**Fundamentals of Small Molecule Virology**

**Brief Historical Timeline**
- **1000 CE**: Variolation used in Far East
- **1798 CE**: Edward Jenner develops the first vaccine ever for smallpox
- **1800s CE**: Modern germ theory begins
- **1885 CE**: Louis Pasteur develops the rabies vaccine
- **1887 CE**: Dmitri Ivanovsky finds tobacco mosaic disease agent through chamberland filter
- **1898 CE**: "Filterable agents" were first viewed under an electron microscope
- **1938 CE**: Martinus Beijerinck demonstrates tobacco mosaic disease agent is infective generationally
- **1940 CE**: Delbruk & Luria found the phage group at Cold Spring Harbor
- **1952 CE**: Hersey & Chase perform groundbreaking experiments proving DNA as genetic information
- **1955 CE**: Salk vaccine for polio
- **1961 CE**: mRNA is discovered (work by Brenner, Jacob, Meselson)
- **1961 CE**: Sabin vaccine for polio
- **1980 CE**: First retrovirus is identified
- **1984 CE**: HIV is identified as the causative agent of AIDS by Gallo and Montagnier
- **1989 CE**: First protease inhibitor approved by FDA
- **1995 CE**: Hepatitis C discovered
- **2003 CE**: Emergence of SARS
- **2006 CE**: HPV vaccine approved
- **2010 CE**: Global eradication of rinderpest
- **2014 CE**: Harvoni approved by FDA
- **2015 CE**: Emergence of MERS
- **2019 CE**: First mRNA vaccines developed for COVID-19 approved under EUA by FDA
- **2020 CE**: Emergence of COVID-19

**Nobel Prizes associated with Virology**
- 1946: Preparation of viral proteins in pure form
- 1951: Combating yellow fever
- 1954: Poliovirus assay
- 1960: Immunological tolerance
- 1965: Genetic control of enzyme and virus synthesis
- 1966: Discovery of tumor-inducing viruses
- 1969: Replication and genetic structure of viruses
- 1975: Interaction between tumor viruses and host genome
- 1989: Discovery of retroviral oncogenes
- 2000: Link between HPV and cervical cancer
- 2008: Discovery of HIV
- 2020: Discovery of HCV

**Table of Contents**
- The objective of this presentation is to provide the basic background of virus life cycles and current mechanisms of action of FDA approved antivirals.

1. **Human Immune Response**
   - Innate immunity
   - Adaptive immunity

2. **Basic Principles and Current Trajectory of Antiviral research**
   - Direct-Action Agents (DAAs)
   - Host-Targeting Antivirals (HTAs)

3. **The Baltimore Classification of Viruses**
   - Class II: dsRNA
   - Class IV: (+) ssRNA
   - Class V: (-) ssRNA
   - Class II: ssDNA
   - Class I: dsDNA
   - Class VI: ssRNA retroviruses
   - Class VII: dsDNA retroviruses

**Topics Omitted**
- Several interesting and important details are left out of this presentation which would be discussed in an introductory virology course. These include:
  1. Structure of viral particles
  2. Viral evasion mechanisms of the immune system

**Helpful Virology Resources**
- Virology Molecular Biology and Pathogenesis by Norkin
- Viruses and Human Disease by Strauss & Strauss
- Virus Hunters by Williams & Knopf

**Why Should We, As Chemists, Care?**
- As much as 90% of all human illness may be caused by viruses
- Billions of people are living with chronic viral infections
- The antiviral R&D market is expected to grow from $46MM to $130MM by 2030.
**Human Host Defenses**

**Physical Barriers Against Infection**

**The Epidermis**
- Outer layers consist of dead cells filled with keratin.
- Cuts, abrasions, bites and stings can allow viral entry.

**The Respiratory Tract**
- Epithelium in the respiratory tract produce mucous and ciliated cells push the mucus out of the respiratory system.
- Additionally, immune surveillance is high in the respiratory system.

**The Gastrointestinal Tract**
- Enzymes in saliva begin the process of breaking down ingested matter.
- The highly acidic stomach, along with digestive enzymes serve to denature and destroy pathogens.
- Other detergents and digestive enzymes are released by the liver and intestinal tract to further destroy potential pathogens.

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**Innate Immune System**

- Second line of defense once the physical barriers are breached.
- The key features of this system are that it is:
  1. Fast.
  2. Non-specific.
- Its role is to slow or stall an infection long enough for the adaptive immune system to respond (~3 days).
- This system responds to many pathogens, not just viruses including bacteria, protozoa, fungi and parasites.
- It is the "quarterback" of immune response. Failure to initiate a strong innate response hinders the adaptive immune response.

**The Cytokine Pathway**

**Step 1:**
Professional Phagocytes (macrophages and neutrophils) identify a pathogen when their Toll-like receptors (TLRs) are activated.

**Step 2:**
The appropriate cytokines are released, recruiting more innate immune effector cells to the region and alerting healthy cells of an ongoing infection.
- Tumor Necrosis Factor Alpha (TNF-α) an inflammatory signal.
- Type I interferons (INF-α and INF-β) antiviral signals
- Type II interferons (INF-γ) immune modulators

**Step 3:**
Type I INFs bind nearby cells JAK receptors and activate their STAT pathways which promote upregulation of ~300 proteins to prepare the cell for defense against the viral pathogen.
- RNA-protein Kinase (PKR)
  - When two molecules of PKR bind to dsRNA, they cross phosphorylate, then activate eIF2α which halts protein synthesis. **WHY?**
- RNA-activated RNaseL
  - dsRNA activates 2'-5' oligo(A) synthetase which activates RNaseL which goes on to destroy all cellular and viral RNA.
  - dsRNA specific adenosine deaminase (ADAR) recognizes dsRNA and catalyzes the deamination of adenosine to inosine on viral and cellular RNA causing synthesis of defective proteins.
- INF activation also induces genes that ultimately lead to programmed cell death

**Antibody-Dependent Cellular Cytotoxicity (ADCC)**

- Neutrophils and Natural Killer (NK) are recruited and further activated by the inflammatory signals.
- These cells recognize viral glycoproteins used for viral entry and endure apoptosis.
- This system is made even more specific by the adaptive immune system's release of specific antibodies.
- Mechanism involves the competition between activating and inhibition signals of apoptosis.

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**Complement System**

Roughly 30 proteins involved in this system that achieve 5 things:
1. Formation of a Membrane Attack Complex (MAC)
2. Binds viral shells to signal destruction by phagocytes
3. Enhances the inflammatory response
4. Can neutralize some viruses
5. Can destroy virus infected cells through MAC-orthogonal mech. It does these thing very quickly through a cascade of protein cleavage. It can occur spontaneously through activation by the adaptive immune system with antigen specific antibody signaling.

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**Figure 4.6** The classical pathway of complement activation. (A) Complement protein C1 binds to the Fc region of either IgG or IgM antibodies that are bound to the target. (B) C1 molecule contains a serine protease activity that is activated when C1 binds to the antibody. The activated C1 then sequentially cleaves complement proteins C4 and C2, to generate a complex designated C3b2a, which binds to the cell surface. (C) C4b2a then activates C3. That is, C4b2a serves as a C3 "converting", cleaving C3 to generate C3a and C3b, the latter of which binds to proteins and carbohydrate groups on the cell surface, forming a complex with C3b2a, designated C3b2a3b. (D) C3b2a3b then cleaves C5 to generate membrane-associated C5b and the free fragment C3a. (E) The last four components of the complement cascade, C6, C7, C8, and C9, then bind to the cell surface to form the MAC that lyses the pathogen.
**Human Host Defenses**

**Adaptive Immune System**

There are 3 key features that make the adaptive immune system particularly well-suited for clearing viral infections.
1. It produces T Cells (CTLs) that are specialized to attack and destroy host cells harboring viruses.
2. It produces B cells that produce specialized antibodies able to recognize extracellular viruses and neutralize them.
3. It produces an immunological memory so it can respond swiftly and aggressively to future challenges by a pathogen.

It takes several days for the adaptive immune system to produce these specialized cells for new infections.

There are two branches of the adaptive immune system:
1. Humoral Immune System
2. Cell-mediated Immune System

**Humoral Immune System (B Cells)**

- This system is capable of generating an astonishing $10^8$ to $10^{16}$ uniquely specific antibodies!
- It accomplishes this with only about 300 genes through modular design.

**B Cell Development**

**Step 1:**
Hematopoietic stem cells (HSCs) produce naïve B cells which express a single, unique IgM membrane bound antibody.

1. B cells undergo a special process called Somatic Recombination where different V,D, and J DNA segments are combined at random to produce the variable region of an antibody.

2. In recombination, imprecise splitting causes random mutations that give rise to additional diversity.

**Step 2:**
A naïve B Cell circulates until it binds its cognate antigen.

1. Binding activates maturation of the B cell and induces clonal expansion (rapid cell division).

2. In this process, somatic hypermutation in the V domain induces random mutations to occur.

3. Cloned B cells with enhanced binding affinity proliferate more aggressively. This positive feedback loop generates incredibly specific antibodies, a process known as affinity maturation.

4. Some proliferated B cells release antibodies and some become memory B cells for use in future infections (Memory).

**Functions of Antibodies**

1. Interfere with viral attachment to host cells
2. Marking and collecting virus particles for destruction
3. Accelerating ADCC of neutrophils
4. Disruption of intracellular replication of virus
5. Complement mediated lysis of antibody/virus complex
6. Complement mediated lysis of antibody/infected cell
7. Inhibition of virus budding and release

**Anatomy of an Antibody**

**T Cell Development**

**Step 1:**
T cells are produced in the thymus and the naïve cells circulate the lymphatic system, searching for an MHC receptor presenting their cognate antigen. Their maturation process is similar to B cell maturation but not identical.

**Step 2:**
At the site of infection, a dendritic cell (which display MHC-1 and MHC-2 receptors for T cell recognition) or macrophage is activated by the innate immune system and leave the infected tissue to the nearest draining lymph node where they wait, eventually coming into contact with the unique T cell for the antigen being presented. This can take several days to occur.

**Step 3:**
The naïve T cell develops into mature Th1 and Th2 cells which go on to activate B cells to stimulate the humoral immune response. Th cells also activate CTL’s to induce the cytopathic element of the this immune response.

**Cell-Mediated Immune System (T Cells)**

Two kinds of T-Cells
1. "killer" T Cells, CTLs, CD8 T Cells
2. "helper" T cells, Th cells, CD4 T Cells

- There are also memory T cells which are immunologically silent. Additionally, 'helper' cells have the capacity to induce apoptosis.
- CTL’s bind Major Histocompatibility Complex 1 (MHC-1).
- MHC-1 are present on all cells (except neurons) and allow CTLs to differentiate healthy cells from unhealthy ones.
- MHC-2 receptors are used by many immune effector cells to share antigen information and activate one another. They are not broadly expressed by all cells.
- MHC-1 complexes display endogenous peptide fragments less than 10 AAs long.
- MHC-2 receptors display exogenous peptide fragments of at least 13 AA's in length.
**General Principles of Antiviral Therapeutics**

Ultimately, all antiviral therapies exist to hinder the virus from succeeding in one of its 3 main goals. Unlike antibiotics and antimicrobials, antivirals do not "kill" the virus, they only **impede its ability to replicate successfully**. A major challenge of antiviral treatment is that the pathogen is replicating **inside the host cells and is often using the host's transcription and translation machinery**.

Broadly, there are two main categories of antivirals:

1. **Direct-acting Agents (DAAs)**
   - These directly target viral proteins/genetic material.

2. **Host-Targeting Antivirals (HTAs)**
   - These activate the host immune system or block viral binding sites.

**DAA Antiviral Mechanisms of Action**

1. **Polymerase inhibition**
   - Viral DNA polymerase inhibitors
   - Viral Reverse Transcriptase inhibitor
   - Nucleoside analog replication inhibitors

2. **Protease inhibitors**
   - Prevent polypeptide cleavage

3. **Integrase Inhibitors**
   - To block retroviral replication

4. **NS5A Inhibitors**
   - HCV RNA replication promoter

5. **Translation Inhibitors**
   - Antisense oligonucleotides

6. **Entry Inhibitors**
   - Block the virus from releasing its contents

7. **Assembly Inhibitors**
   - Prevent final assembly of the capsid and contents

8. **Release inhibitors (neuraminidase inhibitors)**

9. **Attachment Inhibitors**

10. **Methyltransferase inhibitors**

11. **Helicase inhibitors**

**HTA Mechanisms of Action**

1. **Interferon**

2. **Cyclophilin inhibitors**
   - Cyp5b influence transcription, immune response, protein secretion, mitochondrial function

3. **Viral receptor antagonists (CCR 4/5)**
   - Block binding of virus to host cells.

4. **TLR agonists**
   - Stimulate innate immune response

**Helpful Resources for Basic Antiviral Information**


A review: Mechanism of action of antiviral drugs. DOI:10.1177/20587384211002621

Baltimore Class III: ds RNA; The Reoviruses

- Relevant Families include the orthoreoviruses, rotaviruses, orbiviruses, and coltiviruses.
- These are unique, double stranded RNA viruses whose genomes are comprised of multiple segments of dsRNA.
- Non-enveloped, double layered capsid shell. The virus’ σ protein binds the JAMA1 receptor of the tight-junctions in the gut epithelia.

Entry, Replication and Exit

**Step 1:**
The virus particle binds JAMA1 and is endocytosed.

**Step 2:**
In endosome, a pH change causes the capsid to transform into the **infective subviral particle (ISVP)** which perforates the membrane of the endosome and escapes into the cytosol.

**Step 3:**
Inside the cytosol the ISVP changes shape again and pores open on the newly created 'core'

**Step 4:**
Particles-associated RNA polymerases copy the RNA genome inside the core in a **conservative fashion** and the copied RNA is pumped out of the core as mRNA for translation by the host ribosomes. (Note: core proteins "cap" these RNA)

**Step 5:**
Viral proteins are synthesized and assembled with one (+) stranded RNA of each genomic RNA segment. The (-) strand is then synthesized by the viral RNA polymerase. Additionally, these newly assembled particles produce mRNA for cellular translation but these secondary transcripts are uncapped and the host ribosomes have been modified to read them.

**Step 6:**
The virus exits the cell.

Rotaviruses: A case study of Class III

These pathogens are ubiquitous in humans with 95% of children being affected worldwide between the ages of 3 to 5. They are responsible for 1/3rd of all diarrhea related hospitalizations and result in roughly 500,000 deaths annually (mostly children).

Fascinating Read:

Coltiviruses: A case study of Class III

Arthropod-borne virus (transmitted by 'bugs') that has 12 RNA genome segments (the most allowable for a reovirus).

The prototypical virus in this family is the colorado tick fever virus.

No antiviral medications are prescribed for any reovirus disease.
Baltimore Class IV: (+) ssRNA; Picornaviruses

- Relevant Families include the rhinoviruses, enterovirus, hepatovirus, cardiovirus & more
- Ubiquitous in human pathology and nature, these have one of the most straightforward genome replication processes, undoubtedly aiding in their success.
- Smallest members of the RNA viruses, non-enveloped containing (+)-ssRNA
- Capsid shells contain a "canyon" in which a cellular receptor binds, inducing fusion and injection of the RNA genome.

Entry, Replication and Exit

**Step 1:**
The virus particle binds a cellular receptor(s), fuses with the entry receptor creating a pore which the RNA molecule can pass through.

**Step 2:**
Inside the cytosol, the virus genome is capable of directly translating its genome with host ribosomes when they bind internal ribosome entry sites (IRES). Their genomes only have one reading frame and the ribosome synthesizes one long polypeptide containing every viral protein needed for replication and exit. *Why use an IRES, not 5’ cap?*

**Step 3:**
The polypeptide contains proteases which excise themselves and the other peptides to reveal the active enzymes and structural proteins needed for assembly and replication.

**Step 4:**
A (+)-RNA genome molecule interacts with proteins to localize it near intracellular vesicles where replication can begin. Replication is directed by 3 key motifs in the RNA structure:

1. The clover leaf at the 5’ end which serves to localize it to the vesicles and circularize the genome.
2. The cre element which activates Vpg to prime the RNA polymerase 3Dpol
3. An RNA pseudoknot at the 3’ end.

Once VPg is activated, the primed RNA polymerase is transferred to the 3’ end of the genome and transcription begins to synthesize the (-) strand RNA compliment of the viral genome.

**Step 5:**
(-) strand RNA are transcribed into the (+) RNA viral genome at a rate of about 100 to 1

**Step 6:**
Capsid protein pentamers form around the newly synthesized RNA genomes and the final protein cleavages are done to produce the final particle which is released from the cell.

For a complete overview of picornavirus replication watch:  
www.youtube.com/watch?v=mvz3ggZI5-o

Class IV Case Study: Hepatitis C

Replication is highly analogous to picornaviruses, however, they are enveloped viruses and thus have a slightly different entry mechanism involving membrane fusion with an endosome.

In 2017 as many as 71 million people were living with Hepatitis C.

As a chronic condition it can lead to liver failure, liver carcinomas, and ultimately death. Antivirals have essentially cured several of the genotypes of HCV. Most notable is the combinatin therapy of Ledipasvir/sofosbuvir (Harvoni).

The most successful HCV antiviral drugs are NS5A and NS5B inhibitors.

Antivirals are not currently in use for the picornaviruses, although this is an active area of research with some promise:

Antivirals blocking entry of enteroviruses and therapeutic potential. *Journal of biomedical science, 2021*, 20, 10


*Antiviral Research, 2009* 56-63
Baltimore Class IV (Cont.) Coronaviruses

- Largest Genome of any RNA virus with 27 to 30 kb. With such a large genome, their RNA sequences contain 7 to 8 ORFs. ORF1 (which has most of the non-structural protein synthesis) has two overlapping ORFs. As a result these viruses will generate multiple different mRNAs (7 to 8). The longest is genomic the other mRNAs are subgenomic. The theoretical upper limit on RNA genome size is 30 kb based on RNA polymerase accuracy and fidelity.
- In principle, replication is similar to other class IV viruses but it is significantly more complicated:
  1. Their genome encodes for an RNA dependent RNA polymerase, a helicase, proteases and many RNA-processing enzymes not needed by other viruses.
  2. Transcription of genomic RNA undergoes a process called "discontinuous transcription" where the transcribing polymerase can "jump" from a place on the 3' end to a place much further down the 5' end.
  3. A large amount of genomic recombination occurs in the replication cycle, as much as 25% of the entire genome. In addition to the large genome and high polymerase error rate this level of mutation likely confers an evolutionary advantage to the coronavirus.

Baltimore Class V: (-) ss RNA; The Rhabdoviruses

All Class V viruses have the following characteristics:
- 1. Genomes are always associated with many copies of a nucleocapsid N protein and with fewer copies of the viral RNA polymerase molecules
- 2. Nucleocapsids are always helically symmetric reflecting the association of N proteins with the RNA
- 3. All are enclosed within lipid envelopes

What Key challenge will a (-) stranded RNA virus have to overcome compared to a (+) stranded RNA virus?

Entry, Replication and Exit

Step 1:
Generally, the virus glycoproteins bind a hosts receptor(s), inducing endocytosis. Inside the endosome a pH change or other factor induces a membrane fusion event releasing the contents of the viral capsid into the cell.

Step 2:
Before proteins can be translated, sense RNA must be produced. This chicken and egg problem is solved with the virus bringing its RNA polymerase with it. The polymerase begins transcription in the 3' to 5' direction. The genome is arranged such that the sequences closer to the 3' end are transcribed more frequently into mRNA (and thus translated more often). At the junction of each gene sequence there exists an intergenic junction where the polymerase stops transcription roughly 30% of time. As the polymerase continues down the genome each intergenic junction it passes is an opportunity to stop transcription. A clever feedback mechnism is employed: as more N protein is produced it binds nascently forming mRNA via interactions with P protein this prevents the polymerase from stuttering at the intergenic junction and to continue transcription for the length of the minus sense RNA genome, producing a complementary copy of the genome when the proteins are in high enough concentration for exit.

Step 3:
The (+) sense compliment of the genome is transcribed in full length to the (-) RNA genome.

Step 4:
Viral particles are assembled, including the addition of key enzymes like the RNA pol.

Step 5:
These particles are released via budding off the membrane where the viral glycoproteins exist.

Class V Case Study: The Paramyxovirus, Measles. Where did it come from?

Measles kill 1M to 2M annually. It has an R0 of about 15!
One aspect that makes Measles so severe is that one of its host receptor targets is CD46, a ubiquitous receptor in the human body. As a result, measles can have severe complications by infecting the heart, brain, liver, and other organs.

How does a virus with such a high infection rate, high fatality rate, and singular serotype sustain its population in a host?
Sometime in the last 2000 years a rinderpest virus made the jump from live stock to humans when the human population became large enough (500,000) to sustain a virus like measles by producing new susceptible hosts at a rate sufficient to maintain an infected population.

Rinderpest Virus

- Devastating to livestock with a nearly 100% fatality rate in cattle!
- The UN Food and Ag. Org created a program to eliminate the virus using a vaccine.
- It is the first animal (non-human) virus to be eliminated worldwide.
- The program cost $5B in total.
- Would such an aggressive program be tolerated in human society?

Louis Pasteur, Rabies, and Experimental Ethics

- Pasteur developed a rabies vaccine in 1885, long before the identification of any virus itself.
- On July 6th, 1885 Joseph Meister, a child, was bitten by a rabid dog and recieved the first dose of Pasteur's vaccine.
- Pasteur did not have a medical license, claimed he had tested the vaccine on 50 dogs but could only prove 11 vaccinations, and refused to share the production procedure.
- Nevertheless the boy did not contract rabies and went on to be a gatekeeper of the Pasteur Institute of Paris.
**Baltimore Class V (Cont.) Orthomyxoviruses (Influenza)**

- While still a class V virus, they differ in two important ways
  1. They have 6 to 8 individual genomic RNA segments (like reoviruses)
  2. All RNA transcription and replication occurs in the cell nucleus

**What challenge does that pose?**

- Given its name by Italian astrologers who blamed the periodic flu epidemics on the influence of heavenly bodies.
- There are 4 types A, B, C, D. A poses the biggest threat to human health because of its ability to undergo antigenic shift.

**Antigenic Shift** is when two or more different strains of a virus combine to form a new subtype. It is the key contributor to a viruses ability to cause a pandemic through widespread, sudden susceptibility.

Two facts of influenza A that make shift more likely
1. Many host species (which allow for many strains and time for evolution)
2. Its segmented RNA.

If a host becomes coinfected with two strains, segments of one strain can end up in the particles of another strain, creating a new strain.

**Antigenic Drift** is the continuous divergence of a strain as a result of mutations during replication. It is the reason a new vaccine is needed each year for influenza.

**Influenza Antivirals**

**Uncoating Inhibitors**
- Not currently used for contemporary flu strains

[Chemical structures of amantadine and rimantadine]

[Chemical structures and names: Endonuclease inhibitor, Baloxavir marboxil, Process Retro?, MeO2COO, OPRD. 2019. 1298]

**Neuraminidase Inhibitors (viral release inhibitors)**
- Most frequently prescribed
- Fears of resistance developing

[Chemical structures of oseltamivir (tamiflu), zanamivir, peramivir, received emergency use authorization in 2009]

**Baltimore Class II: ss DNA; The Paroviruses**

- "parvo" meaning small. Genome is only 5kb in length and capsids are 18-26nm in diameter.
- Only family of linear single stranded DNA viruses.
- Not particularly pathogenic in humans (only B19), some strains are devastating in dogs and cats

**Entry, Replication and Exit**

**Step 1 (#1-4):** The parovirus binds a host's surface receptor(s), enduring endocytosis. Inside the endosomal pocket the parovirus may use a lipolytic enzyme PLA₂ to escape the endosome and enter the cytoplasm.

**Step 2 (#5):** Being a DNA virus, it will need to enter to nucleus, which is guarded by another membrane. The only way in is through the nuclear pore complex (NPC). The details of this are not exactly known, but it is possible that the entire capsid passes through the pore, given its small size.

**Step 3 (#6-7d):** Inside the nucleus the virus must make dsDNA from its ssDNA, however, DNA synthesis only occurs during S phase of a cell's life. The parovirus waits until cell division begins. While being replicated the virus uses the host replication, transcription, and translation machinery. Being linear the virus needs a mechanism to replicate its extreme 5'ends of its genome. It solves this problem through a "rolling hairpin" mechanism similar to how viruses and bacteria use a rolling circle mechanism.

**Step 4 (#8-12a):** The now double stranded DNA genome can produce mRNA transcripts that leave the nucleus and begin viral protein synthesis. These proteins are then brought back into the nucleus for capsid formation.

**Step 5 (#7e):** With the capsids formed, the ss DNA genome can now be replicated on the capsid surface and packaged into the particles.

**Step 6 (#15):** The completed viral particles exit the nucleus and lyse the cell to exit.
Baltimore Class I: ds DNA; The Polyomaviruses

- Polyomaviruses, along with the other small DNA tumor viruses, papillomaviruses and adenoviruses have the ability to inactivate or block transcription of tumor suppression proteins and genes, activating their host cells into S phase. As a result, many of these viruses cause carcinomas.

**Entry, Replication and Exit**

**Step 1:** The virus binds its host's receptor(s), (for SV40, that receptor is MHC-1) and is endocytosed. Next, the endosome is transported to the ER where the capsid is partially broken down and released into the cytosol.

**Step 2:** Being a DNA virus, it will need to enter to nucleus, which is guarded by another membrane. The virus particle then binds the NPC and injects its DNA through the pore.

**Step 3:** The now double stranded DNA genome can produce mRNA transcripts that leave the nucleus and begin viral protein synthesis. These mRNA are spliced by the host and undergo the usual mRNA processing of eukaryotic cells. Many of these proteins disrupt the cell cycle and natural processes of metabolism. Ultimately, structural proteins are then brought back into the nucleus for capsid formation.

**Step 4:** Inside the nucleus the virus waits (or initiates S phase) making use of 11 of the host proteins to replicate its genome such as DNA Pol, to prime and the other polymerases to replicate and topoisomerase I. Additionally, viral proteins may promote viral DNA replication by binding host replication machinery.

**Step 5:** With the capsids formed, the ds DNA genome can now be replicated and packaged in viromas (viral factories).

**Step 6:** The completed viral particles exit the nucleus and may lyse the cell to exit.

Class I Case Study: Papillomavirus

- Responsible for about 500,000 new carcinomas annually with half as many deaths.
- HPV is the most common STI in humans. As many as 80% of people contract genital HPV of some kind with many genotypes being asymptomatic (even some of the high-risk ones).
- Its replication mechanism is analogous to the polyomaviruses with the unique twist being its cellular preference:

Class I Case Study: Adenovirus

- Military recruits are plagued by pathogenic adenovirus infections, but current research efforts are focused on using the adenovirus family as a vector for recombinant gene therapy.

Class I Case Study: Herpesviruses

- From Greek "to creep" These viruses are ubiquitous human pathogens including VSV (chickenpox), HSV1/2 (herpes), EBV (Mono), and a number of others.
- It is estimated that 50% of people have HSV1 worldwide with ~20% of the population also having HSV2.
- These viruses have an interesting life cycle involving latency in neurons that lead to lifelong recurrent infections. MHC-1 is not present in neurons to avoid autoimmune attack, this makes HSV1/2 impossible for the immune system to completely clear.

Antivirals

- Nucleoside analogs: Current standard of care for α-herpesvirus infections. Acyclovir, patented in 1974 is one of the oldest antivirals and is still in use.
- Helicase Primase Inhibitors: amenamevir, pritelivir, letemovir
- Phosphate analogs to inhibit DNA polymerase: Foscarnet
- Terminase Inhibitors: Blocks DNA cleavage.

Acyclovir

- Conversion to Acv-TP encorporation by viral DNA pol

Pritelivir

- HSV Phase III
Baltimore Class VI: ss RNA Retroviruses

- First retrovirus discovered in 1980 by Robert Gallo. HIV was discovered by Gallo and Montagnier in 1984. (read And the Band Played On by Randy Shilts)
- 7 Classes of retroviruses, 5 are RNA Tumor viruses. One is the lentiviruses (HIV). One is the spumaviruses (foamy viruses)
- 4 noble prizes have been awarded for the study of retroviruses, they are the most well studied class of viruses today.
- Retroviruses have the following characteristics:
  1. There is a reverse transcription step where (+) RNA is replaced by ds DNA
  2. Reverse transcription is catalyzed exclusively by viral reverse transcriptase
  3. Reverse transcripctase is included in the viral particle.
  4. They are the only (+) RNA group whose genome does not serve as mRNA
  5. The copy of the DNA genome is incorporated into the host genome
  6. All mRNA's and progeny genomes/particles are generated by transcription/translation of the incorporated DNA genome.
  7. All retroviral RNAs and mRNAs are transcribed by the host's RNA Pol II
  8. Reverse transcriptase requires an RNA primer. Retroviruses use host tRNAs as primers.
  9. They are the only known viruses that can infect a cell while simultaneously transforming it.
  10. Retroviruses have a diploid, single stranded RNA genome, unlike any other virus.

Entry, Replication and Exit

The retrovirus life cycle is better demonstrated through video than text to a caption. For a high quality explanation watch: www.youtube.com/watch?v=PL6vVyiLuNw

The specifics of retroviral transcription are described as follows:

1. tRNA is opened and used as a primer for reverse transcriptase and the minus strand of DNA is transcribed for about 100 units before reaching the end of the template. The polymerase then "jumps" to the other PBS region where transcription continues in the 3' to 5' direction. This is done to produce a complete LTR region.
2. As the (-) DNA is being transcribed, the RNA template is being hydrolyzed behind it. Except for the pR region which is skipped to be used critically as a primer in the synthesis of the templated DNA section for the synthesis of the (+) strand.
3. After reaching the 5' strong stop sequence, DNA-dependent DNA polymerase begins from the primer at the pR region until it reaches the PBS region where the tRNA primer exists. Hydrolysis of this primer exposes the PBS domain on the (-) strand where retro transcription began and these two complimentary regions hydrogen bond to circularize where the polymerase can now use the PBS region as a primer for the remainder of (+) strand synthesis.
4. After completing transcription, strand displacement synthesis produces the LTRs of both ends of the DNA proviral genome.
5. Integrate directs the proviral genome into the nucleus where it is inserted by integrate into the host genome.

A great video of this process can be found here: www.youtube.com/watch?v=RYwVnzYf4V8

PBS: primer-binding site
PPT: polypurine tract
LTR: long terminal repeats

Antiretroviral Therapies

- Roughly half of all FDA approved antivirals are for HIV.
- There are several classes of antivirals for HIV which are used in combination to reduce the rate of antiviral resistance and further decrease viral load.
- With early intervention and proper management people with HIV can live a natural lifetime, something unthinkable 30 years ago.
- Combination therapies generally involve 3 antivirals with at least 2 having distinct mechanisms of action.

Nucleos(t)ide RT inhibitors (NRTIs) (representative sample)

- Abacavir
- Emtricitabine
- Tenofovir alafenamide

Non-Nucleoside RT inhibitors (NNRTIs) (representative sample)

- Etravirine
- Delavirdine
- Nevirapine
- Efavirenz

Doravirine OPRD 2016 1476
**Protease Inhibitors (PIs)** Why might PI's look the way they do?
- The discovery of protease inhibitors enabled highly active anti-retroviral therapy (HAART), a profound improvement in standard of care.
- Saquinavir was the first FDA approved PI in 1995, followed by ritonavir and indinavir.

**Integrate Strand Transfer Inhibitors (INSTIs)**
- Prevent viral integrate from inserting its genome into the host cell's genome.
- Frequently used for newly diagnosed HIV patients for their minimal side effects.

**How to make?**

**Chemokine coreceptor antagonists (CCR5 antagonists)**
- HTA antiviral medication designed to block viral glycoproteins from binding CD4 cells.

**Fusion inhibitors**
- Enfuvirtide is the only fusion inhibitor currently FDA approved, blocks HIV fusion to host cells.

**Cytochrome P4503A inhibitors**
- Not technically antivirals, but they can increase the half-life of antivirals and improve their effectiveness so they are frequently prescribed.

**Baltimore Class VII: ds DNA retroviruses; Hepadnaviruses**
- These are ds DNA viruses that replicate via an RNA intermediate.
- Interestingly, they share similarities to HIV in their reverse transcriptase, genome organization and size, and capsid protein structure.
- Enveloped virus with a highly unusual partially stranded circular genome with the minus strand being longer than the plus strand. The minus strand also has a polymerase attached to the 5' end. The plus strand has a 5' cap.
- HBV is the prototypical hepadnavirus and is a significant human pathogen more than 350 million people have HBV worldwide. Antiretrovirals are standard of care, akin to HIV.
- As a chronic condition it can lead to liver failure or carcinoma and eventually death.

**Entry, Replication and Exit**
- **Step 1:** The virus binds its host's receptor(s) and is endocytosed. After exiting the endosome the capsid travels to the nucleus and binds importin proteins at the NPC, releasing its genome into the nucleus as a result of phosphorylation mediated disassembly.
- **Step 2:** Inside the nucleus polymerases complete the synthesis of the (+) strand of DNA, filling in the gaps and the completed genome is circularized. This 'mini chromosome' serves as a template for transcription by cellular RNA polymerase II.
- **Step 3:** 4 groups of mRNA are produced by 4 different RNA polymerase promoters. 1. A 3.4 kb pregenomic RNA. 2. Pre-S1 (2.4 kb) 3. Pre-S1 (2.1 kb) 4. X (0.7 kb). The complete genome is only 3.2 kb in length. All mRNA are 5' capped and polyadenylated. None are spliced. This is achieved with the posttranslational regulatory element (PRE). Pregenomic mRNA is also the template for Reverse Transcriptase.
- **Step 4:** In the cytosol, viral mRNAs are translated into viral proteins (RT, structural proteins, glycoproteins, proteases, etc.). The viral capsids are assembled around pregenomic RNA bound to a reverse transcriptase enzyme.
- **Step 5:** Inside the sealed capsid the pregenomic RNA is reverse transcribed by RT into its compliment DNA, however, retrotranscription halts during synthesis of the plus sense strand when all the nucleotides within the capsid are depleted. This gives rise to the observed unusual state of the viral genome which will be finished by the host machinery and circularized upon infection.
- **Step 6:** The viral particle buds from the hepatocellular membrane with its glycoprotein envelope.

Additional Resources:
- Great video: [youtube channel](https://www.youtube.com/watch?v=t3643)

**Roles of hepatocyte nuclear factors in hepatitis B virus infection.** *World Journal of Gastroenterology. 2016. 7017*
"... one can think of the middle of the 20th century as the end of one of the most important social events in history; the virtual elimination of the infectious disease as a significant factor in social life."

Sir MacFarlane Burnet, Nobel Laureate, 1962 (18 years before the discovery of HIV)

The WHO Model List of Essential Medicines lists 31 distinct antiviral medications

The Red Queen Hypothesis