

# Apoptosis: Death comes for the Cell

Joe W. Ramos  
jramos@crch.hawaii.edu



From Ingmar Bergman's *The Seventh Seal*

# Mutations in proteins that regulate cell proliferation, survival and death can contribute to oncogenesis

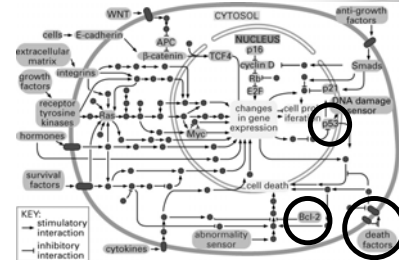


Figure 23-31. Molecular Biology of the Cell, 4th Edition.

Type of cell death	Morphological changes			Biochemical features	Common detection methods
	Nucleus	Cell membrane	Cytoplasm		
Apoptosis	Chromatin condensation; nuclear fragmentation; DNA laddering	Blebbing	Fragmentation (formation of apoptotic bodies)	Caspase-dependent	Electron microscopy; TUNEL staining; annexin staining; caspase activity assays; DNA fragmentation assays; detection of increased number of cells in subG1/G2; detection of changes in mitochondrial membrane potential
Autophagy	Partial chromatin condensation; no DNA laddering	Blebbing	Increased number of autophagic vesicles	Caspase-independent; increased lysosomal activity	Electron microscopy; protein-degradation assays; assays for marker protein translocation to autophagic membranes; MDC staining
Mitotic catastrophe	Multiple micronuclei; nuclear fragmentation	—	—	Caspase-independent (at early stages) abnormal CDK/cyclin B activation	Electron microscopy; assays for mitotic markers (MPF2); TUNEL staining
Necrosis	Clumping and random degradation of nuclear DNA	Swelling; rupture	Increased vacuolation; organelle degeneration; mitochondrial swelling	—	Electron microscopy; nuclear staining (usually negative); detection of inflammation and damage in surrounding tissue
Senesescence	Distinct heterochromatic structure (senescence-associated heterochromatic foci)	—	Fattening and increased granularity	SA- $\beta$ -gal activity	Electron microscopy; SA- $\beta$ -gal staining; growth arrest assays; assays for increased p21, p16, and ARF levels (usually increased); assays for p16 phosphorylation (usually phosphorylated); assays for metalloproteinase activity (usually upregulated)

CDK1, cyclin-dependent kinase 1; MDC, monodansylcadaverine; MPF2, mitotic phosphorylase 2; SA- $\beta$ -gal, senescence-associated  $\beta$ -galactosidase; RE, retinoblastoma protein.

From Okada and Mak, Nat. Rev. Cancer 4:592-603

# Apoptosis: Programmed Cell Death

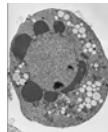


- morphological changes associated with programmed cell death.
- The term was originally used by Wyllie and his colleagues and is from the Greek meaning "dropping away" as the leaves from a tree.



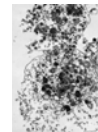
# Apoptosis

- Active cell death
- Requires energy and RNA and protein synthesis
- Characteristic morphological features
- DNA cleaved, chromatin condenses
- Cells shrink
- Formation of apoptotic body
- Cleared by phagocytosis
- No inflammation=no tissue damage



# Necrosis

- Passive cell death
- Cells swell up
- Membrane breaks down and cellular contents leak out
- Nucleus disintegrates
- Cell ghosts
- Inflammatory=tissue damage





## Detection of apoptotic cells

- **Microscopy**

- Cells have classic features (eg. small darkly stained nuclei)
- Detection of free 3' ends of DNA by TUNEL assay (terminal deoxytransferase-mediated dUTP-biotin nick end labeling)



- **Gel electrophoresis**

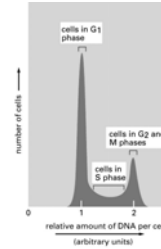
- Detect DNA ladder of 180 bp intervals caused by internucleosomal DNA cleavage



- **Flow cytometry**

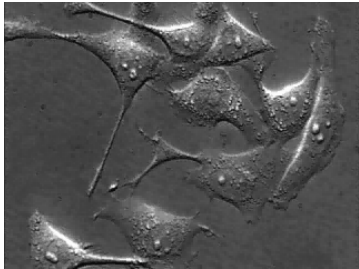
- Measure externalization of phosphatidylserine (PS) with fluorescently labeled Annexin-V
- Measure DNA fragmentation with propidium iodide fluorescence

## Analysis of DNA content with a flow cytometer



Recall the fluorescence intensity of the DNA dye (amount of DNA) is measured for each cell.

Figure 17-12, Molecular Biology of the Cell, 4th Edition.



## Triggers of apoptosis

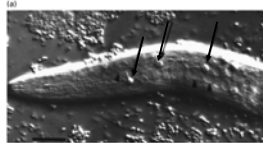


- Programmed cell death in which many more cells are produced than survive (e.g. development of lymphocytes)
- Toxic stimuli (viruses, chemicals, ionizing radiation)
- Extracellular signals (Fas, p75 NGF-R, TNF)
- DNA damage (p53)

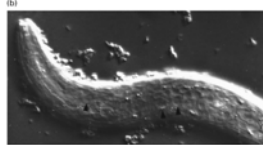
## *C. elegans* has played a key role in our understanding of Apoptosis

1090 total cells  
131 die

**Ced-3=no death**  
**Ced-4=no death**  
**Ced-9=all die**



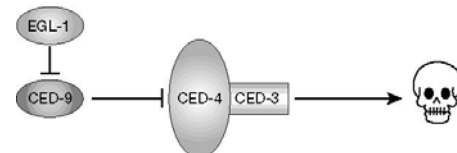
*ced-1* mutant  
(No engulfment)



*ced-1/ced-3*  
(No cells die)

H.R Horvitz and colleagues responsible for much of this work, 2002 Nobel Prize in Medicine with Sulston and Brenner.

## *C. elegans* apoptosis



*CED-9*=Blocks apoptosis  
*CED-4*=linker molecule forms activating complex with *CED-3*  
*CED-3*=Protease that executes cell by chewing up proteins  
*EGL-1*=Proapoptotic by blocking *CED-9* function

Three classes of proteins function in the apoptotic pathway-conserved in vertebrates

	Regulator	Adapter	Effector		
<i>C. elegans</i>	Ced-9	→ Ced-4	→ Ced-3	→ Death	
Vertebrates	Bcl-2	→ Apaf-1	→ Casp9	→ Casp3	→ Death

Mammalian Bcl-2 can substitute for Ced-9 in *c. elegans*



## Death's Methods: A protease cascade

These proteases are called *caspases*

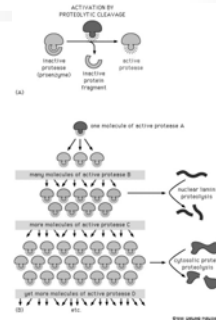


Fig 18-22

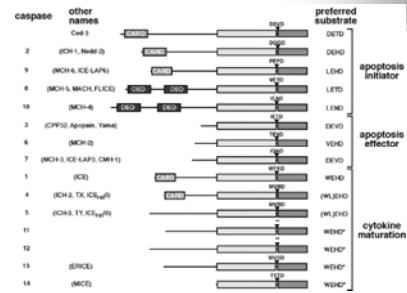


## Caspases

- Caspases are Cysteine directed proteases that cleave after ASpartate residues
- Ced-3 is the *C. elegans* homologue
- At least 14 family members
- Synthesized as proenzymes with low levels of caspase activity (~1-2 % of active form)
- Activated upon after aggregation or cleavage to mature form
  - Caspases -8 and -9 are "initiator" caspases
  - Caspases -3 is the "effector" caspase
  - Caspase activation requires a stimulus
  - They proteolyze cellular proteins to carry out cell death program



## The Caspase Family



## Procaspase activation

(A) procaspase activation

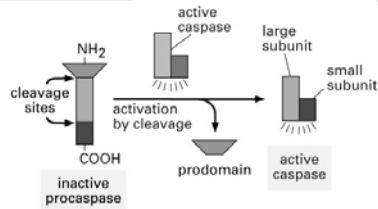


Figure 17-38 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

## Caspase cascade

(B) caspase cascade

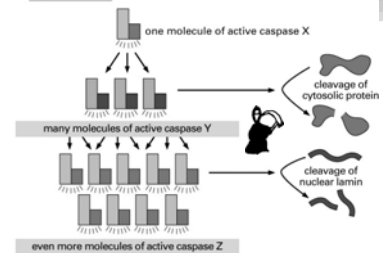
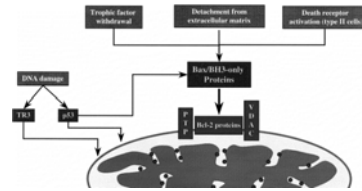


Figure 17-38 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

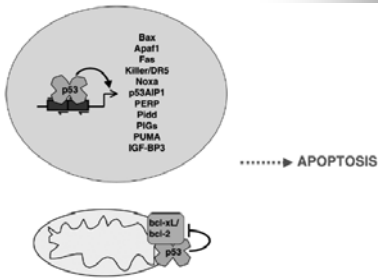
## Two Pathways that Initiate Apoptosis

- Intrinsic/ Mitochondrial Apoptosis
  - Regulated by Mitochondria
  - Cytochrome c release
- Extrinsic/ Death Receptor Apoptosis
  - Activated by ligation of Death Receptors
  - Fas, TNF alpha
- *These pathways intersect at the effector caspases*

## Activation of the Intrinsic Pathway

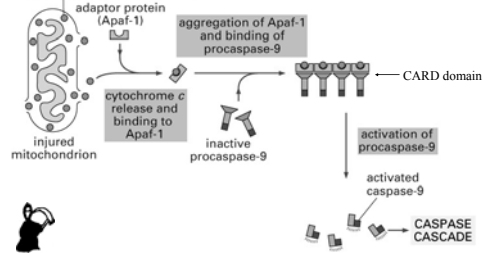


## Two mechanisms for p53 activation of apoptosis

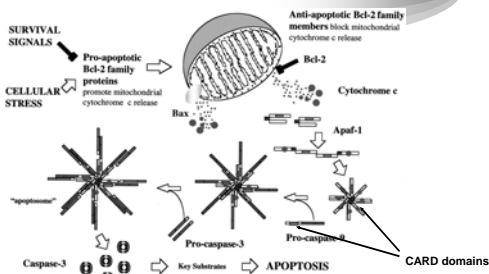


## Intrinsic/Mitochondrial Pathway

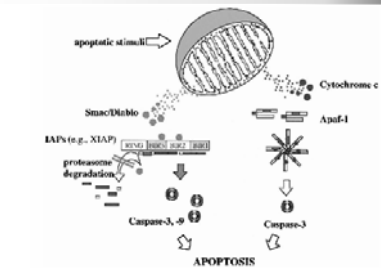
(B) ACTIVATION OF APOPTOSIS FROM INSIDE THE CELL (INTRINSIC PATHWAY)  
cytochrome c (in intermembrane space)



## Intrinsic Pathway: Apaf-1 Induced Apoptosis



## Smac/Diablo and IAPs

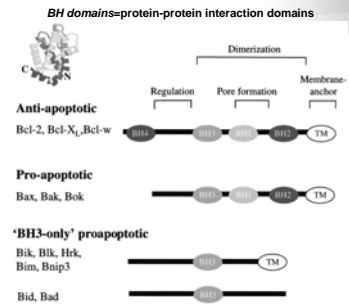


Smac=Second mitochondrial activator of caspases  
IAP=Inhibitor of Apoptosis Proteins

## Bcl-2 family members

- A very large family with 19 members identified
- Bcl-2 (homologous to ced-9) is prototype
- All have the BH3 domain (Bcl-2 Homology)
  - BH-3 is the pro-apoptotic domain exposed on activation
- Act as dimers—either hetero or homodimers
  - Pro-apoptotic dimers (Bax) increase mitochondrial permeability
  - Anti-apoptotic members (Bcl-2, Bcl-XL) form dimers with pro-apoptotic members to inactivate them

## The Bcl-2 Family



## Some trophic factors prevent apoptosis by inducing inactivation of a pro-apoptotic regulator

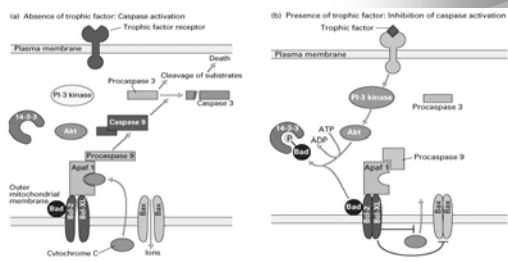
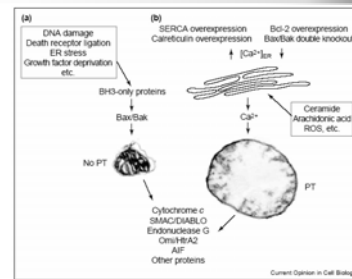


Figure 23-50

## Mitochondrial permeability



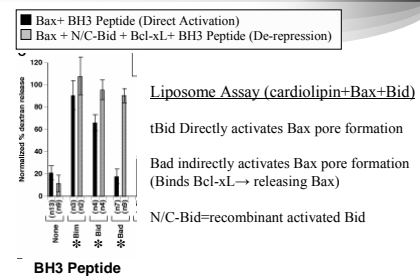
PT=Permeability transition, bursts outer membrane

Cell, Vol 111, 331-342, 1 November 2002

## Bid, Bax, and Lipids Cooperate to Form Supramolecular Openings in the Outer Mitochondrial Membrane

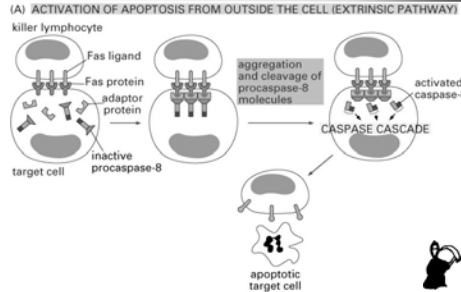
Tomomi Kuwana <sup>1</sup>, Mason R. Mackey <sup>2</sup>, Guy Perkins <sup>2</sup>, Mark H. Ellisman <sup>2</sup>, Martin Latterich <sup>3</sup>, Roger Schneider <sup>4</sup>, Douglas R. Green <sup>1</sup>, and Donald D. Newmeyer <sup>1</sup>

## Bid and Bad have distinct functions to activate apoptosis

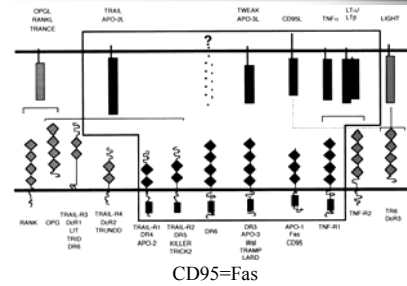


Kuwana et al., Molecular Cell, 17, 525-535, 2005

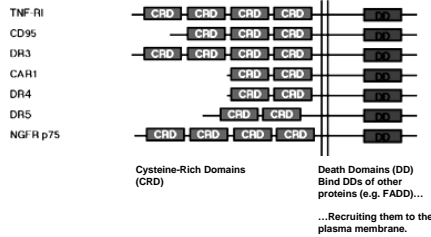
## Extrinsic/Death Receptor Pathway



## Death Receptors and Ligands



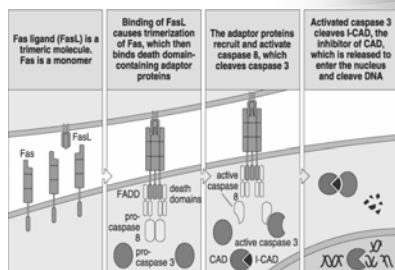
## TNF receptor family



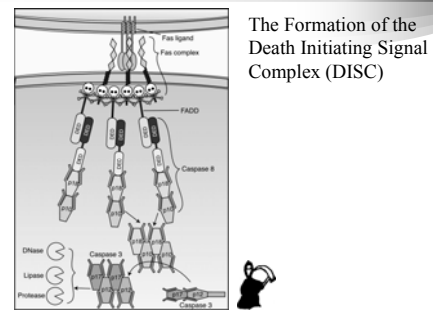
## Fas-FasL Apoptosis

- In response to antigenic stimulation, peripheral T cells expand
- The antigen specific T cells generated must be eliminated (except for the memory cells)
- Upon repeated antigenic stimulation via the T Cell receptor: T cells upregulate Fas and FasL
- Eliminate neighboring T Cells expressing Fas

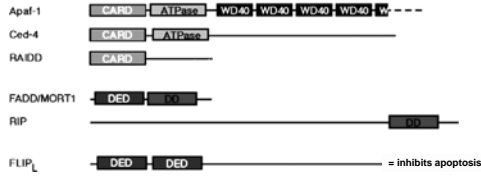
## Activation of Apoptosis by Fas Ligand



## Fas Induced Apoptosis

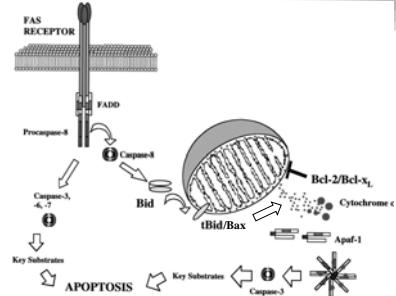


## Adaptor Proteins contain conserved protein interaction domains



-CARD domain of Apaf-1 binds CARD domain of procaspase-9.  
 -DED domain of FADD binds DED domain of procaspase-8.  
 -DED domains of FLIP can bind to the DED domain of FADD and block procaspase-8 recruitment.

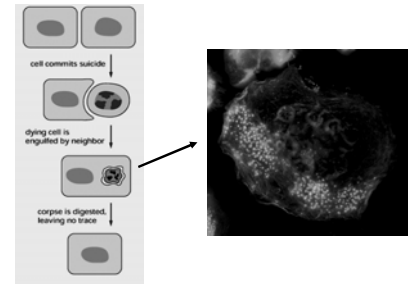
## Fas and the intrinsic pathway: Bid



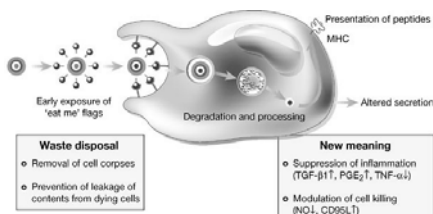
## Proteolytic targets of effector caspases

- Cytoskeletal regulatory proteins
  - Actin
- Nuclear Lamins
- Poly(ADP-ribose) polymerase (PARP)
  - PARP activity depletes ATP, thus cleavage of PARP may maintain store of ATP to drive apoptosis
- DNA-fragmentation factor (DFF)

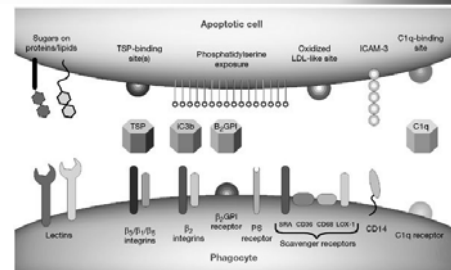
## Removal of apoptotic cell by phagocytosis



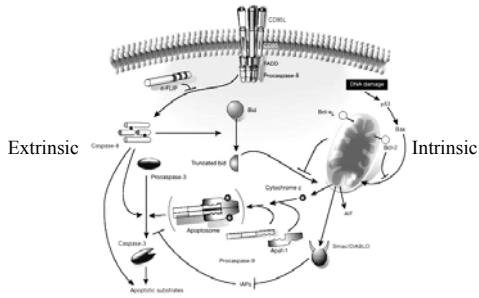
## Removal of cell corpses



## Phagocytosis tags and receptors



## Two roads to activate apoptosis



## TNF $\alpha$ receptors also signal to NF $\kappa$ B

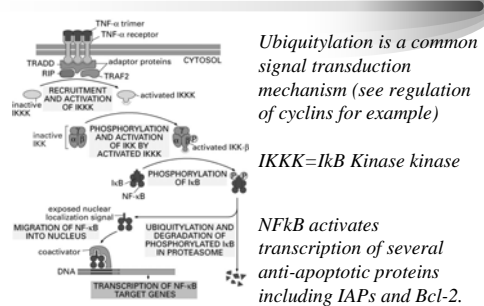
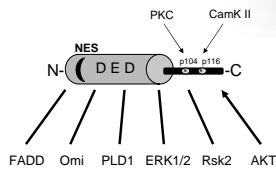


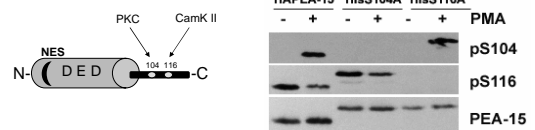
Figure 19-74. Molecular Biology of the Cell, 4th Edition.

## PEA-15 Structure and Binding Partners

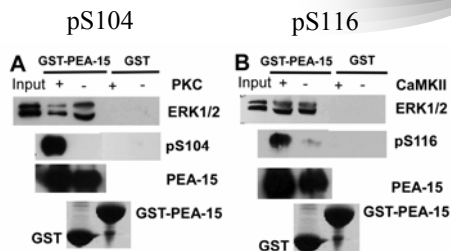


- 15-kDa protein containing 130 amino acids
- N-terminus consists of a Death Effector Domain and NES
- Regulated at Ser104 and Ser116 by phosphorylation

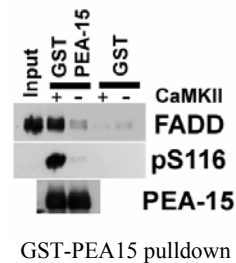
## Characterization of phospho-epitope antibodies

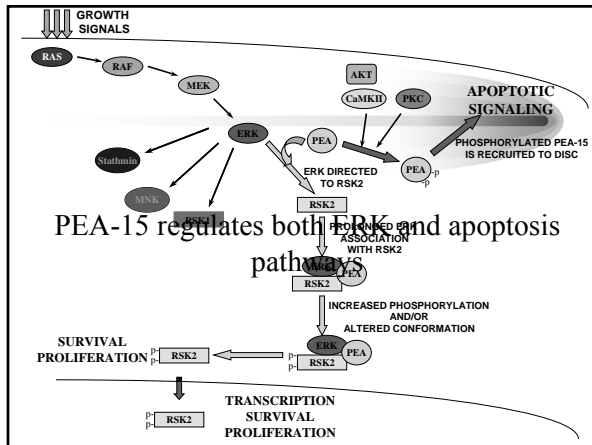
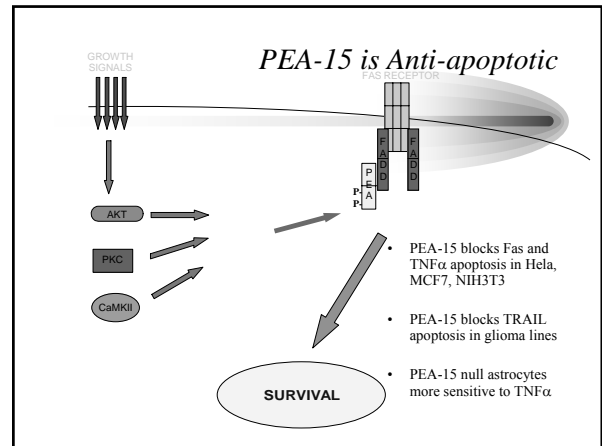
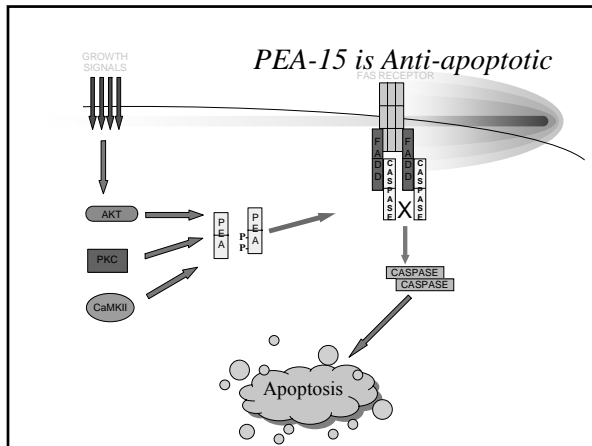


## Effect of PEA-15 phosphorylation on its binding to ERK



## pS116 PEA-15 binds FADD





*Example Question*

- Compare the formation of the Death Initiation Signaling Complex (DISC) of the extrinsic pathway to the formation of the apoptosome of the intrinsic pathway. Drawings could help.
  - What signal initiates the formation of each (an aggregation step)?
  - Where are the complexes formed in the cell?
  - What adaptor proteins mediate the formation of each complex?
  - What are the initiator and effector caspases for each?
  - How are the caspases activated? What do they do?