There are two ways in which cells die:

1. Killed by injurious agents
   - **External forces**
     - Physical agents (trauma, burn, freeze, etc.)
     - Lack of oxygen
     - Chemical agents (drugs, poisons, toxins, heavy metals)
     - Infectious agents (bacteria, viruses, parasites)
   - **Internal factors**
     - Immunologic (anaphylaxis, autoimmune diseases)
     - Genetic (congenital malformations, abnormal proteins)
     - Metabolic (nutritional imbalances)
2. Induced to commit suicide

- programmed cell death
  - apoptosis
  - non-apoptotic PCD

- Cells that die due to external forces primarily undergo necrosis

- Programmed cell death occurs normally in developing and mature tissue and therefore can be physiologic or pathologic

- Necrosis and apoptosis are two death processes that can occur independently, sequentially and/or simultaneously

**Necrosis**

- Dramatic and very rapid form of cell death in which every compartment of the cell disintegrates

- Characterized by marked dysregulation of ion homeostasis causing:
  - cell swelling
  - dilation of mitochondria and ER
  - formation of vacuoles in cytoplasm
  - proteases, primarily calpains and cathepsins B and D (lysosomal proteases) important components of cell degradation
Mitochondrial damage can activate proteases (e.g., caspases) and cytochrome c release, but this is not necessary for necrotic cell death. During the death process, chromatin clumps and the nuclear membrane is disrupted. Gene transcription and protein synthesis stop. ATP is rapidly depleted.

Cells lyse and spill their contents into extracellular fluid. Contents can damage neighboring cells. Spillage of contents causes an inflammatory response.

There are very few cell death triggers that are only capable of inducing either necrosis or apoptosis. Whether a cell undergoes apoptosis or necrosis is determined primarily by the intensity and/or duration of the death-inducing stimulus. Somewhat of a consensus that if stimulus is severe and/or sustained it will induce necrosis. If stimulus less severe with transient stresses it will induce apoptosis. Examples: glutamate/excitotoxicity, trauma, energy failure/ischemia.
Types of Necrosis

1. Coagulative necrosis
2. Liquefactive necrosis
3. Caseous necrosis
4. Fat necrosis

1. Coagulative Necrosis

- Most common form
- Inactivation of hydrolytic enzymes
- Occurs in solid internal organs – heart, kidney and adrenal tissue
- ‘Boiled meat appearance’
- Proteins denatured
- Only outline of cells can be seen
- Results from sudden and severe ischemia
- Eventually, macrophages phagocytose dead tissue and area is replaced with collagenous tissue
2. Liquefactive necrosis

- Characterized by dissolution of tissue
- Necrotic area is soft and fluid filled
- No cell architecture remains
- Infiltration of neutrophils
- Results from enzymatic degradation of tissue
- Occurs in brain ischemia
3. Caseous necrosis

- Form of coagulative necrosis with limited liquefaction
- Necrotic tissue that has appearance of cheese
- Eg - tuberculosis

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4. Fat Necrosis

- Necrosis of fat due to action of enzymes followed by formation of complexes with calcium
- Type of liquefactive necrosis
- Focal outlines of necrotic fat cells surrounded by inflammation
- Eg pancreatitis
Programmed Cell Death

- Essential for development and maintenance of multicellular organisms
  - Formation of fingers and toes
  - Formation of functional synapses requires surplus cells be eliminated
- Required to destroy cells that represent a threat to the integrity of the organism
  - Cells infected with viruses
  - Cells of the immune system – removal of effector cells; defects in immune apoptosis associated with autoimmune diseases
  - Cells with DNA damage – respond by increase production of apoptosis inducer p53
  - Cancer cells – radiation and chemicals used to induce apoptosis in some types of cancer cells
Although PCD is often equated with apoptosis, non-apoptotic PCD also exists.

Developmental studies suggest at least 3 forms of PCD can be distinguished:

1. Type 1 – aka nuclear or apoptotic type
2. Type 2 – aka autophagic
3. Type 3 - cytoplasmic

The various forms occur in specific nuclei and at specific developmental stages. They can also be induced by insults such as DNA damage or accumulation of misfolded proteins.

Oncosis – refers to a specific morphology of cell death – cell swelling. That is typically caused by ischemia and thought to be mediated by failure of ionic pumps.

What is autophagy??

Autophagy is a evolutionarily conserved pathway in which the cytoplasm and organelles are engulfed within double-membraned vesicles (or autophagosomes) in preparation for the turnover and recycling of these cellular constituents.
Recent studies have suggested that death in the nervous system may trigger stem-cell proliferation and survival, thus cell death pathways offer potential points of entry for therapeutics of neurodegenerative diseases.

**Apoptosis or Type 1 PCD**

- Best characterized form of PCD
- Pathways:
  1. Intrinsic – generated by signals arising within the cell
  2. Extrinsic – triggered by death activators binding to receptors at the cell surface
  3. Caspase-independent pathway triggered by reactive oxygen species

(Additional pathway that involves T-cell mediated cytotoxicity and perforin-granzyme-dependent death)
1. Intrinsic Pathway

- Aka mitochondrial pathway
- Pathway is largely conserved from worms to mammals
- Members of the B-cell leukemia/lymphoma 2 (Bcl-2) family of proteins act as gatekeeper of apoptosis and caspases (cysteine aspartate proteases) execute the program.
- Propensity for cells to undergo apoptosis (the apostat) largely determined by balance between anti-apoptotic and pro-apoptotic members of the Bcl-2 family of proteins
Bcl-2

- Bcl family can be pro-apoptotic or anti-apoptotic (Bcl-2 and BclX<sub>L</sub>)
- There are 3 types of pro-apoptotic Bcl-2 proteins
  1. The multi-domain proteins BAX and BAK – have Bcl-2 homology domains 1-3 (BH1-3)
  2. BH3-only proteins BIM and tBID which activate BAX and BAK and likely participate in mitochondrial pore formation
  3. BH3-only de-repressors such as PUMA, NOXA and BAD – these proteins sequester the anti-apoptotic Bcl-2 and Bcl-X<sub>L</sub> (and other proteins with BH1-4 domains) which allows BH1-3 proteins to permeabilize the mitochondrial membrane

- Formation of mitochondrial membrane pore causes release of pro-apoptotic mitochondrial proteins such as cytochrome c and SMAC/Diablo into cytoplasm
- Cytochrome c induces conformational change and heptamerization of the cytosolic protein apoptosis activating factor-1 (APAF-1)
- The heptamer binds caspase-9 forming the apoptosome which results in its activation and cleavage of effector caspases 3 and 7
- Then digestion of structural proteins and degradation of chromosomal DNA by endonuclease and proteases
- Finally, phagocytosis of cell

- Activated effector caspases can be held in check – to a point – by inhibitors of apoptosis (IAPs) such as XIAP.
- SMAC/DIABLO can inhibit XIAPs and thus remove the IAP-mediated block
Apaf-1 proteins

Caspase-9 exists as a monomer and binds to other proteins through the caspase activation and recruitment domain (CARD). This protein-protein interaction results in dimerization of the caspase and leads to its activation. A similar process is involved in the other initiator caspases (8 and 10) and where they bind is called the death effector domain (DED).

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Extrinsic Pathway

- Aka the death receptor pathway
  - FasL/FasR
  - TNFα/TNFR1
  - Apo3L/DR3
  - Apo2L/DR4
  - Apo2L/DR5

- Fas and TNF receptors are integral membrane proteins with receptor domains exposed at surface of cell

- Best characterized is Fas receptor.
  - Fas is bound by trimeric Fas ligand (FasL)
  - This causes recruitment of FADD through Fas’s death domain (DD)

- FADD recruits caspase-8 through FADD’s death effector domain.

- The binding of FADD and caspase-8 causes activation of the caspase-8 and subsequent activation of effector caspase such as caspase-3 or -7

- The extrinsic pathway interacts with the intrinsic pathway via caspase-8 cleavage of BID to produce tBID
Extrinsic pathway

Ligand (FasL, TRAIL, TNF-α)
Adaptors (FADD, TRADD)
Pro-caspase-8
Death Receptor (Fas, TNFR1, DR-5,...)

Apoptosis

Activation of effector caspases (3, 6, 7)
Active Caspase-8
Caspase-Independent Pathway

• Through apoptosis-inducing factor – AIF
• AIF normally located in intermembrane space of mitochondria
• When cell receives signal to die (eg ROS)
  – AIF released from mitochondria
  – Migrates to cell nucleus
  – Binds to DNA
  – Triggers destruction of DNA and cell death
Apoptosis induced by misfolded proteins

- Misfolded proteins are constantly produced
- These proteins trigger a protective stress response known as the unfolded-protein response (UPR)
- Prolonged ER stress and UPR activation ultimately result in activation of cell death pathways
- Misfolded proteins also aggregate and can interact with chaperones and transcription factors
- Misfolded proteins have been implicated in AD, PD, HD, ALS, prion-protein diseases (all show accumulation and aggregation of misfolded proteins)

Mediators of cell death induced by misfolded proteins

- The Bcl-2 family proteins have a key role in communication between the ER and mitochondria (BAX/BAK double KO mice show no caspase activation following ER stress)
- Bcl-2 members suggested to be involved include Bcl-2, Bcl-XL, BAX, BAK, B1-1 and Bik.
- Other ER stress proteins BAP31 and p53-dependent gene products NOXA and PUMA
- In short:
  - The ER membrane protein BAP31 binds Bcl-2 (or Bcl-XL) and a caspase-8 containing pro-apoptotic complex
  - When BAP31 is cleaved, it releases a pro-apoptotic fragment, p20, which induces mitochondrial fission and cytochrome c release
  - There are many pathways involved in ER stress

See: Li et al 2006 J. Biol. Chem 281, 7260-7270

ER stress is coupled to specific independent pathways AND intrinsic and extrinsic apoptotic pathways.
**BIP** is an ER chaperone that also regulates activation of ER-stress transducers IRE1, PERK, and ATF6. Under normal circumstances, BIP is a negative regulator of these stress transducers. When misfolded proteins accumulate and cause ER stress, BIP binds to these proteins and is released from the transducer proteins which as a result become constantly activated.

- Unregulated activation of PERK causes reduction in the amount of new protein.
- Unregulated activation of ATF6 causes increased protein folding capacity of the ER.
- Unregulated activation of IRE1 causes transport of misfolded proteins from the ER to the cytosol and ER-induced protein degradation.

**Caspases**

- **Cysteine-dependent aspartate-specific proteases**
- 14 different caspases in mammals
- All synthesized as inactive procaspases
- All, but initial caspase in a cascade, are activated by proteolytic cleaving by another caspase

**Sequence of Events**

- Prodomain is cleaved off
- Caspase divided into small and large subunits
- Cleaved subunits associate to form active caspase
• Caspases associated with inflammation:
  – 1, 4, 5, 11, 12, 13, 14
• Caspases associated with apoptosis:
  – Executioners: Caspases 3, 6 and 7
  – Initiators: Caspases 2, 8, 9, 10

Caspase substrates

• Other caspases
• DNase
• Regulators of survival pathways
• Regulators of apoptosis (Bcl-2, Bcl-xL, XIAP [good guys], Bid, [bad guy])
• Kinases (Akt, MEKK1, Raf1)
• Structural proteins (laminin, actin)

Apoptosis versus Necrosis

• Two processes are temporally dislocated and likely represent two extremes of a continuum
  – Necrosis process can start only and exclusively when the cell dies and is an irreversible process – no return
  – Secondary necrosis


**Apoptosis**  
**Necrosis**

<table>
<thead>
<tr>
<th>Patterns of death</th>
<th>Single cells</th>
<th>Groups of neighbouring cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>Shrinkage</td>
<td>Swelling</td>
</tr>
<tr>
<td>Plasma Membrane</td>
<td>Blebbing</td>
<td>Preserved continuity</td>
</tr>
<tr>
<td></td>
<td>Preserved</td>
<td>Smoothed</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
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<td>Increased</td>
<td>Swelling</td>
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<td></td>
<td>preserved</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Disordered structure</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Organelle Shape</th>
<th>Contrasted</th>
<th>Swelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>Chromatin:</td>
<td>Swelling</td>
</tr>
<tr>
<td>DNA degradation</td>
<td>Fragmented</td>
<td>Disrupted</td>
</tr>
<tr>
<td>Cell degradation</td>
<td>Phagocytosis</td>
<td>Inflammation</td>
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<tr>
<td></td>
<td>No inflammation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apoptotic bodies</td>
<td>Disruption</td>
</tr>
<tr>
<td>General stimuli</td>
<td>Apoptosis</td>
<td>Necrosis</td>
</tr>
<tr>
<td>----------------</td>
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<td>-----------</td>
</tr>
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<td>Developmental program</td>
<td>Diseases processes</td>
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<tr>
<td>Cellular processes</td>
<td>Programmed cascade of reactions</td>
<td>No proteins synthesis</td>
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<td>Requires protein synthesis</td>
<td>Requires RNA transcription</td>
<td>No RNA transcription</td>
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<tr>
<td>Requires ATP</td>
<td>Energy independent</td>
<td>ATP depletion</td>
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</tbody>
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Necrosis?
Apoptosis?
Most common assays for apoptosis

• Cytomorphological changes
• DNA fragmentation
• Detection of caspases, cleaved substrates, regulators and inhibitors
• Membrane alterations
• Mitochondrial assays
• Tunnel staining = terminal deoxynucleotidyl transferase mediated dUTP nick end labeling.
Diseases associated with the inhibition of apoptosis

Cancer
- Follicular lymphomas
- Carcinomas with p53 mutations
- Hormone-dependent tumors
- Breast / Ovarian / Prostate cancer

Autoimmune disorders
- Systemic lupus erythematosus
- Immune-mediated glomerulonephritis

Viral infections
- Herpesviruses
- Poxviruses
- Adenoviruses

Diseases associated with increased apoptosis

Neurodegenerative disorders
- Alzheimer’s disease
- Parkinson’s disease
- Amyotrophic lateral sclerosis
- Retinitis pigmentosa

Ischemic injury
- Stroke
- Myocardial infarction
- Reperfusion injury

Infectious diseases
- AIDS
- Tuberculosis
- Liver disease (Alcohol)