19. RNA PLUS GENOME THAT SERVES AS MESSENGER RNA: TOGAVIRUSES

FAMILY TOGAVIRIDAE

The virions are spherical and enveloped, with a diameter of 40–70 nm. The genome is a single RNA plus molecule with a molecular weight of 4×10^6. The RNA genome is contained in an icosahedral nucleocapsid that is assembled in the cytoplasm and obtains its lipoprotein envelope during budding through the plasma membrane of the host cell in the final stage of virus maturation.

Genus Alphavirus (Arbovirus group A)

The virus species include: Aura, Bebaru, Chikungunya, Eastern equine encephalomyelitis (EEE), Everglades, Getah, Mayaro, Middleburg, Mucambo, Ndumu, O'nyong-nyong, Pixuma, Ross river, Semliki Forest, Sindbis, Venezuela equine encephalomyelitis (VEE), Western equine encephalomyelitis (WEE) (figure 61), and Whataroa. These viruses multiply in arthropods as well as in vertebrates.

Genus Flavivirus (Arbovirus group B)

Species that are mosquito-borne include: Yellow fever; dengue types 1, 2, 3, and 4; Japanese encephalitis; Spondweni; St. Louis; Uganda S; Wesselsbron; West Nile; and Zika.

Tick-borne species include: Kyasanur Forest disease; Langat; Louping ill;

Y. Becker, *Molecular Virology*

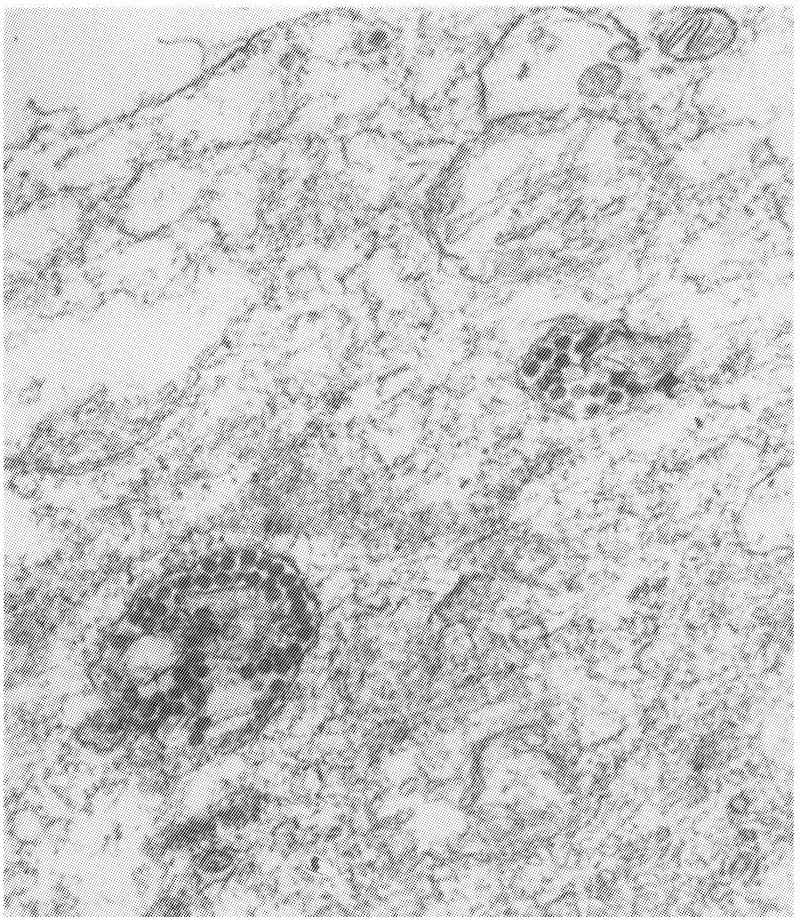

Figure 61. Electron micrograph of Western equine encephalitis (WEE) virus in the cytoplasm of infected cells.
(Courtesy of Dr. Daniel Dekegel, Institut Pasteur du Brabant, Brussels, Belgium.)

Omsk hemorrhagic fever; Royal Farm; Saumarez Reef; and tick-borne encephalitis (European and Far Eastern). There are also a number of species with unknown vectors.

Genus Rubivirus

Rubella virus occurs only in man.

Genus Pestivirus

Mucosal disease virus, border disease of sheep, hog cholera (European swine fever) and other possible members of the Togaviridae are not arthropod-borne.

Toga comes from the Latin *toga* (gown, cloak), alpha from the Greek letter *A*; and flavi, rubi, and pesti from the Latin *flavus* (yellow), *rubeus* (reddish), and *pestis* (plague), respectively.

ORGANIZATION OF THE VIRIONS

The virions have a diameter of 60 nm (alpha- and rubiviruses) and a sedimentation coefficient of 240–300 S. Flavi- and pestiviruses have a diameter of 45 nm and a sedimentation coefficient of 170–220S. Virions have a nucleocapsid complex of 240 capsid proteins (C protein, m.w. 30,000) containing the RNA genome (42 S), enveloped by a lipid bilayer in which the virus-coded glycoproteins are present as spikes. There are about 240 spikes in the envelope of each virion. The spike is made up of three polypeptides: E1 (m.w. 49,000), E2 (m.w. 52,000), and E3 (m.w. 10,000). E1 and E3 have one attached oligosaccharide and E2 contains two attached oligosaccharides. Polypeptides E1 and E2 of the spike are attached to the lipid bilayer by their hydrophobic tails (COOH-terminal ends), while E3 is present on the outer side of the envelope bound to E1 and/or E2 (Garoff and Söderlund 1978). The E2 polypeptide spans the membrane: The terminus of the E2 polypeptide passes through the lipid bilayer; the amino terminal end is present on the outer surface, and the carboxy terminal end on the inner surface of the lipid bilayer envelope. It is possible that each spike is attached to the capsid protein C beneath the lipid bilayer. The viral protein C present in the nucleocapsid is rich in lysine.

The 42 S virion RNA contains 12,000 nucleotides with a poly(A) sequence attached to the 3′ end and a cap ($m^7GpppAp$) present in the 5′ end. The virion RNA serves as messenger RNA after uncoating in the cytoplasm and can be found in the polyribosomes of the infected cell.

Alphaviruses contain neutral lipids arranged in a bilayer structure in the virus membrane: mainly cholesterol, as well as phosphatidylethanolamine, phosphatidylserine, sphingomyelin, and phosphatidylcholine. Oleic acid, palmitic acid, and stearic acid are the major fatty acids in the lipid bilayer. The virion envelope has a lipid composition similar to that of the host cell membrane, but no host cell proteins are found in the viral membrane. The membrane is most likely derived from a segment of the plasma membrane that does not contain host cell proteins.

ANTIGENIC STRUCTURE

The classification of togaviruses is based on immunological cross reactivity, as determined by hemagglutination inhibition, neutralization tests, and agar diffusion, as well as by radioimmunoassay, using specific antibodies against each virus isolate. Members of a genus are serologically related to each other, but not to other members of the family. The protein E1 (a glycoprotein) of Sindbis virus has the ability to hemagglutinate red blood cells, whereas protein E2 is responsible for the ability of the virus to infect cells. Antibodies to the E2

protein, therefore, have the ability to neutralize virus infectivity. The glycoprotein E1 of Sindbis virus is antigenically related to that of WEE, while E2 is specific for both viruses. Relatedness of alphaviruses determined by RNA-RNA hybridization showed that RNA from Chikungunya and O'nyong-nyong viruses had 13% base sequence homology whereas sequence homologies of 1% or less were detected between these two viruses and Semliki Forest and Sindbis viruses (Wengler et al. 1977). Thus the rather low sequence homologies between the nucleic acids of these viruses do not affect their antigenic relationships. This may be explained by the assumption that the antigenic sites are composed of only a small number of amino acids.

MOLECULAR EVENTS IN ALPHAVIRUS REPLICATION

Alphaviruses replicate in the cytoplasm of both vertebrate and invertebrate cell cultures. In chick embryo fibroblasts, the virus reaches maximal yields within five hr. In actinomycin D-treated infected cells, the virus yield is higher than in untreated cultures, possibly due to inhibition of interferon synthesis. Actinomycin D inhibits DNA-dependent RNA synthesis, which shows that this virus does not require host cell nuclear functions for replication.

The virus enters the cell by absorptive endocytosis (Helenius et al. 1980). Inside the lysosomes of the cell, the low pH probably causes the viral membrane to fuse with the lysosomal membrane (White and Helenius 1980). This allows the nucleocapsid to enter the cytoplasm, where the viral genome is uncoated. The parental viral RNA acts as mRNA for the synthesis of the viral RNA-dependent RNA polymerase that binds to the smooth cytoplasmic membranes where replication of viral RNA takes place. The replicative intermediates are RNA molecules that are partially double-stranded and include one complete RNA molecule that serves as template and a number of progeny RNA molecules that are hydrogen-bonded to the RNA template. The RNA polymerase is responsible for the transcription of the viral RNA^+ genomes from the RNA^- strands.

Synthesis of the viral RNA and proteins

In the alphaviruses, two species of single-stranded RNA are synthesized, 42S RNA (m.w. 4.2×10^6) and 26S RNA (m.w. 1.6×10^6). Both RNA molecules contain poly(A) sequences of heterogeneous length, and the 26S molecules represent subgenomic RNA species, having the same polarity as the virion RNA. The 26S subgenomic RNA is homologous to the 3′ end of the viral genome (Kennedy 1976) and functions as mRNA for the structural proteins of the virus. This is an efficient mechanism of reiteration of a portion (about one-third) of the viral genome and allows excess synthesis of the viral structural proteins independently of the synthesis of the nonstructural proteins by the 42S RNA molecules.

The nucleotide sequence of the gene coding for the capsid (C) protein is now known, as is the nucleotide sequence of cloned cDNA made from the 26S

RNA of Semliki Forest virus (Garoff et al. 1980*a,b*). The amino acid sequences of the different membrane proteins deduced from the nucleotide sequences are shown in figure 62. The coding region of the 26S RNA starts with the aminoterminus of the capsid protein C and terminates 3759 nucleotides later when the first stop codon UAA is reached. The regions coding for the E3, E2 and E1 polypeptides were localized in this sequence.

Translation of the 26S RNA

The structural proteins consisting of the four polypeptides E1, E2, and E3, and the capsid protein are translated from the 26S RNA using a single initiation site in the following order: capsid, p62, and E1. The p62 polypeptide is the precursor of the E3 and E2 proteins. Figure 63 shows that the C protein appears after the first cleavage of the precursor polypeptide p130. The C protein molecules subsequently bind to the 42S genomic RNA to form the nucleocapsid. The first cleavage product p97 is further cleaved to yield polypeptide p62 and the glycoprotein E1. P62 is cleaved to yield the glycoproteins E3 and E2 (Kääriäinen and Söderlund 1978).

A scheme for the assembly of the Semliki Forest virus spike glycoprotein E1 in the membrane of the endoplasmic reticulum as suggested by Garoff and associates (1980*b*) is depicted in figure 64.

Translation of the 42S RNA

The viral 42S RNA acts as polycistronic mRNA that is translated by the cellular ribosomes to yield the nonstructural viral proteins. This is demonstrated in figure 65, which shows that the viral RNA is not completely translated. In Semliki Forest virus, the precursor polypeptide is cleaved at three sites to yield four stable proteins of m.w. 70,000, 86,000, 72,000, and 60,000 (ns 70, ns 86, ns 72, and ns 60, respectively); ns 70 is the N-terminal protein. Two large, short-lived, intermediate polypeptides of 155,000 and 135,000 daltons have been identified that are probably precursors to the four stable proteins. Another polypeptide of 220,000 daltons (containing the N-terminus) has also been found.

Biosynthesis of virions

In alphaviruses, the viral nucleocapsid aligns below the plasma membrane and the virus is formed by budding through the plasma membrane. During the budding, the virus obtains the lipids from the host cell plasma membrane (Kääriäinen and Söderlund 1978). The formation of virions is explained schematically in figure 66.

VIRUS MUTANTS

Ts mutants of Sindbis virus can be divided into five complementation groups. Viruses in groups A and B are incapable of virus-specific RNA synthesis (RNA^-) at the restrictive temperature, and the parental RNA fails to enter the

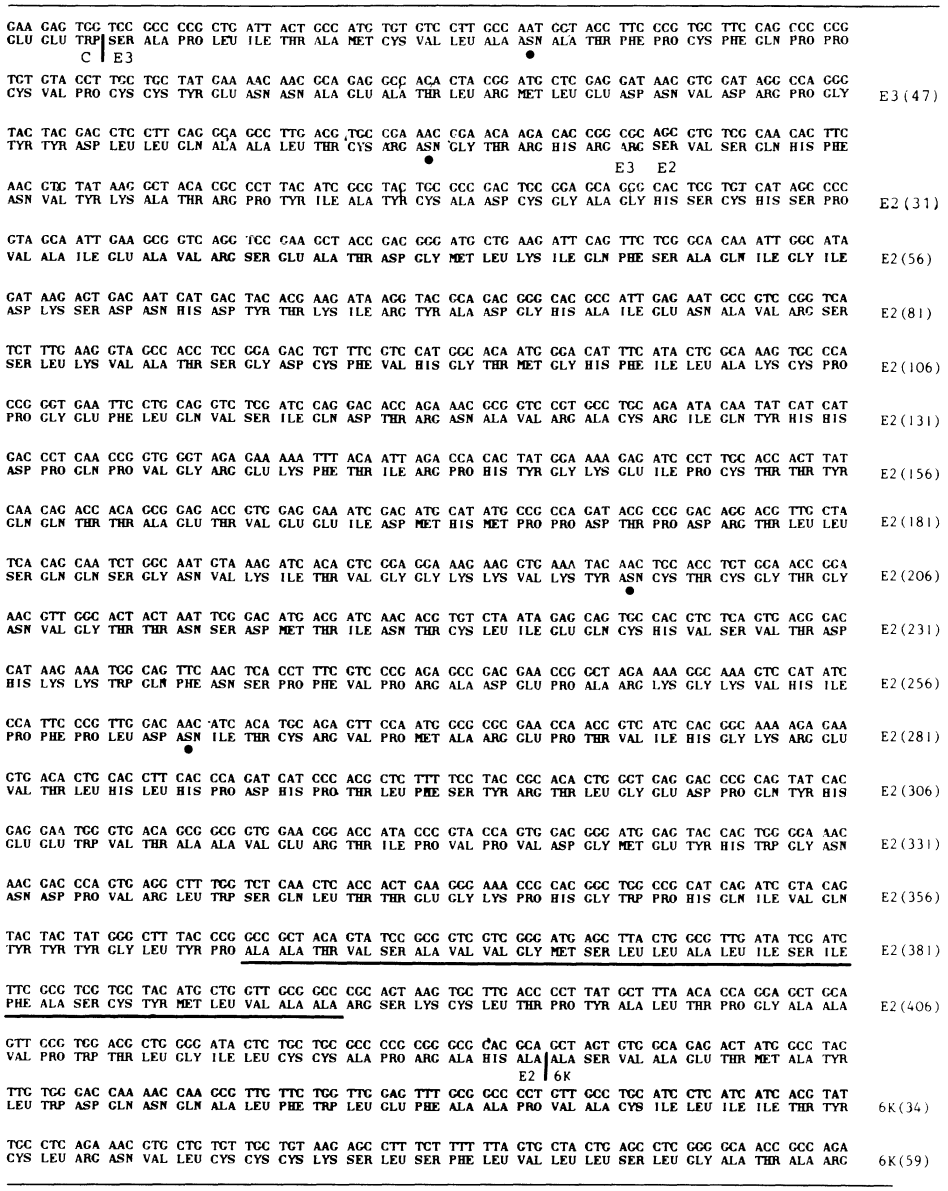

Figure 62. The nucleotide sequence of the membrane protein genes. The deduced amino acid sequences of the membrane proteins are shown. The amino acid residues are numbered from the amino terminus of each polypeptide. The positions are shown in the parentheses to the right. Membrane-spanning segments are underlined and potential glycosylation sites are marked (●).

(Garoff et al. 1980b. Reprinted by permission from *Nature* 288, Fig. 3, p. 239. Copyright © 1980 Macmillan Journals Limited.)

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GCT TAC GAA CAT TCG ACA GTA ATG CCG AAC GTG GTG GGG TTC CCG TAT AAG GCT CAC ATT GAA AGG CCA GGA TAT
ALA TYR GLU HIS SER THR VAL MET PRO ASN VAL VAL GLY PHE PRO TYR LYS ALA HIS ILE GLU ARG PRO GLY TYR
6K | E1

AGC CCC CTC ACT TTG CAG ATC CAG GTT GTT GAA ACC AGC CTC GAA CCA ACC CTT AAT TTG GAA TAC ATA ACC TGT
SER PRO LEU THR LEU GLN MET GLN VAL VAL GLU THR SER LEU GLU PRO THR LEU ASN LEU GLU TYR ILE THR CYS  E1(49)

GAC TAC AAG ACG GTC GTC CCG TCC CCG TAC GTG AAG TGC TCC GGC GCC TCA GAG TGC TCC ACT AAA GAG AAG CCT
GLU TYR LYS THR VAL VAL PRO SER PRO TYR VAL LYS CYS CYS GLY ALA SER GLU CYS SER THR LYS GLU LYS PRO  E1(74)

GAC TAC CAA TGC AAG GTT TAC ACA GGC GTG TAC CCG TTC ATG TGG GGA GGG GCA TAT TGC TTC TGC GAC TCA GAA
ASP TYR GLN CYS LYS VAL TYR THR GLY VAL TYR PRO PHE MET TRP GLY GLY ALA TYR CYS PHE CYS ASP SER GLU  E1(99)

AAC ACG CAA CTC AGC GAC CCG TAC GTC GAT CCA TCG GAC GTA TGC AGG CAT GAT CAC GCA TCT GCT TAC AAA CCC
ASN THR GLN LEU SER GLU ALA TYR VAL ASP ARG SER ASP VAL CYS ARG HIS ASP HIS ALA SER ALA TYR LYS ALA  E1(124)

CAT ACA GCA TCG CTG AAG CCC AAA GTG AGG GTT ATG TAC GGC AAC GTA AAC CAG ACT GTG GAT GTT TAC GTG AAC
HIS THR ALA SER LEU LYS ALA LYS VAL ARG VAL MET TYR GLY ASN VAL ASN GLN THR VAL ASP VAL TYR VAL ASN  E1(149)
                                                                  ●

GGA GAC CAT CCC GTC ACG ATA GGG GGT ACT CAG TTC ATA TTC GGG CCG CTG TCA TCG GCC TGG ACC CCG TTC GAC
GLY ASP HIS ALA VAL THR ILE GLY GLY THR GLN PHE ILE PHE GLY PRO LEU SER SER ALA TRP THR PRO PHE ASP  E1(174)

AAC AAG ATA GTC GTG TAC AAA GAC GAA GTG TTC AAT CAG GAC TTC CCG CGG TAC GGA TCT GGG CAA CCA GGG CGC
ASN LYS ILE VAL VAL TYR LYS ASP GLU VAL PHE ASN GLN ASP PHE PRO PRO TYR GLY SER GLY GLN PRO GLY ARG  E1(199)

TTC GGC GAC ATC CAA AGC AGA ACA GTG GAC AGT AAC GAC CTG TAC GCG AAC ACG GCA CTG AAG CTG GCA CGC CCT
PHE GLY ASP ILE GLN SER ARG THR VAL GLU SER ASN ASP LEU TYR ALA ASN THR ALA LEU LYS LEU ALA ARG PRO  E1(224)

TCA CCC GGC ATG GTC CAT GTA CCG TAC ACA CAG ACA CCT TCA GGG TTC AAA TAT TCG CTA AAG GAA AAA GGC ACA
SER PRO GLY MET VAL HIS VAL PRO TYR THR GLN THR PRO SER GLY PHE LYS TYR TRP LEU LYS GLU LYS GLY THR  E1(249)

GCC CTA AAT ACG AAG GCT CCT TTT GGC TGC CAA ATC AAA ACG AAC CCT GTC AGG GCC ATG AAC TGC CCC GTC GCA
ALA LEU ASN THR LYS ALA PRO PHE GLY CYS GLN ILE LYS THR ASN PRO VAL ARG ALA MET ASN CYS ALA VAL GLY  E1(274)

AAC ATC CCT GTC TCC ATC AAT TTG CCT GAC AGC GCC TTT ACC CGC ATT GTC GAG GCG CCG ACC ATC ATT GAC CTG
ASN ILE PRO VAL SER MET ASN LEU PRO ASP SER ALA PHE THR ARG ILE VAL GLU ALA PRO THR ILE ILE ASP LEU  E1(299)

ACT TGC ACA GTG GCT ACC TGT ACG CAC TCC TCG GAT TTC GGC GGC GTC TTC ACA CTG ACG TAC AAG ACC AAC AAG
THR CYS THR VAL ALA THR CYS THR HIS SER SER ASP PHE GLY GLY VAL LEU THR LEU THR TYR LYS THR ASN LYS  E1(324)

AAC GGG GAC TGC TCT GTA CAC TCG CAC TCT AAC GTA GCT ACT CTA CAG GAG GCC ACA GCA AAA GTC AAC ACA GCA
ASN GLY ASP CYS SER VAL HIS SER HIS SER ASN VAL ALA THR LEU GLN GLU ALA THR ALA LYS VAL LYS THR ALA  E1(349)

GGT AAG GTG ACC TTA CAC TTC TCC ACG GCA AGC GGA TCA CCT TCT TTT GTC GTG TCG CTA TGC ACT GCT ACG GCC
GLY LYS VAL THR LEU HIS PHE SER THR ALA SER ALA SER PRO SER PHE VAL VAL SER LEU CYS SER ALA ARG ALA  E1(374)

ACC TGT TCA GCG TCG TGT GAG CCC CCG AAA GAC CAC ATA GTC CCA TAT CCG GCT AGC CAC AGT AAC GTA GTC TTT
THR CYS SER ALA SER CYS GLU PRO PRO LYS ASP HIS ILE VAL PRO TYR ALA ALA SER HIS SER ASN VAL VAL PHE  E1(399)

CCA GAC ATG TCG GGC ACC GCA CTA TCA TGG CTG CAG AAA ATC TCG GGT GGT CTG GGG GCC TTC GCA ATC GGC GCT
PRO ASP MET SER GLY THR ALA LEU SER TRP VAL GLN LYS ILE SER GLY GLY LEU GLY ALA PHE ALA ILE GLY ALA  E1(424)

ATC CTG GTG CTG GTT GTG GTC ACT TGC ATT GGG CTC GGC ACA TAA GTT ACG GTA GGC AAT GGC ATT GAT ATA GCA
ILE LEU VAL LEU VAL VAL VAL THR CYS ILE GLY LEU ARG ARG

AGA AAA TTC AAA ACA GAA AAA GTT ACG GTA AGC AAT GGC ATA TAA CCA TAA CTC TAT AAC TTC TAA CAA ACC CCA

ACA AGA CCT GCG CAA TTG GCC CCG TGC TCC GCC TCA CGG AAA CTC GGG GCA ACT CAT ATT GAC ACA TTA ATT GGC

AAT AAT TGG AAG CTT ACA TAA GCT TAA TTC GAC GAA TAA TTG GAT TTT TAT TTT ATT TTG CAA TTG GTT TTT AAT

ATT TCC
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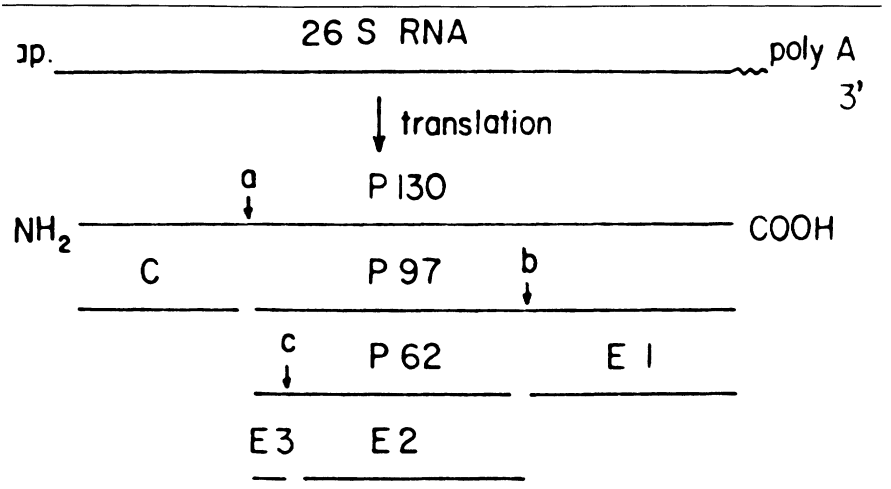

Figure 63. Translation of structural protein as a polyprotein from the 26 S RNA. The cleavage of capsid protein is nascent *(a)*, cleavage between p62 and E1 takes place rapidly after translation *(b)*, whereas p62 is cleaved during the maturation of the virus *(c)*.
(Kääriäinen and Söderlund 1978. Reprinted by permission from *Curr. Top. Microbiol. Immunol.* 82, Fig. 6, p. 35. Copyright Springer-Verlag, 1978, Heidelberg.)

replicative form. Mutants in groups C, D, and E have no apparent lesion in viral RNA synthesis (RNA^+), although in group C nucleocapsids are not assembled; in group D hemadsorption is absent, and in group E infectious virions are not produced, even though hemadsorption and nucleocapsid assembly are normal (Pfefferkorn and Shapiro 1974).

Effect of virus infection on the host cell

Alphavirus infection leads to a decay in the synthesis of cellular proteins and phospholipids. The effect of flaviviruses on the host cell metabolism is milder and leads to a mild cytopathogenic effect.

Defective interfering particles

The defective interfering particles formed during serial passage of high concentrations of virus contain RNA molecules that are smaller than the 42S genome. These particles stimulate the synthesis of abnormal single-stranded RNA molecules that is accompanied by a reduction in the synthesis of 42S and 26S RNA. Double-stranded RNAs of 12–16S have also been found.

DISEASES CAUSED BY TOGAVIRUSES

Alphaviruses and flaviviruses cause encephalitis and are transmitted by mosquitoes and ticks.

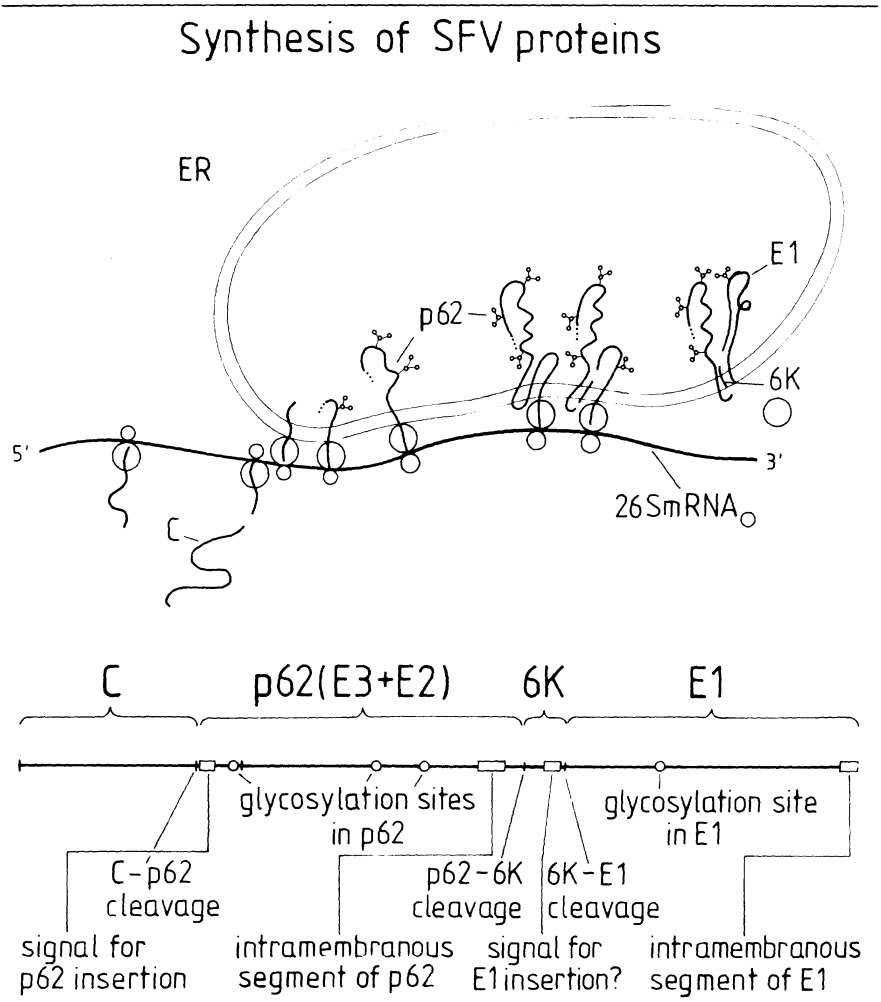

Figure 64. Scheme for the synthesis of Semliki Forest virus (SFV) spike glycoprotein E1 in the membrane of the cytoplasmic reticulum. The signal peptide of the p62 polypeptide mediates insertion into the membrane after the capsid protein C is cleaved. The polypeptide chain continues to grow, cleavage of the 6K peptide occurs, and the E1 polypeptide is synthesized when the signal peptide for E1 emerges.

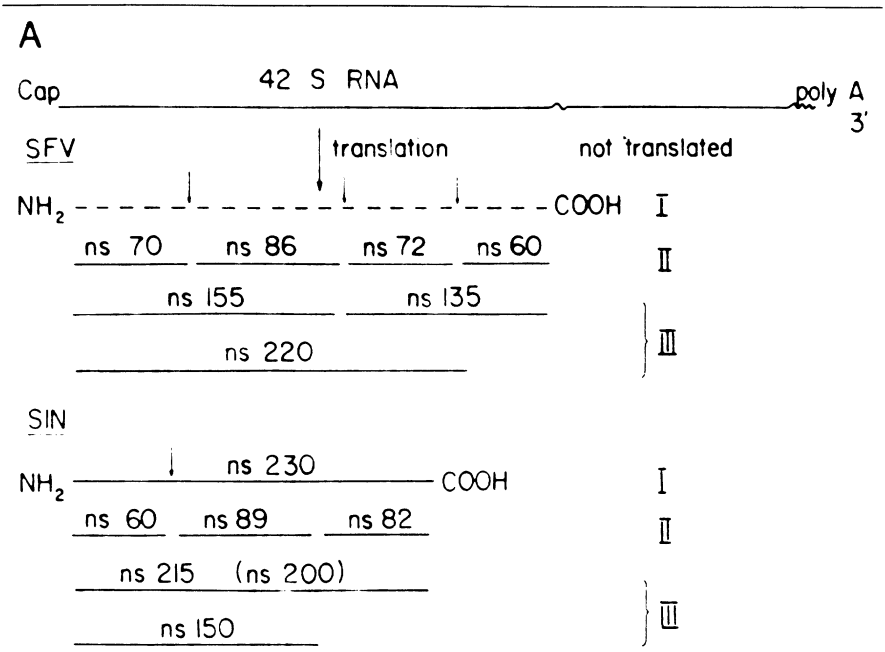

Figure 65. Translation of SFV and Sindbis virus (SIN) nonstructural proteins from 42 S RNA. I indicates the postulated primary translational product; II the stable cleavage products; and III the detected intermediates. Arrows indicate the cleavage sites. Dotted line refers to a protein that has not been found.
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Equine encephalitis

Three members of the genus alphavirus—EEE, WEE, and VEE—cause acute encephalitis in horses. The horses are infected by mosquitoes carrying the virus, and people attending the sick horses can also be infected. In an epidemic in Texas, children and adults who were in contact with infected horses fell ill, and fatal cases were reported among the children.

Yellow fever

Yellow fever virus, a member of the genus flavivirus, has a life cycle in the mosquito and the monkey. In areas in central Africa endemic for yellow fever, people were infected by the bite of the *Aedes aegypti* mosquito carrying the virus. In some infected persons, a fatal disease occurred, but in others the infection was mild, with an incubation period of three–six days, fever, and headache. In severe cases, symptoms included jaundice, haemorrhages in the intestine, vomiting, and a drop in blood pressure followed by coma.

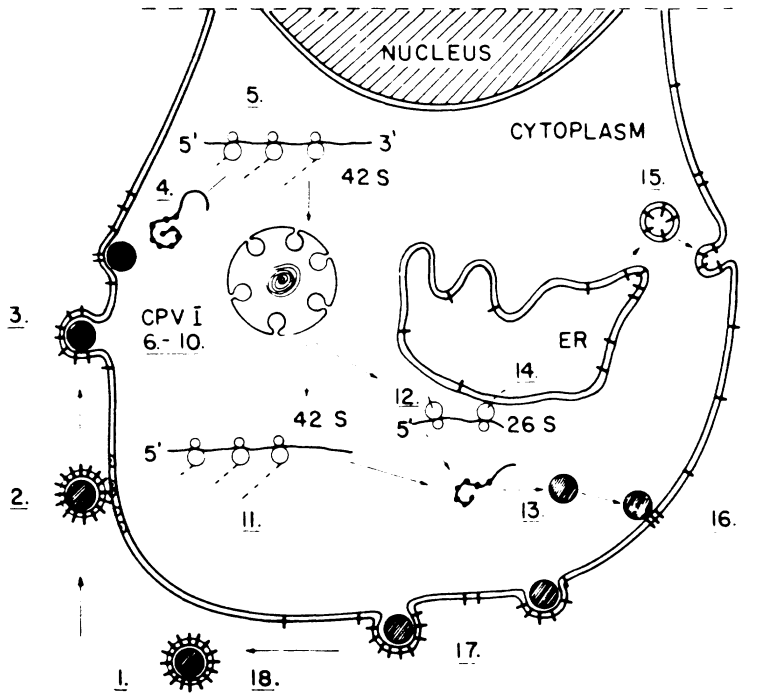

Figure 66. Simplified scheme of α-virus replication. The virus (1) adsorbs to specific receptors (2) at the plasma membrane, fusing (?) with it (3). The nucleocapsid is released to the cytoplasm and uncoated (4). Ribosomes associate with the 42 *S* RNA genome, translating nonstructural proteins which are components of the RNA polymerase (*primary translation,* 5). When enough RNA polymerase has been assembled, the translation of the parental RNA is replaced by *primary transcription,* first producing 42 *S* RNA-negative strands (6) and by their transcription a first progeny of 42 *S* RNA-positive strands (7). These are used as messengers in the production of more nonstructural proteins during the *secondary translation* (8). Part of the positive strands are used parallely as templates for synthesis of more negative 42 *S* RNAs, which in turn are used as templates for synthesis of positive strands (*secondary transcription,* 9). When the concentration of the interconversion protein is high enough, the synthesis of 26 *S* RNA begins (10). The RNA synthesis probably takes place in cytoplasmic vacuoles (CPV I). The translation of 42 *S* RNA takes place in free polysomes (11), and the structural proteins are translated into membrane-bound polysomes (12). The progeny 42 *S* RNA and the capsid protein assemble into nucleocapsids (13). The envelope proteins are protruded into the cisternal side (14) of the endoplasmic reticulum membrane (ER), become glycosylated, first in ER and finally in the Golgi apparatus from which they are transported (15) to the plasma membrane. The nucleocapsid recognizes the spanning part of the envelope protein dimer (p62-E1), preventing its free lateral mobility (16). Increasing interactions between nucleocapsid and envelope proteins lead to protrusion of nucleocapsid into the plasma membrane (17). At this stage, the host cell proteins are excluded from the forming virion and p62 is cleaved to E2 and E3 (?). Finally, the mature virion is released into the medium (18).

(Kääriäinen and Söderlund 1978. Reprinted by permission from *Curr. Top. Microbiol. Immunol.* 82, Fig. 11, p. 50. Copyright Springer-Verlag, 1978, Heidelberg.)

St. Louis encephalitis

During the 1930s, this virus caused epidemics in the central and southern regions of the United States. The disease is accompanied by elevated body temperature, severe headache for several days, followed by rapid recovery. In some individuals, mainly older people, the disease is severe, accompanied by vomiting, loss of coordination, and a lengthy convalescence.

West Nile fever

West Nile virus, which is prevalent in Africa and Southern Asia, has a life cycle in mosquitoes and rodents. Infection causes a rise in temperature after an incubation period of three days, accompanied by severe headache and a skin rash. Fatal cases have been reported.

Rubella

Rubella is usually a childhood infection, but young adults who escaped the disease in childhood can also be infected. The incubation period of rubella is 12–23 days, and a typical skin rash appears about 13 days after exposure to the virus. The disease starts with malaise, fever, headache, and irritation of the conjunctiva, as well as sore throat. The rash starts on the face and progresses downward on the body; the lymph nodes swell. At a certain stage of viremia, the virus spreads throughout the body and into the capillaries of the skin, where it replicates and causes the rash.

During early pregnancy, the virus is transmitted to the fetus. Infection of the fetus during the first trimester of pregnancy leads to damage to the internal ear and the heart due to virus replication in the stem cells. Damage to the eyes, teeth, and CNS can also occur but is less common than damage to the ear and heart. Women infected with rubella virus during the first trimester of pregnancy are advised to have an abortion; infection of pregnant women during the fourth month of pregnancy can also be harmful to the fetus.

A live attenuated rubella virus vaccine is available and is currently being used for the immunization of children. It is advisable that women of childbearing age be tested for immunity to rubella virus by determining the antibody level in the blood. Those women found to lack antibodies should be immunized with the attenuated live rubella virus vaccine at least three months before becoming pregnant to allow the development of the immune response (see chapter 24).

DISEASES CAUSED BY PESTIVIRUSES

These viruses cause diseases in domestic animals.

Hog cholera (European swine fever)

This disease is prevalent in Europe and also in South America. Infection of pigs with hog cholera virus causes fever and rash. In an epidemic form, the disease

causes severe financial losses to the farmers, since the pigs must be eliminated in an infected area in order to block the spread of the virus. Both a live attenuated virus vaccine (Chinese strain) and a killed virus vaccine are available. Countries differ in their policies regarding immunization. In some countries immunization of pigs is compulsory, whereas others, where the disease is under control, immunization is not required so as to allow naturally infected pigs to be identified at the onset of an epidemic. In these countries, the infected animals are eradicated.

The virions contain two antigens, one of which cross reacts with another, unrelated virus—bovine diarrhea virus (BDV)—which infects mainly cattle, but also pigs. A specific diagnostic procedure for the detection of antibodies to the hog cholera virus-specific antigen that does not cross react with BDV antibodies is still needed.

Border disease in sheep seems to be caused by a virus related to hog cholera, but its properties are not yet known.

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