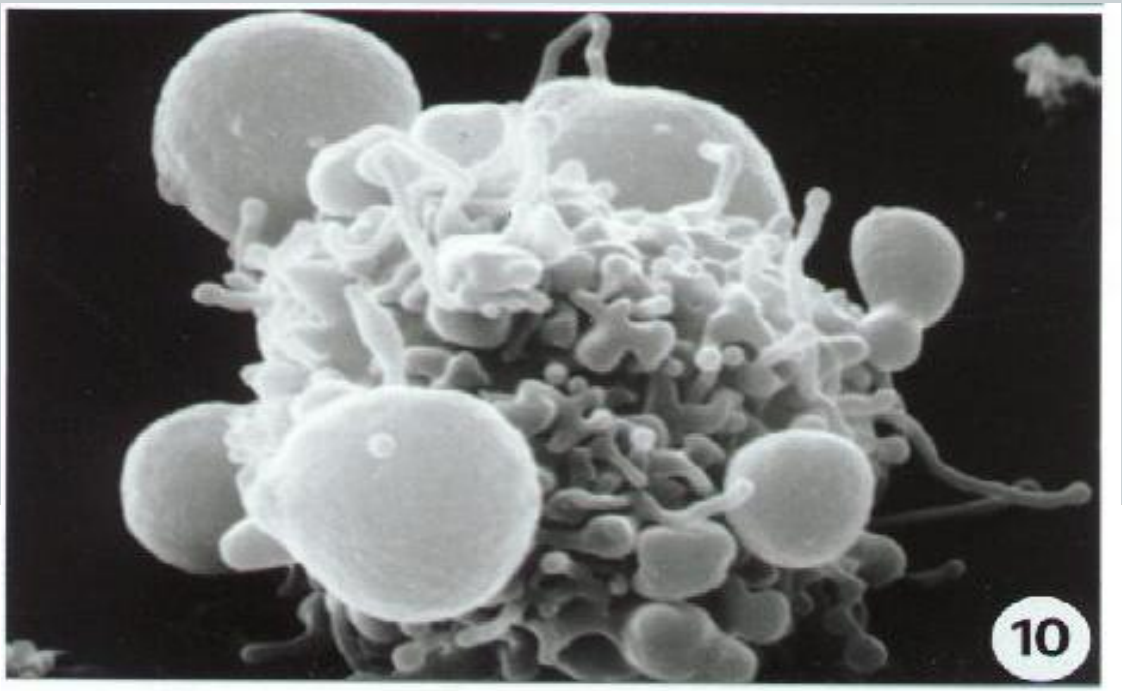
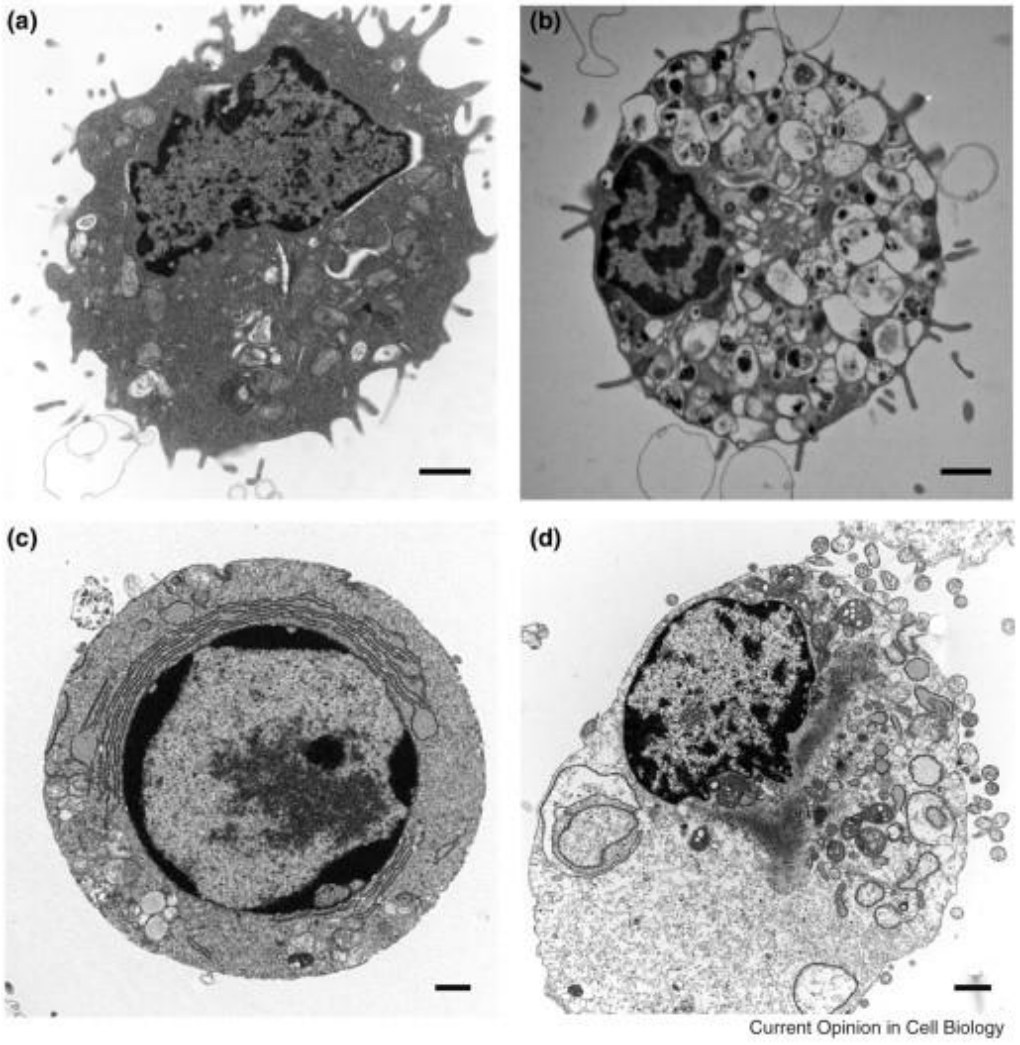


Drug Response

Apoptosis or Cell Signaling

Cell Death



Autophagy

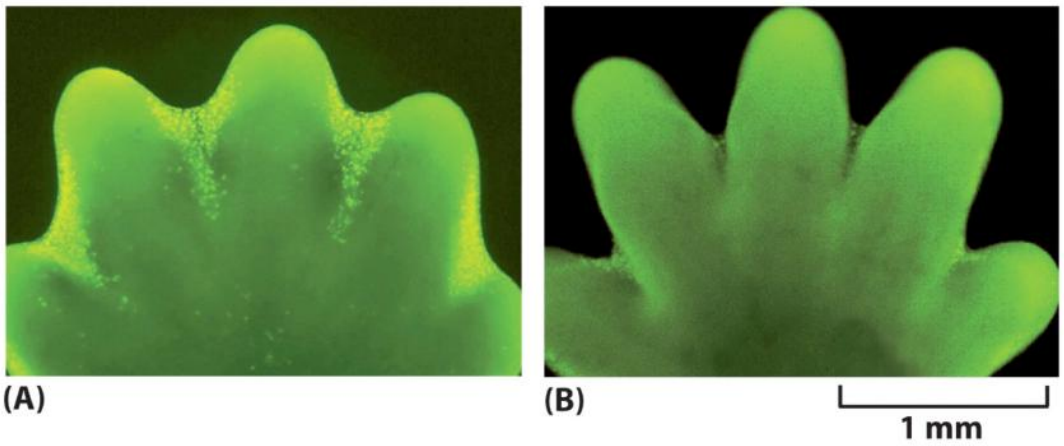
Apoptosis

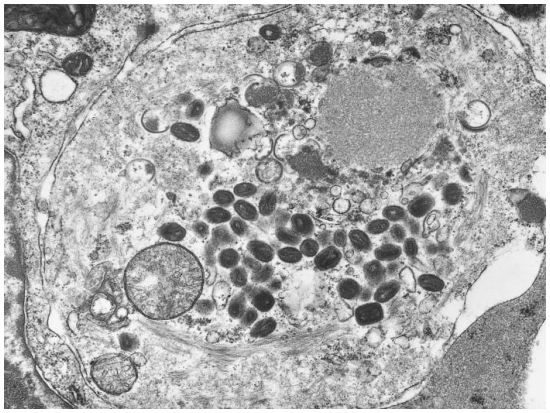
Necrosis

Apoptosis

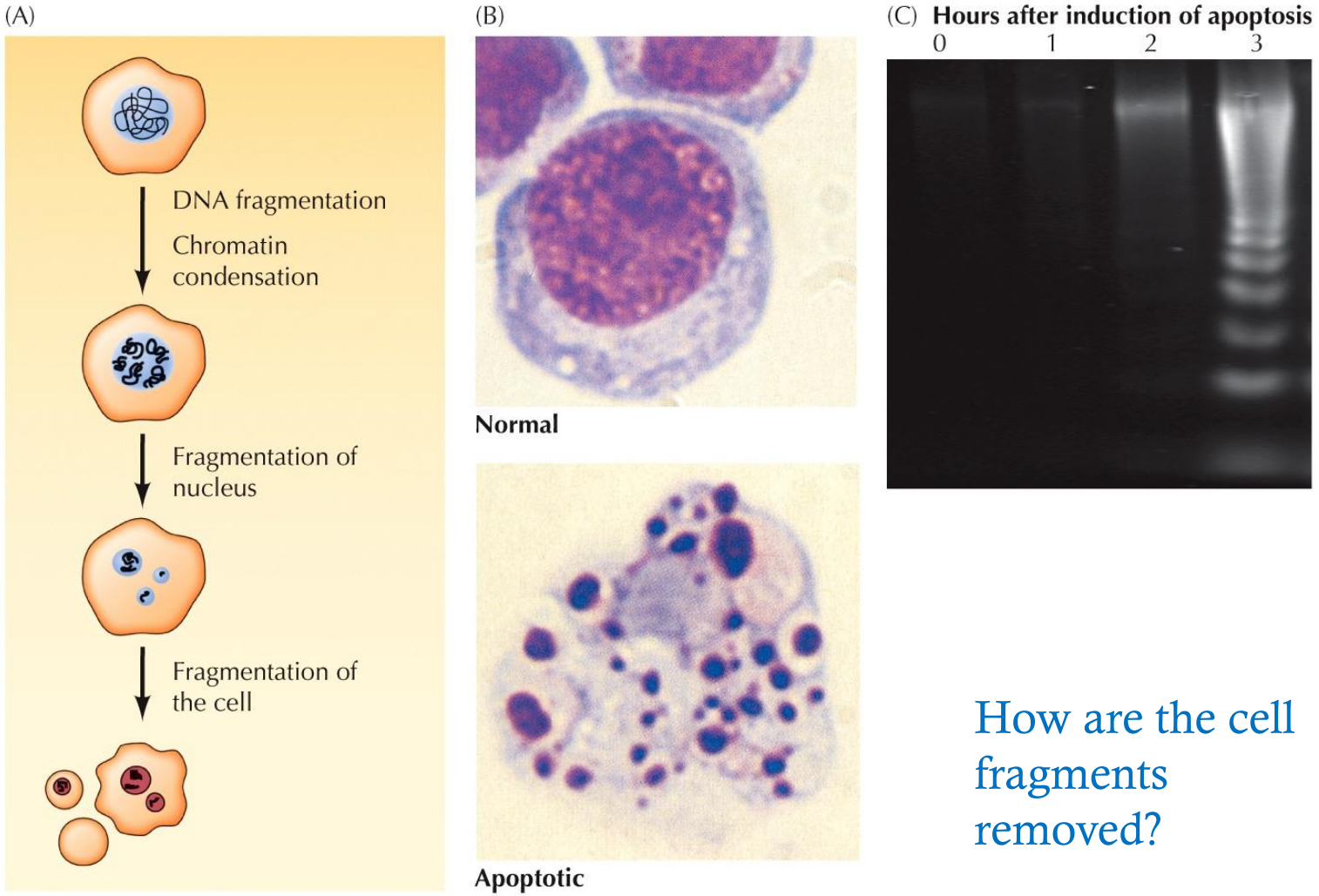
Programmed cell death as an active process, in which the cell proceeds through a series of well defined steps (nuclear condensation, fragmentation, and blebbing).

May occur for a variety of reasons that benefit the organism as a whole:





Apoptosis is a Multistep Process

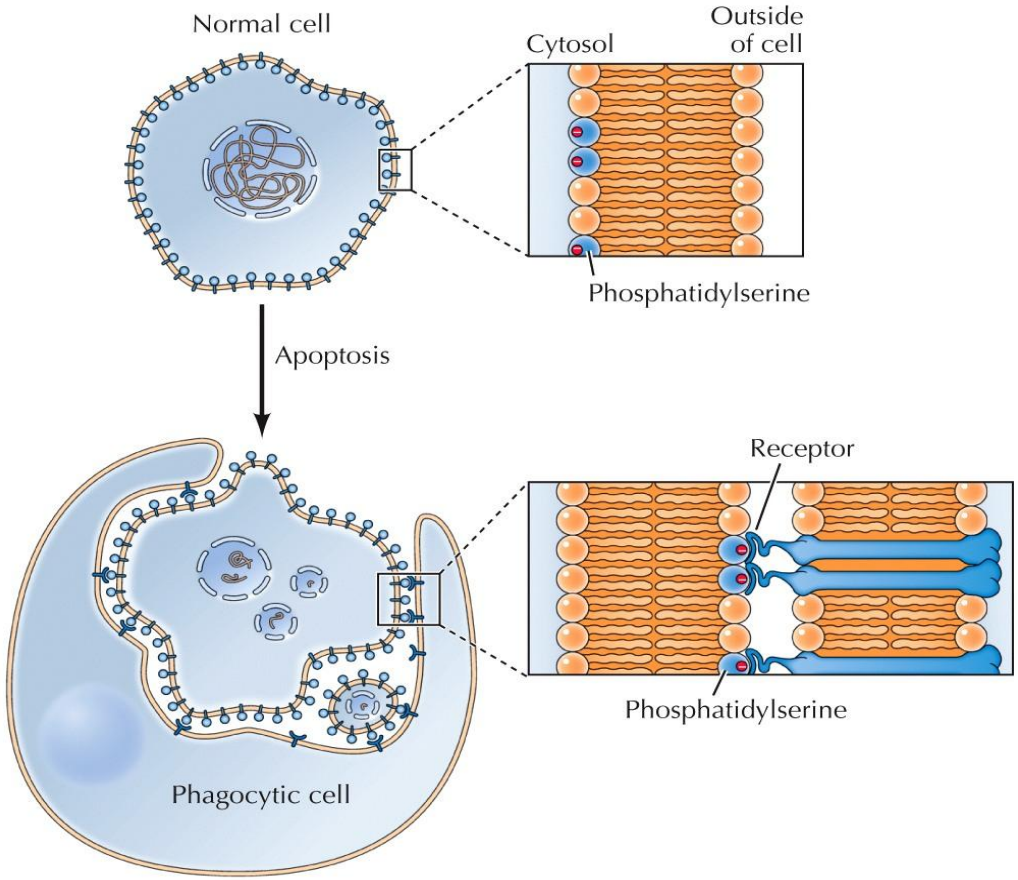


***THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.1**

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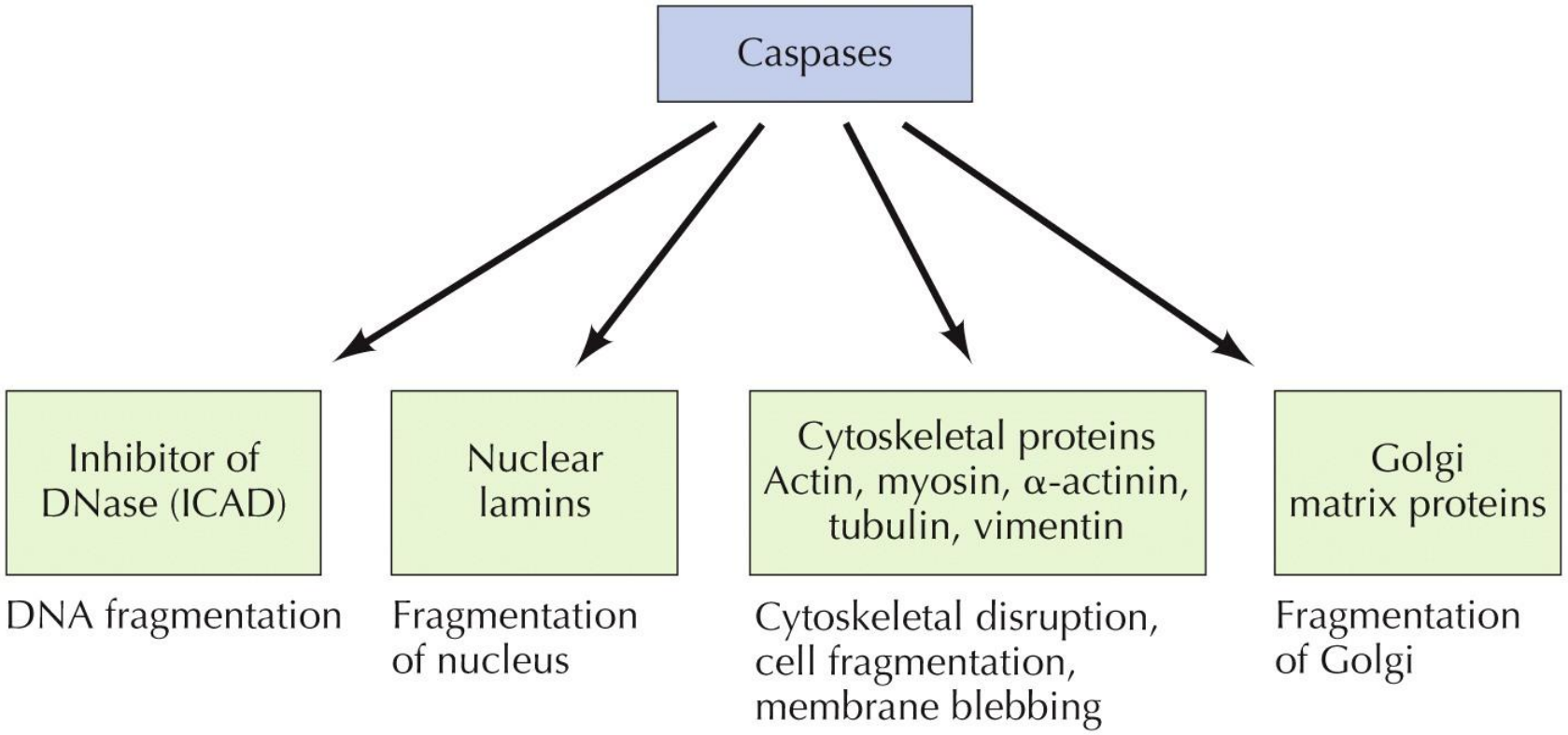
How are the cell fragments removed?

The removal of apoptotic cells is mediated by the expression of "eat me" signals on the cell surface. These signals include **phosphatidylserine**, a phospholipid that flips from the inner to the outer leaflet of the plasma membrane during apoptosis.



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.2**
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Caspases: the executioners of apoptosis

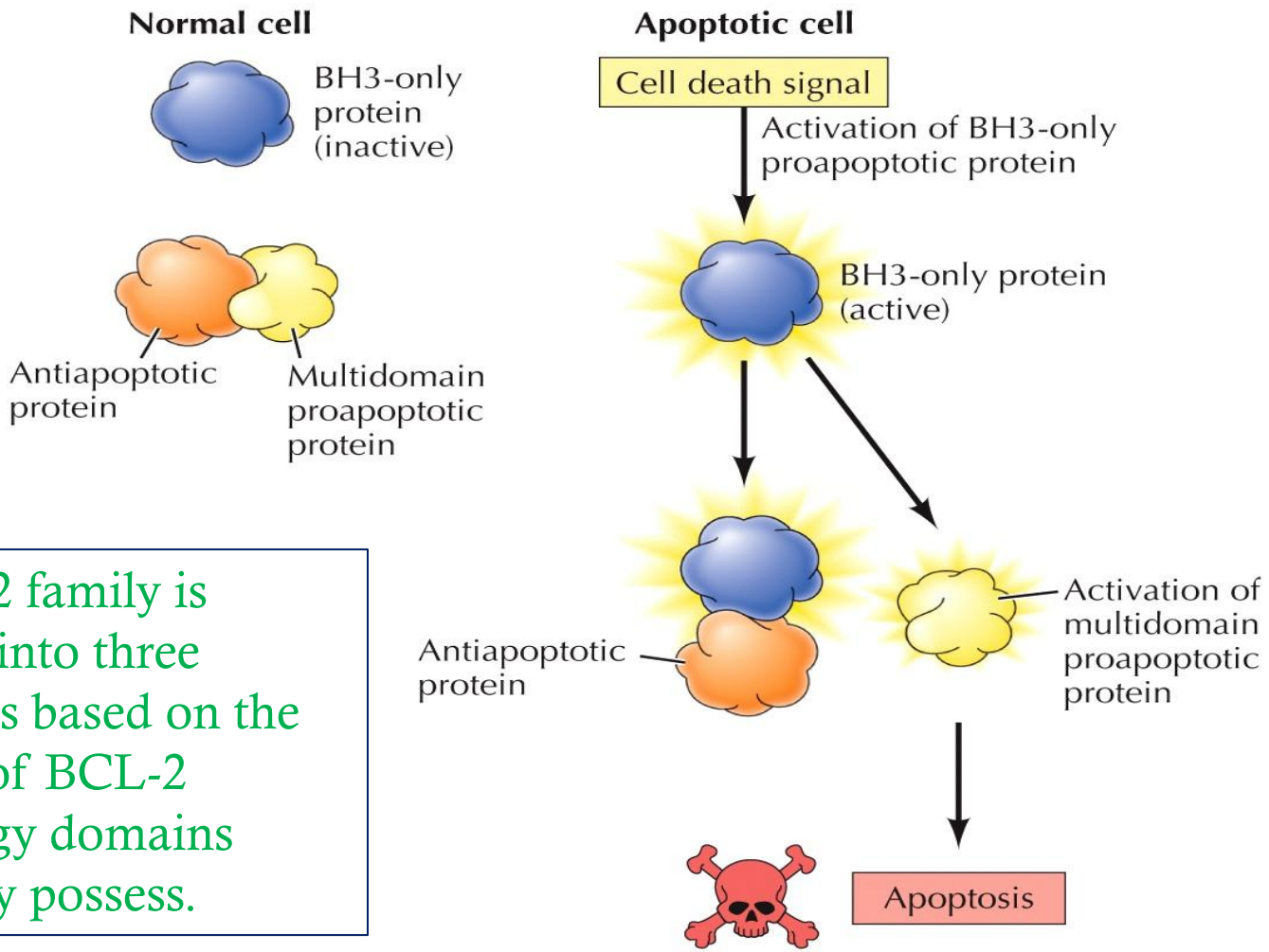


***THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.4**

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Procaspases must be cleaved to form the active caspase.

The Bcl-2 family of proteins are the key **regulators** of apoptosis. Some are anti-apoptotic (Bcl-2) while others are pro-apoptotic (Bax, Bak, Noxa, Puma).



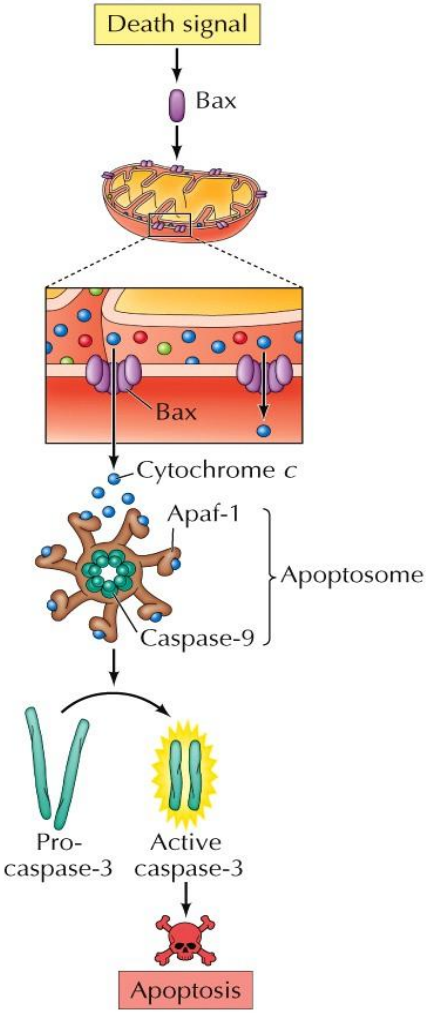
The Bcl-2 family is grouped into three categories based on the number of BCL-2 Homology domains (BH) they possess.

THE CELL: A MOLECULAR APPROACH 6e, Figure 17.6

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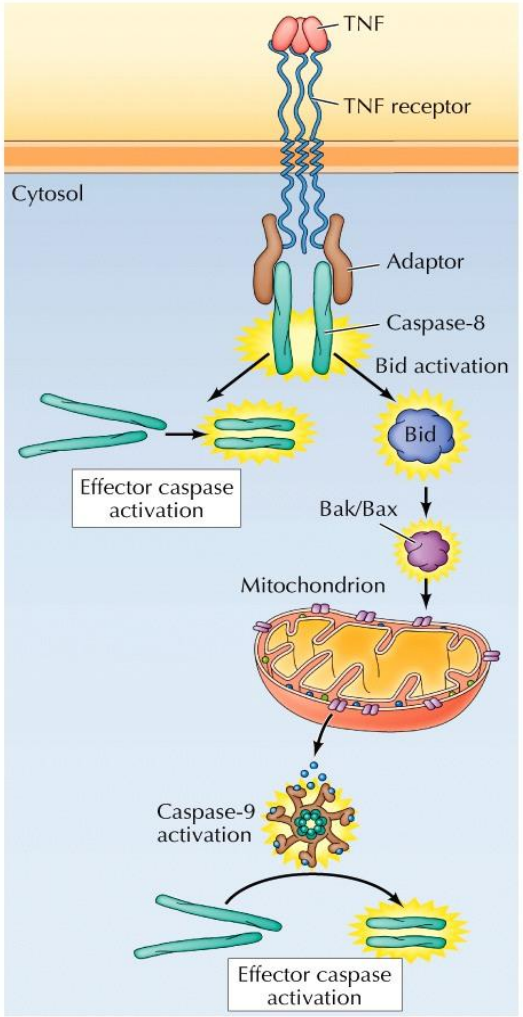
Apoptosis is grouped into two broad **categories**:

Intrinsic



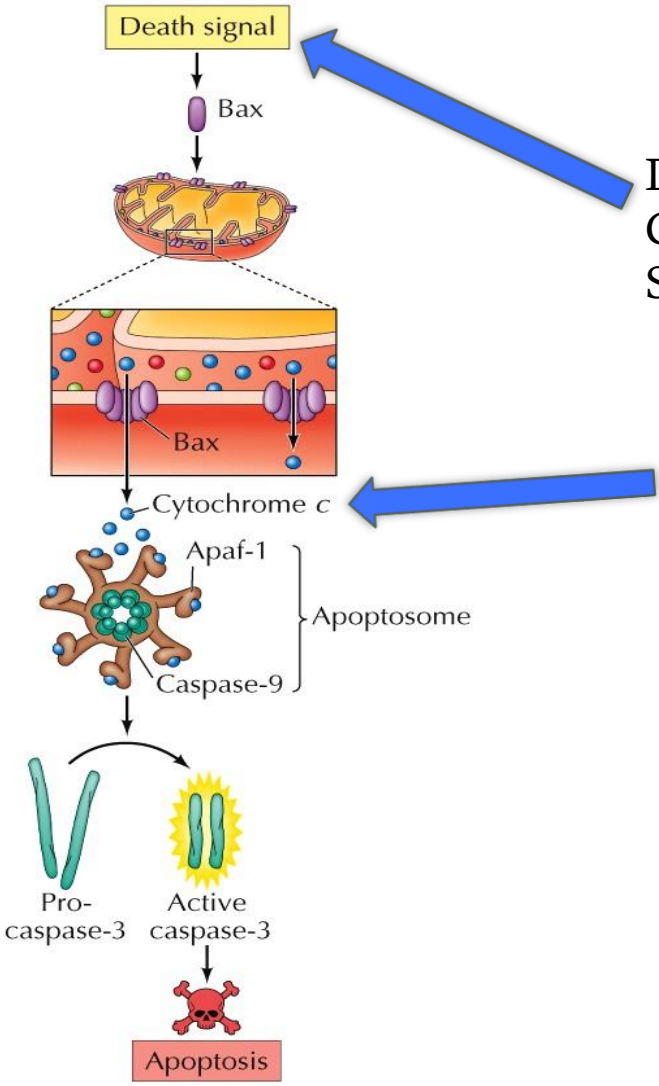
THE CELL: A MOLECULAR APPROACH 6e, Figure 17.7
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Extrinsic



THE CELL: A MOLECULAR APPROACH 6e, Figure 17.11
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Intrinsic Pathway of Apoptosis



DNA damage (increased p53 levels)
Growth factor deprivation (loss of survival signal)
Severe cell stress

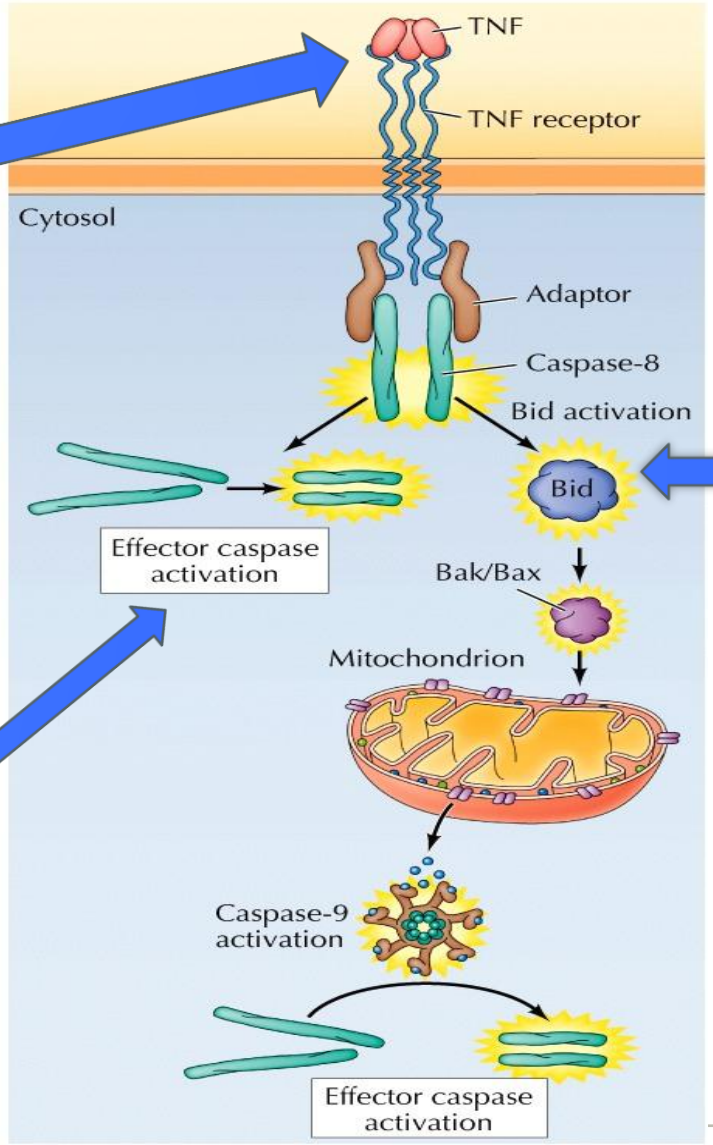
Cytochrome c is necessary for caspase-9 formation, activation of caspase-3, and execution of apoptosis

THE CELL: A MOLECULAR APPROACH 6e, Figure 17.7

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Extrinsic Pathway of Apoptosis

Triggered by external signal for cell death (e.g. immune cell signal for destruction, TNF family of ligands)

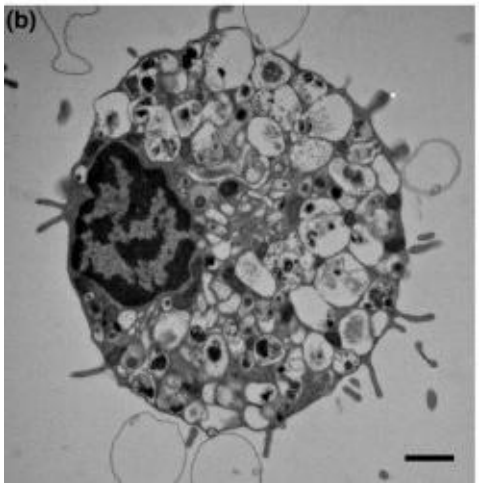


Caspase-8 can also trigger the Bcl-2 family and mitochondrial permeability, similar to the intrinsic pathway

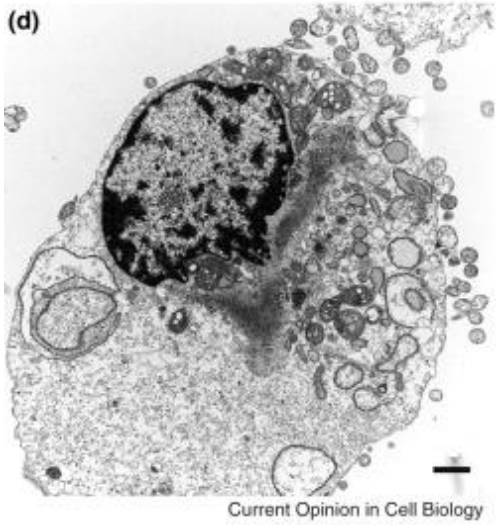
Caspase-8 can directly activate other caspase proteins, leading to apoptosis

Alternative pathways of programmed cell death (?)

Autophagic cell death



Programmed necrosis

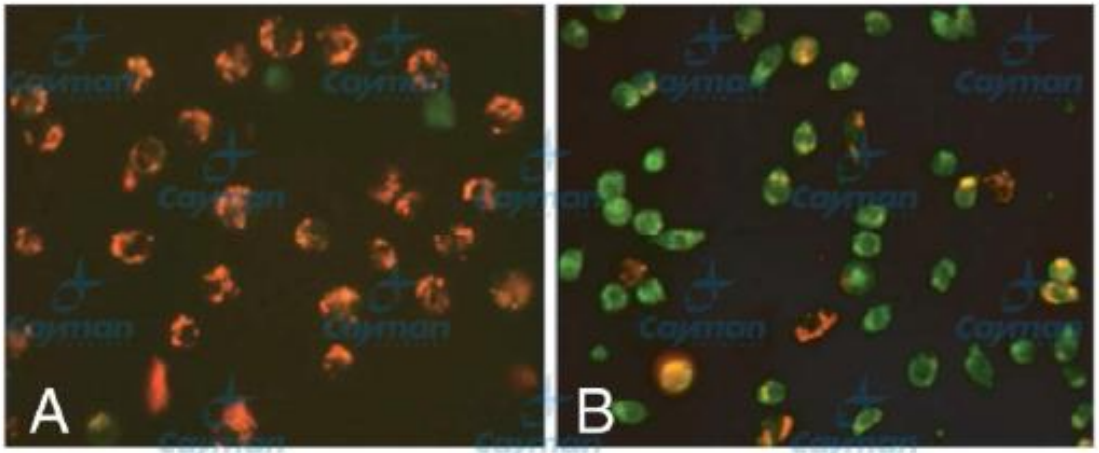


Assays to Detect Apoptosis

Mitochondrial membrane potential

JC-1 Mitochondrial Membrane Potential Assay

Mitochondrial membrane potential, Δψm, is an important parameter of mitochondrial function used as an indicator of cell health. JC-1 is a lipophilic, cationic dye that can selectively enter into mitochondria and reversibly change color from green to red as the membrane potential increases. In healthy cells with high mitochondrial Δψm, JC-1 spontaneously forms complexes known as J-aggregates with intense red fluorescence. On the other hand, in apoptotic or unhealthy cells with low Δψm, JC-1 remains in the monomeric form, which shows only green fluorescence.

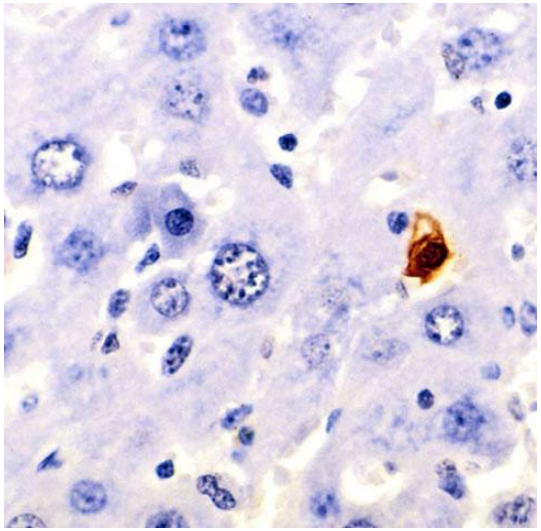


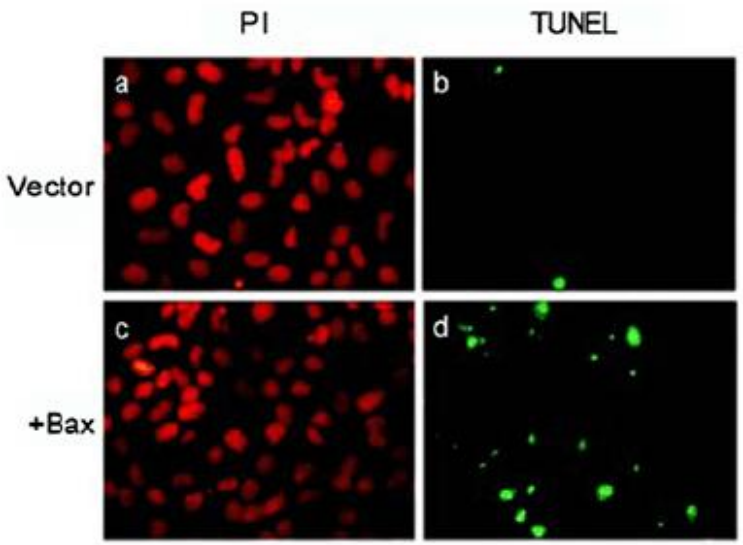
Effect of staurosporine on mitochondrial potential in Jurkat cells. *Panel A:* untreated cells show most of cells had strong J-aggregation (red). *Panel B:* staurosporine-treated cells show a majority of cells stained green due to low ΔΨm.

Assays to Detect Apoptosis

DNA fragmentation

As DNA becomes fragmented, due to DNase degradation, terminal ends of nucleic acids become exposed. The TUNEL assay (terminal deoxynucleotidyl transferase) relies on the binding of fluorescently labeled dUTP to these DNA nicks.





Time course for apoptosis detection requires an understanding of the pathways:

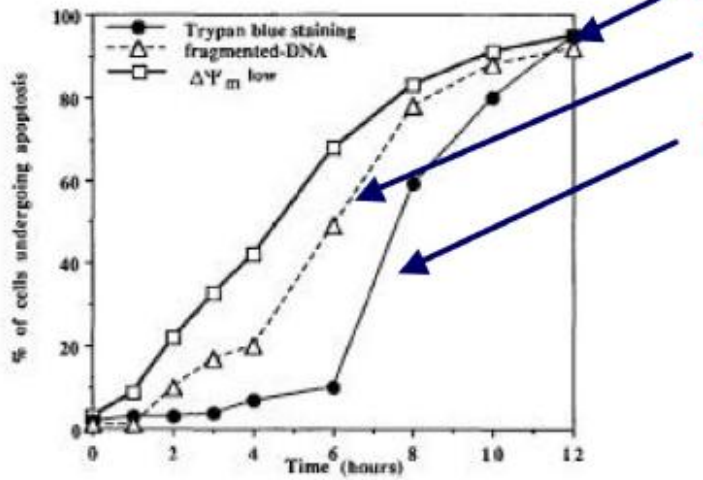


Figure 5. Time-course analysis of early events in dexamethasone-induced apoptosis. Quantification of cells undergoing apoptosis was performed following three parameters: (*a*) percentage of cells exhibiting a low $\Delta\Psi_m$-related fluorescence assessed by $DiOC_6(3)$ staining; (*b*) percentage of cells with fragmented DNA assessed by in situ nick translation; and (*c*) percentage of cells incorporating the trypan blue.

$\Delta\psi_m$

Fragmented DNA

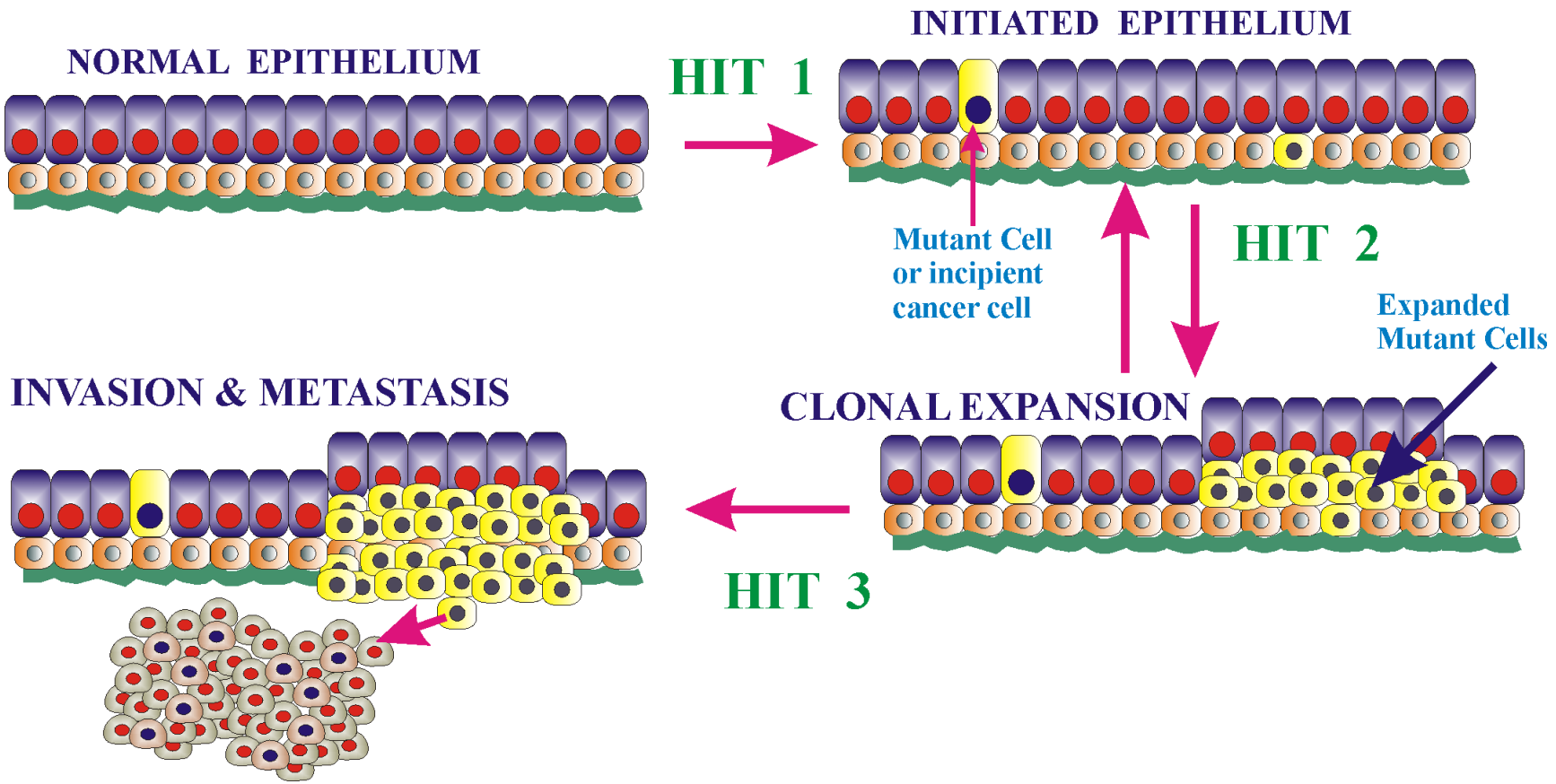
Trypan blue staining

Petit et al. J Cell Biology, 130:157-167

Neoplasia/Cancer

- What is it?
- Molecular Changes
- Cell Assays for studying cancer progression

Cancer: Antisocial Problem



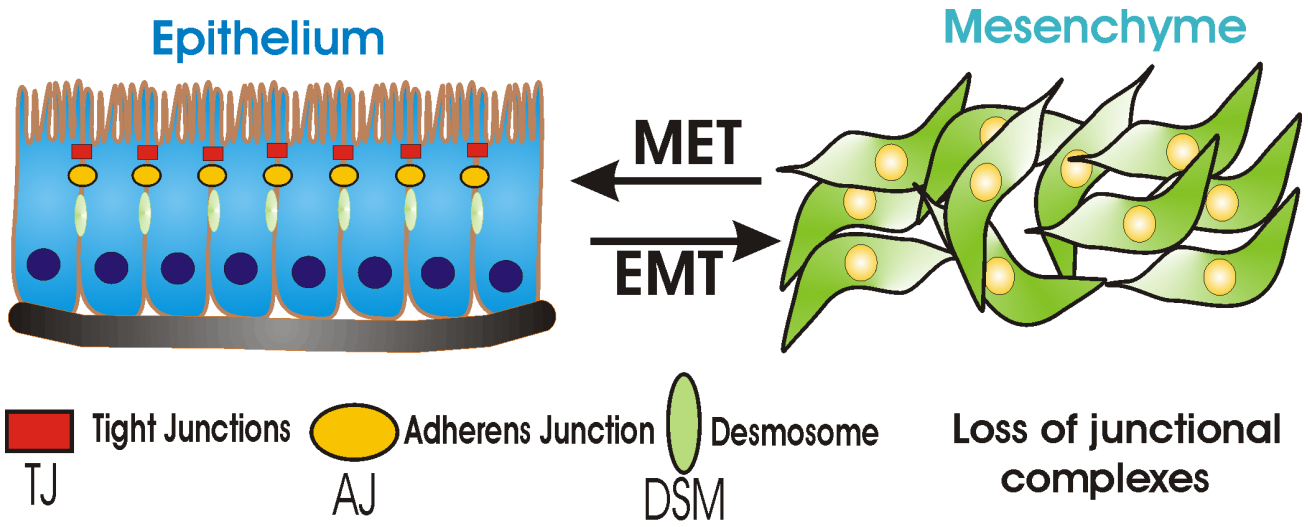
Progression of Cancer

- Benign: neoplastic cells that remain clustered together in a single mass
- Malignant: The cells have acquired the ability to invade surrounding tissue
- Metastasis: an ability to break loose, enter the bloodstream or lymphatic vessels and form secondary tumors.
- Classified according to tissue: Carcinomas, Sarcomas, Leukemias

Molecular Changes/Six Hallmarks

1) Sustained Proliferative Signaling
2) Evading Growth Suppression
3) Activating Invasion and Metastasis
4) Enabling Replicative Immortality
5) Inducing Angiogenesis
6) Resisting Cell Death

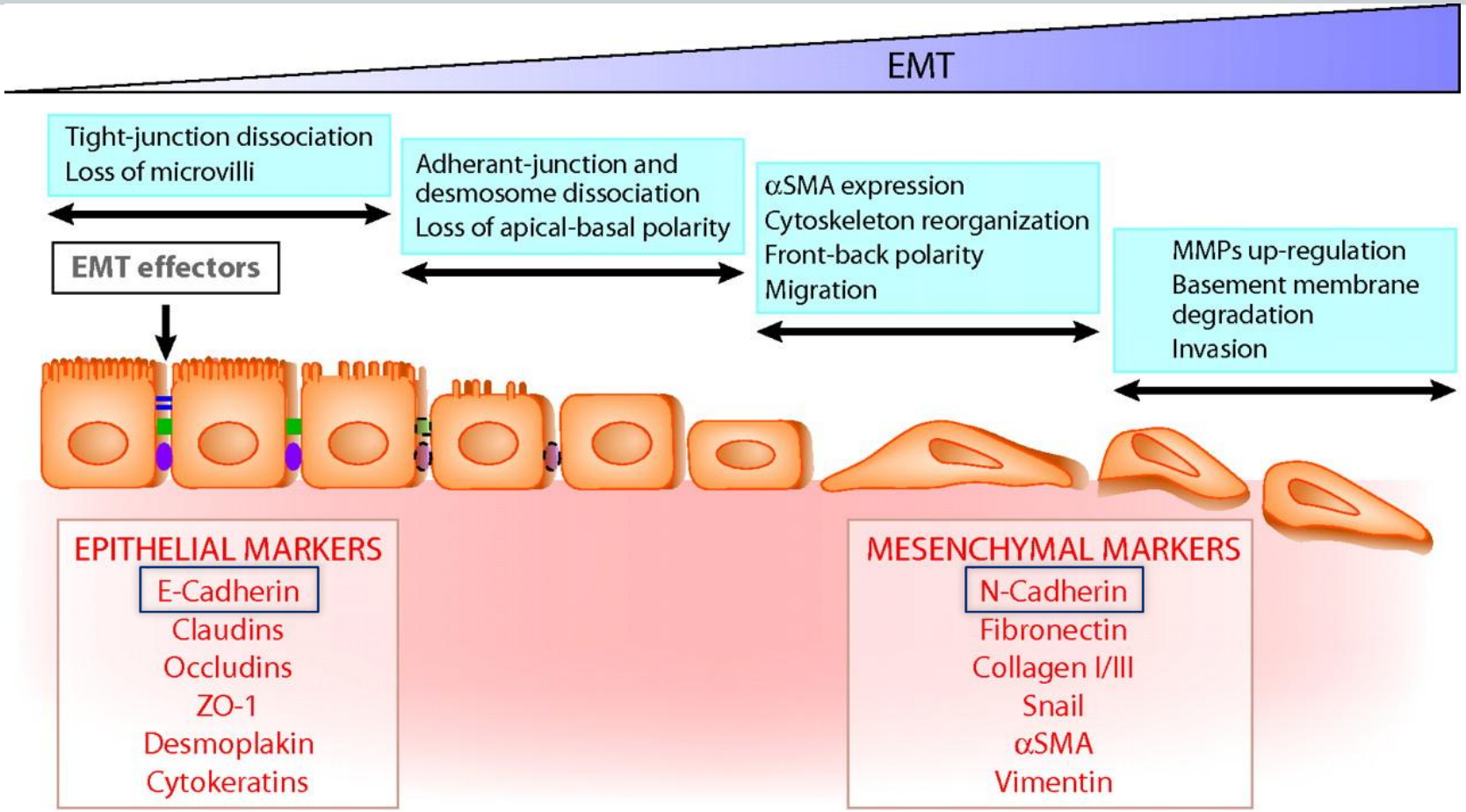
Molecular Changes: Differentiation



Epithelial cells: strong intercellular connections, keratin-based cytoskeleton, distinct cell polarity

Mesenchymal cells: loose intercellular connections, highly developed cytoskeletal structures with vimentin, no clear polarity

Molecular Changes: Differentiation

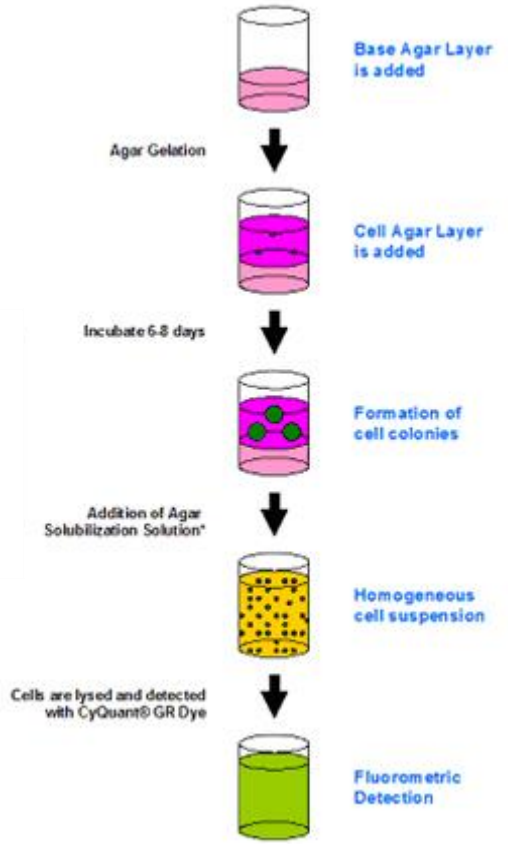


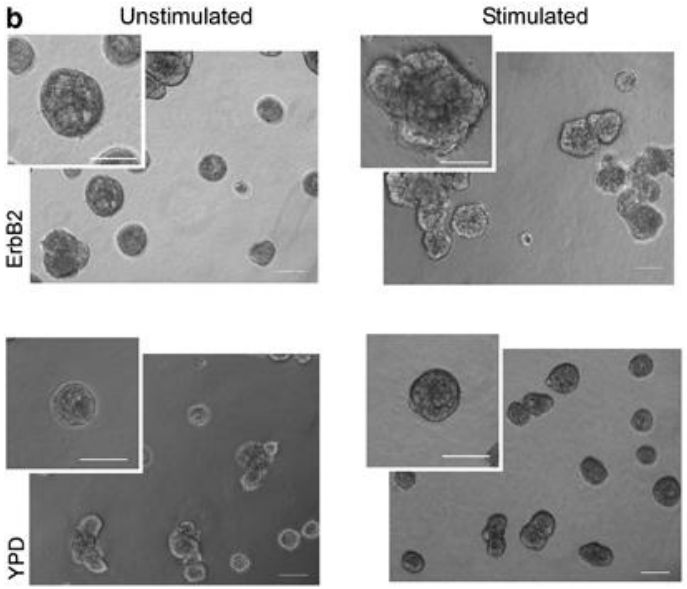
Do you think we could use Flow to analyze EMT?

How do Cell Biologists Study Cancer?

- Tumorigenic Potential
  - Soft Agar Assays
  - Subcutaneous or orthotopic injection of cancer cells
- Cell Polarity
- Examining Molecular Changes
  - Remember in EMT a lot of specific things are happening
  - EMT kits are optimized for many assays (gene expression analysis, epigenetic modification, genotyping, signal transduction pathway activation, etc.)

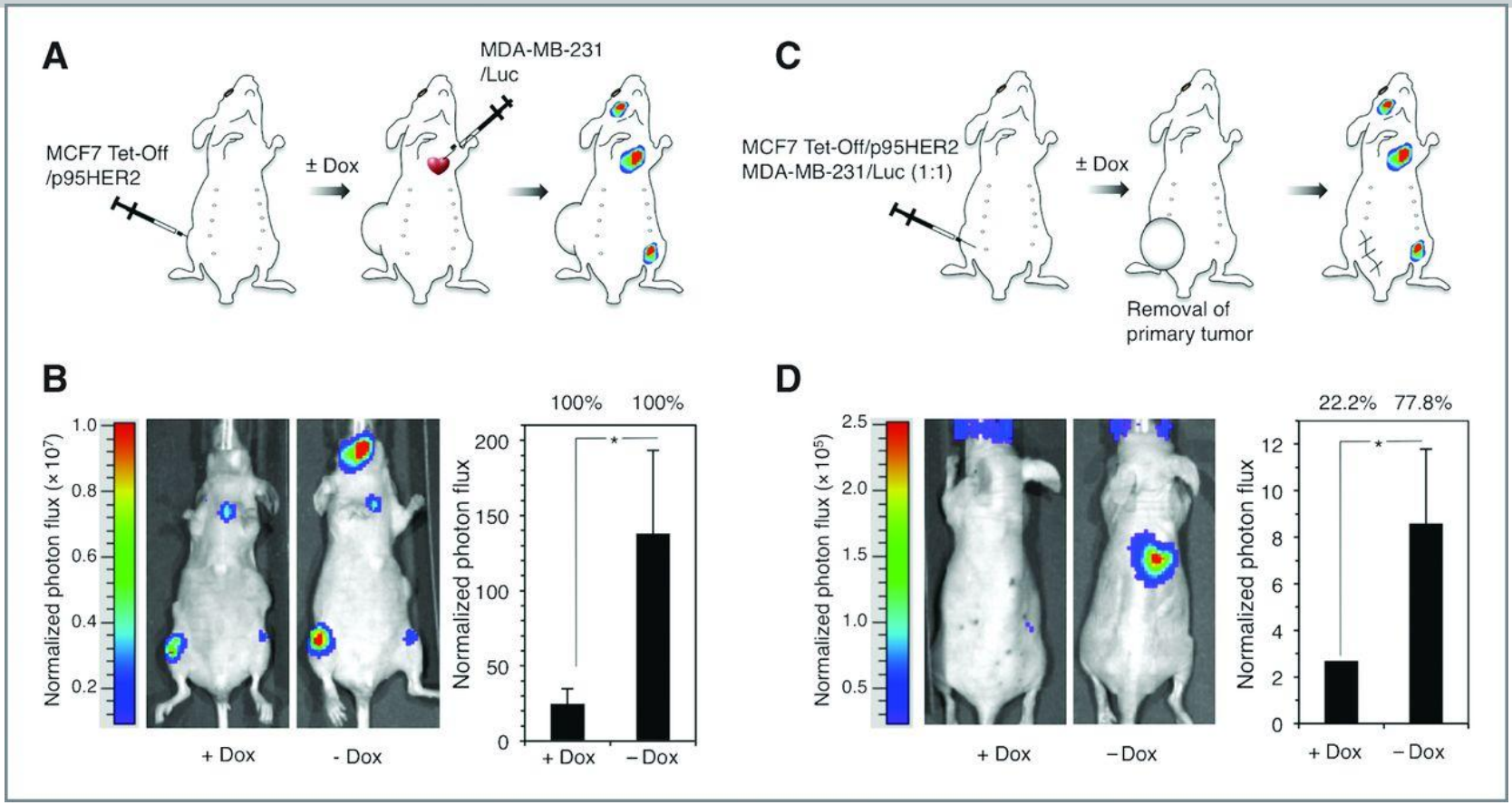
Contact Independent Growth





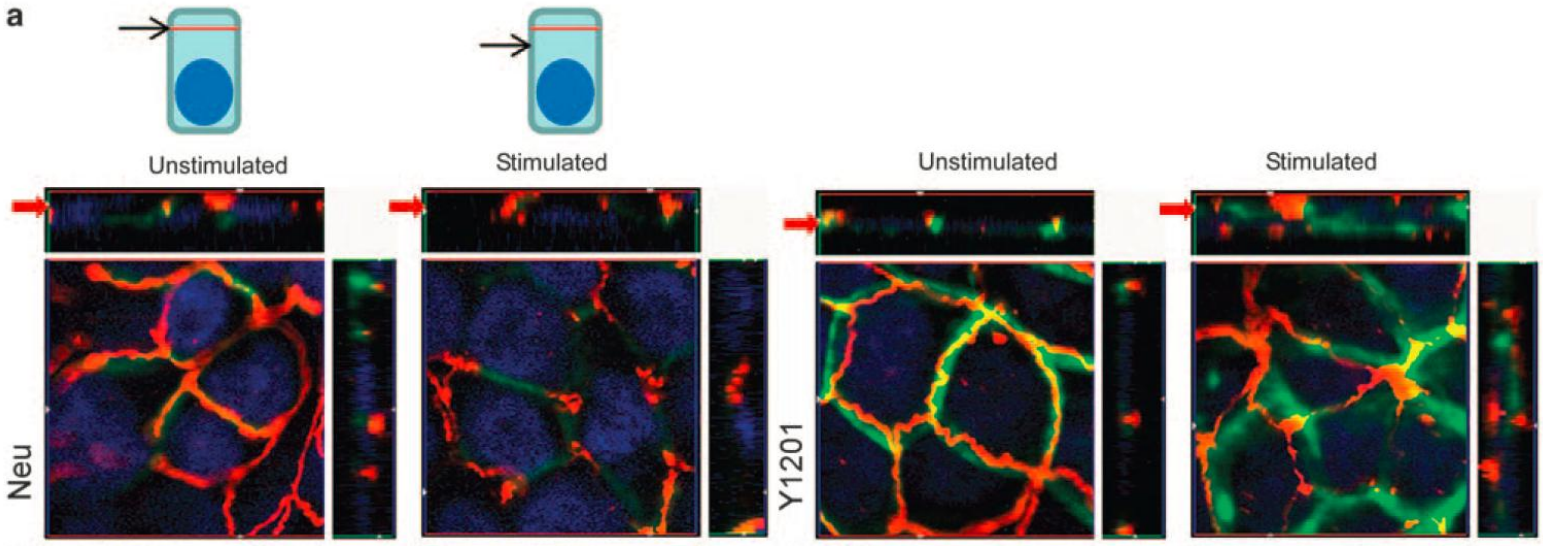
How many foci develop?
How large are they?
What is the morphology?
All of these observations indicate tumorigenic potential.

Animal Injection



The most ideal model to investigate tumorigenicity and metastasis.

Polarity Assays



What indicates polarity?

Remember Tight Junctions maintain polarity.

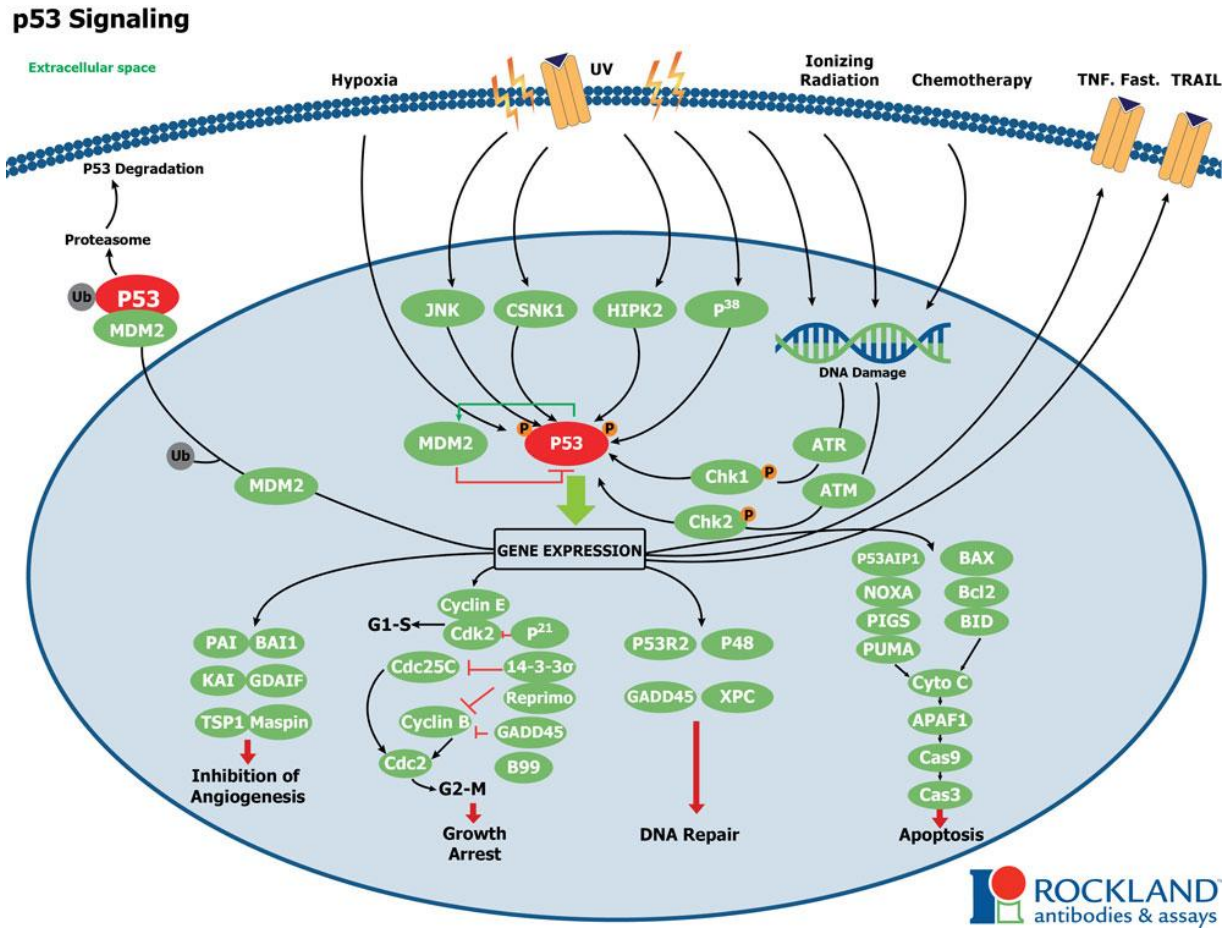
1) Do the tight junction protiens assemble
2) The thickness of the cell indicates polarization as well as whether or not the tight junctions assemble at the top. (Although this harder to observe)

Cell Signaling

Cell signaling governs basic cellular activities and coordinates cell actions through a complex coordination of responses to cellular microenvironment. The disruption or errors found within this communication chain gives rise to various diseases and cancers.

http://www.rockland-inc.com/cell-signaling-pathways.aspx

Cell Signaling



For qPCR, there are many options.

We will examine up to 5 genes per group.

Cadherin switching

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Accepted 17 December 2007
Journal of Cell Science 121, 727-735 Published by The Company of Biologists 2008
doi:10.1242/jcs.000455

Summary

The cadherin molecules at adherens junctions have multiple isoforms. Cadherin isoform switching (cadherin switching) occurs during normal developmental processes to allow cell types to segregate from one another. Tumor cells often recapitulate this activity and the result is an aggressive tumor cell that gains the ability to leave the site of the tumor and metastasize. At present, we understand some of the mechanisms that promote cadherin switching and some of the pathways downstream of this process that influence cell behavior. Specific cadherin family members influence growth-factor-receptor signaling and Rho GTPases to promote cell motility and invasion. In addition, p120-catenin probably plays multiple roles in cadherin switching, regulating Rho GTPases and stabilizing cadherins.

Key words: Cadherin, Biology, Switching

What's Due and What's Coming

- Primer Selection due Friday, March 17th.
- Final Experiment Proposal due Friday, April 7th.