BACTERIOPHAGES

Department of Microbiology

BACTERIOPHAGES

- Bacteria + Phagein (Greek, to eat gluttonously)
- Viruses that are specific to bacteria and infect bacteria and replicate in bacteria are called bacteriophages.
- Bacteriophages have the general characteristics of viruses.
- They have only one of the nucleic acids, DNA or RNA.

How bacteriophages were discovered?

- **Ernest Hankin (1896):** An English bacteriologist, working in India, observed a mysterious antibacterial activity in the **Ganges** and **Yamuna river** waters that could ***pass through porcelain filters***. He hypothesized the *presence of an unseen agent responsible for preventing cholera outbreaks*.
- **Frederick Twort (1915):** A British bacteriologist, Twort observed a "glassy transformation" in bacterial colonies that he was culturing. He noted that *this agent could be passed from one culture to another, was filterable, and destroyed bacteria*. He hypothesized it might be a virus, an enzyme, or a protozoan. He struggled to publish his findings widely.
- **Félix d'Herelle (1917):** A French-Canadian microbiologist working at the Pasteur Institute in Paris, independently discovered similar *filterable agents that lysed (burst) bacteria*, particularly *Shigella*. He coined the term "**bacteriophage**" to describe these agents. D'Herelle's meticulous work and successful application of phages to treat bacterial infections led to him being widely credited with their definitive discovery. He clearly demonstrated their viral nature and their ability to specifically destroy bacteria.

Frederick Twort 1915

English bacteriologist and was the original discoverer in 1915 of bacteriophages (viruses that infect bacteria)

Felix D'Herelle 1917

French-Canadian microbiologist, the co-discoverer of bacteriophages (viruses that infect bacteria) and experimented with the possibility of phage therapy

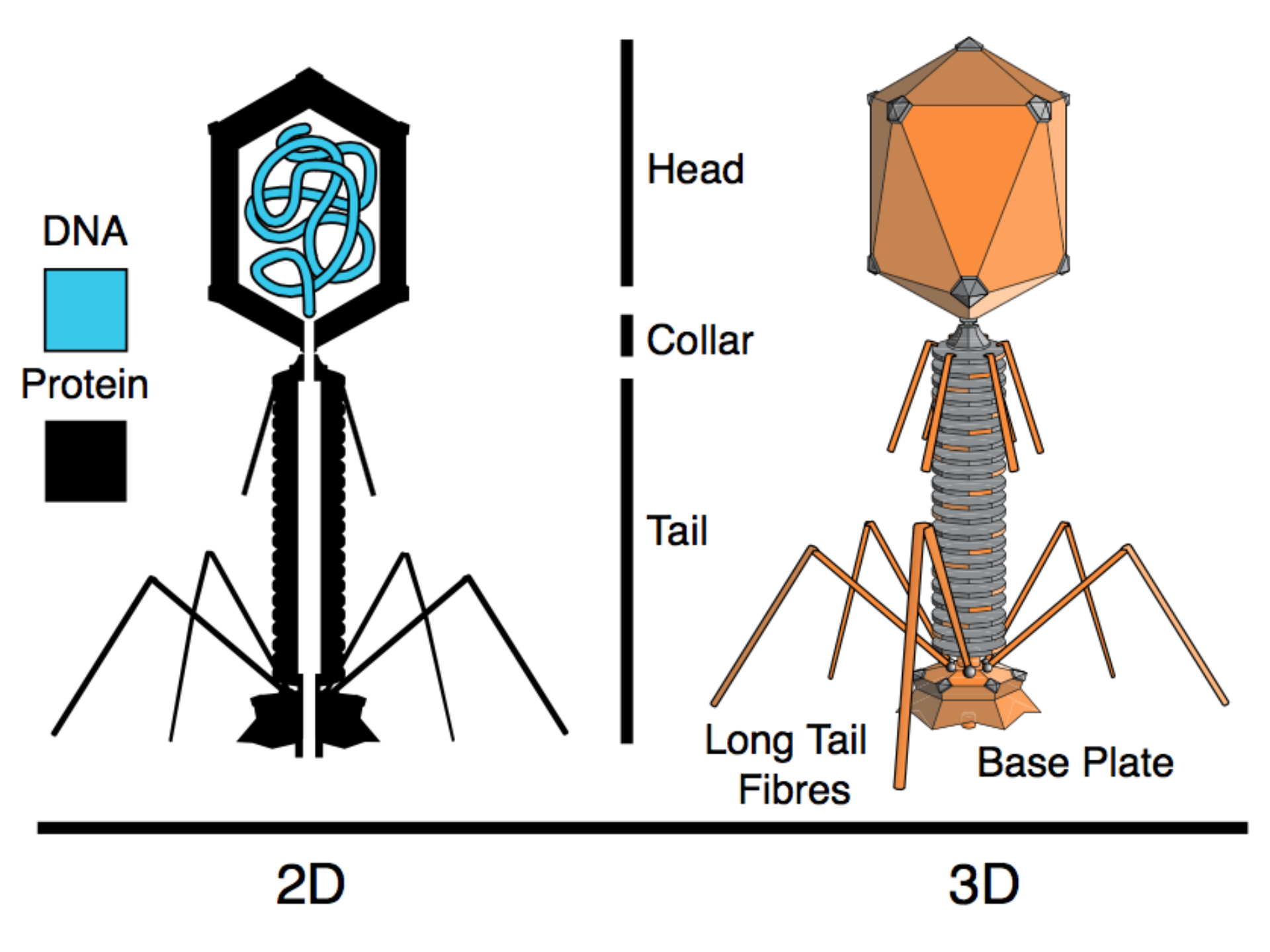
- Depending on the type, they can also have viral structures such as capsids.
- Bacteriophages are classified in various ways due to their,
  - structures,
  - shape,
  - nucleic acid content
- Accordingly, there are enveloped/non-enveloped phages;
- There are DNA phages/RNA phages (single-stranded/double-stranded).

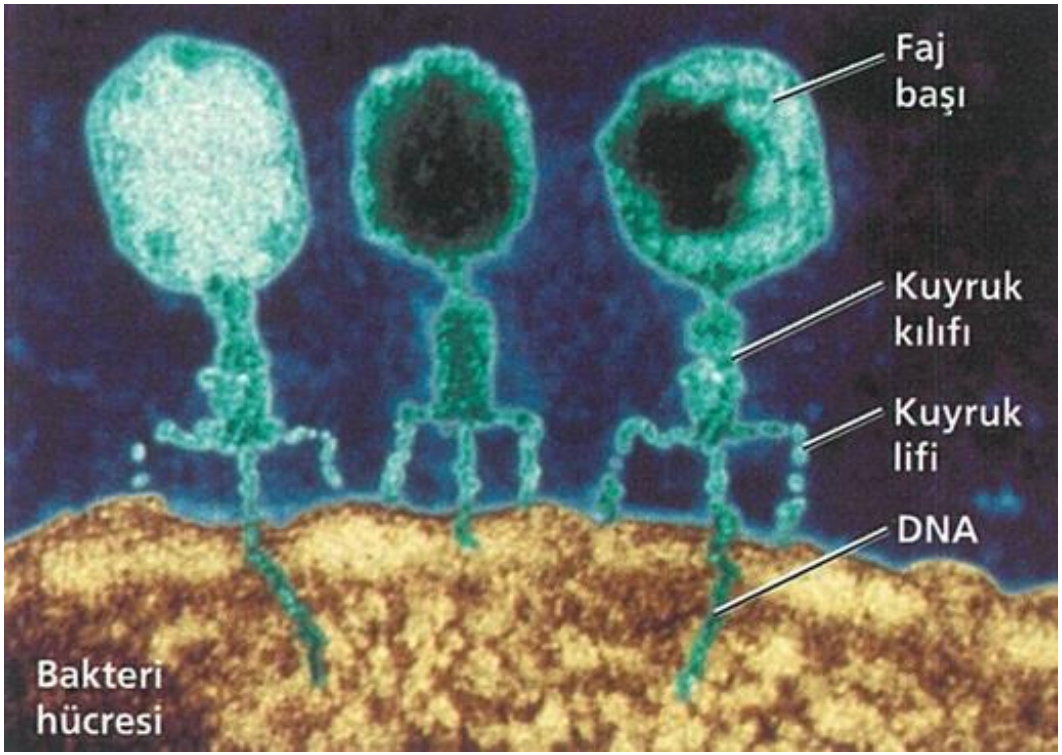
ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	*Myoviridae*	Nonenveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Nonenveloped, noncontractile tail (long)	Linear dsDNA	λ phage, T5 phage, phi, C2, L5, HK97, N15
	Podoviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA	T7 phage, T3 phage, P22, P37
Ligamenvirales	*Lipothrixviridae*	Enveloped, rod-shaped	Linear dsDNA	Acidianus filamentous virus 1
	Rudiviridae	Nonenveloped, rod-shaped	Linear dsDNA	Sulfolobus islandicus rod-shaped virus 1
Unassigned	*Ampullaviridae*	Enveloped, bottle-shaped	Linear dsDNA	
	Bicaudaviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Clavaviridae	Nonenveloped, rod-shaped	Circular dsDNA	
	Corticoviridae	Nonenveloped, isometric	Circular dsDNA	
	Cystoviridae	Enveloped, spherical	Segmented dsRNA	
	Fuselloviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Globuloviridae	Enveloped, isometric	Linear dsDNA	
	Guttaviridae	Nonenveloped, ovoid	Circular dsDNA	
	Inoviridae	Nonenveloped, filamentous	Circular ssDNA	
	Leviviridae	Nonenveloped, isometric	Linear ssRNA	MS2, Qβ
	Microviridae	Nonenveloped, isometric	Circular ssDNA	ΦX174
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA	
	Tectiviridae	Nonenveloped, isometric	Linear dsDNA	

Basic Structure of Bacteriophages

- **Head (Capsid):**
  - **Icosahedral or Polyhedral:** Most phages have an icosahedral (20-sided) or other polyhedral head.
  - **Genetic Material:** Contains the phage's genetic material, which can be DNA (double-stranded or single-stranded) or RNA (though DNA phages are more common and well-studied).
  - **Capsomeres:** The head is made of protein subunits called capsomeres.
- **Collar:**
  - **Neck/Collar:** Connects the head to the tail sheath.
  - **Sheath:** A contractile protein sheath that surrounds the central core. It contracts during infection to push the DNA into the host cell.
  - **Core/Tube:** A hollow tube that runs through the center of the tail, through which the genetic material is injected into the bacterium.
- **Tail:**
  - **Base Plate:**
    - A hexagonal plate at the bottom of the tail.
    - **Tail Pins/Spikes:** Small projections from the base plate that help in attachment.
  - **Tail Fibers:**
    - Long, slender protein fibers extending from the base plate.
    - **Receptor Recognition:** These fibers are crucial for recognizing and binding to specific receptors on the surface of the bacterial cell. This specificity is a key feature of phages.


DNA
Protein
Head
Collar
Tail
Long Tail Fibres
Base Plate
2D
3D


Faj
başı
Kuyruk
kılıfı
Kuyruk
lifi
DNA
Bakteri
hücresi

Where can we find Bacteriophages?

- **Water Environments:**
  - **Oceans:** Phages are incredibly abundant in marine environments, playing a critical role in controlling bacterial populations and nutrient cycling. It's estimated that *phages kill a significant percentage of marine bacteria daily*.
  - **Freshwater:** Rivers, lakes, ponds, and even drinking water systems.
  - **Wastewater and Sewage:** Rich sources of diverse phages due to the high bacterial load.
- **Soil:** A vast and diverse reservoir of phages, interacting with soil bacteria.
- **Animal Gut and Feces:** The gastrointestinal tract of animals, including humans, contains a complex microbiota and a corresponding "**phageome**" (the collection of phages).
- **Fermented Foods:** Foods like yogurt, cheese, and sauerkraut, which rely on bacterial fermentation, also contain phages.
- **Clinical Samples:** Infected tissues, blood, urine, and other bodily fluids from patients with bacterial infections can contain phages targeting the infecting bacteria.
- **Biofilms:** Phages are often found within bacterial biofilms, where they can play a role in their structure and stability.

- There are 1 billion bacteriophages per ml of sea water!!!

- 70% of marine bacteria are infected with bacteriophages!!!

- Phages, which are composed of proteins and have structures such as **capsid** and **envelope**, also **have antigenic properties**.

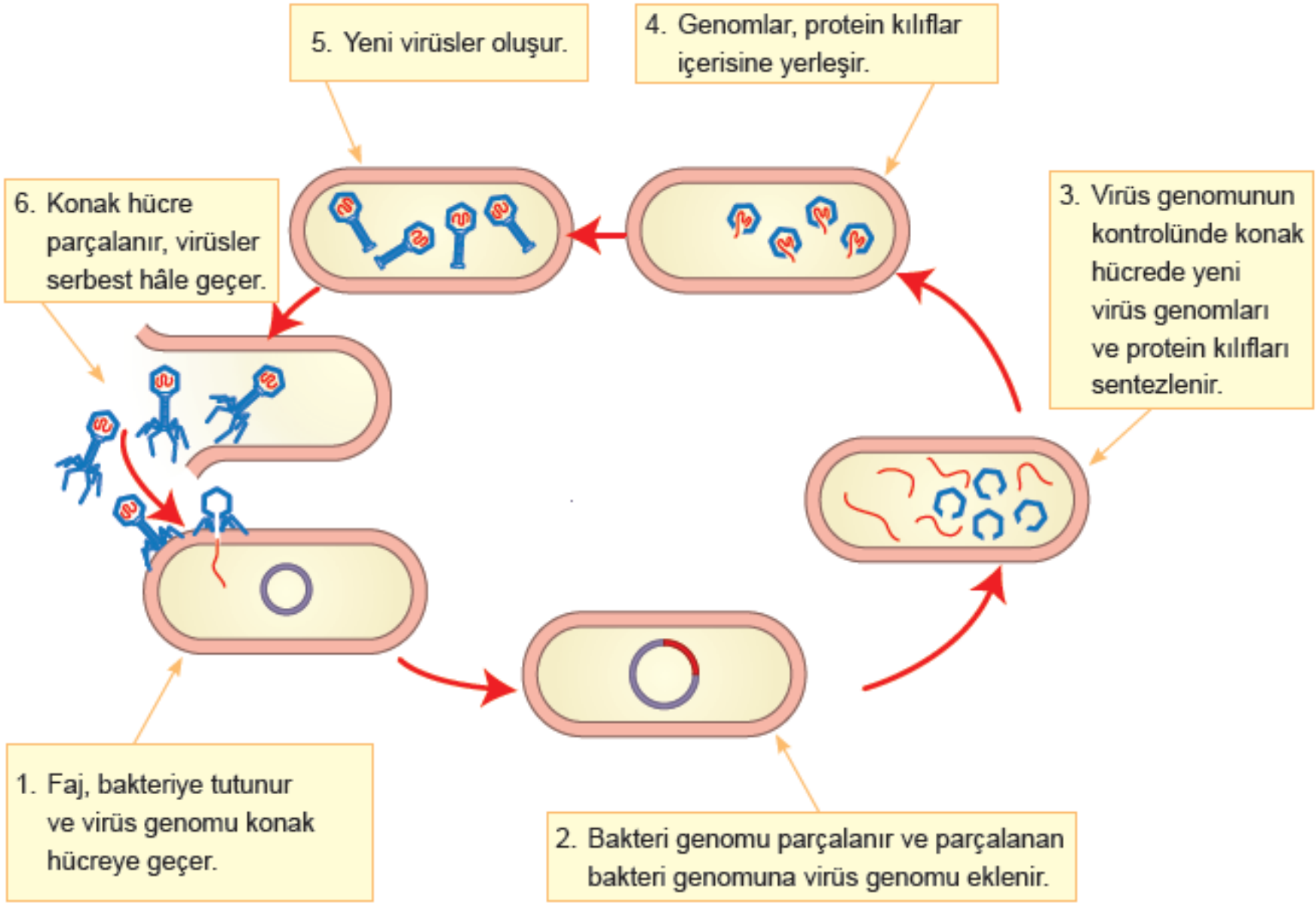
- **Phages have to be inside the bacterial cell to reproduce**.

- They are found in **soil, water sources, intestines of animals**.

- The phage infects and multiplies the bacteria in 4 stages:

- **Adsorption:** It is the **binding step** of the phage on the bacterial cell. Because the surface structure of bacteria is **species-specific**, most phages can infect only a certain species.
- **Penetration:** It is the stage of the phage **inserting its genetic material** or itself into the bacterial cell.

- **Latent period:** During this period, the **genetic material of the phage multiplies** by various pathways and mechanisms, and **phage structures are synthesized and assembled**.
- **Lysis:** This stage is not seen in all phages.
  - Some phages **break out of the bacterial cell after the mature phage particles are formed** and reach a certain number.
  - Some phages can **escape without lysing the cell**.
  - Others **integrate into bacterial DNA** and are **passed on from generation to generation**.



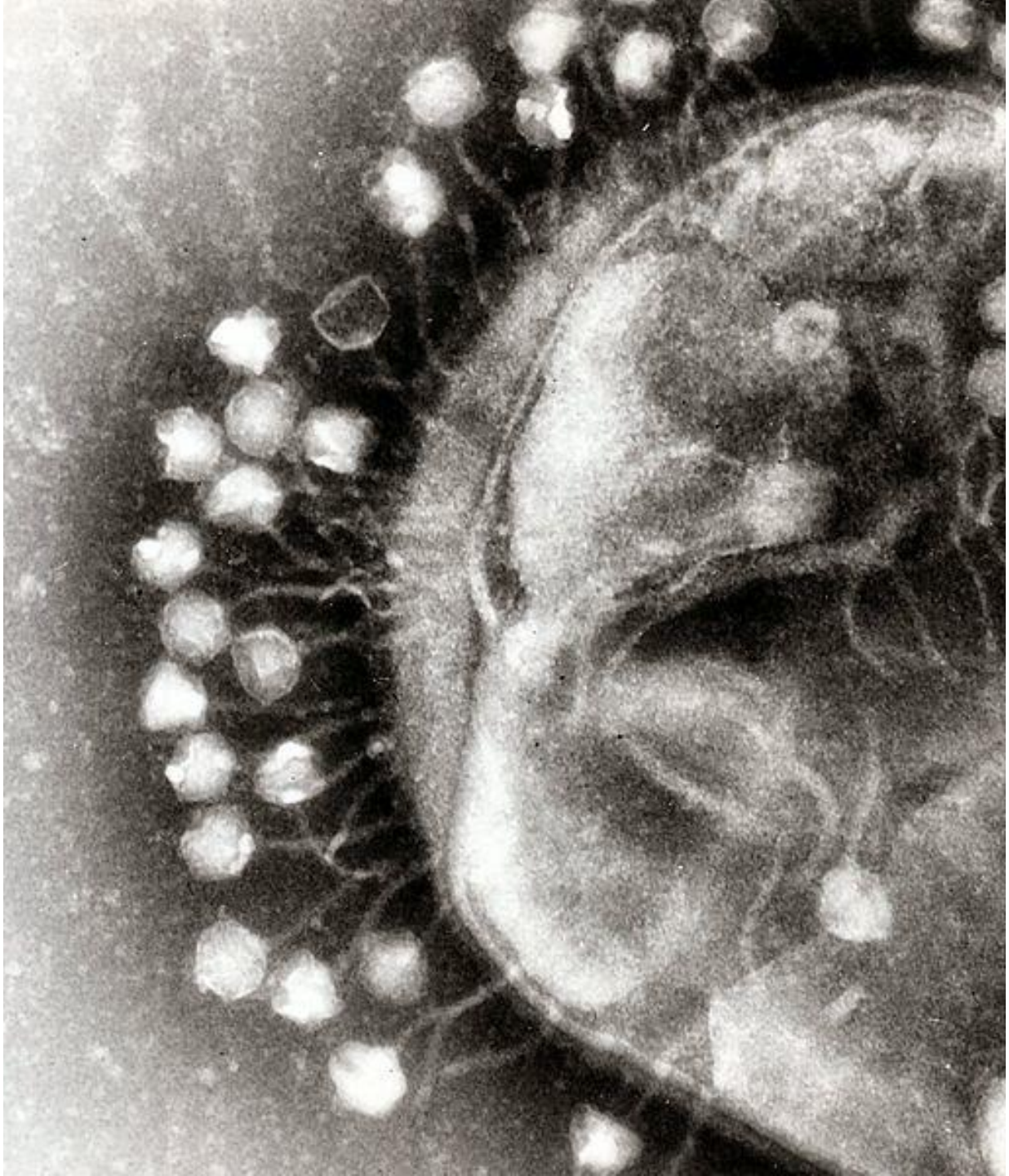
Şekil 1.3.26. Bakteriyofajın hayat döngüsü

Types of infection by phages:

- Phages generally cause 3 types of infection in susceptible bacteria:

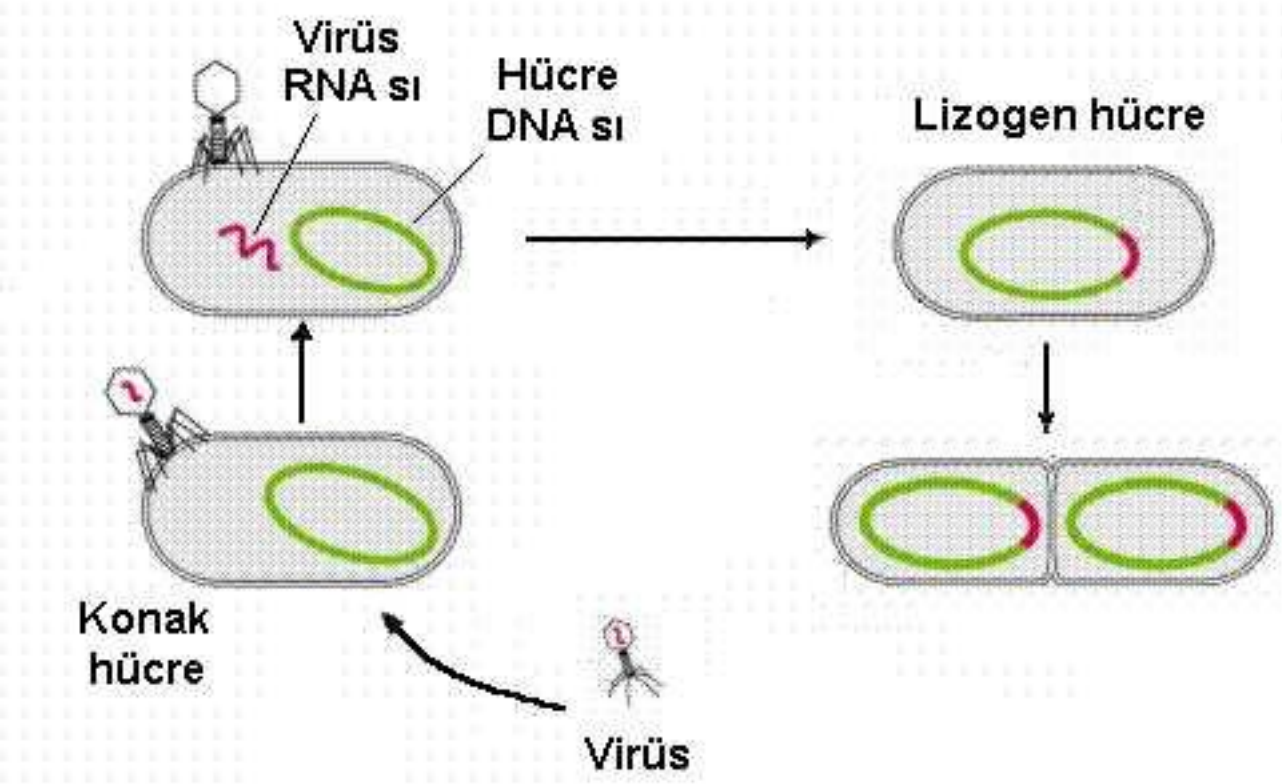
1. Lytic infection: This type of phages **break down the bacteria they infect**. These are also called **virulent phages**. This type of phages are detected by dripping phage liquid on the bacteria grown in solid media. **Plaques** form in areas where phage is instilled. I.e. ***E. coli*** T4 Phage

2. Non-lytic infection: In this type of infections, although the bacteria are invaded by the phage, the phages do not cause the lysis of the bacteria and do not affect the nutrition and reproduction. These types of phages are also called **temperate phages**.



3. Latent infection: In some phages, the phage genetic material integrates into the bacterial genome and becomes a continuation of it. This is also called a **prophage**.

- It replicates together with the bacterial DNA. This situation is called **lysogeny**, and the bacteria in this situation is called **lysogenic bacteria**.
- In this case, phages can become active under some special effects (uv rays, mitomycin) and break down the bacteria and get out of the bacteria. I.e. ***E. coli* lambda phage**


Virüs
RNA sı
Hücre
DNA sı
Lizogen hücre
Konak
hücre
Virüs

Fields of Use of Bacteriophages in Microbiology

1. **Phage Therapy:** The most prominent application, using phages to treat bacterial infections, especially antibiotic-resistant ones (e.g., *MRSA*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*).
2. **Food Safety and Preservation:**
   - **Biocontrol:** Phages can be used to reduce or eliminate pathogenic bacteria (e.g., *Listeria monocytogenes*, *Salmonella*, *E. coli* O157:H7) in food products (meat, poultry, produce) or on food processing surfaces.
   - **Diagnostic Tools:** Phages can be engineered to detect specific bacteria in food samples.
3. **Environmental Biotechnology:**
   - **Bioremediation:** Phages can target and reduce bacterial populations involved in environmental contamination (e.g., degrading pollutants).
   - **Biofouling Control:** Preventing or removing bacterial biofilms from industrial equipment, water pipes, and medical devices.

Fields of Use of Bacteriophages in Microbiology

4. **Agriculture and Aquaculture:**
   - **Plant Disease Control:** Treating bacterial diseases in crops (e.g., fire blight caused by *Erwinia amylovora*).
   - **Animal Health:** Reducing bacterial infections in livestock and aquaculture (e.g., fish farms).
5. **Diagnostic Tools:**
   - **Phage Typing:** Historically used to type bacterial strains based on their susceptibility to a panel of phages.
   - **Detection of Pathogens:** Phages can be used to detect specific bacterial pathogens in clinical samples or environmental samples, often by using engineered phages that produce a detectable signal upon infection.
6. **Molecular Biology Research:**
   - **Vectors for Gene Cloning:** Phages (like lambda phage and M13 phage) are widely used as cloning vectors in genetic engineering.
   - **Genetic Tools:** Understanding phage replication, gene expression, and assembly has provided fundamental insights into molecular biology.
   - **CRISPR-Cas System:** The CRISPR-Cas system, a bacterial adaptive immune system, was first discovered as a defense mechanism against phages. This discovery paved the way for gene-editing technologies.

Advantages of Bacteriophages

1. **Specificity:** Highly specific to their bacterial hosts, meaning they typically don't harm beneficial bacteria (e.g., human gut microbiome) or human cells, unlike broad-spectrum antibiotics.
2. **Self-Replicating and Self-Dosing:** Once they infect bacteria, they replicate, increasing their numbers at the site of infection. This "self-dosing" mechanism can make them very effective.
3. **Overcoming Antibiotic Resistance:** Effective against antibiotic-resistant bacteria, offering a solution to the growing crisis of multidrug-resistant infections.
4. **Penetration of Biofilms:** Many phages produce enzymes (e.g., ***depolymerases***) that can degrade the extracellular polymeric substance (EPS) of biofilms, allowing them to penetrate and destroy bacteria within these protective structures, where antibiotics often struggle.
5. **Low Toxicity:** Generally considered safe for human use as they are non-toxic and do not infect human cells.
6. **Ubiquitous and Renewable Resource:** Easily found in the environment, providing a vast and diverse natural resource for new therapeutic agents.

Disadvantages of Bacteriophages

1. **Narrow Host Range (Specificity):** While an advantage for safety, it's a disadvantage for broad-spectrum treatment. A specific phage might only kill one strain of bacteria, requiring diagnostic testing to identify the infecting strain and select the appropriate phage. This often necessitates "phage cocktails" (mixtures of multiple phages).
2. **Bacterial Resistance:** Bacteria can develop resistance to phages through various mechanisms (e.g., **modifying surface receptors**, **CRISPR-Cas systems**). However, phages can also evolve to overcome this resistance.
3. **Immune Response:** Although phages are generally non-toxic, the human immune system can recognize and clear phage particles, potentially reducing their efficacy over time or causing allergic reactions in some individuals.
4. **Lysogeny and Virulence Genes:** Temperate (lysogenic) phages can integrate their DNA into the host genome and sometimes carry virulence genes that, if transferred to the host bacterium, could make it more pathogenic. Therefore, virulent (lytic) phages are preferred for therapy.
5. **Regulatory Hurdles:** The complex nature of living, replicating biological agents like phages presents significant challenges for regulatory approval (e.g., FDA, EMA) compared to traditional chemical drugs.
6. **Production and Standardization:** Ensuring consistent production, purification, and standardization of phage preparations for clinical use can be challenging due to their biological nature.
7. **Public Perception:** Lack of public awareness or historical skepticism in some regions can be a barrier to acceptance.