

## RECENT ADVANCES IN THE MOLECULAR BIOLOGY OF FLAVIVIRUSES

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### INTRODUCTION

The flaviviruses remain as important causes of morbidity and mortality in the Southeast Asia and Western Pacific region. Dengue and Japanese encephalitis viruses are by far the most important and relevant in our region but others, such as Murray Valley encephalitis virus and West Nile virus, cause periodic outbreaks of human disease. After relatively low activity during 1983 - 1985, dengue disease activity increased in Malaysia during 1986. In other parts of the world, massive dengue outbreaks occurred in Nicaragua in 1985 (> 100,00 cases) and in Brazil in 1986 (> 500,000 cases). In addition, a recent outbreak of Japanese encephalitis in Northern Sri Lanka with 47 fatal cases illustrates the continuing threat posed by the flaviviruses.

Despite the advent and widespread application of recombinant DNA techniques in the last decade, study of the flaviviruses at the molecular level has lagged behind due to several well-known problems; few researchers have been tempted to venture into this world of slow-growing and low-titred viruses. Additionally, the flaviviruses do not shut off, or only partially affect, host cell macromolecular synthesis. Progress has been made, however, and a significant amount of new and important information has been generated recently which may have wide-ranging implications for many aspects of flavivirus biology and disease.

### RECENT ADVANCES IN MOLECULAR BIOLOGY

The excitement was started by the elucidation of the complete nucleotide sequence of the prototype flavivirus, yellow fever virus (17D vaccine strain), by Rice *et al.*<sup>1</sup> This study revealed several important and unique features of the yellow fever genome. It is 10,862 nucleotides in length and contains a single, extremely long open reading frame (a long stretch of triplet codons which are not interrupted by any translational stop codons) of 10,044 nucleotides which could encode a polypeptide of 3348 amino acids (molecular weight of approximately 373,000 daltons)

and thus include all three viral structural proteins (C, M, E) and all twelve nonstructural proteins (e.g. NS1, NS3, NS5). The genes for the viral structural proteins were found at the 5' end of the genome in the sequence of C (core or nucleocapsid protein), M (virion envelope protein) and E (major envelope glycoprotein).

Rice's ground-breaking work is being rapidly confirmed and extended to other flaviviral genomes. The Wengler laboratory in Giessen, Germany have similarly obtained partial nucleotide sequence data with West Nile virus showing the presence of an open reading frame of 924 nucleotides at the 5' end of the genome. Comparison of this nucleotide sequence with partial amino acid sequences of purified viral proteins, indicate that the open reading frame corresponds to the part of genome coding for the viral proteins C, ns2a (present in cell-associated virus), M and the beginning of E.<sup>2</sup> Dalgarno and Weir, in collaboration with Rice, have also sequenced about 60% of the Murray Valley encephalitis (MVE) virus RNA and similarly confirmed the existence of a long open reading frame with considerable sequence homology to yellow fever virus.<sup>3</sup> Similar findings are in the pipeline with dengue-4 virus (Ching Juh-Lai, NIH, USA), dengue-1 virus (Fournier *et al*, Amherst, Massachusetts), St. Louis encephalitis (SLE) virus (Trent *et al*, CDC, Fort Collins, USA) and Japanese encephalitis virus (JEV) (Fournier *et al*, University of Massachusetts, Amherst, USA; Yasui *et al*, Tokyo, Japan). Additionally, the Fournier lab are well into experiments to express the E and NS1 proteins of both JEV and dengue-1 virus in *E. coli* and preliminary immunization studies in mice are being carried out.

### IMPORTANCE OF NS1

The interest in the NS1 (previously NV3) nonstructural protein of flaviviruses is not merely academic or fortuitous but closely linked to another recent report by Schlesinger which demonstrated that immunization of mice with purified NS1 protein resulted

in solid protection from lethal yellow fever virus challenge, in the absence of detectable anti-virion **antibody**.<sup>4</sup> More importantly, this finding has recently been extended and repeated in primates. The importance of these observations cannot be overemphasized as it represents the first report of a nonstructural protein inducing protective immunity to a flavivirus infection *in vivo*.

### IMPLICATIONS

These exciting new developments have some obvious and important implications. From the viewpoint of basic virology, data on the structure and organization of flavivirus genomes is of obvious value in attempts to elucidate the **mechanisms** of viral gene expression, replication and assembly. For example, Rice's results may have put an end to an old controversy regarding the mechanism of translation of flaviviral RNA. The organization of the yellow fever genome suggests that mature viral proteins are produced by post-translational cleavage of a large, **poly-protein** precursor rather than through multiple, internal initiation sites as has been proposed previously by Westaway.<sup>5</sup>

There are also interesting implications for evolution of the flaviviruses. Based on Rice's results, sequence homologies have been found between yellow fever and the polymerase genes of a number of plant and animal viruses. As suggested by Monath, this may point to a common ancestry or **parallel** evolution of the viruses and also conservation of vital **regions** of the genome involved in virus **replication**.<sup>6</sup>

Most importantly, the cloning of flaviviral proteins also paves the way towards identification of important antigenic epitopes and represents the first step towards the development of recombinant vaccines in the form of synthetic **peptides** or by cloning into **heterologous** carrier viruses such as vaccinia. In fact, some of the cloned flavivirus genes have already been inserted into vaccinia virus and expressed *in vitro*. Another intriguing possibility pointed out by Monath<sup>6</sup> would be to use the 17D strain of yellow fever virus, a proven vaccine strain, as a vector for other flaviviral genes. However, the studies of **Schlesinger** quoted above, would seem to warrant a word of caution regarding the design of flaviviral recombinant vaccines. **Schlesinger's** results clearly show that protective immunity to a flavivirus may not depend solely on the antibody response to **virion** structural proteins. It is thus clear that a recombinant vaccine should effectively stimulate the appropriate host

immune response but several other important factors need to be considered. The vaccine may need to possess the following properties: optimum conformation and topology of important antigenic epitopes, ability to stimulate T cell proliferative responses (including T helper cells) and efficient presentation of antigen to macrophages and other **antigen-presenting** cells (including correct association with self MHC antigens). At the same time, an ideal vaccine would perhaps not contain determinants which stimulate T suppressor cells, which can greatly reduce immune responses, or which induce the formation of enhancing antibodies, **which** have been implicated in the **pathogenesis** of dengue haemorrhagic fever.

In relation to the pathogenicity of **flaviviruses**, future studies based on detailed knowledge of genome structure and organization may also be directed towards identification of virulence determinants which may ultimately explain the diverse and complex pathologies and differential tissue tropisms characteristic of the flaviviruses.

Furthermore, the available battery of cloned genomes of the various flaviviruses is of great potential value as diagnostic probes which may result in more rapid, specific, sensitive and economical means of laboratory diagnosis of the various flaviviral infections.

There are thus good reasons to believe that these recent advances in knowledge will herald an exciting era of research where a better understanding of flavivirus biology will lead to more effective control of human disease.

### REFERENCES

1. Rice CM, Lenches EM, Eddy SR, Shin SJ, Sheets RL, Strauss JH. Nucleotide sequence of yellow fever virus : Implications for flavivirus gene expression and evolution. *Science* 1985; 229 : 726-33.
2. Castle E, Nowak T, Leidner U, Wengler G, Wengler G. Sequence analysis of the viral core protein and the **membrane-associated** proteins V1 and NV2 of the flavivirus West Nile Virus and of the genome sequence for these proteins. *Virology* 1985; 145 : 227-36.
3. Dalgarno L, Weir RC, Rice CM. Partial nucleotide sequence of Murray Valley encephalitis virus. *J Mol Biol* 1986; in press.

4. Schlesinger J, Brandriss MW, Walsh EE. Protection against 17D yellow fever encephalitis in mice by passive transfer of monoclonal antibodies to the nonstructural glycoprotein gp48 and by active immunization with gp 48. *J Immunol* 1985; 135 : 2805-9.
5. Westaway E. Replication of flaviviruses. In : Schlesinger RW, cd. *The Togaviruses*, Academic Press, New York, 1980; 531-81.
6. Monath TP. Glad tidings from yellow fever research. *Science* 1985; 229 : 734-5.