Understanding Zika Virus Structure and Replication

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Zika Virus

- A member of the *Flaviviridae* family of RNA viruses
- Genome is single positive strand of RNA ~ 10,600 nt
- Transmitted by mosquitoes
- Virus particle contains a lipid bilayer, one genome RNA, and three distinct types of viral proteins, : 
  E - envelope protein
  M - membrane protein/prM – premembrane protein
  C - capsid or core protein
Flavivirus Life Cycle

A. Virus attachment and entry

B. Virus fusion and disassembly in the endosome

C. Protein translation, polyprotein processing on membranes

D. Viral RNA replication on membranes

E. Immature virus assembly and budding into ER

F. Virus maturation (Furin cleavage of prM)

G. Mature virus release

ER, Nucleus, TGN, Golgi
Flavivirus Proteome

- **5' NTR**: CAP
- **3' NTR**: NS5 polymerase (RdRp)
- **Structural proteins**: 5' NTR, 3' NTR, Capsid
- **Replicase proteins**: NS1, NS2b/NS3, NS3, NS4A, NS4B, NS5
- **PrM-E glycoproteins**: prM, M, E
- **E glycoprotein**: Mature conformation
- **NS2b/NS3 Protease**: prM-E glycoproteins
- **NS3 helicase**: prM-E glycoproteins
- **NS5 Methyltransferase**: prM-E glycoproteins
- **NS5 polymerase (RdRp)**: prM-E glycoproteins

- **Signalase (ER lumen)**
- **unknown protease (ER lumen)**
- **furin (Golgi)**
- **NS2B-NS3 (cytoplasm)**
Flavivirus E Protein

• Envelope (E) protein is the major surface protein

• E protein is responsible for targeting to virus to host cell receptors; it then functions to fuse virus and cell membranes to allow virus entry

• E protein is the major target for antibodies; however in dengue, patients make antibodies to the prM protein
Potential differences between Zika and other flaviviral structural proteins

- Protein size
- Sequence/Structure
- Oligomeric status
- Post-translational modification (N-linked glycosylation)
- Furin cleavage sequence
Percentage amino acid identity of Zika virus (H/PF/2013) with other flaviviruses

<table>
<thead>
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<th>Viruses</th>
<th>prM</th>
<th>E</th>
<th>NS3</th>
<th>NS5</th>
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Dengue virus
Structure determined using cryo-electron microscopy
Maturation of Dengue

Immature = Non-infectious

Mature = Infectious
Maturity, Infectivity and Pathogenesis

- Continuum of particles of variable maturity due to inefficient cleavage of prM by Furin.

- prM cleavage is required for particles to be infectious - Extent of prM cleavage required for the transition from non-infectious to infectious particle is unknown.

- Presence of prM influences interaction of virions and antibodies.

- Antibodies against prM have weak neutralization activity and enhance infection (ADE).

- Patients experiencing secondary infection have been reported to have elevated levels of antibodies against the prM protein.
Cryo-Electron Microscopy of Zika Virus

Sirohi, Chen, Rossmann & Kuhn (Unpublished data)
Unanswered Questions on Zika Virus Structure

- The dengue virus population is pleomorphic - Different states of dengue exist due to variable prM content, temperature dependent transition and protein dynamics. How does the structural heterogeneity of Zika contrast with other flaviviruses, especially dengue?

- Are there unforeseen surprises in Zika structure or it is similar to dengue and West Nile virus structures? What unique features of the surface proteins exist to influence antibody response? If there are subtle or significant changes, how do they affect interaction with neutralizing antibodies and/or cellular receptors?

- How does the structure of Zika influence receptor interactions and govern virus tropism? How will the structure inform function of receptor usage, tropism and entry?
- Easy rapid access to Zika-related news and data
- Consistent annotation of mature peptide predictions
- Zika genotype annotations
- Comparative genomics tools for all Flaviviruses
- Personal workspaces for storing private data and analysis results