Applications of cell culturing

Example of screening in CANCER

Apoptosis
(programmed cell death- PCD)

Necrosis

(A form of cell death characterized by
ATP and NAD^+ depletion, cell swelling, membrane rupture
and disorganized digestion of the cellular contents)

Continued…..

- Apoptosis or programmed cell death, is carefully coordinated collapse of cell, protein degradation , DNA fragmentation followed by rapid engulfment of corpses by neighbouring cells. (Tommi, 2002)

- Essential part of life for every multicellular organism from worms to humans. (Faddy *et al.,*1992)

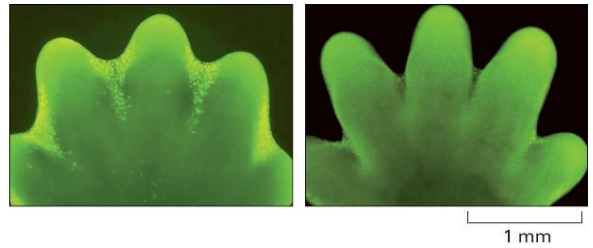
- Apoptosis plays a major role from embryonic development to senescence.

Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

Why should a cell commit suicide?

- **Apoptosis is needed for proper development**

  Examples:
  - The resorption of the tadpole tail
  - The formation of the fingers and toes of the fetus
  - The sloughing off of the inner lining of the uterus



- **Apoptosis is needed to destroy cells**

  Examples:
  - Cells infected with viruses
  - Cells of the immune system
  - Cells with DNA damage
  - Cancer cells

Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

What makes a cell decide to commit suicide?

Withdrawal of positive signals

examples :

- growth factors for neurons
- Interleukin-2 (IL-2)

Receipt of negative signals

examples :

- increased levels of oxidants within the cell
- damage to DNA by oxidants
- death activators :
  - Tumor necrosis factor alpha (TNF-α)
  - Lymphotoxin (TNF-β)
  - Fas ligand (FasL)

Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

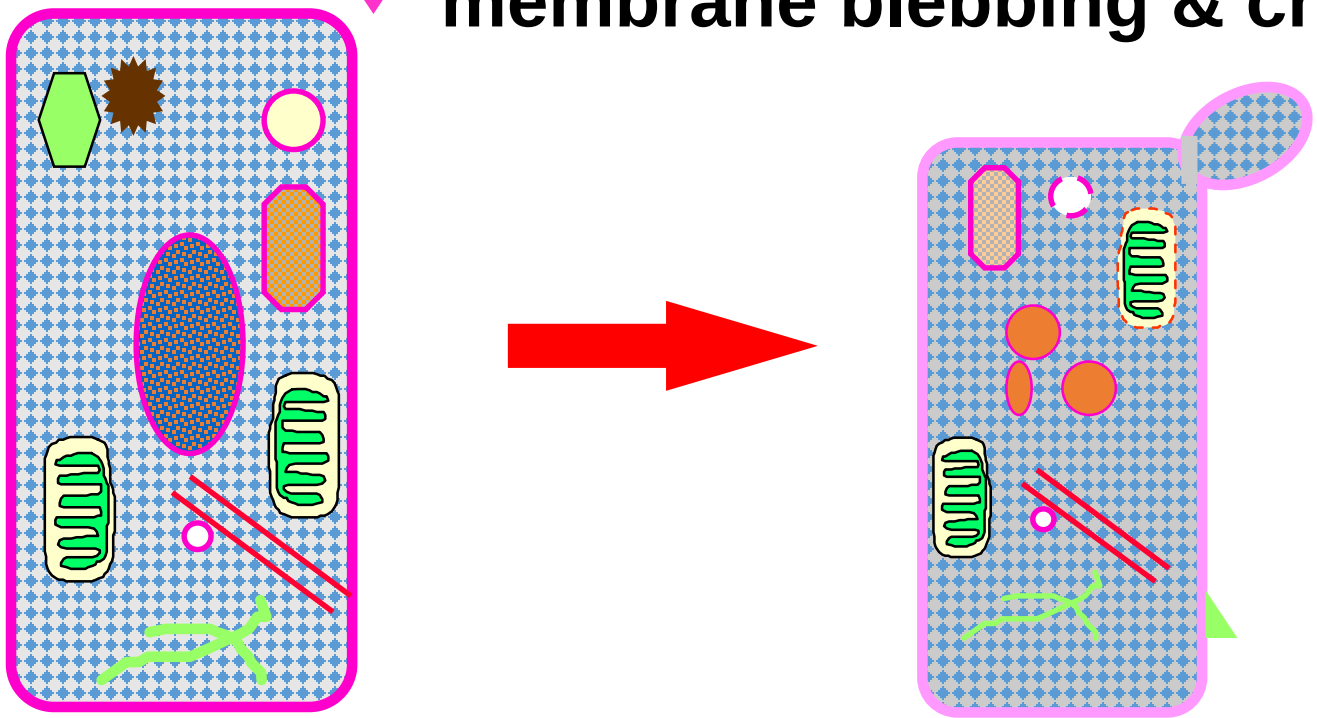
Necrosis *vs.* Apoptosis

Necrosis	Apoptosis
• Cellular swelling	• Cellular condensation
• Membranes are broken	• Membranes remain intact
• ATP is depleted	• Requires ATP
• Cell lyses, eliciting an inflammatory reaction	• Cell is phagocytosed, no tissue reaction
• DNA fragmentation is random, or smeared	• Ladder-like DNA fragmentation
• In vivo, whole areas of the tissue are affected	• In vivo, individual cells appear affected

Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

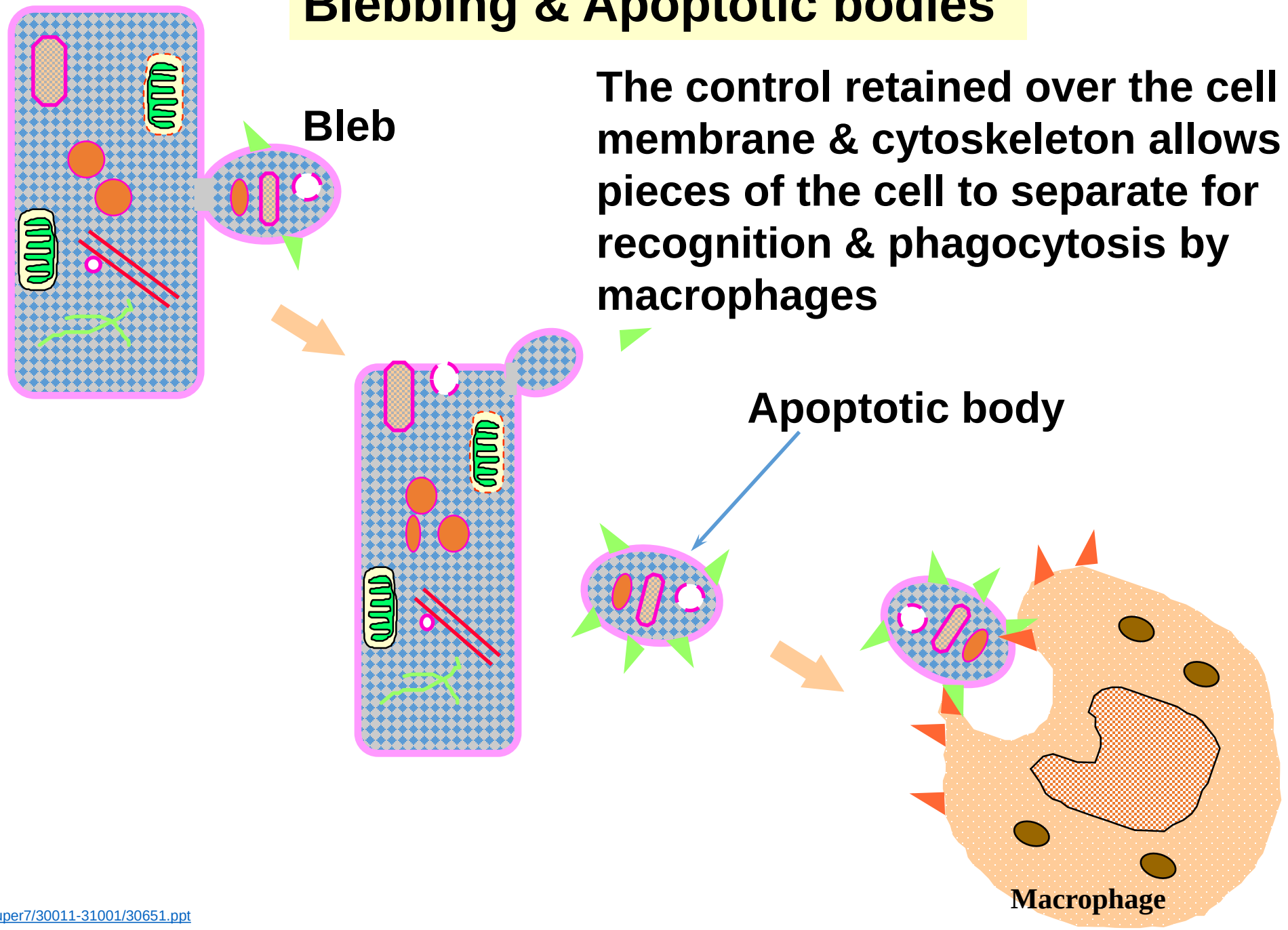
APOPTOSIS: Morphological events

- cell shrinkage
  - organelle reduction
    - mitochondrial leakage
      - chromatin condensation
        - nuclear fragmentation
          - membrane blebbing & changes



Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

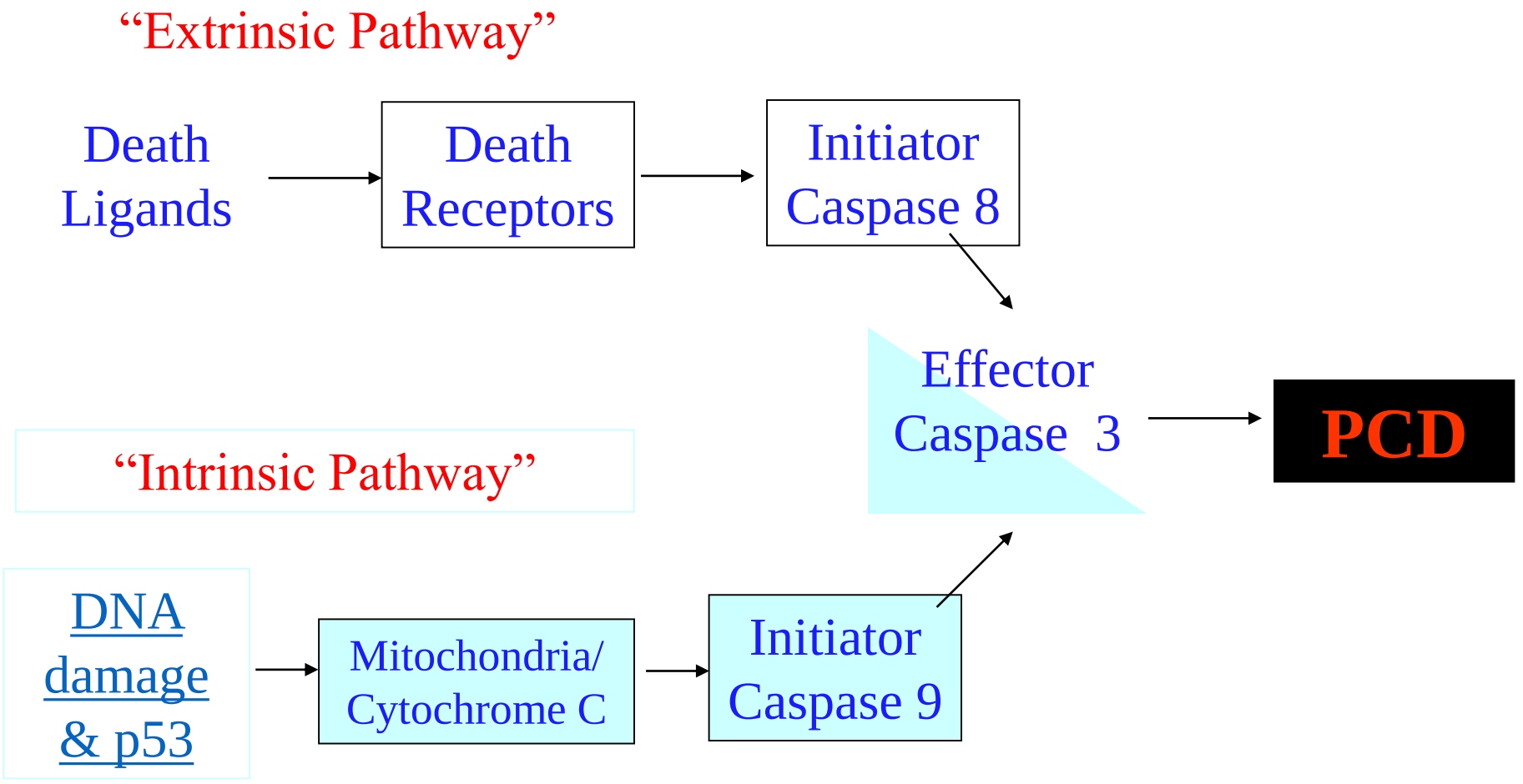
Blebbing & Apoptotic bodies



The control retained over the cell membrane & cytoskeleton allows intact pieces of the cell to separate for recognition & phagocytosis by macrophages

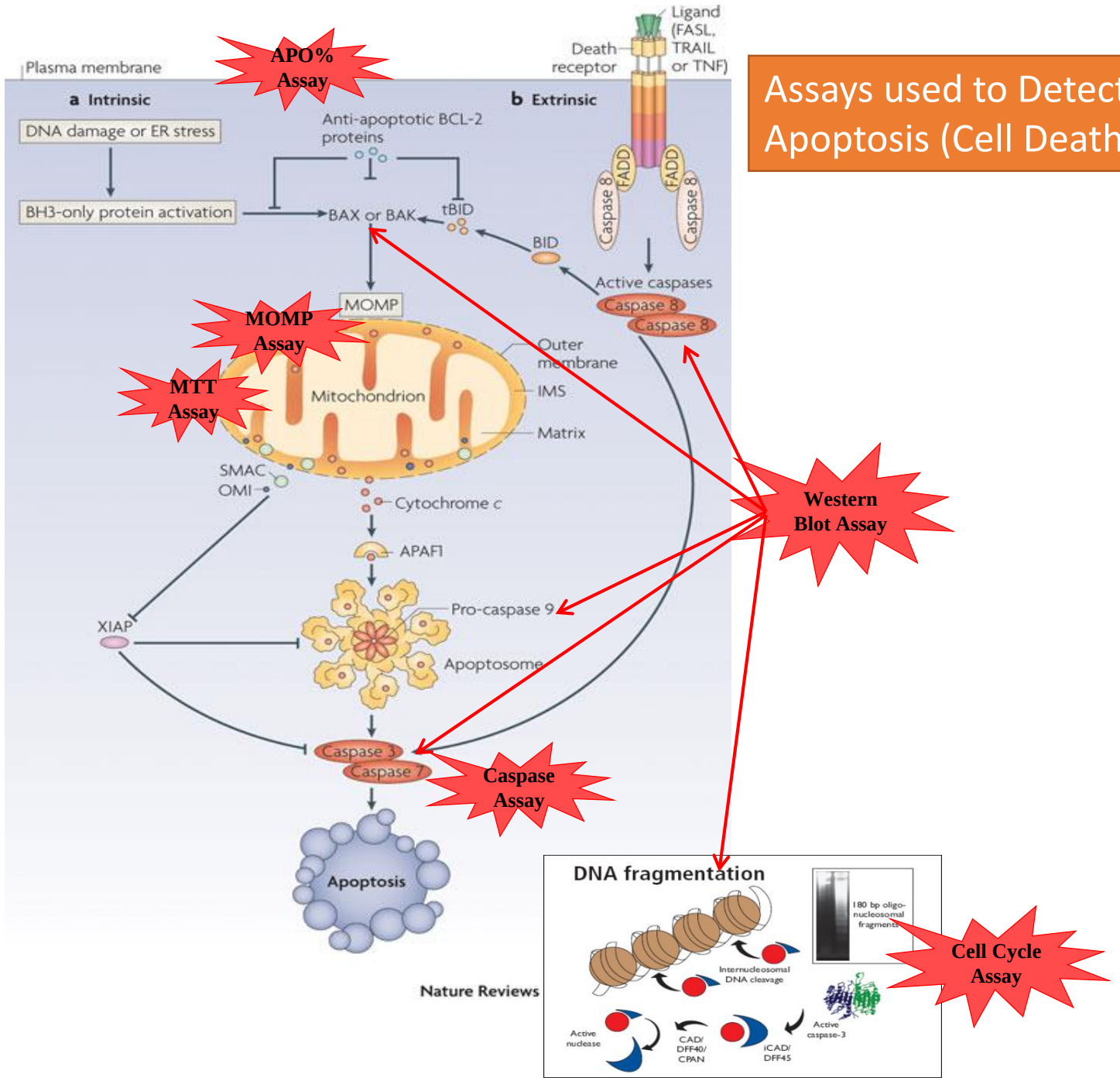
Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

Apoptosis: Pathways

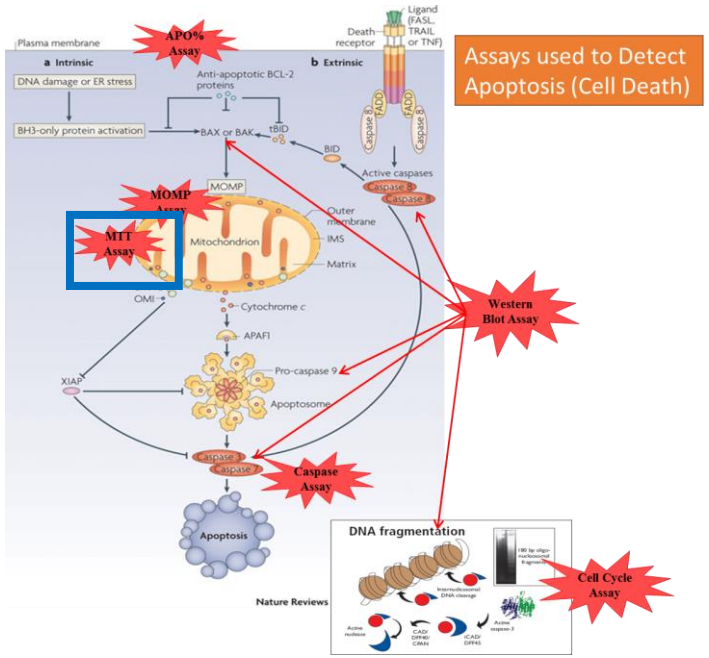


Courtesy: www.pitt.edu/~super7/30011-31001/30651.ppt

Mechanisms of Apoptosis and assays

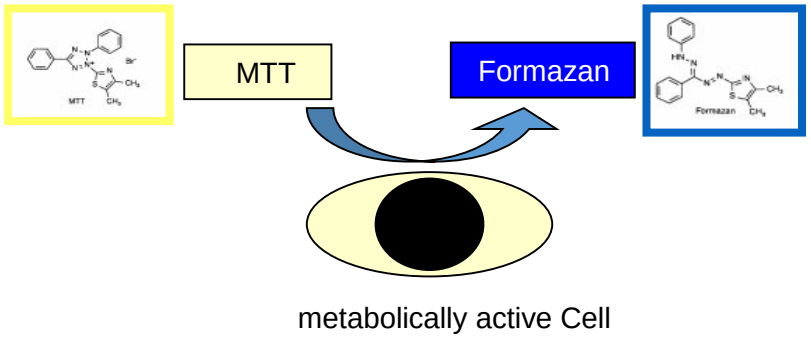


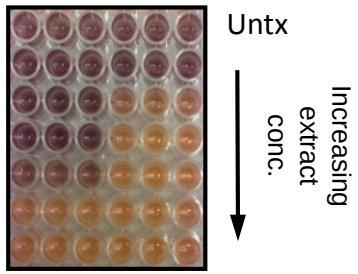
Assays used to Detect Apoptosis (Cell Death)

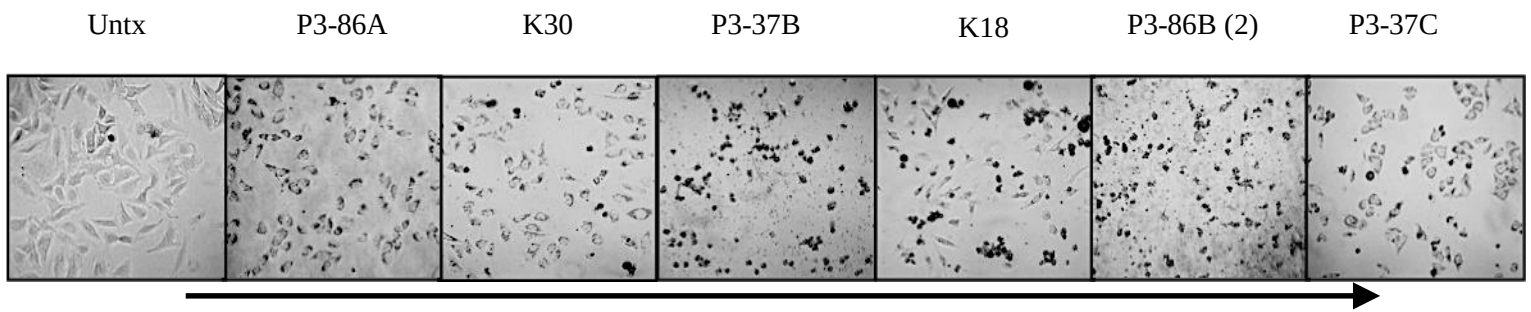


Example - Prescreening of bioactive extracts (Cytotoxicity or Growth Inhibition assay)

The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) into a dark blue formazan product, which is insoluble in water.







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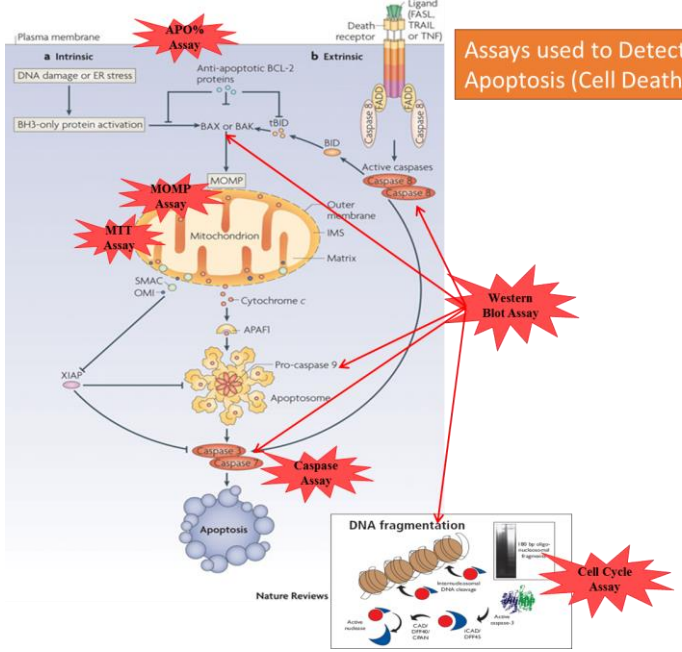
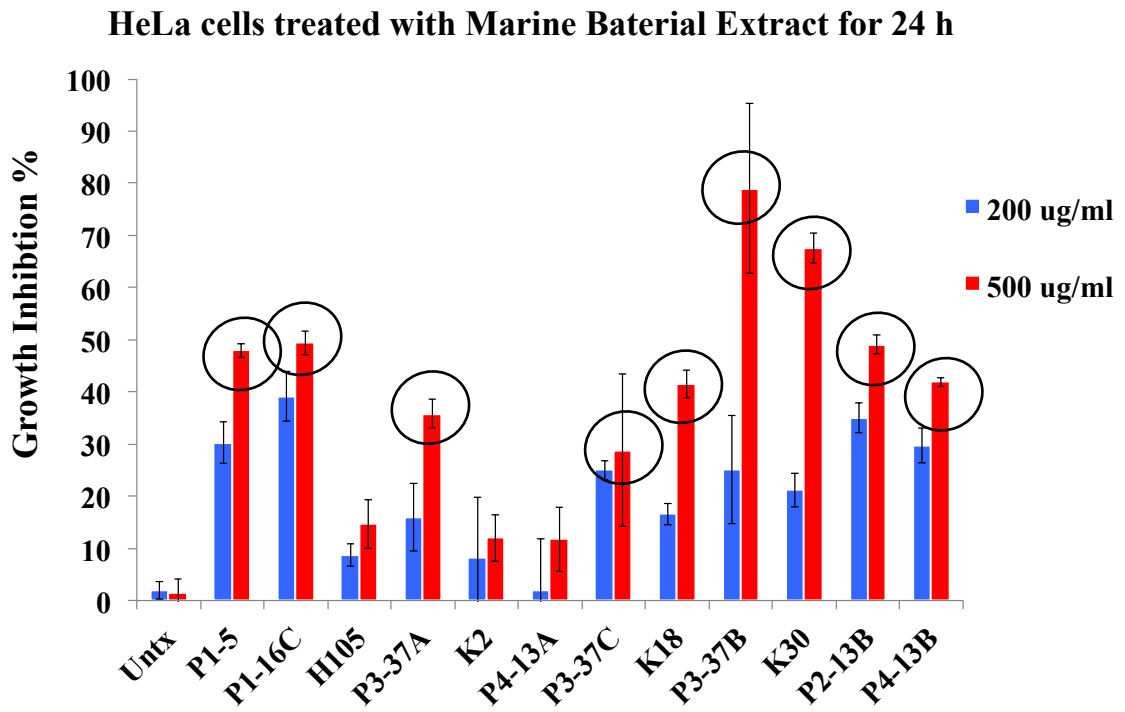


Growth Inhibition

Strains are selected based on their ability to inhibit cell growth



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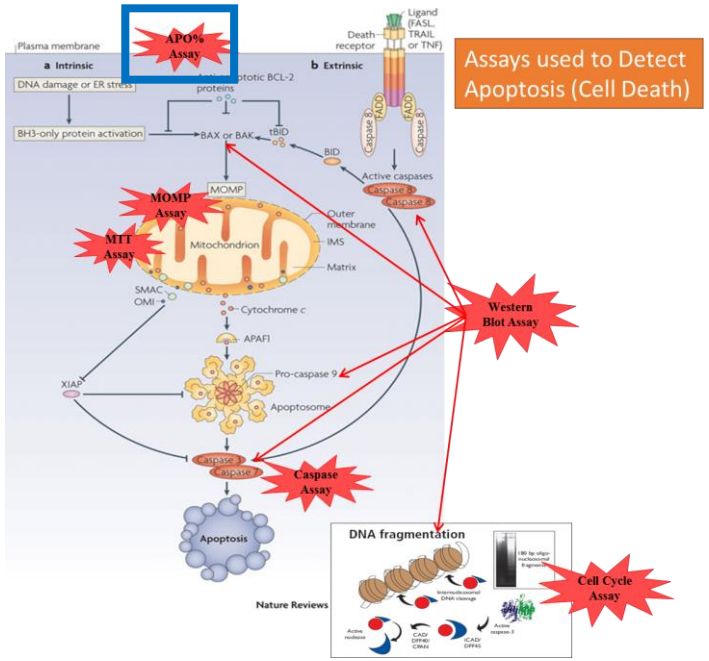
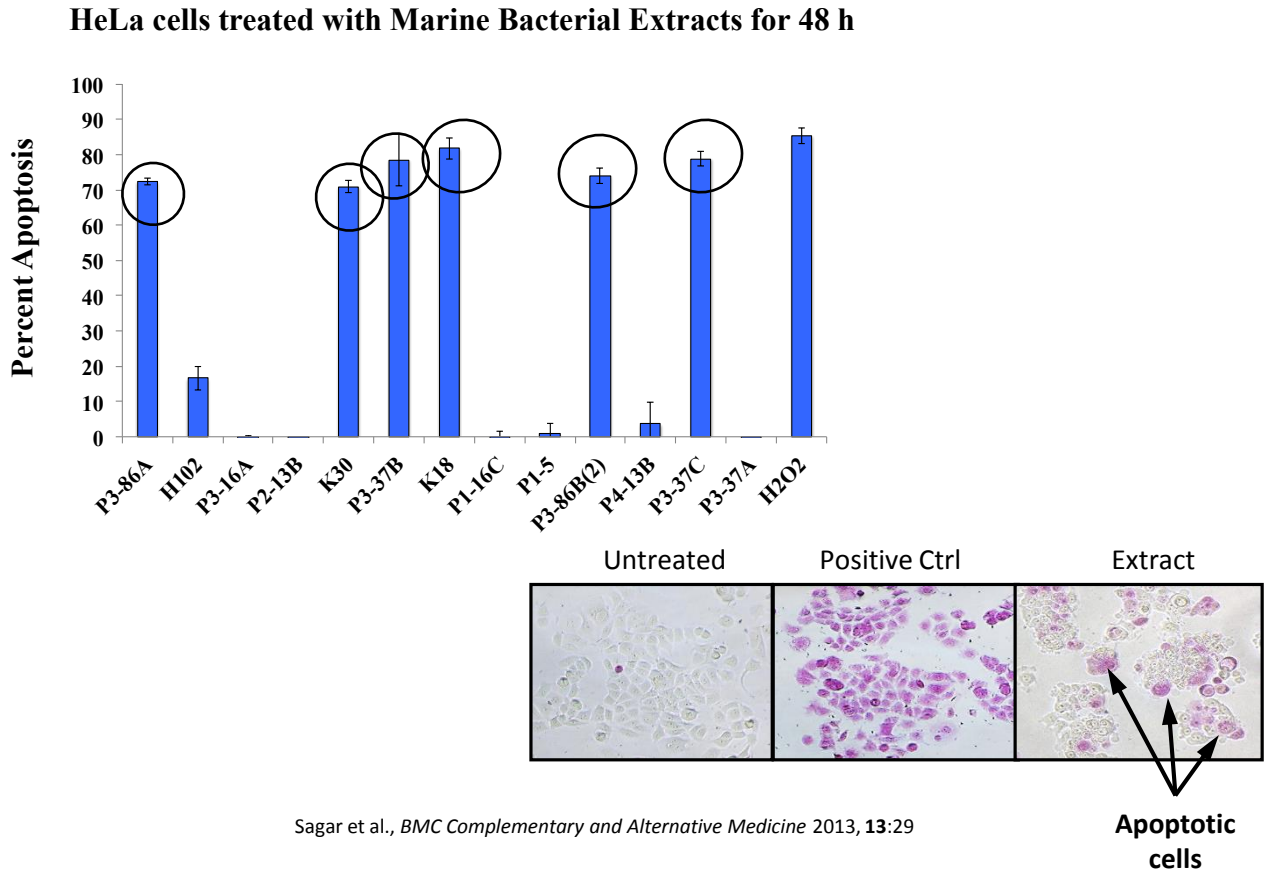


Estimating apoptotic activities of marine microbial extracts

APOPercentage dye binds only to the cells undergoing apoptosis and allows quantitative measurement of dead cells



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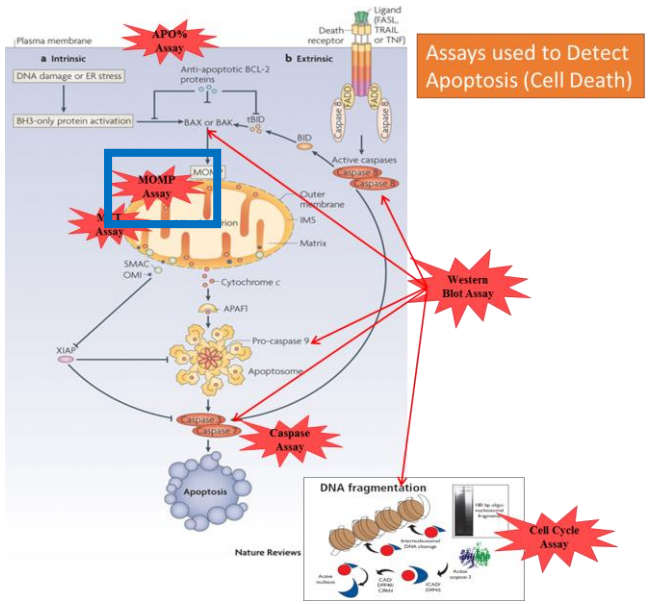


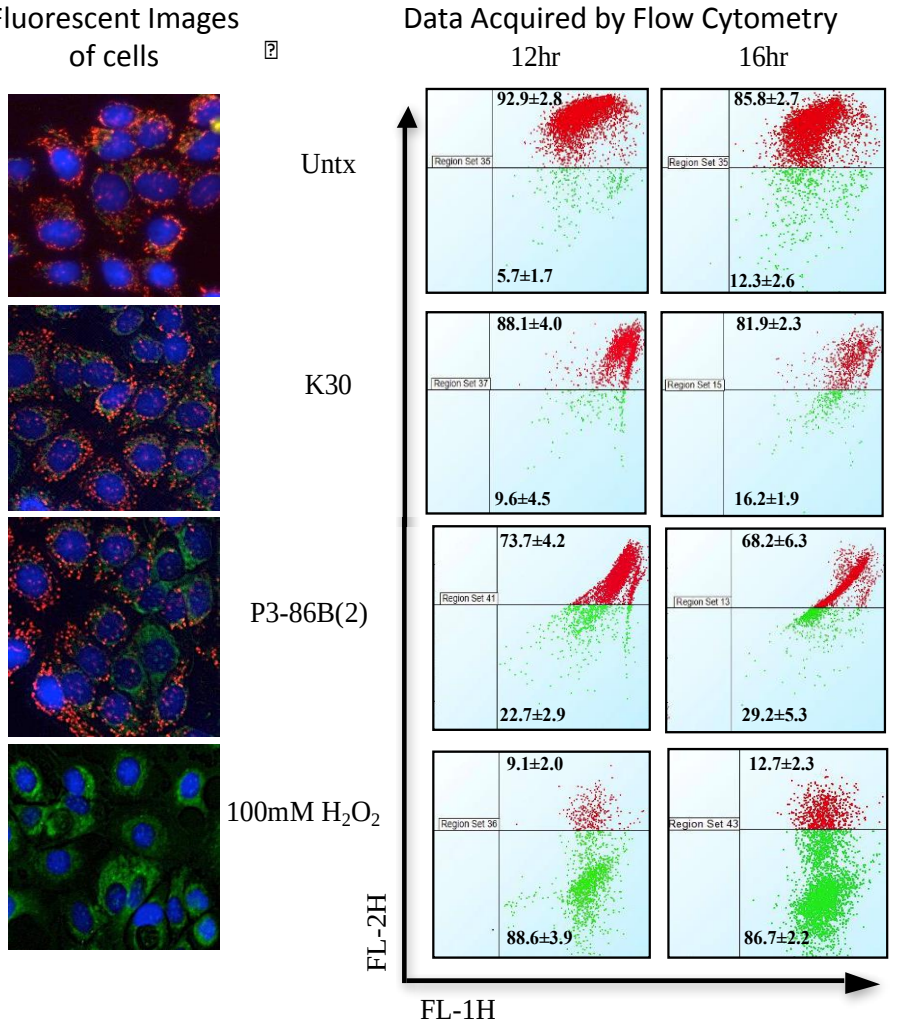
Mitochondrial Outer Membrane Potential (MOMP) in cells treated with microbial extracts

- MOMP is an indicator of cell health
- Measured with JC-1 dye –

red ➡ healthy mitochondria

green ➡ disrupted MOMP

- Disrupted MOMP leads to growth arrest and/or apoptosis



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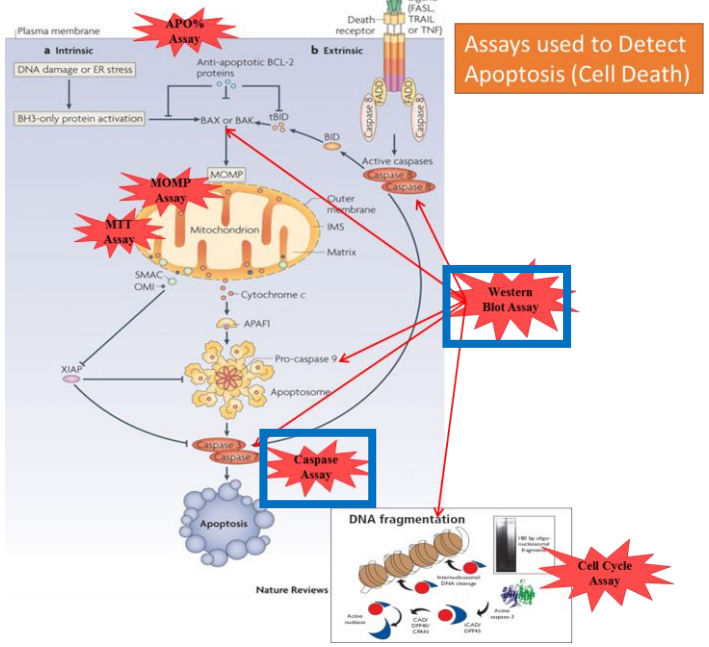
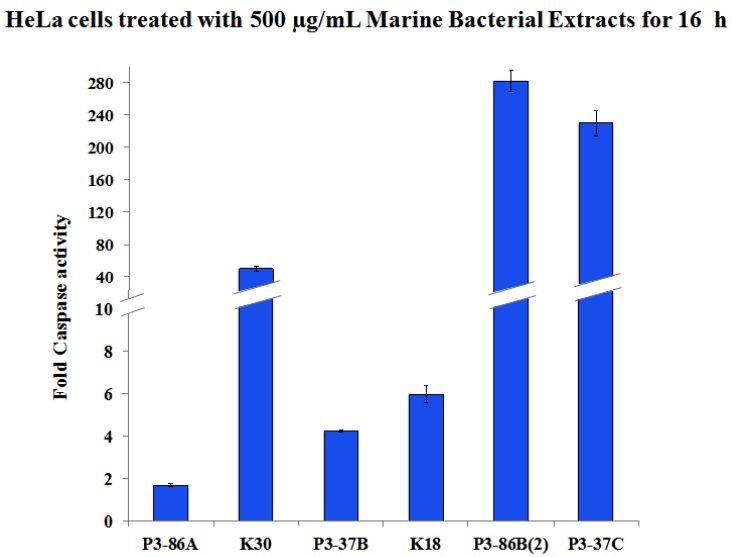
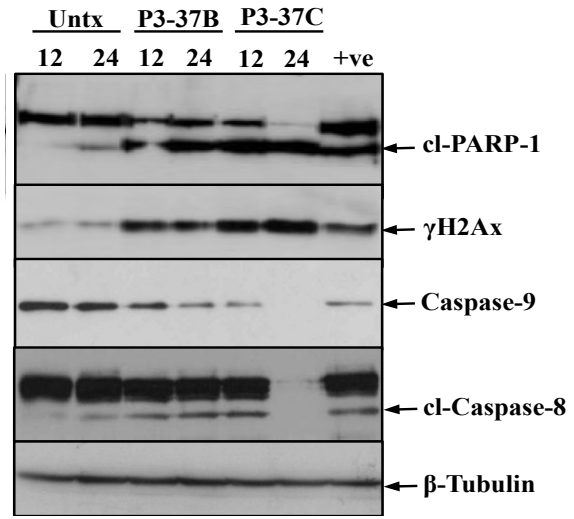


Caspase-3/7 Assay and Western Blotting

Caspase assay allows measurement of activity of enzymes involved in cell death

Western blotting measures expression of proteins involved in apoptosis





cysteine-aspartic proteases- specific cysteine protease activity – a cysteine in its active site nucleophilicly attacks and cleaves a target protein only after an aspartic acid residue

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Understanding mechanism of action

HeLa cells treated with 500 µg/mL concentration of extracts

Extract	PS exposure (percentage of stained cells)	Caspase-3/7 activity (fold change)	MOMP	PARP-1 cleavage	γH2Ax	Caspase-9 reduction	Caspase-8 cleavage
P3-86A	+ (73.95%)	+ (1.7)	+	+	-	-	+
K30	+ (86.74%)	+ (50.0)	-	-	+	+	-
P3-37B	+ (84.89%)	+ (4.2)	-	+	+	+	+
K18	+ (85.85%)	+ (5.9)	-	+	+	+	+
P3-86B(2)	+ (80.01%)	+ (282.1)	+	+	+	+	+
P3-37C	+ (84.77%)	+ (229.7)	-	+	+	+	+

'+' represents a positive observation, whereas '-'represents a negative observation

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