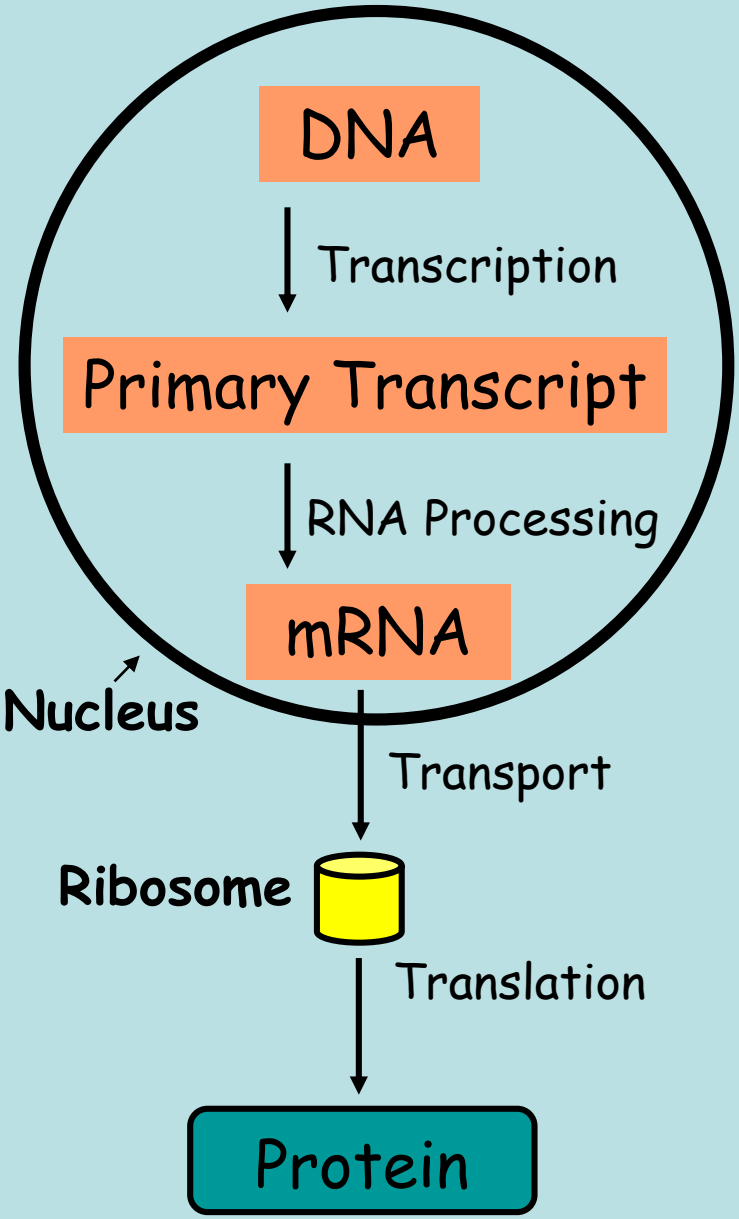
BCHEM 365
Lecture 1
Sept. 05, 2018

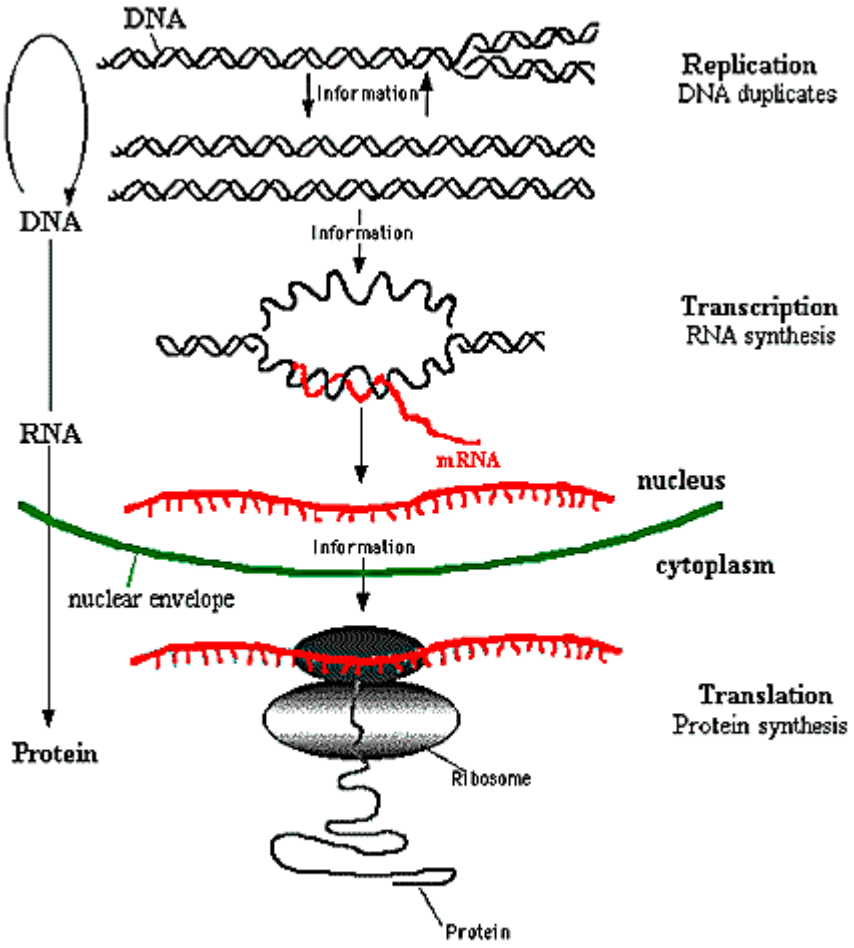
What is Biophysics?

- Biophysics (also biological physics)
- An interdisciplinary science
- Employs and develops theories and methods of physical sciences for study of biological systems, especially **macromolecules**
- From molecular level to whole organisms and ecosystems
- Molecular biophysics addresses questions in biochemistry and molecular biology
- Study of biophysics leads to understanding of the interactions between nucleic acids, proteins, regulation of protein biosynthesis, and membrane structure and function



The fundamental dogma of molecular genetics

The Central Dogma of Molecular Biology



The Central Dogma of Molecular Biology

Macromolecules

- Are polymers present in a cell. MW ranges from 10^4 to 10^{12} Daltons
- Made up of proteins, glycoproteins, nucleic acids
- Determine a cell's shape, size and function
- Knowledge of the properties of macromolecules contributes to understanding of living processes, and their manipulation for the production of useful products that find use as diagnostics, therapeutic agents, degradation of wastes, etc.

Identification of macromolecules and Investigation into their structure and function

- Molecular biologists routinely carry out
  - Identification of genes and their location
  - Investigation of structure and function of genes and gene products
  - Perform molecular separations
  - Locate the position and movement of macromolecules in the cell
  - Modification of macromolecules for specific functions
  - Design macromolecules for many purposes

- The most popular techniques used:
  - Gel electrophoresis
  - Radiolabeled tracers
  - Nucleic acid hybridization

Common techniques

- Gel electrophoresis was used to provide evidence of DNA as the genetic material
- Gel electrophoresis is used to separate different nucleic acids or proteins
- Molecular biologists and biochemists use labeled (radioactive) tracers to detect tiny quantities of substances (e.g. to measure the appearance of a transcript during gene expression). The radiotracer techniques to cover in this module include:
  - Autoradiography
  - Phosphor imaging
  - Liquid scintillation counting

Common techniques

- Nucleic acid hybridization combines electrophoresis and labeled probes to identify specific DNA or map and quantify transcripts. Methods include
  - Southern blots
  - Northern blots
  - DNA fingerprinting/profiling/typing/testing
  - DNA sequencing
  - Restriction mapping
  - S1 mapping

Common techniques

- For detecting protein and DNA-protein interactions, a combination of electrophoresis and labeling with antibodies or radioactive material is used in
  - Immunoblotting (Western blotting)
  - Gel mobility shifts
  - DNase footprinting
  - Microscopy

What is the nature of genetic material?

Griffith's experiment- 1928

- Frederick Griffith laid the foundation for identification of DNA as genetic material
- In his experiment, he performed transformation in the bacterium *Pneumococcus* (*Streptococcus pneumoniae*). Two strains occur: wild and mutant
- The wild type is spherical, surrounded by a mucous coat- **capsule**
- Cells of the wild type form large glistening colonies characterized as Type III **smooth** (Type III-**S**)
- This wild type is **virulent**

Wild = spherical, capsule around it, large, smooth and glistening colonies, virulent

Griffith's experiment

- A **mutant** strain was identified
- Had no protective coat
- Cells formed small, rough colonies, Type II (**R**) -(Type II-**R**)
- Cells were **avirulent** because no protective coat. Why?
- Because host's white blood cells engulf them, and without the protective coat, are destroyed before they can multiply to cause disease
- He found that heat-killed virulent colonies alone could not cause disease
- However, heat-killed virulent **S** strain mixed together with live avirulent **R** strain caused disease

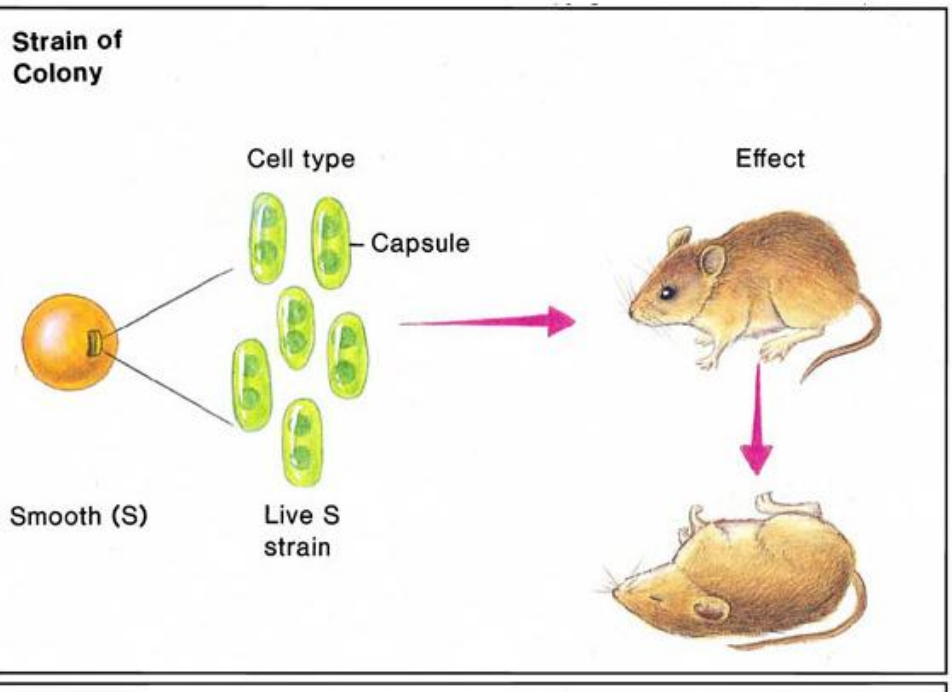
Mutant= no capsule, small and rough colonies, avirulent

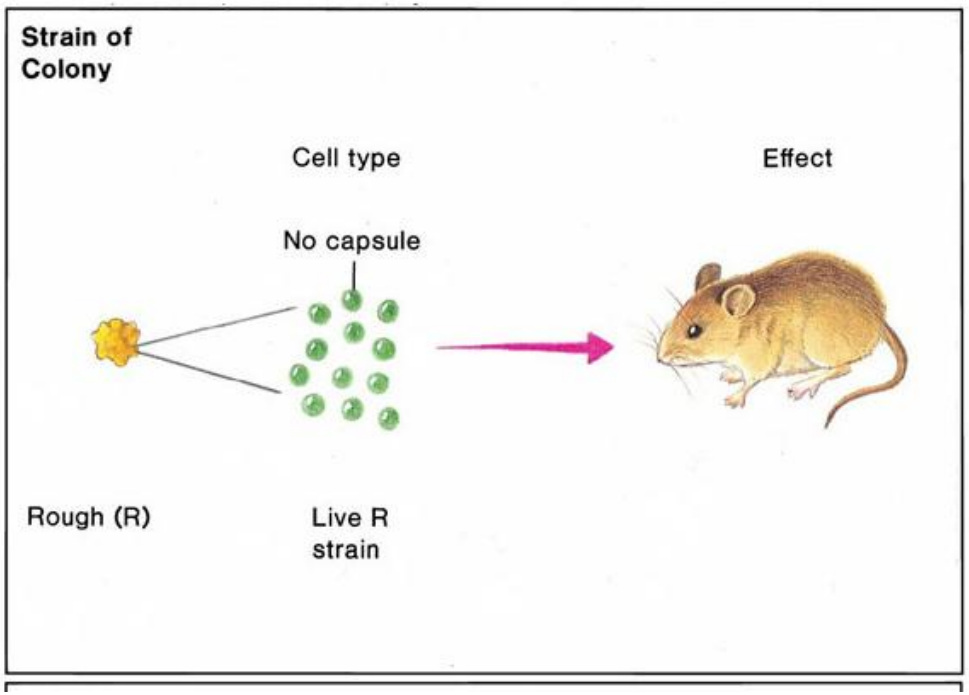
Griffith's interpretation and conclusion

- Somehow, the virulent trait passed from dead, virulent cells to the live, avirulent cells, leading to transformation
- The transformation of avirulence to virulence in Type II-R was not short-lived but was passed on to their descendants as a heritable trait
- The mechanism of transfer, now termed horizontal gene transfer (HGT) is still not understood though similar mechanisms have also been discovered.
- In HGT, living bacterial cells which are competent (ability to pick up naked DNA) pick up foreign DNA across their cell membrane and incorporate it into their own genome by genetic recombination, hence, a transformation.
- Meaning the **transforming substance** in the heat-killed bacteria was probably the gene for virulence

1928

Griffith's transformation experiments

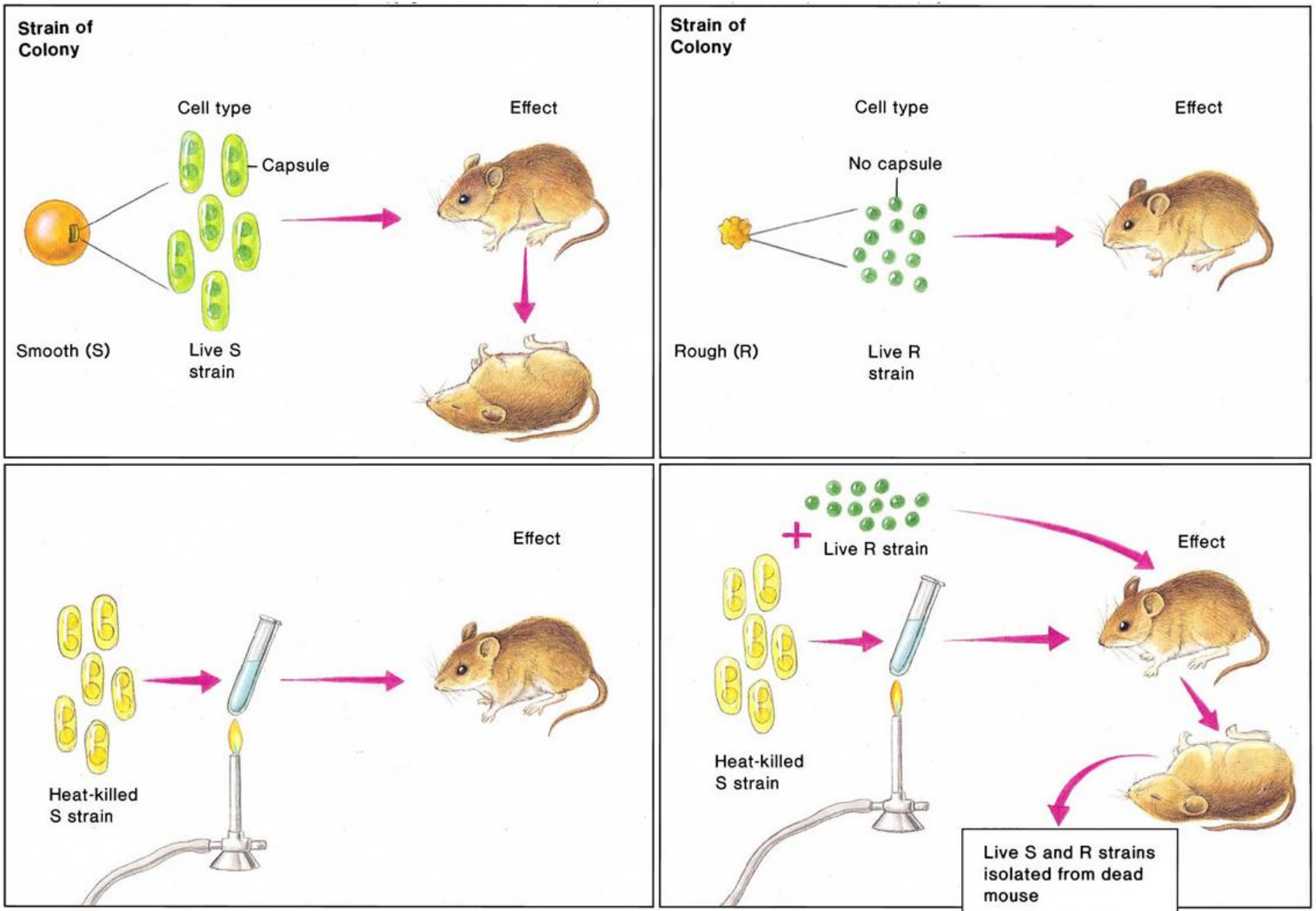




S = smooth colonies (virulent)
Contain protective coat

R = rough colonies (avirulent)
No protective coat
Engulfed by white blood cells
Cannot cause disease

Griffith's transformation experiments



1928 Frederick Griffith

Conclusion: Molecules that carry heritable information are present in the S strain cells, likewise the R strain carried material that could store the heritable material received

Neither live Type II-R strain nor heat-killed Type III-S strain alone could cause the disease and kill the mice

The Type III-S strain contained some material that could transform the Type II-R strain bacteria

What is this transforming material?

Information missing here is the chemical nature of the transforming substance

Oswald Avery, Colin MacLeod, Maclyn McCarty Experiment - 1944

- The researchers demonstrate that the transformation of *Streptococcus pneumoniae* from avirulent Type II-R to a virulent Type II-S is the result of the transfer of DNA from dead Type II-S organisms to live Type II-R ones organisms.

- At that time it was believed that protein was the genetic material

- How did they do this?
- 1. They prepared serum from infected mice and heat-killed the bacteria in it
- 2. Removed protein from extract with the organic solvent chloroform, mixed it with live **R** strain and infected healthy mice with it. Mice got diseased. Extract from diseased mice shows **S** + **R** strain. Result: transformation occurred
- 3. They treated extract with trypsin and chymotrypsin to destroy proteins; repeated steps as before
- Result: transformation occurred
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Oswald Avery, Colin MacLeod, Maclyn McCarty Experiment - 1944

- 4. They treated extract with RNase, repeated steps as before. Result: transformation occurred

  Interpretation

  These steps ruled out RNA and protein as transforming material
- 5. Treated extract with deoxyribonuclease (DNase); repeated steps as before. Extract from diseased mice shows only R avirulent strain.
- Result: No transformation occurred. Transformation ability destroyed by DNase. Transforming material may be DNA

Direct evidence that transforming substance is DNA

1. Ultracentrifugation –sedimented rapidly
   means transforming material has high molecular weight
2. Electrophoresis – showed high mobility (i.e., moved fast down the gel under electric current), meaning the transforming principle has high charge-to-mass ratio
3. UV light absorption spectrum– maximum at 260 nm

4. Elemental chemical analysis – nitrogen to phosphorus ratio of 1.67 (expected for pure DNA)
   Protein: rich in nitrogen and low in phosphorus (would have a higher ratio)
   Even a slight contamination with protein would have raised the ratio

Conclusion: DNA is the transforming material

Today, we know that the "transforming principle" Griffith observed was the DNA of the Type III-S strain

Hershey-Chase Experiment provided evidence that genes were made of DNA

1952 Hershey-Chase Experiment

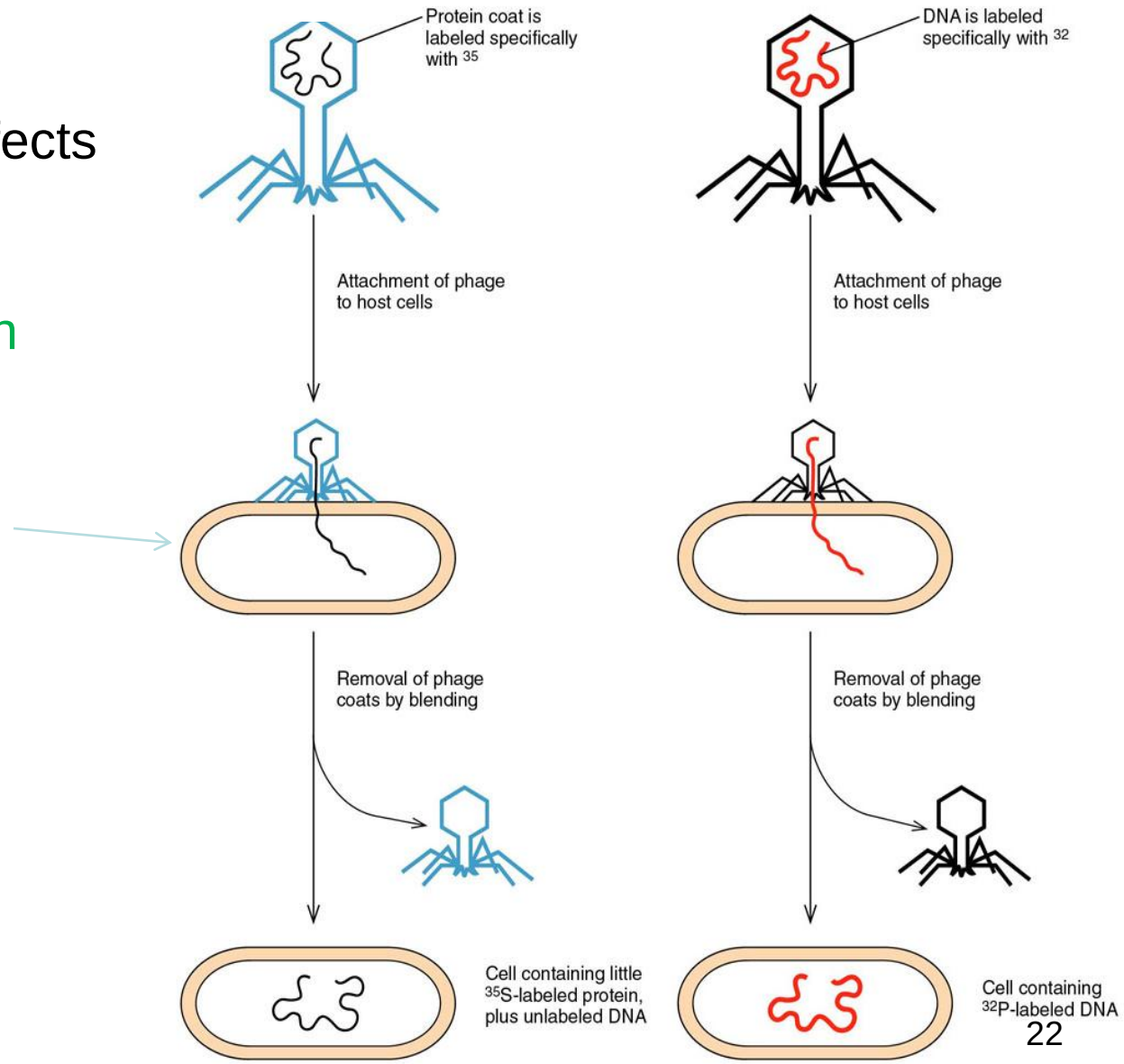
- A.D. Hershey and Martha Chase used the virus, bacteriophage T2 to infect *E. coli*
- During infection, phage genes enter host cells and direct the synthesis of new phage particles
- Phage made up of DNA surrounded by a protein coat
- First part: they labeled the phage protein with ^{35}S (blue) leaving the DNA unlabeled (black)
- Second part: labeled phage DNA with ^{32}P (red), leaving the protein unlabeled (black)

1952 Hershey-Chase Experiment

Known: that phage infects bacteria

Do the genes reside in protein or DNA?

E. coli



1952 Hershey-Chase Experiment

- Since phage genes must enter the cell, the type of label found within the infected cell would indicate the nature of the genes
- In (a) most of the labeled protein remained on the outside
- In (b) most of the labeled DNA entered the infected cells

- Conclusion: genes of this phage are made of DNA

Chemical nature of polynucleotides

DNA consists of

Nitrogenous bases
- adenine (A)
- cytosine (C)
- guanine (G)
- thymine (T)

Phosphoric acid

Sugar: deoxyribose

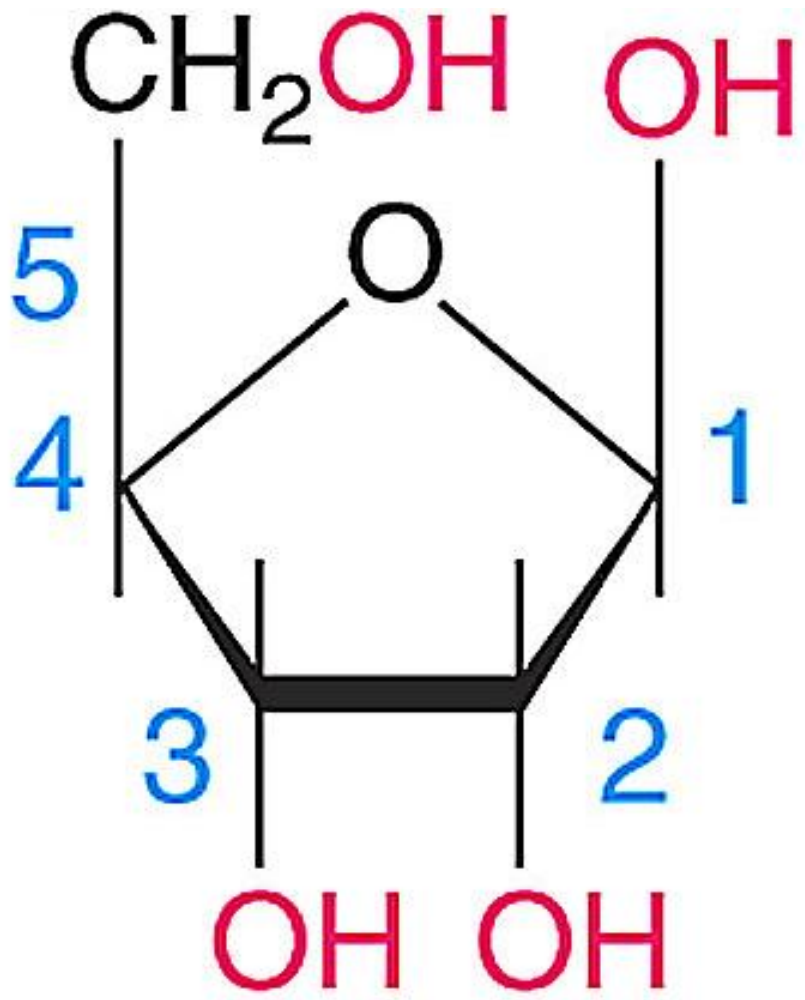
RNA consists of

Nitrogenous bases
- adenine (A)
- cytosine (C)
- guanine (G)
- uracil (U)

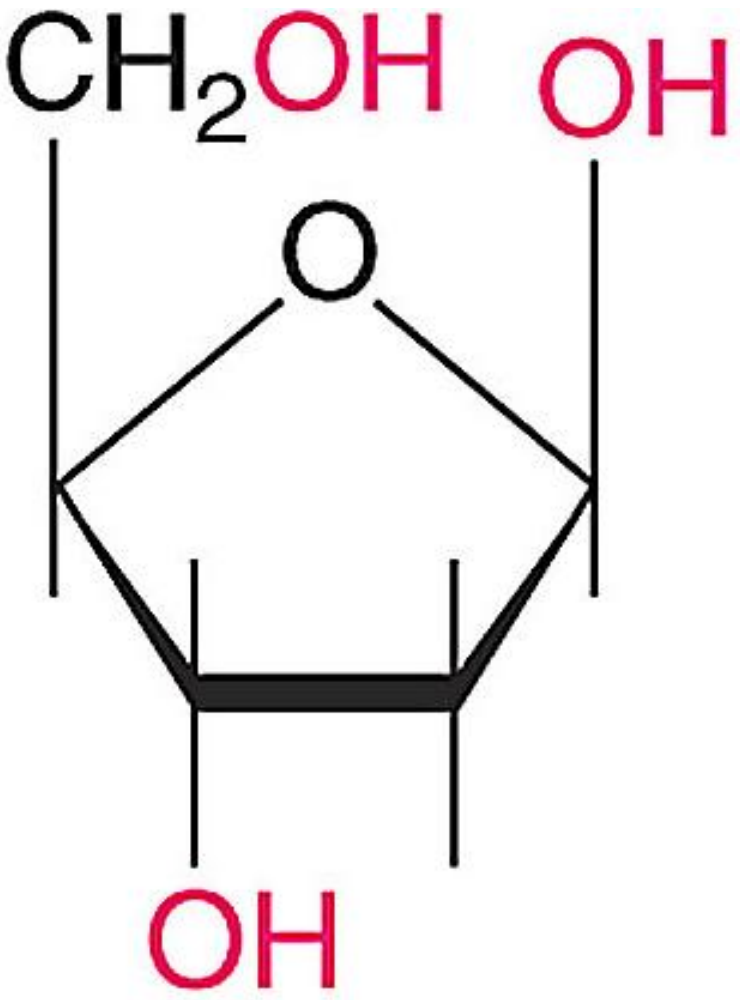
Phosphoric acid

Sugar: ribose

Sugars of nucleic acids

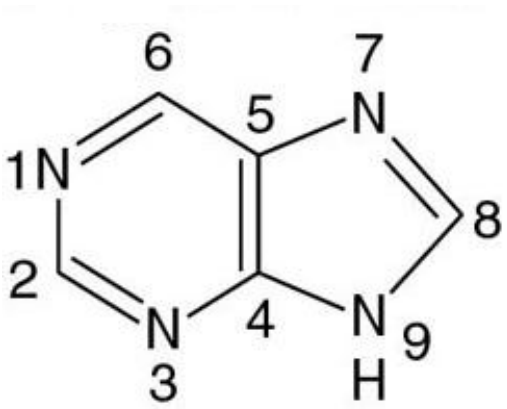


Ribose



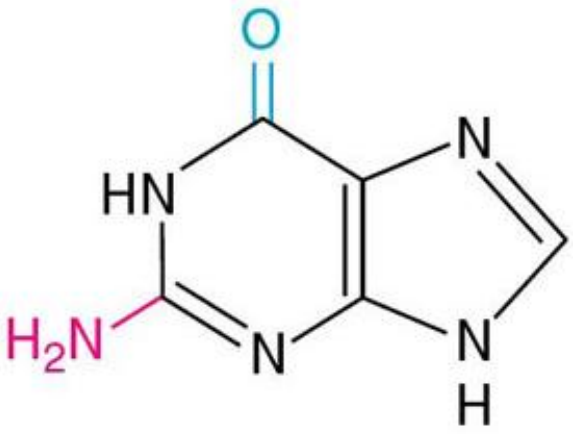
2-deoxyribose

Bases

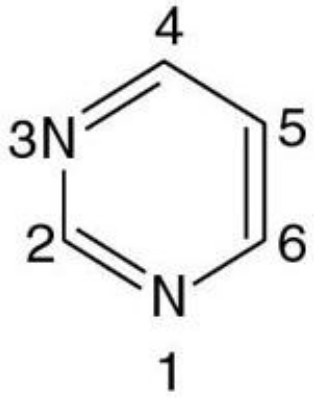


Purine

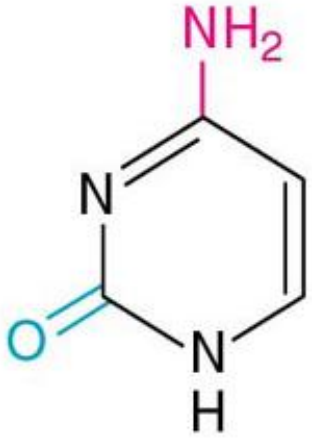
Adenine



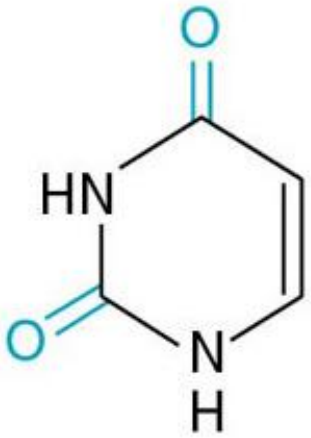
Guanine



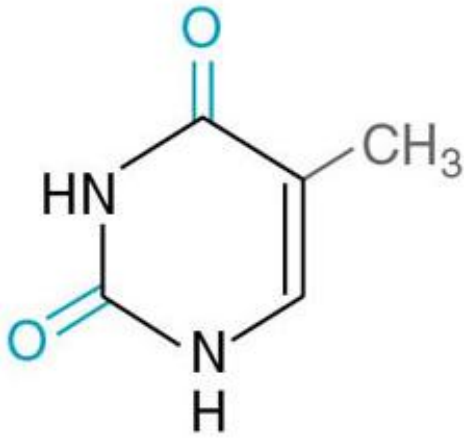
Pyrimidine



Cytosine



Uracil



Thymine

Nomenclature

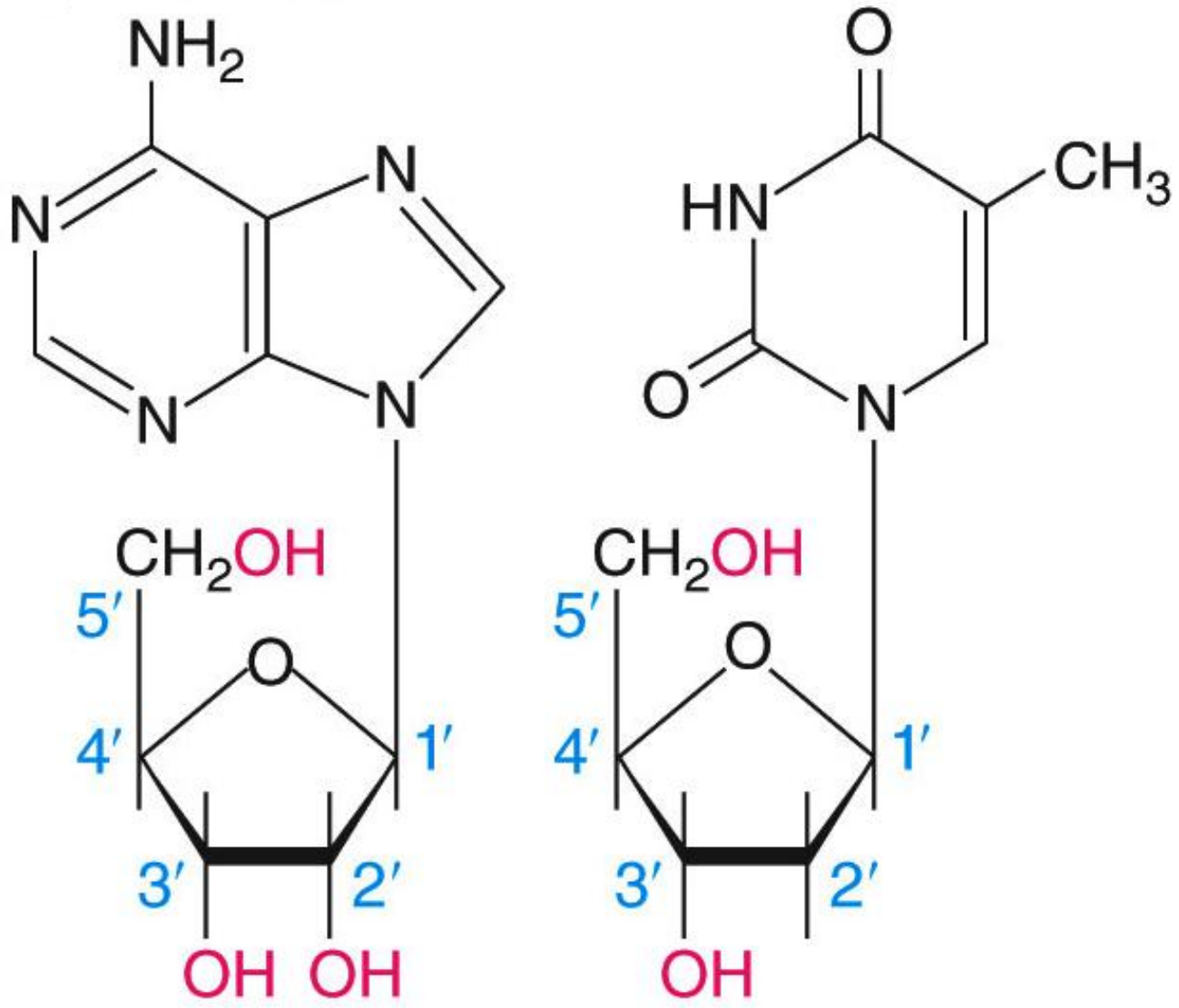
bases

(no sugar or phosphate)

nucleosides = sugar + base

nucleotides = sugar + phosphate group + base

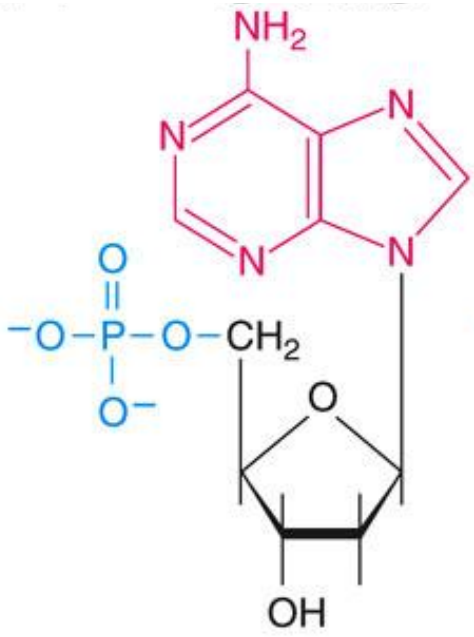
Nucleosides



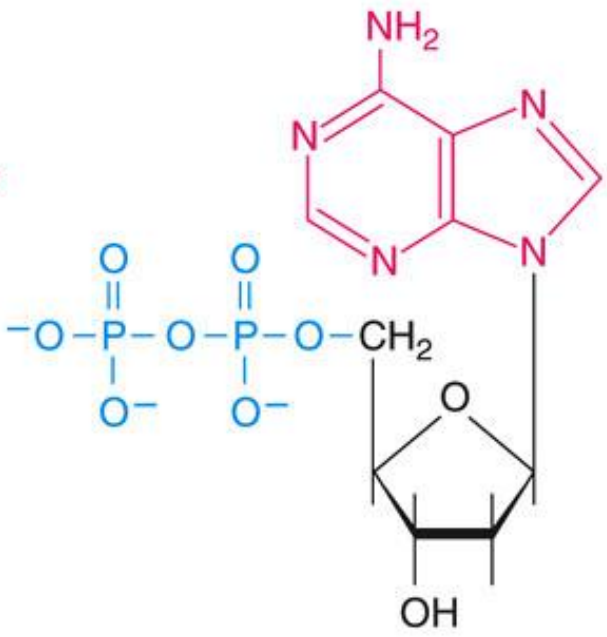
Adenosine

2′ -Deoxythymidine

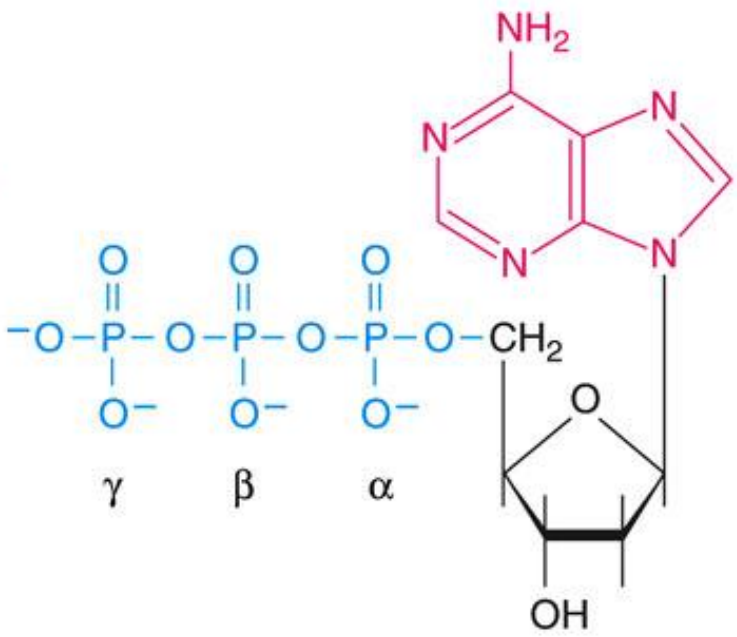
Nucleotides



Deoxyadenosine-5′-monophosphate (dAMP)



Deoxyadenosine-5′-diphosphate (dADP)



Deoxyadenosine-5′-triphosphate (dATP)

A trinucleotide