E -MODULE
ON

DNA STRUCTURE AND REPLICATION

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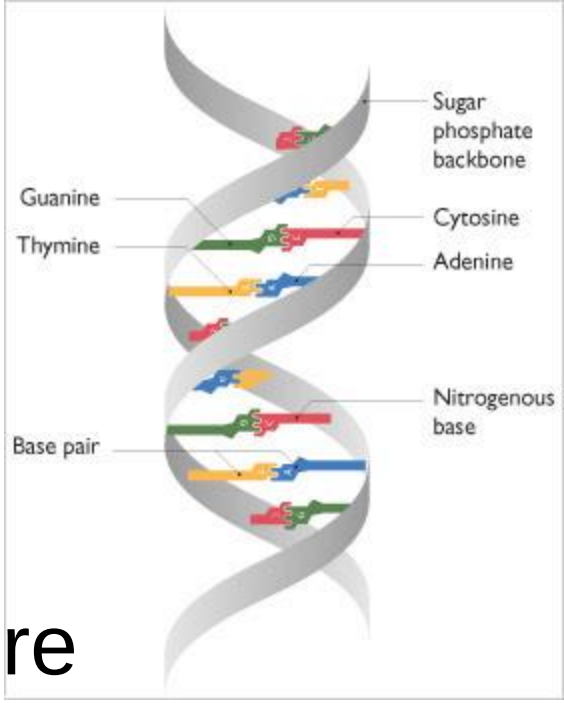
JALANDHAR

2005



We know:

- DNA is the hereditary material
- DNA has a double helix structure
  - Made of four bases; A,T,C,G
  - Sugar-Phosphate backbone
- DNA replication is semi-conservative
- How to do PCR

How did we gain all this knowledge?

Timeline of Experiments

1871 – Miescher – identifies Nucleic Acid

1928 – Griffith – “transformation” of bacteria

1944 – Avery, MacLeod and McCarty – DNase expt

1950 – Chargaff – G&C, A&T

1952 – Hershey and Chase – Blender expt

1953 – Franklin – picture of DNA

1953 – Watson and Crick – Double Helix structure and base pairing

Friedrich Miescher

1871

- Isolates a type of acid
- From white blood cells of bandages
- Coins the term Nucleic Acid – because it was found in the Nucleus of the cell

- Most people thought proteins were hereditary material, so no one cared.

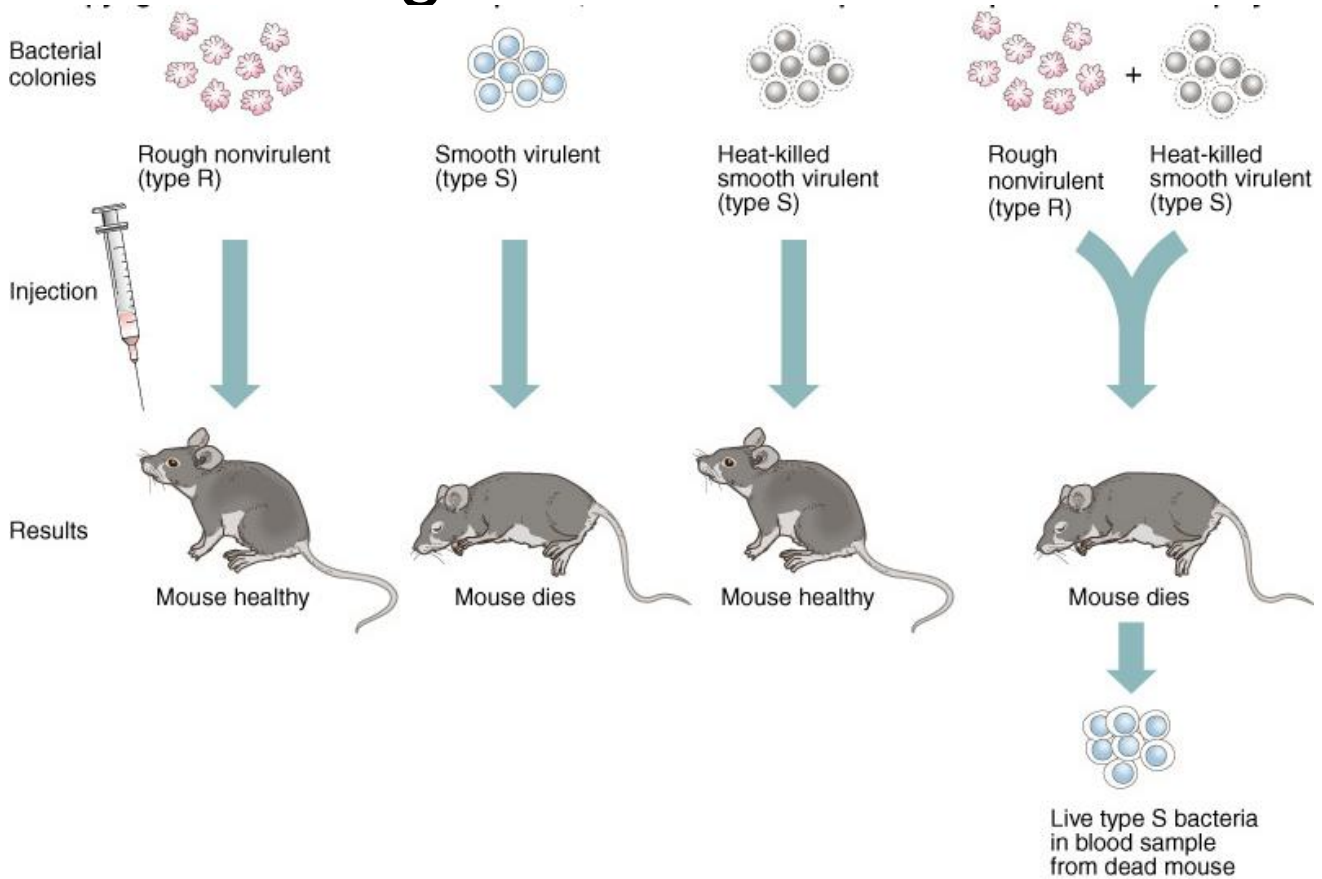
Proteins?

- Scientists had noted that chromosomes moved and divided as cells divided
- Chromosomes had something to do with heredity
- Chromosomes are made of both Nucleic Acids and Proteins
- Choice of either 4 bases or 20 amino acids
- Code of all life? – 20 amino acids

Frederick Griffith

1928

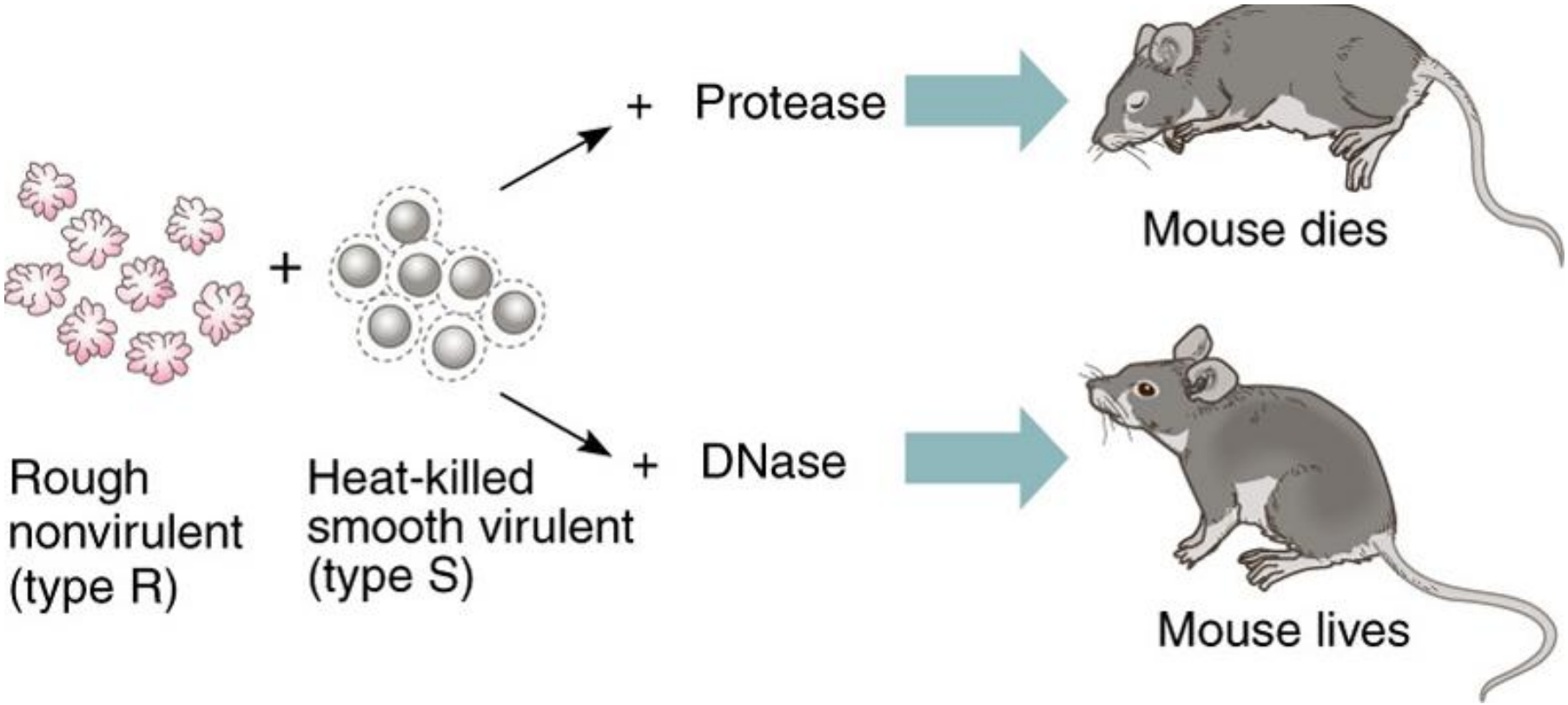
- Transforming factor in bacteria



Avery, MacLeod, McCarty

1944

- DNase vs. Protease

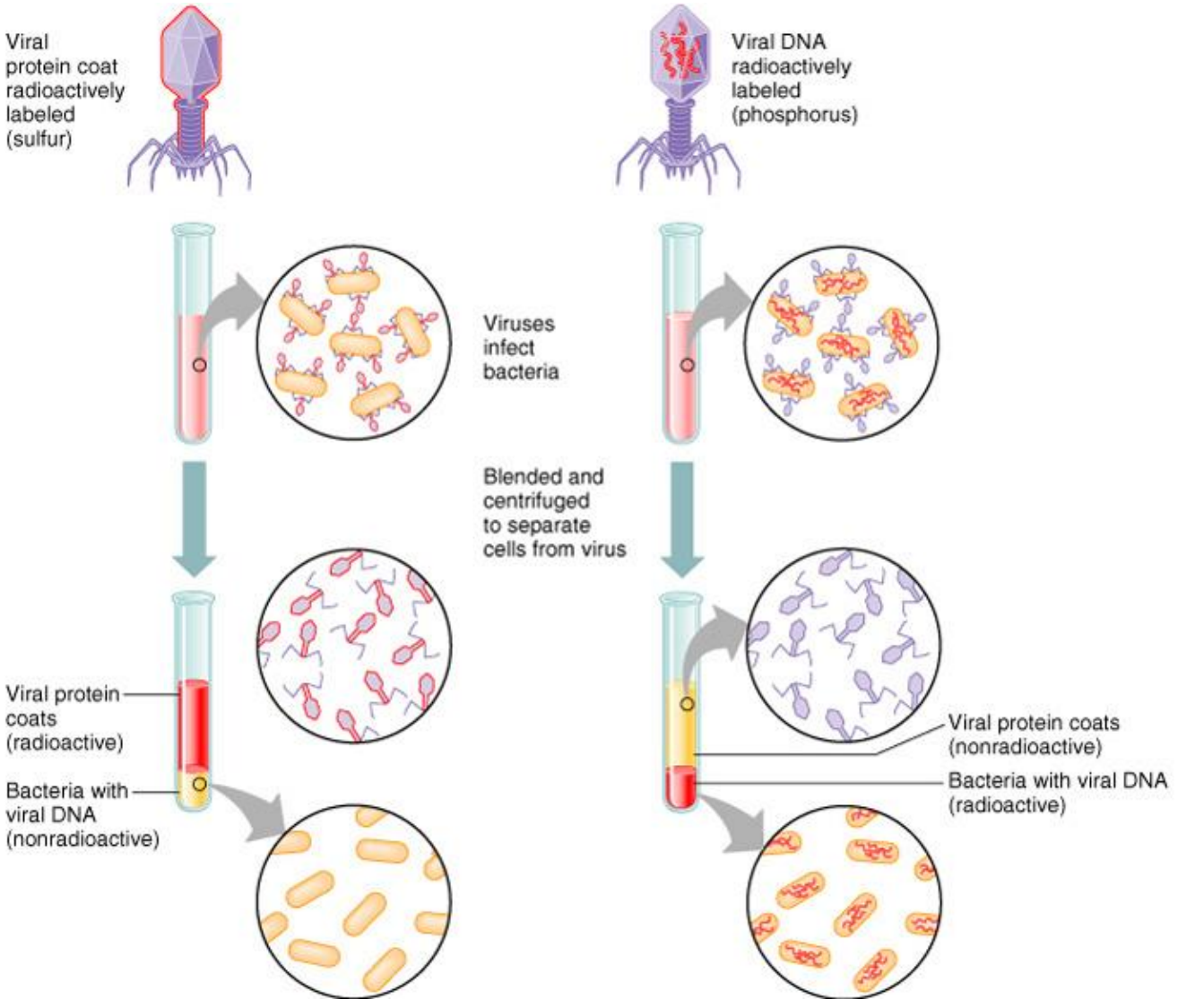


Hershey and Chase

1952

- Blender

Experiment



By 1950's

- DNA was the hereditary molecule
- Protein was not
- DNA had transforming ability
- Scientists began examining DNA's structure

Erwin Chargaff

1950

- Found that strands were always same distance apart
- Also, that the amount of A always the same as amount of T
- Amount of G always same as amount of C

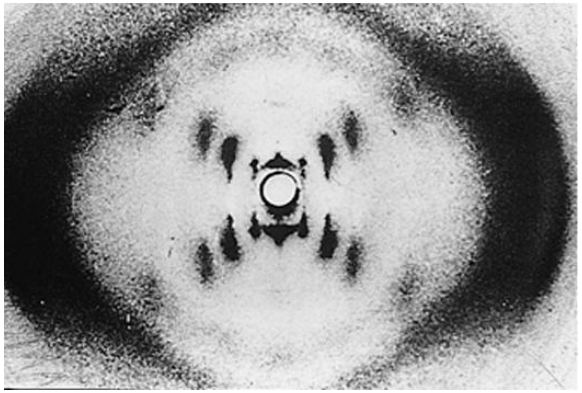
Therefore: Purines must always bind Pyrimidines

A binds to T; G binds to C

Rosalind Franklin

1953

- Crystallized DNA and X-ray diffraction



- From picture it was clear that DNA was in a helix
- With symmetrically organized bases in center

Watson and Crick

April 25th, 1953

- Seen Franklin's picture:
  - Assumed Sugar-Phosphate backbone
- Knew Chargaff's rule:
  - A&T, G&C must bind each other
- Determined turning radius of beta-Helix
- Realized it must be two complementary strands because of base pairing
- Determined it was a double Helix

Watson and Crick

James Watson
American

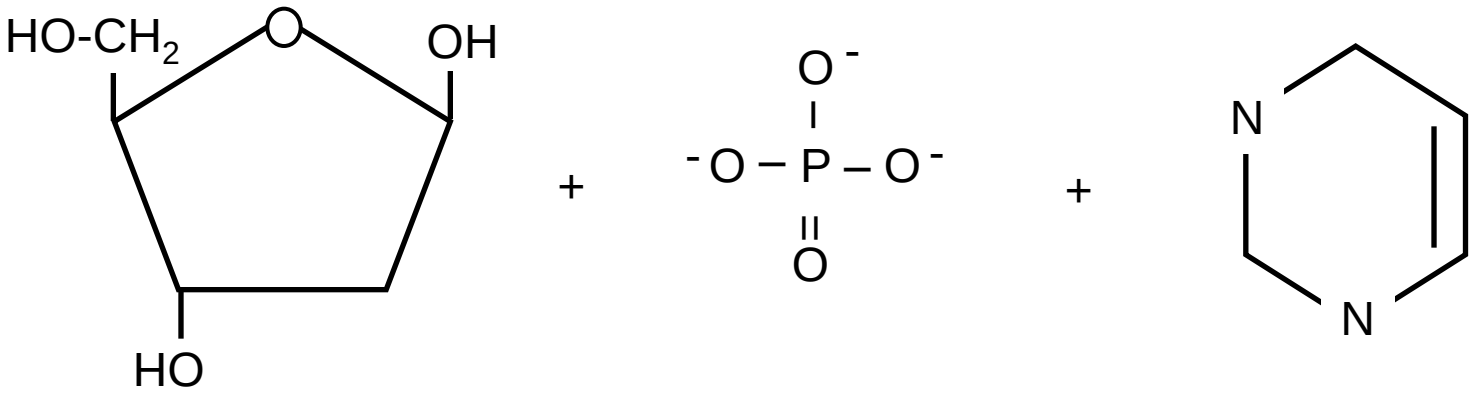
Francis Crick
English

Summary of DNA

- String of Nucleotides
- deoxyribose Sugar-Phosphate backbone
- 4 Bases:
  - A, G are Pyrimidines
  - T, C are Purines
  - A = T
  - G ≡ C
- Two complementary strands (double helix)

What is DNA?

- Deoxyribonucleic Acid:
  - String of nucleotides
    - Nucleotides made up of three parts:

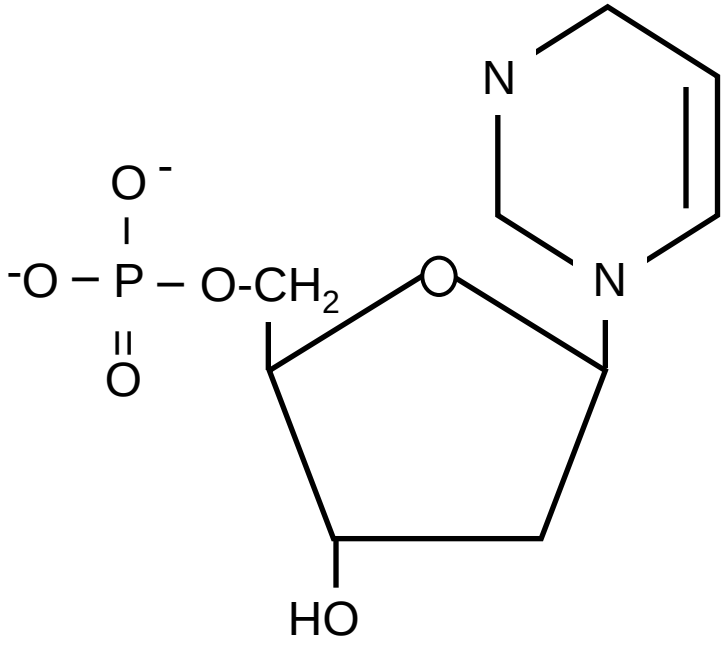


deoxyribose (a sugar)

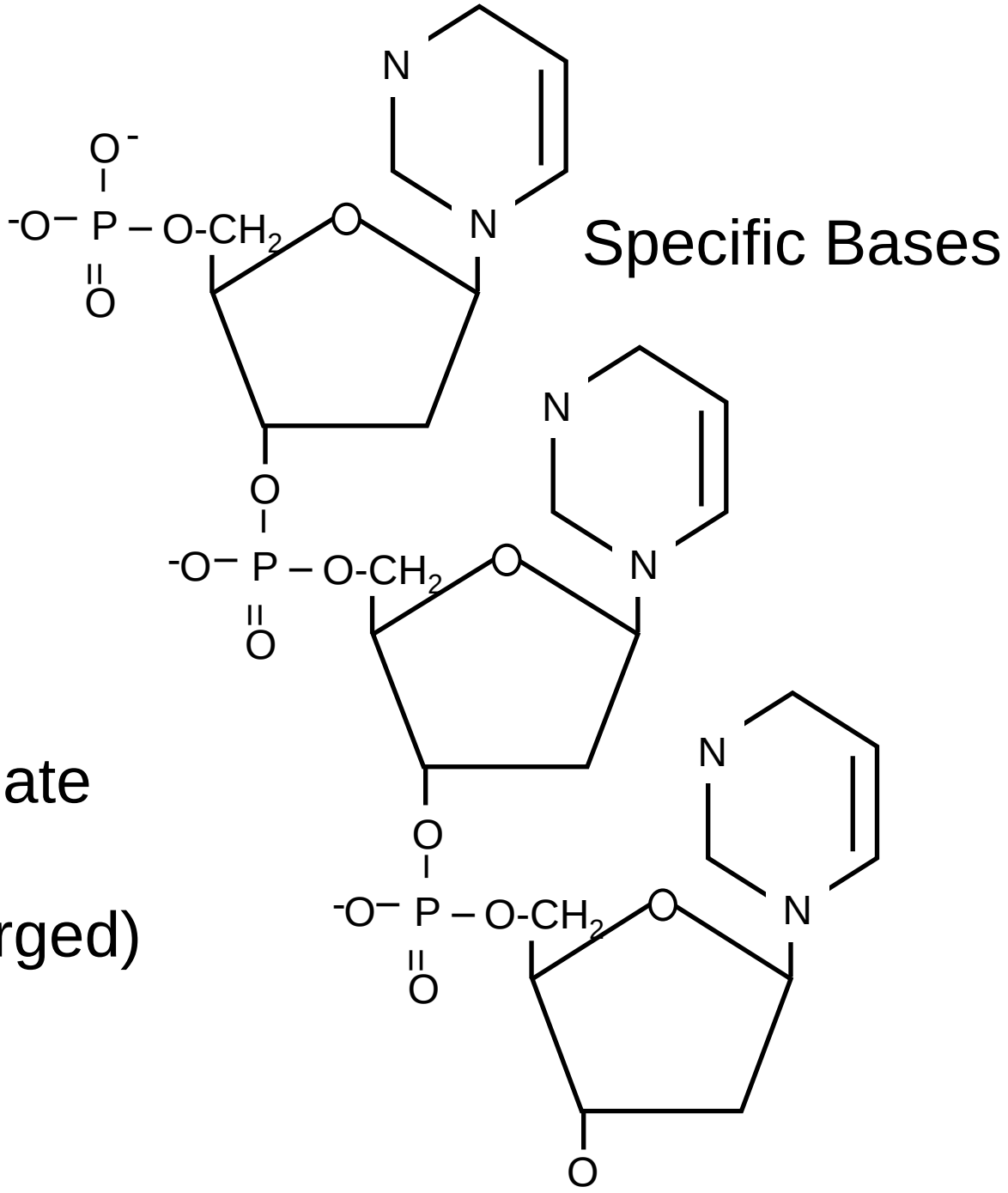
phosphate

cyclic amine (base)

Nucleotide



DNA



Sugar-Phosphate Backbone (negatively charged)

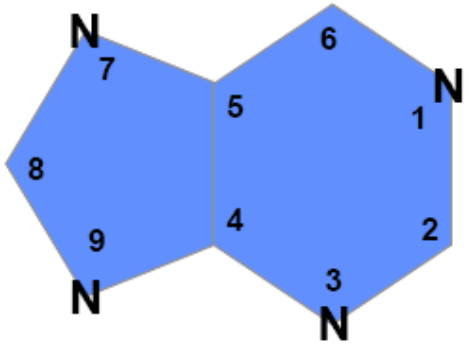
The Five Bases

- A = Adenine
- T = Thymine
- G = Guanine
- C = Cytosine

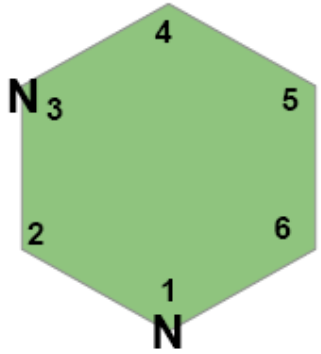
- RNA only:
  - U = Uracil (replaces T)

DNA review

Nitrogenous bases



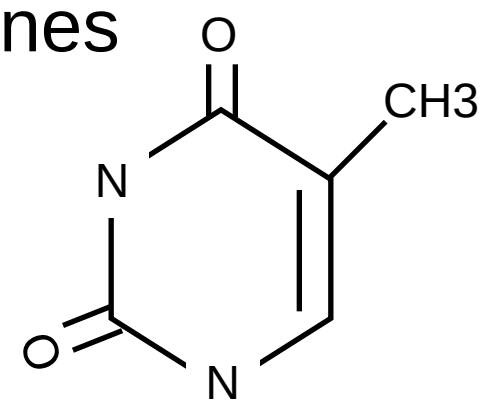
purine



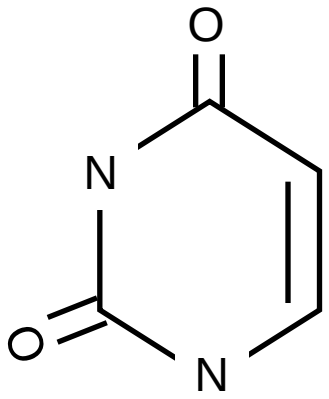
pyrimidine

Structures of Bases

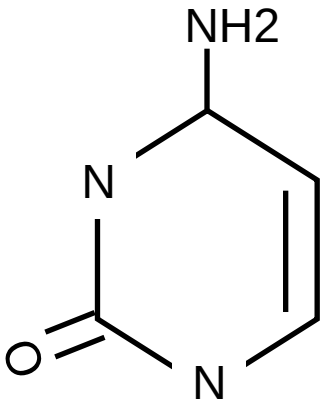
Pyrimidines



T

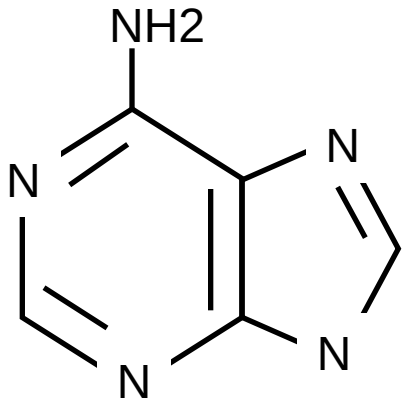


U

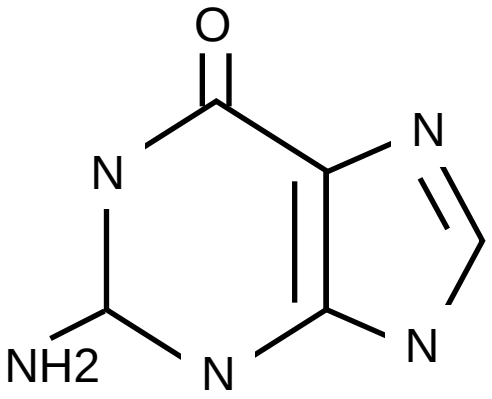


C

Purines



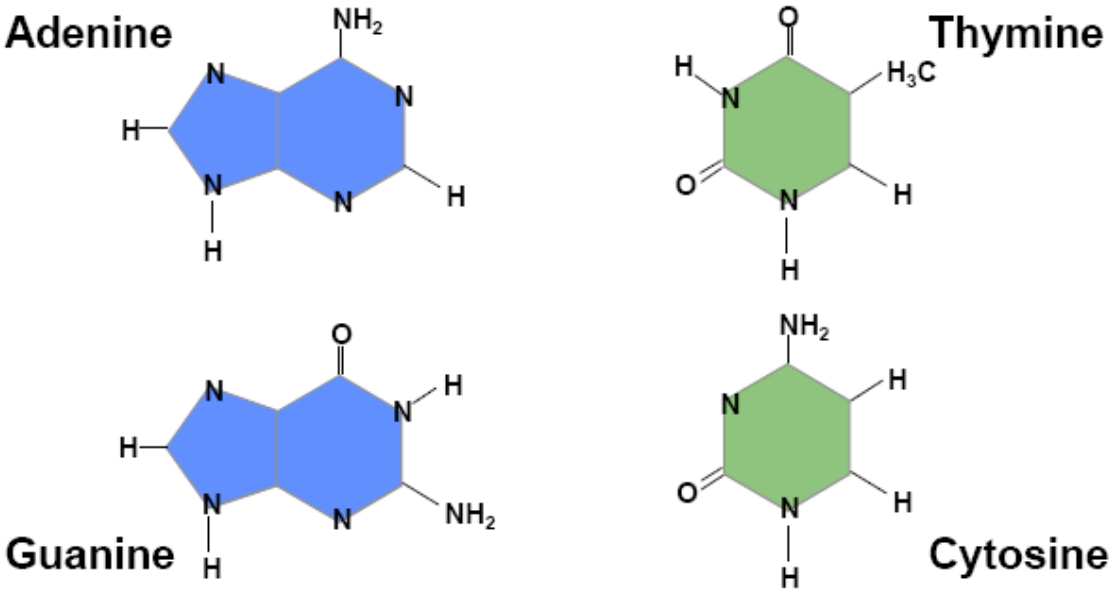
A



G

DNA review

Four bases in DNA

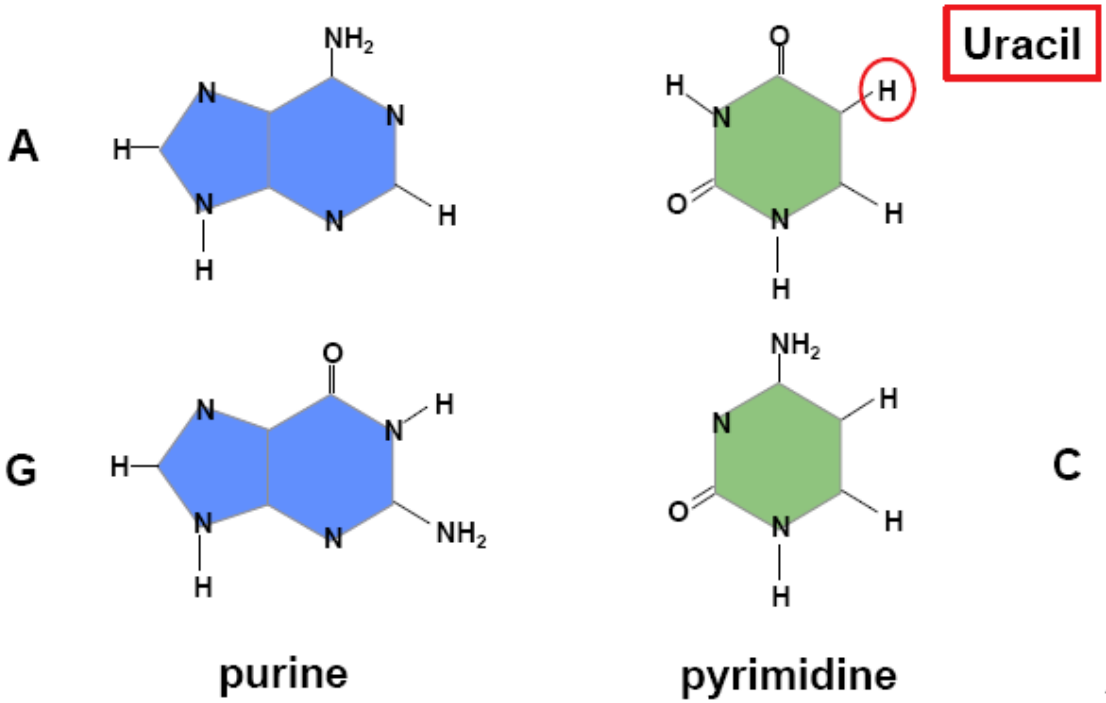


purine

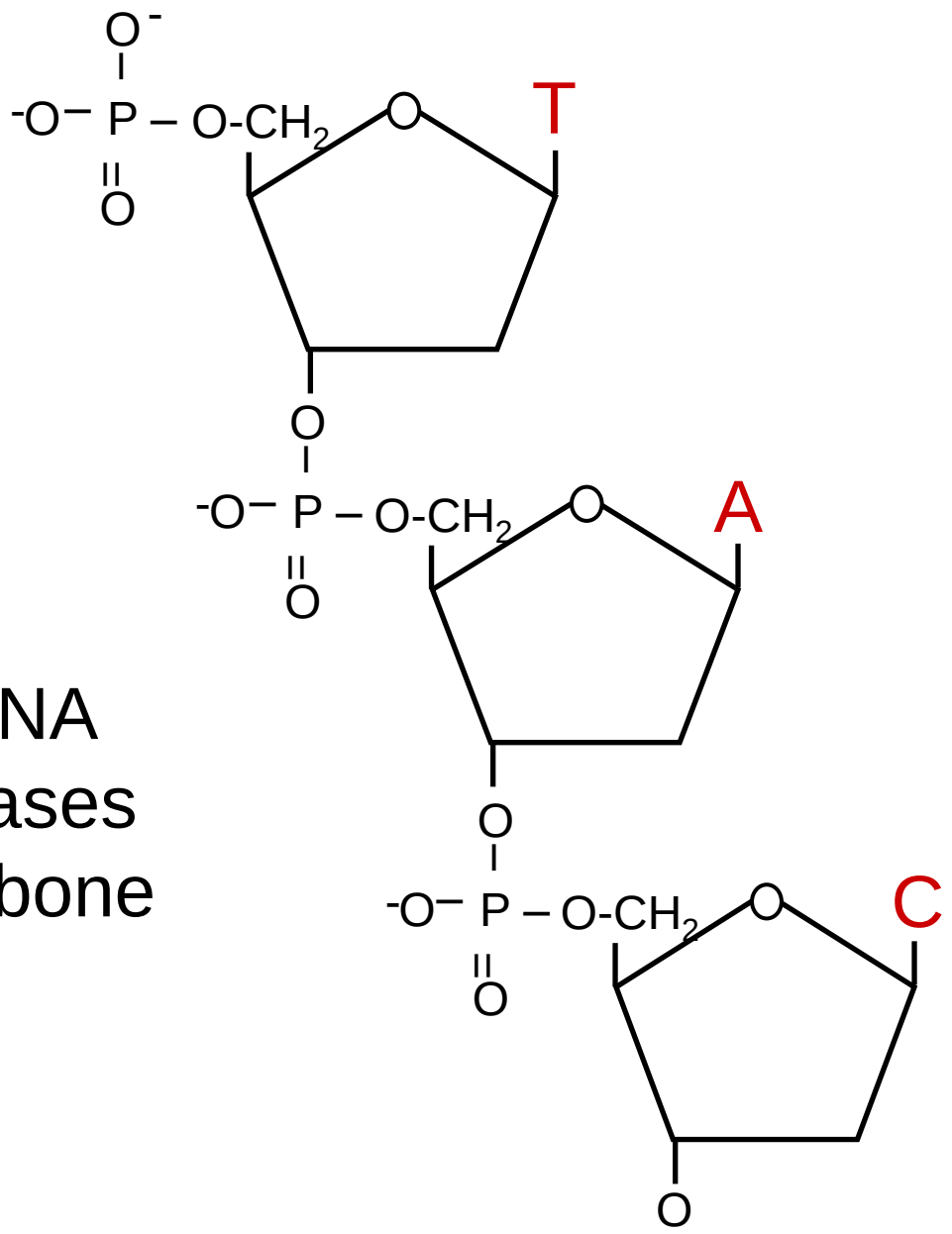
pyrimidine

DNA review

Thymine is replaced by Uracil in RNA



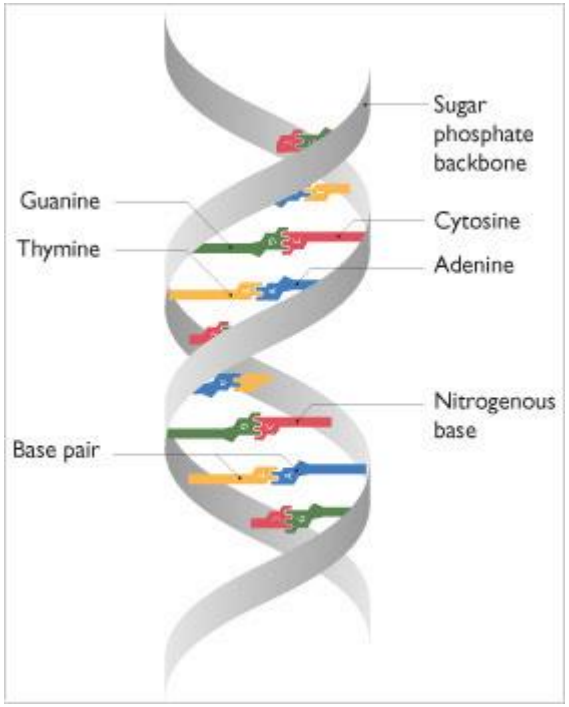
DNA



Sequence of DNA is order of the bases attached to backbone

Double Helix

- Sugar-Phosphate backbone is on outside
- Bases are inside - Hydrogen-bonding to opposing base on opposite strand

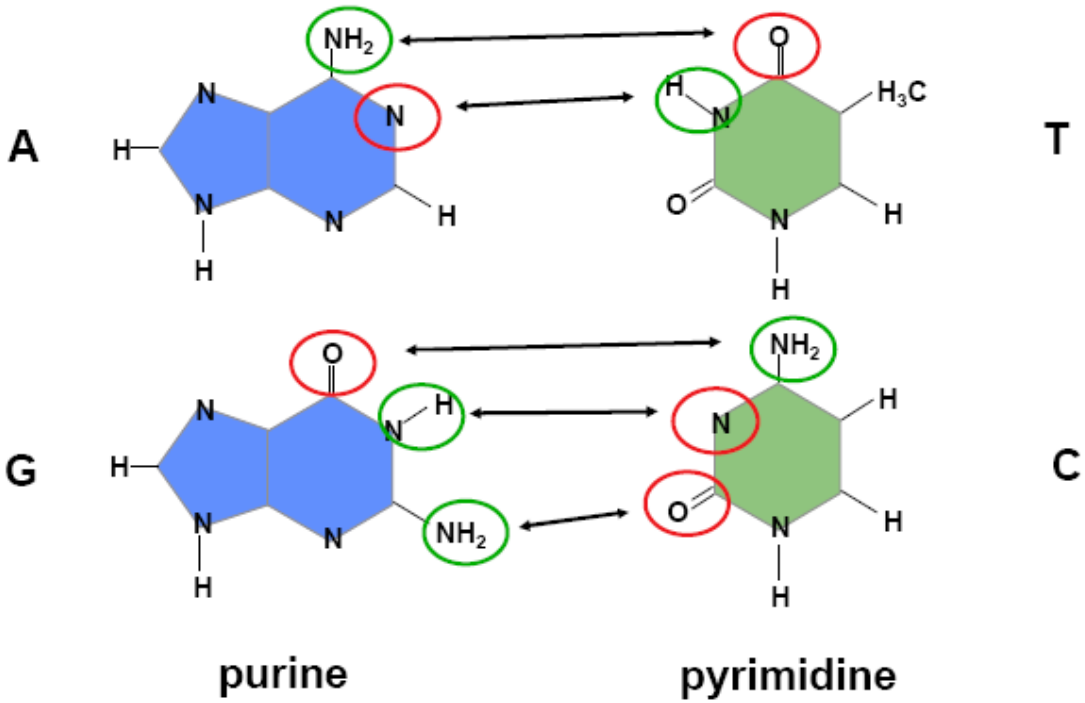


- Forming Base Pairs

DNA review

A-T base pairs have two hydrogen bonds

G-C base pairs have three hydrogen bonds



Base Pairing

1. A Purine must always be base paired to a Pyrimidine

2. A = T – with two Hydrogen Bonds

3. G = C – with three Hydrogen Bonds

Therefore:

Strands must be complementary

Complementary Strands

Double Helix has two strands:

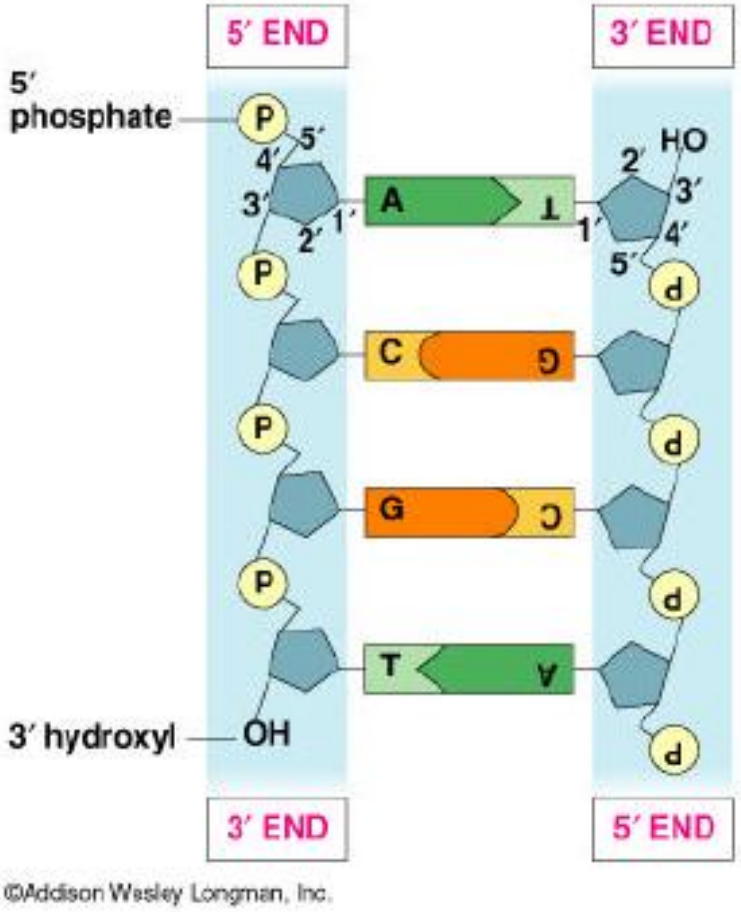
- Complementary – means when you read the message on one strand, you automatically know the message on other strand
- Not identical, because in reverse
- “Antiparallel” strands
- Exact same message on both strands

Antiparallel Strands

- DNA strands match up in opposite directions
- DNA always “read”

  5’ to 3’ direction

- In the end, both strands have the exact same message



DNA Replication

Makes logical sense how DNA replication happens:

1. DNA has two strands with identical information
2. Must open up
3. Exposing unpaired bases
4. Bases are matched perfectly (compliment)
5. Forming two double helixes from one

Three Theories:

1. Semi-conservative
   - The one Watson and Crick just suggested
   - Strands split
   - New strand forms from reading other
2. Conservative
   - Makes copy of double strand, from double strand (without splitting)
3. Dispersive
   - Double helix breaking and reforming at random

Meselson and Stahl

1957

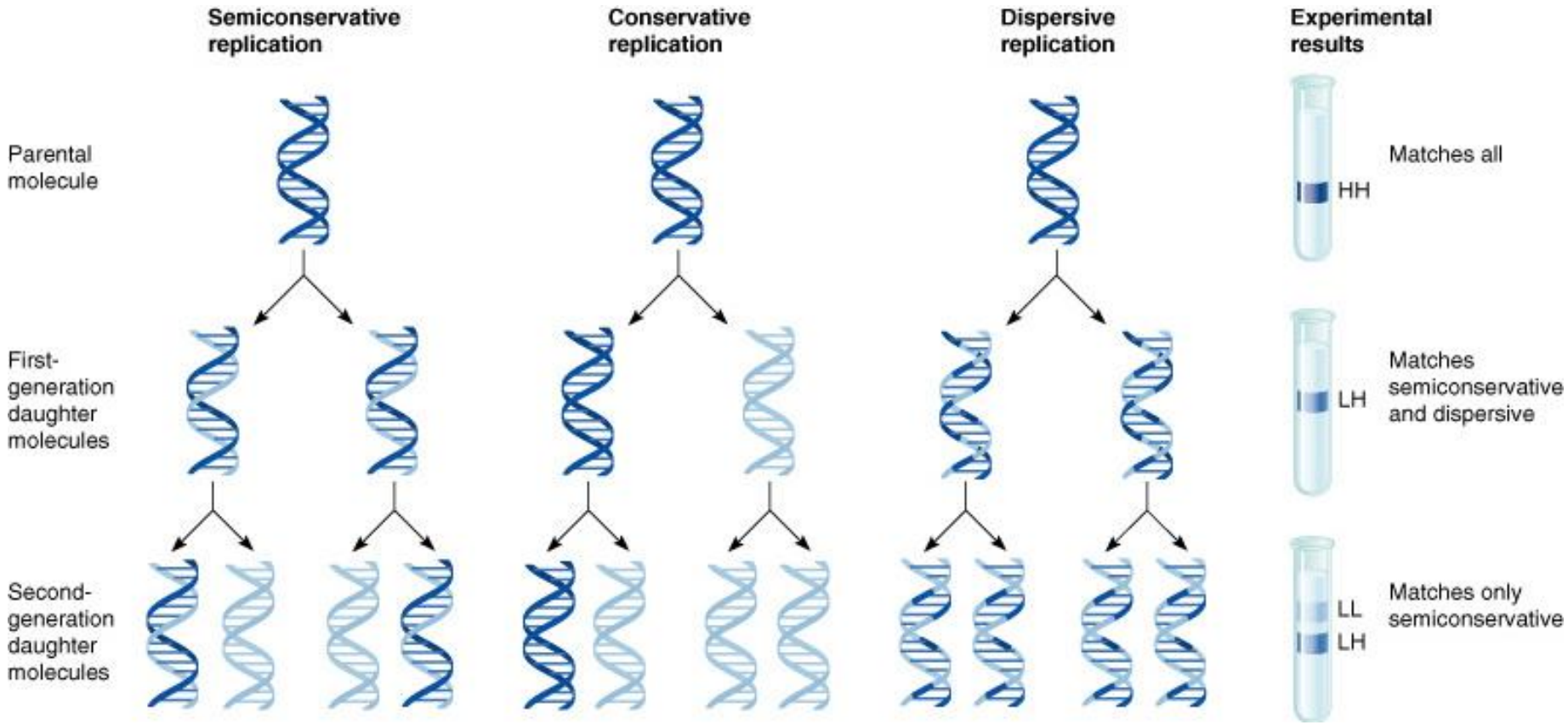
- Density Shift Experiments
- Labeled DNA with “heavy” Nitrogen
- Follow label:

Does it split equally = Semi-conservative

Does it stay together = Conservative

Does it split randomly = Dispersive

Meselson and Stahl

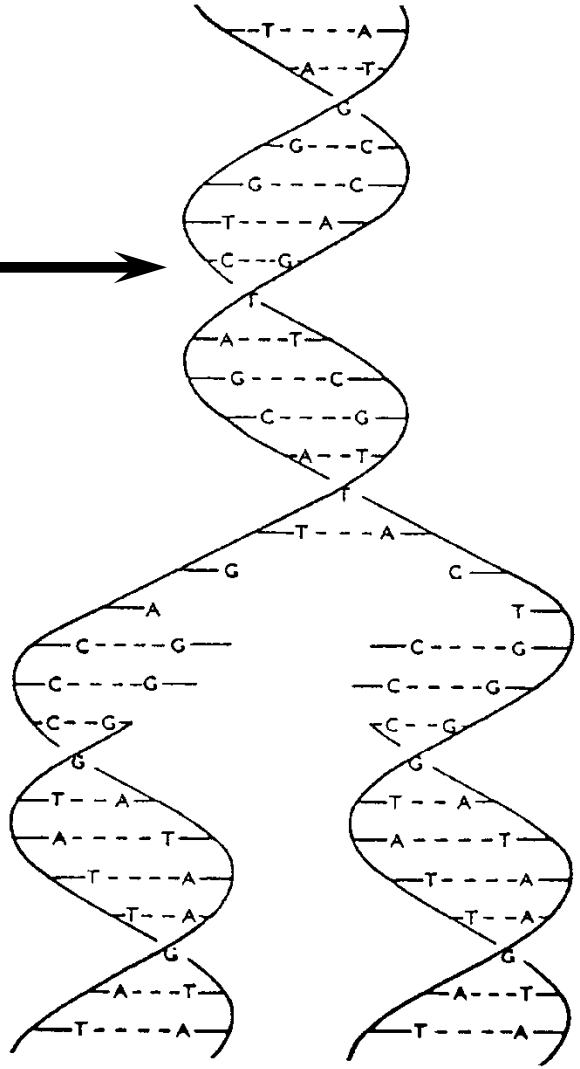


Semi-conservative

Parental DNA strands →

Each of the parental strands serves as a template for a daughter strand

Daughter DNA strands →



DNA Replication

- DNA from cell to cell, has to be replicated with fidelity
- Mistakes – mutations, cancer, etc.
- Only one mistake in a billion nucleotides is made by the replication machinery
- DNA replication is a result of the coordination of > 10 protein complexes and enzymes

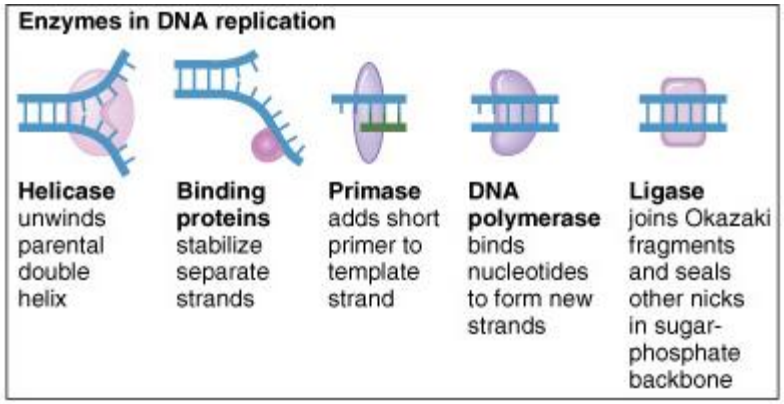
Enzymes

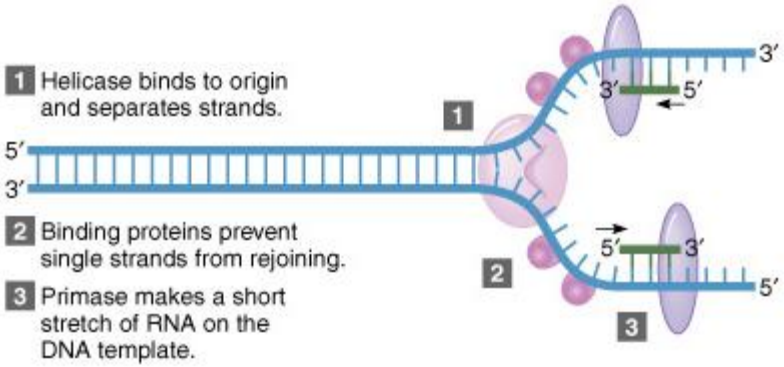
- Helicases – unwind the DNA
- Primase – attracts complimentary bases to form a “primer” sequence
- DNA Polymerase – add bases to the primer strand by reading the code
  - Therefore, extends the new strand
  - According to the original strand’s sequence
- Ligase – seals the sugar-phosphate backbone back together

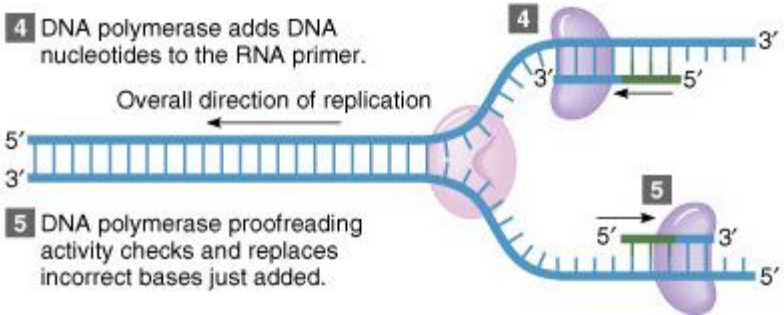
Synthesis

- Two strands
- Opposite directions
- One strand can be “read” directly because it is 5’ to 3’
  - Leading Strand
- Other strand is 3’ to 5’
  - Lagging Strand
  - Discontinuous synthesis of this strand

DNA Replication





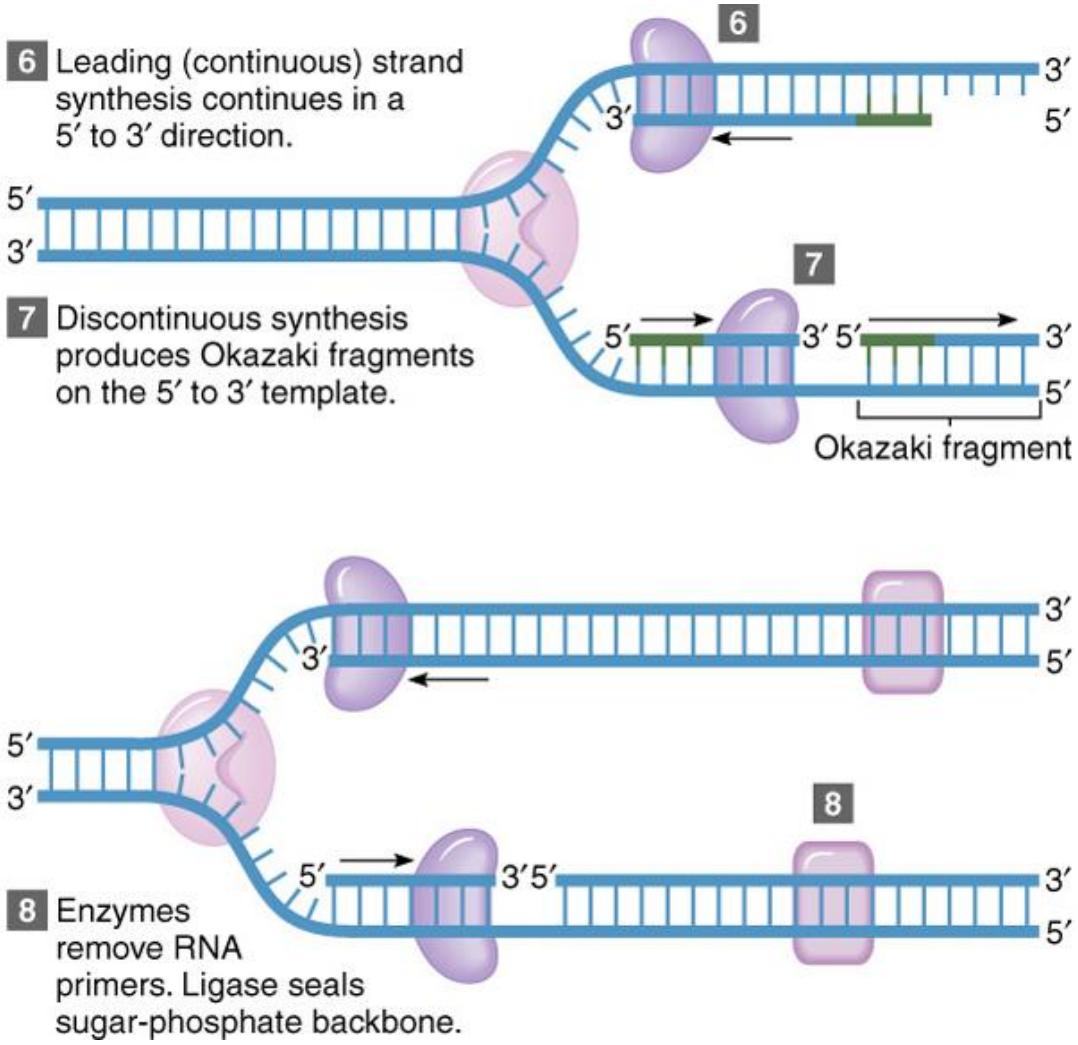


Notes:

1. Enzymes and their functions

2. Direction of replication
- Always 5’ to 3’

DNA Replication



Notes con't:

3. Difference between leading and lagging strands

Error Correction

DNA Polymerase – can also “proofread” the newly formed strand:

1. Excise bases that are “mismatched”

2. Replace with the correct base

3. Then move forward to next base

Summary of DNA Replication

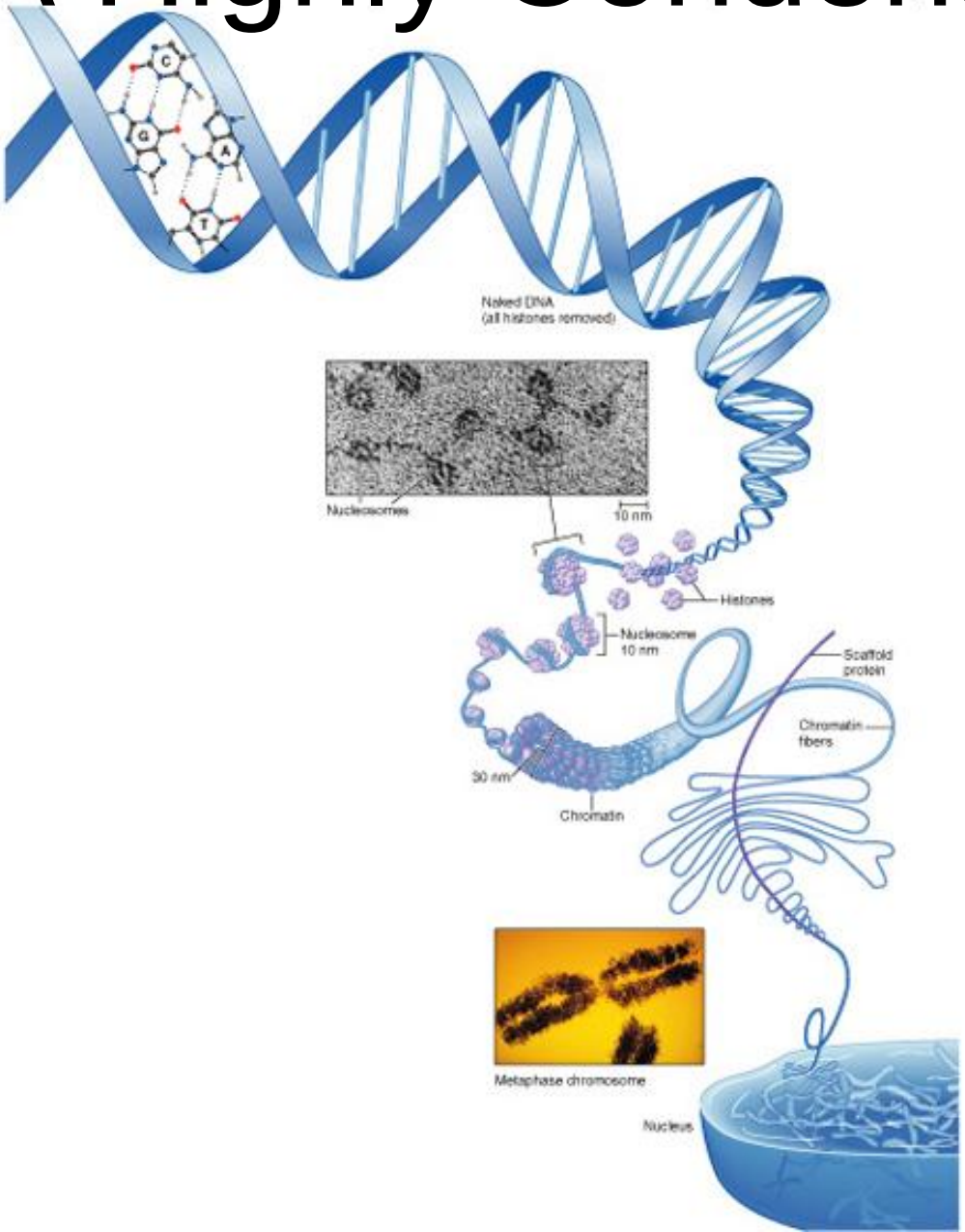
- DNA is a double helix
- Bases in middle make up sequence
- Semi-conservative replication
  1. Strands separate
  2. Each one is copied
  3. One “lagging” and one “leading”
  4. Forming two double helixes from one
  5. Proteins do all the copying
  6. Incredibly accurate

DNA Highly Condensed

- DNA is a very long molecule
- To fit into the nucleus it must be condensed
- Double helix coils around Histones
- Forms “beads on a string”
- Beads = Nucleosomes
- Nucleosomes coil into Chromatin
- Chromatin is condensed into Chromosomes

→ Chromosomes are BOTH – DNA and Protein!

DNA Highly Condensed



PCR

1977

- PCR – Polymerase Chain Reaction
- Kary Mullis
- Idea just came to him while he was thinking about DNA Replication (on LSD)
- Won Noble Prize
- Brought three women to the ceremony:

His wife, his mistress and the hooker from the night before

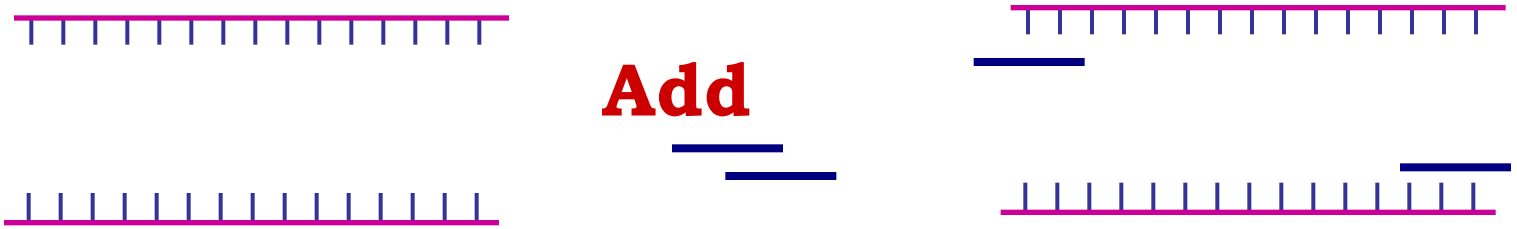
PCR

1. Separate the Double Helix
2. Bind primers (2) to sequence you want to replicate
3. DNA Polymerase copies between two primers
4. Rinse and Repeat
5. Copies DNA between two primers exponentially

PCR

1. Separate the Double Helix

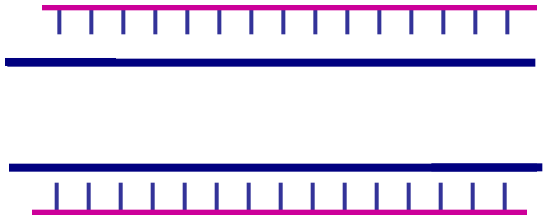
2. Bind primers (2) to sequence you want to replicate



PCR

3. DNA Polymerase copies between two primers

Add DNA Pol.



4. Repeat Many Times

PCR Needs

- You must know the sequence you are trying to amplify
- Primers:
  - One for each side, exact sequence correct
- Excess of four dNTPs:
  - 4 nucleotides
- Heat insensitive DNA Polymerase:
  - So that reagents can be heated and cooled repeatedly

PCR Uses

- Forensics
  - ID a body
  - ID a criminal
  - Free the innocent
- Genetic Tests
  - Testing for specific polymorphism/mutation
- Paternity Tests
- Research