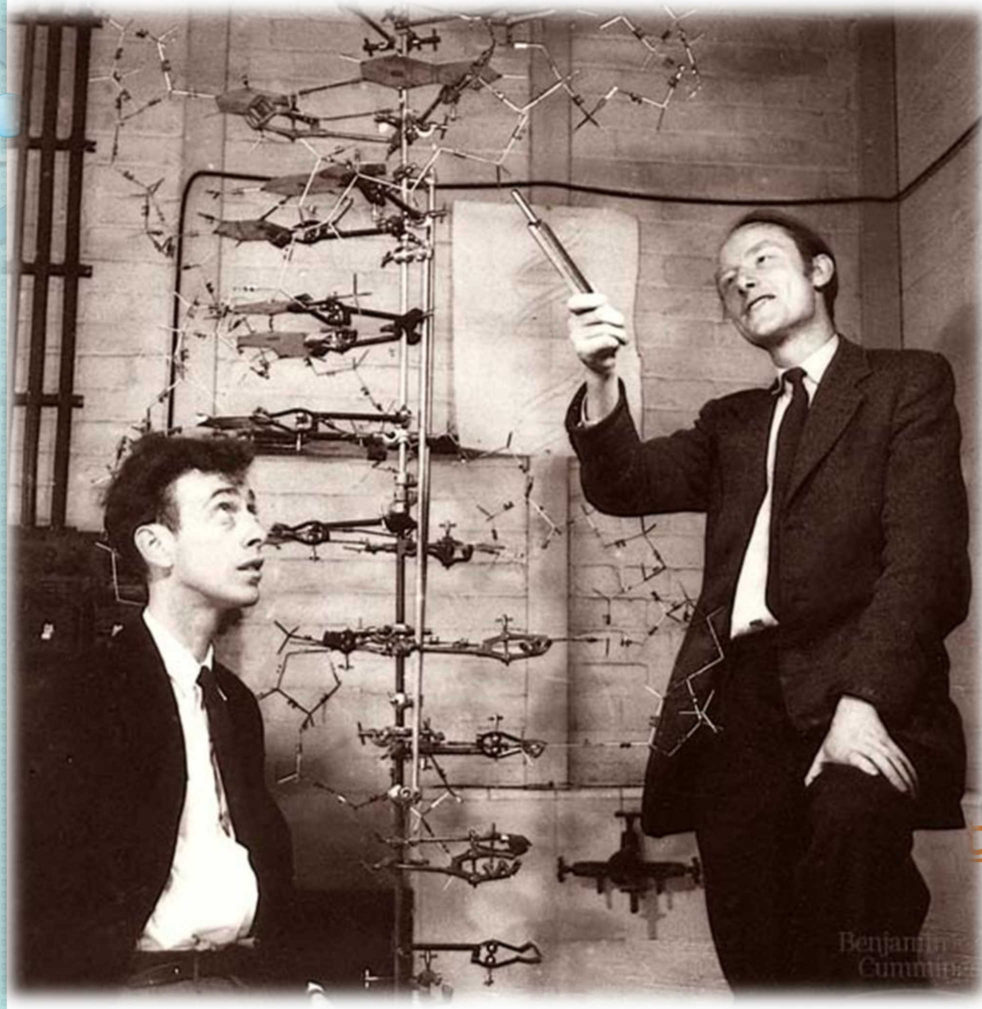

Genetics Material

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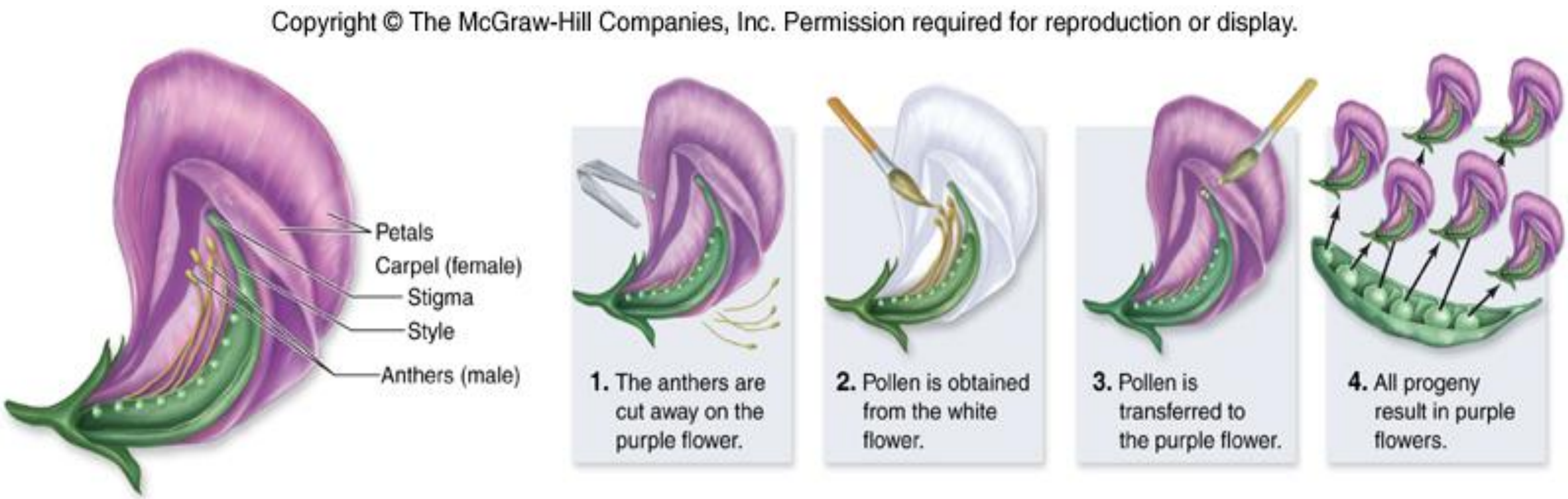
Chemical Composition of the Body

"Because living things, including humans, are composed only of chemicals, it is absolutely essential for a (physiology) student to have a basic understanding of chemistry."

Sylvia Mader..

What is the genetic material?

- George Mendel:



- Thomas Morgan, in his experiments with fruit flies, described genetic recombination, and demonstrated that traits were to inherited together to varying degrees.

What is the genetic material?

- Chromosomes, threadlike structures, first observed by Nageli in 1842.
- Walter Flemming was the first to follow the process of mitosis and replication of chromosomes.
- Thomas Morgan, in his experiments with fruit flies, described genetic recombination, and demonstrated that traits were to inherited together to varying degrees.
- Alfred Sturtevant extended Morgan's ideas, used observed recombination rates to produce the first genetic maps.

What are genes made of?

- Miescher first isolated "nuclein" from the nuclei of white blood cells in 1869.
- By the early 1900's, nuclein was known to be a long polymer of nucleic acids, and by the 1920's DNA and RNA were separately isolated.
- Initially, biologists were not very interested in DNA - it was thought to have a simple sequence, like synthetic polymers:

http://www.chemheritage.org/EducationalServices/nylon/chem/chem.html

The function of genes

- Beadle and Tatum produced strong evidence via mutation experiments with the mold *Neurospora* that genes direct the production of proteins (1941)
  - Produced mutant strain using irradiation
  - Some mutant strains would not grow on conventional media, but would grow on media with supplements (e.g. vitamin B_6)
  - The role of proteins as enzymes, and the part they play in metabolism, was already understood at this time; the evidence suggested that some inherited mutations knocked out specific elements of metabolic machinery (i.e. proteins).

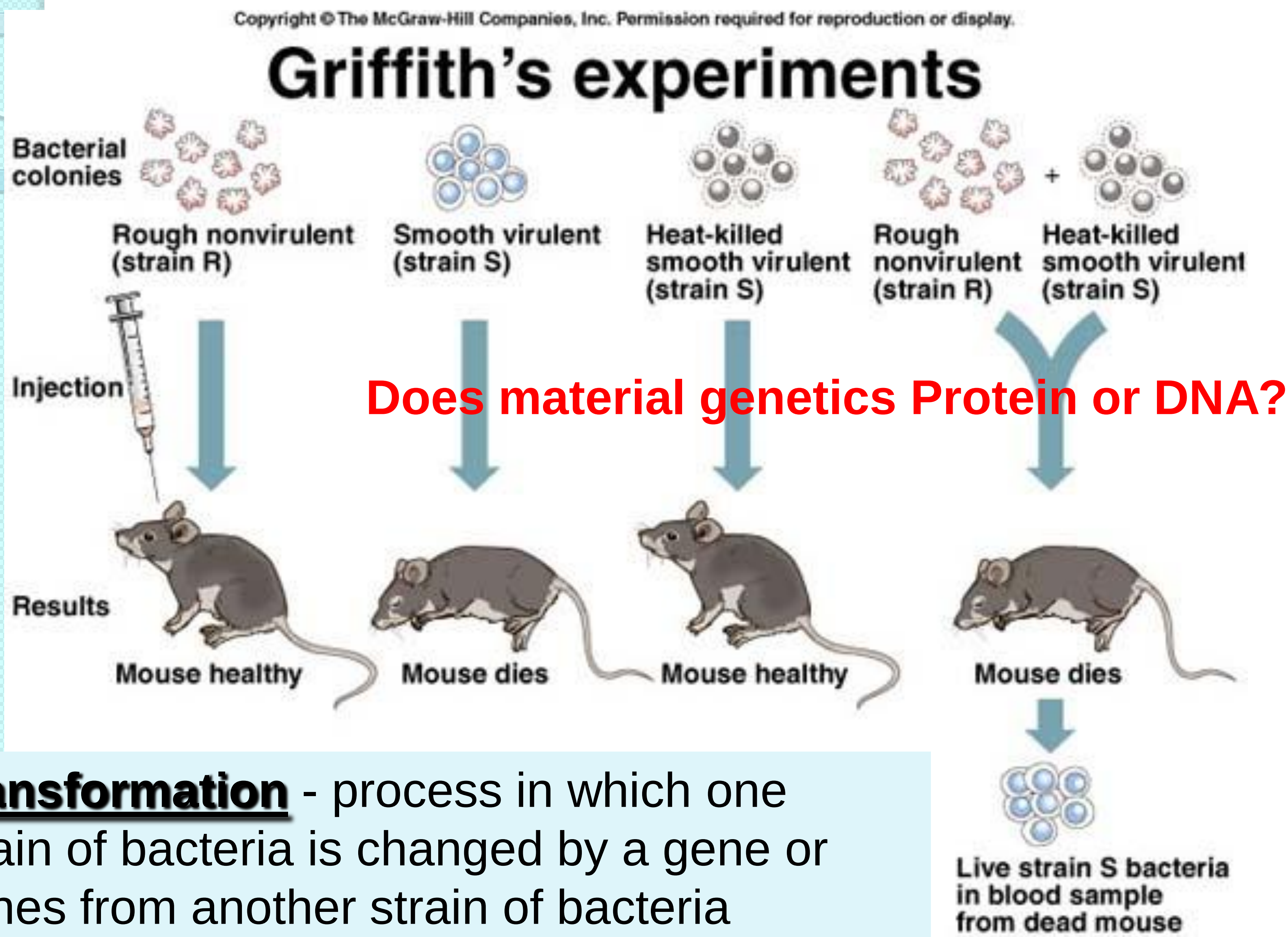
The genetics material: early studies

- <1940s protein chemistry
- 1868 F Miescher, nuclei cell have nuclein
- 1910 Levine tetranucleotide hypothesis as DNA structure
- 1927 Grifftith, transformation studies *Diplococcus pneumoniae*, *virulent and avirulent* strains
- 1944 O Avery, C McLeod, M McCarty: *transforming principle* in Bacteria, the event led to acceptance of DNA as the genetics material

Genes are made of DNA

- Griffith showed that bacteria could be "transformed"
  - pneumococcus colonies come in two varieties, "rough" (R) and "smooth" (S). S colonies are infectious, R are not.
  - Kill S colony with heat, mix dead bacteria with R cells, inject into mouse. Mouse gets sick and dies; can isolate S bacteria from carcass.
- Avery isolated the chemical components of S bacteria, demonstrated that the transforming factor was DNA.

Frederick Griffith 1928

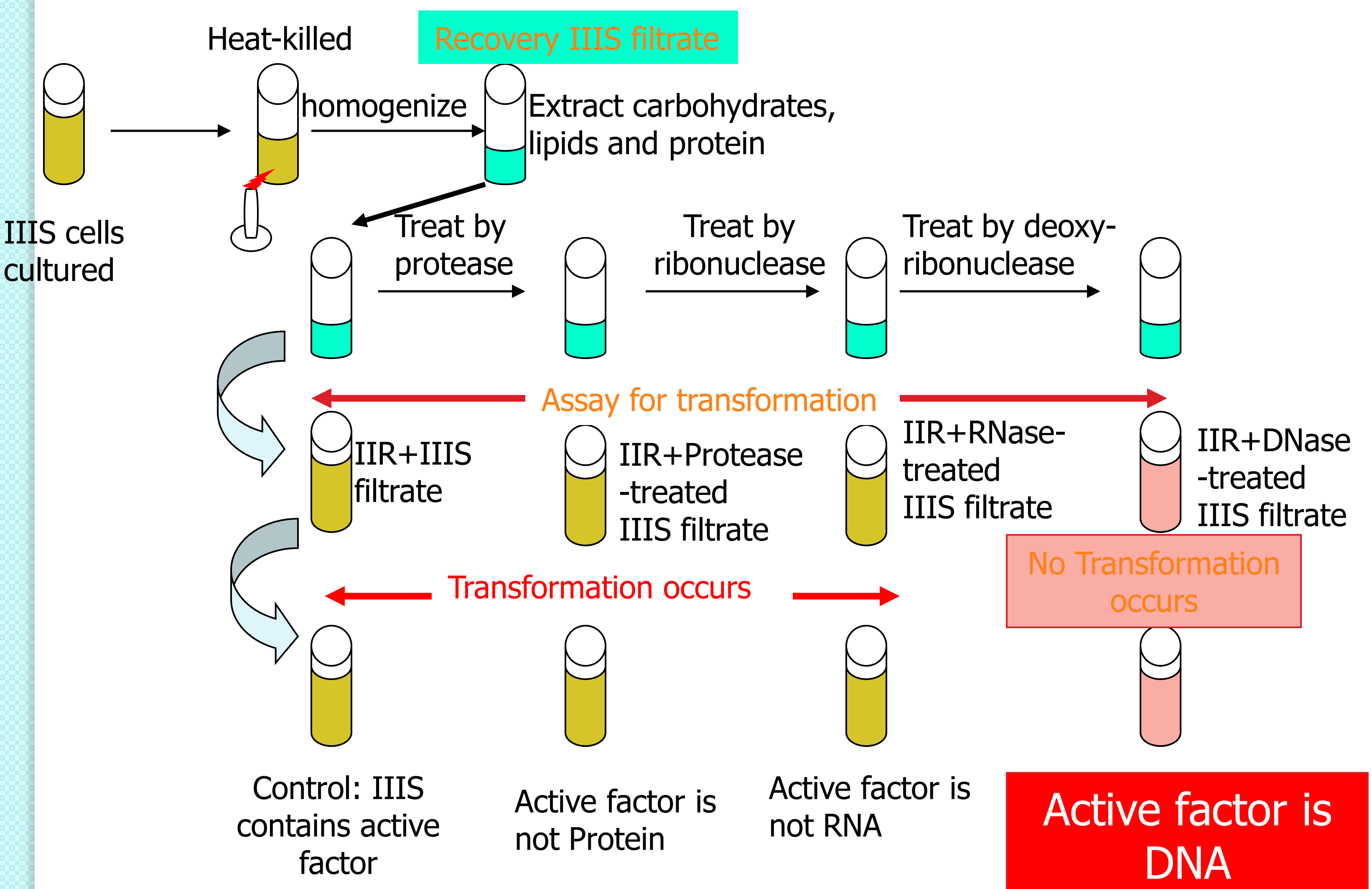


Does material genetics Protein or DNA?

Transformation - process in which one strain of bacteria is changed by a gene or genes from another strain of bacteria

Avery, C McLeod, M McCarty's Experiment

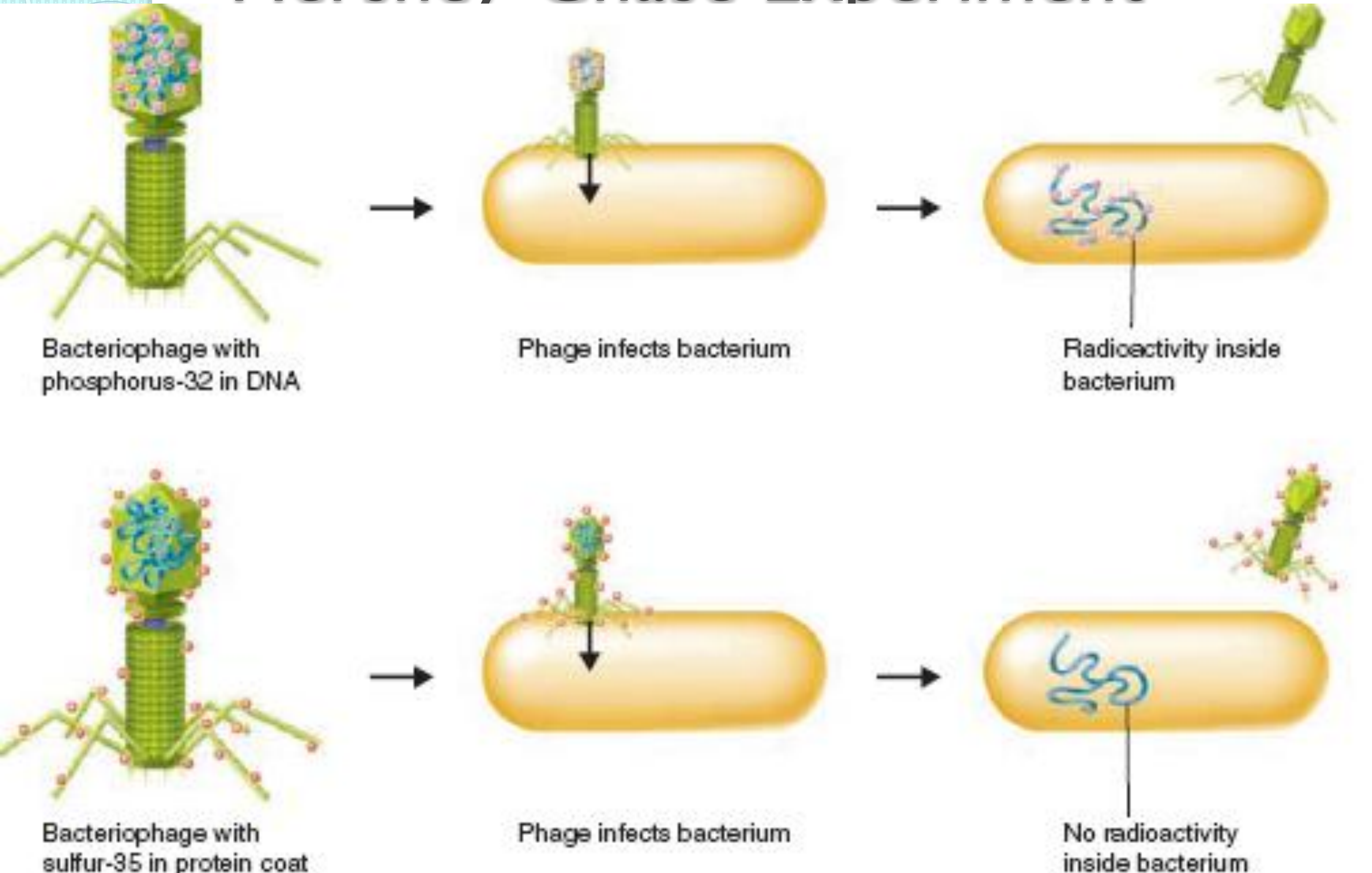
DNA is transforming factor



Hershey-Chase Experiment 1952

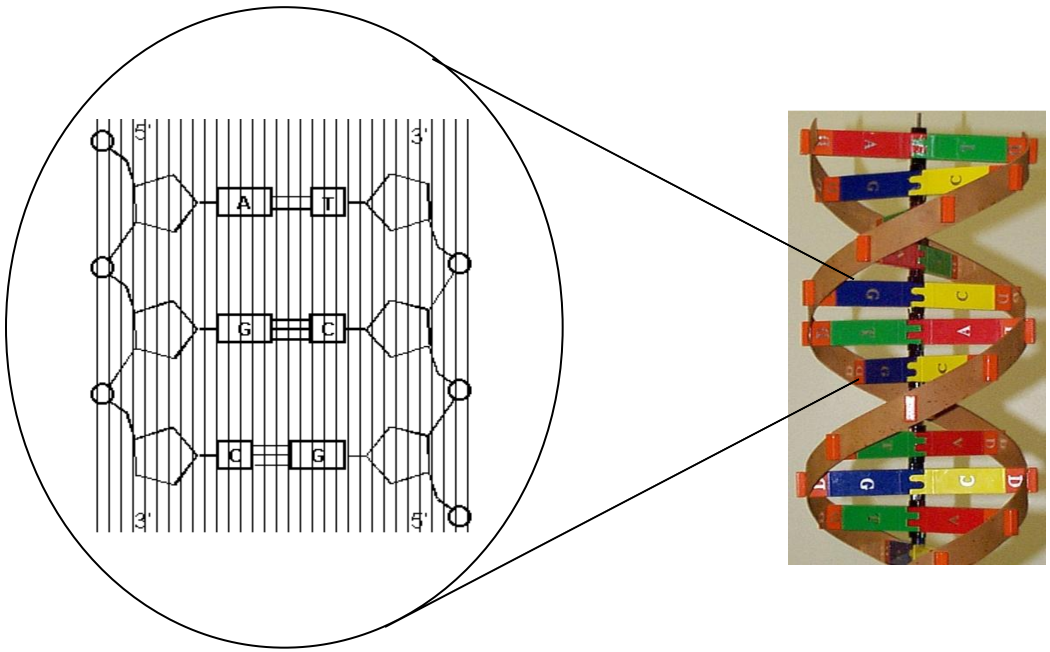
- Good scientists are naturally skeptical.
- Hershey-Chase are testing to see if DNA is the molecule that carries genetic information.
- **Bacteriophage** - virus that infects bacteria

Hershey-Chase Experiment



- DNA, what is it?
- RNA, what is it?
- DNA Replication, how?
- Differences and Similarities

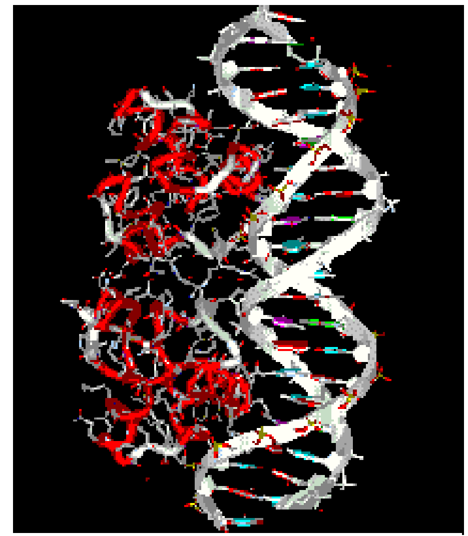
DNA: The Facts

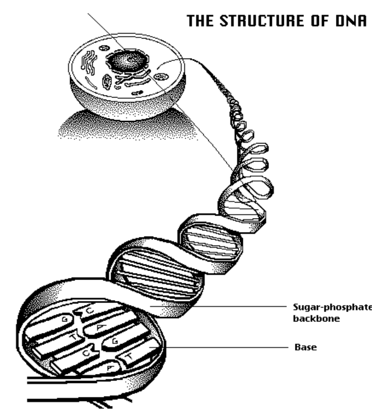


DNA has a Double Helix shape. This shape is due to hydrogen bonds.

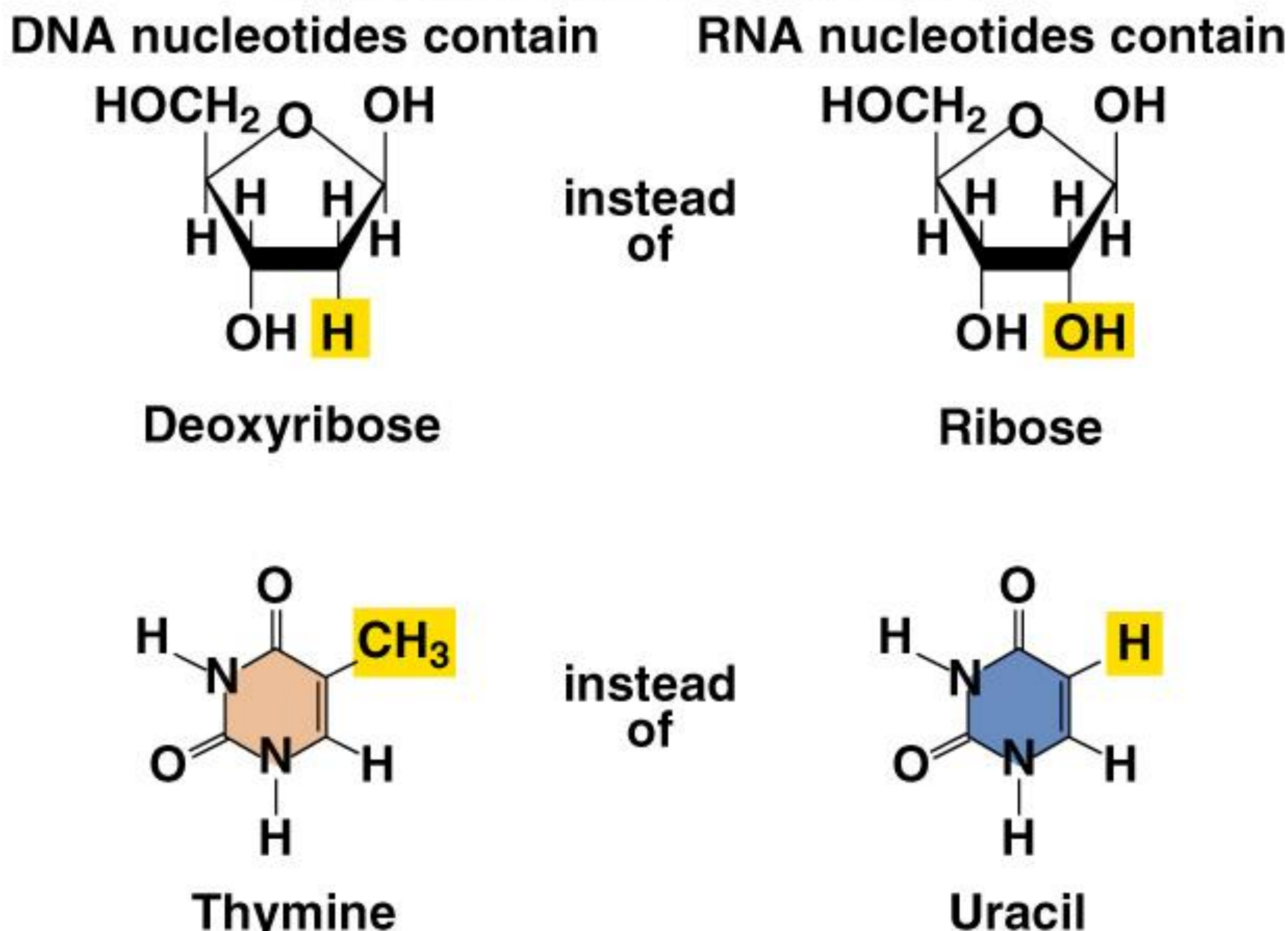
D.N.A. STRUCTURE

- DNA is also known as deoxyribonucleic acid. It is a polymer, which is made up of smaller, similar molecules, which coil together to form chains. DNA is described as a (double helix). This is because it forms a 3D Structure. A DNA molecule can be copied perfectly over and over again.



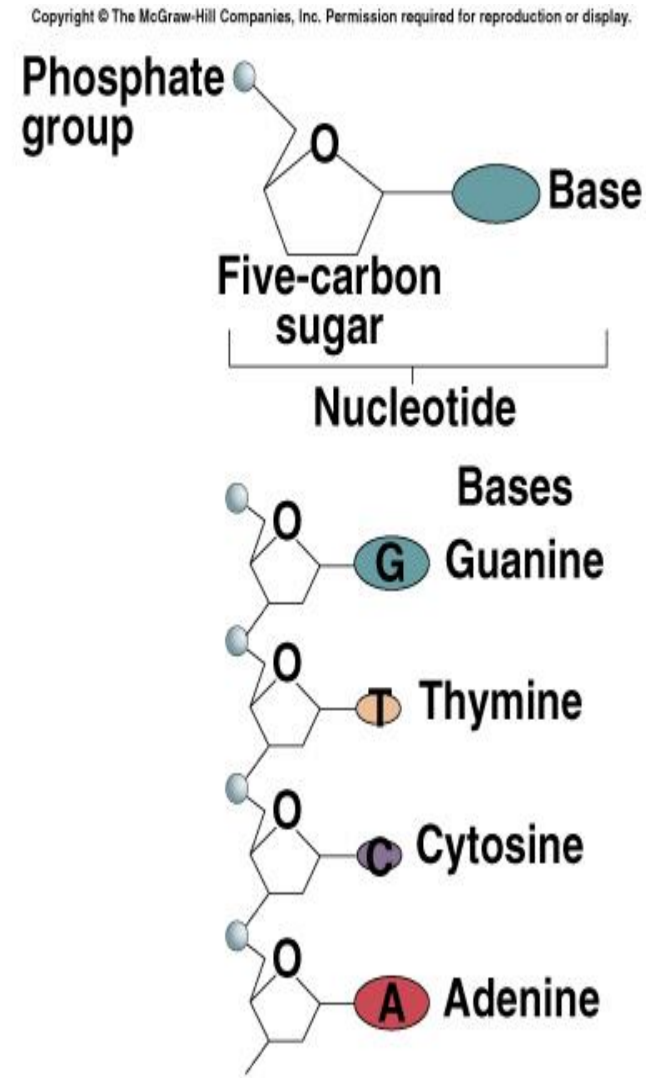


Nucleotides



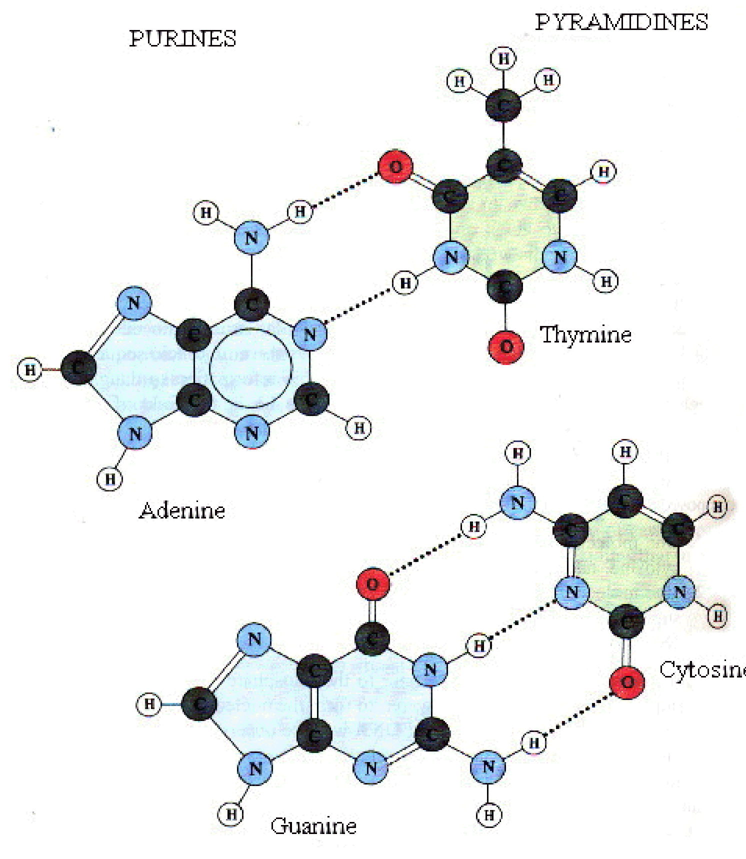
“backbone” of nucleic acid

- The “backbone” of the nucleic acid is formed by the sugar and phosphate pairs.
- Nitrogen containing base.
  A Pentose sugar.
  A phosphate group.
- The “rungs” are formed by paired nitrogenous bases.
  - Nitrogenous bases complementary pair
    - A + T (U)
    - C + G..

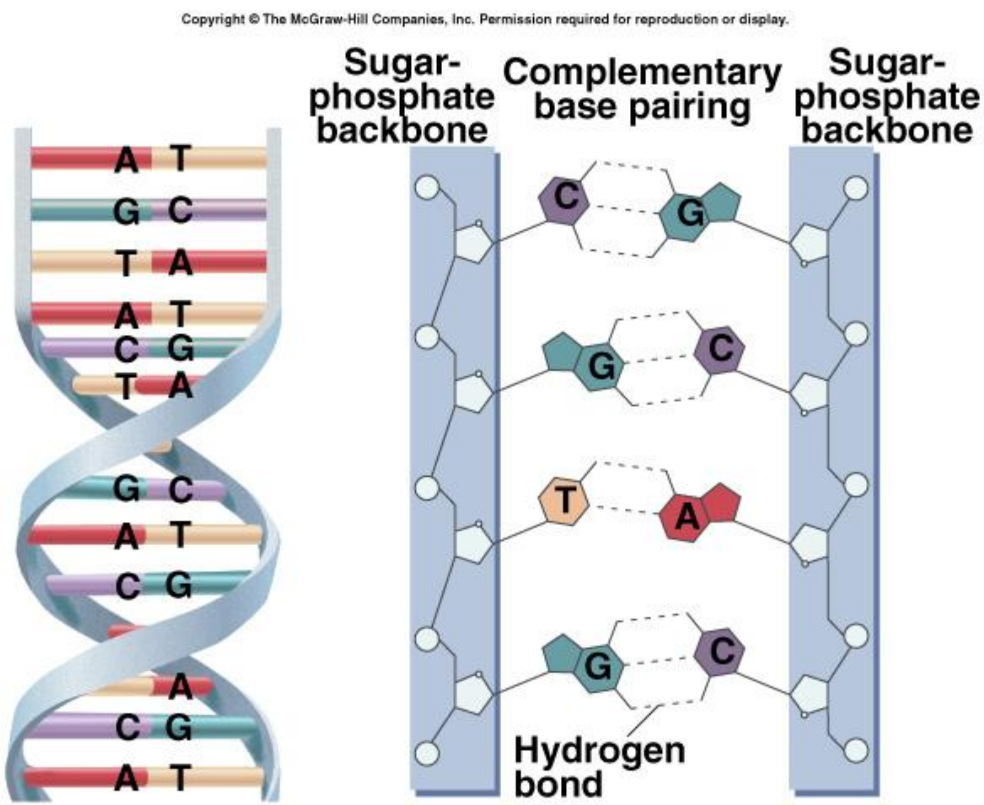


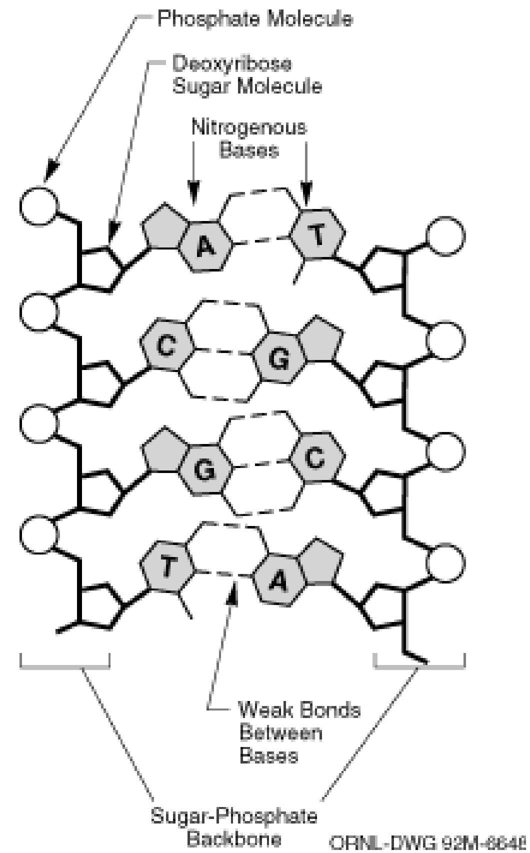
Hydrogen bonds

- Hydrogen bonds are special (polar) covalent bonds that are very important to physiology
- Bonds formed between the hydrogen end (+ charged) of a polar molecule and the – end of any other polar molecule or highly electronegative atom (e.g. P, N, O) are called hydrogen bonds.
- These hydrogen bonds are very important because they alter the physical and chemical properties of many molecules (especially water)..



The Essential Structure of DNA





Why DNA structure is ds?

- Pauling & Carey → structure of nucleat acid
- Chargaff demonstrated that the ratio of A/T in genomic DNA was a constant, and likewise G/C
- Wilkins and Franklin collected x-ray diffraction data for fibers of DNA, and determined that it had a helical structure.

↓

Watson & Crick

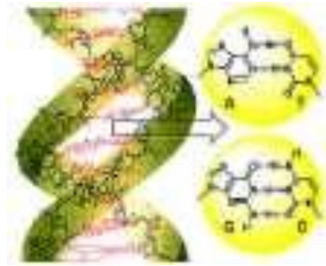
Chargaff : the ratio of A/T in genomic DNA

They also concluded that this percentage of bases in a DNA molecule is independent of age, nutritional state, environment of the organism studied.

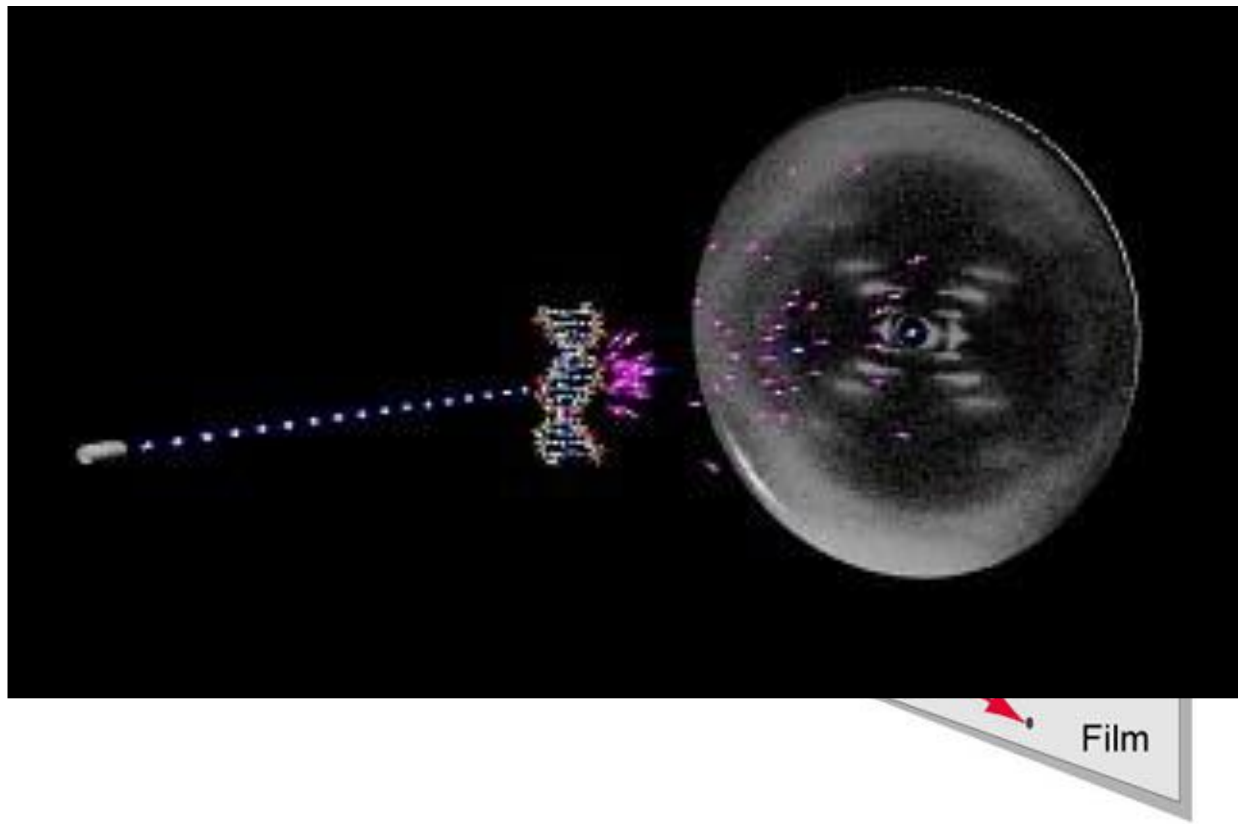
Species	Adenine	Thymine	Guanine	Cytosine
Human	31.0	31.5	19.1	18.4
Fruit fly	27.3	27.6	22.5	22.5
Corn	25.6	25.3	24.5	24.6
Mold	23.0	23.3	27.1	26.6
Escherichia	24.6	24.3	25.5	25.6
Bacillius Subtillis	28.4	29.0	21.0	21.6

It appears that human and e-coli bacteria obey a Chargaff's rule which states that ***In every species, the percent of Adenine almost exactly equals that of Thymine, and the percent of Guanine is essentially identical to that of Cytosine.***

Rosalind Franklin 1950
X-Ray Diffraction of DNA



- Clues from the X-Ray
  - Coiled (forming Helix)
  - Double-stranded
  - Nitrogeneous bases are in the center



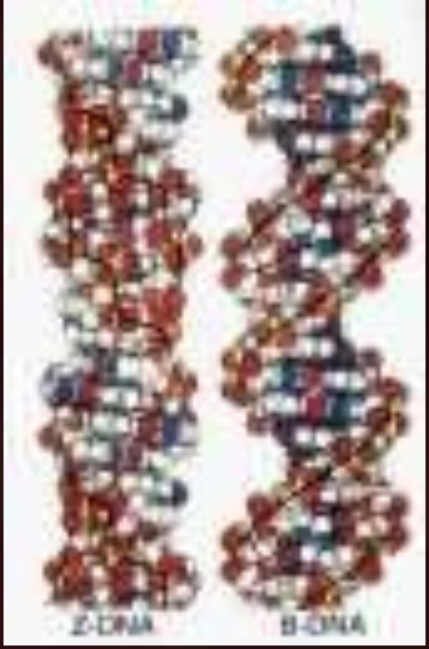
Watson & Crick

- Francis Crick – British physicist
- James Watson – American Biologist
  - Building a 3D model of DNA
  - Franklin's X-Ray opened their eyes to the Double Helix
- **Watson and Crick's model of DNA was a double helix, in which two strands were wound around each other.**

Structure of DNA

- **Watson & Crick** put these clues together with simple MOLECULAR MODELING studies to deduce THE STRUCTURE OF DOUBLE-STRANDED DNA, and also to suggest the mechanism for copying DNA

- Here's the original paper: **http://www.nature.com/genomics/human/watson-crick/index.html**



A- and B-DNA – right-handed helix,
Z-DNA – left-handed helix

B-DNA – fully hydrated DNA in vivo,
10 base pairs per turn of helix

RNA Structure

- RNA is generally single stranded
  - Can fold and create complicated structure
  - Multiple types of RNA, each with a different function
- Sugar-phosphate groups form the backbone of the molecule
  - Nucleotides are organized 5' to 3'
- Bases form the center of the molecule

RNA: Ribonucleic Acid

- Material Genetik pada virus
- Terdapat di nukleus, sitoplasma
- Bentuk Linier, single strand
- Struktur kimiawi:

1. Gula penthose, disebut **ribonucleosa**
2. Asam phosphat
3. Basa Nitrogen:

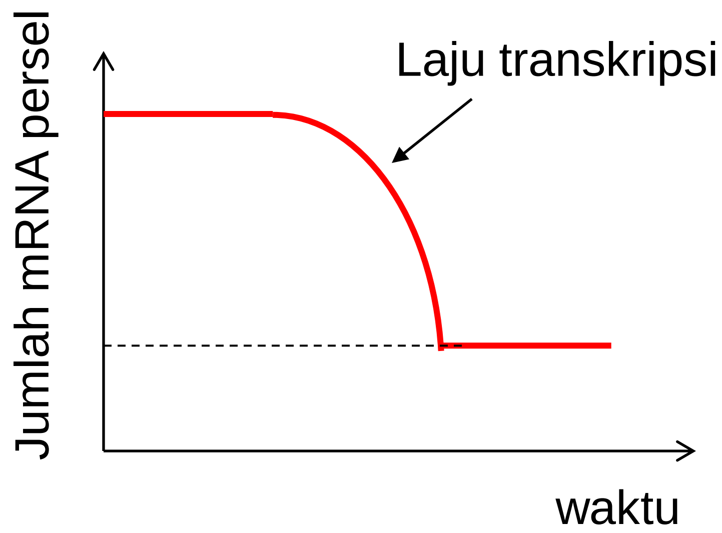
} Backbone RNA

Purin: Adenin, Guanin

Pyrimidin: Sitosin, **Urasil**

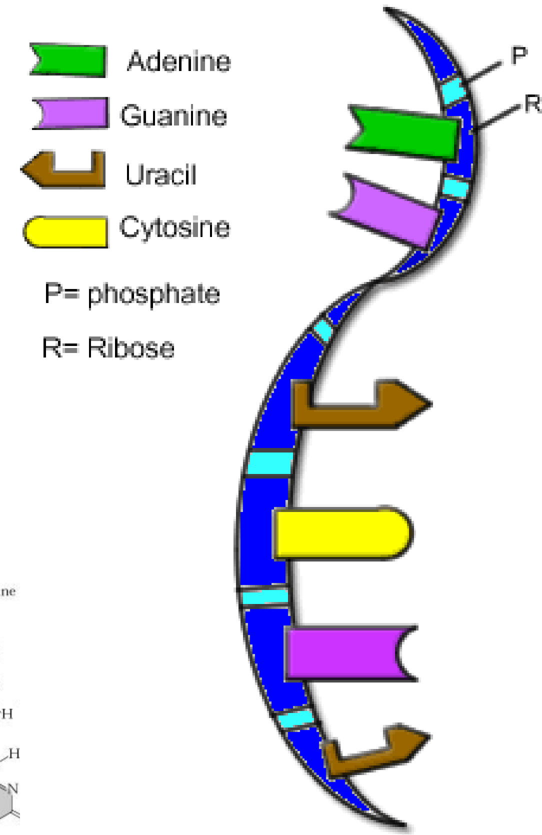
- Type RNA:
- mRNA, messenger RNA
- rRNA, ribosomal RNA
- tRNA, transfer RNA

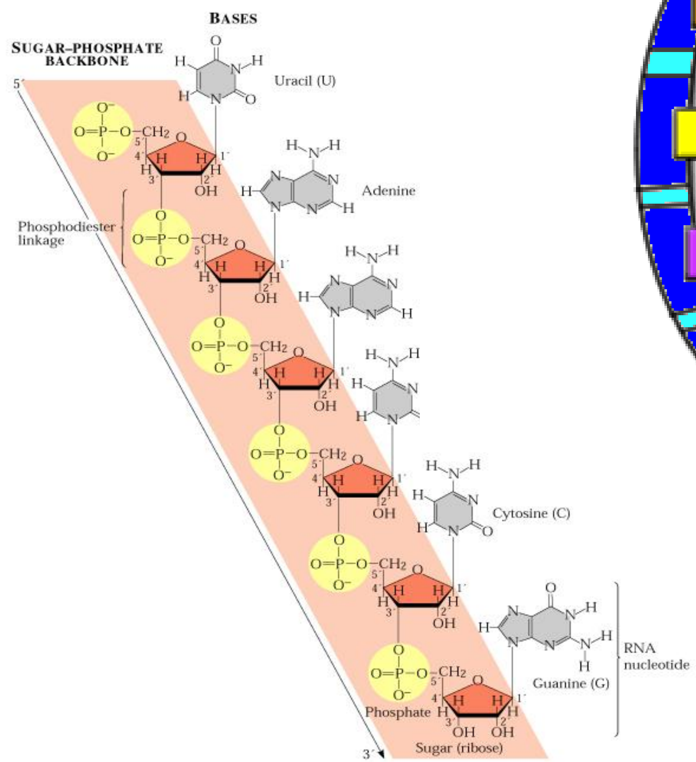
- mRNA mempunyai half life yang pendek → mempertahankan homogenitas



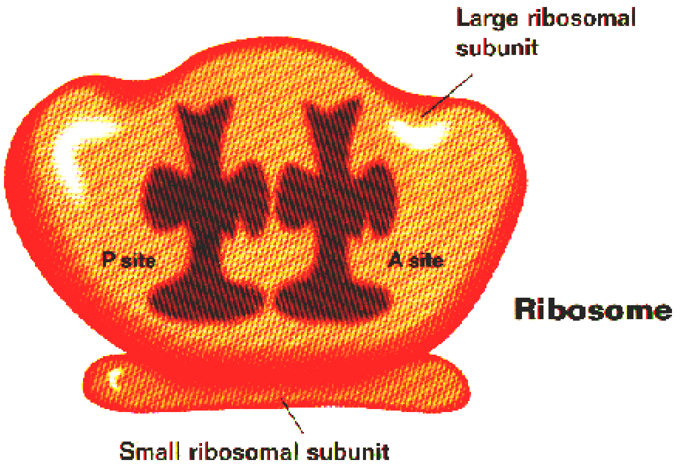
Types of RNA

- mRNA: messenger RNA. It is the copy of RNA that is made in the nucleus and travels outside the cell

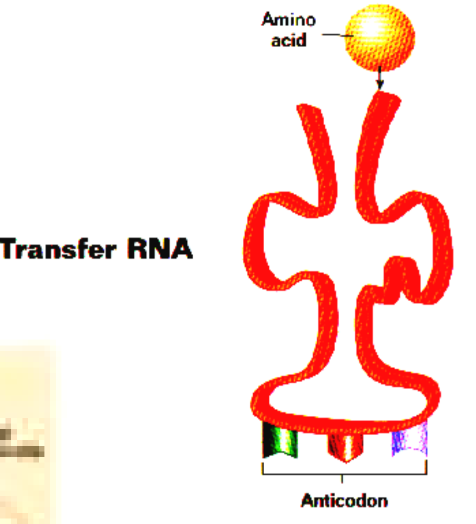


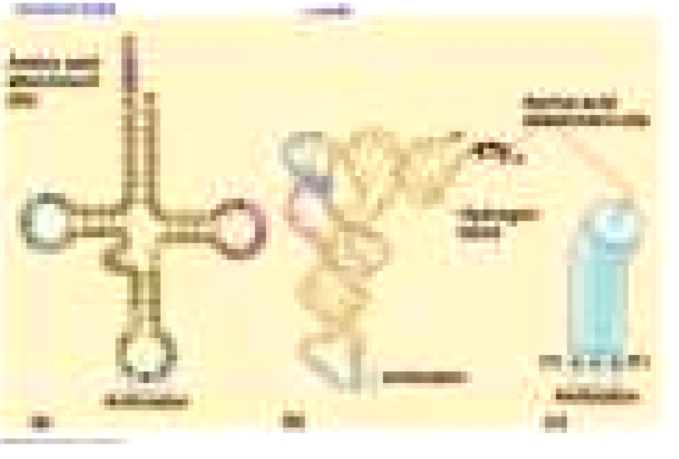


- rRNA: the ribosome itself. It has two parts- large and small and 2 binding sites: P and A



- tRNA: transfer RNA. It contains an anti-codon on one side and an amino acid on the other





Genes code for proteins using symbolic information

- Gene sequences code for protein sequences via a symbolic code, the *genetic code*. This code is used nearly universally by living organisms; it is one of the most ancient shared characteristics of living things.
- The "words" of the genetic code are nucleotide triplets called *codons*. Each codon codes for at most one amino acid.
- Codons that do not code for any amino acids, called *nonsense or stop* codons, terminate a coding region of the gene. They serve as "punctuation marks"

Kode Genetik

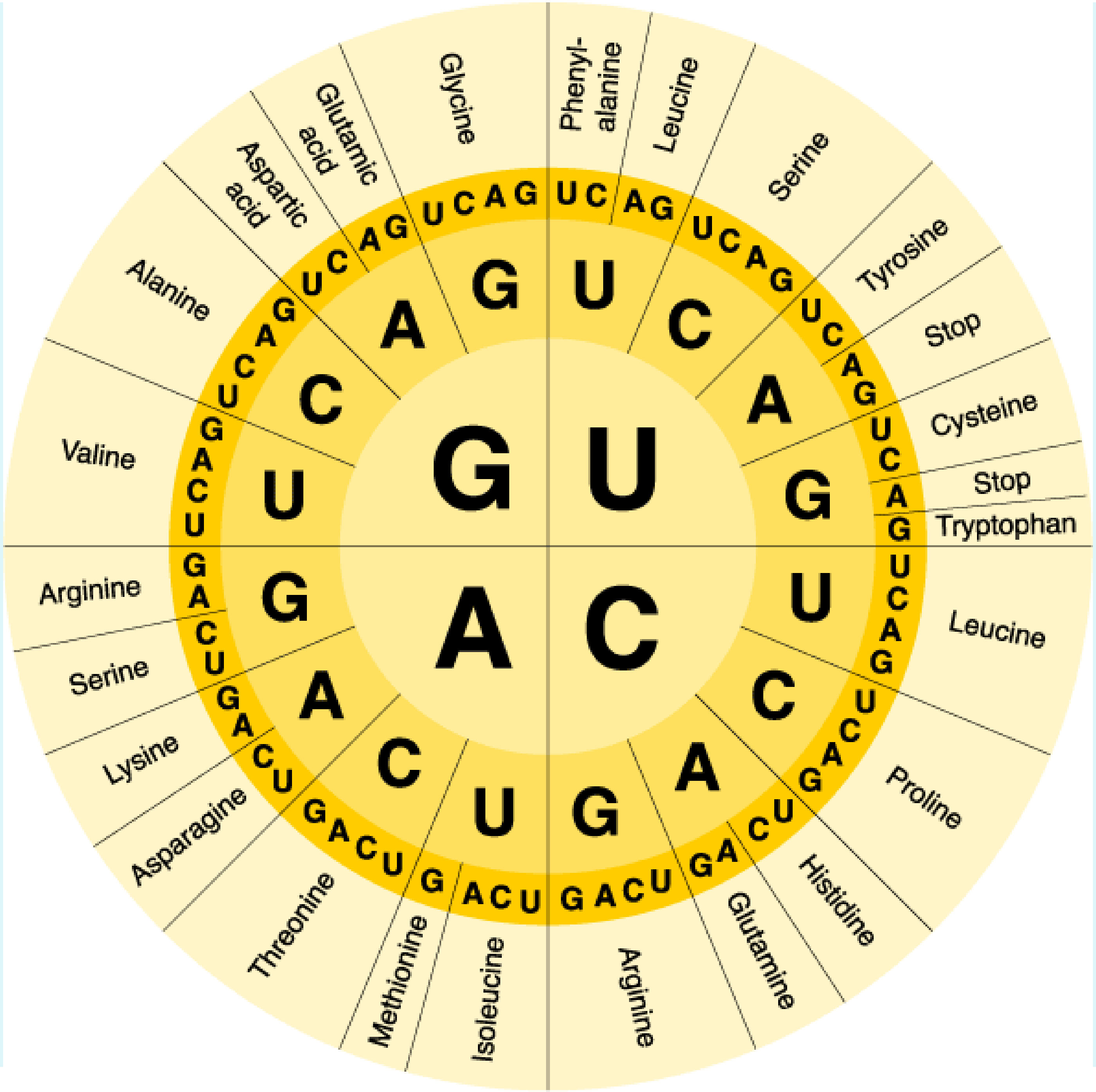
- Codon tersusun atas 3 nukleotida (triplet) yg mengkode informasi untuk satu asam amino, terbentuk 64 macam

- dari 64 mengkode 20 asam amino, beberapa asam amino dikode lebih dari 1 codon

- bersifat UNIVERSAL untuk semua organisme

- Start codon, initiation codon, kodon awal/pembuka adalah AUG (RNA) atau ATG (DNA)

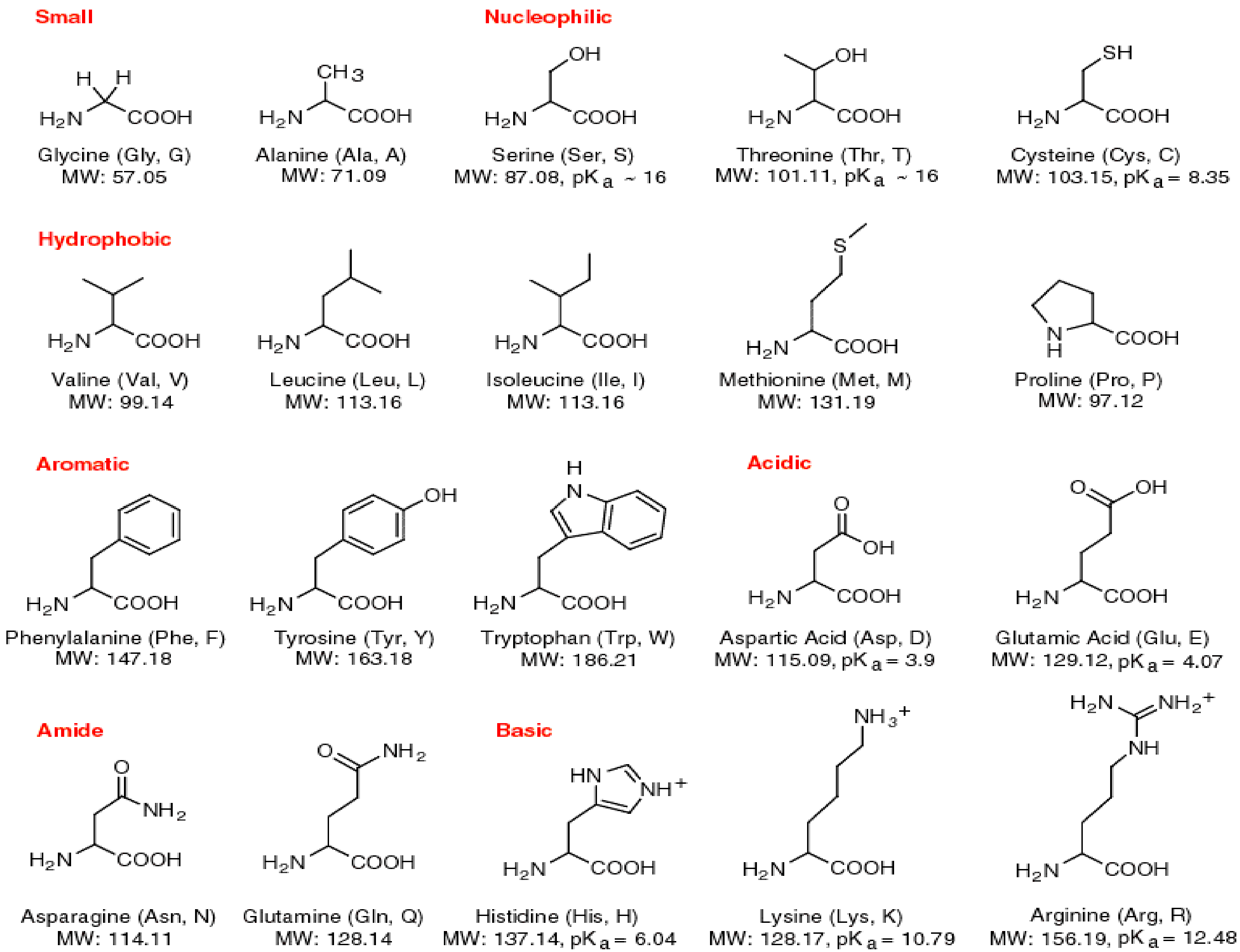
- Stop codon or termination codon adalah UAA, UAG dan UGA. Karena ketiga kodon ini tidak mengkode asam amino apapun disebut juga nonsense-codon


G
U
A
C
Phenyl-
alanine
Leucine
Serine
Tyrosine
Stop
Cysteine
Stop
Tryptophan
Leucine
Proline
Histidine
Glutamine
Arginine
Isoleucine
Methionine
Threonine
Asparagine
Lysine
Serine
Arginine
Valine
Alanine
Aspartic
acid
Glutamic
acid
Glycine

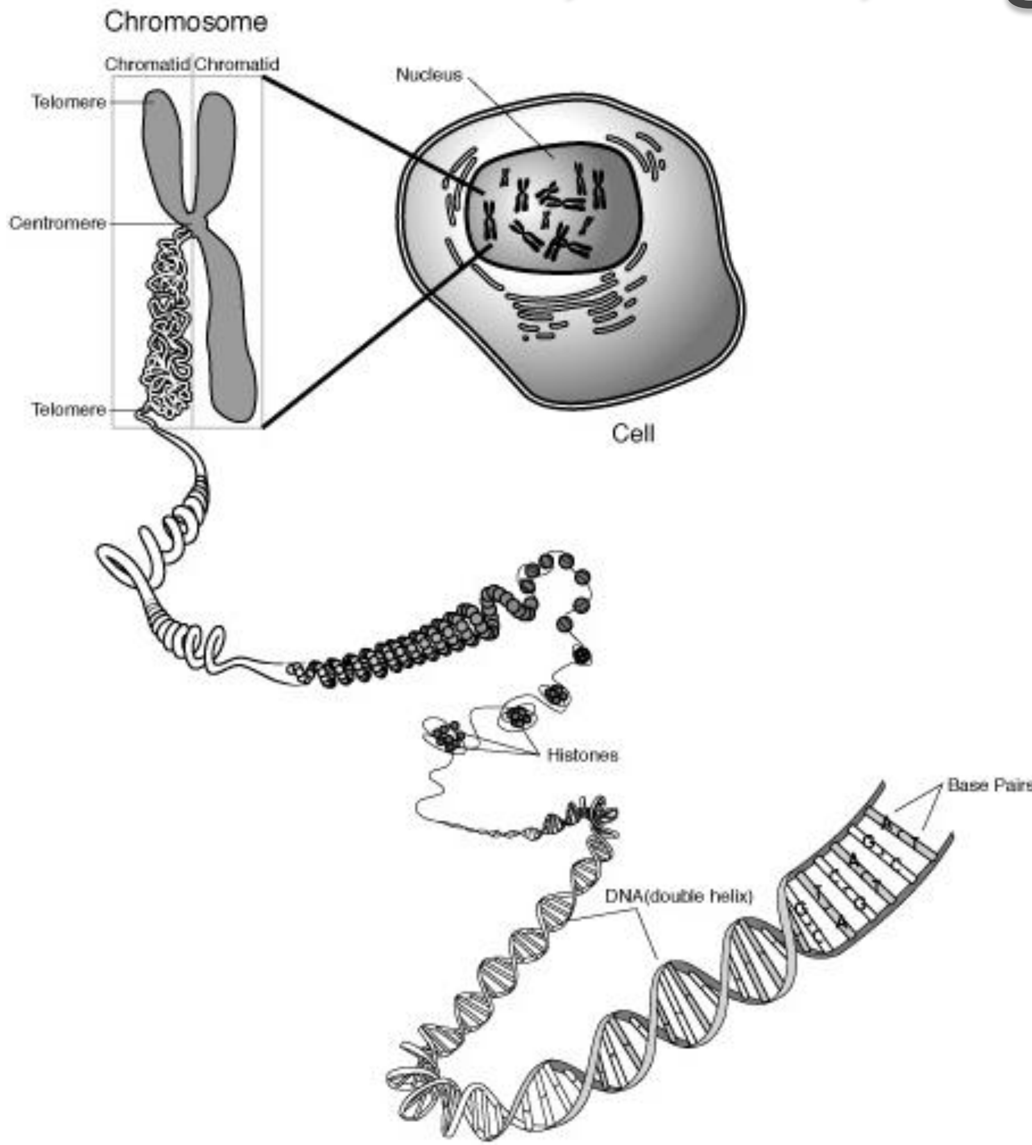
Second letter

First letter	U	C	A	G	Third letter
U	UUU UUC Phenyl-alanine UUA UUG Leucine	UCU UCC UCA UCG Serine	UAU UAC Tyrosine UAA Stop codon UAG Stop codon	UGU UGC Cysteine UGA Stop codon UGG Tryptophan	U C A G
C	CUU CUC CUA CUG Leucine	CCU CCC CCA CCG Proline	CAU CAC Histidine CAA CAG Glutamine	CGU CGC CGA CGG Arginine	U C A G
A	AUU AUC AUA Isoleucine AUG Methionine; start codon	ACU ACC ACA ACG Threonine	AAU AAC Asparagine AAA AAG Lysine	AGU AGC Serine AGA AGG Arginine	U C A G
G	GUU GUC GUA GUG Valine	GCU GCC GCA GCG Alanine	GAU GAC Aspartate GAA GAG Glutamate	GGU GGC GGA GGG Glycine	U C A G

Structure of Amino Acid subclass



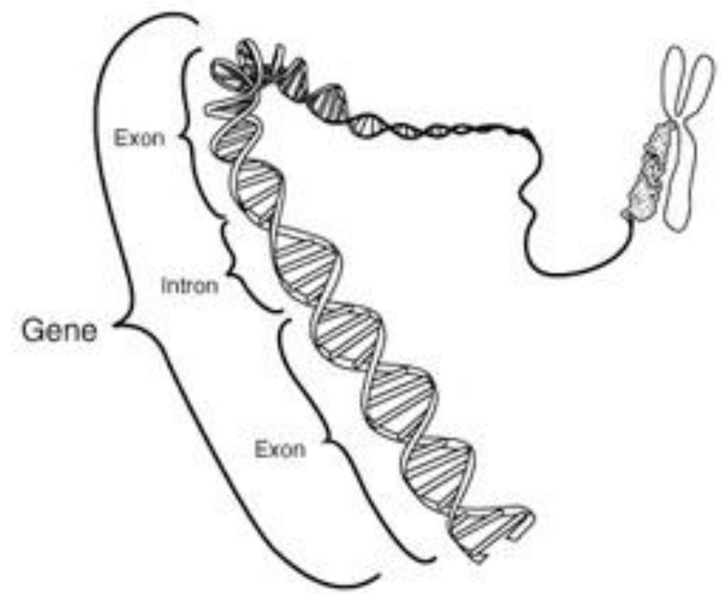
Chromosome, DNA, & gene



http://www.accessexcellence.org/AB/GG/chromosome.html

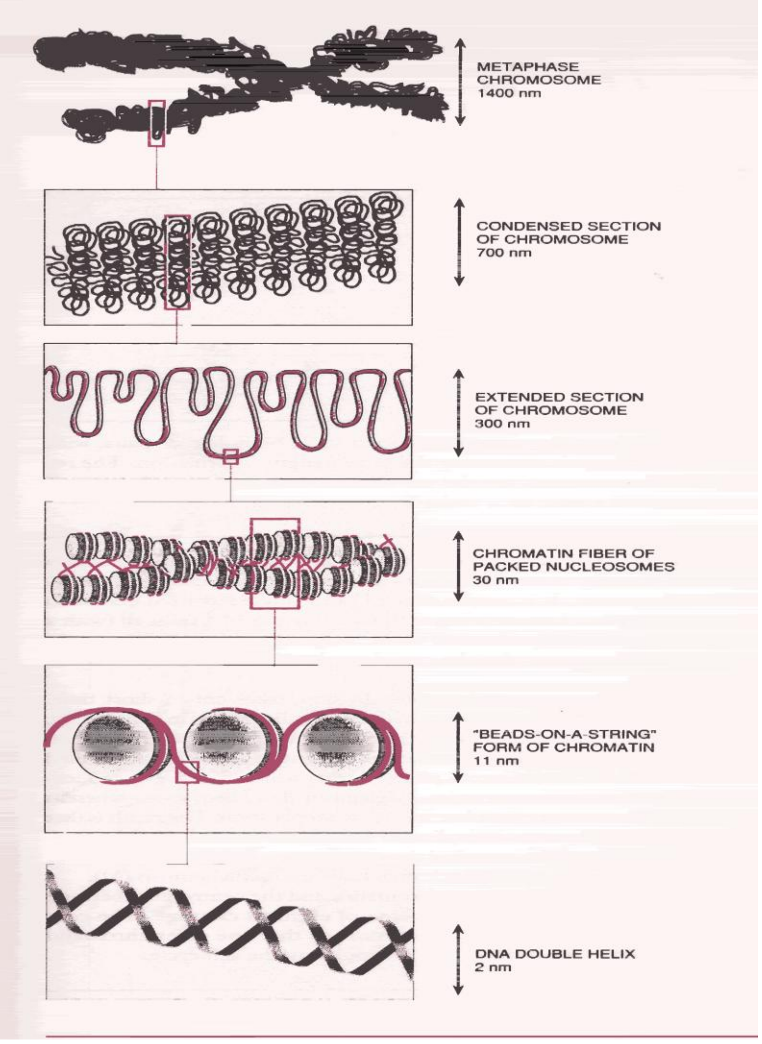
Genes

- Genes are short sections of chromosomes

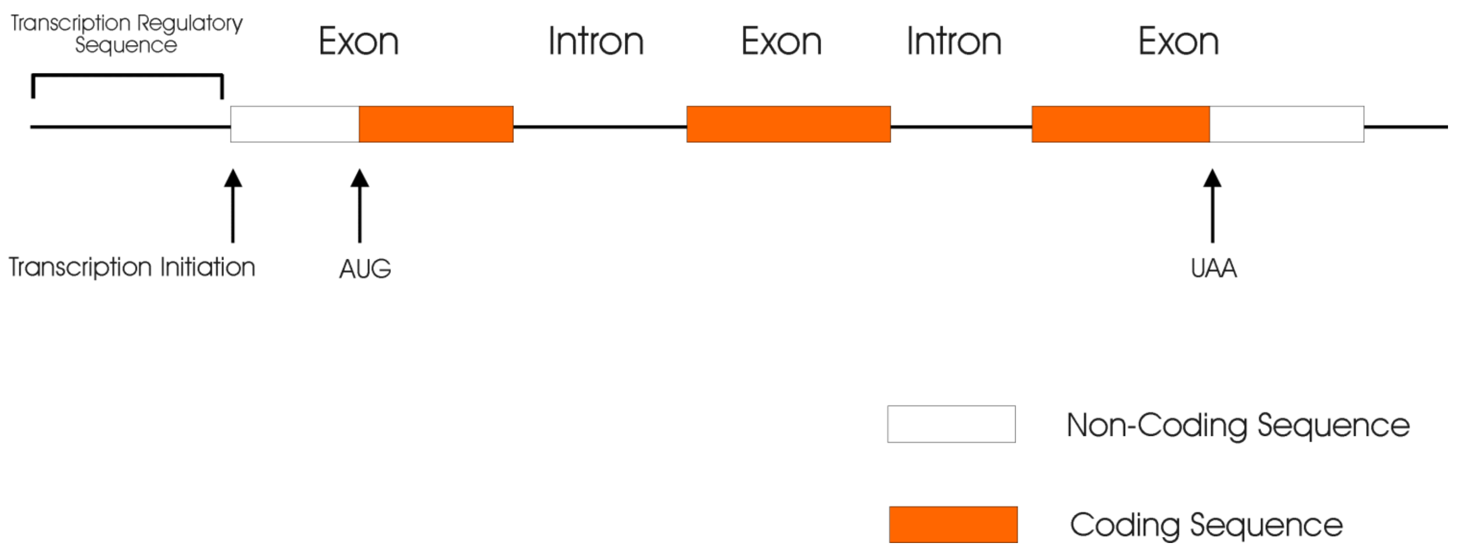


http://www.accessexcellence.org

Chromosomal Structure of the Genetic Material



Structure of a Typical Eukaryotic Gene – the β-Globin Gene

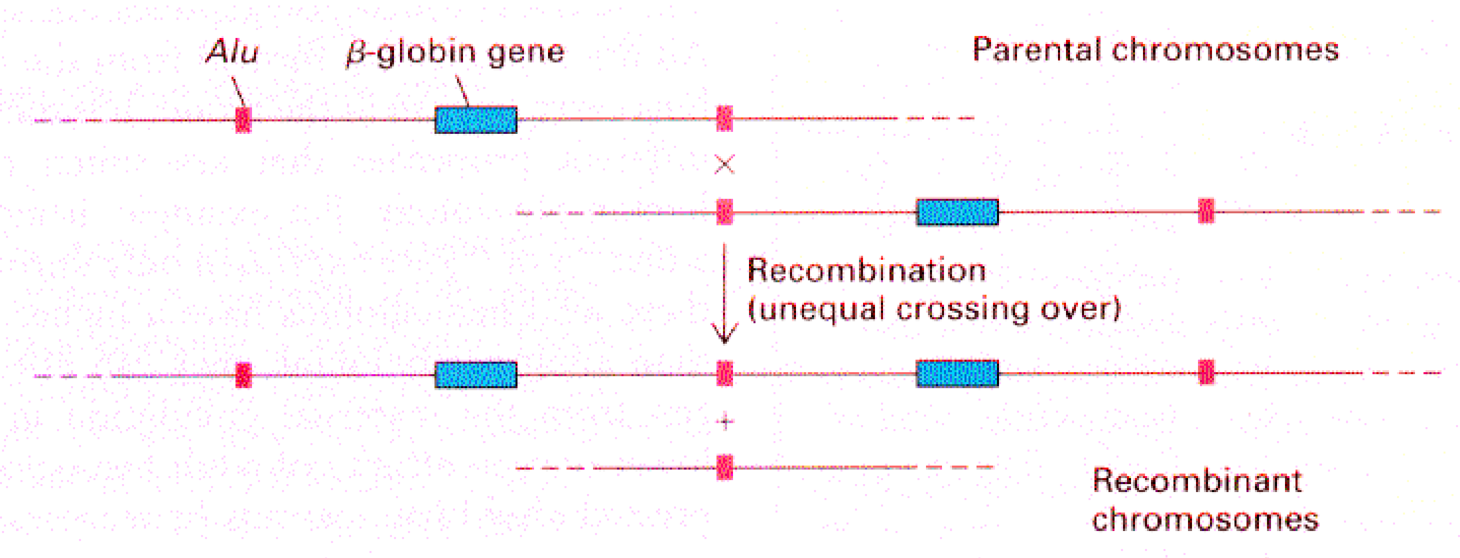


Prokaryotic gene structure

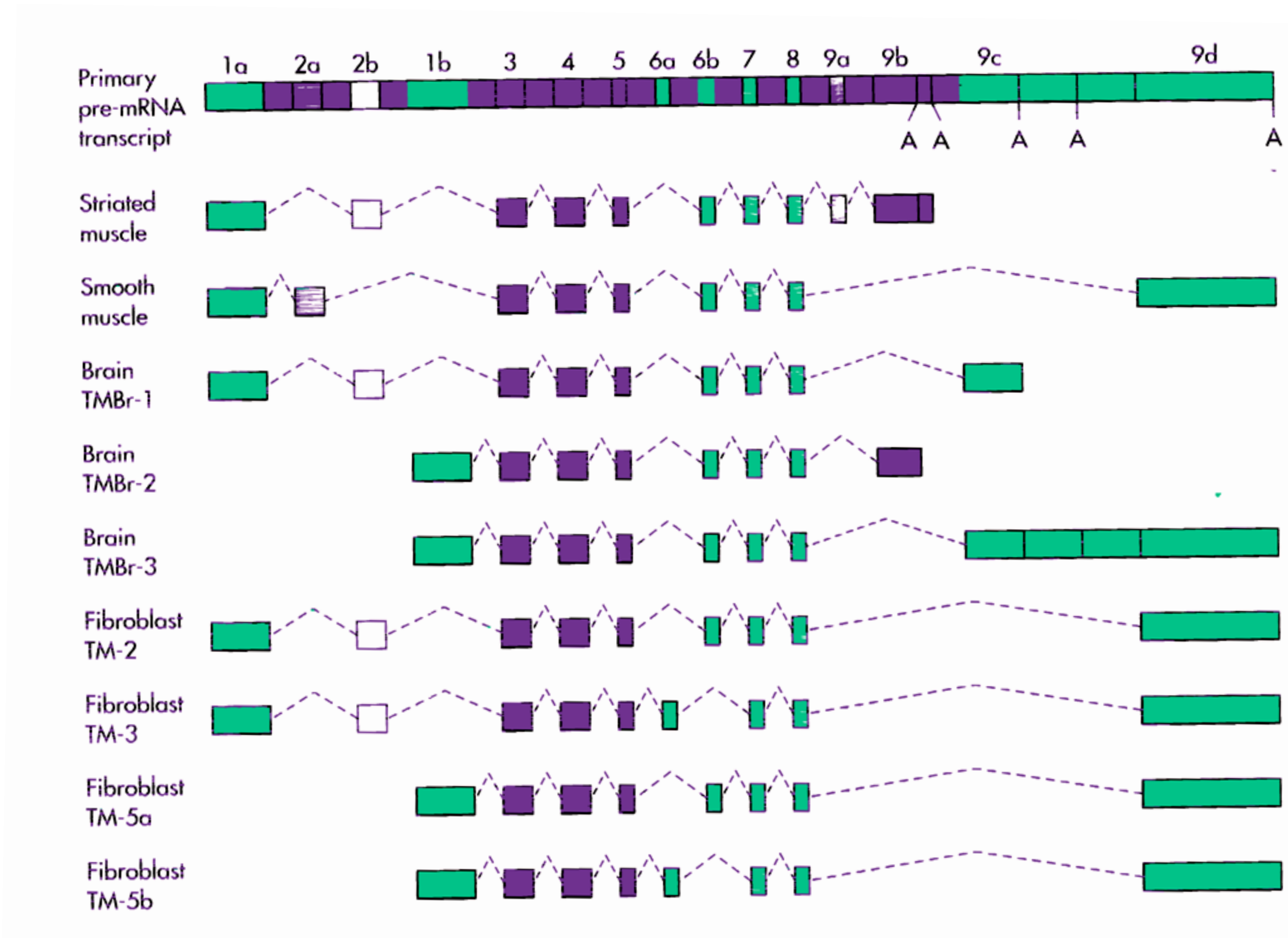
Intronless,
polysistronic

P	O	A	B	C	UTR

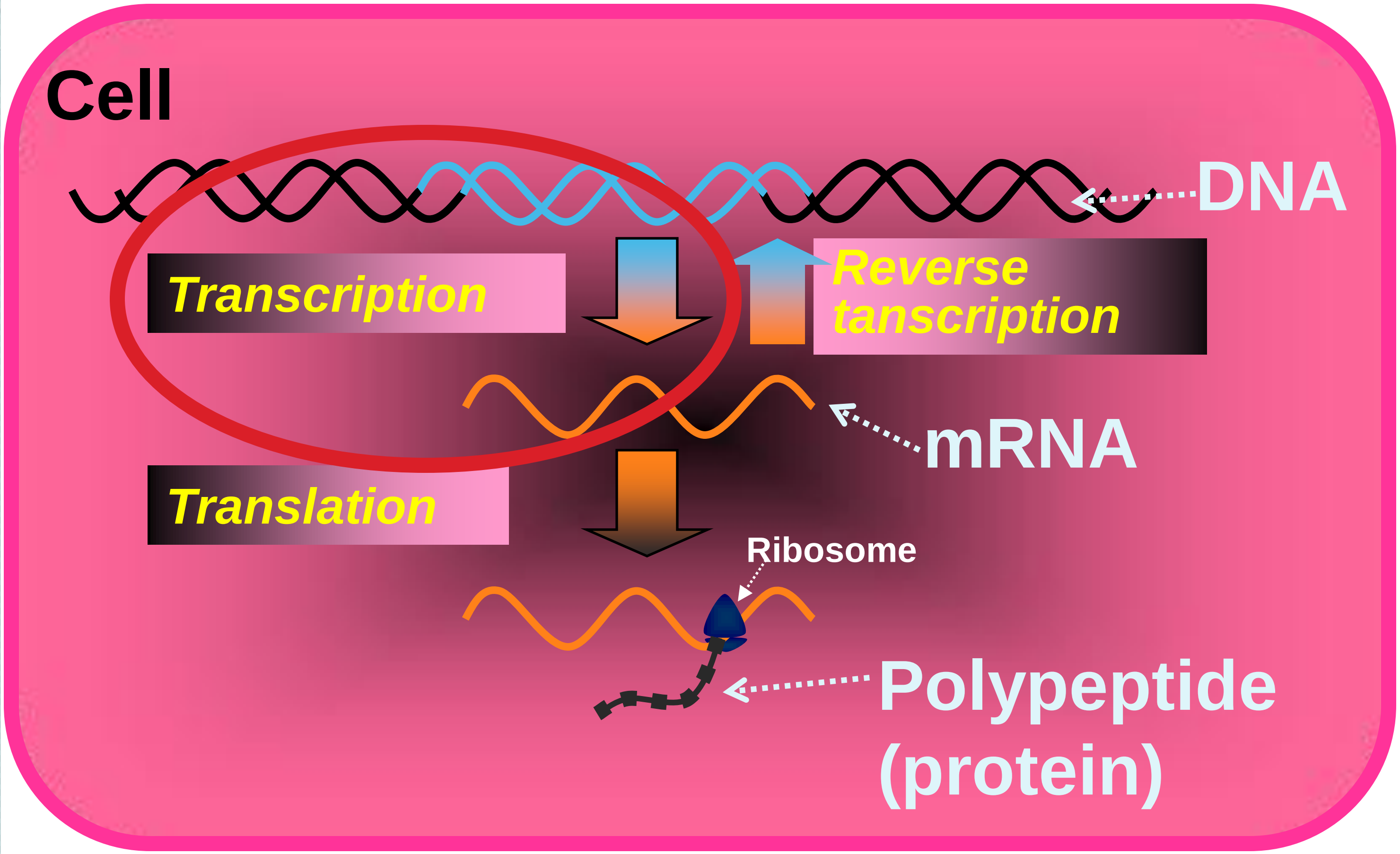
Unequal Crossing Over as a Mechanism for Gene Duplication and Gene Loss



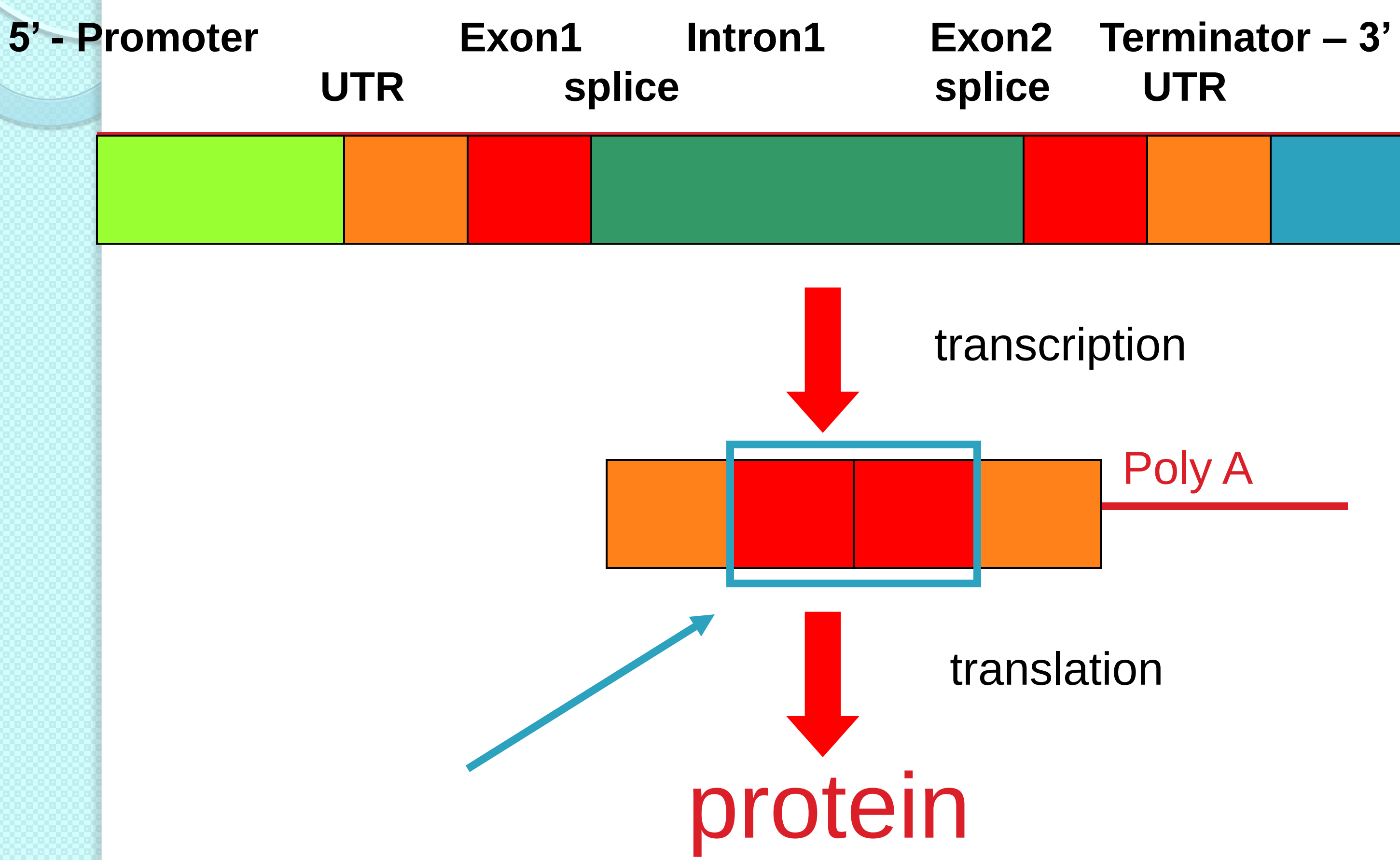
The Impact of the Complexity of Gene Structure on Gene Expression



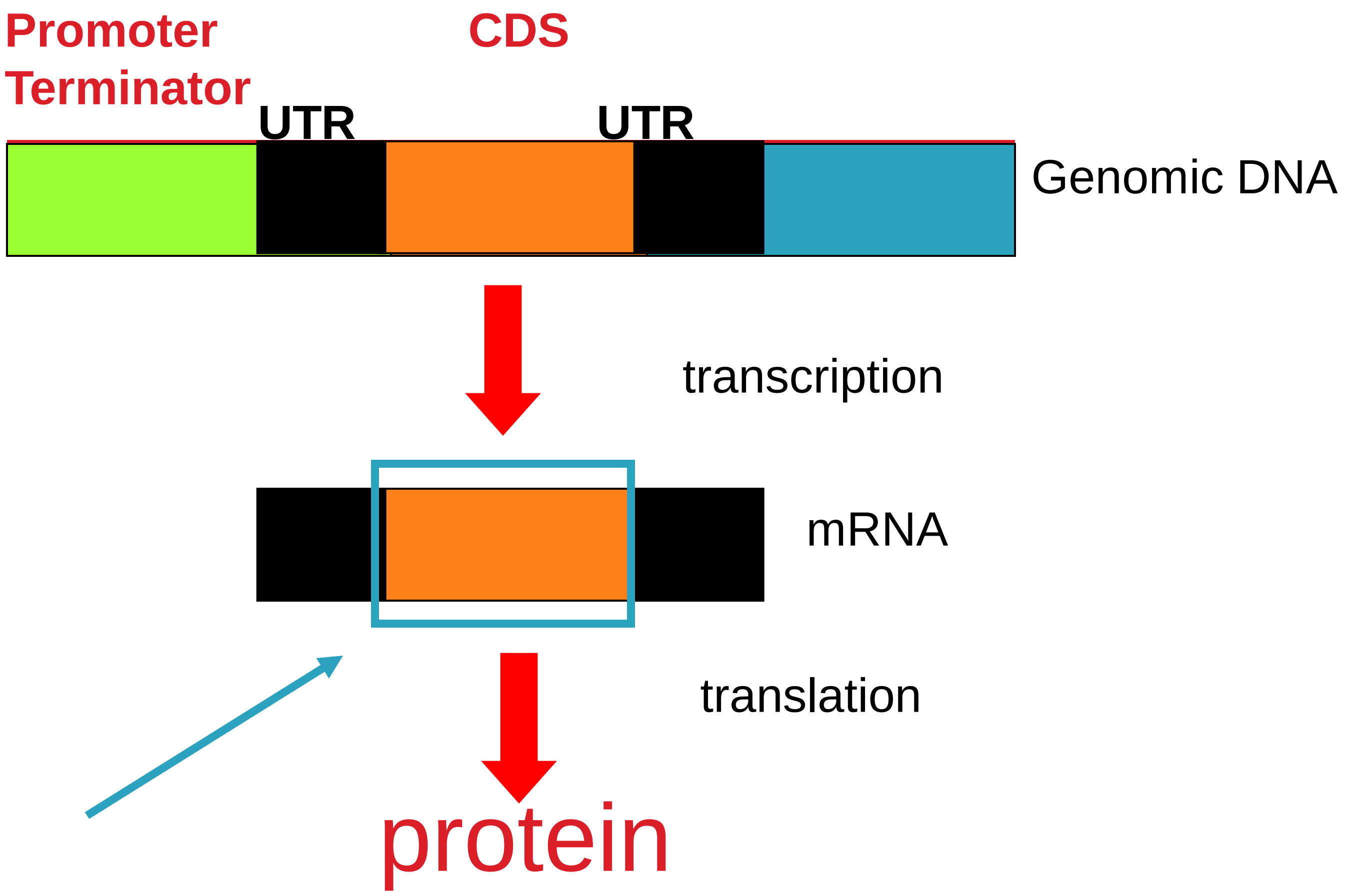
The Central Dogma of SYNTHESIS PROTEIN



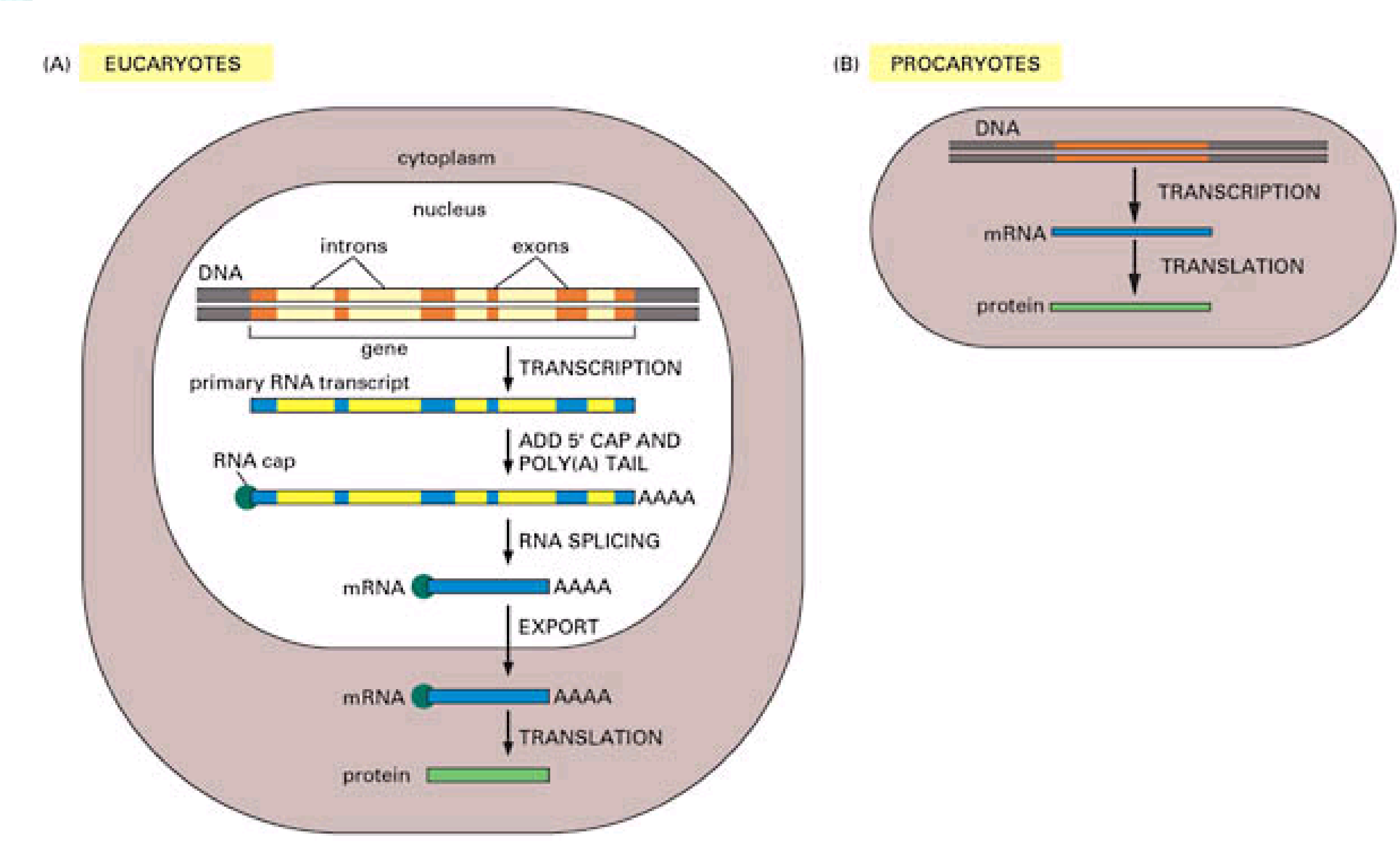
Eukaryotic Gene Structure



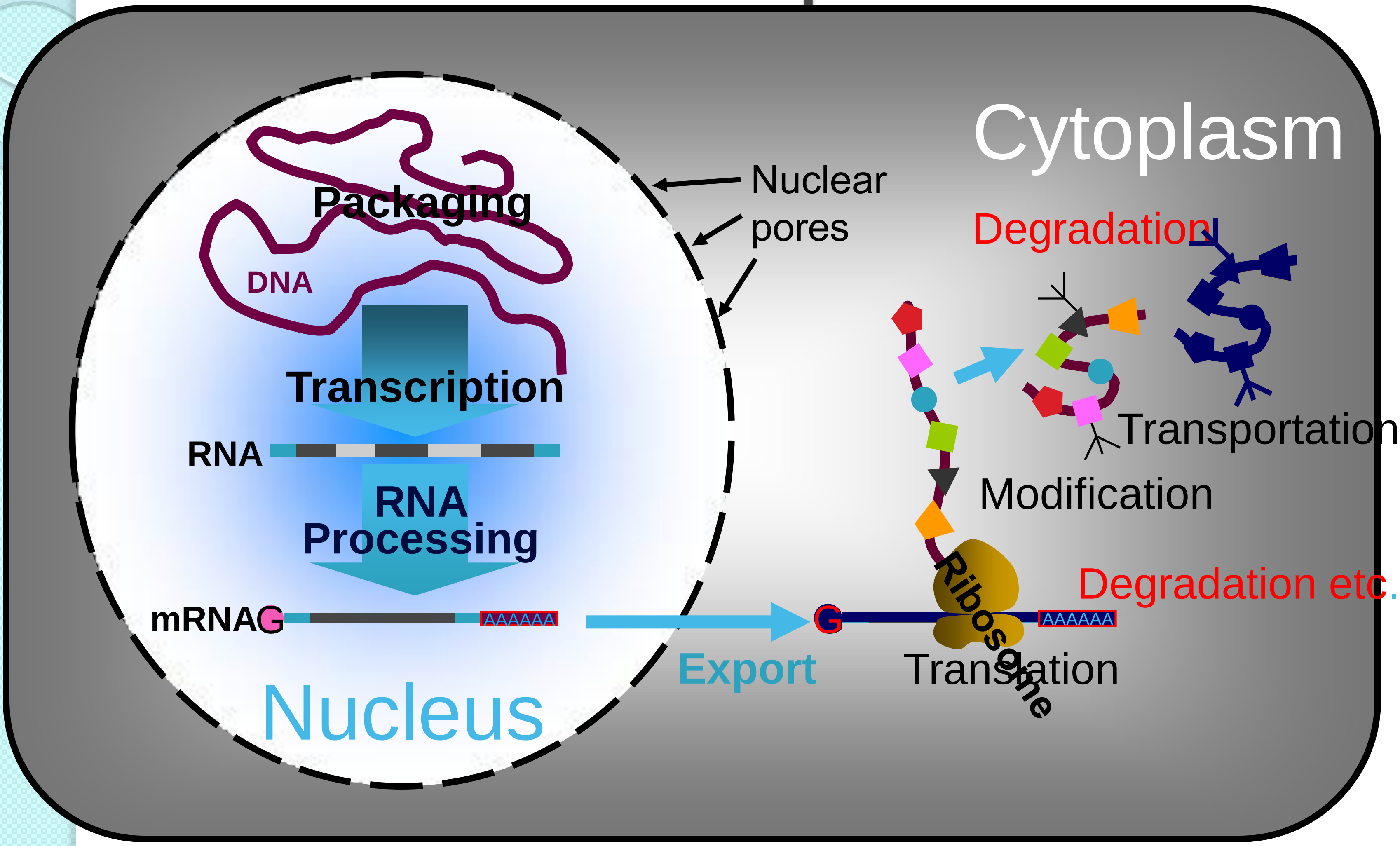
Prokaryotic Gene Structure



From Gene to Protein



Control of Gene Expression



How do DNA Replicate?

- Replication is the process by which copies of DNA
- Cells of living organisms and made on daily basis and most of the older cells die as well.
- So there are many generation and dying of cell.
- are made

The Replication Challenge

- Size of an average human chromosome

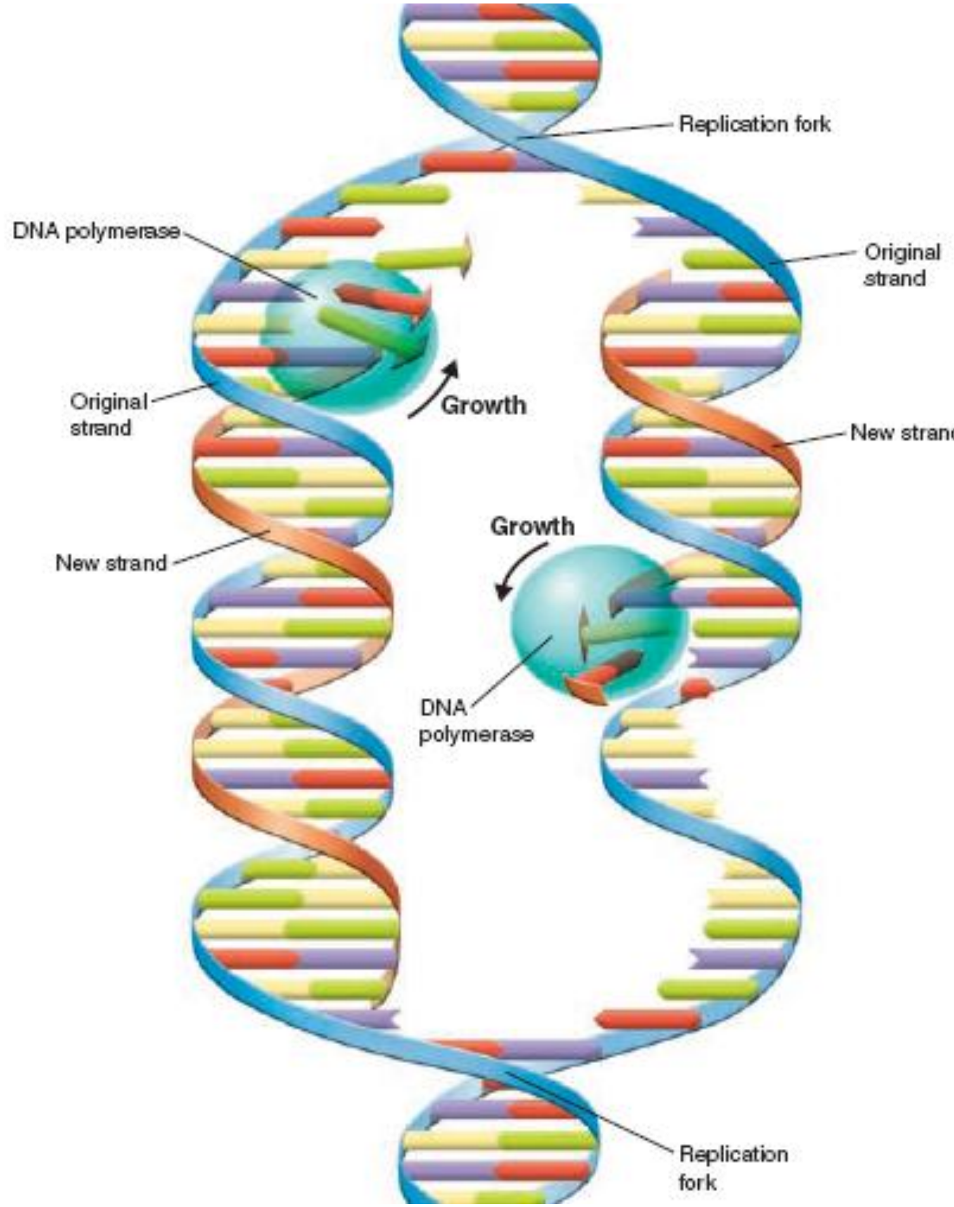
  130 million bp

- Rate of replication

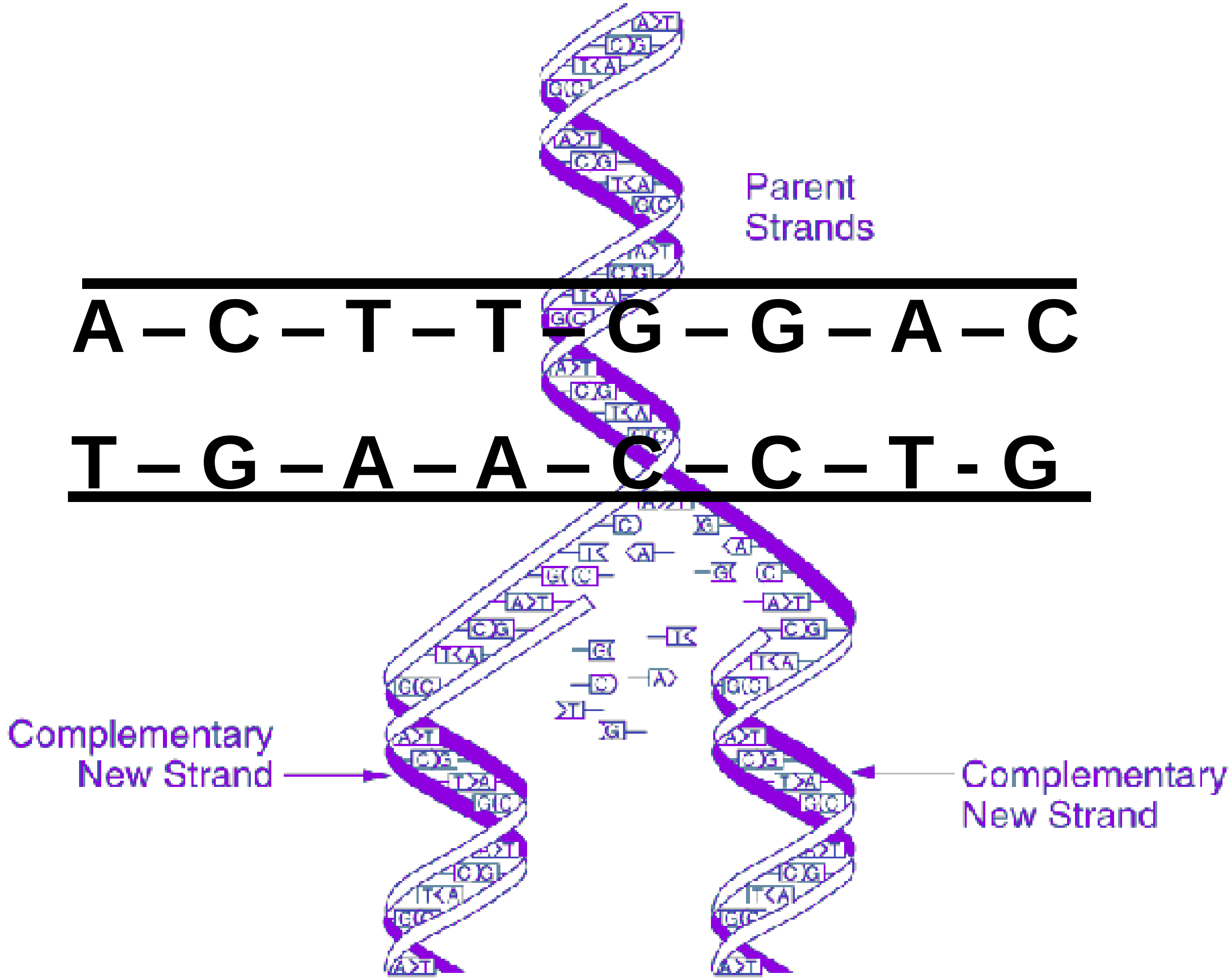
  ~ 50 bp per sec

- Fidelity of replication

1. Enzymes unwind DNA
2. Enzymes split "unzip" double helix
3. The enzyme, ***DNA polymerase,*** finds and attaches the corresponding N-base
4. Each "old" stand serves as a template and is matched up with a new stand of DNA
5. New helixes wind back up.



DNA Replication



A – C – T – T – G – G – A – C

T – G – A – A – C – C – T - G

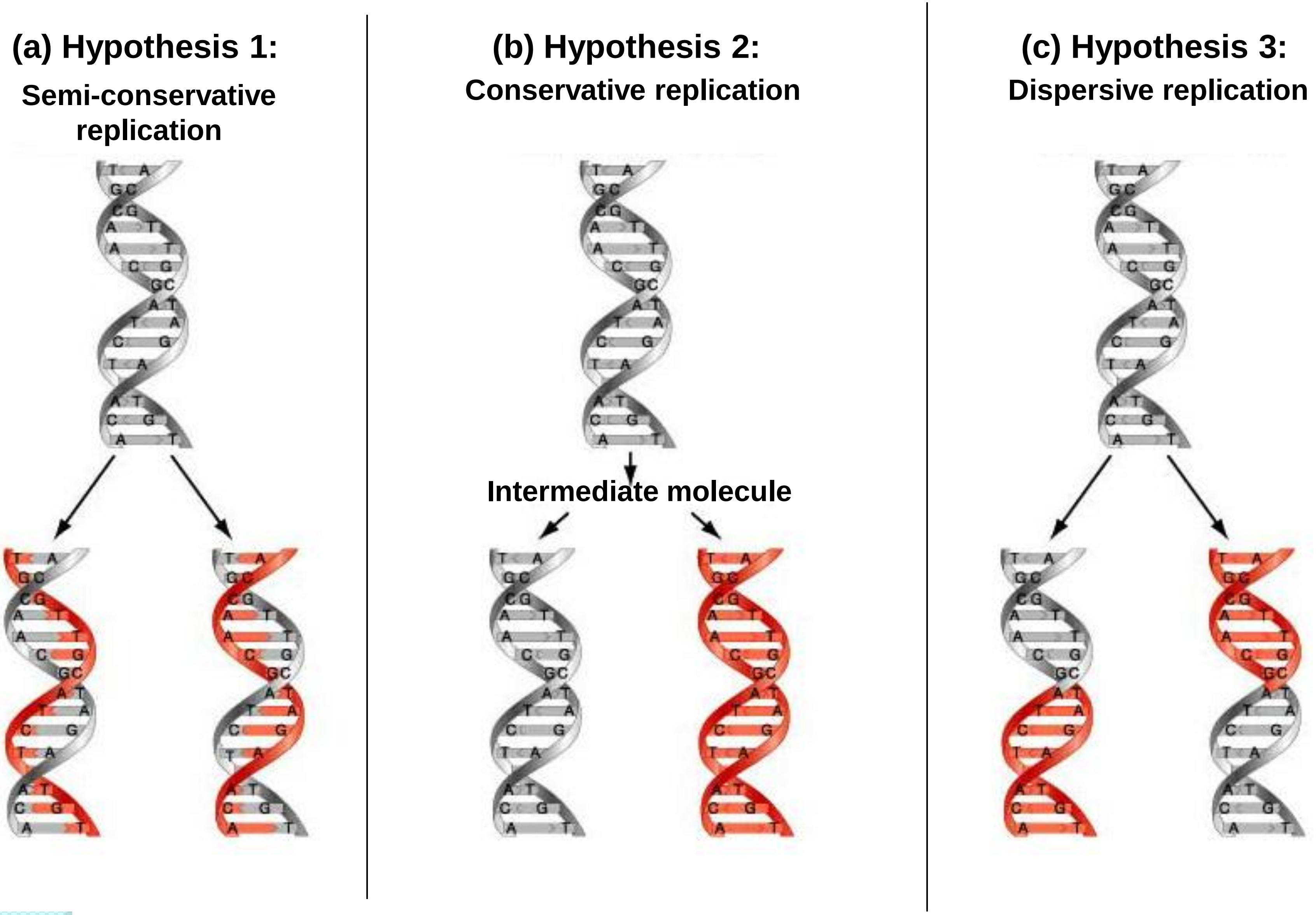
Models for DNA replication

1) Semiconservative model:
Daughter DNA molecules contain one parental strand and one newly-replicated strand

2) Conservative model:
Parent strands transfer information to an intermediate (?), then the intermediate gets copied. The parent helix is conserved, the daughter helix is completely new

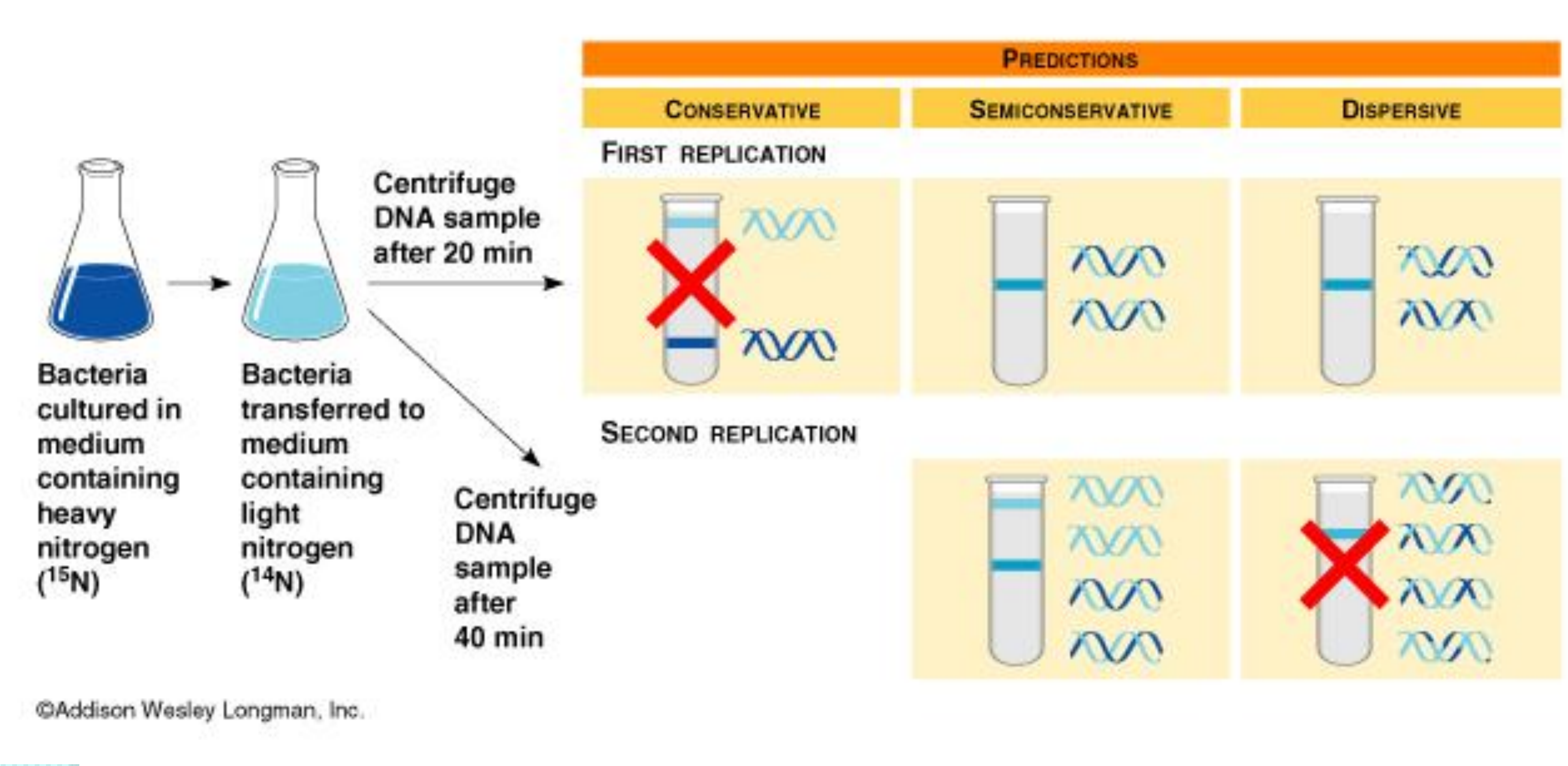
3) Dispersive model:
Parent helix is broken into fragments, dispersed, copied then assembled into two new helices. New and old DNA are completely dispersed

MODELS OF DNA REPLICATION

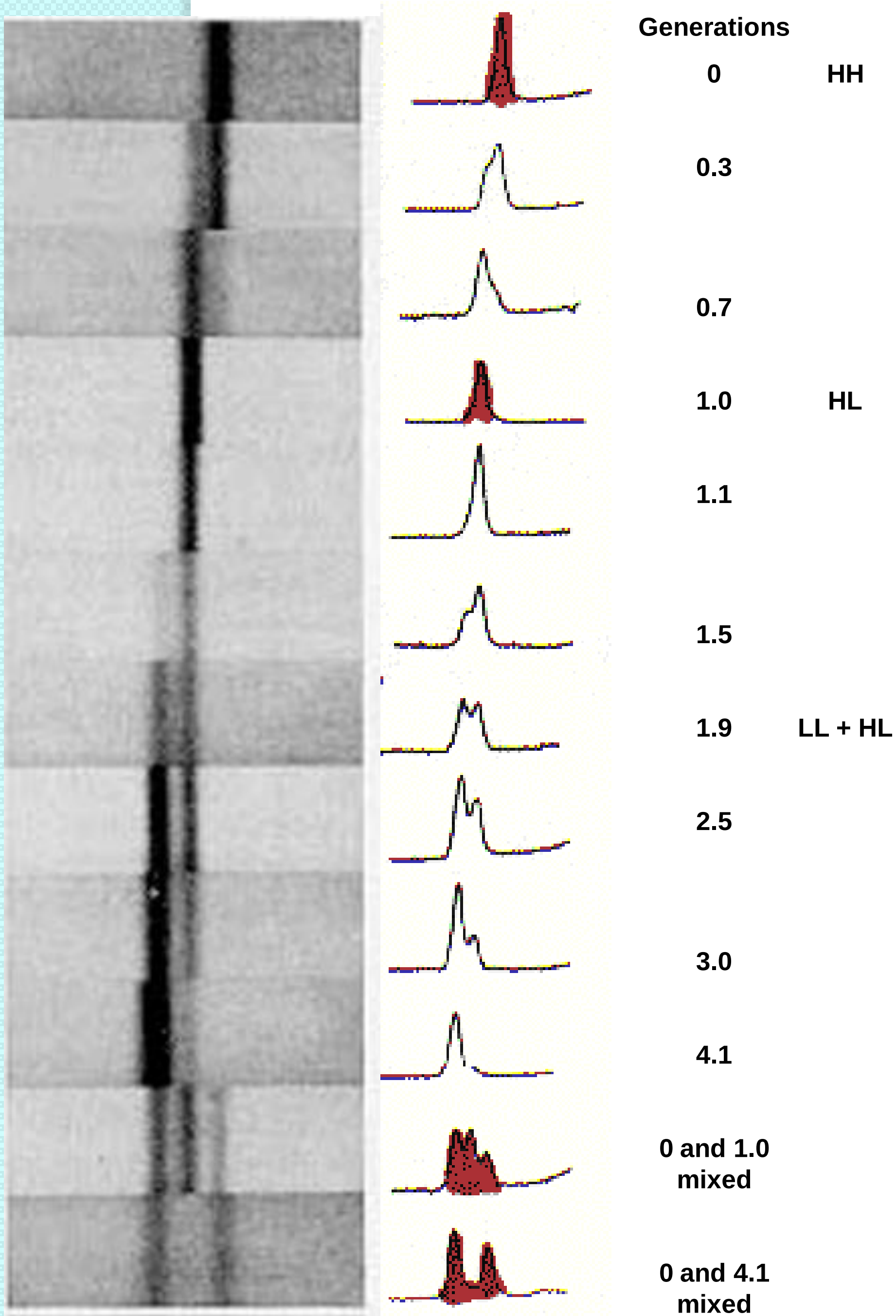


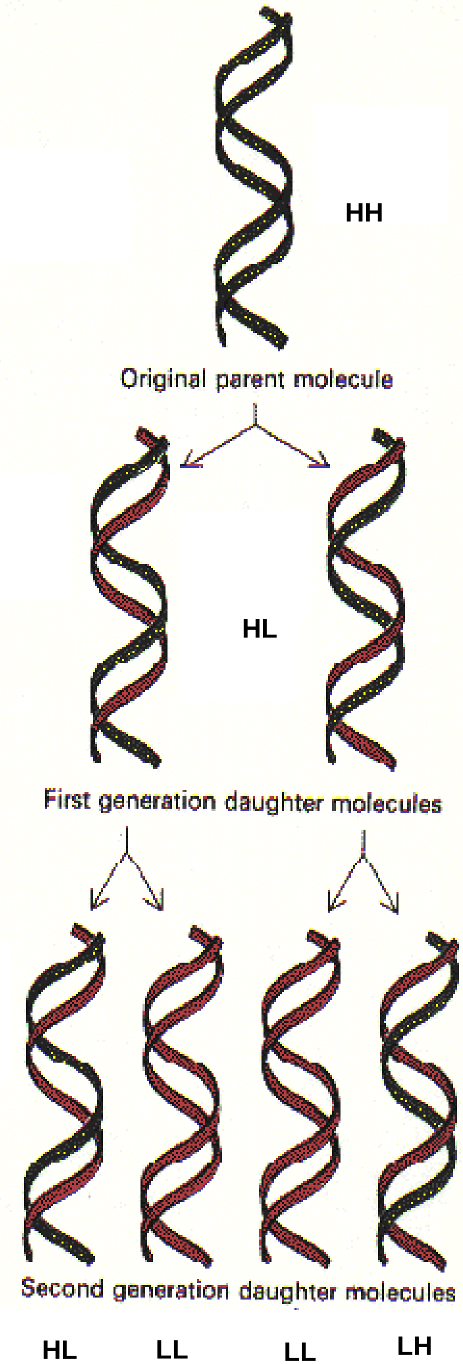
Meselson and Stahl

Semi-conservative replication of DNA



Isotopes of nitrogen (non-radioactive) were used in this experiment



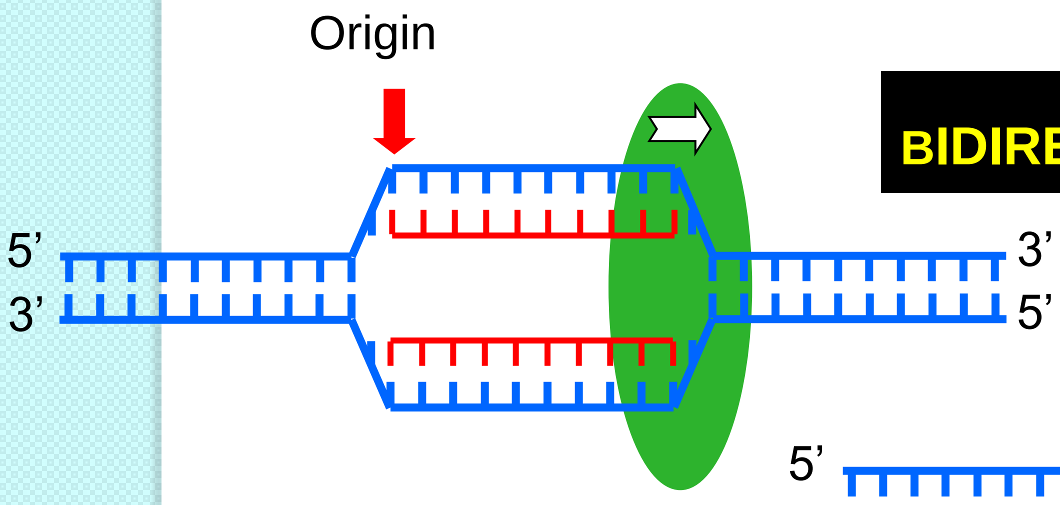


Equilibrium Density Gradient Centrifugation

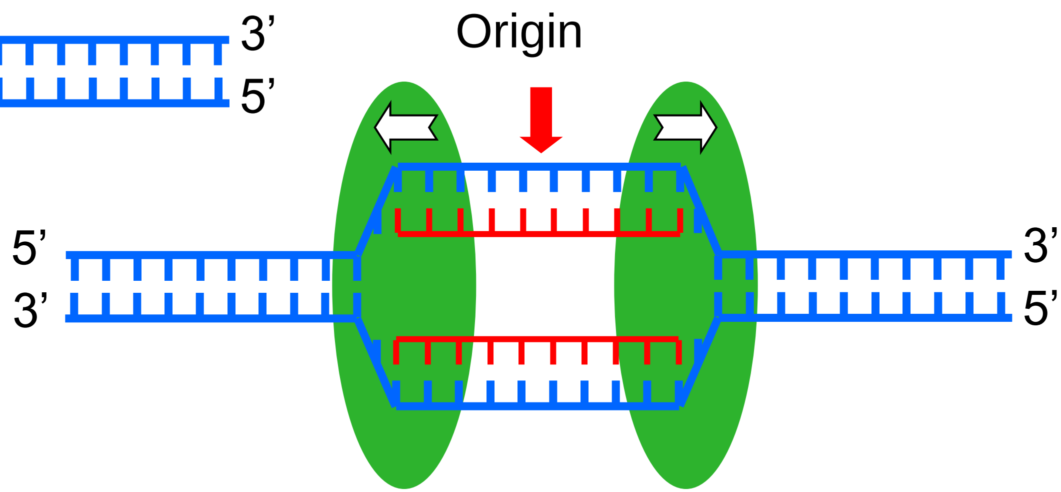
Detection of semiconservative replication in *E. coli* by density-gradient centrifugation. The position of a band of DNA depends on its content of ^{14}N amd ^{15}N. After 1.0 generation, all the DNA molecules are hybrids containing equal amounts of ^{14}N and ^{15}N

Replication can be Uni- or Bidirectional

UNIDIRECTIONAL REPLICATION

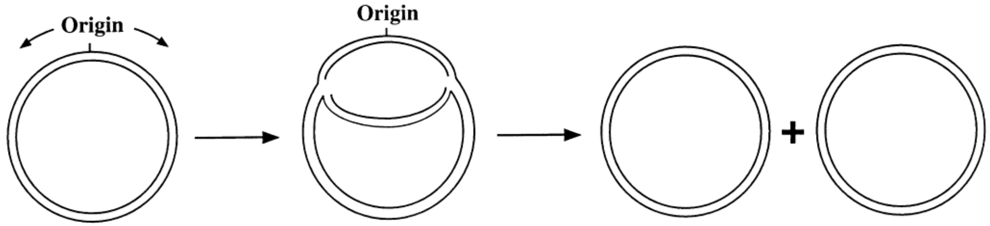


BIDIRECTIONAL REPLICATION

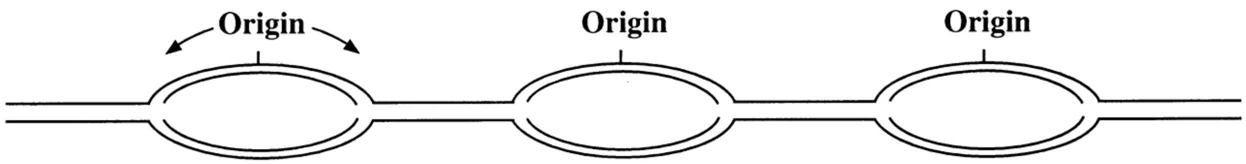


Replication of the Genetic Material

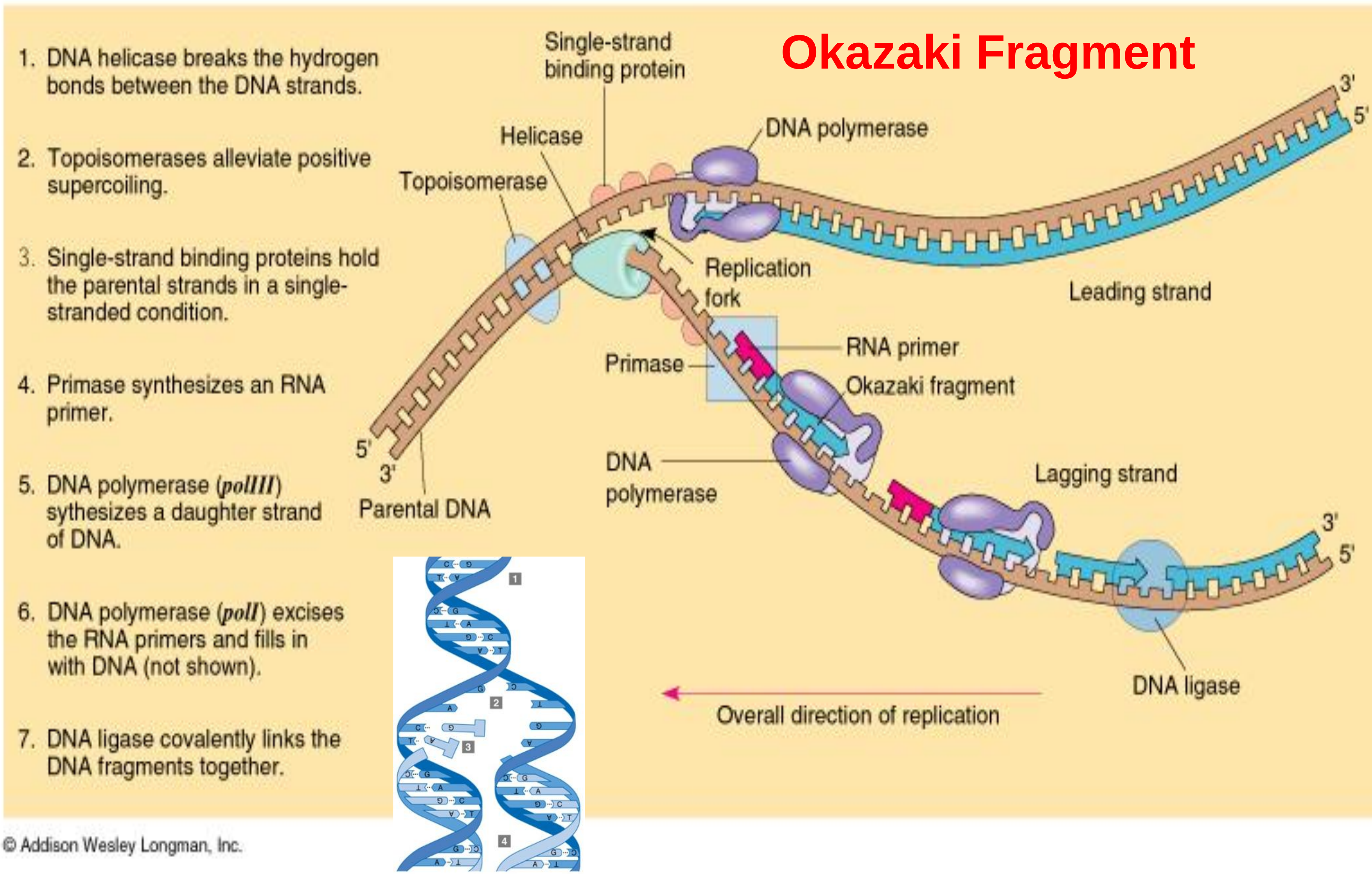
Small chromosomes use a single origin



Replication of large chromosomes requires multiple origins



The Mammalian DNA Replication Apparatus



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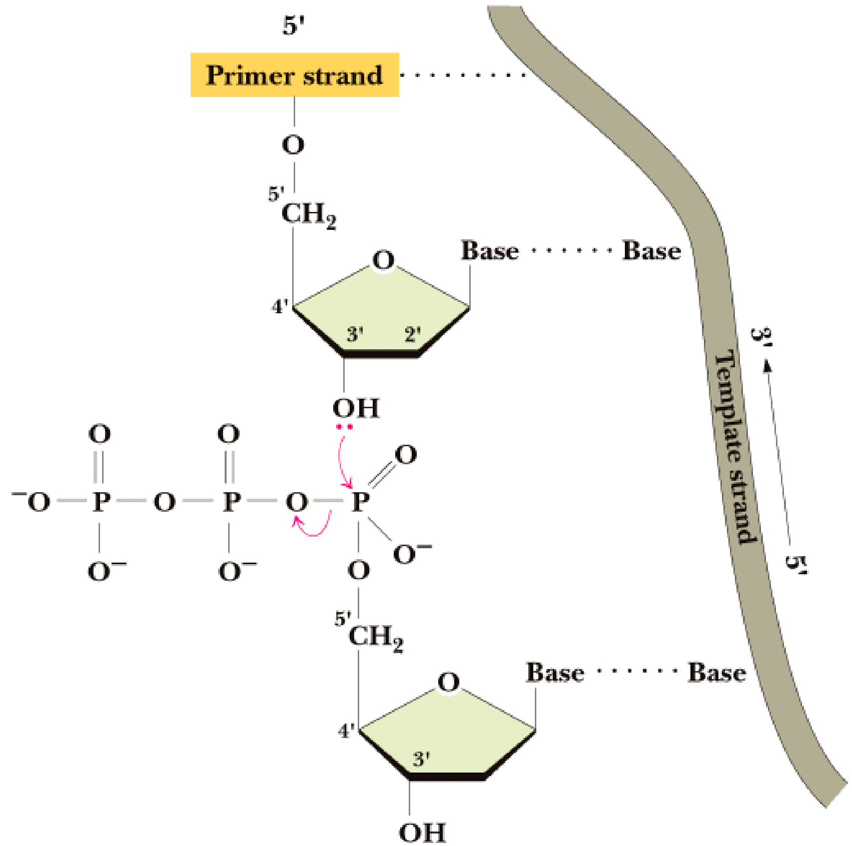
The 5’ to 3’ DNA polymerizing activity

Subsequent hydrolysis of PPi drives the reaction forward



Nucleotides are added at the 3'-end of the strand

Why the exonuclease activities?

- The 3'-5' exonuclease activity serves a proofreading function
- It removes incorrectly matched bases, so that the polymerase can try again.

The DNA Polymerase Family

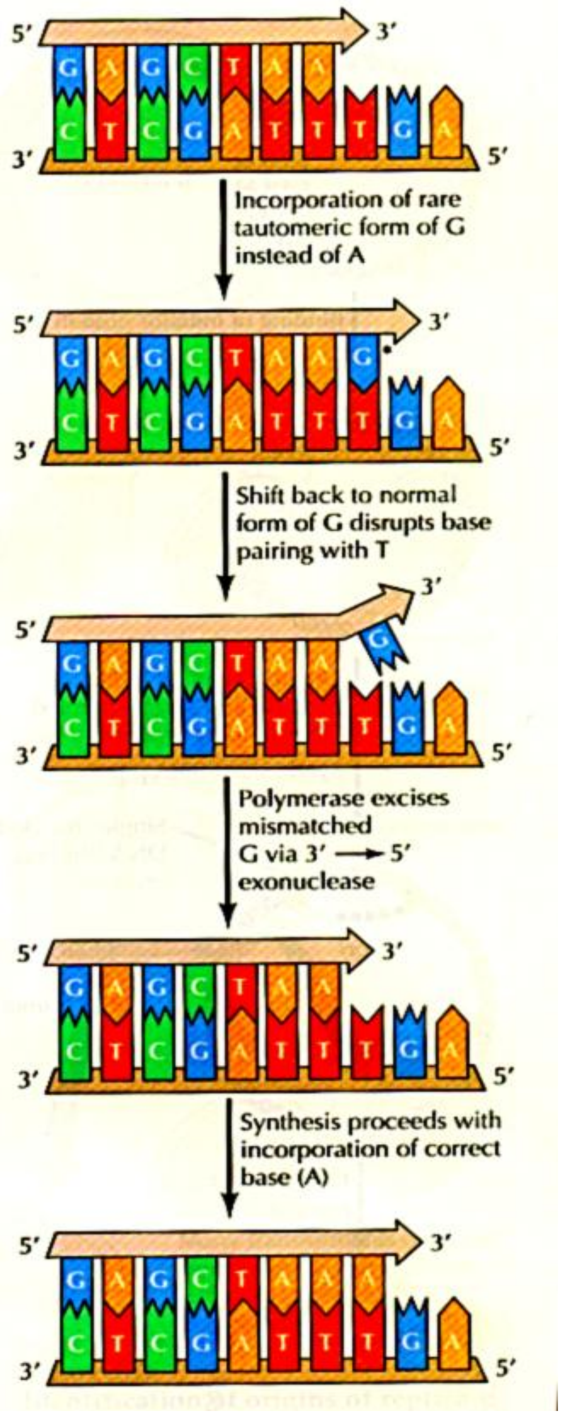
A total of 5 different DNAPs have been reported in E. coli

- DNAP I: functions in repair and replication
- DNAP II: functions in DNA repair (proven in 1999)
- DNAP III: principal DNA replication enzyme
- DNAP IV: functions in DNA repair (discovered in 1999)
- DNAP V: functions in DNA repair (discovered in 1999)

DNA Polymerase III

The "real" replicative polymerase in E. coli

- It's fast: up to 1,000 dNTPs added/sec/enzyme
- It's highly processive: >500,000 dNTPs added before dissociating
- It's accurate: makes 1 error in 10^7 dNTPs added, with proofreading, this gives a final error rate of 1 in 10^{10} overall.



Proof reading activity of the 3' to 5' exonuclease.

DNAPI stalls if the incorrect ntd is added - it can't add the next ntd in the chain

Proof reading activity is slow compared to polymerizing activity, but the stalling of DNAP I after insertion of an incorrect base allows the proofreading activity to catch up with the polymerizing activity and remove the incorrect base.