Programmed Cell Death (Apoptosis)

Programmed cell-death (PCD) is death of a cell in any form, mediated by an intracellular program

(Trends in Cell Biology)
The number of cells in this community is tightly regulated not simply by controlling the rate of cell division, but also by controlling the rate of cell death.

In humans, approx. $10^{10}-10^{11}$ cells die every day

For every cell, there is a time to live and a time to die. There are two ways in which cells die:

- They are killed by injurious agents.
- They are induced to commit suicide.

If cells are no longer needed, they commit suicide by activating an intracellular death program. This process is therefore called programmed cell death.
“DEATH” is important to the “LIFE”

- Apoptosis (programmed cell death): a physiological process of cellular autodestruction, or cell suicide.
- Strictly controlled in response to integrity of prodeath signalling.
- Critical roles of apoptosis in development, maintenance of homeostasis and host defence in multicellular organisms.
CELL DEATHS

Necrosis: Death by Injury
  
  Cells are damaged by injury, such as
  
  - Mechanical damage
  - Exposure to toxic chemicals

Programmed Cell Death: Cell Suicide

  Cells are induced to commit suicide

Types of PCD

- Apoptosis or Type I cell-death
- Autophagic or Type II cell-death

(Cytoplasmic: characterized by the formation of large vacuoles which eat away organelles in a specific sequence prior to the nucleus being destroyed)
Necrosis

- Passive cell death;
- Cell swells up;
- Plasma membrane breaks up and cellular contents leak out (because the ability of the plasma membrane to control the passage of ions and water is disrupted);
- Nucleus disintegrates;
- Cell ghosts;
- Tissue damage due to inflammation
**Apoptosis**

- Active cell death;
- Cell shrinks;
- Develop bubble-like blebs on their surface;
- Have the chromatin (DNA and protein) in their nucleus degraded;
- Have their mitochondria break down with the release of cytochrome c;
- Break into small, membrane-wrapped, fragments.
- The phospholipid phosphatidylserine (normally hidden within the plasma membrane) is exposed on the surface.
  - This is bound by receptors on phagocytic cells like macrophages and dendritic cells which then engulf the cell fragments.
- The phagocytic cells secrete cytokines that **inhibit** inflammation (e.g., IL-10 and TGF-β)
Features of Apoptosis Vs Necrosis

**Apoptosis**
- Chromatin condensation
- Cell Shrinkage
- Preservation of Organelles and cell membranes
- Rapid engulfment by neighboring cells preventing inflammation
- Biochemical Hallmark: DNA FRAGMENTATION

**Necrosis**
- Nuclear swelling
- Cell Swelling
- Disruption of Organelles
- Rupture of cell and release of cellular contents
- Inflammatory response
**CELL DEATHS**

Major Factors

- Accidental
  - Necrosis
- Genetic
  - Apoptosis

**Necrosis:**
The sum of the morphologic changes that follow cell death in a living tissue or organ.

**Apoptosis:**
a physiological process that includes specific suicide signals leading to cell death.
CELL DEATH.

The electron micrographs show cells that have died by
(A) necrosis or
(B and C) apoptosis.
Happy cells → Commitment to die and cell suicide → Phagocytosis of dying cell → Degradation of cell

Apoptic RLY?
http://www.oryyowl.com/
Morphological Features of Apoptosis
Why should a cell commit suicide?

There are two different reasons

1. Programmed cell death is as needed for proper development as mitosis is.

   **Examples:**

   • The resorption of the tadpole tail at the time of its metamorphosis into a frog occurs by apoptosis.

   • The formation of the fingers and toes of the fetus requires the removal, by apoptosis, of the tissue between them.

   • The formation of the proper connections (synapses) between neurons in the brain requires that surplus cells be eliminated by apoptosis.
Apoptosis during the metamorphosis of a tadpole into a frog. As a tadpole changes into a frog, the cells in the tadpole tail are induced to undergo apoptosis; as a consequence, the tail is lost. All the changes that occur during metamorphosis, including the induction of apoptosis in the tail, are stimulated by an increase in thyroid hormone in the blood.
Sculpting the digits in the developing mouse paw by apoptosis.

(A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green* dots between the developing digits.

(B) This interdigital cell death eliminates the tissue between the developing digits, as seen one day later, when few, if any, apoptotic cells can be seen.
Apoptosis regulates nerve cell targeting
2. Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism

Examples:

a) Cells infected with viruses

One of the methods by which cytotoxic T lymphocytes (CTLs) kill virus-infected cells is by inducing apoptosis (some viruses mount countermeasures to thwart it)

b) Cells of the immune system

As cell mediated immune responses wane, the effector cells must be removed to prevent them from attacking body constituents. CTLs induce apoptosis in each other and even in themselves. Defects in the apoptotic machinery is associated with autoimmune diseases such as lupus erythematosus and rheumatoid arthritis.

*Immune response against its own tissues*
c) Cells with DNA damage

Damage to its genome can cause a cell

- to disrupt proper embryonic development leading to birth defects
- to become cancerous

Cells respond to DNA damage by increasing their production of p53 (A potent inducer of apoptosis).

Mutations in the p53 gene, producing a defective protein, are so often found in cancer cells (that represent a lethal threat to the organism if permitted to live).

d) Cancer cells

Radiation and chemicals used in cancer therapy induce apoptosis in some types of cancer cells.
What makes a cell decide to commit suicide?

The balance between:
Withdrawal of positive signals and receipt of negative signals

1. Withdrawal of positive signals

The continued survival of most cells requires that they receive continuous stimulation from other cells and, for many, continued adhesion to the surface on which they are growing.

Positive signals:
• growth factors for neurons
• Interleukin-2 (IL-2), an essential factor for the mitosis of lymphocytes
2. **Receipt of negative signals**

- Increased levels of oxidants within the cell
- Damage to DNA by these oxidants or other agents like
  - Ultraviolet light
  - x-rays
  - Chemotherapeutic drugs
- Accumulation of proteins that failed to fold properly into their proper tertiary structure
- Molecules that bind to specific receptors on the cell surface and signal the cell to begin the apoptosis program.

These **death activators** include:

- **Tumor necrosis factor-alpha (TNF-α)** that binds to the **TNF receptors**;
- **Lymphotoxin** (also known as **TNF-β**) that also binds to the **TNF receptor**;
- **Fas ligand (FasL)**, a molecule that binds to a cell-surface receptor named **Fas** (also called **CD95**).
Detection of apoptotic cells

- **Microscopy**
  - Cells have classic features (e.g. small darkly stained nuclei)

- Detection of free 3' ends of DNA by TUNEL assay
  (terminal deoxytransferase-mediated dUTP-biotin nick end labeling)

- **Trypan Blue Exclusion Assay**
  Dead cells take up dye, dye binds to intracellular proteins
  Checks damage/leakage of plasma membrane
  (mostly stains necrotic cells)
• **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 (not very specific to apoptotic cells)
  A DNA stain is added to distinguish necrotic cells

- Measure DNA fragmentation with propidium iodide fluorescence (not specific for apoptotic cells)
Annexin V Staining

Schematic representation of the Annexin V assay.

Translocase and floppase inactivated, scramblase activate.
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)
DNA damage e.g. radiation

↑p53

↓

cell cycle arrest

DNA repair

cell cycle arrest

irreparable damage

APOPTOSIS elimination of damaged and potentially cancerous cells
Examples of Diseases Associated with Decreased rates Apoptosis

- Cancer
  - Follicular lymphomas
  - Carcinomas with p53 mutations
  - Hormone-dependent tumors
- Breast cancer
- Prostate cancer
- Ovarian cancer
- Autoimmune disorders
- (mixed increase and decrease)
- Viral infection
Examples of Diseases-Injuries Associated with Increased Apoptosis

• AIDS (non-infected cells often increase in apoptosis).
• Neurodegenerative disorders (Diseases of Aging)
  - Alzheimer’s
  - Parkinson’s
• Toxin-induced liver disease
  - Alcohol
Apoptosis and Cancer

Development of Cancer:

Increase in cell viability and decrease in apoptosis

Proto-oncogenes regulate apoptosis

Cancer therapy: Induce apoptosis
Oncogenes

Stimulate Proliferation
Inhibit Differentiation
Inhibit Apoptosis

Tumor Suppressor Genes

Inhibit Proliferation
Promote Differentiation
Stimulate Apoptosis
Induction of apoptosis:

- Cell surface death receptor mediated pathway (extrinsic)

- Mitochondrial-initiated pathway (intrinsic)
Apoptosis: Mediated by Intracellular Proteolytic Cascade

Cells that die as a result of acute injury typically swell and burst. They spill their contents all over their neighbors a process called cell necrosis causing a potentially damaging inflammatory response.

By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors. The cell shrinks and condenses.

- The cytoskeleton collapses,
- The nuclear envelope disassembles,
- The nuclear DNA breaks up into fragments,
- The cell surface is altered, displaying properties that cause the dying cell to be rapidly phagocytosed, either by a neighboring cell or by a macrophage (a specialized phagocytic cell), before any leakage of its contents occurs.
The intracellular machinery responsible for apoptosis

Involves a family of proteases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids called **CASPASES** (*Caspases*: cysteine-dependent aspartate-specific proteases)

CASPASES are synthesized in cell as inactive precursors, or **PROCASPASES**, which are usually activated by cleavage at aspartic acids by other caspases.

Once activated, caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade.

- Some of the activated caspases cleave other key proteins in the cell.
- Some cleave the nuclear lamins, for example, causing the irreversible breakdown of the nuclear lamina;
- Another cleaves a protein that normally holds a DNA-degrading enzyme (a DNAse) in an inactive form, freeing the DNAses to cut up the DNA in the cell nucleus.
The intracellular machinery responsible for apoptosis

CASPASES (cysteine-dependent asparate-specific proteases)

CASPASES are synthesized in cell as inactive precursors, or PROCASPASES, which are usually activated by cleavage at aspartic acids by other caspases.
Activation of the intracellular cell death pathway, like entry into a new stage of the cell cycle, is usually triggered in a complete, all-or-none fashion.

The protease cascade is not only destructive and self-amplifying but also irreversible, so that once a cell reaches a critical point along the path to destruction, it cannot turn back.
The caspase cascade involved in apoptosis.
Each suicide protease is made as an inactive proenzyme (procaspase), which is usually activated by proteolytic cleavage by another member of the caspase family. Two of the cleaved fragments associate to form the active site of the caspase. The active enzyme is thought to be a tetramer of two of these units.
Each activated caspase molecule can cleave many procaspase molecules, thereby activating them, and these can then activate even more procaspase molecules. In this way, an initial activation of a small number of procaspase molecules can lead, via an amplifying chain reaction (a cascade), to the explosive activation of a large number of procaspase molecules.
All nucleated animal cells contain the seeds of their own destruction, in the form of various inactive procaspases that lie waiting for a signal to destroy the cell.

The caspase activity is **tightly regulated** inside the cell to ensure that the death program is held in check until needed.

**Activation of Procaspases: Binding to Adaptor Proteins**

How are procaspases activated to initiate the caspase cascade? What causes activation of caspases?
The activation is triggered by adaptor proteins that bring multiple copies of specific procaspases, known as *initiator procaspases*, close together in a complex or aggregate.

In some cases, the initiator procaspases have a small amount of protease activity, and forcing them together into a complex causes them to cleave each other, triggering their mutual activation.

In other cases, the aggregation is thought to cause a conformational change that activates the procaspase. Within moments, the activated caspase at the top of the cascade cleaves downstream procaspases to amplify the death signal and spread it throughout the cell.
Caspases and the initiation of apoptosis

Caspase: Cysteine Aspartate Specific Protease

Activation through induced proximity

Inactive Initiator Caspase (e.g. Casp -8, -9)

Active Initiator Caspase

Inactive Executioner Caspases (e.g. Casp -3)

Active Executioner Caspases

Substrate cleavage: DNA, Cytoskeletal proteins…

Apoptosis
## Members of CASPASE family

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<thead>
<tr>
<th>Subfamily</th>
<th>Role</th>
<th>Members</th>
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<tbody>
<tr>
<td>I</td>
<td>Apoptosis activator</td>
<td>Caspase-2</td>
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<td>III</td>
<td>Inflammatory mediator</td>
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Targets of Caspases

More than a dozen kinases, including focal adhesion kinase (FAK), PKB, PKC, Raf1. FAK Inactivation is presumed to disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbours.

Lamins. Cleavage of lamins leads to the disassembly of the nuclear lamina and shrinkage of the nucleus.

Proteins of the cytoskeleton, such as those of intermediate filaments, actin, tubulin. Cleavage and consequent inactivation of these proteins leads to changes in cell shape.

An endonuclease called caspase activated DNAase (CAD), which is activated following caspase cleavage of an inhibitory protein. Once activated, CAD translocates from the cytoplasm to the nucleus where it attacks DNA, severing it into fragments.
Induction of apoptosis:

- Cell surface death receptor mediated pathway (extrinsic)

- Mitochondrial-initiated pathway (intrinsic)
Death Signals

**Extrinsic signals**

Binding of death inducing ligands to cell surface receptors called death receptors.

Ligands can either be soluble factors or can be expressed on the surface of cells such as cytotoxic T lymphocytes (when T-cells recognize damaged or virus infected cells and initiate apoptosis in order to prevent damaged cells from becoming neoplastic (cancerous) or virus-infected cells from spreading the infection).

Induced by cytotoxic T-lymphocytes using the enzyme granzyme.

**Intrinsic signals** following cellular stress

From exposure to radiation or chemicals or to viral infection
Terms

- DED: death effector domain
- CARD: caspase recruitment domain
- CAD: caspase-activated deoxyribonuclease (CAD)
- ICAD: CAD inhibitor
- FADD: Fas-associated death domain
- FLICE: Fas-like interleukin-1β converting enzyme
- DISC: death inducing signaling complex
- AIF: apoptosis inducing factor
- Apaf-1: Apoptotic protease-activating factor-1
- Apoptosome: Cyt. C Apaf-1, dATP/ATP and pro-caspase 9
- Smac: second mitochondria-derived activator of caspase.
Key intracellular regulators of apoptosis

1. Bcl-2 proteins (20 members): B cell leukemia/lymphoma

   some are anti-apoptotic (Bcl-2, bcl-XL), inhibit apoptosis by heterodimerization with pro-apoptotic members, by blocking Cytochrome C release or by binding to Apaf-1.

   Others are pro-apoptotic (Bax, bak, Bad, Bid)

2. Caspases: 14 identified; caspase active site QACxG

3. Apaf-1: apoptosis protease activating factor 1
   necessary for caspase (caspase-9,2) activation;
   form apoptosome with cytochrome c, Smac/DIABLO, and caspase-9.

4. IAPs: Inhibitor of apoptosis (8 members in human)
   Inhibit effector caspase activity and promote degradation.
   Also found in viruses

5. Smac/Diablo inhibits IAPs (Negative Regulation of Apoptosis)
**Extrinsic Pathway**

Procaspe activation can be triggered from outside the cell by the activation of death receptors on the cell surface.

Death receptors are cell surface receptors that transmit apoptotic signals initiated by specific ligands such as
- Fas ligand,
- TNF alpha (tumor necrosis factor) and
- TRAIL (TNF-related apoptosis inducing ligand)

Killer lymphocytes, for example, can induce apoptosis by producing a protein called Fas ligand, which binds to the death receptor protein Fas on the surface of the target cell.

The clustered Fas proteins then recruit intracellular adaptor proteins that bind and aggregate procaspase-8 molecules, which cleave and activate one another.

The activated caspase-8 molecules then activate downstream procaspases to induce apoptosis.

Some stressed or damaged cells kill themselves by producing both the Fas ligand and the Fas protein, thereby triggering an intracellular caspase cascade.
The Fas/CD95/Apo-1 system in health and disease

Target cells induced to express FAS

"Worn-out" cell

Virus infected cell

Tumor cell

Cytotoxic T cell carrying the Fas ligand on its surface

Target cell killed through apoptosis upon cell-cell binding
Activation of Apoptosis by Fas Ligand

Fas ligand (FasL) binds to Fas. Both are present at cell surfaces as homotrimers.

Binding of FasL causes a conformational change in Fas, which then binds death domain-containing adaptor proteins.

The adaptor proteins recruit and activate caspase 8, which cleaves caspase 3.

Activated caspase 3 cleaves I-CAD, the inhibitor of CAD, which is released to enter the nucleus and cleave DNA.
Fas-mediated death signaling

Ligand Binding

Receptor trimerization and DISC formation

Activation of pro-caspase-8 and downstream caspases

FasL

Fas

FADD

DD

DED

(caspase 8)

Activation of the caspase cascade

CAD

IGAD

Cleavage of cellular death substrates

*DISC: death-inducing signaling complexes
When cells are damaged or stressed, they can also kill themselves by triggering procaspase aggregation and activation from within the cell.

Mitochondria are induced to release the electron carrier protein cytochrome c into the cytosol.

Cytochrome c binds and activates an adaptor protein called Apaf-1.

DNA damage, for example, can trigger apoptosis.

This response usually requires p53, which can activate transcription of genes that encode proteins that promote the release of cytochrome c from mitochondria. These proteins belong to the Bcl-2 family.
Another important family of intracellular apoptosis regulators is the **IAP (inhibitor of apoptosis) family**. These proteins are thought to inhibit apoptosis in two ways:

- They bind to some procaspases to prevent their activation, and
- They bind to caspases to inhibit their activity.

When mitochondria release cytochrome c to activate Apaf-1, they also release a protein that blocks IAPs, thereby greatly increasing the efficiency of the death activation process.

The intracellular cell death program is also regulated by extracellular signals, which can either activate apoptosis or inhibit it.

These signal molecules act by regulating the levels or activity of members of the Bcl-2 and IAP families.
Cytocrome C leakage

In a normal cell, cytochrome c is present only in mitochondria

When programmed cell death is induced, the mitochondria swell and leak, releasing cytochrome c, which binds to Apaf-1

- Apaf-1
- Caspase
- CAD
- l-CAD

Diagram showing the release of cytochrome c from mitochondria.
caspase 9 and the induction of apoptosis.
Formation of Apoptosomes

First stage of apoptosome formation

2:1 ratio of Apaf-1 and cytochrome c

Recruitment of pro-caspase-9

Caspase Activation
Mitochondria associated caspase-dependent pathway
Caspase-dependent Apoptosis
Sensitivity of the cell to apoptotic stimuli depends on

• Expression of pro-apoptotic proteins
• Anti-apoptotic proteins (Bcl-2 or the inhibitor of apoptosis protein)
• The severity of stimulus (viral infection, cell stress, DNA damage), and
• The stage of the cell cycle
Caspases and chromatin breakdown

1) **Inactivation of enzymes involved in DNA repair.**

The enzyme poly ADP-ribose polymerase, or PARP, is an important DNA repair enzyme (first proteins identified as a substrate for caspases). The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3.

2) **Breakdown of structural nuclear proteins.**

Lamins are intra-nuclear proteins that maintain the shape of the nucleus and mediate interactions between chromatin and the nuclear membrane. Degradation of lamins by caspase 6 results in the chromatin condensation and nuclear fragmentation.

3) **Fragmentation of DNA.**

The fragmentation of DNA into nucleosomal units is caused by an enzyme known as CAD, or caspase activated DNase. Normally CAD exists as an inactive complex with ICAD (inhibitor of CAD). During apoptosis, ICAD is cleaved by caspases, such as caspase 3, to release CAD. Rapid fragmentation of the nuclear DNA follows.
Breakdown of Chromatin during Apoptosis
Removal of apoptotic cell by phagocytosis
Cellular morphology changes during apoptosis

**Normal cell**

- Cell shrinkage
- Membrane ruffling
- DNA condensation
- DNA fragmentation

**Different stages of apoptosis**

Phagocytic cells will recognize phosphatidyl serine on the surface of the apoptotic cell and engulf and degrade it.
Morphology of Apoptosis

*In vivo*: Phagocytosis

*In vitro*: Secondary necrosis
How do we recognize Programmed Cell Death?

"Now wait just a minute here . . . How are we supposed to know you're the REAL Angel of Death?"