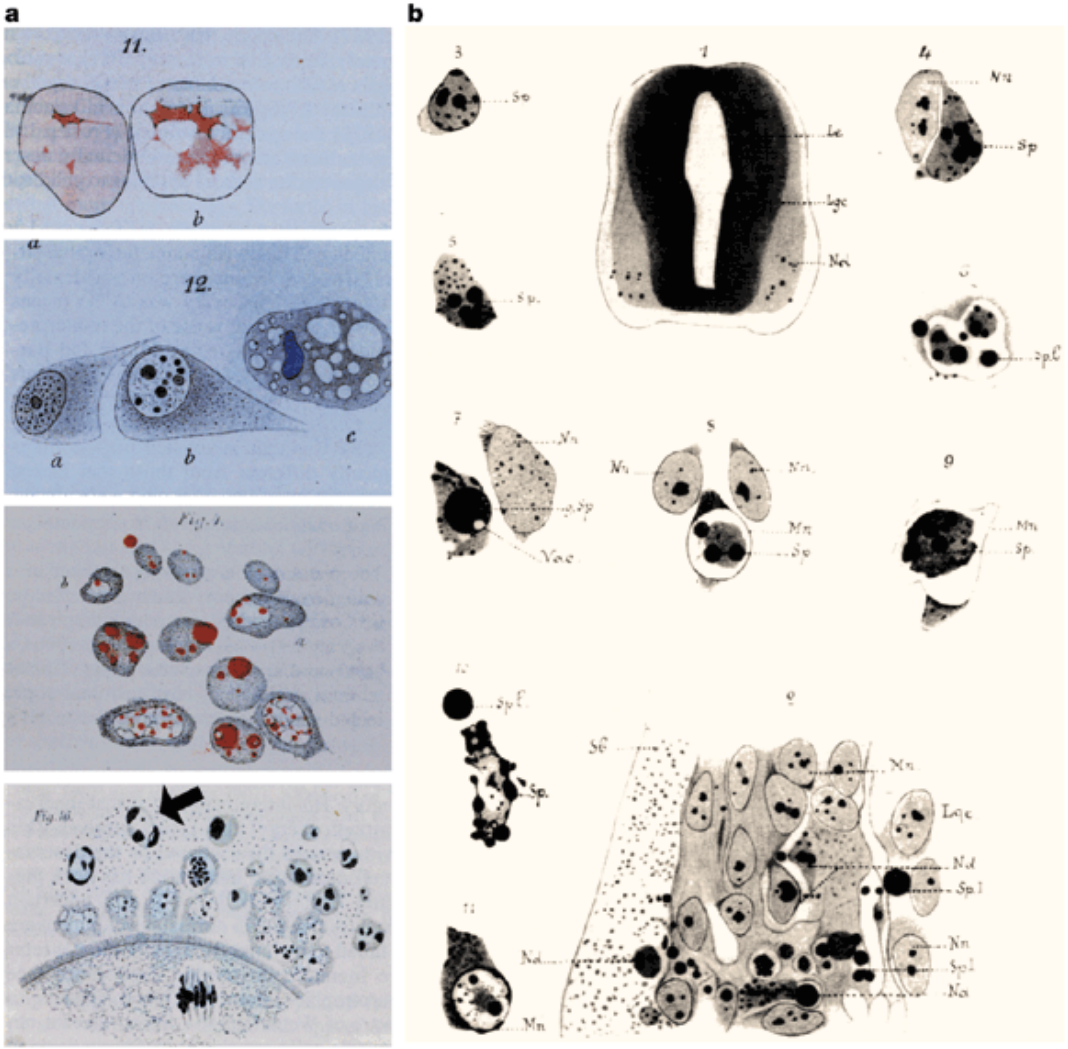
Apoptosis: Mechanisms (Day 1)

- Historical Perspective
- Serine-threonine kinases, survival signaling downstream of PTEN/PI3K/Akt
- Bcl-2 family members, potential usage of BH3 domains as drug targets
- Mitochondrial pathway, apoptogenic factors

Apoptosis: Drug targets (Day 2)

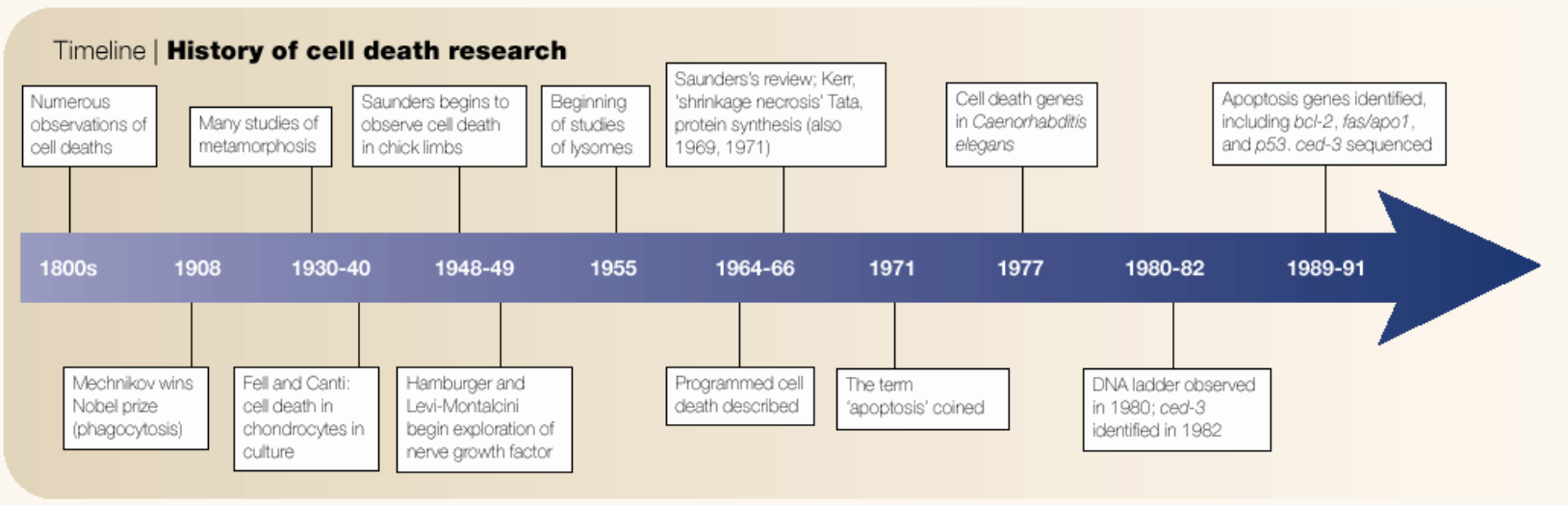
- Caspases, apoptosome, inflammasome
- TNFR, CD95/Fas, ApoL
- TRAIL and decoy receptors
- Bcl-2/xL: Antisense, inhibitors of protein-protein interactions, SAR
- Caspases: Why it did not work

Historical Observations of Apoptosis in Development

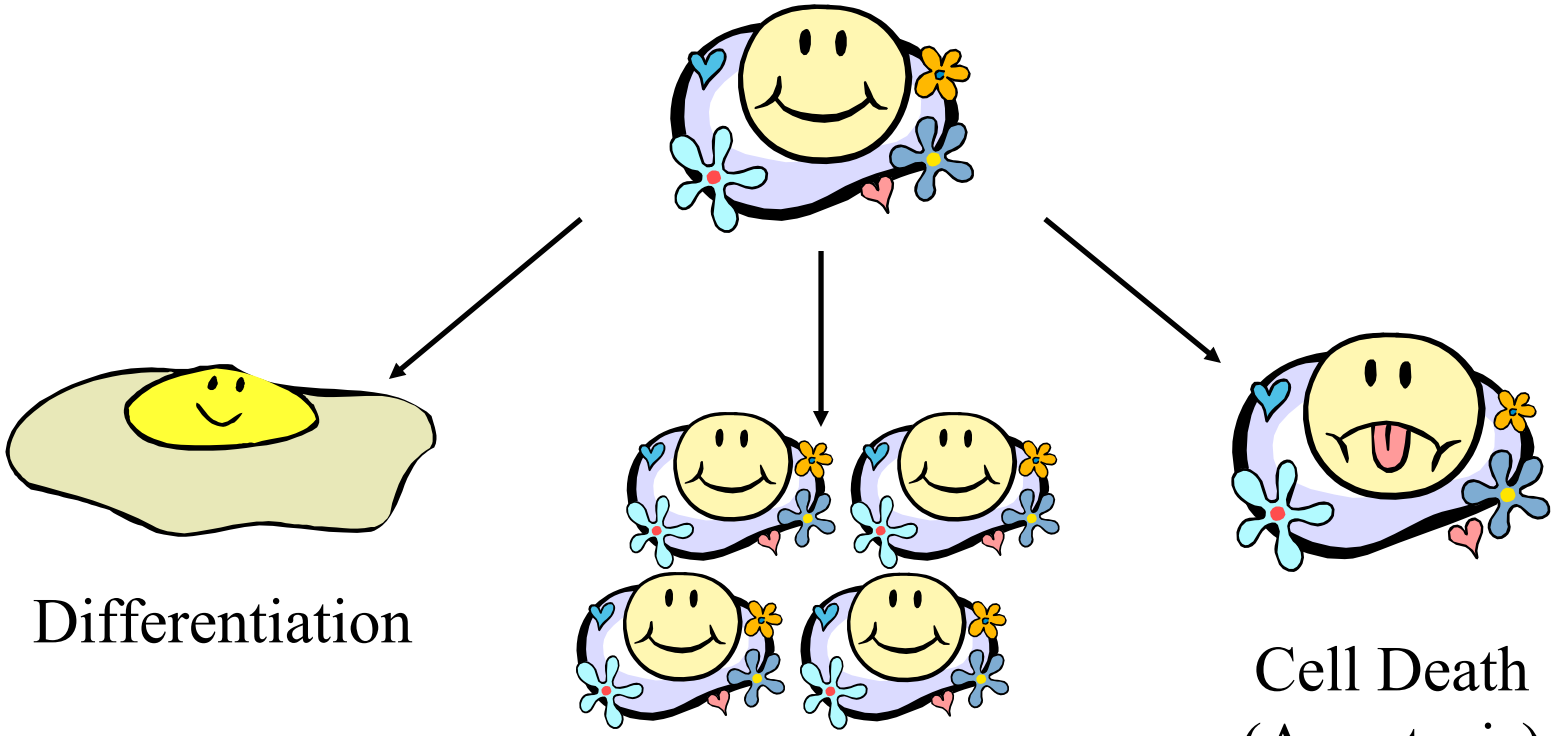


Nature Reviews | Molecular Cell Biology

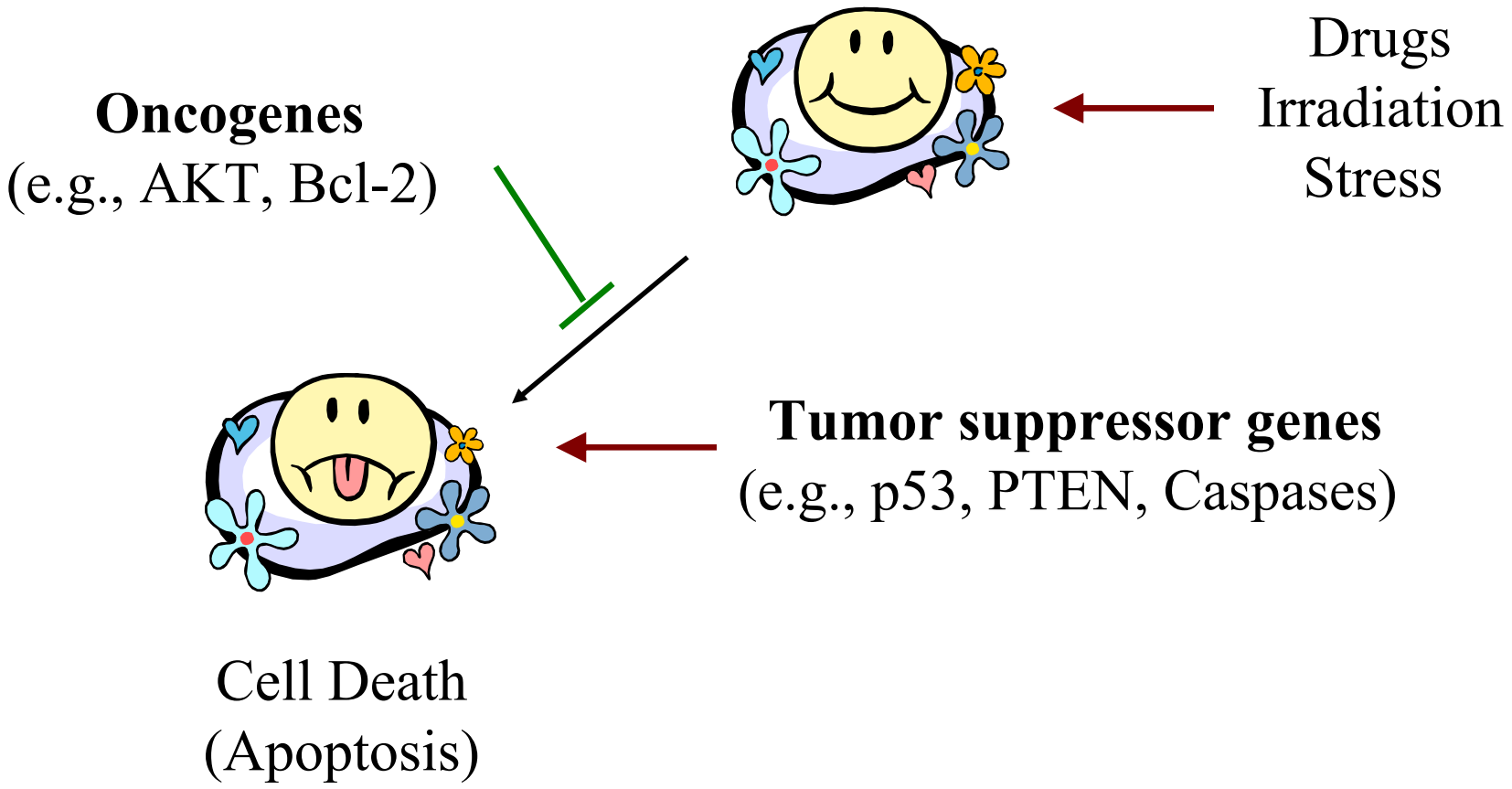
Historical Timeline of Apoptosis Research



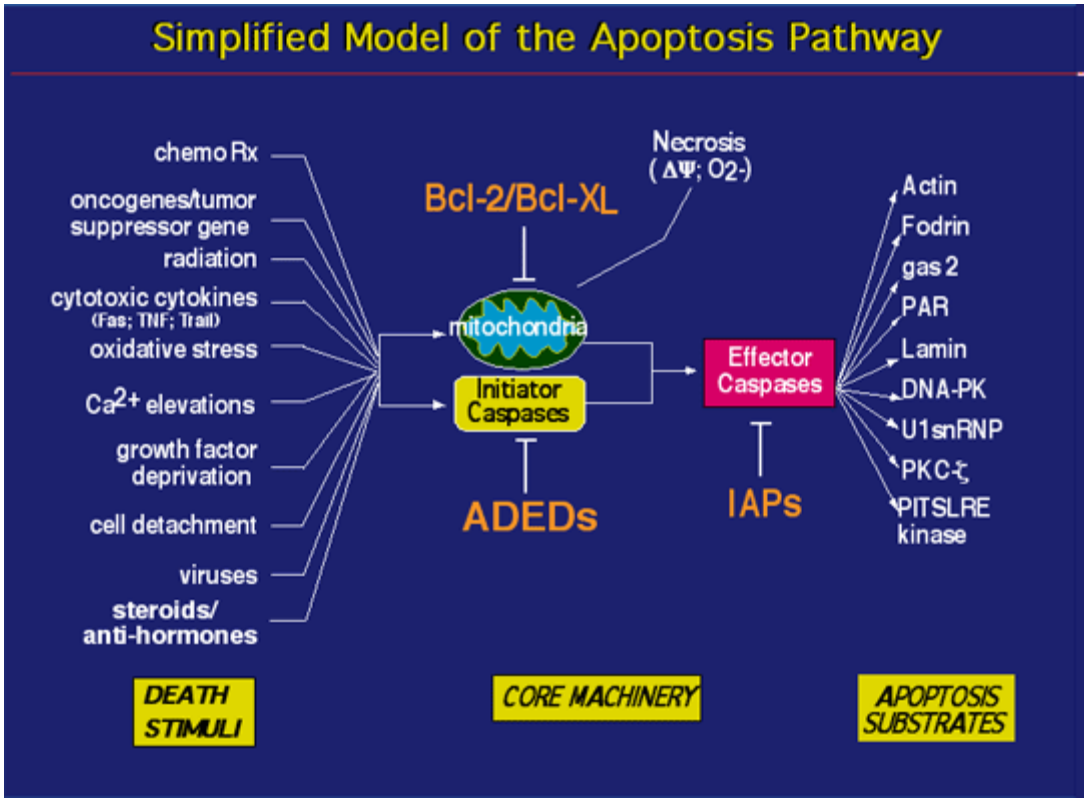
Cell Death is a Physiological Response of Cells To Changes in the Extracellular Environment



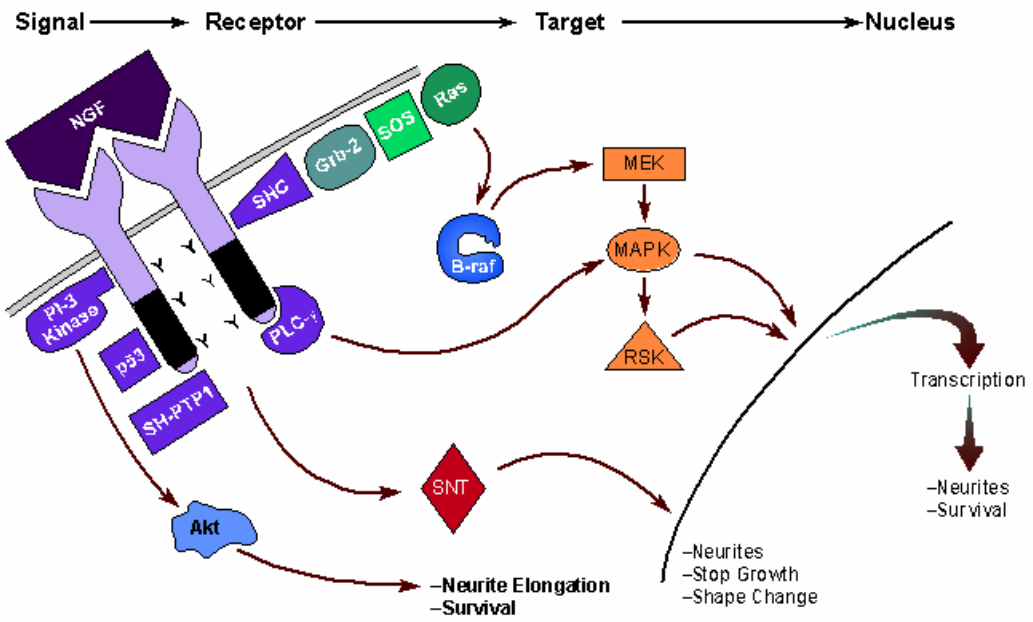
Endogenous Genes Modify Cell Death



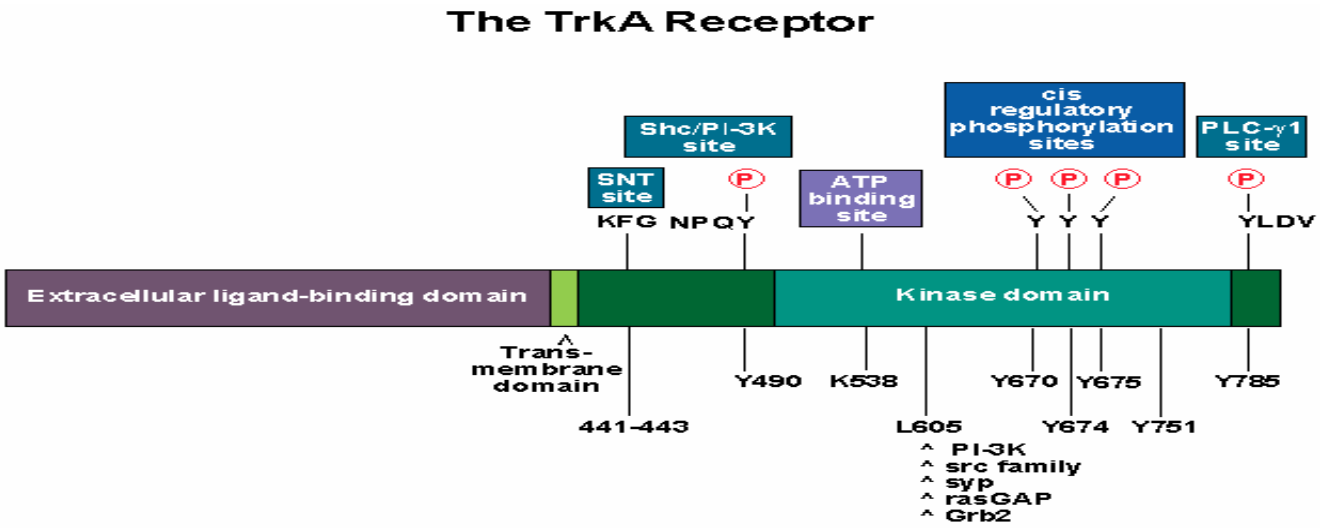
Mitochondria Conduct Apoptotic Signaling



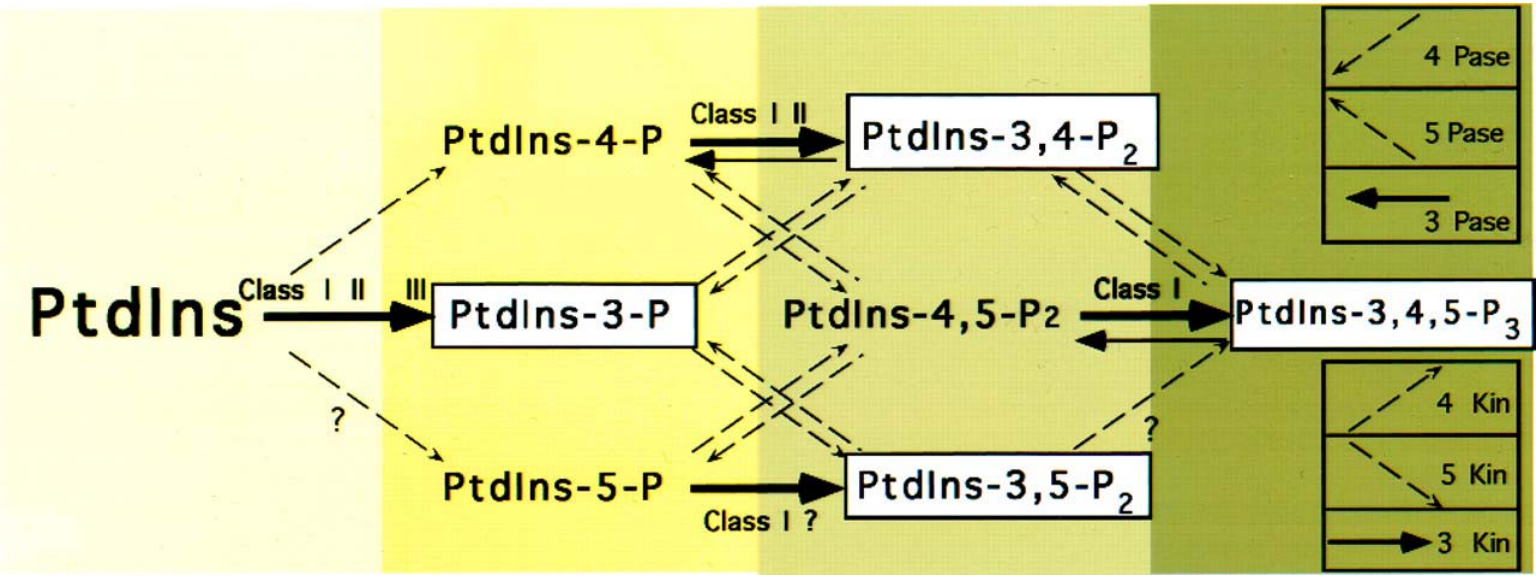
Cellular Receptors Transduce Extracellular Signals



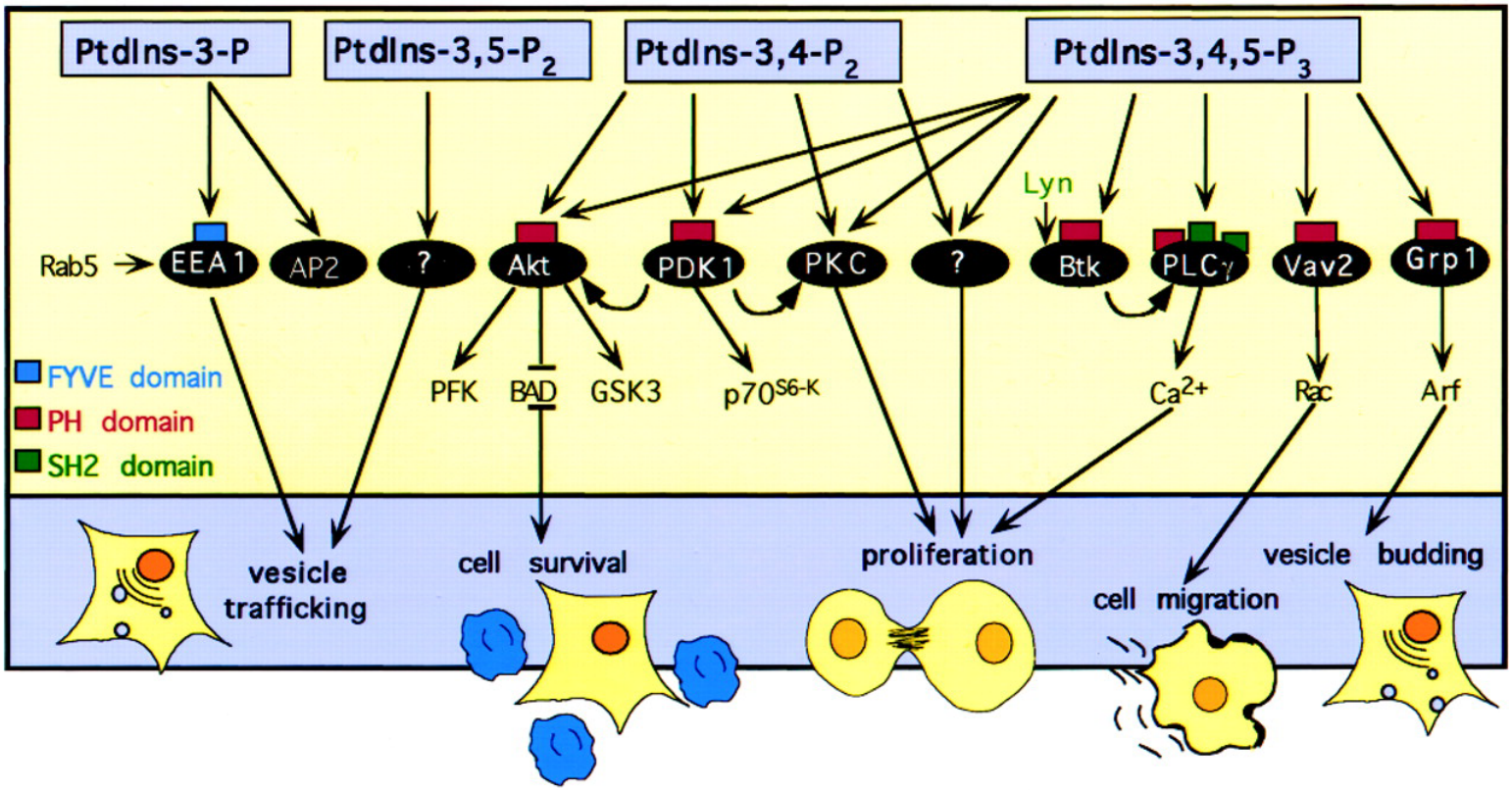
Cellular Receptors Initiate Intracellular Cascades



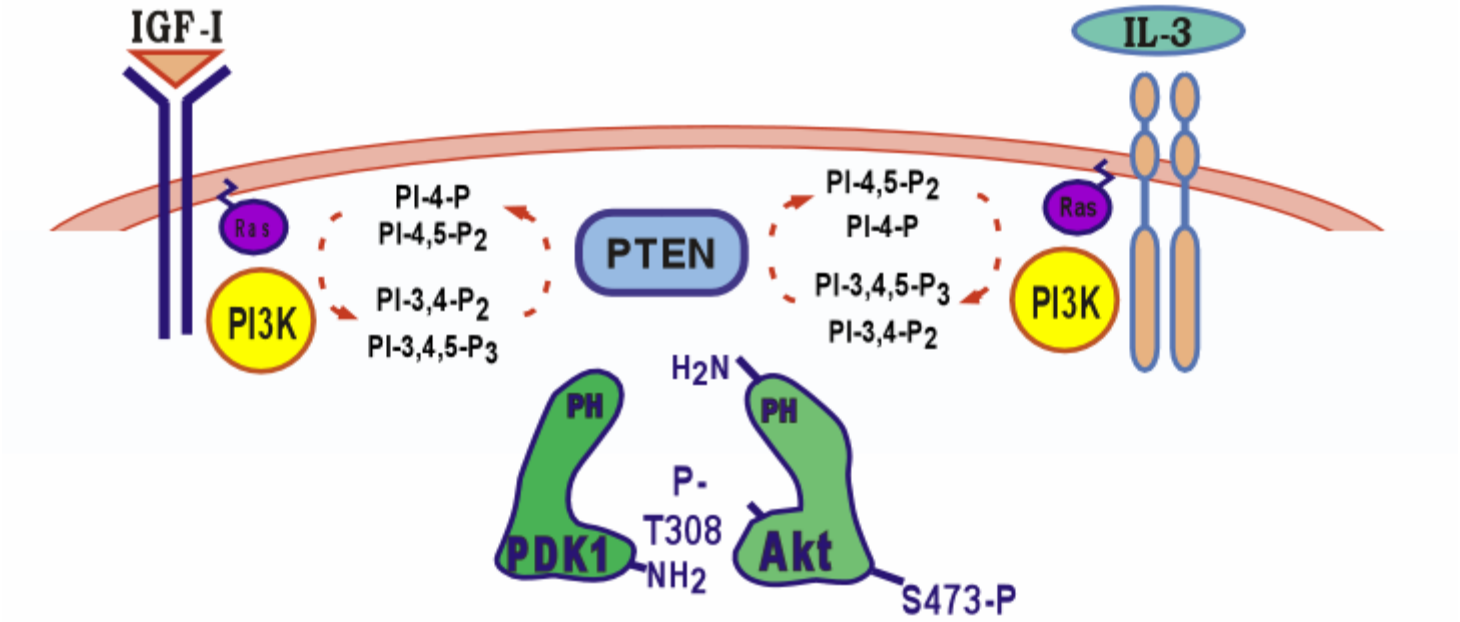
PtdIns-Dependent Second Messengers Function In Distinct Intracellular Pathways…



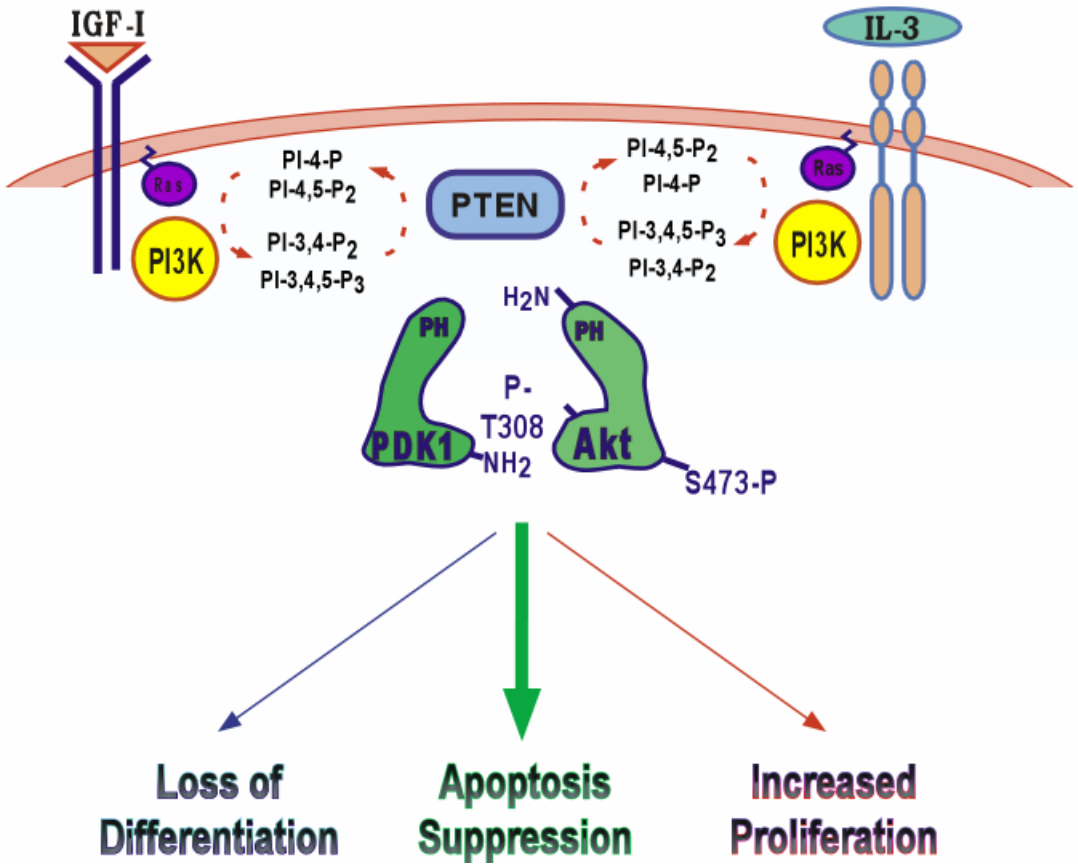
… Depending on Distinct Regulatory Domains Of Target Proteins



PIP3-Dependent Cell Responses are Mediated by PTEN/PI3K/AKT



Multiple Cell Functions are Mediated by PTEN/PI3K/AKT



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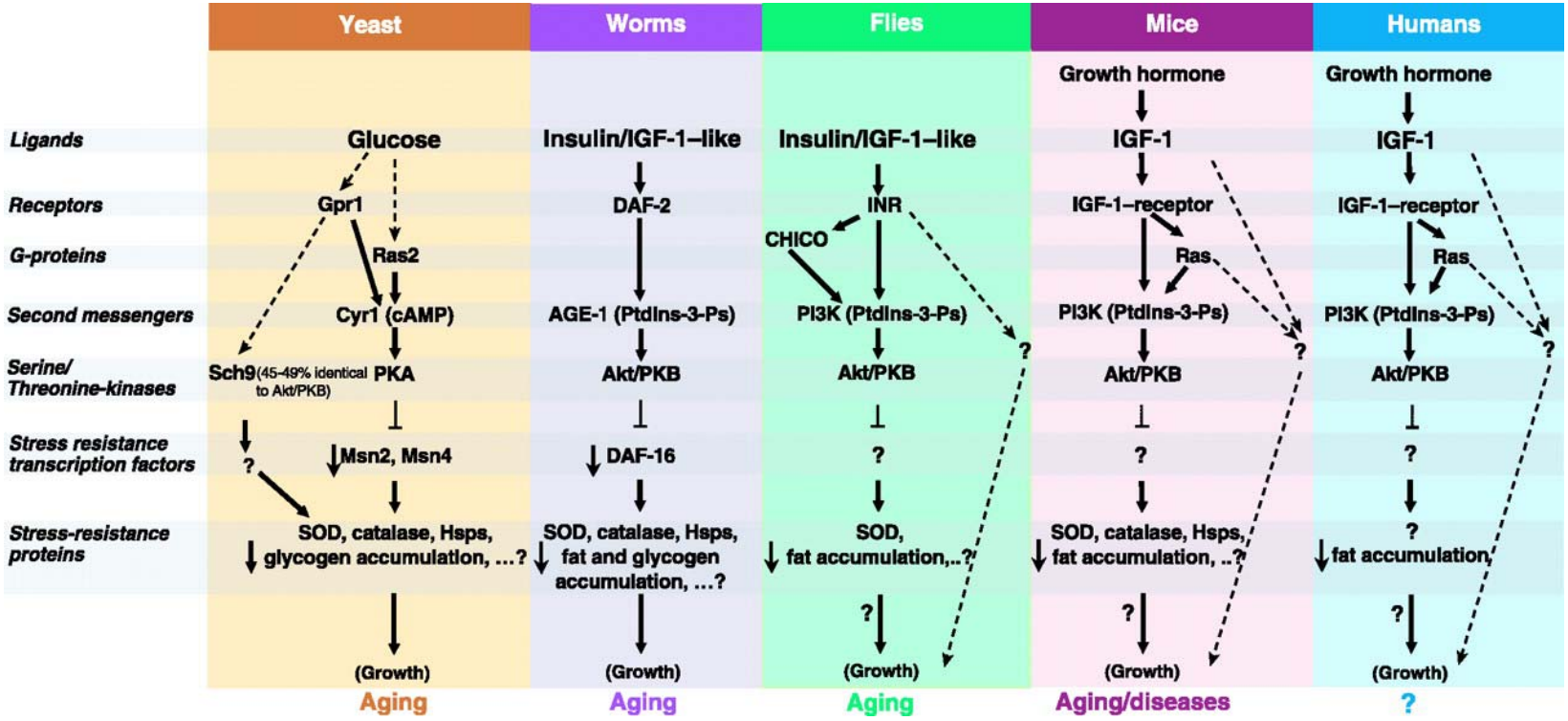
PTEN/PI3K/AKT Signal Transduction is Evolutionarily Conserved



Loss of AKT Signaling Leads to Lifespan Extension and Reduced Cell Size (=Smaller Organisms)

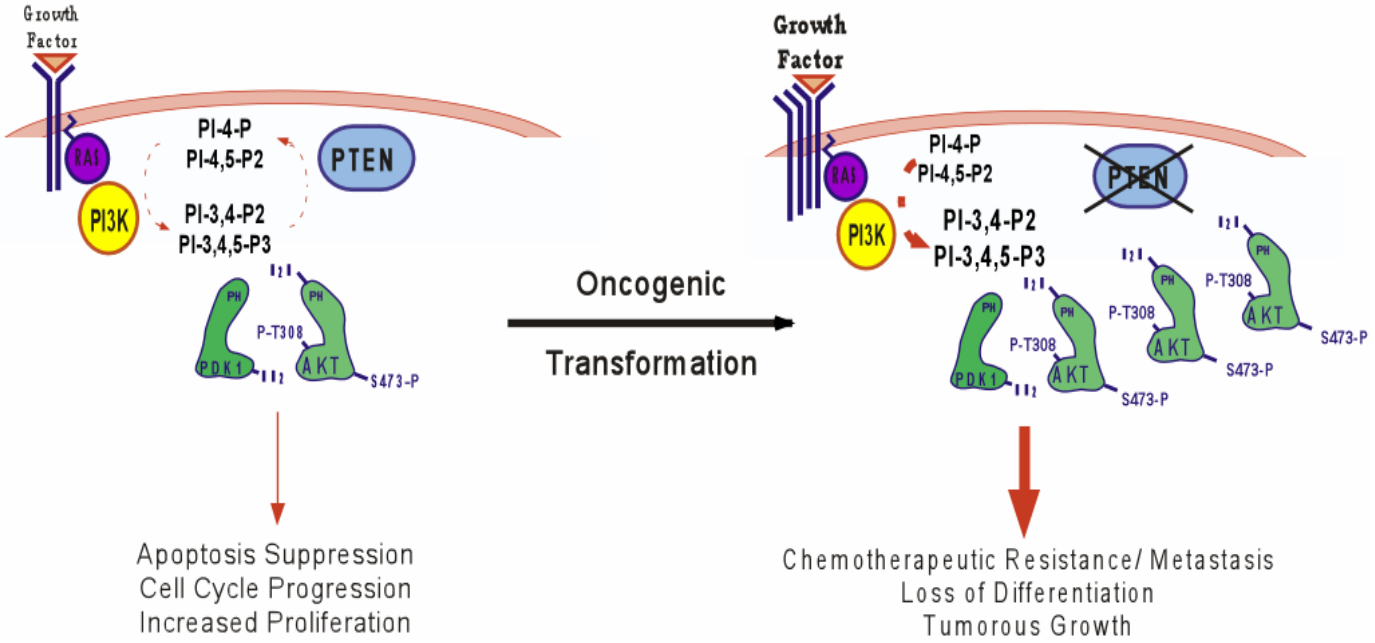
Yeast (sch9) → Fly (Dakt) → Mouse (Akt1,2,3)

Decreased Akt Gene Function = Mini-Mice

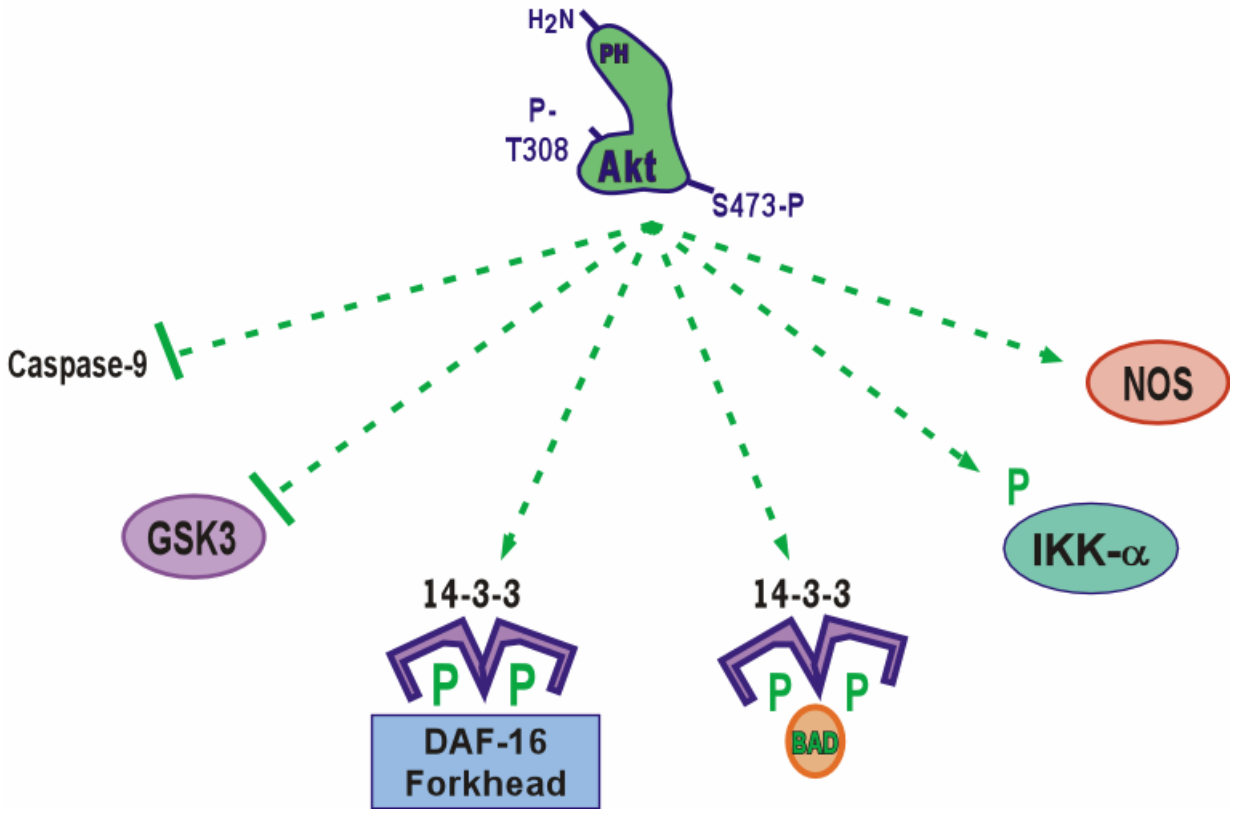
Akt1 KO
(~20% smaller)

Akt1 wt
Littermate

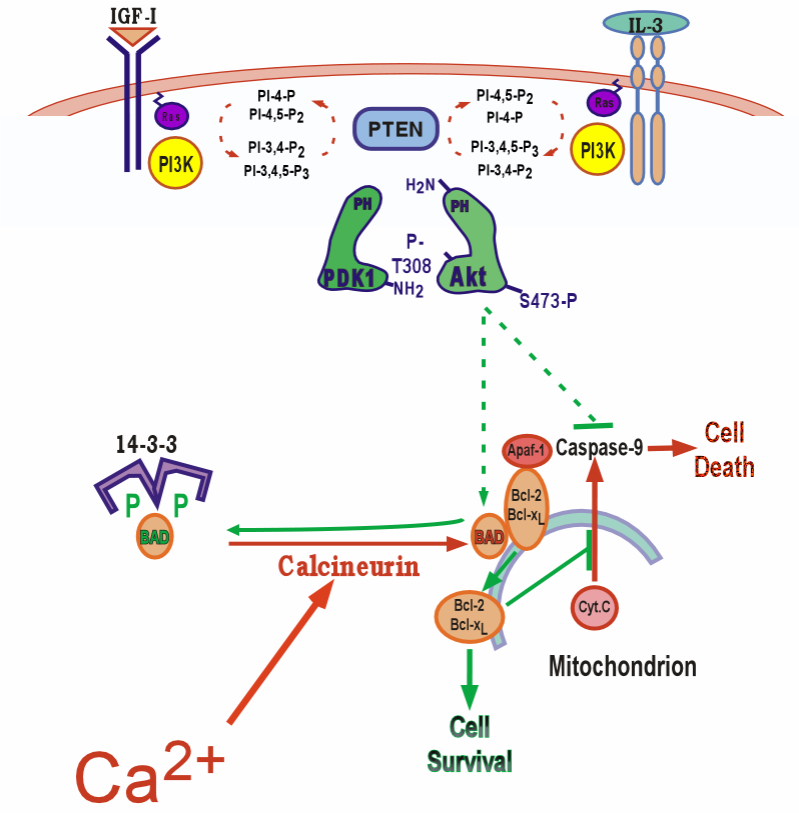
Increased Strength of AKT Signaling = Cancer



AKT Regulates Distinct Downstream Substrates



AKT: A BAD Kinase Makes Good



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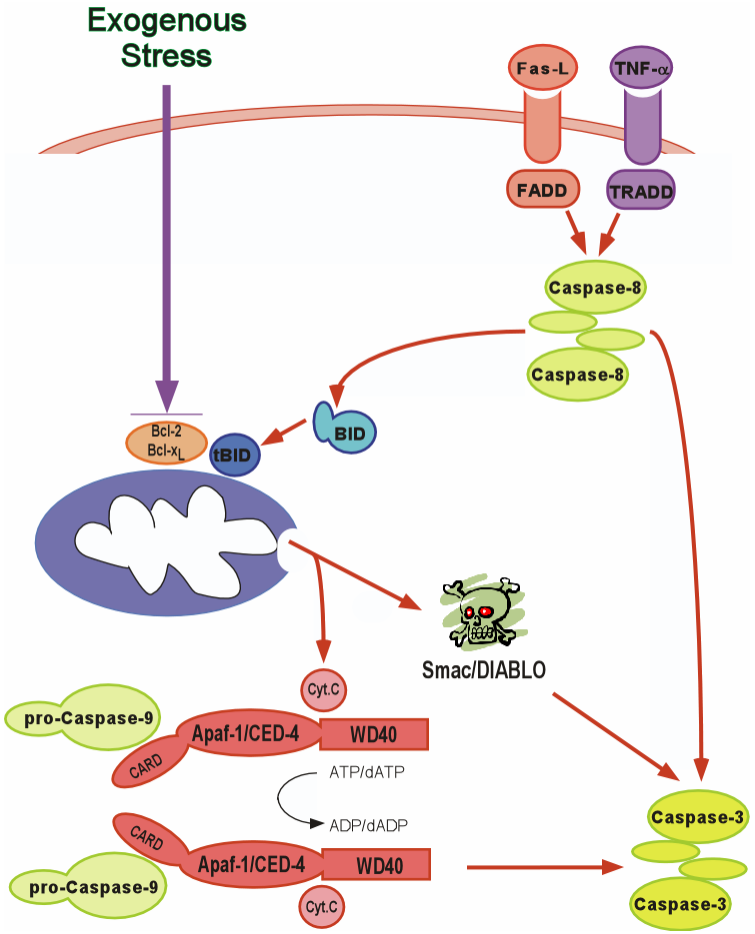
Mitochondria = Pandora's Box of Cell Death



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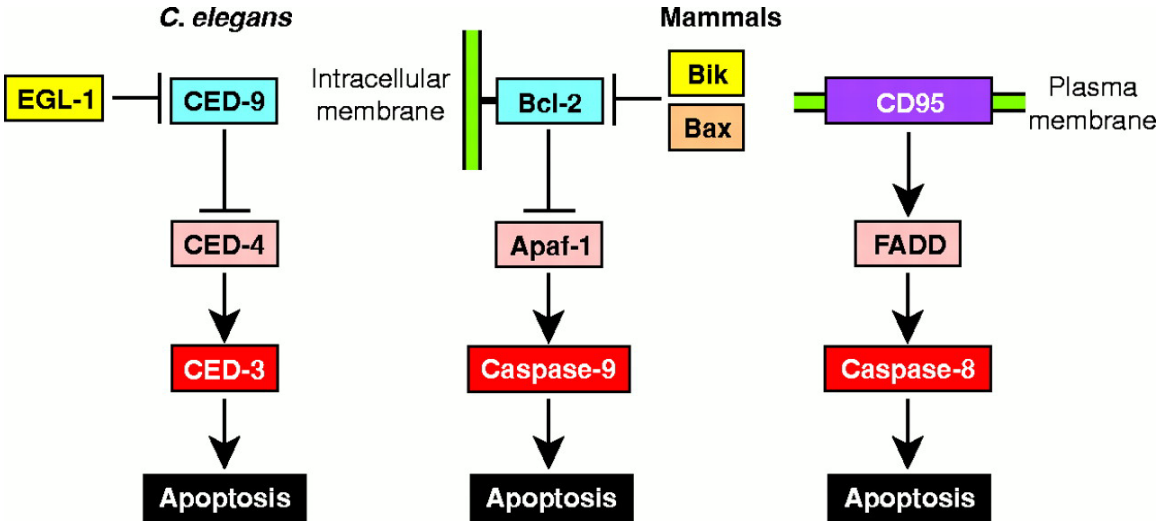
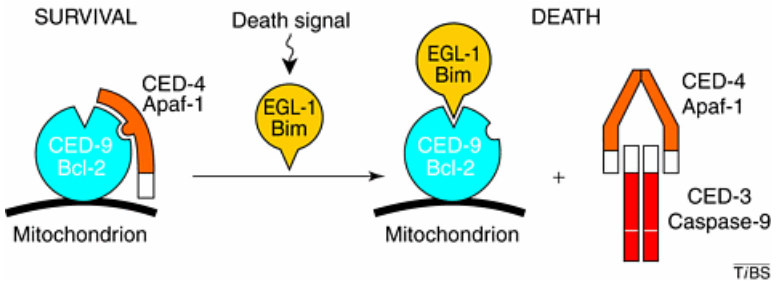
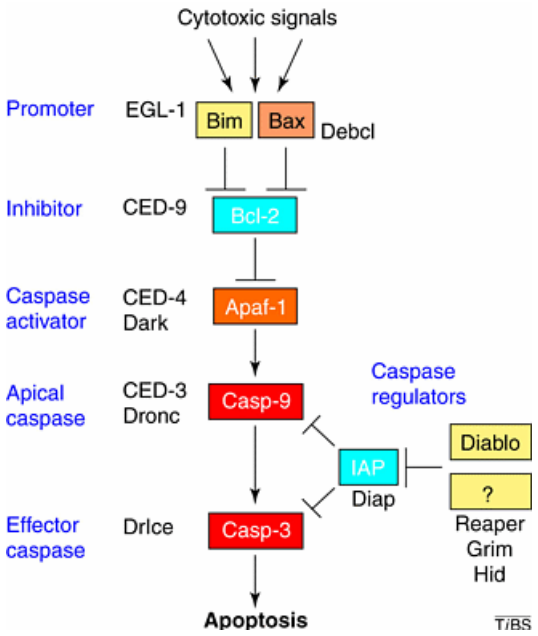
Bcl-2 Proteins as Conserved Gatekeepers Of Mitochondrial Integrity



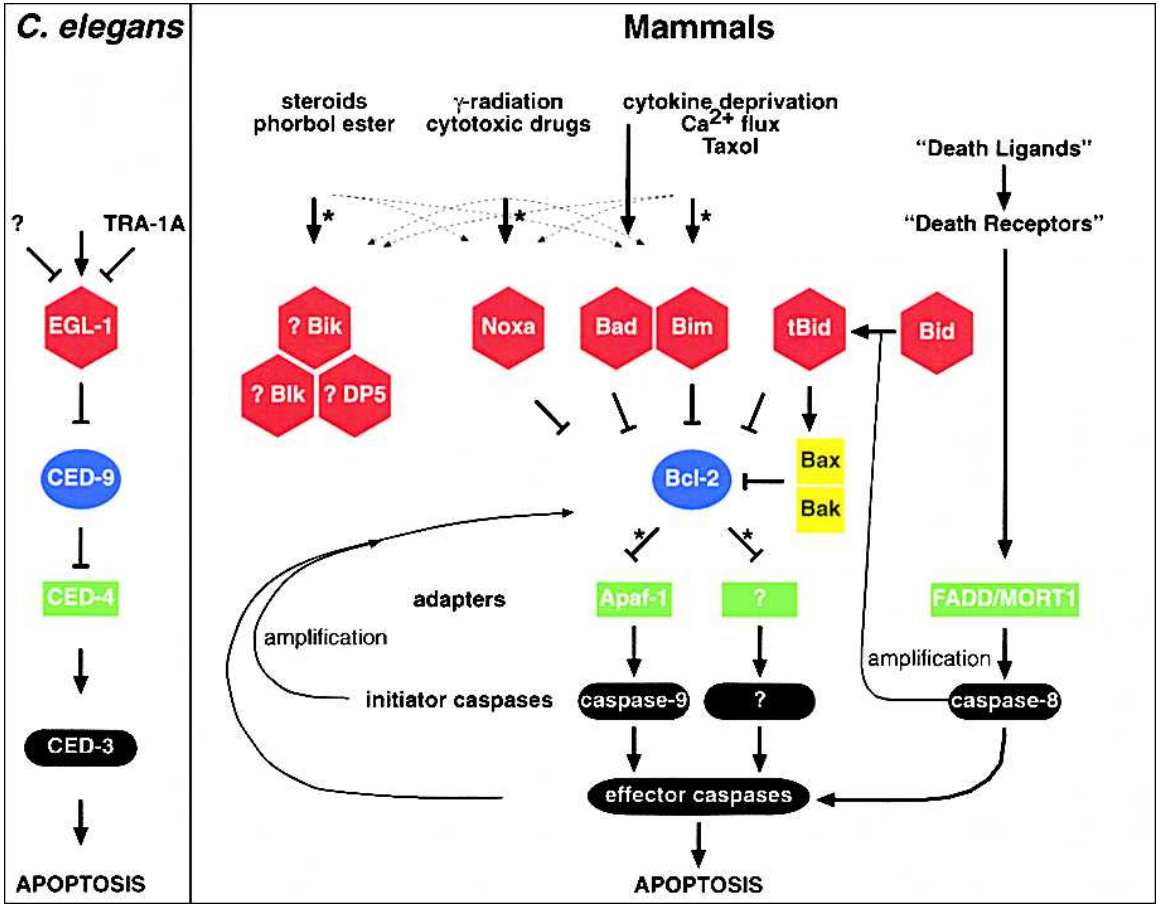
Death Signals Inhibit the Anti-Apoptotic Bcl-2 Family Member CED-9 in Lower Eukaryotes



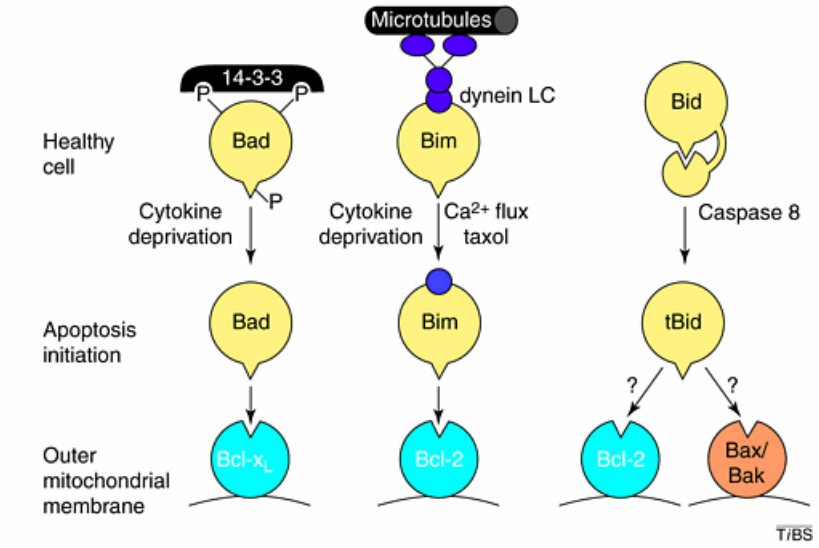
Conservation of a Common Death Pathway Involving Bcl-2-Type Proteins



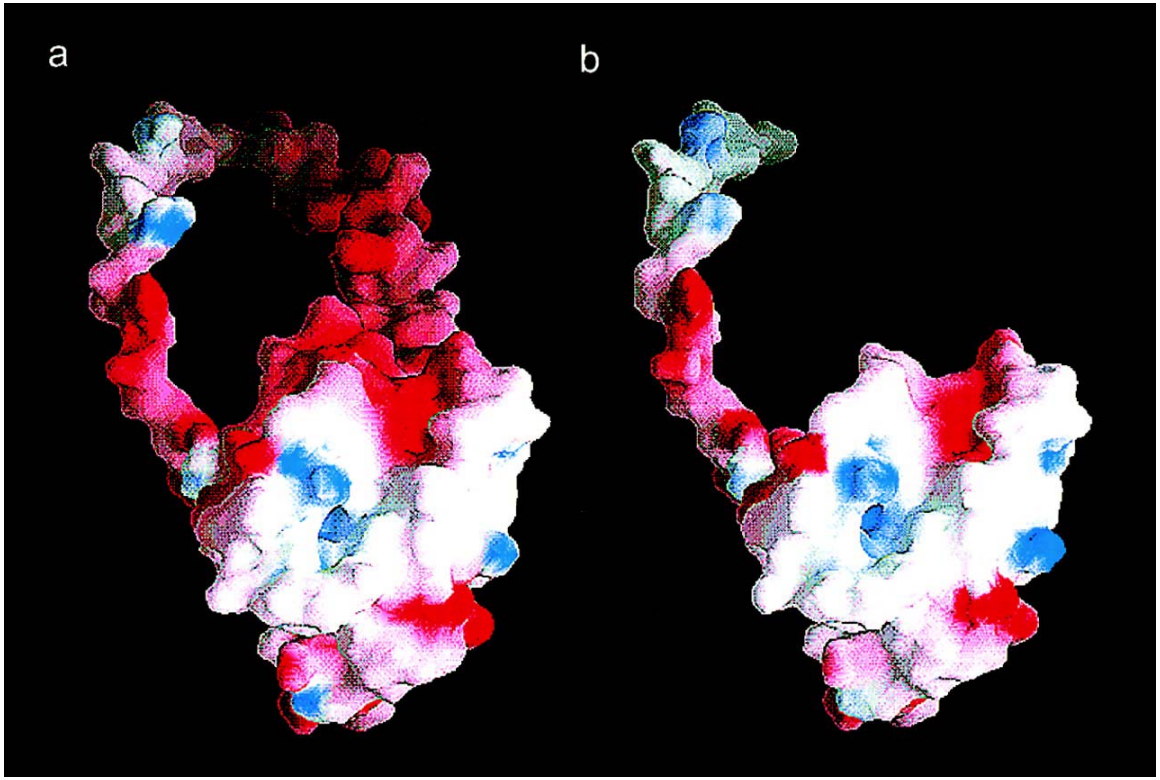
Pro-Apoptotic Bcl-2 Family Members in Mammals Comprise Functionally Distinct Sentinels…



…That Sense Cellular Stress and are Activated by Multiple Mechanisms…

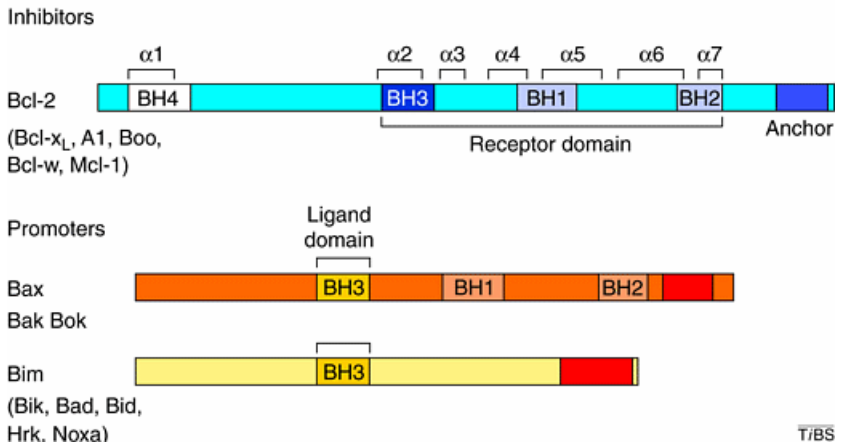


…Including Cleavage by Cellular Proteases

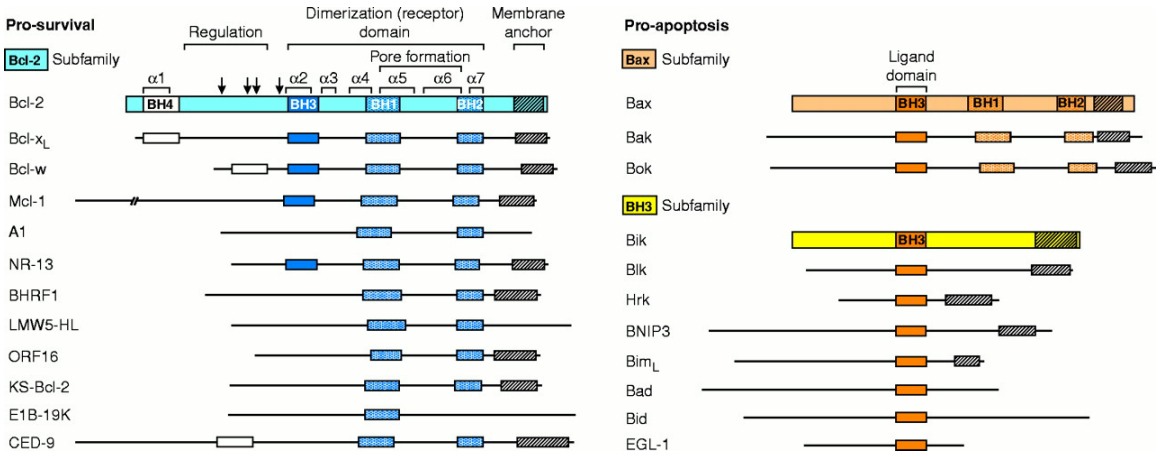


BID (inactive) → tBID (active)

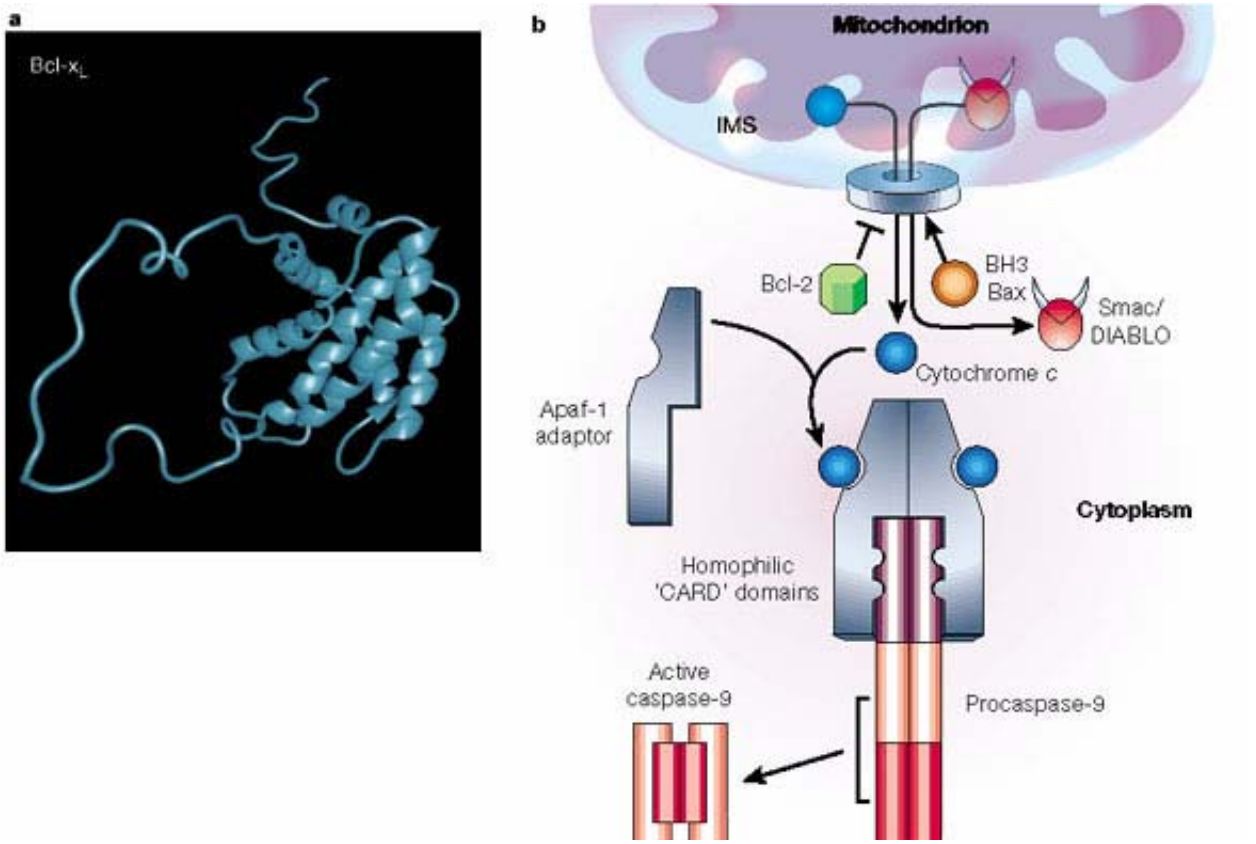
Bcl-2-Type Proteins are Characterized by the BH3 Domain of Protein-Protein Interactions…



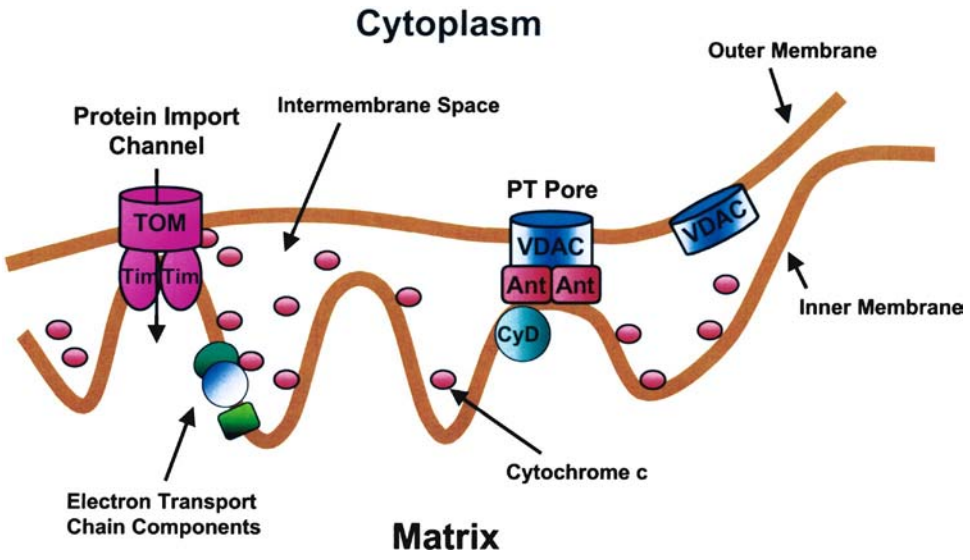
…but Comprise a Family of Structurally and Functionally Diverse Proteins



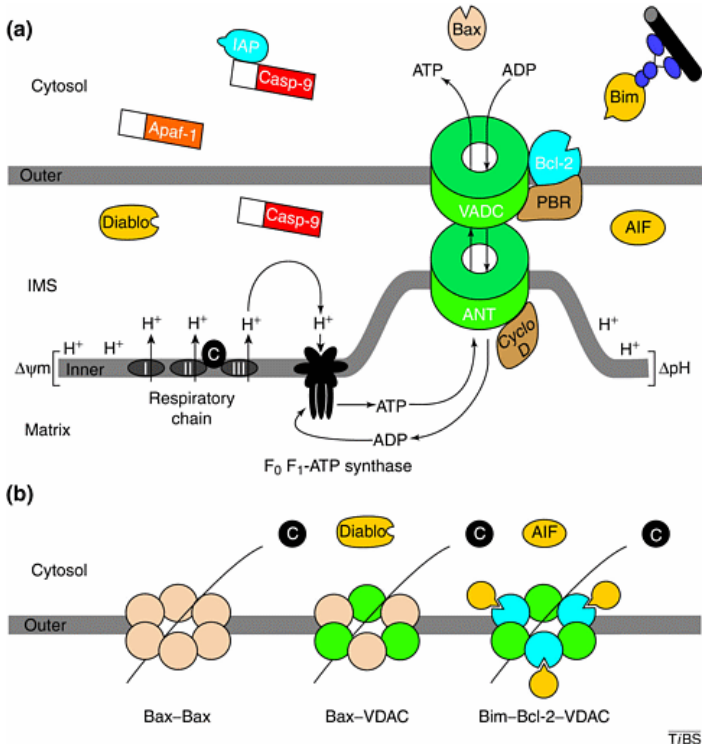
Inhibition of Bcl-2/Bcl-x_L Results in the Release of Apoptogenic Factors



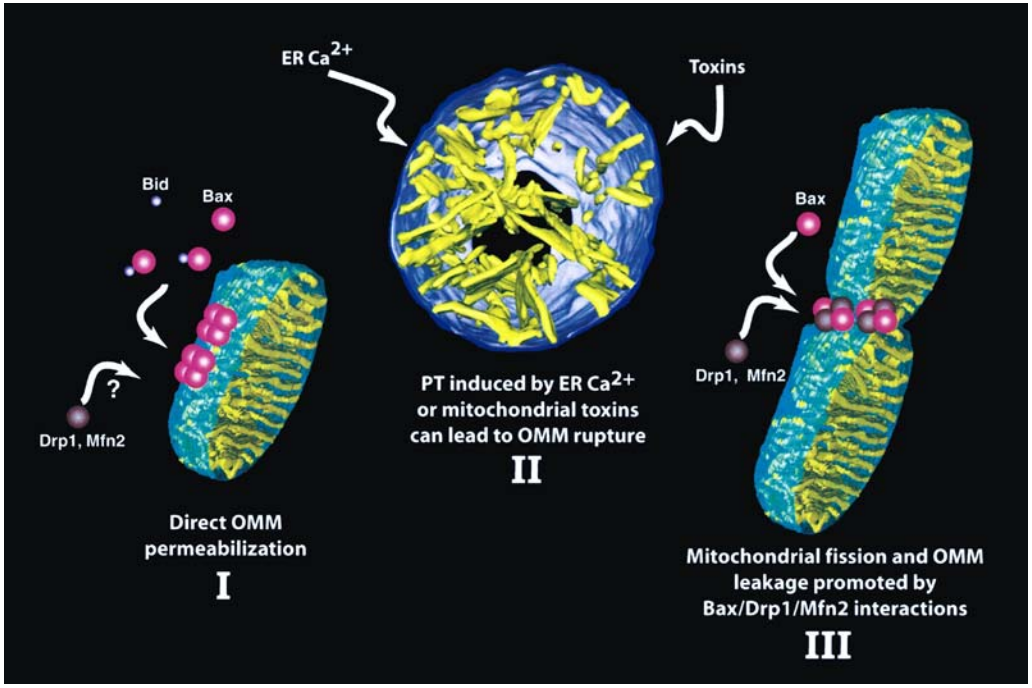
Bcl-2/Bcl-x_L Retain Apoptogenic Proteins in the Mitochondrial Intermembrane Space…



…by Regulating Mitochondrial Channels and Inhibiting Pore Formation by Bax/Bak



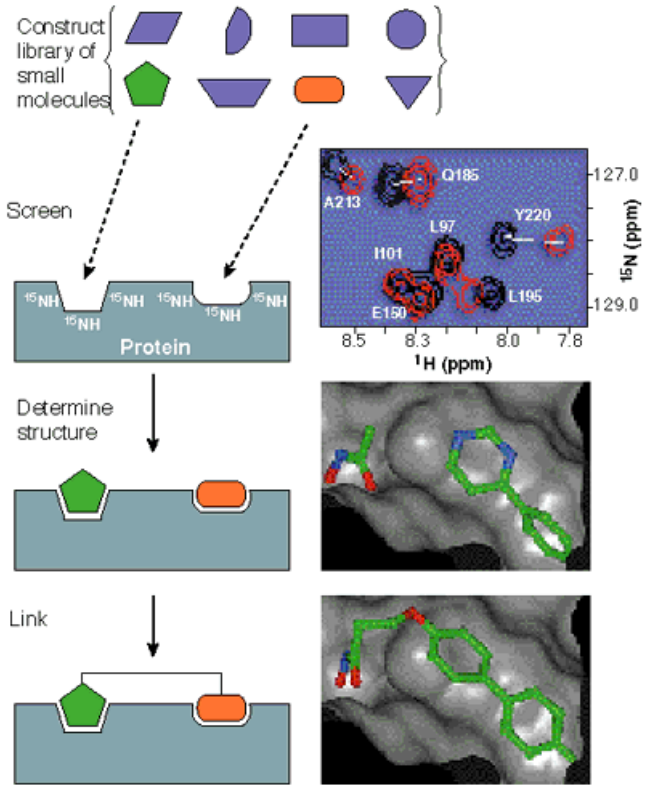
Mitochondrial Breakdown Results from the Permeabilization, Rupture or Leakage of the Outer Mitochondrial Membrane (OMM)



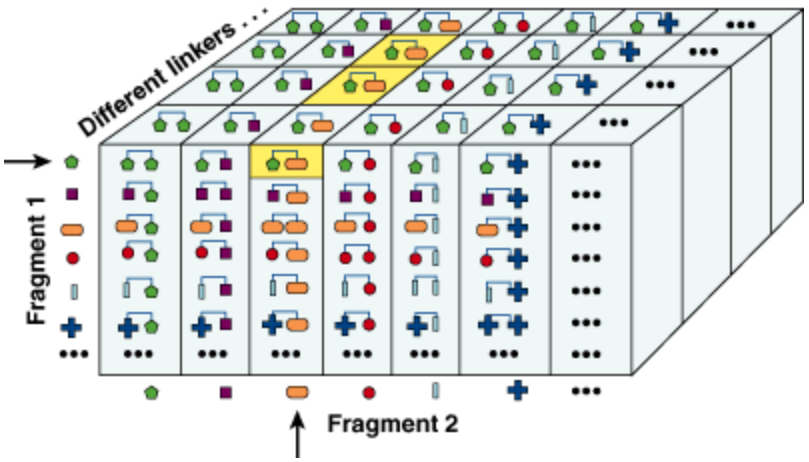
Bcl-2/Bcl-x_L as Drug Targets

- Antisense Oligonucleotides [e.g. Genta=G-3139 (5'-d(P-thio)TCT-CCC-AGC-GTG-CCC-CAT-3')
- Natural Compounds (Antimycin A_3 binds to the hydrophobic groove in Bcl-x_L and inhibits protein-protein interactions)
- SAR by NMR = structure-activity relationships by nuclear magnetic resonance

SAR-by-NMR Method: Screening for Small Molecules and Improving their Affinities using Linkers and NMR



Advantage over Conventional Library Methods: Fewer Compounds have to be Synthesized



WHY BRAIN CELLS *REALLY* DIE

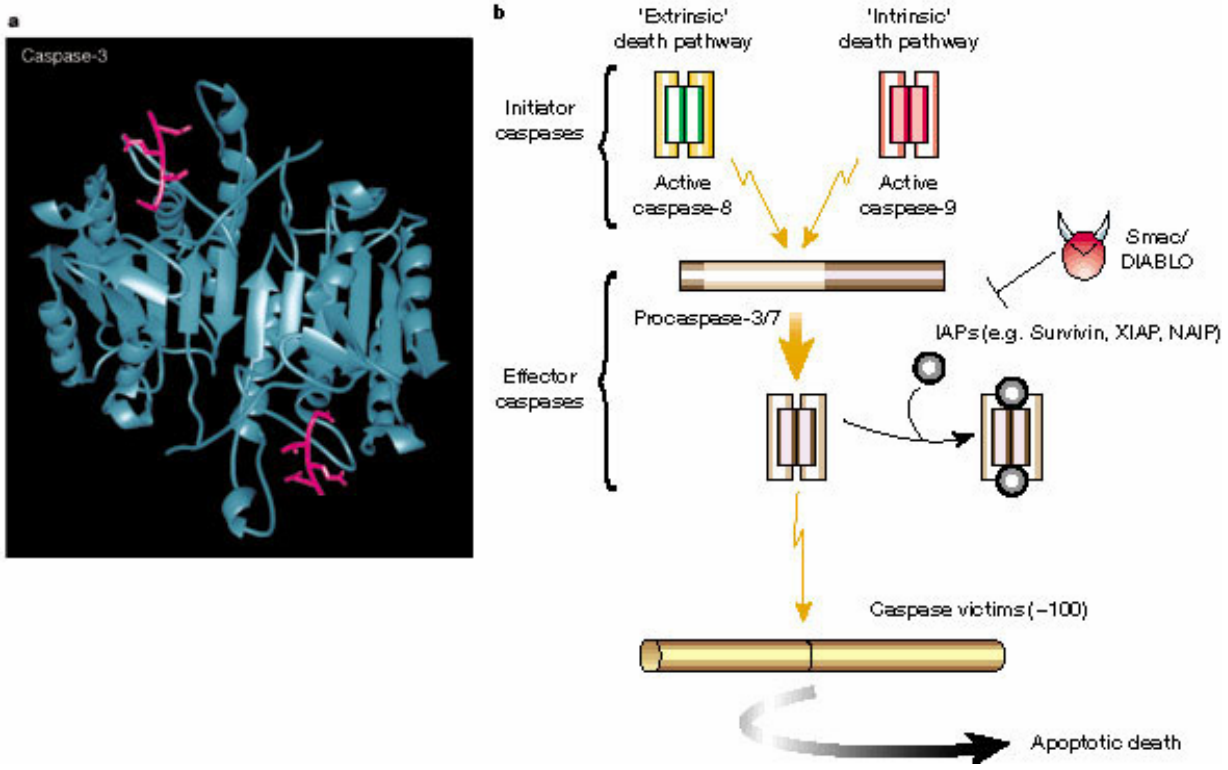
Every sitcom watched leaves a small deposit of crud inside brain cells, which eventually clog up and die.

Any argument with your kids causes massive brain-cell implosion.

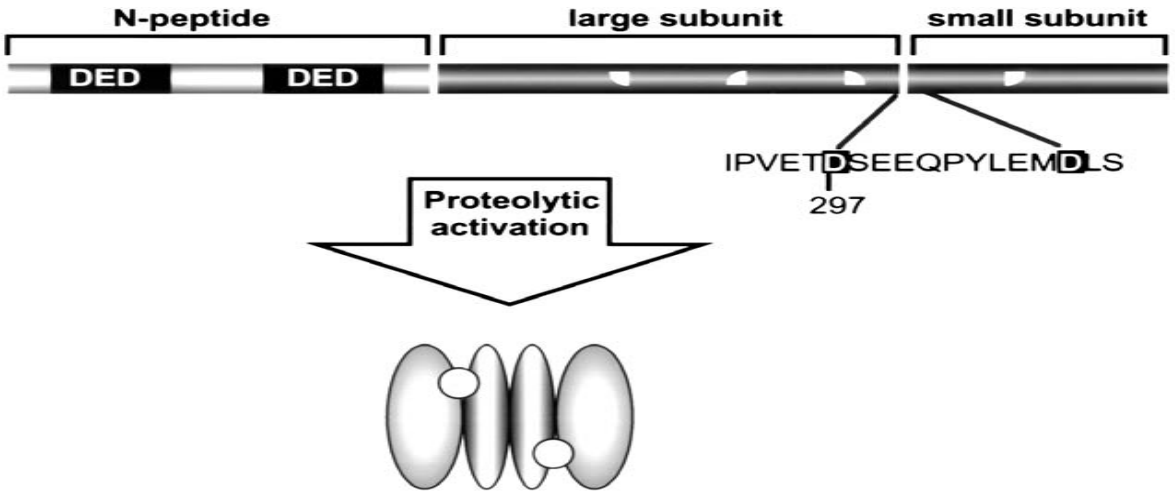
You pick up a beauty magazine and realize, once again, that looks are everything. Two hundred thousand brain cells commit hari-kari.

Running on a treadmill: great for the heart, but shakes brain cells loose, which then fall out of your ears.

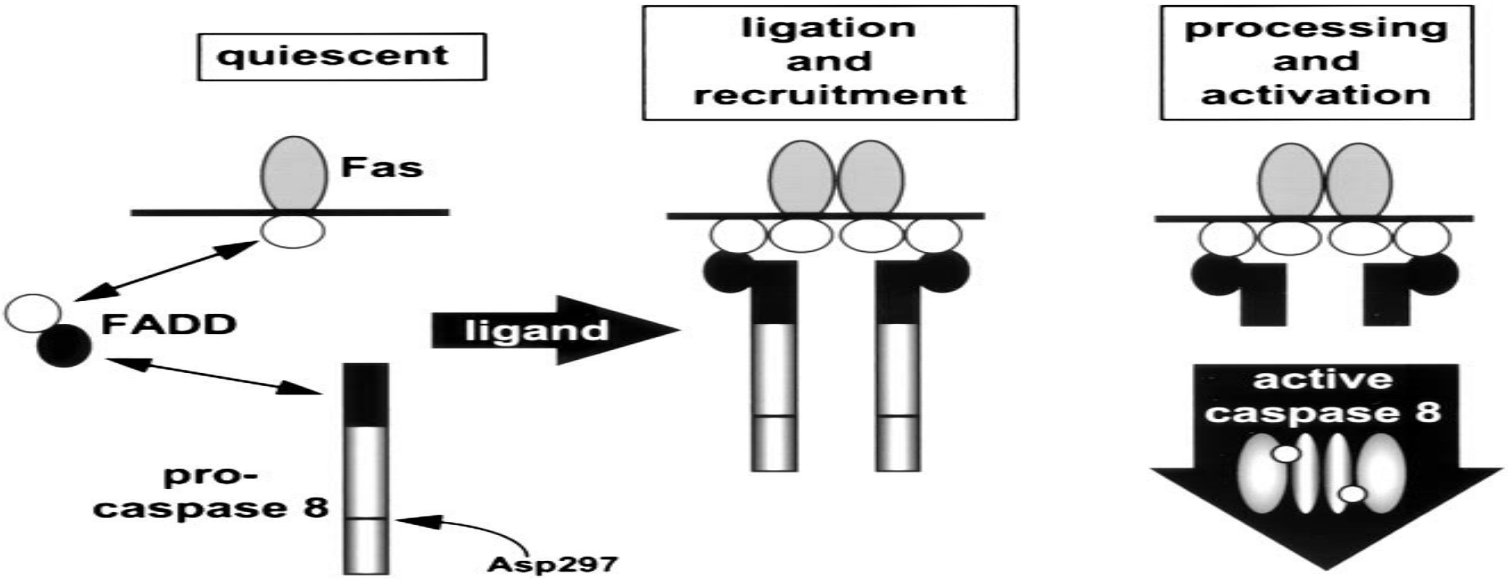
Sequence-Specific Proteases (Caspases) Execute Apoptotic Disassembly of Cellular Protein



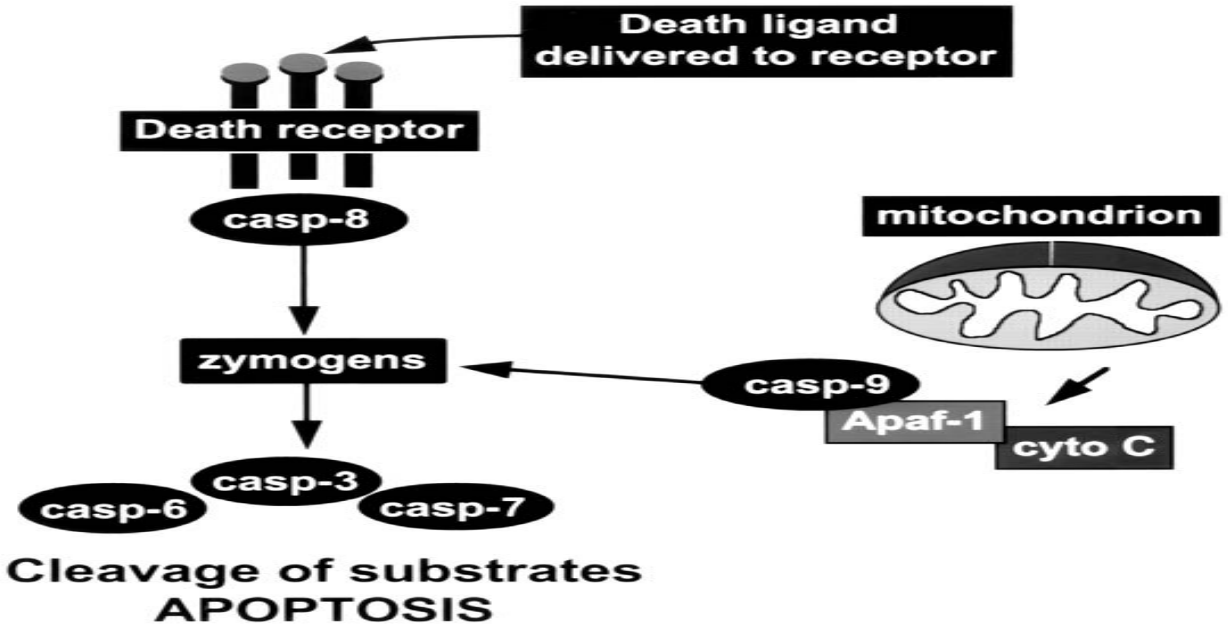
Caspases Contain Similar Catalytic Subunits And Distinct N-terminal Regulatory Domains



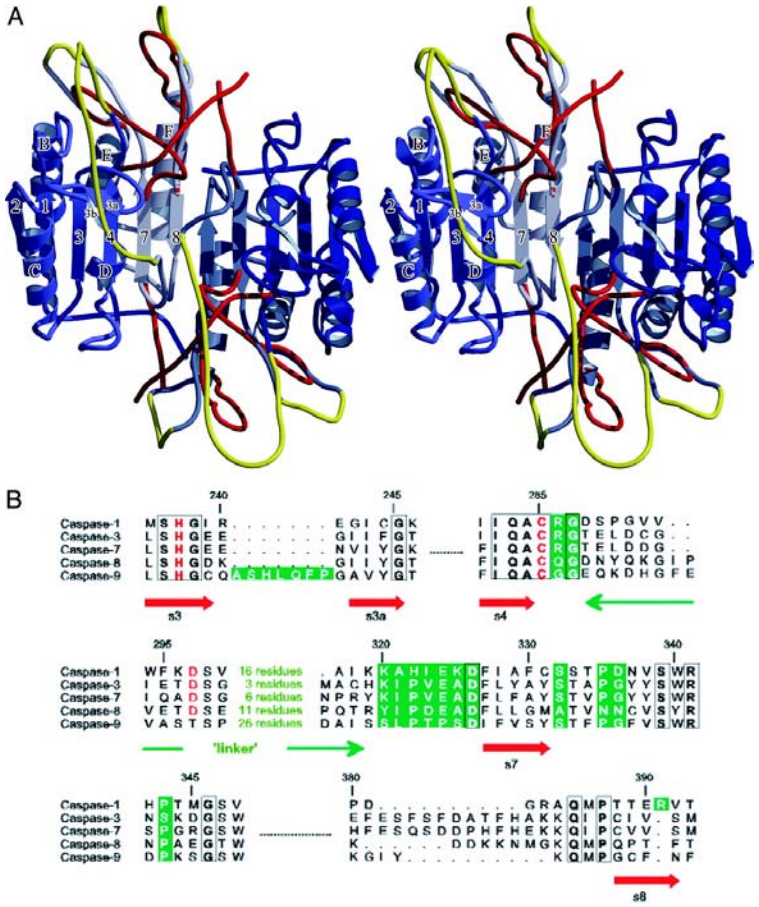
Initiator Caspases (Caspase-8,9) are Activated According to the Forced-Proximity-Model…



…Downstream of Death Signals (Caspase-8) Or Mitochondrial Damage (Caspase-9)



Executioner Caspases (Caspase-3,6,7) Form Structural Heterodimers



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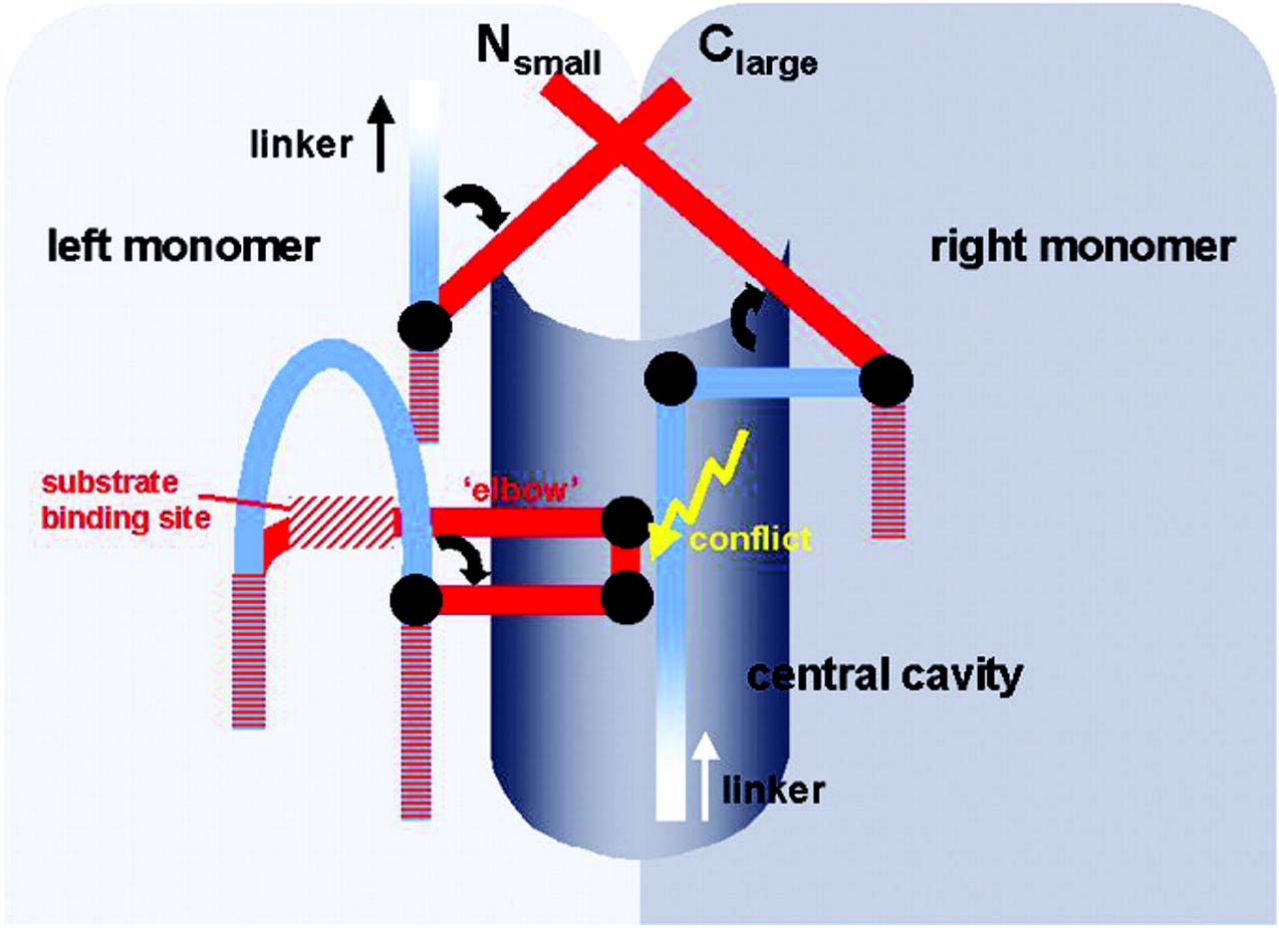
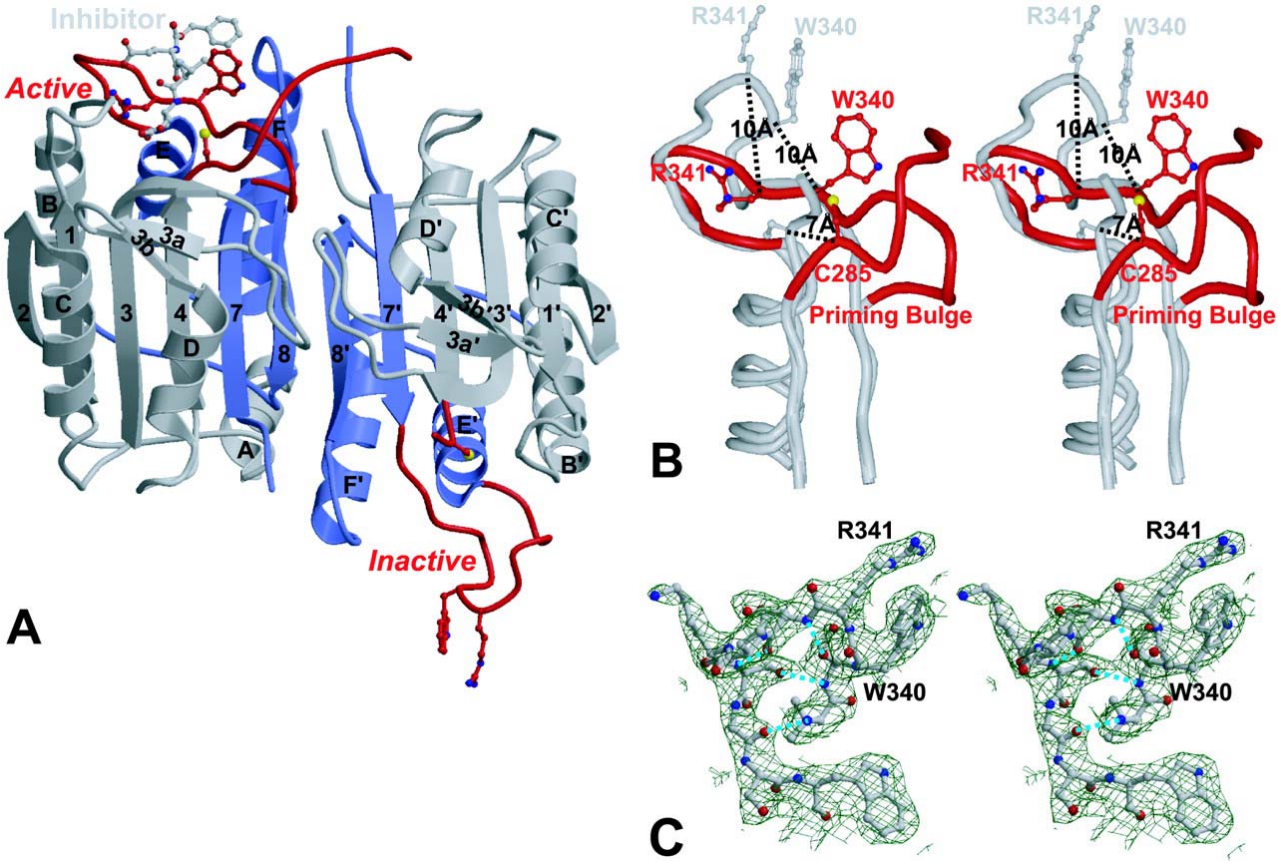
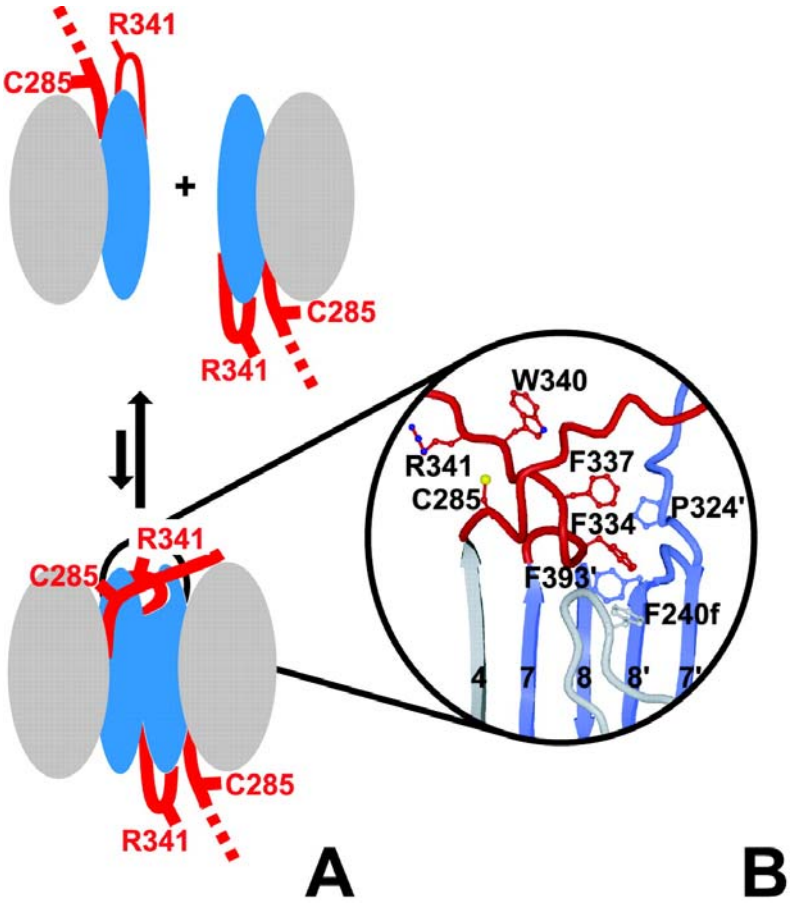
Executioner Caspases Require Processing by Upstream Proteases for Dimer Formation (=Activation)



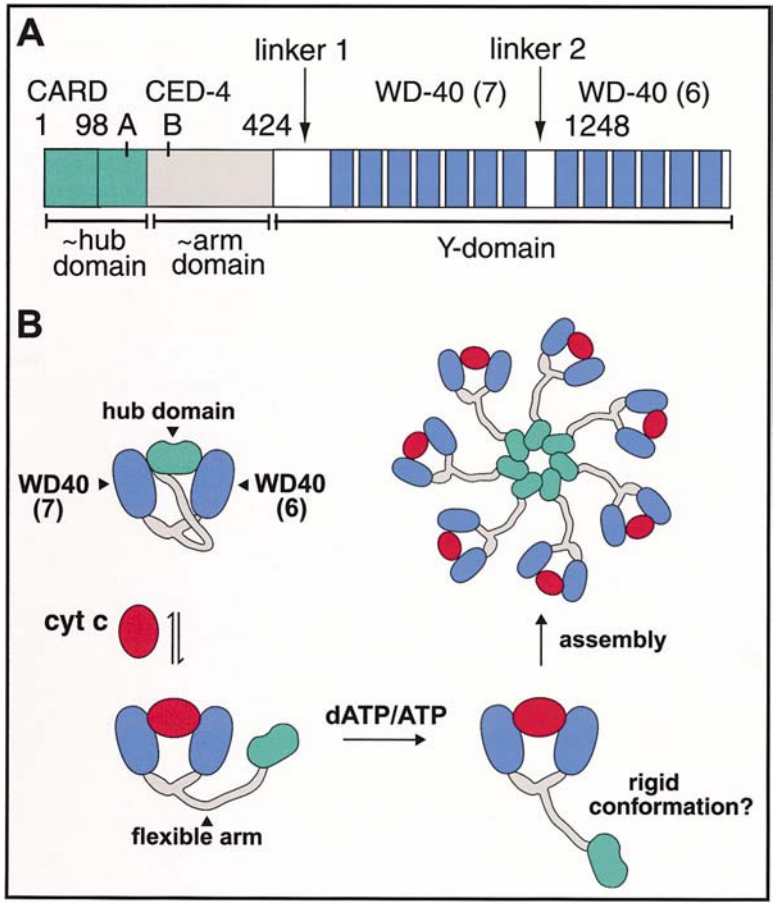
Caspase-9 Differs from Other Caspases by Having Only a Single Active Site…



…that is Formed by the Induced Complex Formation of Caspase-9 Molecules



Apaf-1 Mediates the ATP/dATP- and Cytochrome *c*-Dependent Multimerization of Caspase-9



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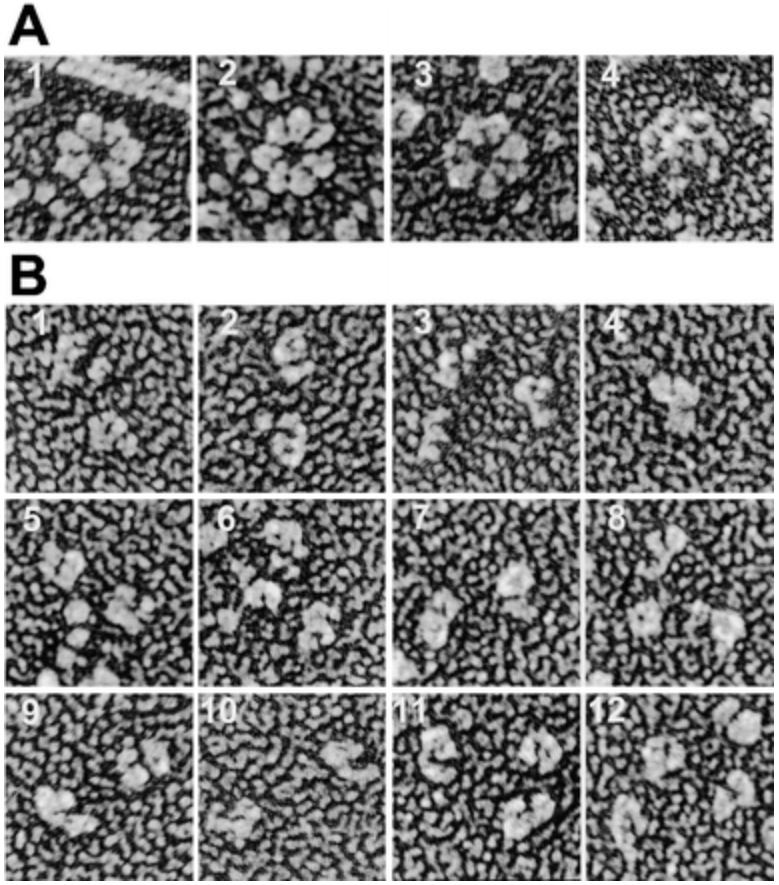
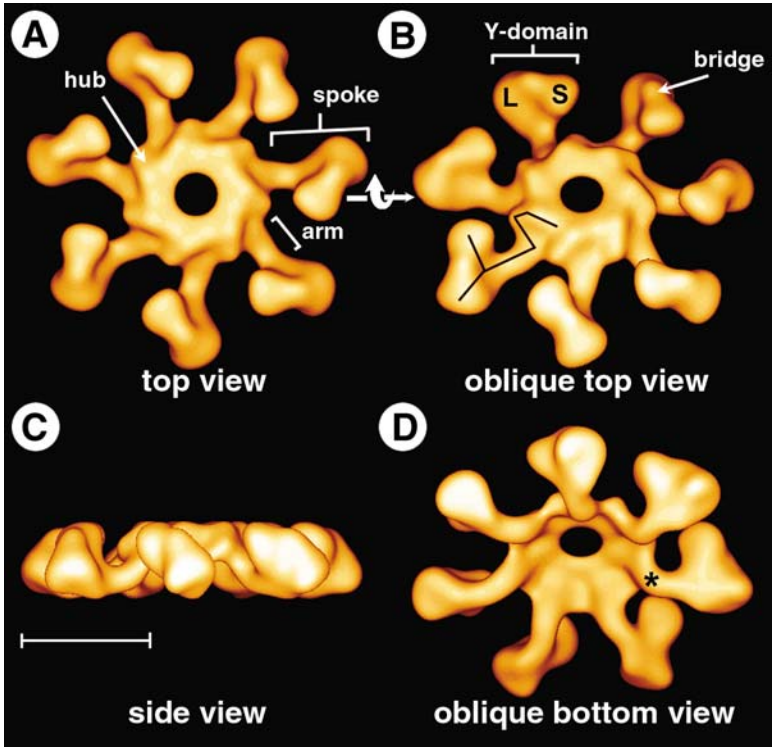
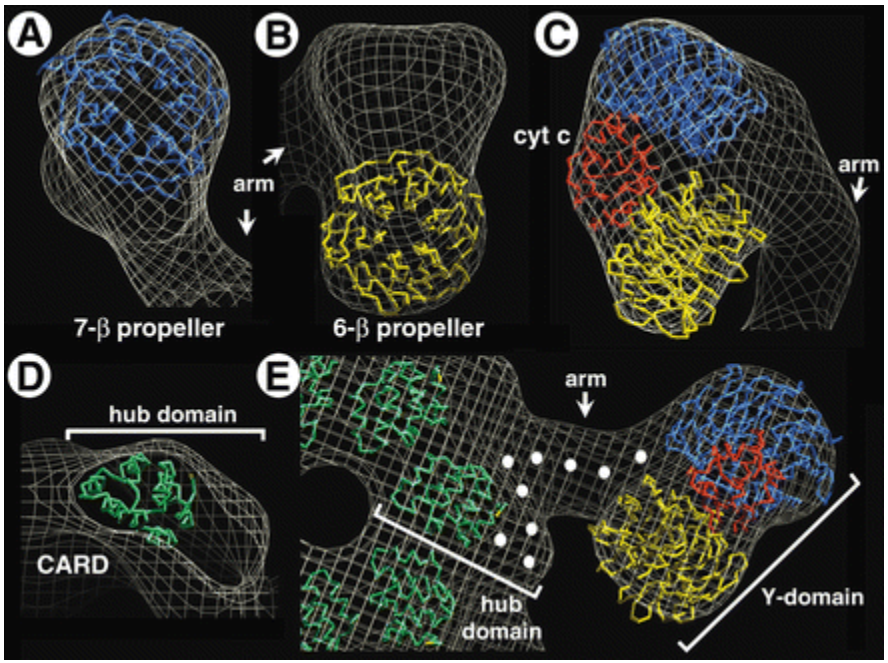
Electron Microscopy Photographs of Apaf-1 In Complex with dATP and Cytochrome *c*



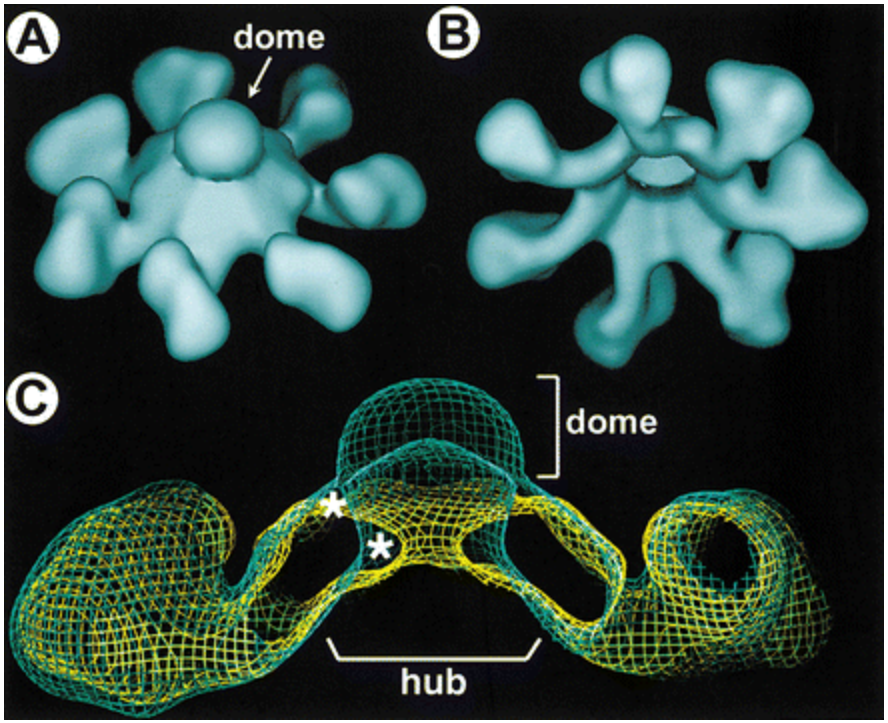
Apaf-1 Complexes (=Apoptosomes) Form Propeller Structures with Heptagonal Symmetry (=7-fold)



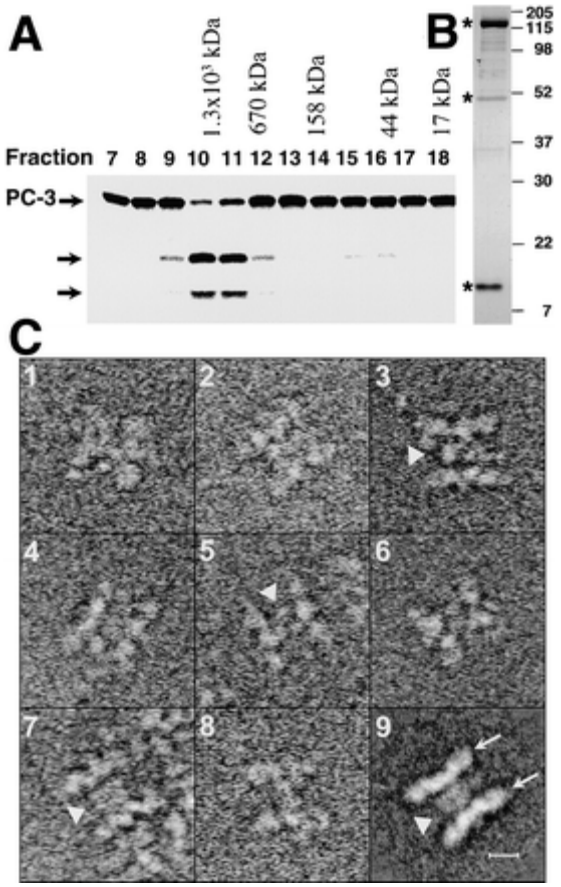
Components of the Apoptosome are Localized In Defined Parts of the Propeller Structure



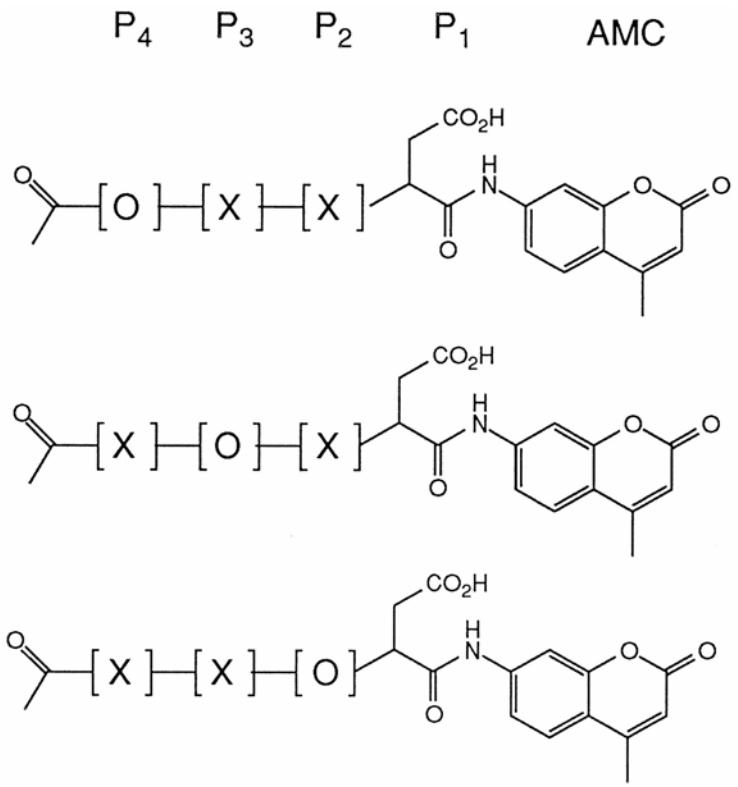
Caspase-9 Molecules are Localized to the Hub And Form a Dome that is the Basis for the…



…Formation of the '*Evil Oreo*' of Apoptosis



Caspase Activity is Determined with Fluorogenic Tetrapeptides Resembling Protease Cleavage Sites



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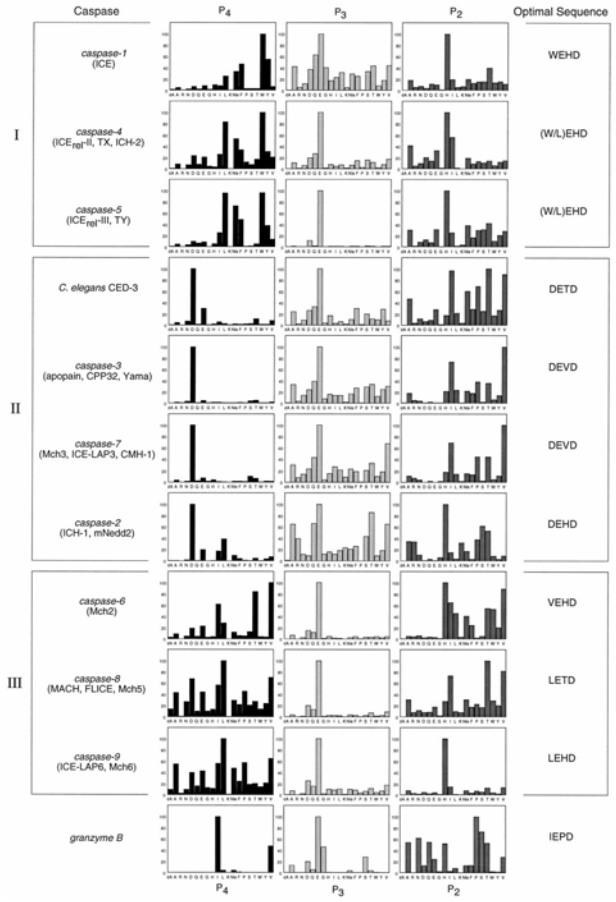
Systematical Permutations of Tetrapeptide Sequences Reveal Synthetic Substrate Peptides…



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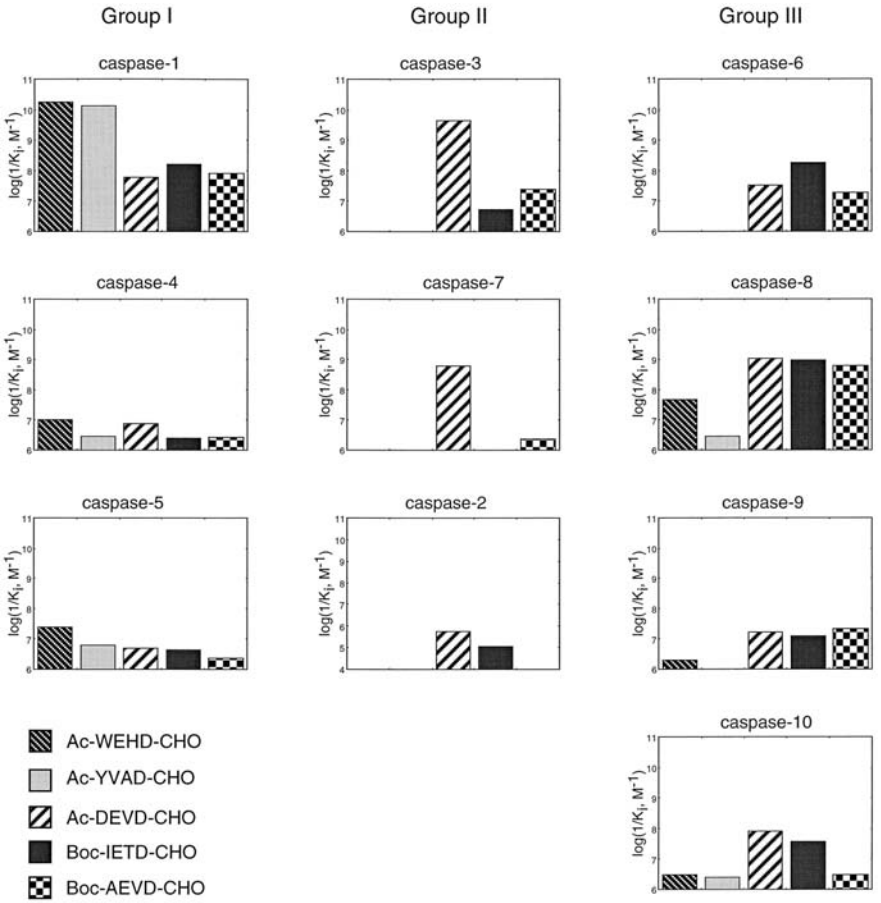
…With Increased Specificity for Selected Caspases And Groups of Caspases…



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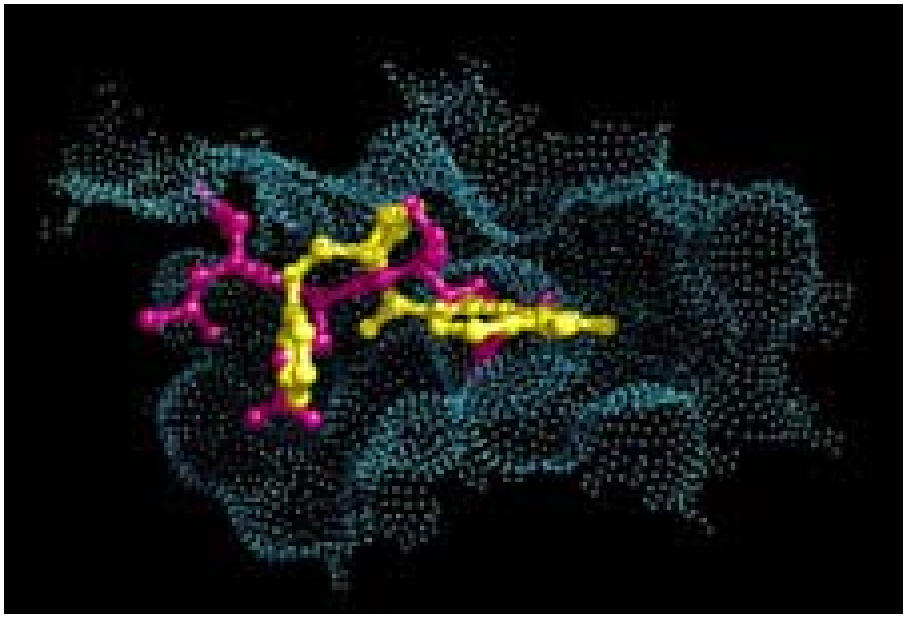
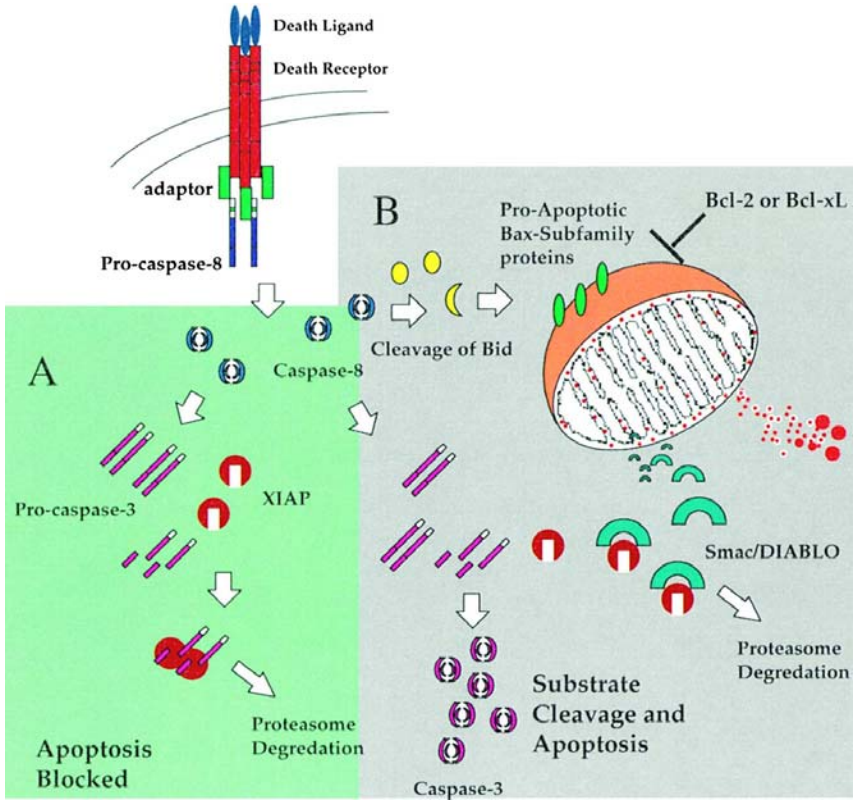
…That Form the Rational for the Generation of Peptoid Caspase Inhibitors

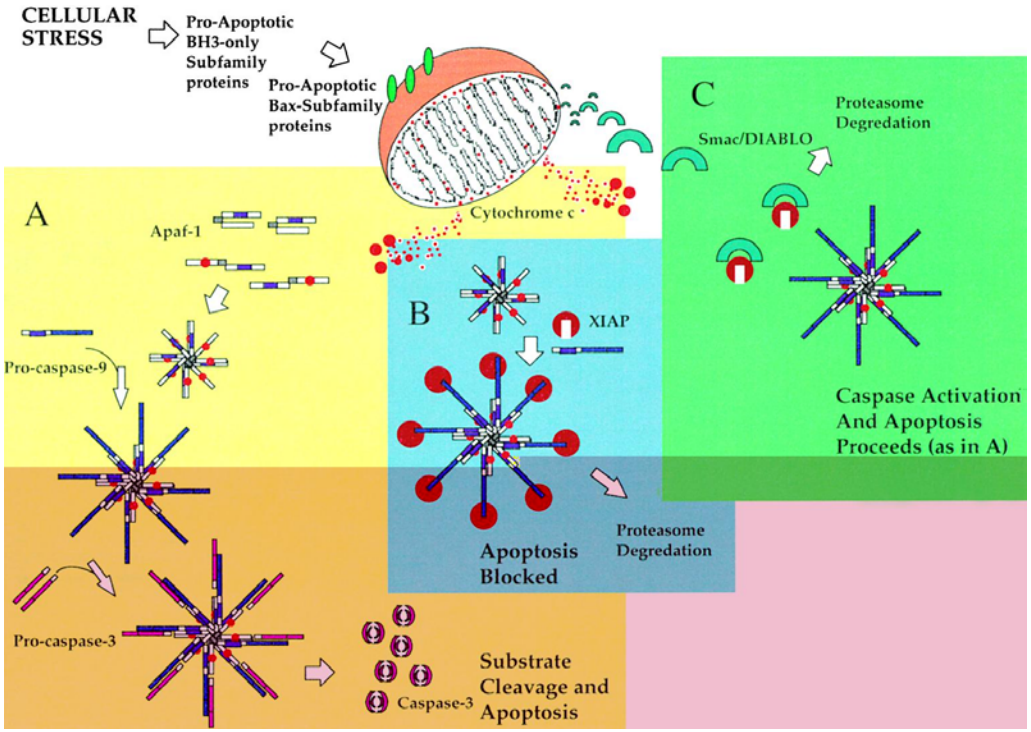


Unfortunately: Limited Practical Use because of Hepatobiliar P_1-Asp Transporter System (=Secretion)

In Summary: Death Receptor Signals (Extrinsic) Induce Caspase-8 Activation while …



…Cellular Stresses (Intrinsic) Cause Mitochondrial Damage Leading to Caspase-9 Activation



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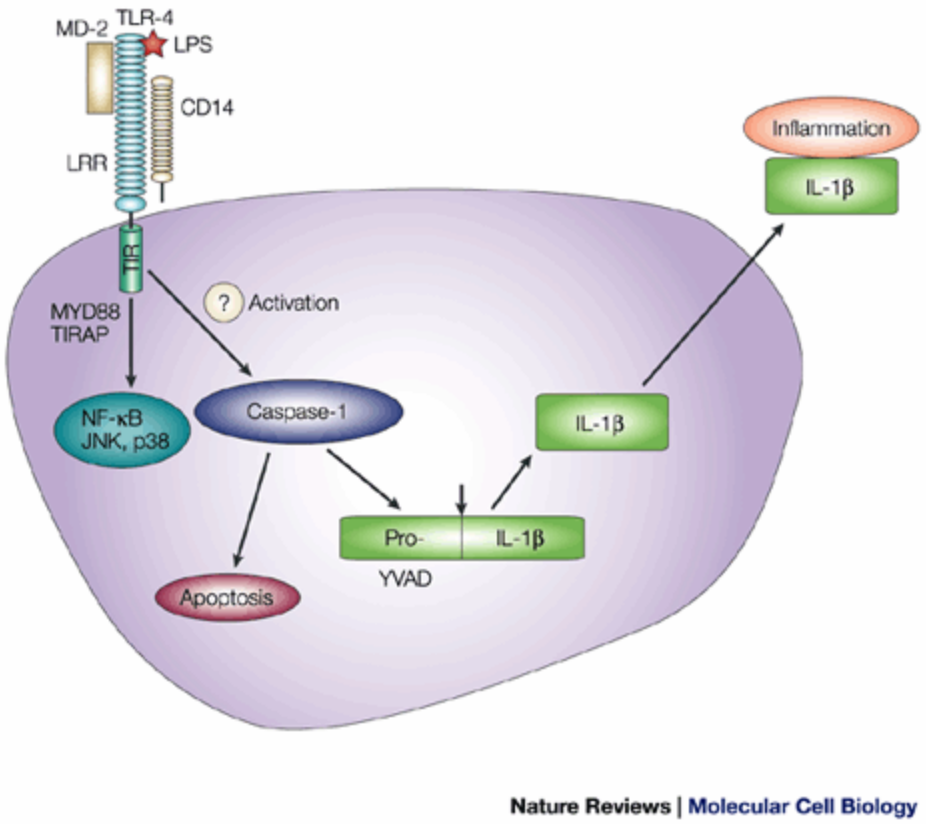
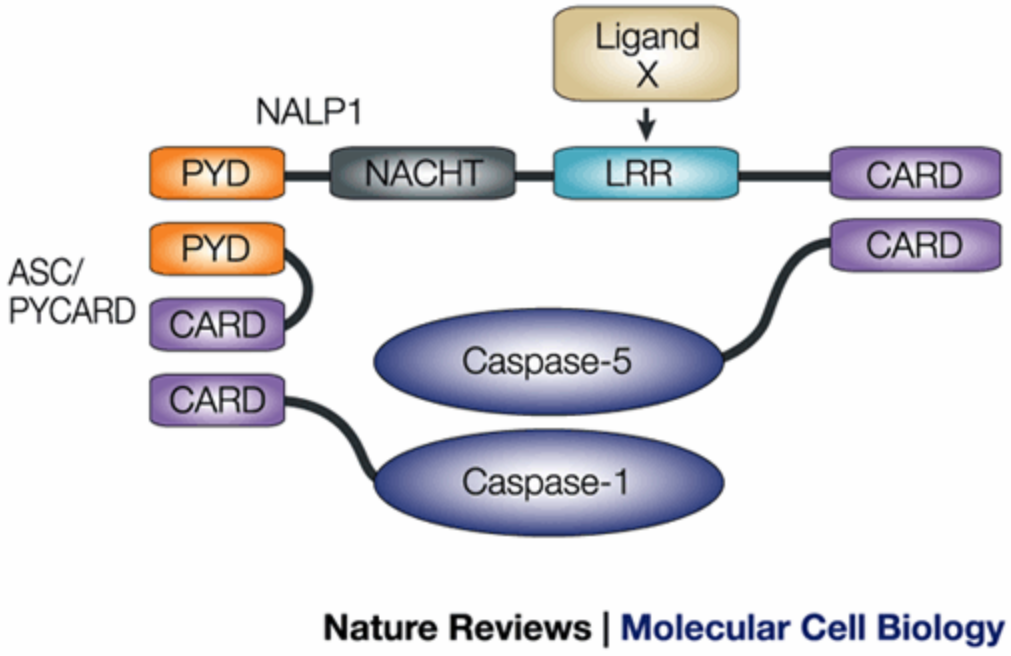
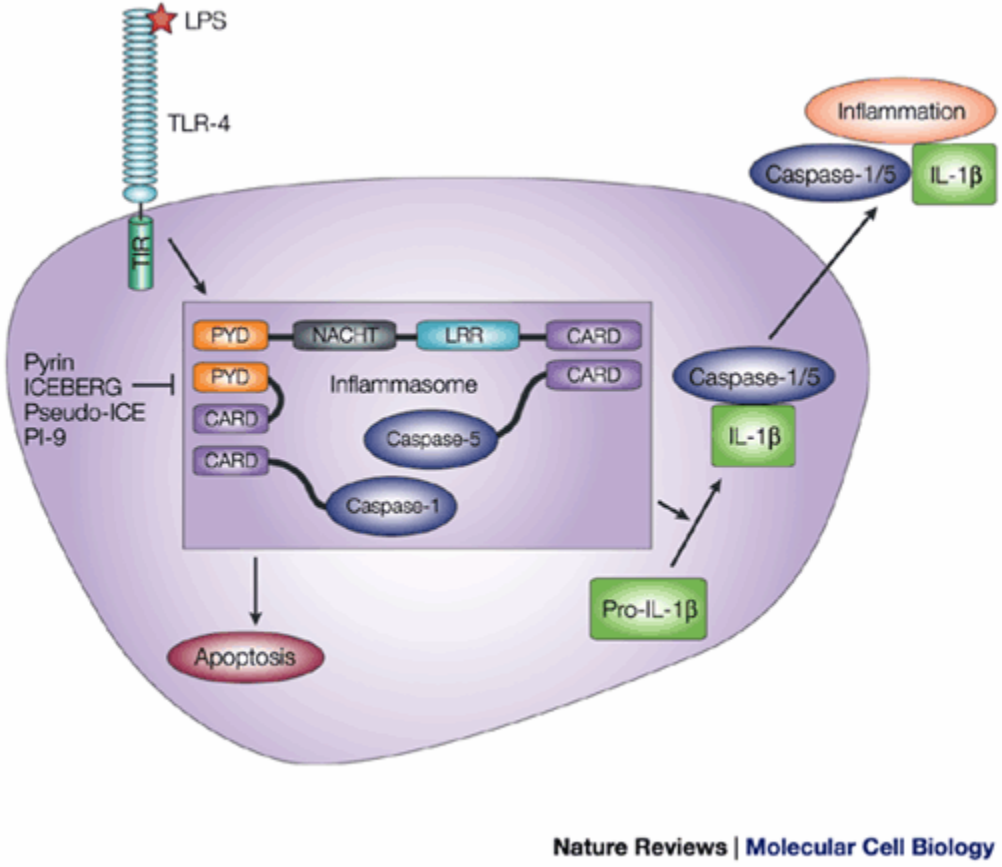
Other Caspases (Caspase-1,5) are Involved in Inflammatory Cell Responses…



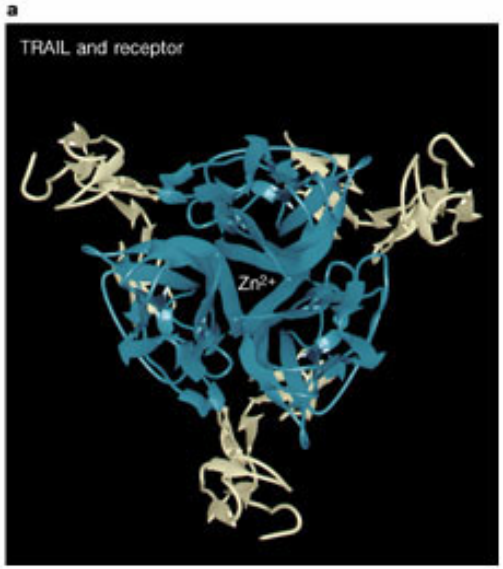
…That Involve Multimolecular Complexes…

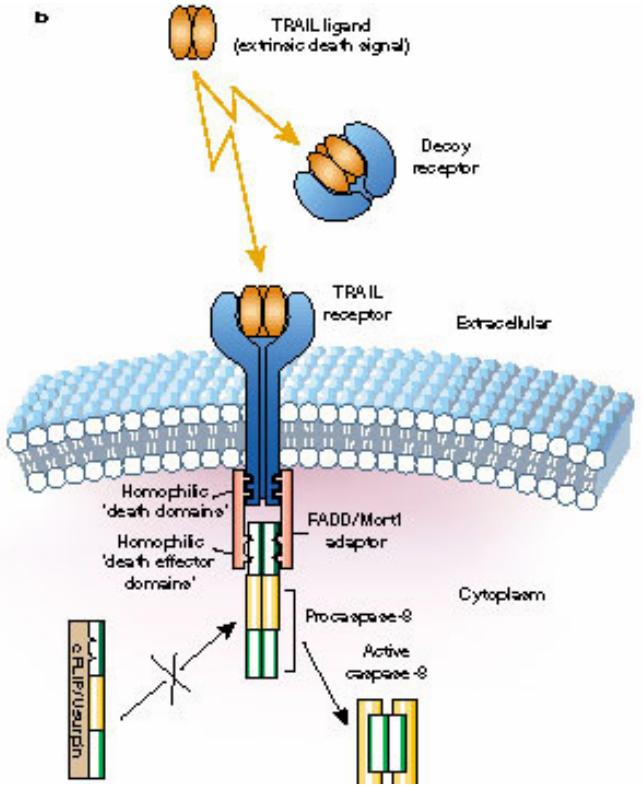


…And Lead to the Assembly of an Inflammasome

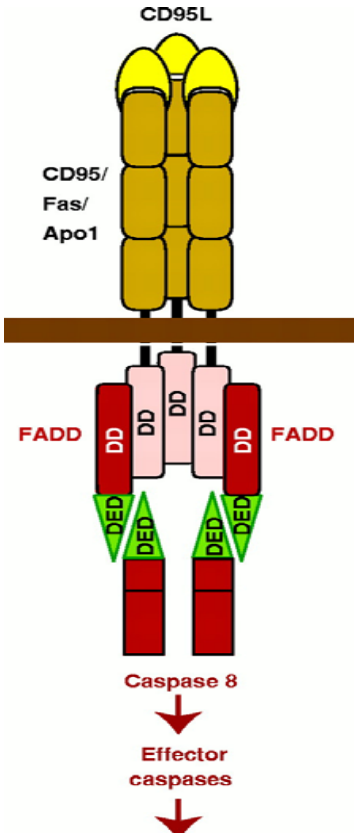


Extrinsic Cell Death Pathways Depend on Death-Receptor Ligands such as TRAIL

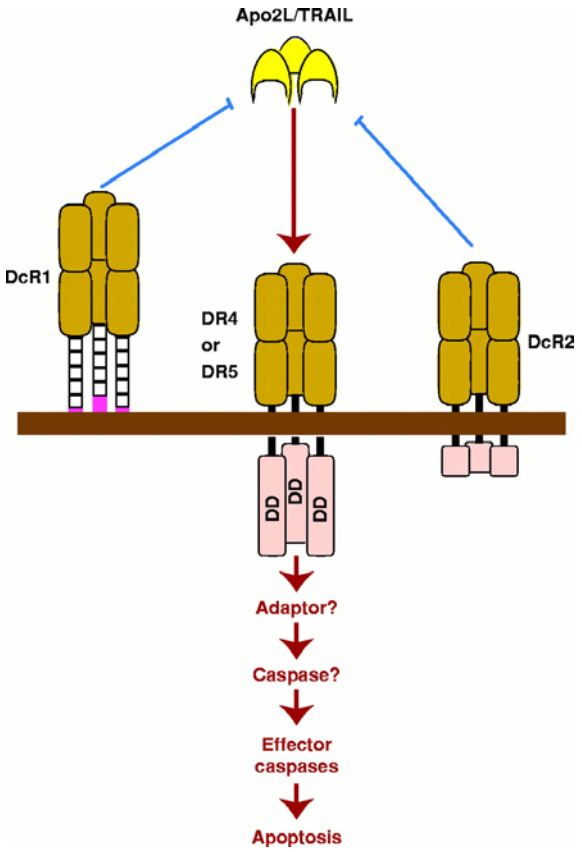




CD95/Fas/Apo1 → Caspase-8 Activation (=Death)

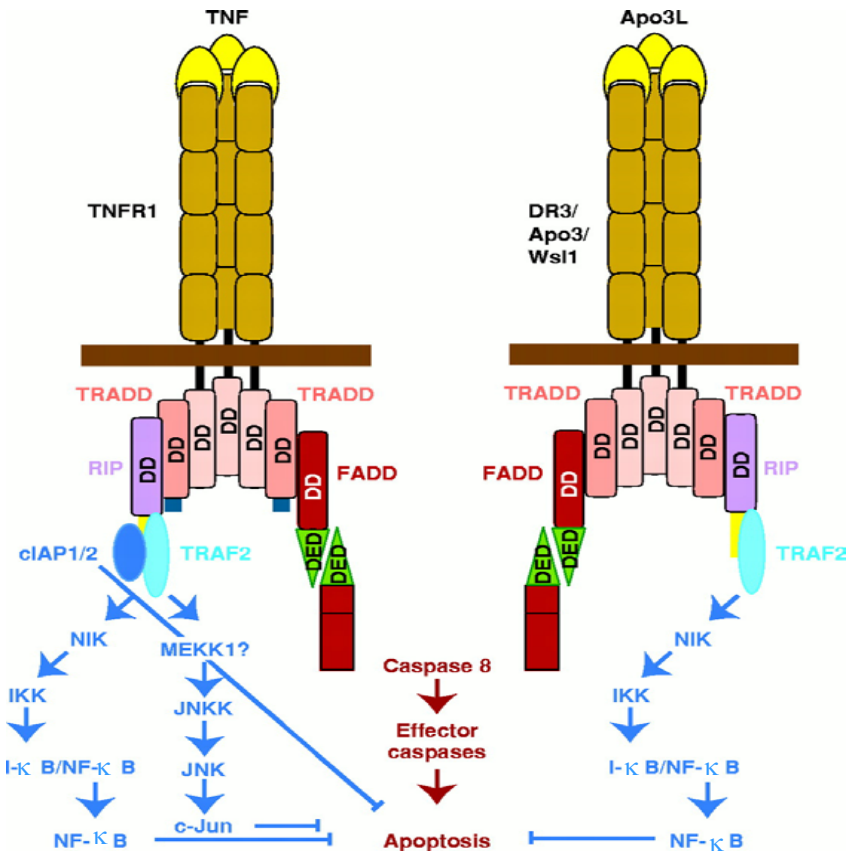


Apo2L/TRAIL → Caspase-8 Activation (=Death)



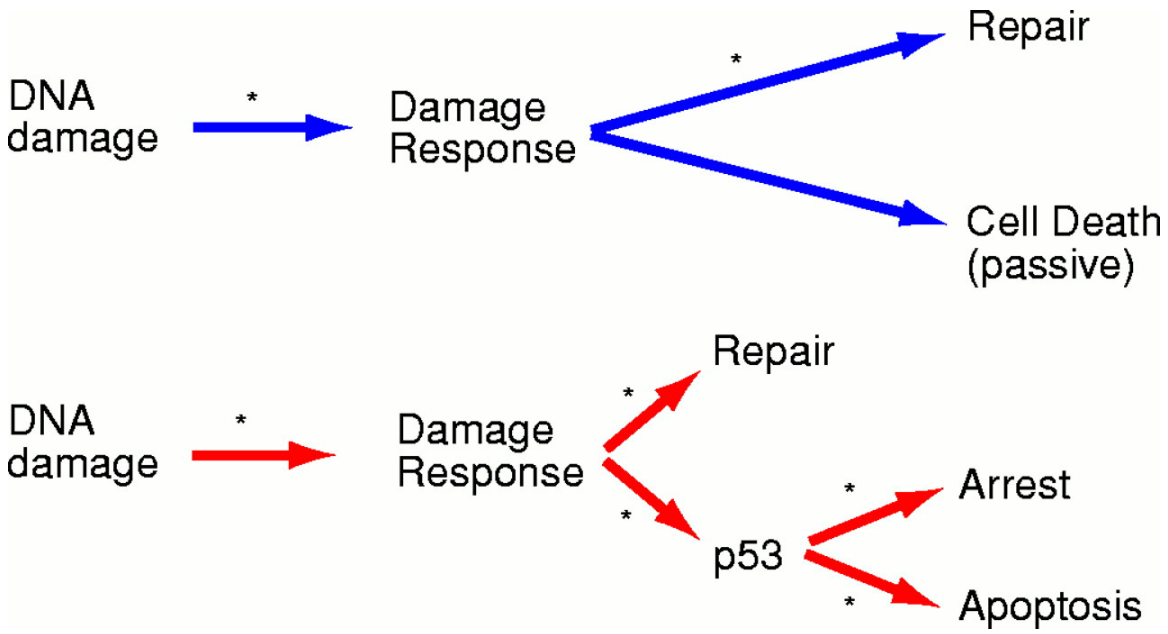
Increased Sensitivity in Cancer Cells (Lack of Decoy Receptors)

Apo3L/TNF → Caspase-8 Activation (=Death) + NF-κB Activation (=Survival)



G9600 TFF 2003

Outlook: DNA Damage Responses



Outlook: Role of p53