5

Assembly of *Bunyaviridae*

I. Introduction

The *Bunyaviridae* family is a large group of over 200 distinct viruses sharing morphological, biochemical and serological properties and formerly included in the now obsolete *Arbovirus* group. They are transmitted by arthropods and occur most frequently in tropical countries. Many of them may cause lethal encephalitis in man and other mammals. Like many other viruses, including togaviruses, they replicate in insects without causing disease. The family has been subdivided in four genera: *Bunyavirus, Nairovirus, Phlebovirus,* and *Uukuvirus.* Clear differences between genera have been reported, such as genetic organization, NC shape, ability to shut off host protein synthesis, glycoprotein spike arrangement, etc. Mature virions have a spherical or slightly oval shape and are 80 to 110 nm in diameter (Holmes, 1971) with a central core formed by a helical NC (von Bonsdorff *et al.,* 1969).

Today, more and more information is being generated on the molecular aspects of *Bunyaviridae* replication and maturation, although only a few representative viruses of this large family have been successfully grown in culture (Bishop *et al.,* 1980). Since there are more dissimilarities between genera than was previously thought, we will specify for which virus or group of viruses information has been obtained.

II. Molecular Organization

The *Bunyavirus* genome (Fig. 5-1) consists of three unique segments of single-stranded RNA of negative polarity, coding for three major structural proteins, a virion-associated RNA polymerase catalyzing the synthesis of a positive mRNA strand (Ranki and Pettersson, 1975; Bouloy and Hannoun, 1976) and probably some non-structural viral polypeptides (Ulmanen *et al.,* 1981; Ushijima *et al.,* 1981; Fuller and Bishop, 1982; Smith and Pifat, 1982). So far, an RNA polymerase has not been formally demonstrated in every member of the family, and non-structural poly-

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peptides have not been detected in cells infected with the prototype *Bunyavirus* itself, Bunyamwera virus (Lazdins and Holmes, 1979).

The total length of the virus genome is between 14 and 18 K-bases. The three segments, which are neither polyadenylated nor capped (Obijeski *et al.*, 1976; Obijeski and Murphy, 1977 and 1980; Gentsch *et al.*, 1977; Pettersson *et al.*, 1977; Abraham and Pattnaik, 1983), are designated according to their respective lengths: L for large (7.5 Kb to 9 Kb), M for medium (5.5 Kb to 7 Kb) and S for small (0.9 Kb to 1.5 Kb) (Fig. 5-1). These segments are encapsidated and may circularize. The L segment is thought to encode at least the putative RNA polymerase L, since its coding capacity is larger than that required for the L protein. In the *Bunyavirus* genus, the M segment encodes the two glycoproteins, G_1 and G_2, and the S segment contains overlapping open reading frames and codes for the N protein and a non-structural protein, NS_s (Gentsch and Bishop, 1978, 1979; Akashi and Bishop, 1983; Bishop *et al.*, 1983). The segmented nature of the genome explains the frequent genetic reassortments observed in *Bunyaviridae* (Ushijima *et al.*, 1981). However, genomic exchange is restricted to members of the same genus and sometimes the same subgroup (Bishop *et al.*, 1981).

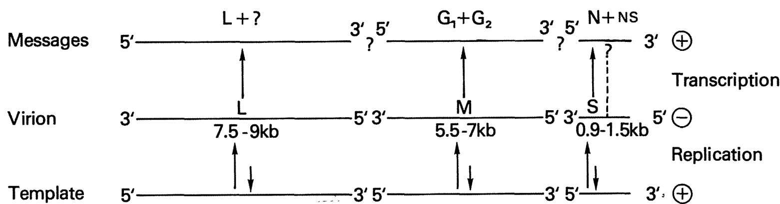

Fig. 5-1. Representation of the replication of the most studied *Bunyaviridae*. The genome consists of three negative strand segments, L, M, and S. The approximate numbers of bases are indicated below the genomic segments. The L segment codes for the putative RNA polymerase, L, and perhaps another unidentified protein. The M segment codes for the two glycoproteins, which may first be synthesized as a single precursor. The S segment codes for N protein and a second nonstructural protein, NS_s, at least in some species of *Bunyaviridae*. It is not known whether these mRNAs are capped and poly (A) tails have not been found

Naked virion RNA is not infectious. RNA replication yields full-length positive-stranded RNA copies which are encapsidated and serve as templates for the production of negative strand virion RNA segments. Virion RNA segments are also transcribed to give full-length or subgenomic complementary mRNAs (Fig. 5-1). It is not known whether the 5′ ends of these mRNAs are capped, but the 3′ ends are apparently not polyadenylated (Ulmanen *et al.*, 1981). In Uukuniemi and snowshoe hare viruses, incomplete transcripts of the S segment code for two proteins (N and NS_S), possibly by overlapping reading frames (reviewed by Strauss and Strauss, 1983; Bishop *et al.*, 1983).

Bunyaviridae replication and transcription are insensitive to α-amanitin, actinomycin D and rifampicin (Bishop and Shope, 1979). Virus production is blocked in enucleated cells, but this may be due to damage to other cellular organelles during enucleation (Pennington and Pringle, 1978). Thus, the role of the nucleus in *Bunyaviridae* replication is still unsettled.

Virus Proteins

The structural and functional properties of *Bunyaviridae* proteins are summarized in Table 5-1.

Table 5-1. *Proteins of* Bunyaviridae[1]

Species	Gene segment	$MW \times 10^{-3}$	Molecules per virion	Location and putative function
N	S	19– 50	2100	NC protein
L	L	120–200	20–25	Polymerase, transcriptase
G_1	M	75–120	630	Surface glycoprotein
G_2	M	30– 63	630	Surface glycoprotein
NS	S	11[2]	–	?

[1] After Strauss and Strauss (1983).
[2] Fuller und Bishop (1982).

(1) *N Protein* (MW: 19 K to 50 K). N protein is involved in the early stages of replication and is the structural protein that binds to both positive and negative RNA strands (but not to mRNAs) to form NCs.

(2) *L Protein* (MW: 120 K to 200 K). The polymerase and transcriptase activities are probably carried by this largest virus protein which exists in only a few copies per virion.

(3) *Glycoproteins G_1 and G_2* (MW: 75 K to 120 K; 30 K to 63 K). Attribution of distinct functions to each glycoprotein has not been achieved yet. These proteins are undoubtedly responsible for attachment of the virus to cells during the adsorption-penetration phase.

(4) *Nonstructural proteins* (MW: 11 K; others?). Several polypeptides have been described, which are specified by the virus but are present only in infected cells (McPhee and Della-Porta, 1981; Ulmanen *et al.*, 1981; Fuller and Bishop, 1982; Smith and Pifat, 1982). It appears that the non-structural proteins differ from one virus to another. In addition, some bunyaviruses do not code for these proteins. Their functions are unknown, but, by analogy with other virus families, they are thought to play roles in virus replication and transcription, or to modify host functions.

III. Intracellular Synthesis of Virus Components

N protein is produced in cells early after infection (Bishop and Shope, 1979). The two glycoproteins, G_1 and G_2, are synthesized from the same mRNA segment on membrane-bound ribosomes. A large 110K precursor is apparently inserted into the membrane and is immediately cleaved, possibly by the same enzyme, the signal peptidase, that cleaves off the insertion signal peptide (Ulmanen *et al.*, 1981).

IV. Assembly of Virus Components

Budding occurs on smooth membranes of the endoplasmic reticulum and of the Golgi apparatus. As a result, virions are shed into intracytoplasmic cisternae (Fig. 5-2) (Murphy *et al.*, 1978 a; Smith and Pifat, 1982). Thus, the *Bunyaviridae* differ

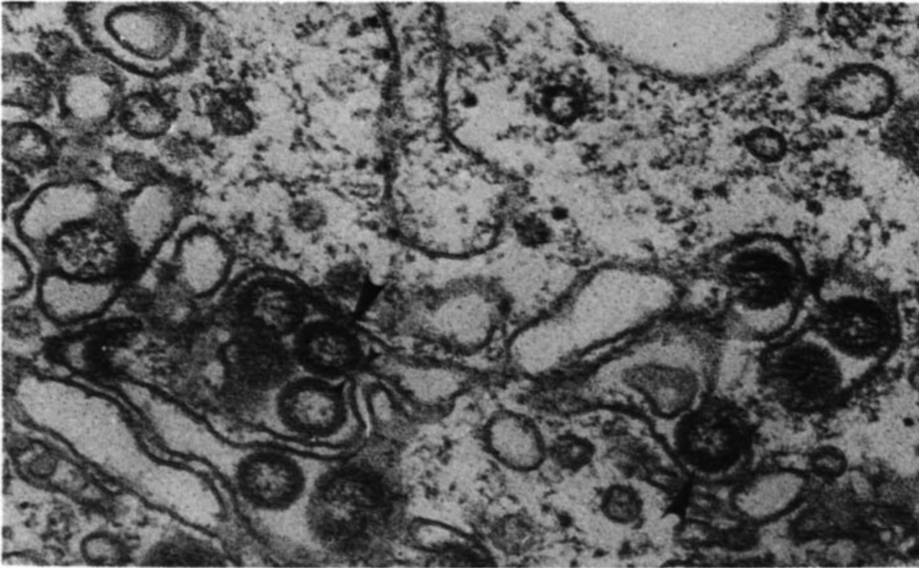

Fig. 5-2. Maturation of a phlebovirus (Punta Toro virus) in Vero cells 16 hours after infection. Viruses are budding into smooth membrane vesicles. The envelope bilayer with its spikes is very distinct (large arrowheads) and in continuity with the vesicular membrane (small arrowheads). Electron-dense material under the virion envelope probably corresponds to NC. (Courtesy of Dr. J. F. Smith, from Smith and Pifat, 1982; reproduced with permission of Academic Press, New York)

from all other negative strand enveloped RNA viruses by their site of budding and this may be correlated with the absence of a matrix (M)-like protein in the virions (Smith and Pifat, 1982). Indeed, an M protein appears to be essential for normal assembly of *Rhabdo-*, *Paramyxo-*, and *Orthomyxoviridae* which never bud into Golgi cisternae (Compans and Klenk, 1979) (see Chapters 2, 3, 4).

Even though NC components are synthesized earlier in infection than glycoproteins (Lazdins and Holmes, 1979), NC cannot be identified morphologically in the cytoplasm outside of the budding sites where spikes are also visible. Virus maturation is a continuous process of involution of localized areas of smooth vesicle membranes where NC and viral spikes are accumulating on opposite sides of the virus bud. NC is closely associated with the membrane during budding. No spikes can be seen in the membrane adjacent to budding sites. Since *Bunyaviridae* contain no M protein, at least one of the glycoproteins, G_1 nor G_2, must be transmembranous and establish direct contact with the NC on the cytoplasmic side of the plasma membrane (Lyons and Heyduk, 1973; Murphy *et al.*, 1973; Smith and Pifat, 1982) as in the case of coronaviruses (see Chapter 7).

V. Virus Release and Organization of the Virion

Virions are transported to the extracellular space in Golgi-derived vesicles and released by the normal process of exocytosis, *i.e.* the fusion of the vesicle with the plasma membrane (Murphy *et al.*, 1968 a, b; Holmes, 1971; Lyons and Heyduk, 1973; Murphy *et al.*, 1973; Smith and Pifat, 1982). In the *Bunyavirus* genus, absence of virus antigens on the plasma membrane, as determined by immunoferritin labeling before and after virus release, indicates that (1) virus components are not accumulating in the plasma membrane in amounts detected by this technique and (2) transport vesicles do not contain in their membranes virus antigens which could be transferred to the plasma membrane during exocytosis. Thus, virus components are clustering at the budding site in the Golgi apparatus. There is, however, one image published by Murphy *et al.* (1973) which demonstrates virus budding from the plasma membrane of a mouse neuron *in vivo,* but this is a unique event so far.

Thus, in contrast to most other enveloped viruses, some *Bunyaviruses* appear to display virus antigens only on budding and free virions and not on the infected cell

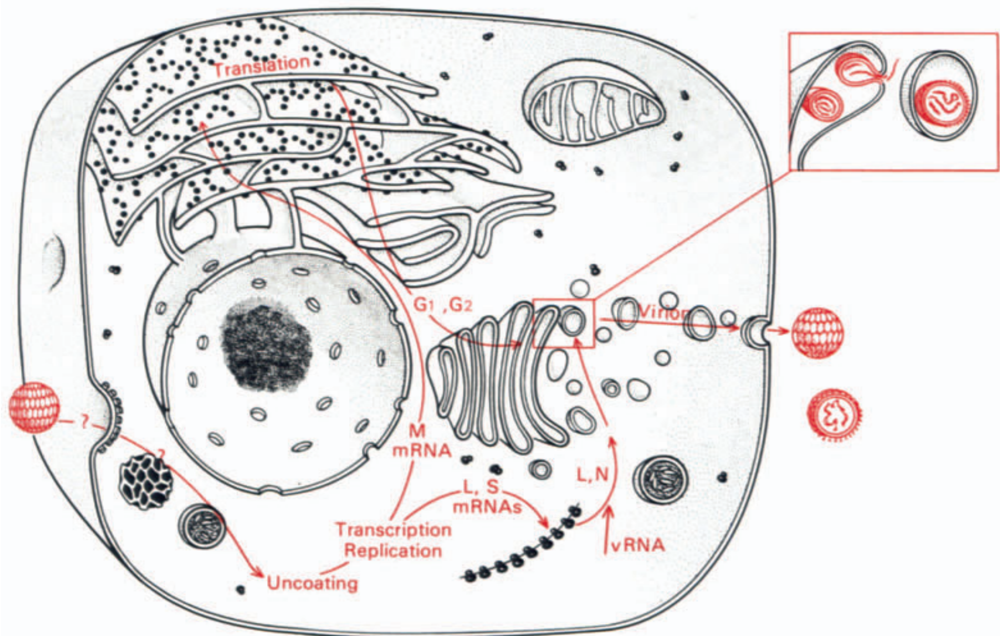

Fig. 5-3. Summary of the putative events of transcription and assembly of *Bunyaviridae.* The mechanism of entry is still unknown. L and S mRNAs are translated on polysomes and the resulting proteins move to the site of assembly in the Golgi apparatus and smooth membrane vesicles. M mRNA is translated in the rough ER. The translation product is probably a precursor protein which is immediately cleaved into the glycoproteins, G_1 and G_2. It is presumed that the three segments are contained in three NCs which are packaged into the virion as shown in the box. Virus glycoproteins are detected at the budding sites, but are apparently not seen at the cell surface. The virus travels to the cell surface in transport vesicles. The remarkable hexagonal arrays of spikes as seen in negative staining (Fig. 5-5) are shown on the envelope of the complete virus

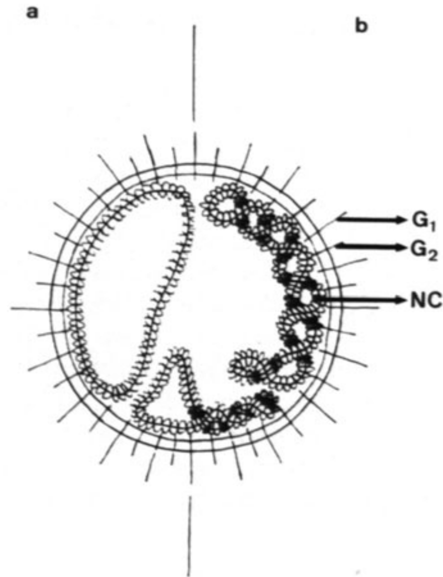

Fig. 5-4. Representation of a bunyavirion, showing its three encapsidated RNA segments which may form circles *(a)* or supercoils *(b)*. It is not known whether G_1 and G_2 reside on different spikes. G_2 has a lower molecular weight than G_1

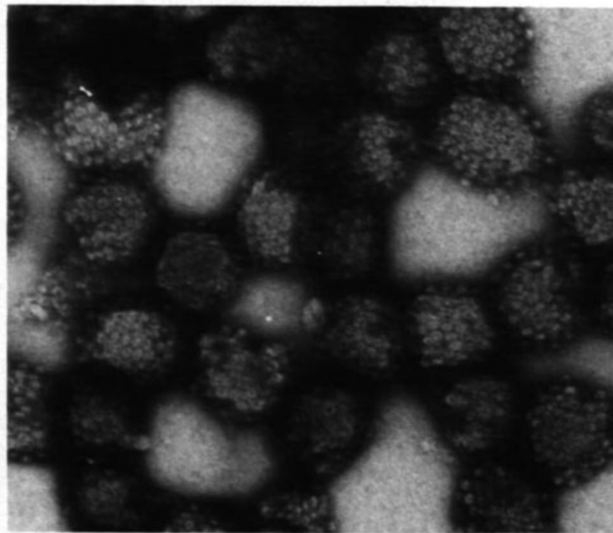

Fig. 5-5. Negative staining of virus Unkuniemi after glutaraldehyde fixation. Spikes are organized in a hexagonal array Magnification: X120,000. (Courtesy of Dr. C. H. von Bonsdorff)

plasma membrane. Therefore, the host's immune system may not recognize infected cells and this may play a role in the pathogenicity of these viruses.

Fig. 5-3 is a summary of the events of replication and assembly of *Bunyaviridae* in the cell. The structure of the complete virus is schematized in Fig. 5-4. From studies on isolated NCs, it appears that NC may form supercoiled circles (Pettersson and von Bonsdorff, 1975; Obijeski *et al.*, 1976; Hewlett *et al.*, 1977) or superhelical filaments (Saikku *et al.*, 1971; Dahlberg *et al.*, 1977). The NC is contained in a lipid bilayer envelope which contains radial spikes 5 to 10 nm long on the outer surface (Holmes, 1971; Obijeski and Murphy, 1977). Spikes are arranged in a hexagonal array in some members of the family, such as Uukuniemi virus (von Bonsdorff and Pettersson, 1975), Punta Toro virus and Karimabad virus (Smith and Pifat, 1982) and this organization is more visible after glutaraldehyde fixation (Fig. 5-5) as represented schematically in Fig. 5-3.