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Review

RNA Structure Duplications and Flavivirus Host Adaptation

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Flaviviruses include a highly diverse group of arboviruses with a global distribution and a high human disease burden. Most flaviviruses cycle between insects and vertebrate hosts; thus, they are obligated to use different cellular machinery for their replication and mount different mechanisms to evade specific antiviral responses. In addition to coding for viral proteins, the viral genome contains signals in RNA structures that govern the amplification of viral components and participate in triggering or evading antiviral responses. In this review, we focused on new information about host-specific functions of RNA structures present in the 3′ untranslated region (3′ UTR) of flavivirus genomes. Models and conservation patterns of RNA elements of distinct flavivirus ecological groups are revised. An intriguing feature of the 3′ UTR of insect-borne flavivirus genomes is the conservation of complex RNA structure duplications. Here, we discuss new hypotheses of how these RNA elements specialize for replication in vertebrate and invertebrate hosts, and present new ideas associating the significance of RNA structure duplication, small subgenomic flavivirus RNA formation, and host adaptation.

Trends

Recent advances in molecular virology provide new hypotheses of how RNA structures in mosquito-borne flavivirus genomes mediate host adaptation, viral replication, and evasion of antiviral responses.

Dengue virus RNA structures play different functions during infection in vertebrate and invertebrate hosts.

Conflicting requirements of viral RNA elements shape the composition of viral populations obtained in human or mosquito cells.

Viral RNA structures can modulate the type and extent of host antiviral responses.

Complex RNA structures present at the viral 3′ untranslated region (3′ UTR) that stall the host exoribonuclease XRN1 and generate small virus-derived RNAs are duplicated in mosquito-borne flaviviruses.

Conservation of RNA structure duplication in the 3′ UTR of insect-borne viruses is associated with mechanisms of host adaptation.

Flaviviruses

The *Flavivirus* genus includes a large number of taxonomically recognized species, many of which are important human pathogens such as dengue, yellow fever, Japanese encephalitis, West Nile and other viruses that cause fever and encephalitis. Dengue virus (DENV) is the most important viral disease in humans transmitted by insects. It is responsible for about 390 million infections each year, without vaccines or antivirals available for its control. Yellow fever virus (YFV) is endemic in a number of African and South American countries, and causes 200 000 cases and 30 000 deaths in Africa even with effective vaccines available (http://www.who.int/csr/disease/yellowfev/YellowFeverBurdenEstimation_Summary2013.pdf). Other diseases caused by flaviviruses include West Nile encephalitis and Zika fever, which are considered emerging diseases with important outbreaks around the world [1].

Despite the similar organization of flavivirus genomes and their mechanisms of replication, they possess differences in their host ranges and transmissibilities. In this regard, flaviviruses are divided into four large ecological groups: the mosquito-borne group (MBFV), the tick-borne group (TBFV), the vertebrate-specific flavivirus group, referred to as 'no known vector viruses' (NKFV), and the ones that have been only isolated from insects, which constitutes a growing group of viruses known as insect-specific flaviviruses (ISFV) (for a recent review see [2]) (Figure 1A).

Flaviviruses are small, enveloped viruses with a single, positive-strand RNA genome of 10 to 12 kb. A type I cap structure is present at the 5′ end followed by the conserved dinucleotide 5′-AG-3′ [3]. The cap structure of flaviviruses contains a methyl group at the N7 position, and a second methyl

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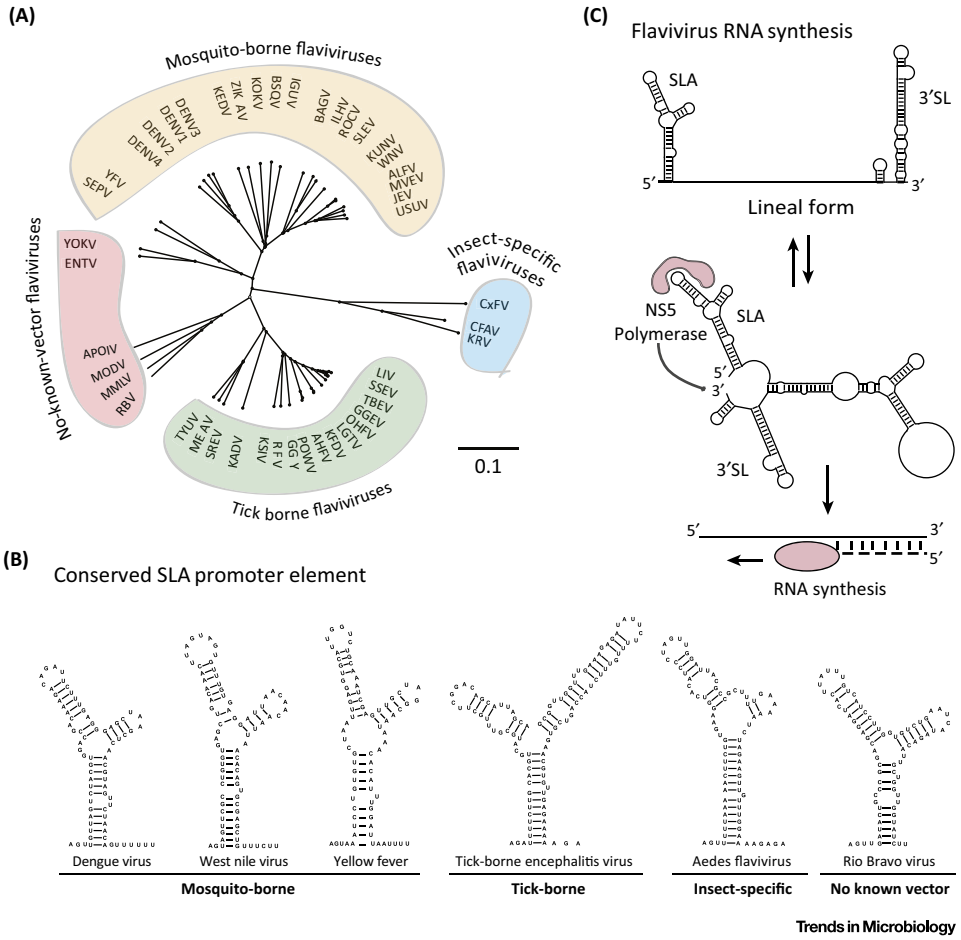

Figure 1. Conserved Features and Mechanism of Flavivirus RNA Synthesis. (A) Schematic representation of the distance tree of the four ecological groups of flaviviruses drawn using the neighbor-joining method and jukes-cantor substitution model. (B) Predicted RNA structure and sequence of stem–loop A structure (SLA) elements of different flaviviruses. (C) General mechanism of viral RNA synthesis that involves the promoter element SLA at the 5′ end of the viral RNA, cyclization of the viral genome, and polymerase initiation at the 3′ end.

group at the ribose 2′OH position of the first nucleotide, m7GpppAmpN2 [4,5]. The 3′ end of the genome lacks a polyadenylate tail and terminates in a conserved 5′-CU-3′ [6]. The genome encodes a single open reading frame flanked by highly structured 5′ and 3′ untranslated regions (UTR). The 5′ UTRs are about 100 nucleotides long while the 3′ UTRs are in general between 400 and 700 nucleotides, while in some exceptional cases they can be over 900 nucleotides [2,7,8]. Although different flavivirus groups contain conserved RNA structures in the 5′ and 3′ UTRs, only two RNA elements are conserved in all flavivirus genomes. These are the Y shape stem–loop A structure (SLA) present at the 5′ end of the viral genome (Figure 1B), and the small hairpin 3′ stem–loop (sHP-3′ SL) located at the 3′ end of the viral RNA. These two essential RNA structures participate in the basic mechanism of viral RNA synthesis (Figure 1C). Sequence and structural features of the SLA as the promoter for viral polymerase binding and activation were first described in DENV and then extrapolated to other flaviviruses [9–12]. The 3′ SL was the first RNA structure described in flaviviruses and it was originally observed in the genome of MBFVs [13–15]. Sequence conservation analysis within each flavivirus group indicates that both the SLA and the 3′ SL are the most conserved regions of the viral genomes (Figure 2). In addition, an important conserved feature in the genome of all flaviviruses is the presence of inverted complementary sequences that mediate genome cyclization, which allows the polymerase, bound to the promoter SLA, to reach