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Short communication

RNA secondary structures in the proximal 3′UTR of Indonesian Dengue 1 virus strains

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ABSTRACT

The characteristics of DENV-1 viruses, isolated during the 2001–2002 outbreak in Indonesia were studied. The secondary structure of the 3′UTR of different DENV-1 strains derived from Indonesian patients was compared with the 3′UTR of previously described DENV-1 sequences. The complete 3′UTR of DENV-1 was sequenced from 13 patients suffering from the severe form of dengue virus infection (dengue hemorrhagic fever). Prediction of RNA secondary structure of the 3′UTR revealed some previously unidentified conserved structures in the proximal region of the 3′UTR, the role of which in viral replication is still unknown. In addition our data suggest that some structural elements previously described in the distal part of the 3′UTR are partly dependent on the proximal part of the UTR. Our data support the existence of previously unidentified conserved secondary structures in the proximal part of the 3′UTR and their roles need to be further investigated.

Dengue viruses belong to the genus *Flavivirus* of the family *Flaviviridae*. They can be further subdivided into four antigenically distinct serotypes (DENV-1–4). With an estimated 100 million infections and 200 000 deaths per year, mainly among children (Anon., 2004), dengue is the most prevalent mosquito borne virus and among the most important public health problems world-wide.

Flaviviruses are single stranded positive sense RNA viruses coding three structural and seven non-structural proteins. The genome of dengue viruses is approximately 11 kb and contains a single open reading frame, which is flanked by 5′ and 3′ untranslated regions (UTRs). The length of the UTRs varies among different dengue serotypes (89–101 nt and 432–466 nt for 5′ and 3′UTR, respectively). The exact function of the UTRs has not been completely elucidated; however several studies have suggested that both the 5′ and 3′UTRs of flaviviruses are important for virus replication, translation and virulence (Alvarez et al., 2005; Mandl et al., 1998; Men et al., 1996; Proutski et al., 1997, 1999; Zeng et al., 1998). The 3′UTR of dengue viruses consists of a variable region of approximately 100 nt proximal to the stop codon of the NS5 gene and a distal region of approximately 300 nt. The secondary structure of the distal part of the 3′UTR of dengue viruses and other flaviviruses has been studied extensively and is shown to contain conserved structural elements among all flaviviruses, although some differences have been observed between mosquito and tick-borne flaviviruses (Hahn et al., 1987; Proutski et al., 1997; Rauscher et al., 1997). Those structures are believed to play an important role in the synthesis of minus strand RNA and consequently in virus replication. The 3′distal part of the UTR forms a long stable hairpin (Hahn et al., 1987; Proutski et al., 1997; Rauscher et al., 1997; Zeng et al., 1998), which stabilizes viral genome and enhance initiation of translation. The proximal part of the 3′UTR shows a high degree of sequence variation, however, the structural elements of this region have not been extensively described.

In this study we characterized the secondary structure of the 3′UTR of DENV-1 strains isolated from Indonesian patients and compared them with the structure of the 3′UTR of DENV-1 strains available in GenBank (accession numbers available from the authors upon request). To this end, we used the Genetic Algorithm (GA) incorporated in the STAR program, which is also able to predict pseudoknots. The validity of the predictions obtained by this program has been described elsewhere where experimental data confirmed *in silico* predictions (Gultyaev et al., 1995; van Batenburg et al., 1995). Our computations were executed with default settings of population size 10 and standard growth increments. During validation of our predictions the same strains were modeled with a different algorithm (mFOLD available from

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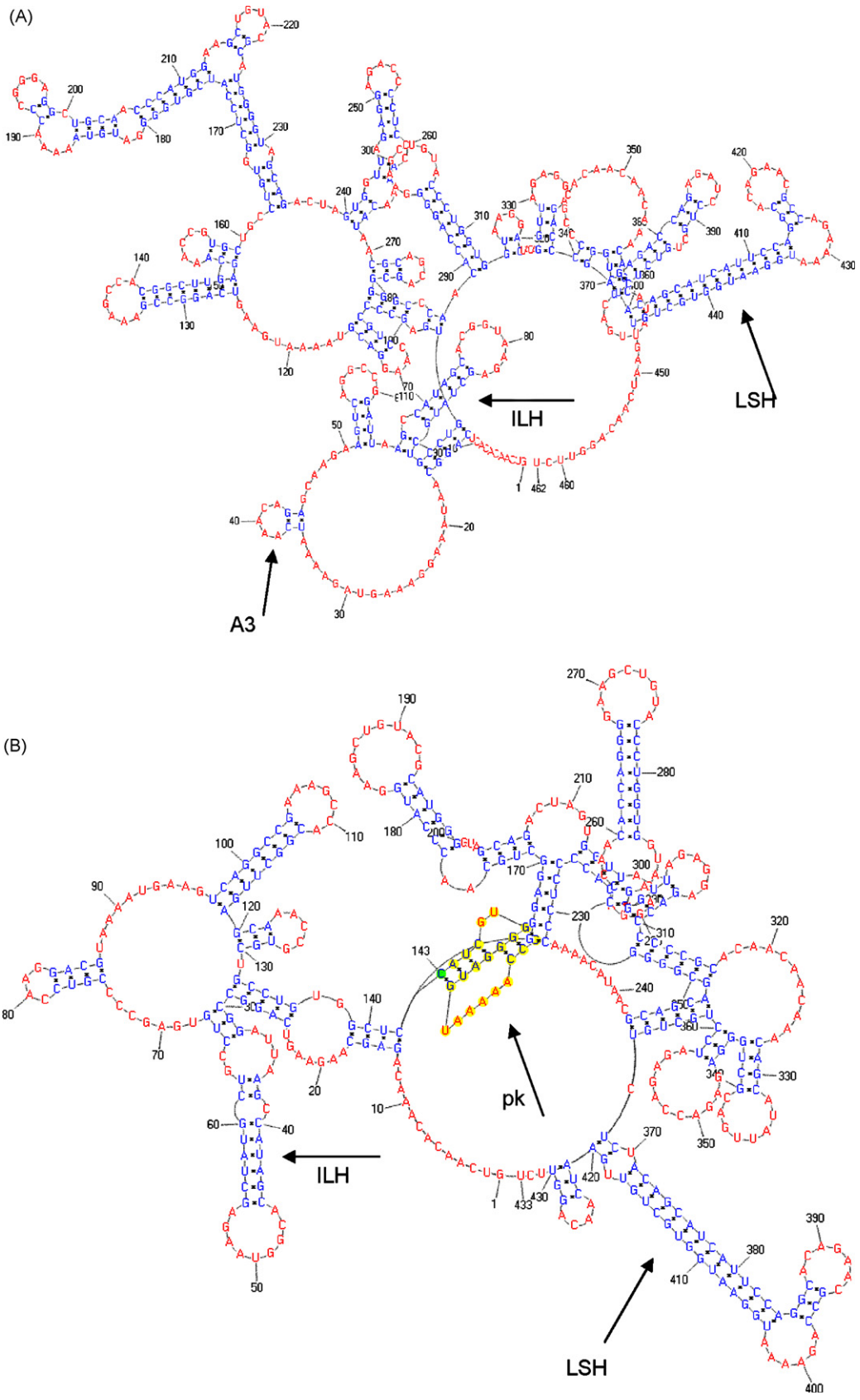

Fig. 1. Effect of the proximal DENV-1 3′UTR to the formation of structural elements in the distal part. The Abidjan strain is depicted in this figure: (A) wild type sequence; (B) 29-nt deletion introduced *in silico*. Only the conserved structural elements ILH (identified in this study) and the long stable hairpin (LSH, reference 9) are shown with arrows. The formation of a pseudoknot (pk) is highlighted and shown with an arrow (B).