DNA IS GENETIC MATEIAL EXPERIMENTAL PROOF

Properties of genetic material

- Genetic material must show the following properties:
- It should store genetic information and transmit it as needed by the cell.
- It should duplicate with minimal errors and the stored information should be transferred to the daughter cells with minimal errors.
- It should have both physical and chemical stability.
- Occasionally it should undergo certain changes without a major loss to the parental information.

History:

- **Miescher** isolated nuclei from pus (white blood cells) in 1869
- Found a novel phosphorus-bearing substance = nuclein
- Nuclein is mostly chromatin, a complex of DNA and chromosomal proteins
- It was found to be **acidic** and called **nucleic acids**
- **Nucleic acids** are of two types **DNA** (**d**eoxyribo**n**ucleic **a**cid) and **RNA** (**r**ibo**n**ucleic **a**cid)
- **Feulgen** discovered specific stain for DNA: nucleic acid was found to be localized in nuclei and chromosomes in contrast to RNA (cytoplasm)

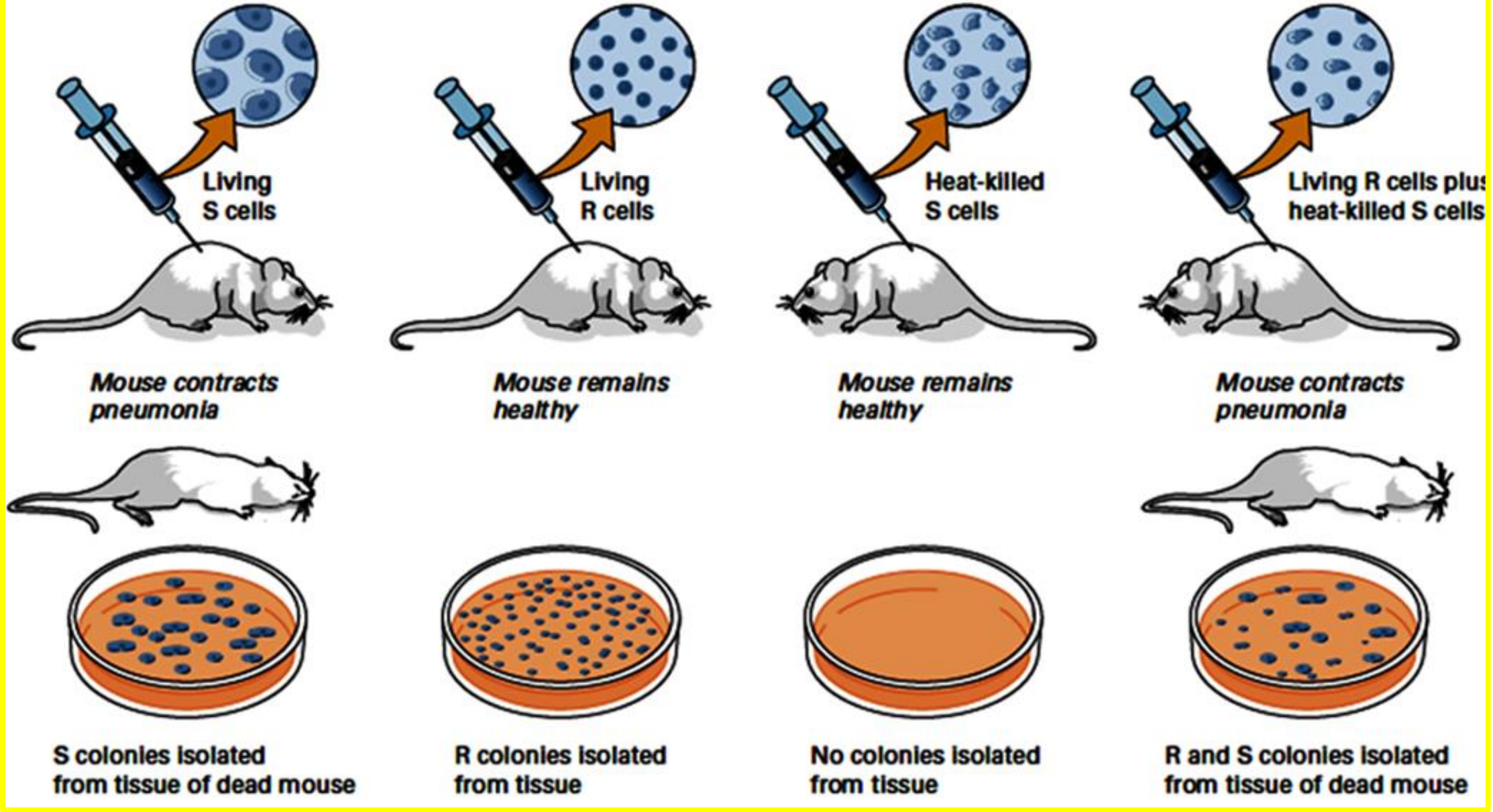
Experiments which prove that DNA is genetic material:

- The observations of a group of simple experiments identified DNA as the genetic material.
- They are
  1. **Transformation Experiments**
  2. **Chemical experiments and**
  3. **Blendor experiments**

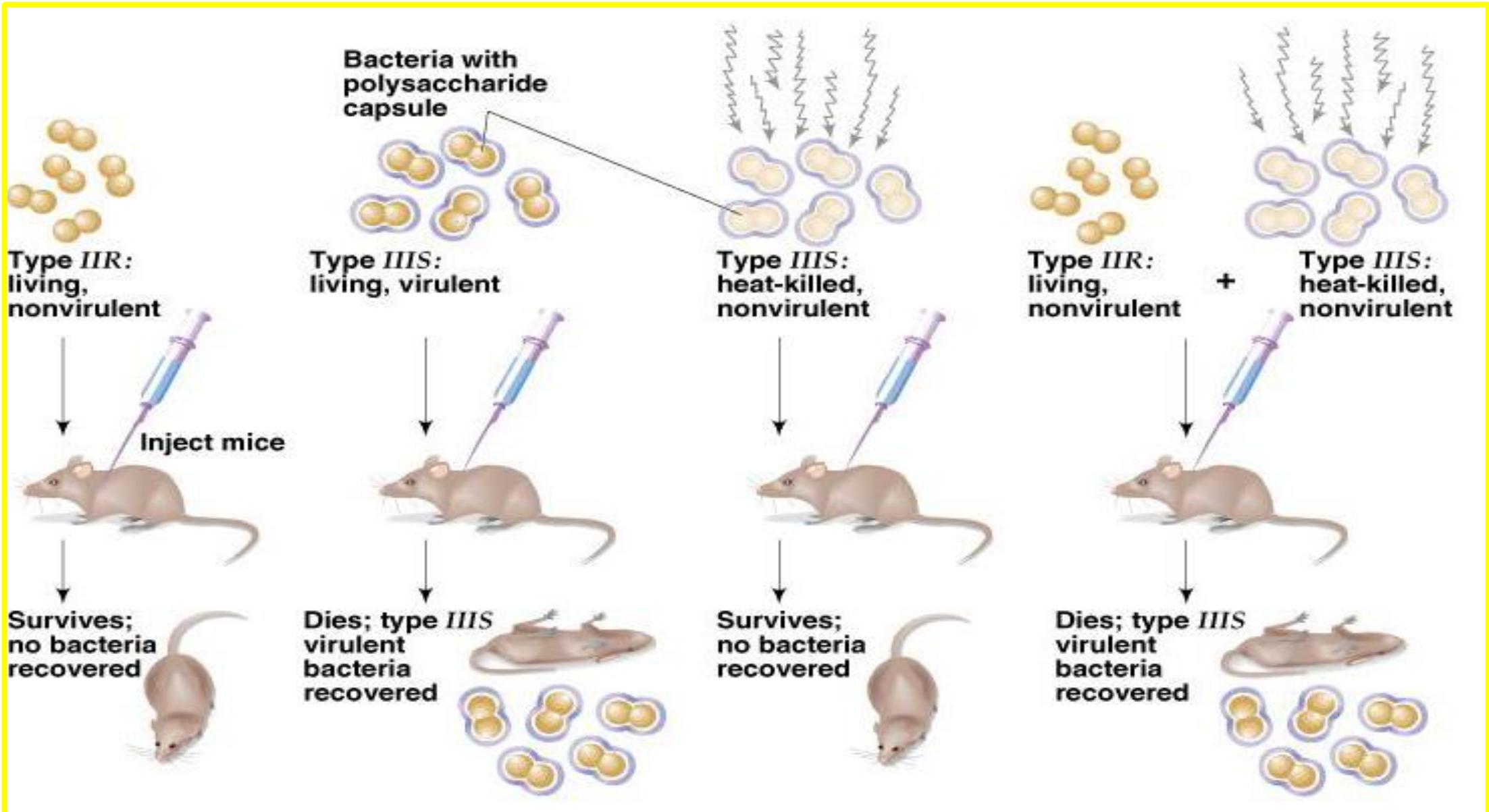
1. Transformation Experiments

- Fred Griffith (1928) has isolated 2 strains of – *Streptococcus pneumoniae* or *Pneumococcus* viz., virulent strain (V^+) and avirulent strain (V^-).
- The virulent strain is surrounded by a smooth **polysaccharide capsule** and when cultured on agar plates they produced **smooth** edged **colonies**. Hence the name '**S**' strain.
- The **virulence** of the '**S**' strain is due to the **Polysaccharide capsule** surrounding it and Griffith observed that when '**S**' strain bacteria were injected, the **mice** died of **pneumonia**.
- The avirulent strain lacks the **capsule** and hence produces **rough** edged colonies on an agar plate and hence the name '**R**' strain.

1. Transformation Experiments



1. Transformation Experiments



1. Transformation Experiments

- When ‘R’ strain bacteria were injected, the mice were alive.
- When ‘S’ strain bacteria were killed by exposing them to heat then they did not kill the mice.
- But when heat killed ‘S’ type and live ‘R’ type were injected together, the mice died of pneumonia and bacteria isolated from mice were all ‘S’ type.

1. Transformation Experiments

- This suggests that live '**R**' bacteria somehow got transformed into '**S**' type and killed the mice.
- Griffith concluded that something from the dead type 'S' was transforming type **R** into type **S**.
- He called this process transformation.
- The substance that allowed this to happen was termed the **transformation principle**
- Griffith did not know what is **transformation principle.**

1. Transformation Experiments

- Later it was shown that some '**S**' cells grew even when a cell extract prepared from broken '**S**' cells freed from intact bacteria and polysaccharide capsule is added to live '**R**' cells.
- From this it was concluded that the **cell extract** contained a **transforming** principle, the **nature** of which was **unknown**.
- The nature of the transforming principle was determined using experimental approaches that incorporated various biochemical techniques

1. Transformation Experiments

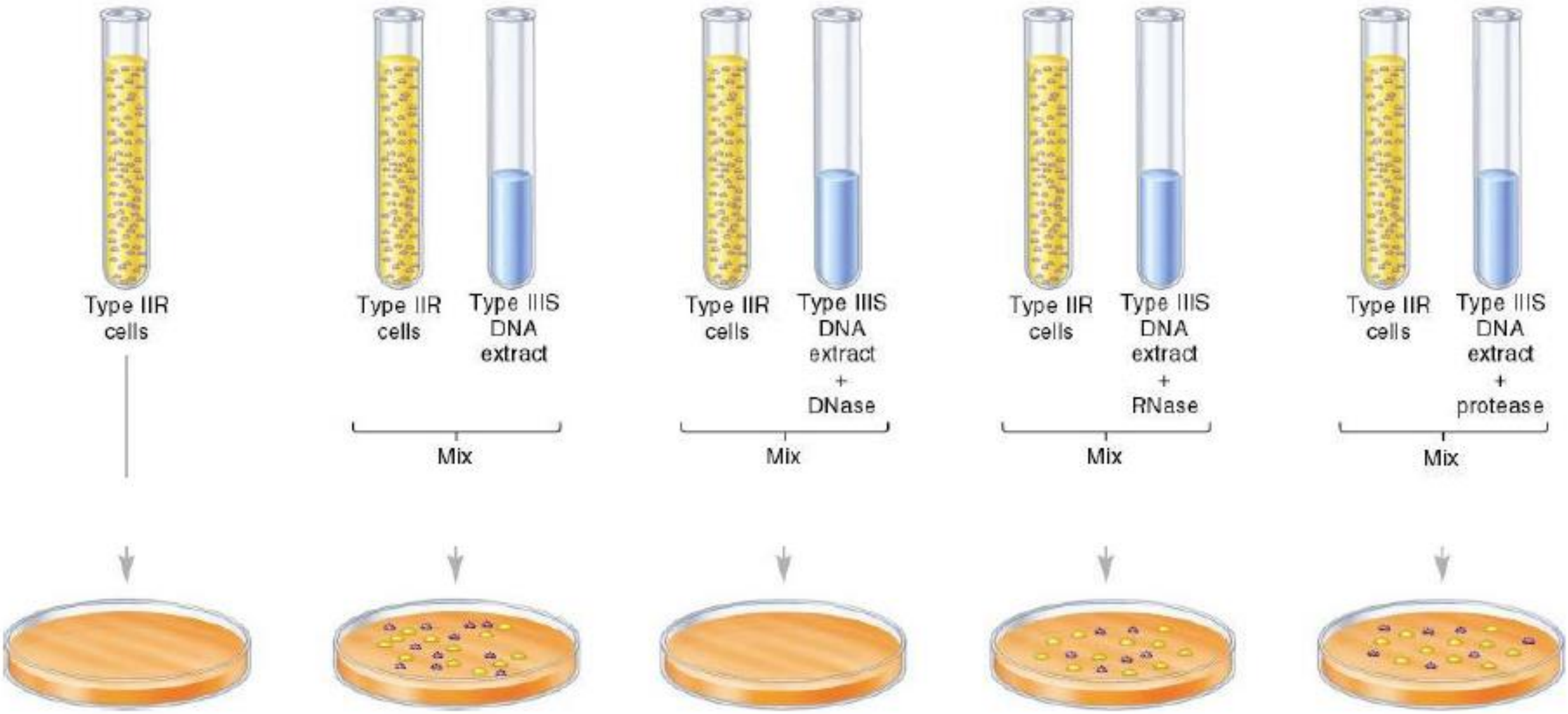
- Later in 1944, Avery, McLeod and McCarty demonstrated that the transforming principle was DNA.
- They isolated DNA from ‘S’ type of bacteria and added to the ‘R’ type of bacteria.
- It was found that some of the ‘R’ type of colonies (1 in 10^4) got transformed into ‘S’ type.

1. Transformation Experiments

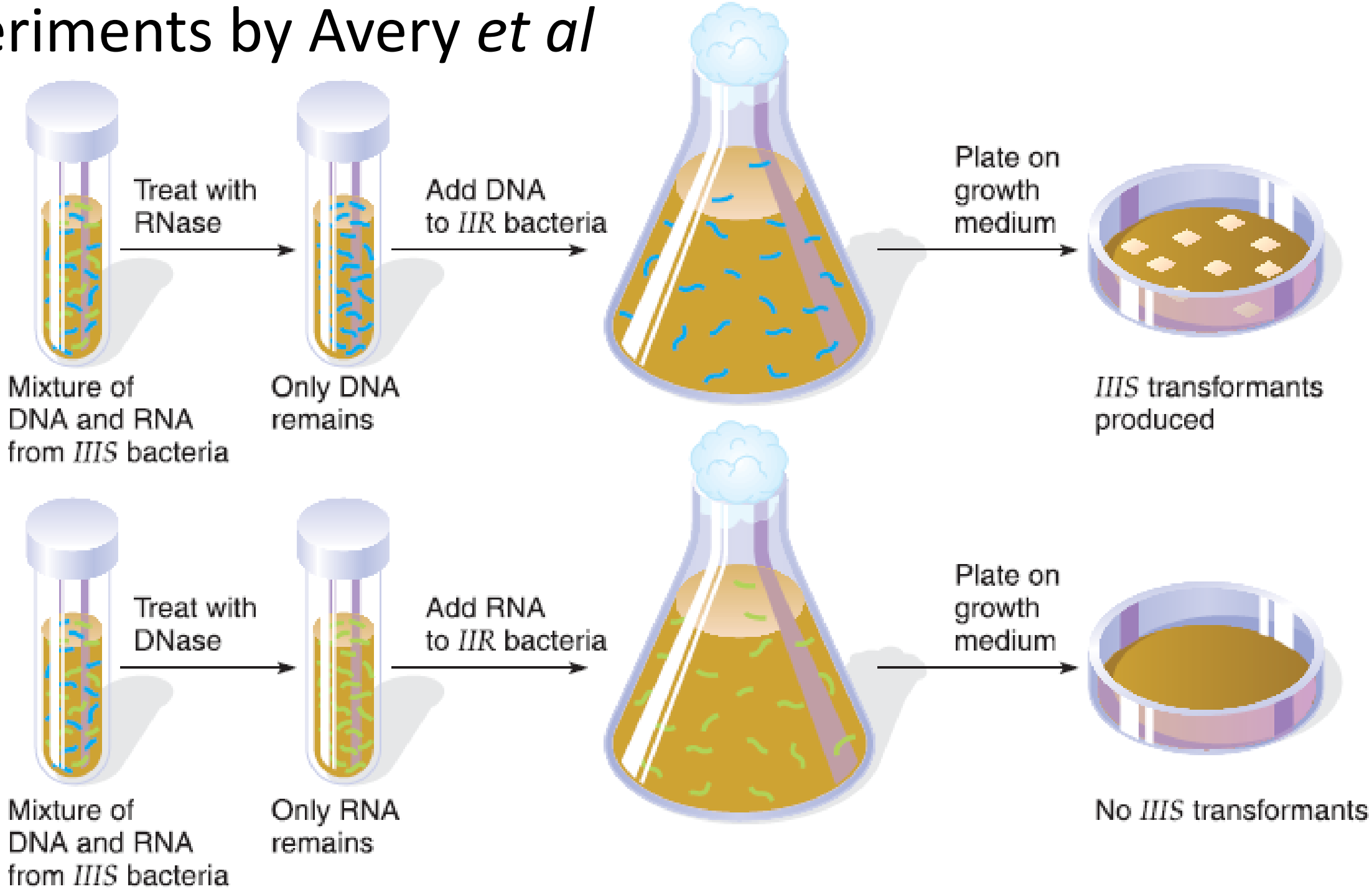
- To know the nature of the transforming principle the below mentioned four experiments were carried out by Avery *et al.*

1. The chemical analysis of transforming principle (TP) suggested that its major component is a deoxyribose containing nucleic acid.
2. Physical measurements of TP viz., density, viscosity etc., are similar to those of DNA.
3. When TP was treated with proteolytic enzymes or RNases the activity was not lost which confirms that TP is neither RNA nor a protein.
4. Treatment of TP with DNases resulted in the loss of activity of TP.

Experiments by Avery *et al*



Experiments by Avery *et al*



1. Transformation Experiments

- Though from the above information Avery, McLeod and McCarty can conclude DNA as the genetic material, they did not do so.
- At that time DNA was thought to be a tetra nucleotide and the proposal that DNA is the genetic material is not acceptable by many scientists.
- Hence Avery, MacLeod and McCarty concluded that "**Nucleic acids** have a specific **biological** role which is to be discovered".

Bacterial Transformation is the process of adding a foreign **DNA** fragment from a **donor** genome into genome of a **recipient** cell.

OR

Bacterial transformation is the uptake of **DNA** by a naked **bacterial** cell.

2. Chemical Experiments

- The **hypothesis** that DNA is a tetranucleotide arose from the belief that DNA has **equimolar** concentration of adenine, thymine, guanine and cytosine *i.e. [A]=[G]=[T]=[C]*.
- This is because the **techniques** used were not very **refined** and DNA was mostly taken either from higher **mammals** or eukaryotes but not from **wide** range of organisms.

2. Chemical Experiments

- **Erwin Chargaff** in 1950 using more refined **techniques** found out that the ratio of **4 nitrogen bases** occur in **different** ratios in **different** organisms.
- Using the information Chargaff has framed ***"Chargaff Rules"*** which are as follows:

2. Chemical Experiments

Chargaff Rules

1) The **base** composition of **DNA** generally **varies** from species to species.
2) **DNA** specimens isolated from different **tissues** of the **same** species have same base composition.
3) The base composition of **DNA** in a given species does not change with an organism's **age**, **nutritional** state or changing **environment**.
4) In all cellular DNAs regardless of species the number of **A** residues is equal to that of **T**. Similarly the number of **G** residues is equal to that of **C**. So the number of **Purines** is equal to the number of **Pyrimidines** (A + G = T + C).

3. Blender Experiments

- Alfred Hershey and Marsha Chase provided further evidence that DNA is the genetic material in 1952.
- They studied the bacteriophage T2
- It is relatively simple since its composed of only two macromolecules

  (1) DNA and (2) Protein

3. Blender Experiments

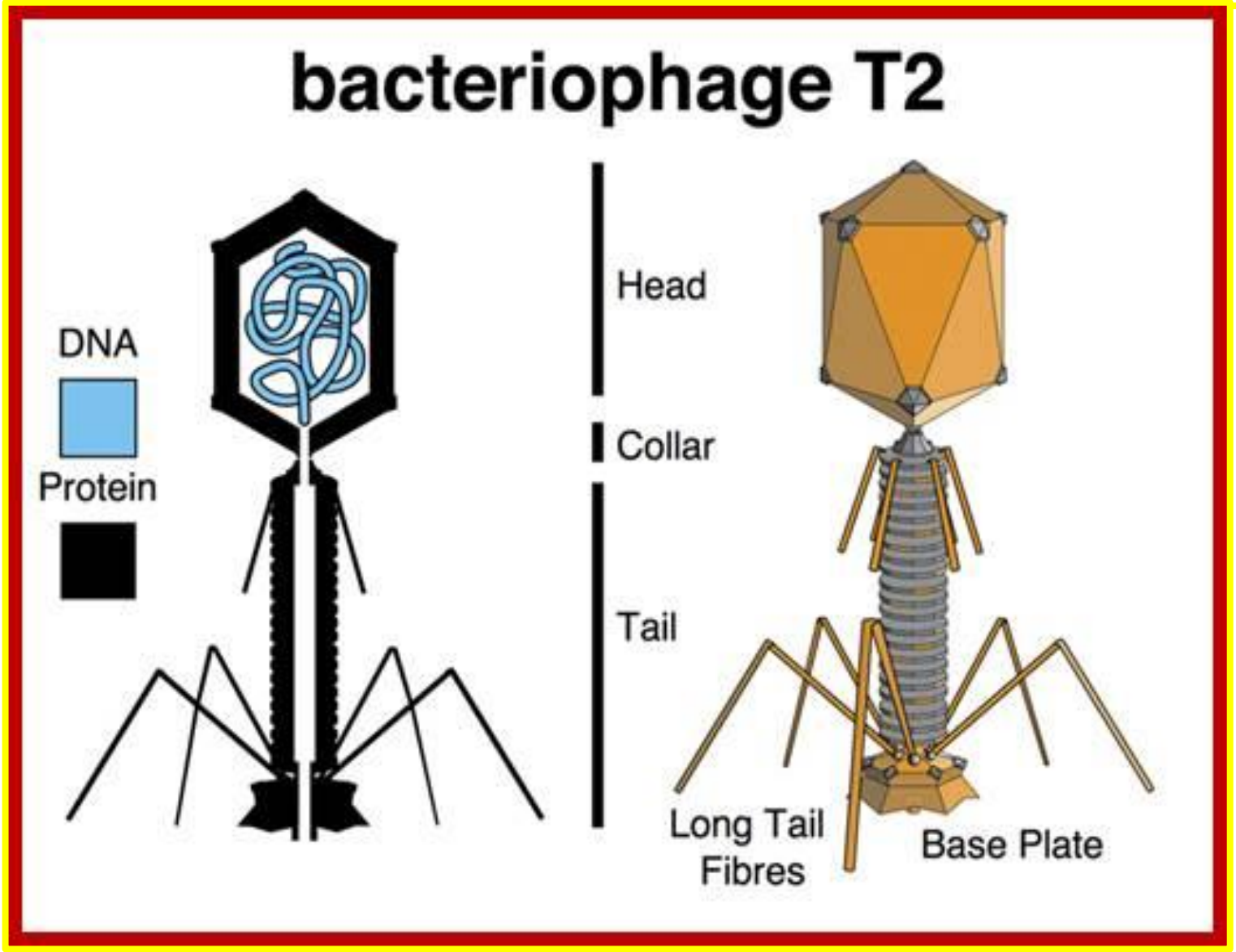
- Alfred Hershey and Martha Chase performed this experiment in 1952.
- As a kitchen blender was used as a major piece of apparatus this experiment is known as Blender Experiment.
- T_2 phage contains DNA encased in a protein coat. DNA is the only phosphorus containing substance in the phage.
- The amino acids methionine and cystein, which contain sulphur are seen in the outer protein coat only.

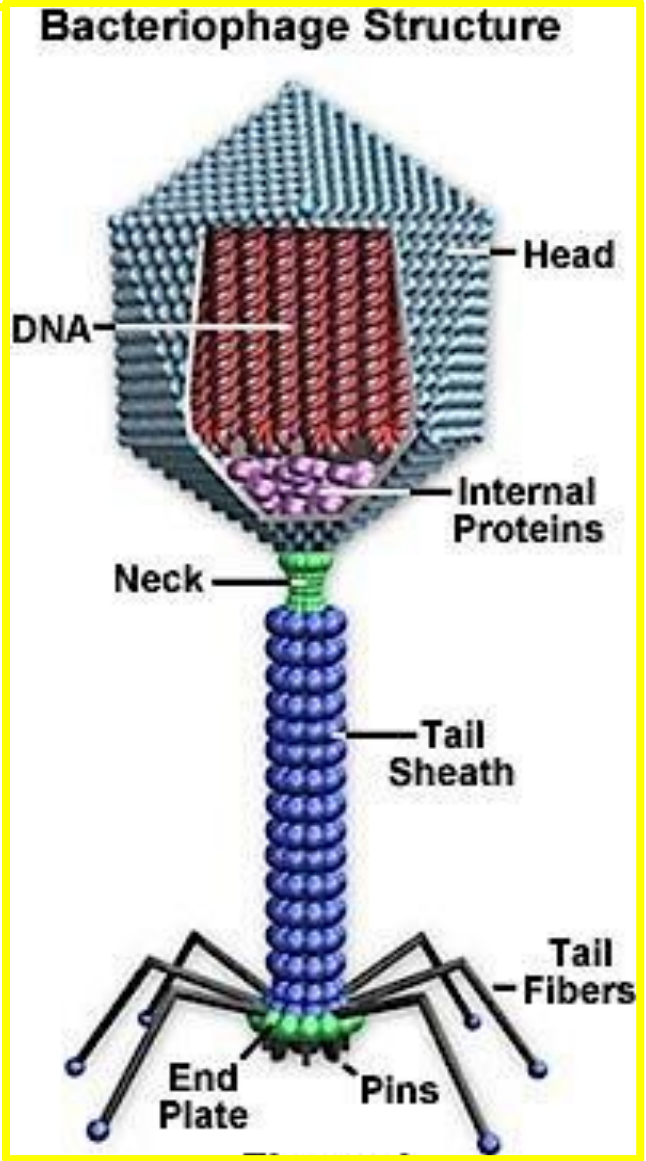
3. Blender Experiments

Summary of Blender Experiments

1. T2 bacteriophage is composed of DNA and proteins:
2. Set-up two replicates:
   - Label DNA with 32P
   - Label Protein with 35S
3. Infected E. coli bacteria with two types of labeled T2
4. **32P** is discovered within the **bacteria** and **progeny** phages, hence DNA is genetic material.
5. **35S** is not found within the bacteria but released with phage ghosts.

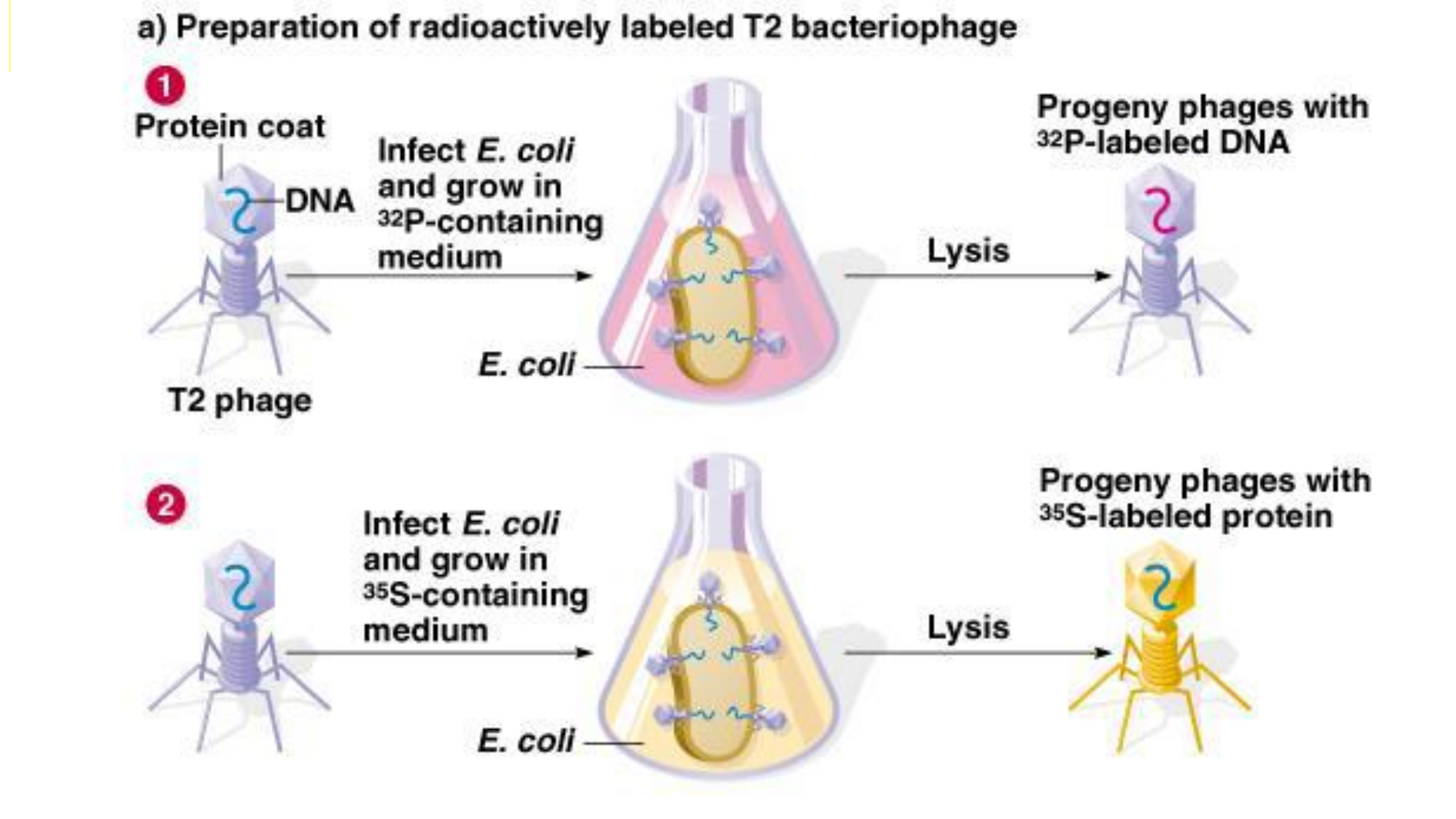
3. Blender Experiments





3. Blender Experiments

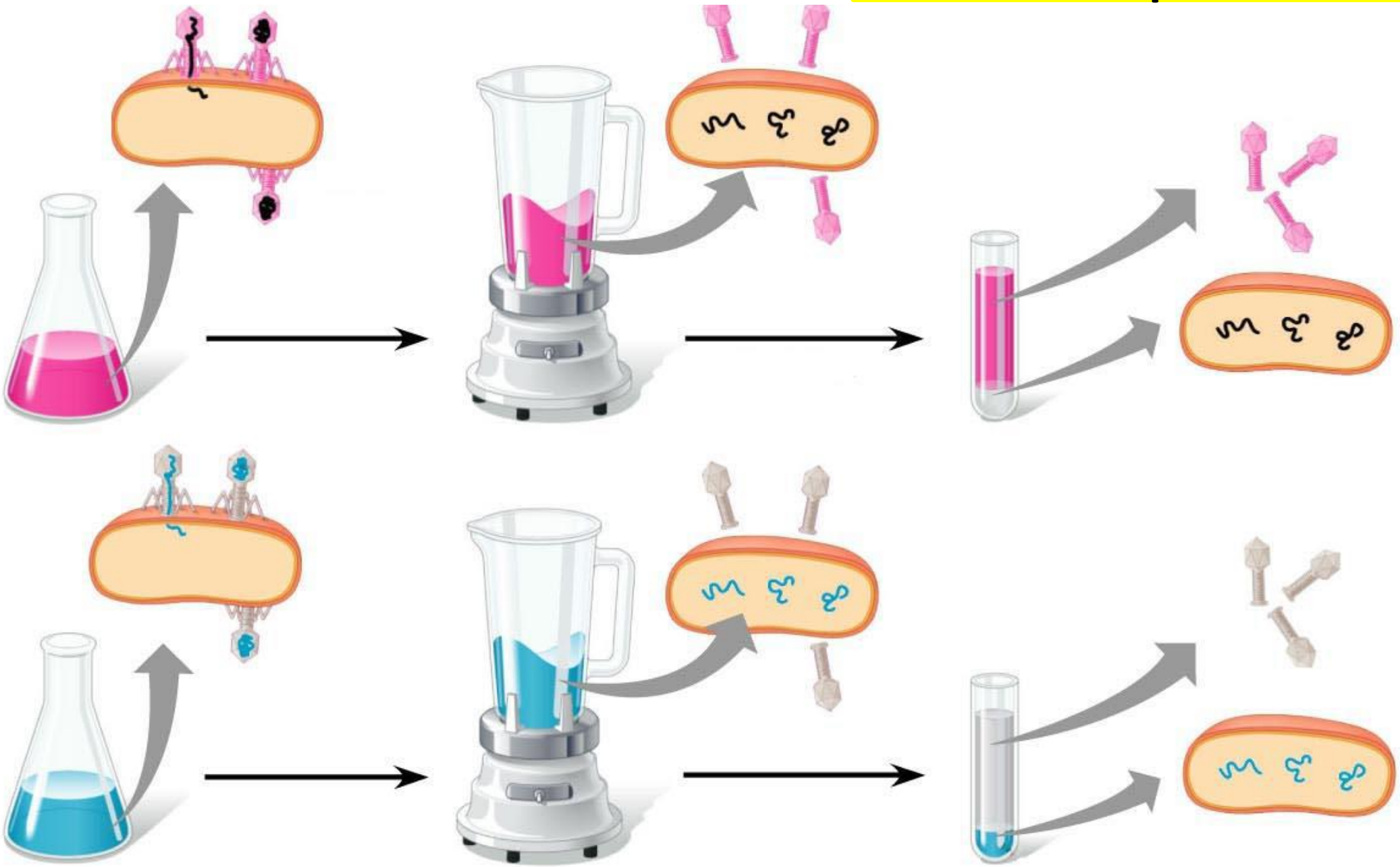
- *E. coli* lawn was developed in a nutrient medium that contains ^{35}S as the sole source of sulphur.
- One phage population was grown on this lawn and the outer protein coat of the phage has been radio labeled with ^{35}S.
- Similarly DNA of a second phage population was labeled with ^{32}P (DNA is the only phosphorous containing molecule in phage).


a) Preparation of radioactively labeled T2 bacteriophage
1
Protein coat
DNA
T2 phage
Infect E. coli and grow in 32P-containing medium
E. coli
Lysis
Progeny phages with 32P-labeled DNA
2
Infect E. coli and grow in 35S-containing medium
E. coli
Lysis
Progeny phages with 35S-labeled protein

3. Blender Experiments

- Each T_2 phage has a long tail by which it attaches to the sensitive bacteria.
- The phage particle then creates a hole and injects its genetic material into the bacteria.
- Hershey & Chase have shown that an attached phage can be separated from the bacterium by shaking it vigorously using a kitchen blender.

3. Blender Experiments



3. Blender Experiments

First experiment, the ^{35}S labeled phage

- In the first experiment, the ^{35}S labeled phage were adsorbed to bacteria for some time.
- The unadsorbed phage particles were separated by centrifugation.
- The pellet which contains phage adsorbed to the bacteria are suspended in water and then shaken vigorously using a kitchen blender.
- Then it is subjected to centrifugation resulting in a pellet and supernatant.

3. Blender Experiments

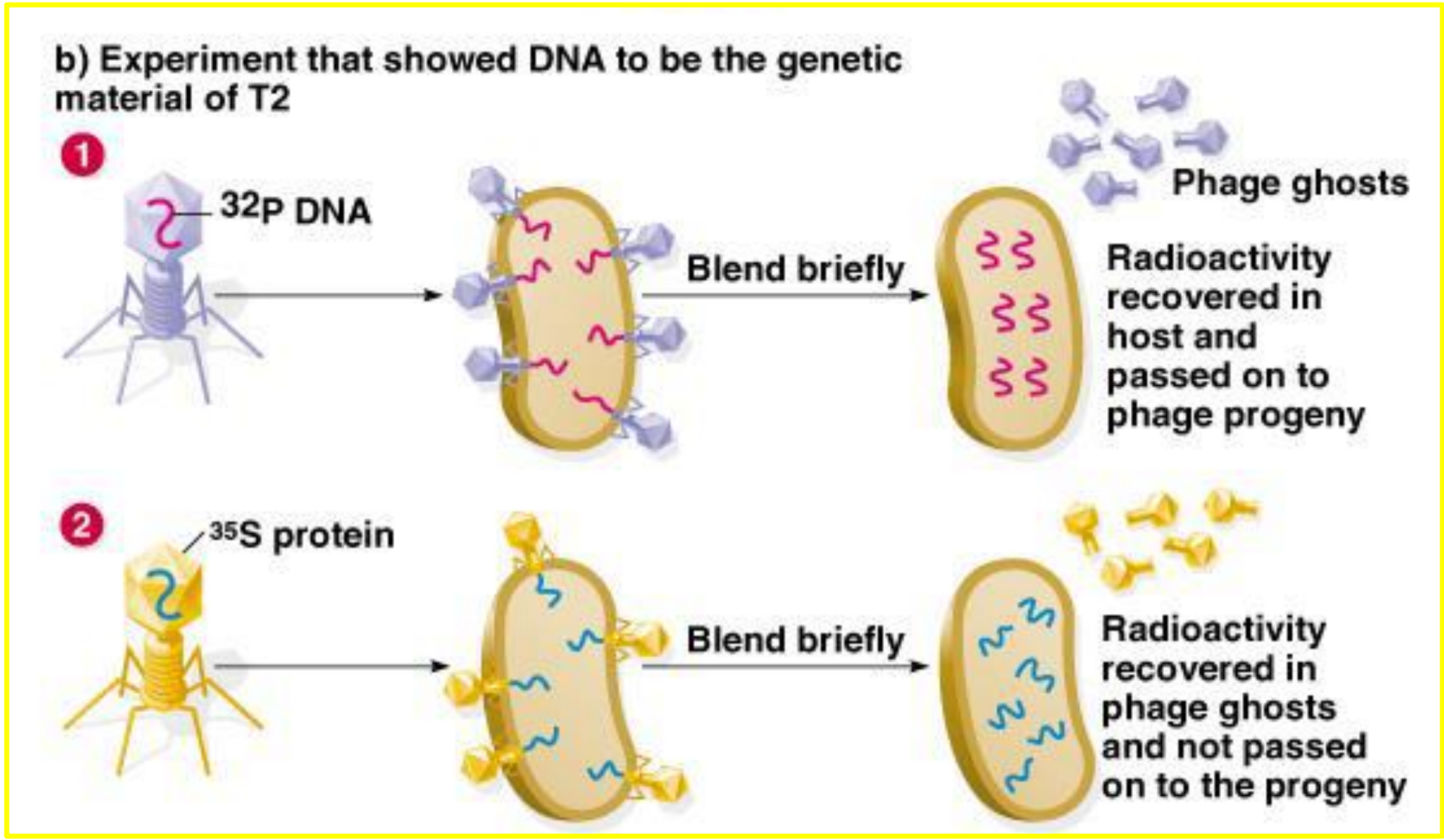
First experiment, the ^{35}S labeled phage

- The pellet which contains the bacteria is separated from supernatant which the phage particles were present.
- It was found that 80% of the radioactivity was associated with supernatant and this supernatant did not generated plaques in the bacterial lawn, which confirms that protein is not the genetic material.

3. Blender Experiments

First experiment, the ^{35}S labeled phage

- **Note**: The phage particles attach very tightly to the bacteria and during blending though the phage particles will be separated, the tail fibers of the phage particles remain associated with bacteria. They account for the 20% of radioactivity in the pellet.

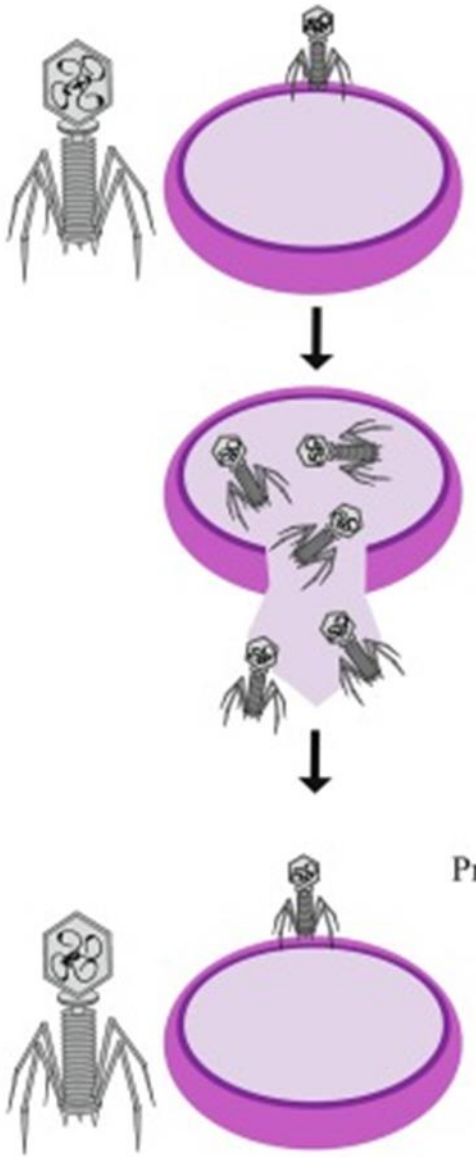

b) Experiment that showed DNA to be the genetic material of T2
1
32P DNA
Blend briefly
Phage ghosts
Radioactivity recovered in host and passed on to phage progeny
2
35S protein
Blend briefly
Radioactivity recovered in phage ghosts and not passed on to the progeny

3. Blender Experiments

Second experiment ^{32}P labeled phage

- In the second experiment the **^{32}P** labeled phages were adsorbed to the bacteria, **70%** of radioactivity is seen with the **pellet** while 30% is seen with supernatant.
- The **pellet** was suspended and applied over bacterial lawn, which produced **plaques** indicating that DNA is the genetic material.
- **Note**: **1/3** of the **radioactivity** seen in the **supernatant** is due to the **breakage** of bacterial cells during **blending** and the rest is due to **defective phage** particles that cannot **infect** the bacteria.

3. Blender Experiments



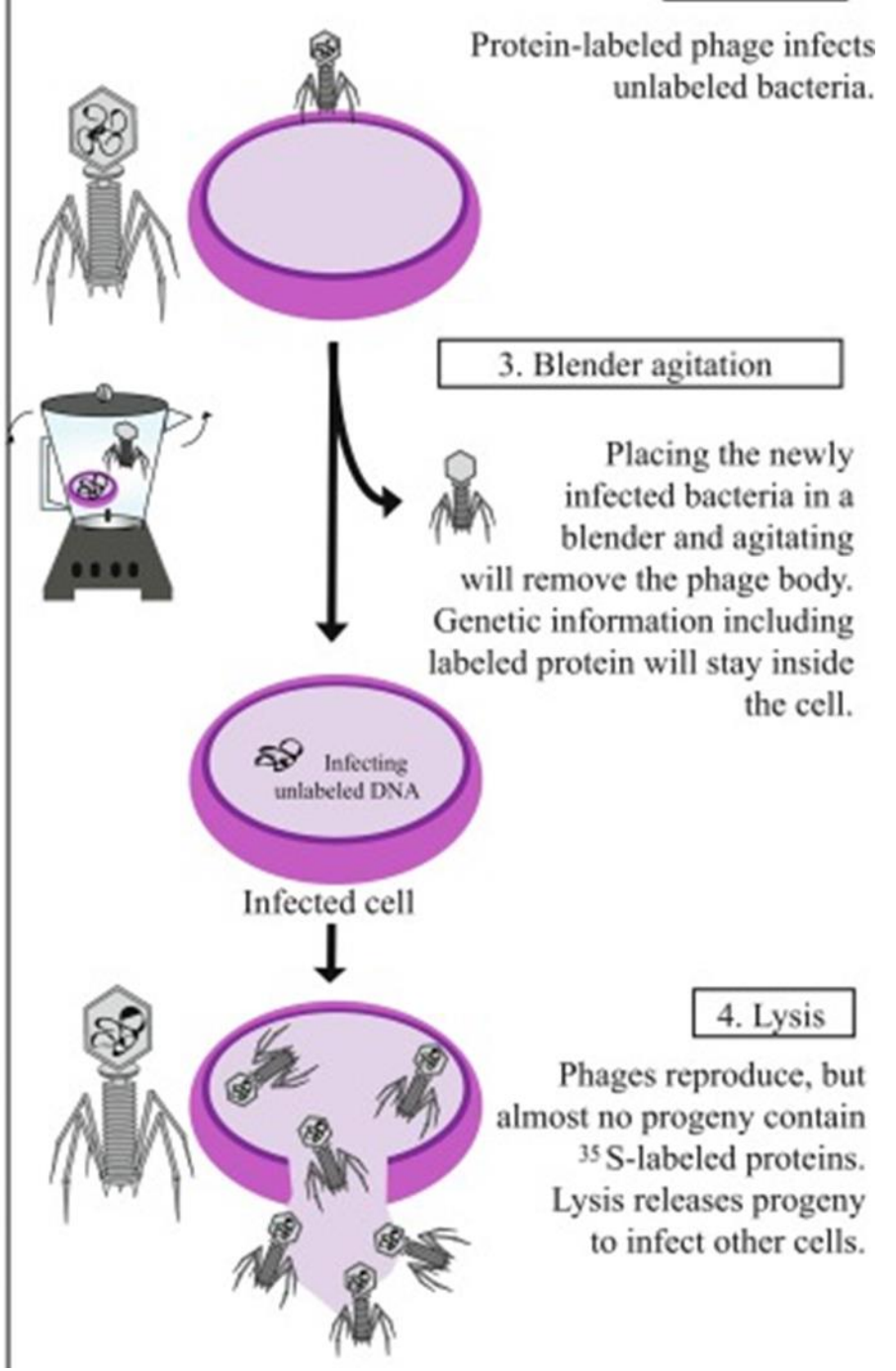
1. Label

Bacteriophage infects *E. coli* cell growing in media containing ^{35}S, which labels proteins.

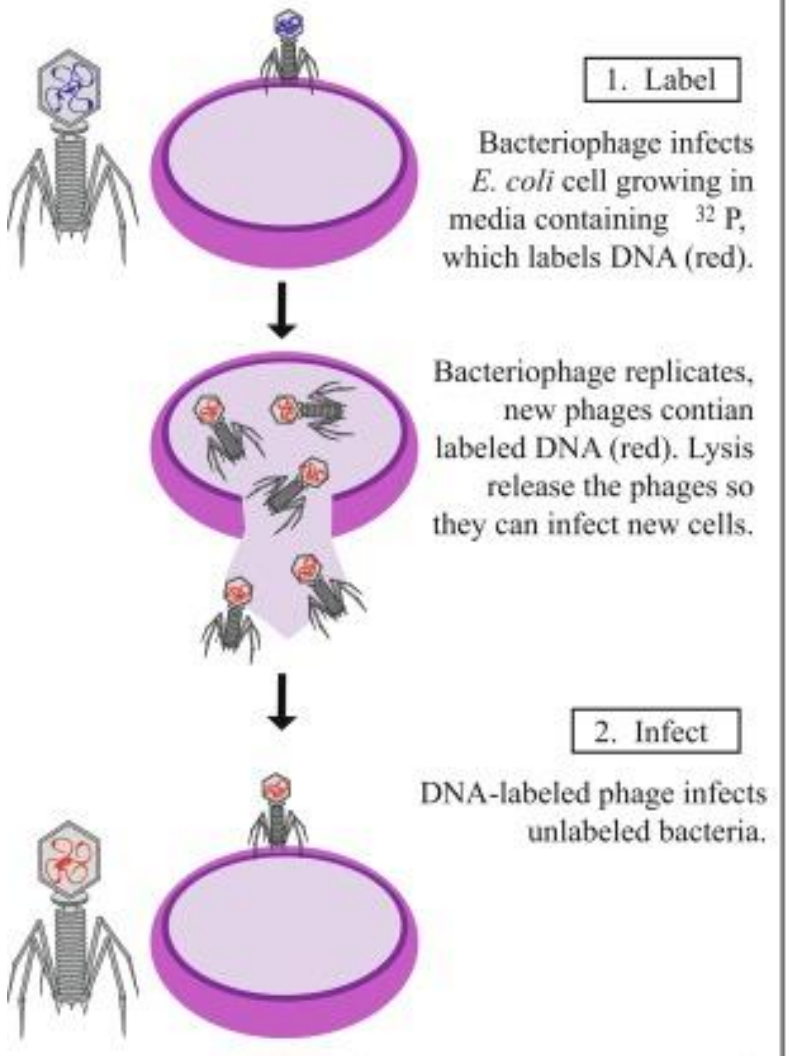
Bacteriophage replicates, new phages contian labeled proteins. Lysis release the phages so they can infect new cells.

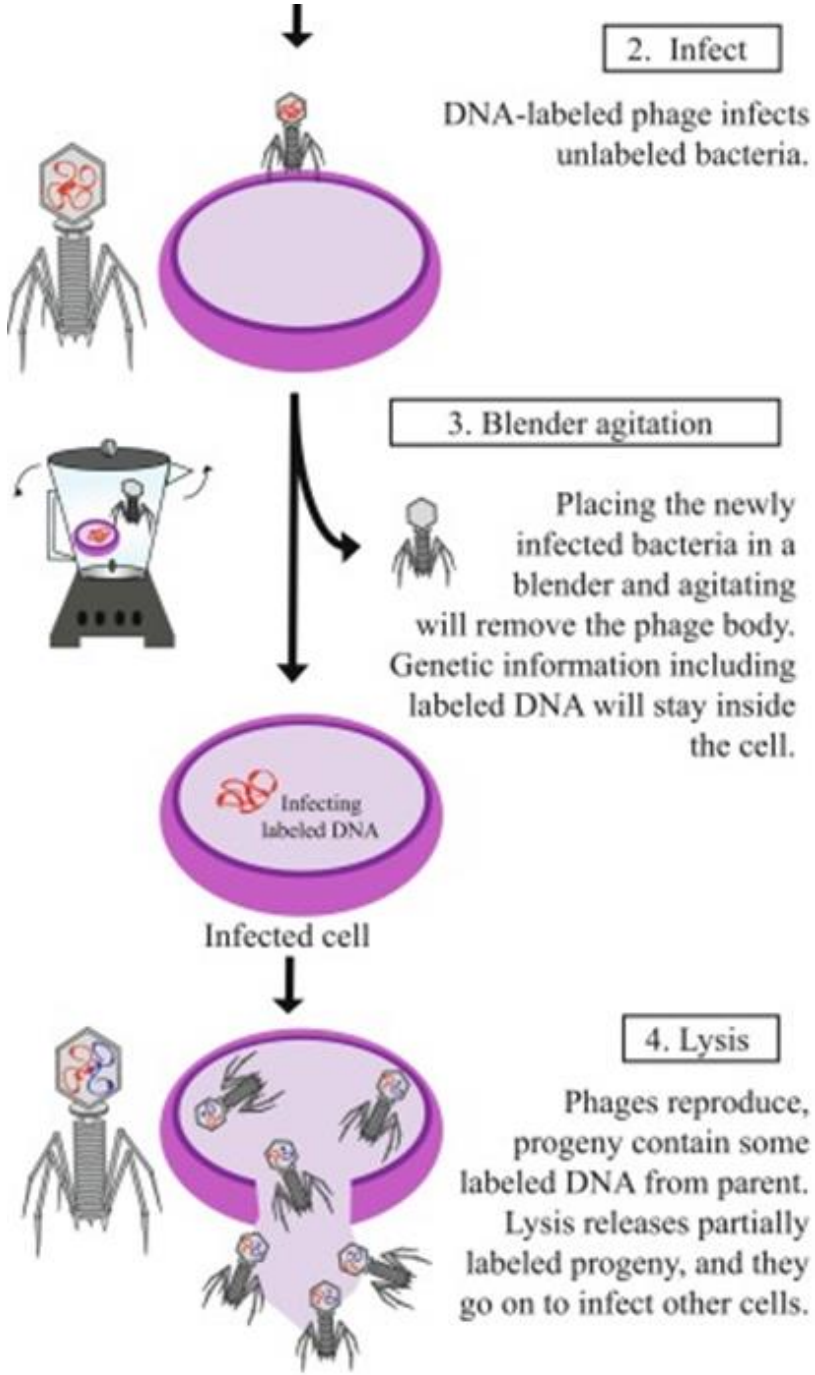
2. Infect

Protein-labeled phage infects unlabeled bacteria.



3. Blender Experiments





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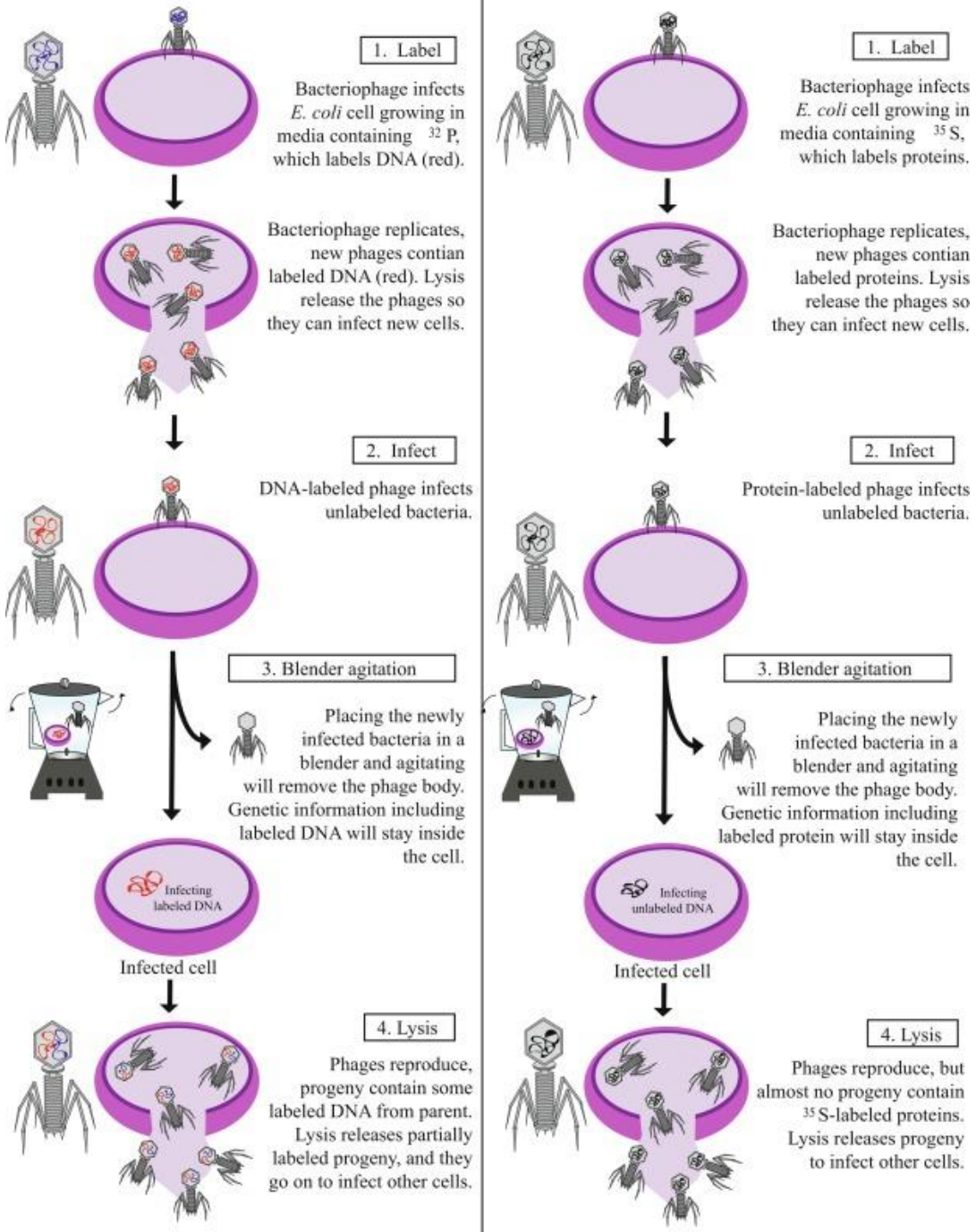

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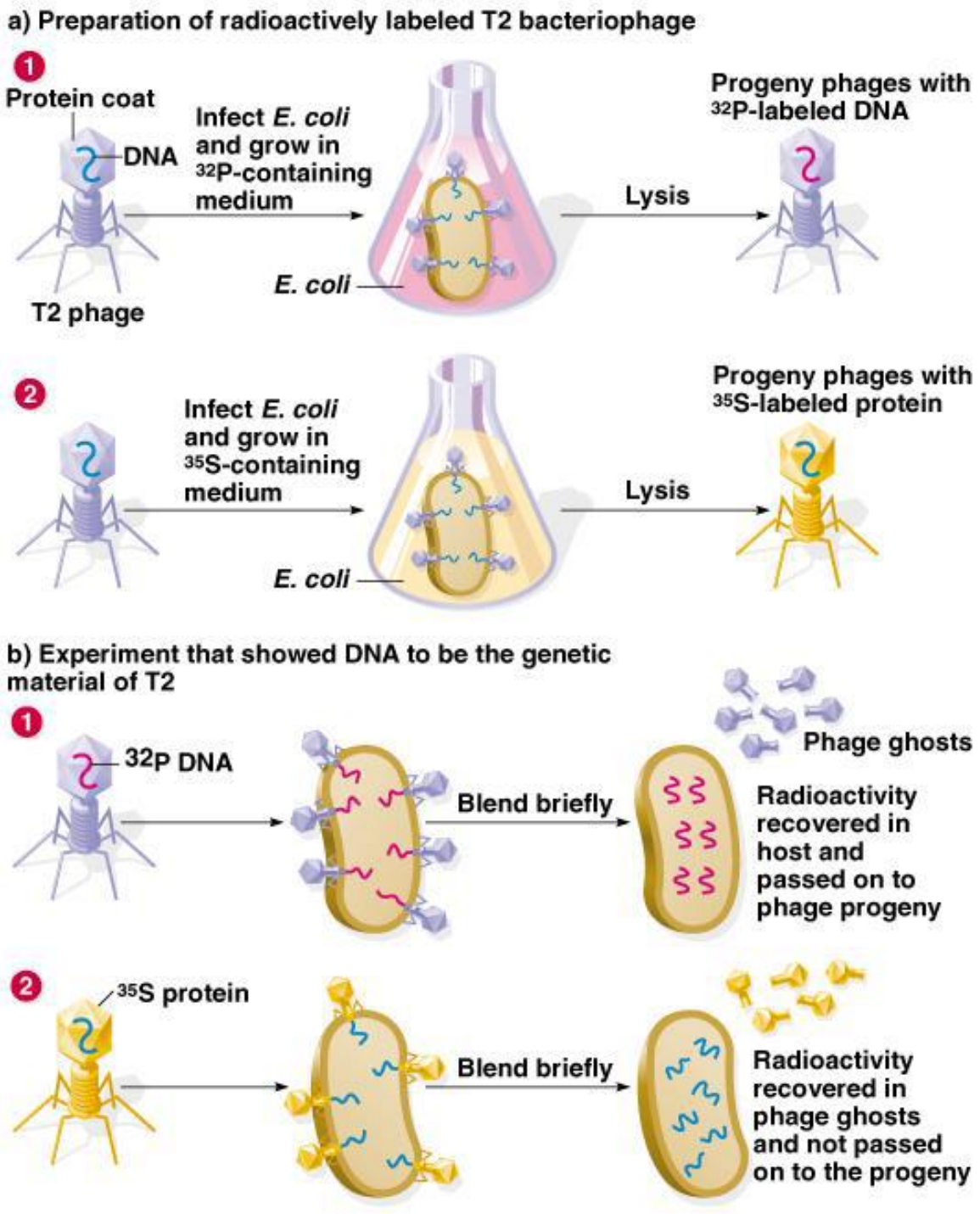


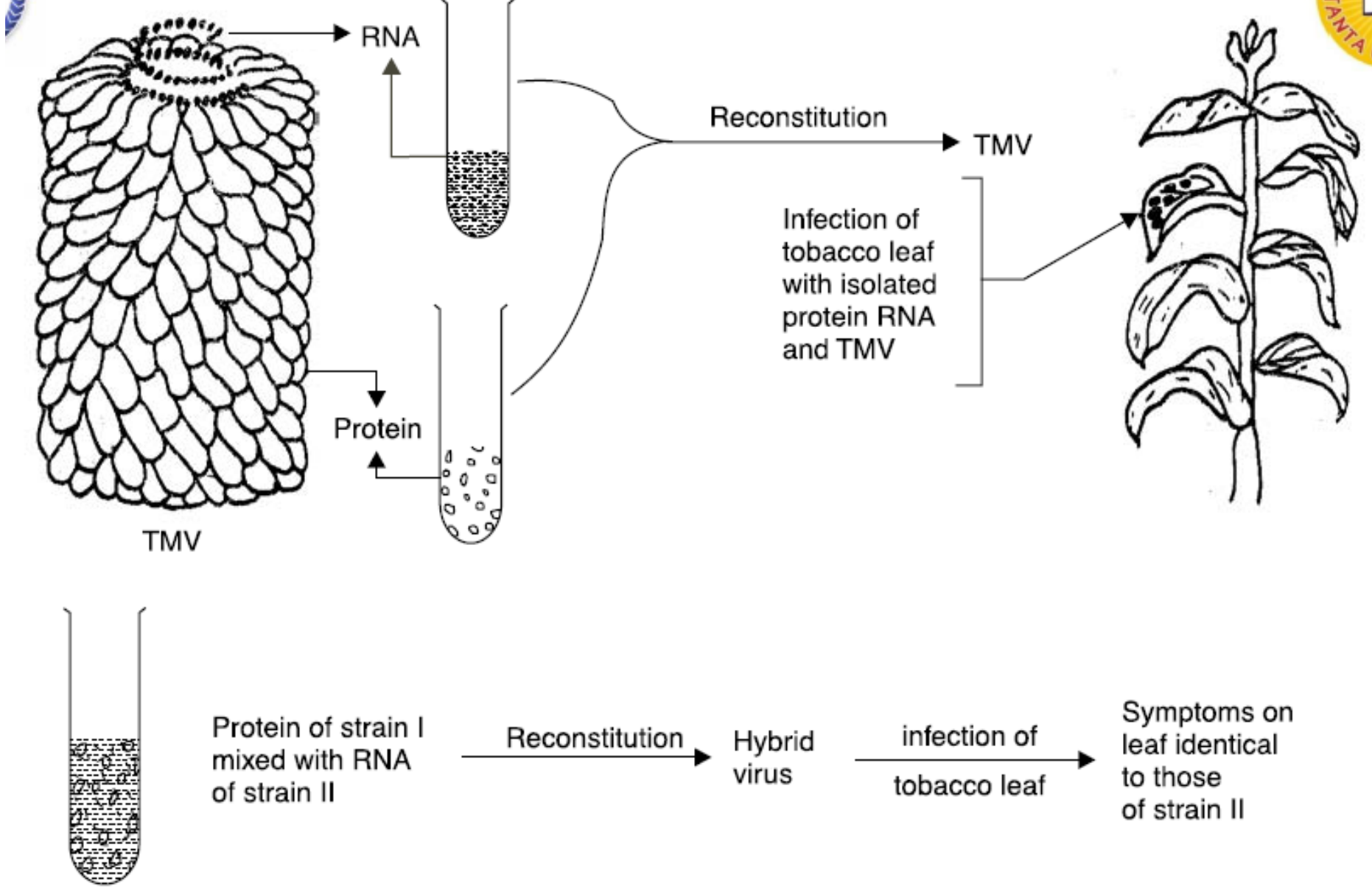
3. Blender Experiments

- **Transfer Experiments:**
- These are extensions of **Blender Experiments.**
- These experiments that support the interpretation that the genetic material in T2 Phage contains **^{32}P** and not **^{35}S**.
- The principle of this experiment is that at least some amount of parental genetic material should be present in the progeny.
- It was found that no ^{35}S but around 50% of ^{32}P is transferred to the progeny.
- This also confirms that DNA is the genetic material.

Genes of some viruses are made of RNA

- **Fraenkel-Conrat and his colleagues in 1950's** found that virus (isolated from infected tobacco plants) consisted of ribonucleoprotein (a compound of protein and RNA).

Genes of some viruses are made of RNA



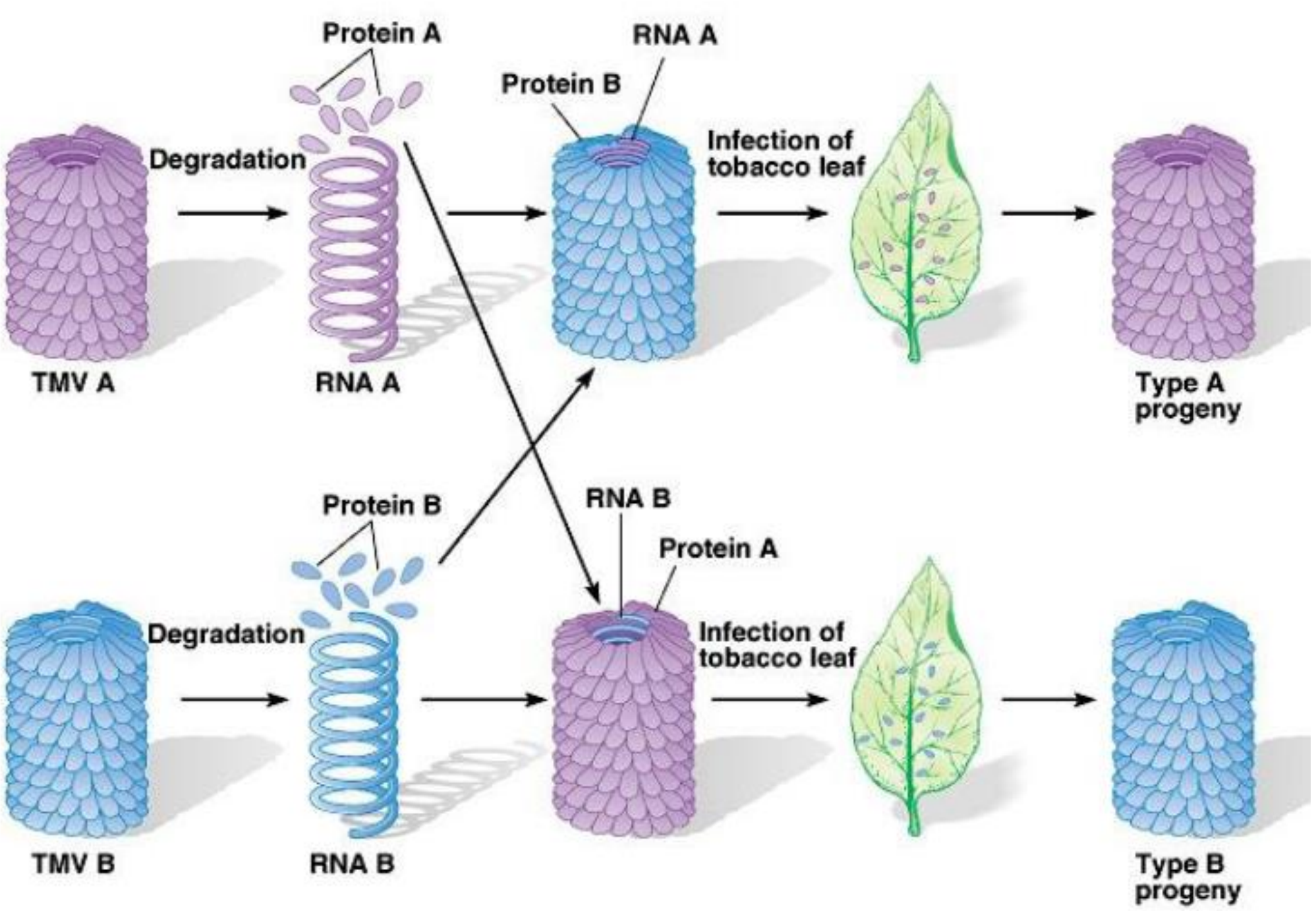
The conclusion was obvious that genes of RNA viruses are made of RNA and not protein

Genes of some viruses are made of RNA

- **Fraenkel-Conrat Experiment**
- 1.Protein and RNA were separated from two tobacco virus strains.
- 2.Hybrid viruses were reformed by combining the protein of one strain with the RNA of the other strain.

Genes of some viruses are made of RNA

Tobacco Mosaic Virus Experiment Fraenkel-Contrat & Singer- 1957 demonstrated that RNA is the genetic material of TMV

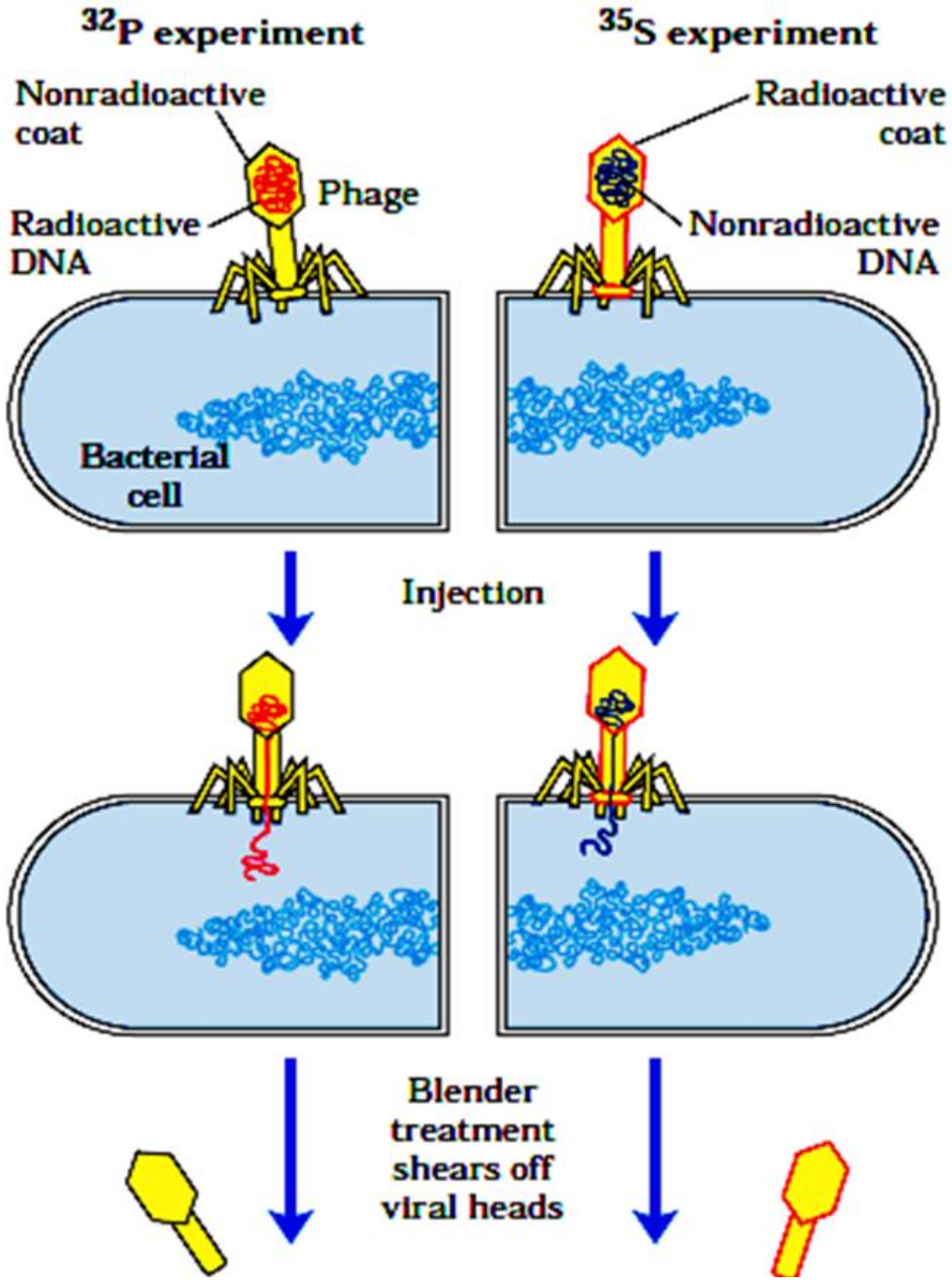


Genes of some viruses are made of RNA

- **Fraenkel-Conrat Experiment**
- 3.Hybrid viruses were used to infect healthy tobacco plants: the new viruses had protein coats identical to the original virus from which RNA had been isolated & symptoms were identical to those of the strain from which RNA had been isolated to reconstitute the hybrid virus.
- 4.The same results were obtained with various combinations of nucleic acid from 4 different strains and of protein from 3 strains.
- This confirms that **RNA** is the **genetic material**.

Thank you

3. Blender Experiments



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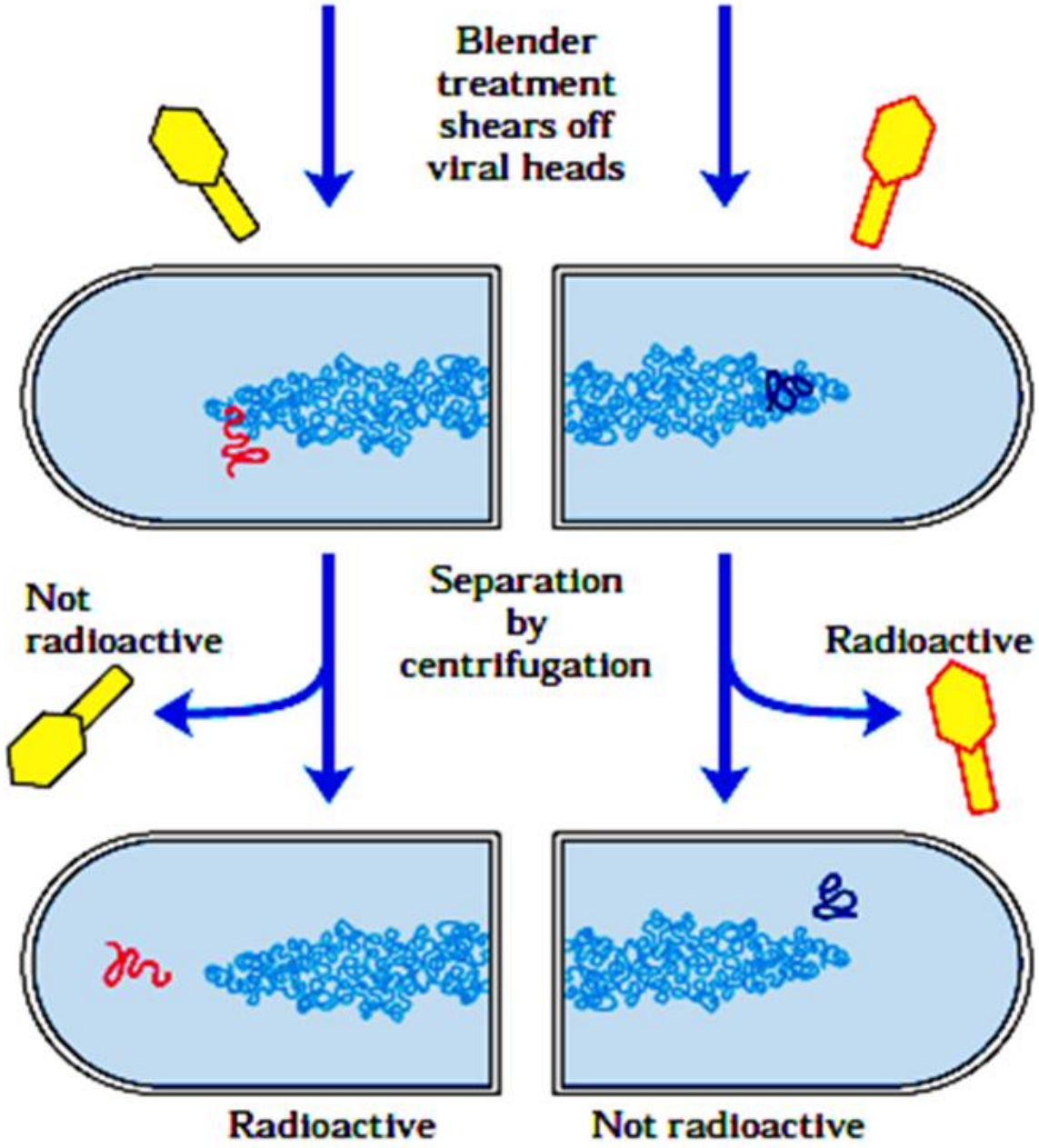

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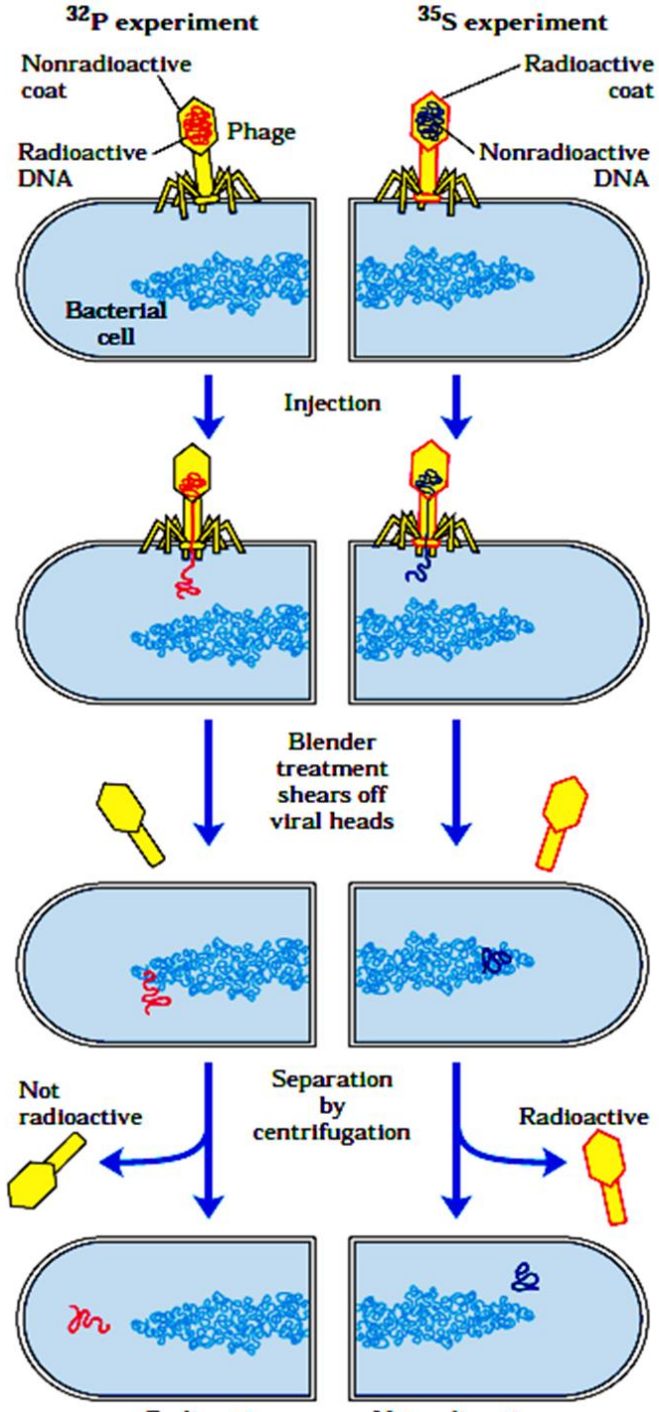

3. Blender Experiments

Outline of Hershey and Chase's Experiment