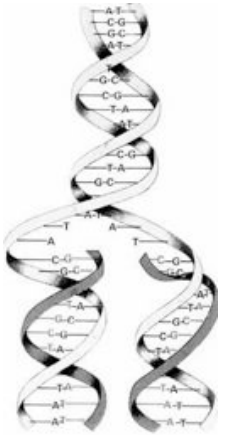
Topics

- DNA
  - Replication
  - Mutations
  - Chromosomal structure

DNA Replication: 2 basic steps

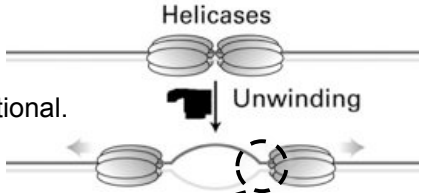
1) DNA double helix is unwound

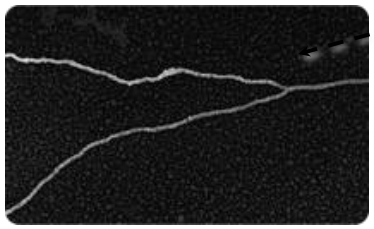
2) DNA synthesis: New nucleotides form complementary base pairs with the template strand.
Are covalently linked within the newly synthesized strand



1) DNA **Helicases** unwind the DNA double helix to allow replication to occur.

Replication is Bidirectional.

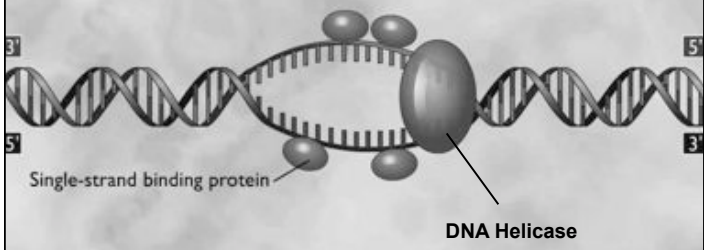




The replication machinery congregate at a **replication fork**.

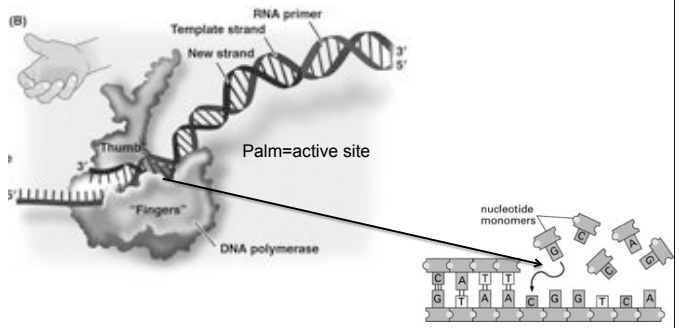
1) DNA double helix is unwound

Single-stranded binding protein (SSBP) prevents the DNA from reforming the double helix



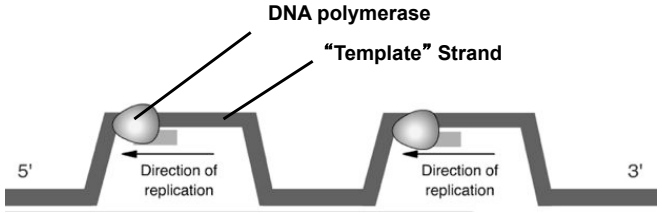
2) DNA synthesis: DNA Polymerase

DNA polymerases are the major proteins that synthesize the new strand, although there are many other proteins involved.
Its active site brings together dNTP substrate and template DNA.



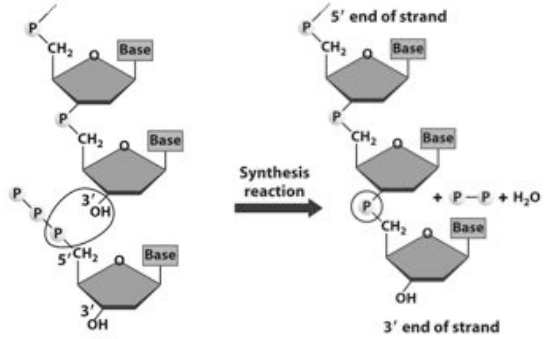
DNA Polymerase

DNA can **only** be synthesized in the **5' to 3' direction**

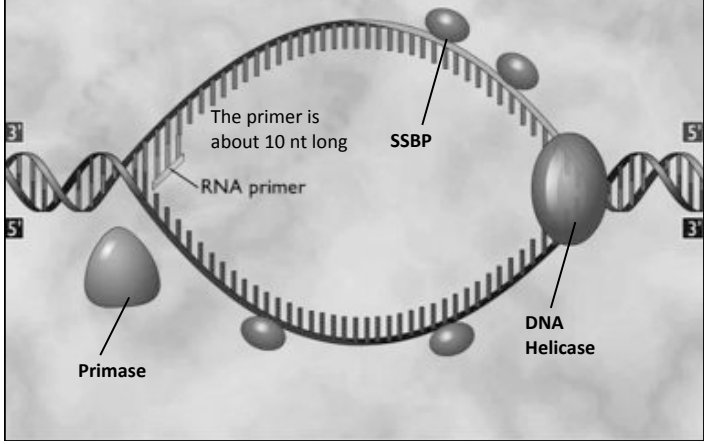


DNA Polymerase

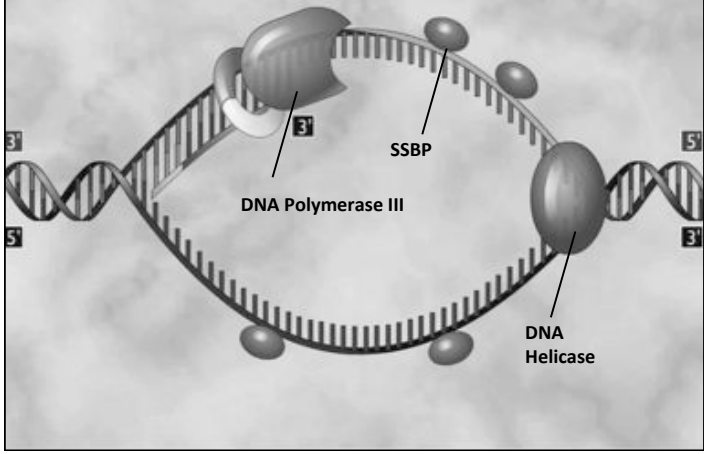
DNA can only be synthesized in the 5' to 3' direction, **adding 1,000bp/sec**

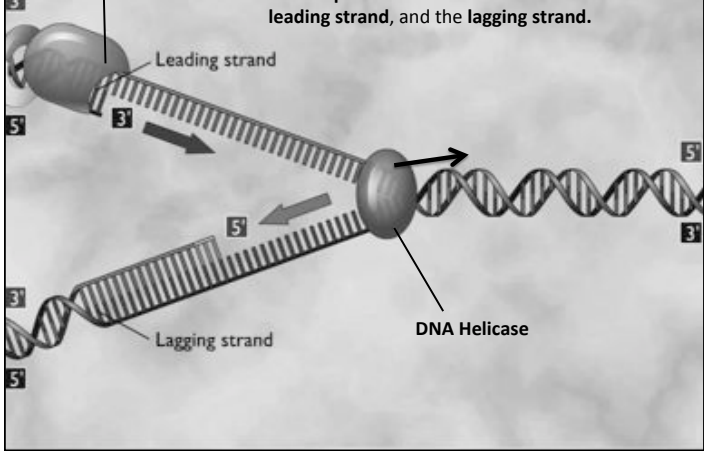


RNA Primase synthesizes an **RNA primer** so that DNA polymerase has a free 3' OH end to begin adding free nucleotides



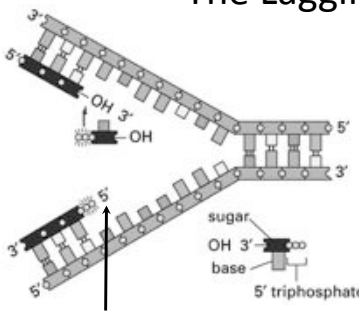
DNA Polymerase III begins synthesizing a new strand in the 5' to 3' direction



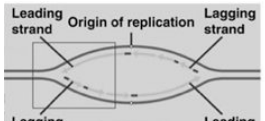


- There are two strands being synthesized due to the **antiparallel** nature of DNA strands. The **leading strand**, and the **lagging strand.**

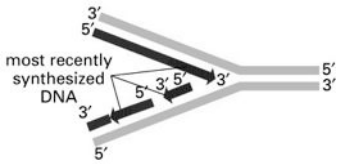
The Lagging Strand

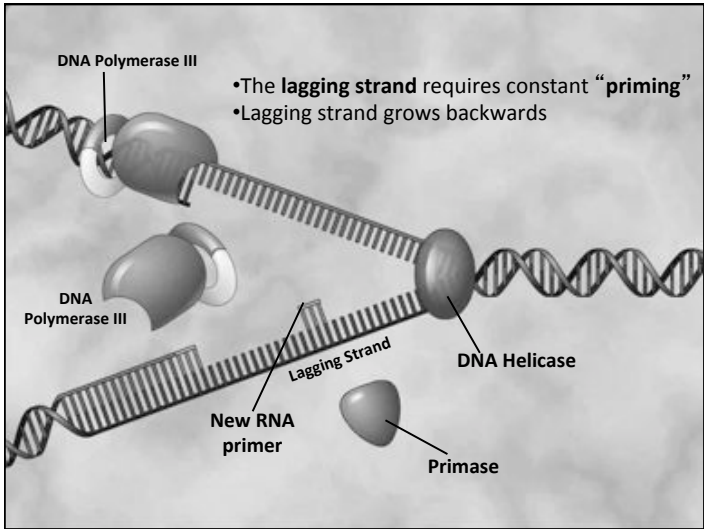


DNA polymerase cannot add nucleotides to this end

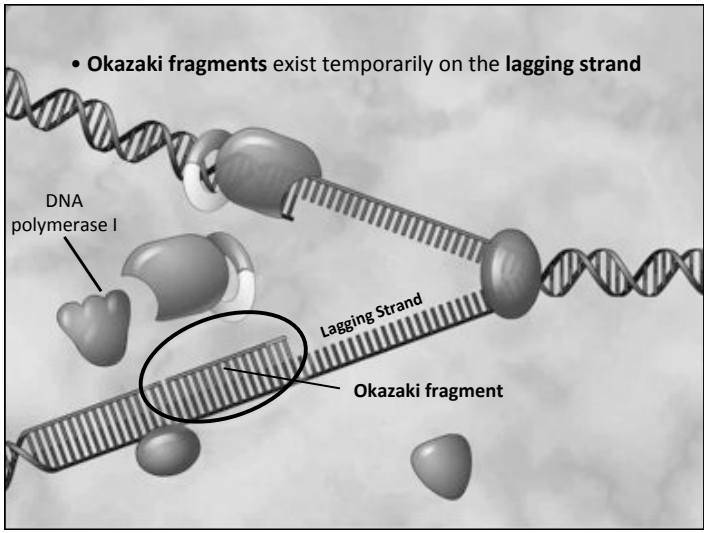


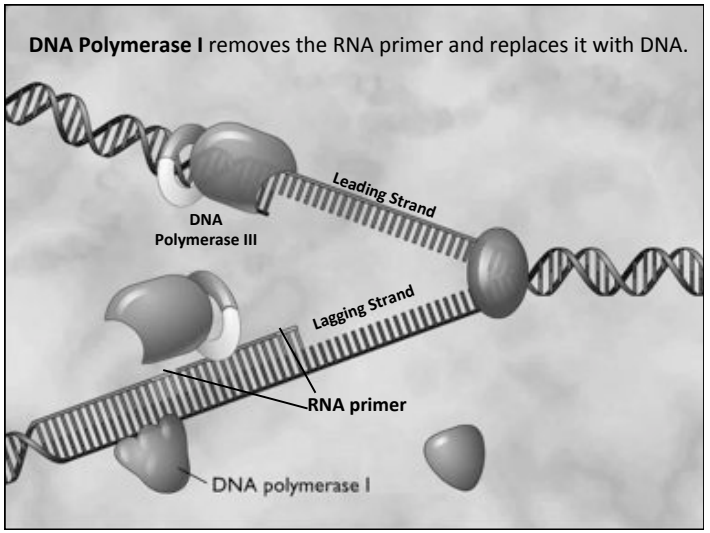
The **"lagging strand"** must be synthesized away from the **replication fork**

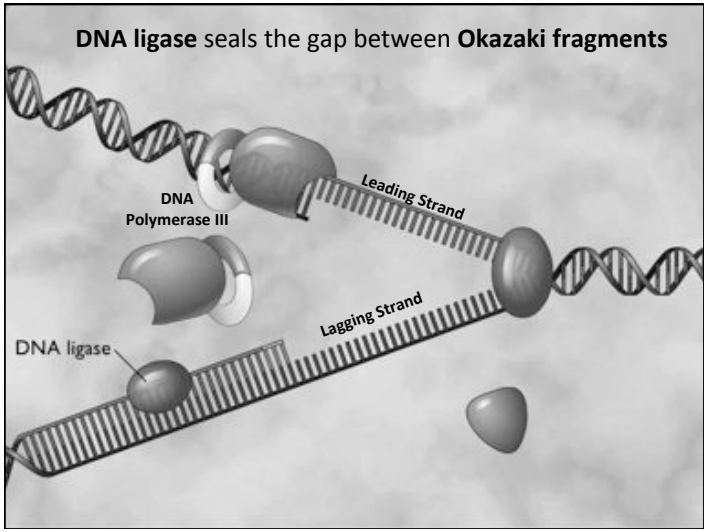


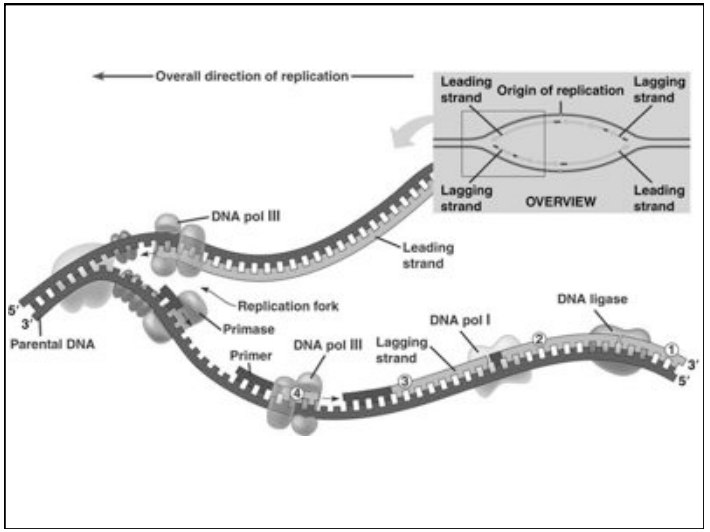


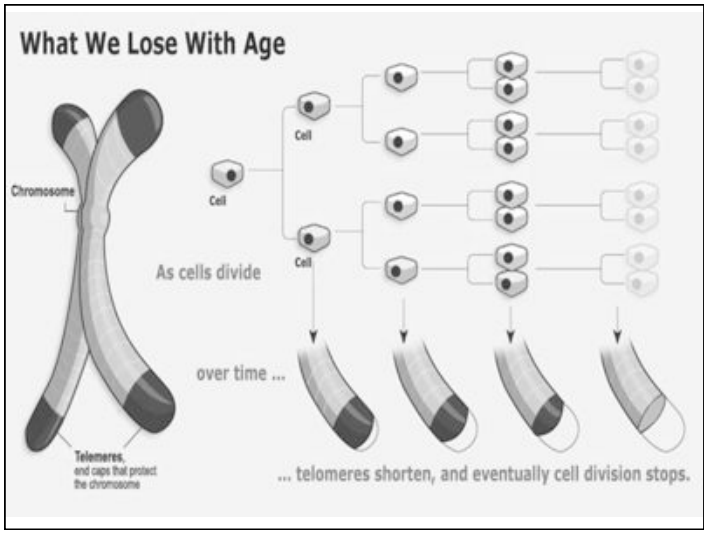
- The **lagging strand** requires constant "**priming**"
- Lagging strand grows backwards


• Okazaki fragments exist temporarily on the lagging strand
DNA polymerase I
Lagging Strand
Okazaki fragment

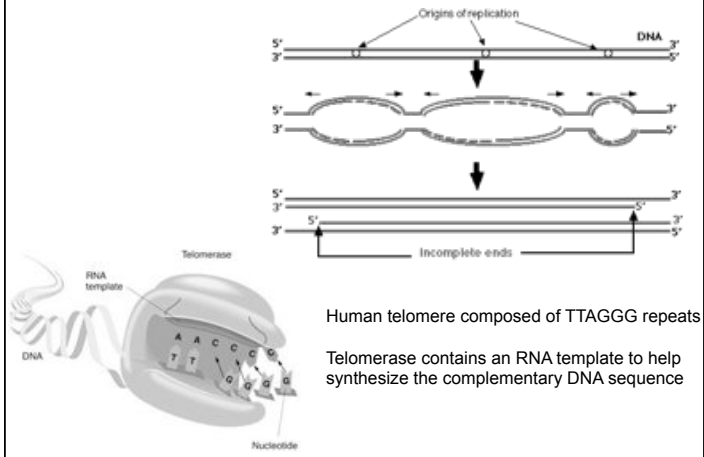

DNA Polymerase I removes the RNA primer and replaces it with DNA.
Leading Strand
DNA Polymerase III
Lagging Strand
RNA primer
DNA polymerase I


DNA ligase seals the gap between Okazaki fragments
DNA Polymerase III
Leading Strand
Lagging Strand
DNA ligase


Overall direction of replication
Leading strand
Origin of replication
Lagging strand
Lagging strand
OVERVIEW
Leading strand
DNA pol III
Leading strand
Replication fork
Primase
Primer
DNA pol III
Lagging strand
DNA pol I
DNA ligase
5'
3'
Parental DNA
3'
5'



Telomeres protect chromosomal ends



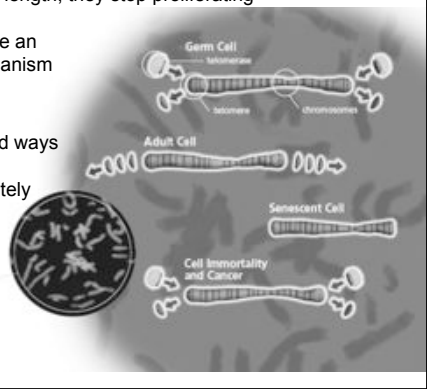
Human telomere composed of TTAGGG repeats

Telomerase contains an RNA template to help synthesize the complementary DNA sequence

Telomeres: The Cancer Connection

- Not all adult cells continue extending their telomeres,
- When they reach a critical length, they stop proliferating
- Natural process of aging
- Shorter telomeres might be an energy sparing mechanism

Many cancer cells have found ways to "hijack" telomerase extend their DNA indefinitely



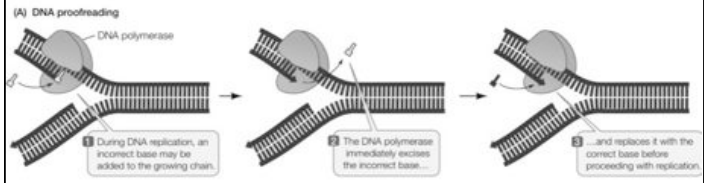
DNA replication errors

Error rate: 1 error/100,000 bp (10^{-5})
Would equal 40,000 errors after every single cell division
Is that tolerable for long term survival?

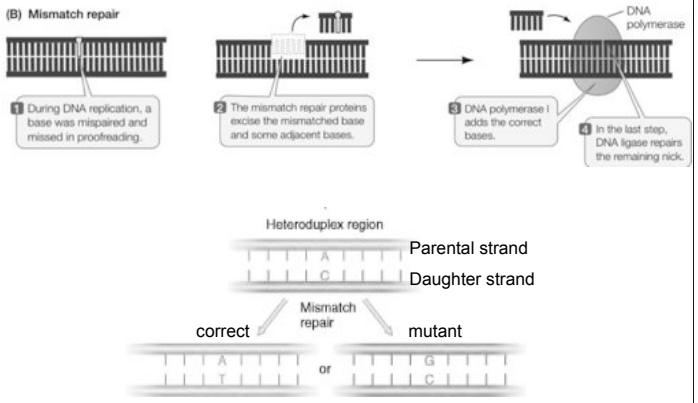
Actual frequency: 1 error/10,000,000,000 bp (10^{-9} to 10^{-10})
Less than 1bp error at each cell division

Why is the actual error rate so low?
1) Proofreading
2) Mismatch repair

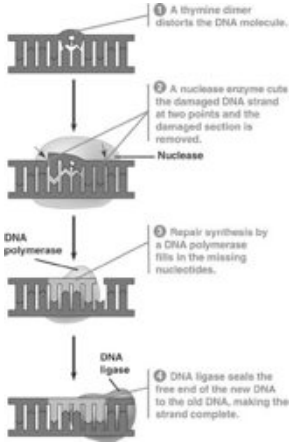
DNA Proofreading



Mismatch repair



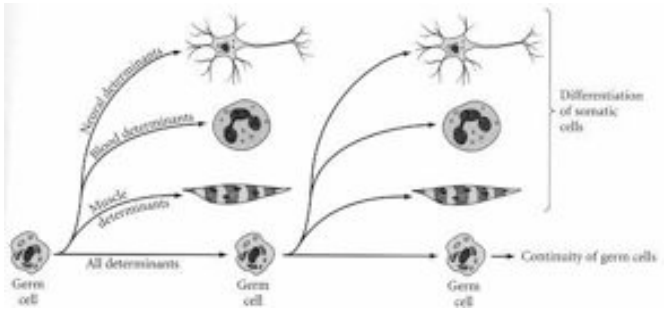
Nucleotide excision repair



Mutations during replication

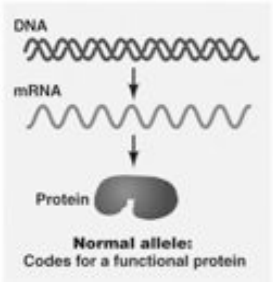
Germline mutations: occur in the specialized cells that give rise to gametes (egg, sperm). This will be passed on to a new organism at fertilization.

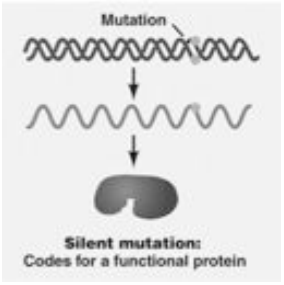
Somatic mutations: in all other cell types. Passed on to future generations of THAT cell. Not passed on through reproduction.



Mutation Effects

May or **may not** affect the expression of a protein

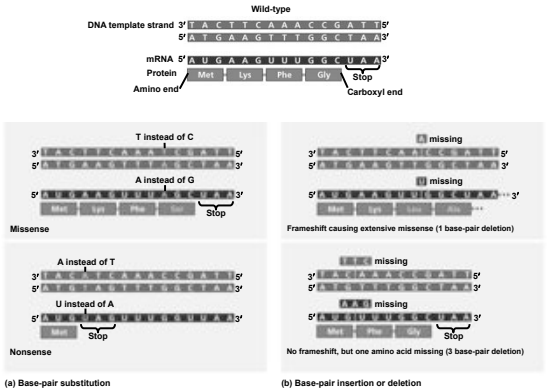




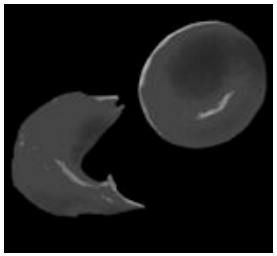
Define: **coding regions**

Most genome mutations are **silent**

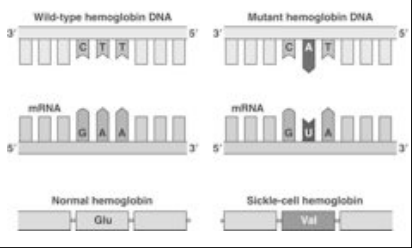
Small Scale Mutations- Point Mutations



Point Mutations



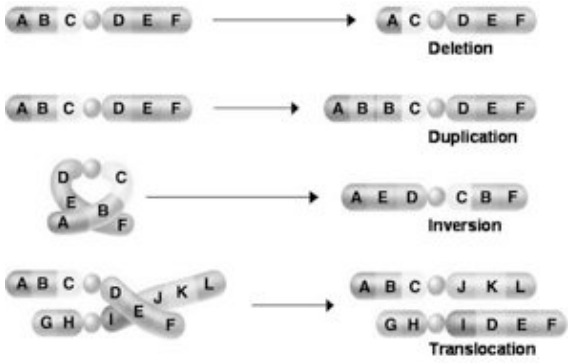
Substitution: Sickle-cell anemia
Single bp mutation in B-globin gene
results in a different protein



Large Scale Mutations- Chromosomal

Extensive changes in chromosomal structure
Whole chromosomes can break and grossly misjoin

Usually fatal



Eg. Duplication:

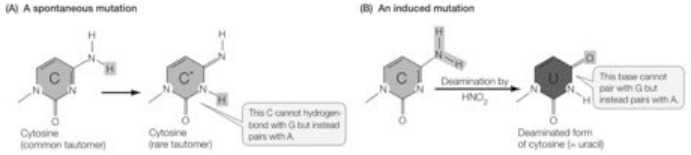
Eg. Deletion
Jacobsen syndrome:

Conditional Mutations
Phenotype expressed under restrictive conditions

Spontaneous vs Induced Mutations

A) Permanent change not due to external influence

B) Permanent change due to external influence

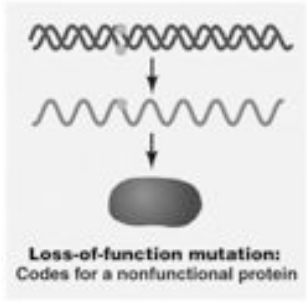


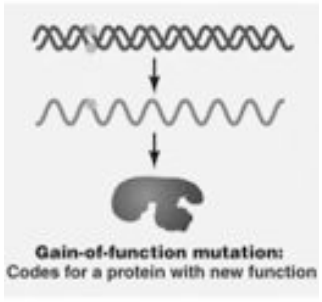
Replication error
Spontaneous reactions (deamination)
Random strand breakage

Chemical mutagens
Radiation

Mutations

May or may not affect the expression of a protein

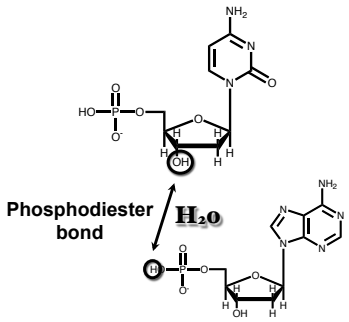


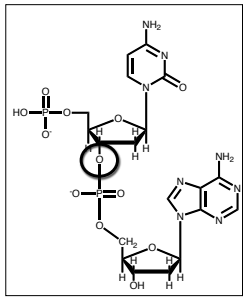


Or loss of expression

Usually is inherited
Common in cancer

DNA is a polynucleotide

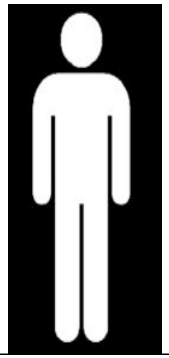




DNA structure

How does 2 meters of DNA fit in a cell?

Man = 2 meters tall



Cell = 10uM diameter
1/1,000,000 the size of a….

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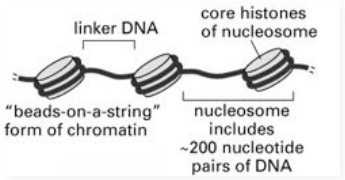
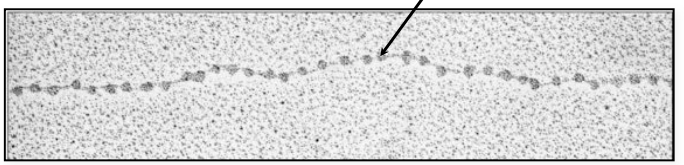
Nucleosomes

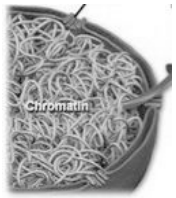


Basic unit of packaged DNA
DNA segment wrapped around a **histone** (protein) core

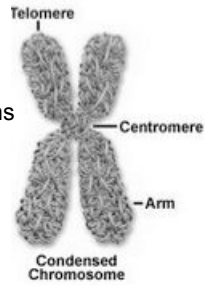
Nucleosome –
"beads on a string"



Chromatin = DNA plus accessory proteins (e.g. histones)
Uncondensed, cells are not dividing



Chromosome = DNA plus accessory proteins (e.g. histones)
Condensed and visible during cell division



DNA structure

DNA is capable of being unwound into **chromatin** and further compressed into **chromosomes**

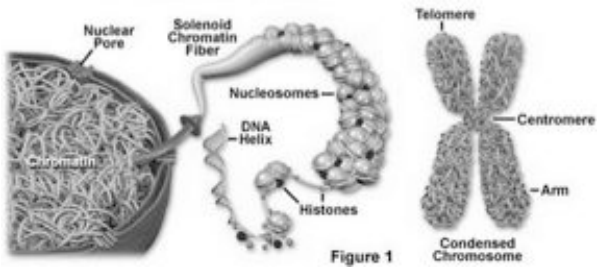


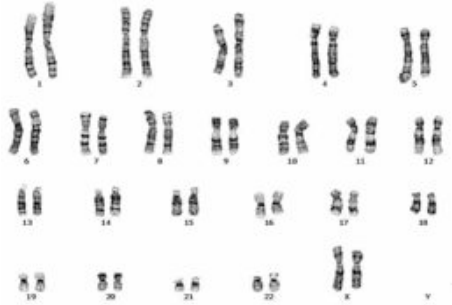
Figure 1

Human Chromosome Spread

Humans are **diploid**, two (slightly different) copies of each chromosome

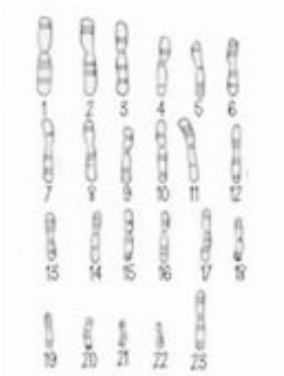
The two parental copies are considered **homologous**

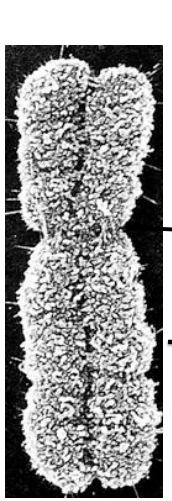
Karyotype: number and size of chromosomes for a given organism



Human Chromosome Spread

What about this karyotype?





DNA Replication

The chromosomes are duplicated to create identical **sister chromatids**, which are joined at the **centromere**

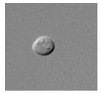
Centromere

Sister chromatids are different from **homologous chromosomes**

Mitosis

During **mitosis** the sister chromatids are separated into respective daughter cells

The Cell Cycle



- Most cells undergo **division** to produce **daughter cells**
- Humans begin as one cell and by adulthood reach 200,000,000,000,000
- The amount of time required is variable.
  - Some mammalian cells might take weeks or months to divide
  - Under proper conditions bacteria can divide every ***20 minutes!***
- Careful control of cell duplication is *critical* for survival
  - Cells can be "grown to death"
  - There are also things worse than cell death....