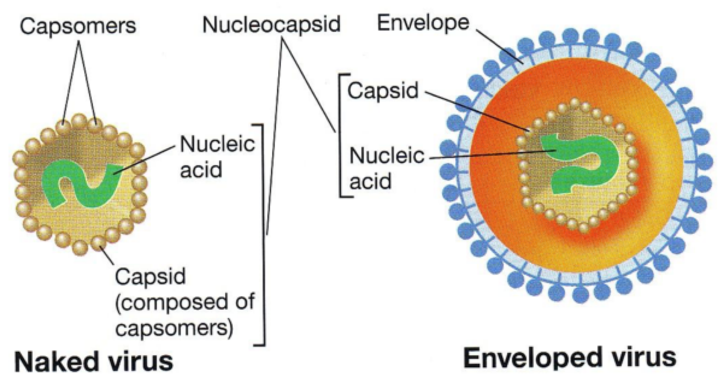
BIOL 409/609

Virology

Third Attempt!

Viral Structure

- **Capsid** - proteinacous structure that surrounds viral genome
- Made up of smaller monomeric subunits - **CAPSOMERES**
- Shape of capsid determined by capsomeres, capsomeres determined by viral genome
- **Nucleocapsid** consists of virus genome surrounded by capsid
- Enveloped viruses have a lipid bilayer surrounding capsid - **envelope**
- Envelope derived from host cell membranes



Defining Characteristics of Viruses

- Several unifying themes for all viruses
  - Infectious, obligate intracellular parasite
  - Genetic material is DNA or RNA, not both
  - Viral genome replicated in host cell, redirects host cell machinery to produce structural viral components (proteins, nucleic acids, sometimes lipids)
  - Progeny virions assembled de novo from individual components within host cell
  - Assembly of progeny virion within host cell facilitates transmission, disassembly following infection marks beginning of next infectious cycle

Cellular Resources Used by Viruses

- **Viruses require cellular gene products and organic molecules**
  - **Organic molecules include nucleotides, amino acids, carbohydrates, and lipids**
  - **Host cell provides energy required for synthesis of progeny virion (ATP)**
  - **Many viruses rely on host DNA, RNA, and protein synthesis machinery**
  - **Trace elements essential for progeny replication concentrated in subcellular compartments within host cell**
- **Viruses lack the coding capacity for production of enzymes that are readily available in the host cell**

Impact of Viruses on Humans

- Disease most obvious (small pox, hepatitis, HIV, SARS, influenza, West Nile)
- Extremely diverse species, infect all life forms (bacteria, algae, plants, other animals)
- Cassava mosaic virus – African famine of 1920
- Photosynthetic algae produce 40% of atmospheric oxygen, 20% of algal death due to virus infection
- Foot-and-mouth disease devastates livestock herds in England today
- Distemper and feline lukemia virus impact domesticated pets

Impact of Viruses on Humans

- Harmful impact receives most publicity
- Viruses also contribute to advances in science and medicine
- First bacteriophages prompted interest in development of living antibiotics
- Analysis of eukaryotic DNA viruses allowed discovery of eukaryotic RNA polymerase
- Replication of DNA viruses allowed characterization of eukaryotic DNA polymerase
- mRNA splicing characterized on viral introns
- Isolation of oncogenes from viruses lead to understanding of control of cell cycle

Historical Landmarks in Virology

- **Unseen harmful agents transmitted in many ways – air, water, food, direct contact – known for millennia**

- **Causative agent often referred to as virus – Latin for poison**

- **No distinction between disease caused by cellular microorganism versus true virus**

- **Distinction became possible due to landmark developments and discoveries by pioneering microbiologists**

A Historical Perspective

- 1796 - First successful vaccination - Edward Jenner
    - Smallpox epidemics still prevalent
    - Cowpox caused similar disease in cattle - mild disease in humans
    - Jenner found infecting humans with cowpox protected against smallpox infection
    - Due to antigenic similarity between two viruses
    - Understood that immunity conferred, lacked understanding of nature of infectious agent

A Historical Prospective

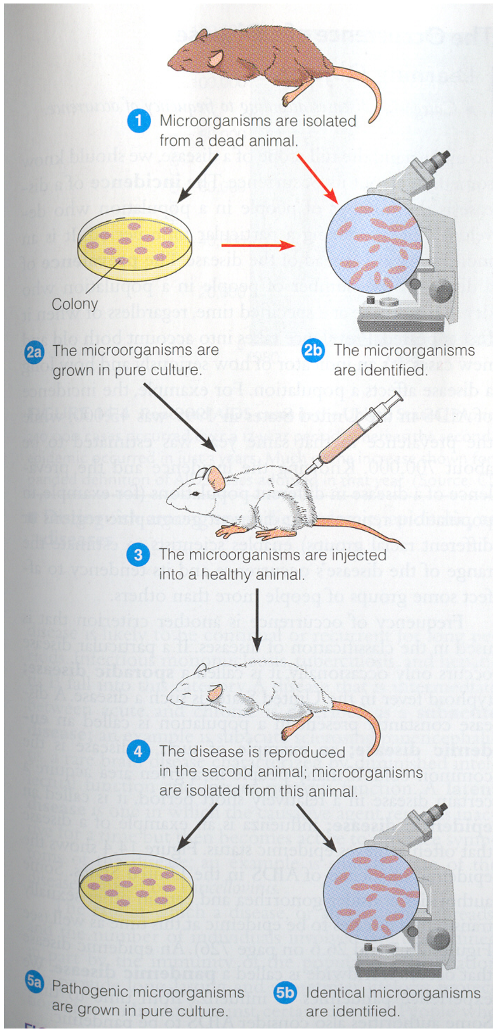
- 1864 - Fermentation and Pasteurization - Louis Pasteur
  - Discovered source of fermentation
  - Yeast convert sugar to alcohol
  - Bacteria cause spoiling - convert alcohol to vinegar
  - Found heating perishables prevented spoiling - kills bacteria (Pasteurization)

A Historical Perspective

- Discovery of microorganisms allowed development of the Germ Theory of Disease
- 1876 - The Germ Theory of Disease proved correct - Robert Koch
    - Anthrax decimating cattle industry
    - Isolated rod shaped bacteria from infected animal
    - Caused disease when introduced to healthy animals
    - Could isolate the same bacteria from experimental animals
    - Procedure now referred to as Koch’s postulates

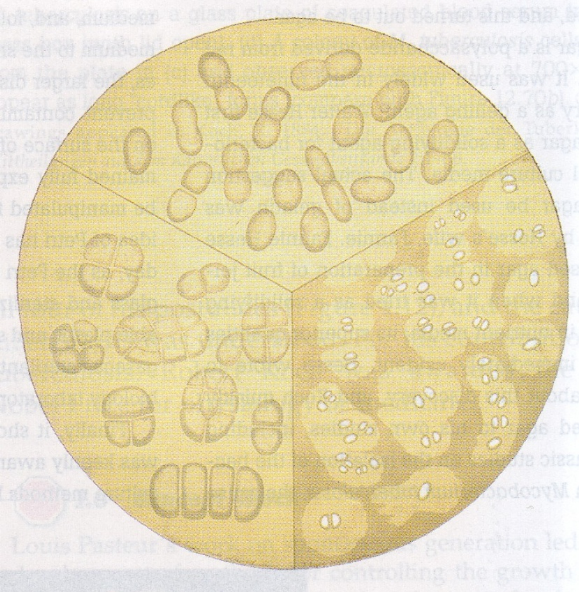
A Historical Perspective

- Isolate organism from animal
- Culture *in vitro*
- Infect healthy animal
- Determine if disease is reproduced
- Isolate organism from infected animal
- Determine if it is the same organism



Discovery of Viruses

- Martinus Beijerinck – 1851 – 1931, developed enrichment cultivation
- Allowed for isolation of pure cultures from environmental samples
- First to identify viruses - TMV
- Demonstrated ability to pass through filters
- Demonstrated necessity of integrating into cell
- First true virus discovered



Start of a New Controversy

- Cultivation of TMV led to discovery
- Purified viruses could be crystalized
- Attribute previously attributed to only non-viable compounds
- Called into question whether or not viruses constitute a life form
- Many scientists believed they were complex catalytic compounds
- Further characterization revealed chemical composition
- Led to realization that they were comprised of nucleic acid and protein
- Ultimately used to identify true source of genetic material

Initiation of the Great Debate

* DNA as genetic material - was not always accepted
    * Only 4 bases
    * Seemed too simplistic
* Proteins the initial favorite
    * 20 amino acids
    * More than enough to account for genetic variability
* Required several decades of research to prove DNA was genetic material

Developing the System to Resolve the Debate

- Max Delbruk, Emory Ellis, and Salvador Luria intensively studied bacterial viruses (phage) in the 1930’s and 1940’s

- Developed cultivation techniques, demonstrated phenomenon of heritable traits

- Contain only DNA and protein, created system to conclusively determine which was the bearer of genetic information

- Techniques used by Alfred Hershey and Martha Chase to provide compelling evidence to end the controversy

- Involved radio -labeling each component and determining which was passed on from parent to progeny

Resolution of the Debate

* Bacteriophage - only 2 components, DNA and protein

* Labeled phage with 35S - only labels protein

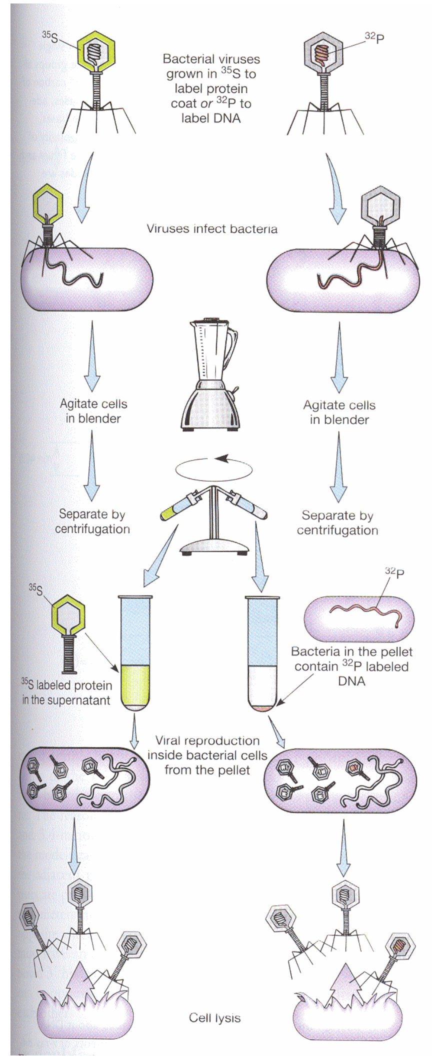
* Infected bacteria - sheared off phage - pelleted

* Pellet was cold - phage produced were cold

* Repeated experiment with 32P - labels only DNA

*Pellet was HOT - so were some phage produced

* Proved DNA was genetic material



Impact of Virology on Scientific Community

- Cultivation techniques for eukaryotic viruses led to rapid advances in molecular and cellular biology

- Led to observation that viral infection could facilitate transformation of eukaryotic cells in tissue culture

- Further investigation revealed mechanism to be inappropriate expression of normal cellular genes

- Genes referred to as oncogenes

- Two well characterized mechanisms lead to inappropriate expression

- Acquisition of normal gene by virus through copy choice recombination or integration of viral genome

Impact of Virology on Scientific Community

- First viral genomes inducing transformation via integration comprised of RNA
- Established that a dsDNA copy of the genome integrates into the host chromosome
- Investigation of the mechanism leading to conversion of the RNA viral genome to DNA led to discovery of reverse transcriptase
- Discovery revolutionized molecular biology
- Allowed for creation of cDNA libraries
- Facilitated expression of eukaryotic genes in prokaryotic systems

Cultivation of Viruses

- Obligate intracellular parasites, requires appropriate host cell for growth
- Straightforward for bacteriophage
- More problematic for animal and plant viruses
- Lack of development of sterile cultivation conditions added to complications during early studies
- Fungal and bacterial contamination commonly over took cell cultures
- Initial studies conducted in whole animals where possible
- Development of aseptic technique now allows common use of cell culture

Cultivation of Viruses

- **Laboratory animals historically used for virus cultivation**

- **Artificial means of transmission selected for mutants that were no longer infectious by normal route**

- **Cultivation of polio in chimpanzees resulted in variant that was no longer infectious via natural oral administration**

- **Cell culture not used where possible, not all viruses produce infectious virions in culture (hepatitis, Norwalk virus)**

- **Animal models still commonly used to investigate viral pathogenesis**

- **Instrumental in development of several vaccines (hepatitis, polio)**

Growth of Bacteriophages

- Can be propagated in liquid culture - will result in clearing

- Can be propagated on solid media - results in plaque formation

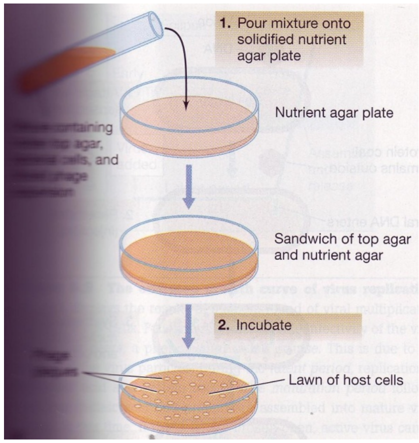
- Mix bacteria with phage in melted agar, allow infection to occur

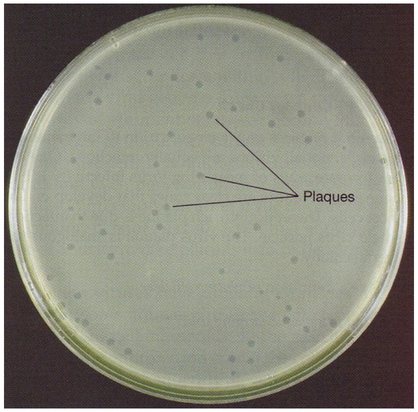
- Pour agar onto solidified agar plate, allow poured layer to solidify

- Virus will replicate and lyse bacteria

- Results in generation of “holes” in otherwise confluent monolayer of bacteria – plaques

- Each plaque assumed to develop from infection by single virus – plaque forming unit





Cultivation of Eukaryotic Viruses

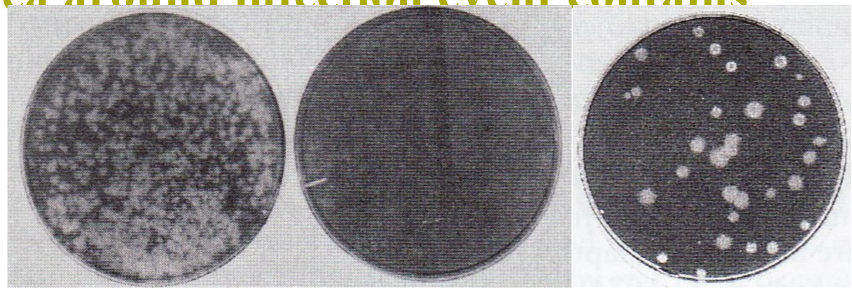
- Ability to grow cells in tissue culture led to development assays for medically relevant viruses

- One method uses the plaque assay, requires confluent monolayer of cells

- Viral titer added, adsorbed, and removed

- Tissue culture overlaid with agar, prevents viral diffusion, only immediately adjacent cells infected

- Results in foci of infection, area around infection event contains dead cells
- Creates “hole” in otherwise confluent lawn of healthy cells - plaque



Detection of Virus Particles

- Certain viruses (influenza) contain adhesins that bind sialic acid on the surface of RBC

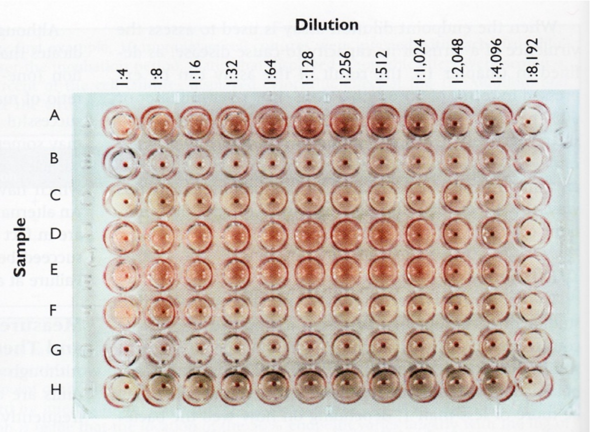
- Presence of virus particles cause cross-linking of RBC

- Possible to detect by hemeagglutination assay

- Two fold serial dilutions of sample suspected of containing virus made

- Mixed with dilute suspension of RBC
- Presence of virus facilitates cross-linking, prevents settling of RBC in well

- Dilution factor qualitatively assesses amount of virus



Absolute Quantification of Viral Load

- Development of electron microscopy allowed direct visualization of viruses

- Possible to determine the precise number of viral particles used in infection

- Plaque assay determines how many of the viruses are capable of establishing a productive infection

- Allows for determination of plating efficiency

Plating Efficiency

- Total number of virus particles present rarely reflects total number of infectious particles

- Total number of particles capable of establishing an infection and creating a plaque always lower than total number of particles present

- Ratio of infectious particles to defective particles denotes plating efficiency

- Variable between viral species

- Bacteriophage plating efficiencies approach 50%, eukaryotic viruses range between 0.1% and 1.0%

- Analogous to total count vs. viable count for bacteria

Mechanisms Contributing to Discrepancy

- Several factors contribute to difference in total vs. viable count
- Enveloped viruses non-infectious if membrane ruptured, contains spike proteins that allow attachment, capsid still visible
- Many RNA viruses mutate at rapid level, inactivate genes essential for viral life cycle, as many as 99.9% of progeny non-infectious
- Viral capsid may assemble in absence of viral genome, appears normal by electron microscopy
- Cells may actively inhibit viral replication (PKR and 2’-5’ polyadenylase)

DNA Virus Life Cycle – Polyoma Virus

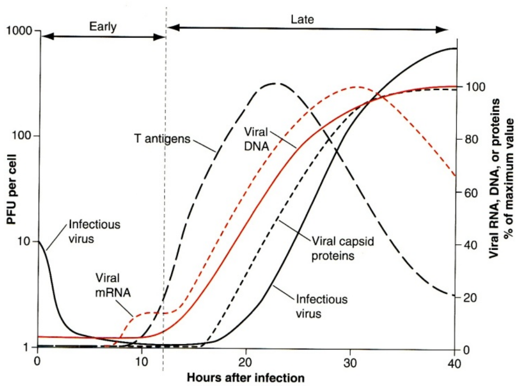
• Cultivation led to understanding of temporal nature of gene expression

• Early viral infection led to production of viral RNA

• Gene products produced facilitated replication of viral DNA – early genes

• Replication of viral genome facilitated production of structural proteins – late genes

• Expression of late genes facilitates assembly of mature viral particles

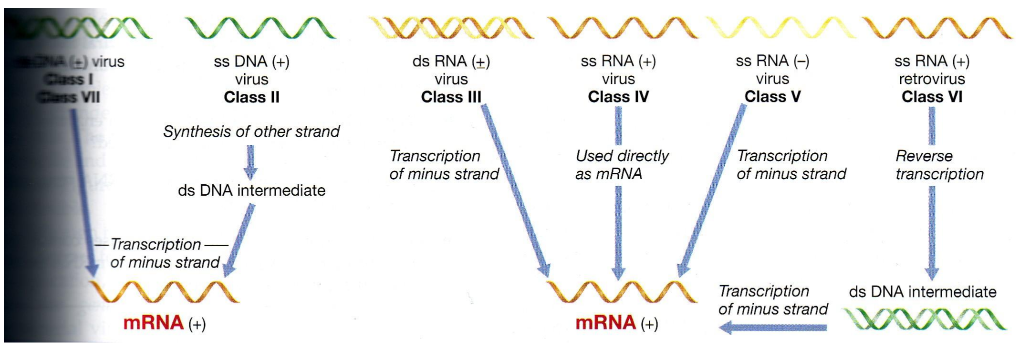


The Baltimore Classification System

• Seven general classes, based on replication scheme for genome

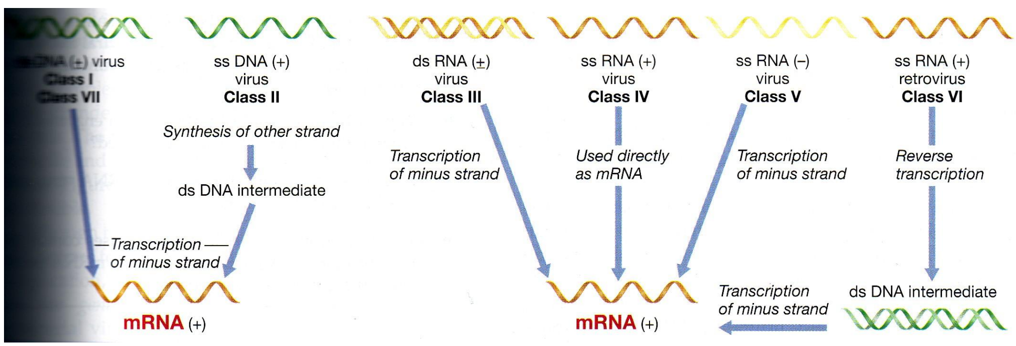
• Class I possesses double stranded DNA for genome, minus strand used for synthesis of (+) strand mRNA

• Class II possesses (+) strand single stranded DNA for genome, used as template to produce double stranded intermediate, minus used for synthesis of (+) strand mRNA, also used as template for (+) strand packaged in progeny viruses



Classification Scheme for Viruses

- Class III possesses double stranded RNA genome, not suitable for protein synthesis
    - Mature particles contain RNA dependent RNA polymerase
    - Uses (-) strand as template for synthesis of mRNA
    - Both strands used as template for additional genomes



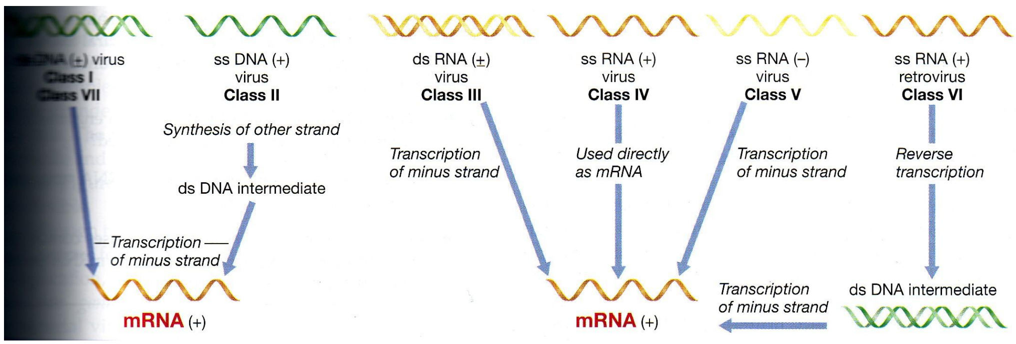
The Baltimore Classification System

• Class IV possesses single (+) strand RNA genome, suitable for protein synthesis

• Viral proteins produced immediately following uncoating

• Results in production of RNA dependent RNA polymerase

• Creates (-) strand to use as template for production of new viral genome, new genomes also serve as mRNA



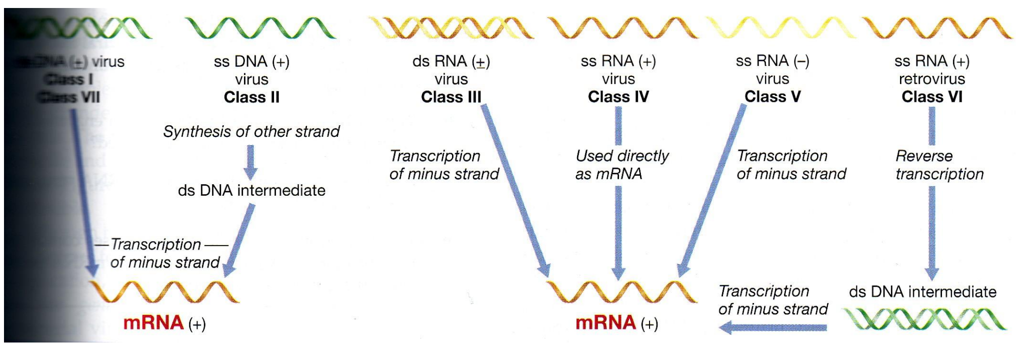
The Baltimore Classification System

- Class V possesses single (-) strand RNA genome, not suitable for protein synthesis

  - Mature particles contain RNA dependent RNA polymerase

  - Uses (-) strand as template for synthesis of mRNA

  - mRNA used for protein synthesis and as template for production of new viral genomes



The Baltimore Classification System

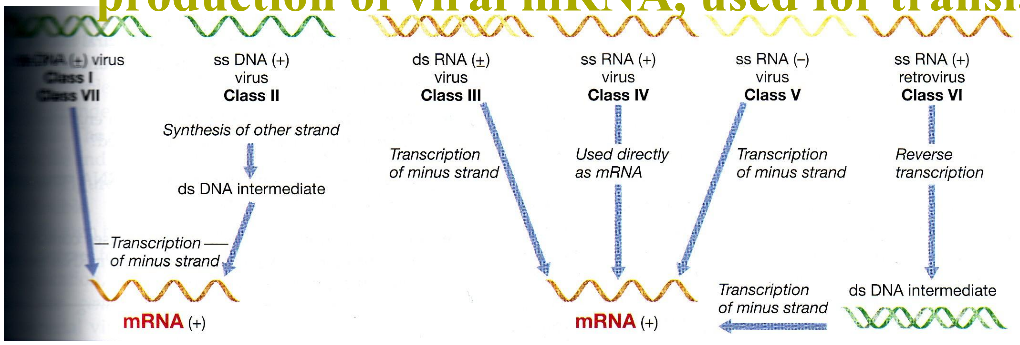
- Class VI possesses single (+) strand RNA genome, suitable for protein synthesis, mature particles contain reverse transcriptase

  - (+) strand not initially used for protein synthesis

  - Reverse transcriptase converts to double stranded DNA

  - Double stranded DNA integrates randomly into host genome

  - May remain latent for years, integrated viral genome used for production of viral mRNA, used for translation and assembly



The Baltimore Classification System

- Class VII – gapped strand DNA viruses
- DNA translocates to nucleus
- Transcribed to pre-genomic mRNA
- Used to produce viral proteins
- Used as template for viral encoded reverse transcriptase to produce additional viral genomes
- Unique replication system required additional classification category