Intervirology *24:* 183–192 (1985)

0300–5526/85/0244–0183$2.75/0

Flaviviridae[1]

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Key Words. *Flavivirus* · Viral taxonomy

Summary. The family ***Flaviviridae*** comprises the genus *Flavivirus*, which contains 65 related species and two possible members. They are small, enveloped RNA viruses (diameter 45 nm) with peplomers comprising a single glycoprotein E. Other structural proteins are designated C (core) and M (membrane-like). The single strand of RNA is infectious and has a molecular weight of about 4×10^6 and an m^7G 'cap' at the 5′ end but no poly(A) tract at the 3′ end; it functions as the sole messenger. The gene sequence commences 5′–C–M–E... The replication strategy and the mode of morphogenesis are distinct from those of the ***Togaviridae*** which are slightly larger and morphologically similar in some respects. Flaviviruses infect a wide range of vertebrates, and many are transmitted by arthropods.

The flaviviruses are a large group (currently 65 species) of small, enveloped viruses that contain a single strand of positive-sense RNA, molecular weight about 4×10^6 [1]. They were classified in 1974 by the International Committee on Taxonomy of Viruses (ICTV) as the genus *Flavivirus* of the family ***Togaviridae*** [2]. The genus was included with three others (*Alphavirus, Pestivirus* and *Rubivirus*) and with several additional members described in the ***Togaviridae*** by the Arbovirus Study Group in 1978 [3]. In addition to their smaller size (45 nm) as compared to the larger togaviruses (50–70 nm) [4], several fundamental differences have become apparent in regard to flavivirus structure, replication strategy, and gene sequence. Based on such criteria, the ***Togaviridae*** Study Group recently proposed the creation of a new family, the ***Flaviviridae***, and this was approved by the ICTV in September 1984. The prefix of the family name is derived from *flavus* (yellow) and refers to the type species, yellow fever virus.

[1] Report of the ***Togaviridae*** Study Group, Vertebrate Virus Subcommittee, International Committee on Taxonomy of Viruses.

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Received: March 11, 1985

Table I. Comparative taxonomic features of *Flavivirus* and *Alphavirus* genera

Property	*Alphavirus* genus	*Flavivirus* genus	References
Virus particle:			4
Size of virion/core by negative stain (nm)	60–65/35–39	40–50/20–30	
Symmetry of nucleocapsid/core	icosahedral	unknown	
RNA:			5–8
Size (daltons $\times 10^6$)	4.2–4.4	4.2–4.4	
m^7G-ppp (5′ end)	type 0	type 1	
poly(A) (3′ end)	yes	no	
Structural proteins (kdaltons):			9–12
Envelope – glycosylated	E1 and E2, 50–59	E1, 51–59	
Core	C, 30–34	C, 13–16	
Membrane-like		M, 8	
Translation strategy:			12–14
Subgenomic mRNA(s)	1	0	6, 12, 15
Polyprotein(s) + cleavage	yes	none detected	12, 16
Enhanced late synthesis of structural proteins	yes	no	10, 12
Translation of genome in cell-free systems	nonstructural proteins	structural proteins	17–19
Location of genes for structural proteins	near 3′ end of genome	near 5′ end of genome	12, 20, 21
Morphogenesis:			4, 14
Intracellular cores or nucleocapsids	visible and isolatable	not visible and not isolatable	
Maturation site	plasma membrane	within cisternae	
Morphogenesis	budding	undefined (condensation?)	

Table I lists the major features [4–21] that distinguish flaviviruses from the alphaviruses; the latter comprise the best characterized genus of the ***Togaviridae*** and provide a useful yardstick for comparison. Flaviviruses have only one envelope protein, in contrast to alphaviruses (and other togaviruses) which incorporate two or three [9–12]. The cap and tail structures of the genomic RNAs also differ, as does the gene sequence. Analyses of cell-free translation products of the genomic RNA of several flaviviruses suggested that the genes for the structural proteins were located at the 5′ end [17–19]. Subsequently, nucleotide sequencing of the genome of yellow fever virus located these genes in the sequence C–M–E at the 5′ end in a very long open reading frame [20]. In contrast, the genes for the structural proteins of alphaviruses are located near the 3′ end of the genome [12].

A significant difference in replication strategy from alphaviruses is indicated by the consistent reports of an absence of subgenomic mRNA in flavivirus-infected cells [6, 15]. The flavivirus genome is thus the largest mRNA that is completely translated in eukaryotic cells. Although the total coding con-

tent of alphaviruses is similar, these togaviruses specify a 26S mRNA that represents the 3′ one-third of the genome, from which the structural proteins are translated [12]; rubella virus (*Rubivirus* genus) employs a similar strategy [22]. In further contrast, no polyproteins have been detected in flavivirus-infected cells. Results of translation mapping experiments do not yield a gene sequence based on a single site of initiation, as do other positive-strand RNA viruses [16, 21]. At least six unrelated nonstructural proteins (relative molecular mass ranging from 10×10^3 to 98×10^3) are translated continuously [10, 23, 24]; among closely related species the two largest products have similarities in amino acid sequences, as do the E and C proteins [25]. Complex and high mannose glycans are found attached to the polypeptides related to E in cells [26].

In flavivirus-infected cells both smooth and rough endoplasmic reticulum undergo extensive proliferation and form characteristic organelles [4, 14]. Flavivirus RNA synthesis appears to occur in the perinuclear region in foci rather than on membranes dispersed more peripherally throughout the cytoplasm, as observed with the alphaviruses [27, 28]. RNA synthesis involves both a replicative intermediate and a replicative form [29, 30]. Complementation occurs between chemically induced temperature-sensitive mutants (RNA^+ and RNA^-) for two species [31, 32]. All flavivirus-specified proteins are translated on membrane-bound polysomes of increased density (relative to membrane-bound polysomes that translate cell proteins) and remain associated with membranes [33, 34]. Visible changes are induced in the rough endoplasmic reticulum, producing a unique appearance in electron micrographs [4, 14] and by immunofluorescence [28]. Whereas free nucleocapsids and budding at the plasma membrane are regularly observed during morphogenesis of alphaviruses, neither of these features have been unambiguously identified in many studies of flavivirus-infected cells [4, 14]. In the latter, virions mature by an undefined process (possibly condensation) and accumulate within cisternae of the endoplasmic reticulum [4, 14, 35]. In mosquito cell cultures some members induce syncytium formation and establish persistent infections by temperature-sensitive mutants [36, 37].

Within the *Flavivirus* genus, most species also are arboviruses in the biological sense. They infect a wide range of vertebrate hosts, causing asymptomatic infections and a variety of diseases, e.g., yellow fever, dengue, and several encephalitides. Some strains of mice, and cells derived from them, have genetically controlled resistance to flaviviruses [38]. Epitopes on the envelope protein E induce monoclonal antibodies which react with (1) type, (2) complex, or (3) group specificity, measurable by hemagglutination-inhibition or neutralization tests (1 and 2) or by only the former test (3) [39–42]; most species are included in seven serological complexes or subgroups. Transovarial transmission of some species occurs in arthropods, and transplacental transmission occurs in some mammals.

The *Aedes albopictus* cell fusing agent (CFA) virus [43] and simian hemorrhagic fever (SHF) virus [44, 45] were originally classified as additional possible members of the ***Togaviridae*** [3]. They are still incompletely characterized, but appear to share several features with flaviviruses, e.g., size of virion and of RNA, absence of subgenomic mRNA in infected cells, and morphogenesis within cisternae of endoplasmic reticulum with no

apparent evidence of budding. They are unrelated antigenically to flaviviruses. Pending further information, they are now classified as possible members of the ***Flaviviridae***.

In summary, the ***Flaviviridae*** comprise the single genus *Flavivirus* and two possible members. Flaviviruses are enveloped viruses about 45 nm in diameter and contain infectious, single-stranded linear RNA of molecular weight about 4×10^6 which is capped at the 5′ end but lacks a poly(A) tract at the 3′ end. The peplomers of the envelope comprise a single species of glycoprotein E that is embedded in host-derived lipid and is associated with a membrane-like protein M, which surrounds the RNA encased in the core protein C. The gene sequence commences 5′–C–M–E... The replication strategy and the mode of morphogenesis are distinct in several respects from the replication processes of the well-characterized members of the ***Togaviridae***, in which the *Flavivirus* genus was formerly classified.

1 Taxonomy
1.1 Family: ***Flaviviridae***.
1.1.1 Genus: *Flavivirus*.
1.1.2 Type species: Yellow fever virus, strain Asibi.
1.2 Taxonomic status: Family with one genus.
1.3 Relationship with other groups: Some similarities to togaviruses.
2 The virion
2.1 Chemical composition
2.1.1 Nucleic acid
2.1.1.1 RNA
2.1.1.2 Single-stranded
2.1.1.3 Linear
2.1.1.4 Number of pieces: One.
2.1.1.5 Sedimentation coefficient: 44S (relative to 49S RNA of Sindbis virus).
2.1.1.6 Molecular weight: 4×10^6.
2.1.1.7 Percentage weight of virion: 6%.
2.1.1.8 Base composition: G, 26; A, 30; C, 22; U, 22 [dengue-2, St. Louis encephalitis (SLE)].
2.1.1.9 Nearest neighbor analysis: Not known.
2.1.1.10 Homology studies: Oligonucleotides from T1 RNase digests of dengue-1 to -4 are distinct, as are those of topotype varieties of SLE, dengue-1, and dengue-2.
2.1.1.11 Infectivity: RNA is infectious.
2.1.1.12 Other features: 44S RNA serves as mRNA, with a type 1 cap at the 5′ terminus and no poly(A) tract at the 3′ end [West Nile (WN), dengue-2]; a common heptanucleotide occurs in the genome of WN and SLE viruses in both strands of the replicative form and within 16 nucleotides of the 3′ end which terminates in CUOH.
2.1.2 Proteins
2.1.2.1 Percentage weight of virion: 66% (SLE).
2.1.2.2 Number of polypeptides: Three.
2.1.2.3 Molecular weights of polypeptides: E, $(51–59) \times 10^3$; C, $(13–16) \times 10^3$; M, $(7–9) \times 10^3$.
2.1.2.4 Number of protein subunits in virion: Not known.
2.1.2.5 Enzymes: None.
2.1.2.6 Other functional proteins: E functions as hemagglutinin. A nonstructural glycoprotein [NV2, mol.wt. $(17–19) \times 10^3$] is incorporated in some virion preparations in lieu of or in addition to M.
2.1.3 Lipids
2.1.3.1 Percentage weight of virion: 17% (SLE).

2.1.3.2 Other features: Composition is host dependent.
2.1.4 Carbohydrates
2.1.4.1 Percentage weight of virion: 9% (SLE).
2.1.4.2 Other features: E incorporates both high mannose and complex glycans.
2.2 Physicochemical properties
2.2.1 Density: 1.19–1.20 g/cm^3 in sucrose; 1.22–1.24 g/cm^3 in cesium chloride.
2.2.2 Sedimentation coefficient: 200S (range from 175S to 218S).
2.2.3 Weight in daltons: $(60–70) \times 10^6$ (based on RNA content of 6%).
2.2.4 Stability of infectivity
2.2.4.1 pH: Stable at pH 7–9.
2.2.4.2 Heat: Rapidly inactivated at 50°; more slowly at 37°. Dengue-2: 50% inactivated at 50° in 10 min.
2.2.4.3 Lipid solvents: Sensitive.
2.2.4.4 Radiation: Rapidly inactivated by UV light.
2.2.4.5 Other agents: Very sensitive to ionic and nonionic detergents; sensitive to trypsin.
2.3 Structure
2.3.1 Nucleocapsid
2.3.1.1 Symmetry: Not known.
2.3.2 Envelope
2.3.2.1 Dimensions: 35–45 nm.
2.3.2.2 Composition: Lipid bilayer membrane incorporating E (a glycoprotein) and membrane-like M protein.
2.3.3 Cores
2.3.3.1 Dimensions: 25–30 nm diameter; 120–140S.
2.3.3.2 Composition: RNA and C protein.
2.4 Morphology
2.4.1 Overall shape: Spherical, with very fine indistinct surface projections that form a continuous halo in some preparations.
2.4.2 Dimensions: 45–50 nm.
2.4.3 Surface projections: 5- to 10-nm spikes, apparently with terminal knobs 2 nm in diameter, but appearance varies with different negative-contrast materials.
2.4.4 Special features in thin sections: Lucent envelope surrounds a dense core; surface projections are not visible. Virion diameter 36–44 nm, core diameter 25–30 nm.
2.4.5 Other features: Not established.
3 Replication
3.1 Site of accumulation of viral proteins: Cytoplasmic membranes. M protein not found in cells. Envelope protein (E of Kunjin virus) accumulates in perinuclear region early in infection.
3.2 Nonstructural proteins
3.2.1 Number and molecular weights of polypeptides: 6 or 7. Nomenclature and molecular weight range: 10×10^3 (P10, formerly NV1); glycoprotein $(17–19) \times 10^3$ (GP17–19, NV2); $(19–21) \times 10^3$ (P19–21, NV2½); $(24–32) \times 10^3$ (p24–32, NVX); glycoprotein $(44–48) \times 10^3$ (GP44–48, NV3); $(67–75) \times 10^3$ (P67–75, NV4); and $(91–98) \times 10^3$ (P91–98, NV5). Peptide maps show no relationship to one another or to intracellular equivalents of E and C. Among closely related species (Kunjin, Murray Valley encephalitis, WN), the similarity of amino acid sequences in peptide maps of the two largest nonstructural proteins (NV4, NV5) varies between 0.5 and 0.9 (expressed as an overlap index), which is equivalent to the variation among the E and C proteins.

3.2.2 Virus-induced enzymes: RNA polymerase.

3.3 Mode of nucleic acid replication

3.3.1 General account: Viral RNA, single strand, molecular weight about 4×10^6, transcribed by virion-induced polymerase into complementary minus-strand 44S RNA. This remains in a double-stranded replicative form (20S) which recycles as template from which progeny RNA is apparently copied by semi-conservative and asymmetric replication via a replicative intermediate (20–28S).

3.3.2 Effects of inhibitors: Insensitive to actinomycin D; insensitive to guanidine (dengue); inhibited by 6-azauridine or 5-fluorouridine.

3.4 Site and mechanism of maturation: Morphogenesis appears to occur within cisternae of endoplasmic reticulum. No precursor or core particles identifiable in cells.

3.5 Other features: Replication is associated with extensive proliferation of smooth and rough endoplasmic reticulum, forming characteristic organelles. RNA synthesis occurs in foci in the perinuclear region. Protein synthesis is associated with visible reorganization of the rough endoplasmic reticulum, and the virus translation products remain incorporated in cytoplasmic membranes. Structural and nonstructural proteins are translated in constant proportions from genomic RNA, which functions as sole messenger. The gene sequence commences 5′–C–M–E... No polyproteins are detected in the cytoplasm. Translation mapping experiments do not yield a gene sequence for the nonstructural proteins based on a single site of initiation. In infected cells protein synthesis inhibitors (cycloheximide, puromycin) selectively block translation of the two largest nonstructural proteins. A subviral particle comprising only the structural proteins E and M and the small nonstructural glycoprotein is released from infected vertebrate cells as a slow-sedimenting hemagglutinin. Host cell RNA and protein syntheses continue throughout infection. The latent period is 12–15 h.

4 Cooperative interactions

4.1 Recombination: No.

4.2 Multiplicity reactivation: No.

4.3 Phenotypic mixing: No.

4.4 Other interactions: Complementation between temperature-sensitive mutants (dengue, SLE).

5 Host range

5.1 Natural: Many vertebrates and invertebrates (mosquitoes for some, ticks for others; some flaviviruses have no known invertebrate host).

5.1.1 Resistance gene(s): A resistance gene specific to flavivirus infection is present in some strains of mice.

5.2 Experimental

5.2.1 In vivo: Wide range of vertebrates and invertebrates.

5.2.2 In vitro: Wide range of vertebrate and invertebrate cells.

6 Pathogenicity

6.1 Association with diseases: Members of the genus cause asymptomatic infections and varied clinical syndromes including undifferen-

tiated fever, fever with rash, hemorrhagic fevers, and encephalitis.

6.2 Tissue tropisms: Tropisms vary among members of genus and among strains.

6.3 Cytopathology: In some vertebrate cells; in invertebrate cells either none or syncytium formation.

7 Geographic distribution: Worldwide.

8 Transmission

8.1 Vertical: Transovarial transmission of tick-borne encephalitis (TBE) virus occurs in ticks and of dengue, Japanese encephalitis, Koutango and yellow fever viruses in mosquitoes; transplacental transmission of TBE and Powassan viruses occurs in goats and of SLE virus in mice.

8.2 Horizontal: Exceptional.

8.3 Vectors

8.3.1 Biological: Proved with most; not established with some.

8.3.2 Mechanical: Possibly.

9 Antigenic properties

9.1 Number of distinct antigenic molecules in virion: At least two – one core protein (C) and one envelope glycoprotein (E).

9.2 Antigen(s) involved in virus neutralization: Type-specific epitopes in E are identified by using monoclonal antibodies.

9.3 Number of distinct nonstructural antigens: Two are well characterized – the largest nonstructural protein (NV5) and the soluble complement-fixing antigen (SCF or NV3). Others probably exist (refer to 3.2.1).

9.4 Specificity of different antigens: Antisera prepared against core and envelope do not cross-react. Envelope protein E contains epitopes that induce monoclonal antibodies with reactivity patterns corresponding to (1) type or subtype/strain specificity, (2) subgroup or complex/subcomplex specificity, and (3) group specificity. In patterns (1) and (2) the reaction is measurable by hemagglutination-inhibition and/or by neutralization test, and in pattern (3) it is measurable by the former test. These same specificities are observed both by antibody-dependent plaque enhancement and by immunofluorescence of infected cells. Topographical analysis of epitopes in E by competition-binding assays defines a continuum of overlapping domains, nearly all in one or two clusters.

9.5 Antigenic properties used for classification: Complement fixation, hemagglutination inhibition, neutralization, immunoprecipitation, solid-phase binding assays.

10 Classification

10.1 Definition of family: Enveloped RNA viruses 40–50 nm in diameter, with one envelope protein (usually a glycoprotein) and a positive-sense, single-stranded RNA of molecular weight 4×10^6. Absence of a poly(A) tract at the 3′ end of the RNA appears to be a general feature. Structural proteins are translated from genomic RNA in vitro; subgenomic mRNA is not synthesized. Morphogenesis of virions occurs within cisternae of modified endoplasmic reticulum, which proliferates during infection.

10.2 Definition of genus *Flavivirus*: RNA has a type 1 cap at the 5′ end; no poly(A) tract at the 3′ end. Molecular weights of structural proteins are $(51–59) \times 10^3$ (E), $(13–16) \times 10^3$ (C), and $(7–9) \times 10^3$ (M); the gene sequence commences 5′–C–M–E... No polyproteins have been identified in the cytoplasm, and translation mapping experiments do not yield a gene sequence based on a single site of initiation. Most species are arboviruses, and all are serologically interrelated. Hemagglutination by virions is pH dependent.

10.2.1 Type species: Yellow fever virus, strain Asibi.

10.2.2 Other members of genus: Alfuy, Apoi, Aroa, Bagaza, Banzi, Bouboui, Bukalasa bat, Bussuquara, Cacipacore, Carey Island, Cowbone Ridge, Dakar bat, dengue-1, -2, -3 and -4, Edgehill, Entebbe bat, Gadgets Gully, Ilheus, Israel turkey meningoencephalitis, Japanese encephalitis, Jugra, Jutiapa, Kadam, Karshi, Kokobera, Koutango, Kunjin, Kyasanur Forest disease, Langat, louping ill, Meaban, Modoc, Montana myotis leukoencephalitis, Murray Valley encephalitis, Naranjal, Negishi, Ntaya, Omsk hemorrhagic fever, Phnom-Pehn bat, Powassan, Rio Bravo, Rocio, Royal Farm, Saboya, Saumarez Reef, Sal Vieja, San Perlita, Sepik, Sokoluk, Spondweni, St. Louis encephalitis, Stratford, Tamana Bat, Tembusu, tick-borne encephalitis (subtypes: European and Far Eastern), Tyuleniy, Uganda S, Usutu, Wesselsbron, West Nile, Zika.

10.3 Species or subgroup: Mosquito-borne, tick-borne and viruses without a known vector.

10.4 Possible members of family: Cell fusing agent (CFA) virus and simian hemorrhagic fever virus which have some flavivirus-like characteristics.

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