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**STRUCTURE AND FUNCTIONAL STUDIES ON DENGUE-2 VIRUS GENOME**

**FINAL Report**

**Radha Krishnan Padmanabhan, Ph.D.**

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**University of Kansas Medical Center  
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## ABSTRACT (March 1, 1982 - August 31, 1985).

1. Conditions were established for the attachment of oligo(A)<sub>10</sub> tail to the 3'-end of Dengue RNA in order to facilitate cDNA synthesis by oligo d(T) priming as proposed in the original research project.
2. We also showed that random primers generated by exhaustive digestion of calf thymus DNA could also serve as efficient primers for cDNA synthesis. A library of clones were obtained using M13 mp8 vector. Some of these M13 clones were sequenced to a total of 1.5 kilo base pairs.
3. We found that oligo d(T) alone could prime cDNA synthesis by reverse transcriptase without necessitating the oligo(A) addition. 3' terminal labeling and sequence analysis of the RNA suggested the presence of poly A tail at the 3' end of the genome (Report Number three). These experiments could not rule out the possibility that our dengue viral preparations was contaminated with small quantities of a messenger RNA from the host. The latter might have been very efficiently labeled compared to the viral RNA due to the lack of secondary structure and would have given rise to erroneous results. Therefore other possibility that the priming occurred at internal regions, possibly at purine rich regions could not be ruled out.
4. Eleven cDNA clones were obtained by oligo d(T) priming, ranging in size from 0.95 -2.0 kilo base pairs.
5. Nine clones were characterized by restriction enzyme analysis and Southern hybridization.
6. Three clones were chosen for structural analysis. Complete DNA sequence analysis of these three clones totalling 4586 nucleotides were obtained.
7. Computer analysis of the primary DNA sequences of DEN-2 cDNA clones showed that the DNA sequences had the coding potential for a 885 and 643 amino acid long polypeptides.
8. Computer alignment of the deduced polypeptide sequences with that of yellow fever virus polyprotein precursor published by James H. Strauss and coworkers showed that the dengue viral polypeptides mapped in the non-structural region of the yellow fever virus genome.
9. Hydrophobicity profiles of the dengue viral polypeptides and the corresponding yellow fever virus polyprotein were compared. The data indicated that the profiles are strikingly similar in several regions although the amino acid sequence homology ranged from 15-51% in these regions, suggesting that the polypeptides of these two flaviviruses share many common functions in the life cycle of these two viruses.
10. The results indicated that our clone specifically hybridized to the viral RNA from the dengue virus infected cells and not to the RNA from the uninfected cells.
11. Dr. Erik Henchal used our clones for diagnostic studies and showed that greater than 1000 pfu of virus can be detected in 24 hours and as little as 160 pfu in 48 hours by hybridization and autoradiography (Henchal et al., 1985, 34th Annual Meeting of ASTMH - Miami, Florida, Nov. 1985). These cDNA clones showed a great promise for the rapid diagnosis of dengue viral infections among patients under the clinical laboratory setting.

## FOREWORD

The investigators have abided by the National Institutes of Health Guidelines for Research involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements, as indicated in the Memorandum of Understanding and Agreement, reviewed originally by Dr. Larry H. Baker (late), Chairman of the Institutional Biosafety Committee and by Dr. Stanley Barban, Scientific Administrator, Office of Recombinant DNA Activity, N.I.H.

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## OBJECTIVES:

The overall objective of this project is to analyze the structure of Dengue-2 virus RNA using recombinant DNA technology in order to understand the biological role of the specific antigens coded by the RNA genome after infection of the host cell. The proposed project is to be carried out in the following steps. 1) Synthesize the cDNA copy of the viral RNA using reverse transcriptase; 2) Clone the cDNA using E.coli host/vector system in order to get large amounts of cDNA; 3) Physically map these cDNA clones using several restriction endonuclease; 4) Location of the genes for the various viral antigens on the cDNAs is to be identified; 5) DNA sequence analysis of cDNA coding for specific viral genes is to be carried out; 6) Finally, the gene for V3 (the glycoprotein E) is to be expressed in E.coli.

## INTRODUCTION:

Dengue viruses are members of the flavivirus family which contain positive stranded, non-segmented RNA genome of approximately 11 kilobases in length. The viruses are classified into four different serotypes which are distributed throughout the world and are causative agents for dengue and dengue haemorrhagic fever in humans. The virus replicates in vertebrate as well as invertebrate cells. The nucleocapsid contains the single-stranded plus RNA associated with a nucleocapsid protein, C. The viral envelope consists of one large glycoprotein, E with an approximate size of 59 Kd and a small non-glycosylated membrane associated protein,  $\mu$ M. The structural components of flaviviruses (Schlesinger, 1977; Russell, et.al., 1980) and their replication strategy (Westaway, 1980) have been reviewed.

The 42S Dengue viral RNA has a 5' end that is capped and a 3' end that is non-polyadenylated (Wengler et al., 1978; Cleaves and Dubin, 1979; Wengler and Wengler, 1981). The absence of poly(A) tract at the 3' end seems to be unique among the plus-stranded viruses (reviewed in Strauss and Strauss, 1983). Westaway (1977) proposed that

three structural polypeptides, C, E, and M as well as five non-structural polypeptides are separately initiated and terminated during translation. On the other hand, Wengler et.al. (1979) and Svetkin et al. (1981) reported that in an in vitro translation system, only a single initiation site appeared to be used. The knowledge of the entire sequence of the flavivirus genome and the deduced amino acid sequence of the polypeptides is required to understand the translation strategy used by the viral genome. The complete nucleotide sequence of the yellow fever virus RNA (17D vaccine strain), another member of the flavivirus family has been determined (Rice et al., 1985). The viral RNA is 10,862 nucleotides in length and contains a single long open reading frame of 10,223 nucleotides.

I report the molecular cloning of Dengue virus type 2 (DEN-2) genome, the characterization of cDNA clones and the nucleotide sequences of three cDNA clones which total to 4,586 nucleotides in length. Translation of these DNA sequences and the alignment of the putative polyprotein segments with the yellow fever polyprotein using the dot matrix homology program allowed us to map our cDNA clones with reference to the yellow virus genome. Significant homologies exist between these two viruses in the regions encoding non-structural proteins NS3 and NS5, suggesting an important role for these two proteins in the life cycle of the viruses.

## RATIONALE

Dengue and Dengue hemorrhagic fever are major health problems in tropical regions of the world. Current serological methods of detection of Dengue viral infections in humans is time-consuming and not very specific to a serotype due to extensive cross reactivities among dengue viruses. Due to recent advance in recombinant DNA technology, it has been possible to develop specific DNA probes for use in nucleic acid hybridizations with viral genomes present in extremely small amounts in clinical samples from the infected patients for the purpose of rapid diagnosis. These sensitive techniques have been used successfully in the diagnosis of human viruses such as

herpes simplex viruses, cytomegalovirus, Epstein-Barr virus, adenoviruses, hepatitis B virus and rotaviruses. A number of complementary DNA (to Dengue Virus RNA) probes some of which have already been developed by us are needed for use in rapid diagnosis of Dengue virus infections.

#### METHODS USED FOR THE PROJECT

This is the summary of the methods used to synthesize the cDNA to DEN-2 RNA and clone these cDNA molecules in E.coli host/vector system. Detailed methods used can be found in the Report Number 1-4 submitted annually.

(a) Isolation of RNA. Dengue-2 (DEN-2) virus (New Guinea strain) was grown in C6/36 clone of mosquito cell line (*Aedes albopictus*), mouse LLC cells or suckling mouse brain and the virus particles were purified by sedimentation on sucrose gradients as described (Smith et al., 1970; Trent and Qureshi, 1971). The virus particles were digested with proteinase K (500 µg/ml) at 37°C in the presence of 0.5% sodium dodecyl sulfate for a period of one hr. The mixture was extracted once with an equal volume of phenol saturated with 1M sodium acetate, pH 4.5, twice with anhydrous ether and then precipitated with two volumes of ethanol. Viral RNA was recovered by centrifugation in a siliconized glass tube (Corex brand from Corning Glass Co.). The pellet of RNA was dissolved in 20mM sodium acetate, pH 4.5.

(b) Synthesis of the cDNA copy of DEN-2 RNA. DEN-2 RNA (5 µg) was denatured with methylmercurichydroxide (Bailey and Davidson, 1976) and the denatured RNA was used as a template for cDNA synthesis by reverse transcriptase using oligo-d(T) as primer as described by Maniatis et.al. (1982). The conditions used for the second strand synthesis were the same as described by Gubler and Hoffman (1983). The size of the double-stranded cDNA was estimated by electrophoresis on alkaline agarose gel as



described by McDonnell et.al. (1977). Oligo(dC) tailing of double stranded cDNA was carried out in a reaction mixture (50  $\mu$ l) containing 100mM sodium cacodylate (pH 6.9), 1mM  $\text{CoCl}_2$ , 0.2mM dithiothreitol, unlabeled dCTP (64  $\mu$ M), 35 Ci of ( $\lambda$   $^{32}$ -P)dCTP and 17 units of terminal transferase. An average of 13 residues were added to each 3' end of cDNA. The cDNA was then fractionated by agarose gel electrophoresis and recovered from the gel by adsorption to glass powder in the presence of 6M  $\text{NaClO}_4$  and subsequent elution from glass powder using 10mM Tris-HCl, pH 7.5 containing 1 mM EDTA (Vogelstein and Gillespie, 1979).

(c) Cloning of the cDNA copy of DEN-2 RNA. The vector used for cloning the cDNA was the derivative of pUC13 constructed by Dr. Ray Wu (Cornell University, Ithaca, New York). The PvuII site present in pUC13, 200 bp upstream from the Hind III site (Vieira and Messing, 1982) was converted into a StuI site in pUC13'-1. The vector DNA (10  $\mu$ g) was linearized with PstI and then oligo(dG) tail was added to the 3' end using the terminal transferase under the same conditions as above except that (3H)dGTP (40  $\mu$ M) was used. It was estimated that about 10 dG residues were added to each 3' end of the vector DNA. Annealing of oligo(dG)-tailed vector (300 ng) and oligo(dC)-tailed DEN-2 cDNA (30 ng) was carried out by incubating the reaction mixture at 58°C for 60 min. The reaction mixture (25  $\mu$ l) contained 10mM Tris-HCl, pH 7.5, 1 mM EDTA and 150 mM NaCl. E.coli JM83 were made competent for transformation following the procedure described by Morrison (1979) and the cells were transformed with the annealed DNA following the procedure described by Guo et.al (1983). The transformed cells were plated on yeast extract tryptone agar plates containing ampicillin (100  $\mu$ g/ml), isopropyl thiogalactoside (4  $\mu$ g/ml) and X-gal (40  $\mu$ g/ml). The transformants (white colonies) were picked for further characterization.

(d) Screening of the transformants. The transformants were screened by colony hybridization (Grunstein and Hogness, 1975) as well as by restriction endonuclease cleavage of the plasmids isolated from these colonies. The probe used for colony hybridization was prepared by using calf thymus random primers (Taylor et.al., 1976; Rice et al. 1981) and reverse transcriptase on DEN-2 template. The plasmids isolated from these colonies were digested with PstI and the sizes of the cDNA released were estimated by electrophoresis on agarose gels.

(e) Generation of subclones of DEN-2 cDNA by Bal-31 nuclease digestion for DNA sequence analysis. The subcloning of DEN-2 cDNA clones was carried out according to Guo et.al. (1983). The plasmid was linearized with SalI and was digested with Bal-31 in a reaction mixture containing 20 mM Tris, pH 8, 12 mM MgCl<sub>2</sub>, 12 mM CaCl<sub>2</sub>, 600 mM NaCl, 1 mM EDTA and 10 units of Bal-31. The reaction was stopped by EDTA (25 mM). The DNA was phenol extracted and ethanol precipitated. The DNA was treated with Klenow's DNA polymerase and was subsequently ligated to SalI linkers (Maniatis et.al., 1982). The progressively shortened cDNA and vector DNA molecules were separated by digestion with PstI, followed by electrophoresis on low melting agarose. The cDNA molecules were eluted and were cloned between Sal-I and PstI sites of pUC13'-I using JM 83 as the host strain. The transformants were screened for the inserts to give a ladder of decreasing sizes which were spaced approximately 200 bases apart. A set of clones were chosen which exhibited this ladder for DNA sequence analysis.

(f) DNA sequence analysis of cDNA clones. The subclones of DEN-2 cDNAs generated by Bal-31 were digested with BamHI (site located at the shortened end of cDNA) to linearize it and labeled with ( $\lambda$ -<sup>32</sup>P dAMP) in a reaction catalyzed by DNA polymerase (Klenow's fragment) in the presence of unlabeled dNTPs. The 5-termini were labeled with polynucleotide kinase and ( $\lambda$ -<sup>32</sup>P) ATP as described by Maniatis et.al. (1982). The

labeled DNA was purified by electrophoresis on low melting agarose after digesting the DNA with PstI. The DNA was sequenced following the procedure of Maxam and Gilbert (1977).

## RESULTS AND CONCLUSIONS

(a) cDNA synthesis primed by oligo d(T). Studies carried out by different groups have shown that flavivirus RNAs do not contain a 3'terminal poly(A) tract (Wengler et al. 1978; Wengler and Wengler, 1981; Cleaves and Dubin, 1979; Vezza, et al., 1980; Duebel et al., 1983). The studies reported by Blok (1984, 1985) and by us for DEN-2 indicate that cDNA could be synthesized to DEN RNA from all four serotypes using oligo d(T)<sub>12-18</sub> as primer for reverse transcriptase. The 3' terminal sequences of DEN-2 genome is unknown at present. If it is similar to yellow fever virus RNA which contains purine rich regions (Rice et al., 1985), it is possible that oligo d(T) priming for cDNA synthesis was initiated from these regions.

(b) Screening of transformants. The transformation of Ecoli JM83 by the annealed mixture of pUC13'-1 vector and the cDNAs gave rise to eleven positive colonies when screened by colony hybridization (Grunstein and Hogness, 1975). These clones were screened further for the sizes of the DEN-2 cDNA insert by digestion with PstI. Nine clones, pVV1, 2, 4, 7, 8, 9, 11, 15 and 17 were found to release the cDNA insert, which ranged from 900 bp to 1950 bp. The clone, pVV17 had the largest cDNA insert (1.95 Kb). Two clones, pVV14 and pVV18 had an insert size of about 1.6 Kb each but seem to have lost one of the PstI sites.

Southern hybridization analysis of these cDNAs showed that the cDNA from pVV17 clone hybridized to the other cDNAs from clones, pVV2,4,7,8,11 and 15 but not to those from pVV1 and 9 (see Report Number Three). However, the cDNAs from pVV1 and

9 hybridized to each other and restriction enzyme cleavage analysis indicated that these clones overlapped by about 450 bp. Three clones pVV1, 9 and 17 were chosen for further structural characterization. In order to establish that these clones were derived from DEN-2 RNA, hybridization of nick-translated cDNAs from these clones to the total RNA isolated from uninfected and DEN-2-infected mosquito (C6/36) or mouse (LLC) cells were carried out. The probes hybridized specifically to the RNA isolated from the infected cells or from the virions used as control.

(c) Sequencing strategy. For the nucleotide sequence analysis of these three cDNAs, the strategy described by Guo et al. (1983) was used. In summary, the target DNA to be sequenced was progressively shortened from one end by digestion with Bal-31 nuclease. Then, synthetic restriction enzyme linkers were added to the shortened ends, followed by digestion with restriction enzymes to release the nested set of target DNA molecules. These DNA molecules were cloned into pUC13'-1 vector at the polylinker region. The sizes of the progressively shortened cDNA inserts were selected by mini plasmid preparation and restriction enzymes digestion such that they were approximately 200 bp apart. DNA sequence analysis of these subclones were carried out from the site which was progressively shortened by Bal-31 by using the chemical method of Maxam and Gilbert (1977). The nucleotide sequences of the cDNA subclones, generated from the parent cDNA clones (pVV1, 9 and 17) by Bal-31 nuclease overlapped with each other as expected. Any ambiguities in the sequence data were verified by sequencing independent subclones in the desired size range as well as by sequencing the complementary strand from the 5' terminus. The composite nucleotide sequence of the overlapping cDNAs from pVV1 and 9 clones and the sequence of the cDNA from pVV17 are given in Annual Progress Report number 4.

(d) Homology of the putative DEN-2 polypeptides with the polyprotein of yellow fever virus genome. Translation of the DNA sequence in all six possible reading frames indicated that there is one long open reading frame (ORF) in reading frame 3 for the entire length of both DNA sequences. Other reading frames contained no significant ORFs. The two putative polypeptides coded by these regions which are 885 and 643 amino acids in length, respectively are also shown in Annual Progress Report Four.

Since the complete nucleotide sequence of the yellow fever virus RNA is known (Rice et al., 1985) it was possible to align and map the DEN-2 polypeptides using the facility of Protein Identification Resource (PIR) on-line system (Georgetown; Washington D.C.). Fig. 1 (a and b) shows the alignment of the DEN-2 polypeptide 1 and 2 with the polyprotein of yellow fever in the region of 1046-1942 and 2132-2778, respectively.

The comparison of putative C-terminus of yellow fever virus NS1 which was assigned from the molecular weight estimates (Rice et al., 1985) with the corresponding region of DEN-2 virus genome shows that there is a fairly conserved region between amino acid no. 1047-1131 of YF polyprotein and the corresponding position 1-85 of the putative DEN-2 polypeptide 1 (see Fig. 1a and 2a). Fig 2a and 2b show that this region is essentially hydrophilic except for two small hydrophobic domains. However, in the C-terminal domain of NS1 between 1132- 1187 of the YF polyprotein, there is little amino acid sequence homology. Yet, there are two hydrophobic domains in this region which seems fairly conserved. The comparison of the region encoding the putative ns2a (1188-1355), ns2b (1356-1484) and a major portion of ns4a (2132-2394) of YF (Rice et. al., 1985) with the corresponding regions of DEN-2 (Figs 1a & 2a) show more similarities when their hydrophobicity plots are compared rather than their primary sequences. The percent homology between YF and DEN-2 sequences in the coding regions for the non-structural polypeptides varies from 15% in ns2a region to a value of about 51% in the NS3 and NS5 regions. The values of ns2b and ns4b are intermediate, being 32% and 37% respectively.

The putative coding regions for NS3 and NS5 appear to be fairly conserved in the two viruses examined (Fig. 1a & 1b; Fig. 2a-d), which suggest an essential role for these proteins in the life cycle of the virus.

Since the ORFs span through the entire length of the cDNA clones and the two putative DEN-2 polypeptides show homology to the region of the yellow fever polyprotein encoding non-structural proteins, it appears that the genetic organization of the DEN-2 genome is similar to that of yellow fever virus. The conserved domains of YF and DEN-2 support the notion that all flaviviruses descended from a common ancestor.

Assuming that the non-structural proteins will be produced by a similar proteolytic cleavage of the polyprotein precursors of yellow fever and DEN-2 viruses, these putative cleavage sites in DEN-2 polypeptides 1 and 2 were compared for homology with the assigned cleavage sites in the yellow fever virus polyprotein (see Table I). The sequences at these potential sites are very similar for YF and DEN-2 and in general contain double basic amino acids except for ns2a and NS3. For NS3 cleavage of DEN-2, the site corresponding to position 1484 of YF polyprotein, KQRA (Table I) does not contain double basic amino acids. However, another potential site, VKKQ containing this general feature is located two amino acids upstream. The exact cleavage sites of any of these flavivirus proteins can be accurately determined only from the knowledge of the NH<sub>2</sub>-terminal as well as the COOH-terminal sequence data. Further work is in progress to determine the complete nucleotide sequence of the DEN-2 genome in order to understand the genomic organization of this flavivirus.

During the last contract period, it was possible to analyze the structure of the dengue virus genome coding for the non-structural proteins. These results gave valuable information regarding the evolutionary relationship of dengue virus with another flavivirus (yellow fever virus). The sequence data is useful in the synthesis of oligonucleotide probes to be used as primers for the cloning and sequence analysis of the region encoding the structural proteins, especially the glycoprotein E. Such studies are in progress.

## APPENDIXES

Figure 1 Alignment of DEN-2 polypeptides 1 and 2 with the yellow fever virus polyprotein.

- a. For alignment, the polyprotein sequence of yellow fever virus genome published by Rice et al. (1985) between amino acid #1050-2000 and the DEN-2 polypeptide 1 from Fig. 6a (in Report Number Four) were used in the program from the Protein Identification Resource on-line system (Georgetown).
- b. alignment between the polyprotein from yellow fever virus between the amino acid #2150-2800 and the DEN-2 polypeptide 2 (from Fig. 6b of Report Number Four) is shown.

Figure 2 Hydrophobicity plots for the putative DEN-2 polypeptides 1(A) and 2(C) and the corresponding regions of YF polyprotein (B & D, respectively as shown in Fig. 1a & b). The plots were generated by the hydrophobicity program of PIR (Georgetown) on-line system, using the values of Kyte and Doolittle (1982). All other parameters were set to the program's default's values. Positive values represent hydrophobic regions, which are often buried in the interior of proteins. The locations of the assigned cleavage sites of YF polyprotein to give rise to non-structural proteins are indicated by vertical arrows (Rice et al., 1985). From left to right, in Fig. 2b, the arrows define one or both the boundaries for NS1, ns2a, ns2b, and NS3 respectively; and in 2D, for ns4a, ns4b and NS5, respectively.

DEN2 1 -----DLQLENDDFCEGTTVVVTEBCGNRPSLRTTASGKLIEMCCRSCLPPLRYRDEGDCWYQNEIRPLKEK  
 YF 1000 KECEWPLTHTIGTSVEEENFNPRSIGPVSNNHNPBYKVTNDPNDVPLEVKREACPSTSVIIDGNCBGRKSTRSTTDSOKVIPENCCRSCTHPPVSFHSBGCWYPNEIRPKTH  
 DEN2 74 EENLVNSLVTAGNDQIMFSLBVLGNALFLEENLRTRVSTKMAILLVAVSFTLITGNISFRDLGRVYVYVATNTDDIGNSVTYLALLAAFKVRPTLDBDYSLRKLTSELNMTTIGI-  
 YF 1120 ESHLVRSMVTAGEIHAVPFGLVSNHIANEVVLRKRDSPKQMLVGGVLLGAMLVGVVTLDBLLKLTVAVGLHFHEHNSGDANYMALIAAFSIRPGLLIGFGLRTLWSPRERLVLTGAA  
 DEN2 193 -VLLSQSTIPETILELTDALALGHDSKIVRKNEKYQLAVTINAILCVPNAVILQNAKVSCTILAVVSVSPLFLTSBOKADNIPALTIKGLNPTAIFLTLS---RTNKRSMPLG  
 YF 1240 NVEIALBSVNSGLMKYLNAVSLCILTINAVASRKAQNTILPLNALLTPVTHAEVRLAAMFFCAVVIIGVLDNFKBTQNKTIPLVALTITSYLGLTQPFGLCAFLATRFORRSIPVN  
 DEN2 308 EAINVGVNSILASSLLKNDIPHTGPLVAGLLTVCYLTVRSABLELERAADVKMEDRAEISGSSPILSITISEGDSIKNEEEERTLILIKTBLVIGSLFPVSPITAAANYLME  
 YF 1360 EALAAAGLVBLAGLAFQENEFLOPIAVGOLLNMLVSAQRVQGLEKKLBEVNEEEAEIGSSARYDVALSEDEFKLLSEEKVPVQVWVNTSLALVGAALHPFALLVLAGMLFHV  
 DEN2 428 VKKORAGVLWVPSPPPVSQAE--LEDBAYRIKKSILGYSQIGADYKESTFHTINVTBQVLMKKRLEPSMADYKDLVSCBGNKLEGEWKEGEEVQVLALEPGKHPRAVQTKP  
 YF 1480 RGARRSGVLDIPTPKIEECEMLEGDIYGFQBTFLBAGDRGVVYAGDVFHTINVTBQVLMKKRLEPSMADYKDLVAYBGNKLEGRVQEEVQVLAIAVPGKVVVWVQTKP  
 DEN2 546 GLFKT-NAGTIGAVBLDFSPGTSBPIDICKKVVOLYQNDVYTRDQAVSIAQTEKSJEMPEIEDDIFAKKL--TJNDLHPGAKTKRYLPAIVRGAIKRSLRTLILAPTRVAAE  
 YF 1600 SLFKVRNGEIGAVLBYPGTSBPVNDQDEVIQLYQNDILVQNDVFAIGQTEVKEEGKELOEIPHLKQNTTVLDFHPGAKTRFLPQILAEARRRLRTLILAPTRVVLSE  
 DEN2 663 NEEALRGLPIRYQTPAIRAENTBREIVLNCMATFTWLLSPVRYVNYLIIINDEANFTDPAQIAAGYISTRVENDEAGIFHTATPPGSDPFPQSNAPINDEEREIPERSNSGQEN  
 YF 1720 NKEAFNSLDVKFHTQFSAHQDREVIDANCAHTLYNMLEPTRVVREVIINDEANFLDPAQIAAGYIAAGYISTRVENDEAGIFHTATPPGSDPFPQSNAPINDEEREIPERSNSGQEN  
 DEN2 783 VTBKQKTVFVFPSTKAGNDTANCLNKHKVTQLBRKTSQDEYVKTRTNDVYVTTDISEGANFKAERVTDPRCKPKPVILTDEERVILAGPIPVTHSS-----  
 YF 1840 ILADKPTANFLPSIRANVVAALNKAQKVVVLRKTFEREYPTIKKPKDFILATDIAENBAMLCVERVLCRTAFKPVLVQGRKVAIKQPLRISASSAARRRRIQRNPNRQDS  
 DEN2 ----- 005  
 YF 1960 YYYEPTBEHNNWNLKLEAGILLNDEYRQDVAFLYQVE 2000



DEN2 1 -----DMLAVLHAEAGGRAYNHALSEPETLETLLELLTLLATV-TGGIFLFLNDRSIOKNTLGNCCIITAGILLNYADIGPWHIAASIILEFFLIVLLIPEPEKORTPOBHO  
 YF 2120 PDLAKKDEANDTISVFLNSEEGRAYRUALSNOPEANTIVULFTLABLLTSDWIFFWPKDIBRNRBRNSTRACBYLNFLDQKPTNHSYVULIFFVLIWVVVPEPGORSIGDHO  
 DEN2 109 LTYVVIAILTVNATRNENDFLEXTKKDLBLSITTDPEHJL--DIDLAPASANTLYHVAITFTVPLANDIENDDWVLTAIANDATVNLKIDNPLKNDIGVPLLAICYSO  
 YF 2240 VAYLJIGJLTLVAVANAELELBLEXTKEDLFOKDLIPSDAPDNDPDLALPDAANTVYVDTVTLSPILANDIKVEYDNLSSIADDAVLFNDKDPFKNDISVILLYSGNS  
 DEN2 227 VNPITLTANFLLVNYAIIOPILAKATREAKKAAASINRPTVORITVIBLPPIYDPIF-ERLQVULLVLCYVPLNRTTUMCEALTLATOPISLNEHPGFHNTTIAVS  
 YF 2340 ITVPLLCBICANLNDLILPBIKADKLABRWFHWEHPPVNDPTVIEEAPENMLVEKELAVLLALBLADWNCRTPFSLAESIVLAGAALOPLEONTLLNNGPWS  
 DEN2 346 NANIFROSYLABALLFSINRITWTRSTONIDETLDEKXKDLBALGDEFBIWKOBIENORTLANESIKRNETDNI-AVDRBAKLRNFVENRNTPEKVVOLCGRGGSYYC  
 YF 2400 NTQWIRNDYAFVGVNYLHKIKTORRDM--KTLBEVNDLALLAKRPFELVCRDIEVEVDTADDLADKVDTOVWVSDTAKLRNFHERDYVLEDRVIBLCCRGGCYA  
 DEN2 465 QDLKIVREVOLTKDOPNEEPINRSTYONLVLGDVWFFTYPERCOTLLCQDSDPPTVEARLAVLALVEMLNNTD-FCIKVLPYDPTERIEALOKYDVALVHPL  
 YF 2590 AAKKEYDVKDFLDRDNEKPNVDBLNDLITFKDCTDINLEPKICFLLCQDSDSDVTEERTVAVLDTVEMLACDNDFCVKVLPYDPTERIEALOKYDVALVHPL  
 DEN2 504 BRNTHERYVDRNDNIVDQVNTDNLHNFTHRKAATYEPVULDSTTRISIEE----- 640  
 YF 2710 BRNTHERYVDRNDNIVDQVNTDNLHNFTHRKAATYEPVULDSTTRISIEE----- 2000

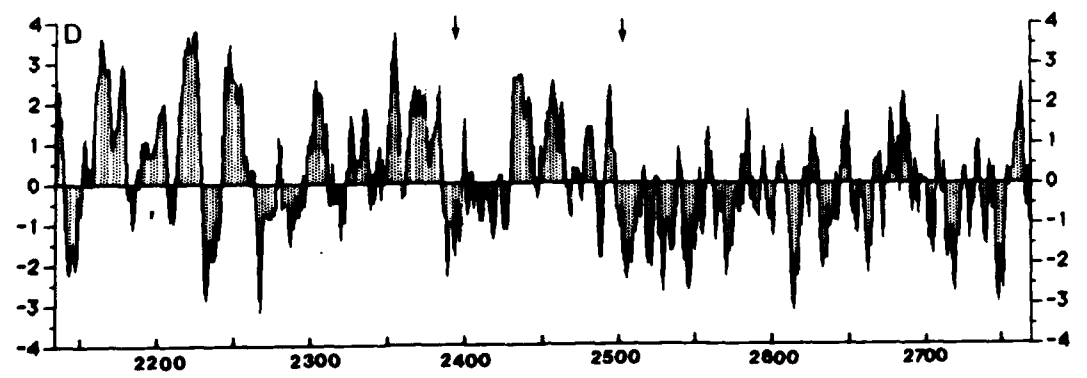
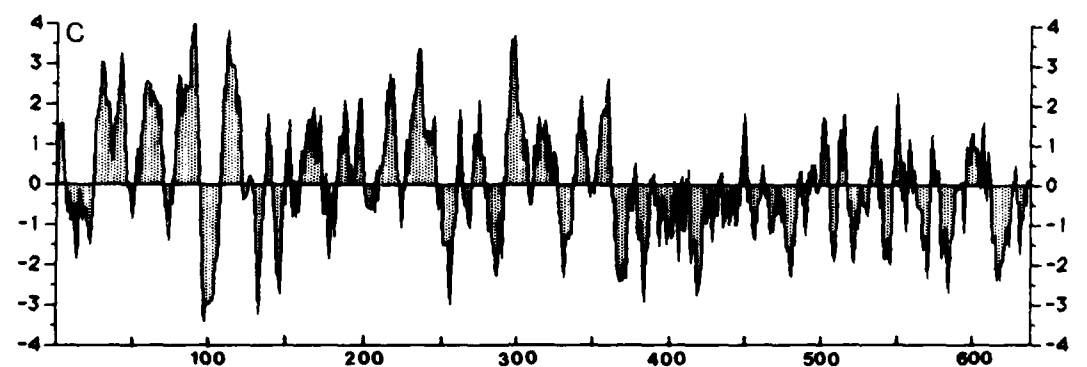
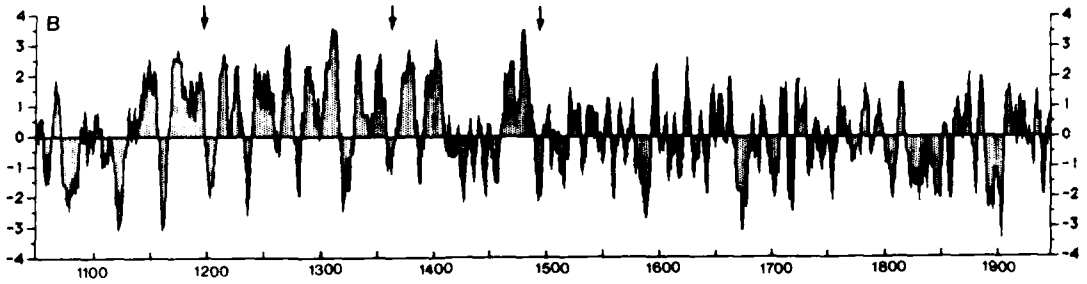
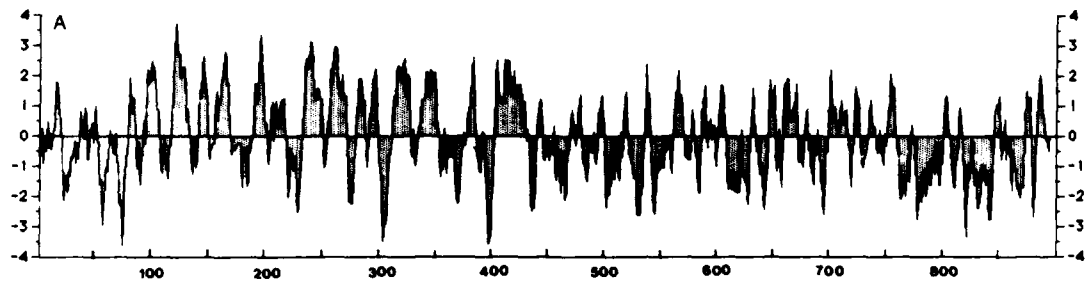


Table I

Protein <sup>1</sup>	site in the YF polyprotein <sup>2</sup>	NH <sub>2</sub> -terminal cleavage sites for	sequence at the the corresponding YFsite in DEN-2 <sup>3</sup>
ns2a (NV2 $\frac{1}{2}$ or NV2)	1187	( TVA↓V )	RVMV
ns2b (NV1 $\frac{1}{2}$ )	1355	( GRR↓S )	KKRS
NS3 (NV4)	1484	ARR↓S	KQRA
ns4b (NV1)	2394	( QRR↓V )	QKRA
NS5 (NV5)	2506	GRR↓G	TRRG

<sup>1</sup>The old (as indicated by parenthesis) and the proposed nomenclature of the non-structural proteins and <sup>2</sup> their tentative (indicated by parenthesis) or confirmed sites for yellow fever virus polyprotein are from Rice et al. (1985).

<sup>3</sup>the hypothetical sites for DEN-2 are based on the homology with the YF sites (Fig. 1a and 1b).

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## List of Publications

### Manuscript

Yaegashi, T., Vakharia, V., Page, K., Feighny, R. and Padmanabhan, R. (1986)  
Molecular Cloning and DNA sequence analysis of Dengue Virus type 2  
genome. Submitted to Gene.

### Abstracts of Presentations at National and International Meeting

1. Kalyan, N., Nohara, M., Henschel, E.A., and Padmanabhan, R. Structural analysis of Dengue-2 virus genome. Fed. Proc. 42, #7, pp 1972 (Abs. #1257) 1983.
2. Kalyan, N., Nohara, M., Henschel, E.A. and Padmanabhan, R. Structural analysis of Dengue-2 virus genome. Presented at the annual meeting of American Society for Tropical Medicine and Hygiene (Dec. 4-8, 1983) held in San Antonio, Texas.
3. Kalyan, N., Nohara, M., Henschel, E.A. and Padmanabhan, R. Structural analysis of Dengue-2 virus genome. Presented at the XV International Congress of Genetics held in New Delhi, India (Dec. 12-21, 1983) Abstract #240, pp. 137.
4. Vakharia, V., Yaegashi, T., Feighny, R., Kohlekar, S., Padmanabhan, R. Structural analysis of Dengue Virus type 2 genome. Presented at the International workshop on the molecular biology of flaviviruses held at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD (Nov. 29 - Dec. 1, 1984) Abstracts, pp. 6.



5. Vakharia, V., Yaegashi, T., Feighny, R., Kohlekar, S., Feighny, R., Schoepp, R., Dalrymple, J.M. and Padmanabhan, R. Structural analysis of Dengue-2 virus genome. Poster presentation at the annual (1984) meeting of the American Society of Tropical Medicine and Hygiene. Abstracts.
  
6. Henschel, E.A., Narupti, S., Nisalak, A., Feighny, R., Padmanabhan, R., McAda, P., Mason, T. and Fourneir, M. Rapid diagnosis of flavivirus infections using cDNA nucleic acid hybridization. Presented at the annual (1985) meeting of the American Society of Tropical Medicine and Hygiene. Abstract #192.

List of Personnel received contract support

**Research Assistants**

Joanne Lee

C. Subramaniam

Baljit Kaur

**Period of Support**

March 1982 - August 1982

June 1982 - August 1982

March 1983 - June 1984

**Research Associates**

Dr. Tazuko Yaegashi

Dr. Masatoshi Nohara

Dr. Narender Kalyan

Dr. Vikram Vakharia

Dr. Suhas Kohlekar

May 1984 - August 1985

May 1982 - June 1983

July 1982 - December 1984

October 1983 - February 1985

March 1984 - February 1985

**Medical Students**

Parvesh Kumar

January 1983 - August 1983

**Graduate Students**

Mr. John R. Lowe

September 1982 - February 1985

**Undergraduate Students**

David Coussens

Stuart Litwer

Ronnie Pelton

Steve Copenhaver

Summer 1982 (2 months)

Summer 1982 (2 months)

Summer 1983 (2 months)

Summer 1983 (2 months)