Family: Picornaviruses
genus: enterovirus (polio)

- Greek: *pico* = ’small’ : small RNA virus

Polio virus capsid crystal structure

http://viperdb.scripps.edu

2006 Pulitzer Prize for History

Very large diversity of picornaviruses

genus

- Rhino
- Polio
- Coxsackie
- Other Entero
- Apotho
- Cardio
- Hepato

- cause of common cold (but not the only one)
- Poliovirus
- Entero
- Coxsackie D68
- Foot-and-Mouth disease
- Encephalomyocarditis
- Hepatitis A

% nucleotide identity

redrawn from http://www-micro.msbl.ac.uk/3035/Picornaviruses.html
General properties of Picornaviruses

- +strand RNA virus (genome is infectious)
- No viral enzymes enter the cell with the virus
- RNA encodes a single open reading frame
- Replicate entirely in cytoplasm
- Induce double-membrane vesicles which are sites of viral replication
- Shut off host protein synthesis
- Non-enveloped
- Very stable
  - e.g., polio is resistant to 2% SDS and pH2
  - The fact that there are no enzymes in the virion and no lipid membrane helps explain this

The Poliovirus Life Cycle

CD155 (Ig superfamily)

Receptor Binding → Inside of the Cell → Final Cleavage and Assembly

Uncoating → Protein Cleavage → Protein Synthesis

RNA Packaging → RNA Replication

Capsids enter by endocytosis and then RNA is extruded into the cytoplasm just under the plasma membrane
Picornavirus genomes are +strand RNA (7-8 kb) that encode a single large polyprotein.

- The 5’ UTR contains an RNA structure for initiation of translation.
- The 3’ UTR terminates with a virally encoded polyA tail.

The polyprotein is sequentially cleaved by specific proteases.

Precursor proteins often have different roles than the final products.

- Picornavirus genomes are +strand RNA (7-8 kb) that encode a single large polyprotein.
- A virally encode 22 amino acid protein (VPg) is covalently attached at the 5’ end of the RNA and serves as the primer for the RNA polymerase.
- The 5’ UTR contains an RNA structure for initiation of translation.
- The 3’ UTR terminates with a virally encoded polyA tail.

The polyprotein is sequentially cleaved by specific proteases.

Precursor proteins often have different roles than the final products.
Picornaviruses favor translation of their own mRNA by competition for cellular ribosomes

Preventing ribosomes from initiating on cellular mRNA

Recruiting translation initiation factors to the Internal Ribosome Entry Site (IRES) on the viral RNA

Normal translation of host mRNA

The eIF4F complex binds the 5’ cap of mRNA and recruits the 40S ribosome in a complex with eIF3 and eIF2-Met-tRNA-GTP which then scans the 5’ UTR until the initiator AUG is reached

links the 5’ and 3’ ends through interaction with PABP
Two mechanisms by which polio inhibits normal protein synthesis

- Cleavage of PABP by the 3C protease (prevents recycling) of initiated ribosomes
- Cleavage of 4G by the 2A protease

Protein synthesis in polio virus-infected HeLa cells

By 4 hrs after infection almost all proteins synthesized are viral

By 2 hrs after infection eIF4G is completely cleaved
Features of the poliovirus 5′ noncoding region

- Highly structured RNA
- IRES (internal ribosome entry site)
- "clover-leaf" regulatory site
- No 5′ cap
- Poly(rC) binding protein

Picornaviruses recruit translation initiation machinery to their own mRNA

Picornavirus tropism is sometimes explained by the presence or absence of different host-encoded proteins that bind the 5′ non-coding region

K.M. Bedard, B.L. Semler / Microbes and Infection 6 (2004) 702–713
Switch from translation to replication
Translation must be inhibited for RNA synthesis to begin

- Viral precursor protein 3CD interacts with a cloverleaf-type RNA structure located at the 5’ end of viral RNA
- Binding of 3CD ejects the host proteins from the IRES
- Translation is inhibited; RNA replication by 3D begins
VPg is modified by addition of uridines on a specific tyrosine at an internal stem-loop sequence (cre) by 3D.

Which is transferred to the 3' poly A tail.

3D elongates negative strand RNA using VPg as a primer on the polyA site.

The negative strand RNA then serves as a template for productions of positive strand genomic RNAs.

Recombination can occur during replication by template switching of the polymerase.

The Poliovirus Life Cycle

Virion release by either cell lysis or Autophagic exit Without Lysis (AWOL).

RNA is packaged into preformed virions.
**Polio virus replication summary**

- +RNA enters cell without viral proteins except VPg at 5’ end
- initial translation ★
- cleavage of viral proteins by viral proteases
- host-cell protein synthesis shut off; induction of sites of virus replication
- buildup of 3CD
- switch to -strand replication by 3D using uridylated VPg as a primer
- +strands made using -strand template
- lots more translation
- packaging of +strands into pre-formed virions
- Both cell lysis and subversion of autophagosomes releases virions (without lysis).
- entire process takes 5-10 hrs

**Polio: spread**

- Virus enters through the mouth and initially grows in the epithelial cells in the intestine
- Most infections in the few months of life
- Excreted in feces over a period of several weeks after infection.
- Poor sanitation = facile spread. Commonly found in sewage. Very stable in the environment.
- Three serotypes. No cross-protection, but life-long immunity for each one separately (i.e. need 3 infections/vaccines to be protected from polio)
Stages of poliovirus replication

Ingested polio replicates in oropharyngeal and intestinal mucosa

Reaches the blood through the lymph nodes

Virus multiplication in skeletal muscle and brown fat?

This is the stage blocked by antibodies (maternal or IPV)

Polio: disease

- Most infections are mild or asymptomatic

- CNS infection in 0.5 to 1% of cases resulting in paralysis of limbs (Poliomyelitis)

- 30% of those are permanent. ~40% of those who recover suffer a “post-polio syndrome” 30-40 years later.

- 5-10% of those paralyzed die when breathing muscles become immobile
Poliomyelitis: an epidemic in developed countries
~1925 to ~1958

- Episodic epidemics in US during the summers
- Improved sanitation delayed the age of first exposures of infants to polio virus
- *First exposures were no longer protected by maternal antibodies*
- Increased viremia (in lymph nodes, likely) leads to increased chance of CNS infection
- Epidemic only waned after introduction of mass vaccination programs in late 1950’s (IPV) and early 1960’s (OPV)

Oral Polio Vaccine (OPV): “Sabin vaccine”

- Mixture of 3 serotypes (now two as of 2016 recommendation by WHO)
  - Attenuated through passage in mouse brains, then further passage in monkey cells
  - Attenuating mutations: 9 for type 1, 3 for type 2, and 5 for type 3 (one in the IRES along with others in the structural proteins). Appears to reduce initial viremia.
  - However, two mutations in serotypes 2 and 3 are sufficient for reversion to WT kinetics.
Oral Polio Vaccine (OPV): “Sabin vaccine”

- Easy to administer without training (oral liquid)
- Cheap (8¢/dose)
- Replication in intestine induces mucosal immunity and prevents new infections
- Virus is shed (“contact immunity”)
- Virus is shed: infection of immunocompromised hosts or naïve populations
- Reversion to wild-type (about 10 cases per year of vaccine-related poliomyelitis in US until ~1995 when only inactivated virus used)

Inactivated Polio Vaccine (IPV): “Salk vaccine”

Made by formalin-inactivation of virus

- No virus spread from vaccine
- No risk of vaccine-related poliomyelitis
- Induces serum antibodies that protect against infection of the CNS
- Does not protect against infection of the intestine. Vaccinated people can still be infected (but won’t get poliomyelitis). Does not stop spread.
- Needs to be injected (trained personnel)
- Cost (5X that of OPV plus cost of needles and trained health worker)
Cases of Poliomyelitis in the U.S. from 1951-2004

why were these decisions made?

Polio: “the endgame”
Wild polio type 2 has disappeared

**Wild Poliovirus Cases**, Previous 12 Months (July 2014)

- Excludes cases caused by vaccine-derived polioviruses and viruses detected from environmental surveillance.
- Data in WHO HQ as of 08 July 2014

Good progress in the past year!

- **YEAR-TO-DATE 2017**
  - Jan 1 - Jun 21, 2017
  - 6 OPV
  - 6 cOPV

- **2016 TOTAL**
  - Jan 1 - Oct 31, 2016
  - 37 WPV
  - 5 cIPV

The plan for final eradication calls for switch from OPV to IPV worldwide when there are no cases of wild polio detected (by 2018)

http://polioeradication.org/
Rhinoviruses: responsible for ~1/2 of “common colds”
Over 150 types. Almost no cross protection.

Clade A and B discovered by culture, Clade C discovered using molecular techniques
Unlike polio, they are unstable at low pH
Grow at 33-35°C, so limited to large airways

Symptoms thought to be largely immune-mediated

Things you should know about picornaviruses

- General characteristics of family
- Diversity of family
- Replication steps including polyprotein cleavage, inhibition of host cell protein synthesis, and switch from translation to replication
- Vaccine issues
- Other picornaviruses

Paper for next time:
- What are the problems with the current vaccines that they are trying to address?
- What was their strategy?
- What is the evidence that it worked?
- What would be be path forward?