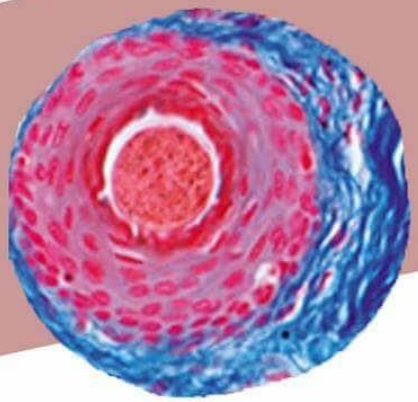
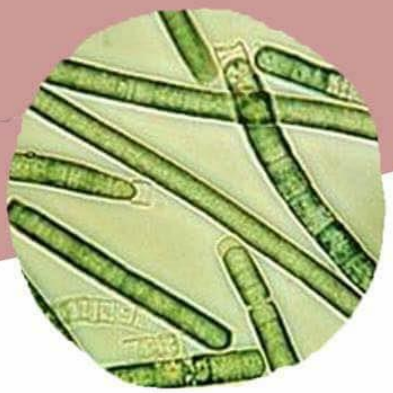
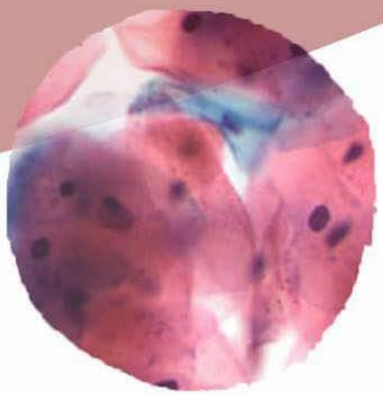
INTRODUCTION TO PATHOLOGY

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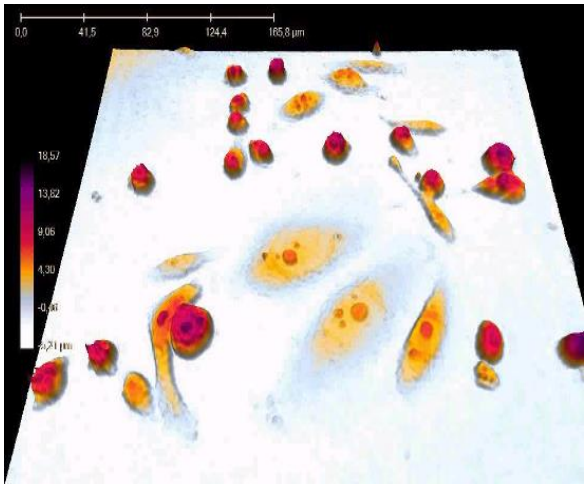
Sheet# 4

The term “Apoptosis” is of Greek origin, accordingly, apoptosis translates to "falling off" or “separation”. Apoptosis is different from necrosis in that it can be physiological, it does not induce inflammation, the cell shrinks, and there is no leakage of cellular contents into the extracellular fluid.



As depicted in the figure above, the cell in the center is undergoing apoptosis and the apoptotic bodies (in red) are falling away from the center and are being cleared by phagocytes to make sure none of their contents is spilled out.

Apoptosis is defined as programmed cell death, or a pathway of cell death where cells activate enzymes that degrade their nuclear DNA, and nuclear and cytoplasmic proteins. Other definitions include:

- A genetically determined process of cell self-destruction.

- A form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area, therefore, not inducing inflammation.

Table: Comparison between apoptosis and necrosis.

Feature	Apoptosis	Necrosis
Plasma membrane	intact, altered structure	disrupted
Cellular contents	intact, release in apoptotic bodies	enzymatic digestion, leakage
Adjacent inflammation	no	frequent
Physiologic vs. pathologic	often, but not always, physiologic	always pathologic
Cell size	reduced; due to budding parts of the cell away	enlarged; ATP- dependent ion pumps no longer function, ions move down their concentration gradient(Na, Ca influx)
Nucleus	Fragmentation into nucleosome size fragments (Karyorrhexis)	Karyolysis, Karyorrhexis, Pyknosis *

* karyolysis: DNA is degraded, therefore, it can no longer bind the basophilic dye and its color becomes faded.

*Pyknosis: the nucleus shrinks.

Causes of Apoptosis

Causes of apoptosis might be physiologic or pathologic, the physiologic causes include:

- Programmed cell death during *embryogenesis* occurs throughout the formation of certain organs that develop canal like structures. The cells die off by apoptosis in order to form these canals.

- *Hormone withdrawal*: At the end of the menstrual cycle, the body withdraws its hormones and the cells die off by apoptosis. Apoptosis is favorable because if necrosis occurs it will not be suitable to have an inflammation at the end of each cycle. Another example is after weaning the child, the lactating cells no longer have to produce milk and undergo apoptosis.

- *Steady state population*: For example, the cells in the intestine must have a certain number, and when the epithelial cells on the surface are exposed to wear and tear forces they die off by apoptosis. Same thing occurs to white blood cells when they become old, they die off by apoptosis and are replaced by new cells in order to maintain their number within the normal range.

- *End of function/ life*: At the end of an infection, white blood cells undergo apoptosis because they no longer receive survival signals; therefore, they go back to their normal level in blood.

- *Self reacting lymphocytes*: These lymphocytes attack the body's own tissues (autoimmune disease), that is why the body induces these harmful cells to die by apoptosis.

Pathologic causes:

- *DNA damage* due to radiation or chemical toxins or even extremes of heat. When the damage is beyond repair, it will cause apoptosis. However, when the damage is too severe apoptosis and necrosis might coexist.

- *Protein misfolding/ER stress*: When a mutation causes protein misfolding, the misfolded proteins accumulate in the cell and induce it to kill itself by apoptosis. In Alzheimer's disease, for example, the neuronal cells are killed and the patient loses their compatibility.

Note: study the following table.

Table 1–2 Diseases Caused by Misfolding of Proteins

Disease	Affected Protein	Pathogenesis
Cystic fibrosis	Cystic fibrosis transmembrane conductance regulator (CFTR)	Loss of CFTR leads to defects in chloride transport
Familial hypercholesterolemia	LDL receptor	Loss of LDL receptor leading to hypercholesterolemia
Tay-Sachs disease	Hexosaminidase β subunit	Lack of the lysosomal enzyme leads to storage of GM_2 gangliosides in neurons
Alpha-1-antitrypsin deficiency	α-1 antitrypsin	Storage of nonfunctional protein in hepatocytes causes apoptosis; absence of enzymatic activity in lungs causes destruction of elastic tissue giving rise to emphysema
Creutzfeld-Jacob disease	Prions	Abnormal folding of PrP^{sc} causes neuronal cell death
Alzheimer disease	A_β peptide	Abnormal folding of A_β peptides causes aggregation within neurons and apoptosis

Shown are selected illustrative examples of diseases in which protein misfolding is thought to be the major mechanism of functional derangement or cell or tissue injury.

- *Cytotoxic T lymphocytes*: these cells kill virally infected cells and tumor cells by inducing them to undergo apoptosis rather than necrosis to minimize inflammaton.

- *Duct obstruction* in the pancreas, kidney and parotid causes the parencymal cells to die off by apoptosis.

Mechanisms of Apoptosis

There two pathways for apoptosis: mitochondrial (intrinsic) pathway and death receptor (extrinsic) pathway.

The Mitochondrial (Intrinsic) Pathway:

The mitochondria contains certain proteins that when leaked out into the cytoplasm will turn on a set of enzymes called **caspases**, the reason they are called caspases is because they are cysteine proteases that cleave off after an aspartic residue. These initiator caspases, in turn, activate another set of enzymes; therefore, it is a cascade of reactions. These activated enzymes include endonucleases that degrade cell's nuclear DNA and proteases that target cytoskeletal proteins.

Apoptosis is balanced through pro- and anti- apoptotic proteins and the choice between survival and death is determined by the permeability of mitochondria which is controlled by Bcl-2 group of proteins. Whenever the cell is deprived of survival signals and growth factors, or when it recognizes DNA damage and accumulation of misfolded proteins, BH3 sensor proteins are activated.

The BH3 sensor proteins activate Bax and Bak pro-apoptotic proteins, these pro-apoptotic proteins open a set of channels in the mitochondria that allow the leakage of pro-apoptotic cytochrome C into the cytoplasm. This, in turn, activates caspase 9. The BH3 proteins also inhibit the Bcl-2 and Bcl-XL anti-apoptotic proteins, and by inhibiting the inhibitor (Bcl-2) activation occurs. Additionally, the BH3

proteins affect how the signal continues through enhancing the production of pro-apoptotic (Bax, Bak, cytochromeC) proteins and increasing their transcription by inhibiting the production of anti-apoptotic proteins.

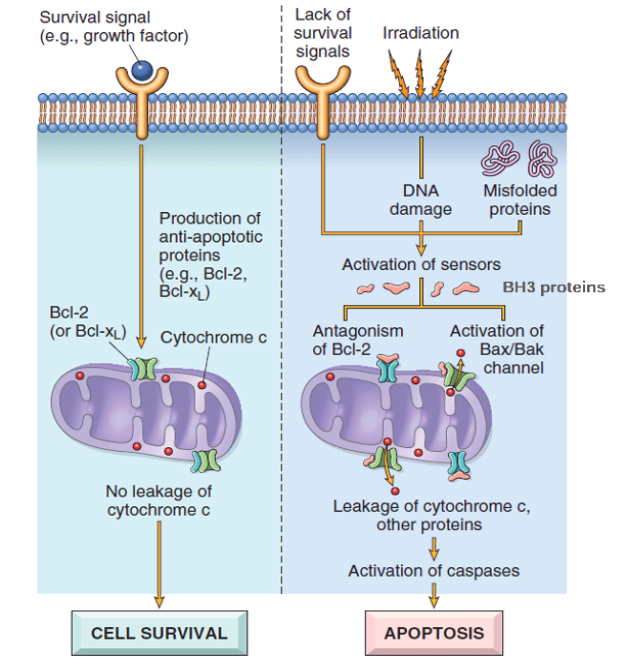
<u>The Death Receptor (extrinsic) Pathway:</u>

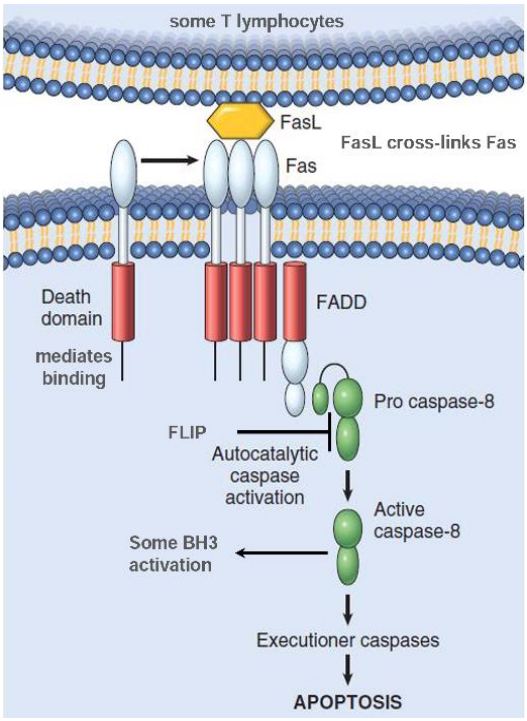
In this pathway caspase 8 is activated, however, the same downstream effect occurs, a cascade of reactions occur that end up activating endonucleases and proteases and forming apoptotic bodies.

There are two main death receptors: type 1 TNF receptor and Fas (CD95) receptor. *Only Fas is mentioned because it is simpler.

Fas receptors are typically singlets, which means that they are useless without a ligand. When the ligand (Fas-L) is present on another cytotoxic T lymphocyte, the Fas receptors trimerize and the three receptors are cross-linked to each other and become activated. When activated the Fas and Fas-L complex attracts FADD protein, this, in turn, stimulates pro-caspase8 to cleave itself (autocatalytic caspase activation) resulting in the activation of caspase 8 and initiating the cascade of reactions.

In addition, caspase 8 can stimulate BH3 proteins, thereby, also turning on the intrinsic death pathway.



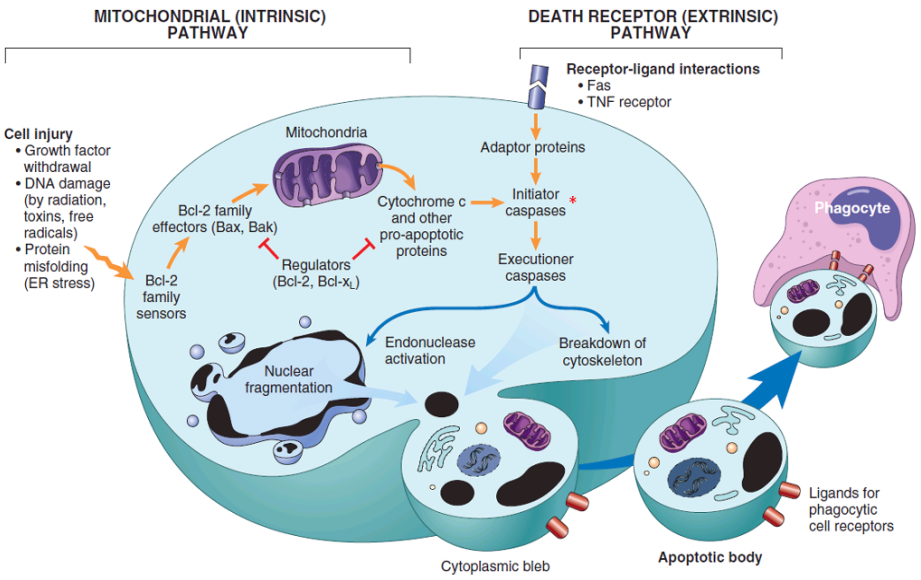


Almost all biological systems have ways of balancing pathways. In this case, cells have a protein called FLIP. This protein inhibits the activation of caspase 8, which accordingly balances the signals dealing with shutting down the extrinsic pathway.

Viruses have evolved many distinct strategies to avoid the host's apoptotic response. Some viruses have proteins that mimic the action of FLIP. When the cytotoxic T lymphocytes identify the infected cells, the extrinsic pathway can no longer be activated and is put to a halt.

The extrinsic pathway is also used to kill self-reacting lymphocytes.
Any mutation related to the Fas receptors and ligands will result in an autoimmune disease.

Figure; when an injurious stimulus occurs, BH3 sensor proteins are turned on and result in the activation of Bax and Bak which inhibit inhibitor proteins and allow the leakage of cytochrome C, thus, constructing the downstream effect of caspases. On the other hand, when a death receptor ligand binds its receptor, it directly activates caspases and the following cascade of reactions.



Clearance of Apoptotic Bodies

Both of these pathways conclude with the formation of apoptotic bodies. These waste bodies have to be cleared efficiently and quickly because they are not cell functioning or self-sustaining organisms. If they are left unremoved for a long time, eventually, they will fail to stay intact and will spill their contents to the outside causing inflammation. That is why the biological systems frequently use biological redundancies, i.e. more than one mechanism to get the same end result.

The mechanisms to clear away apoptotic bodies include:

- Phosphatidylserines are actively held facing the inner side of the cell membrane. However, when a cell undergoes apoptosis, phosphatidylserine is no longer restricted to the cytosolic side. Instead, the membrane lipid is rapidly exchanged between the two sides. When the phosphatidylserines flip to the extracellular (outer) surface of the cell, they act as a signal for macrophages to engulf the cells.

- Apoptotic cells produce glycoproteins that act as signals to the phagocytes.

- Apoptotic cells use soluble factors that induce phagocytes to take them in.

- Phagocytes, themselves, produce proteins that bind apoptotic cells and target them for engulfment.

All of these mechanisms make sure that the apoptotic cells are eliminated as quickly as possible so that inflammation is not induced.

Clinical examples

- *Growth factor deprivation*: When hormones are withdrawn, the intrinsic pathway is turned on, sensor proteins are activated, inhibitors are antagonized, channels are stimulated and proapoptotic proteins leak out into the cytoplasm.

-*DNA damage*: DNA is replicated during the S phase which comes after the G1 phase. In the first growth phase (G1), the P53 protein detects any mutation and does not allow the cell to continue to the S phase unless the damage is repaired. However, if P53 accumulates it induces expression of sensors and channels and stimulates them, it also inhibits anti-proapoptotic proteins, therefore, and it induces the intrinsic death pathway.

P53 protein mutations are one of the causes of cancer, when the protein is mutated the cycle continues despite the presence of DNA damage and there will be no cycle arrest at G1.

- *Misfolded proteins*: cells respond to protein misfolding by refolding them in chaperones. The cell tries to increase the activity and expression of chaperons and stop the production of these misfolded proteins. However, if they are not refolded properly, they start to accumulate and the cell starts degrading them using the ubiquitin-proteasomal pathway, which is an adaptive process, but if the damage persists for too long the cell undergoes apoptosis.

- *Cytotoxic T lymphocytes* kill off virally infected cells and tumor cells. They have a Fas ligand that identifies infected and tumor cells and cause the Fas receptor to trimerize and start off the extrinsic death pathway.

Some virally infected cells produce FLIP or they just mimic it. Cytotoxic T lymphocytes overcome the effect of FLIP by producing granzymes. These enzymes can go from T lymphocytes to targeted cells by bypassing caspase 8 and directly cleaving to activate executioner caspases.

Necroptosis (for your information, not included in the exam**)**

Necroptosis can be both pathologic and physiologic. Physiologically, it occurs during the formation of the mammalian bone growth plate. Pathologically, it occurs in acute pancreatitis, it is also part of the reperfusion injury, parkinsons disease and it is a backup against viruses that encode caspase inhibitors (e.g., cytomegalovirus).

The TNF type 1 receptor rather than activating caspase 8 (apoptosis), especially if that pathway is inhibited by FLIP, activates necrosomes that will cause changes in mitochondria enabling it to inhibit production of ATP and increasing ROS production, therefore, it is programmed cell death resulting in necrosis rather than the formation of apoptotic bodies.