Growth and reproduction are two of the characteristics of life. Cell division is the process by which all the cells of a multicellular organism are formed during growth and development. Cell division is used for replacement of cells and tissues during one's lifetime. Asexual reproduction, a means of making more individuals for many groups of organisms, is also accomplished by cell division. For those organisms that have sexual reproduction in their life cycle, a special cell division, in which chromosome number is reduced, also occurs.

We shall focus on the processes of cell reproduction in eukaryotic organisms. The process of cell division in prokaryotic organisms, all of which are unicellular organisms, is called binary fission, and will be briefly illustrated; the single molecule of DNA and absence of a nucleus in the prokaryotic cell account for a number of differences in the "mechanics" of the process by which prokaryotes divide and increase their numbers.

The collection of one's genetic information, or DNA, is known as the genome. In eukaryotic organisms, the genome consists of a number of chromosomes. Each species has a fixed chromosome number, a number that does not change from generation to generation.

For organisms that have a sexual reproduction in their life cycles, their genome includes two sets of genetic information, one set contributed by each parent at fertilization. Cells having two sets of genetic information are said to be diploid. (Details later)

Our genetic molecule, DNA, is identical in each cell within a multicellular organism, so that when cells divide, new cells formed must have exactly the same DNA as the original cell. To ensure that chromosomes and DNA remain the same in new cells (the genome remains constant) when cells divide, it is crucial to have a mechanism that exactly duplicates (replicates) the DNA of the original cell and distributes, or segregates, the copied DNA equally to the new cells.

To form new cells, we must also divide up the cytoplasm and critical organelles, such as mitochondria and chloroplasts, of the original cell into the new cells formed. Cells must also "know" when it's time to divide; there must be appropriate signals to initiate the process, and checkpoints to ensure that cell division is proceeding accurately.
We will look at the mechanism by which cells duplicate DNA in a later unit. At this time we shall focus on the eukaryotic cell cycle, which includes mitosis, the process in eukaryotic organisms by which the duplicated chromosomes are equally distributed to new nuclei, cytokinesis, the distribution of the cytoplasm of the original cell into new cells, and also look at some of the controls of cell division. In a later unit we will see how normal cell division controls are affected by cancer. We will also address in our next section the process of meiosis, which reduces chromosome number by half at one point of any sexually reproducing organism's life cycle to provide for genetic variation from generation to generation.

We will start our discussion of cell division with the vocabulary of our genetic molecule, and in particular, the vocabulary of the chromosome.

**Chromosome Structure**

DNA, as we know, is comprised of a double chain of nucleotides. It's estimated that human DNA may consist of 140 million nucleotides per cell. Our chromosomes average about 5 cm in length and the total DNA is about 2 meters. Chromosomes fit into the nucleus because they are tightly coiled.

Our chromosomes are associated with a complex of proteins called histones. There are 5 different classes of histone proteins, all of which are positively charged so that they are attracted to the phosphate groups of the nucleotides. Our DNA and its associated chromosomes comprise the chromatin material.

Prior to cell division, each 150 or so nucleotides in a chromosome coil around a complex of 8 histone protein molecules (2 from each of 4 of the classes of histone proteins) forming a nucleosome. The 5th histone protein is on the exterior of the nucleosome and may function as a clamp to hold the DNA to the histone core. Nucleosomes further coil into "beaded" supercoils that subsequently fold into loops, which coil even more into the, finally, visible chromosome during cell division.

Some DNA stays highly condensed in cells following division and is known as heterochromatin. Heterochromatin may stay so tightly condensed that its DNA cannot be read (or expressed). The DNA that can be expressed is called euchromatin. Mutations that prevent the protein condensin from coating chromosomes, which helps chromosomes condense during prophase, is one cause of sterility. *(How DNA gets expressed is discussed in later sections.)*
Chromosome Terms before and after DNA Duplication (Replication)

Note: Duplication and replication mean exactly the same thing.

• An unduplicated chromosome is **one chromosome**. A chromosome consists of two "arms" that extend from a narrow region called the **centromere**. The centromere region is often visible in duplicated chromosomes. The two ends of a chromosome are telomeres (see later).

• When a chromosome duplicates (during the S phase of Interphase of the cell cycle), it becomes **one duplicated chromosome**, and the two copies remain attached to each other. Note it is still **one chromosome**.

• The two **exact** copies of the duplicated chromosome, which remain attached by the protein **cohesin**, are called **"sister" chromatids**. They are identical to each other. **It is essential that you remember this!**

• **Kinetochore** (made of protein and DNA) form at the centromere region of the duplicated chromosome during cell division. The three-layered kinetochores attach to microtubules of the spindle during mitosis. The kinetochores of the two sister chromatids face in opposite directions.

• After the identical sister chromatids are separated during mitosis, each (called a "daughter" chromosome now) becomes a single unduplicated chromosome again.

• **Remember: "Sister" chromatids are not two chromosomes.** The two sister chromatids comprise **one duplicated chromosome** that consists of two identical chromatids. Once sister chromatids are separated they are no longer chromatids!
To summarize - When cells divide:

- We form two new cells from the original cell.
- The new cells formed must have all of the genetic material for the organism, so we need a mechanism that exactly duplicates the DNA of the original cell's nucleus and distributes or segregates the copied DNA equally to two new nuclei. **Mitosis** is the process by which the duplicated chromosomes are equally distributed to new nuclei. *(We will see how DNA duplication is accomplished later.)*
- We must also separate the cytoplasm and critical organelles, such as mitochondria and chloroplasts, of the original cell into the new cells formed so that the new cells can survive, grow and function. The distribution of the cytoplasm of the original cell into new cells is called **cytokinesis**.
- There must be appropriate signal controls for cell division to occur.

**Mitosis and the Eukaryotic Cell Cycle.**

**Mitosis** is one part of the **cell cycle.** The cell cycle includes all activities of a cell from the time it is formed until (and if) it divides or dies.

The processes, or events, of cell division can be related to the normal lifetime of a cell. For our convenience, these events are divided into "stages" of a cell's **cell cycle.** The cell cycle starts when a cell is formed and continues until it divides. Some cells never divide, others divide frequently, and some only when damaged and replacement is needed. Cell division is a brief part of the life cycle; most of the life of a cell is spent in normal activities of growth and maintenance, a stage called **interphase.** The events of interphase and cell division are:

**Interphase**
- Gap 1 (**G1**)
- DNA Synthesis
- Gap 2 (**G2**)

**Cell Division** (or cell reproduction)
- **Mitosis**
  - Prophase
  - Prometaphase
  - Metaphase
  - Anaphase
  - Telophase
- **Cytokinesis**
Cell Cycle Details

Interphase

Growth (called G₁ or First Gap in the cell cycle)
- Normal growth and cell activities
- The chromosomes are stretched out and grainy in appearance. They stain well, and early on this material was called chromatin.

DNA Duplication (called S for synthesis in the cell cycle)
- DNA duplication takes place forming the duplicated chromosomes
- DNA duplication is triggered in the G₁ phase. Once started this process cannot be reversed; the cell is committed to divide.

Preparation for Division (called G₂)
- Condensation of chromosomes is initiated during the G₂ phase of the cell cycle, although condensed chromosomes are not visible until well into prophase of mitosis.
- Synthesis of materials, such as tubulin for the spindle microtubules needed for mitosis and cytokinesis also takes place during G₂.
- Duplication of the centrosome (in organisms that have a centrosome) and its centrioles (in organisms that have centrioles) occurs.
- As G₂ progresses duplicated centrosomes migrate in the cytoplasm to either side of the nuclear envelope and the microtubule organizing center, (centrosome) is initiated. The orientation of the centrosomes (or microtubule organizing center) determines the "poles" of the dividing cell.
- Tubulin dimers aggregate in the centrosome regions and/or poles of the cell in preparation for spindle formation during prophase.
- In plants, a preprophase band of microtubules formed near the center of the cell determines the plane of division.

Note: If a cell never divides, following G₁ it will be in a “permanent” state of G₀ (or non-dividing state).
The Stages of Mitosis: Mitosis is a continuum. Humans have decided to separate the process into stages for the convenience of our discussions. Some humans even separate the stages into sub-stages and intermediate stages. For our purposes, cell division in eukaryotes involves three events:

DNA Duplication
- Process of duplicating the genetic material of the nucleus (Discussed later)
- This occurs during Interphase, when growth and/or normal metabolic activities take place.

Mitosis
- Process of distributing the duplicated DNA equally to the two new nuclei.

Cytokinesis
- Process of separating the cytoplasm contents

Mitosis consists of five stages (phases):
Prophase, Prometaphase, Metaphase, Anaphase, Telophase

Prophase
Chromosome Condensation
As the cell starts prophase, the nucleoli disperse and "disappear". The duplicated chromosomes continue condensing and thickening as mitosis progresses and become visible as threadlike structures. Motor proteins are involved in the condensation of chromosomes. The duplicated chromosomes are firmly attached at their centromeres throughout this condensation and coiling, although cohesin molecules degrade along the remainder of the duplicated chromosome separating most of the sister chromatids.

The kinetochores, important in chromosome movement, are formed in the centromere regions of each duplicated chromosome at this time.

Microtubule Organization and the Mitotic Spindle
- Microtubules and associated proteins form the spindle apparatus during prophase. Some of the cell's cytoskeleton will disassemble to provide spindle microtubules. The spindle also synthesizes additional microtubules from the tubulin dimers synthesized during interphase.
- Microtubules radiating from the centrosomes in animal cells are called asters. Centrosomes migrate toward the poles by lengthening microtubules during prophase. Plant cells also have less visible asters and spindle formation, but lack centrosomes in the microtubule organizing center.
Prometaphase (Late Prophase)

Nuclear Envelope

• The nuclear envelope degrades in prometaphase into small vesicles, used to synthesize new nuclear membrane material in the new cells.

Spindle Apparatus

• The spindle apparatus will extend from the poles of the cell through the center of the cell to the opposite pole of the cell by the end of prometaphase. Elongating polar microtubules go from each pole toward the equator of the cell where they overlap with polar microtubules from the opposite centrosome or aster forming the framework of the mitotic spindle and stabilizing the spindle complex.

• Kinetochore microtubules from the opposite poles attach to kinetochores of each chromatid of the duplicated chromosomes. The organization of the kinetochore microtubules is such that each duplicated chromosome has kinetochore microtubules from one pole attaching to one of the sister chromatids, and kinetochore microtubules from the opposite pole attaching to the other sister chromatid. If there are mistakes in attachment, chromatids are not separated correctly during mitosis.

• The spindle microtubules pull on the kinetochore regions of the sister chromatids in a "tug-of-war" which eventually leads to a migration of the duplicated chromosomes to the equatorial plane of the cell. Both sets of kinetochore microtubules are pulling towards their respective "pole" that assists the ultimate migration of the duplicated chromosomes toward the equator. Viewed "live", chromosome motion seems random and aimless.

• As the cell proceeds into metaphase aster microtubules have also attached to the plasma membrane, anchoring the "poles".
Metaphase
- In metaphase, the spindle apparatus has moved the chromosomes to the equator of the cell, aligning the centromeres of each duplicated chromosome along the equator of the cell.
- Centromeres of each sister chromatid are aligned with each other and each sister chromatid is connected at its kinetochore to microtubules from its respective pole.

This alignment of chromosomes along the equatorial plane of the cell is often called the **metaphase plate**. The metaphase plate is quite distinctive when viewed from the "top of a cell" or polar view as opposed to the view typically shown in textbooks.
Anaphase

- To initiate anaphase, the remaining cohesin proteins at the centromere regions of the sister chromatids degrade so the sister chromatids can be separated. The hydrolytic enzyme, **separase**, catalyzes this reaction. If the chromatids are not aligned properly, the separase precursor is inhibited, and mitosis does not proceed. This is called the **spindle checkpoint** (see later).

- Separated chromosomes being moved away from the equator is the first visual sign of anaphase. By definition, each sister chromatid is now a single unduplicated chromosome, or "daughter" chromosome.

- The kinetochore motor protein, **cytoplasmic dynein**, hydrolyzes ATP to move the chromosomes along the kinetochore microtubules. Most cells take about 10–60 minutes to complete anaphase.

- In addition, kinetochore microtubules decompose at their kinetochore ends, pulling the chromosomes, centromere first, away from each other and toward the respective poles of the cell as the kinetochores shorten.

- Polar microtubules lengthen, moving the poles of the cell further apart, and, in animal cells elongating the cell.

- Since sister chromatids are identical, each of the two clusters of chromosomes being pulled to the two poles of the cell has one copy of each original chromosome. As the chromosomes are pulled to the poles, they begin to lengthen out.
Telophase

- The two sets of daughter chromosomes aggregate at the poles of the cell, uncoil and the aggregate of chromosomes becomes indistinct as chromatin.
- The cell, if it is an animal cell, continues to elongate by lengthening the polar microtubules (decreasing the overlap).
- At each pole membrane vesicles and membrane fragments form new nuclear envelopes around each group of chromosomes resulting in two distinct nuclei in the cell.
- The spindle microtubules disperse and the spindle apparatus disappears.
- New nucleoli form, concluding mitosis.

Mitosis in Blood Lily
Mitosis and the Cell Cycle - 11

Cytokinesis: Separation of the Cytoplasmic Contents
Speaking precisely, mitosis describes events of chromosomes and nuclei. Most cells accompany mitosis with cytokinesis, the separation of the cytoplasm of the original cell into two new cells. This is not always the case. Some organisms (including many fungi and algae) are "multinucleate"; they just have one cell body with many nuclei. Some animal tissues are also multinucleate.

Cytokinesis typically coincides with the events of late anaphase and telophase, so that at the completion of mitosis, the original cell is separated into two cells, each with a nucleus and DNA identical to that of the original cell. Although the end result of cytokinesis is always two new cells, the mechanism of separation is different in plants and animals, so we shall discuss them separately.

Cytokinesis in Animal Cells
The cells of animals lack cell walls. Cytokinesis in animal cells is started with the formation of a cleavage furrow, a depression or pinching in of the plasma membrane.

This is caused by a ring of microfilaments, the contractile ring, composed of the protein, actin, associated with myosin, which forms across the middle of the cell after the chromosomes are separated in anaphase. This ring contracts, pinching or drawing in the plasma membrane toward the center of the cell, which eventually pinches the cell in two. The additional membrane surface needed is supplied by membrane material synthesized during Interphase. Cytokinesis, hence cell division, can be disrupted by mutations that affect microfilament function.
Cytokinesis in Plant Cells
Each cell of a plant is surrounded by a rigid cell wall. Plant cells can not form a cleavage furrow. Instead, plant cells are separated by the cell plate formation.

Cell plate formation involves making a cross wall at the equator of the original cell and plasma membrane. Kinesin motor proteins move Golgi vesicles containing wall material along microtubules to the equator of the cell. The vesicles contents contribute to a disk-like structure that is called the phragmoplast. As cellulose and other fibers are deposited, the cell plate results creating a boundary and new cell wall between the two new cells. Membrane material from the original vesicles fuses to each side of the cell plate forming new cell membranes on the dividing sides of the original cell.

In both plants and animals, organelles within the cytosol are distributed into the two new cells formed by cytokinesis.

Variations in Mitosis in Eukaryotes
The process of mitosis in most eukaryotes is remarkably the same. However, some protists, such as dinoflagellates and diatoms, and some yeasts do not degrade the nuclear membrane during mitosis. Spindles may not form at all (some protists), may form within the nucleus (yeasts and diatoms), or may form outside of the nucleus and pass through nuclear pores (dinoflagellates).
Binary Fission in Prokaryotes
The process of cell division in prokaryotes, called binary fission, parallels that of eukaryotes. Bacteria have just one long continuous, or circular molecule of DNA with some associated proteins, although the DNA is routinely folded and compacted to fit into the cell. That, and the absence of a nucleus in the prokaryotic cell, account for a number of differences in the "mechanics" of the process. Polar proteins attached to the DNA molecule facilitate the folding needed for the DNA to fit within the cytoplasm of the cell. The rate of cell division in prokaryotes is related to environmental conditions, dividing more rapidly when conditions are good, and less rapidly in poor environments. Binary fission is used to increase population numbers.

- The single DNA molecule attaches to the plasma membrane prior to duplication and cell division at a site on the chromosome identified as the origin, or ori. (The termination region of replication is the "ter" and signals the completion of DNA duplication.)
- As the DNA is duplicated using a complex of enzymes (very similar to that of eukaryotes), the duplicated molecule's ori region is attached to the plasma membrane.
- The cell elongates by synthesizing new membrane and wall material between the two ori regions, separating the two DNA molecules.
- After a period of elongation, in which the original cell about doubles its length, plasma membrane is pulled inward (much as occurs with eukaryotic animal cells), pinching off the two halves of the original cell. New cell wall material is also synthesized.

Prokaryote Cell Cycle

E coli dividing  Pseudomonas dividing

Binary Fission
Regulating the Cell Cycle
The control of cell division is one of the most active areas of biological research, in part because cancers are diseases that involve cells that have lost their cell division controls. In humans, some cells routinely divide: taste bud cells and cells lining the digestive tract divide about every three days, skin cells monthly and red blood cells are produced by the millions each day. Other cells in mature humans never divide. Nerve cells and muscle cells are two examples (although one can increase one’s muscle mass by making muscles work). Much of our human brain development occurs in the first two years. Liver cells divide only if damage occurs. (Animal growth and development is discussed in Biology 212).

Plants have an open pattern of growth. They are making new cells "constantly", not for replacement, but for continued growth. Plants have a tissue, meristem, that is specialized for cell division. However, there are numerous exceptions in plants, including some that are a part of normal development. In addition, many plant cells have a remarkable ability to dedifferentiate and become "embryonic", something that rarely happens in animals. Many plant cells die at maturity, but continue to do their function (Plant growth is discussed in Biology 213).

But what tells a cell to divide - or not to divide? In cell fusion studies during the 1970's, researchers learned that there are definite cell-cycle controls that direct and coordinate the events of the cell cycle. The cell cycle is subject to both internal and external chemical control mechanisms.

Cell-cycle Control Checkpoints
As is common, most of what we know comes from research on animal processes. For cell division, we also have research on yeast cells, and the processes are remarkably similar. The animal cell cycle has at least three "checkpoints" (in G₁, G₂ and mid-mitosis) where the cell cycle remains in that stage until over-ridden by chemical signals, which we shall discuss. Cells have both external and internal signal transduction pathways controlling cell division.
There are three well-studied checkpoints for cell division that function during G₁, G₂ and in Mitosis (M).

- The first checkpoint, called the G₁/S checkpoint, or sometimes restriction (R) point, is in G₁ and determines whether DNA replication should proceed. Cells that never leave G₁ are said to be in a non-dividing cycle called G₀. Cells will stay in G₁ until they receive a signal to proceed with DNA duplication.

- The second checkpoint, G₂/M, is in G₂ just prior to mitosis and determines if mitosis will begin.

- The third checkpoint, which is in metaphase of mitosis (M), is the spindle or APC (anaphase-promoting complex) checkpoint.

The checkpoint signals involve protein kinases, called cyclin-dependent kinases (Cdk), which are activated by proteins called cyclins, whose concentration is cyclical (hence cyclin). Cyclin is a cyclin-dependent kinase allosteric regulator molecule. {Kinases are enzymes with serine, threonine or tyrosine amino acids that that function in cell signal relay pathways when phosphorylated by ATP. Kinase-relay pathways are discussed with cell communication and signaling.}
Cyclin concentration rises and falls during the cell cycle. When levels are high, cyclins combine with the cyclin-dependent kinases (CdkS) forming a complex (cyclin-Cdk).

When activated by their regulator cyclin, the cyclin-dependent kinases phosphorylate proteins needed for DNA synthesis and for mitosis. The phosphorylated protein changes shape to initiate important steps in the cell cycle.

The first cyclin-Cdk complex discovered was called MPF for "maturation (or mitosis) promoting factor", a cyclin-Cdk complex that activates the start of mitosis in G2. Its activity coincides with peak levels of cyclins. MPF activates kinase transduction relay pathways that promote a number of mitosis activities, including degradation of the nuclear membrane, spindle formation and chromosome condensation.

There are a variety of different Cdk and cyclin molecules that function in the cell cycle. For example:

- The cyclin/Cdk at the spindle (M) checkpoint activates the Anaphase promoting complex, APC.
- The cyclin/Cdk at G1/S phosphorylates the protein, RB to start a transduction cascade for DNA duplication. Until phosphorylated, RB blocks DNA duplication.

The Cdk-cyclin complexes involved at the cell division checkpoints promote their own degradation by activating proteolytic enzymes that destroy cyclin at each checkpoint. Enzymes that dephosphorylate are phosphatases. This fluctuation in the level of cyclins regulates the cell cycle.
Cell Control Checkpoint Details

G₁/S (or R {restriction}) checkpoint
Nutritional status and cell size signals at the G₁/S checkpoint ensure that the cell has sufficient resources and volume to divide. External signals include a number of growth factors that promote cell division (see below). Such signals promote the synthesis of G₁ cyclin molecules to complex with Cdk (the Cdk/G₁ cyclin complex), which activates the synthesis of proteins needed for DNA duplication. DNA integrity is also monitored at G₁. (p21, a protein that blocks DNA duplication by binding to the G₁/S cyclin/Cdk is activated when DNA is damaged. Mutations in p21 are involved in some cancers.)

G₂/M Checkpoint
The MPF cyclin-dependent kinase that operates at the G₂/M checkpoint triggers cell activities that check the integrity of the duplicated DNA molecules and commits the cell to mitosis.

At this checkpoint, a phosphorylated Cdk (called cdc2) must be dephosphorylated to activate mitosis by appropriate signals. Once cdc2 is dephosphorylated, the MPF activates its own phosphatase enzyme. The checkpoint works by assessing the balance of kinases that add phosphates (maintaining inhibition) with the phosphatases that remove phosphates. When DNA damage is detected, the balance favors MPF phosphorylation activity, and mitosis does not take place. (One DNA damage-sensing protein, p53, when mutated and non-functional, is associated with nearly half of human cancers.)

The Spindle Checkpoint – the Anaphase-Promoting Complex (APC)
The spindle checkpoint is regulated by the kinetochores of the sister chromatids. They delay anaphase until the kinetochores of all the duplicated chromosomes are attached to kinetochore microtubules and oriented correctly to their poles with the appropriate tension between the poles. Proteins associated with the kinetochores have a signal pathway that blocks the enzyme that hydrolyzes securin, the separase inhibitor. Separse cannot hydrolyze the remaining cohesin holding sister chromatids together until its inhibitor is removed. Once cohesin is degraded, anaphase commences. This checkpoint ensures that each cell formed will have the correct complement of chromosomes; no stray chromosome can avoid the metaphase plate and impact mitosis.
External Signals and Cell Division Control

Cell division, like many other cell activities, cannot occur if essential nutrients are not available. As mentioned in the G₁ checkpoint discussion, Growth factors are a class of proteins involved with signal transduction pathways that can promote or arrest cell division, so that cells divide when needed and do not divide inappropriately (except when cancers happen).

It takes a certain concentration of growth factor(s) for cell division to proceed. It also takes sufficient appropriate nutrients to divide so that growth factors and nutrient supply are both important in regulating the rate of cell division. Absence of appropriate growth factors is one means of keeping a cell in G₀.

In plant tissue culture, a cell will remain undivided until and unless an appropriate mixture of nutrients and growth regulators (plant hormones) is provided. Early plant tissue culture succeeded when researchers added coconut endosperm (coconut milk) to the culture medium. The endosperm is rich in both plant hormones and nutrients because its normal job is providing nutrients to the developing embryo.

External Factors and Growth Density-dependent Inhibition

Normal growth is also regulated by external environmental conditions. In animal cell cultures, cell division is halted when the cell population gets too dense and exceeds the nutrient and growth factor concentration needed to continue to divide. Crowded cells stop dividing. As in population biology and ecology, such regulation of cell division is known as a density-dependent activity or in this case a density-dependent inhibition. It’s important to note that density-dependent inhibition is chemical. Physical contact is less important in stopping cell division.
Anchorage Dependence
Animal cell division in cultures is also arrested when cells do not have an appropriate substrate or an extracellular matrix of cells to attach to during division. Normally, membrane proteins and components of the cytoskeleton trigger anchoring signal pathways that have a role in regulating cell division. Such cell division control is known as anchorage dependence.

Cancers and Cell Controls
In cancers, the normal controls of the cell cycle are disrupted. Cancer cells seem to lack cell density and cell anchoring controls. Abnormal cell cycle patterns are also present in cancers, as are significant changes in chromosomes. Although most cells cease division after 20 – 30 cycles, cancer cells may divide indefinitely. Tumor cells removed from a woman named Henrietta Lacks in 1951 are still dividing. Cancer cells also continue to divide when growth factors are missing. It is likely that for some cancers, signal pathways activated normally by a growth factor remain active, or that cancer cells synthesize their own growth factors. (Cancer and gene regulation will be discussed later in Biology 211.)

Cell Death and the Cell Cycle
Death of cells is a normal part of multicellular organisms. Cells have two common ways of dying. If a cell is damaged by injury (mechanical damage) or toxins, or if the cell lacks sufficient nutrients to function, it will generally swell and burst. This is called necrosis. Our immune system responds to necrosis with inflammation and other appropriate responses to "clean up" the damaged area.

Alternatively cells may be are programmed to die when they are no longer needed or when they are damaged genetically beyond repair. This set of genetic events is called apoptosis. Apoptosis is common and normal during development, particularly of the nervous system and for morphological changes in development, such as the formation of digits or reabsorption of parts used by the embryo, but not the adult. Apoptosis is also part of the normal functioning of the immune system and in the normal sloughing off of epithelial tissues, such as the linings of the digestive tract.
During apoptosis, the cell's DNA is destroyed, organelles are destroyed and the cell shrinks and becomes lobed in appearance, a process called **blebbing**. The cell components are packaged into vesicles to be engulfed by the cells of the immune system.

![Normal WBC and WBC in Apoptosis](image)

Apoptosis is important in cells in which there has been genetic damage and cell division would perpetuate the damage, possibly leading to abnormal growth and cancers. Apoptosis is common in all animals and the regulatory genes are so similar that an apoptosis gene from one organism spliced into a second will function in the second organism. Apoptosis genes are activated when defective DNA is discovered. Apoptosis may have a role in both Alzheimer's and Parkinson's diseases, and failure of apoptosis is involved in some cancers. In some plants, apoptosis is used in the hypersensitive response in response to some pathogens. *(Plant defense responses are discussed in Biology 213.)* Plant apoptosis does not involve blebbing. The cell contents are digested within the central plant vacuole, and the digested molecules released from the cell.

**Gene Control of Apoptosis**

By studying a small nematode worm, *Caenorhabditis elegans*, researchers were able to identify genes involved in apoptosis called ced genes. *(ced = cell death).* The ced genes are coded, but the proteins in the cytosol are inactive until called into action for apoptosis during normal development or when cells are damaged. The activated ced proteins hydrolyze nucleic acids and proteins of the cell. In *C elegans*, the ced-4 and ced-3 proteins are involved in apoptosis. A third protein, ced-9, located on the mitochondrial membrane, inhibits ced-3 and ced-4. When ced-9 is inhibited, ced-4 initiates apoptosis.

Humans have a set of enzymes, called capases, which function in the same manner. The protein, Bcl-2 inhibits apoptosis similarly to ced-9 in *C elegans*. Apaf1 activates apoptosis.

Apoptosis can be triggered by signals when either DNA is too damaged or proteins are not being assembled correctly in the ER. Capases are then released from the mitochondria through holes formed in the mitochondrial membranes. Cytochrome c, an electron carrier for cell respiration promotes apoptosis by facilitating the movement through the mitochondrial membrane.
Ced 9 Control of Apoptosis

Capase Activation in Apoptosis

Comparing Apoptosis in C elegans and Human neuron
When and Where Does Mitosis Occur?

Growth
All growth (increase in numbers of cells) in individual organisms takes place by mitosis, from the fertilized egg (zygote) to death.

Repair and Replacement
Mitosis is used for replacement of damaged cells or tissues, as well as for the routine replacement of cells that is a part of normal growth, development and maintenance. When cells die, they are replaced by mitosis. The rate of cell replacement varies with tissue type. Some human cells, such as those that line the digestive tract, are replaced every 1 – 3 days. Our red blood cells last about four months. Some, such as nerve cells, are never replaced.

Non-Sexual (Asexual) Reproduction
Mitosis is used for all asexual reproduction or propagation. This is especially common in plants, fungi and protists. Animals less commonly reproduce asexually. There are many claims for the world’s largest organism based on the ability to make more. Asexual reproduction produces offspring genetically identical to the original parent, as would be expected of any mitosis. Cloning is a variant of asexual reproduction.

Asexual Reproduction in Aspen, Fungi, Protist and Hydra (an Animal)
Cloning – A Variant of Asexual Reproduction
The zygote of any organism has total genetic competence. It is said to be **totipotent** because its DNA, or genome, has all of the instructions for the organism that is going to develop. In multicellular organisms, as growth and development take place (all by mitosis), cells and tissues differentiate for their specialized functions and their DNA becomes determined; different genes are activated or repressed in different tissues, so we have differential or selective gene expression depending on the cell and tissue type, although each cell retains the total complement of DNA found in the zygote.

The idea of cloning is to produce a genetically identical organism using a single cell from a "mature" multicellular organism. For cloning to succeed, that single cell must be able not only to divide, but also to differentiate its cells into the tissues and organs of the "adult". One compelling reason for cloning research today is not to have more "copies" of any one individual, but for the production of stem cells, which have the ability to generate many kinds of tissues for treatment of diseases and injuries (*Stem cell research and applications discussed in our section on Genetic Technology*).
However, our initial start in this subject dates from the 1950's when FC Steward at Cornell University successfully cultured carrots from single cells taken from the root of the carrot. This was the first laboratory "clone". Although the plant cells used were differentiated root tissue cells, they were totipotent – no genetic material had been permanently lost or "turned off". That plants naturally have cells that dedifferentiate to form new meristem regions was a good clue to this. Most cells of plants retain the ability to "dedifferentiate" and become embryonic-like. *(Plant growth and development is discussed in Biology 213.)*

Cloning many kinds of plants turned out to be fairly easy – provide the totipotent cell with the right mix of hormones, nutrients, chemical signals and a growth medium, such as agar, and genetically identical plants grow. In the 1970's one could buy test-tube plants to share with one's friends as a novelty it was so successful. This method, known as tissue culture, is a common way of cloning plants. Cloning is used in agriculture, forestry and orchid cultivation today, and augments the myriad ways plants have for "natural" asexual propagation.

### Animal Cloning

Cloning animals has not been so easy, although experiments have been ongoing for decades. In contrast to plant cells, nuclei in animal tissues do undergo "permanent" changes during development so that taking one cell and treating it with hormones and nutrients and other chemical signals does not result in the cell developing into a new organism.
Early animal cloning research used nuclear transplantation – a process that takes a nucleus from an embryo and transplants it into an egg cell whose nucleus has been destroyed, typically by UV light. Thomas King and Robert Briggs did extensive nuclear transplant experiments on frog embryos in the 1950s while doing research on genetic determination in embryo cell lines, and also by John Gurdon in the 1970s. How and if the embryo developed helped researchers identify how long an embryonic cell line remained totipotent. Only early embryo nuclei were successful in the transplant studies. Cells transplanted from tadpoles typically did not develop. The totipotency of very early human embryos provides for some genetic screening that requires cell DNA observation.

Similar studies with mammalian embryo nuclear transplants were also successful, but efforts with adult or fully differentiated cells did not succeed until 1997.

All cloning in animals still involves nuclear transplantation into an egg cell. The cytoplasm of the egg cell is essential, and plays significant roles in early development. In animal cloning, the new organism has a nucleus from the "parent", but the nucleus is injected into an egg cell from which the egg cell nucleus has been removed. This is called cell fusion. The fused cell, or "clone", is then implanted into a surrogate mother for development.

In 1996, Ian Wilmut cloned a sheep using the nucleus from an altered mammary cell of an adult sheep and an enucleated ovum from a second donor. They induced the nuclei of the mammary cells to dedifferentiate by growing isolated mammary cells in a nutrient-poor medium that forced the cells into a stalled G1 growth phase prior to implantation into the egg cell (ovum). At that time, they used mitosis-stimulating chemicals to initiate division. Once the diploid egg cell divided and started developing, the embryos were transplanted into a surrogate "mother" and one survived, the now famous Dolly, who aged prematurely and is no longer alive.

Since Dolly, many mammals have been "cloned" using this technique. It was announced in 2001 that cloned human embryos had been formed, but not implanted in surrogate mothers. Researchers in South Korea in 2004 developed human embryonic clones through the blastocyst stage. Many such announcements have occurred during the past decade.
Apart from ethical concerns with cloning, the nuclear transplantation method of animal cloning has a very low success rate, and many, if not most, embryos fail to develop and many that do have abnormalities, as did Dolly.

Both donor cell and donor nucleus undergo trauma, and implantation has additional risks. In addition, during normal development, the chromatin within cells is altered by DNA methylation and histone modifications to deactivate many genes. The DNA of donor cells for cloning has been affected by these generally non-reversible changes. Although donor nuclei are treated to "dedifferentiate" prior to transplanting into egg cells, the reversal appears to be incomplete in cases studied. Some newer techniques cause less trauma to the donor nucleus and using a mixture of chemicals from rapidly dividing cells promotes more stable DNA in the donor nucleus. This technique is called chromatin transfer. None the less, cloning is ever increasing, and used for many purposes, including increasing the populations of endangered species, ensuring that organisms whose genome humans value for "economic" reasons increase their numbers, and even replicating the genome of desired pets. However, clones are never "identical" despite the common DNA, showing the role of specialization within cell lines during development and the role of the environment.