Apoptosis

分子醫學研究所
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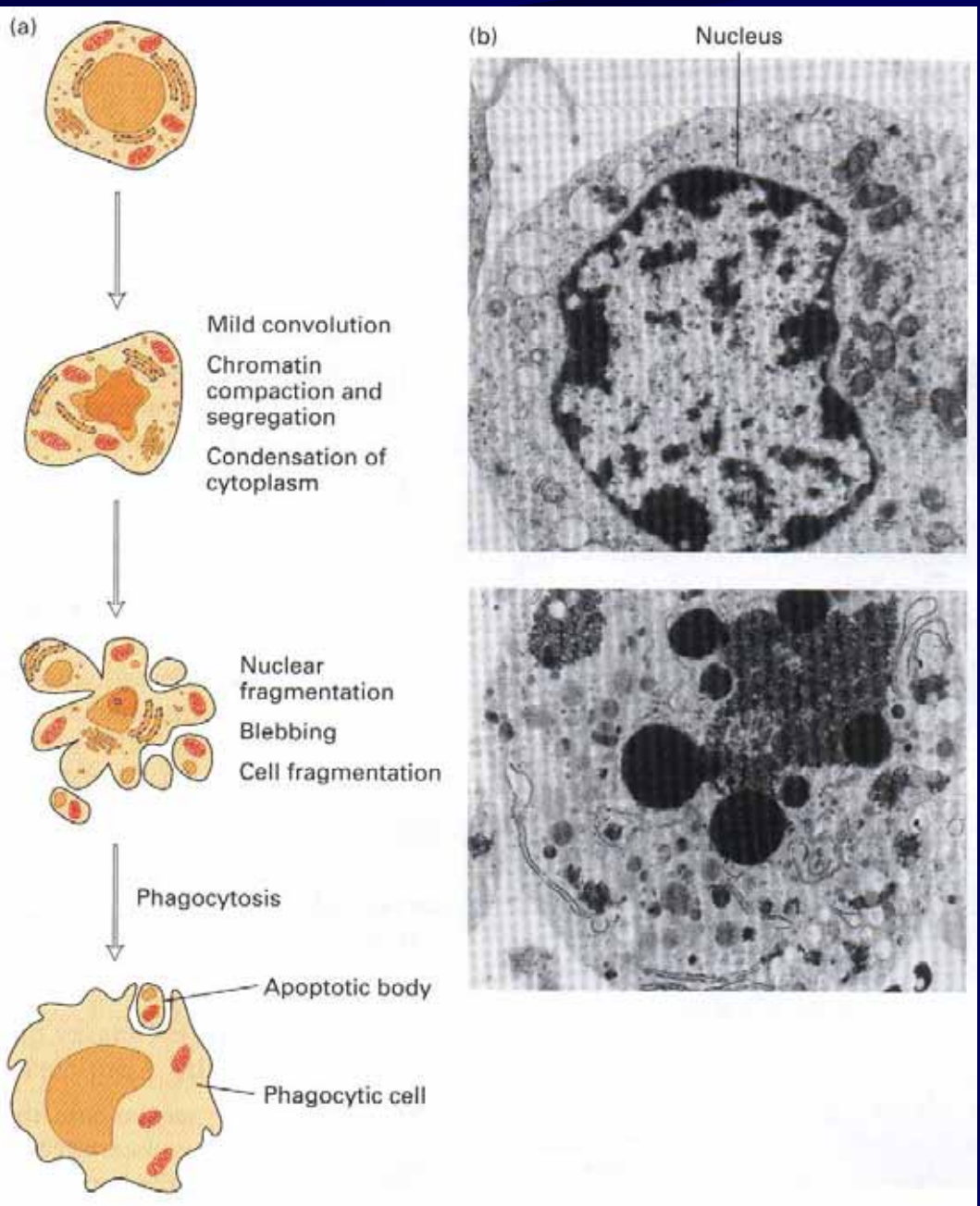
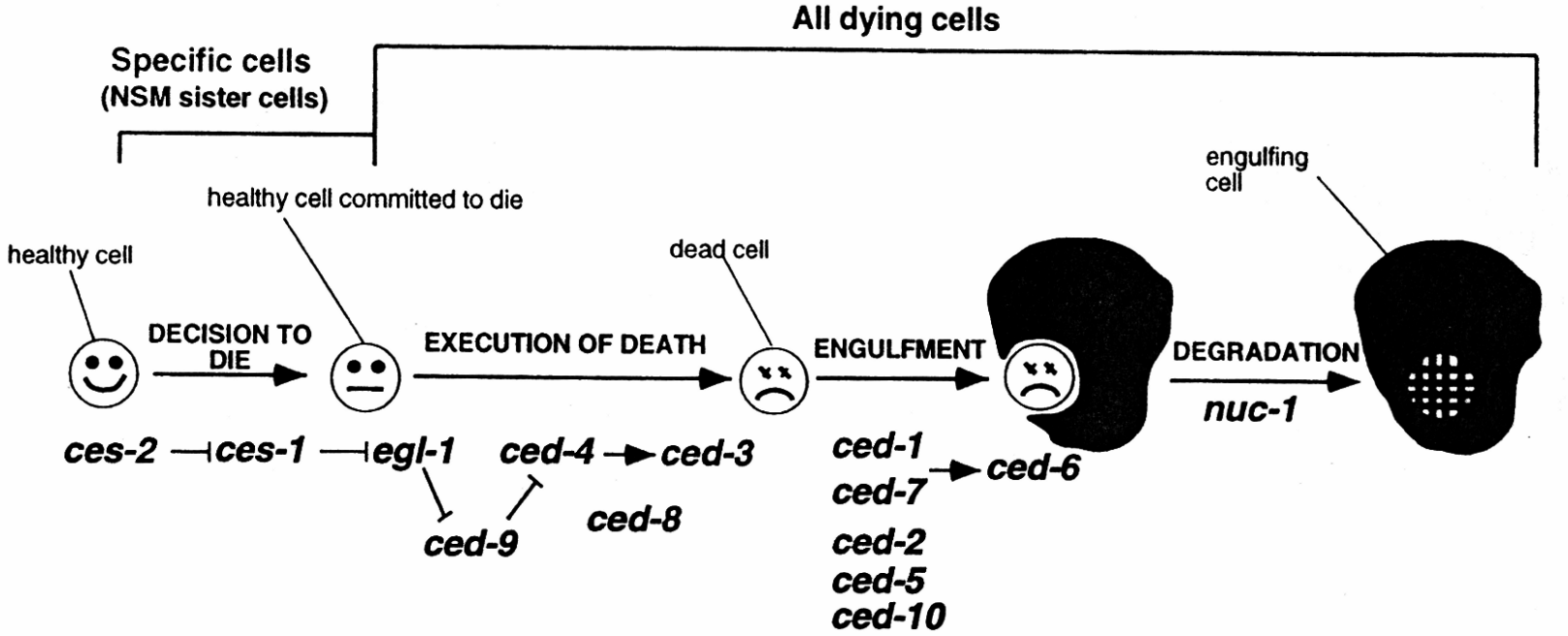
The hallmarks of apoptosis



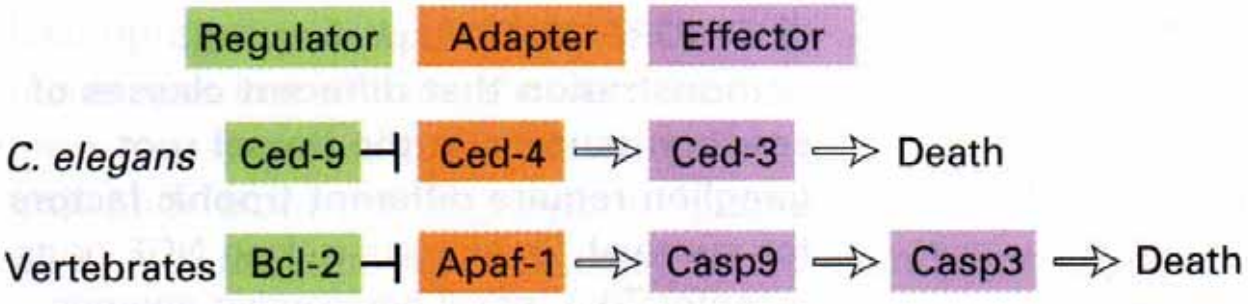
Morphology of Cell Corpse

C. elegans embryo at 3-fold stage

C. elegans apoptotic pathway

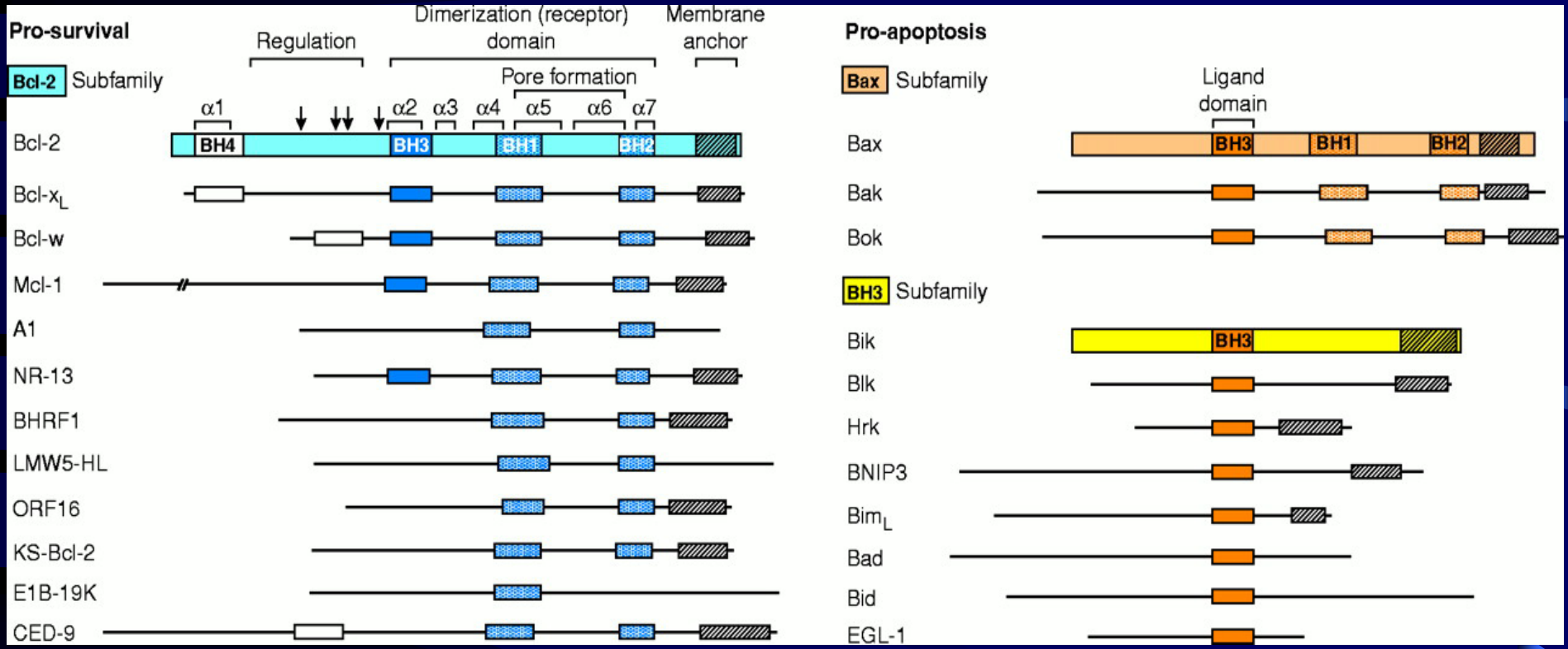


Apoptosis Core Pathway



▲ **FIGURE 23-49 Overview of the apoptotic pathway in *C. elegans* and vertebrates.** Three general types of proteins are critical in this conserved pathway. Regulators either promote or suppress apoptosis; the two regulators shown here, CED-9 and Bcl-2, both function to suppress apoptosis in the presence of trophic factors. Adapters interact with both regulators and effectors; in the absence of trophic factors, they promote activation of effectors. A family of cysteine proteases serve as effector proteins; their activation leads to degradation of various intracellular substrates and eventually cell death. [Adapted from D. L. Vaux and S. J. Korsemeyer, 1999, *Cell* **96**:245.]

Bcl2 Superfamily



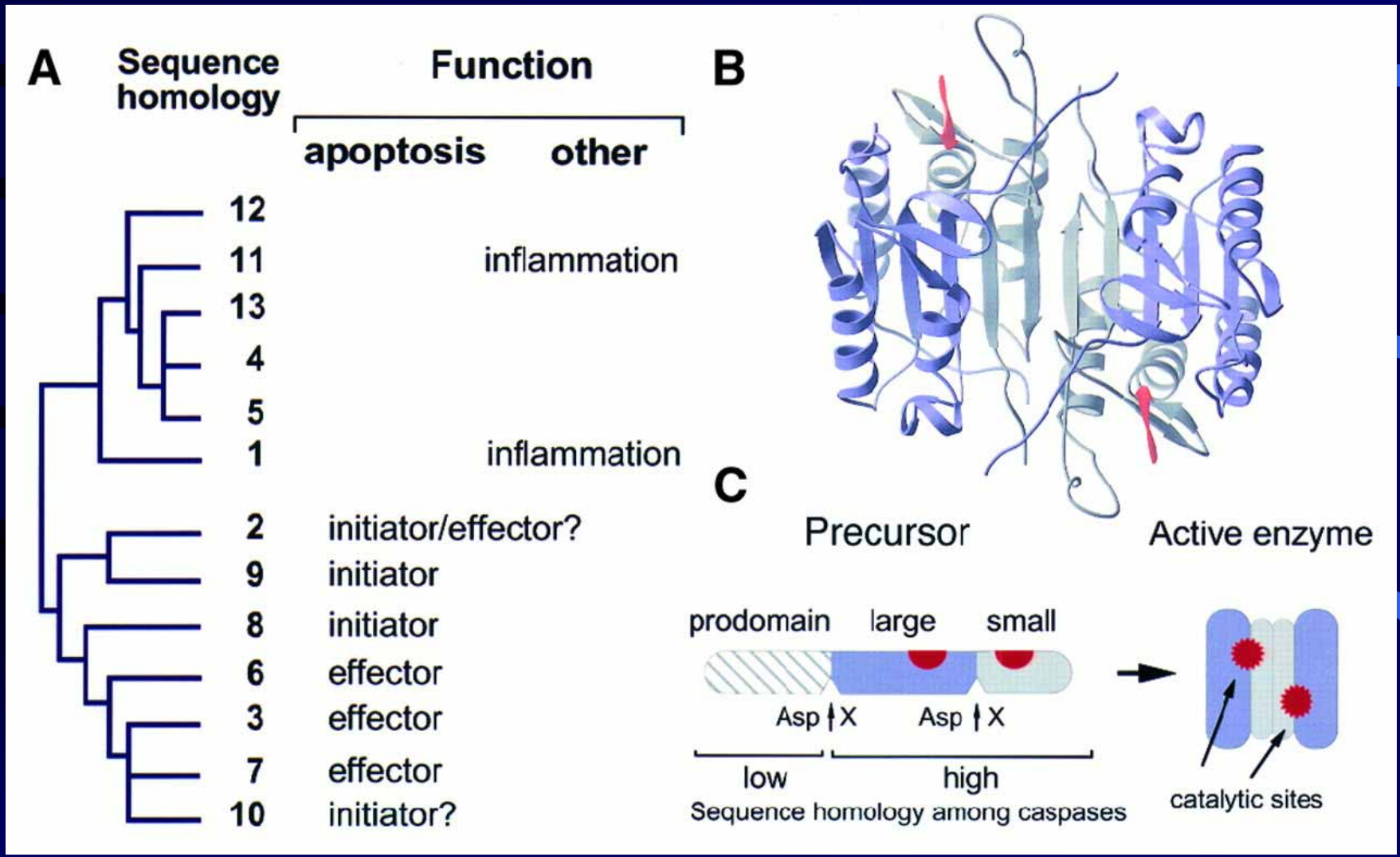
Science 281;1323,1998

Function of Bcl2 family proteins:

Regulation of mitochondria homeostasis and permeability

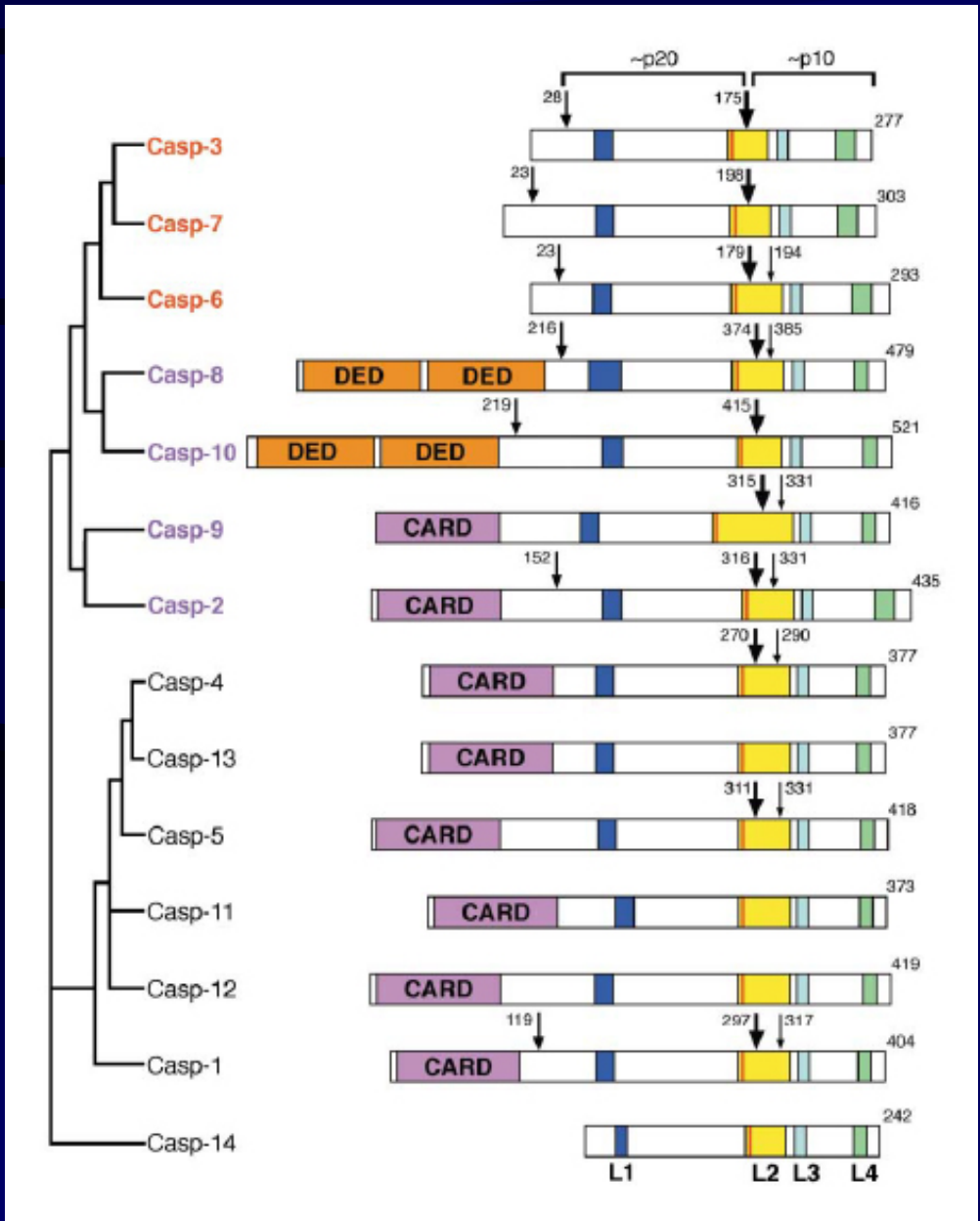
Caspases Family

Cysteine proteases which cleaves peptide at c-terminal of aspartic acid



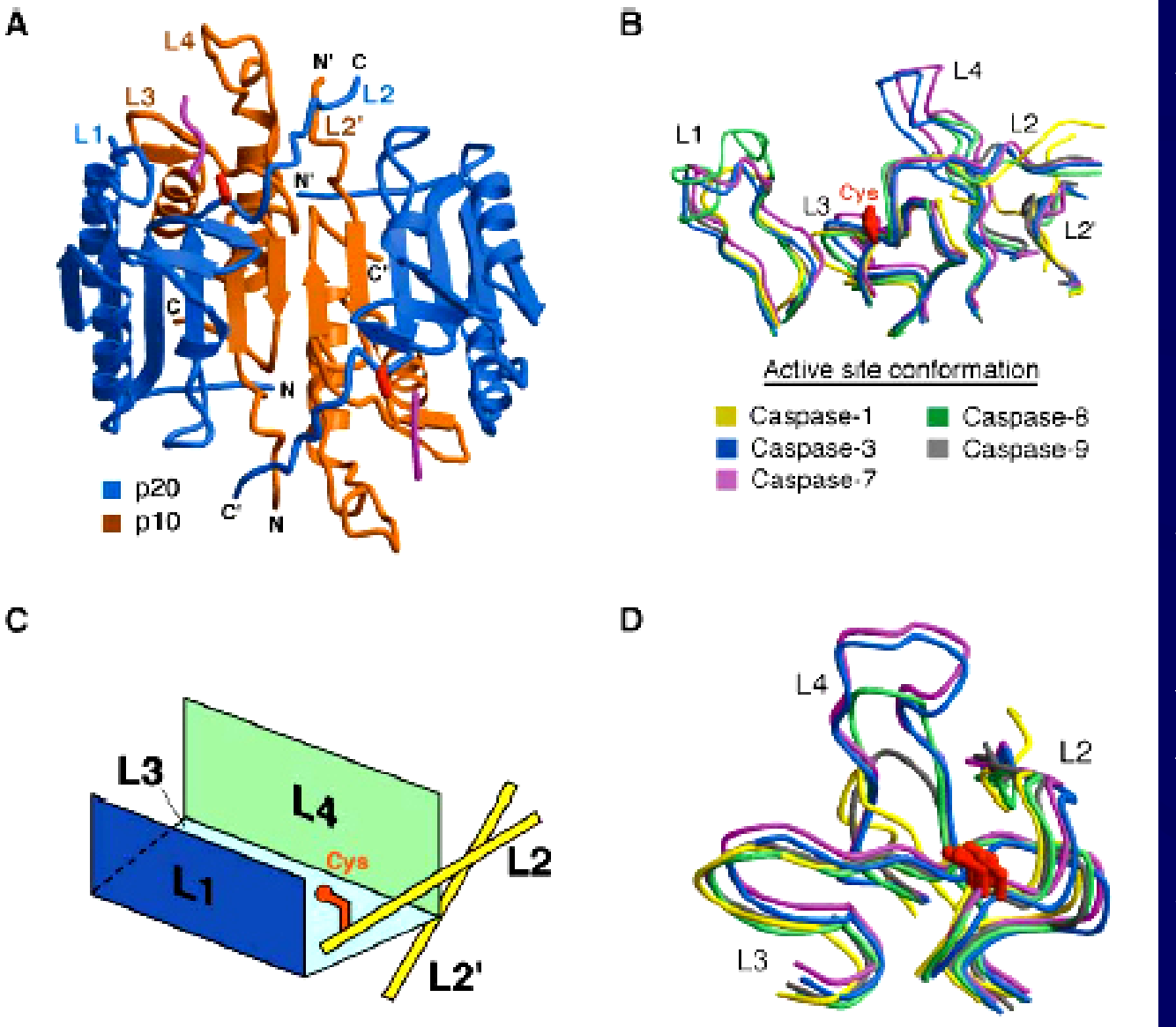
Science 281;1313,1998

Two types of prodomain: CARD and DED



Mol Cell 9: 459, 2002

Structure of Caspases



A: Overall structure

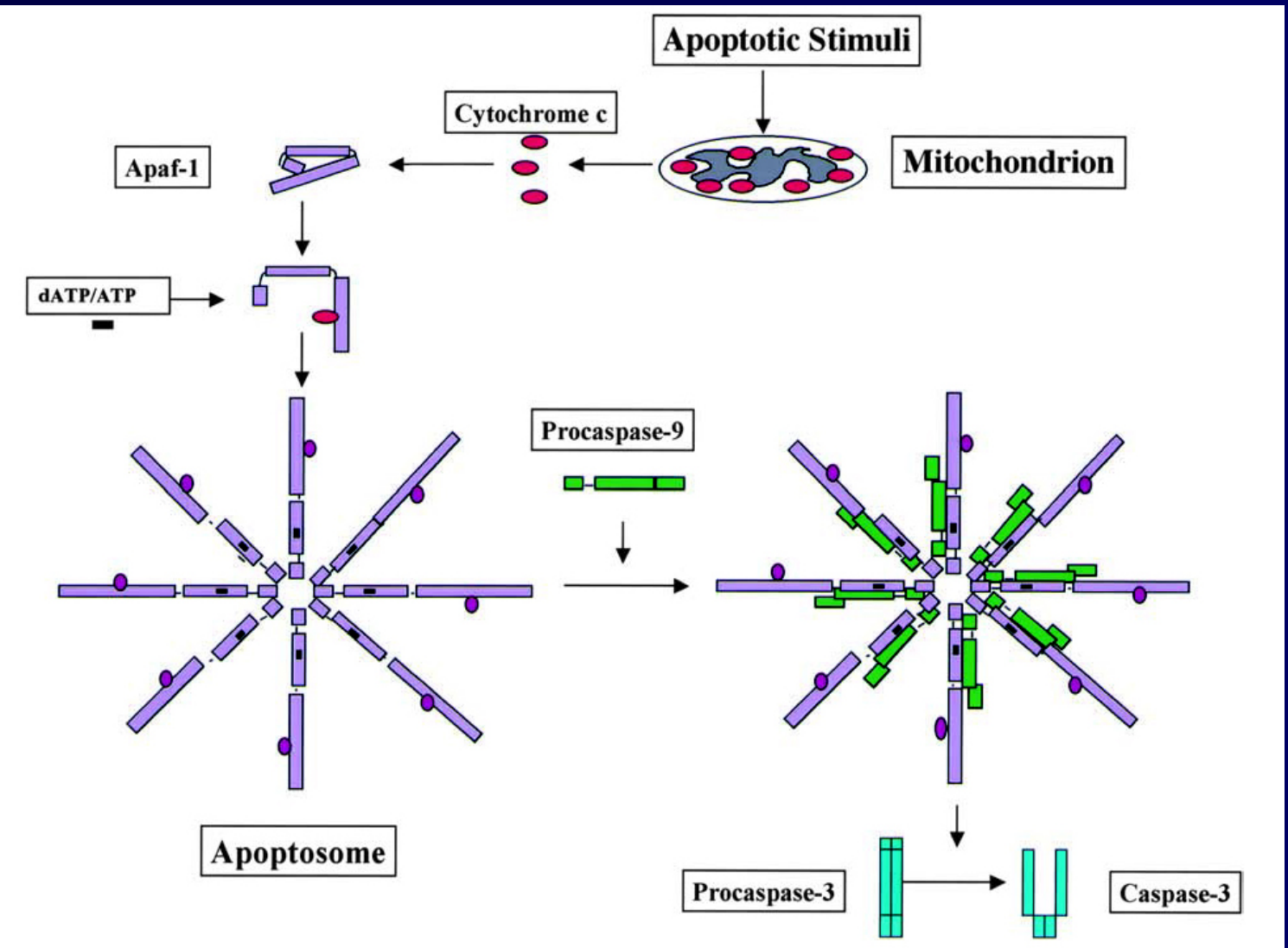
B: Active site conformation

C: Schematic diagram of substrate binding groove

D: L4 loop determines the substrate specificity (e.g., short L4 loop prefers bulkier P4 residue, whereas extended L4 in caspase 3 and 7 prefers Asp)

Mol Cell 9: 459, 2002

Mechanism of procaspase-9 activation: the story of Apaf-1, Apaf-2 and Apaf-3

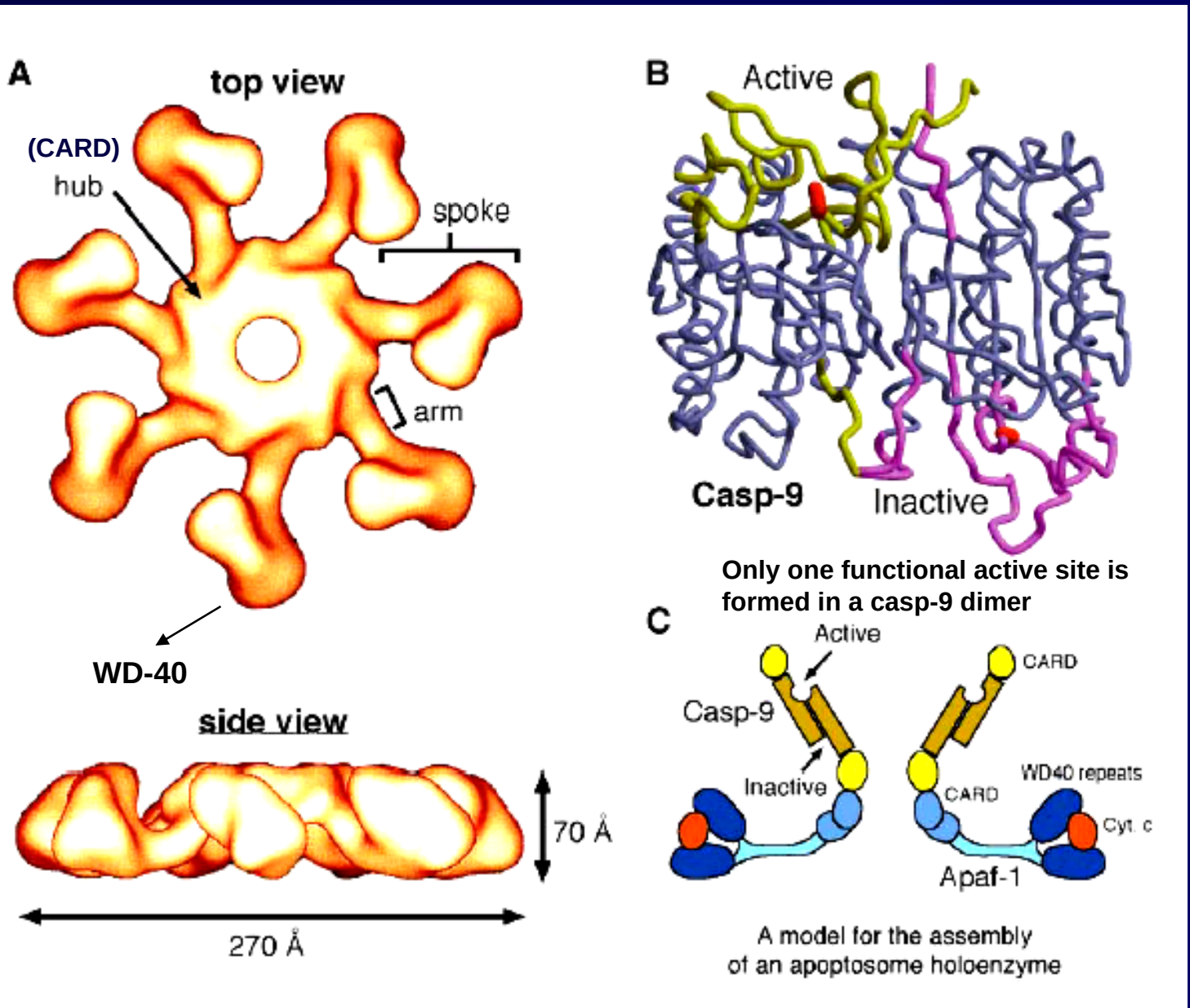


Apaf-1 contains: CARD domain, CED-4 like domain and WD40 repeats.

Cyto c binding to WD40 of Apaf-1 -> dATP/ATP binding -> oligomerization -> CARD domain exposure -> procaspase-9 binding and autoactivation (holoenzyme theory)

Gene & Dev 15: 2922, 2001

Structure of caspase-9 and apoptosome



A and B: Two EM views of hepatameric apoptosome

C: Structure of caspase-9

D: A model for casp-9 activation: The high local concentrations of casp-9 within apoptosome drives the recruitment of additional casp-9 monomers, which are activated upon dimerization

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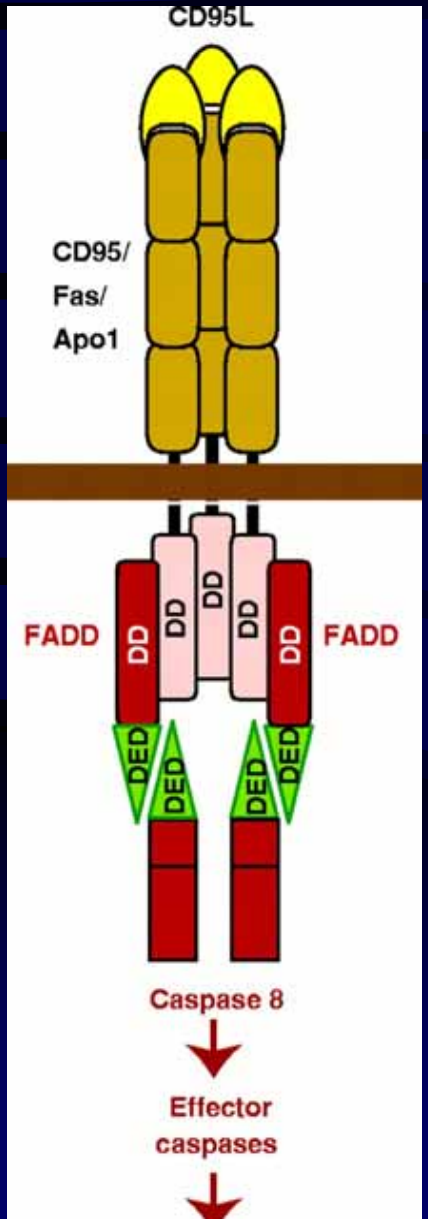
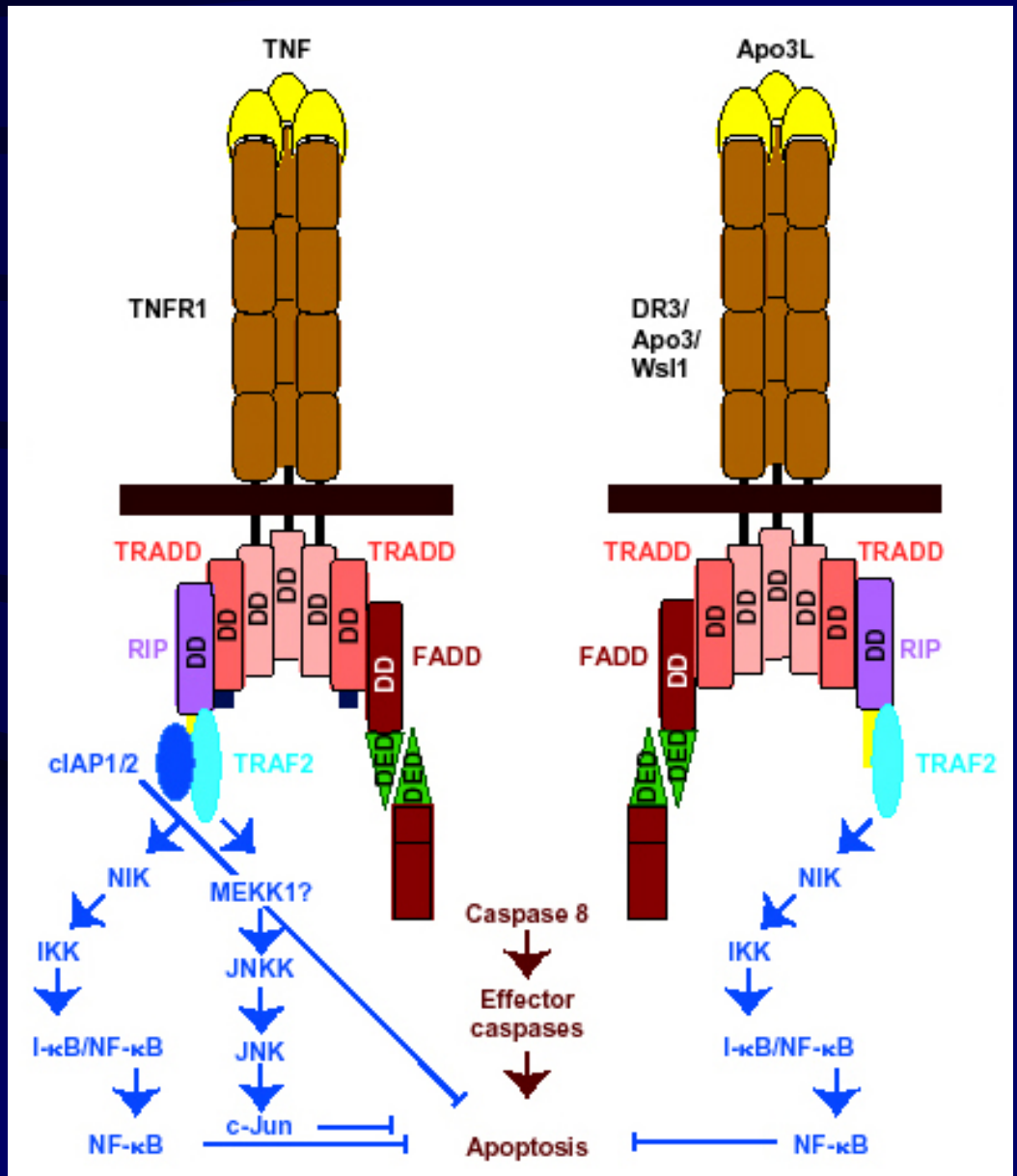
Death receptor family (1): Fas and TNF signal transduction

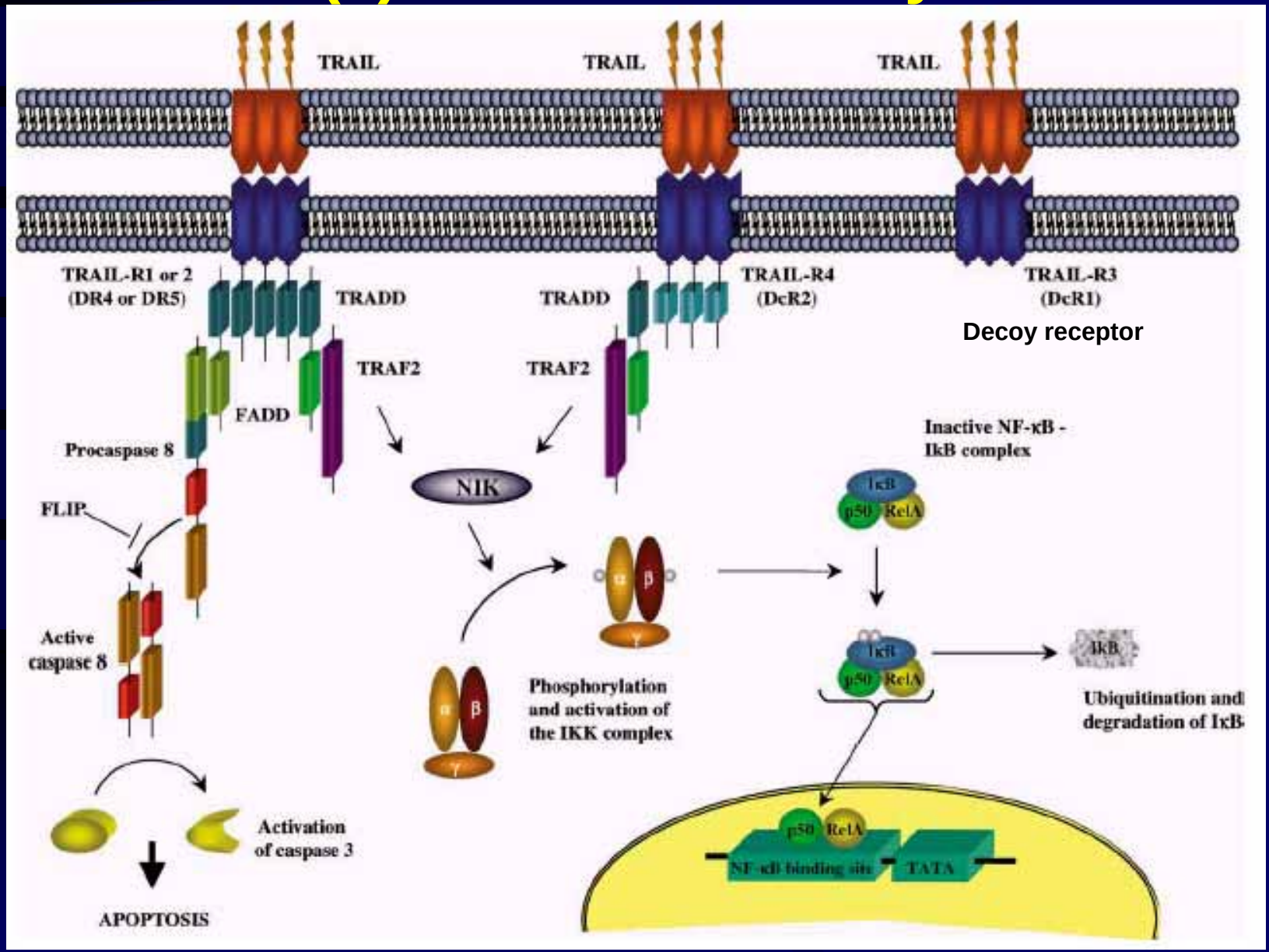




TNF transduces both apoptosis and survival signals.

Science 281;1305,1998

(2) TRAIL subfamily



TRAIL selectively kills certain tumor cells

Cytokine & Growth Factor Rev 12: 199, 2002

Two types of apoptotic pathways

- Extrinsic (mitochondrial-independent) pathway
- Intrinsic (mitochondrial-dependent) pathway

Fas type I and type II cells

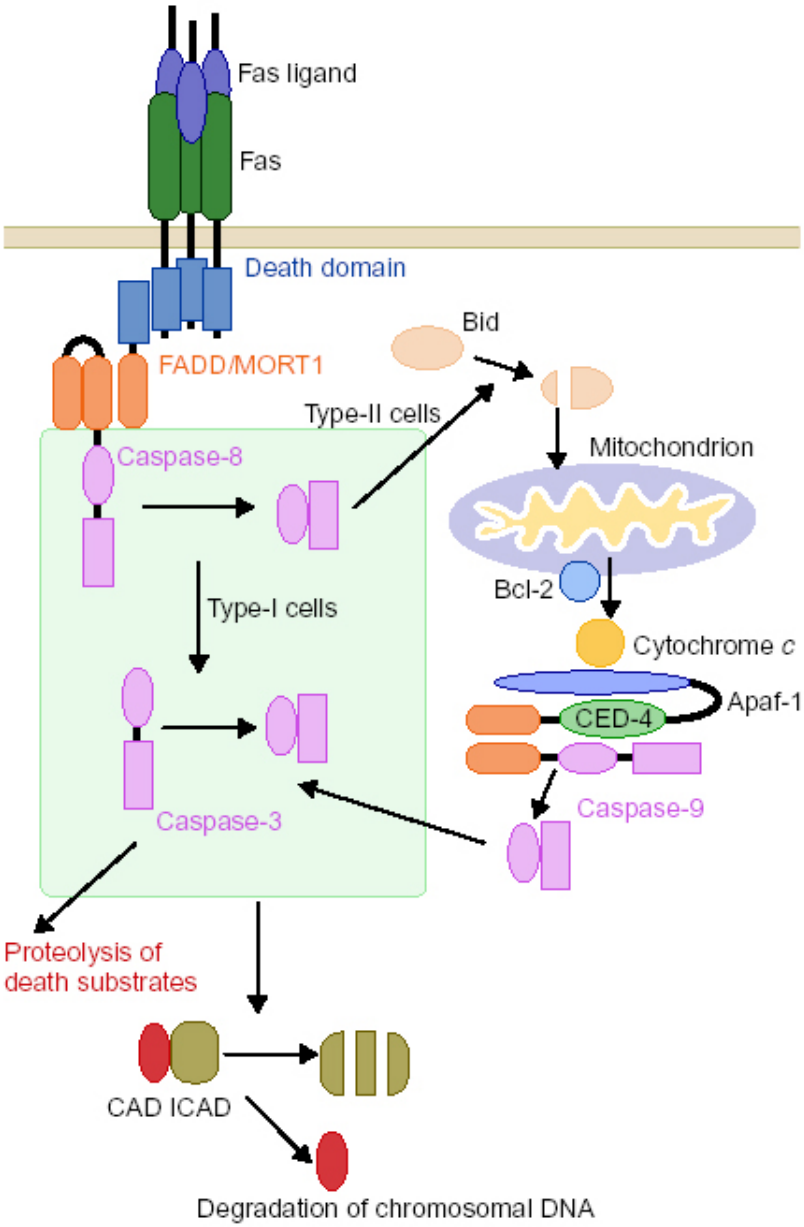
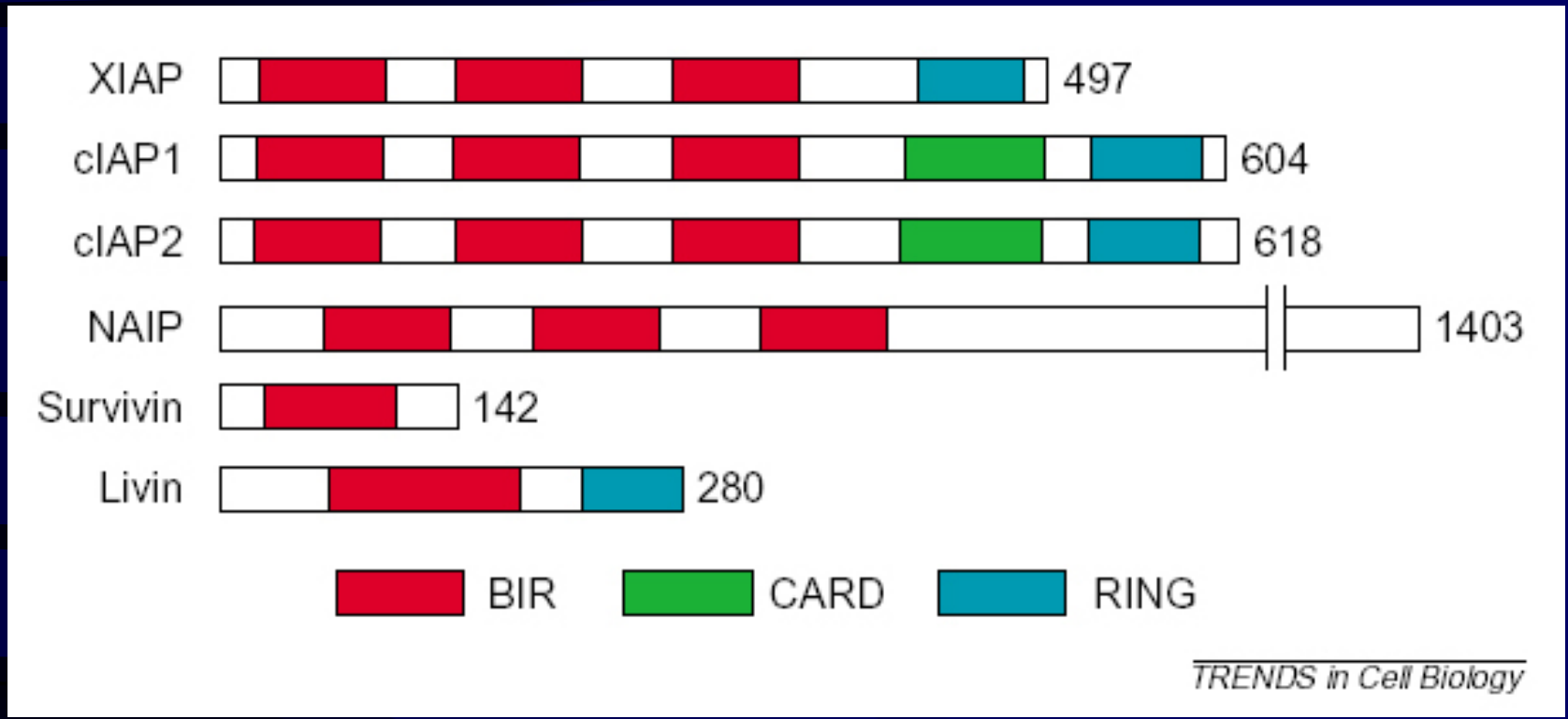


Figure 2 **Two signalling pathways for Fas ligand (FasL)-induced apoptosis. Binding of FasL to Fas recruits procaspase-8 through the FADD adaptor, which results in processing of procaspase-8 into the active enzyme. In type-I cells such as thymocytes, caspase-8 directly cleaves caspase-3, which is an effector caspase. In type-II cells such as hepatocytes, caspase-8 cleaves Bid and the truncated Bid stimulates the release of cytochrome *c* from mitochondria. Cytochrome *c*, together with Apaf-1 and ATP, then activates caspase-9, which in turn then activates caspase-3. One of the substrates of caspase-3 is ICAD (inhibitor of caspase-activated DNase (CAD)). Cleavage of ICAD by caspase-3 activates CAD, which causes DNA degradation in nuclei.**

Cross-talk between mitochondrial-dependent and -independent pathways

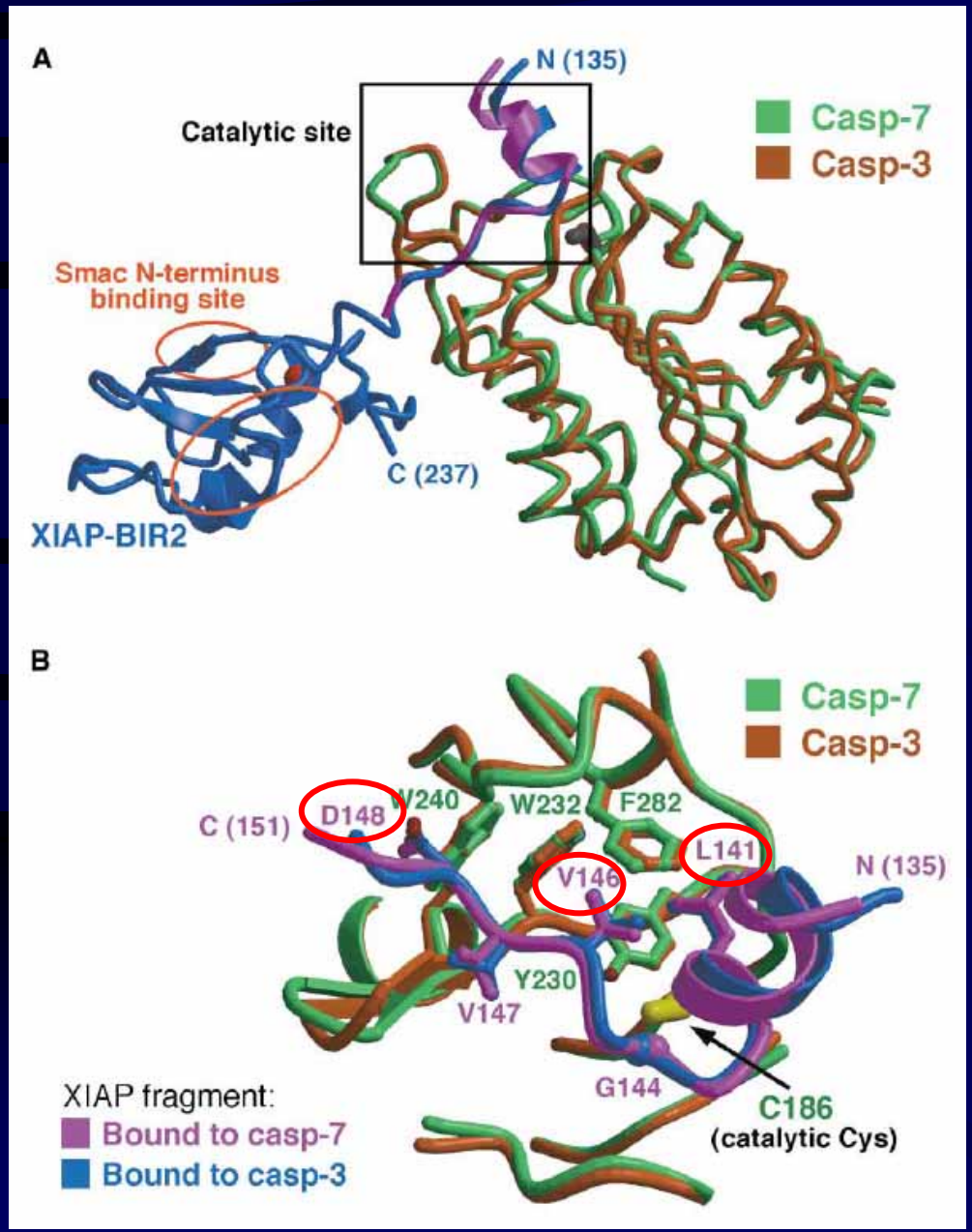
IAP (inhibitor of apoptosis) family



IAP family proteins inhibit both initiator and effector caspases

BIR-2 domain (and its N-terminal sequence) is required for effector caspase inactivation.

BIR-3 domain is required for initiator caspase inactivation



Structural basis of IAP-mediated inhibition of effector caspases

A: IAP-bound caspase-3 or –7 -> interaction of a segment N-terminal to the BIR-2 domain of XIAP to the active site of caspase.

B: Close-up view of the active sites D148 occupies P4 pocket and L141 and V146 interact with hydrophobic pocket of caspase.

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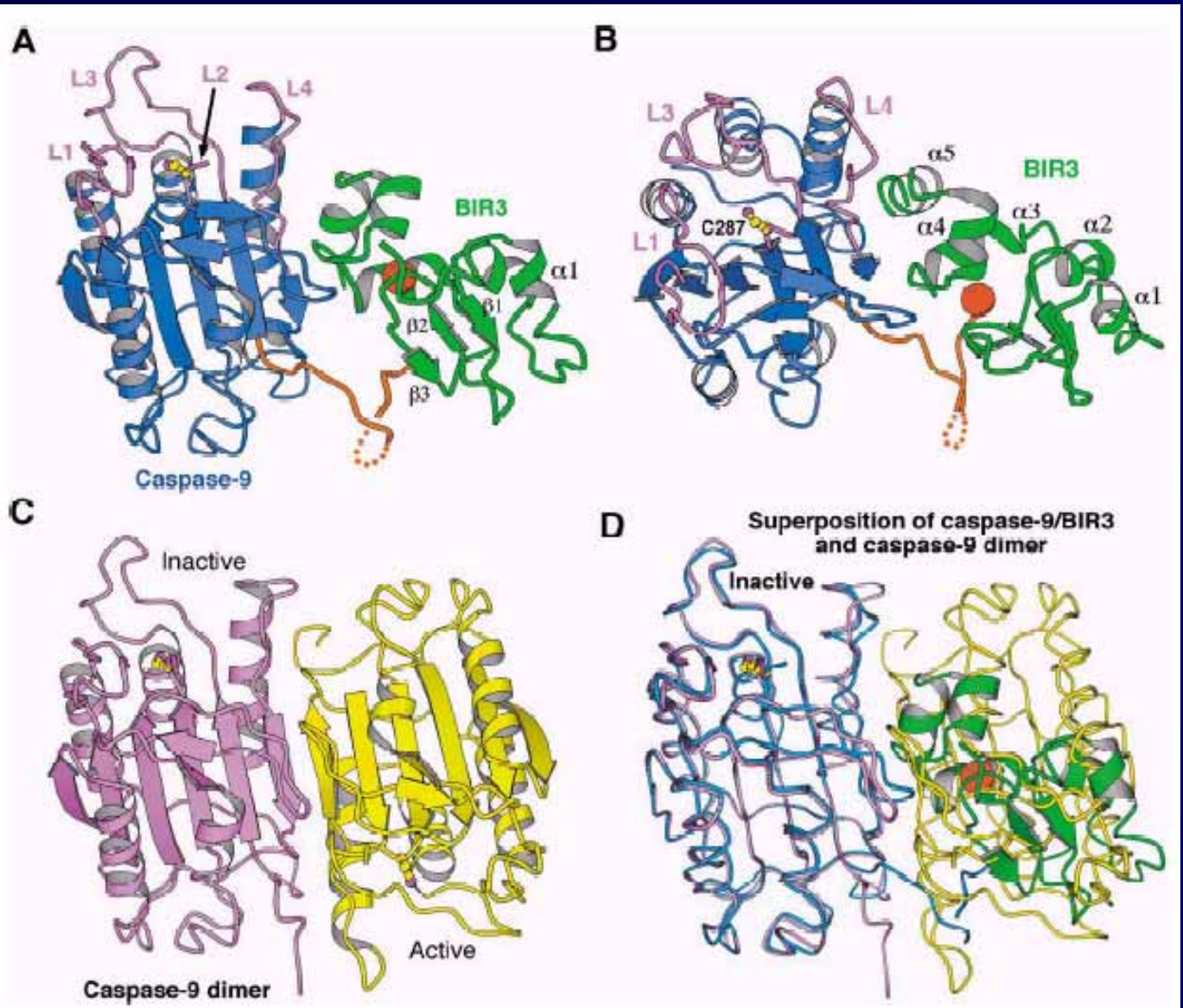
Structural basis of IAP-mediated inhibition of caspase-9



Structural of Caspase-9/BIR3 of XIAP complex

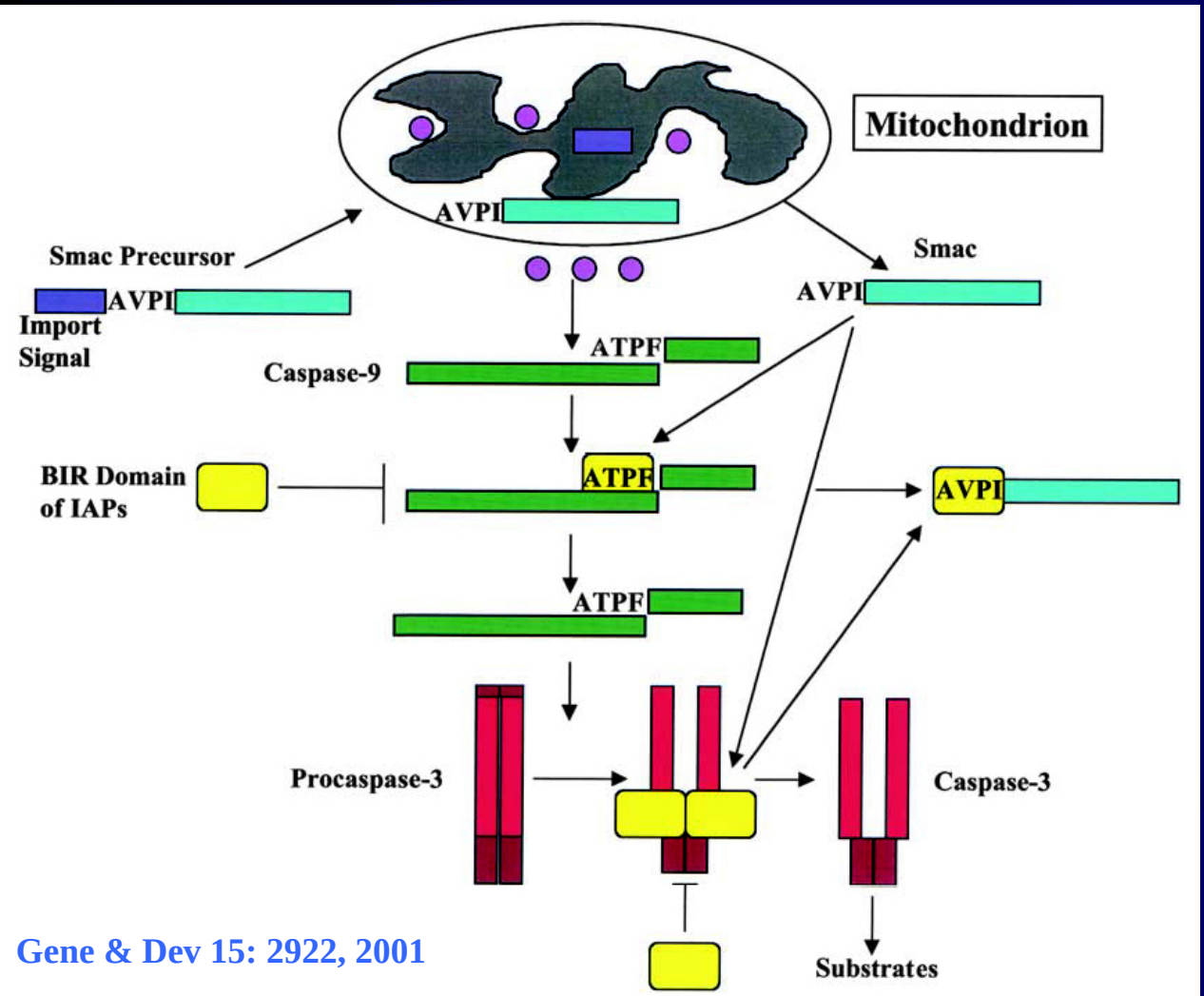
The structure of BIR3 bound caspase-9 closely resembles the inactive half of the caspase-9 dimer.

The surface of caspase-9 that interacts with BIR3 also mediates its homodimerization

XIAP-BIR3 traps caspase-9 in an inactive conformation

Mol Cell 11: 519, 2003

Smac/IAP/Caspase-9: Paper wraps stone blunts scissors



Gene & Dev 15: 2922, 2001

Smac/IAP system: allows tight control of apoptosis

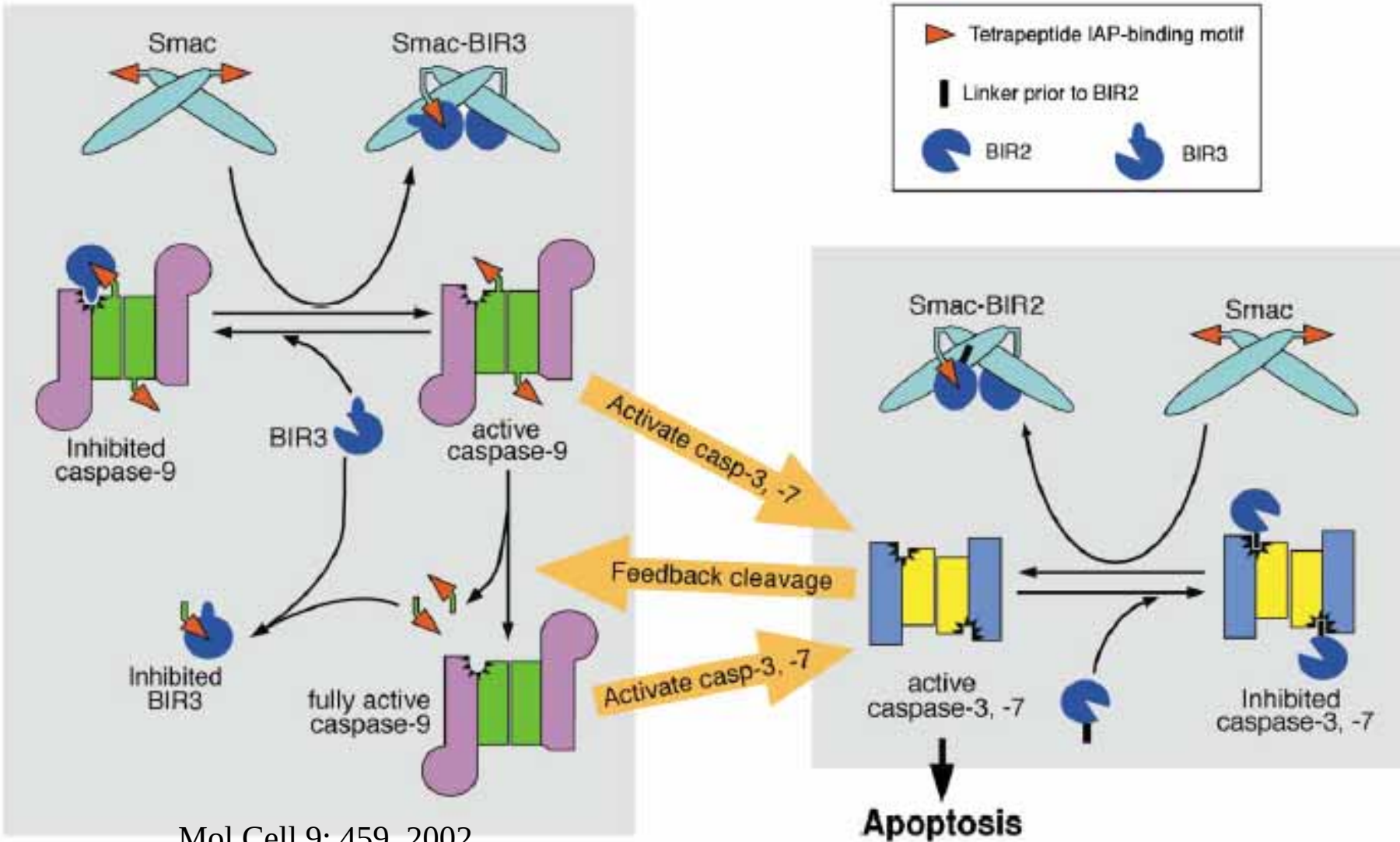
AVPI binds to BIR-2 or BIR-3 domain of IAP and is analogue to ATPF (the IAP binding site in Casp-9)

Smac-IAP binding displaces caspase-9 or -3 binding to IAP

Reaper/Grim/Hid: *Drosophila* apoptotic proteins

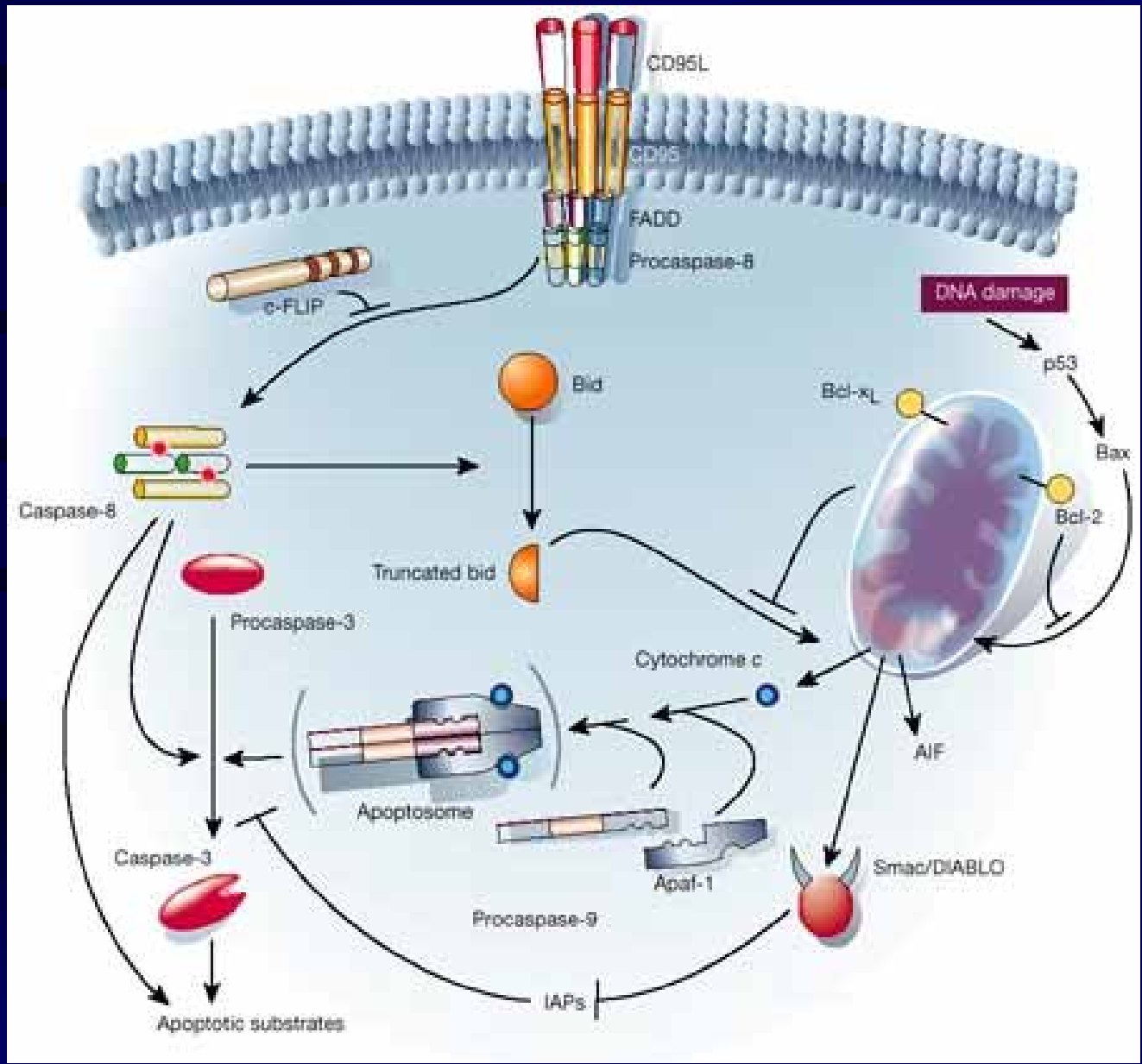
Smac/DIABLO	A	V	P	I	A	Q	K	S
Reaper	A	V	A	F	Y	I	P	D
Grim	A	I	A	Y	F	L	P	D
Hid	A	V	P	F	Y	L	P	E
Sickle	A	I	P	F	F	E	E	E
hCasp-9	A	T	P	F	Q	E	G	L
mCasp-9	A	V	P	Y	Q	E	G	P
xCasp-9	A	T	P	V	F	S	G	E
HtrA2/Omi	A	V	P	S	P	P	P	A

Mechanisms of caspase activation & inhibition



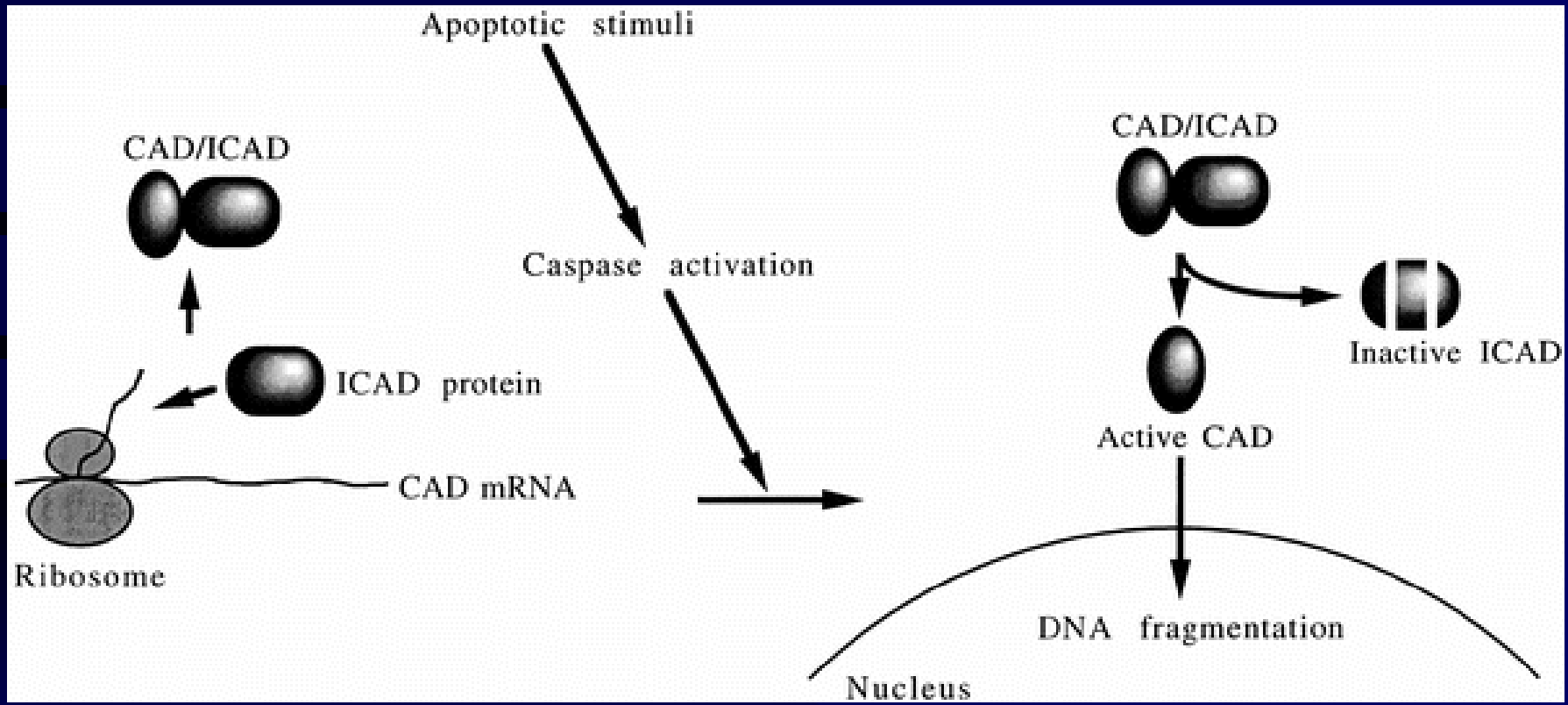
Mol Cell 9: 459, 2002

Summary of apoptotic pathways

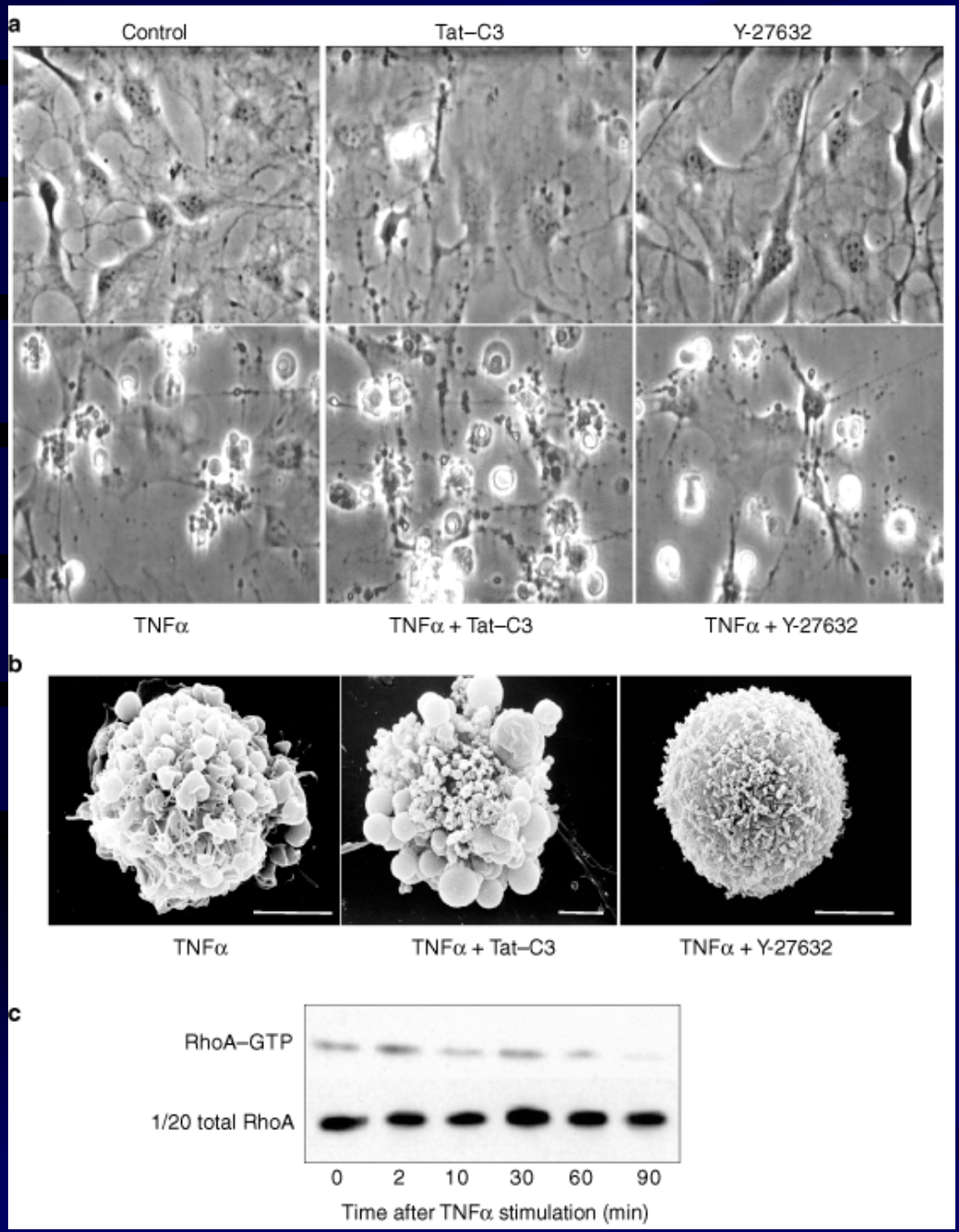


Nature 407;770,2000

Caspase activates CAD：
The Nuclease for DNA Fragmentation



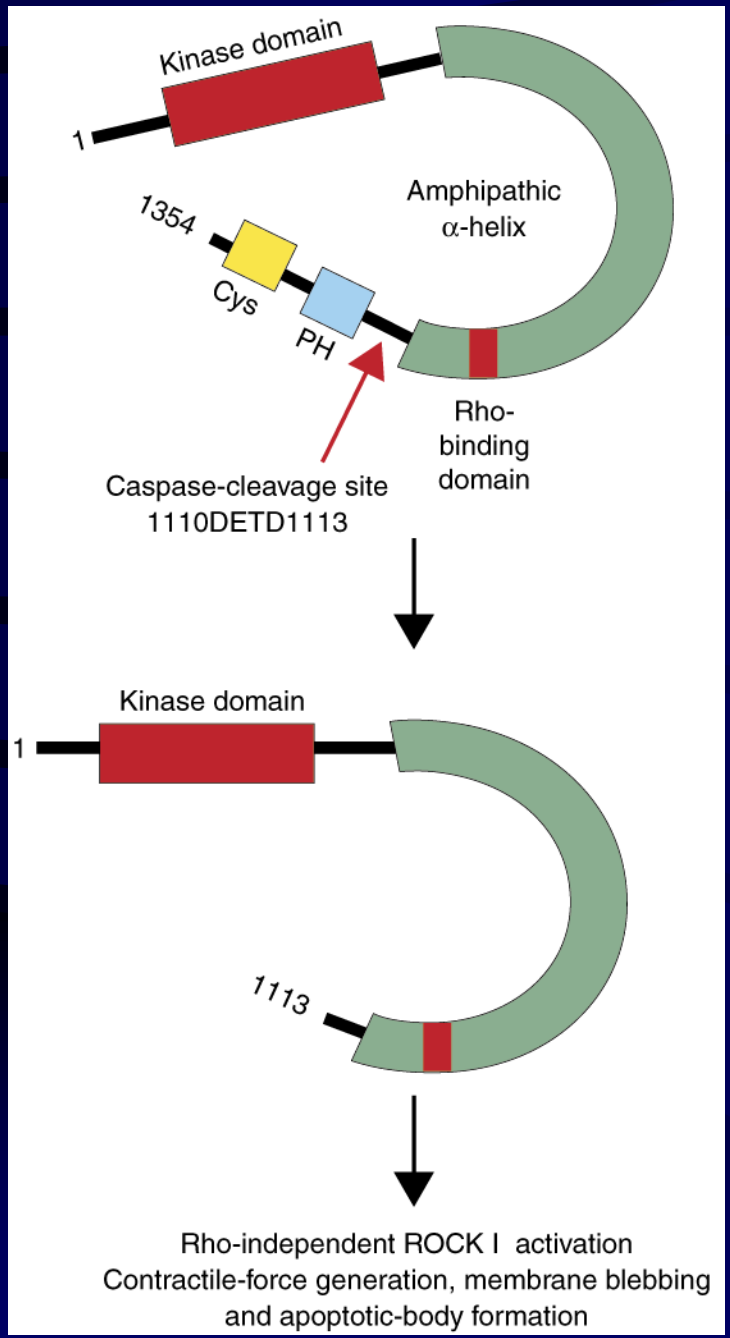
Nature 391;43,1998



Rock II is responsible for membrane blebbing

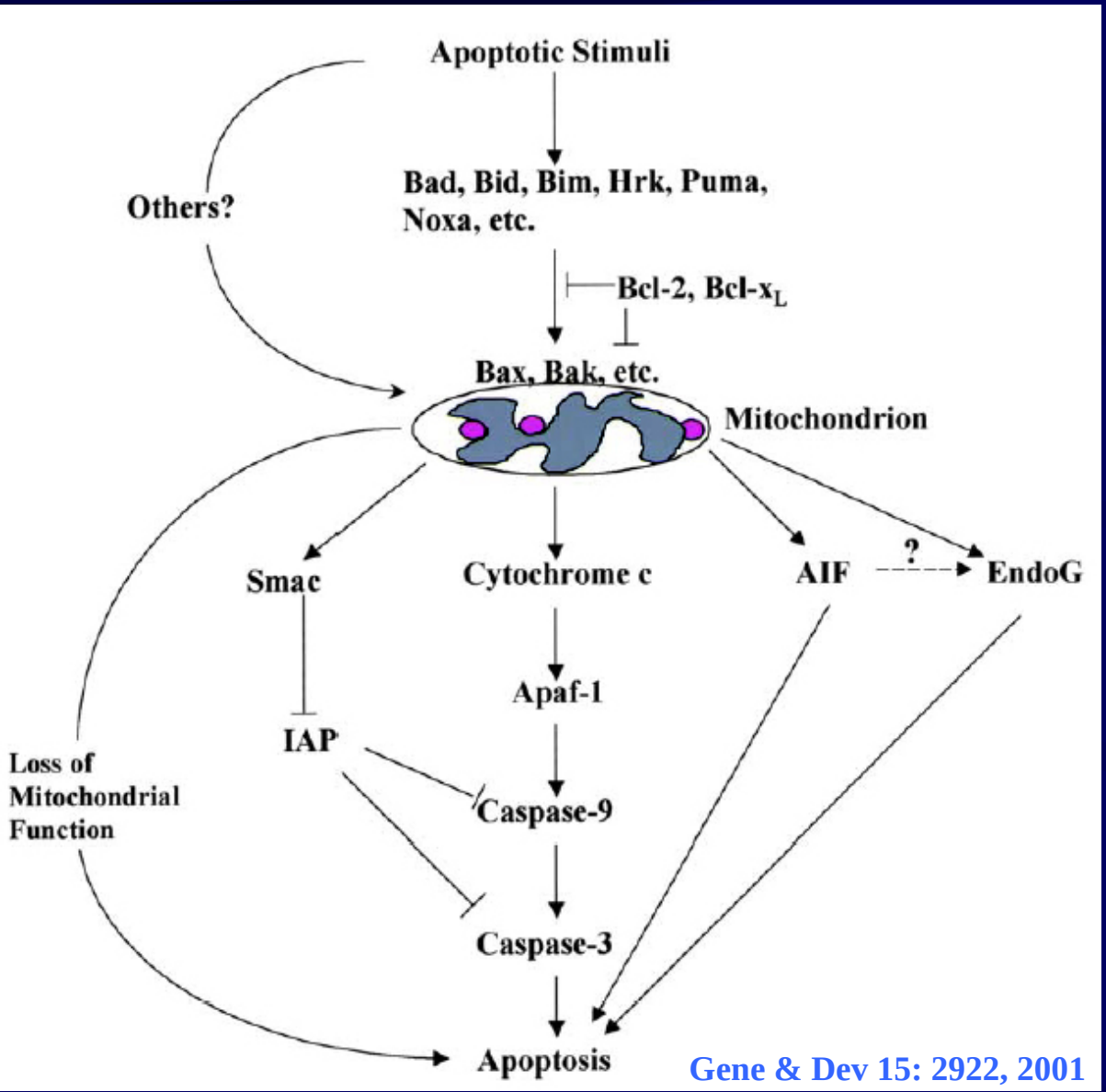
Nature Cell Biology 3;339,2001

Rock II is activated by Caspase



Nature Cell Biology 3;339,2001

Other apoptotic proteins released from mitochondria

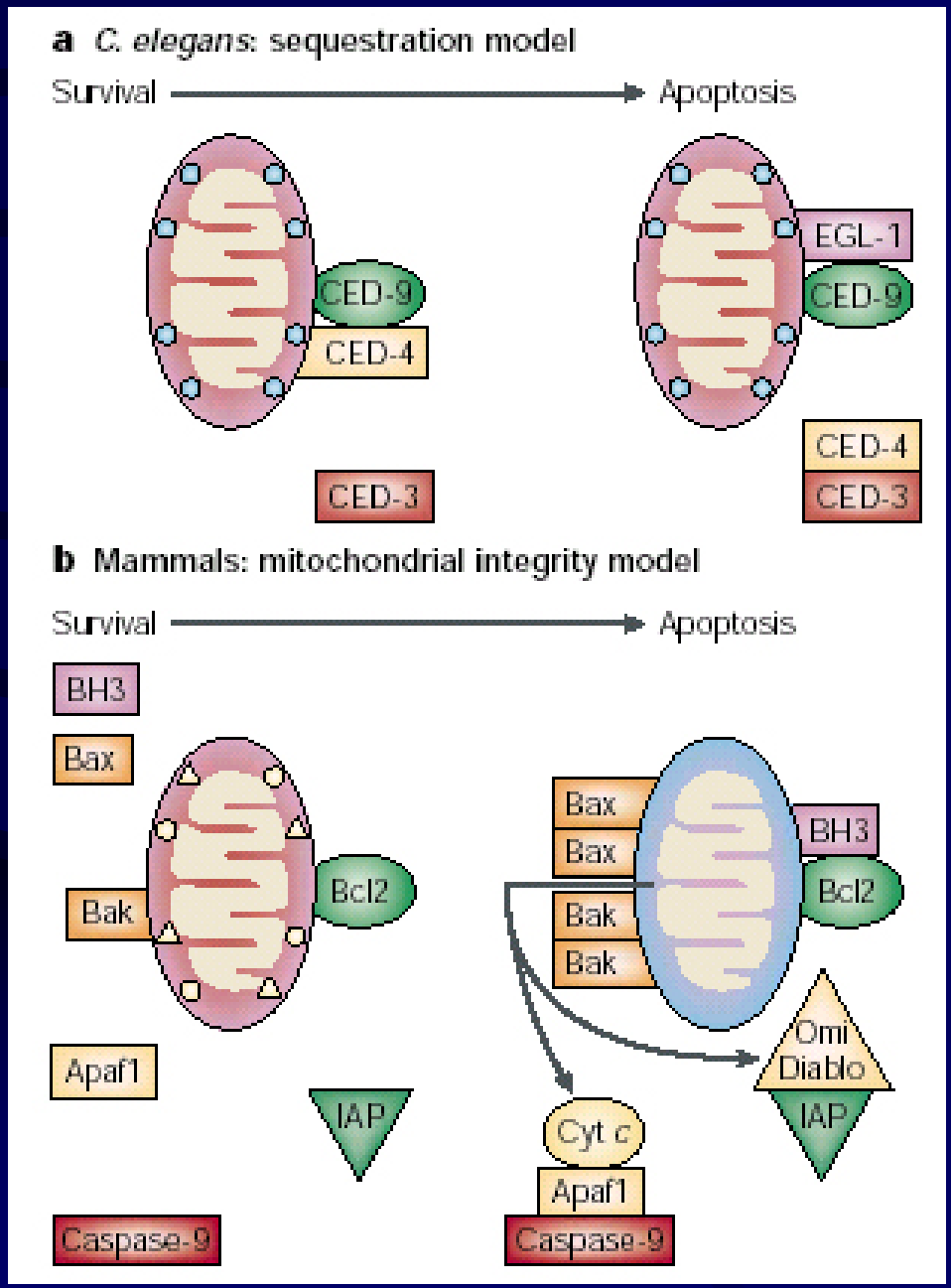


Gene & Dev 15: 2922, 2001

AIF: 1. Flavoprotein with oxidoreductase activity. 2. Normally in the mitochondria. Upon apoptosis, AIF is released and then translocated to nucleus, where it causes chromatin condensation and large-scale DNA fragmentation.

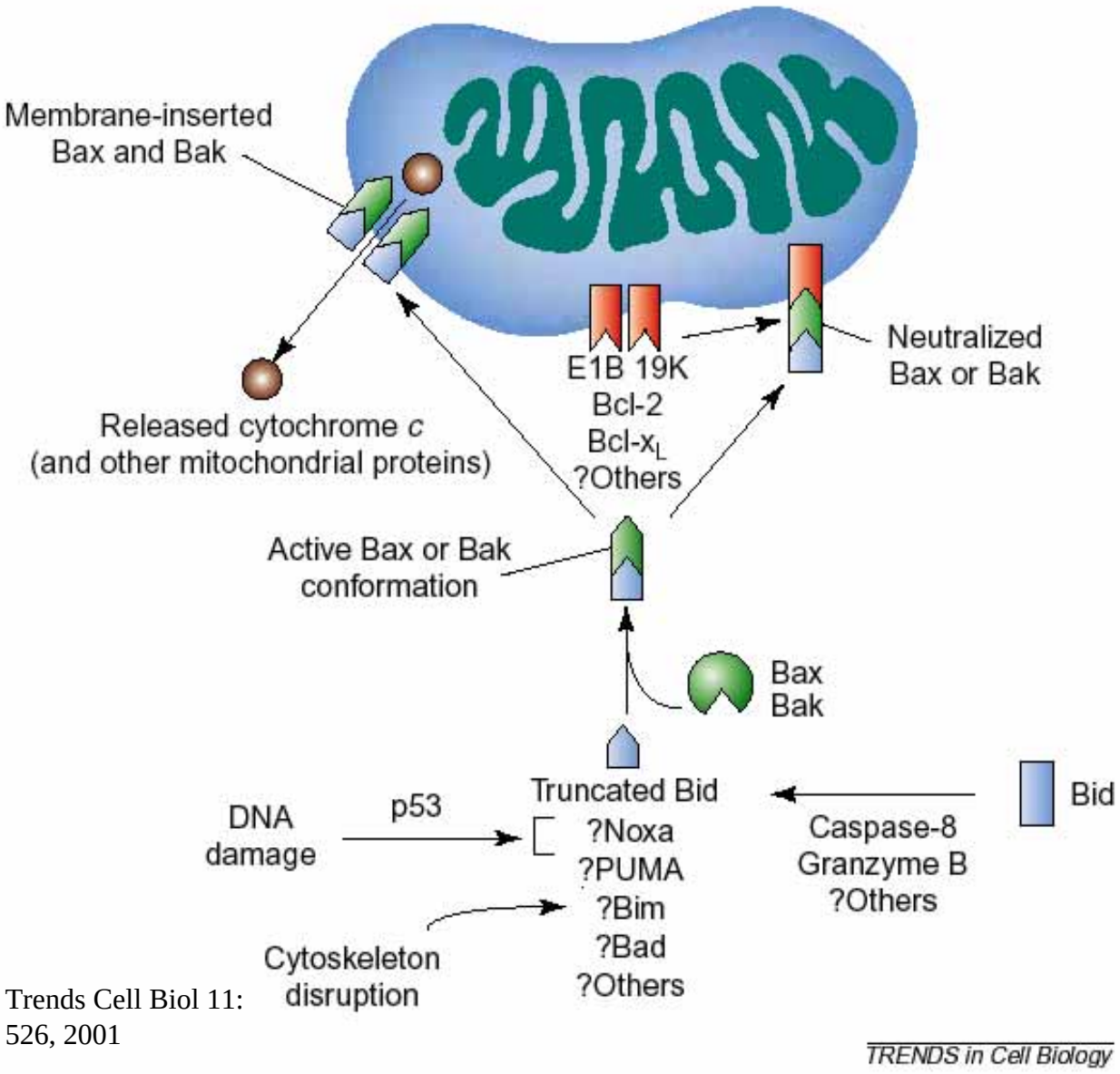
EndoG: 1. mitochondria endonuclease. 2. Released upon apoptosis. 3. EndoG activity is caspase-3 independent

How Bcl-2 family proteins regulate apoptosis



Nat. Rev. Cancer 2: 647, 2002

A model for the role of mammalian Bcl-2 family members in apoptosis



Trends Cell Biol 11: 526, 2001

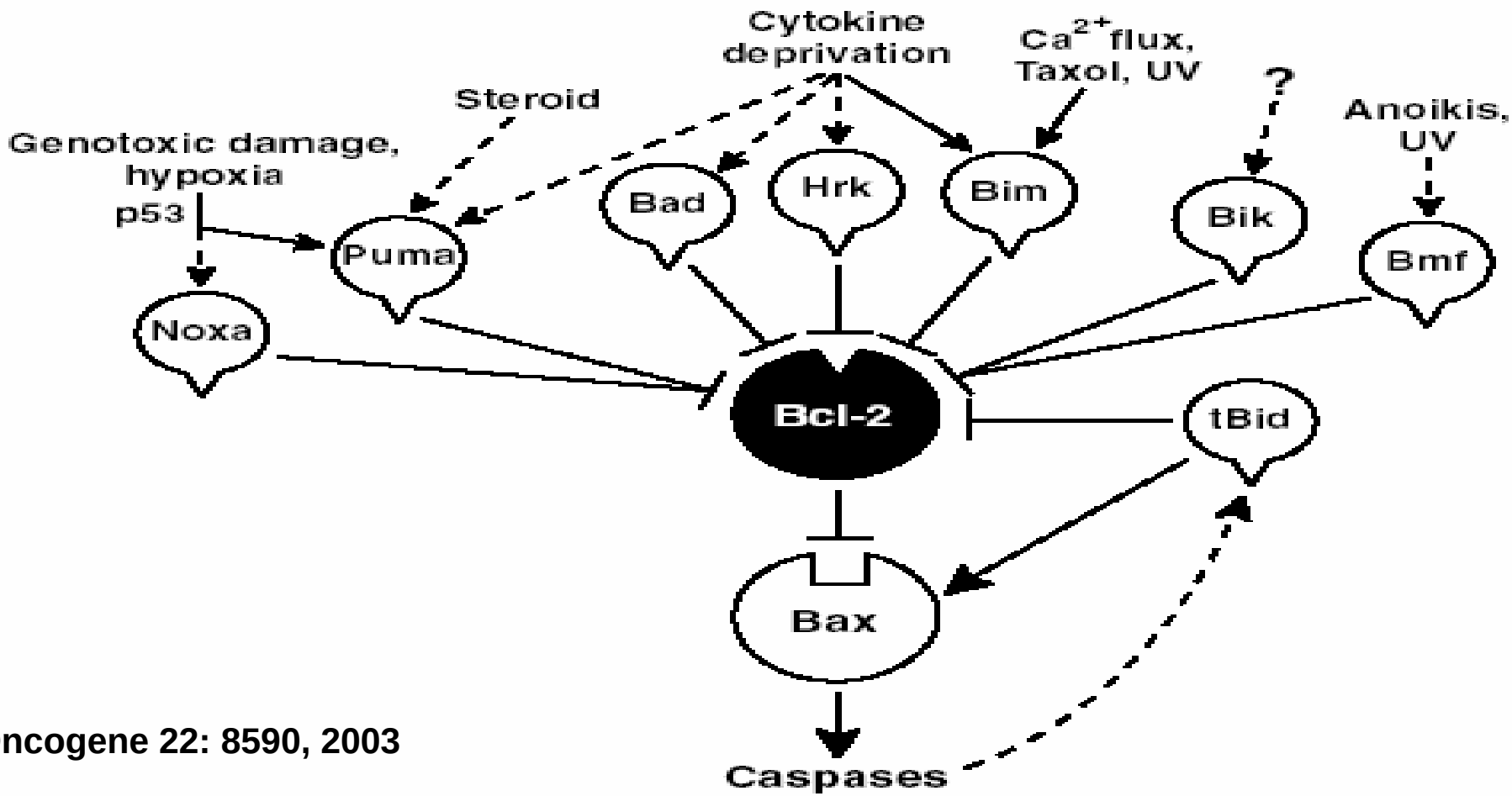
Bax or Bak translocates from cytosol to mitochondria -> oligomerization and insert to mito. membrane.

Bcl-2 or Bcl-xL: heterodimerization with Bax or Bak and neutralize its activity

BH3-only proteins can be activated by various apoptotic signals

Bax or Bak is required for the induction of apoptosis by the BH3-only proteins

Activation of BH3 only proteins



Oncogene 22: 8590, 2003

Activation can be transcriptional (Hrk, Noxa, Puma) or post-translational (Bim, Bmf, Bad, Bik, Bid)

The permeability transition pore

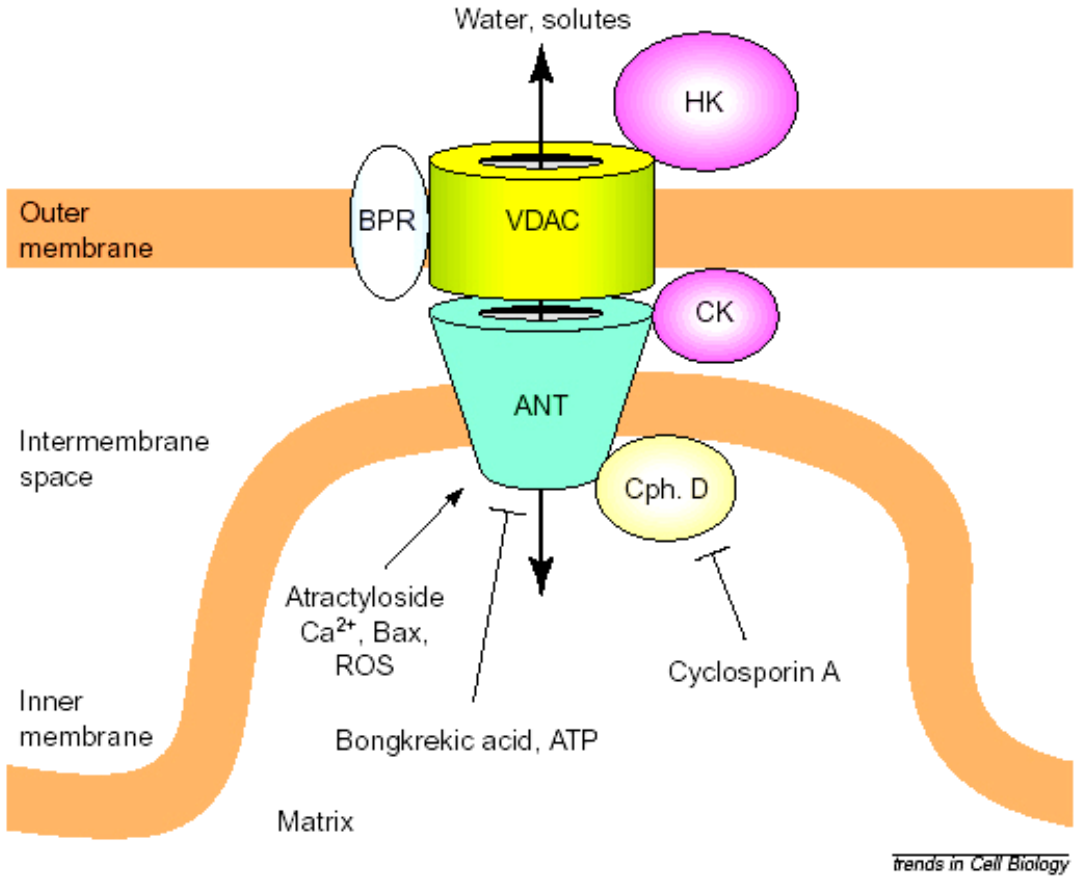
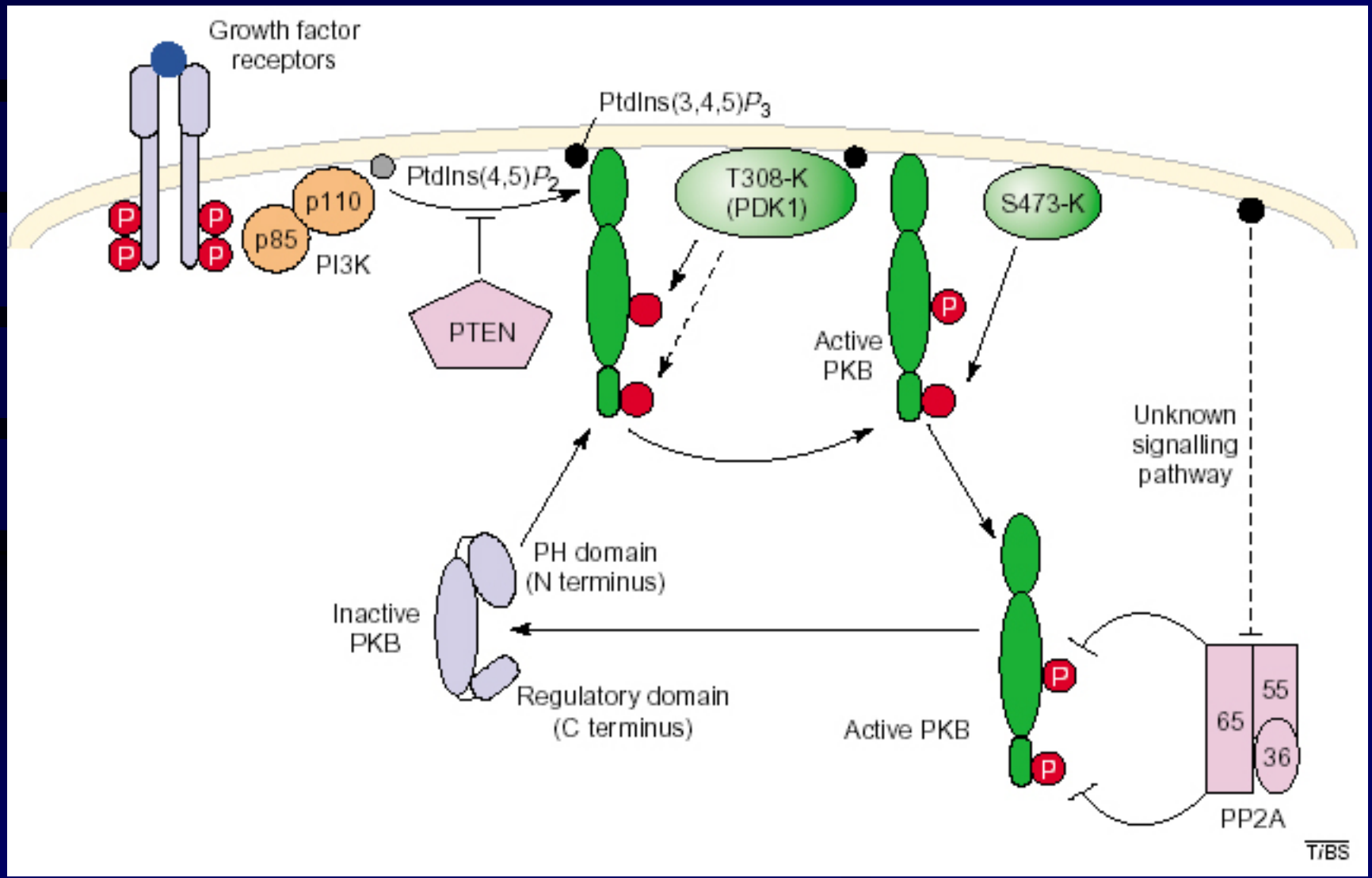


FIGURE 5

The possible components of mitochondrial permeability transition pore (PTP). In the open configuration, water and solutes enter the matrix, causing matrix swelling and rupture of the outer mitochondrial membrane. Abbreviations: ANT, adenine nucleotide translocator; BPR, benzodiazepine peripheral receptor; CK, creatine kinase; HK, hexokinase; VDAC, voltage-dependent anion channel; Cph. D, cyclophilin D. The different inducers [atractyloside, Ca^{2+}, Bax, reactive oxygen species (ROS)] and inhibitors (cyclosporin A, bongkrekic acid, ATP) of PTP opening are represented according to their site of action.

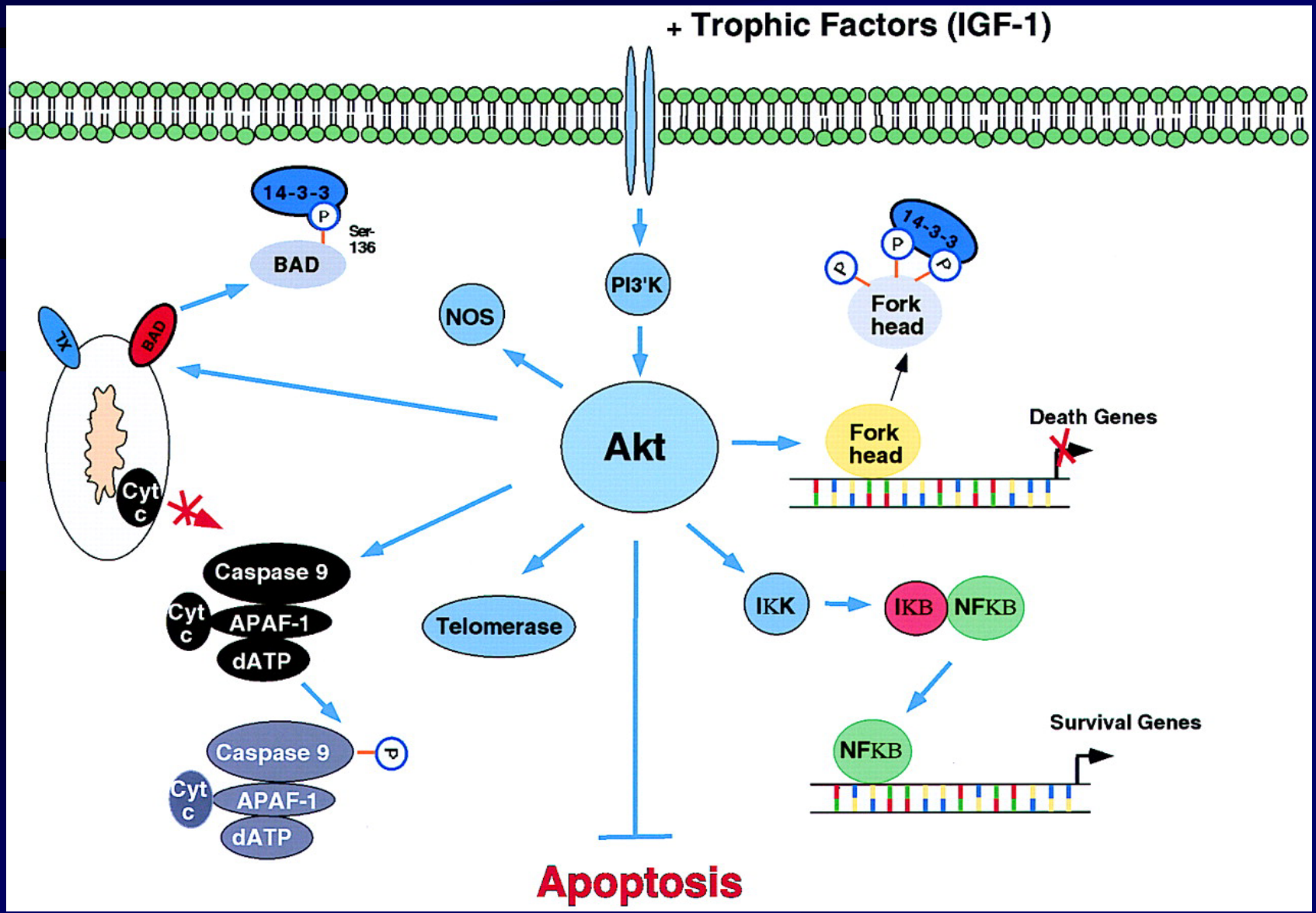
Trends Cell Biol.
10: 369, 2000

Activation of Akt by receptor tyrosine kinase pathway



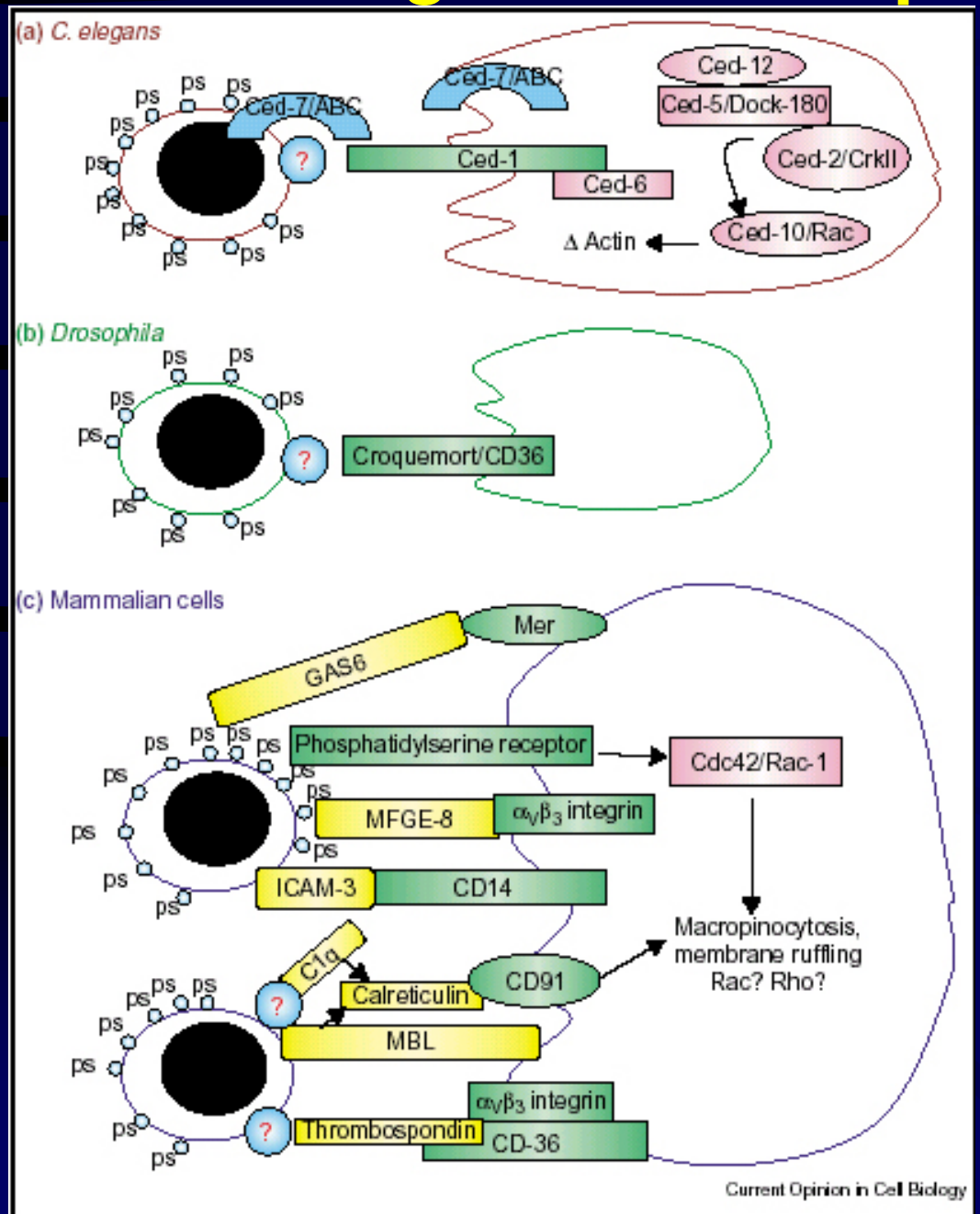
TIBS 26: 657, 2001

Akt-the master of anti-apoptotic protein



Gene & Development 13;2905,1999

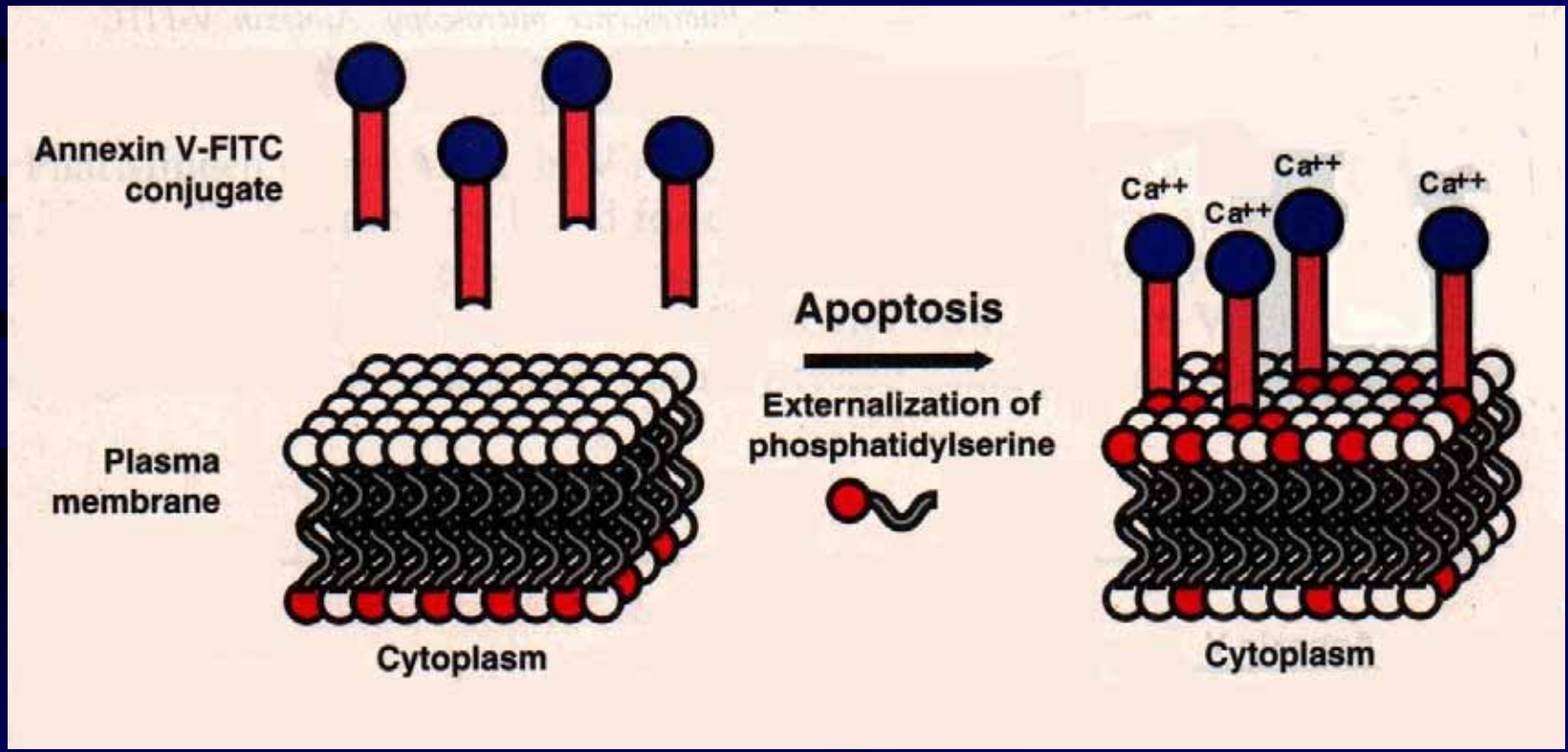
Engulfment of apoptotic cells



1. Surface changes on the apoptotic cells
2. Tethering and/or stimulating receptors
3. Signal transduction for apoptotic cell uptake
4. Digestion of nucleic acids in apoptotic cells (nuc-1 in worms and acid-activated DNase II in mammals)

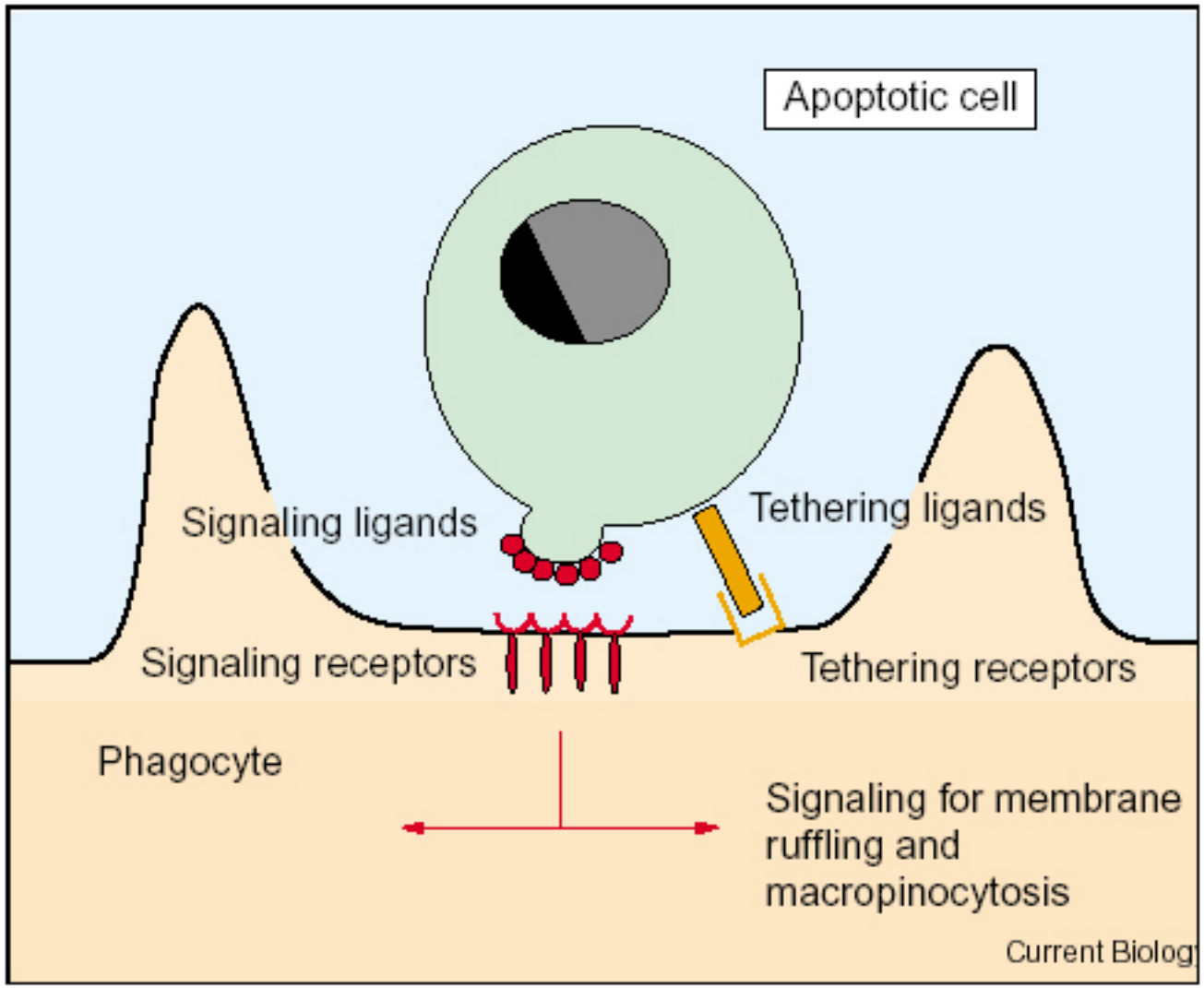
14: 734, 2002

Annexin V binds to PS in the presence of calcium



A phospholipid scramblase may be activated during apoptosis

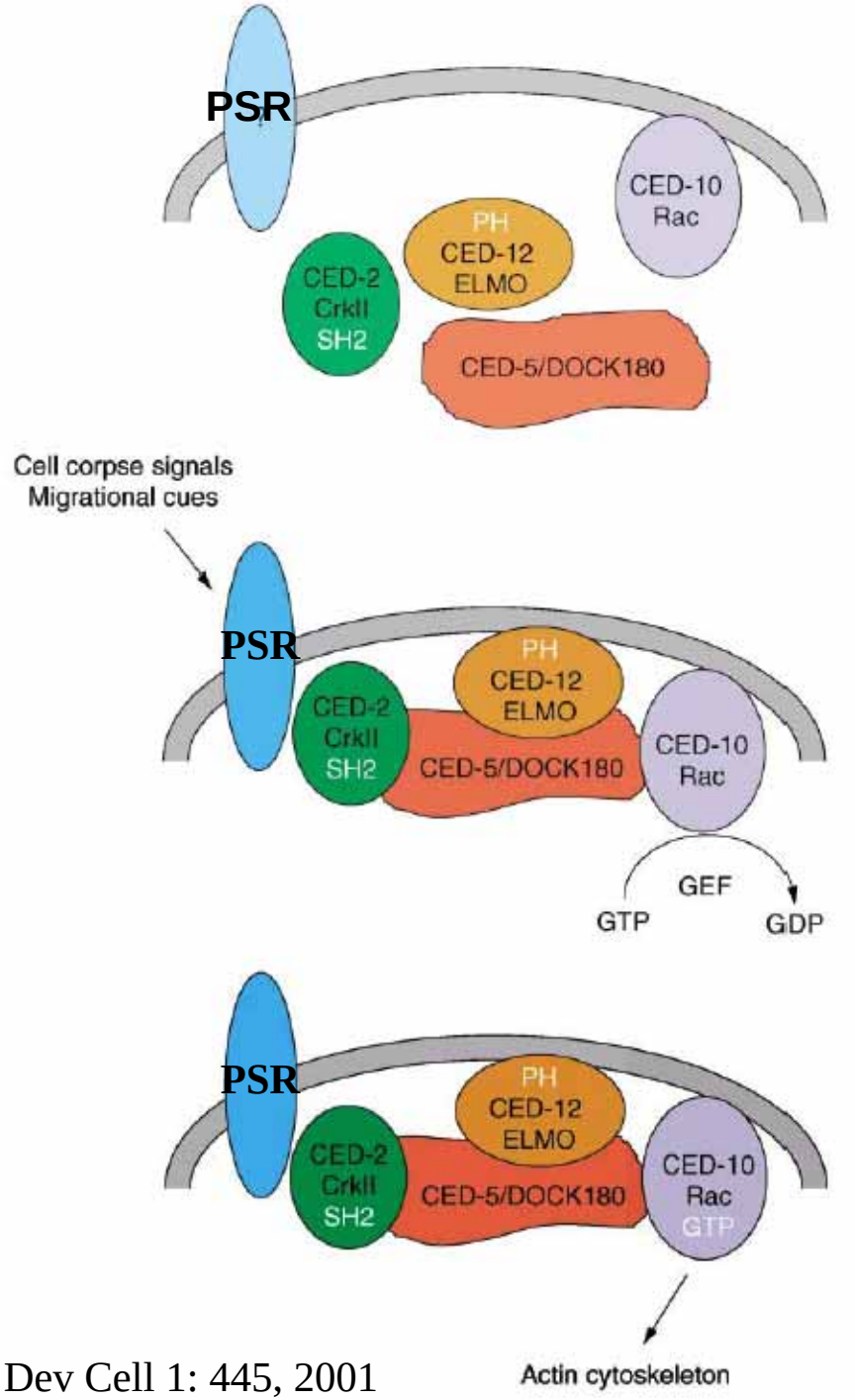
Two component uptake mechanism for apoptotic cells



For example, ligation of some apoptotic cell recognition receptor (CD36 or α_v integrins) induces tethering of cells to macrophages but without uptake. Inclusion of PS on target cell surface initiates uptakes.

However, PS on target cell surface without the tethering ligand is ineffective.

11: R795, 2001



Dev Cell 1: 445, 2001

Signaling pathways for engulfment

Two alternative paths for uptake:

1. CED-1, CED-6 and CED-7
2. CED-2, CED-5, CED-10 and CED-12

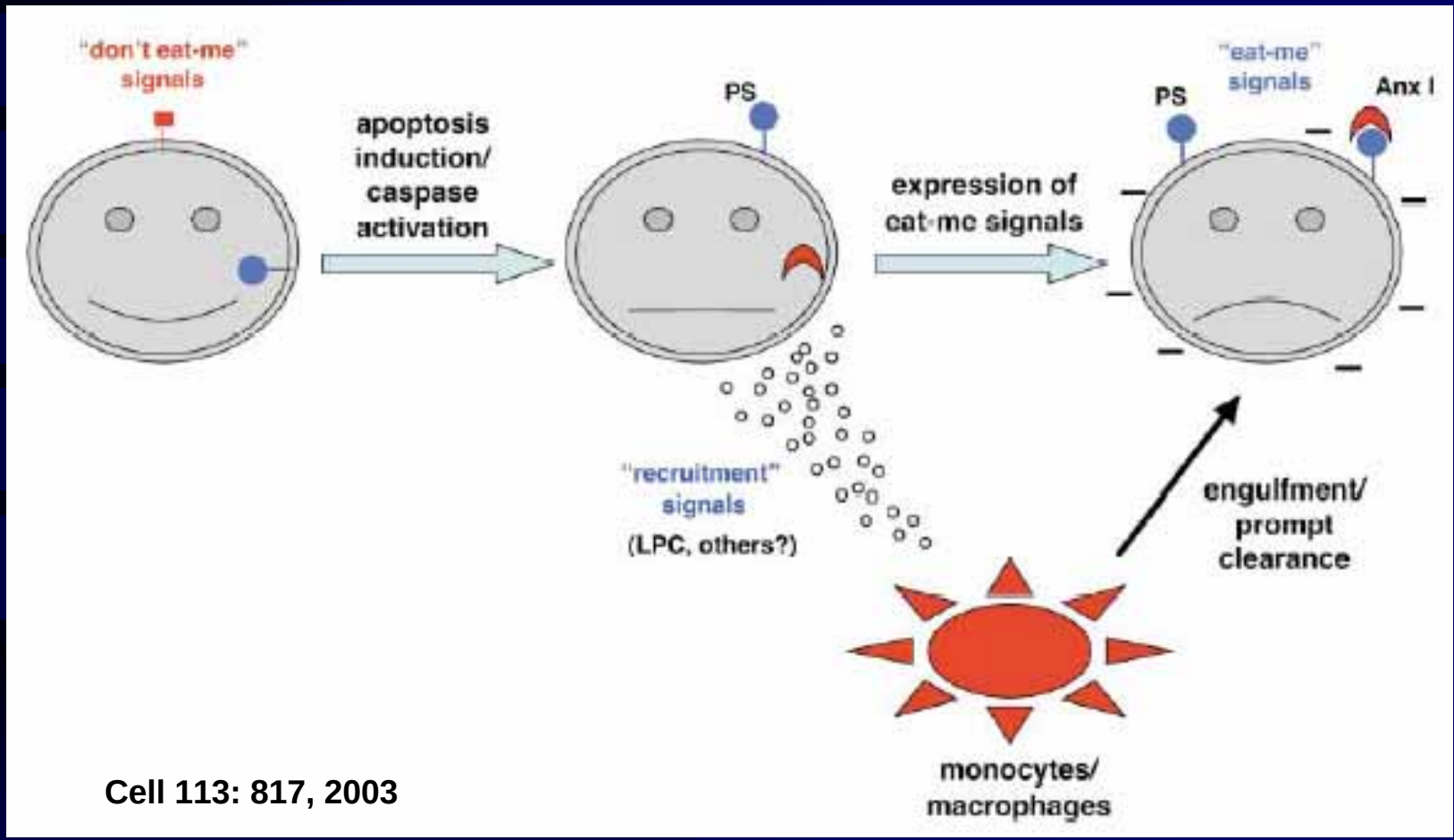
CED-1: transmembrane protein

CED-7: ABC transporter

CED-6: adaptor protein with pY binding motifs. Binds CED-1

CED-2/CED-5/CED-10/CED-12: form a membrane-localized complex in respond to cell corpse signal. CED-10 (Rac-1) activation induces lamellipodia formation and actin assembly.

Soluble factors secreted from apoptotic cells can help the recruitment of phagocytes



Lysophosphatidylcholine (LPC) is released from apoptotic cells to attract monocytes and macrophages.

Calcium-independent phospholipase A2 (iPLA2) is activated by a caspase 3-dependent cleavage, and in turn promotes LPC release.