Apoptosis – the death of cells that promotes life

Order of Volvocales

unicellular

Chlamydomonas

Gonium

Pandorina morum

multicellular

Chlamydomonas
-like cells

Eudorina elegans

Pleodorina californica

Volvox carteri

Reproductive cells

Somatic cells

differentiation

cell growth

http://www.chembio.uoguelph.ca/edu
 cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Dictostelium discoideum

migrating slug
24mm
Slug (pseudo-plasmodium; grey)

14 hours
15 hours
16 hours
17 hours
18 hours
19 hours
20 hours
21 hours
22 hours
23 hours
24 hours

AGGREGATION
MIGRATION
CULMINATION

Dark
Illuminated area

No food

fruiting body
differentiation / Cell growth

stalk cells die

solitary amoebae, eating bacteria

gilbert 2.17
Apoptosis – the death of cells that promotes life

Frog

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Mouse development

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Developmental principles

**cell division**

1. Mitogens stimulate cell division

**cell growth + cell differentiation (specialization)**

2. Growth factors stimulate cell growth (increase in cell mass)

=> The term growth factor is often used inappropriately

**cell number + size**

determine the 3 dimensional shape + total cell mass of an organism

molecular biology of the cell
Apoptosis – the death of cells that promotes life

Developmental principles

cell division & cell growth & cell differentiation

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

C. elegans fate map
Programmed Cell Death (PCD) is a term originally used to describe cells that die at predictable time and places during development.
Apoptosis – the death of cells that promotes life

Programed cell death in C. elegans

_Egl1_ (egg laying defective)
- gain of function mutation causing unscheduled death of two neurons innervating the vulva, and hence the egg laying defect.

_ced-4_ (cell death determine)
- cell death determining factor

_ced-3_
- found to induce cell death

_ced-9_
- promotes survival of cells

_ced-1,-2,-5,-6,-7,-10_
- phagocytosis or clean-up genes

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Genes involved in PCD – gain and loss of function experiments

- ced-9 → ced-4
- egl-1 → ced-9 × ced-4
- ced-4 → ced-3
- Ced-1, 2, 5, 6, 7, 10

molecular biology of the cell 4th edition
Apoptosis – the death of cells that promotes life

Development - PCD

- **cell division**
- **cell growth**
- **cell death**

1. **Mitogens**
2. **Growth factors**
3. **Survival + death factors**

Determine the 3 dimensional shape + total cell mass of an organism

molecular biology of the cell
Apoptosis – the death of cells that promotes life

PCD in frog development

Herbivores, water
- large intestine
- horny teeth
- gills regress

Carnivores, land
- short
- jaw
- fly-catching tongue
- lungs enlarge

Herbivores, water
tadpole
hindlimb growth
forelimb growth
Tail regression (PCD)
adult bullfrog

Carnivores, land

Gilbert 2.4
Apoptosis – the death of cells that promotes life

PCD in limb development

(A) DUCK LEG PRIMORDIUM
Minimal cell death

interdigital tissue - web

Area of cell death

(B) CHICK LEG PRIMORDIUM
Extensive cell death

Anterior zone

Posterior zone

Interior zone

Gilbert
Apoptosis – the death of cells that promotes life

PCD in limb development of the mouse

Defect in apoptosis
Apoptosis – the death of cells that promotes life

PCD in brain development of the mouse

Caspase9

Apoptosis defect
Apoptosis – the death of cells that promotes life

PCD versus apoptosis

- **Programmed Cell Death (PCD)** is a term originally used to describe cells that die at predictable times and places during development.

- **Apoptosis** describes the controlled dying of cells which contrast with necrosis.

- Since nearly all PCD is apoptotic these terms are sometimes used interchangeably.
Apoptosis – the death of cells that promotes life

Apoptosis in the immune system

<-molecular biology of the cell24F3,
BoIS->
Apoptosis – the death of cells that promotes life

Apoptosis in the immune system, T-cell development

Maturation

Detection of foreign antigen but not of self antigen

molecular biology of the cell24f6
Apoptosis – the death of cells that promotes life

Apoptosis in the immune system, T-cell development

Apoptosis ensures that only T-cells with no or weak recognition of self-proteins will survive
=> no autoimmune defects in the normal organism

molecular biology of the cell24f61
Apoptosis – the death of cells that promotes life

Apoptosis in the immune system, T-cell development

Thymus section
red for apoptotic cells
black for macrophages
Anxa5 Deficiency - Spleen

Cell removal of apoptotic cells by phagocytes

Lymphocyte cultures (7d in culture)
Apoptosis – the death of cells that promotes life

Apoptosis and cell removal

**C. elegans**

1. Cell commits suicide
2. Dying cell is engulfed by neighbor
3. Corpse is digested, leaving no trace

**Mammals**

1. **Apoptosis**
   - Mild convolution
   - Chromatin compaction and segregation
   - Condensation of cytoplasm
2. Nuclear fragmentation
3. Blebbing
4. Apoptotic bodies
5. Phagocytosis
6. Apoptotic body
7. Phagocytic cell

*Source: Molecular Biology of the Cell 4th Edition*
Apoptosis – the death of cells that promotes life

Apoptosis versus necrosis

**controlled**

**APOPTOSIS**
- Mild convolution
- Chromatin compaction and segregation
- Condensation of cytoplasm

- Nuclear fragmentation
- Blebbing
- Apoptotic bodies

- Phagocytosis
- Apoptotic body
- Phagocytic cell

**uncontrolled**

**NECROSIS**
- Chromatin clumping
- Swollen organelles
- Flocculent mitochondria

- Disintegration
- Release of intracellular contents
- Inflammation

molecular biology of the cell
# Apoptosis – the death of cells that promotes life

## Features of apoptosis versus nekrosis

**Apoptosis:**
- A normal physiological response to specific suicide signals, or lack of survival signals.

**Nekrosis:**
- A pathological response to cellular injury

### Apoptosis:
- Chromatin condenses, laddering of DNA
- Cytoplasm shrinks without membrane rupture
- Blebbing of plasma and nuclear membranes
- Cell contents are packaged in vesicles, internal organelles still functioning
- Epitopes appear on plasma membrane marking cell as a phagocytic target

### Nekrosis:
- Chromatin clumps
- Plasma membrane lyses
- Cell contents spill out, mitochondria swell and rupture
- General inflammatory response is triggered
Apoptosis – the death of cells that promotes life

Induction of apoptosis

- Genetically determined apoptosis in development
- Withdrawal of growth factors
- Withdrawal of survival factors
- Elimination of lymphocytes recognising self proteins.
- Killing of infected or tumor cells by T-lymphocytes
- DNA Damage (e.g. UV exposure)

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Mechanism of apoptosis

2620 Hay
Apoptosis – the death of cells that promotes life

Caspases – Cys catalytic Asp targeting proteases

1. each caspase is synthesized as a zymogen that contains an N-terminal prodomain, a large subunit and a small subunit.

2. Maturation involves caspase-mediated cleavage at aspartate residues

3. each mature active caspase is an \( \alpha_2\beta_2 \) tetramer comprising two identical large (17–22 kDa) and two identical small (10–12 kDa) subunits

4. Procaspase can be a substrate for the active caspase, and autacatalytic activation is common among caspases. positive feedback loop

kaufmann review
Apoptosis – the death of cells that promotes life

Caspases – the activation cascade

molecular biology of the cellch1738
Apoptosis – the death of cells that promotes life

In vivo substrates of effector caspases

Nuclear: Lamins, nucleoplasmin, the SR protein 70K U1, hnRNP C, RNA Pol I, upstream binding factor, p53, regulator MDM2, pRB, p27 Kip and p21Cip

DNA related: MCM3, Repair enzymes including Rad51, poly-ADP-ribose polymerase (PARP), topoisomerase, inhibitor of caspase activated DNase, (iCAD/DFF45)

Cytoskeleton: actin, gelsolin, spectrin, keratin

Cytoplasmic: β-catenin, Bcl-2

Protein kinases: DNA dependent protein kinase, protein kinase C, CAM kinase, focal adhesion kinase, MAP and ERK kinases, Raf1, Akt1/protein kinase B, ROCK I.

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Overview caspase family

Initiator caspases (-2, -8, -9, -10)
- long prodomains with homotypic protein–protein interaction motifs:
  - death effector domain (DED)
  - caspase activation and recruitment domain (CARD)
- for the transduction of various signals into proteolytic activity

Effector caspases (3, -6, -7)
- short prodomains, lack of intrinsic enzymatic activity
  - Proteolytic activation by caspase-mediated cleavage
  - cleave most known apoptotic substrates

Cytokine maturation caspases (-1, -4, -5, -11, -12, -13, -14)
- involved in cytokine maturation
Apoptosis – the death of cells that promotes life

In vivo effects of effector caspases

- general disablement of DNA related processes such as replication, repair and transcription

  cleavage of *iCAD*, the inhibitor of *CAD*, Caspase Activated DNase or DNA fragmentation factor

  ⇒ DNA cleavage at internucleosomal linker regions leads to laddering of DNA at the nucleosomal repeat length, ca. 200 bp.
  ⇒ Chromatin condenses and migrates to nuclear membrane.

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

In vivo effects of effector caspases

• cytoskeleton reorganization, weakening of adhesion to neighbouring cells, disruption of key signalling pathways

Caspase mediated cleavage converts the rho-activated protein kinase ROCK I, making it constitutively active instead of rho-dependent.

=> promotes myosin light chain phosphorylation and contractile activity of actomyosin
=> characteristic membrane blebbing effect of apoptosis (Coleman et al., 2001).
Apoptosis – the death of cells that promotes life

In vivo substrates of effector caspases

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Apoptosis – the death of cells that promotes life

Activation of the effector caspases

Type I or receptor mediated pathway
- lymphoid cell lines

Type II or mitochondrial mediated pathway
- All other cells
Apoptosis – the death of cells that promotes life

Receptor mediated apoptosis

Janeway + Molcellbiol
Apoptosis – the death of cells that promotes life

Receptor mediated apoptosis

caspase activation and recruitment domain (CARD)
deathefector domain (DED)

Janeway + Kaufmannreview
Apoptosis – the death of cells that promotes life

Mitochondrial mediated apoptosis

Receptor mediated apoptosis

Mitochondrial mediated apoptosis

Janeway + Molcellbiol
Apoptosis – the death of cells that promotes life

Mitochondrial mediated apoptosis

Janeway + Molcellbiol
Apoptosis – the death of cells that promotes life

Mitochondrial mediated apoptosis

- Apoptosis
- CARD
- WD40
- autoinhibited form of Apaf-1
- Aoptotic protease activating factor - 1
- Cleavage of procaspase 3
- => Apoptosis
Apoptosis – the death of cells that promotes life

Receptor + mitochondrial apoptotic pathways
Apoptosis – the death of cells that promotes life

T-cell mediated apoptosis of a tumor cell

cytotoxic T cell

tumor cell
Apoptosis – the death of cells that promotes life

PCD in limb development

Gruß Apaf1
Apoptosis – the death of cells that promotes life

Defects in apoptosis pathways affect embryonal development

Apaf-1 KO

Caspase-9 KO
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control

The Bcl-2 Family

Protein

<table>
<thead>
<tr>
<th>Protein</th>
<th>size (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2</td>
<td>239</td>
</tr>
<tr>
<td>Bcl-x_L</td>
<td>233</td>
</tr>
<tr>
<td>Bcl-w</td>
<td>193</td>
</tr>
<tr>
<td>A1</td>
<td>172</td>
</tr>
<tr>
<td>Mcl-1</td>
<td>350</td>
</tr>
<tr>
<td>Bax</td>
<td>191</td>
</tr>
<tr>
<td>Bax/Net</td>
<td>192</td>
</tr>
<tr>
<td>Bcl-x_S</td>
<td>170</td>
</tr>
<tr>
<td>Bak</td>
<td>211</td>
</tr>
<tr>
<td>Bad</td>
<td>197</td>
</tr>
<tr>
<td>BIK/Nbk</td>
<td>160</td>
</tr>
<tr>
<td>Bid</td>
<td>195</td>
</tr>
<tr>
<td>Hrk/OP5</td>
<td>91</td>
</tr>
<tr>
<td>Bik</td>
<td>150</td>
</tr>
<tr>
<td>Bim</td>
<td>196</td>
</tr>
</tbody>
</table>

http://www.chembio.uoguelph.ca/edu/cmatochm736/apoptosis.htm
**Apoptosis – the death of cells that promotes life**

*Keeping apoptosis under control - Bcl2 family*

**Group I: anti-apoptotic proteins**

(Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1/Bfl1, Boo/Diva and Nrf3)

- four short, conserved **Bcl-2 homology (BH) domains**, known simply as BH1–BH4.
- a C-terminal **hydrophobic tail** attachment to the cytosolic surface of intracellular membranes (outer mitochondrial membrane and endoplasmic reticulum).

**Function:**

- **prevent cell death**
- by binding to and sequestering pro-apoptotic Bcl-2 family members from groups II and III
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control - Bcl2 family

Group II: pro-apoptotic proteins
(Bax, Bak and Bok/Mtd)

- similar in structure and sequence to group I proteins
- but lack of the N-terminal BH4 domain

Function:

- Bax and Bak are profoundly promote cell death
- actively induce release of cytochrome c from mitochondria
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Bcl2 family

Bcl2 deficiency in the mouse – increase in apoptosis:
- small size,
- increased postnatal mortality,
- polycystic kidneys, apoptotic
- involution of thymus and spleen,
- reduced numbers of motor, sympathetic and sensory neurons

Bax deficiency in the mouse – decrease in apoptosis:
- hyperplasia of lymphocytes
- reproductive failure with abnormal germ cells and gonadal morphology
- reduced cell death in the CNS

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Bcl2 family

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Balance of factors
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control - Bcl2 family

Group III: apoptotic mediator proteins
(Bid, Bad, Bik, Bim, Blk, Bmf, Hrk, Bnip3, Nix, Noxa and Puma)
• contain a single BH3 domain.

Function:
• act by binding to Group I and/or Group II proteins via their BH3 domains
• respond to a wide variety of pro-apoptotic stimuli (removal of trophic factors to cytoskeletal alterations to DNA damage),
• Interpretation of pro and anti-apoptotic signals into a single life-versus-death output.

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Bcl2 family

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Bcl2 family

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Bcl2 family

Group I: anti-apoptotic proteins
INHIBITORS

Group II: pro-apoptotic proteins
ACTIVATORS

Group III: apoptotic MEDIATOR proteins

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – Bcl2 family

Kaufmann
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – IAPs, Inhibitors of apoptosis

http://www.chembio.uoguelph.ca/edu/cmats/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – IAPs, Inhibitors of apoptosis

(baculovirus IAP repeat domains)
zinc-finger-like BIRs bind to the surfaces of caspases, allowing sequences between the BIRs to block the catalytic grooves of target enzymes

RING domains can act as ubiquitin ligases, facilitating ubiquitination and presumed proteasomal degradation of the bound caspases

Catalytic blockade of caspases
Degradation of caspases
Binding of caspases

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – hsp70

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Keeping apoptosis under control – hsp70, 90

Hsp70

Sequestration of Apaf1

Inhibition of apoptosome assembly

Inhibition of Caspase 9 recruitment

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Summary – apoptosis in mammals

**Apoptosis** describes the controlled dying of cells.

- **Genetically** determined in development
- Withdrawal of **growth factors**
- Withdrawal of **survival factors**
- **Elimination** of lymphocytes recognising self proteins.
- **Killing of infected or tumor cells** by T-lymphocytes
- **DNA Damage** (e.g. UV exposure)
Apoptosis – the death of cells that promotes life

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*Bcl2 Family - regulates cytochrom C release*
Apoptosis – the death of cells that promotes life

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*Bcl2 Family* - regulates cytochrom C release

*IAP Family* - regulates caspase activity
Apoptosis – the death of cells that promotes life

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**IAP Family** - regulates caspase activity

**Hsp Family** - regulates apoptosome assembly
Apoptosis – the death of cells that promotes life

Summary

Apoptosis:

a normal physiological response to specific suicide signals, or lack of survival signals.

- Chromatin condenses, laddering of DNA
- Cytoplasm shrinks without membrane rupture
- Blebbing of plasma and nuclear membranes
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- Epitopes appear on plasma membrane marking cell as a phagocytic target

Nekrosis:

a pathological response to cellular injury

- Chromatin clumps
- Mitochondria swell and rupture
- Plasma membrane lyses
- Cell contents spill out
- General inflammatory response is triggered
Apoptosis – the death of cells that promotes life

Summary

Apoptosis is essential for the development:

- Xenopus tail regression
- Limb buds interdigital tissue
- Brain development
- Immune system homeostasis
- Immune defence

molecular biology of the cell
Apoptosis – the death of cells that promotes life

Detection of apoptosis

Peripheral B-Cell Cultures (7d in culture)
Apoptosis – the death of cells that promotes life

Detection of apoptosis, in vivo by macrophages

Macrophage
## Apoptosis – the death of cells that promotes life

### Detection of apoptosis in the laboratory

<table>
<thead>
<tr>
<th>Early</th>
<th>Late events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-caspase cleavage:</td>
<td></td>
</tr>
<tr>
<td>• initiator caspase cleavage by Western blot analysis</td>
<td></td>
</tr>
<tr>
<td>• Immunohistochemistry</td>
<td></td>
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<tr>
<td><strong>Annexin V staining:</strong></td>
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<tr>
<td>• FACS analysis using Annexin V conjugated to a fluorescent dye to detect PS on the outer membrane</td>
<td></td>
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<tr>
<td>Pro-caspase cleavage:</td>
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<td>• Immunohistochemistry</td>
<td></td>
</tr>
<tr>
<td><strong>Death substrate cleavage:</strong></td>
<td></td>
</tr>
<tr>
<td>• PARP, nuclear lamin, ICAD cleavage by Western blot analysis</td>
<td></td>
</tr>
<tr>
<td><strong>DNA fragmentation:</strong></td>
<td></td>
</tr>
<tr>
<td>• inter chromatin fragmentation by agarose gel electrophoresis (&quot;DNA ladder&quot;)</td>
<td></td>
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<tr>
<td>• DNA fragmentation by TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling)</td>
<td></td>
</tr>
<tr>
<td><strong>Loss of plasma membrane integrity:</strong></td>
<td></td>
</tr>
<tr>
<td>• loss of dye exclusion (eg., trypan blue, PI-staining</td>
<td></td>
</tr>
<tr>
<td><strong>Morphological</strong> (phase contrast microscopy, electron microscopy):</td>
<td></td>
</tr>
<tr>
<td>• nuclear blebbing</td>
<td></td>
</tr>
<tr>
<td>• cell shrinkage</td>
<td></td>
</tr>
</tbody>
</table>
Apoptosis – the death of cells that promotes life

Detection of apoptosis, in vivo by macrophages
Apoptosis – the death of cells that promotes life

Detection of apoptosis – membrane architecture

Phospholipids are asymmetrically distributed in the membrane

Phosphatidyl-

-ethanolamine -serine -choline sphingomyelin

MBOC 10-14, 10-12
Apoptosis – the death of cells that promotes life

Detection of apoptosis – changes in membrane architecture during apoptosis

**Early apoptotic event**
- Phosphatidylserine rearrangement

**late apoptotic/necrotic event**
- Loss of plasma membrane integrity:
  - phosphatidylserine
  - other phospholipids
  - DNA
  - Nucleus
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Anxa5 as an early marker for apoptosis

Early apoptotic event
Phosphatidylserine rearrangement

phosphatidylserine  other phospholipids
Annexin 5  fluorescent dye

Anxa5 (+)
Apoptosis – the death of cells that promotes life

Detection of apoptosis – changes in membrane architecture during apoptosis

viable cell  early apoptotic cell  late apoptotic cell / necrotic cell

No ANXA5/PI  Anxa5 (+)  Anxa5 (+)

phosphatidylserine  other phospholipids  Annexin 5

fluorescent dye (Fitc, Alexa 488)  PI (Propidium iodide)  => DNA

BB
Apoptosis – the death of cells that promotes life

Detection of apoptosis – PI as a late marker for apoptosis & necrosis

**viable cell**

**early apoptotic cell**

**late apoptotic cell / necrotic cell**

No ANXA5/PI → Anxa5 (+) → Anxa5 (+) PI (+)

**phosphatidylserine**

other phospholipids

**Annexin 5**

**fluorescent dye**

(Fitc, Alexa 488)

**PI**

(Propidium iodide)

=> DNA
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, Forward scatter (FSC)
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry FSC Forward scatter (FSC)

Cell size
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry Forward scatter (FSC)
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, Side scatter (SSC)

Cell granularity and surface
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, detection of fluorochroms
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, viable cells

1. Size + form + granularity

Flow cell

Anxa5 (++)
Early + late apoptosis

PI (+)
Late apoptosis + necrosis

Viable cells

Laser

PI (+)
A5 (+)
Late apoptosis + necrosis

Anxa5 Fite

PI

PI (+)
A5 (+)
early apoptosis

basicflowcytometry.ppt
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, early apoptotic cells

Flow cell

Laser

PI

Anxa5 FITC

early apoptosis
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, early apoptotic cells

Flow cell

Laser

PI

Anxa5 FITC

Late apoptosis + necrosis
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, UV induction of apoptosis

Flow cell

Anxa5 Fitc

PI

unstained

3h

6h

24h

After UV induction

Laser

viable

early

late + necrosis

basicflowcytometry.ppt
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, UV induction of apoptosis

Anxa5/Pi staining in flow cytometry is a tool for detecting viable, early apoptotic and late apoptotic & necrotic cells

Flow cell

basicflowcytometry.ppt
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, UV induction of apoptosis

DNA Damage → Damage Response → p53 → Procaspsase-9

UV light → Repair → p21^{CIP} → Arrest → G1-Block

Bax ↓ Bcl-2

Kaboom

Apoptosis

http://www.chembio.uoguelph.ca/edu/cmat/chm736/apoptosis.htm
Apoptosis – the death of cells that promotes life

Detection of apoptosis – Flow cytometry, UV induction of apoptosis

1. untreated
   - unstained
   - ANXA5-Fitc & PI

2. 56°C for 30 min
   - unstained
   - ANXA5-Fitc & PI

3. UV light 180 sec, t= ~30 to 60 min
   - ANXA5-Fitc & PI

4. UV light 180 sec, t= ~200 to 300 min
   - ANXA5-Fitc & PI

viable = viable?
controls
dead = dead?
samples
Apoptosis – the death of cells that promotes life

Detection of apoptosis – How to determine the cell numbers

Neubauer counting chamber

1/10 dilution:
10µl cell suspension + 90 µl PBS

16 cells x 10(dil) x 10000 = ? 1.6 x 10^6 cells / ml
(1,600,000)