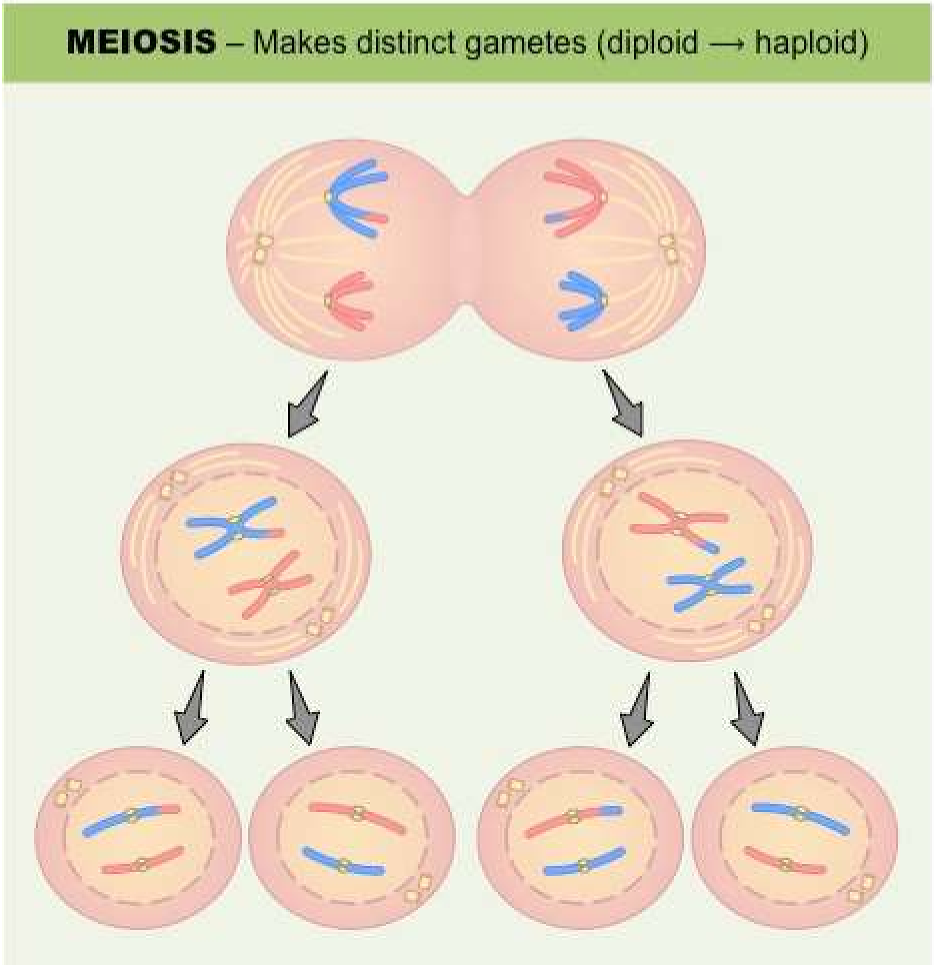
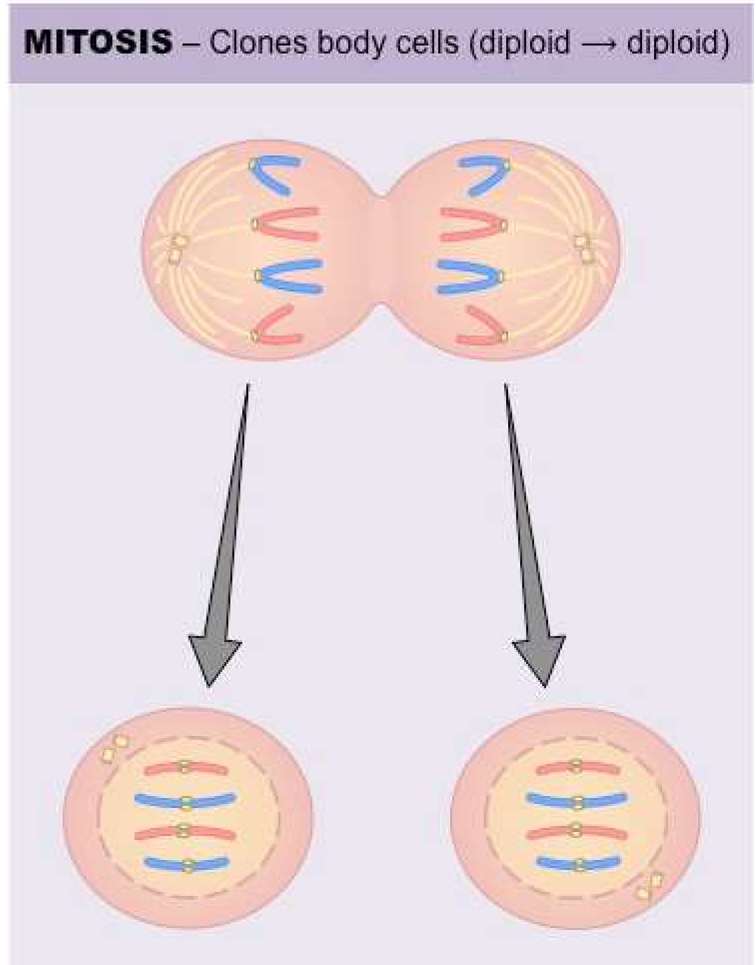
Chapter 3.2

DNA Replication & The Cell Cycle

About Cell Division

There are two types of cell division: mitosis and meiosis.

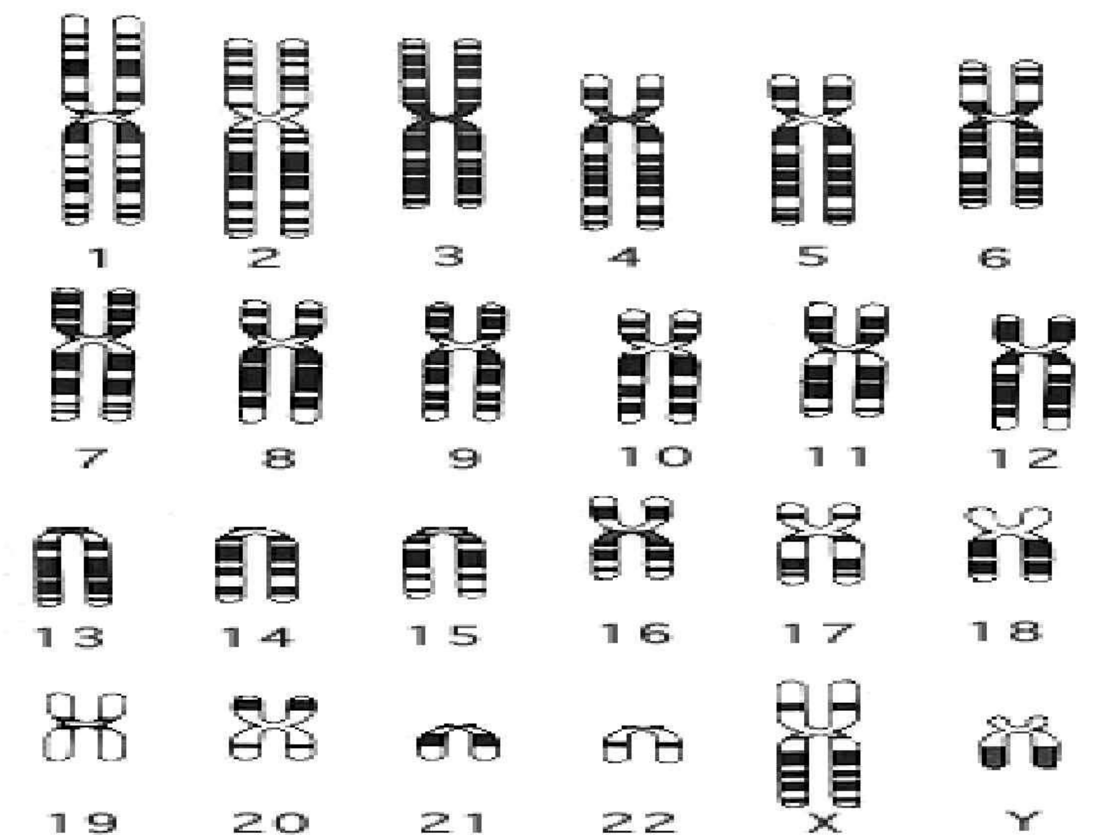




About Mitosis

- Mitosis: conserves chromosome number (humans = 46)
  - Human “somatic cells” divide by mitosis
  - Somatic cells are called “diploid cells” /// 46 chromosomes
  - Humans have 23 pairs of chromosomes /// total of 46 chromosomes
  - 23 come from our mother
  - 23 come from from father

Human Karyotype



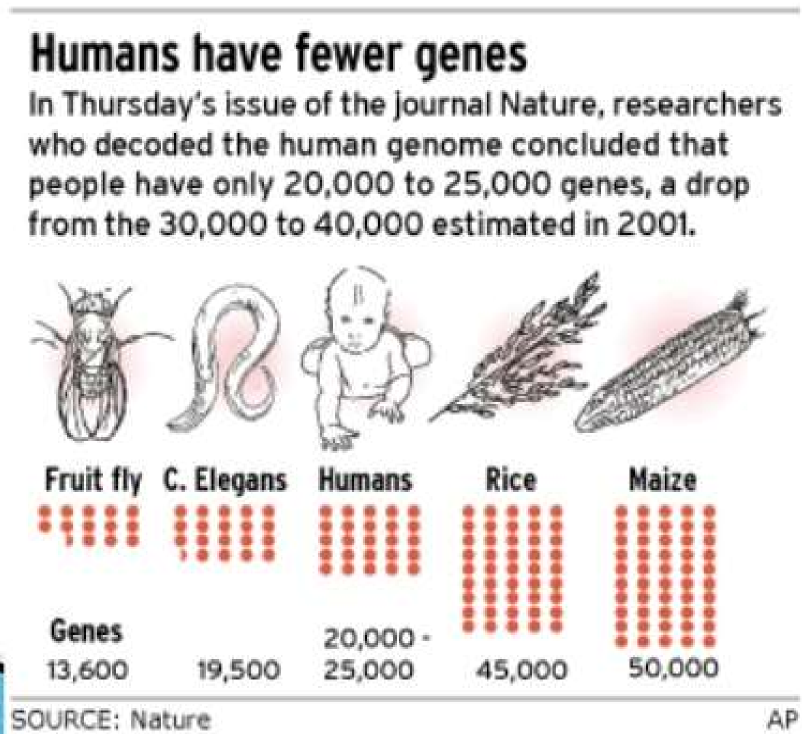
Typical karyotype of somatic cell. Twenty-two autosome chromosomes and one pair of sex chromosomes

About Meiosis

- Meiosis: cell division that reduces chromosome number by ½
- Human gonadal tissue produce gametes by meiosis (testes produce sperm and ovaries produce eggs)
  - Gametes are “haploid cells” // these are different than our somatic cells
  - Haploid cells only have 23 chromosomes
  - An egg and a sperm combine their chromosomes to create a new diploid cell // called the zygote
- *We will review mitosis in this power-point presentation. We will look at meiosis when we cover the reproductive system. /// There are similar step in mitosis and meiosis.*

Human Genom Project

- The human genome project was completed in 2003.
- It was established that human genome contains about 20,000 to 25,000 genes.

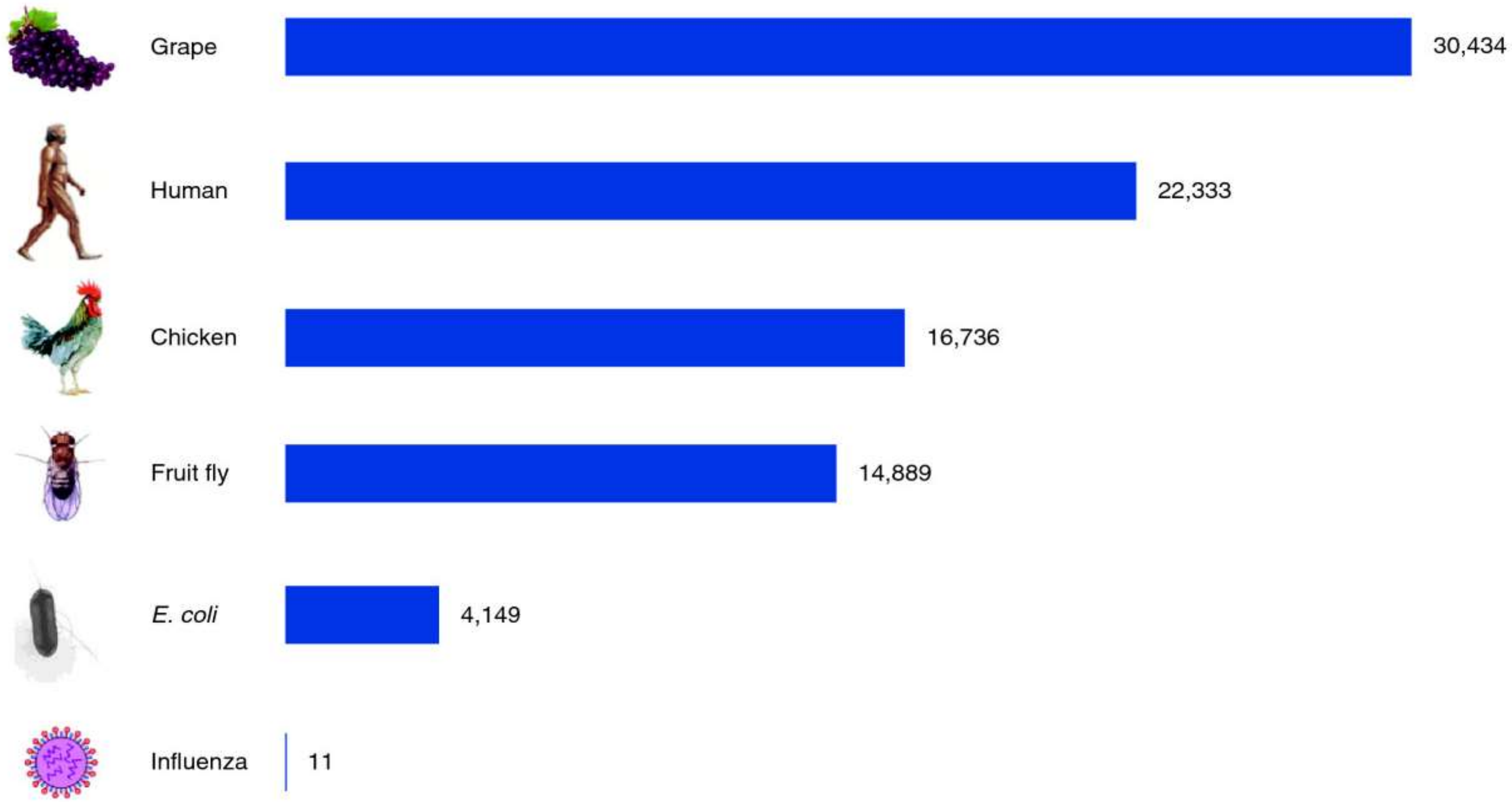


Genus species are defined by the number of chromosomes. The number of genes is just an index of how many different proteins the species is able to make.

Humans have 46 chromosomes.

In fact, each species of plants and animals has a set number of chromosomes. A fruit fly has 8 chromosomes, while a rice plant has 12 and a dog, 39. Gorilla 48, monkey 54, strawberry 56, carp 100, black mulberry 308.

Number of Genes



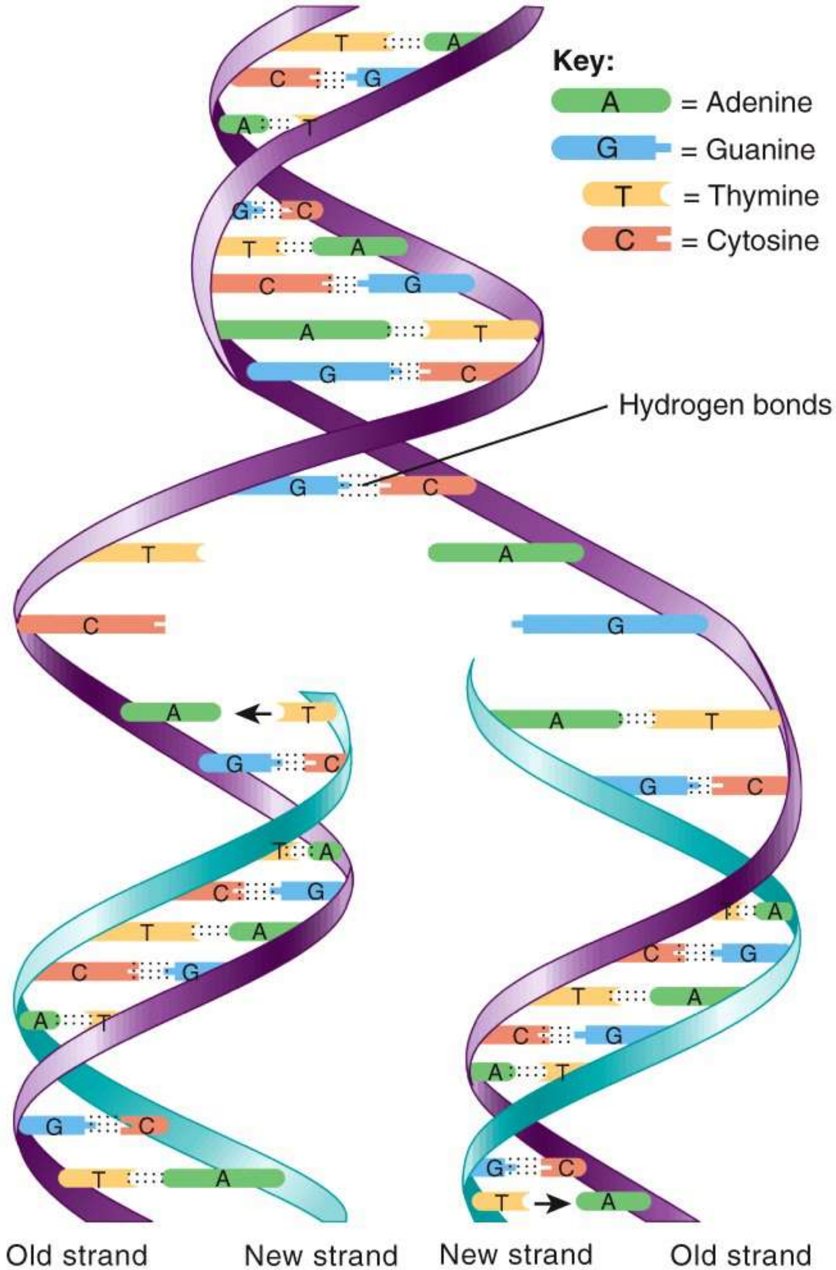
What type of molecule is made by the information contained in the gene?

DNA Replication and the Cell Cycle

- The cell cycle has two phases: interphase and mitotic phase.
- Before a cells divides by mitosis, the cell must first double its DNA (the genetic material)
- During interphase – DNA's chromosomes are organized as uncoiled strands now called chromatin // humans have 46 of these strands
- Parent cell (i.e. the original cell) must double its DNA so it can give each new cell (the daughter cell) a complete copy of DNA (the chromosomes) // this occurs in mitosis // 46 x 2 = 92 (or 46 identical chromosome pairs)
- Since DNA controls all cellular function, this replication process must be very exact – no mistakes in duplication process /// a mistake = mutation

These Two Laws Allow for Accurate DNA Replication

- **Law of Complementary Base Pairing**
  - we can predict the base sequence of one DNA strand if we know the sequence of the other
  - enables a cell to reproduce one strand based on the information in another
- **Law of Semi-Conservative Replication**
  - New cell will contain DNA molecule with one side new nucleotides and the other side of the DNA molecule will have “half” of the original DNA molecule



DNA is a macromolecule constructed by two strands of nucleic acid connected by hydrogen bonds.

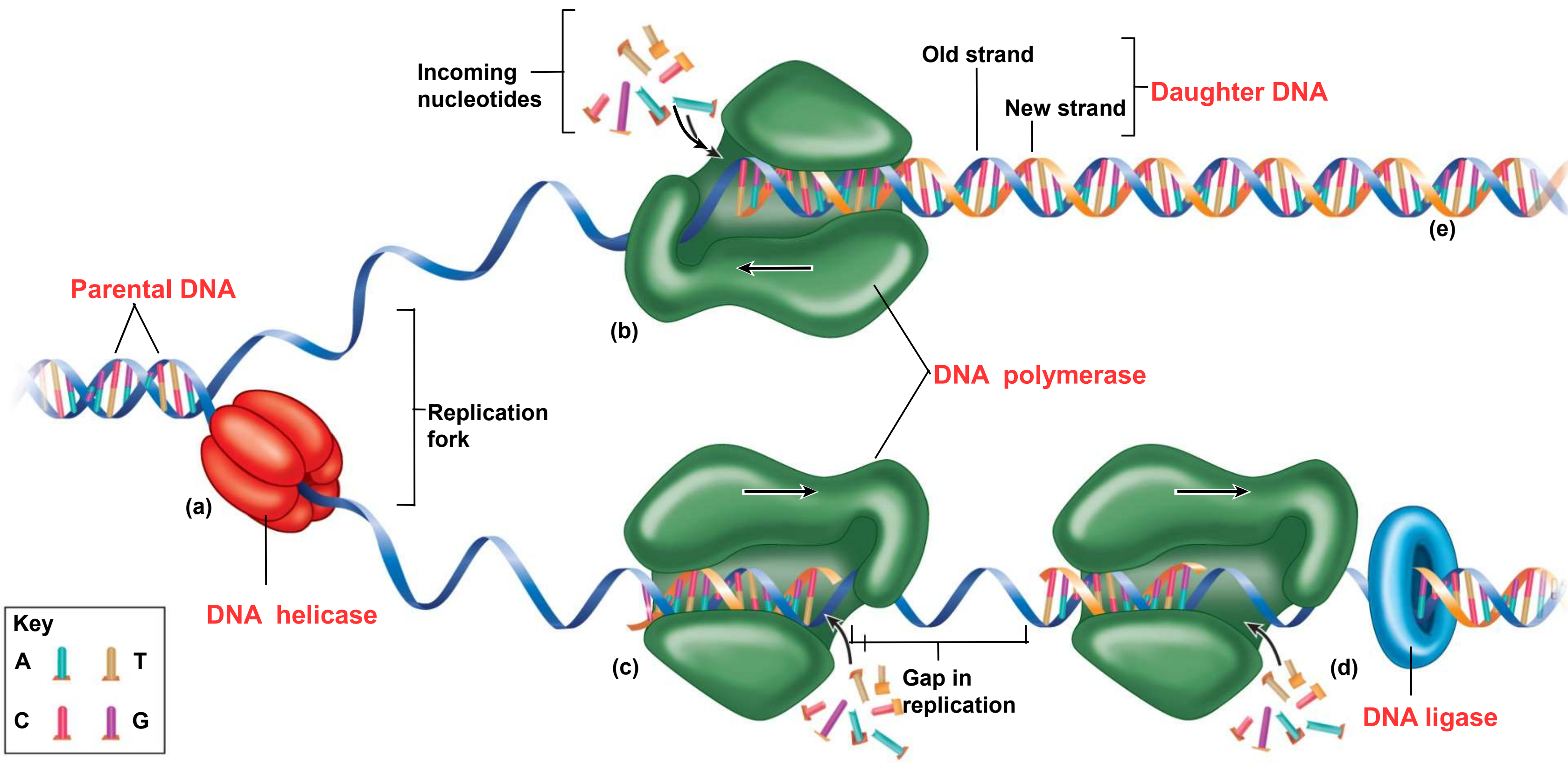
These four nucleotide molecules are used to make a strand of DNA.

Adenine forms hydrogen bonds only with thymine.

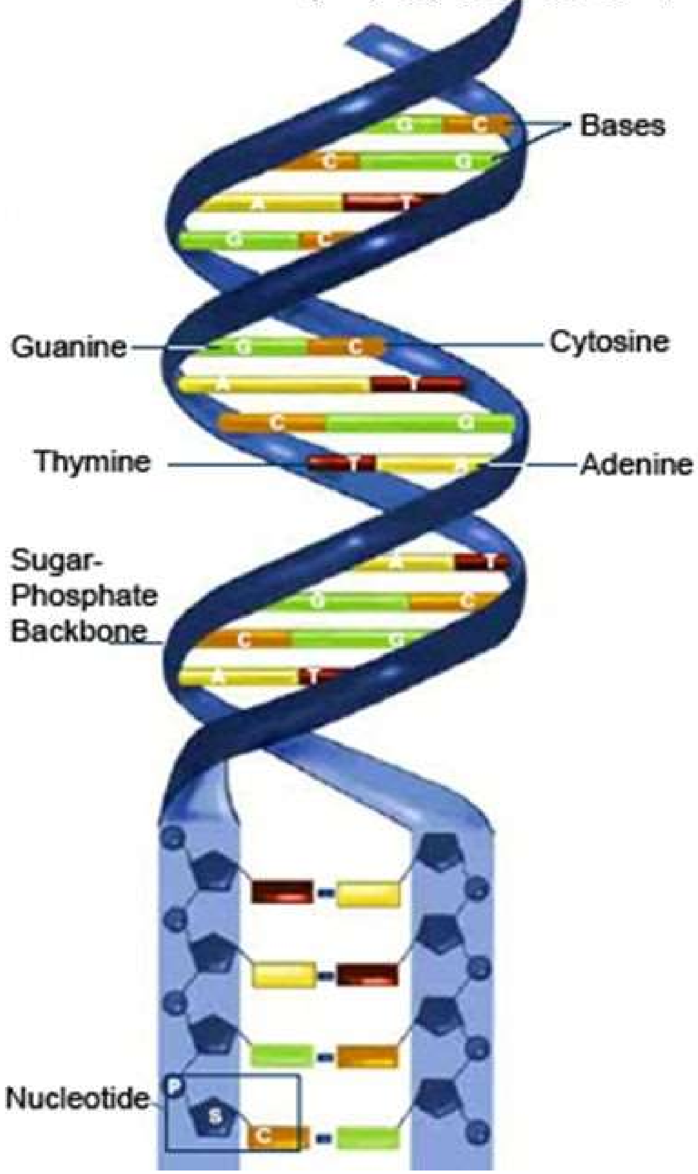
Guanine forms hydrogen bonds only with cytosine.

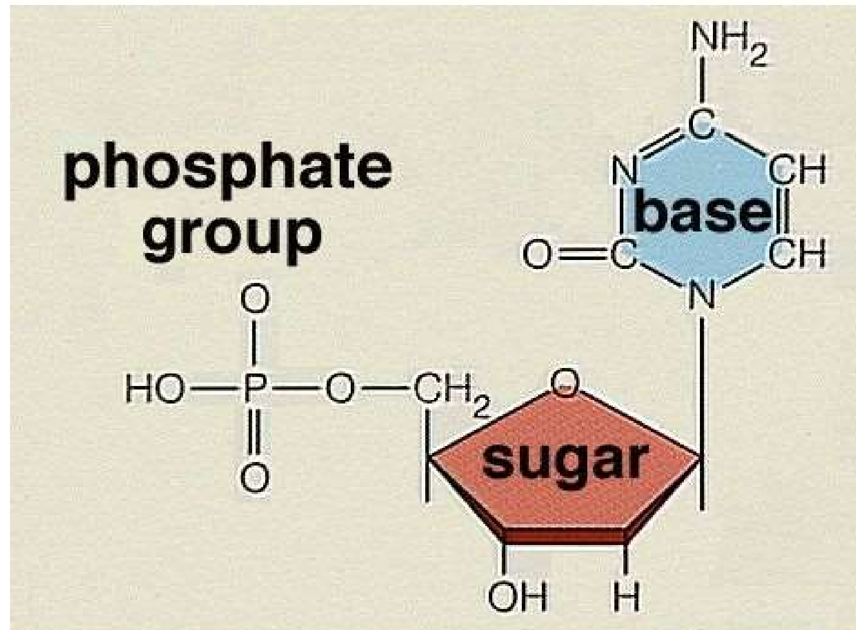
If you have only a single strand of nucleic acids and an assortment of nucleotides, then they will hydrogen bond with their appropriate nucleotide to make a new strand.

DNA Replication



Nucleotides Form DNA





Nucleotide Structure

Steps of DNA Replication

Double helix unwinds from histones

Enzyme DNA helicase opens one short segment of helix at a time // exposing its nitrogen bases

Replication fork – the point where the DNA is opened up (like two separated halves of a zipper)

DNA polymerase molecules move along each strand /// read the exposed bases /// matches complementary free nucleotides

The two separated strands of DNA are copied by two separate polymerase molecules, one on each strand - proceeding in opposite directions

- the polymerase molecule moving toward the replication fork makes a long, continuous, new strand of DNA
- the polymerase molecule moving away from the replication fork makes short segments of DNA at a time …DNA ligase joins them together

Steps of DNA Replication

from the one old *parental DNA* molecule, two new *daughter* DNA molecules are made

semiconservative replication - each daughter DNA consists of one new helix synthesized from free nucleotides and one old helix conserved from the parental DNA

new histones are synthesized in cytoplasm

- millions of histones are transported into the nucleus within a few minutes after DNA replication

- each new DNA helix wraps around them to make a new nucleosome

- each DNA polymerase works at a rate of 100 base pairs per second

- would take weeks if only one polymerase to replicate one chromosome

- thousands of polymerase molecules work simultaneously on each DNA molecule

- all 46 chromosomes are replicated in 6 - 8 hours

Errors and Mutations

- DNA polymerase may make mistakes
  - multiple modes for correction of replication errors
  - double checks the new base pair and tend to replace incorrect nucleotides /// biochemically unstable pairs replaced with more stable correct pairs
  - result is only 1 error per 1 billion bases replicated
- Mutations
  - changes in DNA structure due to replication errors or environmental factors (radiation, viruses, chemicals)
  - some mutations cause no ill effects. others kill the cell, turn it cancerous or cause genetic defects in future generations.

Cell Cycle

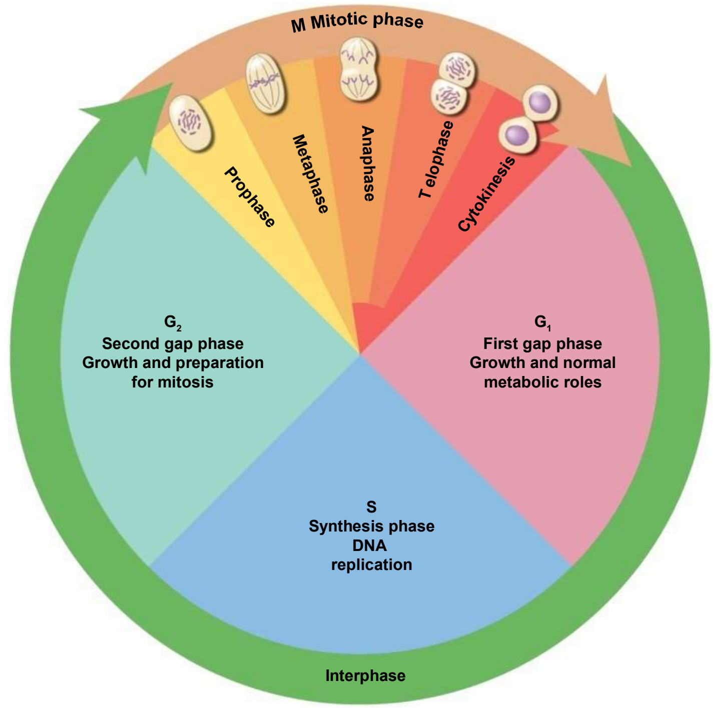
- **Cell Cycle** – a cell's life cycle that extends through several phases to produce two identical cells

- During the cell cyclel there are two phases = mitotic phase and interphase // each phase is broken into sub-phases

- Interphase occurs when the cell is doing what it is programed to do.

- During embryonic development, different genes are turned on or off to determine what the mature cell will become. For example, some epithelial cells become skin tissue and other epithelial cells become liver tissue.

- The mitotic phase occurs when the cell is ready to form an identical copy of itself. At the end of the mitotic phase, each new cell wll have a nucleus and half of the original cells organelles and cytoplasm.



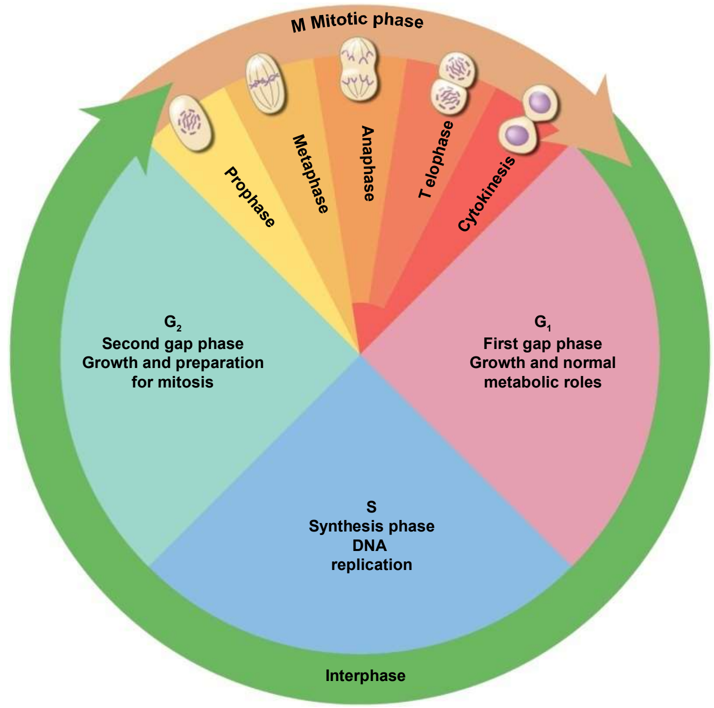
Cell Cycle

- Interphase // broken into three phases /// interphase is when the cell is "doing its work" and it is during interphase that the cell also doubles its DNA /// there is also an optional fourth phase called G_0 (G zero)

- **G_1 phase**, the first gap phase
  - Newly formed daughter cells lack sufficient cytoplasm
  - make more organelles and cytoplasm as well as nucleotides needed to replicate DNA in S phase

- **S phase**, synthesis phase
  - duplicates centrioles
  - DNA replication occurs

- **G_2 phase**, second gap phase
  - interval between DNA replication and cell division
  - finishes centriole duplication
  - synthesizes enzymes that control cell division
  - repairs DNA replication errors



G_0 (G zero) phase

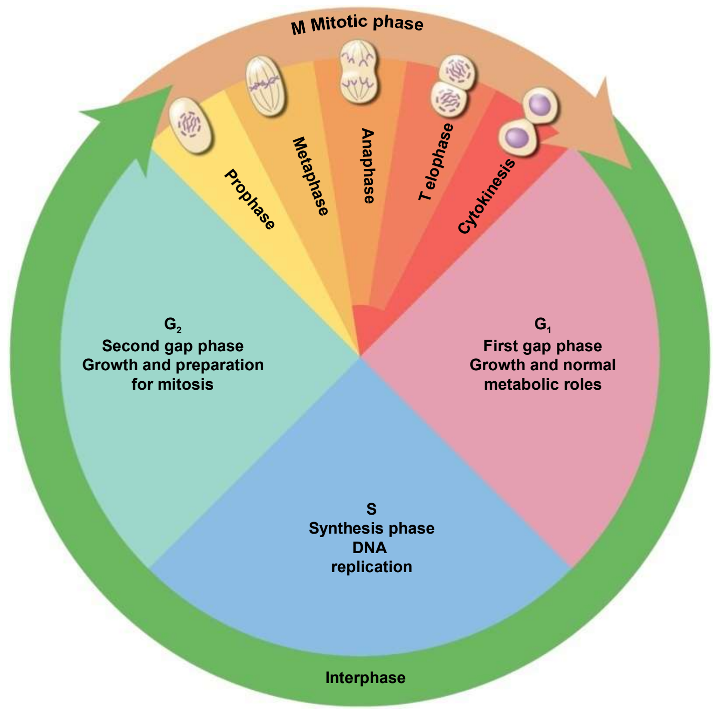
- Cells that leave the cell cycle to "rest" / temporary pause
- Also skeletal muscle and most nerve cells are examples of cells in G zero

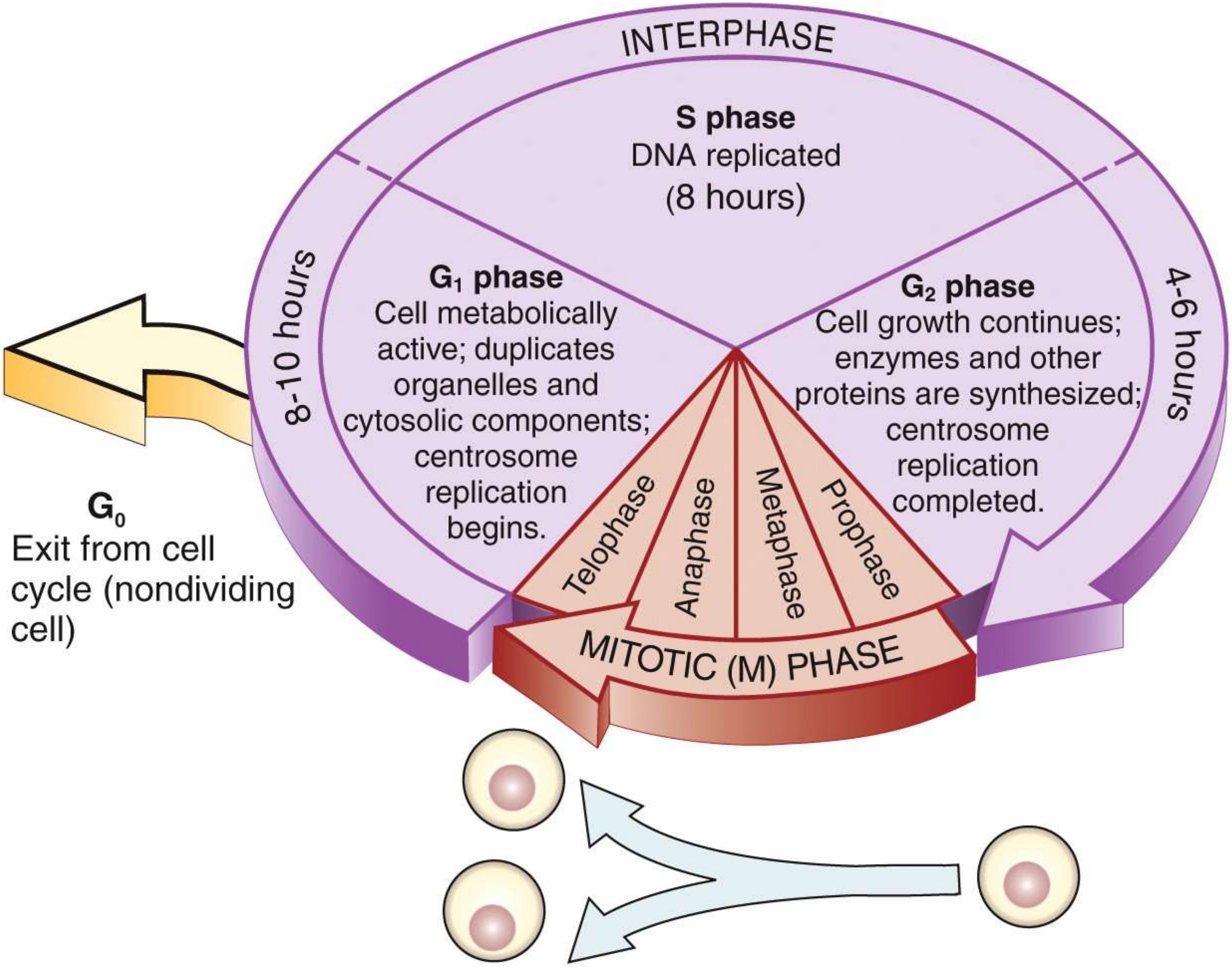
Cell Cycle

- **M phase = Mitotic Phase**
  - At end of M phase, original cell creates two new nuclei, each with 46 chromosomes
  - cell must double DNA so each daughter cell will receive same amount of DNA as parent cell
  - after DNA is duplicated the cell pinches in two to form new daughter cells

- ***Interphase***
  - collection of G_1, S, and G_2 phases
  - S phase is when a copy of the original DNA is made

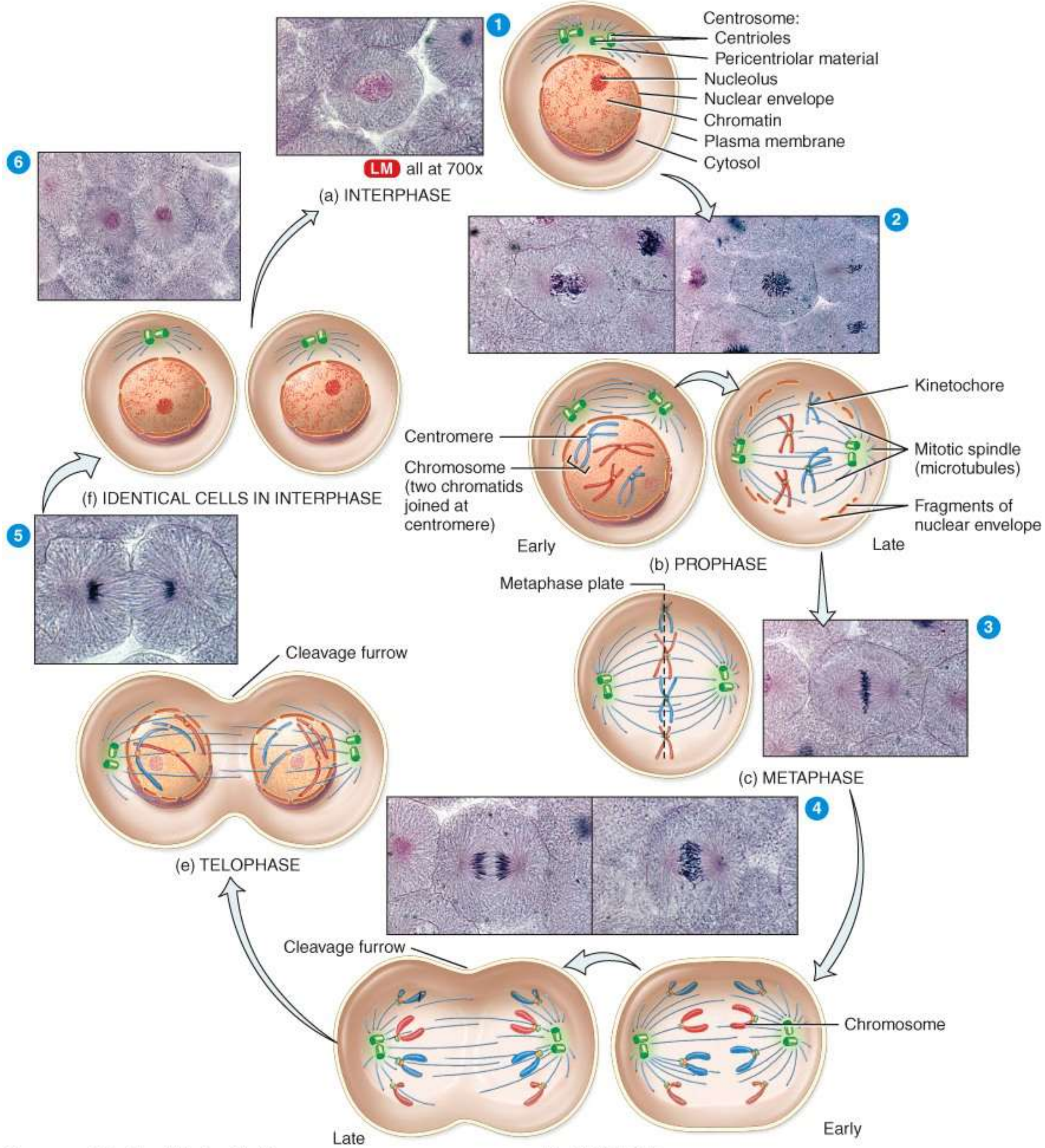
- Cell cycle duration varies between cell types




INTERPHASE
S phase
DNA replicated
(8 hours)
8-10 hours
G$_1$ phase
Cell metabolically active; duplicates organelles and cytosolic components; centrosome replication begins.
4-6 hours
G$_2$ phase
Cell growth continues; enzymes and other proteins are synthesized; centrosome replication completed.
Telophase
Anaphase
Metaphase
Prophase
MITOTIC (M) PHASE
G$_0$
Exit from cell cycle (nondividing cell)

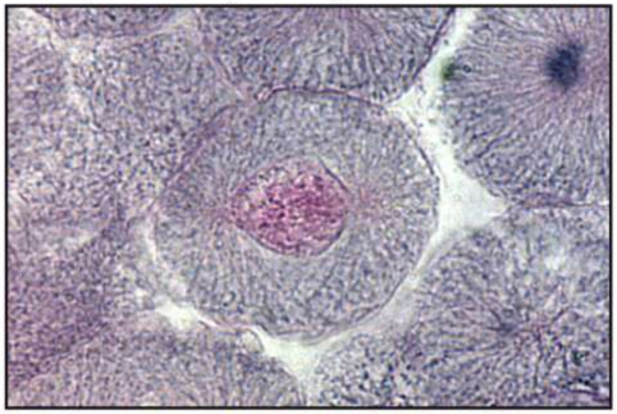
Mitosis

- Functions of mitosis
  - development of the individual from one fertilized egg to some 50 trillion cells
  - growth of all tissues and organs after birth
  - replacement of cells that die
  - repair of damaged tissues
- Four phases of mitotic phase
  - Prophase
  - Metaphase
  - Anaphase
  - Telophase
  - (Note: after mitosis cytokinesis occurs)



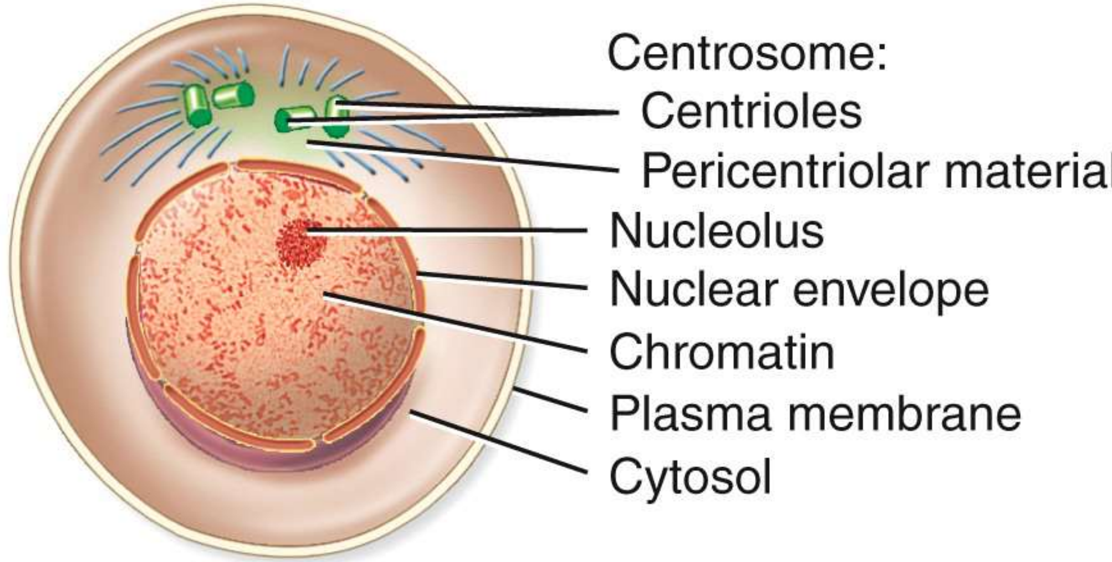
Photos courtesy Michael Ross, University of Florida

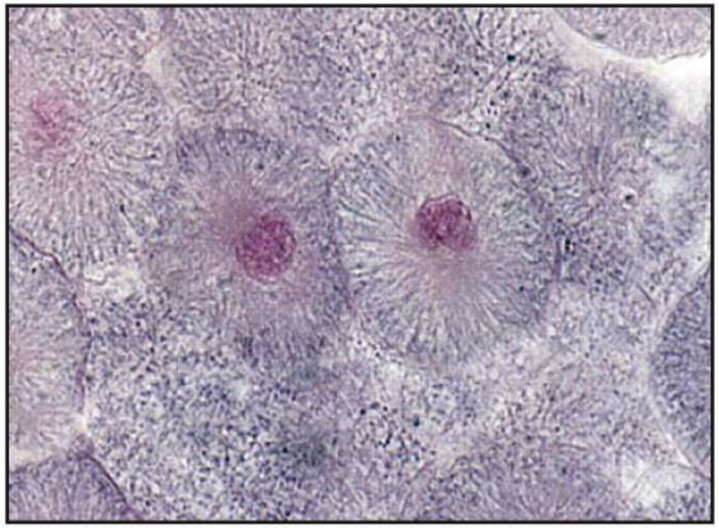
Courtesy Michael Ross, University of Florida



LM all at 700x

(a) INTERPHASE





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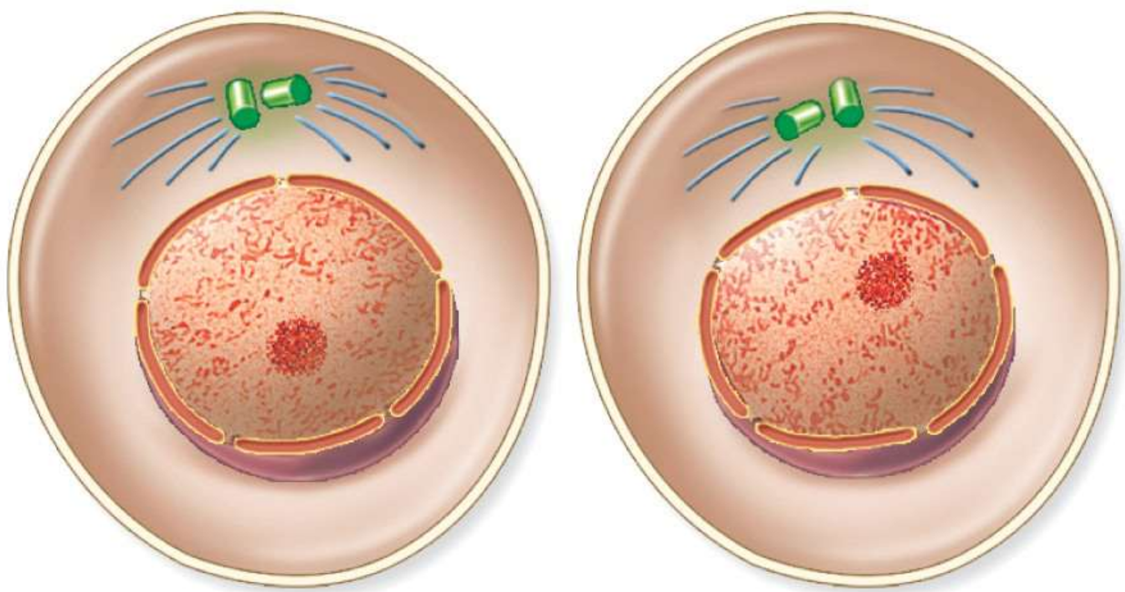
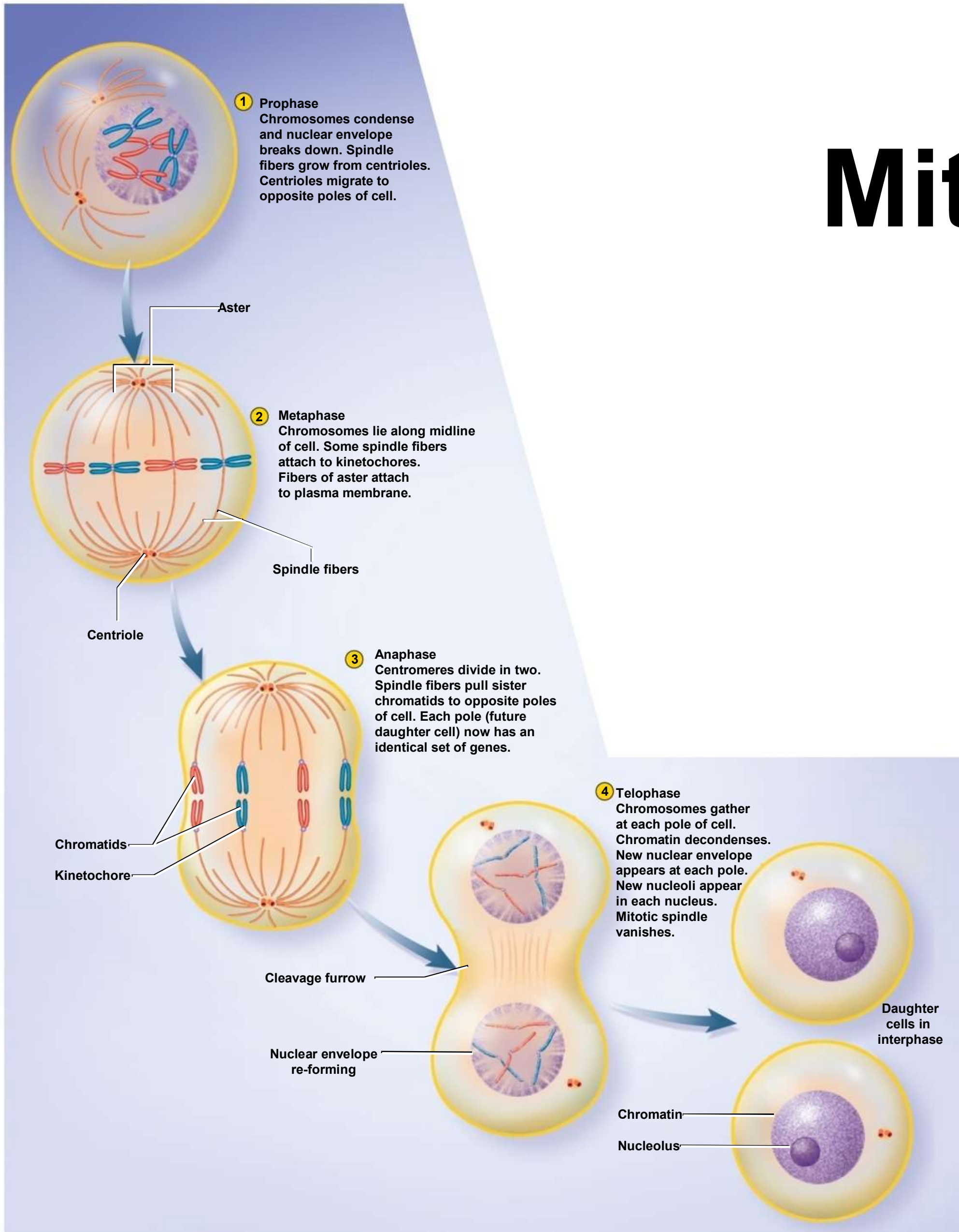


(f) IDENTICAL CELLS IN INTERPHASE

Mitosis



1 Prophase
Chromosomes condense and nuclear envelope breaks down. Spindle fibers grow from centrioles. Centrioles migrate to opposite poles of cell.
Aster
2 Metaphase
Chromosomes lie along midline of cell. Some spindle fibers attach to kinetochores. Fibers of aster attach to plasma membrane.
Spindle fibers
Centriole
3 Anaphase
Centromeres divide in two. Spindle fibers pull sister chromatids to opposite poles of cell. Each pole (future daughter cell) now has an identical set of genes.
Chromatids
Kinetochore
4 Telophase
Chromosomes gather at each pole of cell. Chromatin decondenses. New nuclear envelope appears at each pole. New nucleoli appear in each nucleus. Mitotic spindle vanishes.
Cleavage furrow
Nuclear envelope re-forming
Daughter cells in interphase
Chromatin
Nucleolus

Mitosis: Prophase

- Note: DNA is a polymer of nucleic acids /// the polymer can exist in different forms – either as diffused chromatin or condensed chromosomes

- DNA during S phase appears as chromatin /// chromatin shortens and thickens then coiling into compact rods called chromosomes during prophase --- Makes it easier to distribute to daughter cells than when in form of chromatin

- Homo sapiens have 46 chromosomes

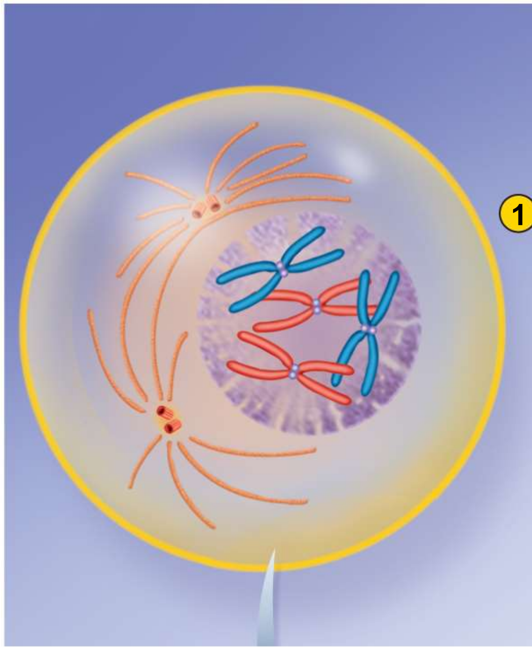
- At end of S phase /// individual chromosomes are copied but held together /// each pair are now called sister chromatids = similar chromosome // <u>this now doubled total amount of DNA</u> (now equal to 92 chromosomes)

- In prophase nuclear envelope disintegrates and releases sister chromatids into the cytosol

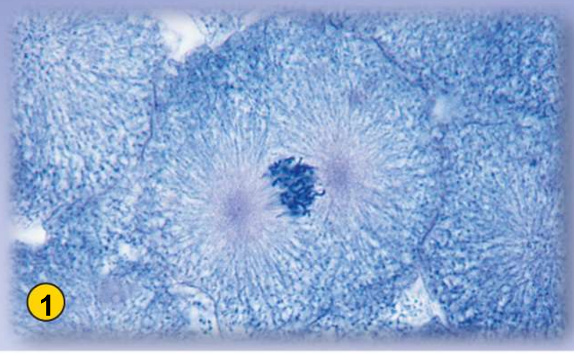
Mitosis: Prophase (cont.)

- centrioles sprout elongated microtubules – called the spindle fibers
- push centrioles apart as they grow
- pair of centrioles migrate to opposite poles of the nucleus
- after centrioles reach opposite sides /// spindle fibers grow toward chromosomes and attach to the kinetochore on each side of the centromere
- Note: Kinetochore = point where chromatids attach to each other
- spindle fibers then tug the chromosomes back and forth until they line up along the midline of the cell

Mitosis: Prophase



1 **Prophase**
Chromosomes condense and nuclear envelope breaks down. Spindle fibers grow from centrioles. Centrioles migrate to opposite poles of cell.



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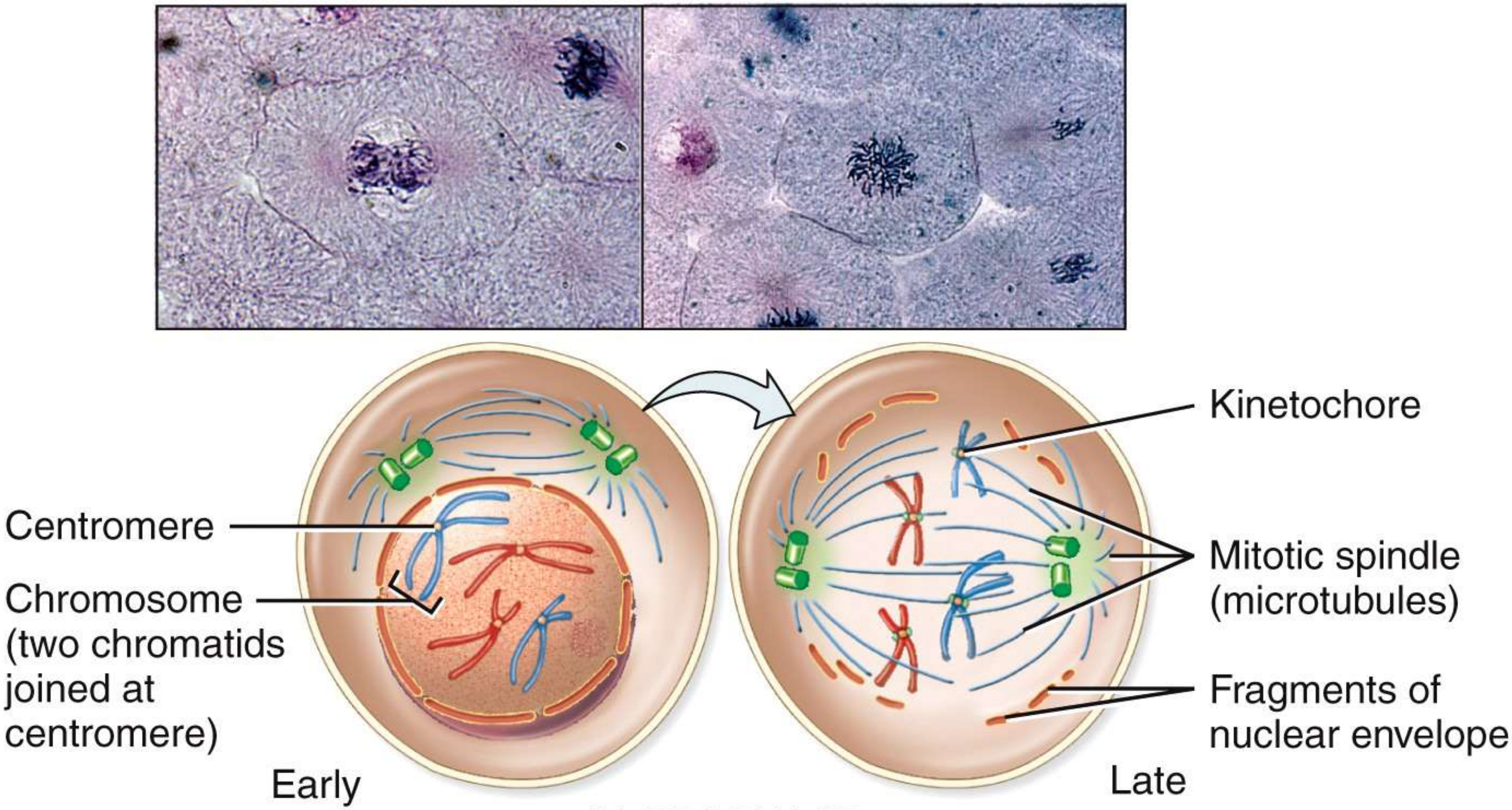
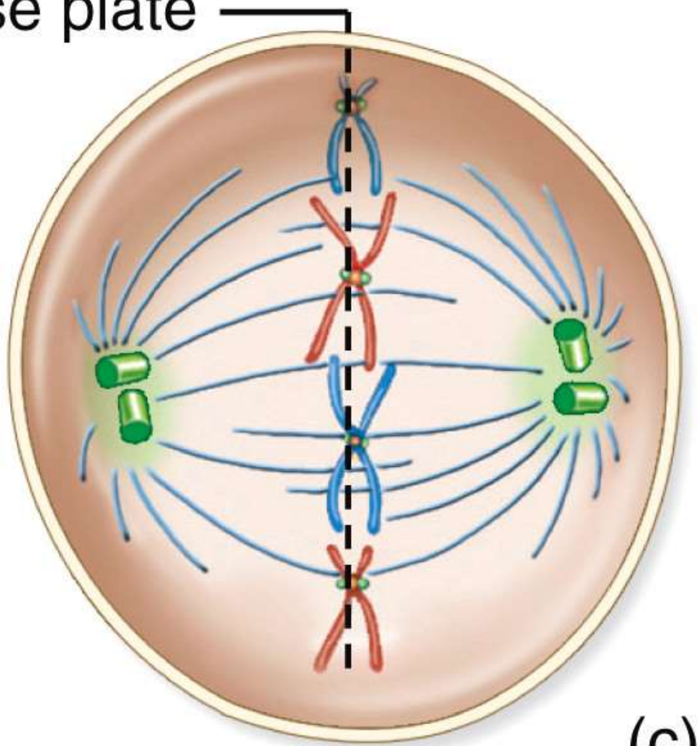


(b) PROPHASE

Mitosis: Metaphase

- Now chromosomes are aligned on cell equator
  - oscillating slightly and awaiting signal that stimulates each of them to split
- Mitotic spindle – lemon-shaped array of spindle fibers
  - long spindle fibers (microtubules) attach to chromosomes at kinetochore
  - shorter microtubules (aster fibers) anchor centrioles to plasma membrane at each end of cell



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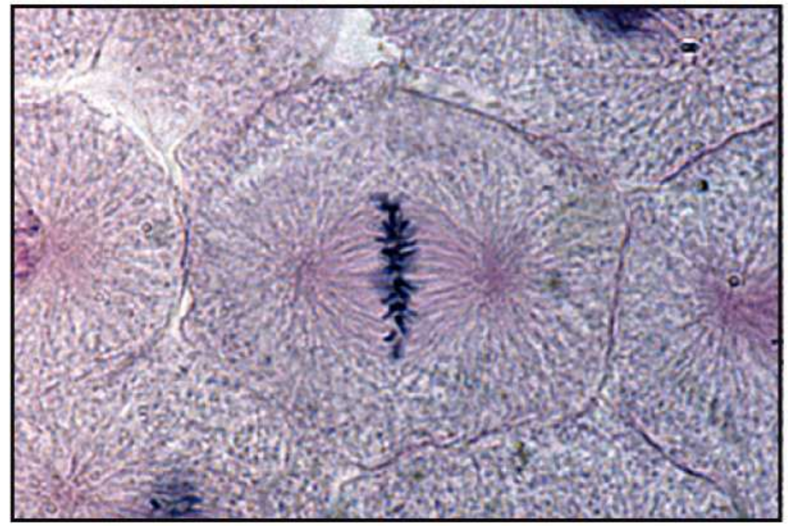


(c) METAPHASE

Mitosis: Anaphase

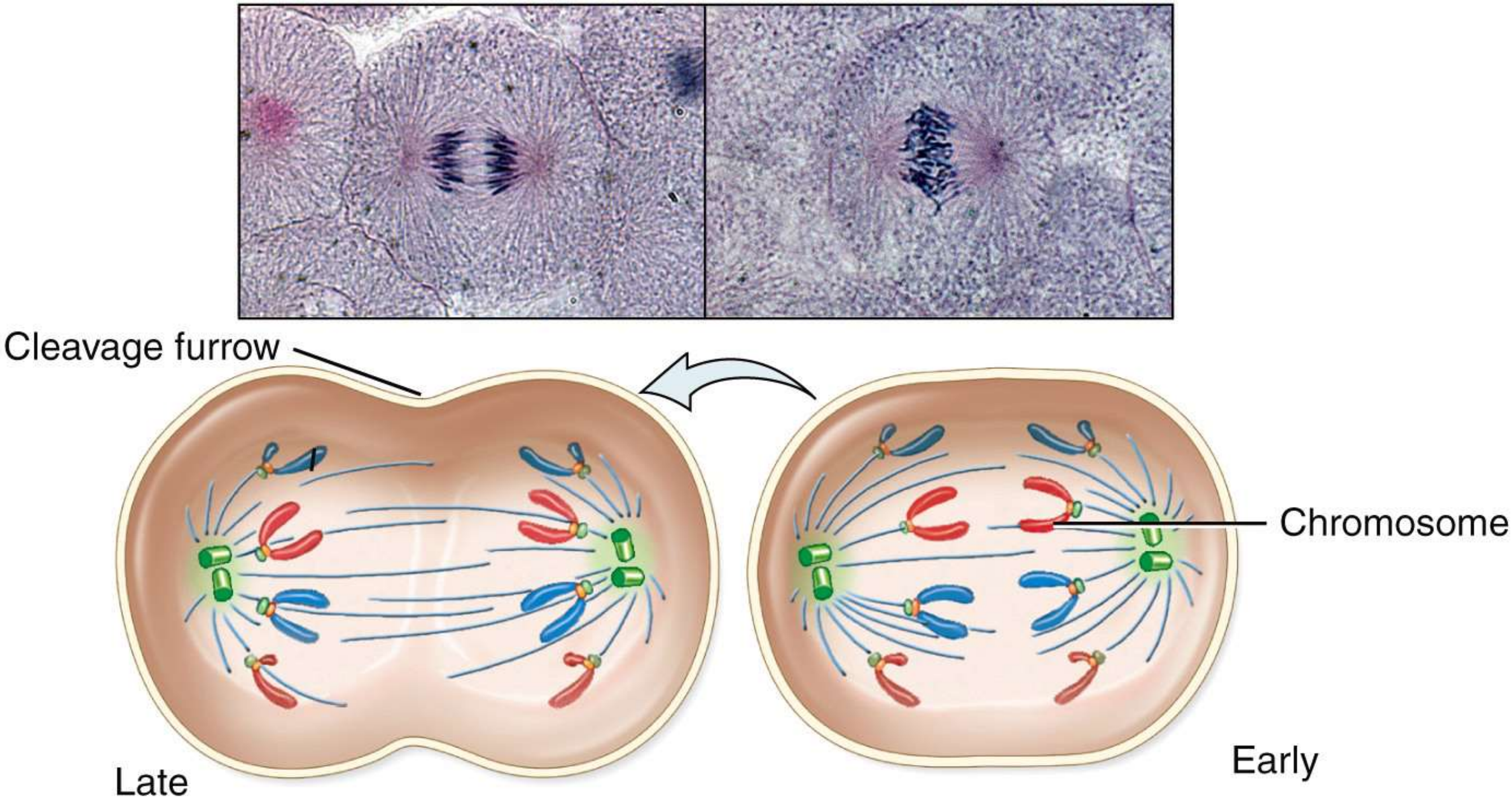
- Begins with activation of an enzyme that cleaves two sister chromatids /// separates the two chromatids at centromere (point of chromatid attachment)

- Each chromatid now becomes a daughter chromosome which migrate towards opposite poles of the cell with centromere leading the way

  – motor proteins in kinetochore crawling along the spindle fiber as the fiber itself is ‘chewed up’ and disassembled at the chromosomal end

  – Each daughter cell following mitosis are genetically identical

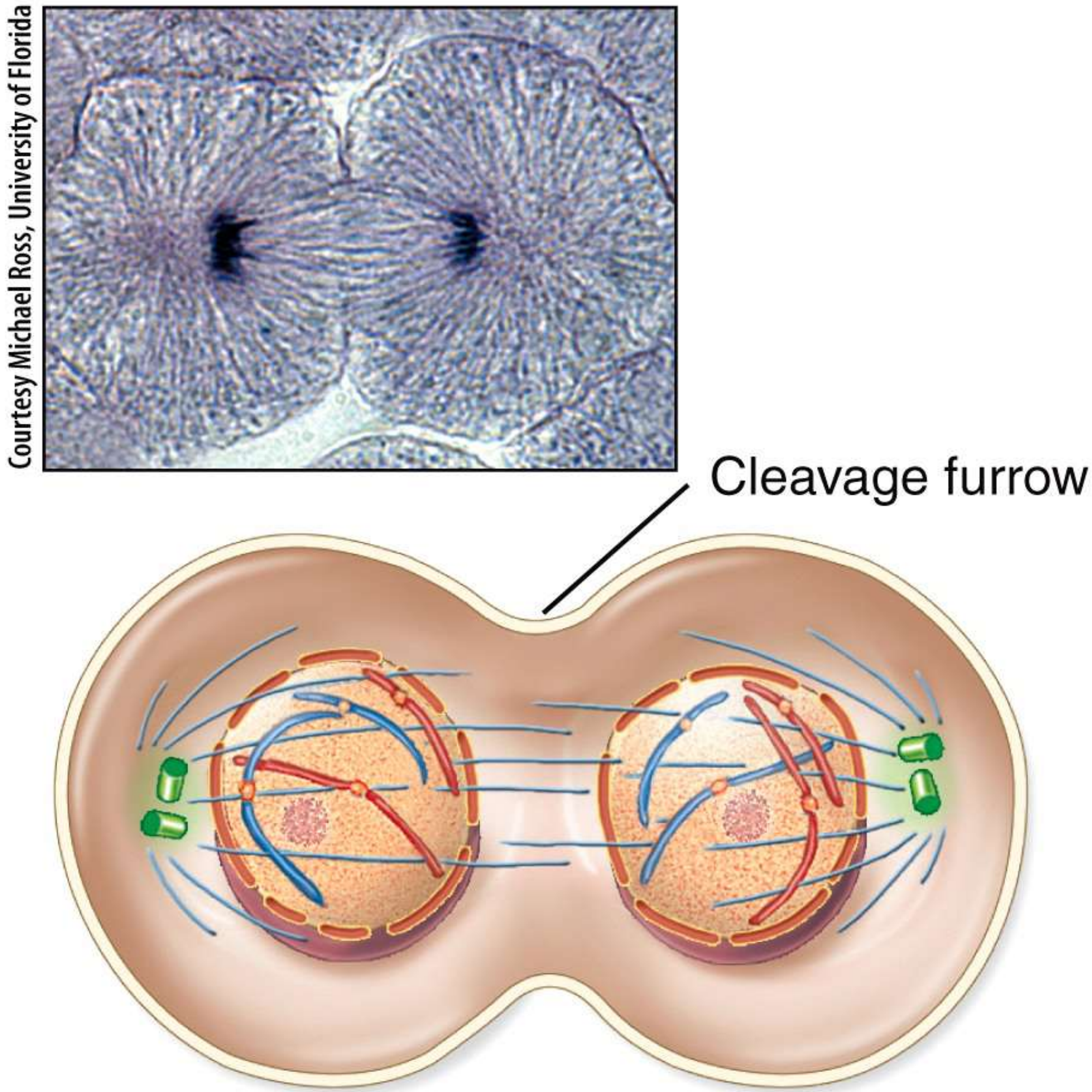
Courtesy Michael Ross, University of Florida



(d) ANAPHASE

Mitosis: Telophase

- chromatids (the daughter chromosome) cluster on each side of the cell
- <u>rough ER produces new nuclear envelope around each cluster</u>
- chromatids begin to uncoil and form the chromatin
- remaining mitotic spindle breaks up and vanishes
- <u>each nucleus forms nucleoli</u>
  - this indicates that cell has already begun making RNA and preparing for protein synthesis


Courtesy Michael Ross, University of Florida
Cleavage furrow

(e) TELOPHASE

Cytokinesis

- the division of cytoplasm into two cells
  - telophase is the end of mitosis but overlaps with cytokinesis
  - early traces of cytokinesis visible in anaphase
- achieved by motor protein (myosin) pulling on microfilaments of actin in the terminal web of cytoskeleton
- creates the cleavage furrow around the equator of cell
- cell eventually pinches in two

Timing of Cell Division

When may cells divide by mitosis?

- If they have enough cytoplasm for two daughter cells
- If they have replicated their DNA
- If they have adequate supply of nutrients
- If they are stimulated by growth factor
  - chemical signals secreted by blood platelets, kidney cells, and other sources
- If neighboring cells die. This opens up space in the tissue for new cells

Timing of Cell Division

What will inhibit mitosis?

- When nutrients or growth factors are withdrawn
- If existing cells are too closely packed with neighboring cells / no room for new cells
- **Contact inhibition** – the cessation of cell division in response to contact with other cells

Classical Genetics VS Modern Genetics VS Epigenetics

- Gregor Mendel is considered the father of Classical Genetics. He worked with pea plant's in the mid 1850s to demonstrate patterns of heredity. He coined the terms dominant and recessive traits. Mendel's early work predated our understanding of molecular biolody and DNA's role as the informational molecule responsible for heredity by almost 80 years.

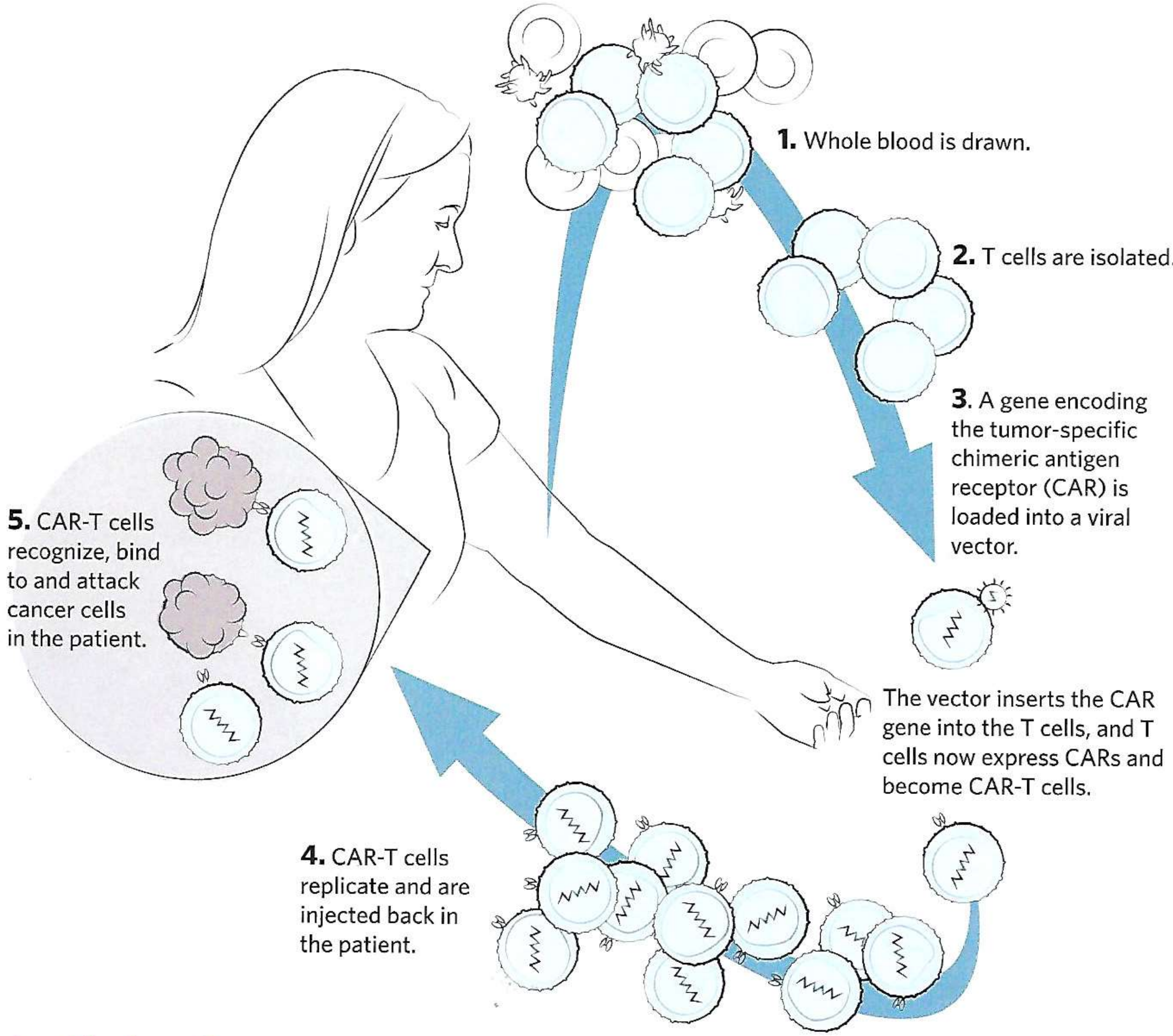
- Charles Darwin wrote On the Origin of Species in 1859. He explained how Evolution and Natural Selection create new species.

- Darwin's and Mendel's early work is known as Classical Genetics which was updated and renamed Modern Genetics in the 1940's to incorporate the role of DNA .

- A consensus definition of the concept of an epigenetic trait is a "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence". This was formulated at a Cold Spring Harbor meeting in 2008

How CAR-T Therapy Works

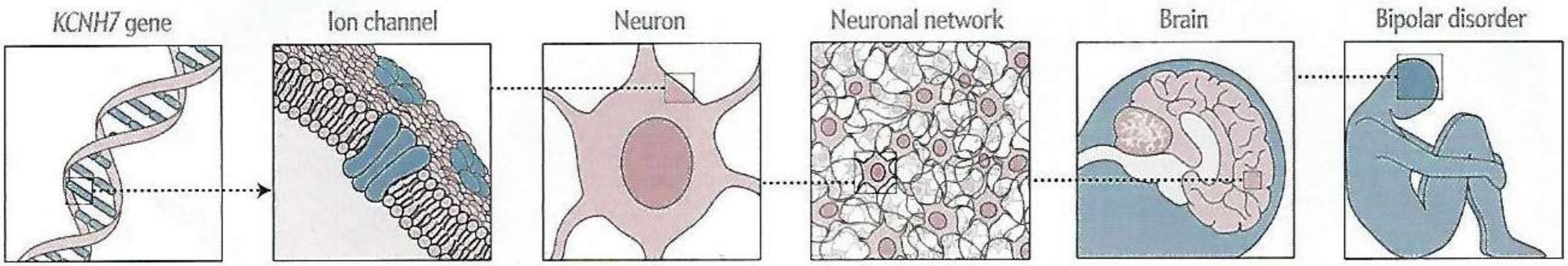
CAR-T—the initial class of T cell therapies—harnesses the patient's own immune system to fight certain types of cancer



Scientific American

How Genetic Mutations Lead to Disease

Gene mutations can disrupt biology at multiple levels (molecules, cells, tissues and organs) to cause disease. Certain mutations are particularly prevalent in Amish and Mennonite populations. For each patient the clinic sees, it applies advanced technologies to identify the individual's genetic variants, understand their causal links to disease, and devise ways to alleviate or prevent the mutations' harmful effects. In related work, the clinic and its collaborators recently identified a gene mutation linked to bipolar disorder among the Amish, and they are now constructing a picture of how it might impair emotional regulation (*below*). This knowledge could lead to a deeper understanding of bipolar disorder in the general population and to new strategies for prevention and treatment.



Gene

A gene consists of a sequence of DNA "letters" that spell out the amino acids needed to make a protein. Proteins are the main workhorses of cells. A mutation in a gene can alter the functioning of the encoded protein. The bipolar study pinpointed a mutation in a gene called *KCNH7*.

Protein

To function properly, proteins must have the right structure, location and abundance in each cell. *KCNH7* encodes a protein that spans the cell membrane, forming a channel that regulates the flow of potassium ions. The mutant is altered at just a single amino acid, but this subtle change affects potassium movement across the membrane.

Cell

All cells contain the same genes, but many genes are expressed (that is, give rise to proteins) only in select cell types. The ion channel encoded by *KCNH7* is used by neurons throughout the brain. Potassium currents critically shape each neuron's electrical behavior, and the mutant alters the cells' firing patterns.

Tissue

Tissues can contain a mixture of cell types. Brain tissue, for instance, includes neurons and supporting cells called glia. The mutant *KCNH7* gene would be expected to disrupt the operation, not only of individual nerve cells, but of whole neuronal circuits, such as those regulating emotions and behavior.

Organ

Nerve cells throughout the brain make the ion channel encoded by the *KCNH7* gene, but the channel is most abundant in brain regions underlying emotions and cognition. Consistent with that finding, mutation of the gene has been tied to mania observed in laboratory animals.

Behavior

Bipolar disorder is marked by a spectrum of behaviors that can include depression, mania and psychosis. New insight into how the *KCNH7* mutation affects each level of biology—from misspelled protein to perturbed brain function—could lead to fresh ideas for interrupting the chain of events underlying the disorder.