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Review paper

Genomics, proteomics and evolution of dengue virus

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Abstract

The genome of a pathogenic organism possesses a specific order of nucleotides that contains not only information about the synthesis and expression of proteomes, which are required for its growth and survival, but also about its evolution. Inhibition of any particular protein, which is required for the survival of that pathogenic organism, can be used as a potential therapeutic target for the development of effective drugs to treat its infections. In this review, the genomics, proteomics and evolution of dengue virus have been discussed, which will be helpful in better understanding of its origin, growth, survival and evolution, and may contribute toward development of new efficient anti-dengue drugs.

Key words: dengue virus; serotypes; proteomics; genomics; evolution

Introduction

Dengue virus (DENV) of Flaviviridae family has emerged as the fatal pathogen, which is transmitted in human population by the nimble (day-biting) of *Aedes aegypti* female mosquito and causes a serious health problem called dengue fever. Both types of dengue fever, dengue hemorrhagic fever [1–8] and dengue shock syndrome, are deadly infections of five different serotypes of this virus (DENV 1–5) [9–14]. DENV contains 10 723 nucleotides in a single-strand positive RNA genome, which encodes a large polyprotein precursor of 3391 amino-acid residues. The polyprotein of DENV comprises three structural proteins (C, prM and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [15–19]. Each protein performs a specific function and helps to produce new virus particle by using the host cell machinery. All the serotypes of DENV cause severe and critical health problems. Each serotype provides specific lifetime immunity and short-term cross-immunity. There is genetic variation within each serotype and some genetic variants of each serotype emerge to be more virulent or have greater 'epidemic' potential. The complete genome of 'only four different serotypes' have been sequenced and the three-dimensional structures of many of these viral proteins have been determined [20, 21], which are being used for the screening of novel antiviral compounds against DENV. The above-stated facts emphasize that the genomic, proteomic and evolutionary information about this human fatal pathogen is not only required to understand the origin, growth, survival and evolution but also for the development of effective drugs to treat its infections.

Genomics

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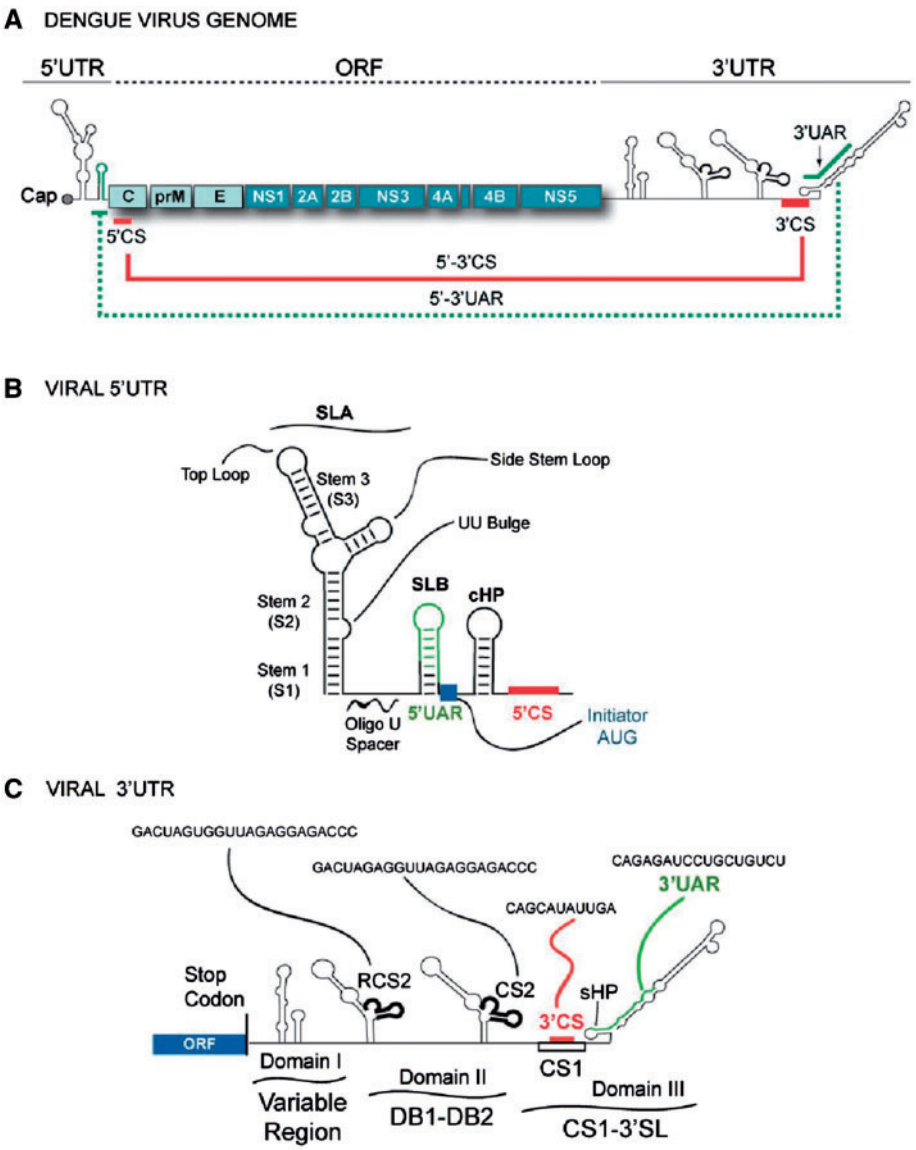

Figure 1. Diagrammatic representation of the DENV genome [38]. (**A**) 5′ and 3′ UTRs and the open reading frame of the DENV indicating structural proteins, C, prM, E, and nonstructural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 [38]. The location of the complementary sequences 5′–3′CS (solid lines) and 5′–3′UAR (dashed lines) also have been shown. (**B**) Predicted secondary structure of the 5′ terminal region of the viral genome [38]. Structural elements that are located at the 5′ end are shown as stem loop A (SLA), stem loop B (SLB), oligo (U) track spacer, translation initiator AUG, capsid region hairpin (cHP) and the 5′CS element. (**C**) Representation of predicted RNA elements at the 3′ UTR of the DENV genome. The predicted secondary structures of the three defined domains are shown: DI comprising variable region (VR), DII comprising DB structures (DB1 and DB2) and DIII consisting conserved sequence CS1 and 3′SL. In addition, the location and sequence of each of the conserved elements corresponding to RCS2, CS2, 3′CS and 3′UAR are also indicated [38].

change in the specific order of its nucleotides can originate new strains/species, which may be more virulent than its parent strains/species. In the case of DENV, all the types are serologically distinct from each other but each one contains a single-stranded RNA genome, which is translated in to a single polyprotein. The polyprotein is cleaved by host- and virus-derived proteases to produce the structural (capsid-premembrane/membrane-envelope; C-prM/M-E) and nonstructural (NS1-NS2ANS2B-NS3-NS4A-NS4B-NS5) proteins [19] (Figure 1A). The 5′ and 3′ ends of each serotype of DENV RNA genome include untranslated regions (UTRs), which are essential for replication and translation, and possibly interact with cellular factors involved in these functions [22–24]. The 5′ UTRs of DENV (Figure 1B) are found to be 95–101 nt long and consist of two RNA domains having different functional activity during its genome synthesis. First domain of 5′ UTRs is about 70 nt in length and fold into a large stem loop (stem loop A, SLA, Figure 1B). This large stem-loop is known as the promoter for RNA-dependent RNA polymerase (RdRp) domain of DENV NS5 protein. The binding of RdRp domain to large stem loop is essential for DENV RNA synthesis [25, 26]. The second domain is reported to fold into a short stem loop (stem loop B, SLB, Figure 1B). The SLB contains important sequence patterns for long-range RNA–RNA binding and RNA duplication [27–30]. The 3′ UTRs of DENV are approximately 450 nt in length and divided into three domains (Figure 1C). First domain of 3′ UTRs is the

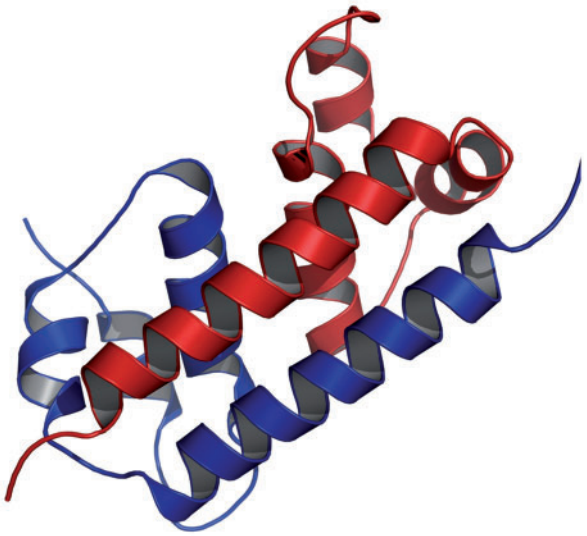

Figure 2. Three-dimensional structure of capsid protein (PDB code: 1R6R) [49] that has a large dimerization surface contributed by two pairs of helices.

Figure 3. The structure of membrane protein (M/prM) separated from membrane-envelope protein complex structure (PDB ID: 3C6E) [53] and visualized in PyMol.

most changeable region, which is found immediately after the termination codon [31] (Domain I, Figure 1C). It exhibits extensive size variation among DENV serotypes, ranging from >120 nt to <50 nt [32–38]. Second domain of DENV 3′ UTRs contains a characteristic dumbbell (DB) structure, which is duplicated in tandem (Domain II, Figure 1C) [32, 33, 36]. The DB structure has two conserved sequences named CS2 and RCS2 (repeated CS2), which are present in all vector-borne flaviviruses [39–43]. Third domain of DENV 3′ UTRs is the most conserved region, having a CS1 element followed by a terminal stem-loop structure (3′SL) (Domain III, Figure 1C). CS1 contains important sequence patterns for long-range RNA–RNA binding between the ends of the DENV genome [39, 38]. The above-described information will be helpful in understanding of the DENV genome structure.

Proteomics

The comprehensive study of structures and functions of the proteins produced by the genome of an organism can be defined as the proteomics of that organism [44–46]. Proteins are the essential elements of any organism, as they are the most important component for its growth and survival [47]. The structural proteomics of DENV was reviewed by Perera and Kuhn in 2008 [20], in which they have described only few proteins of DENV owing to unavailability of atomic structures of many DENV proteins. In the present review, all the structural (capsid, membrane and envelope) and nonstructural (NS1-NS2ANS2B-NS3-NS4A-NS4B-NS5) proteins of DENV (visualized using Pymol program [48]), which are reported till date have been described.

Structural proteins

Capsid protein

The capsid (C) protein of DENV is essential for specific encapsidation of its RNA genome. The solution structure of the 200-residue homodimer of DENV-2 C protein was reported by Ma et al., in 2004 [49], and their atomic coordinates can be accessed using the Protein Data Bank (PDB code: 1R6R). This structure (Figure 2) presents three-dimensional picture of capsid protein that possesses a large dimerization surface contributed by two pairs of helices, one of which has a characteristic of a coiled-coil [50, 49]. This structure exposes an alternate fold for which dimerization highlights characteristics likely to be functionally essential. Owing to the high positive charge of the dimer, it is improbable to oligomerize this molecule into a protein-only core. On the other hand, this solution structure suggests a region of interactions with other viral components in the virus particle [49, 51].

Membrane protein (M/prM)

The membrane protein of DENV plays an important role in the arrangement and maturation of DENV particle. The structure of the membrane protein (residues 1–81) possesses seven antiparallel β-strands stabilized by three disulfide bonds [20]. The 180 copies each of the envelope protein and M protein are found in the glycoprotein covering of the mature DENV virion. The proteolytic cleavage of pr peptide from the M peptide is accomplished by furin, a host protease, during the maturation of viral particle. The M protein then functions as a transmembrane protein under the E-protein shell of the mature viral particle. The pr peptide stays connected with the E protein until the viral molecule is discharged into the extracellular environment. This pr peptide acts like a cap, covering the hydrophobic fusion loop of the E protein until the viral particle has left the cell [20, 52]. The three-dimensional structures of membrane proteins were solved at pH 5.5 (2.2Å) and 7.0 (2.6Å) [53]. Both different pH values did not affect the tertiary conformation of both structures of membrane protein because no structural variation was observed between both solved structures. Figure 3 represents the crystal structure of the precursor membrane protein-envelope protein heterodimer from the dengue 2 virus at neutral pH.

Envelope protein (E protein)

DENV envelope protein is present on the viral surface and essential for the initial attachment of the virus to the host cell. DENV infectivity depends on E protein binding to target cell heparan sulfate [54]. The atomic structure of the envelope protein in dimer form has been determined (Figure 4) [52, 55–57]. Each monomer of E protein consists of three different domains.

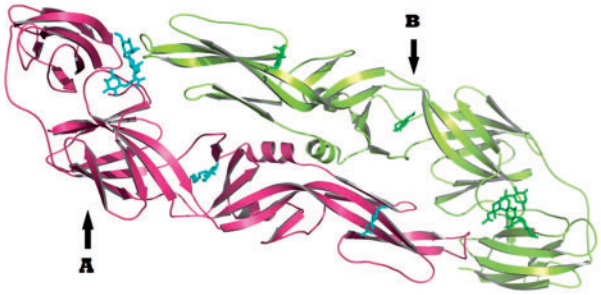

Figure 4. The structure of envelope protein in dimeric form (PDB ID: 1OKE) [55]: each monomeric unit is represented by A and B, respectively.

Domain I (DI) is found at the N-terminal region but structurally it is a central domain. Domain II (DII) is an elongated, finger-like arrangement that has a hydrophobic fusion peptide; therefore, it is called a fusion domain. The Domain III (DIII) is the predicted receptor binding domain. The DII bears a loop at its tip with a hydrophobic pocket (residues 98–109 in dengue type 2). This hydrophobic pocket opens and closes through a conformational change in a beta-hairpin at the interface between two domains [57]. The dissociation of the E protein dimeric form present at the viral membrane shell on acidification, binds liposomes and irreversibly trimerizes. Three fusion loops are found at one end of the E protein trimeric form, to introduce into the host-cell membrane, and domains DI and DIII at the other [55]. The most important difference between the post-fusion DEN-1 E protein and DEN-2 E protein structures is in regions having conserved amino-acid sequences, namely, in the polar cluster between domains I and III and in the fusion loop [58]. DENV gets inside a host cell when the viral E protein attaches to a receptor protein and counters by structural changes to the reduced pH of an endosome [59]. The structural change encourages fusion of DENV and host-cell membrane [55, 60]. The DENV E gene is considered as the molecular marker for viral pathogenicity because the E gene sequences of DENV pathogenic strain and nonpathogenic strain are different [61]. Antibodies that deactivate one type of DENV may be ineffective against other DENV strain owing to the structural variations in E gene [60, 62]. These structural variations in the E genes of DENVs provide notable information for the discovery of new DENV particles and development of its strain-specific anti-dengue drugs.

Nonstructural protein 1

The nonstructural protein 1 (NS1) protein of flaviviruses is a flexible conserved N-linked nonstructural glycoprotein (~48 kDa) with six invariant intramolecular disulfide bonds that are expressed on the cell surface and secreted into the extracellular space, where it has immune evasion activities [21, 63, 64]. The critical role of NS1 protein in DENV replication has been also reported [65]. NS1 is integrated as a monomer, the dimerization occurs in the lumen of the endoplasmic reticulum (ER) after post-translational modification, is handled in the trans-Golgi arrange and emitted into the extracellular space as a hexameric lipoprotein molecule [66]. NS1 hexamers have a focal lipid-rich center and are held together by weak hydrophobic interactions that separate into dimers in the vicinity of nonionic cleansers [67–72]. NS1 can be discharged at abnormal states into the extracellular environment, gathering up to 50 μg/ml in the sera of some DENV-contaminated patients [73–75]. The expression of NS1 protein is also reported on the plasma membrane

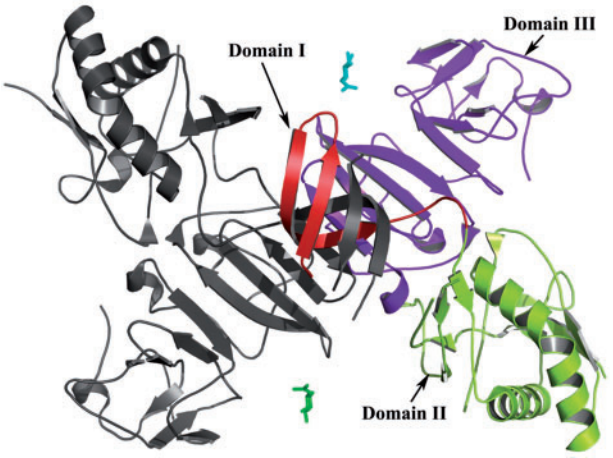

Figure 5. The crystal structure of NS1 was retrieved from protein data bank (PDB ID: 4O6B) [78] and visualized in PyMol program. The dark gray color is representing the one monomer unit of dimer and the domains are highlighted in the other colorful monomer unit. The firebrick, limon and purple colors represent DI, DII and DIII, respectively.

surface through some molecular mechanisms. Through recognition of sulfated glycosaminoglycans, the emitted NS1 binds to the plasma membrane of cells [76, 77].

The atomic structure, of baculovirus-derived recombinant dengue-2 virus NS1 protein by single-particle electron microscopy has been determined at 23 A° resolution [70]. The barrel-like arrangement of three dimeric elements has been found in this structure that contains the hexamer and offers more insights into the overall arrangement of oligomeric NS1.

The high-resolution crystal structure of DENV NS1 dimer has been recently solved [78, 79], which provides significant information about the complex NS1 fold (Figure 5). The dimer form of NS1 protein has three domains. The first domain (amino acids 1–29), a small 'β-roll' dimerization domain that has two intertwined β-hairpins, each stabilized by a disulfide linkage (Cys4-Cys15). The second domain (amino acids 30–180), also known as a 'Wing' domain, has an α/β sub-domain and a discontinuous connector that sits against the β-roll. The third domain (amino acids 181–352), a 'β-ladder' domain, is composed of 18 anti-parallel β-strands (nine contributed by each monomer) gathered in a continuous β-sheet that runs along the whole length of the dimer left panel). The projection created by the β-roll and the connector sub-domain provides one side of the dimer hydrophobic, and has been suggested to face the ER membrane and to bind with other transmembrane DENV proteins [78–80]. On the other hand, within the NS1 hexameric structure, the β-roll faces the interior of the lipoparticle, where it combines with the central lipid core. The above described crystal structure suggests that the NS1 hexamer in crystal structures is similar to a solution hexamer visualized by single-particle electron microscopy [78]. The crystal structure of a C-terminal fragment (residues 172–352) of DENV NS1 protein has been also determined at 2.7 Å resolutions [81]. The interactions between NS1 protein, E protein and prM protein have been recently identified, which suggests a novel role of NS1 for the production of infectious DENV particles, that is linked to NS1 interaction with the structural proteins, but independent from NS1 secretion [79].

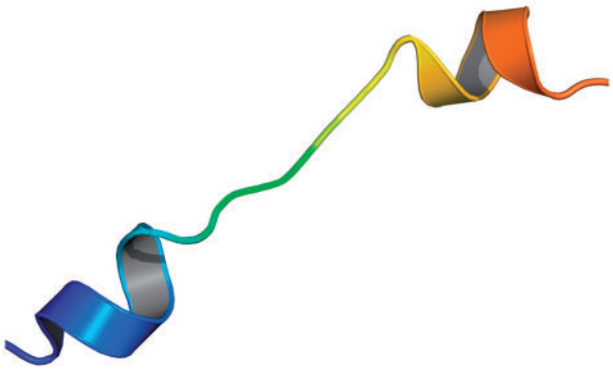

Figure 6. Structure of DENV NS2A protein (first membrane segment, PDB ID: 2M0S [85]) is showing two helices connected by a Pro85-mediated 'helix breaker'.

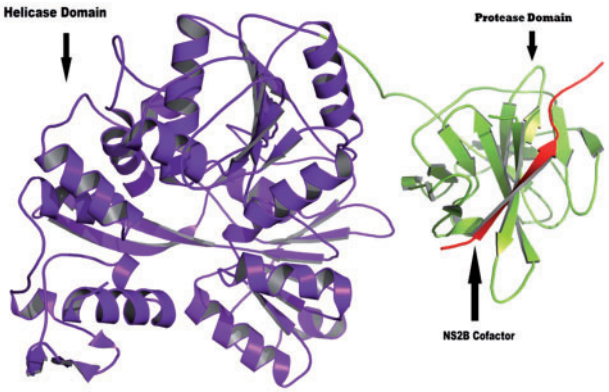

Figure 7. The structure of the complete NS3 molecule retrieved from Protein Data Bank (PDB ID: 2VBC [89]). A small red color beta sheet represents NS2B co-factor, Limon color represents NS3 protease domain and a large purple color molecule represents the helicase domain of the NS3 protein.

Nonstructural protein 2A

In the family of flaviviruses, most of the species contain a hydrophobic nonstructural protein 2A (NS2A) protein of 22 kDa [82, 83]. The NS2A protein of DENV is an important constituent of the viral replication complex that plays a vital role in virion assembly and antagonizes the host immune response [84]. Two different sets of NS2A protein have been reported [83]. One set, located in the viral replication complex, has been found to be accountable for DENV RNA synthesis, and the other one, located in the virion assembly site, has been found to be involved in the virion assembly [83]. DENV hydrophobic NS2A protein (in DENV-2) consists of five fundamental transmembrane segments (residues 69–93, 100–118, 143–163, 165–186 and 189–209) that cover the lipid bilayer of the membrane of ER [21, 85]. In addition, NS2A molecule has two membrane-associated segments (residues 32–51 and 120–140) that interact with the membrane of the ER without crossing the lipid bilayer. The first membrane segment of DENV-2 NS2A (residues 69–93) (Figure 6), analyzed by nuclear magnetic resonance, is composed of two helices connected by a Pro85-mediated 'helix breaker' [21, 85].

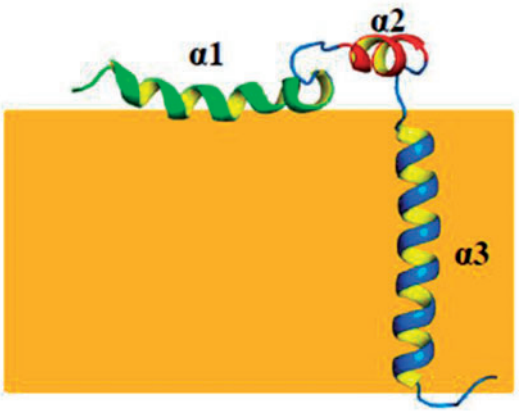

Figure 8. Ribbon representation of structural model of NS4A (17–80) [108]. The three helices are shown in different colors. The membrane is shown as a brown box. The permission for reusing this picture has been taken by the publisher (American Society for Microbiology) with License Number 3831900083249 on March 18, 2016.

Nonstructural protein 2B and nonstructural protein 3

The nonstructural protein 3 (NS3) of DENV is a multifunctional enzyme carrying activities involved in viral RNA replication and capping: helicase, nucleoside 5′-triphosphatase (NTPase), and RNA 5′-triphosphatase (RTPase) [86–88]. The crystal structure of complete NS3 molecule with 18 residues of the nonstructural protein 2B (NS2B) cofactor has been solved (PDB code: 2VBC) at a resolution of 3.15 Å [89]. This structure contains two domains: serine protease N-terminal domain and the ATPase/helicase domain located at the C terminus of NS3 molecule (Figure 7). The protease domain of this protein interacts with a NS2B cofactor (Figure 7) for its activation and forms a complex called NS2B-NS3 protease. The NS2B-NS3 protease complex mediates the cleavage of the DENV polyprotein [90]. DENV NS2B-NS3 proteases from all different serotypes share similar structure–activity relationships. Therefore, NS2B-NS3 protease is the most 'powerful therapeutic target' for the development of effective drugs against all the serotypes of DENV [91–96]. The carboxyl-terminal part of NS3 possesses three different enzymatic activities: helicase activity [97], NTPase activity [98–101] and RTPase activity [86, 102–105]. Recently, it has been reported that the C-terminal 50 amino-acid residues of DENV NS3 protein are important for NS3-NS5 interaction and viral replication [106].

Nonstructural proteins 4A and 4B

Of the four nonstructural proteins (NS2A, NS2B, NS4A and NS4B), nonstructural proteins 4A and 4B (NS4A and NS4B) are the integral membrane proteins of DENV, which play multiple functions in DENV replication and virus–host interactions. Through the molecular communication with DENV NS4A, cellular vimentin regulates the formation of DENV replication complex [107]. Both NS4A and NS4B proteins are crucial elements of the ER membrane-associated replication complex [108, 109]. The structure of NS4A (Figure 8) protein contains 127 amino acids (DENV-2) and possesses two transmembrane domains (TMDs) [108, 110]. The first TMD of 48 amino acids has been found to be involved in the formation of an amphipathic helix that mediates oligomerization [111]. The 248 amino acids containing NS4B protein possesses three TMDs [112]. NS4A regulates the ATPase activity of NS3 helicase in West Nile Virus (WNV) [113], while NS4B interacts with the helicase domain of NS3 and dissociates it from single-stranded RNAs in DENV [114].

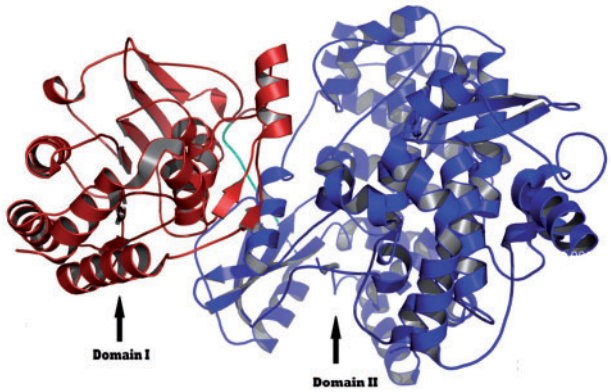

Figure 9. Structure of NS5 protein (PDB ID: 4V0Q) [117] after removing ligand molecules: red color represents DI (SAM-dependent MTase domain) and blue color represents DII (RdRp domain).

A 23-amino-acid-long conserved peptide of 2000 (2K) molecular weight links NS4A and NS4B proteins (NS4A-2K-NS4B). In different flaviviruses, the 2K peptide controls NS4A's activity in modulating the ER membrane through distinct mechanisms. The viral protease cleaves the linkage between NS4A and 2K, which is the precondition for the host signalase to cleave the linkage between 2K and NS4B [108, 110, 115]. A region of NS4A, from 40 to 76 amino acids, interacts with a region of NS4B, from 84 to 146 amino acids. A functional analysis study reports that these amino acid residues have a correlation between viral replication and NS4A-NS4B interaction, which demonstrates the biological importance of the NS4A-NS4B interaction. Mutations in these amino acids sequence regions prohibit the interaction of NS4A with NS4B, which may lead to inhibition of the replication [108, 116]. The functional importance of NS4A-NS4B interaction suggests that inhibitors of this interaction could be pursued for anti-dengue drug design.

Nonstructural protein 5

Nonstructural protein 5 (NS5) protein of DENV is the biggest protein of 900 amino acid residues and reported as the most conserved protein in the genus Flaviviruses. The crystal structure of the full-length DENV NS5 protein was determined at a resolution of 2.3 Å in the presence of bound S-adenosyl-L-homocysteine (SAH) and guanosine triphosphate (GTP) [117]. The complete structure of DENV NS5 contains two different domains at each end (Figure 9). DI is found at the N-terminal region (residues 1–262 in DENV3) of the NS5 protein and belongs to the S-adenosyl-L-methionine (SAM)-dependent methyltransferase (MTase) superfamily [118]. The MTase domain of NS5 caps the DENV RNA genome, a step required for its stability and translation into DENV polyproteins by host cell [119]. DII is found at the C-terminal region (residues 273–900) of DENV NS5 and also known as the RdRp domain that synthesizes the antigenome and offspring genome [108, 120]. The most important domain of NS5 protein, RdRp, consists of three different subdomains (Finger, Thumb and Palm) that are found to be structurally conserved across viral RdRps [121]. Region of RdRp domain, from 316 to 415 residues, has the functional nuclear localization sequences that are essential for interactions with other viral and host proteins [106, 108, 122–124]. The above facts suggest that NS5 plays essential enzymatic roles through its N-terminal MTase and C-terminal RdRp domains, and forms an important target for the discovery of antiviral drugs against DENV.

Evolution

Genomic sequences of any organism contain rich evolutionary informations about the origin of that species and the functional constraints on macromolecules such as proteins/enzymes [125]. Evolution in the genomic RNA sequences of DENV originates five distinct serotypes, DENV 1–5 [13], whose respective genomes (only DENV 1–4) share 60% sequence identity to each other [121, 126–129]. In 1943, scientists of Japan first isolated the DENV by inoculation of serum of patients in suckling mice [130, 131]. The blood samples for this experiment were collected from the patients infected during the 1943 dengue epidemic in Nagasaki, Japan. In 1944, DENV was also isolated in Calcutta from serum samples of US soldiers [9, 132]. The serotype isolated in Japan and Calcutta, India was the first serotype of DENV (DENV-1). DENV-2 was first isolated in Trinidad in 1953 [10]. DEN-3 was first isolated in Americas (in Puerto Rico) in 1963 [11] and subsequently caused epidemics in Jamaica and the eastern Caribbean during that year [133]. In 1981, DENV-4, first reported in Americas was also of an Asian origin [12]. The fifth and most recent expansion to the current serotypes of DENV is DENV–5, which has been reported in October 2013 [13, 14]. The genome sequencing and sequence alignment of each serotype of DENV confirmed the homology of all the serotypes as well as its conserved genetic organization, and allowed for the more precise and broad classification of DENV into genetically distinct groups or genotypes within each serotype [23, 134]. The evolutionary history of DENV has been assessed by reconstructing a molecular time scale of its evolution. This has been recently achieved by estimating the rates of nucleotide substitution using a maximum likelihood method that analyses the amount of evolutionary change, which has occurred between viruses sampled at different times [129, 135]. A large number of envelope gene (E gene) sequences were analyzed by using maximum likelihood method, which calculated the rate of DENV-1, -2, -3 and -4, evolution at 4.55×10^{-4}, 6.07×10^{-4}, 9.01×10^{-4} and 6.02×10^{-4} subs/site/year, respectively [23, 136, 137]. These observed rates of DENV evolution often conform to a molecular clock, although some serotype- and genotype-specific rate differences were observed, and were found to be similar to those calculated for DENV in a detailed study of a number of RNA viruses [138, 139]. Interestingly, the calculated times of DENV divergence were extensively dissimilar from prior findings [140, 141], introducing the emergence of human scourge DENV transmission at more recent times (approximately 300 years ago) [23, 137, 139]. A phylogenetic study of various DENV strains from four different serotypes presents its evolutionary history (Figure 10) [23]. This tree contains two major related and one out grouped cluster. Cluster I contains two different subclusters of DENV-1 and DENV-3 strains, while Clusters II and III contain DENV-2 and DENV-4 strains, respectively. This phylogeny provides significant evolutionary relatedness of all the serotypes of DENV (except DENV-5) at molecular level. The above-stated facts provide essential information for our understanding about the evolution of DENVs.

Although basic and translational research about DENV has substantially improved, our knowledge includes the concerns about the DENV genetic diversity and causes for the emergence of its five viral serotypes and function of almost every gene in the viral genome RNA. However, to tackle the global pandemic, new efforts are needed. In this regard, we have briefly summarized the essential information about the genomic, proteomics and evolutionary biology of DENV, which would assist in the future innovation and development of new efficient anti-dengue

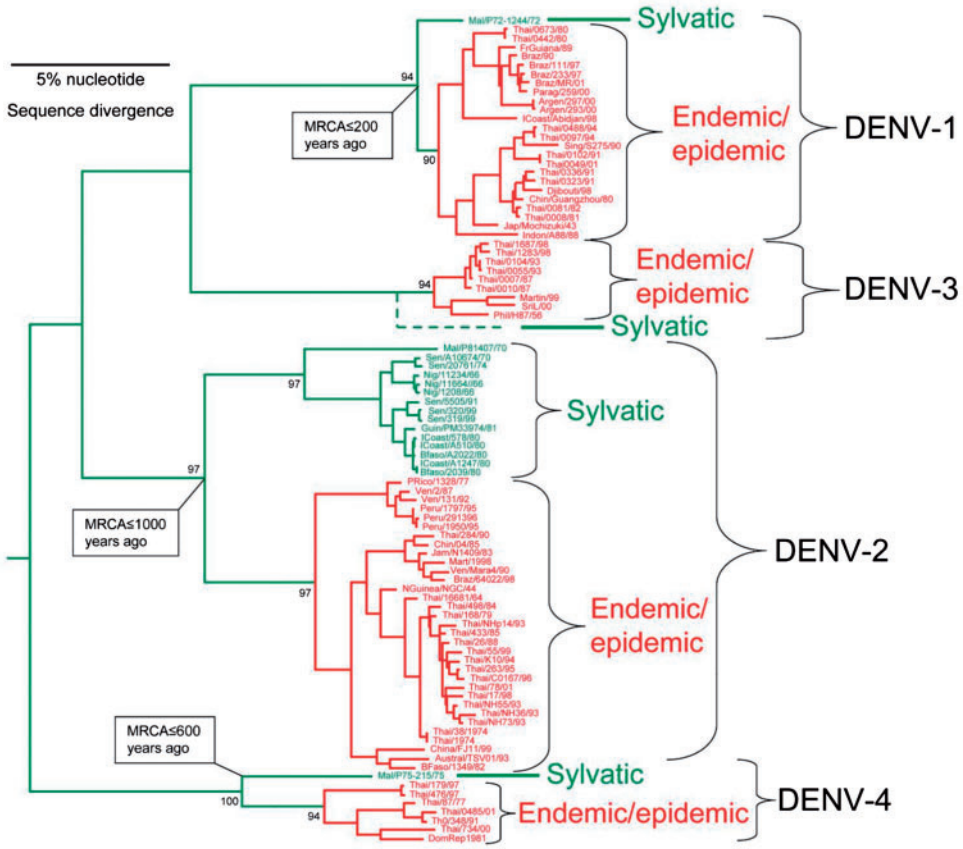

Figure 10. Phylogenetic tree of DENV strains from four different serotypes derived from complete open reading frames available in the GenBank library [23]. The phylogeny was inferred using Bayesian analysis (1 million reiterations) and all horizontal branches are scaled according to the number of substitutions per site [23]. Bayesian probability values are shown for key nodes. Virus strains are coded by abbreviated country of collection/strain name/year of collection [23]. The permission for reusing this picture has been taken from the publisher (Elsevier) with License Number 3832350978638 on March 19, 2016.

drugs, and vaccines against the individual virus populations. Also, some studies also predicted that although the viral strains have characteristic phenotypic features such as virulence, and for positive selection at immunologically important sites but stochastic processes are also responsible in shaping viral genetic diversity, with pedigree extinction a common incidence. Hence, we need to explore the genetics of structural and nonstructural proteins in different biological factors and environs, and indeed also compare the effect of these factors on whole genome RNA sequences, as this virus is prone to random mutations. Such information is essential to predict and understand the evolution of new serotypes of DENV strains. Finally, predicting, tracking and implementation of major research findings in the genomics, proteomics and evolutionary biology of DENV are required to reverse the inclination in dengue.

Key Points

- Dengue virus (DENV) belongs to Flaviviridae family and causes a serious health problem called Dengue Fever. A total of five different serotypes of dengue virus (DENV 1–5) have been reported till date, while the genomic sequence data are available only for four different serotypes.
- Single-stranded positive RNA genome of dengue virus encodes a large polyprotein precursor, which gives rise to three structural proteins (C, prM and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).
- NS2B-NS3 proteases from all different serotypes share similar structure–activity relationships, and therefore, NS2B-NS3 protease is the most 'powerful therapeutic target' for the development of effective drugs against all the serotypes of DENV.
- Phylogenetic tree of DENV strains from four serotypes divides them into three different clusters. Cluster I contains two different subclusters of DENV-1 and DENV-3 strains, while Clusters II and III contain DENV-2 and DENV-4 strains, respectively.

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