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Ari Helenius' Lecture Part 1:
Cell Biology of Virus Entry

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Contents

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1. Keywords and Terms

Virus, host cell, virus entry, virus replication, genome, capsid, lipid bilayer envelope, binding, endocytosis, uncoating, glycolipid, filopodia, signal transduction, Simian virus 40, Human Papilloma Virus-16

2. Lecture Notes

What is a virus?

Viruses are obligate intracellular parasites. This means that they cannot replicate on their own but instead have to rely on host cells and the cellular biosynthetic machinery for reproduction. They enter the cytosol or nucleus of a host cell, and force the cell to

synthesize new virus particles according to instructions that they provide in the form of RNA or DNA.

A virus particle is generally very small and structurally quite simple. In addition to proteins and sometimes also lipids, it contains as an essential component one or more molecules of DNA or RNA. These constitute the genome of the virus that carries genes necessary for the production of new viruses in the host cell. The number of genes varies from a handful to a few hundred depending on the virus.

In a virus particle isolated from the extra cellular space, the genome is present in a highly condensed form and protected by a coat of proteins in the so called viral capsid. Capsids take the form of a helix or they have an icosahedral protein shell that encloses the genome. In enveloped viruses, the capsid is further covered by a lipid bilayer membrane, the viral envelope. The membrane contains additional viral proteins that are usually glycosylated and often form spike-like projections required for cell attachment and penetration into the host cell.

Virus particles carry the viral genome and accessory proteins from the infected host cell to an uninfected cell. Importantly, the particle must also protect the genome in transit so that it can be delivered in a replication competent form. The target cell can be a neighboring cell in the same tissue and organism, or a cell in another organism. The lifecycle of a typical virus occurs in four stages: Entry and uncoating, replication, assembly, and release. Once infected, the new host cell produces and releases thousands of new virus particles. The cell often suffers from the infection and eventually dies. Many viruses are therefore dangerous pathogens.

There are different types of animal viruses

There are many different types of viruses. Here we focus on animal viruses. They differ in size, shape, composition, and in the type of cells and host animals that they infect. Both DNA and RNA viruses come in both enveloped and non-enveloped forms.

Viruses as a health risk

Infectious diseases are the second most common cause of human deaths globally, and about half of these are attributed to viruses. There are a number of long established viruses in the human populations that cause diseases such as polio and measles. In

addition, there are re-emerging viral diseases already known as human pathogens but expanding due to local and global changes in the world. Finally, there are emerging viruses, such as SARS virus and HIV-1, which have their origin in other species and are new to the human population.

Virus transmission

Viruses are infectious and spread from organism to organism. The most common routes of virus transmission are directly via contact, via aerosols, through contaminated surfaces, by exchange of body fluids, etc. Viruses can also be transferred by insects, and as contaminants in food and water. There are many factors that contribute to the rate and mode of transmission, including population density and fitness, trade and travel, hygiene, and effective vaccination and other counter measures.

Studying virus entry

The study of virus entry requires an interdisciplinary approach including, in addition to virological methods, approaches from cell biology, molecular biology, biochemistry, systems biology, etc. Light and electron microscopy are important tools, together with *in vitro* systems, and a variety of perturbations through inhibitors, mutant viruses and mutant cells, expression of dominant and constitutively active proteins, and siRNA silencing. Most studies so far have been performed in tissue culture cells.

Example 1: Entry of Semliki forest virus (SFV)

SFV is a simple enveloped RNA virus that is transmitted by mosquitoes. It serves as an important model virus for analyzing entry. A variety of experiments including scanning electron microscopy have demonstrated that after attaching to the cell surface, the SFV particles are internalized by endocytosis in clathrin-coated vesicles and delivered to the lumen of endosomal vacuoles called endosomes. Here they are exposed to lowered pH, and this triggers a conformational change in the spike glycoproteins. This change activates a membrane fusion activity in the viral proteins, and the virus fuses its envelope membrane with the limiting membrane of the endosome. As a result, the viral capsid is delivered to the cytosol where the RNA is rapidly uncoated and used as an mRNA to translate viral proteins. Within a few hours, the single particle that entered manages to convert the host cell into a virus factory.

The entry program of a typical animal virus

Virus entry occurs in multiple stages beginning with virus binding to cell surface receptors. Different viruses use different cell surface molecules as their receptors. Following binding, most viruses move laterally on the cell surface. During this process, they may activate cellular signaling pathways to prime the host cell for uptake. Although some viruses can penetrate directly through the plasma membrane, the majority undergo endocytosis first. The primary endocytic vesicles deliver the viruses to secondary organelles such as endosomes whose conditions favor virus penetration. Once in the cytosol, the capsids are often transported on microtubules to the location of uncoating and replication. For most DNA viruses the target is the nuclear pore complex through which the genome is transported into the nucleus where replication is initiated.

Of course there are exceptions. Some viruses fuse directly with the cell surface by-passing the need for endocytosis. Others fail to initiate cellular signaling pathways and enter pre-formed endocytic carriers. Viruses that replicate in the cytosol are transported to specific cytosolic locations for uncoating and replication rather than to the nuclear pore complex.

Entry involves multiple steps where the movement of the virus deeper into the cell is coupled to step-wise uncoating of the particle according to a built-in disassembly program. Almost all steps depend on cellular factors. The viruses respond to cues such as low pH by undergoing changes made possible by the metastable structure of the capsids or spike glycoproteins. The incoming virus and the cell engage in a sort of biochemical dialogue that allows the critical events in the program to occur in the right place at the right time. For all of this to be possible, the viruses must speak the language of the cell, and the cell has to inadvertently support the entry program.

Differences between enveloped and non-enveloped virus infection strategies

For transmission of the genome form cell to cell, enveloped viruses use the same mechanism as cells to move macromolecules between membrane-bounded compartments, i.e. vesicle-mediated transfer. The virus envelope serves as the transport vesicle. The cargo, in this case the viral capsid, is packaged into the vesicle during budding and membrane fission from the plasma membrane or the limiting membrane of an intracellular transport vesicle. The envelope membrane thus formed

later fuses with a membrane in the uninfected cell releasing the capsid into the cytosol. Thus transfer is achieved without the capsid having to pass directly through a hydrophobic interior of a membrane.

Non-enveloped viruses work differently. They are typically released from infected cells by a lytic event. The released particles are then endocytosed and penetrate into the cytosol of another cell either by lysing an endosomal membrane, or by generating a channel in a cell membrane through which the genome can slip into the cytosol.

Virus binding

Viruses use many different types of cell surface receptor molecules. Their identity and tissue distribution dictates which cell-type and species can be infected, and ultimately what type of disease the virus causes. The receptors are normal cellular proteins, lipids, or carbohydrates on the cell surface that end up inadvertently playing a role in assisting a pathogen. Often viruses use multiple receptors, and receptor binding is multivalent. Molecules that serve merely to bind the viruses and concentrate them on the cell surface are called attachment factors, whereas the receptors mediate in addition conformational changes in the virus, trigger signaling, or induce endocytosis.

Example 2: Simian Virus 40 (SV40)

SV40 is a non-enveloped DNA virus that replicates in the nucleus. It is a member of the polyoma virus family. It has an icosahedral capsid - 42nm in diameter - composed of 72 homo-pentamers of the surface protein VP1. Binding to the cell surface is mediated by attachment of VP1 pentamers to the carbohydrate moiety of GM1, a ganglioside. Gangliosides and other glycolipids are components of the plasma membrane. The crystal structure of VP1 in complex with GM1 shows that each VP1 in the pentamer can bind one GM1 receptor molecule.

Cell free systems can be used to study viruses

Viruses on the surface of cells display multiple modes of movement ranging from random diffusion to directed movement (‘surfing’) along surface specializations such as filopodia. In some instances, artificial membranes can be used to investigate particular aspects of movement. Using this artificial system it was recently shown that the SV40

bound to GM1 receptors moves along the membrane by sliding and wobbling rather than rolling.

Example 3: Human papilloma virus 16 (HPV16)

HPV16 is a member of the papillomaviridae. It is a non-enveloped DNA virus that binds to cell surface heparansulfates, enters cells by endocytosis, and penetrates after acid-activation. HPV16 replicates in the nucleus, and is a major causative agent of cervical cancer. Using electron microscopy the virus was found to bind to cellular filopodia, long motile finger-like projections from the cell body. When visualized by fluorescence live cell imaging, it was determined that HPV16 particles are moving or "surfing" on the filopodia. Movement occurs in a directed fashion towards the cell body, and is dependent upon retrograde movement of actin within the filopodia. Endocytosis of the virus particles appears to occur at the base of filopodia.

Summary of events during virus binding

Virus binding occurs by multivalent association of the virus with its receptor. This results in receptor clustering, which in turn activates a trans-bilayer coupling (outside-in) initiating cellular signaling pathway(s). This activation informs the cell of the presence of the virus particle, resulting in an endocytic "reflex" by the cell and the uptake of the virus particle. This is followed by transport of the particle into cytoplasmic vacuoles followed by virus penetration.

Words you should be able to define

Virus, host cell, entry, uncoating, replication, virus genome, capsid, non-enveloped virus, enveloped virus, envelope glycoprotein, attachment factor, virus receptor, signal transduction, endocytosis, endosome, pH-activation, fusion, fission, filopodia.

3. Recommended Reading

1. Helenius, A. (2007) Virus entry and uncoating, Fields Virology, 5th edition, Lippincott Raven, Philadelphia.

2. Marsh, M., and A. Helenius. (2006) Virus entry: open sesame. Cell 124:729-40.

4. Review Questions

1. What are the major components of non-enveloped and enveloped viruses?
2. What is the basic function of a virus particle?
3. What health risk classification is given to HIV and SARS and why?
4. What factors influence transmission of viruses?
5. What type of techniques can be used to study virus entry?
6. What are the major stages of virus entry
7. Where do viruses encounter low pH and how does this serve as a “cue” for virus entry?
8. What is the major difference between enveloped and non-enveloped virus entry?
9. What are the differences between viral attachment factors and viral receptors.

5. Answers to Review Questions

1. For both non-enveloped and enveloped viruses, the major components are nucleic acids which make up the viral genome and proteins that form the viral capsids. In addition, enveloped viruses have a lipid bilayer.

2. To transport the viral genome and accessory proteins from an infected cell to the cytosol or the nucleus of a non infected cell in a replication competent form.

3. These viruses are emerging viruses. They are given this classification because they are not historically associated with infection in the human population.

4. Some of the things that influence virus spread are mentioned in the lecture, including population density and fitness, time of health care response, and effectiveness of

treatment. Transmission can also be influenced by the virus pathogen, its rate of replication, pathogenicity, and mode of spread.

5. Light microscopy of live and fixed cells or tissues, electron microscopy, inhibitors of cellular factors, mutant cell lines, dominant negative and constitutively active proteins, siRNA silencing, mutant viruses, and virus perturbations such as detergent or protease treatment.

6.
    a. Binding
    b. lateral movement
    c. activation of signaling
    d. virus endocytosis
    e. penetration (capsid release into the cytosol)
    f. genome uncoating

7. Viruses encounter low pH in endosomes after internalization. Low pH triggers conformational changes in the virus resulting in its activation of membrane fusion, endosome lysis activity, or pore formation.

8. With their lipid bilayer, enveloped viruses can utilize fusion/fission to transport the viral capsids from cytosol to cytosol. Non-enveloped viruses have to form pores or induce lysis of carrier vesicles for capsid or genome release.

9. Attachment factors are only responsible for binding of viruses to the cell surface. Virus receptors initiate conformation changes in the virus particle, or mediate signal transduction, and virion endocytosis.

6. Discussion Questions

1. Throughout this lecture, it is stressed that viruses are obligatory intracellular parasites that require cellular factors for all aspects of the viral lifecycle. What does this imply about the origin of viruses?

2. What causes viruses to “emerge” in the human population?
3. If you were to design new antiviral drugs would you target viral or cellular factors and why?
4. What cellular factors not present in the artificial system may contribute to these major differences in virus motility?
5. What advantage might surfing along filopodia give a virus during human infection?

7. Answers to Discussion Questions

1. The origin of viruses is not discussed in this lecture. Although there is no definitive answer it is a highly thought-provoking question. There are three main hypotheses; degeneracy (or regression), cellular origin, and co-evolution. The degeneracy hypothesis suggests that viruses were once small parasitic cells that over time lost the genes not required for their parasitism. The lack of evolutionary virus-cell intermediates weakens this hypothesis. The hypothesis suggesting a host cell derived origin maintains that viruses were formed when portions of cellular nucleic acid (DNA or RNA) “escaped” and became migratory moving from cell to cell. This hypothesis is favored for the origin of large DNA viruses. The co-evolution hypothesis states that viruses and cells evolved together. The requirement for cellular machinery for virus infection, receptor usage, and cell-type specificities lends support to this hypothesis. It also dictates that cells must have preceded viruses.
2. It is important to realize that newly emerging viruses are not new but rather modified versions of viruses that have existed in other species in nature. Viruses can jump species (i.e. Avian and Swine Flu). The encounters between species that lead to this type of reaction can occur as the result of climate change, deforestation, social unrest, and the increase in trade and travel. Many other factors that contribute to the emergence of new human viruses can be discussed.
3. This is not addressed in the lecture but all the tools needed to think about this are presented. There is also not a set answer, but rather arguments for both strategies.
    a. **Viral targets:** The current strategy behind most antiviral agents is to target viral proteins and activities. The rationale behind this choice is that viral proteins are dissimilar to host proteins and targeting them thereby reduces the likelihood of toxicity or off-target effects on the host. Targeting viral proteins also offers the advantage of common targets in multiple viral strains or virus species. In addition, targeting viral factors has been shown to work (agents against HIV

provide a good example). There are also several disadvantages to targeting viral components, including the potential for viral resistance and mutation (HIV again one example).

b. **Cellular targets:** Development of antiviral agents that target cellular proteins is now emerging as a potential way to go in the future. As indicated throughout the lecture viruses depend on critical cellular factors for each stage of their life cycle. This offers many potential targets against which inhibitors can be made. Since many viruses use the same cellular factors, one inhibitor may be used to target multiple virus strains and types. In addition, viruses cannot easily gain resistance against such drugs by undergoing point mutations.

c. In the lecture the movement of SV40 on CV1 cells and on artificial lipid bilayers containing the virus receptor was presented. Tracking of viruses on the cell surface showed multiple modes of behavior including random diffusion, directional movement, slow drifting, start and stop, and stationary binding. In contrast, on artificial lipid bilayers all viruses displayed random movement.

4. The most obvious answer is virus induced signaling. The in vitro system lacks the effector molecules needed to cause these different modes of movement. An important consideration is the role of the cytoskeleton and what role this may play in start/stop and stationary modes. Is there actin caging upon receptor activation, and if so how does it affect movement? Is there a critical mass of receptor clustering required for endocytosis? It is important to consider how receptor binding and trans-bilayer coupling can affect membrane curvature and virus movement.

5. In most instances, tissue culture experiments do not reflect the situation viruses encounter within a host organism. Tissues are formed from tightly opposed cells that have polarity. In many instances virus receptors are expressed on the basolateral surface of cell and are not easily accessible to viruses entering from the outside world. Some viruses can induce rupture of cell layers while others must take advantage of wounds or micro-abrasions to gain access to their receptors. During the process of wound-healing, filopodia are formed at the leading edge of cells as they migrate together to close the abrasion. This would indicate that viruses that enter polarized cells from the basolateral surface, such as HPV16, have adapted to surf on filopodia taking advantage of this naturally occurring wound healing process to efficiently reach their host cells and receptors.

8. Explain or Teach These Concepts to a Friend

1. Explain how Avian and Swine Influenza viruses “learn” to infect humans. (This will require additional background reading)
2. Explain the entry stages of a typical animal virus.
3. Explain how and why viruses activate cellular signaling pathways.
4. Explain how the use of artificial systems can be used to simplify the study virus binding and entry (This may require some background reading)

9. Research the Literature on Your Own

1. Entry of Herpes simplex virus 1 (HSV1) is complicated by the use of multiple receptors, several viral glycoproteins, and different cell type-dependent entry mechanisms. What are different attachment factors and virus receptors used by HSV1? (Hint: check the literature on “HSV and Glycosaminoglycans”)
2. It is known that many viruses can use different cell surface receptors to promote their uptake. What advantage might this be to the virus with regards to infection in a human host? (Hint: check the literature on “virus receptors and tropism”)
3. What evidence is there that influenza virus uses clathrin-mediated endocytosis for entry? Using the techniques presented for “studying virus entry” as a guide, determine what cellular factors are used by this virus during its clathrin-mediated uptake.
4. It was shown in the lecture that HPV16 uses filopodia to enter the cell body. Other viruses have been demonstrated to use this mechanism as well. Movement has been shown to depend on retrograde actin flow within the filopodia. What is known about the mechanisms used by viruses to associate with filopodial actin (Hint: check the literature on “viruses and filopodia”)