# #19 Apoptosis Chapter 9

Neelu Yadav PhD
Neelu.Yadav@Roswellpark.org

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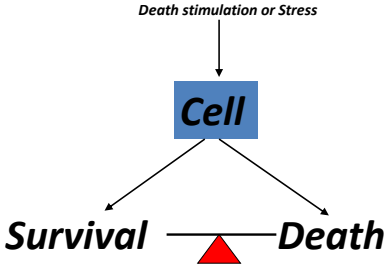
**Why cells decide to die?**

- Stress, harmful, not needed
- Completed its life span

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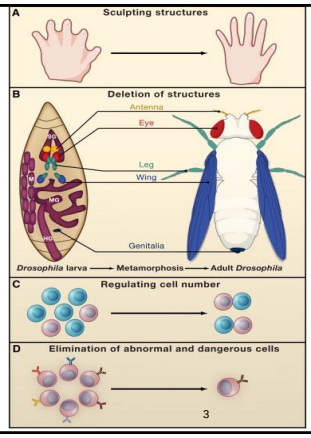
**Functions of PCD during Development**

Involution of the mammary gland after lactation




Fuchs and Steller.
Cell Volume 147, Issue 4 2011 742 - 758

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## Types of Cell Death

- Cell Death
  - Controlled cell death
    - Physiological cell death or Programmed cell death
      - Autophagic cell death (Type II)
      - Apoptotic cell death (Type I)
        - Caspase-independent
        - Caspase-dependent
          - Extrinsic pathway (Death receptor-mediated)
          - Intrinsic pathway
  - Uncontrolled cell death
    - Necrotic cell death **(?)** (Type III)

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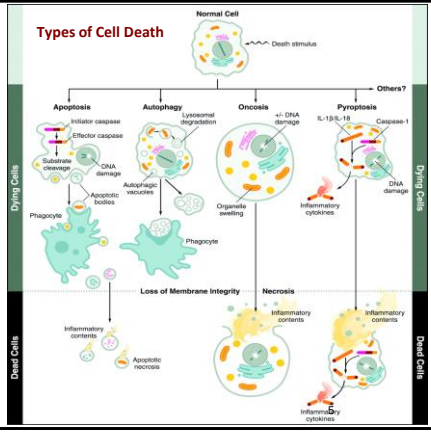
## Types of Cell Death




Fink and Cookson, Infect Immun. 2005

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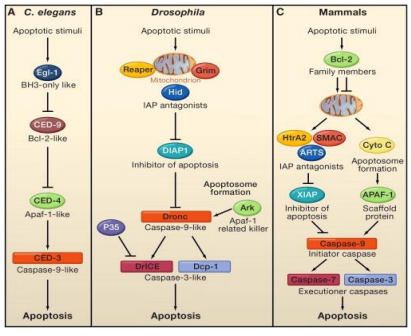
## Evolutionary Conservation of the Core Apoptotic Machinery




Yaron Fuchs , Hermann Steller
Cell Volume 147, Issue 4 2011 742 - 758

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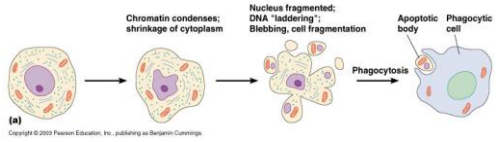
## What is the difference between key cell death mechanisms

| Apoptosis | Autophagy | Necrosis |
|---|---|---|
| -Genetically programmed | Genetically programmed | Not (?) genetically |
| -Extra and intracellular | Extra and intracellular | Acute injury (?) (Extra) |
| -Cell shrinkage | Autophagic vacuoles | Cytoplasm swelling |
| -Blebbing | Blebbing | Disruption of Memb |
| -Organelle intact | Sequesteration | Disruption |
| -Chromatin condensation | Partial condensation | Non-condensed |
| -DNA fragmentation (laddering) | No laddering | No laddering |
| -Phagocytosis with | Phagocytosis with | Release of intracellular |
| no inflammation | no inflammation | 7 |

## What is Apoptosis?

- A predominant form of cell death
- A sequence of events involving various class of proteins
- Genetically programmed and evolutionarily conserved

### Hallmarks of Apoptosis

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## Why should we study apoptosis?

- Intact death program is required for successful embryonic development and maintenance of normal tissue homeostasis

-Insufficient or evasion of apoptosis manifest as:
- Cancer
- Autoimmunity (proliferation of immune system cells that recognize and attack self antigens)
- Viral infections (removal of virally infected cells by apoptosis)

-While accelerated cell death is related to:
- Neurodegenerative diseases
- Immunodeficiency, infertility
- Hematologic diseases

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## Major Players in Apoptosis

**-Bcl-2 family proteins**

-Antiapoptotic
-Proapoptotic multi-domain proteins
-Proapoptotic BH3-only domain protein

**-Caspases**

-Initiator caspases
-Executioner caspases

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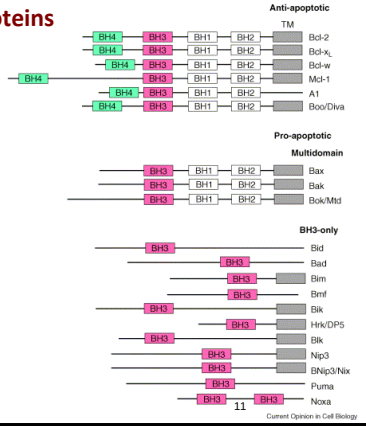
## Bcl-2 family proteins

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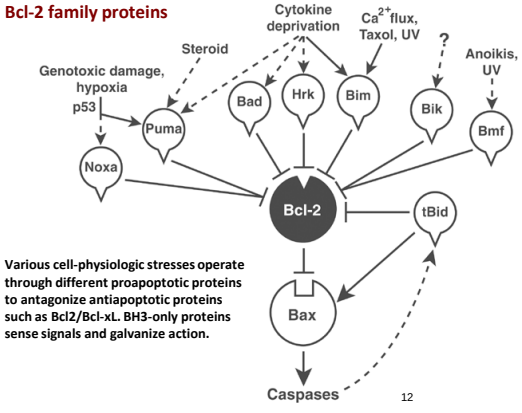
Current Opinion in Cell Biology

## Bcl-2 family proteins




**Various cell-physiologic stresses operate through different proapoptotic proteins to antagonize antiapoptotic proteins such as Bcl2/Bcl-xL. BH3-only proteins sense signals and galvanize action.**

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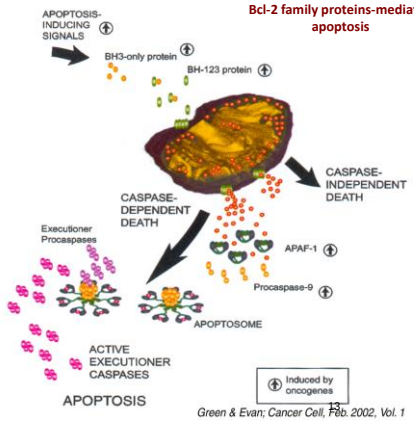
**Bcl-2 family proteins-mediated apoptosis**




Green & Evan; Cancer Cell, Feb. 2002, Vol. 1

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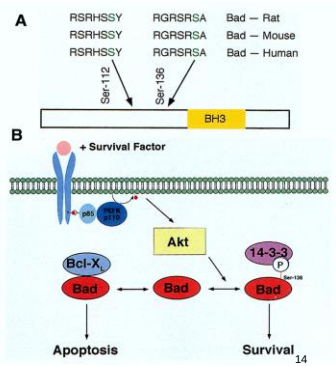
**BAD: Phosphorylation sequesters it in the cytosol**

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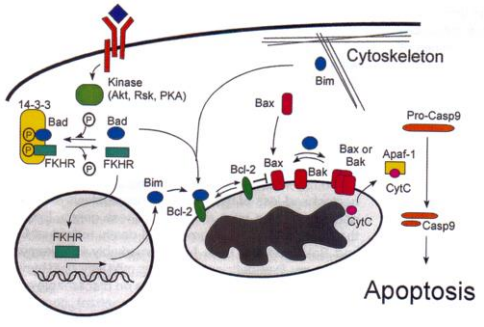
**Bcl-2 family proteins-mediated apoptosis**




Rathmell & Thompson; Cell, Vol. 109, S97-S107, April, 2002

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## Regulation of Bcl-2 and Bcl-xL: Post-translational modifications

**Bcl-2 must change conformation (or be 'activated') on the mitochondria to inhibit Bax (Dglugosz PJ, EMBO J. 25, 2287, 2006).*

**Bcl-2 and Bcl-xL (and other prosurvival members) are the guardians of the mitochondria: they are inactivated when BH3-only proteins juxtapose their BH3 domain to Bcl-2 (protein-protein interaction)..*

**These proteins are transcribed into multiple splice variants.*

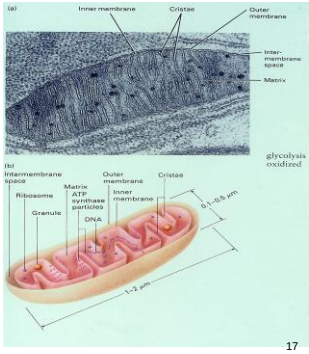
**Bcl-2 is phosphorylated by many apoptotic stimuli. Phosphorylation of Bcl-2 within the flexible loop generally inhibits its anti-apoptotic activity. But it has also been reported that phosphorylation is required for its apoptosis-inhibitory effect.*

**Bcl-2 and Bcl-xL can also be cleaved by caspases during apoptosis. Cleaved proteins turns themselves into apoptotic killers.*

**Bcl-xL may undergo deamidation: deamidation of Asn imparts susceptibility to apoptosis by disrupting the ability of Bcl-xL to block the proapoptotic activity of BH3-only proteins.*

16 *D. Tang*

## Mitochondria

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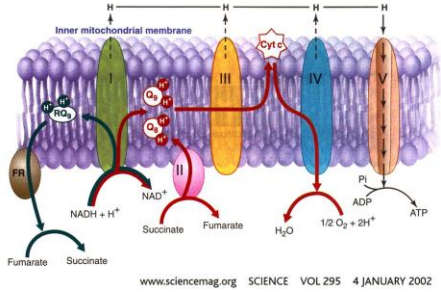
## Mitochondrial respiratory chain




www.sciencemag.org SCIENCE VOL 295 4 JANUARY 2002

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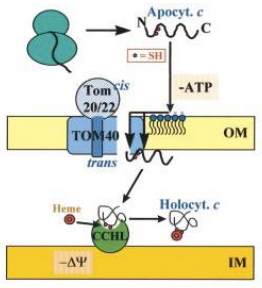
## Apocytochrome c and holocytochrome c

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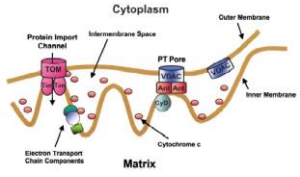
## Mitochondrial Porin or PTP (permeability transition pore)




Newmeyer, D.D., and Ferguson-Miller, S. Cell 112, 481-490, 2003

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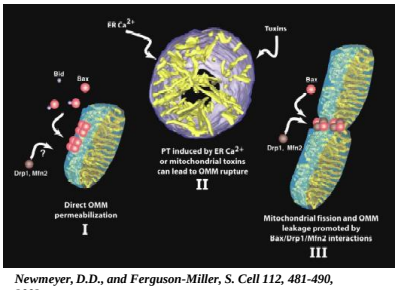
## OMM permeabilization, PTP, and Mitochondrial fission




*Newmeyer, D.D., and Ferguson-Miller, S. Cell 112, 481-490, 2003*

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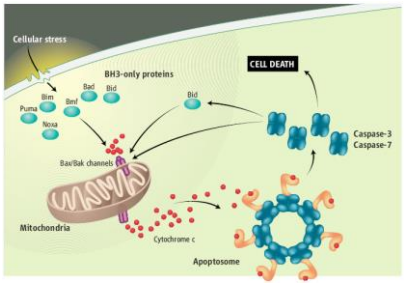
## Casp-3/7 may be involved in feedback cyt. c release




Lakhani SA et al., Science 311, 847, 2006
Adrain and Martin Science 311, 785, 2006

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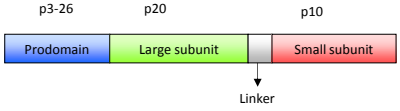
## Caspases

Types of caspases:

-Initiator caspases: caspase-2, -8, -9 and -10

-Executioner Caspases: caspase-3, -6 and -7

**Caspase Structure:**




**Caspase prodomains:**

Prodomains:In executioner caspases: ~3kd

In initiator caspases: 10-26 kd

23 *D. Chandra*

## Caspases

| Zymogen | Prodomain, length and motif[a] | Active subunits | Activation adapter | Tetrapeptide preference[b] |
|---|---|---|---|---|
| | kDa | kDa | | |
| Apoptotic initiators | | | | |
| Caspase-2 (51) | Long, CARD | 20/12 | RAIDD | DEXD[b,c] |
| Caspase-8 (55) | Long, DED | 18/11 | FADD | (L/V/D)EXD[c] |
| Caspase-9 (45) | Long, CARD | 17/10 | APAF-1 | (I/V/L)EHD |
| Caspase-10 (55) | Long, DED | 17/12 | FADD | Unknown |
| Apoptotic executioners | | | | |
| Caspase-3 (32) | Short | 17/12 | NA[d] | DEXD |
| Caspase-6 (34) | Short | 18/11 | NA | (V/T/I)EXD |
| Caspase-7 (35) | Short | 20/12 | NA | DEXD |
| Cytokine processors | | | | |
| Caspase-1 (45) | Long, CARD | 20/10 | ?CARDIAK | (W/Y/F)EHD |
| Caspase-4 (43) | Long, CARD | 20/10 | Unknown | (W/L/F)EHD |
| Caspase-5 (48) | Long | 20/10 | Unknown | (W/L/F)EHD |
| mCaspase-11[e] (42) | Long | 20/10 | Unknown | Unknown |
| mCaspase-12 (50) | Long | 20/10 | Unknown | Unknown |
| Caspase-13 (43) | Long | 20/10 | Unknown | Unknown |
| mCaspase-14 (30) | Short | 20/10 | NA | Unknown |
| Invertebrate caspases | | | | |
| CED-3 (56) | Long, CARD | 17/14 | CED-4 | DEXD |
| DCP-1[e] (36) | Short | 22/13 | NA | Unknown |

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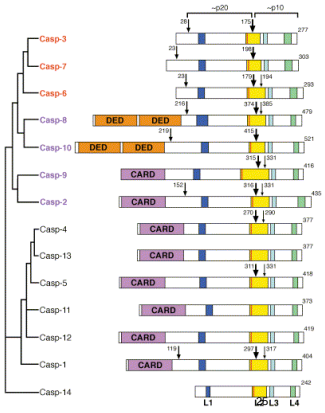
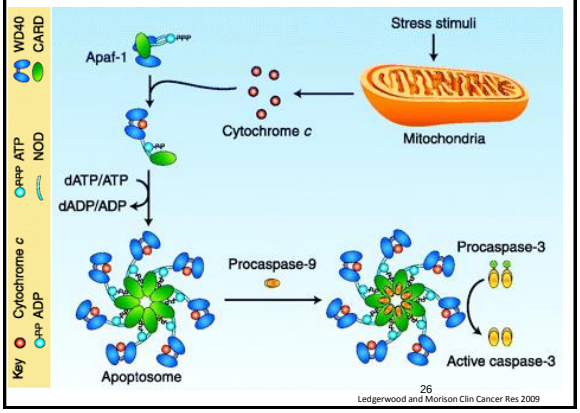
## Caspases:

Executioner caspases

Initiator caspases




Mol. Cell 9, 459-470, 2002

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Ledgerwood and Morison Clin Cancer Res 2009

## Caspase substrates

| Substrate class | Putative function | Examples |
|---|---|---|
| Pro- and anti-apoptotic proteins | Signal amplification<br>Inhibitor inactivation | Procaspases, Bcl-2, Bcl-$X_L$, Bid, p28Bap31 |
| Components of the apoptotic machinery | Induction of the apoptotic phenotype | ICAD, gelsolin, PAK2, MEKK1, PKCδ |
| Structural proteins and associated molecules | Dissolution of cell integrity<br>Cellular packaging | Lamins, NuMa, SAF-A, fodrin, Gas2, keratins, actin, Rabaptin-5, β-catenin, FAK |
| Homeostatic proteins | Disruption of macromolecular synthesis and cellular repair mechanisms<br>Termination of survival signals | DNA-$PK_{CS}$, PARP, U1–70kDa, RFC-140, HnRNPs, D4-GDI, transcription factors |
| Other | Unknown<br>?Apoptosis induction | Huntingtin, presenilins, atrophin-1, ataxin-3, $cPLA_2$ |

**Proteosome components (Mol Cell 14, 81-93, 2004)**
**Mitochondrial complex I p75 subunit (Cell 117, 773-786, 2004)**

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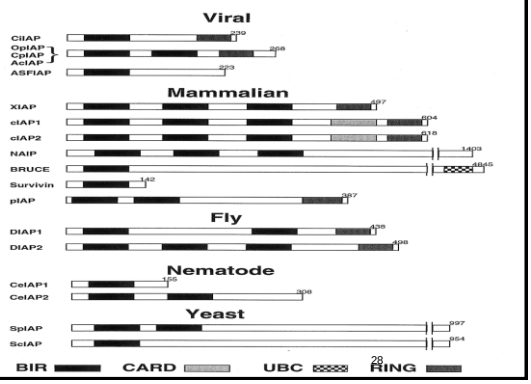
## IAPs

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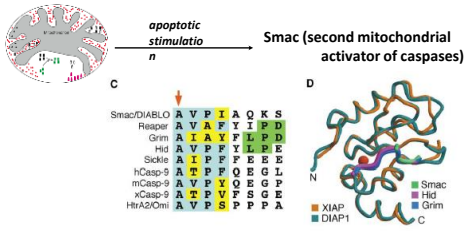
## IAPs function as critical prosurvival molecules

*IAPs (especially XIAP) are generally overexpreessed in multiple types of cancer cells




Shi, Y. Mol. Cell 9, 459-470, 2002

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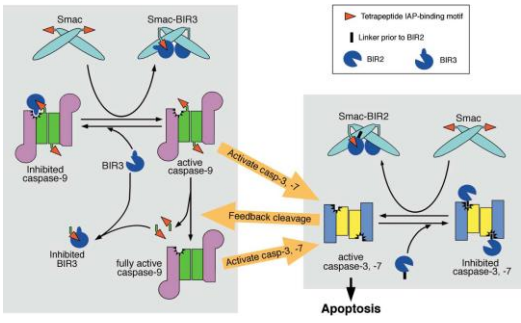
## Mechanisms of Caspase Activation and Inhibition




Shi, Y. Mol. Cell, 9, 459-470, 2002

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## Known apoptotic pathways to activate caspases

- Intrinsic pathway
- Extrinsic pathway or death-receptor pathway
- Other alternative pathways
- Caspase-independent apoptosis

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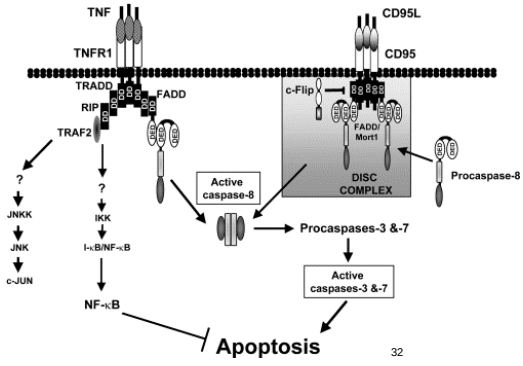
## Extrinsic Apoptotic pathway (death receptor)

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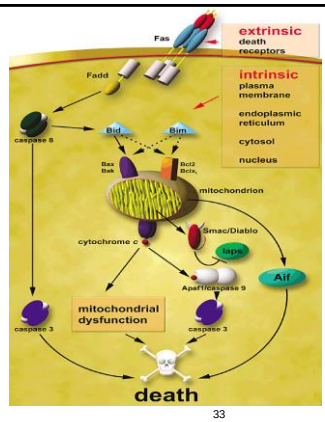
## Crosstalk between two pathways

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## How to detect apoptosis ?

-Cell morphology by using microscopy
-Cytochrome c release by immunolabelling and Western
-Western Blot for caspases and their substrate
-DAPI and Annexin-V-staining
-Detection of apoptosome using gel filtration

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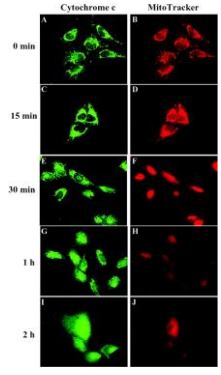
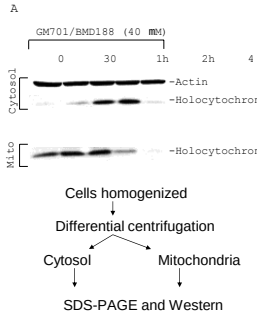



**Immunolabeling, subcellular fractionation and Western blot**




Cells homogenized
↓
Differential centrifugation
↓
Cytosol | Mitochondria
↓ | ↓
SDS-PAGE and Western

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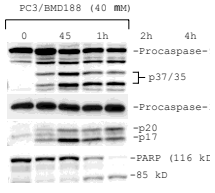
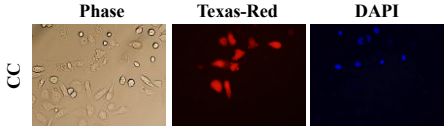
## Caspase cleavage and DAPI labeling

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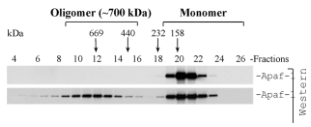
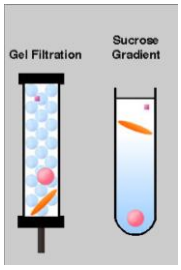
## Detection of Apoptosome

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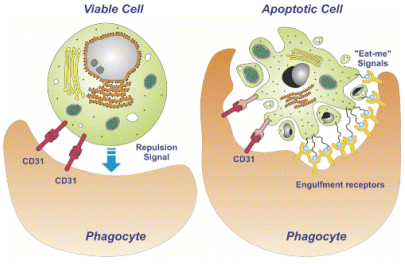
## What happens to the apoptotic cells?

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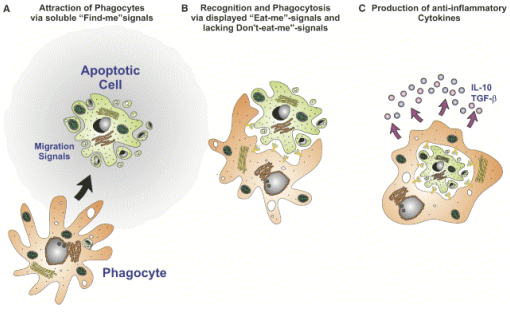
## What happens to the apoptotic cells?

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## Apoptosis and Tumorigenesis

1) Upregulation of Bcl2, BclxL, IAPs, Flip etc.

2) Downregulation/ mutation of Bax, Bak, BH3-only protein, Death-receptors, Apaf-1 etc.

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## Apoptosis based strategies for cancer therapy

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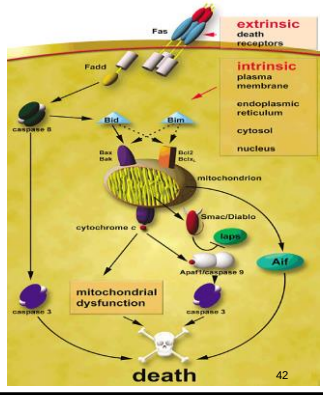
## Exploiting apoptotic machinery for cancer therapy

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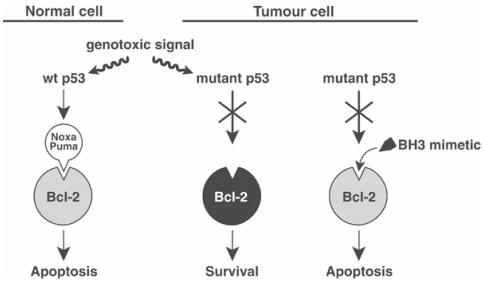
## Importance of BH3-only protein mimetics in cancer therapy

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## Take Home message from apoptosis:

-A predominant form of cell death from worm to mammals

-Bcl-2 family proteins make decision to die or live

-Caspases are the main soldiers in the battle field to execute apoptosis

-Mitochondrion is the center point for all activities

-Targeting Bcl-2 family proteins have enormous potential in cancer therapy

-Finding N-terminal smac/DIABLO mimetics will counter IAPs

-Applications of death ligands such as TNF-α, TRAIL etc.

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## Other apoptosis/survival functions of Bcl2-family proteins and caspases

- Bcl-2 and Bcl-xL can also be cleaved by caspases during apoptosis and this cleaved fragments function as proapoptotic proteins.
- These proteins also undergo posttranslational modifications, which can also impact on apoptosis sensitivity.
- Pro-apoptotic BH3-only proteins such as Bad, Bid, Puma, Noxa have also been shown to possess prosurvival roles
- Caspase-3 activation is involved in cancer cell invasion
- Caspase-8 is required by NF-kB activation and promotes cell motility

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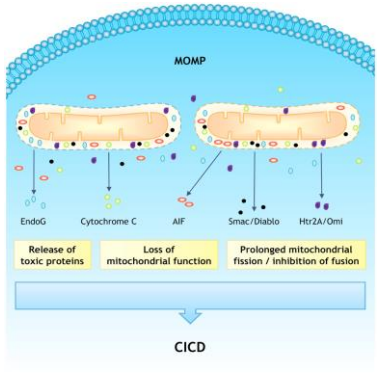
## Caspase-independent cell death




Tait and Green, Oncogene 2008

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## Autophagy

- First recognized under EM early 1960s.
- Also called macroautophagy: Self-eating to survive.
- A unique form of membrane trafficking in which membrane compartments (autophagosomes) engulf both organelles and cytosolic macromolecules and deliver them to the lysosome for degradation.

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## Autophagy

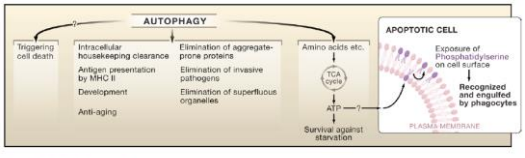


Figure 1. The Many Functions of Autophagy
Autophagy has many protective roles but may also be involved in programmed cell death. Qu et al. (2007) now show that autophagy, through the production of ATP, is important for presenting a signal (phosphatidylserine) on an apoptotic cell to ensure its clearance. This appears to be important in morphogenesis during early mouse embryonic development.

**Yoshimori T et al., Cell 128, 833-836, 2007**
**Qu X et al., Cell 128, 931-46, 2007**

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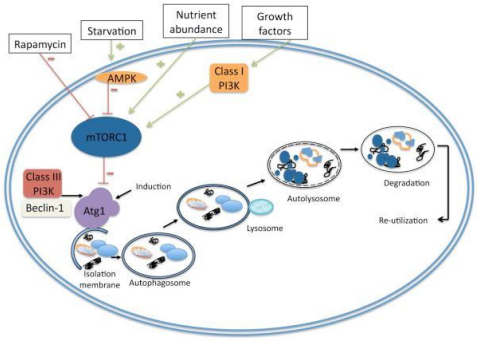
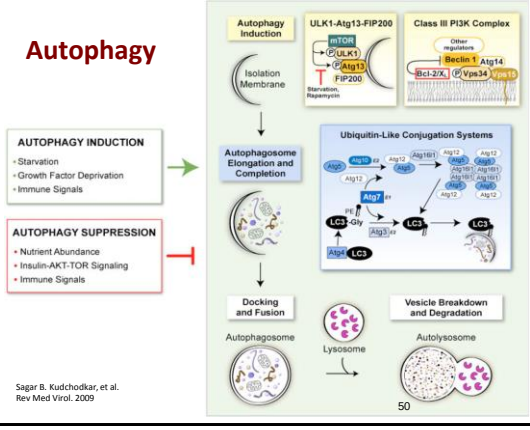
## Autophagy




Martin Lotz, et al. Nat Rev Rheumatol. 2011



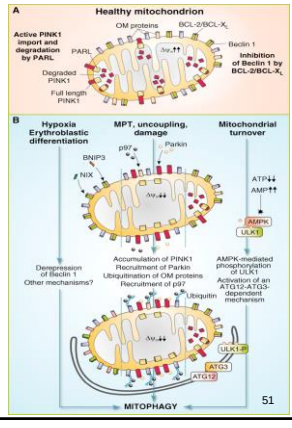
## Autophagy




Sagar B. Kudchodkar, et al. Rev Med Virol. 2009

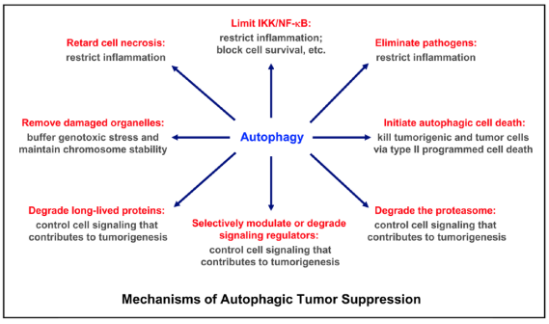


## Mitophagy




Green et al., Science 2011

## Autophagy and Cancer




Mechanisms of Autophagic Tumor Suppression

Gutian Xiao. Cytokine Growth Factor Rev. 2007

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