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Research paper

Molecular cloning and characterization of the genes encoding the proteins of Zika virus

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ABSTRACT

Zika virus (ZIKV) encodes a precursor protein (also called polyprotein) of about 3424 amino acids that is processed by proteases to generate 10 mature proteins and a small peptide. In the present study, we characterized the chemical features, suborganelle distribution and potential function of each protein using Flag-tagged protein expression system. Western blot analysis revealed the molecular weight of the proteins and the polymerization of E, NS1, and NS3 proteins. In addition, we performed multi-labeled fluorescent immunocytochemistry and subcellular fractionation to determine the subcellular localization of these proteins in host cells. We found that 1) the capsid protein colocalizes with 3 different cellular organelles: nucleoli, Golgi apparatus, and lipid droplet; NS2b and NS4a are associated with the Golgi apparatus; 2) the capsid and NS1proteins distribute in both cytoplasm and nucleus, NS5 is a nuclear protein; 3) NS3 protein colocalizes with tubulin and affects Lamin A; 4) Envelope, PrM, and NS2a proteins co-localize with the endoplasmic reticulum; 5) NS1 is associated with autophagosomes and NS4b is related to early endosome; 6) NS5 forms punctate structures in the nucleus that associate with splicing compartments shown by SC35, leading to reduction of SC35 protein level and trafficking of SC35 from the nucleus to the cytoplasm. These data suggest that ZIKV generates 10 functional viral proteins that exhibit distinctive subcellular distribution in host cells.

1. Introduction

The recent outbreak of the Zika virus (ZIKV) has attracted attention worldwide and ZIKV infected cases are now spreading from the Americas to many other countries and its infection might be linked to some severe medical sequelae (Mlakar et al., 2016; Petersen et al., 2016; Weaver et al., 2016). The recent reports that Zika virus infection is probably associated with microcephaly of the neonates and Guillain–Barré syndromes (GBS) in adults spurred researchers to seriously reevaluate the medical significance of this agent as a pathogen (Mlakar et al., 2016; Petersen et al., 2016; Costa et al., 2016; Oehler et al., 2013). Since its first isolation from an infected monkey in 1947 in Uganda, only a few studies had been taken before the recent outbreak in Brazil. Detailed information about ZIKV that can be acquired via in depth investigation at the molecular, epidemical, and clinical levels will be critical for the elucidation of its role as a pathogen of serious diseases in humans.

The ZIKV, together with the West Nile virus, Yellow fever virus, Japanese encephalitis virus, Dengue fever virus, and many other viruses, forms the genus *Flavivirus* that belongs to family *Flaviviridae* (Wikan and Smith, 2016; Plourde and Bloch, 2016). The family *Flaviviridae* consists of many other viruses that have been summarized in a 2010 review (Bollati et al., 2010). A growing number of strains of ZIKV have been isolated from > 60 countries (Ramos da Silva and Gao, 2016; Dick et al., 1952). In earlier studies, it was determined that it causes only a mild arthropod-borne disease in humans, known as Zika fever, and so it had been rarely taken into clinical consideration seriously until recent epidemic outbreaks. The first ZIKV was isolated from a monkey and it is known that ZIKV can be transmitted to humans via mosquito bite or occasionally by sexual contact (Ramos da Silva and Gao, 2016; Boorman and Porterfield, 1956; Haddow et al., 1964; Marchette et al., 1969; Musso et al., 2015). It was then isolated from humans in Nigeria many years later (Moore et al., 1975). Since 2007,

Abbreviation: ZIKV, Zika virus; C, Capsid; Env, Envelope; NS proteins, nonstructural; Ctr, Centrosome; Mito, Mitochondria; ER, Endoplasmic reticulum; Golgi, Golgi apparatus; ICC, Immunocytochemistry; WB, western blot

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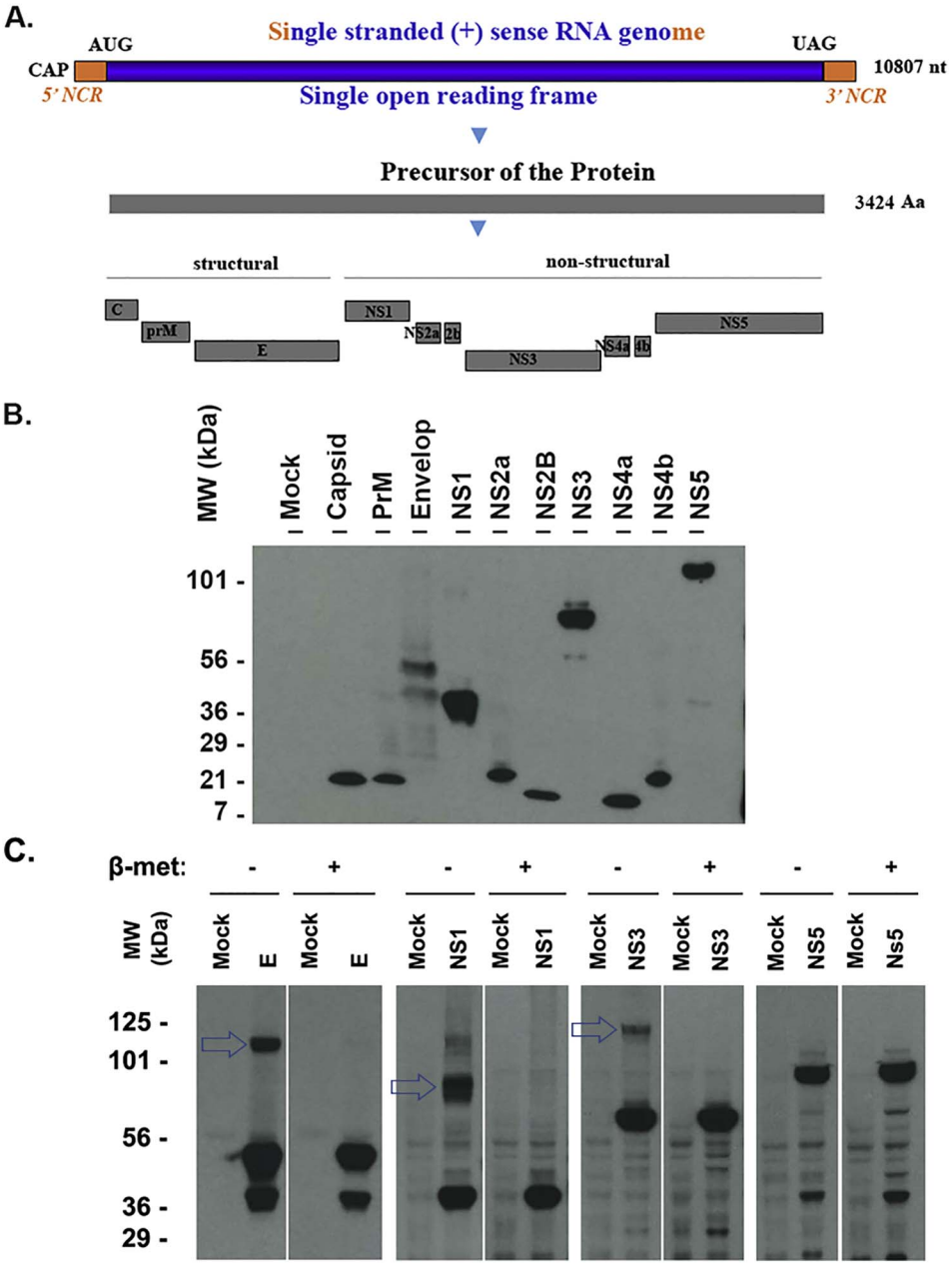

Fig. 1. Expression of the ZIKV proteins that are detected by western blot. **A.** Genomic structure and gene production of ZIKV (MR766 strain, GenBank Sequence Accession: LC002520). AUG: translation start codon; UAG: translation stop codon; NCR: noncoding RNA sequence; nt: nucleotide; Aa: amino acid; **B.** Western blot assay to examine ZIKV proteins. The ZIKV protein-expressing plasmids were transfected into HEK 293T cells for 24 h. The whole cell lysate samples were applied to run a PAGE and the transferred membrane was blotted with anti-FLAG antibody. The names of the protein were shown on the top and the size marker was shown on the left. **C.** western blot assay to examine the protein polymerization. HEK293T cells were transfected with the plasmid-expressing ZIKV protein E, NS1, NS3, or NS5 for 24 h. The whole cell lysate samples were collected in a SDS-lysis buffer with or without reducer (beta-mercaptoethanol) and examined for ZIKV protein using anti-FLAG antibody. The experiments have been performed for more than 3 times, one representative is shown.

Table 1
Nuclear localization signals of ZIKV proteins.

Capsid	RKERKRRGADT
NS1	RLKRAHLIEM
NS3	RRVLPEIVREAIKKRLRTV
	KYGEKRVLKPRWMDARVCSDHAALKSFKE
NS5	ELGKRKRPRVCTKEEFINKVRSN

The Aa sequences of the ZIKV proteins were input into cNLS Mapper software, the NLS sequences with high score > 5 were recorded and listed in the table.

ZIKV-caused epidemic outbreaks with different scales have occurred in Micronesia, French Polynesia, Cook Island, and Easter Island, ZIKV has become an emerging arbovirus (Musso et al., 2014). More recently, a pandemic of ZIKV infection occurred in South America. ZIKV infection has been related to the increasing number of cases of microcephaly and GBS in the areas of epidemics (Ramos da Silva and Gao, 2016; Stratton, 2016). Recent studies using mouse models demonstrated that ZIKV infection directly inhibited neuron stem cell proliferation, which supports the hypothesis that ZIKV is causatively related to microcephaly (Aliota et al., 2016; Hickman and Pierson, 2016; Lazear et al., 2016; Rossi et al., 2016; Werner et al., 2016). Phylogenetic studies have led to the classification of the ZIKVs into Asian and African lineages (Faye et al., 2014; Lanciotti et al., 2016). The recent cases of microcephaly and GBS linked to ZIKV are mostly, if not all, caused by Asian strains (Weaver et al., 2016). During the evolution of ZIKV, the virus developed new molecular relationships with factors of host cells (Faye et al., 2014; Qin, 2016; Singh et al., 2016; Shen et al., 2016; Wang et al., 2016). It is likely that interactions of viral proteins and viral genomic RNA with host factors determine the fate and/or efficiency of infection, pathogenicity, transmission, and epidemic potential.

This family of viruses has an enveloped, icosahedral capsid that contains a single stranded RNA genome (about 11,000 nucleotides) with positive sense (Faye et al., 2014). Therefore, the infected viral RNA can be directly translated to a large polyprotein precursor, which is co- and post-translationally processed by viral and cellular proteases into structural and nonstructural proteins. The three structural proteins are critical for the formation of envelope and capsid, and the seven

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