Flavivirus family

- More than 70 enveloped RNA viruses – human and animal pathogens
- Dengue, Japanese encephalitis, West Nile and Yellow fever virus
- Hemorrhagic fevers: DENV and YFV  Encephalitis: WNV and JEV
- Arthropod-borne viruses – Aboviruses
General properties of Flaviviruses:
Virion structure

- 50 nm in diameter
- Enveloped
- Icosahedral capsid
- (+)ssRNA virus

C = capsid
M = membrane
E = Envelope

General properties of Flaviviruses:
Genome Structure

~10-11 kb  + ssRNA  Nonsegmented
General properties of Flaviviruses: Genome Structure

- **5’ \(7\text{mGpppAm}\)**
  - Stabilizes the viral RNA
  - Initiate translation
  - Subvert innate antiviral defenses

- **5’ Noncoding regions (5’ NCR)**
  - Bifurcating 5’ stem-loop (5’ SL) — conserved structure across flaviviruses
    - Influence viral genome translation
  - Promoter to initiate RNA replication — binding to NS5

- **3’ Noncoding regions (3’ NCR)**
  - Differs between mosquito-borne viruses and tick-borne viruses
    - Secondary structures
    - Sequence duplications
    - 3’ stem loop region
      - Enhances translation of reporter mRNAs
      - Interacts with several important viral proteins

Where else have we seen RNA motifs implicated in the viral life cycle?
### General properties of Flaviviruses: Genome organization

<table>
<thead>
<tr>
<th>Viral protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Capsid protein associates with RNA genome</td>
</tr>
<tr>
<td>M</td>
<td>Small proteolytic fragment of the precursor (pr)M produced during viral maturation</td>
</tr>
<tr>
<td>E</td>
<td>Mediates binding and fusion during virus entry. Covers completely the virus envelope.</td>
</tr>
<tr>
<td>NS1</td>
<td>Integral to virulence and pathogenesis. Plays role in genome replication (with NS4). Inhibits TLR signaling.</td>
</tr>
<tr>
<td>NS2A and B</td>
<td>Assembly of viral replication complexes.</td>
</tr>
<tr>
<td>NS3</td>
<td>The N-terminal serine protease functions with its essential cofactor NS2B in the processing of the polyprotein, while the C-terminal NTPase/helicase performs ATP-dependent RNA strand separation during replication</td>
</tr>
<tr>
<td>NS4A and B</td>
<td>RNA replication (with NS1), membrane rearrangement</td>
</tr>
<tr>
<td>NS5</td>
<td>RNA-dependent RNA polymerase, methyl transferase activity</td>
</tr>
</tbody>
</table>

How is this similar and different to alphaviruses?

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Translation of the polyprotein.</td>
</tr>
<tr>
<td>2</td>
<td>Co- and post-translational processing to produce the structural proteins and the nonstructural proteins</td>
</tr>
<tr>
<td>3</td>
<td>Synthesis of complementary, negative-sense RNA by the RNA-dependent RNA polymerase NS5 and other viral replicase components.</td>
</tr>
<tr>
<td>4</td>
<td>Synthesis of progeny genomes by transcription of the negative strand.</td>
</tr>
<tr>
<td>5</td>
<td>Progeny genomes are packaged into nucleocapsids and bud intracellularly to acquire envelope.</td>
</tr>
</tbody>
</table>
- Concentrate viral and host components and improve the efficiency of replication
- Anchor the viral replication complex
- Conceal the viral RNA replicative intermediates from host cell surveillance mechanisms

Structural features of flavivirus NS5
Varied mechanisms of NS5-mediated antagonism of IFN-I-dependent signaling in flavivirus-infected cells

Summary of Flavivirus life cycle
Yellow fever clinical features and pathogenesis

**INCUBATION:**
~3-6 days

**PERIOD OF INFECTION:**
~3 days: Fever, headache, myalgia, nausea, vomiting
Replication in draining lymph nodes, primary serum viremia, cytokine release

**PERIOD OF REMISSION:**
~24 hours: Defervescence, mitigation of symptoms
Virus clears from serum, antibody appears

**PERIOD OF INTOXICATION:**
Fever, jaundice, kidney failure, hemorrhaging
Replication in the liver hepatocytes, secondary serum viremia, cytokine storm

**DEATH:**
20-50% of people that develop severe disease

Yellow fever endemic regions

Endemic to tropical regions in Africa and the Americas

200,000 cases per year, 30,000 deaths
South America

Jungle (Sylvatic) → Nonhuman Primate → mosquito → human → Aedes aegypti → urban

Africa

Aedes africanus → simian host → human → Aedes aegypti → urban

Simian hosts in South America}

Simian hosts in Africa
**Introduction of Yellow Fever to the Americas**

Phylogenetic tree constructed using prM/E sequences

- Why does the tree indicate this pattern of emergence?
  1. YF isolates are monophyletic with respect to region
  2. YF from South America branches with YF from West Africa to the exclusion of YF from East Africa

Using average mutation fixation rates to extrapolate the ages of nodes...

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**History of Yellow Fever (YF)**

- YF was imported into the Western Hemisphere on slave ships from West Africa
- Yellow fever spreads to Europe
- 2,200 deaths in Cadiz, Spain
- 1648

- First recorded epidemic in the Yucatan peninsula
- 26,000 people infected in New Orleans
- Other outbreaks in US eastern coast
- 1700

- 1800
- Carlos Finlay starts working on the mosquito vector hypothesis
- Nobody believed him...

- 1819
- 5,162 deaths in Havana, Cuba
- New epidemics in Europe
- 1880

- 1881
- U.S. Yellow Fever Commission arrives in Cuba
- Walter Reed, Aristides Agramonte, James Carroll, and Jesse Lazear

- 1898
- Spanish-American war
- For every soldier who died in battle, 13 died of yellow fever

- 1898
- Jesse William Lazear contracts YF and dies

- 1900
- Walter Reed reports that YF is transmitted by mosquitoes
- Determined YF was caused by a filterable agent
1901-1925: The “golden age” of anti-mosquito efforts

- **1899**: General William Gorgas to institute a sanitation campaign in Havana against the urban mosquito vector, eliminating the disease in 1902.

- **1904**: The Panama canal construction had been hampered severely by yellow fever infection among the workers.

- **1906**: Sanitation campaigns led by General Gorgas were directed to eradicate the mosquitoes *Aedes aegypti* and *Anopheles*, the carriers of yellow fever and malaria, respectively, from the canal zone. Canal was completed by 1914.

- **1926**: New epidemics in South America reveal yellow fever in the absence of *Aedes aegypti*. Seminal discovery that yellow fever was a zoonosis, maintained by sylvatic mosquito species and nonhuman primates in the Amazon jungle.

**Last yellow fever outbreak in the USA: New Orleans, 1905**

Improved sanitation and mosquito abatement programs eliminated epidemic urban YF from North American and European cities, with the last outbreak occurring in New Orleans in 1905.
Why is YF not present in all the areas where Aedes aegypti is?

Vaccination requirement for travelers
Yellow fever vaccine "YF-VAX"

- Developed in 1937 by Max Theiler
- Attenuated virus still retained its capacity to replicate and induce and immune response
- Loss of mosquito competence
- Best preventive measure against outbreaks
- Safe and affordable
- A single-dose provides life-long protection
- Seroconversion to YF within two weeks
- Established protocol for generating other vaccines.

Development of YF vaccine "17D"

- Asibi strain
  - Deadly to Rhesus monkeys
    - Passaged 53X in monkeys
    - Intermittent passages in A. aegypti
  - Vaccine parental strain
    - Passaged in mouse embryonic tissue 18X
      - 71
    - Passaged in minced in whole chicken embryo 50X
      - 121
    - Passaged in chicken embryos without nervous tissue 152X
      - 17D-204

Marked reduction in vicro- and neurotropism!

Explain in terms of fitness landscapes
Differences between Asibi and 17D

- 32 amino acid changes
- 0.94% difference between strains

<table>
<thead>
<tr>
<th>REGION</th>
<th>TOTAL NT/AA</th>
<th>CHANGE NT/AA</th>
<th>% CHANGE NT/AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' Noncoding</td>
<td>118 / -</td>
<td>0 / -</td>
<td>0 / -</td>
</tr>
<tr>
<td>Capsid</td>
<td>363 / 121</td>
<td>2 / 0</td>
<td>0.55% / 0</td>
</tr>
<tr>
<td>N</td>
<td>267 / 89</td>
<td>0 / 0</td>
<td>0 / 0</td>
</tr>
<tr>
<td>Envelope</td>
<td>225 / 75</td>
<td>2 / 1</td>
<td>0.89% / 1.89%</td>
</tr>
<tr>
<td>M</td>
<td>1479 / 493</td>
<td>15 / 12</td>
<td>1.01% / 2.43%</td>
</tr>
<tr>
<td>NS1</td>
<td>1227 / 409</td>
<td>5 / 2</td>
<td>0.41% / 0.49%</td>
</tr>
<tr>
<td>NS2a</td>
<td>501 / 167</td>
<td>6 / 5</td>
<td>1.20% / 2.99%</td>
</tr>
<tr>
<td>NS2b</td>
<td>390 / 130</td>
<td>4 / 2</td>
<td>1.03% / 2.31%</td>
</tr>
<tr>
<td>NS3</td>
<td>1859 / 623</td>
<td>9 / 2</td>
<td>0.48% / 0.32%</td>
</tr>
<tr>
<td>NS4a</td>
<td>861 / 297</td>
<td>6 / 3</td>
<td>0.70% / 0.78%</td>
</tr>
<tr>
<td>NS4b</td>
<td>336 / 112</td>
<td>2 / 1</td>
<td>0.60% / 0.69%</td>
</tr>
<tr>
<td>NS5</td>
<td>2715 / 905</td>
<td>11 / 4</td>
<td>0.40% / 0.44%</td>
</tr>
<tr>
<td>3' Noncoding</td>
<td>511 / -</td>
<td>6 / -</td>
<td>1.17% / -</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10862 / 3411</td>
<td>68 / 32</td>
<td>0.63% / 0.97%</td>
</tr>
</tbody>
</table>

Vaccine production

https://www.youtube.com/watch?v=lZhuUrXNpSM&t=81s
2016 YFV outbreak in Angola, Democratic Republic of Congo and Uganda

- First outbreak in 28 years!
- Urban transmission = quick spread in dense unvaccinated population!

Lessons on YF control:
1. Potential large scale outbreaks will become more common due to urbanization in Africa
2. Vaccine production needs to be scaled up.
3. International regulations to required YF vaccination certificates for travellers

2017 YFV outbreak in Brazil

Low natural immunity in the regions = YFV kills 1/3 of those who contract it

- Hundreds were vaccinated daily!
- Vaccine production is difficult to scale up
- Vaccine is too good and doesn’t generate profits
Things You Should Know About Flaviviruses

• Differences & similarities to other RNA viruses
• Differences between sylvatic and urban transmission cycles
• Causes for urban outbreaks
• Importance of vaccination in naïve populations
• Current epidemic in South America