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Bacteriophages and Their Structural Organisation

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1. Introduction

Viruses are extremely small infectious particles that are not visible in a light microscope, and are able to pass through fine porcelain filters. They exist in a huge variety of forms and infect practically all living systems: animals, plants, insects and bacteria. All viruses have a genome, typically only one type of nucleic acid, but it could be one or several molecules of DNA or RNA, which is surrounded by a protective stable coat (capsid) and sometimes by additional layers which may be very complex and contain carbohydrates, lipids, and additional proteins. The viruses that have only a protein coat are named “naked”, or non-enveloped viruses. Many viruses have an envelope (enveloped viruses) that wraps around the protein capsid. This envelope is formed from a lipid membrane of the host cell during the release of a virus out of the cell.

Viruses interacting with different types of cells in living organisms produce different types of disease. Each virus infects a certain type of cell which is usually called “host” cell. The major feature of any viral disease is cell lysis, when a cell breaks open and subsequently dies. In multicellular organisms, if enough cells die, the entire organism will endure problems. Some viruses can cause life-long or chronic infections, where the viruses continue to replicate in the body despite the host's defence mechanisms. The other viruses cause lifelong infection because the virus remains within its host cell in a dormant (latent) state such as the herpes viruses, but the virus can reactivate and produce further attacks of disease at any time, if the host's defence system became weak for some reason (Shors, 2008).

Viruses have two phases in their life cycle: outside cells and within the cells they infect. Viral particles outside cells could survive for a long time in harsh conditions where they are inert entities called virions. Outside living cells viruses are not able to reproduce since they lack the machinery to replicate their own genome and produce the necessary proteins. Viruses can infect host cells, recognising their specific receptors on the cell surface. The viral receptors are normal surface host cell molecules involved in routine cellular functions, but since a portion of a molecular complex on the viral surface (typically spikes) has a shape complementary to the shape of the outer soluble part of the receptor, the virus is able to bind the receptor and be attached to the host cell's surface. After receptor-mediated attachment to its host the virus must find a way to enter the cell. Both enveloped and non-enveloped viruses use proteins present on their surfaces to bind to and enter the host cell

employing the endocytosis mechanism (Lopez & Arias, 2010). The endocytic vesicles transport the viral particles to the perinuclear area of the host cell, where the conditions for viral replication are optimal. The other way of infection is to inject only the viral genome (sometimes accompanied by additional proteins) directly into the host cytoplasm.

The viruses are very economical: they carry only the genetic information needed for replication of their nucleic acid and synthesis of the proteins necessary for their reproduction (Alberts et al., 1989). Interestingly, the survival of viruses is totally dependent on the continued existence of their host, since after infection the viral genome switches the entire active host metabolism to synthesise the virion components. Without living host cells viruses will not be able to produce their progeny.

With the discovery of the electron microscope it became possible to study the morphology of viruses. The first studies immediately revealed that viruses could be distinguished by their size and shape, which became the important characteristics of their description. Viruses may be of a circular or oval shape, have the appearance of long thick or thin rods, which could be flexible or stiff. Some viruses have distinctive heads and a tail. The smallest viruses are around 20 nm in diameter and the largest around 500 nm.

The viruses that infect and use bacteria resources are classified as bacteriophages. Often we refer to them as “phages”. The word “bacteriophage” means to eat bacteria, and is so called because virulent bacteriophages can cause the complete lysis of a susceptible bacterial culture. Bacteriophages, like bacteria, are very common in all natural environments and are directly related to the numbers of bacteria present. As a consequence they represent the most abundant ‘life’ forms on Earth, with an estimated 10^{32} bacteriophages on the planet (Wommack & Colwell, 2000). Phages can be readily isolated from faeces and sewage, thus very common in soil. Sequencing of bacterial genomes has revealed that phage genome elements are an important source of sequence diversity and can potentially influence pathogenicity and the evolution of bacteria. The number of phages that have been isolated and characterised so far corresponds to only a tiny fraction of the total phage population. Since bacteriophages and animal cell viruses have many similarities phages are used as model systems for animal cell viruses to study steps of the viral life cycle and to understand the mechanisms by which bacterial genes can be transferred from one bacterium to another.

1.1 Discovery of bacteriophages

Bacteriophages were discovered more than a century ago. In 1896, Ernest Hanbury Hankin, a British bacteriologist (1865–1939), reported that something in the waters of rivers in India had unexpected antibacterial properties against cholera and this water could pass through a very fine porcelain filter and keep this distinctive feature (Hankin, 1896). However, Hankin did not pursue this finding. In 1915, the British bacteriologist Frederick Twort (1877–1950), Superintendent of the Brown Institution of London, discovered a small agent that killed colonies of bacteria in growing cultures. He published the results but the subsequent work was interrupted by the beginning of World War I and shortage of funding. Felix d'Herelle (1873–1949) discovered the agent killing bacteria independently at the Pasteur Institute in France in 1917. He observed that cultures of the dysentery bacteria disappear with the addition of a bacteria-free filtrate obtained from sewage. D'Herelle has published his discovery of “an invisible, antagonistic microbe of the dysentery bacillus” (d'Herelle, 1917).

In 1923, the Eliava Institute was opened in Tbilisi, Georgia, to study bacteriophages and to develop phage therapy. Since then many scientists have been involved in developing techniques to study phages and using them for various purposes. In 1969 Max Delbrück, Alfred Hershey and Salvador Luria were awarded the prestigious Nobel Prize in Physiology and Medicine for their discoveries of the replication of viruses and their genetic structure.

1.2 Why do we need to study bacteriophages?

The first serious research of phages was done by d'Herelle which inspired him to do first experiments using phages in medicine. D'Herelle has used phages to treat a boy who had bad dysentery (d'Herelle, 1917). After administration of phages the boy successfully recovered. Later d'Herelle and scientists from Georgia (former USSR) have created an Institute to study the properties of bacteriophages and their use in treating bacterial infections a decade before the discovery of penicillin. Unfortunately a lack of knowledge on basic phage biology and their molecular organisation has led to some clinical failures. At the end of 1930s antibiotics were discovered; they were very effective, and nearly wiped out studies on the medical use of phages. However, a new problem of bacterial resistance to antibiotics has arisen after many years of using them. Bacteria adapted themselves to become resistant to the most potent drugs used in modern medicine. The emergence of modified pathogens such as *Mycobacterium tuberculosis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, and methicillin-resistant *S. aureus* (MRSA) has created massive problems in treating patients in hospitals (Coelho et al., 2004; Hanlon, 2007; Burrowes et al., 2011) and the time required to produce new antibiotics is much longer than the time of bacterial adaptation. Modern studies on the phage life cycle have revealed a way for their penetration through membrane barriers of cells. These results are important in the development of methods for using bacteriophages as a therapeutic option in the treatment of bacterial infections (Brussow & Kutter, 2005). Phages, like many other viruses, infect only a certain range of bacteria that have the appropriate receptors in the outer membrane. The antibiotic resistance of the bacteria does not affect the infectious activity of a phage. Knowledge of the phage structure, understanding the mechanism of phage-cell surface interaction, and revealing the process of switching the cell replication machinery for phage propagation would allow the design of phages specific for bacterial illnesses.

2. Classification of bacteriophages

All known phages can be divided in two groups according to the type of infection. One group is characterised by a lytic infection and the other is represented by a lysogenic, or temperate, type of infection (Figure 1). In the first form of infection the release of DNA induces switching of the protein machinery of the host bacterium for the benefit of infectious agents to produce 50-200 new phages. To make so many new phages requires nearly all the resources of the cell, which becomes weak and bursts. In other words, lysis takes place, causing death of the host bacterial cell. As result new phages are released into the extracellular space. The other mode of infection, lysogenic, is characterised by integration of the phage DNA into the host cell genome, although it may also exist as a plasmid. Incorporated phage DNA will be replicated along with the host bacteria genome and new bacteria will inherit the viral DNA. Such transition of viral DNA could take place

through several generations of bacterium without major metabolic consequences for it. Eventually the phage genes, at certain conditions impeding the bacterium state, will revert to the lytic cycle, leading to release of fully assembled phages (Figure 1). Analysis of phages with lysogenic or lytic mode of infection has shown that there is a tremendous variety of bacteriophages with variations in properties for each type of infection. Moreover, under certain conditions, some species were able to change the mode of infection, especially if the number of host cells was falling down. Temperate phages are not suitable for the phage therapy.

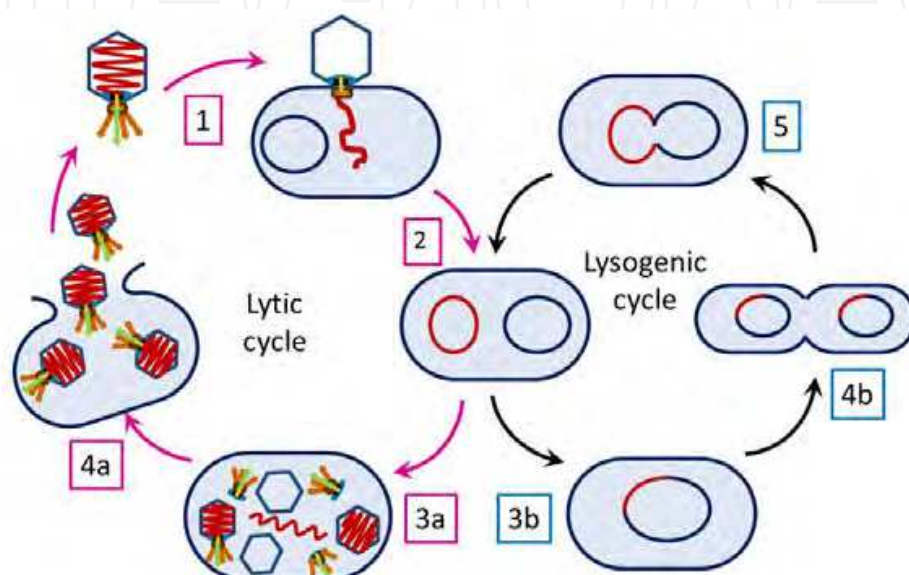


Fig. 1. Two cycles of bacteriophage reproduction. 1 - Phage attaches the host cell and injects DNA; 2 - Phage DNA enters lytic or lysogenic cycle; 3a - New phage DNA and proteins are synthesised and virions are assembled; 4a - Cell lyses releasing virions; 3b and 4b - steps of lysogenic cycle: integration of the phage genome within the bacterial chromosome (becomes prophage) with normal bacterial reproduction; 5- Under certain conditions the prophage excises from the bacterial chromosome and initiates the lytic cycle. (Copyright of E.V. Orlova)

Classification of viruses is based on several factors such as their host preference, viral morphology, genome type and auxiliary structures such as tails or envelopes. The most up-to-date classification of bacteriophages is given by Ackermann (2006). The key classification factors are phage morphology and nucleic acid properties. The genome can be represented by either DNA or RNA. The vast majority of phages contain double strand DNA (dsDNA), while there are small phage groups with ssRNA, dsRNA, or ssDNA (ss stands for single strand). There are a few morphological groups of phages: filamentous phages, isosahedral phages without tails, phages with tails, and even several phages with a lipid-containing envelope or contain lipids in the particle shell. This makes bacteriophages the largest viral group in nature. At present, more than 5500 bacterial viruses have been examined in the electron microscope (Ackermann, 2007) (Figure 2).

Pleomorphic and filamentous phages comprise ~190 known bacteriophages (3.6% of phages) and are classified into 10 small families (Ackermann, 2004). These phages differ significantly in their features and characteristics apparently representing different lines of origin. Pleomorphic phages are characterized by a small number of known members that are divided into three

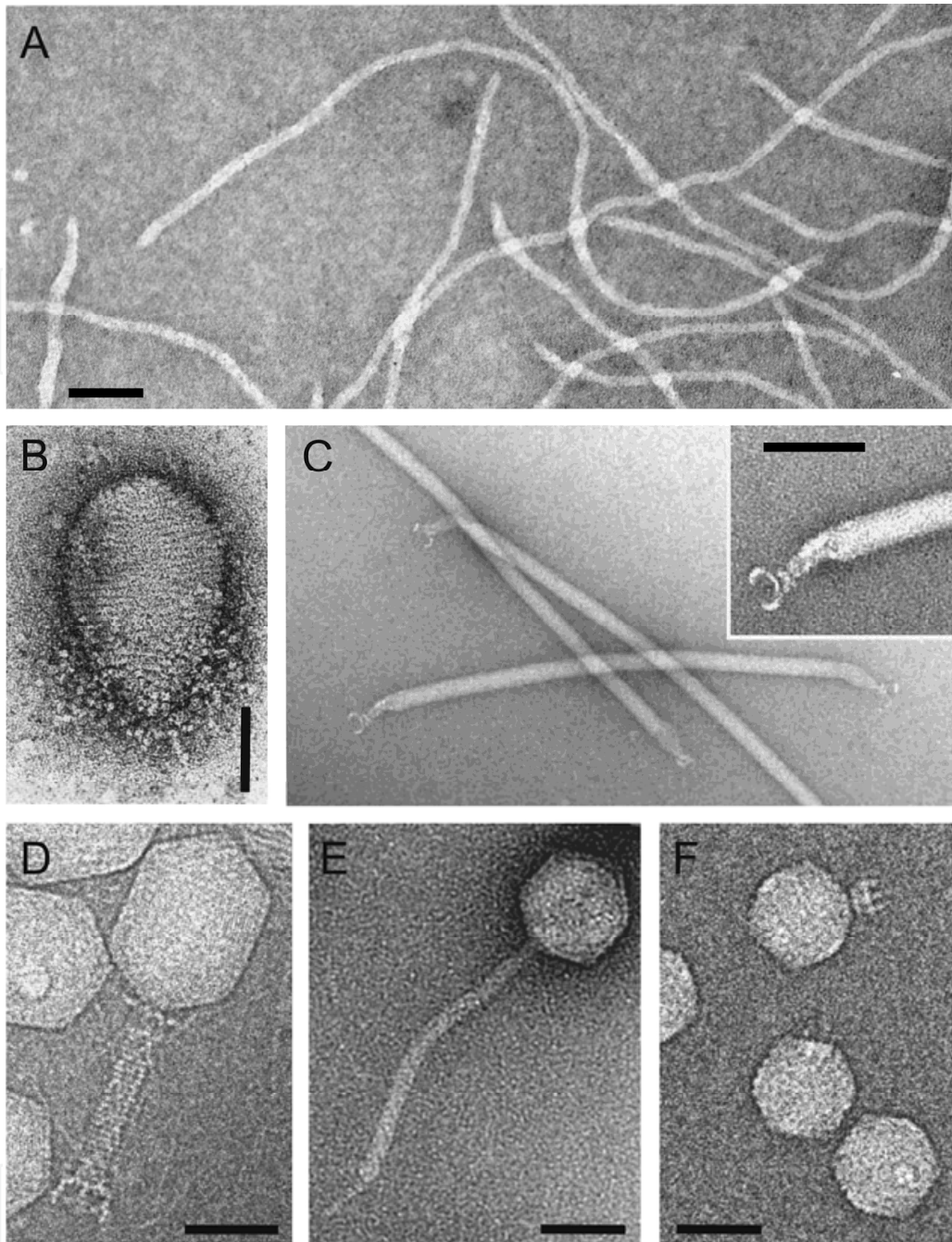


Fig. 2. Images of bacteriophages. A – filamentous phage B5 (*Inoviridae*) infects *Propionibacterium freudenreichi*; negatively stained with 2% uranyl acetate (UA) (Chopin et al., 2002, reproduced with permission of M.C. Chopin); B – *Sulfolobus neozealandicus* droplet-shaped virus (*Guttaviridae*) of the crenarchaeotal archaeon *Sulfolobus*, negatively stained by 2% UA (Arnold et al., 2000, reproduced with permission of W. Zillig); C – *Acidianus* filamentous virus 1 (*Lipothrixviridae*) with tail structures in their native conformation, negatively stained with 3% UA (Bettstetter et al., 2003, reproduced with permission of D. Prangishvili); D – Bacteriophage T4 (*Myoviridae*), in vitreous ice (Rossmann et al., 2004, reproduced with permission of M.G. Rossmann); E – Bacteriophage SPP1 (*Siphoviridae*), negative stain 2% UA (Lurz et al., 2001, reproduced with permission of R. Lurz); F – Bacteriophage P22 (*Podoviridae*) in vitreous ice (Chang et al., 2006, reproduced with permission of W. Chiu). Bars are 50 nm.

families that need further characterization. *Plasmaviridae* (dsDNA) includes phages with dsDNA that are covered by a lipoprotein envelope and therefore can be called a nucleoprotein granule. Members of the *Fusseloviridae* family have dsDNA inside a lemon-shaped capsid with short spikes at one end; *Guttavirus* phage group (dsDNA) is represented by droplet-shaped virus-like particles (Figure 2B, Arnold et al., 2000).

There are phages with helical or filamentous organization. The *Inoviridae* (ssDNA) family includes phages that are long, rigid, or flexible filaments of variable length and have been classified by particle length, coat structure and DNA content. The *Lipothruxviridae* (dsDNA) phages are characterized by the combination of a lipoprotein envelope and rod-like shape (Figure 2C). The *Rudiviridae* (dsDNA) family represents phages that are straight rigid rods without envelopes and closely resemble the tobacco mosaic virus.

The next group of phages have capsids with an isosahedral shape. Phages from the *Leviviridae* family have ssRNA genome packed in small capsids and resemble enteroviruses. The known phages that form *Corticoviridae* family contain three molecules of dsRNA and, which is unusual, RNA polymerase. Phages with icosahedral symmetry for the capsids and a DNA genome compose the next three families *Microviridae*, *Cystoviridae* and *Tectiviridae*. The first includes small virions with a single circular ssDNA. The second family is currently represented only by a maritime phage, PM2, and has a capsid formed by the outer layer of proteins with an inner lipid bilayer (Huiskonen et al., 2004). The capsid contains a dsDNA genome. The last family, *Tectiviridae*, is characterised by presence of the lipoprotein vesicle that envelops the protein capsid with dsDNA genome. These phages have spikes on the apical parts of the envelope.

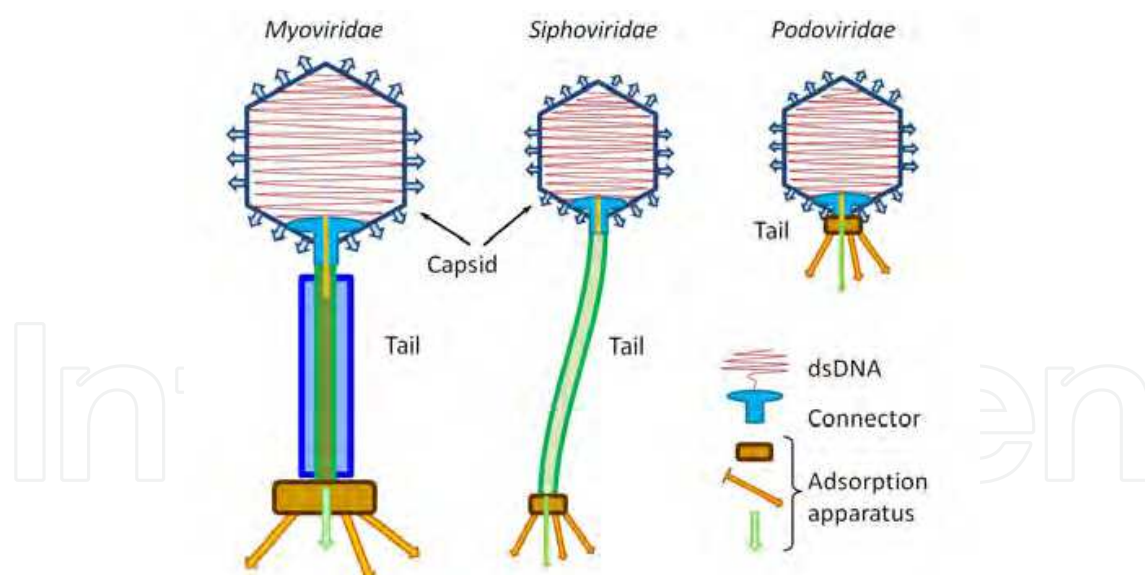


Fig. 3. Tailed phage families (copyright of E.V. Orlova).

The tailed phages were classified into the order *Caudovirales* (dsDNA) (Figure 2D,E,F) (Ackermann, 2006). Tailed phages can be found everywhere and represent 96% of known phages and are separated into three main phylogenetically related families. Tailed phages are divided into three families: A - *Myoviridae* with contractile tails consisting of a sheath and a central tube (25% of tailed phages); B - *Siphoviridae*, long, noncontractile tails (61%); C - *Podoviridae*, short tails (14 %). Since the tailed phages represent the biggest population of

bacteriophages they are easy to find and purify; they are the most studied family both biochemically and structurally. For this reason the following part of the review will concentrate on results and analysis of the tailed bacteriophages.

3. Organisation of tailed bacteriophages

3.1 General architecture of bacteriophages

The basic structural features of bacteriophages are coats (or capsids) that protect the genome hidden inside a capsid and additional structures providing interface with a bacterium membrane for the genome release. The *Caudovirales* order of bacteriophages is characterised by dsDNA genomes and by the common overall organisation of the virus particles characterized by a capsid and a tail (Figure 3). Different phage species can vary both in size from 24-400 nm in length and genome length. Their DNA sequences differ significantly and can range in the size from 18 to 400 kb in length.

Structures obtained by electron microscopy (EM) do not typically provide detailed information on the atomic components owing to methods used for visualisation of particles. However, EM has allowed visualisation of these minuscule particles and morphological analysis. Each virion has a polyhedral, predominantly icosahedral, head (capsid) that covers the genome. The heads are composed of many copies of one or several different proteins and have a very stable organisation. A bacteriophage tail is attached to the capsid through a connector which serves as an adaptor between these two crucial components of the phage. The connector is a hetero-oligomer composed of several proteins (Lurz et al., 2001; Orlova et al., 2003). Connectors carry out several functions during the phage life cycle. They participate in the packaging of dsDNA into the capsid, and later they perform the function of a gatekeeper: locking the capsid exit of the phage, preventing leakage of DNA which is under high pressure and later, after a signal transmitted by the tail indicating that the phage is attached to the bacterium, the connector will be open allowing the release of DNA into the bacterium (Plisson et al., 2007). The tail and its related structures are indispensable phage elements securing the entry of the viral nucleic acid into the host bacterium during the infectivity process. The tail serves both as a signal transmitter and subsequently as a pipeline through which DNA is delivered into the host cell during infection. The tails may be short or long, the latter are divided into contractile and non-contractile tails. The long tails are typically composed of many copies of several proteins arranged with helical symmetry. All types of tails have outer appendages attached to the distant end of the tail and often include a baseplate with several fibres and a tip, or a needle that has specificity to the membrane receptors of the bacterium (Leiman et al., 2010). As soon the receptor has been found by the tail needle, which happens during multiple short living reversible attachments to the bacterium, the baseplate and tail fibres are involved in the binding of the phage to the bacterial outer membrane that makes the attachment irreversible (Christensen, 1965; São-José et al., 2006). The docking (irreversible attachment) of the phage induces opening of the phage connector and release of the genome through the tail tube into the bacterial cell.

3.2 DNA and its packaging

The virions of the bacteriophage *Caudovirales* have a genome represented by linear molecules of dsDNA. The length of genome varies significantly between the phages. DNA is

translocated through the central channel of the portal protein located at one vertex of the capsid. The portal complex provides a docking point for the viral ATPase complex (*terminase*). The terminase bound to the portal vertex forms the active packaging motor that moves the viral dsDNA inside the capsid. Encapsidation is normally initiated by an endonucleolytic cleavage at a defined sequence (*pac*) of the substrate DNA concatemer although some phages like T4 do not use a unique site for the initial cleavage. Packaging proceeds evenly until a threshold amount of DNA is reached inside the viral capsid. At the latter stages of packaging the increasingly dense arrangement of the DNA leads to a steep rise in pressure inside the capsid that can reach ~6 MPa (Smith et al., 2001). The headful cleavage of DNA is imprecise leading to variations in chromosome size of more than 1 kb (Casjens & Hayden, 1988; Tavares et al., 1996). The mechanism of packaging requires a sensor that measures the amount of DNA headfilling and a nuclease that will cleave DNA as soon the head is full. Termination of the DNA packaging is coordinated with closure of the portal system to avoid leakage of the viral genome. In tailed bacteriophages this is most frequently achieved through the binding of head completion proteins (or adaptor proteins). The complex of the portal dodecamer and these proteins composes the connector (Lurz et al., 2001; Orlova et al., 2003). After termination of the first packaging cycle initiated at *pac* (initiation cycle), a second packaging event is initiated at the non-encapsidated DNA end created by the headful cleavage and additional cycles of encapsidation follow. Some packaging series can yield 12 or more encapsidation events revealing the high processivity of the packaging machinery (Adams et al., 1983; Tavares et al., 1996).

4. Methods for study of bacteriophages

Microbiology and bacteriology were the first methods used to investigate viruses. Studies related to the life cycle of prokaryotic and eukaryotic microorganisms such as bacteria, viruses, and bacteriophages are combined into microbiology. This includes gene expression and regulation, genetic transfer, the synthesis of macromolecules, sub-cellular organization, cell to cell communication, and molecular aspects of pathogenicity and virulence. The earlier studies of phages were based on microbiological experiments including immunology. Nowadays the research of the biological processes is not limited to biochemical analysis and microbiology. To understand processes of virus/cell communication and interaction one often needs information on the molecular level and conformational changes of the components under different conditions. Gel filtration or Western blotting provides information for a protein on the macromolecular level such as size, molecular mass, binding to an antibody etc. These experiments will display how the proteins will change their characteristics with several chemical modifications and analysing what kind of change occurred, one could draw a conclusion for the structure. At the cellular level, optical microscopy can reveal the spatial distribution and dynamics of molecules tagged with fluorophores.

4.1 X-ray crystallography and NMR of phages

The methods of X-ray crystallography and NMR spectroscopy provide detailed information on molecular structure and dynamics. However, X-ray crystallography requires the growth of protein crystals up to 1 mm in size from a highly purified protein. Crystal growth is an experimental technique and there are no rules about the optimal conditions for a protein

solution to result in a good protein crystal. It is extremely difficult to predict good conditions for nucleation or growth of well-ordered crystals of large molecular complexes. In practice, the best conditions are identified by screening multiple probes where a wide variety of crystallization solutions are tested. Structural analysis of viral proteins by crystallographic methods was very successful when separate proteins were studied. Protein crystals contain trillions of accurately packed identical protein molecules. When irradiated by X-rays, these crystals scatter X-rays in certain directions producing diffraction patterns. Computational analysis of that diffraction produces atomic models of the proteins. Viruses are much bigger than single proteins and may comprise thousands of components; it is difficult to pack them into crystals, and when successful, crystals have large unit cell dimensions (unit cell is an elementary part, from which the crystal is composed). Because of that the diffraction from virus crystals is far weaker than that of single proteins. It was an extremely challenging task to crystallise viruses for crystallographic studies although some icosahedral viruses were crystallised and the atomic structures have been obtained (Harrison, 1969; Grimes et al., 1998; Wikoff et al., 2000). Nowadays X-ray analysis has provided a wealth of information on atomic structures of many small protein components of large viruses including bacteriophages (Rossmann et al., 2005).

Nuclear Magnetic Resonance (NMR) is another very powerful method of structural analysis allowing studying dynamics of samples in solution. NMR methodology, combined with the availability of molecular biology and biochemical methods for preparation and isotope labelling of recombinant proteins has dramatically increased its usage for the characterization of structure and dynamics of biological molecules in solution. In NMR, a strong, high frequency magnetic field stimulates atomic nuclei of the isotopes H^1 , D^2 , C^{13} , or N^{15} and measures the frequency of the magnetic field of the atomic nuclei during its oscillation period before returning back to the initial state. NMR is able to obtain the same high resolution using different properties of the samples. NMR measures the distances between atomic nuclei, rather than the electron density in a molecule. Protein folding studies can be done by monitoring NMR spectra upon folding or denaturing of a protein in real time. However, NMR cannot deal with macromolecules in the mega-Dalton range, the upper weight limit for NMR structure determination is ~ 50 kDa.

4.2 Electron microscopy of tailed bacteriophages

For microbiological research, light microscopy is a tool of great importance in studies of the biology of microorganisms. However, light microscopy is not able to provide a high enough magnification to see viruses. The modern development and use of synchrotrons has revealed the structures of spherical viruses, nonetheless obtaining virus crystals remained problematic, especially for bacteriophages. EM has become a major tool for structural biology over the molecular to cellular size range. Bacteriophages do not have exact icosahedral symmetry since they have different appendages facilitating interactions and infection of the host cells, a fact that makes them very challenging objects for crystallography and their size makes them unsuitable for NMR. Members of the *Caudovirales* phage family with dsDNA genome are especially difficult to crystallise because they have tails. Here EM has become a tool of choice for structural analysis of these samples.

The simplest method for examining isolated viral particles is negative staining, in which a droplet of the suspension is spread on an EM support film and then embedded in a heavy metal salt solution, typically uranyl acetate (Harris, 1997). The method is called negative staining because the macromolecular shape is seen by its exclusion of stain rather than by binding of stain. During the last two decades other methods became widely used and demonstrated their efficiency when samples were fixed in the native, hydrated state by rapid freezing of thin layers of aqueous sample solutions at liquid nitrogen temperatures (Dubochet et al., 1988). Such rapid cooling traps the biological molecule in its native, hydrated state but embedded in glass-like, solid water – vitrified ice. This procedure prevents the formation of ice crystals, which would be very damaging to the specimen. EM images of particles are used to calculate their three-dimensional structures (Jensen, 2010).

EM was a major tool used in analysis of phage morphology and initiated a process of classification of viruses. The development of cryogenic methods has enabled EM imaging to provide snapshots of biological molecules and cells trapped in a close to native, hydrated state. High symmetry of the complexes is an advantage, but single particles of molecular mass ~ 0.5 -100 MDa with or without symmetry (e.g. viruses, ribosomes) can now be studied with confidence and can often reveal fine details of the 3D structure. The resulting images allow information not only on quaternary structure arrangements of macromolecular complexes but the positions of their secondary structural elements like helices and beta-sheets (Rossmann et al., 2005).

4.3 Hybrid methods

The components of bacteriophages and their interactions have to be identified and analysed. This can be done by localisation of known NMR or X-ray structures of individual viral proteins and nucleic acids combined with biochemical information to identify them in the EM structures. Electron cryo-microscopy and three-dimensional image reconstruction provide a powerful means to study the structure, complexity, and dynamics of a wide range of macromolecular complexes. One has to use different approaches for several reasons: there are limitations of the individual methods; some complexes do not crystallise; phages, being multi-protein complexes, have different conformational organisation at different conditions. Therefore all known structural and biochemical methods have to complement each other to generate structural information. When atomic models of components or subassemblies are accessible, they can be fitted into reconstructed density maps to produce informative pseudoatomic models. If atomic structures of the components are not known, it is helpful to perform homology modelling so that the generated models could be fitted into the EM maps. Fitting atomic structures and models into EM maps allows researchers to test different hypotheses, verify variations in structures of viruses and effectively increase the EM map resolution creating pseudo-atomic viral models (Lindert et al., 2009).

5. Examples of bacteriophage structures

In spite of the great abundance of the tailed phages, details of their organisation have emerged only during the last decade. The progress in structural studies of phages as a whole entity was slow because of their flexibility and complex organisation. The additional hindrance arises from intricate combination of different oligomerisation levels of the phage elements. Fully assembled capsids have at least 5-fold symmetry or more often, icosahedral

symmetry where multiple structural units form a regular lattice with 2, 3, and 5 rotational symmetries. All known portal proteins were found to be dodecameric oligomers; tails have overall 6- or 3-fold rotational symmetry, the multiple repeats of major proteins have helical arrangement. The proteins related to the receptor sensor system at the far end of the tail could be in 6, 3, or only one copy. The information on the relative amount of different protein components has been revealed by biochemical and structural methods such as X-ray analysis of separate components. Development of hard and software has led to new imaging systems of better quality, new programs allowing processing of bigger data sets comprising hundreds of thousand images. The modern strategy is based on hybrid methods where structure determination at high resolution of isolated phage components is combined in three-dimensional maps of lower resolution obtained by electron microscopy. Electron microscopy by itself has reached such level of quality that for the complexes with icosahedral symmetry it has become nearly routine to obtain structures at 4-5 Å resolution (Hryc et al., 2011; Zhou, 2011; Grigorieff & Harrison, 2011)

5.1 Phage T4

The T4 phage of the *Myoviridae* family infects *E. coli* bacteria and is one of the largest phages; it is approximately 200 nm long and 80-100 nm wide with the capsid in a shape of an elongated icosahedron. The phage has a rigid tail composed of two main layers: the inner tail tube is surrounded by a contractile sheath which contracts during infection of the bacterium. The tail sheath is separated from the head by a neck. Phages of *Myoviridae* family have a massive baseplate at the end of the tail with fibres attached to it. The tail fibres help to find receptors of a host cell and provide the initial contact; during infection the tail tube penetrates an outer bacterial membrane to secure the pathway for genome to be injected into the cell.

The capsid of the T4 phage is built with three essential proteins: gp23* (48.7 kDa), which forms the hexagonal capsid lattice; gp24* (the * designates the cleaved form of the protein when the prohead matures to infectious virus) forms pentamers at eleven of the twelve vertices, and gp20, which forms the unique dodecameric portal vertex through which DNA enters during packaging and exits during infection. 3D-reconstruction has been determined at 22 Å resolution by cryo-EM for the wild-type phage T4 capsid forming a prolate icosahedron (Figure 4, Fokine et al., 2004). The major capsid protein gp23* forms a hexagonal lattice with a separation of ~140 Å between hexamer centres. The atomic structure of gp24* has been determined by X-ray crystallography and an atomic model for gp23* was built using its similarity to gp24* (Fokine et al., 2005). The capsid also contains two non-essential outer capsid proteins, Hoc and Soc, which decorate the capsid surface. The structure of Soc has been determined by X-ray crystallography and shows that Soc has two capsid binding sites which, through binding to adjacent gp23* subunits, reinforce the capsid structure (Qin et al., 2010). The failure of gp24* to bind Soc provides a possible explanation for the property of osmotic shock resistance of the phage (Leibo et al., 1979). The 3D maps of the empty capsids with and without Soc (Iwasaki et al., 2000) have been determined at 27 and at 15 Å resolution, respectively.

Single molecule optical tweezers and fluorescence studies showed that the T4 motor packages DNA at a rate of up to 2000 bp/sec, the fastest reported to date of any packaging motor (Fuller et al., 2007). FRET-FCS studies indicate that the DNA gets compressed during the translocation process (Ray et al., 2010).

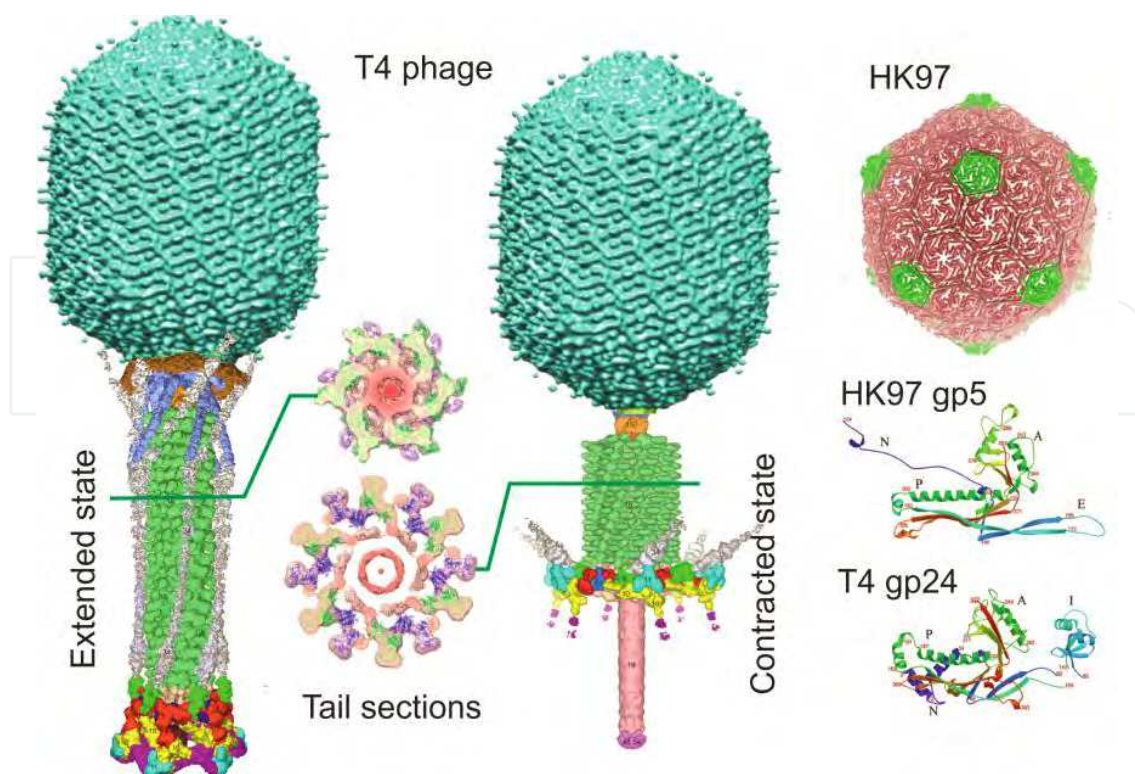


Fig. 4. Structures of T4 (cryo-EM) and HK97 (X-ray analysis) phages (reproduced with permission of M.G. Rossmann and J. E. Johnson). Ribbon diagrams compare the structure of HK97 (gp5) with the structure of the T4 gp24 capsid protein. Phages and sections are on a different scale.

Tails of *Myoviridae* phages have a long, non-contractible tube surrounded by a contractile sheath. Bacteriophage T4 has a tail sheath that is composed of 138 copies of gp18 (Leiman et al., 2004). The tail tube inside the sheath is estimated to be assembled from as many gp19 subunits as there are gp18 subunits in the sheath (Moody & Makowski, 1981). The tail sheath has helical symmetry with a pitch of 40.6 Å and a twist of 17.2° (Kostyuchenko et al., 2005). The tail sheath contraction can be divided into several steps. Previous studies of partially contracted sheath showed that conformational changes of the sheath are propagated 'upwards' starting from the disk of the gp18 subunits closest to the baseplate (Moody 1973). The cryo-EM reconstructions showed that during contraction, the tail sheath pitch decreases from 40.6 Å to 16.4 Å and its diameter increases from 24 nm to 33 nm (Figure 4, Leiman et al., 2004; Kostyuchenko et al., 2005). The combination of X-ray model and EM structures show that gp18 monomers remain rigid during contraction and move about 50 Å radially outwards while tilting 45° clockwise, viewed from outside the tail. During contraction of the tail the interactions between neighbouring subunits within a disk are broken so that the subunits from the disk above get inserted into the gaps formed in the disk below (Aksyuk et al., 2009).

The baseplate with the cell-puncturing device of the T4 phage is an ultimate element of the phage. This is an extremely complex multiprotein structure on the far end of the tail and represents multifunctional machinery that anchor the phage on the bacteria surface and provide formation of the DNA entrance into the bacteria. This important part of the phage structure is of ~27 nm in height and 52 nm in diameter at its widest part. The baseplate

conformation is coupled to that of the sheath: the dome shape conformation is associated with the extended sheath, whereas the flat “star” conformation is associated with the contracted sheath that occurs in the T4 particle after attachment to the host cell. Short treatment of bacteriophage T4 with 3 M urea resulted in the transformation of the baseplate to a star-shape and subsequent tail sheath contraction (Kostyuchenko et al., 2003). During that switch the baseplate diameter increases to 61 nm and the height decreases to 12 nm although the protein composition of the baseplate does not change. It is composed of ~150 subunits of a dozen different gene products (Leiman et al., 2010). Proteins gp11, gp10, gp7, gp8, gp6, gp53, and gp25 form one sector of 6-fold structure. The central hub of the baseplate is formed by gp5, gp27, and gp29 and probably includes gp26 and gp28. Assembly of the baseplate is completed by attaching gp9 and gp12 to form the short tail fibres, and also gp48 and gp54 that are required to initiate polymerization of the tail tube, a channel for DNA (Leiman et al., 2010).

T4 tail has three types of fibrous proteins: the long tail fibres, the short tail fibres, and whiskers. Long tail fibres and short tail fibres are attached to the baseplate and whiskers extending outwardly in the region of the tail connection to the capsid. The long tail fibres, which are ~145 nm long and only ~4 nm in diameter, are primary reversible adsorption devices (Figure 4, Kostyuchenko et al., 2003). Each fibre consists of the rigid proximal halves, formed by gp34, and the distal ones composed by gp36 and gp37. The distal part of the fibre has a rod-like shape about 40 nm long that is connected to the first half of the fibre through the globular hinge. Gp35 forms a hinge region and interacts with gp34 and gp36. The N-terminal globular domain of gp34 interacts with the baseplate. Short tail fibres are attached to the baseplate by the N-terminal thin part, while the globular C-terminus binds to the host cell receptors (Boudko et al., 2002). The structure of this domain of the short tail fibres was determined by X-ray crystallography (Tao et al., 1997)

5.2 HK97

HK97 is a temperate phage from *Escherichia coli* which was isolated in Hong Kong by Dhillon (Dhillon & Dhillon, 1976). It shares a host range with the Lambda phage (Dhillon et al., 1976). HK97 has an isometric head and a long, flexible, non-contractile tail representing *Siphoviridae* family (Dhillon et al., 1976). The HK97 phage has multi step pathway of self-assembly revealing two forms of procapsids of ~470 Å in diameter. Capsid protein gp5 (42 kDa) forms capsids, with icosahedral symmetry characterised by $T = 7$ (Hendrix, 2005). A part of the gp5 (102 amino acids from the N terminus) plays the role of a scaffold, which is cleaved by gp4 (the phage protease) at maturation of the capsid (Conway et al., 1995). The first low resolution structures have shown conformation changes reflecting transition of the HK97 procapsids into expanded capsids (Conway et al., 1995). The diameter of procapsids during transition into the heads increases from 470 Å to 550 Å while the thickness of the capsid shell changes from 50 Å to ~25 Å.

The first atomic structure of a capsid for the tailed phage was only published in 2000. Gp5, if expressed alone, assembles into a portal-deficient version of prohead I. Co-expressing gp5 with the gp4 protease, which cleaves gp5 scaffolding domain, produces Prohead II that expands into the icosahedral head II (the diameter is ~650 Å) without DNA and portal complex; and it was used for the crystallisation. The crystal structure of the dsDNA bacteriophage HK97 mature empty capsid was determined at 3.6 Å resolution using

icosahedral symmetry (Wikoff et al., 2000). The capsid crystal structure shows how an isopeptide bond is formed between subunits, arranged in topologically linked, covalent circular rings (Figure 4). The structure of the HK97 gp5 coat protein has revealed a new category of virus fold: it is mixture alpha-helices and beta-sheets organised into three domains that are not sequence contiguous (Figure 4). Domain A is located close to the centre of the hexamers and pentamers of the capsid. Domain P (peripheral) provides contacts between adjacent molecules within pentamers and hexamers. The third domain, represented by the E-loop, is an extension through which each subunit of the HK97 capsid is covalently linked to two neighbouring subunits. The bond organization explains why the mature HK97 particles are extraordinarily stable and cannot be disassembled on an SDS gel without protease treatment (Popa et al., 1991; Duda, 1998).

5.3 SPP1

SPP1 is a virulent *Bacillus subtilis* dsDNA phage and belongs to the *Siphoviridae* family. The virion is composed of an icosahedral, isometric capsid (~60 nm diameter) and a long, flexible, non-contractile tail. The SPP1 genome length is 45.9 kb. The procapsid (or prohead) of SPP1 consists of four proteins: the scaffold protein gp11, the major capsid protein gp13, the portal protein gp6, and a minor component gp7. The inside of the capsid is filled with gp11 which exits the procapsid during DNA packaging. Gp13 and the decoration protein gp12 form the head shell of the mature SPP1 capsid.

The portal protein is located at a 5-fold vertex of the icosahedral phage head and serves as the entrance for DNA during packaging. The structure of gp6 as a 13-subunit assembly was determined by EM and X-ray at 10 and 3.4 Å resolution correspondingly (Orlova et al., 2003; Lebedev et al., 2007). The 13-mer portal complex has a circular arrangement with an overall diameter of ~165 Å and a height of ~110 Å. A central tunnel pierces the assembly through the whole height. The portal protein monomer has four main domains: crown, wing, stem, and clip. The crown domain consists of three alpha-helices connected by short loops and has 40 additional C-terminal residues that are disordered in the X-ray structure. Mutations in the crown indicate the importance of this area for DNA translocation (Isidro et al., 2004a, b). The wing region is formed by alpha-helices flanked on the outer side by a beta-sheet. The stem domain connects the wing to the clip domain. It consists of two alpha-helices that are conserved in phi29 and SPP1 phages; a similar arrangement of helices was found in the P22 portal protein (Simpson et al., 2000; Guasch et al., 2002; Lebedev et al., 2007; Olia et al., 2011). The clip domain forms the base of the portal protein and is expected to be exposed to the outside of the capsid during viral particle assembly. The three-dimensional structures of the portal proteins of SPP1, phi29, and P22 phages demonstrate a strikingly similar fold. Although there is no detectable amino-acid sequence similarity between proteins, they have a nearly identical arrangement of two helices forming stem domains and in the clip domain which form a tightly packed ring of three stranded beta-sheets each made up of two strands from one subunit and one strand from an adjacent subunit.

After termination of DNA incorporation the portal pore needs to be rapidly closed to prevent leakage of the viral chromosome. In SPP1 this role is played by the head completion proteins gp15 and gp16 that bind sequentially to the portal vertex forming the connector (Lurz et al., 2001). Disruption of the capsids yielded connectors composed of gp6, gp15 and gp16. The connector is an active element of the phage that is involved into packaging the

viral genome, serves as an interface for attachment of the tail, and controls DNA release from the capsid. The connector of *Bacillus subtilis* bacteriophage SPP1 was found to be a 12-fold cyclical oligomer (Lurz et al., 2001), though isolated gp6 is a cyclical 13mer. The structure of the connector was determined at 10 Å resolution, using cryo-EM (Figure 5, Orlova et al., 2003). Both the isolated portal protein and the gp6 oligomer in the connector reveal a similar arrangement of four main domains, the major changes take place in the clip domain through which gp15 contacts gp6. The connector structure shows that gp15 serves as an extension of the portal protein channel where gp16 binds. The central channel is closed by gp16 physically blocking the exit from the DNA-filled capsid (Orlova et al., 2003). Structures of SPP1 gp15 and gp16 monomers were determined by NMR and together with gp6 were docked into the EM map of the connector (Figure 5, Lhuillier et al., 2009). The channel of the connector will be opened when the virus infects a host cell. Comparison of the structures before and after assembly, provides details on the major structural rearrangements (gp15) and folding events (gp15 and gp16) that accompany connector formation.

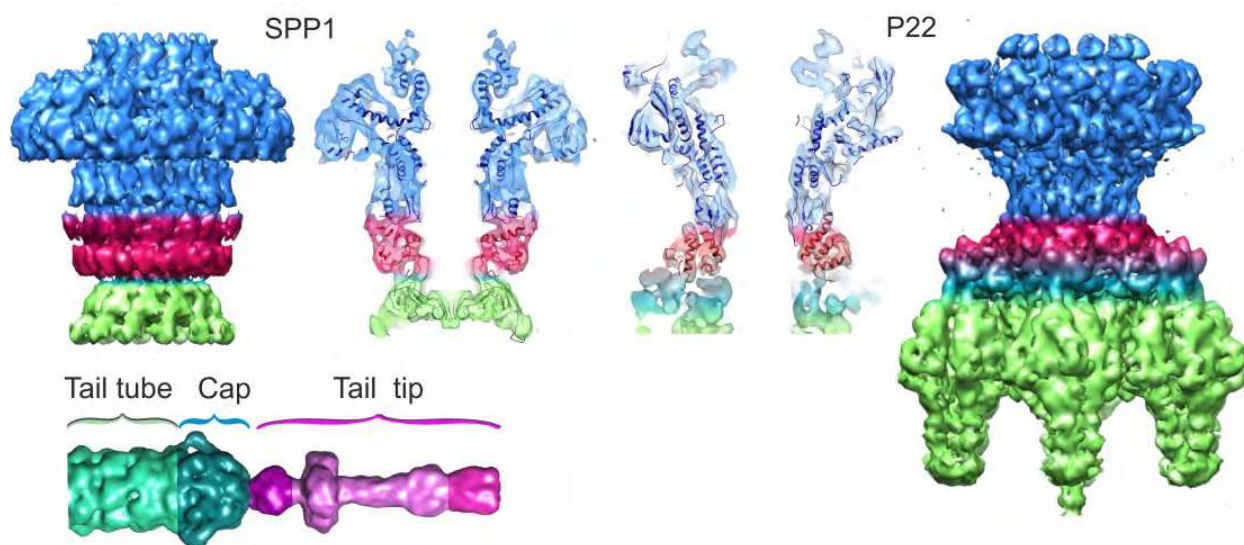


Fig. 5. Surface representation of SPP1 phage connector (top left, Orlova et al., 2003), tail tip (bottom left, Plisson et al., 2007) and P22 phage tail machine (top right, Lander et al., 2009). The fit of the atomic coordinates into each connector is shown as a cut open view adjacent to its corresponding surface view. The portal proteins are shown in blue, the adaptor proteins in crimson, gp16 of SPP1 and the tail spikes of P22 are shown in green. (Copyright of E.V. Orlova)

The 160-nm-long tail of the SPP1 phage is composed of two major tail proteins (MTPs), gp17.1 and gp17.1*, in a ratio of about 3:1. They share a common amino-terminus, but the latter species is ~10 kDa more than gp17.1. The polypeptide sequence, identical in the two proteins is responsible for assembly of the tail tube while the additional module of gp17.1* shields the structure exterior exposed to the environment. The carboxyl-terminus domain of MTPs shares homology to motifs of cellular proteins (Fraser et al., 2006) or to phage components (Fortier et al., 2006) involved in binding to cell surfaces. Structures of the bacteriophage SPP1 tail before and after DNA ejection were determined by negative stain electron microscopy. The results reveal extensive structural rearrangements in the internal wall of the tail tube. It has been

proposed that the adsorption device–receptor interaction triggers a conformational switch that is propagated as a domino-like cascade along the 160 nm -long helical tail structure to reach the head-to-tail connector. This leads to opening of the connector, culminating in DNA exit from the head into the host cell through the tail tube (Plisson et al., 2007).

The tail tip is attached to the cap structure that closes the tail tube (Figure 5). The absence of a channel for DNA traffic in the tip implies that it must dissociate from the cap for DNA passage to the cytoplasm during infection. The structural data show that the tail tip does not have a channel for DNA egress and that the signal initiated by interaction of the tip with the bacterial receptor causes release of the tip from the tail cap. Reconstructions were performed for two states of the tail: before and after DNA ejection. The cap structure was reconstructed separately from the tip and the main area of the tail. The reconstructions of the cap together with the first four rings of the tail tube demonstrate that the tail external diameter (before DNA ejection) tapers from ~110 to ~40 Å at the capped extremity and changes symmetry from six-fold to three-fold. This arrangement provides a sturdy interface between the tail tube and the three-fold symmetric tip. Opening of the dome-shaped cap involves loss of the tip and movement of the cap subunits outwards from the tail axis, creating a channel with the same diameter as the inner tail tube (Plisson et al., 2007).

5.4 Phi29

The *Bacillus subtilis* bacteriophage phi29 (*Podoviridae* family) is one of the smallest and simplest known dsDNA phages. The bacteriophage phi29 (Figure 6) is a 19-kilobase (19-kb) dsDNA virus with a prolate head and complex structure. Proheads consist of the major capsid protein gp8, scaffolding protein gp7, head fibre protein gp8.5, head–tail connector gp10, and a pRNA oligomer. Mature phi 29 heads are 530 Å long and 430 Å wide, and the tail is 380 Å long. The packaging of DNA into the head involves, besides the portal protein, other essential components such as an RNA called pRNA and the ATPase p16, required to provide energy to the translocation machinery. Once the DNA has been packaged, pRNA and p16 are released from the portal protein. In the mature phi29 virion, the narrow end of the portal protrudes out of the capsid and attaches to a toroidal collar (gp11). The collar has a diameter of about 130 Å and is surrounded by 12 appendages that function to absorb the virion on host cells (Anderson et al., 1966). A thin, 160 Å -long tube, with an outer diameter of 60 Å, leads away from the centre of the collar (Hagen et al., 1976). The outer end of the tail (gp9) has a cylindrical shape and bigger diameter of ~ 80 Å.

The three-dimensional structure of a fibreless variant has been determined to 7.9 Å resolution allowing the identification of helices and beta-sheets (Figure 6, Morais et al., 2005, Tang et al., 2008). For the prolate capsid phi29 there was not the advantage of using icosahedral symmetry for structural analysis, its cryo-EM three-dimensional reconstructions have been made of mature and of emptied bacteriophage phi29 particles without making symmetry assumptions (Xiang et al., 2006). Possible positions of secondary elements for gp8 indicate that the folds of the phi29 and bacteriophage HK97 capsid proteins are similar except for an additional immunoglobulin-like domain of the phi29 protein: the gp8 residues 348–429 are 32% identical to the group 2 bacterial immunoglobulin domain (BIG2) consensus sequence (Morais et al., 2005; Xiang et al., 2006). The BIG2 domain is found in many bacterial and phage surface proteins related to cell adhesion complexes (Luo et al., 2000; Fraser et al., 2006). The asymmetrical reconstruction of the complete phi29 has

revealed new details of the asymmetric interactions and conformational dynamics of the phi29 protein and DNA components (Tang et al., 2008).

The DNA packaging motor is located at a unique portal vertex of the prohead and contains: the head-tail connector (a dodecamer of gp10); the portion of the prohead shell that surrounds the connector, a ring of 174-base prohead RNAs (pRNA), and a multimer of gp16, an ATPase that first binds DNA-gp3 and then assembles onto the connector/pRNA complex prior to packaging. The wide end of the portal protein contacts the inside of the head, whereas the narrow end protrudes from the capsid where it is encircled by the pentameric pRNA. The structure of the isolated phi29 portal complex has been studied by atomic force microscopy and electron microscopy (EM) of two-dimensional arrays (Carazo et al., 1985) and X-ray crystallography (Simpson et al., 2000; Guasch et al., 2002). X-ray crystallographic studies of the phi29 portal showed that it is a cone-shaped dodecamer with a central channel (Simpson et al., 2000). The three-dimensional crystal structure of the bacteriophage phi29 portal has been refined to 2.1 Å resolution (Guasch et al., 2002). This 422 kDa oligomeric protein is part of the DNA packaging motor and connects the head of the phage to its tail. Each monomer of the portal dodecamer has an elongated shape and is composed of a central, mainly alpha-helical domain (stem domain) that includes a three-helix bundle, a distal a/b domain and a proximal six-stranded SH3-like domain (Simpson et al., 2000). The portal dodecamer has a 35 Å wide central channel, the surface of which is mainly electronegative. The narrow end of the head-tail portal protein is expanded in the mature virus. Gene product 3, bound to the 5' ends of the genome, appears to be positioned within the portal, which may potentiate the release of DNA-packaging machine components, creating a binding site for attachment of the tail (Tang et al., 2008).

The process of DNA packaging is an extremely energy consuming act because electrostatic and bending repulsion forces of the DNA must be overpowered to package the DNA to near-crystalline density. Force-measuring laser tweezers were used to measure packaging activity of a single portal complex in real time where one microsphere has been used to hold on to a single DNA molecule as they are packaged, and the other was bound to the phage and fixed (Smith et al., 2001). These experiments have demonstrated that the portal complex is a force-generating motor which can work against loads of up to 57 pN, making it one of the strongest molecular motors reported to date. Notably, the packaging rate decreases as the prohead is filled, indicating that an internal force builds up to 50 pN owing to DNA confinement. These results suggest that the internal pressure provides the driving force for DNA injection into the host cell for the first half of the injection process.

The structure of the phi29 tail has revealed that 12 appendages protruding from the collar like umbrella with 12 ribs that end in 'tassels' (Xiang et al., 2006). Two of the 12 appendages are extended radially outwards (the 'up' position), whereas the other 10 have their tassels 'hanging' roughly parallel to the virus major axis. The adsorption capable 'appendages' were found to have a structure homologous to the bacteriophage P22 tail spikes. Two of the appendages are extended radially outwards away from the long axis of the virus, whereas the others are around and parallel to the phage axis. The appendage orientations are correlated with the symmetry mismatched positions of the five-fold related head fibres. The tail in the mature capsids, that have lost their genome have an empty central channel (Xiang et al., 2006). Comparisons of these structures with each other and with the phi29 prohead indicate how conformational changes might initiate successive steps of assembly and infection.

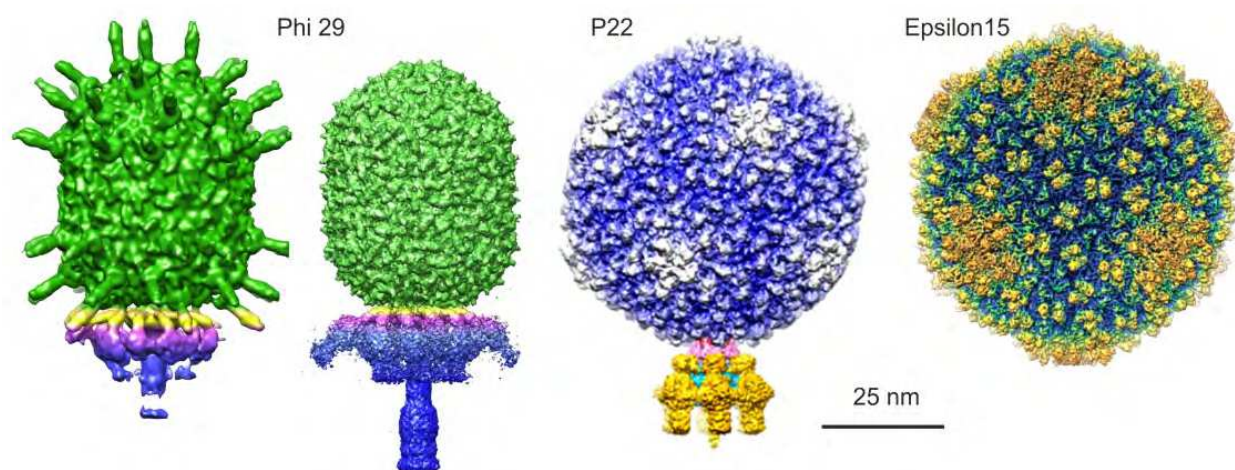


Fig. 6. Surface representation of EM structures. The Phi29 capsid is in green, the tassels are shown in magenta and blue, the tail is also in blue. The complete phage is shown at 16 Å (left) and a mutant phage without spikes at 8 Å (right, Tang et al., 2008) (Image copyright of E.V. Orlova). P22 phage is shown at 7 Å resolution (reproduced with permission of J. E. Johnson) and Epsilon15 is at 4.5 Å (Tang et al. 2011; Jiang et al., 2008, reproduced with permission of W. Chiu)

5.5 P22

Bacteriophage P22 infects *Salmonella enterica serovar Typhimurium* and is a prototypical representative of the *Podoviridae* family. The mature P22 virion presents an icosahedral $T = 7l$ capsid about 650 Å in diameter. The bacteriophage P22 procapsid comprises hundreds of copies of the gp5 coat and gp8 scaffolding proteins, multiple copies of three ejection proteins (gp7, gp16, gp20, also known as pilot proteins), and a unique multi-subunit gene 1 (gp1) portal (Prevelige, 2006).

Single-particle cryo-EM has been used to determine the P22 procapsid structure initially at low resolution then improved from 9 Å to 3.8 Å resolution (Figure 6, Jiang et al., 2006; Jiang et al., 2008; Chen et al., 2011). The procapsids were isolated from cells infected with mutants defective in DNA packaging and representing the physiological precursor prior to DNA packaging and capsid maturation. Coat protein gp5 is organized as pentamers and skewed hexamers as previously reported for the GuHCl treated procapsid (Thuman-Commike et al., 1999; Parent et al., 2010). The high resolution structure allowed C α backbone models for each of the seven structurally similar but not identical copies of the gp5 protein in the asymmetric unit to be built. The analysis has shown that gp5 has fold similar to the HK97 coat protein (Jiang et al., 2008).

The first structures of the P22 assembly-naïve portal formed from expressed subunits (gp1) were obtained at ~ 9 Å resolutions by cryo-EM (Lander et al., 2009). Later two atomic structures were obtained for the P22 portal protein: one is for a fragment of the portal, 1–602 aa (referred to as the ‘portal-protein core’), bound to 12 copies of tail adaptor factor gp4 (Olia et al., 2006; Lorenzen et al., 2008). The second was the full-length P22 portal protein (725aa) at 7.5 Å resolution. To solve three independent crystal forms of the complex gp1/gp4 to a resolution of 9.5 Å, the EM structure of P22 tail at 9.4 Å resolution has been extracted computationally from the P22 tail complex and used as molecular replacement model. The

high resolution atomic structure of the P22 portal protein has been obtained using a combination of multi- and intra crystal non-crystallographic-symmetry averaging, and by extension of EM phases to the resolution of the best diffracting crystal form (3.25 Å). The P22 portal complex is a ~0.96 MDa ring of 12 identical subunits, symmetrically arranged around a central channel of variable diameter, with an overall height of ~350 Å (Lander et al., 2009; Olia et al., 2011). A lower-resolution structure of the full-length portal protein unveils the unique topology of the C-terminal domain, which forms a ~200 Å long alpha-helical barrel. This domain inserts deeply into the virion and is highly conserved in the *Podoviridae* family. The quaternary structure of the P22 portal protein can be described as a funnel-shaped core ~170 Å in diameter, connected to an ~200 Å long, mostly α -helical tube formed by the C-terminal residues 603–725, which resembles a rifle barrel (Olia et al., 2011). The portal core is similar in topology to other portal proteins from phage SPP1 (Lebedev et al., 2007) and phi29 (Simpson et al., 2000; Guasch et al., 2002), but presence of the helical barrel is the first example of a dodecameric tube in a portal protein. Gp4 binds to the bottom of the portal protein, forming a second dodecameric ring ~75 Å in height (Olia et al., 2011).

In *Podoviridae*, the mechanisms of bacteria cell penetration and genome delivery are not well understood. P22 uses short, non-contractile tails to adsorb to the host cell surface. The tail machine comprises the tail spike, gp9; the tail needle, gp26; and the tail factors gp4 and gp10 (Tang et al., 2005). Protein gp4 serves as an adaptor between portal protein and tail elements. The tail has a special fibre known as the “tail needle” that likely functions as a cell membrane piercing device to initiate ejection of viral DNA inside the host. The structure of the intact tail machine purified from infectious virions has been obtained by cryo-EM at ~9 Å resolution (Figure 5, Lander et al., 2009). The structure demonstrated that the protein components are organized with a combination of 6-fold (gp10, trimers of gp9), and 3-fold (gp26, gp9) symmetry (Lander et al., 2009). The combined action of an adhesion protein (tailspike) and a tail needle (gp26) is responsible for binding and penetration of the phage into the host cell membrane (Bhardwaj et al., 2011a). Gp26 probably plays the dual role of portal-protein plug and cell wall-penetrating needle, thereby controlling the opening of the portal channel and the ejection of the viral genome into the host. In Sf6, a P22-like phage that infects *Shigella flexneri*, the tail needle presents a C-terminal globular knob (Bhardwaj et al., 2011b). This knob, absent in phage P22 but shared in other members of the P22-like genus, represents the outermost exposed tip of the virion that contacts the host cell surface. In analogy to P22 gp26, it was suggested that the tail needle of phage Sf6 was ejected through the bacterial cell membrane during infection and its C-terminal knob is threaded through peptidoglycan pores formed by glycan strands (Bhardwaj et al., 2011a; 2011b).

5.6 Epsilon 15

The Gram-negative *Salmonella anatum* is the host cell for bacteriophage Epsilon15 (ε15, *Podoviridae* family). The ~40kb Epsilon15 dsDNA is packed within the isometric icosahedral capsid with a diameter of ~680 Å. The virion capsid contains 11 pentons and 60 hexons made from the major capsid protein gp7 and a small decoration protein gp10 (12-kDa). Single-particle cryo-EM was used about ten years ago to determine the first structures of icosahedral viruses to subnanometre resolutions (Jiang et al., 2006). A 9.5 Å density map was generated from EM data using icosahedral symmetry. In the average subunit map, the locations of three helices were identified. Now the structure of the epsilon15 capsid has been

refined to a 4.5 Å resolution (Figure 6, Jiang et al., 2008). The quality of the map allowed tracing the backbone chain of gp7. Comparison of the models has shown local discrepancies between subunits at the N-terminus and the E-loop in different subunits of gp7 within the hexamers of the capsid. Interestingly, a connection between E-loops of neighbouring subunits possibly exists, but the resolution was not sufficient to reveal it. Moreover, additional density was located between the gp7 monomers. This density has been assigned to the gp10 decoration protein that consists mainly of beta-sheets and two short alpha-helices. A back-to-back dimer of gp10 is positioned at the two-fold axes and makes contact with six gp7 subunits through the N-termini and the E-loops. It was suggested that gp10 'staples' the underlying gp7 capsomeres to cement the gp5 cage so that it withstands the pressure from packed dsDNA (Jiang et al., 2008).

The Epsilon15 capsid volume can accommodate up to 90kb dsDNA. Since the Epsilon15 genome is only ~40kb, there is ample space for a protein core of this size in the capsid chamber. The core has a cylindrical shape with a length of ~200 Å and diameter of ~180 Å. The protein core may facilitate the topological ordering of the dsDNA genome during packaging and/or release as suggested for T7 core. At the virion's tail vertex, six tails pikes attach to a central 6-fold-symmetric tail hub of the length ~170 Å. This hub may be equivalent to *Salmonella typhimurium* bacteriophage P22's hub. The hub is connected to the portal ring inside the capsid. The Epsilon 15 genome winds around the core, with a short segment of terminal DNA passing through the axis of the core and portal (Jiang et al., 2006)

6. Conclusions

Bacteriophages represent an example of amazing molecular machines with powerful motors energised by ATP hydrolysis and puncturing devices allowing to inject viral genome into the host cells. As more and more phage structures been studied a general theme emerges pointing to a common bacteriophage ancestor from which they all inherited essentially the same capsid protein fold and other elements of their organisation: capsids, tails, portal complexes, tail fibres, and other components. The number of phages that were discovered, purified, and studied by biochemical, and biophysical methods increased tremendously during the last decade. New technologies used for their studies both on the microbiological and molecular levels made it possible to analyse their evolutionary relationship and origins of the host range specificity. One of the powerful techniques in the structural biology of phages is the modern cryo-EM that recently allowed to reach close to atomic resolution level of details in the EM reconstructions (Hryc et al., 2011; Zhou, 2011; Grigorieff & Harrison, 2011). Understanding of the mechanisms which determine the host-range is required to solve many practical questions related to infectious human and animal diseases caused by bacteria, and quality food and its production (e.g. dairy products). A study conducted in Japan has demonstrated the efficiency of phages against bacterial infections of cultured fish (Nakai & Park, 2002). The use of bacteriophage as antimicrobial agents is based on the lytic phages that kill bacteria via lysis, which destroys the bacterium and makes its adaptation nearly impossible. High bacteriophage resistance for external factors is important for the stability of phage preparations. However, this stability is disadvantageous for industry when maintenance of the active bacterial strains is important.

Comparative studies demonstrate that bacteriophages have many common features on the molecular level and common principle of interaction with a bacterium cell, although

components that trigger adsorption of phages to the host cell and the genome release are host dependent. Phage infection also depends on the availability of specific receptors on the cell surface, and investigation of the structure and biosynthesis of the bacterial cell membrane may be undertaken using phage-resistant mutants. Therefore there is a need to carry out further studies on phages, identifying receptors of targeted bacteria and environmental features that affect phage activity (Jończyk et al., 2011). The growing interest of the pharmaceutical and agricultural industries in phages requires more information on phage interactions, survivability and methods of their preservation. Structural studies revealed many similarities between bacteriophages and animal cell viruses. The chances of success in using bacteriophages as model systems for animal cell viruses and eventually as medical therapy are much better given our current extensive knowledge of bacteriophage biology following the advances in their molecular structural biology.

7. Acknowledgments

The author is grateful to Dr Helen White for the helpful comments during the preparation of this manuscript. EVO was also supported by BBSRC grant BB/F012705/1

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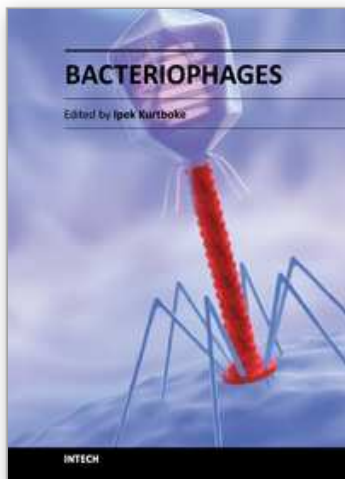
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Bacteriophages

Edited by Dr. Ipek Kurtboke

ISBN 978-953-51-0272-4

Hard cover, 256 pages

Publisher InTech

Published online 14, March, 2012

Published in print edition March, 2012

Bacteriophages have received attention as biological control agents since their discovery and recently their value as tools has been further emphasized in many different fields of microbiology. Particularly, in drug design and development programs, phage and prophage genomics provide the field with new insights.

Bacteriophages reveals information on the organisms ranging from their biology to their applications in agriculture and medicine. Contributors address a variety of topics capturing information on advancing technologies in the field. The book starts with the biology and classification of bacteriophages with subsequent chapters addressing phage infections in industrial processes and their use as therapeutic or biocontrol agents. Microbiologists, biotechnologists, agricultural, biomedical and sanitary engineers will find Bacteriophages invaluable as a solid resource and reference book.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

E.V. Orlova (2012). Bacteriophages and Their Structural Organisation, Bacteriophages, Dr. Ipek Kurtboke (Ed.), ISBN: 978-953-51-0272-4, InTech, Available from:

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