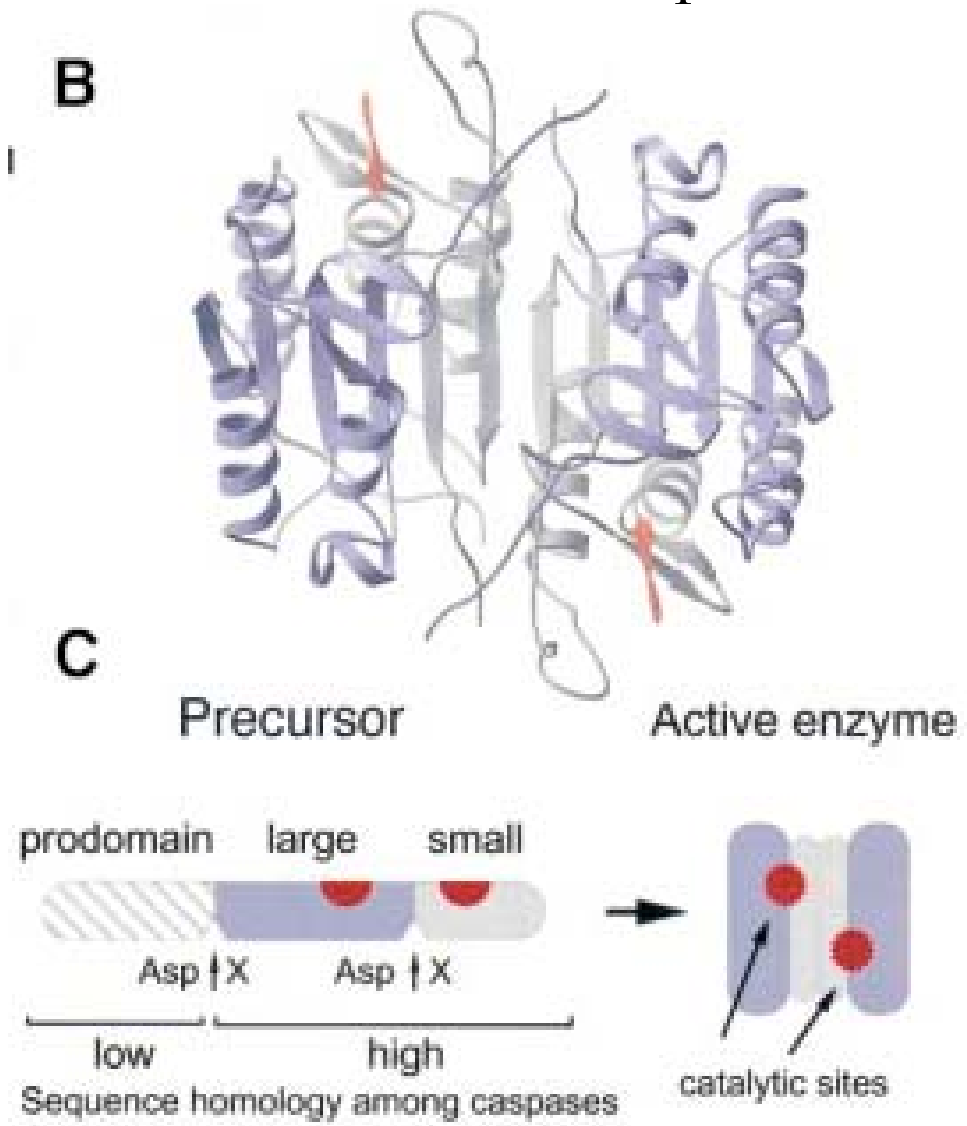
Apoptosis is programmed cell death



Apoptosis pathway was initially elucidated in the nematode C. elegans

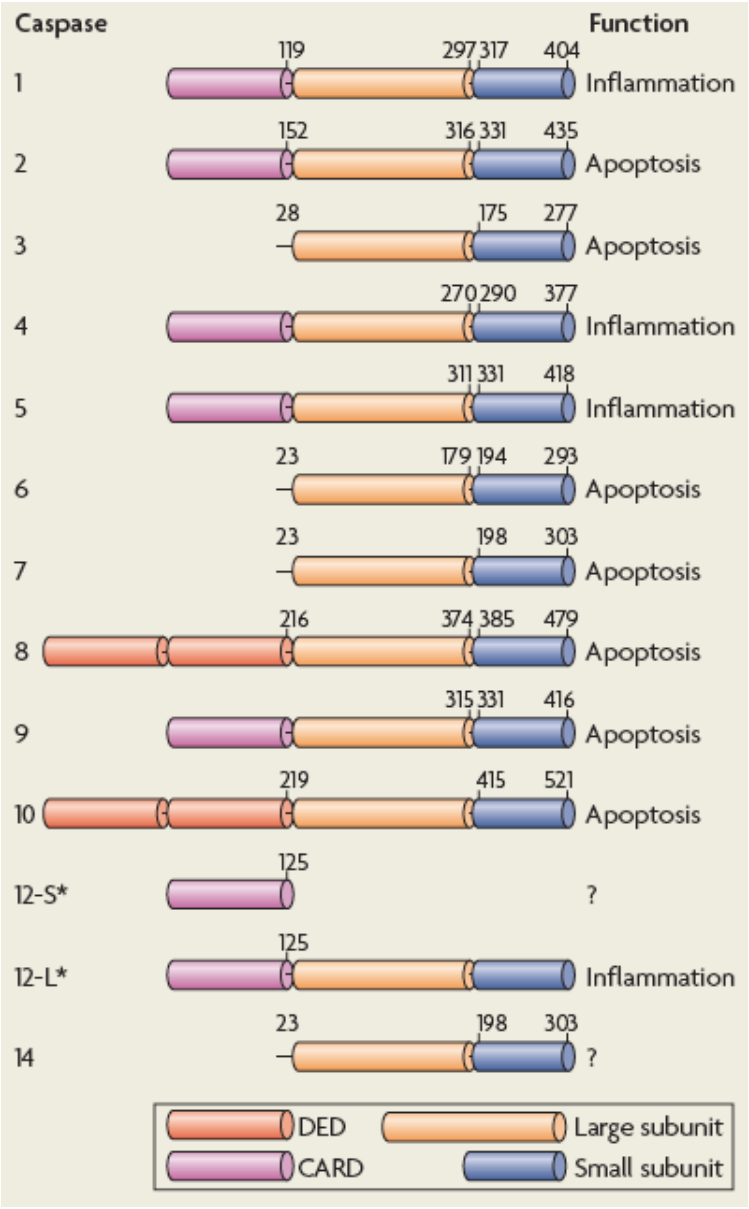
Degterev and Yuan, 2008. Nature Reviews MCB 9: 378

- The structure of caspases



Thornberry and Lazebnik (1998) Science 281: 1312

The caspase family



Box 2 of Taylor et al. (2007) Nature Reviews MCB 9:231

How caspases induce apoptotic morphology

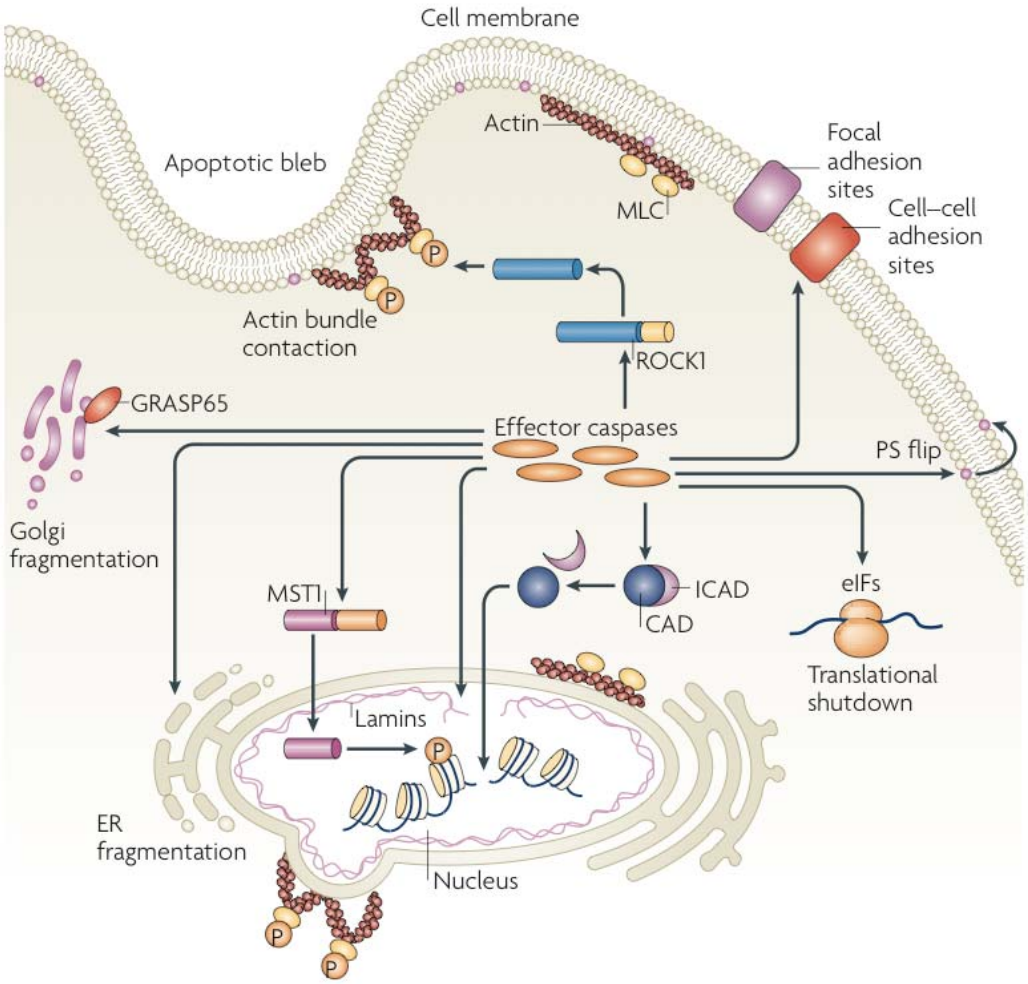


Fig. 3 of Taylor et al. (2007) Nature Reviews MCB 9:231

Caspases responsible for apoptotic controlled autodigestion

- Proteases that cause ordered dismantling of cell
- Present as inactive precursors
- Activation through proteolytic cascade
- Activity must be tightly regulated

Models of apoptotic cell death

- Extrinsic
- Intrinsic

Extrinsic Pathway

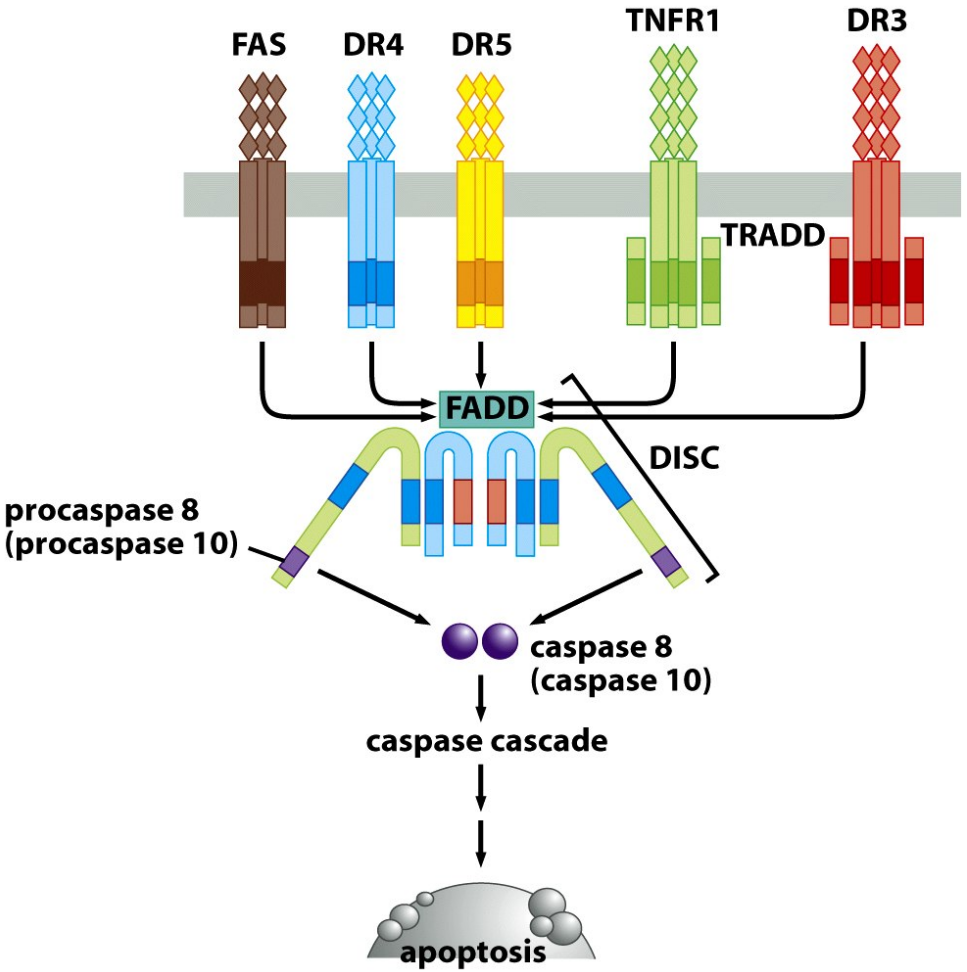


Figure 9.31a *The Biology of Cancer* (© Garland Science 2007)

Intrinsic Pathway

- Mitochondrial-mediated apoptotic pathway

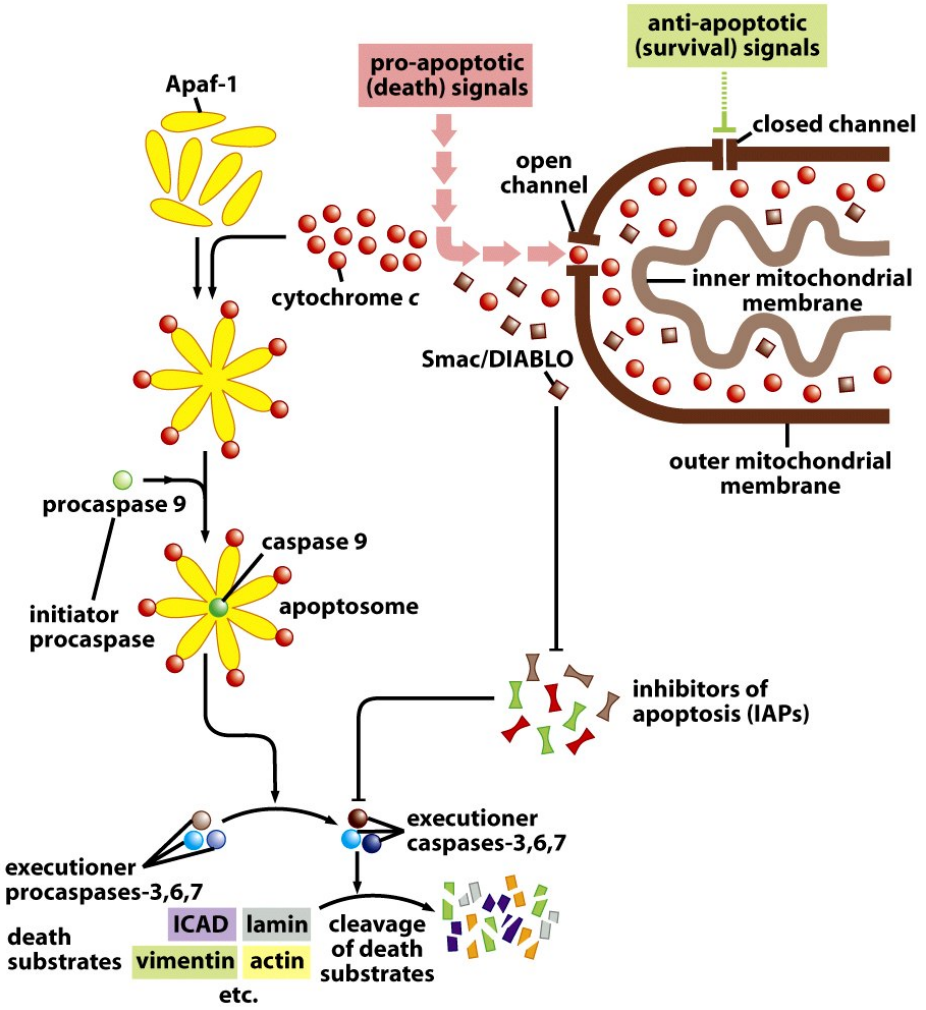
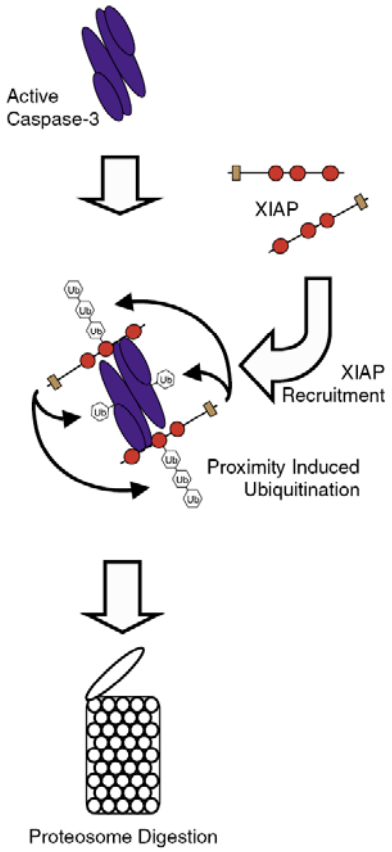
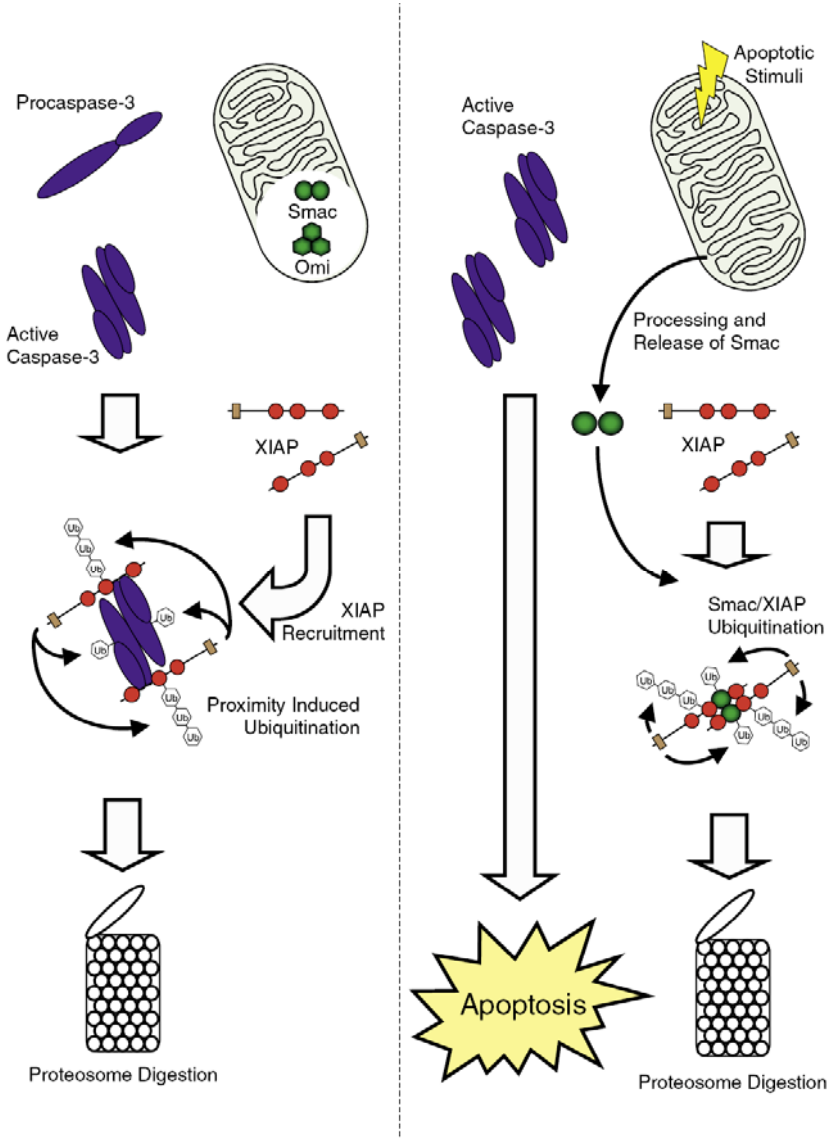


Figure 9.29 *The Biology of Cancer* (© Garland Science 2007)

Inhibitor or Apoptosis proteins (IAPs)

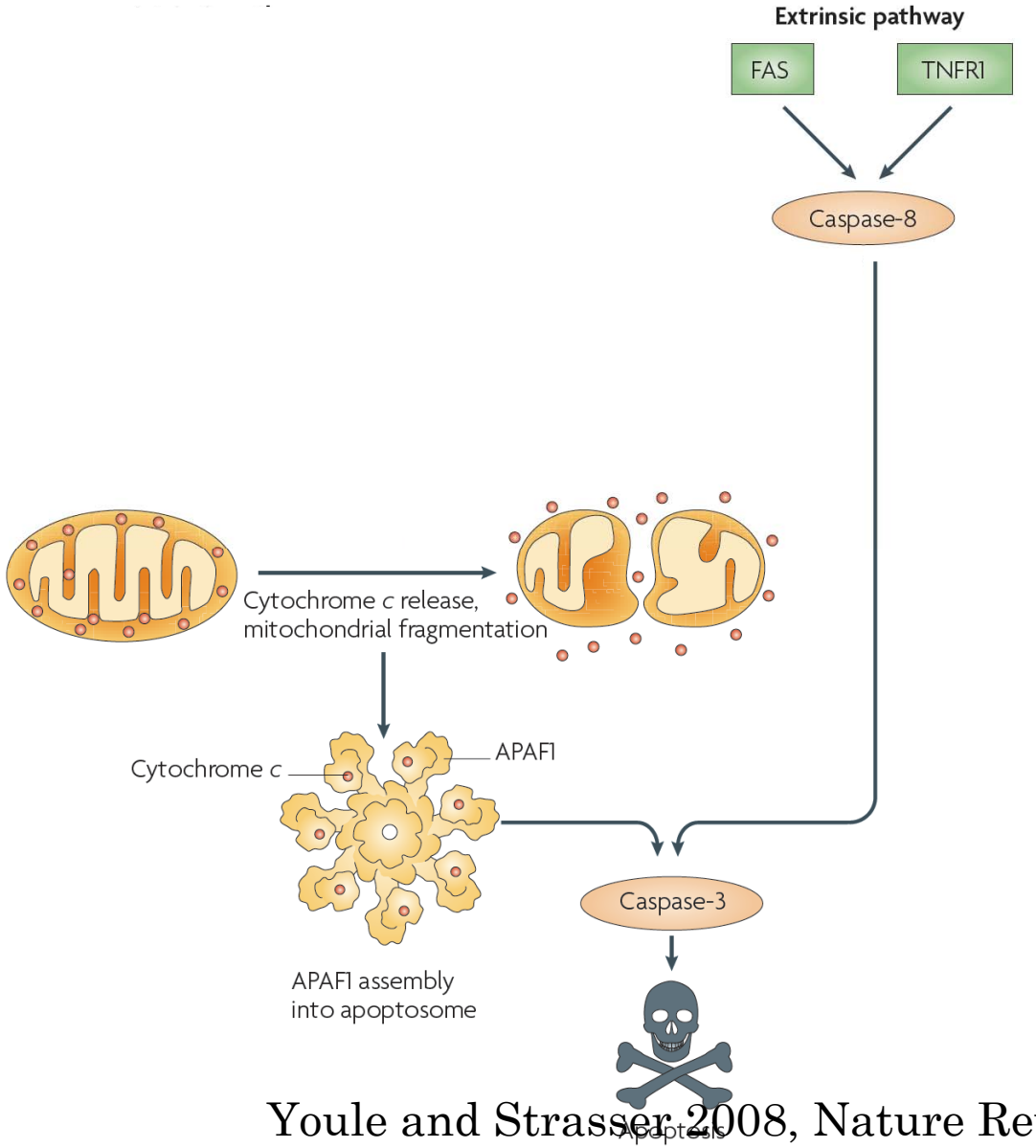


Smac inhibits IAPs



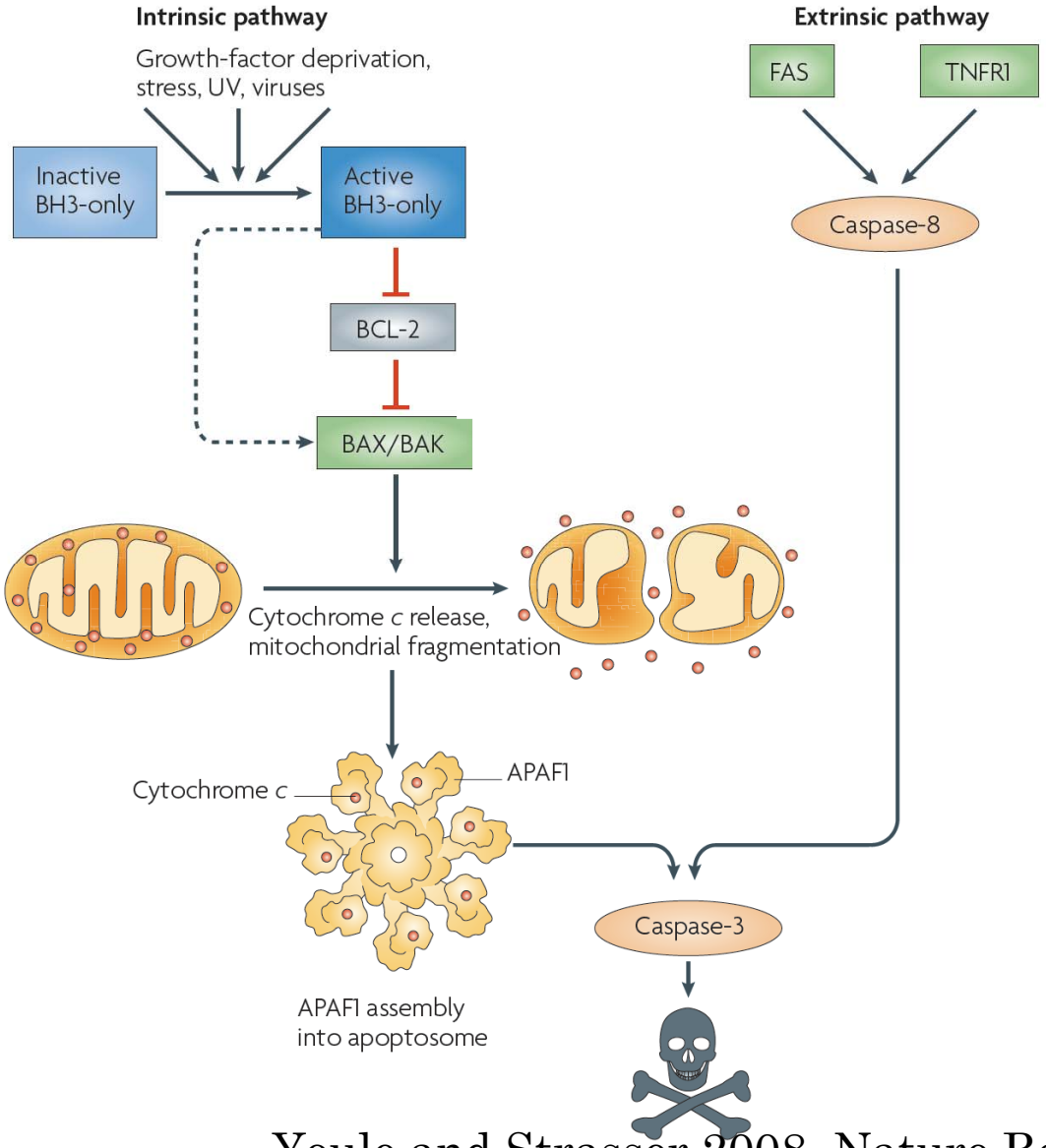
Liston (2003)

Connection between extrinsic and intrinsic pathway



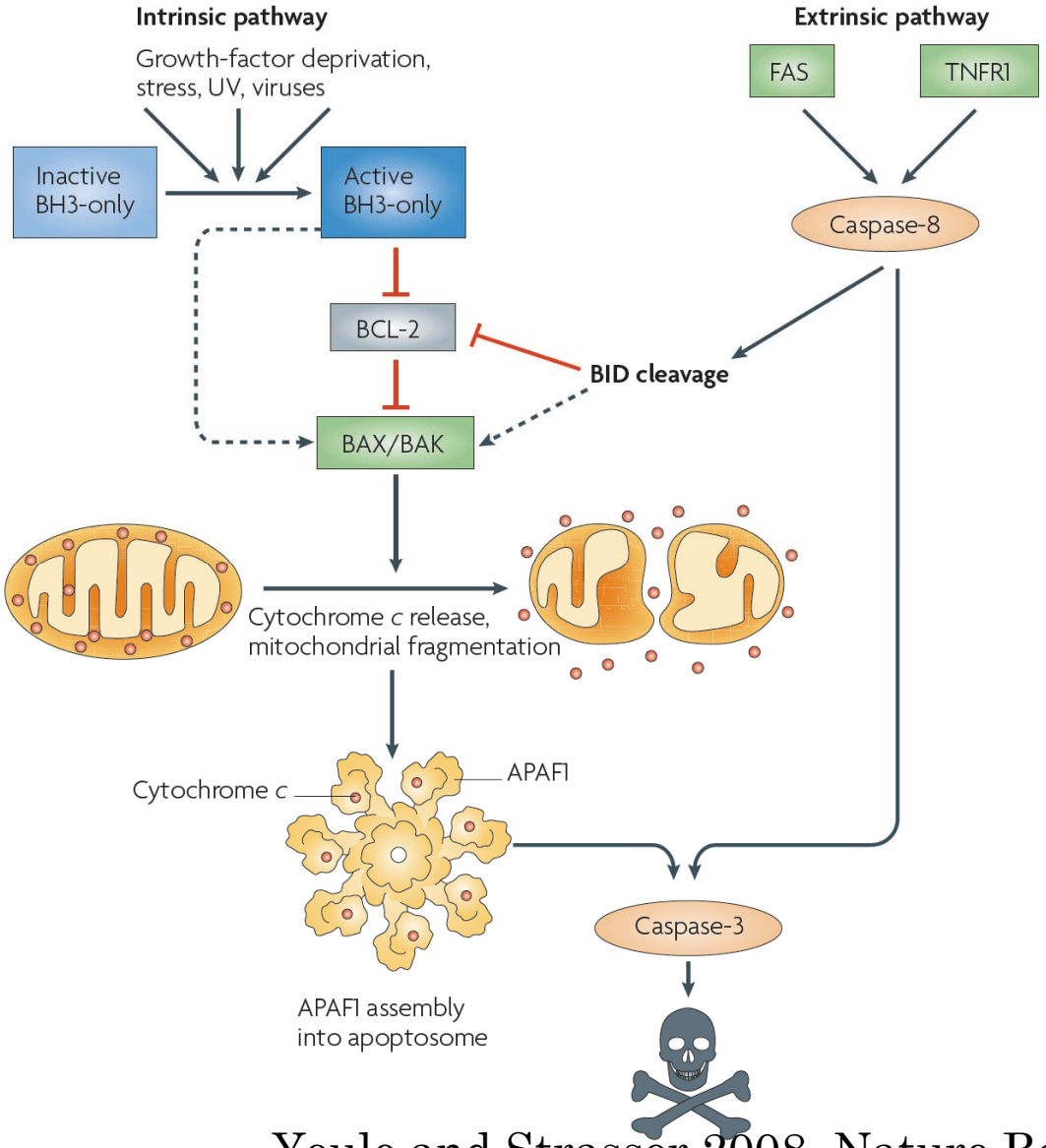
Youle and Strasser 2008, Nature Reviews MCB 9: 47

Connection between extrinsic and intrinsic pathway



Youle and Strasser 2008, Nature Reviews MCB 9: 47

Connection between extrinsic and intrinsic pathway



Youle and Strasser 2008, Nature Reviews MCB 9: 47

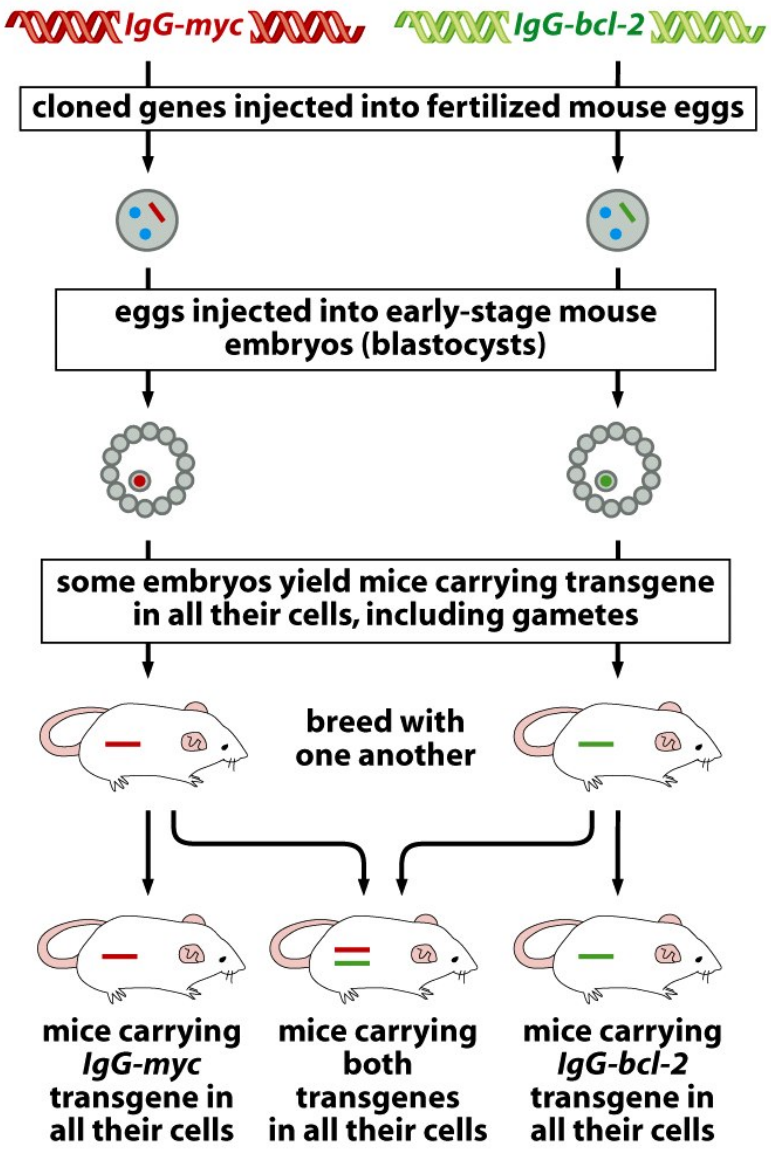
Bcl-2 family proteins and cancer

- Bcl-2-family proteins play central roles in cell death regulation
- Alterations in their expression and function contribute to the pathogenesis and progression of cancer

Bcl-2 (B-cell lymphoma-2)

- The BCL-2 (B-cell lymphoma-2) gene was discovered at the t(14;18) chromosome translocation breakpoint in B-cell follicular lymphomas (Tsujimoto et al., 1985)
- Translocation placed Bcl-2 under the control of the immunoglobulin heavy chain gene promoter and enhancer→excessive transcription of Bcl-2
- Introduced a new paradigm for carcinogenesis
  - unlike previous oncogenes, instead of promoting cell proliferation, overexpression of Bcl-2 inhibits cell death

Bcl-2 studies



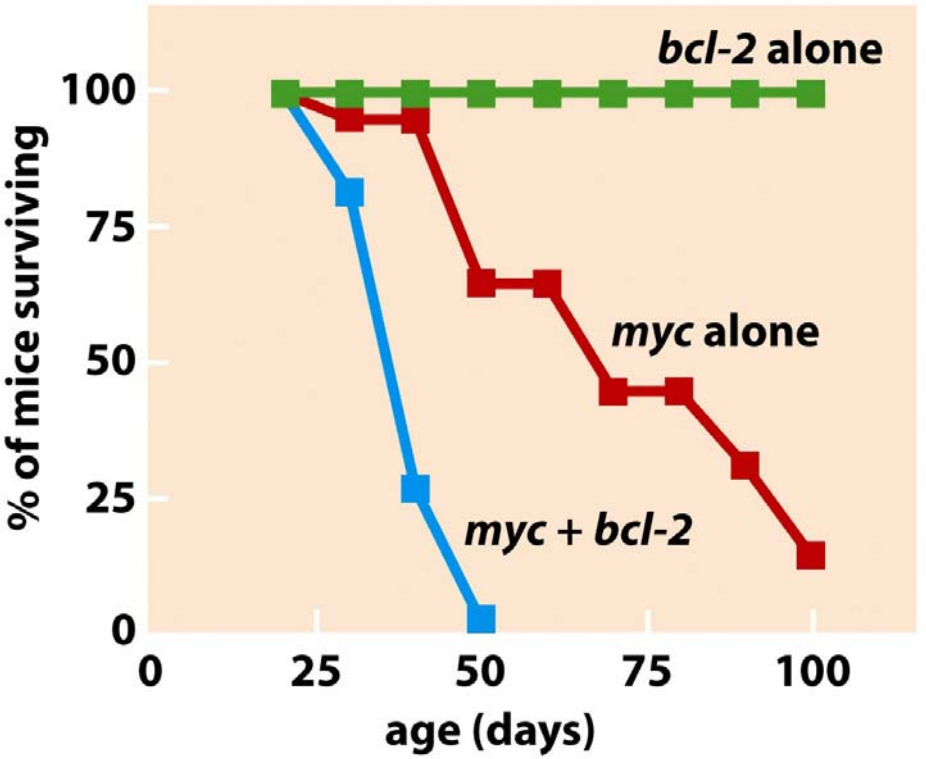
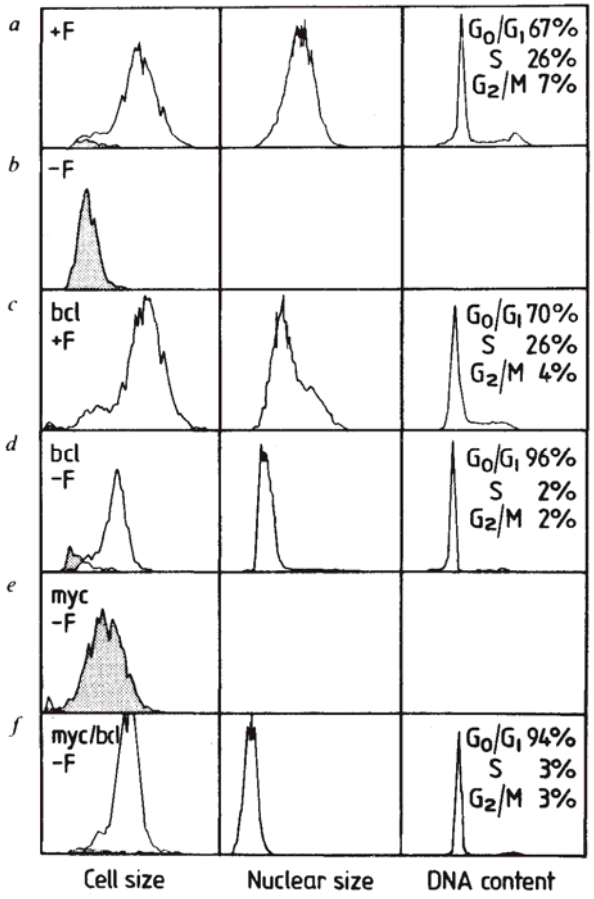


Figure 9.22a *The Biology of Cancer* (© Garland Science 2007)

Does Bcl-2 directly promote proliferation? *How can you test this?*

Indicated parameters of live cells



Shaded: dead cells

-Established haemopoietic cell lines that require growth factor (IL-3) to survive and proliferate.
-Cells were grown for 4 days in IL-3-free media (-F)or media that contained IL-3 (+F).
-On day 4, the authors used flow cytometry to measure cell viability, cell size, nuclear size and DNA content.
-Small cell and nuclear size of live cells indicates that cells are in Go.
-DNA content indicates whether cells are in Go/G1, S or G2/M.
-**Conclusion:** Bcl-2 can bypass death signal in cell lines deprived of IL-2.
-cMyc cannot bypass death signal in cell lines deprived of IL-3.

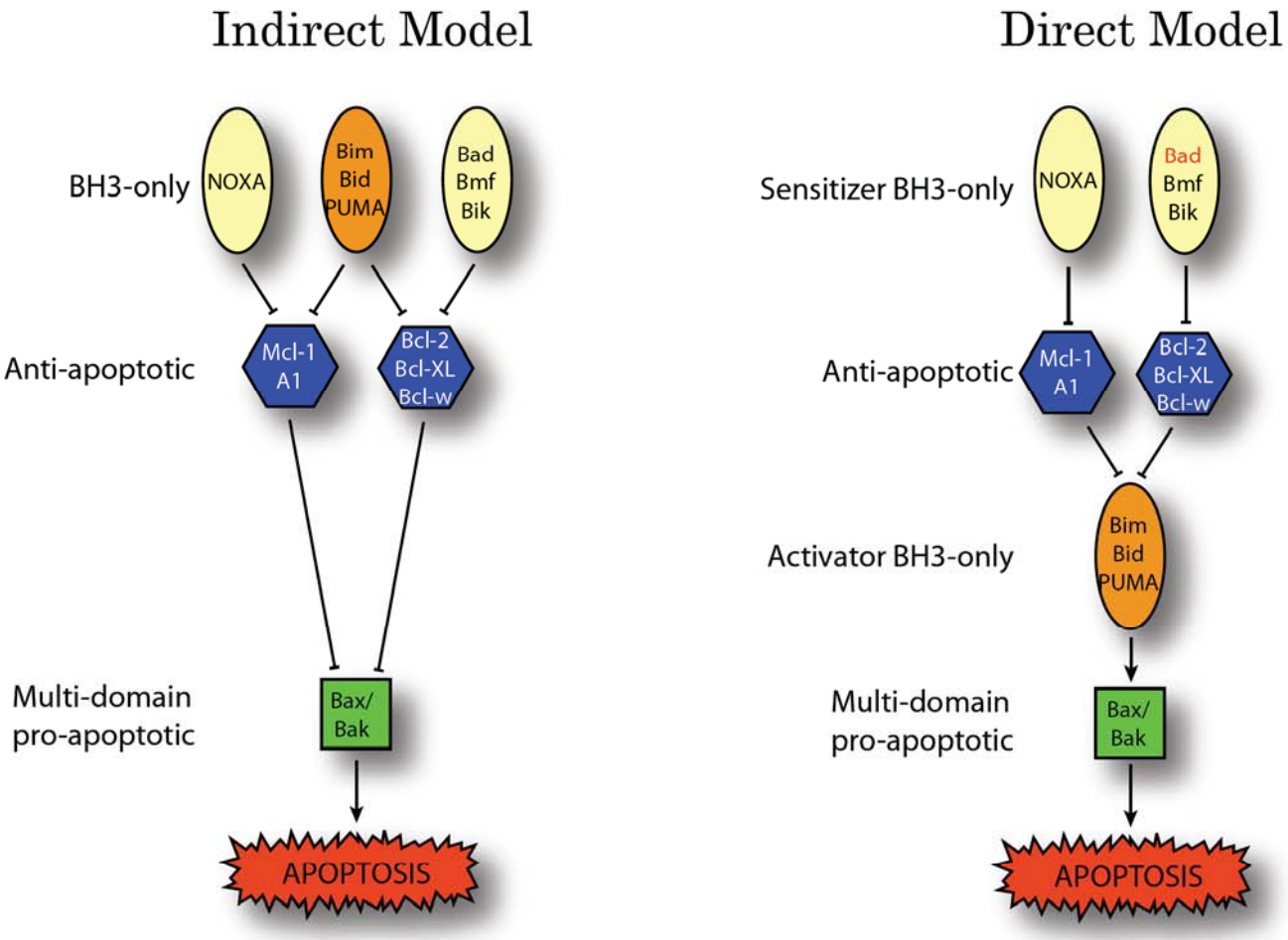
Concluding statement in abstract: *These results argue that bcl-2 provided a distinct survival signal to the cell and may contribute to neoplasia by allowing a clone to persist until other oncogenes, such as c-myc, become activated.*
-Paradigm shift in cancer biology

Vaux et al., 1988. Nature 335: 29

Enhanced expression of BCL-2- gene in cancer

- Bcl-2 in t(14;18) translocation in non-Hodgkin's lymphomas
- Enhanced Bcl-2 copy number
  - Small cell lung carcinoma (Ikegaki et al, 1994)
  - B-cell lymphoma (Monni et al., 1997)
- Loss of endogenous microRNAs that normally repress BCL-2 gene expression (Cimmino et al., 2005)—chronic lymphocytic leukemia
  - Genes encoding miR15 and miR16 are deleted or inactivated by mutations in >70% of these leukemia
- Gene hypomethylation (Hanada et al., 1993)

Protein-protein interactions between Bcl-2 family members dictate function



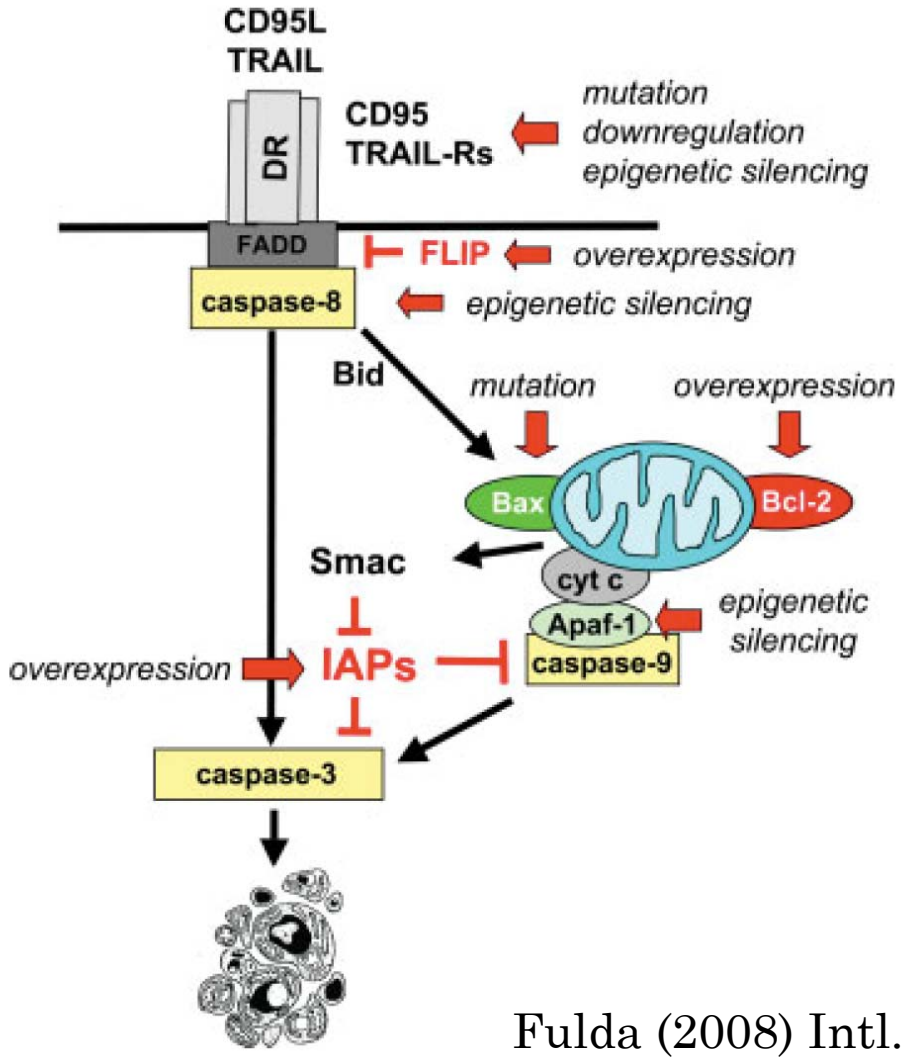
ax/Bak are crucial for mitochondrial outer membrane permeabilization (MOMP)

-Double KO cells are highly resistant to most apoptotic inducers

Bax/Bak are responsible for mitochondrial permeabilization

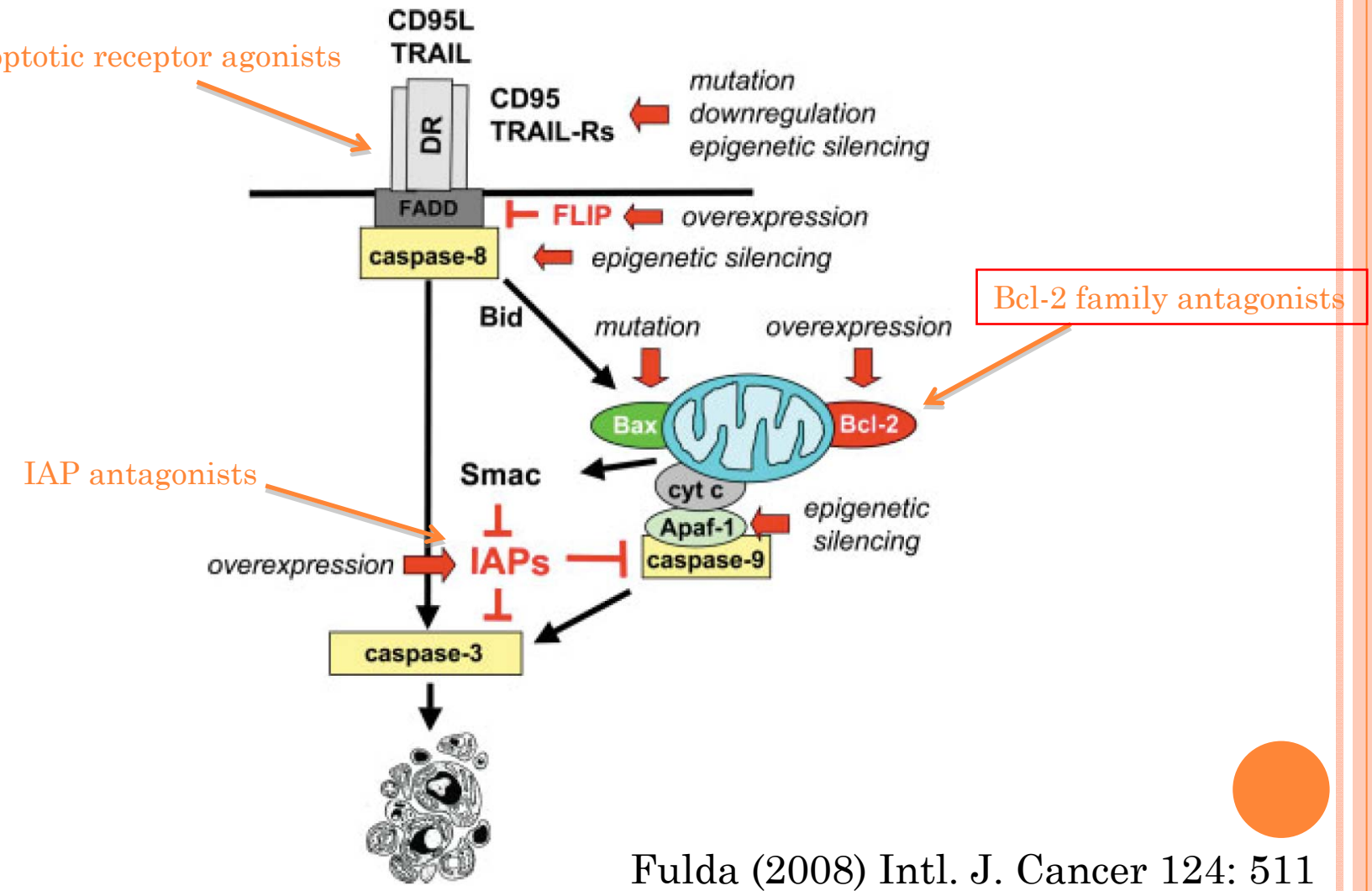
- Bax is present in the cytosol as an inactive monomer in healthy cells
- Apoptotic stimulus induces Bax translocation to the outer mitochondrial membrane
- At the membrane, Bax oligomerizes and forms pores
  - Facilitate MOMP→release of cyt c, SMAC
- However, Bax pore-forming ability at the mitochondrial membrane can be inhibited by anti-apoptotic proteins

Aberrant apoptotic machinery in cancer



Fulda (2008) Intl. J. Cancer 124: 511

Rational drug design



Fulda (2008) Intl. J. Cancer 124: 511

Bcl-2 and resistance to chemotherapy

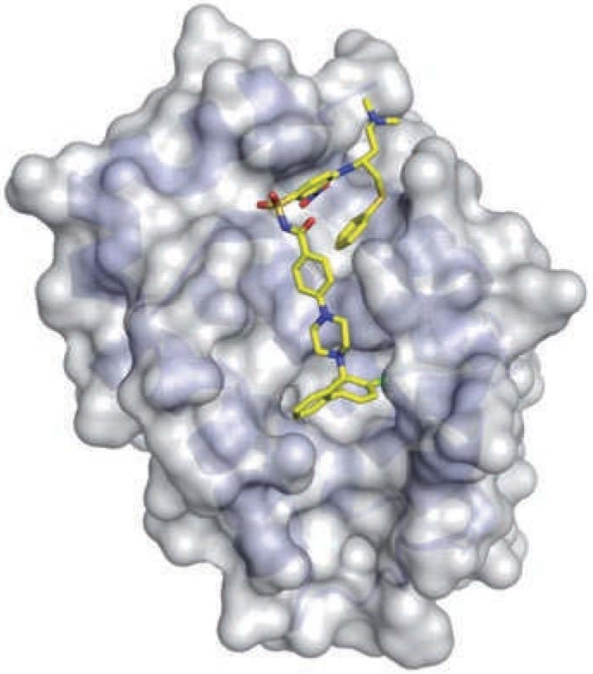
- Overexpression of Bcl-2 and other anti-apoptotic proteins inhibits cell death induced by cytotoxic anticancer drugs
- Essentially all anti-cancer drugs depend on Bcl-2/Bax-dependent mechanism for killing cancer cells (reviewed by Debatin et al., 2002; Reed, 2008)
  - Single or double-strand breaks, microtubule depolymerization or stabilization, gucocorticoid receptor activation, inhibition of estrogen and androgen receptors
    - Imparts intrinsic chemoresistance
- Explains why expression of a variety of Bcl-2 family proteins are of prognostic significance for many types of cancer treated by chemotherapy

Mechanisms for Bcl-2 family antagonism

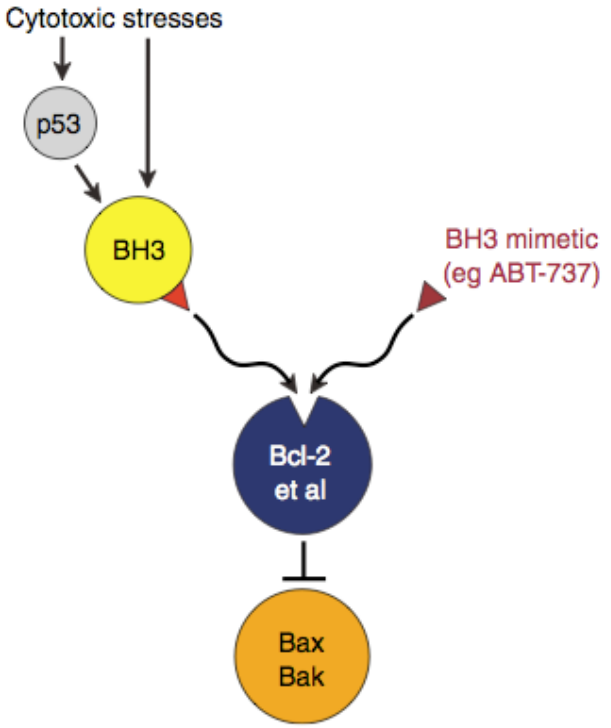
- Bcl-2 anti-sense oligonucleotides
  - Result in degradation of Bcl-2 mRNA
    - Oblimersen (Genta)
- Small molecule inhibitors (BH3-mimetics)
  - Bind to hydrophobic BH3-binding pocket and release pro-apoptotic proteins
    - ABT-737 (Abbott)
    - Obataclax (Gemin-X)

ABT-737 inhibits Bcl-2, Bcl-XL and Bcl-w

ABT-737 bound to Bcl-XL

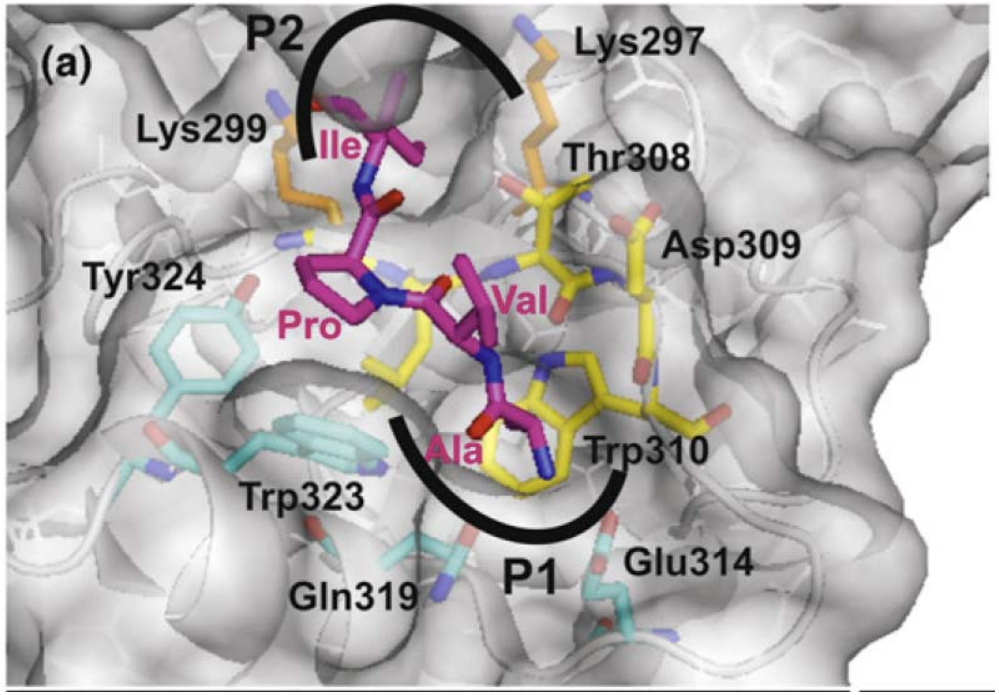


Lessene et al. (2008) Nature Reviews Drug Discovery 7: 989



Adams and Cory (2007) Oncogene 26: 1324

Smac peptide bound to XIAP



Mannhold et al. (2010)

Drug development programmes

Table 1 | **Selected apoptosis drug development programmes**

Drug name/code	Sponsor(s)	Target/mechanism	Clinical stage
BCL-2-targeting programmes			
Oblimersen	Genta	BCL-2 antisense	Phase III
Obatoclax	Gemin X Pharmaceuticals	Pan-BCL-2 antagonist*	Phase II
AT-101	Ascenta Therapeutics	Pan-BCL-2 antagonist*	Phase II
SPC2996	Santaris Pharma	BCL-2 antisense	Phase I/II
ABT-263	Abbott/Genentech	BCL-2 inhibitor*	Phase I/II
PARA programmes			
rhAPO2L/TRAIL	Amgen/Genentech	Recombinant human TRAIL	Phase II
Apomab	Genentech	Anti-DR5 mAb	Phase II
Mapatumumab	HGS/GSK/Takeda	Anti-DR4 mAb	Phase II
AMG-655	Amgen	Anti-DR5 mAb	Phase II
LBY135	Novartis	Chimeric anti-DR5 mAb	Phase I/II
Lexatumumab	HGS/Kirin	Anti-DR5 mAb	Phase I/II
TRA-8/CS-1008	Daiichi Sankyo	Anti-DR5 mAb	Phase I
IAP-targeting programmes			
YM-155	Astellas Pharma	Survivin antagonist*	Phase II
LY-2181308	Eli Lilly/Isis Pharmaceuticals	Survivin antisense	Phase II
AEG35156	Aegera Therapeutics	XIAP antisense	Phase I/II
HGS1029	HGS	IAP inhibitor*	Phase I

*Small molecule. GSK, GlaxoSmithKline; HGS, Human Genome Sciences; IAP, inhibitor of apoptosis protein; mAb, monoclonal antibody; TRAIL, TNF-related apoptosis-inducing ligand (also known as APO2L); XIAP, X-linked IAP.

Storey (2008) Nature Reviews Drug Discovery 7: 971