

BT601-Virology

Final term Handouts (Reading Tabs)

Lecture 106 – 215 (Prepared by Mehwish Mughal)

LECTURE 106: CELL CULTURE

Growing Animal Viruses in Cell Culture

Cell culture is a process by which cells are grown under controlled conditions.

Propagation of cell cultures

All procedures involving cell lines should be performed in a Class II biological safety cabinet under strict aseptic conditions to minimize the risk of contaminating the cells with bacteria, yeasts, and mycoplasmas.

Media: Most media in use today are chemically defined, but are supplemented with 5-20% serum. The medium used for any particular cells will be determined by the cell type and the procedures to be performed with these cells. The culture medium is the most important component of the culture environment, because it **provides the necessary nutrients, growth factors, and hormones** for cell growth, as well as regulating the pH and the osmotic pressure of the culture.

Serum is vitally important as a source of growth and adhesion factors, hormones, lipids and minerals for the culture of cells in basal media. In addition, serum also regulates cell membrane permeability and serves as a carrier for lipids, enzymes, micronutrients, and trace elements into the cell.

pH: Most normal mammalian cell lines grow well at pH 7.4, and there is very little variability among different cell strains.

CO₂: The growth medium controls the pH of the culture and buffers the cells in culture against changes in the pH

Temperature The optimal temperature for cell culture largely depends on the body temperature of the host from which the cells were isolated, and to a lesser degree on the anatomical variation in temperature (e.g., temperature of the skin may be lower than the temperature of skeletal muscle). Most human and mammalian cell lines are maintained at 36°C to 37°C for optimal growth.

Buffer: Culture media may require buffering. A buffer may be incorporated in the medium to stabilize the pH, but in open dishes, exogenous CO₂ may still be required by cell lines,

particularly at low cell concentrations, to prevent the total loss of dissolved CO₂ and bicarbonate from the medium.

History

Although animal cell culture was first successfully undertaken by Ross Harrison in 1907, it was not until the late 1940's to early 1950's that several developments occurred that made cell culture widely available as a tool for scientists.

- Cell culture was first successfully undertaken by Ross Harrison in 1907
- Roux in 1885 for the first time maintained embryonic chick cells in a cell culture
- 1878: Claude Bernard proposed that physiological systems of an organism can be maintained in a living system after the death.
- 1885: Roux maintained embryonic chick cells in a saline culture.
- 1897: Loeb demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma.
- 1903: Jolly observed cell division of salamander leucocytes in vitro.
- 1907: Harrison cultivated frog nerve cells in a lymph clot held by the hanging drop' method and observed the growth of nerve fibers in vitro for several weeks. He was considered by some as the father of cell culture.

APPLICATIONS OF CELL CULTURE

Cell culture has become one of the major tools used in cell and molecular biology. Some of the important areas where cell culture is currently playing a major role are briefly described below:

Model Systems: Cell cultures provide a good model system for studying 1) basic cell biology and biochemistry, 2) the interactions between disease-causing agents and cells, 3) the effects of drugs on cells, 4) the process and triggers for aging, and 5) nutritional studies.

Toxicity Testing Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types. Especially important are liver- and kidney-derived cell cultures.

Cancer Research: Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied. In addition, it is possible, by the use of chemicals, viruses and radiation, to convert normal cultured cells to cancer causing cells. Thus, the mechanisms that cause the change can be studied. Cultured cancer cells also serve as a test system to determine suitable drugs and methods for selectively destroying types of cancer.

Virology: One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production. Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.

Genetic Engineering: The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists wishing to study the cellular effects of the expression of these genes (new proteins). These techniques can also be used to produce these new proteins in large quantity in cultured cells for further study.

Gene Therapy: Cells having a functional gene can be replaced to cells which are having non-functional gene

LECTURE 107: VIRAL IDENTIFICATION AFTER GROWING

Virus Detection

Following is the list of some virus detection methods

- Plaque formation
- Cytopathic Effects
- Fluorescent Antibody technique
- Nucleic Acid hybridization
- Electron Microscopy

1. Plaque formation

Bacteriophages can be grown in the presence of a dense layer of bacteria (also called a **bacterial lawn**) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask. The soft 0.7% agar allows the bacteriophages to easily diffuse through the medium. For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a clear zone called a **plaque** is detected (Figure1). As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.



Figure1: These plates contain bacteriophage T4 grown on an *Escherichia coli* lawn. Clear plaques are visible where host bacterial cells have been lysed. Viral titers increase on the plates to the left.

2. Cytopathic effects:

Animal and plant viruses are detected by **cytopathic effects**. **Cytopathic effects (CPEs)** are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis Figure 2&3.

Further pathological changes include viral disruption of the host genome and altering normal cells into transformed cells, which are the types of cells associated with carcinomas and sarcomas. The type or severity of the CPE depends on the type of virus involved.

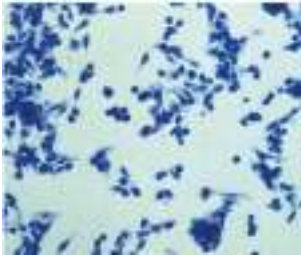


Figure 2: Cell enlargement, rounding, and distinctive grape-like clusters in *Adenovirus* infected cells

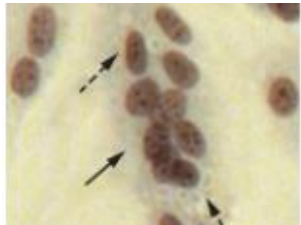


Figure 3: Syncytium and faint basophilic cytoplasmic inclusion bodies in *Paramyxovirus* infected cells

3. Fluorescent antibody technique

The Fluorescent antibody technique is based on the observation that in animals infected by virus have virus proteins (antigen) present in their tissues. For example, rabies virus is present in nervous tissue (and not blood like many other viruses), the ideal tissue to test for rabies antigen is brain. The most important part of a dFA test is fluorescently-labeled anti-rabies antibody. When labeled antibody is incubated with rabies-suspect brain tissue, it will bind to rabies antigen. Unbound antibody can be washed away and areas where antigen is

present can be visualized as fluorescent-apple-green areas using a fluorescence microscope. If rabies virus is absent there will be no staining.



Figure 4: Positive dFA

Nucleic Acid hybridization

A technique in which single-stranded nucleic acids (DNA or RNA) are allowed to interact so that complexes called **hybrids** are formed by molecules with similar, complementary sequences.

Through nucleic acid hybridization, the degree of sequence identity between nucleic acids can be determined and specific sequences detected in them. The hybridization can be carried out in solution or with one component immobilized on a gel or, most commonly, on nitrocellulose

paper. Hybridizations are done in all combinations: DNA-DNA (DNA can be rendered single-stranded by heat denaturation), DNA-RNA or RNA-RNA.

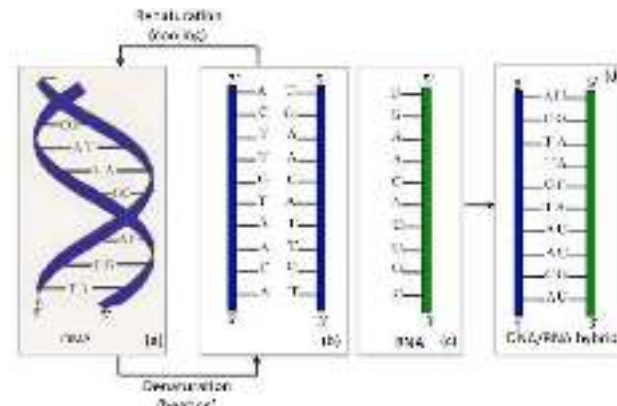


Figure 5: Nucleic Acid hybridization

Electron microscopy

Electron microscopy (EM) is a technique for obtaining high resolution images of biological and non-biological specimens. It is used in biomedical research to investigate the detailed structure of tissues, cells, organelles and macromolecular complexes. The high resolution of EM images results from the use of electrons (which have very short wavelengths) as the source of illuminating radiation.

LECTURE 108: MULTIPLICATION OF VIRUSES: VIRUS ATTACHMENT

Introduction

Because viruses are obligate intracellular parasites, the genome must enter a cell for the viral reproduction cycle to occur. The physical properties of the virion are obstacles to this seemingly simple goal. Virus particles are too large to diffuse passively across the plasma membrane. Furthermore, the viral genome is encapsidated in a stable coat that shields the nucleic acid as it travels through the harsh extracellular environment. These impediments must all be overcome during the process of viral entry into cells. Encounter of a virus particle with the surface of a susceptible host cell induces a series of events that lead to entry of the viral genome into the cytoplasm or nucleus.

The first step in entry is adherence of virus particles to the plasma membrane, an interaction mediated by binding to a specific **receptor** molecule on the cell surface.

General Principles

Infection of cells by many, but not all, viruses requires binding to a receptor on the cell surface. Exceptions include viruses of yeasts and fungi, which have no extracellular phases, and plant viruses, which are thought to enter cells in which the cell wall has been physically damaged, for

example by insects or farm machinery. A receptor is a cell surface molecule that binds the virus particle and participates in entry. It may induce conformational changes in the virus particle that lead to membrane fusion or penetration, and it may also transmit signals that cause uptake. The receptor may also bring the bound particle into endocytic pathways.

Receptors for viruses comprise a variety of cell surface proteins, carbohydrates, and lipids, all with functions in the cell unrelated to virus entry.

Many virus receptors have been identified in the past 30 years and include immunoglobulin-like proteins, ligand-binding receptors, glycoproteins, ion channels, gangliosides, carbohydrates, proteoglycans, and integrins.

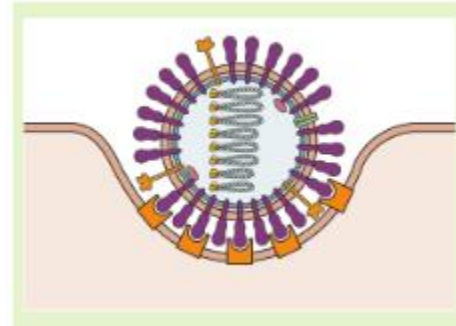


Figure: Binding of viruses

The receptor may determine the **host range** of a virus, i.e., its ability to infect a particular animal or cell culture. For example, poliovirus infects primates and primate cell cultures but not mice or mouse cell cultures. Receptors can also be determinants of tissue **tropism**, the predilection of a virus to invade and reproduce in a particular cell type. Nonenveloped viruses bind via the capsid surface or projections.

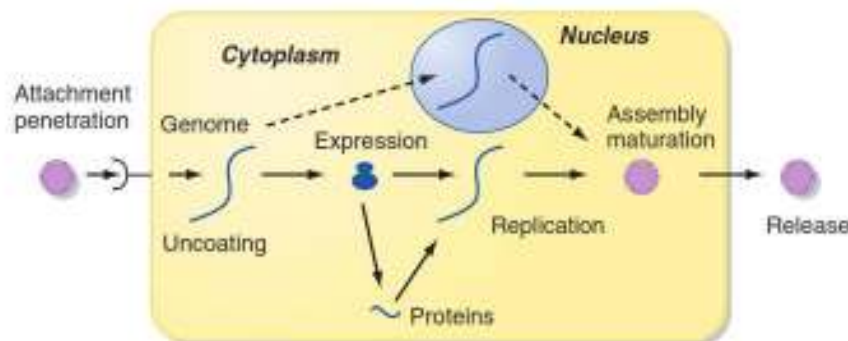


FIGURE 4.6 A generalized scheme for virus replication.

This diagram shows an outline of the steps which occur during replication of a "typical" virus which infects eukaryotic cells. See the text for more details.

LECTURE 109: MULTIPLICATION OF VIRUSES: VIRUS ENTRY

Entry in the target cell normally occur in a very short time after attachment of the virus to its receptor in the cell membrane. Unlike attachment, virus entry is generally an energy-dependent process—that is, the cell must be metabolically active for this to occur.

Three main mechanisms are involved:

1. **Membrane Fusion** or **Hemifusion State**: The cell membrane is punctured and made to further connect with the unfolding viral envelope.

2. **Endocytosis:** The host cell takes in the viral particle through the process of endocytosis, essentially engulfing the virus like it would a food particle.
3. **Viral Penetration:** The viral capsid or genome is injected into the host cell's cytoplasm.

Membrane Fusion:

Fusion of the virus envelope (so this is only applicable to enveloped viruses) with the cell membrane, either directly at the cell surface or following endocytosis in a cytoplasmic vesicle (Figure 1), which, requires the presence of a specific fusion protein in the virus envelope—for example, influenza hemagglutinin or retrovirus TM glycoproteins. These proteins promote the joining of the cellular and virus membranes which results in the nucleocapsid being deposited directly in the cytoplasm. There are two types of virus-driven membrane fusion: one pH dependent, the other pH independent.

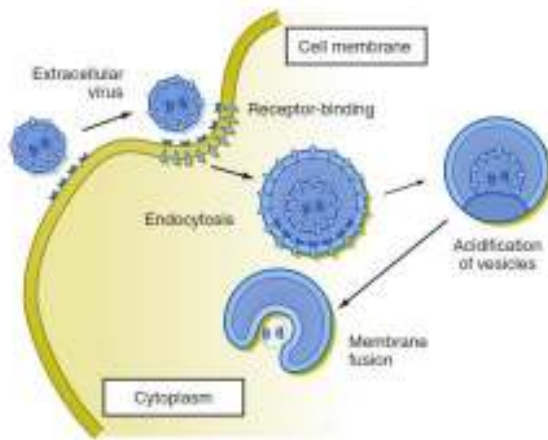


Figure 1: Virus-induced membrane fusion. This uptake process is dependent on the presence of a specific fusion protein on the surface of the virus which, under particular circumstances (e.g., acidification of the virus-containing vesicle), becomes activated, inducing fusion of the vesicle membrane and the virus envelope.

Endocytosis: Endocytosis of the virus into intracellular vacuoles (Figure 2). This is probably the most common mechanism of virus entry into cells. It does not require any specific virus proteins (other than those already utilized for receptor binding) but relies on the normal formation and internalization of coated pits at the cell membrane. The formation of coated pits results in the engulfment of a membrane-bounded vesicle by the cytoplasm of the cell. The lifetime of these initial coated vesicles is very short. Within seconds, most fuse with endosomes, releasing their contents into these larger vesicles. Moreover, as endosomes fuse with lysosomes, the environment inside these vessels becomes increasingly hostile as the pH falls, while the concentration of degradative enzymes rises. This means that the virus particle must leave the vesicle and enter the cytoplasm before it is degraded.

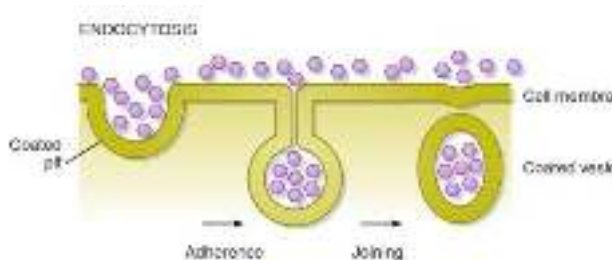


FIGURE 2 Endocytosis of virus particles: The processes of endocytosis and exocytosis are involved in both the take up and release of enveloped virus particles from host cells. Viruses modify these normal cellular processes by encoding proteins which promote endocytosis.

Entry via genetic injection

Attaching to the surface of the cell via receptors

- Injecting only its genome into the cell
- Leaving the rest of the virus on the surface
- This is restricted to viruses in which only the gene is required for infection of a cell

Example includes the Bacteriophages; for example, when the tail fibers of the T2 phage land on a cell, its central sheath pierces the cell membrane and the phage injects DNA from the head capsid directly into the cell.

LECTURE 110: MULTIPLICATION OF VIRUSES: UNCOATING

Uncoating is a general term for the events that occur after entry, in which the virus capsid is completely or partially removed and the virus genome is exposed, usually in the form of a nucleoprotein complex.

In one sense, the removal of a virus envelope that occurs during membrane fusion is part of the uncoating process. Fusion between virus envelopes and endosomal membranes is driven by virus fusion proteins. These are usually activated by the uncloaking of a previously hidden fusion domain as a result of conformational changes in the protein induced by the low pH inside the vesicle.

LECTURE 111: BIOSYNTHESIS OF DNA VIRUSES

Replication of genetic information is the single most distinctive characteristic of living organisms, and nowhere in the biosphere is replication accomplished with greater economy and apparent simplicity than among viruses. To achieve the expression, replication, and spread of their genes, different virus families have evolved diverse genetic strategies and replicative cycles.

Most DNA viruses produce functional viral mRNAs by taking the transcriptional machinery of the cell. This machinery includes RNA pol II, multiple transcription factors, poly(A) polymerase, guanylyltransferase, methyltransferases, and the pathway of mRNA export from the nucleus. Even viruses with unusual genome structures such as parvoviruses and hepadnaviruses use these cellular components because their genomes are rendered into dsDNA before transcription. Only DNA viruses that replicate in the cytoplasm use virus-specific enzymes for transcription and posttranscriptional modification of their mRNAs.

Synthesis of viral proteins

Viral protein synthesis: virus mRNA translated on cell ribosomes into two types of virus protein

a. Structural: proteins which make up the virus particle are manufactured and assembled

b. Non – structural: not found in particle, mainly enzymes for virus genome replication

Viral nucleic acid synthesis new virus genome synthesized, templates are either the parental genome or with single stranded nucleic acid genomes, newly formed complementary strands

LECTURE 112: Biosynthesis of RNA viruses

RNA genomes share a common requirement: they must be efficiently copied within the infected cell to provide both genomes for assembly into progeny virus particles and messenger RNAs (mRNAs) for the synthesis of viral proteins.

The synthesis of these RNA molecules is a unique process that has no parallel in the cell.

The genomes of all RNA viruses except retroviruses encode an **RNA-dependent RNA polymerase** to catalyze the synthesis of new genomes and mRNAs.

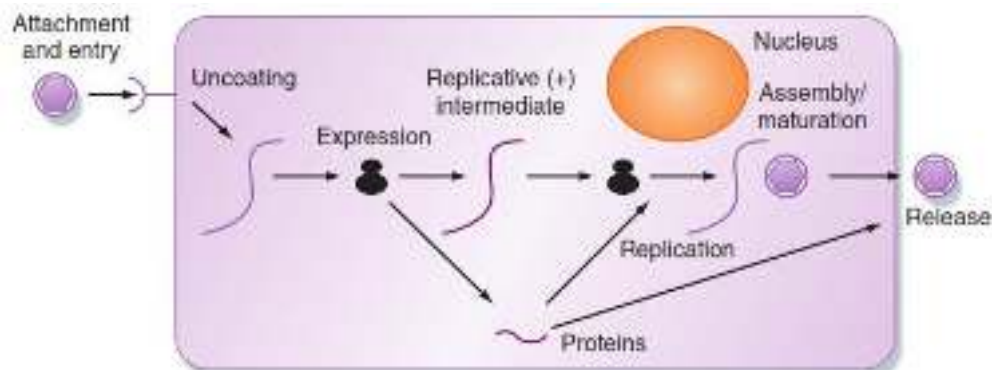


Figure: Schematic representation of the replication of class V viruses (Single-stranded (-)sense RNA)

Virus particles that contain (-) strand or double-stranded, RNA genomes must contain the RNA polymerase, because the incoming viral RNA can be neither translated nor copied by the cellular machinery.

In contrast, viral particles containing a (+) strand RNA genome lack a viral polymerase; the deproteinized RNAs of these viruses **are** infectious because they are translated in cells to produce, among other viral proteins, the viral RNA polymerase. An exception is the retrovirus particle, which contains a (+) stranded RNA genome that is not translated but rather copied to DNA by reverse transcriptase.

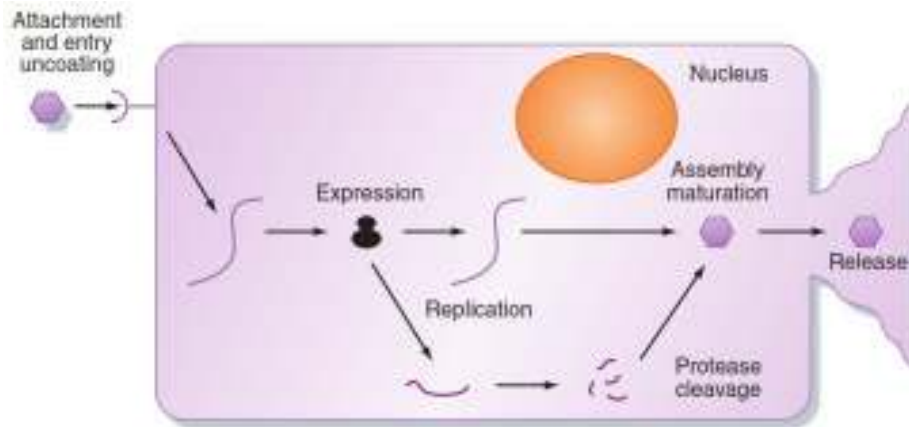


Figure: Schematic representation of the replication of class IV viruses (Single-stranded (+) sense RNA)

LECTURE 113: Viral diagnostic tests: Immunofluorescence Assay

Immunofluorescence is an assay which is used primarily on biological samples and is classically defined as a procedure to detect antigens in cellular contexts using antibodies. The specificity of antibodies to their antigen is the base for immunofluorescence.

The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as **fluorescence**. In immunofluorescence test, fluorescent dye which illuminates in UV light are used to detect/show the specific combination of an antigen and antibody. The dye usually used is **fluorescein isothiocyanate**, which gives yellow-green fluorescence. Immunofluorescence tests are also termed as **fluorescent antibody test (FAT)**.

Fluorescent dyes, such as fluorescein isothiocyanate and lissamine rhodamine, can be tagged with antibody molecules. They emit blue-green and orange-red fluorescence, respectively under ultraviolet (UV) rays in the fluorescence microscope. This forms the basis of the immunological test. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1. Direct immunofluorescence test
2. Indirect immunofluorescence test

Direct immunofluorescence test

Direct immunofluorescence test is used to detect unknown antigen in a cell or tissue by employing a known labeled antibody that interacts directly with unknown antigen. If antigen is present, it reacts with labeled antibody and the antibody coated antigen is observed under UV light of the fluorescence. It involves use of labeled antiviral antibody.

The specimen is placed on slide; fluorescent labeled antibody is then added to it and allowed for some time for Antigen-Antibody reaction. The preparation is then washed which will allow the

removal of other components except the complex of antigen and fluorescent labeled antibody. On microscopy (Fluorescence Microscopy), Antigen- Antibody complex are observed fluorescing due to the dye attached to antibody.

The need for preparation of separate labeled antibody for each pathogen is the major disadvantage of the direct immunofluorescence test.

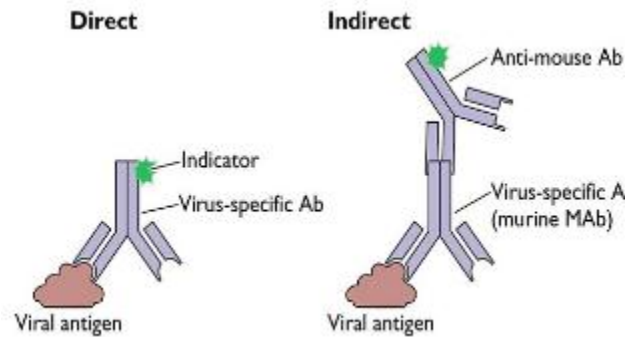


Figure: Presentation primary and secondary immunofluorescence assays

Indirect immunofluorescence test

Indirect fluorescence is a double antibody technique. The unlabeled antibodies which have bound to the antigens are visualized by a fluorescent antiglobulin reagent directed at the unlabeled antibodies. The indirect immunofluorescence test is used for detection of specific antibodies in the serum and other body fluids for sero-diagnosis of many infectious diseases.

The first step in the indirect immunofluorescence test is the incubation of a fixed antigen (e.g., in a cell or tissue) with unlabeled antibody, which becomes associated with the antigen. After careful washing, a fluorescent antibody (e.g. fluorescent labeled anti-IgG) is added to the smear. This second antibody will become associated to the first, and the antigen–antibody complex can be visualized on the fluorescence microscope.

The indirect method has the advantage of using a single labeled antiglobulin (antibody to IgG) as a “universal reagent” to detect many different specific antigen–antibody reactions. The test is often more sensitive than the direct immunofluorescence test.

Applications of Immunofluorescence assay

- Immunofluorescence may also be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules.
- Immunofluorescence has been widely used in biological research and medical research.
- This technique can even be used to visualize structures such as intermediate-sized filaments.
- Immunofluorescence aid to evaluate whether or not cells in a particular sample express the antigen in study. In cases where an immune-positive signal is found, immunofluorescence also helps to determine which subcellular compartments are expressing the antigen.

- Immunofluorescence can be used on cultured cell lines, tissue sections, or individual cells.

LECTURE 114: Biochemical Changes Induced in Virus-Infected Cell

Virus induced changes leading to cell death or accelerated growth, Answer hidden in different proteins coded by viral genome.

Two types of proteins:

1. Early proteins
2. Late proteins

Early protein synthesis usually due to genetic functioning of parental viral genomes. Include those which inhibit host cellular DNA replication, host cell RNA formation, host cell protein synthesis

Late proteins include viral structural proteins and proteins that function in repression or switching off the formation of virus induced enzymes

- Actinomycin prevents transcription of RNA from DNA template
- Puromycin or parafluoroalanine inhibits translation of genetic message from mRNA into protein

LECTURE 115: Inhibition of Host Cell RNA Synthesis

Altered host cell gene expression is a universal consequence of virus infection. The altered patterns range from inhibition of the synthesis or translation of the majority if not all cellular mRNAs, to differential increases or decreases in expression of particular sets of cellular genes as an infection proceeds.

These changes may be the result of modulation of any of the several reactions by which mammalian pre-mRNAs are produced, used as template for protein synthesis, and turned over. The impact of virus infection on cellular gene expression and the mechanism(s) by which this process is altered vary with the strategies by which viral genes are expressed. For example, transcription of viral genes from DNA templates by the cellular transcriptional machinery and processing of the transcripts in the same manner as cellular pre-mRNAs precludes inhibition of these reactions (although their selectivity may be redirected).

Furthermore, the genomes of such viruses often encode powerful activators of transcription that promote viral gene expression, but can also exert broad effects on cellular mRNA synthesis.

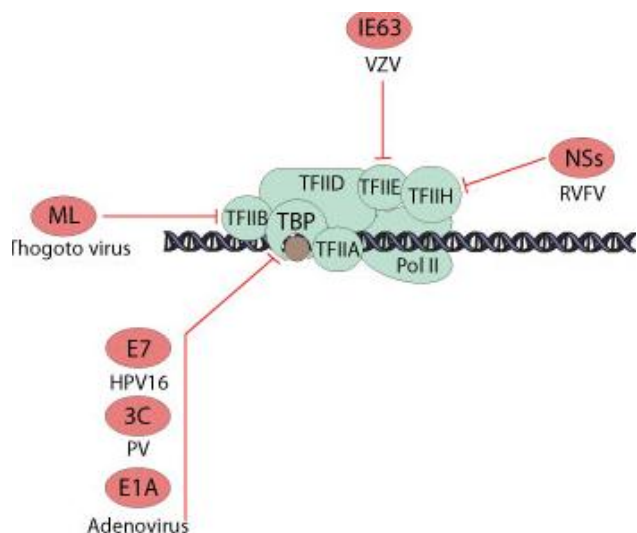
Inhibition of Cellular Gene Expression

Viral genomes typically encode one or more proteins (or RNAs) that inhibit cellular gene expression indirectly, by blocking mechanisms that expedite antiviral responses or modulating

signal transduction pathways of the host cell. However, reactions in the pathways by which cellular mRNAs are produced, translated, or degraded are inhibited directly by proteins of viruses with diverse reproduction strategies. Their impact on cellular gene expression emphasizes the fact that such proteins operate by a considerable variety of mechanisms

Host cell mRNA production can be inhibited following infection of permissive cells by viruses with (-) or (+) strand RNA genomes because the viral genomes are expressed and replicated with minimal dependence on cellular systems. Such inhibition facilitates selective and efficient synthesis of viral proteins by reducing competition of cellular with viral mRNAs for components of the translation machinery, and can also mitigate host responses that impair virus reproduction.

Although the specific reactions that are disrupted by viral proteins vary, the targets are cellular proteins necessary for production of cellular mRNAs in the nucleus or their export to the cytoplasm. Such proteins include essential components of the transcriptional initiation machinery, such as TATA-binding protein (Tbp), which is cleaved and inactivated by the poliovirus protease 3C pro ; and a catalytic subunit of RNA polymerase II targeted for degradation by alphavirus nsP2.



Proteins necessary for splicing or export of viral mRNAs to the cytoplasm can also be destroyed or inhibited by viral proteins

The impact of such inhibition can be both substantial and widespread. For instance, only some 7% of the 5,000 cellular cytoplasmic poly(A)-containing mRNAs examined by microarray hybridization could be detected by 18 h after infection with the alphavirus Sindbis virus.

LECTURE 116: Inhibition of Host Cell Protein Synthesis

In some cases, viral proteins also block translation. This property is illustrated by poliovirus proteins that inhibit not only production of cellular mRNAs but also their translation. Such seeming redundancy ensures efficient synthesis of viral proteins: inhibition of transcription and export of RNAs from the nucleus blocks the flow of newly synthesized cellular mRNAs into the cytoplasm, but those mRNAs made before infection, many of which are quite stable, are still present and potential templates for translation.

The unusual mechanism of initiation of translation of viral mRNAs and concomitant cleavage of the initiation protein eIF4G by the viral protease 2A pro eliminates such potential competition from cellular mRNAs. The very short infectious cycle of poliovirus (and other picornaviruses;

some 8 h) may necessitate particularly effective measures to prevent synthesis of cellular proteins.

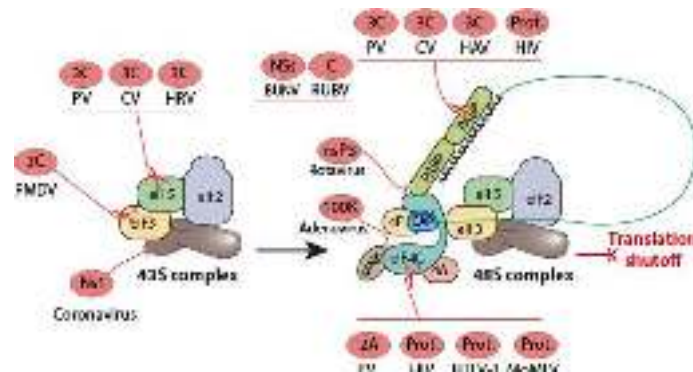
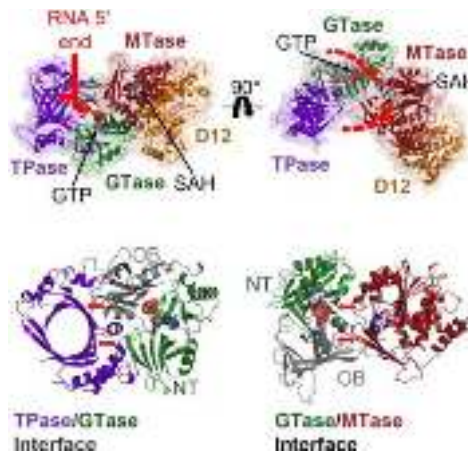


Figure: Viruses have evolved ways of interacting with the host translational machinery to shutoff host gene expression

LECTURE 117: Enzyme Metabolism of Virus Infected Cells

- Enzymes normally speed up different biochemical reactions within host cell
- They help to break down protein, fat or carbohydrate molecules
- Induction of new enzymes is followed by cellular infection by virus
- These enzymes not found in normal uninfected cells
- Or have an additive effect with the already present enzymes
- Some enzymes are induced *de novo* from information encoded by viral genome



- Observed enzyme changes may be secondary from overall disorganization of cell brought by viral development
- Particular enzyme activities thought to be regulated by information encoded in the viral genome:
 - Changes in RNA-synthetase
 - Changes in structural protein

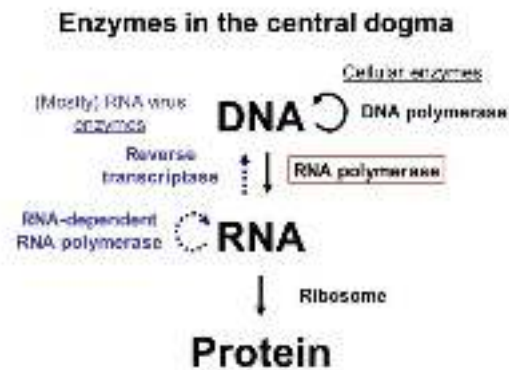
LECTURE 118: Enzymatic Changes in Virus-infected Cells

RNA-dependent RNA Polymerase

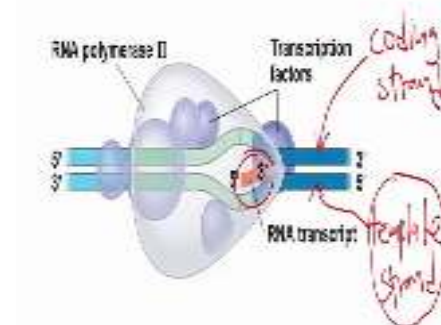
- RNA-dependent RNA polymerase (RdRP), (RDR), or RNA replicase, is an enzyme that catalyzes the replication of RNA from an RNA template
- This is in contrast to a typical DNA-dependent RNA polymerase, which catalyzes the transcription of RNA from a DNA template
- All RNA transcribed from cellular DNA
- Direct transcription
- Instead of formation of Mrna

Cell Infected With Rna Virus

- Which replicate in cytoplasm
- Polymerization of viral RNA from cellular RNA template
- This enzyme not found normally



Transcription initiation



Lecture 119: Enzymatic Changes in Virus-infected Cells: DNA-dependent DNA polymerase

The DNA polymerases are enzymes that create DNA molecules by assembling nucleotides, the building blocks of DNA. These enzymes are essential to DNA replication and usually work in pairs to create two identical DNA strands from one original DNA molecule. During this process, DNA polymerase “reads” the existing DNA strands to create two new strands that match the existing ones.

Every time a cell divides, DNA polymerase is required to help duplicate the cell’s DNA, so that a copy of the original DNA molecule can be passed to each of the daughter cells. In this way, genetic information is transmitted from generation to generation.

Before replication can take place, an enzyme called helicase unwinds the DNA molecule from its tightly woven form. This opens up or “unzips” the double stranded DNA to give two single strands of DNA that can be used as templates for replication.

DNA polymerase adds new free nucleotides to the 3' end of the newly-forming strand, elongating it in a 5' to 3' direction. However, DNA polymerase cannot begin the formation of this new chain on its own and can only add nucleotides to a pre-existing 3'-OH group. A primer is therefore needed, at which nucleotides can be added. Primers are usually composed of RNA and DNA bases and the first two bases are always RNA. These primers are made by another enzyme called primase.

Although the function of DNA polymerase is highly accurate, a mistake is made for about one in every billion base pairs copied. The DNA is therefore “proofread” by DNA polymerase after it has been copied so that misplaced base pairs can be corrected. This preserves the integrity of the original DNA strand that is passed onto the daughter cells.

Structure of DNA polymerase

The structure of DNA polymerase is highly conserved, meaning their catalytic subunits vary very little from one species to another, irrespective of how their domains are structured. This highly conserved structure usually indicates that the cellular functions they perform are crucial and irreplaceable and therefore require rigid maintenance to ensure their evolutionary advantage.

In general, the mutation rate at a specific site in different DNA viruses ranges from 10^{-8} to 10^{-11} per replication, whereas in RNA viruses it is at least hundred-fold higher, between 10^{-3} and 10^{-6} Per replication. The difference in mutation rate observed between RNA and DNA viruses is thought to result primarily from differences in the replication enzymes. Specifically, the DNA dependent DNA polymerases used by DNA viruses contain a proofreading function, whereas the reverse transcriptases used by retroviruses and RNA-dependent RNA polymerases used by RNA viruses lack a proofreading function.

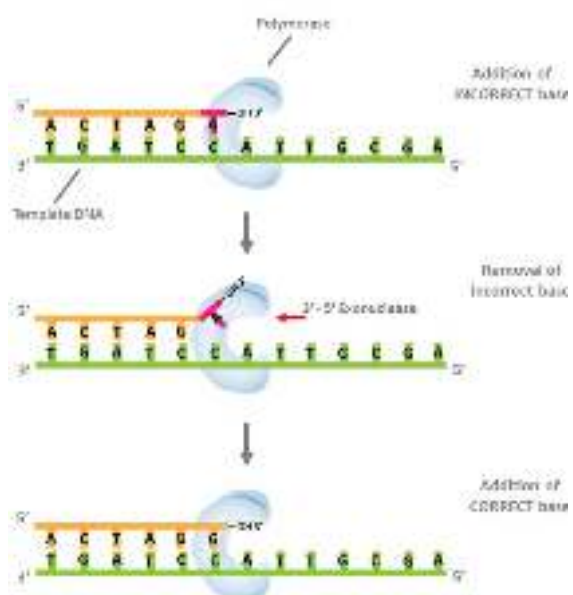


Figure: The 3' - 5' exonuclease activity results in proofreading of the new DNA strand and removal of any mistakes. <https://goldbio.com/articles/article/Somanypolymerasessolittletime>

LECTURE 120: Enzymatic Changes in Virus-infected Cells: Thymidine Kinase

Thymidine kinase (TK) is associated with nucleoside salvage pathway, exists widely in bacterial, eukaryotic, and prokaryotic cells. Cellular TKs, present in cytoplasm and mitochondria are the key enzymes that catalyze the transfer of the γ -phosphate of ATP to 2'-deoxythymidine (dT) in the nucleoside salvage pathway, forming thymidine monophosphate (dTMP) in the presence of magnesium ions (Mg). The vast majority of herpesviruses and some DNA viruses, such as vaccinia virus (VACV), African swine fever virus (ASFV), and Rana grylio virus (RGV) also have a specific gene encoding a viral TK. TKs are crucial targets in antiherpes virus treatments and potential therapeutic targets in antitumor gene therapy strategies.

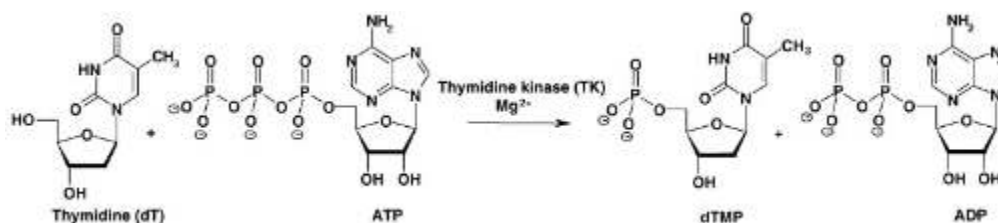
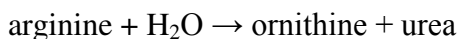


Figure 1: Enzymatic reaction of thymidine kinase

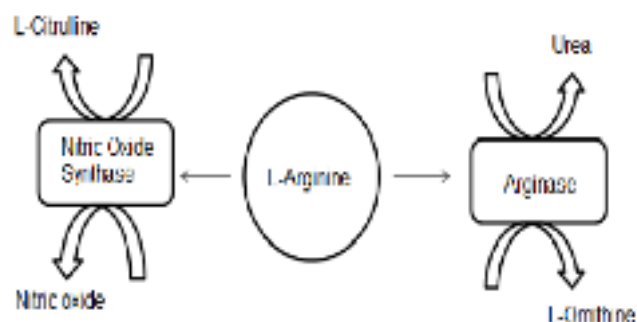
LECTURE 121: Enzymatic Changes in Virus-Infected Cells

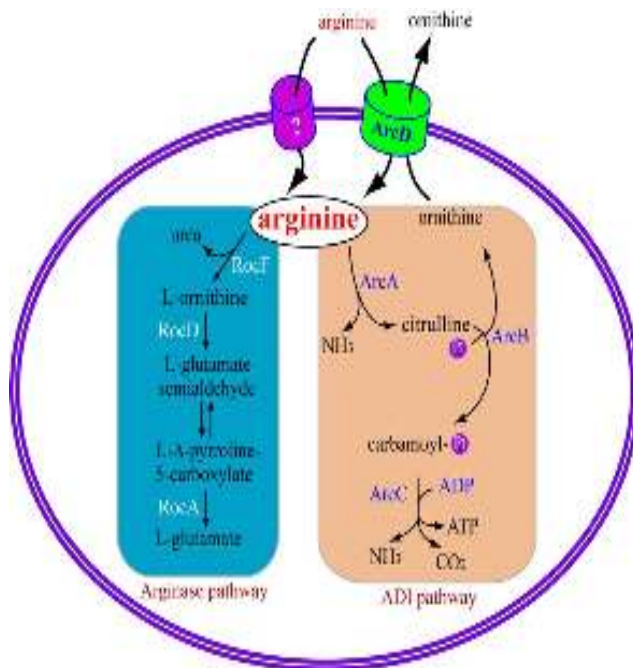
Arginase

- Arginase (EC 3.5.3.1, arginine amidinase, canavanase, L-arginase, arginine transamidinase) is a manganese-containing enzyme
- The reaction catalyzed by this enzyme is:



- It is the final enzyme of the urea cycle
- Rabbit papilloma virus induces high levels of arginine in squamous epithelium
- Squamous epithelium normally contains arginine
- The induced enzyme differs immunologically and physiochemically from normal enzyme
- Released from normal cellular control mechanism





Speculation: net effect is the depletion of cellular arginine which results in reducing the amount of arginine rich histones synthesized

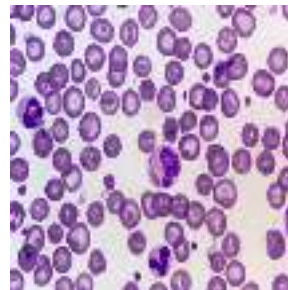
LECTURE 122: Changes in Structural Proteins

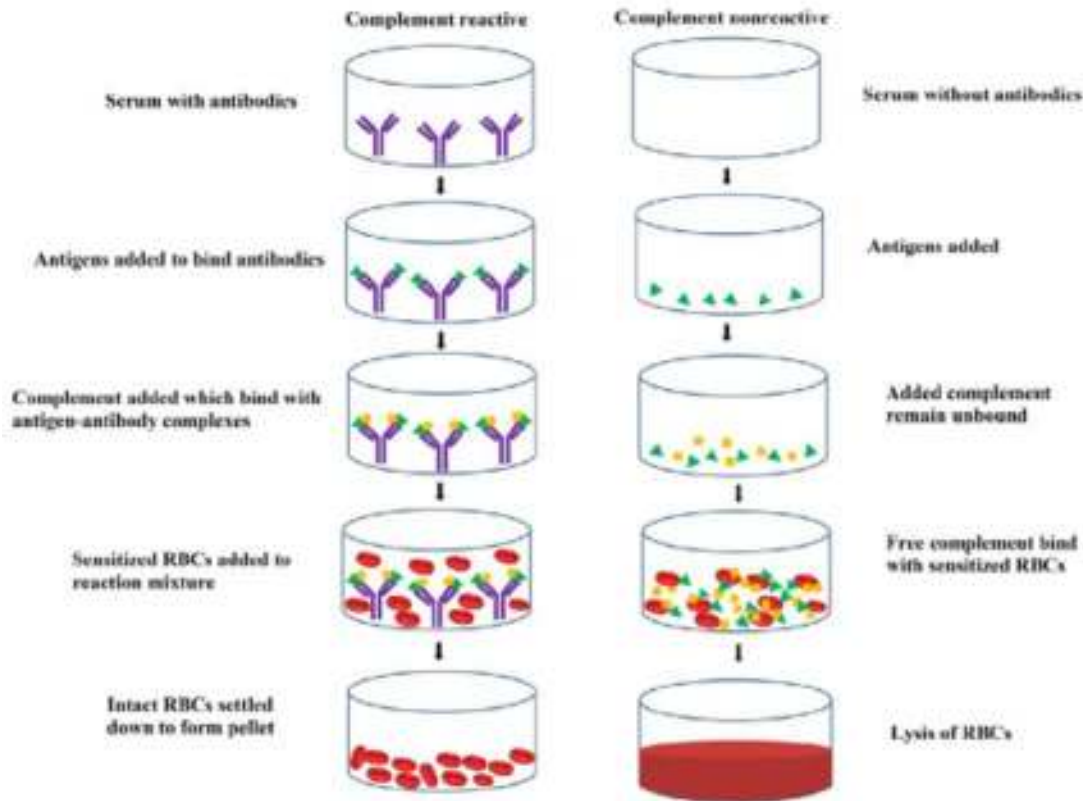
INCLUSIONS

Examination of virus infected cells by light or electron microscope shows viral multiplication and maturation referred as 'inclusions' or 'factories'

EXAMPLE

- Inclusion may be large, in case of poxvirus infection
- Or small incase of picornavirus infection
- Or maybe limited by a membrane
- New proteins are usually associated with tumors
- Property of oncogenic viruses





DETECTION

- Complement fixation
- Fluorescent antibody detection
- Graft detection

CONTROL

- Most probably under control of viral genome
- Possibility of cellular response products to virus

LECTURE 123: Factors Affecting Viral Replication

FACTORS

1. Viral concentration
2. Virus-cell systems
3. Elevated temperatures
4. Incomplete viral genomes

Viral Concentration

- One infectious unit equals to 10^3 to 10^5 noninfectious particles
- Low ratio associated with slow uncoating of viral particles
- Exception of vaccinia virus

Virus-cell Systems

- Blocks in viral development after viral genome starts to function
- E.g herpes simplex virus in dog kidney cells after development of cytopathic effects, inhibition of cell division, synthesis of viral
- Failure of virus to replicate in dog kidney cells

- Possible that viral DNA acted upon cellular nuclease or that sufficient DNA polymerase not present

Elevated Temperatures

- At 40°C, poliovirus inhibits cellular RNA synthesis
- But viral RNA fails to replicate and no viral progeny produced
- Polymerization fails at this temp.

Incomplete Genomes

- Incomplete viral genomes
- Like influenza virus
- Produce nucleoproteins, hemagglutinin
- But no mature virus

LECTURE 124: Viral interference

Viral interference is a phenomenon for which a cell infected by a virus becomes resistant toward a second outgoing infection by a superinfectant virus. Even though other mechanisms are known, it can be assumed that most cases of viral interference occurring in natural conditions are mediated by interferon, a low molecular weight protein produced by the infected cell in response to a stimulus provided by viral nucleic acid(s). The interferon produced by a cell can migrate to other cells not yet involved by the spreading infection, transmitting to them the antiviral-resistant state.

Interferons are secreted by infected cells to warn their neighbors, and once stimulated, cells of the immune system secrete interferons as part of their viral surveillance. Interferons are small proteins that bind to receptors on the cell surface. This signal is transmitted into the cells and leads to production of hundreds of proteins involved in viral defense. Several types of interferons are made by our cells. Interferon-alpha and interferon-beta, are the most common types, and are made by most types of cells, especially cells of the immune system. They send a basic signal to stop growing and focus on defense.

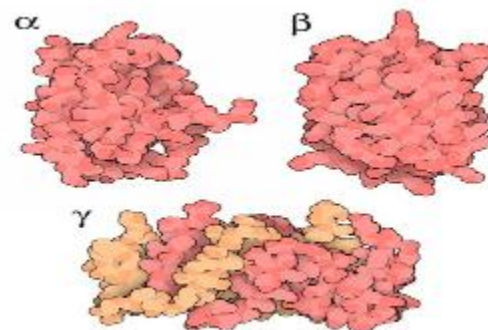
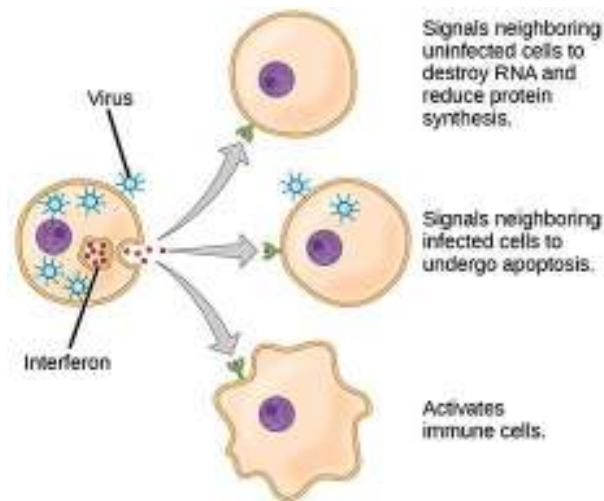


Figure: Types of interferon

One of the actions of interferons is to activate a cellular protein kinase known as PKR that inhibits the initiation of translation. The effects of interferons on the cell are generalized and result in inhibition of the translation of both cellular and virus mRNAs. The effect of the VA RNAs is to promote selectively the translation of adenovirus mRNAs at the expense of cellular mRNAs whose translation is inhibited.



LECTURE 125: Collection of Samples

Failure to provide a suitably selected specimen for the diagnostic laboratory is the single most important cause of unreliable laboratory results. Important factors relating to specimen collection and submission include:

- the timing of specimen collection from a sick individual ;
- the type, quality and quantity of the specimen;
- the time taken for the specimen to reach the laboratory,
- the suitability of the medium in which the sample is stored during transportation and the conditions under which transportation took place.

Ideally, specimens for laboratory examination should be collected as early as possible when viral titers are highest and before secondary bacterial or fungal infections become established. The specimens selected for laboratory examination should relate to the clinical signs or to lesion distribution post mortem. Swabs from the oropharynx or nasopharyngeal aspirates are suitable specimens for investigation of respiratory diseases. In enteric viral diseases, large numbers of virus particles are shed in faeces. In those diseases characterized by viraemia, virus may be demonstrable in cells of the buffy coat layer.

Table: Specimens appropriate for laboratory diagnosis of various clinical syndromes in the live animal.

TABLE 5.2 Specimens Appropriate for Laboratory Diagnosis of Various Clinical Syndromes in the Live Animal

Syndrome	Specimen
Respiratory	Nasal or throat swab; nasopharyngeal aspirate, tracheal wash fluid
Enteric	Feces
Genital	Genital swab
Eye	Conjunctival swab
Skin	Vesicle swab or scraping; biopsy of solid lesion
Central nervous system	Cerebrospinal fluid
Generalized	Nasal swab ^a , feces ^a , blood leukocytes ^a , serum, urine
Biopsy	Relevant organ
Any disease	Blood for serology ^b

^aDepending on presumed pathogenesis.

^bBlood allowed to clot, serum kept for assay of antibody.

LECTURE 126: Preservation of Samples of Virus

Preservation of the infectivity or antigenicity of viruses may be required for particular tests. As many viruses are labile, specimens for virus isolation should be collected into transport medium, refrigerated and transmitted to the laboratory without delay. Samples should be frozen at

-70°C if delay in delivery is anticipated. Freezing in a domestic freezer at -20°C decreases the infectivity of most viruses.

Transport medium consists of buffered isotonic saline containing a high concentration of protein, such as bovine albumin or foetal calf serum, which prolongs virus survival. Antibiotics and antifungal drugs are added in order to inhibit the growth of contaminants.

Packaging and specimen labeling and identification may be a mundane topic, but attention to these details maximizes the likelihood of safe arrival of the specimens at the laboratory and prevents legal sanctions over incorrectly shipped hazardous materials.

Aliquots of specimens should be taken before the specimens are frozen. Repeated freezing and thawing of specimens must be avoided to prevent loss of infectivity.

LECTURE 127: Transport of Samples for Viruses

Purpose Of Sop

- Safety from bio-hazardous material
- Results depend on sample collection and handling

Biosafety consideration

- Zoonotic or transmissible
- Latex gloves, Lab coats, Masks
- New syringes and containers
- Sealed container
- Wash hands after removing gloves
- In event of injury, wash area with soap and water
- Always have a first aid safety kit

Handling and transport of blood sample

- ✓ Collect in EDTA or Heparin containing containers
- ✓ Store at 4°C
- ✓ Transport upright with cushion (to prevent hemolysis)
- ✓ Never freeze blood

Nasopharyngeal swabs transport

- ✓ All respiratory specimens should be transported in proper medium
- ✓ Transport as quickly as possible to the laboratory to reduce overgrowth by oral flora
- ✓ For transit periods up to 24 hours
 - ambient temperature for bacteria
 - 4-8°C for viruses

POSTMORTEM SAMPLES

- **Collection**
 - ✓ Biopsy relevant tissues
 - in formalin for histopathology
 - in transport medium for microbiological testing
 - in sterile saline for isolation of viral pathogens
- **Handling and Transport**
 - in transport media within 24h at ambient temperature
 - in sterile saline at 4-8°C within 48h

LECTURE 128: COMMONLY USED MICROBIOLOGICAL FILTERS

- Berkefeld and Mandler filters
- Chamberland and Sela type filters
- Seitz filters

- Sintered glass filters
- Membrane filters

BERKEFELD AND MANDLER FILTERS

These are made of diatomaceous earth.

Berkefeld is graded into three porosity

- a) V (Viel) grade - is coarse, used for preliminary filtration to remove some bacteria and large particles from a solution.
- b) N (Normal) grade - retains all bacteria while nearly all viruses will pass through.
- c) W (Wenig) grade - have very small pores and will retain all bacteria as well as some large viruses.

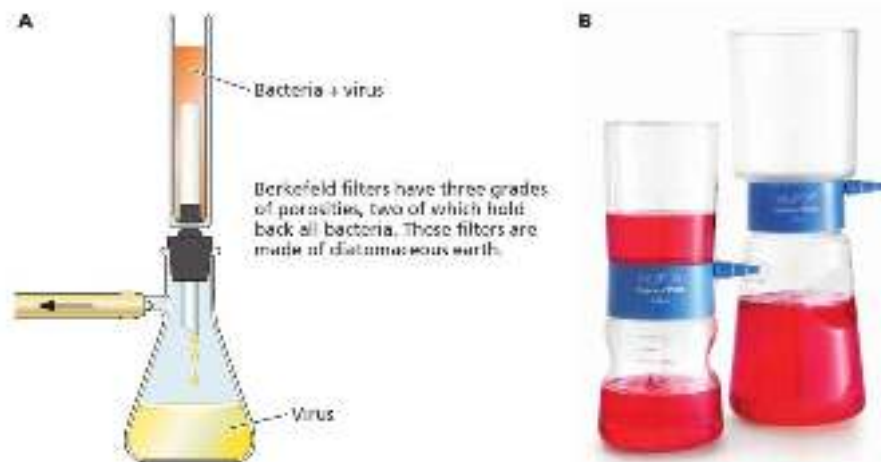


Figure: Filter systems used to characterize/purify virus particles.

(A) The earliest, the Berkefeld filter, was invented in 1891. It was a “candle”-style filter comprising diatomaceous earth, pressed into the shape of a hollow candle. The white candle is in the upper chamber of the apparatus, which is open at the top to receive the liquid to be filtered into the suction flask. The smallest pore size retained bacteria and allowed virus particles to pass through. Such filters were probably used by Ivanovsky, Loeffler, and Frosch to isolate the first plant and animal viruses.

(B) A typical Millipore membrane filter apparatus. Such modern-day filter systems are disposable plastic laboratory items in which the upper and lower chambers are separated by a biologically inert membrane, available in a variety of pore sizes.

Mandler filter is graded into (a) preliminary, (b) regular and (c) fine grades.

LECTURE 129: FILTRATION METHOD

The Sels Filter

- Made up of porcelain
- Seven grades of porosity
- Graded as XF, XFF, No. 10, and No. 01 (coarse grade)
- Graded as No. 015, No. 02, No. 03 which retain bacteria

Seitz Filter

- Asbestos pads
- Manufactured in two grades
- K and EK (coarse)
- Pads in metal holder
- Discarded after one use

Boerner Filter

- Same as Seitz
- Except they are small and fitted in small metal receptacle
- Adapts into small test tube
- Centrifuged to force liquid through them

LECTURE 130: Collodian filter and cellulose filter

Viral filtration is a critical step in the purification of biologics and in the monitoring of microbiological water quality. Cellulosic membrane microfilters have been used routinely for ages in laboratories to perform the so-called “sterile filtration” (cold sterilization), i.e., the absolute removal of bacteria, yeasts and molds but not viruses from heat-sensitive liquid media. However, many virus filtration devices currently implemented are made from cellulose and its derivatives. Graded collodion (cellulose nitrate) membrane filters have been extensively used in ultrafiltration to estimate the size of several medically important viruses such as foot-and-mouth disease, vaccinia, herpes, poliomyelitis and influenza. Later on, commercial mixed cellulose ester filters with very low pore size have also been applied to virus ultrafiltration for grouping by size assessment.

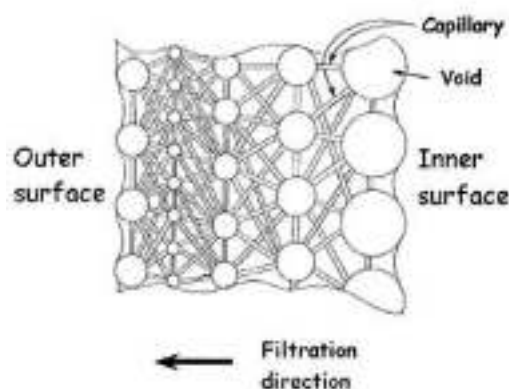


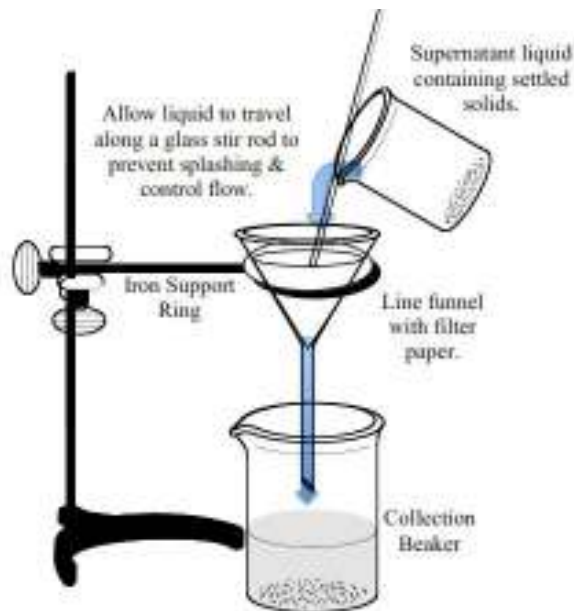
Figure: Schematic representation of the void pore structure of regenerated cellulose hollow fibers used in Planova filters.

LECTURE 131: Filtration Technique

Filtration, the technique used to separate solids from liquids, is the act of pouring a mixture onto a membrane (filter paper) that allows the passage of liquid (the filtrate) and results in the collection of the solid. Two filtration techniques are generally used in lab: "gravity" filtration and "vacuum" filtration.

Gravity Filtration

This is the simplest form of filtration and is utilized widely in chemical laboratories. The process involves placing a filter paper in the funnel which is fixed on an iron ring and liquid is poured into the funnel. As the liquid passes through the filter paper, it gets collected in a beaker placed beneath. The solids which are larger than the pores of the filter paper will get trapped in the paper. Since gravitational force is utilized to allow the fluid to travel downwards, this method is termed as gravity separation.



Vacuum Filtration

The process of vacuum filtration is somewhat similar to gravity filtration in that a funnel is required with a filter paper fixed into it and the fluid is made to traverse through the paper. The difference is that Buchner flask is required to collect the filtrate, the flask, in turn, is connected to an aspirator or a vacuum line with the help of a tube. By turning the vacuum on, the fluid is forced into flask via filter paper. As compared to gravity filtration, this process is faster and more efficient.

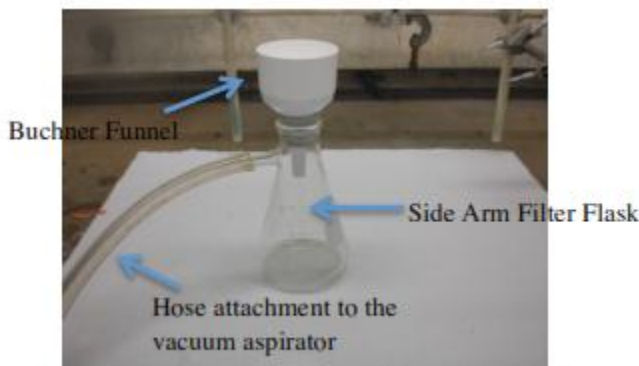


Figure 3. Buchner Funnel on a side arm filter flask.

LECTURE 132: Determination of Viral Size by Filtration

Membrane filtration is a separation process using semipermeable membrane. It works on the principle of differential hydraulic pressure by passing water from one side of the membrane to another. Methods used for the membrane filtration include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO).

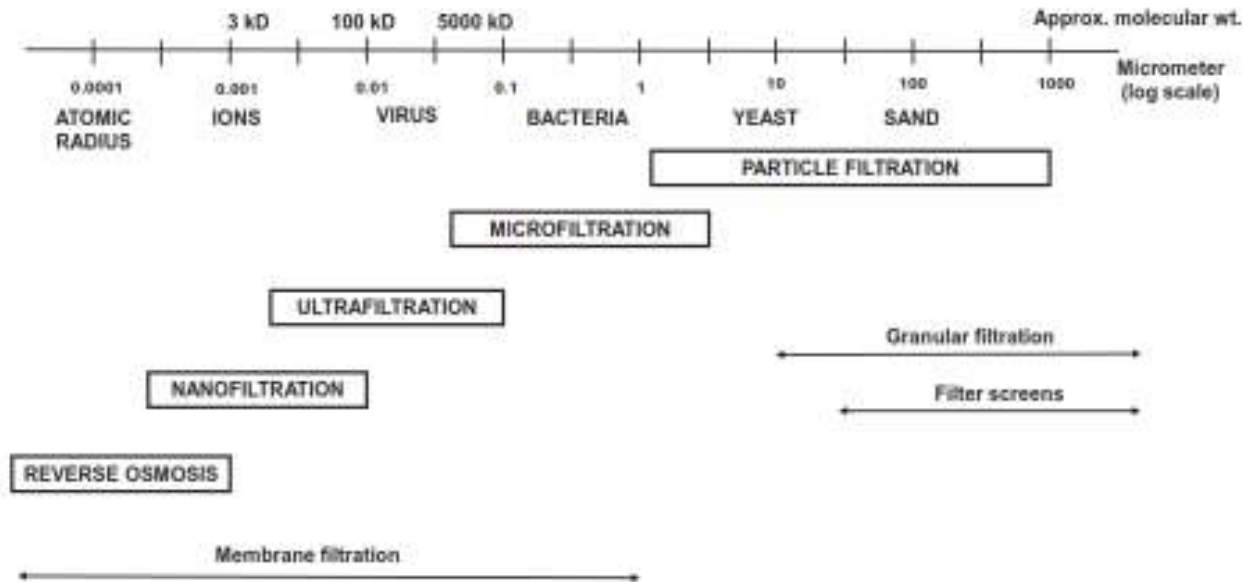


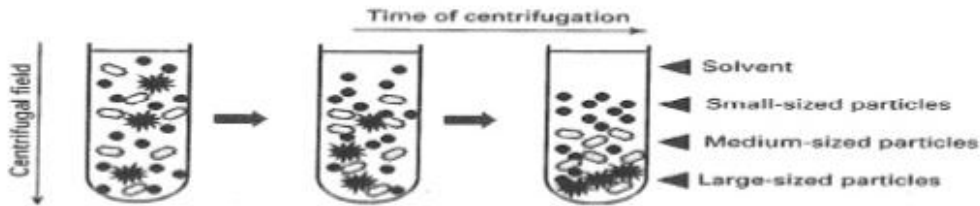
Figure: Size range of different filtration methods

LECTURE 133: CENTRIFUGATION TECHNIQUES

Centrifugation techniques

■ Centrifugation :-

- Is carried out by spinning a biological sample at high rate of speed , thus subjecting it to an intense force (Artificial gravitation field)
 - particles or cells in a liquid suspension, will settle at the bottom of tube due to gravity (1x g) during long period to time.
 - However, the length of time required for such separations is impractical.
 - Other particles which are very small in size, will not separate at all
 - So, high centrifugal force though method called centrifugation must be used in these cases.



♦ Sedimentation

- Is the **motion** of molecules in solutions or, particles in suspensions, in response to an **external force** such as gravity, centrifugal force or electric force.
- Centrifugation increase the rate of sedimentation by spinning the samples and creating a centrifugal force acts on particles in an instrument called a centrifuge.

■ A centrifuge :-

- found in every biochemistry laboratory
- Used primary for the preparation of biological samples
- use for the analysis of the physical properties of biomolecules , Organelles , and cells .
- Most centrifuge techniques fit into one of two categories .

■ Basic principles of centrifugation :-

- ♦ A particle whether it is (a floating solid , a precipitate , a macromolecule , or a cell organelle) is subjected to a centrifugal force when it is rotated at a high rate of speed .
- ♦ Centrifugal force depends on three variable :
 - Mass - Speed - radius .
- ♦ The speed is expressed in revolutions per minute (rpm) .
- ♦ The generated centrifugal force can be measured in terms of earth's gravitational force (g) as relative centrifugal force (RCF)

- Relative centrifugal force (RCF) is the measurement of the force applied to a sample within a centrifuge.
- Centrifugal force is measured as X g gravity (number times gravity or × g).
- An RCF of 500Xg indicates that the centrifugal force applied is 500 times greater than earth gravitational force
- r : the radius in centimeters , measured from the center of the centrifuge axis to the bottom of the test tube shield .
- This can be calculated from the speed (RPM) and the rotational radius (cm) using the following calculation.

$$RCF = 1.119 \times 10^{-5} \times \text{rpm}^2 \times r$$

♦ Example: calculation of RCF

▣ Spins at 20 000 revolutions per minute. Distance from the centre of the liquid to axis of rotation is 35 mm (remember to convert this to centimetres = 3.5 cm).

$$RCF = 1.119 \times r \times (\text{revolutions per minute})^2 \times 10^{-5}$$

$$RCF = 1.119 \times 3.5 \times (20000)^2 \times 10^{-5}$$

$$= 1.119 \times 3.5 \times (4 \times 10^8) \times 10^{-5}$$

$$= 1.119 \times 3.5 \times 4 \times 10^3$$

$$RCF = 15666 \text{ g [where g means times the force of gravity]}$$

Instrumentation for centrifugation

♦ The basic centrifuge consists of two components :-

- 1- An electric motor with drive shaft to spin the sample .
- 2- A rotor to hold tubes or other container of the sample .

Centrifuge components:

Every centrifuge contains :

1. Drive shaft *محور الدوران*
2. Electric motor (Rotating part)
3. Rotor



♦ Different classes of centrifuge are available based on several criteria , including :-

- refrigeration , rotor head (eg : Fixed or swinging-bucket or vertical)
- and maximum speed attainable (i.e. : low-speed , high speed or ultracentrifuge)

LECTURE 134: Determination of essential LIPIDS

Lipids are usually defined as those components that are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. This group of substances includes triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, sterols, carotenoids and vitamins A and D.

LECTURE 135: Determination of NUCLEIC ACID TYPES

From the perspective of the virus, the purpose of viral replication is to allow production and survival of its kind. By generating abundant copies of its genome and packaging these copies into virions, the virus is able to continue infecting new hosts. All viruses must therefore express their genes as functional mRNAs early in infection in order to direct the cellular translational machinery to synthesize viral proteins. The pathways leading from genome to message vary among different viruses (Fig. 1). There are four main categories of viral genomes: dsDNA, ssDNA, dsRNA, and ssRNA. These categories are further divided on the basis of distinct modes of transcription. For some RNA viruses, the infecting genome acts as mRNA. For other RNA and DNA viruses, viral mRNA is synthesized upon entry into the host cell.

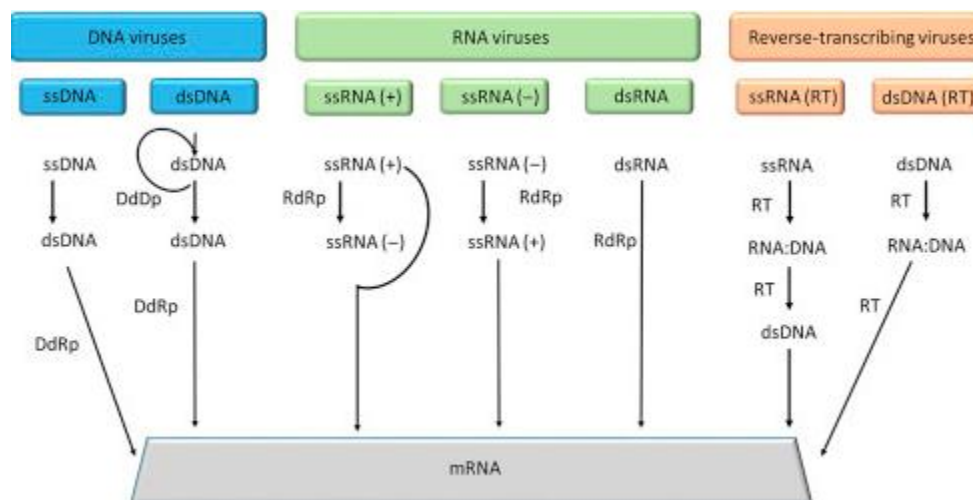


Figure: Summary of replication and transcription modes of different classes of viruses. DdDp, DNA-dependent DNA polymerase; DdRp, DNA-dependent RNA polymerase; RdRp, RNA-

dependent RNA polymerase; RT, reverse transcriptase. The ssRNA(+) can serve as the template for translation and does not undergo any modification prior to translation.

LECTURE 136: Concentration and Purification of Viruses

Virus purification is the physical separation of virus in a concentrated form from the host cell milieu in which it has grown. Viruses need to be purified for many studies in which properties or structure of the virus must be distinguished from those of the host cells or culture medium, such as analyses of structure of viral polypeptides, function of membrane glycoproteins, etc.

Differential centrifugation also separates particles on the basis of their density. Whilst the procedure can be successful in achieving good separation of a variety of particles as long as there are large differences in their mass and/or densities, the yield of such a procedure is likely to be low and the purity of the particles questionable. The technique is however often used as a starting point designed so as to enrich populations of particles before further purification. Virus purification is more likely to be achieved by the technique of density gradient centrifugation, where separation of the components is achieved by sedimentation through a density gradient, (i.e. a solution which increases in density with increasing distance down the centrifuge tube).

LECTURE 137: VIRAL DIAGNOSTIC TESTS

Viruses are too small to be seen except by electron microscopy (EM) and this requires concentrations in excess of 10^{11} particles per ml, or even higher if a virus has no distinctive morphology, some fancy equipment, and a highly skilled operator. Thus viruses are usually detected by indirect methods. Viral diagnostic tests fall into following three categories:

(i) Direct Examination of Specimen

- Electron Microscopy morphology / immune electron microscopy
- Light microscopy histological appearance - e.g. inclusion bodies
- Antigen detection immunofluorescence, ELISA etc.
- Molecular techniques for the direct detection of viral genomes

(ii) Indirect Examination

- Cell Culture - cytopathic effect, haemadsorption, confirmation by neutralization, interference, immunofluorescence etc.
- Eggs pocks on CAM - haemagglutination, inclusion bodies
- Animals disease or death confirmation by neutralization

(iii) Serology

- Detection of rising titres of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection.

IDENTIFICATION OF VIRUSES USING ANTIBODIES

Antibodies are proteins produced by the immune system of higher vertebrates in response to foreign materials (antigens) which those cells encounter. Such antibodies have a region that recognizes and binds specifically to that same antigen. Antibodies are secreted into the body fluids and are most easily obtained from blood. Blood is allowed to clot and antibodies remain in the fluid part (serum) which remains after clotting has removed cells and clotting proteins. This is then known as an antiserum.

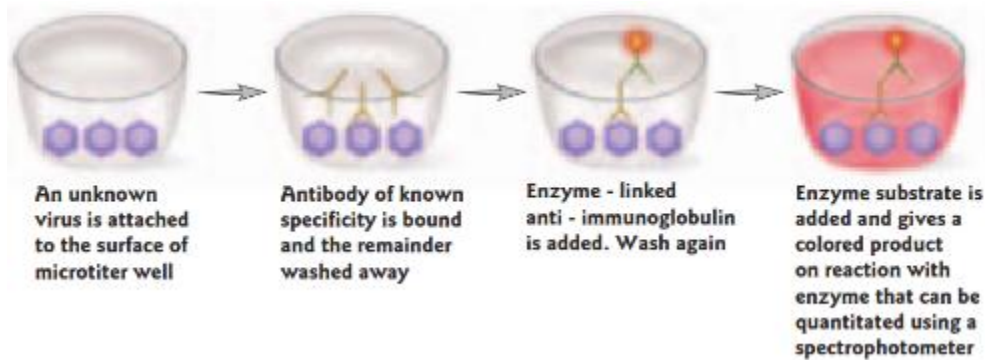


Figure 1: Identification of an unknown virus by ELISA with a specific antiserum. The unknown virus would be used in parallel with a control virus preparation that is known to react with the antibody. The unknown virus is positively identified when the antibody reaction is identical to that using the control virus.

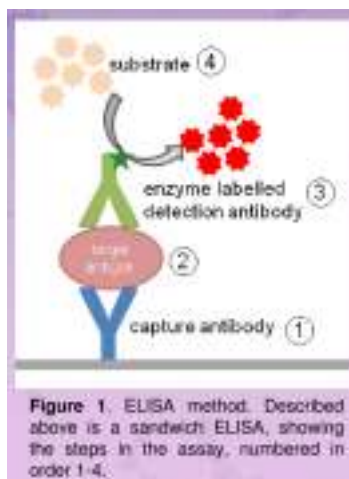


Figure 2: Sandwich ELISA

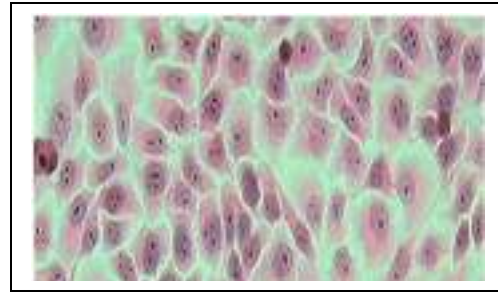
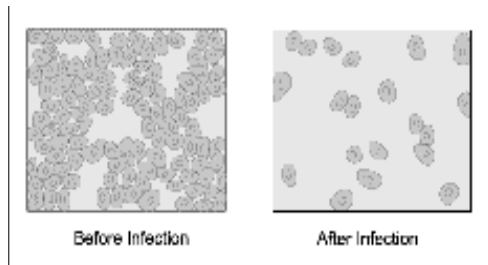
LECTURE 138: VIRAL DIAGNOSTIC PROCEDURES

Histological Examination of Virus infected cells

CLTURE Grown in :

1. Primary cells

2. Semi-continuous cells
3. Continuous Cells



Identification of growing viruses

- Cytopathic effects (CPE)
- Haemadsorption

Cytopathic Effects

- Specific or non-specific signs
- Herpes Simplex Virus produces specific CPE
- Enterovirus don't produce CPE

Haemadsorption

- Cells acquire ability to stick to mammalian RBCs
- Mainly used for influenza and parainfluenza

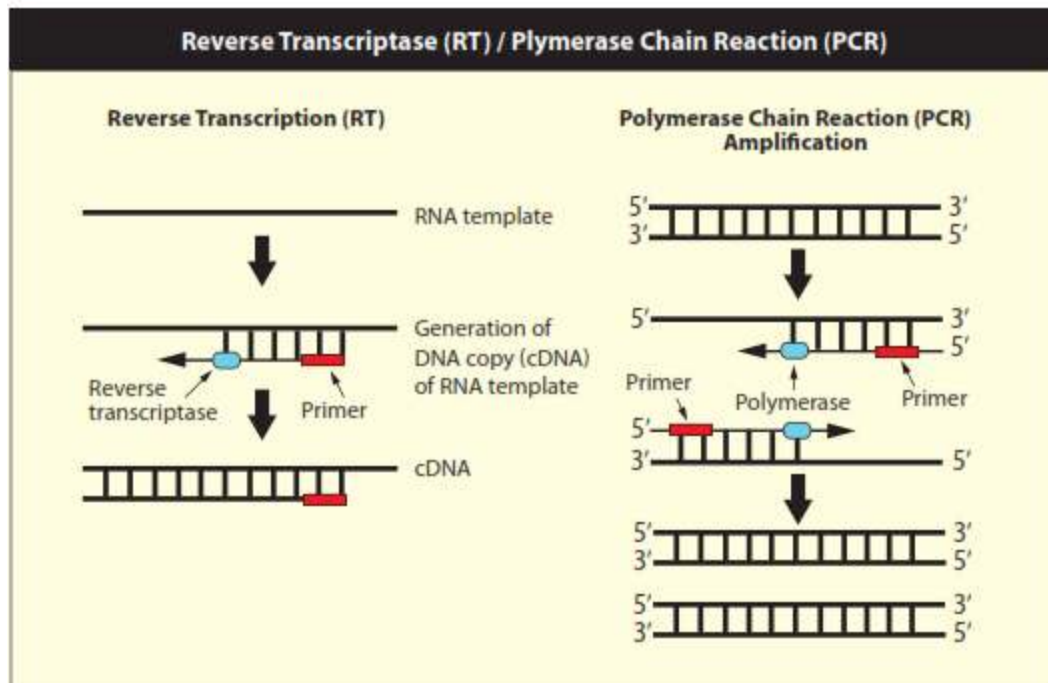
Confirmation of virus

- Neutralization
- Haemadsorption-inhibition
- Immunofluorescence
- Molecular tests (PCR/ELISA)

LECTURE 139: Polymerase Chain Reaction

The area undergoing the most growth is in molecular methods and techniques, particularly in relation to virology. Molecular methods do not rely on the presence of a live virus like virus isolation procedures. These methods detect a piece of the viral genome, making them more sensitive for the detection of viruses.

The technique that has probably had the greatest impact on the field of virology is the polymerase chain reaction (PCR, identification of DNA) and the reverse transcriptase polymerase chain reaction (RT-PCR, identification of RNA) along with the development of better, more standardized methods of nucleic acid purification. The rapid advances and decrease in expense associated with nucleotide sequencing, commercial synthesis of oligonucleotides (primers), and the availability of genetic sequences in public databases have contributed to the increased appeal of these methods.



The PCR involves the use of two primers designed to identify a target. In the presence of a DNA polymerase and other components required for DNA amplification, the target sequence of interest, if present, is amplified. The PCR requires double stranded DNA, RNA template generation of DNA copy (cDNA) of RNA template, primers. The discovery of reverse transcriptase, an enzyme capable of making a DNA copy of RNA, enabled the ability to expand PCR to include a reverse transcription step (RT) which generates a double stranded target (RNADNA), which is then used in the PCR to identify RNA viruses.

LECTURE 140: Ligase Chain Reaction (LCR)

Ligase Chain Reaction is a technique that amplifies the amount of DNA probes. DNA is double-stranded and connected by matching base pairs, kind of like two sections of a zipper being zipped together. **DNA probe** is a section of single-stranded DNA resembling one side of the zipper. This means it has only one side of the base pairs and they aren't matched yet.

LCR sounds similar to the **polymerase chain reaction**, which is also a technique that amplifies the amount of DNA. The difference is that the polymerase chain reaction amplifies a segment of DNA, whereas the ligase chain reaction amplifies a short DNA probe. LCR also uses two enzymes, polymerase and ligase.

LCR is useful anytime scientists need to identify mutations of one base pair in DNA strands. It's also helpful for finding DNA changes in viruses and can lead to the development of better vaccines against viral infections.

Ligase Chain Reaction Process

So, just how does this process take place? Well, first you have to have the appropriate DNA probe that will bind to your target sequence. For instance, if your target sequence is ATGC, then you will need a DNA probe with the complementary base pairs of TACG. The DNA probe will ensure the target sequence is amplified. The LCR process is carried out in three basic steps in a thermal cycler.

1. **Denaturation** - DNA is heated so that the double-stranded structure becomes single-stranded. The hydrogen bonds are broken, allowing the base pairs to be exposed so that the DNA probes can attach.
2. **Annealing** - Two sets of DNA probes attach to each end of the single strands of DNA. This step marks where the duplication of the DNA will begin. From this point, **Taq polymerase**, a polymerase enzyme from a heat-loving bacteria, will add nucleotides based on the already existing DNA strands.
3. **Ligation** - The DNA ligase will fill in the gap in the DNA strand between the probes by filling in the appropriate primer. Ligase will only recognize and ligate primers that properly anneal to the probe.

After the ligation step, the DNA has been doubled. These steps are then repeated until the desired amount of DNA is created. Every time the cycle completes, the amount of DNA present doubles.

Detection and Interpretation of Results

It's possible to go through this whole process and not get your target DNA molecule. That can happen if you use the wrong primers, probes, ligases or polymerases. Therefore, it is important to know what you have to ensure you amplify the DNA you want.

LECTURE 141: Nucleic acid sequence based amplification (NASBA)

Introduction to NASBA

RNA detection is commonly done using RT-PCR, a time consuming process often resulting in false positives due to cross contamination. Alternatively, nucleic acid sequence-based amplification (NASBA) is a one step isothermal process for amplifying RNA. NASBA has proven to be successful in detection of both viral and bacterial RNA in clinical samples.

A NASBA reaction consists of avian myeloblastosis virus (AMV), reverse transcriptase (RT), T7 RNA polymerase and RNase H with two oligonucleotide primers. The amplification of ssRNA is possible only when the denaturation of dsDNA does not occur. As NASBA is an isothermal process, it is thus possible. The NASBA reaction does not get false positives caused by genomic dsDNA, as in the case of RT-PCR.

Mechanism

A schematic representation of the NASBA amplification is shown below:

This mechanism requires two short single stranded DNA fragments primers and three enzymes.

1. The viral RNA strands are represented as the sense strand present in the original samples.
2. Primer P1 binds to the RNA and is elongated by reverse transcriptase (AMV-RT). The RNA strand of the yielded DNA : RNA hybrid is hydrolyzed by RNase H.
3. After the binding of P1, primer P2 can also bind. Primer P2 is then elongated by AMV – RT, yielding a double-stranded DNA molecule.
4. Primer P1 is designed in such a manner that when it forms a double- stranded DNA, it codes for a T7 RNA polymerase Promoter site. This helps in generating antisense RNA copies using a DNA template.
5. The new copies of DNA are generated using RNA. The process is same as followed for sense strand. Here in this case, P2 will bind first.

In a NASBA reaction, DNA is the final product formed. It has a promoter region. This allows T7 RNA polymerase to use it as a template. The reaction sequences starting from the sense and antisense RNA strands are different but they are considered to be kinetically identical. They both are referred to as copy DNA (cDNA).

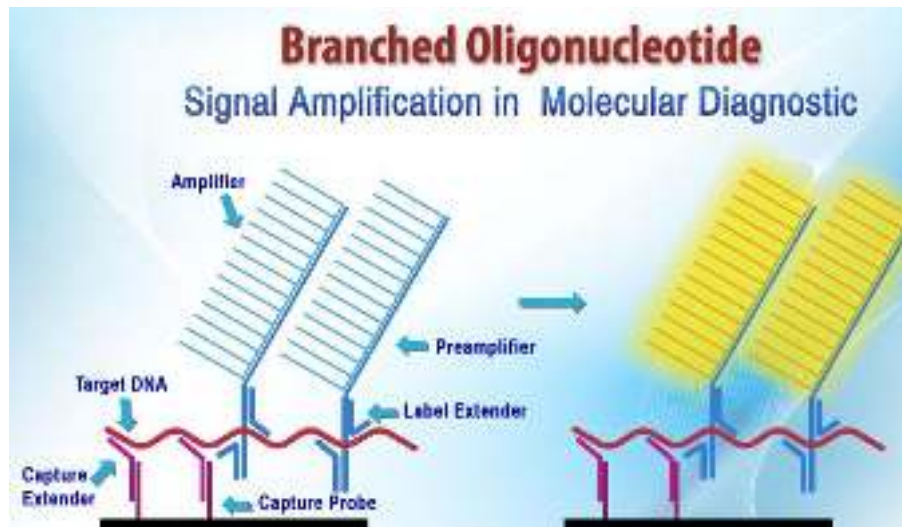
LECTURE 142: Strand Displacement amplification

Strand/Multiple displacement amplification (MDA)

- ❖ A non-PCR based DNA amplification technique
- ❖ This method can rapidly amplify minute amounts of DNA samples to a reasonable quantity for genomic analysis.

LECTURE 143: Branched Chain DNA

Instead of amplifying the target sequence to yield high sensitivity, this method relies on the signal amplification of the branched DNA (bDNA) probe that binds to a specific nucleotide sequence. The bDNA assay is also known as the sandwich nucleic acid hybridization method, because the sequence of interest is captured in between target-specific oligonucleotides. The first-generation of bDNA assays were viral quantification assays, developed for the detection of viral loads (HIV-1 and HCV) in humans. Currently, applications of this method range from microarray validation and biomarker discovery to predictive toxicology, when applied to screening compounds in early preclinical phases.



LECTURE 144: Solid phase immunoassay

Detection of viral antigens or antiviral antibodies can be accomplished by solid-phase methods, in which antiviral antibody or protein is adsorbed to a plastic surface. To detect viral antigens in serum or clinical samples, a “capture” antibody, directed against the virus, is linked to a solid support, a plastic dish or bead. The specimen is added to the plastic support, and if viral antigens are present, they will be captured by the bound antibody. Bound viral antigen is detected by using a second antibody linked to an enzyme. A chromogenic molecule that is converted by the enzyme to an easily detectable product is then added.

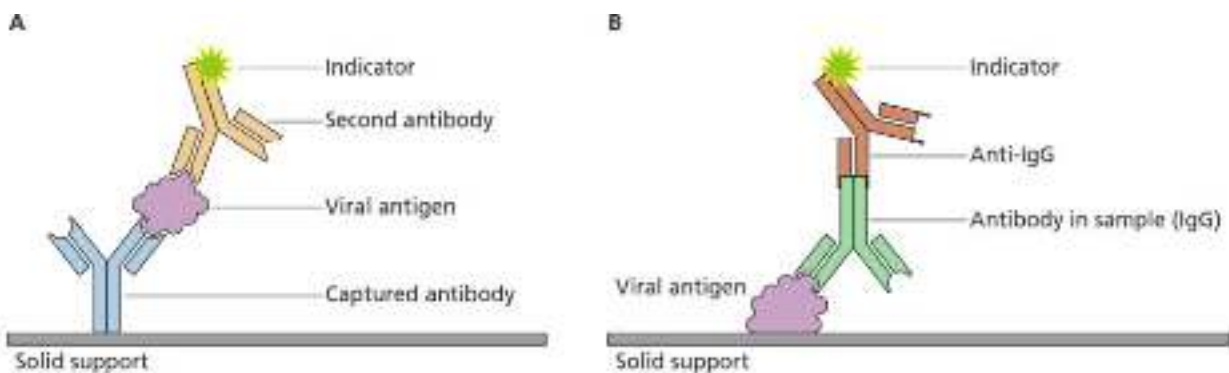


Figure 1: Detection of viral antigen or antibodies against viruses by enzyme-linked immunosorbent assay. (A) To detect viral proteins in a sample, antibodies specific for the virus are immobilized on a solid support such as a plastic well. (B) To detect antibodies to a virus in a sample, viral antigen is immobilized on a solid support.

To detect IgG antibodies to viruses, viral protein is first linked to the plastic support, and then the specimen is added. If antibodies against the virus are present in the specimen, they will bind to the immobilized antibody. The bound antibodies are then detected by using a second antibody directed against a common region on the first antibody. Like other detection methods, enzyme immunoassays are used in both experimental and diagnostic virology. In the clinical laboratory,

enzyme immunoassays are used to detect a variety of viruses including rotavirus, herpes simplex virus, and human immunodeficiency virus.

A modification of the enzyme immunoassay is the lateral flow immunochromatographic assay, which has been used in rapid antigen detection test kits. In this assay, a sample is applied to a membrane and is drawn across it by capillary action. Antigens in the sample react with a specific antibody, which is conjugated to a detector. The antigen-antibody complexes move across the membrane until they are captured by a second antibody. At this point a line becomes visible, indicating that viral antigen is present in the sample. The lateral flow immunochromatographic assay does not require instrumentation and can be read in 5 to 20 min.

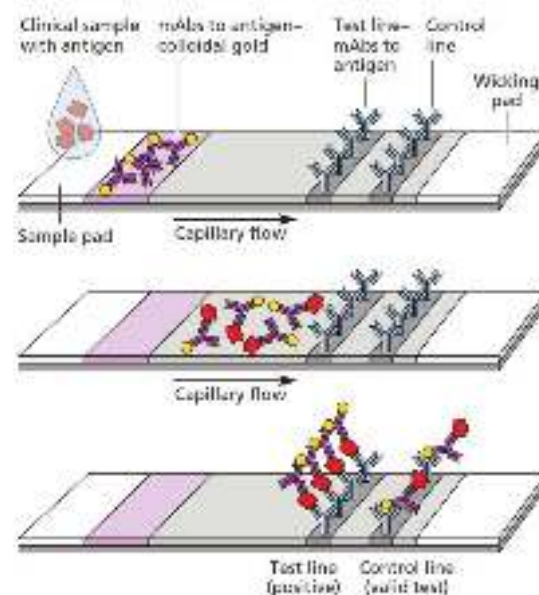


Figure 2: Lateral flow immunochromatographic assay.

LECTURE 145: Immunodiffusion

Immunodiffusion refers to the movement of antigen or antibody within the gel. Add the reactants to the wells. They diffuse to the area of lower/no concentration. A gradient of the reactants' concentration forms as they diffuse into the gel. Immunoprecipitation takes place in a region with an equivalent concentration of both the antibody and antigen. This is represented by bands.

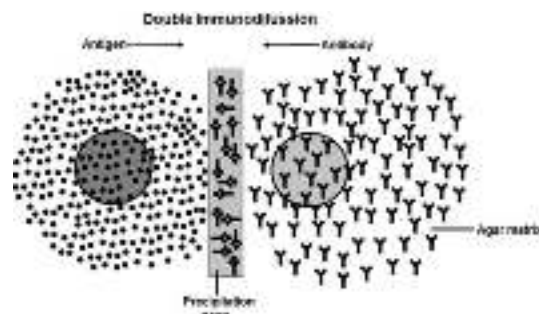


Figure1: A) single immune assay B) Double immunodiffusion

There are different types of immunodiffusion assays:

- Single diffusion in one dimension (Oudin procedure)
- Double diffusion in one dimension (Oakley Fulthorpe procedure)
- Single diffusion in two dimension (radial immunodiffusion or Mancini method)
- Double diffusion in two dimensions (Ouchterlony double immunodiffustin)

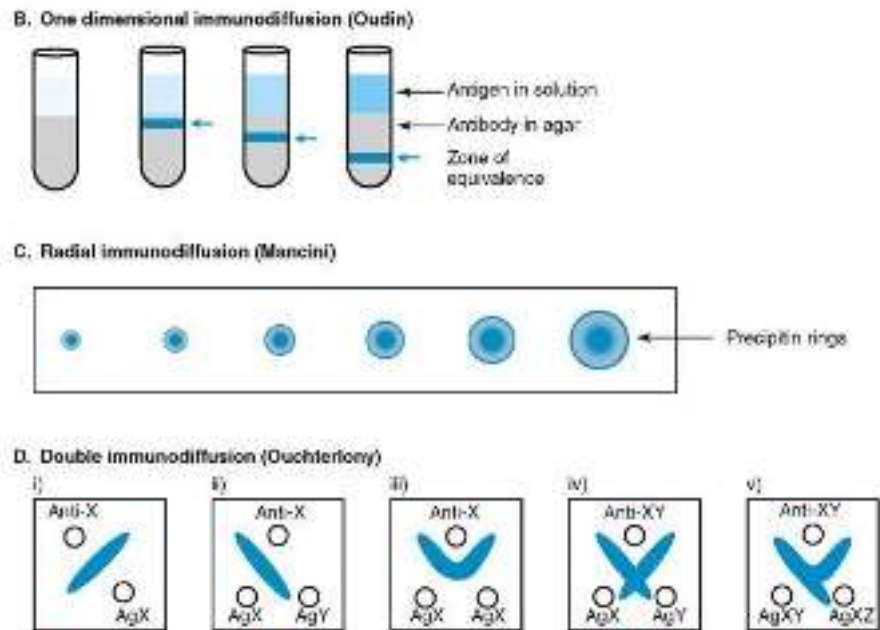


Figure 2: Types of immunoassays

LECTURE 146: Bacteriophages

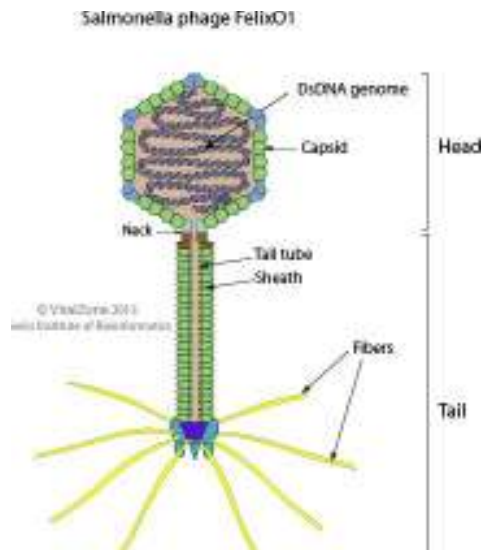
Even bacteria can get a virus! The viruses that infect bacteria are called bacteriophages.

A **bacteriophage**, or **phage** for short, is a virus that infects bacteria. Like other types of viruses, bacteriophages vary a lot in their shape and genetic material.

- Phage genomes can consist of either DNA or RNA, and can contain as few as four genes or as many as several hundred.
- The capsid of a bacteriophage can be icosahedral, filamentous, or head-tail in shape. The head-tail structure seems to be unique to phages and their close relatives (and is not found in eukaryotic viruses).

Bacteriophages, just like other viruses, must infect a host cell in order to reproduce. The steps that make up the infection process are collectively called the **lifecycle** of the phage.

Some phages can only reproduce via a lytic lifecycle, in which they burst and kill their host cells. Other phages can alternate between a lytic lifecycle and a lysogenic lifecycle, in which they don't kill the host cell (and are instead copied along with the host DNA each time the cell divides).



LECTURE 147: Bacteriophage properties

A bacteriophage also known informally as a phage is a virus that infects and replicates within bacteria and archaea. The First twenty years of research on phages was aimed at understanding the basis of these phenomena, and in the process these studies contributed to developing ideas about the nature of viruses more generally. Bacteriophages were originally thought to be lethal agents, killing their host cells after infection. In the early 1920s, a previously unknown interaction was discovered, in which the host cell not only survived the infection but also stably inherited the genetic information of the virus. It was also observed that certain bacterial strains could lyse spontaneously and produce bacteriophages after a period of growth in culture. Such strains were called **lysogenic**, and the phenomenon, **lysogeny**.

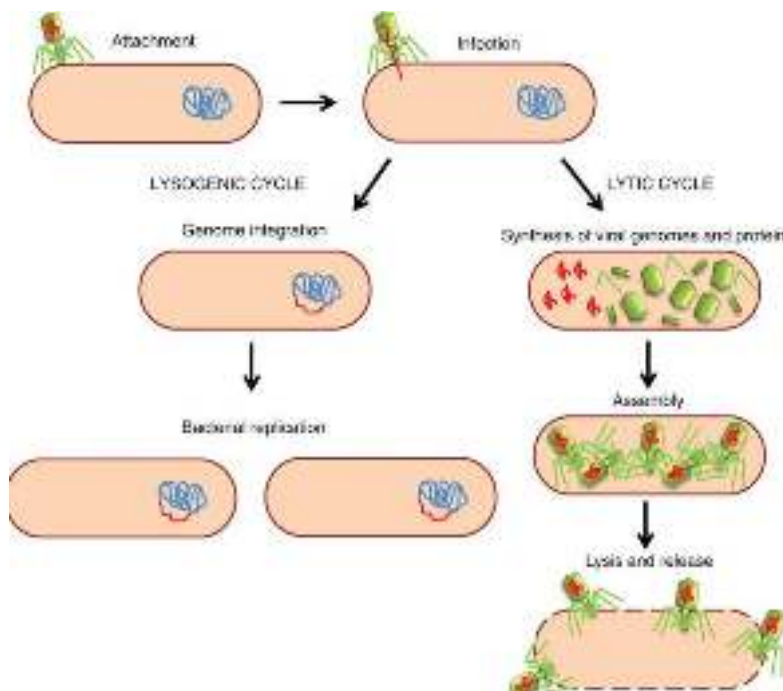


Figure: Main biological cycles of bacteriophages. **Left**, the lysogenic cycle, where the viral genome is integrated into the bacterial chromosome and where both replicate together without producing a viral progeny (the virus remains latent). **Right**, the lytic cycle. After injection of the viral genome inside the bacteria, the host machinery will replicate the viral genome and synthesize proteins that will assemble. Specific viral lysins will facilitate the release of the virions, killing the host.

The structure of phage

All phage have a chromosome encased in a capsid that is composed of phage-encoded proteins. For many phage types, the capsid is attached to a tail structure that is also made from phage-encoded proteins. T4 and P1 contain a linear double-stranded DNA genome enclosed in a capsid and attached to a tail. The T4 genome is 172 kb, while P1 is a smaller phage with a genome of 90kb. The T4 capsid is an elongated icosahedron. T4 has a very elaborate tail structure including a collar at the base of the head and a rigid tail core surrounded by a contractile sheath. The core and sheath are attached to a hexagonal base plate. Also attached to the tail plate are tail pins and six kinked tail fibers. P1 also has an icosahedral capsid, a tail with a contractile sheath, a base plate, and tail fibers. λ contains a linear double-stranded DNA genome of 48.5kb, a capsid, and a tail. The finished capsid is again shaped like an icosahedron whereas the tail is a thin flexible tube that ends in a small conical part and a single tail fiber. M13 contains a circular single-stranded DNA genome of 6407 nucleotides surrounded by five phage-encoded proteins.

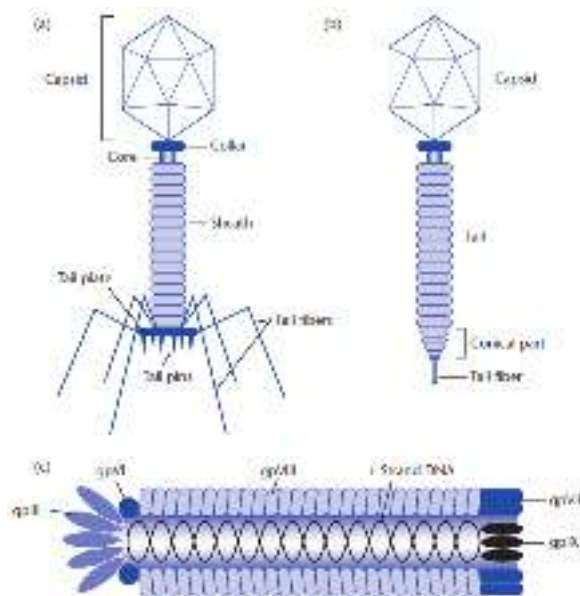


Figure: The structures of (a) T4, (b) λ , and (c) M13.

The process of a phage infecting a bacterium and producing progeny is referred to as a lytic infection. Some phage, like T4, are only capable of lytic growth. Some phage are also capable of maintaining their chromosome in a stable, silent state within the bacteria. This is called lysogeny. Phage that is capable of both a lytic and lysogenic pathway are called temperate phage.

P1 and λ are temperate phage. M13 is unusual because phage continually exit from a bacterium without killing it. For this reason, M13 is not considered to have a true lysogenic state and is not a temperate phage. When the bacterium contains a silent phage chromosome, it is referred to as a lysogen. The incorporated phage genome is referred to as a prophage.

LECTURE 148: The lifecycle of T4

T4 adsorption and injection

For T4, the phage binds to the lipopolysaccharide. The tips of the tail fibers contact the cell first. Once the phage has bound to the cell, the base plate rearranges creating a hole in the base plate. The outer sheath contracts and the internal tube goes through the outer membrane, peptidoglycan, and periplasm and comes close to the cytoplasmic membrane. The DNA is injected and crosses the cytoplasmic membrane in about 30 seconds. Not all phage that have the structure of T4 inject their DNA this way. Some phage such as T7, have tails that cannot contract. The T7 genome is only 40kb but takes 9 to 12 minutes to cross into the cytoplasm. For T7, a small portion of genome (about 8%) crosses both membranes, the peptidoglycan and periplasm, and enters the cytoplasm. After a 4-minute lag during which two proteins encoded by this piece of DNA are synthesized, the rest of the phage DNA enters the cytoplasm. Binding of these two phage proteins to the DNA is thought to pull the DNA into the cytoplasm.

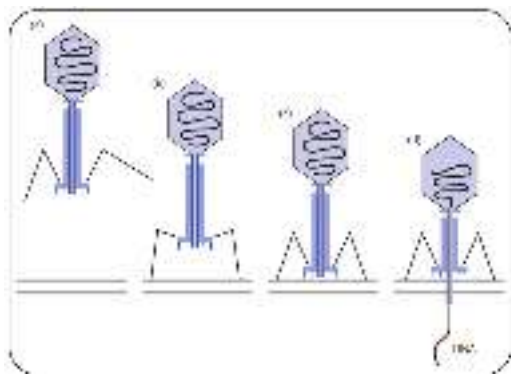
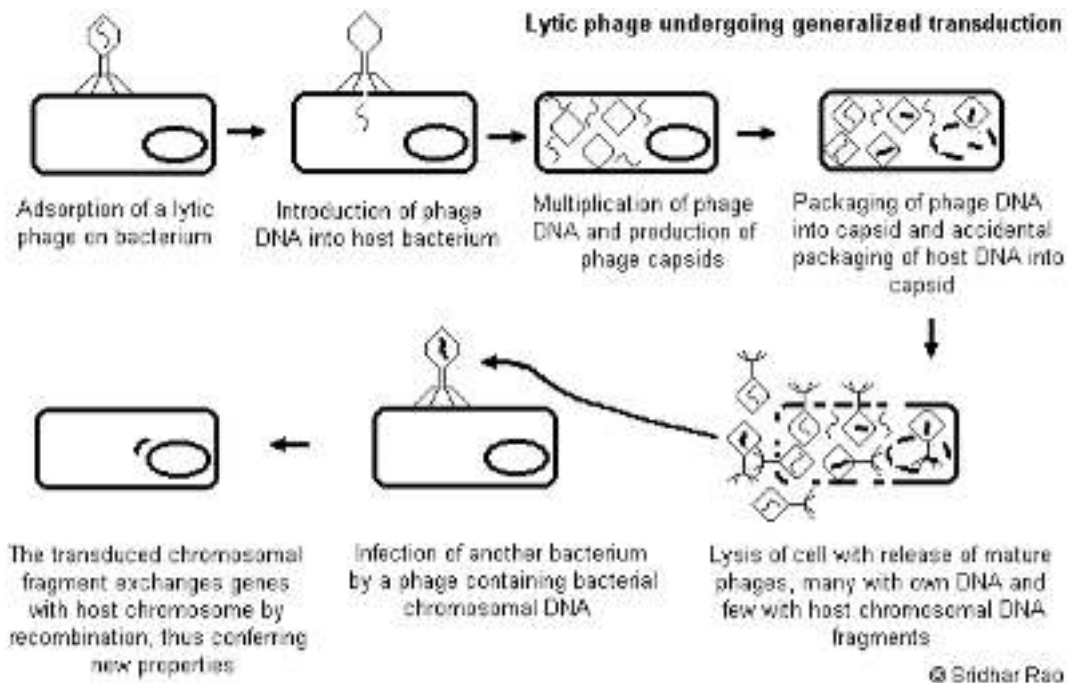


Figure: Injection of T4 DNA into the cell. (a) T4 “looks” for a susceptible bacterium with its tail fibers. (b) The tail fibers recognize the membrane first. (c) The tail spikes interact with the membrane. (d) The tail sheath contracts, driving the internal tail tube through the outer membrane, peptidoglycan, and to the inner membrane where the DNA is released.

Once T4 DNA is in the cell cytoplasm, it specifies a highly organized and coordinated program of gene expression. A group of genes with similar promoters, called the **early genes**, are transcribed and translated by host enzymes. One early gene encoded protein activates a second set of promoters for the **middle genes**. A different early gene encoded protein shuts off synthesis of the early genes. One product of middle transcription is required to activate the **late genes**. The early genes encode the proteins needed for DNA synthesis and late genes encode the proteins needed to build the capsid and tail structures. Many phage stage the expression of their genes in this temporal fashion to ensure proper construction of the phage particles.



LECTURE 149: Phage Growth Curve

A classic experiment developed initially for bacteriophage and still frequently used to determine the essential growth properties of a virus is the one-step growth experiment. The goal of this experiment is to measure the time course of virus replication and the yield of virus per cell during a single round of infection.

At various times after infection, virus from individual dishes is harvested, and at the completion of the experiment, the virus titer in samples representing each time point is determined. The virus yield at each point can be converted to PFU/cell (also called burst size) by dividing the total amount of virus present in the sample by the number of cells originally infected in the sample.

First, during the first several hours of the wild-type infection at the permissive temperature, the titer in the cultures decreases and then increases. This dip in the growth curve is called *eclipse* and results from the fact that early during the experiment, virus attached to the cell surface but not uncoated remains infectious; however, infectivity is lost following uncoating during the first few hours of infection, and infectivity is recovered only after new virus is produced. The infection then enters a rapid growth phase, followed by a plateau. The plateau results from the fact that all infected cells have reached the maximum yield of virus, or have died or lysed, depending on the type of virus infection. The time interval from infection to plateau represents the time required for a single cycle of growth, and the yield of virus at plateau shows the amount of virus produced per cell.

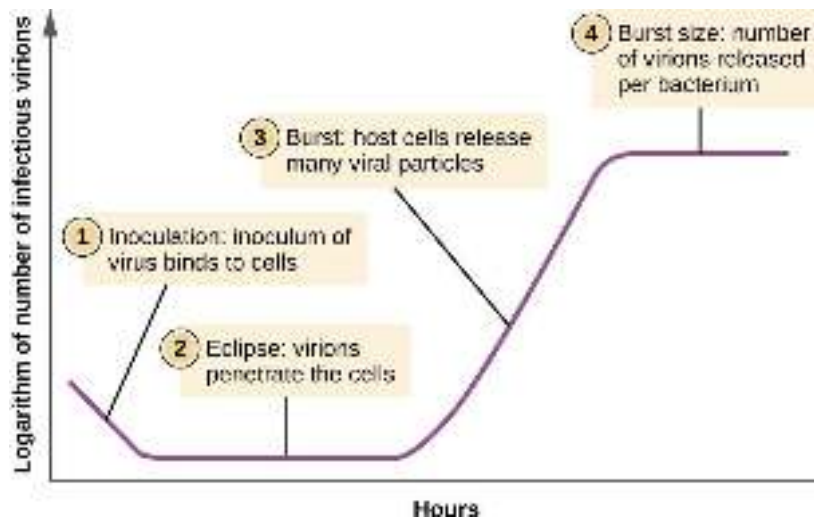


Figure: Growth curve

LECTURE 150: Bacteriophage Growth

- Phage collision with an adsorption-susceptible bacterium,
- Virion attachment,
- Virion nucleic acid uptake,
- Synthesis of phage proteins and nucleic acid,
- A period during which virions mature,
- A virion release step

Phage Relationship with bacteria

The Red Queen hypothesis

- The interaction of phage with bacteria is the primary concern of phage community
- Bacteria have developed mechanisms that prevent phages from having an effect on them, which has led to this evolutionary arms race between the phages and their host bacteria
- Bacterial resistance to phages puts pressure on the phages to develop stronger effects on the bacteria.

The Red Queen hypothesis

- The Red Queen hypothesis describes this relationship, as the organisms must constantly adapt and evolve in order to survive.
- This relationship is important to understand as phages are now being used for more practical and medicinal purposes.

Restriction-Modification System

- Bacteria have developed multiple defense mechanisms
- Foreign DNA trying to enter the bacterial host is restricted by endonucleases that recognize specific base pairs within the DNA
- DNA of the cell is protected from restriction due to methylase
- RM systems have evolved to keep up with the ever-changing bacteria and phage.
- In general, these RM types differ in the nucleotide sequences that they recognize.
- There is an occasional slip where the endonuclease misses the DNA sequence of the phage and the phage DNA is able to enter the cell anyway, becoming methylated and protected against the endonuclease.

Clustered Regularly Interspersed Palindromic Repeats

- CRISPR means that the immunity to phages by bacteria has been acquired via adding spacers of DNA that are identical to that of the DNA from the phage.
- Some phages have been found to be immune to this mechanism as well.
- In some way or another, the phages have managed to get rid of the sequence that would be replicated.
- Phages are also capable of interacting with species other than bacteria, e.g., such as phage-encoded exotoxin interaction with animals
- Phage therapy is an example of applied phage community ecology.

LECTURE 151: VIRUSES AS THERAPEUTICS

Phage therapy, the use of bacteriophages to treat or prevent disease, stretches back a century to the earliest days of the discovery of phages. Long before the discovery of antibiotics, the thought that viruses which lyse bacteria could be used to treat diseases was highly attractive. Yet this idea has never become a widespread practical reality. Devotees of phage therapy defend their cherished belief with almost religious fervor, but there are serious obstacles to be overcome, such as the narrow host range of most phages (a few strains of bacteria, not even an entire species) and the speed at which bacteria develop resistance to infection.

LECTURE 152: Phage ecology

Ecology is the study of the interactions between organisms and their environments. Within our planet's varied environments there exist 10³⁰ or more individual bacteria (Whitman et al., 1998), and perhaps 10 or more times as many bacterial viruses, or bacteriophages (phages) as they are more typically described. Whether these viruses are diffusing, decaying, or finding bacteria to adsorb and infect, what is certain is that phages must participate in enormous numbers of ecological interactions.

LECTURE 153: Lysogeny

The lysogenic cycle, sometimes referred to as temperate or non-virulent infection, does not kill the host cell, instead using it as a refuge where it exists in a dormant state. Following the injection of the phage DNA into the host cell, it integrates itself into the host genome, with the help of phage-encoded integrase, where it is then termed a prophage. The prophage genome is

then replicated passively along with the host genome as the host cell divides for as long as it remains there and does not form the proteins required to produce progeny. As the phage genome is generally comparatively small, the bacterial hosts are normally relatively unharmed by this process.

LECTURE 154: Lysogenic cycle

The **lysogenic cycle** allows a phage to reproduce without killing its host. Some phages can only use the lytic cycle, but some phage like λ lambda, can switch between the two cycles.

In the lysogenic cycle, the first two steps (attachment and DNA injection) occur just as they do for the lytic cycle. However, once the phage DNA is inside the cell, it is not immediately copied or expressed to make proteins. Instead, it recombines with a particular region of the bacterial chromosome. This causes the phage DNA to be integrated into the chromosome.

The lysogenic cycle is complementary to the Lytic cycle for viral entry and reproduction within cells. While the Lytic cycle is common to both animal viruses and bacterial phages, the lysogenic cycle is more commonly found in animal viruses. The following are the steps of the lysogenic cycle:

- 1) Viral genome enters cell
- 2) Viral genome integrates into Host cell genome
- 3) Host cell DNA Polymerase copies viral chromosomes
- 4) Cell divides, and virus chromosomes are transmitted to cell's daughter cells
- 5) At any moment when the virus is "triggered", the viral genome detaches from the Host cell's DNA and enters stage 2 of the Lytic cycle.

Incorporation of Genetic Material

- Once the virus's genetic material is inserted, it attaches itself to that of the host's. Some viruses may not immediately start multiplying or replicating after infecting the cell. In case it does not start replicating, the virus is said to be *dormant*.
- The virus's genetic material is known as *prophage*, while it is in the dormant state.

Similarly, every time, any of the host's progenies multiply, the virus is replicated into each of them.

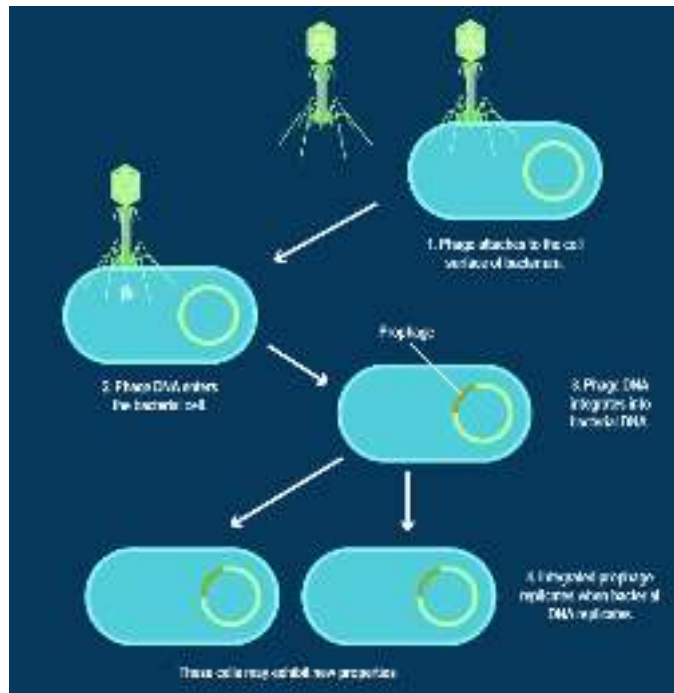


Figure: Depiction of the stages of the bacteriophage lysogenic cycle.

LECTURE 155: SATELLITES

Satellites are small molecules that are absolutely dependent on the presence of another virus for multiplication. Even viruses have their own parasites!

Most satellites are associated with plant viruses, but a few are associated with bacteriophages or animal viruses that are satellites of adenoviruses. Two classes of satellites can be distinguished:

- satellite viruses, which encode their own coat proteins, and
- Satellite RNAs (or “virusoids”), which use the coat protein of the helper virus.

RNA Satellites replicate in the cytoplasm using an RNA-dependent RNA polymerase, an enzymatic activity found in plant but not animal cells. Typical properties of satellites include:

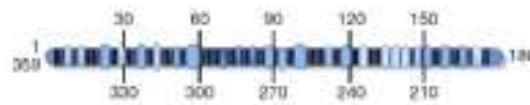
- Their genomes have approximately 5002,000nt of single-stranded RNA.
- unlike defective virus genomes, there is little or no nucleotide sequence similarity between the satellite and the helper virus genome.
- They cause distinct disease symptoms in plants that are not seen with the helper virus alone.

Satellites of plant viruses do not interfere with the replication of their helper viruses. In contrast, some recently discovered satellite-like viruses of **animal viruses** (such as the “Sputnik” virophage) do reduce helper virus production, and have become known as virophages—“eaters of viruses.” A number of different virophages have been found and all replicate in cells

coinfecting with large DNA viruses such as Mimivirus. Inhibition of helper virus replication makes virophages distinct from true satellites, even though their biology more closely resembles satellites than anything else.

LECTURE 156: VIROIDS

Viroids are very small (200-400 nt), rod-like RNA molecules with a high degree of secondary structure (Figure). They have no capsid or envelope and consist only of a single nucleic acid molecule. Viroids are associated with plant diseases and are distinct from satellites in a number of ways. The first viroid to be discovered and the best studied is potato spindle tuber viroid (PSTVd; viroid names are abbreviated “Vd” to distinguish them from viruses). Viroids do not encode any proteins and are replicated by host-cell RNA polymerase II or possibly by the product of an RNA-dependent RNA polymerase gene in some eukaryotic cells. The details of replication are not understood, but it is likely to occur by a rolling circle mechanism followed by autocatalytic cleavage and self-ligation to produce the mature viroid.



LECTURE 157: PRIONS

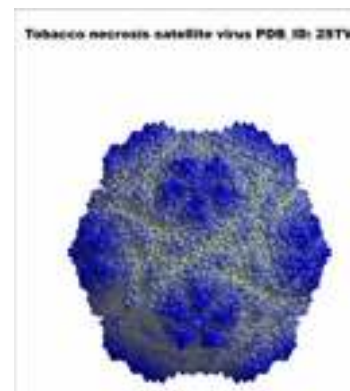
A particular group of transmissible, chronic, progressive infections of the nervous system show common pathological effects and are invariably fatal. Their pathology is similar to that of amyloid diseases such as Alzheimer's syndrome, and to distinguish them from such noninfectious (endogenous) conditions they are known as transmissible spongiform encephalopathies (TSEs). The earliest record of any TSE dates from several centuries ago, when a disease called scrapie was first observed in sheep. Originally thought to be caused by viruses, the first doubts about the nature of the infectious agent involved in TSEs arose in the 1960s.

In 1967, Tikvah Alper was the first to suggest that the agent of scrapie might replicate without nucleic acid, and in 1982 Stanley Prusiner coined the term prion (proteinaceous infectious particle)—which according to Prusiner is pronounced “pree-on.” The molecular nature of prions has not been unequivocally proved, but the evidence that they represent a new phenomenon outside the framework of previous scientific understanding is now generally accepted.

LECTURE 158: DIFFERENCE B/W SATELLITES, VIROIDS, PRIONS

Satellite

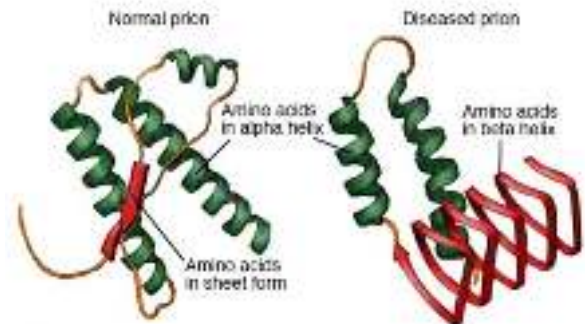
- Smallest
- Most simple infectious
- RNA molecules
- Parasites of viruses
- Satellites require helper viruses for replication
- Satellites do encode protein



- Circular RNA genome absent
- Satellites range between 475-1700 bases

Prions

- Neither have DNA or RNA
- Abnormal or mutated form of protein
- Causes several lethal diseases



Viroids

- Autonomous
- Circular RNA genome present
- 250 to 400 bases
- Positive sense RNA
- Smaller than virus
- Do not need helper virus for replication
- Two families: *Pospoviridae* and *Avsunviridae*
- Usually affects plants

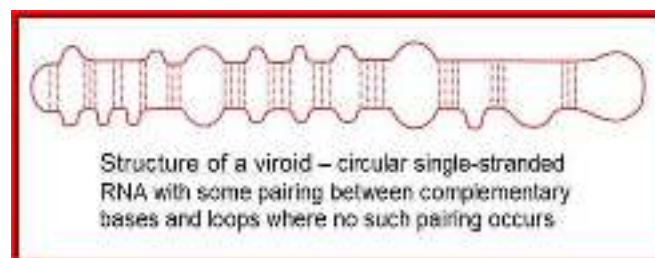


Table 8.1. Satellites and Viroids

Characteristic	Satellites	Viroids
Helper virus required for replication	Yes	No
Protein(s) encoded	Yes	No
Genome replicated by	Helper virus enzymes	Host-cell RNA polymerase II
Site of replication	Same as helper virus (nucleus or cytoplasm)	Nucleus

LECTURE 159: SATELLITES PROPERTIES

- Smallest sub-viral agent
- Most simple infectious
- RNA molecules
- Parasites of viruses
- Require helper viruses for replication
- Satellites do encode protein
- Satellites range between 475-1700 bases

- Present in plants, mammals, arthropods
- Symbiotic relation with helper virus
- 0.22- 1.5kbp genomic size

CLASSIFICATION

1. Single stranded RNA satellite virus
2. Double stranded DNA satellite virus
3. Single stranded satellite DNAs
4. Double stranded satellite RNAs

APPLICATIONS

- Application of mild strains to prevent plant viral diseases
- Transgenic plant production
- To study cell to cell movements in plants

Advantages to Study Satellites

- Ease in manipulation
- High in-vivo stability
- High expression level

Lecture 160: SATELLITE EXAMPLES

Necrosis and Systemic Chlorosis

- Reduced chlorophyll production
- Pale, yellow or yellowish white leaves

Hepatitis Delta Virus

- Associated with human helper virus, hepatitis B virus
- 1.7 kb genome
- Circular single stranded RNA
- Replicated by cellular RNA polymerase

Tobacco Necrosis Virus

- First identified satellite
- 18nm isomeric particles
- Linear positive single stranded RNA genome
- Icosahedral
- 60 identical copies of identical proteins



Satellite Tobacco Mosaic VIRUS

- 60 identical copies of identical proteins
- 1063 nucleotides
- Single stranded RNA genome



Satellite Panicum Mosaic Virus

- Only affects grasses
- Icosahedral virus
- Eight stranded coat protein
- Induces mild mottle in millet plant

LECTURE 161: VIROID PROPERTIES

- The smallest infectious pathogens known so far
- They are composed solely of a short strand of circular, single-stranded RNA
- RNA that has no protein coating
- All known viroids are inhabitants of plants in which they are causing diseases

Nomenclature

➤ Family Pospiviroidae

- Genus *Pospiviroid* species: *Potato spindle tuber viroid* 361 nucleotides
- Genus *Pospiviroid* another species *Citrus exocortis viroid* 368–467 nt
- Genus *Hostuviroid* species: *Hop stunt viroid*, 294–303 nt

➤ Family Pospiviroidae

- Genus *Cocadviroid* species: *Coconut cadang-cadang viroid* 246–247 nt¹
- Genus *Apscaviroid* type species *Apple scar skin viroid* 329–334 nt
- Genus *Coleviroid* type species: *Coleus blumei viroid*

LECTURE 162: Viroid structure:

All viroids share a common structural feature: a conserved central region of the genome believed to be involved in their replication. One group of viroids is capable of forming a hammerhead structure, giving them the enzymatic properties of a ribozyme (an autocatalytic, self-cleaving RNA molecule). This activity is used to cleave the multimeric structures produced during the course of replication. Other viroids use unknown host nuclear enzymes to achieve this objective.

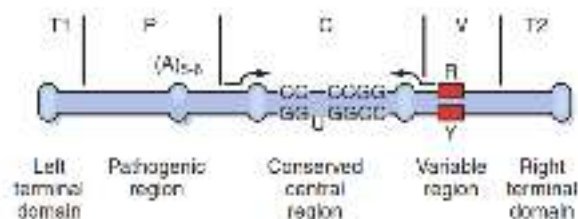
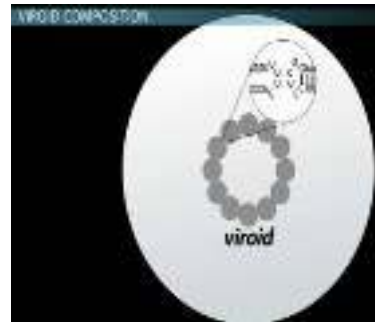


FIGURE 8.2 Functional regions of viroid RNA molecules.

All viroids share common structural features. The central region of the genome is highly conserved and believed to be involved in replication.

- Low molecular weight
- Unique structure
- Cause of disease
- Not encapsidated
- Replicate autonomously



GENOME

- Single stranded
- Covalently closed, circular
- As well as, linear RNA molecules
- Intramolecular complementarity
- Do not manufacture any proteins
- Infectious
- Highly structured
- Produce single specific RNA molecule

REPLICATION

- Rolling circle mechanism in-vivo
- Of two types
- Non-hydrolytic reaction

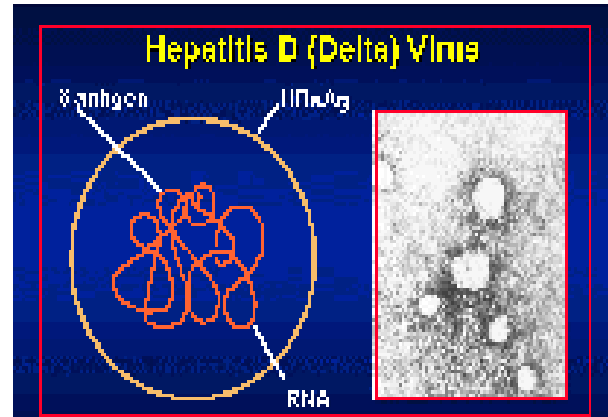
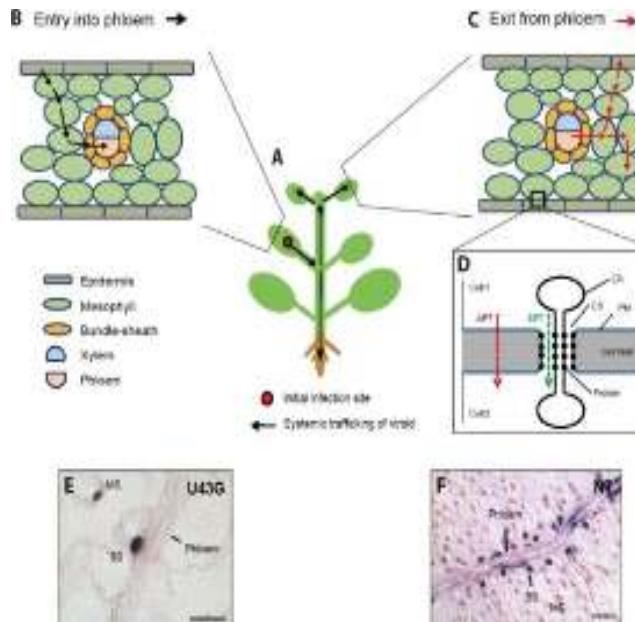
LECTURE 163: VIROID TRANSMISSION

- Mechanical means
- Contaminated machinery, tools, pruning and mechanic agriculture techniques
- Transmitted sometimes through seeds and pollens
- No important natural vectors

THROUGH PLANTS

- Viroids enter pollen and ovule
- Transmitted through seed
- When seed germinates, the new plant gets infected
- Transmitted through aphids
- Mechanical damage
- Leaf to leaf contact
- Often spread by vegetative propagation
- Agriculture and horticulture practices
- Both natural and human driven transmission
- Both horizontal and vertical spread
- Horizontal: parasitic fungi, nematodes, insects
- Vertical: seed transmission,

- Hepatitis D viroid enter human liver cells, if binding protein is present
- Obtains it from hepatitis B virus
- Delta virus enters blood stream and spreads by blood transfusion

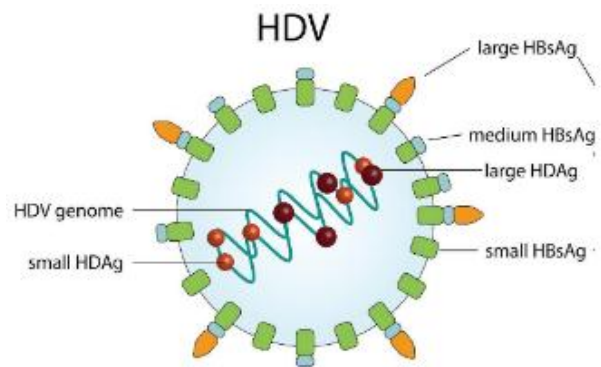


LECTURE 164: VIROID EXAMPLES

HDV requires hepatitis B virus (HBV) as a helper virus for replication and is transmitted by the same means as HBV, benefiting from the presence of a protective coat composed of lipid plus HBV proteins.

Hepatitis delta virus. One circular negative sense ssRNA molecule of 1700 nt in an isometric particle of 36 nm, with lipid and all three surface proteins of the hepatitis B virus helper. Two delta virus encoded proteins form the core. Despite its RNA genome is apparently replicated by host

DNA-dependent RNA polymerase II. RNA has self-cleaving activity.



Hepatitis D viroids

- Only human disease
- Simultaneous infection with hepatitis B virus
- Causes liver cell death
- Sequestering 7S RNA and/or cleaving it

Cucumber Green mottle virus

- Cucumber, melons and watermelons
- Affects young leaves with green and light green spots
- Veins remain green
- With heavy infection, leaves are deformed
- Small fruits

Carnation mottle virus

- Carnation flower plants
- Mild mottling of young leaves
- Faint chlorosis
- Yellow ring spots in heavy infection
- Vein chlorosis 8 to 10 days post infection

Tomato bushy stunt virus

- Economically significant plant pathogen
- Stunted growth, leaf mottling, deformed or absent fruits
- Broad host range
- Necrotic or chlorotic lesions

Potato Spindle Tuber Viroid

- Small circular RNA molecule
- Natural host: potato and tomato
- Transmission by seeds and aphids
- Necrosis of leaves and veins

Chrysanthemum stunt Viroids

- Affects chrysanthemum and pinks
- Light colored flowers
- Leaves reduce in number and size
- Wavy or crumpled leaf surface

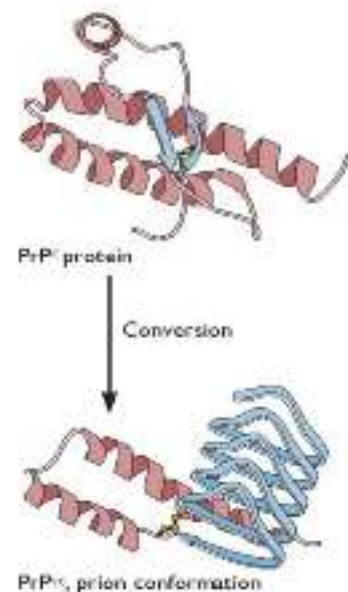
LECTURE 165: PRION STRUCTURE

Normal proteins

- 200 to 250 amino acids
- Twisted into three telephone cord-like coils
- Named as HELICES
- With tails of more amino acids

Mutated proteins

- Built from same amino acids
- But take different shape
- 100 times smaller than smallest virus



Difference from Bacteria and Viruses

- Prions don't contain nucleic acids
- Resistant to heat and chemicals
- Difficult to decompose biologically
- Survive in soil for many years

Structure

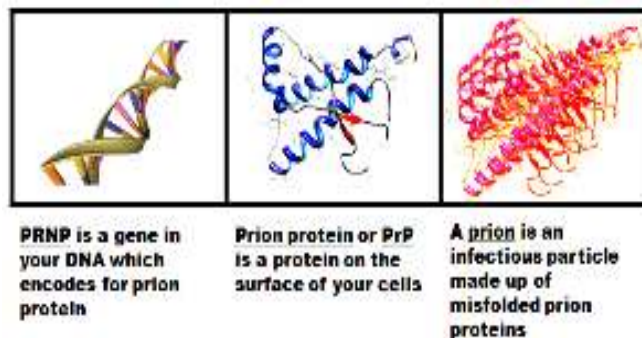
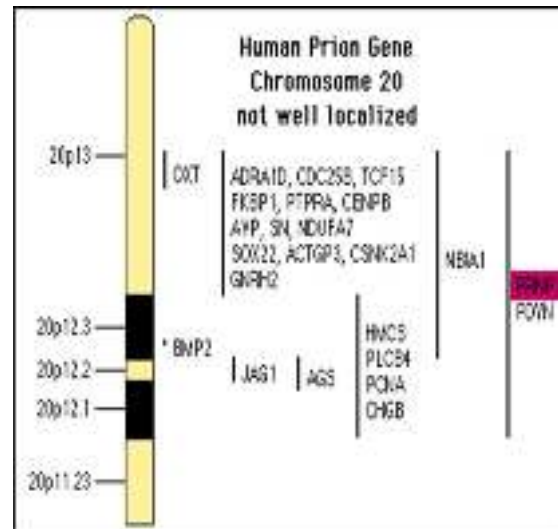
- Misfolded/ abnormally folded protein
- Pathogenic
- Causes similar proteins to misfold also

REPLICATION

- Unique
- Replicate on their own and become infectious
- Occur in two forms: PrP-sen and PrP-res

LECTURE 166: PRION GENOME

- Genome is absent
- No DNA
- No RNA
- Itself a protein
- Misfolded
- That makes other alike proteins misfolded
- causes disease condition in host
- single exon of a single-copy chromosomal gene
- PrP gene in mammals contain one or two 5'-non-coding exon



LECTURE 167: PRION PATHOLOGY OF DISEASES

A protein of 54 amino acids (PrP^{Sc}) is associated with scrapie infectivity. Biochemical purification of scrapie infectivity results in preparations highly enriched in PrP^{Sc}, and purification of PrP^{Sc} results in enrichment of scrapie activity. In 1984, Prusiner determined the sequence of 15 amino acids at the end of purified PrP^{Sc} happened just down to luck? No. It's

down to the species barrier which protects each species from the prions of another. Millions of people were exposed to BSE in the

United Kingdom alone, but fortunately, humans are quite resistant to cattle prions—more resistant than mice are to hamster prions for example. So it wasn't just luck. But it certainly wasn't down to good planning or judgment. This led to the discovery that all mammalian cells contain a gene (Prnp) that encodes a protein identical to PrP^{Sc}, termed PrP^C. No biochemical differences between PrP^C have been determined, although, unlike PrP^C, PrP^{Sc} is partly resistant to protease digestion, resulting in the formation of a 141-amino-acid, protease-resistant fragment that accumulates as fibrils in infected cells. Only a proportion of the total PrP in diseased tissue is present as PrP^{Sc}, but this has been shown to be the infectious form of the PrP protein, as highly purified PrP^{Sc} is infectious when used to inoculate experimental animals. Like other infectious agents, there is a dosage effect that gives a correlation between the amount of PrP^{Sc} in an inoculum and the incubation time until the development of disease. Thus, TSEs, which behave like infectious agents, appear to be caused by an endogenous gene/protein. Susceptibility of a host species to prion infection is codetermined by the prion inoculum and the Prnp gene.

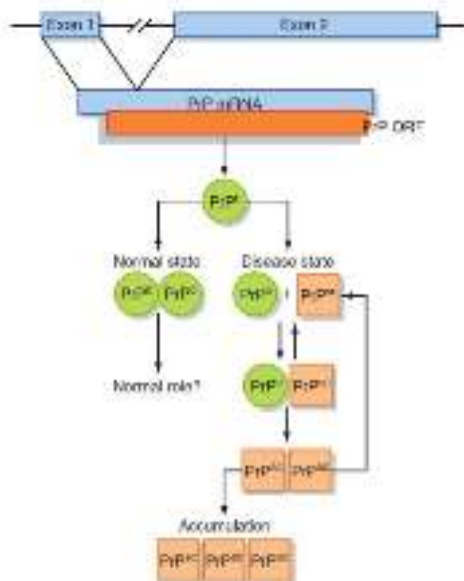


Figure: Schematic diagram of the role of PrP in TSEs. TSEs behave like infectious agents but appear to be caused by a cellular gene/protein.

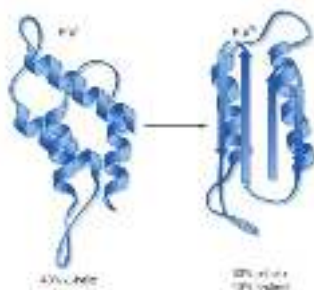
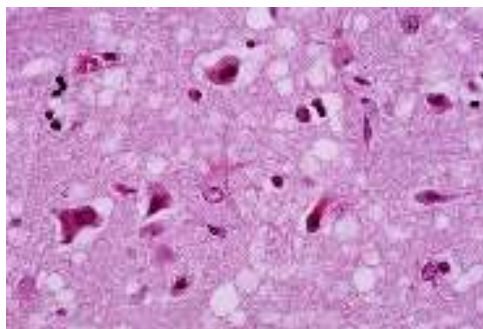


FIGURE: Conformational changes in PrP.

The fundamental difference between the infectious, pathogenic form (PrP^{Sc}) and the endogenous form (PrP^C) results from a change in the conformation of the folded protein, which adopts a conformation rich in β-sheet.

- TSE are caused by abnormal folding of prions
- The normal prion protein, which is designated as PrP^c, is a 35kD membrane glycoprotein, which is water-soluble and proteinase-sensitive.
- Abnormal prions, designated as PrP^{Sc} or PrP^{TSE}
- The unique feature of prion diseases is that they are self-propagating and transmissible.
- Once PrP^{Sc} is generated endogenously or introduced into the body from the environment, it converts normal prions into abnormal ones.
- This conversion begins with the initial production of a small polymer of misfolded prions, (a seed), perhaps no more than 28 molecules.
- This seed converts normal adjacent prions into abnormal ones by an unknown mechanism.
- As more PrP^{Sc} polymers are produced, they, in turn, act as seeds, propagating the conversion of normal to abnormal prions.
- This novel mechanism of disease is also implicated in the propagation of protein misfolding that is involved in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.
- During the incubation period and active clinical phase of prion diseases, PrP^{TSE} is probably present (and can transmit the disease) in all tissues and body fluids, especially brain, nerves, and skeletal muscle.
- Pathology develops only in the brain.
- Initially, intracytoplasmic vacuoles appear in neurons.
- As the disease progresses, vacuolization becomes more pronounced and the cortical neuropil develops a spongy appearance, hence the term spongiform encephalopathy.
- Advanced cases show neuronal loss, gliosis, and brain atrophy.
- Cerebellar atrophy is usually severe.
- Unlike most cerebellar degenerations, there is more pronounced loss of granular neurons
- There is no inflammation. The CSF is normal.
- The changes are confined to the gray matter (the primary pathology involves the neuronal body).



Spongiform encephalopathy

LECTURE 168: TSE IN ANIMALS

A number of TSEs have been observed and intensively investigated in animals. In particular, scrapie is the model for our understanding of human TSEs. Some of these diseases are naturally occurring and have been known about for centuries, whereas others have only been observed more recently and are almost certainly causally related to one another.

Scrapie First described more than 200 years ago, scrapie is a naturally occurring disease of sheep found in many parts of the world, although it is not universally distributed. Scrapie appears to have originated in Spain and subsequently spread throughout Western Europe. The export of sheep from Britain in the nineteenth century is thought to have helped scrapie spread around the world. Scrapie is primarily a disease of sheep although it can also affect goats. The scrapie agent has been intensively studied and has been experimentally transmitted to laboratory animals many times. Infected sheep show severe and progressive neurological symptoms such as abnormal gait; they often repeatedly scrape against fences or posts, a behavior from which the disease takes its name.

The incidence of the disease increases with the age of the animals. Some countries, such as Australia and New Zealand, have eliminated scrapie by slaughtering infected sheep and by the imposition of rigorous import controls. Work has shown that the land on which infected sheep graze may retain the condition and infect sheep up to three years later.

The incidence of scrapie in a flock is related to the breed of sheep. Some breeds are relatively resistant to the disease while others are prone to it, indicating genetic control of susceptibility. In recent years the occurrence of TSE in sheep in the United Kingdom closely parallels the incidence of bovine spongiform encephalopathy (BSE) in cattle. This is probably due to infection with the BSE agent (to which sheep are known to be sensitive) via infected feed. The natural mode of transmission between sheep is unclear.

Lambs of scrapie-infected sheep are more likely to develop the disease, but the reason for this is unclear. Symptoms of scrapie are not seen in sheep less than one and a half years old which indicates that the incubation period of scrapie is at least this long.

ETIOLOGY

- Neurodegenerative disease caused by prions
- Replicate by converting normal proteins into copies of prions
- non-conventional disease agents

HOST SPECIES

- Sheep
- Goat
- Mice
- Rat
- Monkey
- And other Lab animals

Geographical distribution

- World wide
- More in developing countries

Transmission

- Well understood in sheep
- To goats if kept with sheep
- Within herd
- During pregnancy
- Through urine



SYMPTOMS

- Affected sheep will apart from flock
- Trembling
- Convulsions
- Scraping body with wall
- Drink small quantities of water often
- Blindness



LECTURE 169: Transmissible Mink Encephalopathy (TME)

IT is a rare disease of farmed mink caused by exposure to a scrapie-like agent in feed. The disease was first identified in Wisconsin in 1947 and has also been recorded in Canada, Finland, Germany, and Russia. Like other TSEs,

TME is a slow progressive neurological disease. Early symptoms include changes in habits and cleanliness as well as difficulty in eating or swallowing.

TME-infected mink become hyperexcitable and begin arching their tails over their backs, ultimately losing locomotor coordination. Natural TME has a minimum incubation period of 7-12 months, and, although exposure is generally through oral routes, horizontal mink-to-mink transmission cannot be ruled out.

ETIOLOGY

- Caused by prions
- Progressive and fatal neurodegenerative disease

HOST SPECIES

- Minks
- Once signs are evident, death is inevitable
- Raccoons
- Skunks
- Ferrets
- Hamsters

GEOGRAPHICAL DISTRIBUTION

- In USA from 1947 to 1985
- Canada
- Finland
- Germany

TRANSMISSION

- Orally
- Wounds on tongue
- Mucous membrane
- Skin

SYMPTOMS

- Difficulty in eating and swallowing
- Hyperexcitable
- Incoordination
- Clenching of jaw
- Convulsions
- Death



LECTURE 170: Feline Spongiform Encephalopathy (FSE)

FSE was recognized in the United Kingdom in May 1990 as a scrapie-like syndrome in domestic cats resulting in ataxia (irregular and jerky movements) and other symptoms typical of spongiform encephalopathies. By December 1997, a total of 81 cases had been reported in the United Kingdom. In addition, FSE has been recorded in a domestic cat in Norway and in three species of captive wild cats (cheetah, puma, and ocelot). Inclusion of cattle offal in commercial pet foods was banned in the United Kingdom in 1990, so the incidence of this disease is expected to decline rapidly.

ETIOLOGY

- Caused by prions
- Neurodegenerative disease
- Same agent responsible for Bovine Spongiform encephalopathy

HOST SPECIES

- Domesticated cats
- Cheetahs
- Puma
- Tiger
- Golden cat
- Lion
- Leopard

GEOGRAPHICAL DISTRIBUTION

- Most cases in UK
- Australia
- North America

TRANSMISSION

- Eating BSE contaminated tissue
- No horizontal transmission
- Possible maternal transmission
- Iatrogenic transmission in cheetah



SYMPTOMS

- Aggression
- Hiding
- Gait abnormalities
- Hyperesthesia
- Excessive salivation
- Tremors
- Death



LECTURE 171: Chronic Wasting Disease (CWD)

CWD is a disease similar to scrapie which affects deer and captive exotic ungulates (e.g., nyala, oryx, kudu). CWD was first recognized in captive deer and elk in the western United States in 1967 and appears to be endemic in origin. Since its appearance in Colorado, the disease has spread to other states and has also been reported in Canada and South Korea. This disease seems to be more efficiently transmitted from one animal to another than other TSEs, so it seems unlikely that it will ever be eradicated from the regions in which it occurs. CWD prions taken from the brains of infected deer and elk are able to convert normal human prion to a protease-resistant form, a well-studied test for the ability to cause human disease, but the overall risk to human health from this disease remains unclear and there is no evidence that this disease has ever been transmitted to humans.

ETIOLOGY

- Caused by prions
- Contagious neurological disease
- Causes a characteristic spongy degeneration of the brains of infected animals resulting in emaciation, abnormal behavior, loss of bodily functions and death.

Host

- Mule deer
- white-tailed deer
- elk
- moose
- reindeer

Signs & symptoms

- Difficulties in movement
- Weight loss over time
- Listlessness
- Lowering of head
- Excessive salivation
- Nervousness
- Tremors



Transmission

- Contact with infected animals
- Their body tissues
- Body fluids

LECTURE 172: Bovine Spongiform Encephalopathy

BSE was first recognized in dairy cattle of the United Kingdom in 1986 as a typical spongiform encephalopathy. Affected cattle showed altered behavior and a staggering gait, giving the disease its name in the press of “mad cow disease.” On microscopic examination, the brains of affected cattle showed extensive spongiform degeneration. It was concluded that BSE resulted from the use of contaminated foodstuffs. To obtain higher milk yields and growth rates, the nutritional value of feed for farmed animals was routinely boosted by the addition of protein derived from waste meat products and bonemeal (MBM) prepared from animal carcasses, including sheep and cows.

Etiology:

- Caused by prions
- Affects nervous system
- Also called mad cow disease

Signs:

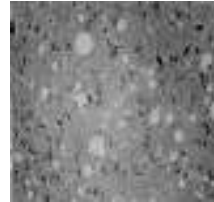
- Apprehension
- Hyperaesthesia
- Frequent licking with progressive paresis, & ataxia
- Circling

HOST = CATTLE

Transmission

- ❖ from the carcasses of scrapie-infected sheep.
- ❖ sheep by products infected with scrapie is used to feed cattle with the meat and bone mill (MBM)

BSE shows spongy appearance of the brain tissue of infected cattle when sections are examined under a microscope.

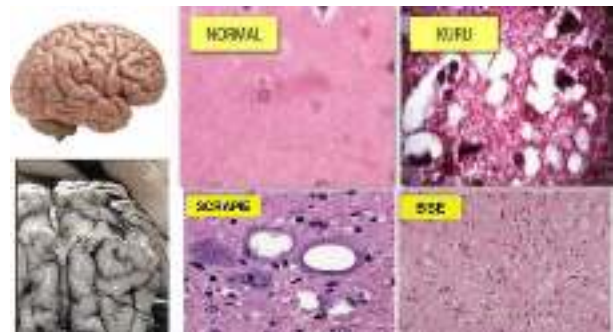


LECTURE 173: HUMAN TSEs

There are four recognized human TSEs. Our understanding of human TSEs is derived largely from studies of the animal TSEs already described. Human TSEs are believed to originate from three sources:

Sporadic: Creutzfeldt Jakob disease (CJD) arises spontaneously at a frequency of about one in a million people per year with little variation worldwide. The average age at onset of disease is about 65, and the average duration of illness is about 3 months. This category accounts for 90% of all human TSE, but only about 1% of sporadic CJD cases are transmissible to mice. Iatrogenic (acquired) TSE: This occurs due to recognized risks (e.g., neurosurgery, transplantation). About 50 cases of TSE were caused in young people who received injections of human growth hormone or

gonadotropin derived from pooled cadaver pituitary gland extracts, a practice that has now been discontinued in favor of recombinant DNA-derived hormone.



Classic CJD (Creutzfeldt-Jakob disease):

- Most prevalent of spongiform diseases
- 1 out of million people
- 10% cases are inherited mutations

in PRPN gene: Strikes people age 50-75.

Symptoms: dementia, muscle twitching, vision problems

Diagnosis:

- Post mortem definitive diagnosis
- Amyloid plaques surrounded by vacuoles
- Prion protein accumulation in cerebellum
- Spongiform appearance in gray matter

Familial: Approximately 10% of human TSEs are familial (i.e., inherited). A number of mutations in the human PrP gene are known to give rise to TSE as an autosomal dominant trait acquired by hereditary Mendelian transmission.

Fatal familial Insomnia:

- All case are inherited mutations in Prp gene
- Strikes people age 36-61
- Disruption of sleep/wake cycle leads to coma, then death.

Disease	Description	Comments
CJD	Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical gray matter, or encephalopathy with prion protein (PrP) immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivascular types)	Three forms: sporadic, iatrogenic (recognized risk, e.g., neurosurgery), familial (same disease in first degree relative).
Familial fatal insomnia (FFI)	Thalamic degeneration, variable spongiform change in cerebrum.	Occurs in families with PrP ^{Sc} Asp-Asn mutation.
Gerstmann–Strausler–Scheinker disease (GSS)	Encephalo/myelo(pathy) with multicentric PrP plaques.	Occurs in families with dominantly inherited progressive ataxia and/or dementia.
Kuru	Characterized by large amyloid plaques.	Occurs in the Fore population of New Guinea due to ritual cannibalism, now eliminated.

Kuru was the first human spongiform encephalopathy to be investigated in detail and is possibly one of the most fascinating stories to have emerged from any epidemiological investigation. The disease occurred primarily in villages occupied by the Fore tribes in the highlands of New Guinea. The first cases were recorded in the 1950s and involved progressive loss of voluntary neuronal control, followed by death less than 1 year after the onset of symptoms. The key to the origin of the disease was provided by the profile of its victims—it was never seen in young children, rarely in adult men, and was most common in both male and female adolescents and in adult women.

The Fore people practiced ritual cannibalism as a rite of mourning for their dead. Women and children participated in these ceremonies but adult men did not take part, explaining the age/sex distribution of the disease. The incubation period for kuru can be in excess of 30 years but in most cases is somewhat shorter. The practice of ritual cannibalism was discouraged in the late 1950s and the incidence of kuru declined dramatically. Kuru has now disappeared.

Kuru: Struck members of Fore tribe in 1950s and 1960s

Signs:

- Muscle weakness
- loss of coordination
- tremors
- inappropriate episodes of laughter or crying

Transmission: Transmitted by ritual cannibalism as part of funeral ceremonies.

LECTURE 174: MOLECULAR BIOLOGY OF PRIONS

The evidence that prions are not conventional viruses is based on the fact that nucleic acid is not necessary for infectivity, as they show: Resistance to heat inactivation: Infectivity is reduced but not eliminated by high-temperature autoclaving (135 C for 18 min). Some infectious activity is even retained after treatment at 60°C, suggesting that an inorganic molecular template is capable of nucleating the biological replication of the agent.



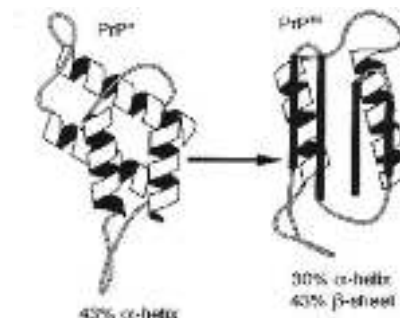
Resistance to radiation damage: Infectivity was found to be resistant to shortwave ultraviolet radiation and to ionizing radiation. These treatments inactivate infectious organisms by causing damage to the genome. There is an inverse relationship between the size of target nucleic acid molecule and the dose of radioactivity or ultraviolet light needed to inactivate them; that is, large molecules are sensitive to much lower doses than are smaller molecules. The scrapie agent was found to be highly resistant to both ultraviolet light and ionizing radiation, indicating that any nucleic acid present must be less than 80 nt.

Resistance to DNase and RNase treatment, to psoralens, and to Zn catalyzed hydrolysis, all these treatments inactivate nucleic acids.

Sensitivity to urea, detergents, phenol, and other protein-denaturing chemicals.

Prion protein:

- Abnormal isoform of normal protein
- Located in cell surface (brain, nervous system)
- Protects cell against oxidative stress (free radicals lacking electrons)
- PrP^c = prion protein cellular (normal form)
- PrP^{sc} = scrapie form (infectious form)



PrP ^c	PrP ^{Sc}
Rich in alpha helices	Rich in beta sheets
Soluble	Insoluble
PK (protease) sensitive - Definitive shelf life - Degrades when no longer needed	PK resistant
No aggregation Cannot polymerize into aggregates	Aggregation - Allow polymerization of fibrils - Due to beta sheets

LECTURE 175:

HISTORY OF CELL CULTURE MEDIA

In 1912, Carrel demonstrated that the long-term cultivation of the cells that have been obtained from the connective tissues of chick fetuses is possible (for several months) with a periodic exchange of the medium. In 1913, he discovered that adding embryonic extract to blood plasma can dramatically increase cellular proliferation and extend the culture period of fibroblasts from the chick embryo heart.

It is rare for healthy somatic cells that are derived from animals to acquire unlimited proliferative capacity during cultivation. They typically stop growing after a certain number of divisions. As a result, researchers would conduct studies by using cells that had been sampled from animals in each experiment. In 1940, Wilton R. Earle et al. used carcinogens to successfully create immortal mouse fibroblasts (L cells), revealing that proliferation from a single cell is possible. In 1951, George O. Gey and his coworkers created an infinitely proliferating human cell line from a tissue of a patient with uterine cervical cancer (HeLa cells).

DEVELOPMENT OF CULTURING METHOD FOR VIRUSES

- First cell line developed in 1912 by Carrel and his coworkers
- From fragments of explanted chicken embryo heart
- Maintained by washing with Ringer solution
- Developed in 1940s and 1950s
- By John Franklin Enders, Thomas Huckle Weller and Frederick Chapman Robbins

FIRST CULTURE

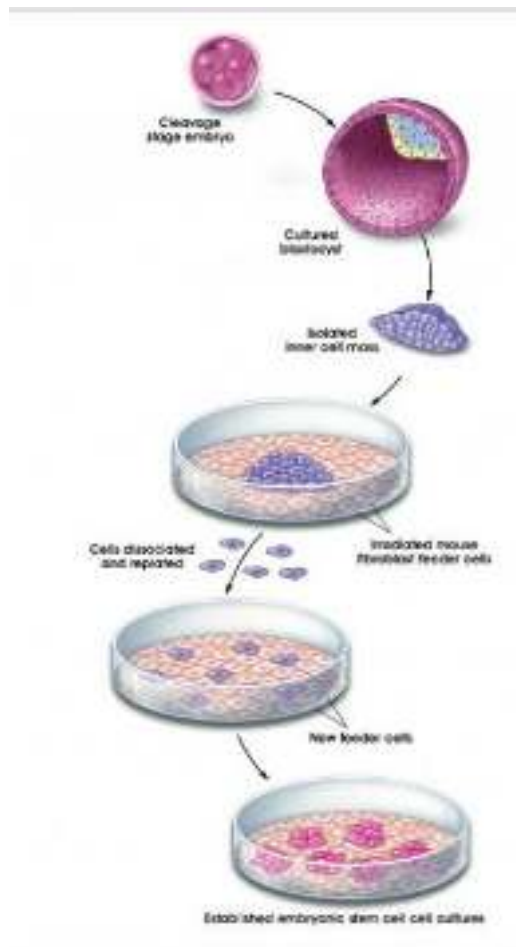
- Received Nobel Prize
- Culturing viruses in Monkey kidney cells

MEDIA

- Early use was naturally derived products from serum
- Later use of chemical-based synthetic media

OBSERVATIONS

- Using modern microscopy techniques
- Possibility to observe inclusions in cells as a result of virus



L-CELL LINE

- Developed by Earle in 1948
- From subcutaneous mouse tissue

HELA CELL LINE

- In 50s and 60s
- Diploid cell lines
- From human tissue

VERO CELL LINE

- In 50s and 60s
- Diploid cell lines
- From Simian tissue

EARLIEST DERIVED CELL LINES

Name	Species and tissues	Morphology	Year of origin
L929	Mouse connective tissue	Fibroblast	1948
HeLa	Human cervix	Epithelial-like	1951
CHO	Chinese Hamster Ovary	Epithelial	1957
MDCK	Canine kidney	Epithelial	1958
WI-38	Human lung	Fibroblast	1961
BHK-21	Syrian Hamster Kidney	Fibroblast	1961
Vero	African Green monkey kidney	Epithelial	1962

LECTURE 176: CELL CULTURE:-

The most commonly used cell cultures in virology derive from primates, including humans and monkeys; rodents, including hamsters, rats, and mice; and birds, most notably chickens. Cells to be cultured are obtained by mincing tissue and dispersing individual cells by treatment with proteases (e.g. trypsin) and/or collagenase to disrupt cell–cell interactions and interactions of cells with the extracellular matrix.

With the exception of cells from the hemopoietic system, normal vertebrate cells will grow and divide only when attached to a solid surface. Dispersed cells are therefore placed in a plastic flask or dish, the surface of which has been treated to promote cell attachment. The cells are incubated in a buffered nutrient. Medium in the presence of blood serum, which contains a complex mixture of hormones and factors required for the growth of normal cells. The blood serum may come from a variety of sources, although bovine serum is most commonly used. Under these conditions, cells will attach to the surface of the dish, and they will divide and migrate until the surface of the dish is covered with a single layer of cells, a monolayer, where upon they will remain viable but cease to divide. If the cell monolayer is “wounded” by scraping cells from an isolated area, cells on the border of the wound will resume division and migration until the monolayer is reformed, whereupon cell division again ceases.

INTRODUCTION

Cell culture is the process by which prokaryotic, eukaryotic or plant cells are grown under controlled conditions. But in practice it refers to the culturing of cells derived from animal cells.

MAJOR DEVELOPMENT’S IN CELL CULTURE TECHNOLOGY

- First development was the use of antibiotics which inhibits the growth of contaminants.

- Second was the use of trypsin to remove adherent cells to subculture further from the culture vessel
- Third was the use of chemically defined culture medium.

IMPORTANCE

Virology: Cultivation of virus for vaccine production, also used to study their infectious cycle.

Genetic Engineering: Production of commercial proteins, large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles

Gene therapy: Cells having a functional gene can be replaced to cells which are having non-functional gene

LECTURE 177:-

Slide Culture Technique

Preparation of a slide culture can be useful in the study and identification of an unknown fungal isolate. While the 'sticky tape' preparation is quick and easy to perform, it can be disruptive, with salient features altered or destroyed. The slide culture when performed properly is more gentle allowing for the examination of intact structures & features as they naturally developed.

Rapid **method** of preparing fungal colonies for examination and identification.

Permits the fungi to be studied virtually insitu with as little disturbance as possible.



PROCEDURE

The technique is quite easy to perform and the basic steps of procedure are given below:

- (1) Start by getting a plate of fungal media is a sterile scalpel however they can be relatively pricey for such a small job. Cut out small blocks of agar somewhat about 1/2 to 3/4 of an inch square.
- (2) Remove an agar square that you have cut into the plate using cutting tool (scalpel or cover slip). Place the agar block onto a clean glass microscope slide.
- (3) The slide can then be placed in a clean petrie dish which will prevent contamination and preserve moisture during incubation.

(4) Using a sterile instrument (loop, needle, etc), transfer some of the fungus (spores, etc) from the specimen being cultured to each of the four sides of the agar block.

(5) After inoculation, place a clean cover slip on the surface of the agar block.

(6) A few drops of sterile water can be added to the petrie dish as an additional source of moisture which may be beneficial to slow growing fungi which may dry out with prolonged incubation. In general this is not necessary as the dish will be partially sealed up.

(7) The plate is now partially sealed with Parafilm™ or a bit of cellulose tape. If fully sealed the plate may fog up and moisture condense on specimen. Incubate the slide at an appropriate temperature (room temperature to 30°C for most fungi) and for an appropriate length of time.

Application of Stain

- Place a drop of lactophenol cotton blue stain on a clean microscope slide.
- Remove the cover glass from the slide culture and discard the block of agar.
- Add a drop of 95% ethanol to the hyphae on the cover glass.
- when alcohol has evaporated place the cover glass, mold sidedown, on the drop of lactophenol cotton blue stain on the slide.
- Examine the slide under microscope

LECTURE 178:-

Laboratory flask: Laboratory flasks are vessels or containers that fall into the category of laboratory equipment known as glassware.

Erlenmeyer flask: An Erlenmeyer flask, also known as a conical flask or titration flask, is a type of laboratory flask which features a flat bottom, a conical body

Round-bottom flask: Round-bottom flasks (also called round-bottomed flasks or RB flasks) are types of flasks having spherical bottoms

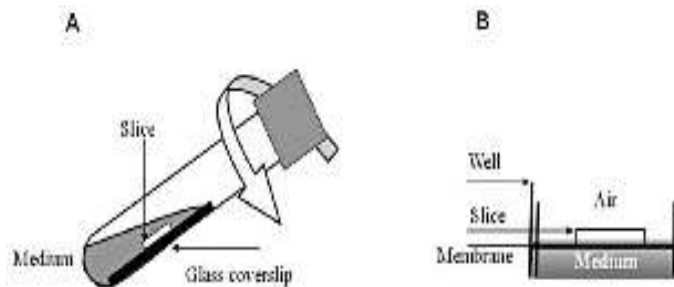


Fernbach flask: A Fernbach flask is a type of flask suited for large volume cell culture where the culture requires a large surface area to volume ratio.



LECTURE 179: ROLLER TUBE CULTURE:-

- Grown in test tubes
- Rotated during incubation



PROCEDURE

- Tissue explants placed in test tubes
- Small amount of plasma and agar distributed over the inside surface of tube, so fragments stay in place
- As the tube is rotated 8 to 12 rpm
- Tissue explants submerged in nutrient media, only a portion of time
- Some cells don't grow satisfactorily if entirely submerged
- Other cells, especially those cultivated through many transfers will stick to glass surface and grown when completely submerged



LECTURE 180: MONOLAYER TISSUE CULTURE

<https://www.youtube.com/watch?v=CMRKKI9XSDU>

INTRODUCTION

Monolayer tissue culture is a technique used in cell biology to grow and maintain cells in a single layer on the surface of a culture vessel, such as a petri dish or a flask. This method allows researchers to study the behavior, growth, and interactions of individual cells in a controlled environment.

PROCEDURE

Here are the key steps involved in monolayer tissue culture:

- 1. Cell Preparation:** Cells are usually obtained from a tissue or an established cell line. These cells are then suspended in a suitable growth medium that contains nutrients, salts, and other essential components for cell growth.
- 2. Substrate Coating:** The surface of the culture vessel needs to be coated with a substrate that promotes cell adhesion. Common substrates include gelatin, collagen, fibronectin, or synthetic materials like poly-L-lysine. This coating provides a surface for the cells to attach and spread.
- 3. Seeding Cells:** The cell suspension is added to the culture vessel and allowed to settle on the coated surface. The cells adhere to the substrate and start spreading, forming a monolayer.
- 4. Culturing Conditions:** The culture vessel is then placed in a controlled environment, typically an incubator, where conditions such as temperature, humidity, and CO₂ levels are carefully regulated. This ensures optimal cell growth and viability.
- 5. Medium Refreshment:** The growth medium is periodically replaced to provide cells with fresh nutrients and remove waste products. The frequency of medium changes depends on the cell type and specific experimental requirements.
- 6. Monitoring and Maintenance:** The cells in the monolayer are regularly monitored under a microscope to assess their confluence (degree of cell coverage) and overall health. If the cells reach confluence, they may need to be subcultured or passaged to maintain an actively growing population.
- 7. Experimental Manipulations:** Monolayer cultures are often used for various experimental purposes, including drug testing, gene expression studies, and cellular behavior assessments. Researchers can manipulate the cells within the monolayer to investigate specific biological processes.

Monolayer tissue culture is widely used in cell biology and provides a simplified system for studying cell behavior in vitro. It is essential to maintain sterile conditions throughout the process to prevent contamination and ensure the reproducibility of experimental results.

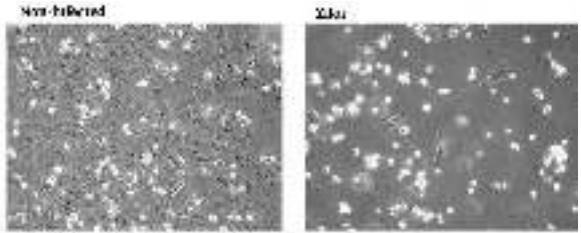
LECTURE 181: Cytopathic Effect

- **Definition:** Cytopathic effect or cytopathogenic effect (CPE) refers to structural changes in host cells that are caused by viral invasion.

When a virus invades a host cell, its structure changes. This is known as the cytopathic effect. This condition occurs when the infecting cell causes the lysis of the host cell or when the cell dies due to its inability to reproduce. A virus causing morphological changes in the host cell is known as cytopathogenic.

The first sign of cytopathic effect or viral infection is the rounding of cells. The inclusion bodies are observed in the cytoplasm and nucleus of the host cell. These can be identified in the blood smear of the patients through light microscopy or electron microscopy. Some viral infections lead to the formation of syncytia. These are large cytoplasmic masses produced by the fusion of infected cells. It is composed of many nuclei.

TYPES OF CYTOPATHIC EFFECTS

- a. **Total Destruction:** This is the most severe type of cytopathic effect. This is seen with enteroviruses. This causes the death of all the cells in the monolayer. This is observed by seeding the cells on a glass surface and a single layer of host cells is formed. The viral infection is introduced into the cells and the cells are observed. Within three days all the cells shrink and detach from the glass.
 - most severe form of CPE. All cells in the monolayer rapidly shrink, become dense (pyknosis), and detach from the glass within 72 hours.
 - typical of most enteroviruses.
- b. **Subtotal Destruction:** A monolayer of host cells is seeded on the glass surface and viral infection is introduced. Only a few cells detach from the glass surface after some time. This type of CPE is caused by togavirus, picornavirus, paramyxovirus, etc.
 - detachment (death) of some but not all of the cells in the monolayer.
 - togaviruses (alphaviruses), picornaviruses,
 - can be observed using the 20X objective
- c. **Focal Degeneration:** In this, there is a direct transfer of the virus from one cell to the other. The host cells are enlarged, rounded, and refractile. After some time, the cells

detach from the surface. This type of cytopathic effect is exhibited by the herpes virus and pox virus.

- Herpesviruses and poxviruses.
- viruses produce localized areas (foci) of infection.
- The focal nature of these lesions is due to direct cell-to-cell transfer of virus rather than diffusion through the extracellular medium.
- Cells initially become enlarged, rounded, and refractile (more easily seen), then eventually detach from the growth surface leaving cleared areas surrounded by rounded up cells as the infection spreads concentrically

d. **Swelling and Clumping:** This is a characteristic of adenovirus. The cells swell and become enlarged, clump together, and eventually detach from the surface.



FIG. 5. Bovine fetal spleen cells 4 days postinfection with a high MOI of the haemagglutinating, an Adenovirus, showing cell rounding and small amounts of clumping. Cells were observed using the 10X objective (100X total magnification).

- typical of adenoviruses Infected cells greatly enlarge and clump together in "grape-like" clusters.

e. **Foamy Degeneration:** A number of large cytoplasmic vacuoles are formed. It can be observed by fixing and staining the host cell. It is observed in retroviruses and flaviviruses.

- production of large and/or numerous cytoplasmic vacuoles.
- retroviruses, paramyxoviruses, and flaviviruses (pestiviruses) may cause vacuolization.
- Vacuolization is difficult to visualize without staining.

f. **Syncytium:** This is also known as cell fusion. In this cytopathic effect, the plasma membranes of the host cells fuse and form an enlarged cell. This cell contains at least four nuclei. This can be detected by fixing and staining the host cells. Herpes virus and paramyxovirus can be detected this way.

- fusion of the plasma membranes of four or more cells to produce an enlarged cell with four or more nuclei.
- paramyxoviruses, herpesviruses may produce syncytia as well as other forms of CPE



FIG.(a) Giemsa-stained bovine fetal spleen cells 4 days postinfection with the bovine respiratory syncytial virus, a Paramyxovirus, showing syncytia (arrows) and faint basophilic cytoplasmic inclusion bodies (dashed arrows). Cells were observed with the 20X objective (200X total magnification).

FIG.(b). Bovine fetal spleen cells infected with the parainfluenza virus type 3, a Paramyxovirus, showing syncytia (arrows).

- g. **Inclusion Bodies:** Insoluble abnormal structures in the nuclei or cytoplasm of the cells can only be detected through staining. Inclusion bodies are areas of altered staining in cells. These inclusions may be large, small, single, multiple, intranuclear, intracytoplasmic, basophilic, or eosinophilic. Chromatin migration causes a thin stained ring around the edge of the nucleus.

Cytopathic Effect in Diagnostics

Cytopathic effects are used to diagnose viral infections. A few cytopathic effects can be seen in unstained, unfixed cells under low-power optical microscope. While a few cytopathic effects such as inclusion bodies can be seen in stained and fixed cells under a light microscope.

The cytopathic effects of a few viruses are specific and therefore can be used to diagnose viral infections in humans and animals. The rate at which a cytopathic effect appears is also used to diagnose the type of infections by virologists.

LECTURE 182: VIRUSES AND CANCER

History: An avian erythroblastosis virus (AEV) was isolated from a spontaneous erythroleukemia in a chicken in 1908 (Ellerman and Bang 1908), and soon after, Peyton Rous isolated Rous sarcoma virus from a chicken fibrosarcoma (Rous 1911). Strains descended from Rous' original isolate are still studied today. These experiments were eventually followed by many others in which tumor inducing viruses were isolated from rodents, cats, cows, primates, and other animals.

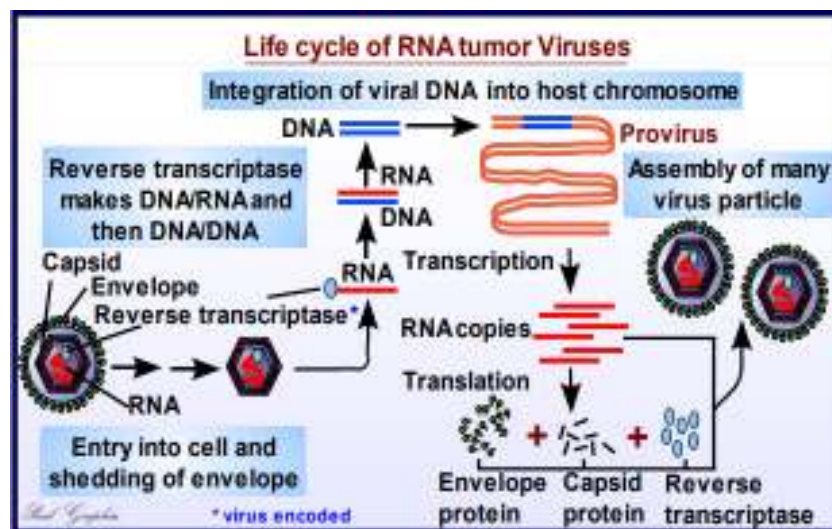
- 1876: Russian researcher reported transmission of a tumor from one dog to another
- 1908, **Wilhelm** and **Olaf Bang** in Copenhagen reported the extract from chicken leukemia can cause tumor.
- In 1909 **Rous** found that extract from chicken sarcoma can cause tumor.

Viruses and cancer: Members of six distinct families of animal viruses, called tumor viruses, are capable of directly causing cancer in either experimental animals or humans (Table). Viruses belonging to five of these families have DNA genomes and are referred to as DNA tumor viruses. Members of the sixth family of tumor viruses, the retroviruses, have RNA genomes in virus particles but replicate via synthesis of a DNA provirus in infected cells. The viruses that cause human cancer include hepatitis B virus (liver cancer), papillomaviruses (cervical and other anogenital cancers), Epstein-Barr virus (Burkitt's lymphoma and nasopharyngeal carcinoma), Kaposi's sarcoma-associated herpesvirus (Kaposi's sarcoma), and human T-cell lymphotropic virus (adult T-cell leukemia). In addition, HIV is indirectly responsible for the cancers that develop in AIDS patients as a result of immunodeficiency, and hepatitis C virus (an RNA virus) is an indirect cause of liver cancers resulting from chronic tissue damage.

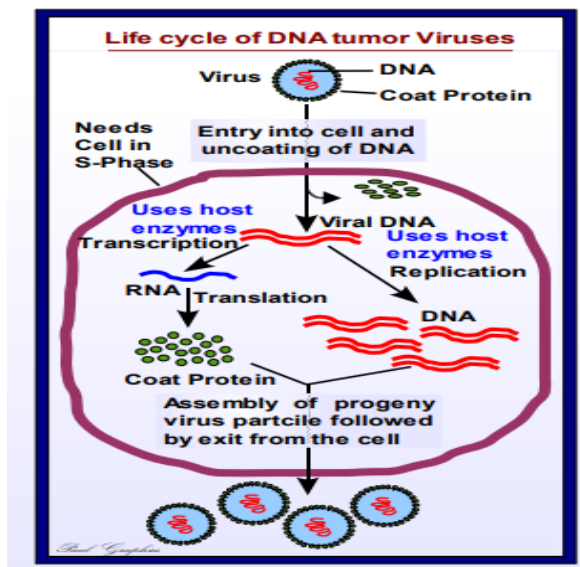
Virus family	Human tumors
Hepatitis B viruses	Liver cancer
SV40 and polyomavirus	None
Papillomaviruses	Cervical carcinoma
Adenoviruses	None
Herpesviruses	Burkitt's lymphoma, nasopharyngeal carcinoma, Kaposi's sarcoma
Retroviruses	Adult T-cell leukemia

Etiology of cancer:

- Viruses (papilloma, Epstein-Barr, Hepatitis B, retrovirus)
- Radiation
- Environmental/industrial carcinogens
- Immunodeficiency syndromes
- Genetic susceptibility syndromes



DNA tumor Viruses



LECTURE 183: CELL TRANSFORMATION BY VIRUSES

Transformation is a change in the morphological, biochemical, or growth parameters of a cell. Transformation may or may not result in cells able to produce tumors in experimental animals, which is properly known as neoplastic transformation; therefore, transformed cells do not automatically result in the development of “cancer.” Carcinogenesis (or more properly, oncogenesis) is a complex, multistep process in which cellular transformation may be only the first, although essential, step along the way.

Normal cells, i.e. cultures established from healthy tissue and then passaged, senesce and die after a fixed number of divisions (about 50). No matter how carefully they are

Cell transformation is a single-hit process; that is, a single virus transforms a single cell (c.f. oncogenesis, which is the formation of tumors and a multi-step process). All or part of the virus genome persists in the transformed cell and is usually (but not always) integrated into the host-cell chromatin.

Transformation is mediated by proteins encoded by oncogenes: Cell-transforming viruses may have RNA or DNA genomes, but all have at least a DNA stage in their replication cycle; that is, the only RNA viruses directly capable of cell transformation are the retroviruses. Certain

looked after, something within the cells limits their lifespan. Immortalized cells, by contrast, are able to be passaged indefinitely in cell culture, allowing them to form permanent cell lines.

Table: Some altered properties of transformed cells

Some altered properties of transformed cells

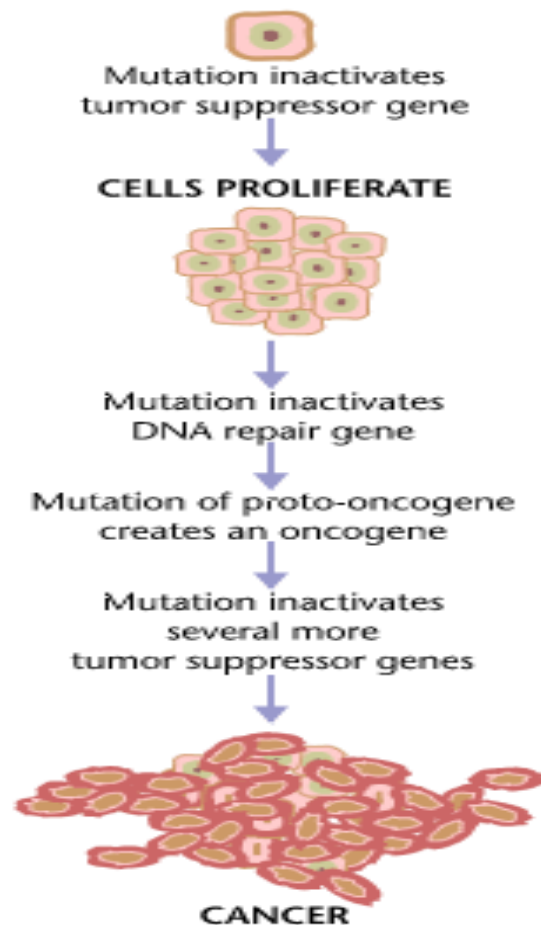
- Multiply for ever (immortalization).
- Grow to higher saturation density.
- Have reduced requirement for serum growth factors.
- Grow in suspension in soft agar.
- Form different cell colony patterns.
- Grow on top of normal cell monolayers.
- Readily agglutinated by lectins.

retroviruses carry homologues of c-oncs derived originally from the cellular genes and known as v-oncs. In contrast, the oncogenes of cell-transforming DNA viruses are unique to the virus genome—there are no homologous sequences present in normal cells.

Genes involved in the formation of tumors can be grouped by their biochemical functions:

- **Oncogenes and proto-oncogenes:** Oncogenes are mutated forms of proto-oncogenes, cellular genes whose normal function is to promote the normal growth and division of cells.
- **Tumor suppressor genes:** These genes normally function to inhibit the cell cycle and cell division.
- **DNA repair genes:** These genes ensure that each strand of genetic information is accurately copied during cell division of the cell cycle.

Mutations in these genes lead to an increase in the frequency of other mutations. The function of oncogene products depends on their cellular location. Several classes of oncogenes are associated with the process of signal transduction—the transfer of information derived from the binding of extracellular ligands to cellular receptors to the nucleus.



TRANSFORMATION OF NORMAL CELL TO CANCEROUS CELL

- **Oncogene** – a gene that when mutated or expressed at abnormally high levels contributes to converting a normal cell into a cancer cell
- **Proto-oncogene** – the “normal” cellular progenitors of oncogenes that function to promote the normal growth and division of cells
- **Proto-oncogene to oncogene:** An alteration occurs in a normal cellular gene (proto-oncogene) that makes the protein hyper-functional (oncogene)

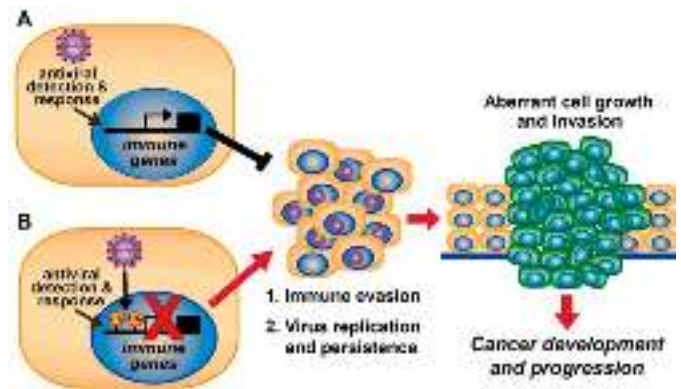
Proteins involved in the cell signaling pathways are products of proto-oncogenes

- Proliferative
- Anti-apoptotic (survival)
- Angiogenic

- Tumor suppressors are recessive – require mutation of both alleles
- Oncogenes are dominant – mutation of 1 allele is sufficient

LECTURE 184: DNA TUMOR VIRUSES

Several families of DNA viruses are capable of transforming cells. They are mostly nuclear proteins involved in the control of DNA replication which directly affect the cell cycle. They achieve their effects by interacting with cellular proteins which normally appear to have a negative regulatory role in cell proliferation.



Types of virus that (very rarely) cause transformation of the infected cell.

Family	Genome	Proportion of genome integrated
Retrovirus	RNA	Whole
Polyomavirus	DNA	Part
Papillomavirus	DNA	Part
Adenovirus	DNA	Part
Herpesvirus	DNA	None; episomal
Hepadnavirus	DNA	Part

Hepatitis B Viruses: The hepatitis B viruses, which have the smallest genomes (approximately 3 kb) of all animal DNA viruses, specifically infect liver cells of several species, including ducks, woodchucks, squirrels, and humans. Infection with hepatitis B virus usually results in acute liver damage. In 5 to 10% of cases, however, the acute infection is not resolved and a chronic infection of the liver develops. Such chronic infection is associated with more than a hundredfold increased risk of liver cancer. Hepatitis B virus infection is particularly common in parts of Asia and Africa, where it is associated with up to a million cases of liver cancer annually.

Polyomavirus: The best studied DNA tumor viruses, from the standpoint of molecular biology, are probably simian virus 40 (SV40) and polyomavirus. Although neither of these viruses is associated with human cancer, they have been critically important as models for understanding the molecular basis of cell transformation.

Papillomaviruses: The papillomaviruses are small DNA viruses (genomes of approximately 8 kb) that induce both benign and malignant tumors in humans and a variety of other animal species. Approximately 60 different types of human papillomaviruses, which infect epithelial cells of several tissues, have been identified. Some of these viruses cause only benign tumors

(such as warts), whereas others are causative agents of malignant carcinomas, particularly cervical and other anogenital cancers.

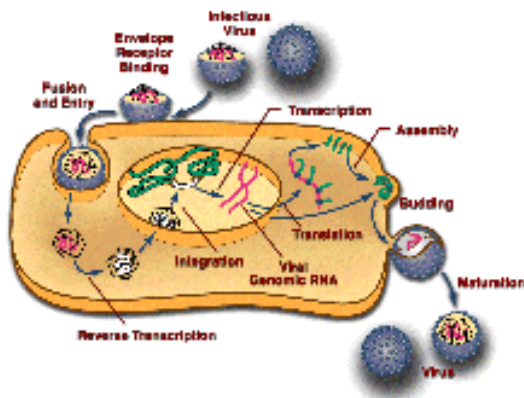
Adenoviruses: The **adenoviruses** are a large family of DNA viruses with genomes of about 35 kb. In contrast to the papillomaviruses, the adenoviruses are not associated with naturally occurring cancers in either humans or other animals.

Herpesviruses: The herpesviruses are among the most complex animal viruses, with genomes of 100 to 200 kb. Several herpesviruses induce tumors in animal species, including frogs, chickens, and monkeys. In addition, two members of the herpesvirus family, Kaposi's sarcoma-associated herpesvirus and Epstein-Barr virus, are associated with human cancers.

LECTURE 185: RNA TUMOR VIRUSES

Oncovirinae:

- First discovered Rous- Sarcoma virus, showing slow neoplasms in chicken.
- Human T-cell lymphotropic virus

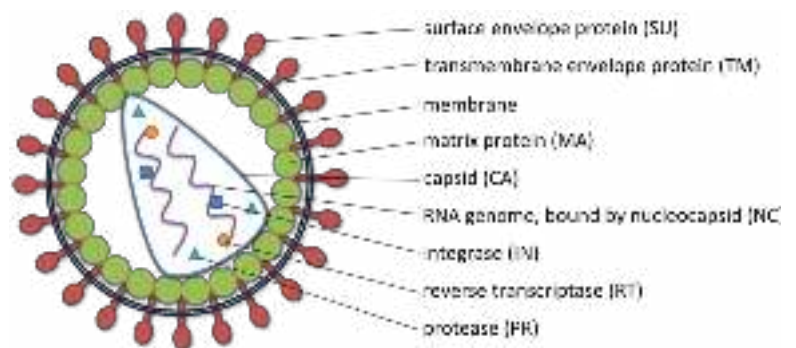


Lentivirinae:

- Long latent period of infections.
- Disease of ungulates.
- HIV that causes AIDS

Spumavirus:

- Foamy appearance of cells.
- Syncytia formation of giant multinucleate cells.
- Human foamy virus.
- Simian foamy virus



Retroviruses: Members of one family of RNA viruses, the retroviruses, cause cancer in a variety of animal species, including humans. One human retrovirus, human T-cell lymphotropic virus type I (HTLV-I), is the causative agent of adult T-cell leukemia, which is common in parts of Japan, the Caribbean, and Africa. Transformation of T lymphocytes by HTLV-I results from expression of the viral gene *tax*, which encodes a regulatory protein affecting expression of several cellular growth control genes.

Different retroviruses differ substantially in their oncogenic potential. Most retroviruses contain only three genes (*gag*, *pol*, and *env*) that are required for virus replication but play no role in cell transformation (Figure). Retroviruses of this type induce tumors only rarely, if at all, as a consequence of mutations resulting from the integration of proviral DNA within or adjacent to cellular genes.

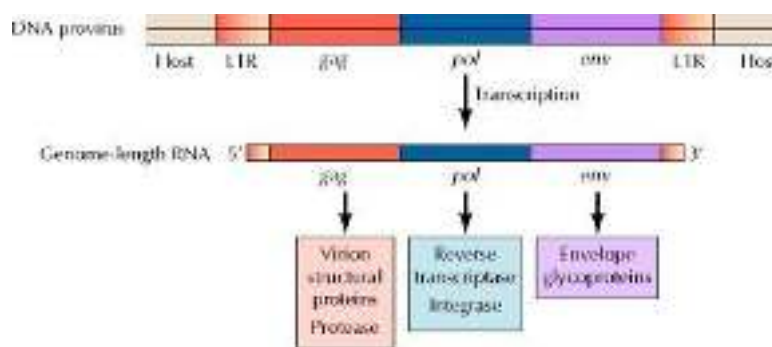
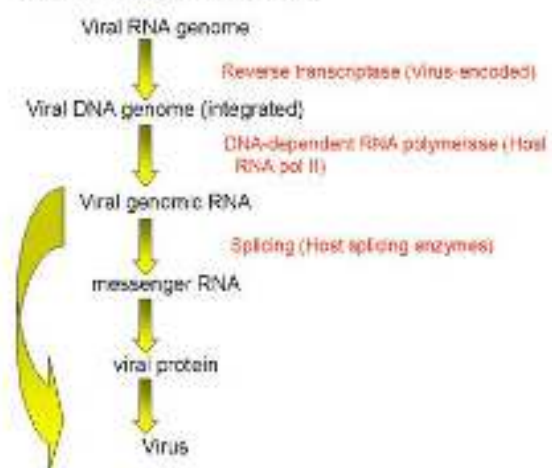


Figure: A typical retrovirus genome

The DNA provirus, integrated into cellular DNA, is transcribed to yield genome-length RNA. This primary transcript serves as the genomic RNA for progeny virus particles, and as mRNA for the *gag* and *pol* genes. In addition, the full-length RNA is spliced to yield mRNA for *env*. The *gag* gene encodes the viral protease and structural proteins of the virus particle, *pol* encodes reverse transcriptase and integrase, and *env* encodes envelope glycoproteins.

Other retroviruses, however, contain specific genes responsible for induction of cell transformation and are potent carcinogens. The prototype of these highly oncogenic retroviruses is Rous sarcoma virus (RSV), first isolated from a chicken sarcoma by Peyton Rous in 1911. More than 50 years later, studies of RSV led to identification of the first viral oncogene, which has provided a model for understanding many aspects of tumor development at the molecular level.

RNA Tumor Viruses



LECTURE 186: PERSISTENT VIRAL INFECTION

Characterized as those in which the virus is not cleared but remains in specific cells of infected individuals. Involve stages of both silent and productive infection without rapidly killing or even producing excessive damage of the host cells.

There are three types of overlapping persistent virus-host interaction

- latent,
- chronic
- slow infection

PATHOGENESIS:

- modulation of virus
- cellular gene expression
- modification of the host immune response.
- Host immunosuppression _____ associated with reactivation of a number of persistent virus infections.

BODILY SITES OF PERSISTENCE:

- Nervous system
- Glands and body surfaces
- Germ cells
- Blood

IMPORTANCE:

- Reactivation
- Tumor causing
- Can cause progressive disease
- Persistence enables the parasite to be successful

LECTURE 187:

LECTURE 188: Plant Viruses and Viroids

Plant viruses are viruses that affect plants. Like all other viruses, plant viruses are obligate intracellular parasites. They do not have the molecular machinery to replicate without a host. Plant viruses can be pathogenic to higher plants.



Pepper mild mottle virus and leaf curl virus

General characteristics

- Most plant viruses are rod-shaped, with protein discs forming a tube surrounding the viral genome
- Isometric particles are another common structure
- They rarely have an envelope

- The great majority have an RNA genome, which is usually small and single stranded (ss), but some viruses have double-stranded (ds) RNA, ssDNA or dsDNA genomes
- Plant viruses are not as well understood as their animal counterparts
- One plant virus has become iconic: tobacco mosaic virus (TMV), the first virus to be discovered
- This and other viruses cause an estimated U.S.\$60 billion loss in crop yields worldwide each year
- Plant viruses are grouped into 73 genera and 49 families
- However, these figures relate only to cultivated plants, which represent only a tiny fraction of the total number of plant species.

TRANSMISSION

To transmit from one plant to another and from one plant cell to another, plant viruses must use strategies that are usually different from animal viruses. Plants do not move, and so plant-to-plant transmission usually involves vectors (such as insects). Plant cells are surrounded by solid cell walls, therefore transport through plasmodesmata. Plants have specialized mechanisms for transporting mRNAs through plasmodesmata.

DEFENSE SYSTEM

Plant defenses against viral infection include, among other measures, the use of siRNA in response to dsRNA. Most plant viruses encode a protein to suppress this response. Plants also reduce transport through plasmodesmata in response to injury.

LECTURE 189: VIRUS INFECTIONS OF PLANTS

Life on Earth depends on the primary productivity of plants—the production of organic molecules from inorganic molecules such as CO₂ (with some an additional contribution from some bacteria). All higher animals depend on the primary productivity of plants for their food.

Plant viruses come in two basic shapes: helical and polyhedral. Helical virus particles have an elongated shape and can be rigid rods or flexible filaments. Polyhedral virus particles are roughly spherical. The genetic material of a plant virus may be enclosed in one particle or split between two or more particles. Here are some examples of plant viruses and their characteristics:

Tobacco mosaic virus (TMV) infects the leaves of tobacco, tomato, bean, and pepper plants and is an example of a helical, rigid rod virus; each particle is about 300 nanometers (nm) in length and 18 nm in diameter.

STRUCTURE OF PLANT VIRUSES

- These are extremely small and can only be observed under an electron microscope.
- The structure of a virus is given by its coat of proteins, which surround the viral genome.
- Assembly of viral particles takes place spontaneously.
- Over 50% of known plant viruses are rod-shaped (flexuous or rigid).

- The length of the particle is normally dependent on the genome but it is usually between 300–500 nm with a diameter of 15–20 nm.
- Protein subunits can be placed around the circumference of a circle to form a disc.
- In the presence of the viral genome, the discs are stacked, then a tube is created with room for the nucleic acid genome in the middle
- The second most common structure amongst plant viruses are isometric particles.
- They are 25–50 nm in diameter.
- In cases when there is only a single coat protein, the basic structure consists of 60 T subunits, where T is an integer.
- Some viruses may have 2 coat proteins that associate to form an icosahedral shaped particle.
- There are three genera of Geminiviridae that consist of particles that are like two isometric particles stuck together.
- A very small number of plant viruses have, in addition to their coat proteins, a lipid envelope.
- This is derived from the plant cell membrane as the virus particle buds off from the cell

LECTURE 190: INFECTION IN PLANTS-II/TRANSMISSION

The mechanism by which plant viruses are transmitted between hosts is therefore of great importance. There are a number of routes by which plant viruses may be transmitted:

Seeds: These may transmit virus infection either by external contamination of the seed with virus particles or by infection of the living tissues of the embryo. Transmission by this route leads to early outbreaks of disease in new crops which are usually initially focal in distribution but may subsequently be transmitted to the remainder of the crop by other mechanisms.

- Plant virus transmission from generation to generation occurs in about 20% of plant viruses.
- When viruses are transmitted by seeds, the seed is infected in the generative cells and the virus is maintained in the germ cells and sometimes, but less often, in the seed coat.
- There does not seem to be a correlation between the location of the seed on the plant and its chances of being infected.
- Examples; families Leguminosae, Solanaceae, Compositae, Rosaceae, Cucurbitaceae, Gramineae.
- Bean common mosaic virus is transmitted through seeds.

Vegetative propagation/grafting: These techniques are inexpensive and easy methods of plant propagation but provide the ideal opportunity for viruses to spread to new plants.

Through sap:

- Viruses can be spread by direct transfer of sap by contact of a wounded plant with a healthy one.
- During agricultural practices, as by damage caused by tools or hands, or naturally, as by an animal feeding.
- Examples, TMV, potato viruses and cucumber mosaic viruses

Plasmodiophorids

- A number of virus genera are transmitted, both persistently and non-persistently, by soil borne zoosporic protozoa.
- These protozoa are not phytopathogenic themselves, but parasitic.
- Transmission of the virus takes place when they become associated with the plant roots.
- Examples; *Polymyxa graminis* and *Polymyxa betae*.

Vectors: Many different groups of living organisms can act as vectors and spread viruses from one plant to another:

Bacteria: (e.g., *Agrobacterium tumefaciens*—the Ti plasmid of this organism has been used experimentally to transmit virus genomes between plants)

Nematodes:

- Soil-borne nematodes also have been shown to transmit viruses.
- They acquire and transmit them by feeding on infected roots.
- Viruses can be transmitted both non-persistently and persistently, but there is no evidence of viruses being able to replicate in nematodes.
- Examples; tobacco ringspot virus and tobacco rattle virus.

Arthropods: insects (e.g., aphids, leafhoppers, planthoppers, beetles, thrips)

Arachnids (e.g., mites) involves plant viruses, either as experimental tools or in terms of finding ways to prevent infection. And as this section describes, the biology of plant viruses has some striking differences from that of animal viruses. So if you think botany is boring, you probably need to find out more about plant viruses.

- Plant viruses need to be transmitted by a vector, most often insects such as leafhoppers.
- One class of viruses, the Rhabdoviridae, has been proposed to actually be insect viruses that have evolved to replicate in plants

Direct plant-to-human transmission

- The Pepper Mild Mottle Virus (PMMoV) may have moved on to infect humans.
- This is a very rare and highly unlikely event
- One possibility is that the virus does not infect human cells directly.
- Instead, the naked viral RNA may alter the function of the cells through a mechanism similar to RNA interference, in which the presence of certain RNA sequences can turn genes on and off

Mechanical: Mechanical transmission of viruses is the most widely used method for experimental infection of plants and is usually achieved by rubbing virus-containing preparations into the leaves, which in most plant species are particularly susceptible to infection. However, this is also an important natural method of transmission. Virus particles may contaminate soil for

long periods and be transmitted to the leaves of new host plants as wind-blown dust or as rain-splashed mud.

LECTURE 191: Macroscopic Signs of Plant Viral Diseases

- Important for virus identification
- Used to name the disease
- Solely cannot be used to characterize the virus as affected by many factors

Typically, virus infections of plants might result in effects such as growth retardation, distortion, mosaic patterning on the leaves, yellowing, wilting, etc.

These macroscopic symptoms result from:

- Necrosis of cells, caused by direct damage due to virus replication,
- Hypoplasia—localized retarded growth frequently leading to mosaicism (the appearance of thinner, yellow areas on the leaves)
- Hyperplasia—excessive cell division or the growth of abnormally large cells, resulting in the production of swollen or distorted areas of the plant.

Systemic lesions

- Appear in different patterns depending upon virus-host combinations
- Appear in sequential patterns and
- May comprise of different symptoms

Symptoms of a viral infection can appear in the plant's leaves, flowers, and/or fruit. These symptoms are very diverse and depend on the virus, the infected plant, and the environmental conditions (e.g., drought, humidity, and temperature). The most common leaf symptoms include mosaic patterns, streaking, yellowing, curling, vein banding, and lesions. The flowers of an infected plant can show changes in color and shape, and the fruit can show discoloration, deformation, ringspots, and mosaic patterns. Examples of symptoms of virus infections on plant leaves, flowers, and fruit are shown in Figure.



Figure: Symptoms of viral infection on plant leaves, flowers, and fruit.

Symptoms

1. Mosaic
 2. Mottle
 3. Yellowing
 4. Chlorosis
 5. Vein clearing
 6. Vein banding
 7. Leaf roll
 8. Leaf curl
 9. Streak
 10. Blistering
- Green vein banding
 - Yellow vein banding

LECTURE 192: SYNTHESIS OF PATHOGEN RELATED PROTEINS

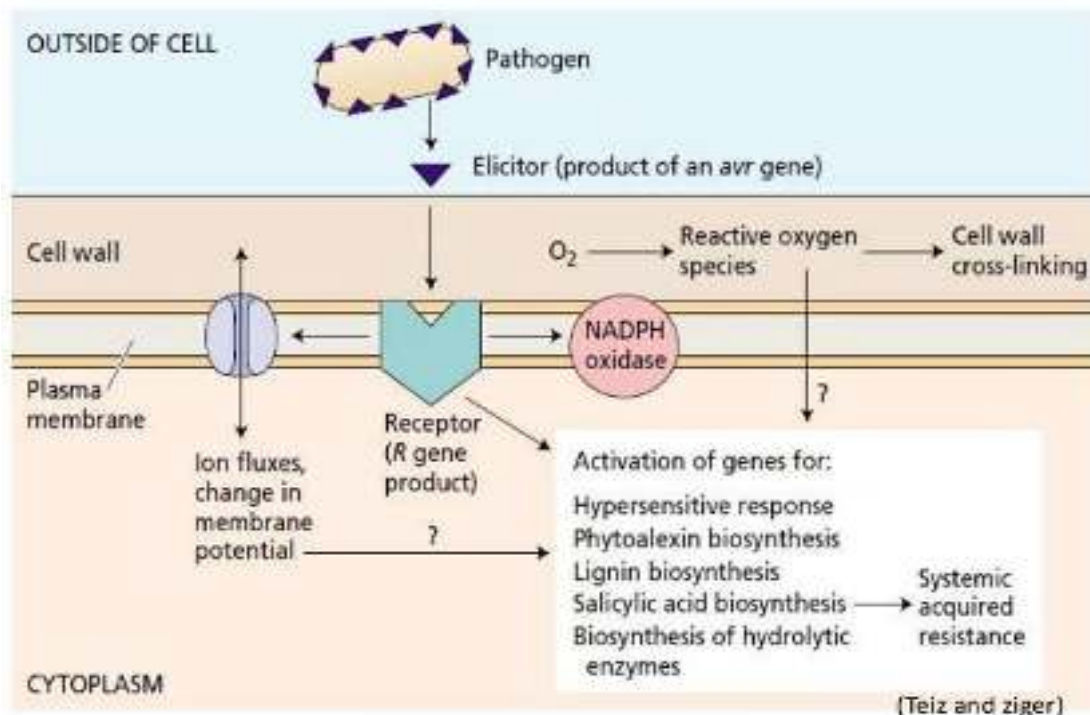
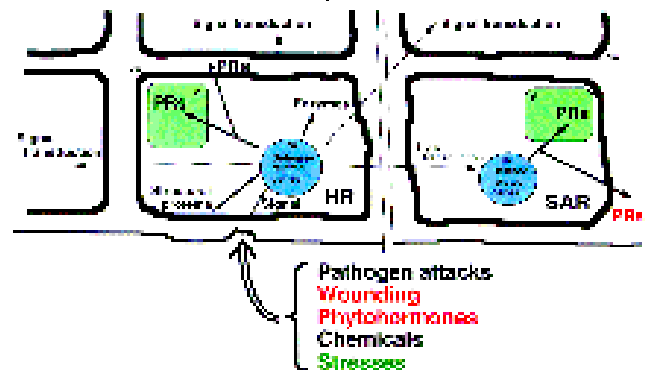
Pathogen Related (PR) Proteins

- Produced as a result of pathogen attack
- Infection activate genes that produce PR proteins

NATURE

- Some are antimicrobial
- Some function as signal to spread news
- Salicylic acid induces production of proteins

Plant Defense Responses



FUNCTIONS

- Antiviral action
- As well as antifungal and antibacterial
- Signals the message to nearby cells and tissues
- Stimulate cross-linkage of molecules
- Peroxidase
- Ribonuclease
- Lysozyme activity
- Hydrolytic and proteinase-inhibitory
- Inactivate viral proteins

LECTURE 193: HOST DEFENSES AND VIRAL COUNTER MEASURES

The responses of host plants to viruses are determined by the interactions of viral proteins and nucleic acids with host factors as well as host surveillance systems that protect plant cells against virus infection. Resistance to viruses can be determined by specific recognition that is mediated by dominant resistance (R) genes, by recessive resistance genes that include defective host factors, or by RNA silencing.

Dominant Resistance Genes

Dominant, monogenic virus resistance traits mediate defense against specific strains of a virus species, an entire virus species, or several virus species within a genus. The *N* gene of tobacco is an example of an *R* gene with broad specificity, because it confers resistance to all tobamoviruses except the Ob strain of TMV.

R gene-mediated resistance is frequently accompanied by (a) programmed cell death, (b) induction of reactive oxygen species, (c) induction of defense-related proteins, (d) accumulation of the phytohormone salicylic acid, (e) accumulation of defense-related secondary metabolites, and (f) fortification of cell walls. These concomitant events are known collectively as the hypersensitive response (HR) that is accompanied by the appearance of macroscopic necrotic lesions (local lesions) at the site of inoculation.

PTGS—An Inducible, Adaptable Innate Immune Response

One of the major functions of the RNA silencing system in plants and other eukaryotes is to protect cells against foreign, nucleic acid-based invaders including RNA viruses, DNA viruses, and viroids. The importance of RNA silencing in antiviral defense is punctuated by the fact that most, if not all, plant viruses encode suppressors of RNA silencing (VSRs) that function by a variety of different mechanisms.

Unlike the genetic resistance described above, antiviral RNA silencing is adaptive because RNA silencing is initiated by dsRNA, irrespective of origin and sequence.

VIRUS RESISTANT PLANTS

Plant viruses



[Tulip breaking virus (TBV)]
(https://en.wikipedia.org/wiki/Tulip_breaking_virus)



[Tobacco mosaic virus (TMV)
symptoms on tobacco leaf]

Modes of Transmission of plant viruses

To transmit from one plant to another plant and from one plant cell to another, plant viruses must use strategies that are usually different from animal viruses. Plant do not move so plant plants transmission usually involves vectors (such as insect).

Modes of Transmission of plant viruses

- Insect – this forms the largest and most significant vector group and particularly include; Aphids, Whiteflies, Hoppers, Beetles etc.
- Through sap : Eg TMV, Potato virus
- Nematodes: Eg Tobacco ring spot virus
- Through seed

Mechanism of Plant resistance to viruses

There are approximately 450 species of plant-pathogenic viruses, which cause a range of diseases. The plant show two types of antiviral defense resistance mechanism are

- 1) R gene mediated
- 2) RNA Silencing

LECTURE 194: PATHOGENESIS OF PLANT VIRAL DISEASES

Initially, most plant viruses multiply at the site of infection, giving rise to localized symptoms such as necrotic spots on the leaves. The virus may subsequently be distributed to all parts of the plant either by direct cell-to-cell spread or by the vascular system, resulting in a systemic infection involving the whole plant. However, the problem these viruses face in reinfection and recruitment of new cells is the same as the one they faced initially—how to cross the barrier of the plant cell wall. Plant cell walls necessarily contain channels called “plasmodesmata” which allow plant cells to communicate with each other and to pass metabolites between them. However, these channels are too small to allow the passage of virus particles or genomic nucleic acids. Many (if not most) plant viruses have evolved specialized movement proteins that modify the plasmodesmata. One of the best known examples of this is the 30-k protein of tobacco

mosaic virus (TMV). This protein is expressed from a subgenomic mRNA, and its function is to modify plasmodesmata causing genomic RNA coated with 30-k protein to be transported from the infected cell to neighboring cells. Other viruses, such as cowpea mosaic virus (CPMV; Comoviridae) have a similar strategy but employ a different molecular mechanism. In CPMV, the 58-/48-k proteins form tubular structures allowing the passage of intact virus particles to pass from one cell to another

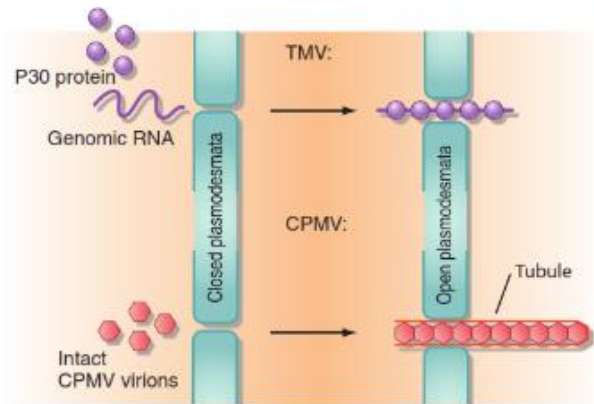


FIGURE 6.1 Plant movement proteins.

Plant movement proteins allow plant viruses to infect new cells without having to penetrate the cell wall

ROUTES

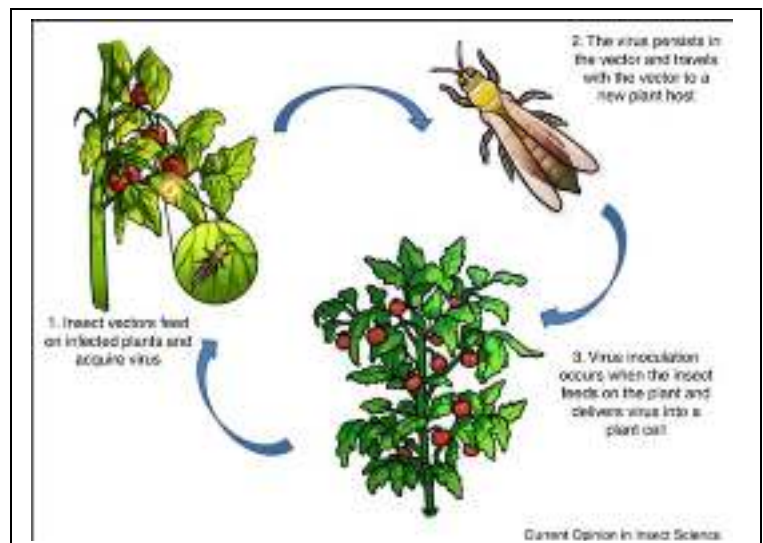
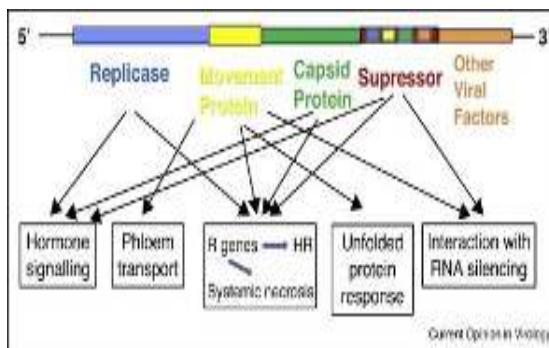
- Seeds
- Grafting/ Vegetative propagation
- Vectors
- Mechanical
- No specific receptors on cells
- Rely on mechanical breach for virus to enter through cell wall
- Usually with the help of insects

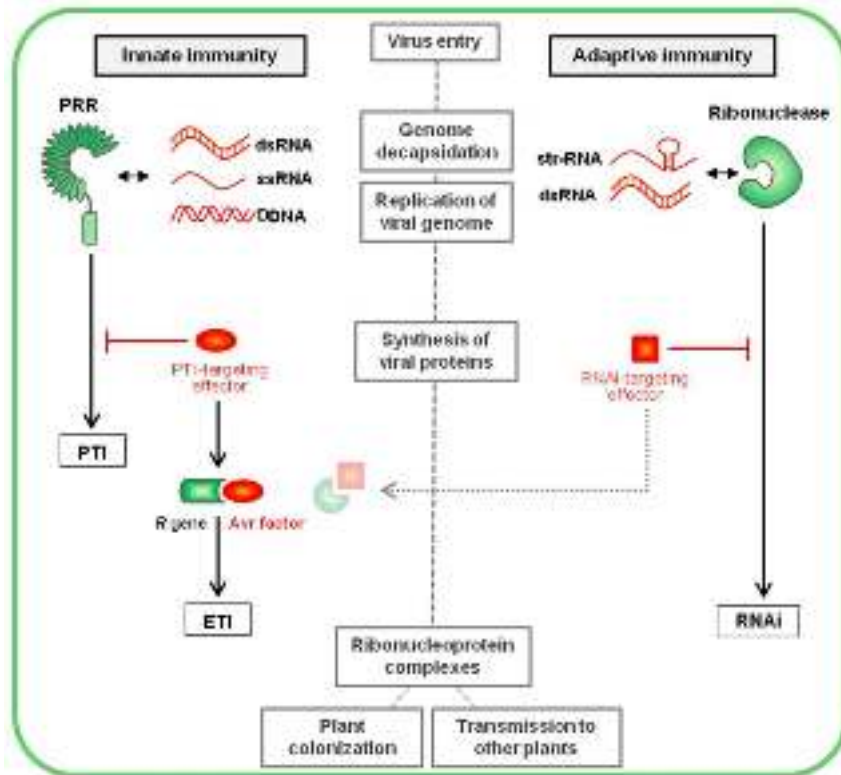
PROPOGATIVE TRANSMISSION

- Multiply in tissues of insects as well as host
- Most multiplication at site of infection
- Localized symptoms

SPREAD

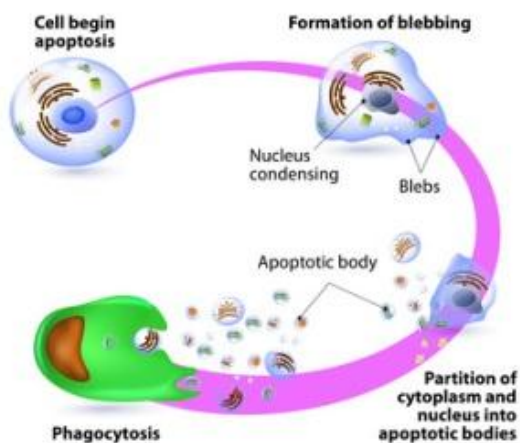
- Direct cell to cell
- Vascular system



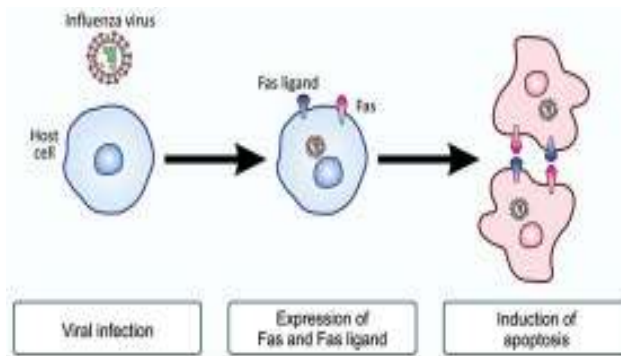


LECTURE 195: VIRUSES AND APOPTOSIS

APOPTOSIS: Apoptosis is a critical mechanism in tissue remodeling during development and in cell killing by the immune system. Apoptosis is a tightly regulated process that relies on complex molecular cascades for its control. It is marked by cell shrinkage, condensation, and clumping of chromatin, a regular pattern of DNA fragmentation, and “blebbing off” of cellular contents into small membrane-bounded vesicles (“blebbing”) which are subsequently phagocytosed by macrophages, preventing inflammation.



- Programmed cell death
- During cell development and cell killing by immune system
- Tightly regulated process
- Relies on complex molecule cascades for its control
- Shrinkage, condensation of chromatin, DNA fragmentation and blebbing



When triggered by the appropriate signals, immune effector cells such as CTLs and NK cells release previously manufactured lytic granules stored in their cytoplasm.

- Immune effector cells like CTL's and NK cells release lytic granules
- Act on target cells by two mechanisms

These act on the target cell and induce apoptosis by **two mechanisms**:

1. Release of cytotoxins such as: (1) perforin (aka cytolysin), a peptide related to complement component C9 which, on release, polymerizes to form polyperforin, which forms transmembrane channels, resulting in permeability of the target cell membrane; and (2) granzymes, which are serine proteases related to trypsin. These two effectors act collaboratively, the membrane pores allowing the entry of granzymes into the target cell. The membrane channels also allow the release of intracellular calcium from the target cell, which also acts to trigger apoptotic pathways.
2. In addition, CTLs (but not NK cells) express Fas ligand on their surface which binds to Fas on the surface of the target cell, triggering apoptosis. Binding of Fas ligand on the effector cell to Fas (CD95) on the target cell results in activation of cellular proteases known as "caspases," which in turn trigger a cascade of events leading to apoptosis.

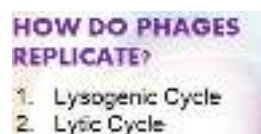
MECHANISM 1

- Release of cytokinins such as, perforin: peptide related to C9, forms a polyperforin and affects permeability of cell membrane
- Granzyme: serine proteases, affect cell pores

MECHANISM 2

- CTLs express a ligand, that binds on surface on target cell
- Triggers apoptosis
- Triggers cascade to initiate apoptosis

LECTURE 196:-



What is Lytic Cycle?

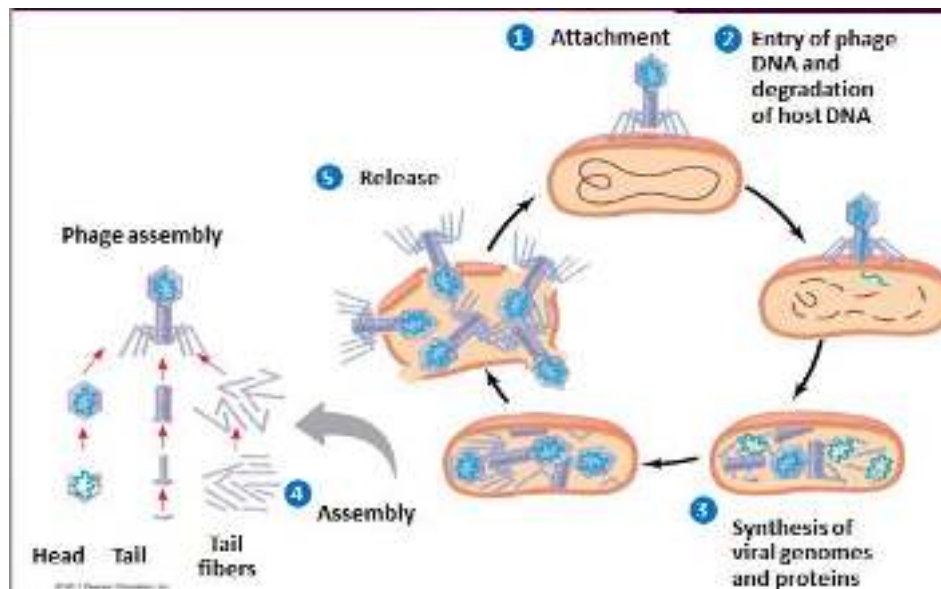
The definition states that virulent phages are those that can multiply only on bacterial cells. At the end of their life cycle, they cause cell lysis, which kills the host bacterium. The viruses that infect bacteria are called bacteriophages. The most common examples of a bacteriophage are T4 and lambda phage. Both these phages can infect the bacterium *Escherichia coli* (*E. Coli*). Phages are obligatory intracellular parasites like other viruses and need a host cell to reproduce. The T4 phage replicates by the lytic cycle, which eventually causes the host cell's death. The lambda phage multiplies using lysogenic cycle, which does not cause the host cell to die. There are five stages of lytic cycle, and they are explained in the following paragraphs using T4 phage as an example.

PROCESS: The bacteriophage reproduces using lytic cycle as its method of reproduction. The five steps of its reproduction are attachment, penetration, biosynthesis, assembly, and release. Let us learn about these steps in detail.

1. **Attachment:** The T4 phage has a complex structure with several tail fibers. These fibers help in the attachment of virus to the cell wall of *E. coli* at the complementary receptor cells. Once attached, weak chemical bonds are formed between the receptor site and the attachment, which helps the virus adhere to the host cell.
2. **Penetration:** Once the T4 phage is attached to the bacterial cell, it injects its double-stranded DNA (or single stranded RNA, depending on the type of phage) into the *E. coli* cell. The T4 phage releases enzymes that weaken the cell wall of the bacterium. This helps in injection of the genetic material of the virus by pressing its sheath against the cell. The empty capsid or virus body remains in the bacterial cell. Some types of phages enter the host cell intact and dissolve their capsid inside the host. This process is known as uncoating.
3. **Biosynthesis:** The host's protein synthesis stops when the host DNA is degraded by the infecting virus. The viral DNA begins to interfere with the transcription and translation of host DNA. The T4 phage uses the host nucleotides to replicate its own DNA. It also uses the host ribosomes, enzymes, and amino acids to synthesize its own enzymes and proteins. During the biosynthesis, there are not complete phages inside the host cell. Thus, this stage is known as the eclipse period.
4. **Assembly:** There are many viral components made in the host cell. These are then assembled into complete viruses. The proteins coded for phage DNA in case of T4 phage act as enzymes,

which are used for construction of new phages. The host's metabolism is used for assembly of the phages, which results in the bacterial cell being filled with new viruses. Slowly, the capsids are assembled and the DNA is packaged within the head. Finally, the tail fibers are attached to the complex viral structure.

5. **Release:** The viruses are released from the host cell after assembly of the new viral particle. The phage produces an enzyme that breaks down the bacterial cell wall from within and allows entry of fluid. The cell becomes full of new phage cells (about 100-200) and fluid that cause cell lysis, thus completing the cycle. The phages released are free to infect more host cells and continue with the process.



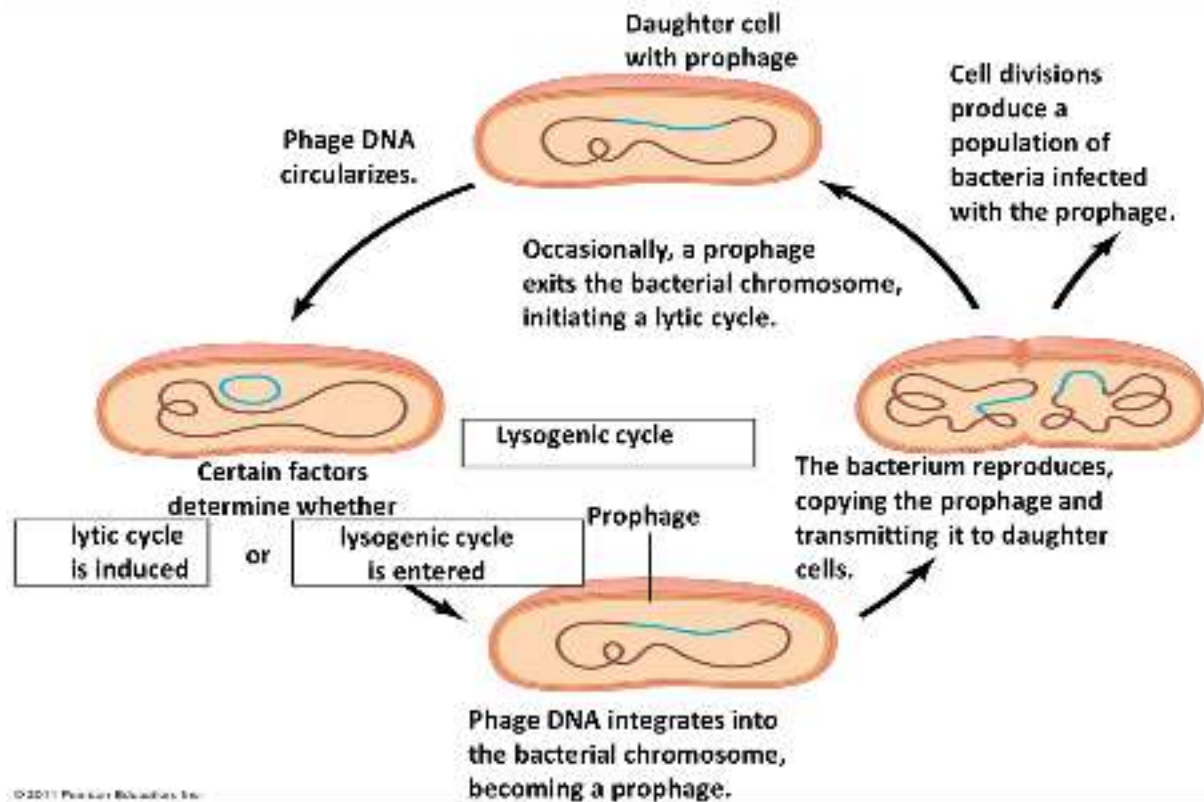
LECTURE 197: LYSOGENIC CYCLE

A lysogen or lysogenic bacterium is a bacterial cell in which a phage exists as DNA in its dormant state (prophage)

STEPS

1. Viral genome enter cells
2. Viral Genome integrates into host cell genome
3. Host DNA polymerase copies viral chromosomes
4. Host cell divides and viral chromosomes divide in to daughter cells

- When virus is triggered, viral genome detaches and enters lytic cell



LECTURE 198: Comparison of Lytic and Lysogenic

• Lytic cycle (virulent phage):

Release of virus bursts and kills host cells (lysis)

Lysogenic cycle (temperate phage)

- Viral DNA integrates into host genome (provirus)
- Can be transmitted to daughter cells.
- Can initiate lytic cycle in response to environmental signals (stress)

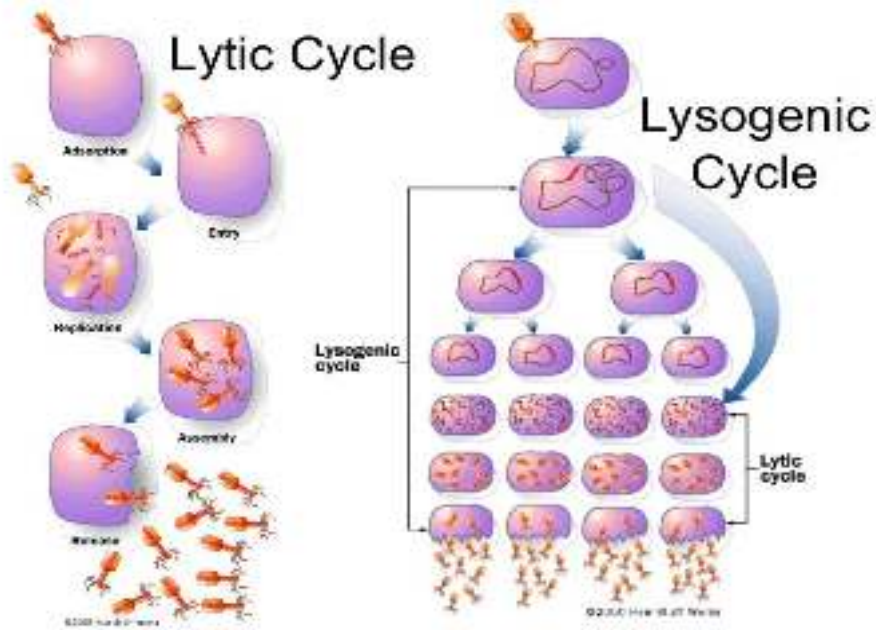
Advantages and Limits

Lytic cycle

- Replication of new viruses is fast
- However, the host is also immediately killed preventing the viral genome from passing onto the next generation of host cells

Lysogenic cycle

- Many more viruses can be made because the viral genome is passed onto future generation of host cells
- However, replication is takes longer because it is dependent on the host cell's replication



LECTURE 199: Interferons (IFNs)

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites, or tumor cells. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to “interfere” with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages, they increase recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes, and they increase the ability of uninfected host cells to resist new infection by virus.

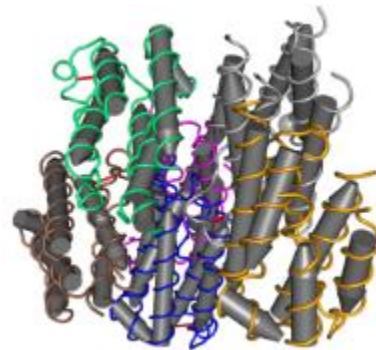


Figure: Interferon: The molecular structure of human interferon-alpha

About ten distinct IFNs have been identified in mammals; seven of these have been described for humans. They are typically divided among three IFN classes: type I IFN, type II IFN, and type III IFN. IFNs belonging to all IFN classes are very important for fighting viral infections. Based on the type of receptor through which they signal, human interferons have been classified into three major types:

Interferon type I: All type I IFNs bind to a specific cell surface receptor complex, known as the IFN- α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains. The type I interferons present in humans are IFN- α , IFN- β and IFN- ω .

Interferon type II: These bind to IFNGR that consist of IFNGR1 and IFNGR2 chains. In humans this is IFN- γ .

Interferon type III: These signal through a receptor complex consisting of IL10R2 and IFNLR1. Acceptance of this classification is less universal than that of type I and type II.

IFNs activate immune cells (natural killer cells and macrophages), increase recognition of infection and tumor cells by up-regulating antigen presentation to T lymphocytes, and increase the ability of uninfected host cells to resist new infection by virus.

USE OF INTERFERON

Interferons have been shown in vitro and in vivo to possess antiviral, antitumour and immunoregulatory properties. Some of the the therapeutic use of interferons are as below

- (a) viral infections, including condylomata acuminata, viral hepatitis and human immunodeficiency virus infection;
- (b) neoplastic disorders, including hairy cell leukaemia, multiple myeloma,
- (c) myeloproliferative disorders, including chronic myelogenous leukaemia and polycythaemia vera;
- (d) rheumatoid disorders, including rheumatoid arthritis and systemic sclerosis;
- (e) other disorders, including multiple sclerosis, chronic granulomatous disease and cryoglobulinaemia.

LECTURE 200: EVASION OF IMMUNE RESPONSE BY VIRUSES

When a virus infects a host, a complex immune response develops to eliminate the invading pathogen. Viruses, in turn, have evolved a profusion of strategies to escape from the immune system. Mechanisms of viral evasion occur at

- cellular level
- systemic level.

Viruses can avoid detection by both innate and adaptive immune responses. Targets of viral evasion proteins

- Pattern recognition receptors in infected cells
- Interferons

- dendritic cells
- T cell receptors
- Antibodies

Viruses can express proteins that directly interfere with host processes or mimic host proteins and compete to bind specific receptors. Pathogens are able to directly deplete immune cells, and prevent their recruitment to the site of infection. In a state of latency, viruses remain dormant and undetectable in host cells.

LECTURE 201: Prevention of Viral Infection

Viruses are a leading cause of disease and death worldwide. Although public health measures and vaccines are the most effective ways to control many viral infections, preventive measures have not succeeded for numerous viral diseases. For some of these diseases, antiviral drugs have been developed. A number of these drugs have been highly successful, saving lives and relieving suffering. The key to the success of any antiviral drug lies in its specificity. Almost any stage of virus replication can be a target for a drug, but the drug must be more toxic to the virus than the host. The first highly successful antiviral drug was acyclovir (ACV), initially developed in the late 1970s against herpes simplex virus (HSV).

- Viruses cause a variety of diseases in animals, including humans, ranging from the common cold to potentially fatal illnesses like meningitis
- Vaccines may be prepared using live viruses, killed viruses, or molecular subunits of the virus.
- The killed viral vaccines and subunit viruses are both incapable of causing disease.
- Live viral vaccines are designed in the laboratory to cause few symptoms in recipients while giving them protective immunity against future infections.
- Polio vaccines.

Antiviral drugs

Have limited success in curing viral disease, but in many cases, they have been used to control and reduce symptoms for a wide variety of viral diseases. For most viruses, these drugs can inhibit the virus by blocking the actions of one or more of its proteins.

- Acyclovir-for genital herpes
- Tamiflu (oseltamivir) –for influenza

LECTURE 202: Viral Vaccines

DEFINITION: A vaccine is a biological preparation that provides active acquired immunity to a particular disease.

- A vaccination is the injection of a killed or weakened organism that produces immunity in the body against organism.

- An immunization is the process by which a person or animal becomes protected from a disease.

There are two aspects of the response to the threat of virus diseases: first, prevention of infection, and second, treatment of the disease. The former strategy relies on two approaches: public and personal hygiene, which perhaps plays the major role in preventing virus infection (e.g., provision of clean drinking water and disposal of sewage; good medical practice such as the sterilization of surgical instruments) and vaccination, which makes use of the immune system to combat virus infections. Most of the damage to cells during virus infections occurs very early, often before the clinical symptoms of disease appear. This makes the treatment of virus infection very difficult; therefore, in addition to being less expensive, prevention of virus infection is undoubtedly better than cure.

History:

1796: Edward Jenner prevented smallpox by vaccination with cowpox

1885 Louis Pasteur successfully prevented rabies in Joseph Meister by post-exposure vaccination.

Vaccination is the most effective way of preventing viral diseases. The concept of vaccination is considered to have been widely introduced by Edward Jenner in 1798 to protect humans against smallpox. Nearly a century later, the concept was shown by Louis Pasteur to have wider applications and, most notably, could be used to prevent rabies.

Why Vaccines?

Vaccines are safe.

Vaccines are effective.

Vaccines work with immune system.

Vaccines protect individuals and the community.

LECTURE 203: Viral Vaccines Types

There are following four basic approaches to produce vaccines. Each uses components of the pathogenic virus that the vaccine is intended to target.

- Live-attenuated vaccines
- Inactivated vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines

1. Live Attenuated Virus Vaccines

Replication-competent, attenuated vaccines are effective for at least two reasons. Progeny virus particles are generally restricted to tissues around the site of inoculation, and this focal restriction generally results in mild or inapparent disease. However, the limited virus reproduction stimulates a potent and lasting immune response. Attenuated (less virulent) viruses are selected by growth in cells other than those of the normal host or by propagation at non-physiological temperatures.

- Live vaccines use a weakened (or attenuated) form of the germ that causes a disease.
- Because these vaccines are so similar to the natural infection that they help prevent, they create a strong and long-lasting immune response.

Live vaccines are used to protect against:

- Measles, Mumps, Rubella (MMR combined vaccine)
- Rotavirus
- Smallpox
- Chickenpox
- Yellow fever

2. Inactivated vaccines

The methods are designed **Inactivated or “Killed” Virus Vaccines**. The inactivated poliovirus, influenza virus, hepatitis A virus, and rabies virus vaccines are examples of effective inactivated vaccines administered to humans. To prepare such a vaccine, virulent virus particles are isolated and inactivated by chemical or physical procedures. These treatments eliminate the infectivity of the virus, but not its antigenicity (i.e., the ability to induce the desired immune response). These vaccines are safe for immune-deficient individuals, as the treated viruses cannot reproduce. Immunization by inactivated vaccines, however, often requires the administration of multiple doses, as the first dose is generally insufficient to produce a protective response.

Inactivated vaccines use the killed version of the germ that causes a disease. Inactivated vaccines usually don't provide immunity (protection) that's as strong as live vaccines.

Inactivated vaccines are used to protect against:

- Hepatitis A
- Flu (shot only)
- Polio (shot only)
- Rabies

3. Subunit Vaccines

A vaccine may consist of only a subset of viral proteins, as demonstrated by the highly successful hepatitis B vaccine. Vaccines formulated with purified components of viruses, rather than the intact particles, are called subunit vaccines. Even fragments of viral proteins may be sufficient to Recombinant DNA Approaches to Subunit Vaccines. **DNA vaccines**, a variant of

the subunit protein approach to immunization, consist simply of plasmids encoding viral genes that can be expressed in cells of the animal to be immunized.

- Use specific pieces of the virus — like its protein, sugar, or capsid
- They give a very strong immune response that's targeted to key parts of the virus.
- One limitation- need booster shots

- Hepatitis B
- HPV (Human Papillomavirus)
- Whooping cough (part of the DTaP combined vaccine)
- Pneumococcal disease
- Meningococcal disease

4. Toxoid vaccines

- Use a toxin (harmful product) made by the virus that causes a disease.
- They create immunity to the parts of the virus that cause a disease instead of the virus itself. That means the immune response is targeted to the toxin instead of the whole virus.
- You may need booster shots
- Toxoid vaccines are used to protect against:
- Diphtheria
- Tetanus

Use a toxin (harmful product) made by the virus that causes a disease. They create immunity to the parts of the virus that cause a disease instead of the virus itself. That means the immune response is targeted to the toxin instead of the whole virus. You may need booster shots

- Toxoid vaccines are used to protect against:
- Diphtheria
- Tetanus

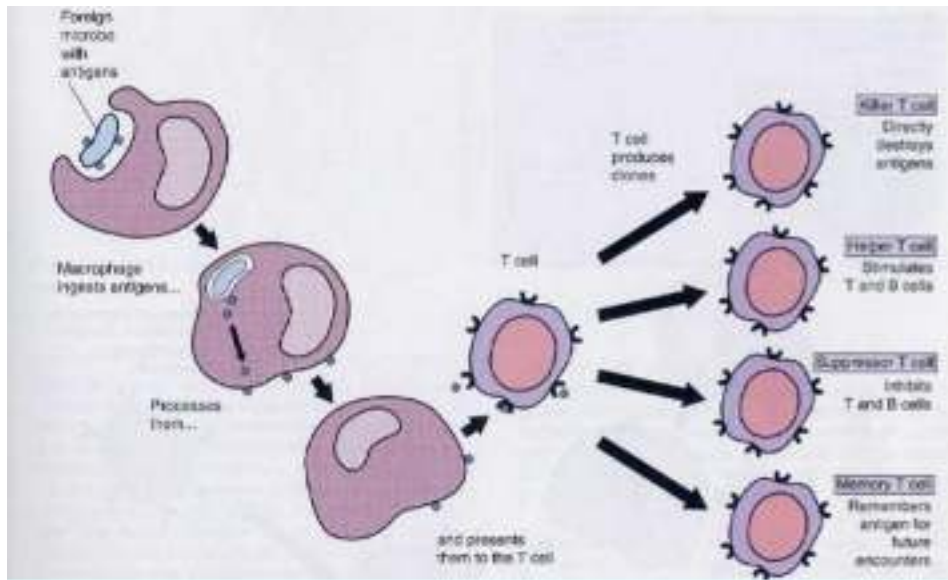
When scientists create vaccines, they consider:

- How your immune system responds to the germ
- Who needs to be vaccinated against the germ
- The best technology or approach to create the vaccine

LECTURE 204: VIRAL VACCINE MECHANISM

Vaccines are made from same organism or parts of them that causes disease.

- Introduced in body.
- Immune system reacts to the vaccine-make antibodies.
- Antibodies destroy vaccine germs
- Stay in body, giving specific immunity.



LECTURE 205: Viral vectors

Several different viruses are being tested as potential vectors. After initial optimism, gene therapy involving virus vectors has fallen from favor, and nonvirus methods of gene delivery including liposome/DNA complexes, peptide/DNA complexes, and direct injection of recombinant DNA are also under active investigation. It is important to note that such experiments are aimed at supplementing defective cellular genes in the somatic cells of patients to alleviate the symptoms of the disease and not at manipulating the human germ line, which is a different issue.

SV 40 Based Vectors

- First animal virus based vector
- Small virus with capsid and circular dsDNA
- Bidirectional replication

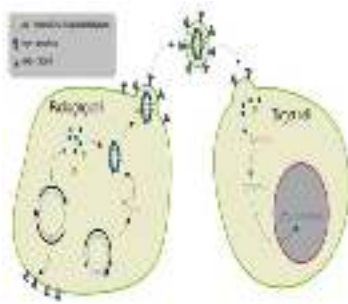
Lytic Infection of Sv40

Three steps:

- Early mRNA synthesis
- Viral DNA replication
- Late mRNA synthesis

Vaccinia Virus Vector

- Large ds DNA
- Contains enzymes for replication
- Generated in vivo



Epstein Barr Virus

- Herpes virus with large genome
- Infects primates and canines
- Used for construction of episomal cDNA libraries

LECTURE 206: Gene therapy

Viruses have also developed as gene delivery systems for the treatment of inherited and acquired diseases. Gene therapy offers:

- Delivery of large biomolecules to cells,
- The possibility of targeting delivery to a specific cell type,
- High potency of action due to replication of the vector,
- Potential to treat certain diseases (such as head and neck cancers and brain tumors) that respond poorly to other therapies or may be inoperable.

Viral-vector gene therapy strategies offer a novel and especially attractive approach to the correction of specific genetic disorders, particularly those with a defined missing or dysfunctional gene. Correction of such disorders requires the long-term expression of the specific protein that is absent or dysfunctional; thus viruses with the capability of safely and stably inserting the target gene into the genome of the affected individual are a logical choice as vectors for this purpose.

- Experimental technique that uses genes to treat or prevent disease.
- Therapeutic delivery of nucleic acid into a patient's cells as a drug to treat disease
- First attempt at modifying human DNA was performed in 1980 by Martin Cline.

Vectors

- Viral
- Non viral
 - electroporation,
 - the gene gun,
 - sonoporation,
 - magnetofection,
 - oligonucleotides, lipoplexes, dendrimers, and inorganic nanoparticles

Hurdles:

- Short-lived nature
- Immune response
- Problems with viral vectors
- Multigene disorders
- Insertional mutagenesis
- Cost effective

LECTURE 207: CHEMOTHERAPY AGAINST VIRAL DISEASES

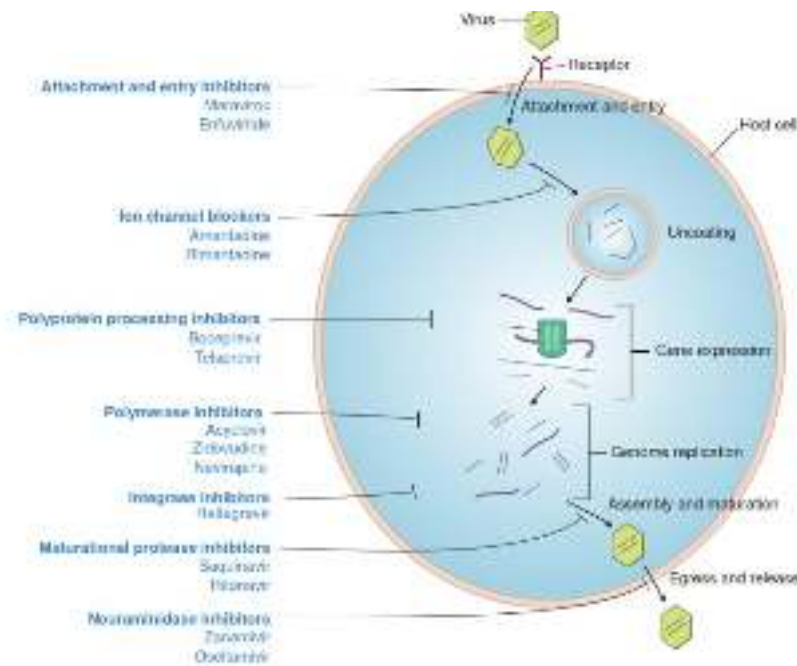


FIGURE. Antiviral drugs block various stages of viral replication. A generic replication cycle of viruses in cells is cartooned, showing the stages of infection, which different drug classes block. These include attachment and entry, uncoating, gene expression, genome replication, assembly and maturation, and egress and release.

CHEMOTHERAPY AGAINST VIRAL DISEASES

IDEAL DRUG

- Water soluble
- Chemically and metabolically stable
- Easily absorbed (apolar)

NOT

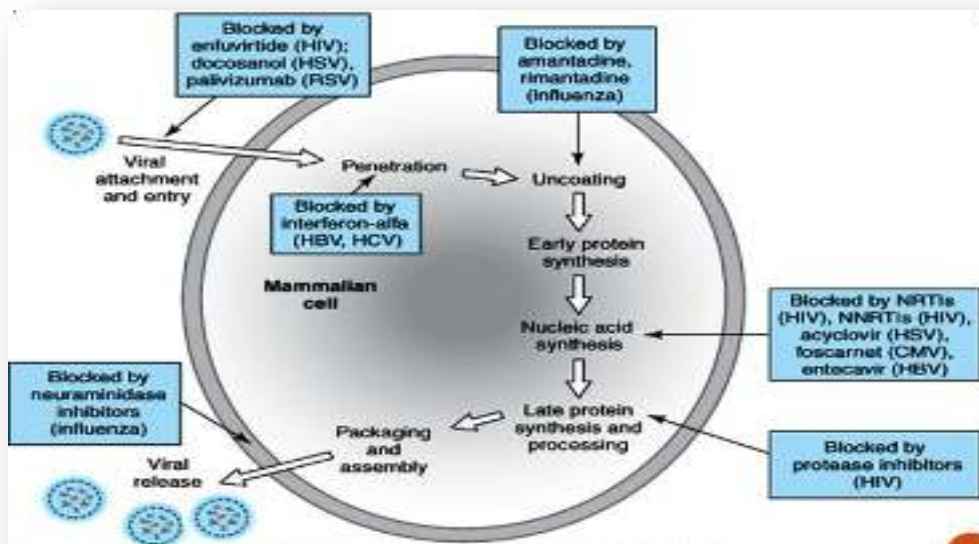
- Toxic
- Carcinogenic
- Allergenic
- Mutagenic
- Teratogenic

A successful drug must interfere with:

- A specific viral function e.g. enzyme necessary for viral life cycle Anti-Viral Chemotherapy
- A cellular function that the virus needs in order to replicate

If interfere with cellular function either:

- It must be crucial to virus but not the cell
- Only the virus-infected cell must be killed.



APPROVED ANTIVIRAL DRUGS

- Amantadine and rimantadine (influenza A virus)
- Ribavirin (respiratory syncytial virus)
- Idoxuridine and trifluridine (topical treatment of herpetic keratitis)
- Vidarabine and acyclovir (systemic treatment of herpes simplex virus and varicella-zoster virus infections)
- Famciclovir and valaciclovir (oral treatment of varicella-zoster virus infections)
- Ganciclovir and foscarnet (cytomegalovirus)
- Zidovudine, didanosine, zalcitabine and stavudine (human immunodeficiency virus)

LIMITATIONS OF ANTIVIRAL DRUGS

- Narrow antiviral spectrum
- Ineffectiveness against the latent virus
- Development of drug-resistant mutants
- Toxic side effects

LECTURE 208: VIRAL TITRATION

One of the most important procedures in virology is measuring the **virus titer**, the concentration of a virus in a sample. This parameter is determined by inoculating serial dilutions of virus into host cell cultures, chicken embryos, or laboratory animals and monitoring for evidence of virus multiplication. Serial dilutions of a virus stock are inoculated into replicate test units, which can be cell cultures, eggs, or animals. The number of test units that have become infected is then determined for each virus dilution. When cell culture is used, infection may be determined by the development of cytopathic effect; in eggs or animals, infection is gauged by death or disease.

When the assay is used to assess the virulence of a virus or its capacity to cause disease the result of the assay can be expressed in terms of 50% lethal dose (LD 50) per milliliter or 50% paralytic dose (PD 50) per milliliter, end points of death and paralysis, respectively.

- To understand the concentration of virus
- Infectivity titer
- Reed and Muench method or Karber formula

STANDARD

- LD50/mL
- Based on mortality of lab mice
- Infectivity titer
- Computation

TITER

- Frequency of production of specific response of host
- Necessary for viral reproduction
- Smallest amount of virus capable of producing manifestation in host

REED AND MUENCH METHOD

Table 1 Calculation of virus titre in mice using the Reed and Muench method

Log ₁₀ virus dilution	Mice		Cumulative total			Percent mortality
	Died	Survived	Died	Survived	Total	
0	10	0	10	0	10	$10/10 \times 100 = 100$
1	10	0	10	0	10	$10/10 \times 100 = 100$
2	10	0	10	0	10	$10/10 \times 100 = 100$
4	10	0	10	0	10	$10/10 \times 100 = 100$
5	10	0	10	0	10	$10/10 \times 100 = 100$
6	6	4	6	4	10	$6/10 \times 100 = 60$
7	1	9	1	9	10	$1/10 \times 100 = 10$

Difference of logarithms: $(6.5-5)/(6.5-7) = 0.25$ (approx 50% end point dilution) $-5 + (0.25 \times 1) = -5.25$, 50% end point dilution $= 10^{5.25}$, the titre of the virus $= 10^{5.25}$ I.U./ml.

KARBER METHOD

Midlength (mm)	Log Midlength (x)	Number of fish (n)	Mature (r)	Proportion of mature fish (p)	X_u $X_{u+0.5}$	$q_i=1-p_i$	$(p \times q)_i$ $(n-1)$
85	1.9264	4	0	0	0.0488	1	0
95	1.9777	142	0	0	0.0436	1	0
105	2.0212	155	0	0	0.0386	1	0
115	2.0657	64	4	0.0625	0.0332	0.9375	0.0009
125	2.0969	51	25	0.4902	0.0334	0.5098	0.0050
135	2.1303	59	24	0.4068	0.0310	0.5932	0.0042
145	2.1614	29	11	0.3793	0.0290	0.6207	0.0084
155	2.1903	40	23	0.5750	0.0272	0.4250	0.0083
165	2.2175	27	15	0.5556	0.0258	0.4444	0.0095
175	2.2430	13	11	0.8462	0.0241	0.1538	0.0108
185	2.2672	4	2	0.5000	0.0228	0.5000	0.0633
195	2.2900	7	6	0.8571	0.0217	0.1429	0.0204
205	2.3118	4	4	1		0	0
Total		588	125	0.6728			0.1488

$$m = 2.3118 - [0.0002/281 - (0.0002 \times 5.6725)] = 2.1244$$

LECTURE 209:

LECTURE 210:

LECTURE 211: FACTORS AFFECTING TITRATION PROCEDURES

INCUBATION TIME

- Precise period
- Most viruses take precise time to reproduce
- Time between inoculum and appearance of symptom

TYPE OF VIRUS

- Plant virus or animal virus
- Conditions provided
- Optimum media

PROCEDURE CONTAMINATION

- Bacterial contamination
- Fungal contamination
- Careless lab work
- Improper cleanliness and sanitation
- Disruption of viral genome

LECTURE 212: BACTERIOPHAGE TITRATION

DEFINITION: Determination of the number of phage particles in a stock.

- Simplest type of titration
- Several dilution preparation
- Plating one or more measured samples of each dilution

PROCEDURE:

- Phage and bacteria introduced on agar surface
- Incubation
- Interruption of continuous bacterial layer by lysis of phage

Phage titration (determination of the number of phage particles in a stock) is an important molecular biology technique. When genetic libraries in phage vectors are screened for positive clones, the plates that are being screened should have approximately 50-500 phage plaques per plate for optimal results. Less plaque will mean that too many plates will have to be screened,

while more plaques will make it difficult to identify individual positive plaques. Plates in the ideal range are produced by preparing sequential dilutions of phage stocks.

CHARGING:

Bacteriophage particles will sometimes adhere to plastic or glass. If particles adhere to pipettor tips during transfers, it may lower the amount of phage particles present in the sample transferred to the next dilution blank. One way to minimize this problem is to “charge” the pipettor by pipetting a sample of the solution up and down in the tip, and discharging the sample back into the original tube.

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LECTURE 213: PLAQUE COUNT IN BACTERIOPHAGE TITRATION

When a suspension of an infective phage (e.g. T4 phage) is spread over the lawn of susceptible bacterial cells (e.g. *Escherichia coli*), the phage attaches the bacterial cell, replicate inside it, and kills it during its lytic release. Lysis of the bacteriophage is indicated by the formation of a zone of clearing or plaque within the lawn of bacteria. In the absence of lytic phage, the bacteria form a confluent lawn of growth. Each plaque corresponds to the site where a single bacteriophage acted as an infectious unit and initiated its lytic cycle. The number of plaques that develop and the appropriate dilution factors can be used to calculate the number of bacteriophages i.e. plaque-forming units (PFU) in a sample.

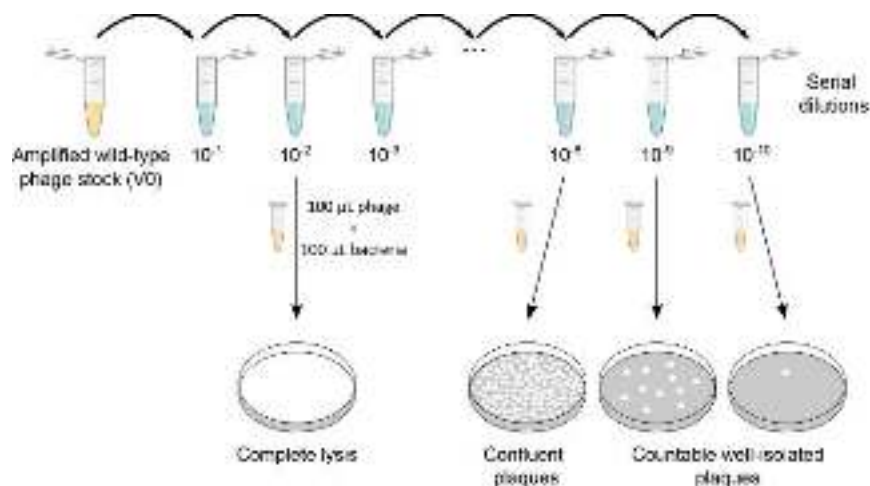


Figure: Phage plaque assay for titer determination

Upper panel: Preparation of ten-fold serial dilutions of phage stock is followed by bacterial culture infection. The corresponding mixtures are plated according to the double agar method and, after incubation, the number of well-isolated plaques allows for pfu/mL determinations.

Lower panel: Representative examples of T7 plaque formation after inoculating the original host at different phage dilutions.

PLAQUE VALUES

- Plaque counts analogous to colony counts for bacteria
- Values directly proportional to amount of phage plated
- Plate cultures prepared by combining phage with host cells in top agar
- Top agar preparations contain lower conc. of agar (7 g/L) than normal solutions used to prepare agar plates (15 g/L)
- Low agar conc. allows progeny phage from lysed cells to diffuse through the media and infect neighboring cells
- After lysis, plaque (zone of lysed cells) is produced on the plate
- The size of the plaques depend on how soon the bacteria in the agar reach stationary phase and stop reproducing
- Plaques will stop spreading at this point

