Molecular biology
second lecture
improve the DNA hereditary material

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References :

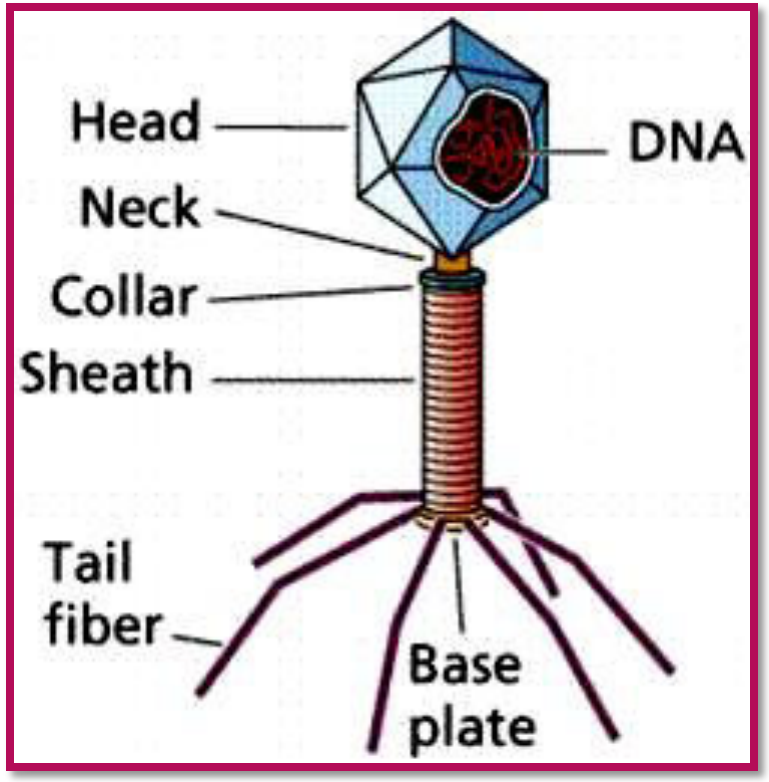
1-Essential of molecular biology by George M. Malacinski 4th edition

2-Second references: Molecular biology (principles and practice)

Hershey–Chase experiments to prove that the DNA IS the genetic material using virus (phage) model

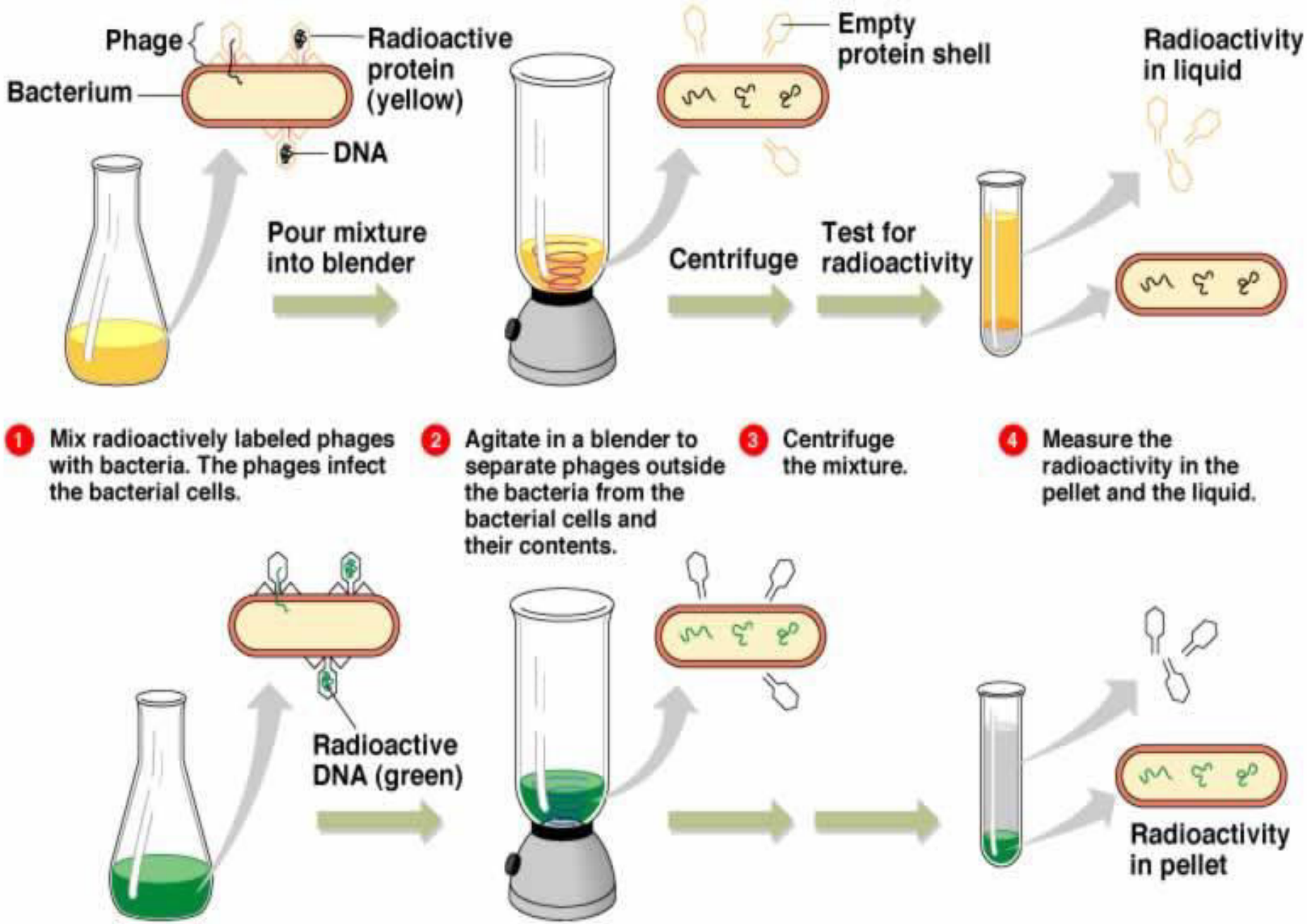
Alfred Hershey and Martha Chase (1952)confirming that DNA was the genetic material (first demonstrated in 1944) using T2 phage virus

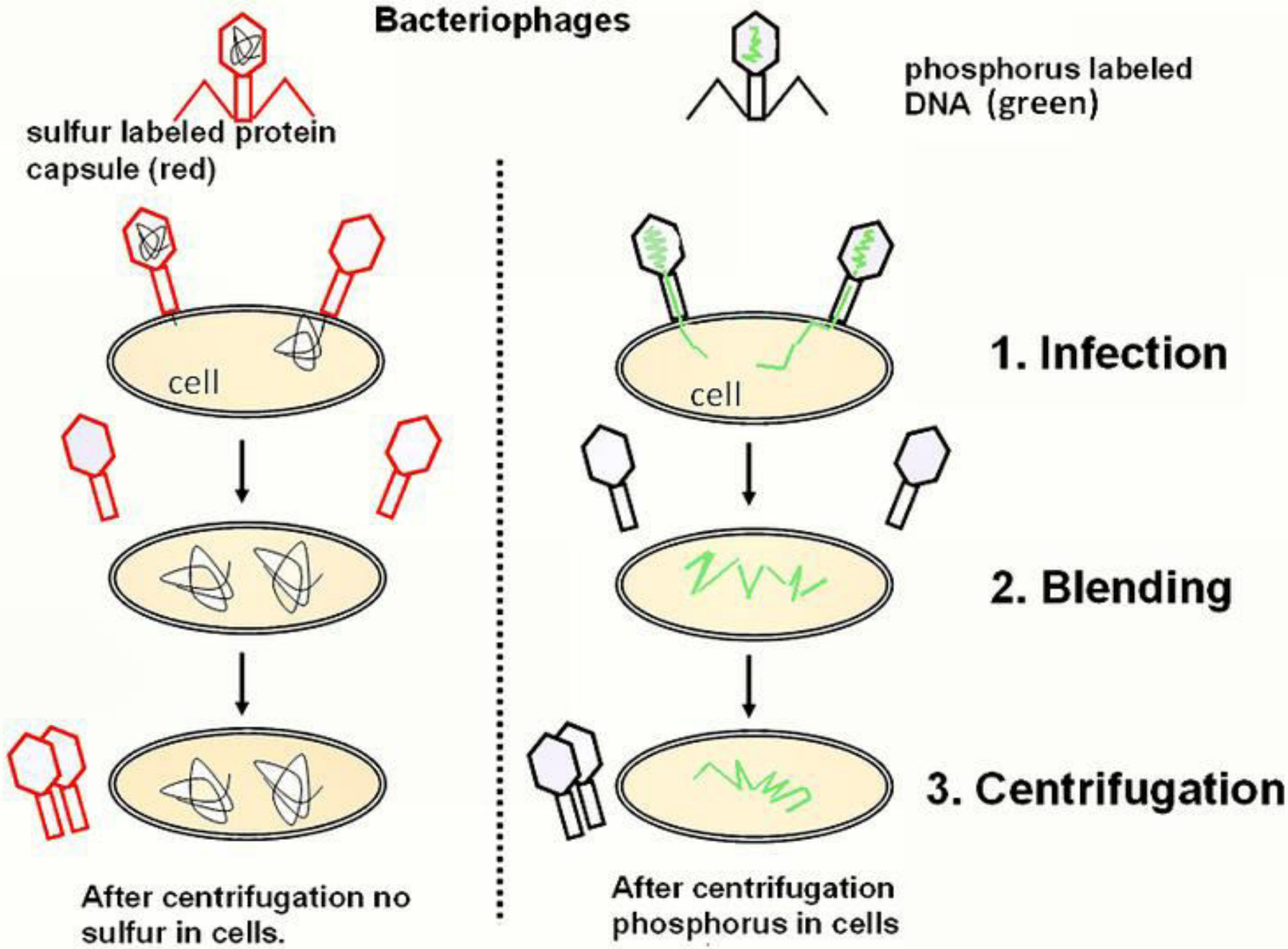
The phage consists of a **protein shell(capsule)** containing its genetic material(DNA). The phage infects a bacterium by attaching to its outer membrane by tail fiber then injecting its genetic material leaving its empty shell attached to the bacterium.



ERPIREMENT:

- They depend on the differences between protein &DNA chemical structure (DNA contains :C, H,O,N and Ph while protein :C,H.O.N,S)
- In their first set of experiments, Hershey and Chase labeled the DNA of phages with radioactive **Phosphorus- P32** (the element phosphorus is present in DNA but not present in any of the 20 amino acids from which proteins are made). They allowed the phages to infect *E. coli*, and through several elegant experiments were able to observe the transfer of P32 labeled phage DNA into the cytoplasm of the bacterium
- In their second set of experiments, they labeled the phages with radioactive **Sulfur-35** (Sulfur is present in the amino acids cysteine and methionine, but not in DNA). Following infection of *E. coli* they then sheared the viral protein shells off of infected cells using a high-speed blender and separated the cells and viral coats by using a centrifuge.
- After separation, the radioactive S35 tracer was observed in the protein shells, but not in the infected bacteria, supporting the hypothesis that the genetic material which infects the bacteria was DNA and not protein.
- Hershey shared the 1969 Nobel Prize in Physiology or Medicine for his “discoveries concerning the genetic structure of viruses.”



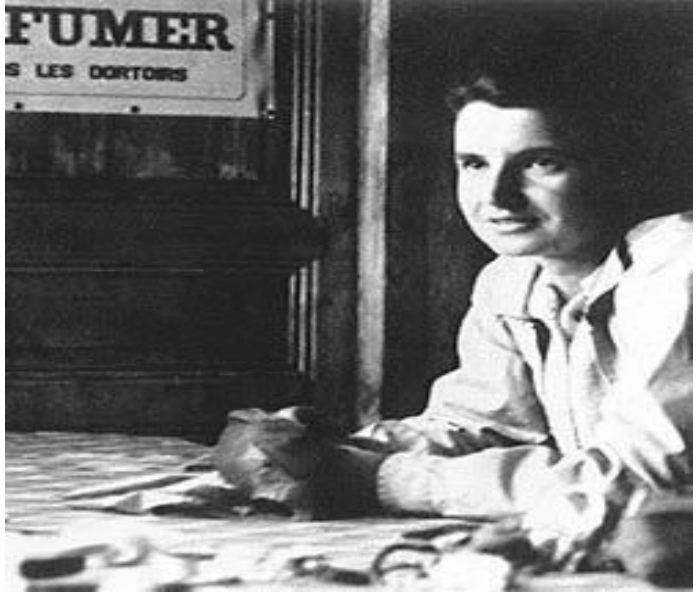

Bacteriophages
sulfur labeled protein capsule (red)
phosphorus labeled DNA (green)
cell
cell
1. Infection
2. Blending
3. Centrifugation
After centrifugation no sulfur in cells.
After centrifugation phosphorus in cells

Another important findings in molecular biology science

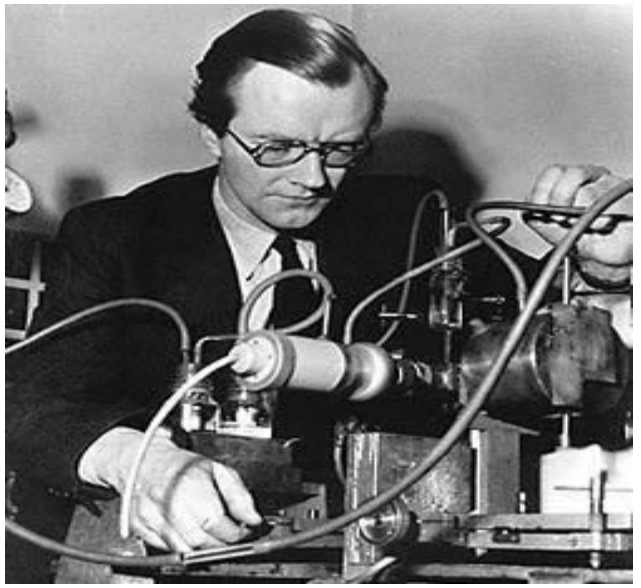
- 1950 **Rosalind franklin& Maurice Wilkins**(using X-ray crystallographic equipment to solve the DNA problem at King's College\London to determine the 3 dimensional structure of the DNA or protein, according to this, A- DNA & B- DNA were described
- George Beadle , Edward Tatum &Joshua Lederberg (1946-1956) Beadle and Tatum's key experiments involved exposing the bread mold *Neurospora crassa* to x-rays causing mutations to cause changes in specific enzymes involved in metabolic pathways. The Nobel Prize in Physiology or Medicine 1958 was divided, one half jointly to Beadle and Tatum "for their discovery that genes act by regulating definite chemical events" and the other half to Joshua Lederberg "for his discoveries concerning genetic recombination and the organization of the genetic material of bacteria
- (1953)James Watson (USA) &Francis Crick\UK discovered DNA molecule (will be discused latter) depending on franklin Wilkins X-ray model .they won the 1962 Nobel Prize in Medicine for their discovery of the structure of DNA. This was one of the most significant scientific discoveries of the 20th century
- Francis Crick in 1958 established the theory of **central dogma of molecular biology** that is to say the genetic information follow from

DNA→RNA→PROTEIN

- **1957 Arthur Kornberg** : was an American biochemist who won the Nobel Prize in Physiology or Medicine 1959 for his discovery of "the mechanisms in the biological synthesis of deoxyribonucleic acid by discovering DNA polymerase enzyme. His primary research interests in biochemistry especially enzymes of DNA replication, and studying the nucleic acids which control heredity in animals, plants, bacteria and viruses
- 1958 The Matthew Meselson **and** Franklin Stahl **experiment** : finding the semi conservative replication (discussed latter) . It has been called "the most beautiful experiment in biology
- **1960 Jacob** andMonod (French biologist) : controlling and regulation the cell activity through operon . They shared the 1965 Nobel Prize in Medicine with André Lwoff
- **1960 Khorana** was a biochemist who shared the 1968 Nobel Prize for Physiology or Medicine with Marshall Nirenberg and Robert Holley for research that helped to show how the nucleotides in nucleic acids, which carry the genetic code of the cell, control the cell's synthesis of proteins
- **1969 Thomas Brock** : is an American microbiologist known for his discovery of hyper thermophiles living in hot springs .In the late 1960s, Brock discovered high-temperature bacteria living in the Great Fountain region with his colleague . they isolated a sample named it *Thermus aquaticus*. By 1976, *T. aquaticus* was found useful for artificially amplifying DNA segments. Brock's discoveries led to great progress in biology, contributed to new developments in medicine and agriculture,



Rosalind franklin 1950



Maurice Wilkins 1950

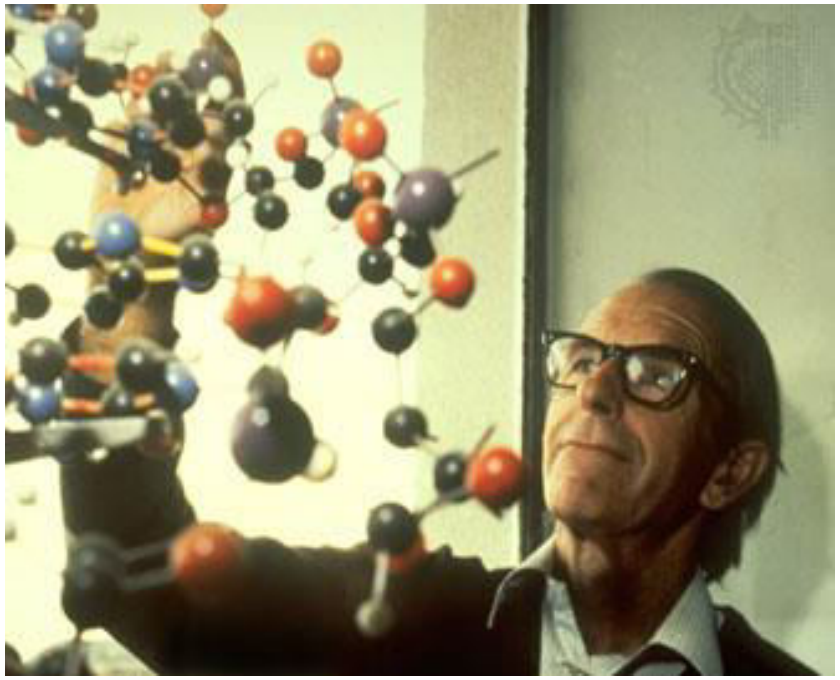
James Watson(USA)&Francis Crick\UK 1953

Matt Meselson and Frank Stahl 1984

- 1970 : **Arber** and **Meselson** discovered **type I restriction enzymes which cleave DNA randomly away from the recognition site**.
- Also In 1970, Smith, Kelly and Welcox isolated and characterized the first type II restriction enzyme, *HindII*, from the bacterium *Haemophilus influenzae* that cleave DNA at specific recognition sequence. Their discovery led to the development of recombinant DNA technology that allowed, for example, the large scale production of human insulin for diabetics using *E. coli* bacteria.
- 1973:Cohen, Paul Berg and Boyer made what would be one of the first genetic engineering experiments. They demonstrated that the gene for frog ribosomal RNA could be transferred into bacterial cells *Escherichia coli* by using a vector (plasmid) then expressed by them
- **Genetic engineering**, also called **genetic modification**, is the direct manipulation of an organism's genome using biotechnology. New DNA may be inserted in the host of interest using molecular cloning methods to generate a modified DNA sequence

- **1977 Frederick Sanger (95years) :**is a British biochemist who was twice awarded the Nobel Prize for Chemistry, the only person to have been so. In 1958 he was awarded a Nobel prize in chemistry "**for his work on the structure of proteins, especially that of insulin**". In 1980, Walter Gilbertand Sanger shared half of the chemistry prize "for their contributions concerning the determination of base sequences in nucleic acids".
- **1980 Maxam–Gilbert sequencing** is a **method of DNA sequencing developed** by Allan Maxam and Walter Gilbert in 1976–1977. This method is based on nucleobase-specific partial chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides.
- **1983 Kary Mullis** American chemist start synthesis a desired DNA sequence and to copy it using polymerase chain reaction **(PCR Technique** discussed latter), a technique which would allow a small strand of DNA to be copied almost an infinite number of times. This has created revolutions in biochemistry, molecular biology, genetics, medicine and forensics. he shared the 1993 Nobel Prize in Chemistry with Michael Smith.
- **2007** The first recorded knockout genes in mouse was created by Mario R. Capecchi, Sir Martin Evans and Oliver Smithies, for which they were awarded the 2007 Nobel Prize in Physiology or Medicinen.

Kary mullis receiving his Nobel price



Frederick Sanger August 13, 1918 (age 95), United Kingdom

Sir Martin Evans Bioscience school cardiff university Nobel price 2007

biological system as experimental models

- **Bacteria** :Prokaryotes unicellular free living cells .only one single chromosome not enclosed inside nucleus but it is free within the cytoplasm called nucleoid .the size of *Escherichia coli(E.coli)* is about 4,639,221 base pair (bp زوج قاعدة)or 4.6 Kbp (كيلو زوج قاعة نتروجينة) .it represent the best model to be used for many reason like easily to be cultured , relatively simple in their needs , short generation time (20 min for *E.coli),*best growth temperature 37cº so it complete DNA replication ,RNA transcription and Protein synthesis within few minutes
- **Bacteriophage** : they represent the simplest form of life These infect the bacteria(there are animal ,plant human viruses) .unlike the bacteria, they are not free living (completely inert)once they enter the host they start replication depending on the machines of the host cell .it now used as cloning vector
- **Yeast:** another experimental model but for Eukaryotic cell thus it contains chromosomes within a true nucleus surrounded with nuclear membrane .great deal of early biochemical research was carried out specially fermentation process ..now for molecular biologist, mutant strains of yeast often used to discover genes that control growth ,division ,and cell behavior.
- **Animal and plant cell :** also could be used as a model in genetic experiments