Tikrit University/ Science College/ Biology Department

Forth class/ Microbiology / Virology

Lecture 3

Viral Life Cycles Have Five Steps

In the early years of virology, one of the fundamental questions about the biology of these unique entities was how they made progeny viruses. The one-step growth experiment devised in 1939 by Max Delbrück and Emory Ellis served as an experimental approach to answering this question. Delbrück and Ellis worked with bacteriophage T4. They knew that T4 killed its host, *Escherichia coli*, and released progeny phages by lysing the cells it infected. In their experiment, *E. coli* cells were mixed with T4. After a short interval, the mixture was greatly diluted so that any virions released upon host cell lysis would not be able to encounter and infect other cells. The diluted culture was then incubated, and over time samples were removed to determine the number of infectious phage particles in the culture. This was determined using a plaque assay. A plot of phage particles versus time shows several distinct periods in the resulting growth curve. The latent period occurs immediately following addition of the phage. During this period, no virions are released. The rise period follows and is characterized by the rapid release of infective phages. Finally, a plateau is reached and no more virions are produced.

The one-step growth experiment is important for several reasons. It employed procedures that are still used today to culture and enumerate viruses, and it ushered in the modern era of phage biology. It also led to

another fundamental question: What is occurring during the latent period? A subsequent set of one-step growth experiments artificially lysed infected cells during the latent period and discovered that intracellular virions could not be detected early in the latent period. In essence, the phages disappeared once inside the cell. This period is called the eclipse period because the infecting virions were concealed or eclipsed within the host cell. These experiments also demonstrated that the number of completed, infective phages within the host cell increases after the end of the eclipse period. Still other experiments eventually showed that a carefully orchestrated series of events occurs during the latent period. These events are the focus of this section.

If a virus is to multiply and give rise to new progeny viruses, it must find and use (and in many cases abuse) a host cell. To accomplish this, a virus must use guile and subterfuge to access an appropriate host, enter the host, and avoid any defenses the host might employ to rid itself of the virus or prevent its multiplication. Once inside a host cell, a virus uses a repertoire of clever tricks to take control of cellular functions, thereby ensuring that viral genomes, mRNAs, and proteins are synthesized. The diversity of tricks viruses use has led to a plethora of distinctive viral life cycles (also called viral replicative cycles). The tricks used by a virus are often related to its virion structure, in particular the nature of its genome. Thus, viruses with a similar type of genome (e.g., dsDNA, ssRNA) often employ similar tricks.

Despite the diversity of viral life cycles, a general pattern of viral replicative cycles can be discerned; it can be divided into five steps. Because viruses need a host cell in which to multiply, the first step is usually attachment (often called adsorption) to a host. This is followed by entry of

either the nucleocapsid or the viral nucleic acid into the host. If the nucleocapsid enters, uncoating of the genome usually occurs before the life cycle continues. Once inside the host cell, the synthesis stage begins. During this stage, viral genes are expressed. That is, the virus's genes are transcribed and translated. This allows the virus to take control of the host cell, forcing it to manufacture viral genomes and viral proteins. Then follows the assembly stage, during which new nucleocapsids are constructed by self-assembly of coat proteins with the nucleic acids. Finally, during the release step, mature virions escape the host.

1-**Attachment (Adsorption):**

All viruses, with the exception of plant viruses, must attach to a potential host cell long enough to gain entry into the cell. Attachment to the host is accomplished by specific interactions between molecules on the surface of the virion (ligands) and molecules on the surface of the host cell called **receptors**. For instance, some bacteriophages use cell wall lipopolysaccharides and proteins as receptors, while others use teichoic acids, flagella, or pili. Binding of an animal virus particle to its receptor often causes conformational changes in virion proteins that facilitate interaction with secondary receptors, entry into the host, and uncoating.

Receptor specificity is at least partly responsible for the preferences viruses have for a particular host. Bacteriophages not only infect a particular bacterial species but often infect only certain strains within a given species. Likewise, animal viruses infect specific animals and, in some cases, only particular tissues within that host. However, if the receptor recognized by a

virus is present in numerous animals, then the virus will infect more than one animal species. Such is the case with rabies viruses.

Viruses have evolved such that they use host receptors that are always present on the surface of the host cell and are important for normal host cell function. Because the cell surface proteins are vital for cellular function, mutations that change them significantly are not tolerated, and this ensures that the virus can infect the host. In some cases, two or more host cell receptors are involved in attachment. For instance, human immunodeficiency virus (HIV) particles bind to two different proteins on human cells (e.g., CD4 and CCR5). Both of these host molecules normally bind cytokines—signaling molecules used by the immune system.

Distribution at the tissue level plays a crucial role in determining the **tropism** of the virus and the outcome of infection. For example, poliovirus receptors are found only in the human nasopharynx, gut, and anterior horn cells of the spinal cord. Therefore, polioviruses infect these tissues, causing disease that ranges in severity from milder forms such as gastrointestinal disease to more serious paralytic disease. In contrast, measles virus receptors are present in most tissues and disease is disseminated throughout the body, resulting in the widespread rash characteristic of measles.

Plant viruses are a notable exception to attachment based on receptor binding as no receptors have been identified for plant viruses. Rather, damage of host cells is required for the virus particles to access and enter the host. This is often achieved by plant-eating insects that carry virions from one plant to another. The virions are deposited in plant tissues as the insect devours the plant. Interestingly, evidence suggests that some viruses alter

their plant hosts to promote activity of the insects and thereby foster transmission to new plants.

2- **Entry into the Host:**

After attachment to the host cell, the virus's genome or the entire nucleocapsid enters the cytoplasm. For many bacteriophages only their nucleic acid enters the host's cytoplasm, leaving the capsid outside and attached to the cell. In contrast to phages, the nucleocapsid of many viruses of eukaryotes enters the cytoplasm with the genome still enclosed. Once inside the cytoplasm, some shed their capsid proteins in a process called uncoating, whereas others remain encapsidated. Because penetration and uncoating are often coupled, we consider them together.

The mechanisms of penetration and uncoating vary with the type of virus, and for many animal viruses, detailed mechanisms of penetration are unclear. However, it appears that one of three different modes of entry is usually employed by animal viruses: fusion of the viral envelope with the host cell's plasma membrane, entry by endocytosis, and release of viral nucleic acid into the cytoplasm of the host cell.

Fusion of viral envelopes with the host cell's plasma membrane often involves viral envelope glycoproteins that interact with proteins in the plasma membrane of the host cell. This interaction sets into motion events that allow the nucleocapsid to enter. For example, after attachment of paramyxovirus virions (single-stranded RNA viruses such as measles virus), membrane lipids rearrange, the adjacent halves of the contacting membranes merge, and a proteinaceous fusion pore form. The nucleocapsid then enters the host cell cytoplasm, where a viral enzyme carried within the

nucleocapsid begins synthesizing viral mRNA while it is still within the capsid.

Virions of nonenveloped viruses and some enveloped viruses enter cells by one of the endocytic pathways, including clathrin-dependent endocytosis and macropinocytosis. The resulting endocytic vesicle contains the virion and fuses with an endosome; depending on the virus, escape of the nucleocapsid or its genome from the endocytic vesicle may occur either before or after fusion with an endosome. Endosomal enzymes can aid in virion uncoating, and low pH often triggers the uncoating process. For some enveloped viruses, the viral envelope fuses with the endosomal membrane, and the nucleocapsid is released into the cytosol (the capsid proteins may have been partially removed by endosomal enzymes). Once in the cytosol, the viral nucleic acid may be released from the capsid upon completion of uncoating or may function while still attached to capsid components. Nonenveloped animal viruses cannot employ the membrane fusion mechanism for release from the endosome. In this case, it is thought that the low pH of the endosome causes a conformational change in the capsid. The altered capsid contacts the endosome membrane and either releases the viral nucleic acid into the cytosol or ruptures the membrane to release the intact nucleocapsid.

3- **Synthesis Stage:**

This stage of the viral life cycle differs dramatically among viruses because the genome of a virus dictates the events that occur. For dsDNA viruses, the synthesis stage can be very similar to the typical flow of information in cells. That is, the genetic information is stored in DNA and

replicated by enzymes called DNA polymerases, recoded as mRNA (transcription), and decoded during protein synthesis (translation). Because of this similarity, some dsDNA viruses have the luxury of depending solely on their host cells' biosynthetic machinery to replicate their genomes and synthesize their proteins.

The same is not true for RNA viruses. Cellular organisms (except for plants) lack the enzymes needed to replicate RNA or to synthesize mRNA from an RNA genome. Therefore, RNA viruses must carry in their nucleocapsids the enzymes needed to complete the synthesis stage, or the enzymes must be synthesized during the infection process.

Some animal and plant viruses carry out the synthesis stage and subsequent assembly step within the host's cytoplasm. To protect these processes from host defenses, some viruses bring about the reorganization of host cell membranes (e.g., membranes of the endoplasmic reticulum, Golgi apparatus, and lysosomes) to form membranous structures that enclose the machineries needed for genome replication, transcription, and protein synthesis. The structures are called **viral replication complexes**, and they appear as vesicles, tubular structures, and other forms in electron micrographs of infected cells. Other viruses carry out synthesis and assembly in defined areas within the cytoplasm that are not enclosed by membranes. These areas of concentrated viral genomes, mRNAs and proteins are called **viroplasms**, and they are also visible in electron micrographs of infected cells. Both viral replication complexes and viroplasms are sometimes referred to as **virus factories**.

One important feature of the synthesis stage is the tight regulation of gene expression and protein synthesis. Genes are often referred to as early, middle, or late genes based on when they are expressed. The proteins they encode are likewise referred to as early, middle, or late proteins. Many early proteins are involved in taking over the host cell. Middle proteins often participate in replication of the viral genome or activation of expression of late genes. Late proteins usually include capsid proteins and other proteins involved in self-assembly and release.

4- **Assembly:**

Several kinds of late proteins are involved in the assembly of mature virions. Some are nucleocapsid proteins, some are not incorporated into the nucleocapsid but participate in its assembly, and still other late proteins are involved in virion release. In addition, proteins and other factors synthesized by the host may be involved in assembling mature virions.

The assembly process can be quite complex with multiple subassembly lines functioning independently and converging in later steps to complete nucleocapsid construction. The baseplate, tail fibers, and head components of bacteriophage T4 are assembled separately. Once the baseplate is finished, the tail tube is built on it and the sheath is assembled around the tube. The phage prohead (procapsid) is constructed with the aid of scaffolding proteins that are degraded or removed after assembly is completed. DNA is incorporated into the prohead by a complex of proteins sometimes called the **"packasome."** The packasome consists of a protein called the portal protein, which is located at the base of the prohead, and an enzyme called terminase, which moves DNA into the prohead. The

movement of DNA consumes energy in the form of ATP, which is supplied by the metabolic activity of the host bacterium. After the head is completed, it spontaneously combines with the tail assembly.

5- **Virion Release:**

Several release mechanisms have been identified. The two most common are release by lysing the host cell and release by budding. Release by lysis is especially common for bacterial viruses and some nonenveloped animal viruses. This process involves the activity of viral proteins. For instance, lysis of *E. coli* by T4 requires two specific proteins. One is lysozyme, an enzyme that attacks peptidoglycan in the host's cell wall. The other, called holin, creates holes in *E. coli's* plasma membrane, enabling T4 lysozyme to move from the cytoplasm to the peptidoglycan.

Budding is frequently observed for enveloped viruses; in fact, envelope formation and virion release are usually concurrent processes. When virions are released by budding, the host cell may survive and continue releasing virions for some time. All envelopes of animal viruses are derived from host cell membranes by a multistep process. First, virus-encoded proteins are incorporated into the membrane. Then the nucleocapsid is simultaneously released and the envelope formed by membrane budding. In several virus families, a matrix (M) protein attaches to the plasma membrane and aids in budding. Most envelopes arise from the plasma membrane. The endoplasmic reticulum, Golgi apparatus, and other internal membranes also can be used to form envelopes.

Interestingly, some viruses are not released from their host cell into the surrounding environment. Rather, their virions move from one host cell

directly to another host cell. Most fungal viruses lack an extracellular phase in their replicative cycles. Instead, they are transmitted by cell division, spore formation, or during mating. Vaccinia viruses elicit the formation of long actin tails that propel nucleocapsids through the plasma membrane, directly into an adjacent cell. In this way, the virus avoids detection by the host immune system. The genomes or nucleocapsids of many plant viruses also move directly from cell to cell through small connections called plasmodesmata that link adjacent cells. This spread of the virus typically involves virus-encoded movement proteins.

DR.WAQAS SAADI MAHMOOD