properties of the C protein is poorly understood. This study aims to characterize the C and RNA interaction. Method: Co-localization study was performed by transfecting in vitro transcribed WN virus RNA and C protein clones into BHK cells and visualized under fluorescence microscopy. RNA binding properties of C protein were further investigated with a Northwestern Blot assay and RNA pull-down assay. Synthesized C protein peptides were used to map out the RNA binding regions on C protein. In addition, C protein immunopurified from BHK cells were used to investigate the effect of phosphorylation of C protein on its RNA binding properties.

*Results:* RNA and C protein have failed to show co-localization in BHK cells by immuno-fluorescence but interactions were observed at the molecular level. It showed that the first 465 and last 693 nucleotides of the WN virus RNA had specific affinity for the full length C protein. In addition, the amino- and carboxy-terminal of the C protein were shown to bind to the virus RNA. It was also found that the C protein had affinity for viral anti-sense RNA. Phosphorylated peptides of C protein and C protein expressed in BHK cells show attenuated binding to viral RNA.

*Conclusion:* C protein interaction with anti-sense indicates that its interaction with viral sense RNA may not be specific. Phosphorylation of C protein could play a role in regulating C and RNA interaction and allow time for viral assembly. Understanding the interaction of C protein with viral RNA and role of phosphorylation in nucleocapsid assembly could help develop anti-viral strategies aimed at disrupting viral assembly.

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**46.007**

## Identification of Critical Molecular Determinants of West Nile Virus PrM Protein: A Potential Site for Antiviral Targeting

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*Background:* West Nile virus (WNV), a member of Flaviviridae family is a viral agent that transmitted by numerous species of mosquitoes and birds. It is known to cause fever, paralysis and encephalitic maladies in infected humans and animals. The WNV particle is made up of premembrane (prM), envelope (E) and capsid proteins. The prM protein mediates the folding of the viral envelope (E) protein and protects the E protein against premature acidification during virus maturation. To date, critical residues responsible for prM function in viral assembly are yet to be defined. There is no antiviral drug or vaccine available for treatment of WNV infection in humans. Understanding the molecular mechanism of virus assembly will identify new vulnerable sites of virus replication and expedite the discovery and development of new therapeutics to treat WNV-induced diseases.

*Methods:* In this study, multiple sequence analysis identified a number of highly conserved residues in the prM protein among the flaviviruses. To investigate the significance of these residues, multi-site and single point mutagenesis work in WNV infectious clone were carried out to generate assembly were carried out using plaque assay, biochemical techniques, immunoflourescence and transmission electron microscopy.

*Results & conclusion:* Our study discovered that Tyrosine 78 is absolutely required for virus infectivity and replication in BHK and C6/36 cells. This is supported by positive and negative complementation assays performed by expressing wildtype and Y78A prM protein in trans. The Tyrosine 78 residue is also highly conserved in Dengue and Japanese Encephalitis, raising the possibility that this amino acid is equally important. Future experiments will explore various molecular strategies targeting Tyrosine 78 residue. This will potentially unravel a broad-spectrum assembly inhibitor against the flaviviruses.

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**46.008**

## The Role of Dengue NS1, Through the Modulation of STAT3 Signaling, in the Pathogenesis of Dengue Virus Infection

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*Background:* In an earlier study, NS1 was also shown to be able to interact with STAT3 β, an acute-phase protein and NS1 over-expression in dendritic cells results in increased production of pro-inflammatory cytokines. These results points towards a role played by NS1 in the pathogenesis of dengue virus infection. Hence, this study aims to further our current understanding of this role played by NS1, in particular, its effects on STAT3 signaling.

*Methods:* First, the yeast-two-hybrid (Y2H) system was used to map the interaction domains between STAT3 β and NS1. This technique was also used to probe for the ability of NS1 to interact with the α-isoform of STAT3. Co-immunoprecipitation and immunofluorescent assays were also used to investigate this interaction.

*Results:* From the Y2H screen, the interaction domain was mapped to the coiled-coiled domain of STAT3. Interestingly, however, STAT3 α, fail to interact with NS1 in the Y2H system. Further experiments in the mammalian system using co-immunoprecipitation and immunofluorescent assays also support this finding that STAT3 α was incapable of interacting with NS1.

*Conclusion:* Since STAT3 α activation is known to negatively regulate STAT1-dependent type I interferon antiviral response and STAT3 β is known to be the dominant negative form of STAT3 α, this unique interaction of NS1 with STAT3 β only, could potentially reduce the inhibitory effect of STAT3 β on STAT3 α, thereby increasing STAT3 α activation, consequently down-regulating the type I interferon antiviral response. This modulation of STAT3 signaling could be one of the strategies employed by dengue virus to evade the host anti-viral immune response.

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