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# AMBISENSE RNA GENOMES OF ARENAVIRUSES AND PHLEBOVIRUSES

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## I. INTRODUCTION

Five families of viruses are recognized as negative-stranded RNA viruses. These are the Arenaviridae, Bunyaviridae, Orthomyxoviridae, Paramyxoviridae, and Rhabdoviridae (Matthews, 1982). All have a lipid envelope, an external fringe of glycoprotein, and internal components consisting of one or more species of single-stranded RNA in addition to structural proteins and RNA polymerase components. Positive-stranded RNA viruses include members of the Picornaviridae, Caliciviridae, Togaviridae, Flaviviridae, Coronaviridae, and the non-enveloped insect and plant virus groups. The togaviruses, flaviviruses, and coronaviruses are enveloped and in that regard structurally resemble negative-stranded viruses except that they lack virion RNA polymerase activities. The picornaviruses, caliciviruses, and the insect and plant nonenveloped, positive-stranded viruses consist of single-stranded viral RNA enclosed in structural proteins. Of the other major families of RNA viruses that have been recognized, the retroviruses are enveloped, diploid, single-stranded RNA viruses that have reverse transcriptase enzyme components within their virus particles; Reoviridae and Birnaviridae members are nonenveloped, multisegmented, double-stranded RNA viruses that have virion RNA polymerase components.

The feature that distinguishes the positive-stranded RNA viruses from the negative-stranded viruses is that their viral RNA functions as a messenger RNA (mRNA). For the picornaviruses, caliciviruses, and flaviviruses, the complete viral-size RNA is the only mRNA species; for at least some togaviruses and coronaviruses there are additional subgenomic, viral-sense RNA species (i.e., mRNA species that represent parts of the viral RNA sequence). Upon introduction into a cell, the viral RNA of positive-stranded viruses is translated by cellular ribosomes and the derived gene products initiate the processes that lead to a productive infection (i.e., replication of the RNA and

synthesis of all the usual RNA, mRNA, protein, and virus forms). Due to these properties, the viral RNA of positive-stranded viruses can be infectious per se. The RNA species of negative-stranded viruses, retroviruses, and the double-stranded RNA viruses are not infectious per se since the infection processes of each of these viruses requires that the virion polymerase transcribes the viral RNA into complementary sequence mRNA species (reoviruses, birnaviruses, negative-stranded viruses), or DNA copies (retroviruses), before the infection can proceed. Therefore, removal of viral protein from the RNA of these viruses eliminates the required enzymes and renders the RNA noninfectious.

Two families of negative-stranded viruses have single species of genomic RNA (Rhabdoviridae, Paramyxoviridae); the others have seven or eight (Orthomyxoviridae), three (Bunyaviridae), or two species (Arenaviridae). In this chapter, the evidence will be reviewed that shows that arenaviruses and members of one genus of the Bunyaviridae (phleboviruses) have some proteins coded in subgenomic, viral-sense mRNA species and other proteins coded in subgenomic, viral-complementary mRNA sequences (i.e., the viruses have genomes with an ambisense coding strategy). This unique feature is discussed in relation to the implications it has on the intracellular infection process and how such a coding arrangement may have evolved.

## II. ARENAVIRIDAE

### A. *The Members of the Arenaviridae*

A list is given in Table I of the known members of the Arenaviridae, their origins, and the vertebrate hosts from which isolates have been reported. The viruses are grouped into the Old World and New World species, although LCM virus (the prototype virus of the family) has been found in Africa, the Americas, Europe, and Asia (Pfau *et al.*, 1974). An alternate designation for the New World arenaviruses that is based on serological considerations is the Tacaribe complex (Table I). Recent information indicates that at the nucleotide and protein sequence levels members of both groups are closely related (see below). For this reason, as well as the observation that LCM virus has been recovered from both Old and New World countries, the serological designation Tacaribe complex viruses will be employed in this review. In reference to the other group, the term LCM complex viruses will be employed since various investigators (Casals, 1978; Casals *et al.*, 1975; Rowe *et al.*, 1970a; Wulff *et al.*, 1978) have reported that LCM and

TABLE I  
THE ARENAVIRIDAE

| Virus                               | Distribution             | Vertebrate hosts <sup>a</sup>  | References   |
|-------------------------------------|--------------------------|--|--|
| Old World species: LCM complex      |                          |  |  |
| Ippy                                | Central African Republic | <i>Arvicanthis</i> sp.,<br><i>Lemniscomys striatus</i> ,<br><i>Mastomys</i> sp.,<br><i>Praomys</i> sp.   | Berge (1975)   |
| LCM <sup>b</sup>                    | Worldwide                | <i>Mus musculus</i>  | Armstrong and Lillie (1934)                              |
| Lassa                               | West Africa              | <i>Mastomys natalensis</i>   | Buckley and Casals (1970),<br>Frame <i>et al.</i> (1970) |
| Mobala                              | Central African Republic | <i>Praomys jacksonii</i>   | Gonzalez <i>et al.</i> (1983)                            |
| Mopeia <sup>c</sup>                 | Mozambique               | <i>Mastomys natalensis</i>   | Wulff <i>et al.</i> (1978)                               |
| New World species: Tacaribe complex |                          |  |  |
| Amapari                             | Brazil                   | <i>Oryzomys goeldi</i> ,<br><i>Oryzomys capito</i> ,<br><i>Neacomys guianae</i>  | Pinheiro <i>et al.</i> (1966)                            |
| Flexal                              | Brazil                   | <i>Oryzomys bicolor</i> ,<br><i>Oryzomys capito</i>  | Pinheiro <i>et al.</i> (1977)                            |
| Junin                               | Argentina                | <i>Calomys laucha</i> ,<br><i>Calomys musculinus</i> ,<br><i>Akodon arenicola</i> ,<br><i>Akodon azarae</i> ,<br><i>Akodon obscurus</i> ,<br><i>Oryzomys flavences</i> | Parodi <i>et al.</i> (1958), Berge<br>(1975)             |
| Latino                              | Bolivia                  | <i>Calomys callosus</i>  | Johnson <i>et al.</i> (1973)                             |
| Machupo                             | Bolivia                  | <i>Calomys callosus</i>  | Johnson <i>et al.</i> (1966)                             |
| Parana                              | Paraguay                 | <i>Oryzomys buccinatus</i>   | Webb <i>et al.</i> (1970)                                |
| Pichinde                            | Colombia                 | <i>Oryzomys albigularis</i> ,<br><i>Thomasomys fuscatus</i>  | Trapido and Sanmartin<br>(1971)                          |
| Tacaribe                            | Trinidad                 | <i>Artibeus literatus</i> ,<br><i>Artibeus jamaicensis</i>   | Downs <i>et al.</i> (1963)                               |
| Tamiami                             | Florida                  | <i>Sigmodon hispidus</i> ,<br><i>Oryzomys palustris</i>  | Calisher <i>et al.</i> (1970)                            |

<sup>a</sup>Other than man (LCM, Lassa, Junin, Machupo, Flexal).

<sup>b</sup>LCM, Lymphocytic choriomeningitis virus-type species of the family.

<sup>c</sup>Mopeia (otherwise identified in the literature as Mozambique virus) is considered a subtype of Lassa virus.

Lassa viruses are serologically more closely related to each other than to the Tacaribe complex viruses.

All of the viruses have been isolated from rodents. The exception, however, is Tacaribe virus, which was isolated from fruit-eating bats (Downs *et al.*, 1963). Several of the viruses (LCM, Junin, Lassa, and Machupo viruses) have been recovered from naturally acquired

human infections (reviewed by Casals, 1975; Berge, 1975; Peters, 1984). Different strains of many of the viruses listed in Table I have been reported, strains that exhibit both biological and biochemical variation (Dutko *et al.*, 1981; Hotchin, 1971; Hotchin *et al.*, 1975; Kirk *et al.*, 1980; Lehman-Grube, 1971; Rivers and Scott, 1935; Riviere *et al.*, 1985a,b; Traub, 1935; Vezza *et al.*, 1980). The genetic basis for the reported biological variation is not understood.

In terms of human disease, Lassa virus is the etiologic agent of Lassa fever in West Africa, Junin virus is the cause of Argentine hemorrhagic fever, and Machupo virus is responsible for Bolivian hemorrhagic fever. LCM virus can also infect humans, usually inducing a mild, influenza-like infection that on occasion may lead to aseptic meningitis (see Johnson, 1985; Peters, 1984). Antibodies to these four viruses as well as to Flexal virus (Pinheiro *et al.*, 1977) have been detected in human sera. Laboratory-acquired infections to LCM, Lassa, Junin, Machupo, Flexal, Pichinde, and Tacaribe viruses have been reported (see Peters, 1984).

Serologically, the members of the Tacaribe group are more closely related to each other than to the members of the Old World virus group (Casals, 1978; Casals *et al.*, 1975; Johnson *et al.*, 1965; Mettler *et al.*, 1963; Rowe *et al.*, 1970b; Wulff *et al.*, 1978); however, studies with polyclonal and monoclonal antibodies have shown that there are cross-reactive epitopes in the proteins of representatives of both groups of viruses (Buchmeier and Oldstone, 1978a; Buchmeier *et al.*, 1981). Sequence analyses have confirmed that, from an evolutionary viewpoint, Lassa, LCM, and Pichinde viruses are in fact closely related (see below).

Reviews concerning the biological aspects of arenavirus replication in animals and the immunological responses to arenavirus infections in relation to the host species, infection route, and virus type are available from other sources (Bro-Jorgensen, 1978; Casals, 1975; Hotchin, 1971, 1974; Lehmann-Grube, 1971, 1973; Murphy, 1977; Oldstone, 1975a,b; Pedersen, 1979; Rawls and Leung, 1979; Welsh *et al.*, 1975; Zinkernagel and Doherty, 1977). Other than providing contextual information, this review primarily concerns the genetic and biochemical information that is available on arenaviruses with regard to the RNA coding strategy. A comprehensive earlier review on the structural features of arenaviruses, including information on the biophysical and antigenic properties, purification, and replication processes, has been published by Pedersen (1979). The extensive literature on the biology of LCM and an able discussion of the subject matter can be found in the review by Lehmann-Grube (1971). Additional information, including the results

of studies with other arenaviruses, can be found in the *Bulletin of the World Health Organization* (Vol. 52, pp. 381–766, 1975).

### *B. Arenavirus Structural Components*

Electron microscopic analyses of intact arenaviruses, as well as thin sections of arenavirus preparations and infected tissues, have shown that the viruses usually contain ribosomes, and that virions are frequently spherical but often pleomorphic, with sizes ranging from 50 to 300 nm, although their average size is 110–130 nm (Abelson *et al.*, 1969; Blechschmidt *et al.*, 1977; Dalton *et al.*, 1968; Gard *et al.*, 1977; Lascano and Berria, 1969, 1971, 1974; Maiztegui *et al.*, 1975; Mannweiler and Lehmann-Grube, 1973; Matthews, 1982; Murphy and Whitfield, 1975; Murphy *et al.*, 1969, 1970, 1973b; Ofodile *et al.*, 1973; Speir *et al.*, 1970; Vezza *et al.*, 1977). The structural proteins of arenaviruses have been reported by a variety of investigators (Buchmeier *et al.*, 1977, 1978; Gangemi *et al.*, 1978; Gard *et al.*, 1977; Grau *et al.*, 1981; Harnish *et al.*, 1981a,b; Kiley *et al.*, 1981; Martinez Segovia and de Mitri, 1977; Pedersen, 1973; Ramos *et al.*, 1972; Vezza *et al.*, 1977, 1978a,b). Embedded in the lipid envelope are 5- to 10-nm-long, club-shaped surface projections of glycoprotein that appear to have a hollow axis (Gard *et al.*, 1977; Murphy and Whitfield, 1975; Vezza *et al.*, 1977; Welsh *et al.*, 1976). The projections consist of either two distinct protein species present in essentially equal numbers (for Pichinde, LCM, Lassa, Mopeia, and Machupo viruses: G1, size:  $50\text{--}72 \times 10^3$  Da; G2, size:  $34\text{--}41 \times 10^3$  Da) or one size class of protein (for Junin, Tacaribe, and Tamiami viruses: G, size:  $35\text{--}44 \times 10^3$  Da). Estimates of the order of 400 molecules of each glycoprotein species have been reported for Pichinde virus preparations (Buchmeier *et al.*, 1977; Vezza *et al.*, 1977). Unlike many other enveloped viruses (e.g., orthomyxoviruses), the surface projections appear to be sparsely arranged. Whether the single size class of glycoprotein for Junin, Tacaribe, and Tamiami viruses means that they only have one protein species, or whether they have two similarly sized proteins, is not known (see Grau *et al.*, 1981). The surface projections can be removed by protease digestion, leaving a spikeless particle that exhibits reduced infectivity (Gard *et al.*, 1977; Vezza *et al.*, 1977). As mentioned previously, the projections are embedded in the viral envelope, which is derived from the host cell plasma membrane. At the site of virus morphogenesis (budding), the plasma membrane has been observed to be denser than at other regions; the reason is not known (Murphy and Whitfield, 1975). How the glycoproteins are embedded in the viral membrane and in what way

they make liaisons with the internal components of the virus particle are also not known. A schematic representation of an arenavirus particle is given in Fig. 1.

The G1 and G2 proteins of Pichinde and LCM viruses each have distinct amino acid sequences (Auperin *et al.*, 1984b; Buchmeier and Oldstone, 1979; Harnish *et al.*, 1981a,b; Romanowski *et al.*, 1985). They have been shown to be derived from a common glycosylated precursor [(GPC) size:  $\sim 80 \times 10^3$  Da; Buchmeier and Oldstone, 1979; Harnish *et al.*, 1981a,b)]. The nonglycosylated forms of the LCM and Pichinde virus GPC primary gene products have been deduced from DNA sequence analyses to have sizes of  $56\text{--}57 \times 10^3$  Da (Auperin *et al.*, 1984b; Romanowski *et al.*, 1985). Tunicamycin studies have indicated a size of  $42 \times 10^3$  Da for the unglycosylated form of Pichinde virus GPC (Harnish *et al.*, 1981a). Whether removal of signal sequences from the initial gene product accounts for the difference is not known. Based on precursor incorporation data, more carbohydrate has been identified in association with Pichinde G1 than with G2 (Buchmeier *et al.*, 1978; Martinez Segovia and De Mitri, 1977; Vezza *et al.*, 1977). This observation agrees with the sequence data for Pichinde virus (Auperin *et al.*, 1984b), which indicate that there are more potential asparagine-linked glycosylation sites on the amino-terminal (G1) half of the glycoprotein primary gene product than on the carboxy-terminal half (G2). The viral glycoproteins are important for the initiation of

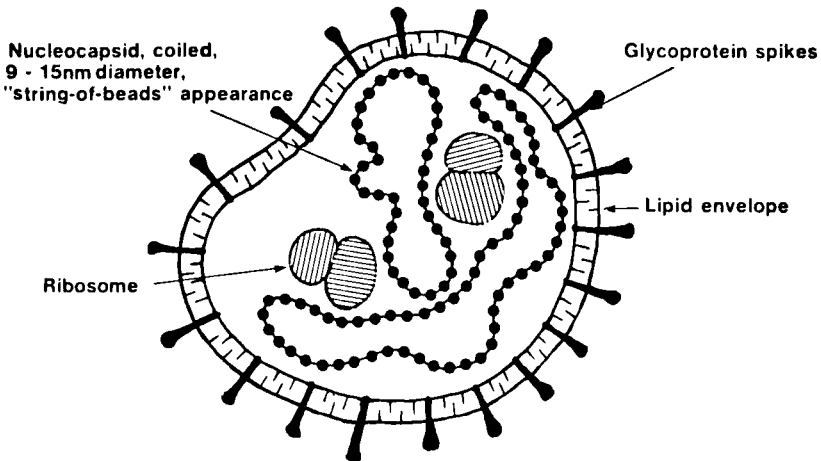


FIG. 1. Schematic representation of an arenavirus particle.

the infection process, since their removal by proteases reduces virus infectivity (VeZZa *et al.*, 1977).

The internal components of arenaviruses include two other viral-coded proteins. There are minor quantities of a large protein, L (size:  $180-200 \times 10^3$  Da), that is believed to be a transcriptase/replicase component, and large quantities of the nucleocapsid protein, N (size:  $63-72 \times 10^3$  Da). The latter constitutes some 60–70% of the total viral protein (Buchmeier and Oldstone, 1978b; Buchmeier *et al.*, 1977; Gard *et al.*, 1977; Ramos *et al.*, 1972; VeZZa *et al.*, 1977). Estimates of some 1500 molecules of N protein per virion have been reported (VeZZa *et al.*, 1977; 1978a,b). It has been shown that the N protein is responsible for the antigenic cross-reactivity among the Tacaribe complex of arenaviruses (Buchmeier and Oldstone, 1978b). The N protein is closely associated with the two viral RNA species (Buchmeier and Oldstone, 1978b; Gard *et al.*, 1977; Ramos *et al.*, 1972; VeZZa *et al.*, 1977). The viral nucleocapsids have been isolated from virus preparations using non-ionic detergents and salt (Farber and Rawls, 1975; Gard *et al.*, 1977; Pedersen and Konigshofer, 1976; VeZZa *et al.*, 1977; Young *et al.*, 1981). The RNA within the viral nucleocapsids is sensitive to digestion with ribonuclease, indicating that the phosphodiester bonds of the viral RNA are accessible to the nuclease and are not protected by the nucleoprotein. The extended forms of the nucleocapsids are long and convoluted, 3–5 nm in diameter, and without any obvious helical symmetry (Gard *et al.*, 1977). For Pichinde virus, 12-nm-diameter helical structures, presumably representing the condensed forms of the nucleocapsids, have been described (Young and Howard, 1983). For Tacaribe virus, positive staining with uranyl acetate has revealed circular nucleocapsid structures of two size classes (1300 and 640 nm; Palmer *et al.*, 1977), consistent with the existence of two viral RNA species but without a simple relationship to the published sizes of the viral RNA species. Similar results have been reported for Pichinde virus (Young *et al.*, 1981).

Almost no information is available on the viral L protein, other than its distinct size and tryptic peptide profile (Harnish *et al.*, 1981a,b, 1983). Other proteins that have been described in arenavirus preparations (P protein and some small proteins that have been observed irregularly; Buchmeier *et al.*, 1977; Gard *et al.*, 1977; Martinez Segovia and De Mitri, 1977; Ramos *et al.*, 1972; VeZZa *et al.*, 1978b; Young *et al.*, 1981) may be alternative or derived forms of the major structural proteins (see Harnish *et al.*, 1981b). Their functions (if any) are not known.

Like other negative polarity RNA viruses, RNA polymerase activities have been identified in extracts of arenavirus preparations



(Boesma and Compans, 1985; Carter *et al.*, 1974; Leung, 1978). The L protein is a candidate for the viral RNA polymerase, but this has not been proven by genetic experiments or by *in vitro* reconstruction analyses.

The genetic information of arenaviruses is resident in two species of RNA, designated small, S (size:  $1.1 \times 10^6$  Da), and large, L (size:  $2.2 \times 10^6$  Da) (Anon *et al.*, 1976; Auperin *et al.*, 1984a,b; Carter *et al.*, 1973a; Dutko *et al.*, 1976, 1981; Kiley *et al.*, 1981; Leung *et al.*, 1977; Pedersen, 1970, 1971, 1973, 1979; Ramsingh *et al.*, 1980; Romanowski and Bishop, 1985; Romanowski *et al.*, 1985; Vezza *et al.*, 1978a,b). For Pichinde and LCM viruses, it has been shown that the L and S RNA species have different sequences, as evidenced by fingerprint analyses (Dutko *et al.*, 1981; Kirk *et al.*, 1980; Vezza *et al.*, 1978a,b, 1980), although their 3' ends are homologous in sequence (Auperin *et al.*, 1981, 1982a,b). From cloning and sequence analyses, it has been found that the Pichinde and LCM S 5' end sequences are complementary to the 3' end sequences for approximately 20 residues (Auperin *et al.*, 1984b; Romanowski *et al.*, 1985). As discussed below, the L RNA is believed to code for the L protein, the 5 RNA for the GPC and N proteins. Whether either RNA codes for nonstructural proteins is not known.

In addition to the protein, RNA, and envelope components of arenaviruses, a variety of other macromolecules have been identified in arenavirus preparations. These include host ribosomes, various RNA species, and enzymes. Reviews of this subject are available elsewhere (Compans and Bishop, 1985; Pedersen, 1979; Rawls and Leung, 1979). The presence of ribosomes is a characteristic feature of arenaviruses that sets them apart from other families of viruses (Dalton *et al.*, 1968; Murphy and Whitfield, 1975). With regard to the question of the origins of the ribosomes and the other minor components, it should be borne in mind that arenaviruses are pleomorphic and form virus particles at the surfaces of cells. It is perhaps not surprising that virus morphogenesis may result in the acquisition of cellular components, including bulky host ribosomes if the processes of viral morphogenesis do not include ways for their exclusion. Other pleomorphic viruses (e.g., paramyxoviruses) do not exhibit this property, possibly because they have another internal structural protein, the matrix protein. Whether the arenavirus ribosomes have any function in the process involved in initiating an infection is not known. Leung and Rawls (1977) have shown that Pichinde virus grown in cells with thermolabile ribosomes yielded viruses that were able to productively infect other cells at temperatures that were nonpermissive for the viral ribosomes. It appears, therefore, that ribosomes are not essential for the infectivity of

arenavirus particles. This does not mean, however, that competent ribosomes incorporated into arenavirus particles do not function upon gaining entry to a permissive cell (e.g., they may become involved in the translation of an associated mRNA or participate in the *de novo* initiation of mRNA translation). Northern analyses have shown that among the minor RNA species that can be identified in extracts of purified virus preparations are the two S mRNA species (unpublished data). If such species are associated with the virion ribosomes, then they may be involved with the continued or *de novo* synthesis of viral proteins after virus penetration.

### C. The Infection Cycle

In the rodent species vertical transmission appears to be frequent (transuterine, transovarian, as well as postpartum involving milk, saliva, or urine routes). Venereal transmission may also be involved in intraspecies infections (Matthews, 1982). Interspecies transmission (e.g., to man) is thought to be caused by the acquisition of virus through contamination, due to either rodent infestations of dwellings (e.g., Lassa) or encounters in the field (e.g., Junin) or the laboratory (see Peters, 1984). For transuterine or neonatally acquired virus infections, rodents usually become persistently infected and develop viremia and viruria, secreting virus throughout their life. An attribute of such infections is the hypoimmune response and coexistence of circulating antibody and virus. Depending on the host and virus species, experimental infections of adult rodents may be either inapparent (e.g., Tacaribe virus) or lethal (e.g., LCM virus). In neonates, experimental infection with Tacaribe virus is lethal; for LCM viruses a persistent infection is frequently induced (Matthews, 1982). LCM virus infection of young rodents induces a chronic immune complex disease involving virus infection of lymphocytes and an overall immunodepression and possible autoimmune effects. Extensive reviews of this subject are available elsewhere (Johnson, 1985; Lehmann-Grube, 1971; Rawls *et al.*, 1981). The abilities of arenaviruses to elicit short-term acute or long-term persistent infections have been studied from the viewpoint of the virus strain, the host species, and *in vitro* culture (see Lehmann-Grube, 1971).

In cell culture, arenaviruses can productively infect a variety of cell lines. Some studies have indicated that the host cell nucleus is required to obtain a productive infection and also that arenaviruses are inhibited by actinomycin D or  $\alpha$ -amanitin (Banerjee *et al.*, 1975–1976; Buck and Pfau, 1969; Carter *et al.*, 1973b; Mifune *et al.*, 1971; Rawls *et al.*, 1976; Stanwick and Kirk, 1971). However, the intracellular pro-

cesses in the replication cycle and their relationships to cellular functions are not understood in detail (see Pedersen, 1979). In broad terms, the viral glycoproteins are involved in the adsorption, penetration, and uncoating process, although how these results are achieved is not known. Following uncoating, it is assumed that the viral polymerase synthesizes mRNA (presumably the S-coded N and the L-coded mRNA species). As described below, RNA replication must proceed before the S-coded GPC mRNA can be synthesized. With the availability of the newly synthesized gene products and replicated viral RNA, viral morphogenesis takes place at the cell surface. The molecular steps involved in these processes have yet to be defined.

#### *D. Genetic Attributes of Arenaviruses*

Intraspecies virus recombination involving the reassortment of the two viral RNA species has been demonstrated using temperature-sensitive (*ts*) mutants of two strains of Pichinde virus (Veza and Bishop, 1977; Veza *et al.*, 1980). Reassortment has also been documented using wild-type or *ts* mutants of LCM virus strains (Kirk *et al.*, 1980; Riviere *et al.*, 1985a; Romanowski and Bishop, 1983). No reassortment has yet been detected between representatives of Pichinde and LCM viruses (Romanowski and Bishop, 1983). Analyses of recombinant arenaviruses have shown that the S RNA species codes for the N protein and GPC (and therefore the two viral glycoproteins, G1 and G2) and that the L RNA codes for the L protein (Harnish *et al.*, 1983; Riviere *et al.*, 1985a). Using LCM virus strains that exhibit different phenotypes, it has been shown that the S RNA codes for functions that result in growth hormone-induced disease and other virulence markers (Kirk *et al.*, 1980; Riviere *et al.*, 1985b).

Genetically diploid LCM reassortants have been detected among the viruses obtained from crosses of complementing *ts* mutants representing the S RNA (i.e., N and GPC mutants) (Romanowski and Bishop, 1983). The diploid viruses readily segregate *ts* mutants upon passage, in agreement with the postulate that they contain complementing S RNA species representing both *ts* genotypes (see Fig. 2). Whether the propensity to produce polyploid viruses has a biological consequence is not known. Conceivably, it could confer genetic stability in nature by acting against the biological cloning of variants.

#### *E. Ambisense Coding Arrangement of the S RNA Species*

In order to determine how arenaviruses code for gene products, the S RNA species of Pichinde virus and that of a viscerotropic strain of

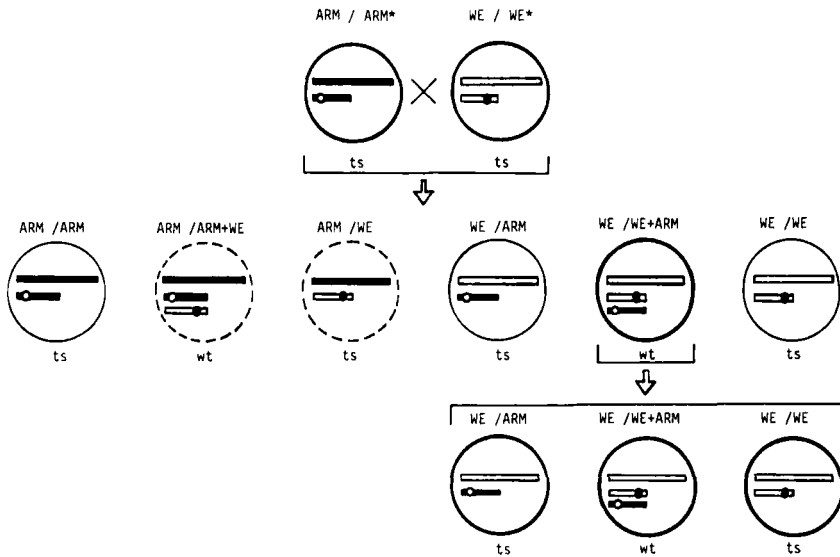


FIG. 2. Reassortment between LCM virus S RNA *ts* mutants representing different gene products. Mutants of the WE and Armstrong (ARM) strains of LCM virus (top line) were used in dual virus infections to generate reassortants with a *ts* or wild-type plaque assay phenotype (second line). On passage, the wild-type viruses segregated into *ts* and wild-type virus clones (see bottom line), indicating that they were diploid with respect to the S RNA and contained complementing S RNA coded gene products (Romanowski and Bishop, 1983).

LCM virus (LCM-WE) have been cloned into DNA and sequenced (Auperin *et al.*, 1984a,b; Romanowski and Bishop, 1985; Romanowski *et al.*, 1985). Analyses of the  $1.1 \times 10^6$  Da sequences of each S RNA have, like the earlier genetic and biochemical studies, confirmed that two gene products are coded by the arenavirus S RNA. One (the  $62-63 \times 10^3$  Da N protein) is coded in a viral-complementary sequence corresponding to the 3' half of the viral RNA (Auperin *et al.*, 1984a; Romanowski and Bishop, 1985). The other (the  $56-57 \times 10^3$  Da primary gene product corresponding to GPC) is coded in the 5' half of the viral RNA in a viral-sense sequence (Auperin *et al.*, 1984b; Romanowski *et al.*, 1985) (Fig. 3). Comparison of the gene products of Pichinde and LCM viruses indicates that the N proteins of the two viruses exhibit 51% direct amino acid sequence homology; the GPC primary gene products have only 39% sequence homology (Fig. 4). It is of interest to note that in classical serological procedures such as the complement fixation test which assesses antigenic epitopes on the viral nucleocapsid protein, different arenaviruses have been shown to be related to each other to different extents (Casals *et al.*, 1975). By these pro-

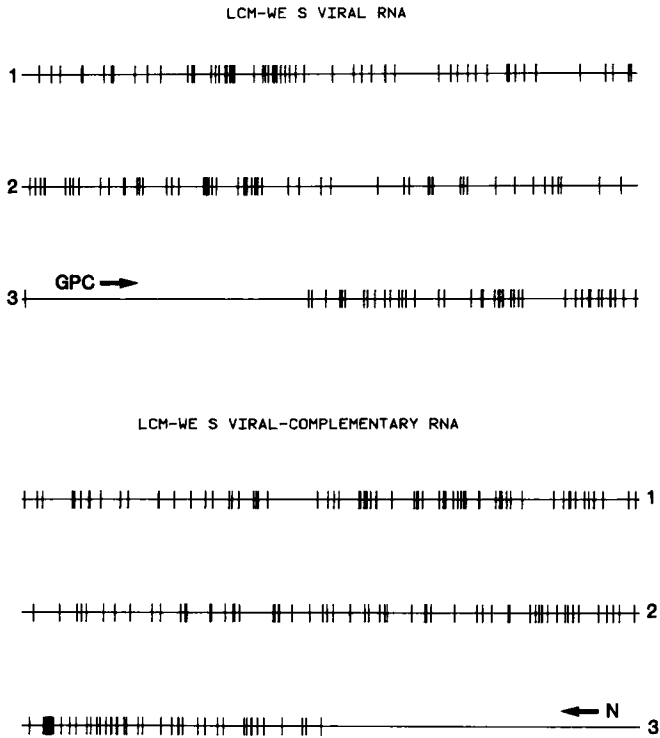


FIG. 3. The arrangement of translation termination codons (vertical bars) in the three possible reading frames of the viral (top three lines, 5' is on the left side) and viral-complementary (bottom three lines, 5' is on the right side) sequences of LCM-WE virus S RNA. The positions of the S-coded gene products (GPC and N) are shown.

cedures LCM virus is reported to exhibit little relationship to members of the New World arenaviruses; however, in such tests LCM virus has been shown to be distantly related to Lassa virus (Casals *et al.*, 1975). A recent report of the DNA sequence of the part of the Lassa virus S RNA that codes for the viral nucleoprotein (i.e., the 3' half; Clegg and Oram, 1985) indicates that the Lassa viral N protein is related by primary sequence to both LCM and Pichinde viral N proteins. Some 62% of the Lassa and LCM N protein amino acids are identical and about 50% of the Lassa and Pichinde N protein residues (Clegg and Oram, 1985). This result, as well as the Pichinde-LCM viral N protein relationships, was not expected from the serological experiments, nor from monoclonal antibody studies which have indicated that few N protein monoclonals of those that have been characterized are cross-reactive between these viruses (Buchmeier *et al.*, 1980, 1981). The fact, though, that some are cross-reactive is in agreement with the reported

sequence relationships. The observation that most of the analyzed monoclonals are not cross-reactive may suggest that the conserved regions of the viral N proteins may not be as antigenic as the nonconserved regions. The same may well be true for the viral glycoproteins.

Using the appropriate single-stranded S DNA probes, it has been shown that extracts of Pichinde virus-infected cells contain two subgenomic S RNA species in addition to the full-length viral and viral-complementary S RNA species (Auperin *et al.*, 1984b). The subgenomic RNA species are each approximately half the size of the viral S RNA.

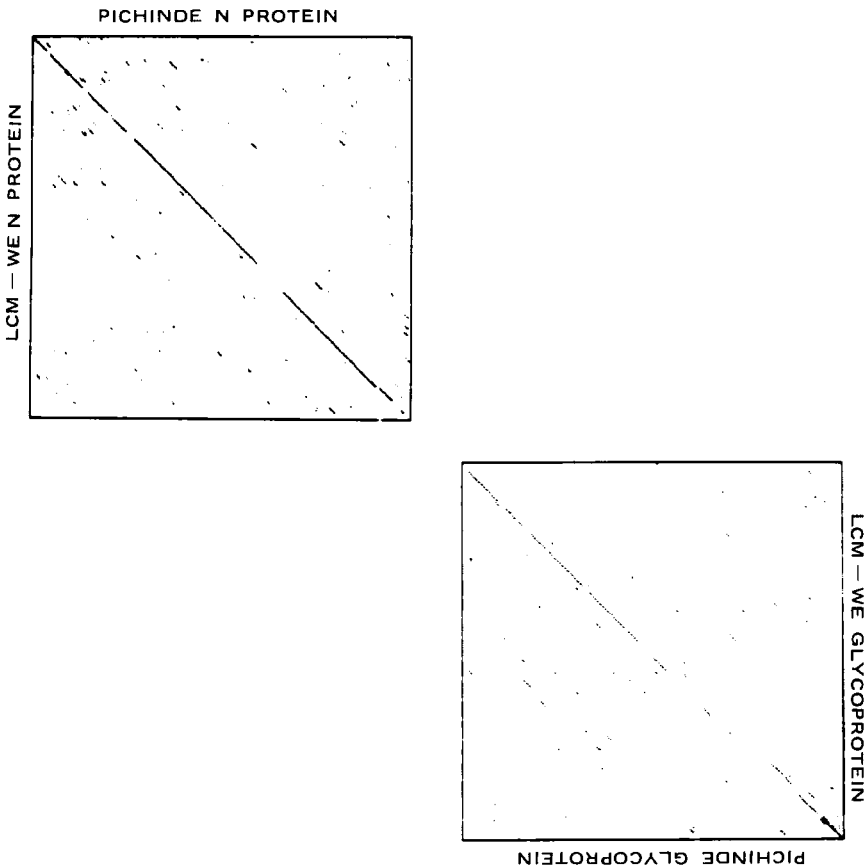


FIG. 4. Diagon comparisons of the Pichinde and LCM-WE viral N proteins (top) and glycoproteins (bottom), indicating regions of extensive homology (Romanowski *et al.*, 1985). The arrangement shown corresponds to the RNA alignment (3' to 5') with the N and G amino termini at the extreme top left and bottom right corners, respectively (Romanowski *et al.*, 1985).

They correspond to a viral-complementary N mRNA (as demonstrated by immune precipitation of N protein from *in vitro* translation products of the mRNA) and a viral-sense subgenomic species that is deduced to be the GPC mRNA species. The inability to bind Pichinde N mRNA to oligo(dT)-cellulose columns (Auperin *et al.*, 1984b) indicates that the N mRNA species lacks 3' polyadenylated sequences. Tryptic peptide analyses have shown that for Pichinde virus the protein order in the GPC precursor is G1 (amino half), then G2 (carboxy half) (unpublished data).

It has been observed that, in general, the flanking sequences to the AUG initiation codons of the Pichinde and LCM S and L genes that have been characterized (Auperin *et al.*, 1982a,b, 1984a,b; Romanowski and Bishop, 1985; Romanowski *et al.*, 1985) conform at least in the -3 and +4 positions to the consensus CG/ACCAUGG sequence identified by Kozak (1978, 1984) for eukaryote translation initiation. It has been pointed out (P. Young, personal communication) that in the leader sequence of the LCM and Pichinde S and L mRNA species, there is a conserved six-nucleotide sequence (GAUCCU) that is complementary to a 3' terminal sequence (CUAGGA) present in 18 S ribosomal RNA (Atmadja *et al.*, 1984). This complementarity may be important in arenavirus mRNA-ribosome interactions and subsequent translation of the mRNA.

In view of the observations that have been made concerning the coding arrangement, the arenavirus S RNA is described as having an ambisense strategy, to denote the fact that both viral and viral-complementary sequences are used to make gene products. Only partial sequence information has been reported for the L RNA of the WE strain of LCM virus (Romanowski and Bishop, 1985; ~1000 nucleotides from the 3' end). The limited data that have been obtained suggest that there is a gene product coded in the L RNA viral-complementary sequence (presumably the L protein). Northern analyses have so far only identified a single L mRNA species of approximately the same size as the viral L RNA (unpublished data). It may be, therefore, that the arenavirus L RNA has a simple negative strand coding arrangement. Alternatively, it may have an ambisense arrangement. Until the complete L RNA has been cloned and sequenced and the clones have been used to identify all of the L mRNA species, the answer will not be known.

One implication of the arenavirus S RNA coding arrangement is that the GPC subgenomic mRNA species and the viral glycoproteins cannot be made in infected cells until after viral RNA replication has commenced and a replicative intermediate, full-length, viral-complementary RNA is produced that can function as a template for GPC mRNA synthesis (Fig. 5). This is unlike the organization of the negative-

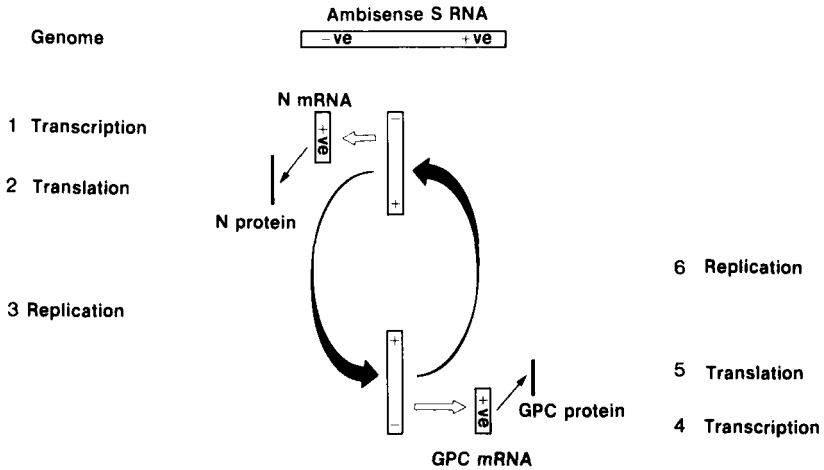


FIG. 5. Coding, transcription, and replication strategies of arenavirus S RNA species.

stranded rhabdoviruses and paramyxoviruses which have been shown to synthesize all their (viral-complementary) mRNA species in a consecutive manner from the viral RNA. It is also unlike the negative-stranded, segmented-genome orthomyxoviruses that also only code for proteins in their viral-complementary sequences. An advantage of the arenavirus S RNA strategy is that it allows the syntheses of the two S-coded mRNA species to be regulated independently so that different quantities of each can be made. Another feature is that the GPC mRNA (and protein) species are not synthesized until the time that they are required (i.e., after the onset of RNA replication and before initiation of the processes of viral morphogenesis).

It is quite possible that GPC mRNA synthesis may be curtailed by competition with viral RNA synthesis if, for instance, the availability of N protein regulates the process. Whether this occurs and contributes to the establishment (and maintenance) of persistently infected cells *in vivo* or *in vitro* remains to be determined. Conceivably, the curtailment of glycoprotein synthesis would prevent virus morphogenesis (or at least the synthesis of virus particles coated with the homologous viral protein) but may allow viral RNA replication to proceed. It may also render the infected cell subliminal to effective recognition by the host immune procedures. While these observations are speculative, the procedures and tools for the analysis of such hypotheses are now available.

The intergenic region of the Pichinde and LCM viral S RNA species has a unique feature, that of an inverted complementary sequence



that may be arranged into a hairpin configuration. The corresponding sequence for Lassa virus has not yet been reported, although it is apparently similar (D. A. Auperin, personal communication). The data for the intergenic region of Pichinde and LCM viruses is exemplified in Fig. 6. Although there is no information on the transcription initiation and termination processes of mRNA synthesis from the S (or L) RNA species, in unpublished experiments using oligonucleotides representing viral and viral-complementary sequences of the intergenic region of Pichinde S RNA, we have found that transcription of both N and GPC mRNA species terminates near the top of the intergenic hairpin (see Fig. 6). Thus, oligonucleotides complementary to the 3' half of the intergenic hairpin anneal to the S mRNA species (both N and GPC mRNA), whereas those representing the 5' half do not. Both types of oligonucleotide anneal to complete size S RNA species (viral RNA and replicative intermediate S RNA). How transcription termination in the intergenic region is effected is not known.

In agreement with the absence of binding to oligo(dT)-cellulose (Auperin *et al.*, 1984b), there are no polyuridylylate tracts in the intergenic region of the viral S RNA (or viral-complementary S RNA) that could serve as templates for polyadenylation of the 3' ends of the S mRNA species.

Concerning the question of how arenaviruses with an ambisense coding strategy may have arisen, unless one invokes an origin from a DNA source in which the arrangement of proteins coded on opposite strands of nucleic acid has been maintained, the simplest explanation is that a chimeric RNA was derived at some stage of arenavirus evolution. Such a chimeric RNA could have been formed during the processes of RNA replication and could represent a consolidation of genetic information (i.e., a virus with three RNAs each coding for a single gene product giving rise to an arenavirus with a consolidated genome and, subsequently, only two RNAs by the exclusion of the redundant third RNA species). Such consolidation of genetic information could occur by a viral replicase copying the coding strand of one RNA species and, instead of terminating, continuing RNA synthesis on the noncoding strand of another RNA. This would result in a chimeric RNA molecule composed of two genes coded on opposite strands of the RNA. Together with the subsequent loss of the redundant RNA species, a virus would be generated in which all the original genetic information had been conserved. Of course, the reverse situation may have occurred, i.e., the formation of a virus with three RNA species from a virus having two, one of which originally had an ambisense coding arrangement. Whatever the origins, the ambisense coding arrangements that have been observed for arenaviruses (and phleboviruses,



see below) open yet another dimension to the way in which viruses replicate in cells.

### III. BUNYAVIRIDAE

#### A. Structural and Genetic Properties of Bunyaviridae Members

##### 1. Viral Morphologies and Structural Components

Most of the viruses that have been assigned to the Bunyaviridae on morphological grounds have been grouped into four genera (*Bunyavirus*, *Nairovirus*, *Phlebovirus*, and *Uukuvirus*) on the basis of serological and biochemical relationships (Tables II–V; see Berge *et al.*, 1970, 1971; Berge, 1975; Bishop and Shope, 1979; Bishop *et al.*, 1980; Karabatsos, 1978). A fifth genus (*Hantavirus*, including Hantaan virus and related species) has been proposed (Table VI; McCormick *et al.*, 1982; Schmaljohn and Dalrymple, 1983). There are also many viruses that have yet to be assigned to a genus (Table VI); their inclusion in the family is based solely on common morphological and morphogenetic features by comparison with recognized members of the family.

Other than the Hantaan-related viruses, almost all of the viruses assigned to the Bunyaviridae are considered to be arthropod-borne viruses (arboviruses, i.e., they are believed to replicate in, and be transmitted by, arthropods, although formal demonstration of this postulate has only been obtained for a few members). By contrast, Hantaan and related viruses do not appear to involve arthropods in their transmission cycles.

As indicated in the schematic diagram shown in Fig. 7, Bunyaviridae viruses are spherical (~100 nm in diameter), enveloped in lipid, and in possession of an external layer of glycoproteins (Behbehani *et al.*, 1967; Bishop and Shope, 1979; Chastel *et al.*, 1979; Holmes, 1971; Lyons and Heyduk, 1973; McPhee and Westaway, 1981; Murphy *et al.*, 1968a,b, 1973a; Nuttall *et al.*, 1981; Obijeski and Murphy, 1977; Obijeski *et al.*, 1976a; Pettersson *et al.*, 1971; Robeson *et al.*, 1979; Saikku and von Bonsdorff, 1968; Saikku *et al.*, 1970; Smith and Pifat, 1982; Southam *et al.*, 1964; von Bonsdorff and Pettersson, 1975; von Bonsdorff *et al.*, 1969). For uukuviruses and phleboviruses, glutaraldehyde fixation of virus preparations led to the demonstration of hexagonal arrangements of the surface components (Robeson *et al.*, 1979; Smith and Pifat, 1982; von Bonsdorff and Pettersson, 1975), in contrast to the results obtained with bunyaviruses for which an amorphous arrangement has been reported (Obijeski *et al.*, 1976a; F. Murphy, personal communication).

NUCLEOCAPSID

(three, internal, helical, circular)

- 1. L RNA- N+L protein
  - 2. M RNA- N+L protein
  - 3. S RNA- N+L protein
- L protein is transcriptase?  
Arrangement ?

GLYCOPROTEINS

(two, G1, G2, external)

Penetration through envelope ?  
Arrangement ? Uukuniemi virus  
with hollow cylindrical surface  
spikes

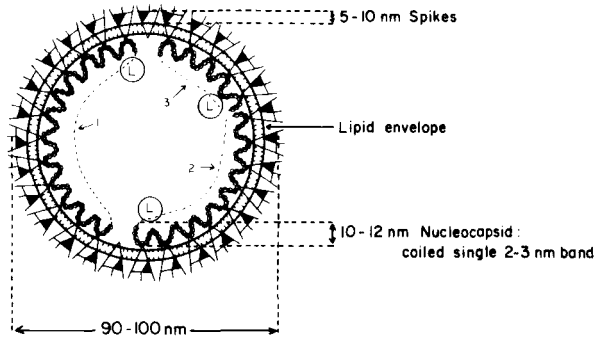


FIG. 7. Schematic representation of a bunyavirus particle.

Distinct morphological features of members of the *Nairovirus* genus have been recognized after glutaraldehyde fixation (Chastel *et al.*, 1979; Clerx and Bishop, 1981; Clerx *et al.*, 1981).

Generic differences have been reported for the major structural components of members of the Bunyaviridae (RNA and proteins, Figs. 8 and 9). The viral glycoproteins consist of two species that are now designated in relation to their relative sizes as G1 and G2 (Fig. 9; Bishop and Shope, 1979; Bishop *et al.*, 1980; Bouloy and Hannoun, 1976b; Cash *et al.*, 1980; Clerx and Bishop, 1981; Clerx *et al.*, 1981; Dalrymple *et al.*, 1982; El Said *et al.*, 1979; Gentsch and Bishop, 1976; Gentsch *et al.*, 1977a; Klimas *et al.*, 1981b; McLerran and Arlinghaus, 1973; McPhee and Westaway, 1981; Obijeski and Murphy, 1977; Obijeski *et al.*, 1976a; Pennington *et al.*, 1977; Pettersson *et al.*, 1971, 1977;

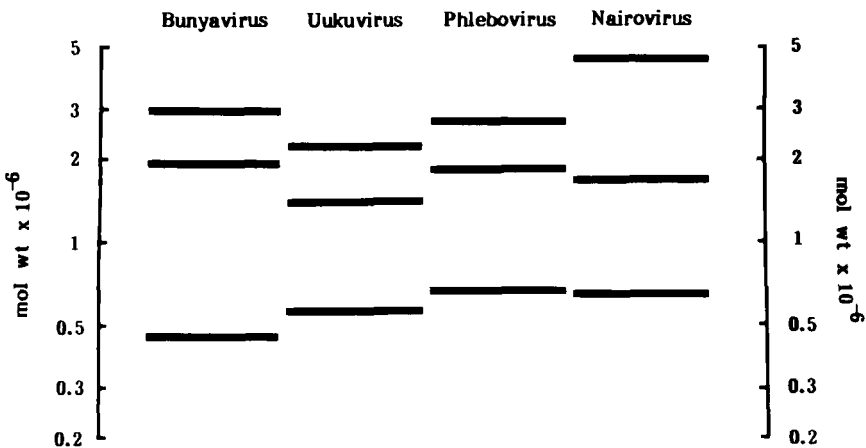


FIG. 8. RNA species characteristic of four of the Bunyaviridae genera.

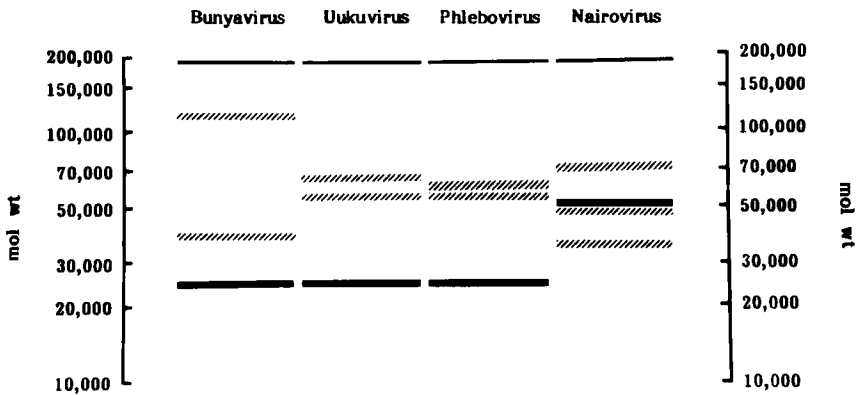


FIG. 9. Protein species characteristic of four of the Bunyaviridae genera.

Robeson *et al.*, 1979; Rosato *et al.*, 1974a,b; Smith and Pifat, 1982; Ushijima *et al.*, 1981; von Bonsdorff and Pettersson, 1975; White, 1975). Two or three glycoproteins have been reported for Hazara virus, a member of the *Nairovirus* genus (Clerx *et al.*, 1981; Foulke *et al.*, 1981).

Bunyaviridae viruses have internal components consisting of three nucleocapsids (Bouloy and Hannoun, 1976b; Obijeski *et al.*, 1976b; Pettersson and von Bonsdorff, 1975; Samsø *et al.*, 1975). Electron microscopic analyses of the nucleocapsids have revealed that they consist of coiled strands 2–3 nm in diameter that are occasionally observed to be circular and sometimes supercoiled with diameters between 7 and 12 nm (Obijeski *et al.*, 1976b; Pettersson and von Bonsdorff, 1975; Pettersson *et al.*, 1971; Saikku *et al.*, 1971; Samsø *et al.*, 1975; von Bonsdorff *et al.*, 1969). Each nucleocapsid consists of a single species of RNA [large (L), medium (M), and small (S)], nucleoprotein (N), and minor quantities of a large 180–200 × 10<sup>3</sup> Da protein (L protein) (Bouloy and Hannoun, 1976b; Obijeski *et al.*, 1976b; Pettersson *et al.*, 1971; Rosato *et al.*, 1974b; Saikku *et al.*, 1971; Samsø *et al.*, 1975; White, 1975).

The viral RNA species of several representative members of the family have been characterized by a variety of procedures and shown to consist of three molecules of single-stranded RNA with distinct sizes and sequences (Fig. 8; Bishop and Shope, 1979; Bouloy *et al.*, 1973/1974; Cash *et al.*, 1979; Clerx and Bishop, 1981; Clerx *et al.*, 1981; Clerx-van Haaster and Bishop, 1980; Clerx-van Haaster *et al.*, 1982a,b; Clewley *et al.*, 1977; El Said *et al.*, 1979; Gentsch and Bishop, 1976; Gentsch *et al.*, 1977a,b; Hewlett *et al.*, 1977; Klimas *et al.*, 1981a,b; McPhee and Westaway, 1981; Obijeski *et al.*, 1976b; Pettersson and Kaariainen, 1973;

Pettersson *et al.*, 1977; Robeson *et al.*, 1979; Ushijima *et al.*, 1980, 1981). Cloning and sequence analyses have shown that the 3' and 5' ends of each viral RNA are conserved and complementary in sequence (Akashi and Bishop, 1983; Akashi *et al.*, 1984; Bishop *et al.*, 1982; Cabradilla *et al.*, 1983; Clerx-van Haaster and Bishop, 1980; Clerx-van Haaster *et al.*, 1982a,b; Collett *et al.*, 1985; Eshita and Bishop, 1984; Ihara *et al.*, 1984, 1985b; Obijeski *et al.*, 1980; Parker and Hewlett, 1981). The complementarity may account for the circular forms of the extracted viral RNA species (Hewlett *et al.*, 1977; Samso *et al.*, 1976) and the viral nucleocapsids (Obijeski *et al.*, 1976b; Pettersson and von Bonsdorff, 1975; Samso *et al.*, 1975).

In summary, members of the Bunyaviridae exhibit a common morphology consisting of an enveloped virus particle with external glycoproteins and three internal nucleocapsids. Unlike orthomyxoviruses, paramyxoviruses, or rhabdoviruses, members of the Bunyaviridae lack other structural proteins such as a membrane or matrix protein. Unlike arenaviruses, members of the Bunyaviridae do not encapsidate host cell ribosomes. The viruses of the Bunyaviridae family exhibit a common pattern of morphogenesis that is not shared by these other RNA viruses, a morphogenesis that involves budding into the Golgi apparatus of infected cells.

The known biochemical characteristics that distinguish the bunyaviruses, nairoviruses, hantaviruses, phleboviruses, and uukuviruses are discussed below. This is followed by a brief review of the genetic attributes of the viruses prior to a discussion of what is known concerning their coding arrangements and the perceived hierarchy of evolutionary relationships.

## 2. *Bunyaviruses*

Some 151 virus serotypes, subtypes, and varieties have been described as bunyaviruses (Table II). The viruses have been placed into 16 serogroups to reflect the results of serological comparisons (see Bishop and Shope, 1979). In general, members of each serogroup are serologically more closely related to each other by particular tests (e.g., neutralization, hemagglutination inhibition) than to members of other serogroups, although, depending on the test, distant serological relationships have been demonstrated between representative members of different bunyavirus serogroups (see Bishop and Shope, 1979; Klimas *et al.*, 1981b). This latter observation, plus the common biochemical features that are discussed below, is the rationale for the inclusion of all 16 serogroups into the *Bunyavirus* genus. No serological relationships have been detected between bunyaviruses and members of other Bunyaviridae genera.

TABLE II  
PROPOSED SEROLOGICAL CLASSIFICATION OF VIRUSES OF FAMILY BUNYAVIRIDAE, GENUS *Bunyavirus*<sup>a</sup>

|  |                                  |  |                              |
|--|----------------------------------|--|------------------------------|
| <i>Anopheles A group</i>                         | <i>Bwamba group</i>              | <i>Gamboa group</i>                                      | <i>Simbu group</i>           |
| Anopheles A                                      | Bwamba                           | Gamboa   | Simbu                        |
| CoAr 3624 <sup>b</sup>                           | Pongola                          | Pueblo Viejo (75-2621 <sup>b</sup> )                     | Akabane                      |
| ColAn 57389 <sup>b</sup>                         |                                  | Alajuela <sup>b</sup>                                    | Yaba-7 <sup>b</sup>          |
| Las Maloyas                                      | <i>C group</i>                   | San Juan (78V2441 <sup>b</sup> , 75V-2374 <sup>b</sup> ) | Manzanilla                   |
| Lukuni   | Caraparu                         |  | Ingwavuma                    |
| Trombetas <sup>b</sup>                           | Caraparu (BeH5546 <sup>b</sup> , |  | Inini                        |
| Tacaiuma   | Trinidad <sup>b</sup> )          | <i>Guama group</i>                                       | Mermet                       |
| H-32580 <sup>b</sup>                             | Ossa                             | Guama  | Buttonwillow                 |
| SPAr 2317 <sup>b</sup> (Virgin River)            | Apeu                             | Ananindeua   | Nola                         |
| CoAr 1071 <sup>b</sup> (CoAr 3627 <sup>b</sup> ) | Vinces                           | Moju   | Oropouche                    |
|  | Bruconha <sup>b</sup>            | Mahogany Hammock   | Facey's Paddock <sup>b</sup> |
|  | Madrid                           | Bertioga   | Utinga                       |
| <i>Anopheles B group</i>                         | Marituba                         | Cananea  | Utive <sup>b</sup>           |
| Anopheles B                                      | Murutucu                         | Guaratuba  | Sabo                         |
| Boraceia   | Restan                           | Itimirim   | Tinaroo                      |
|  | Nepuyo (63U11 <sup>b</sup> )     | Mirim  | Sathuperi (Douglas)          |
|  | Gumbo Limbo                      | Bimiti   | Shamonda                     |
|  | Oriboca                          | Catu   | Sango                        |
|  | Itaqui                           | Timboteua  | Peaton                       |

|  |  |                           |  |
|--|--|---------------------------|--|
| <i>Bunyamwera group</i>  |  |                           |  |
| Bunyamwera   | <i>California group</i>                      | <i>Koongol group</i>      | Shuni                                  |
| Batai (Calovo)   | California encephalitis                      | Koongol                   | Aino (Kaikalur, Samford <sup>b</sup> ) |
| Birao  | Inkoo  | Wongal                    | Thimiri                                |
| Cache Valley (Tlacotalpan)                                     | La Crosse (snowshoe hare)                    |                           |  |
| Maguari (CbaAr 426 <sup>b</sup> )                              | San Angelo                                   | <i>Minatitlan group</i>   | <i>Tete group</i>                      |
| Playas   | Tahyna (Lumbo <sup>b</sup> )                 | Minatitlan                | Tete                                   |
| Xingu <sup>b</sup>   | Melao  | Palestina                 | Bahig                                  |
| Germiston  | Keystone                                     |                           | Matruh                                 |
| Hlesha   | Jamestown Canyon (South River <sup>b</sup> , | <i>Olifantsvlei group</i> | Tsuruse                                |
| Lokern   | Jerry Slough)                                | Olifantsvlei (Bobia)      | Batama                                 |
| Northway   | Serra do Navio                               | Botambi                   |  |
| Santa Rosa   | trivittatus                                  |                           | <i>Turlock group</i>                   |
| Shokwe <sup>b</sup>  | Guaroa                                       | <i>Patois group</i>       | Turlock                                |
| Tensaw   |  | Patois                    | Lednice                                |
| Kairi  | <i>Capim group</i>                           | Abrás                     | Umbre                                  |
| Main Drain   | Capim  | Babahoyo                  | M'Poko                                 |
| Wyeomyia   | Acara  | Shark River               | Yaba-1 <sup>b</sup>                    |
| Anhembi (BeAr 314206 <sup>b</sup> , BeAr 328208 <sup>b</sup> ) | Moriche                                      | Zegla                     |  |
| Macaua <sup>b</sup>  | Benevides                                    | Pahayokee                 |  |
| Sororoca   | BushBush                                     |                           |  |
| Taiassui <sup>b</sup>  | Benfica                                      |                           |  |
|  | GU71U344 <sup>b</sup>                        |                           |  |
|  | Juan Diaz                                    |                           |  |
|  | Guajara (GU71U350 <sup>b</sup> )             |                           |  |

<sup>a</sup>Viruses are classified in three steps indicated by degrees of indentation—complex, virus, and subtype; viruses in parentheses are varieties.

<sup>b</sup>These viruses are not in the published or working *International Catalogue of Arboviruses* (Berge, 1975; Karabatsos, 1978).



The limited genetic studies that have been reported for bunyaviruses (see below) suggests that viruses within a bunyavirus serogroup are more likely to be genetically compatible (e.g., capable of RNA segment reassortment) than are members representing different bunyavirus serogroups. Whether this conclusion extends to all bunyavirus serogroups (or to members of serogroups of other genera) remains to be determined.

DNA cloning studies have revealed that the S RNA species of snowshoe hare (SSH), La Crosse (LAC), and Aino bunyaviruses are of the order of  $3 \times 10^5$  Da (Akashi and Bishop, 1983; Akashi *et al.*, 1984; Bishop *et al.*, 1982, 1984b; Cabradilla *et al.*, 1983). From such analyses it has been determined that the S RNA of SSH bunyavirus codes for two proteins that are read from overlapping reading frames in viral-complementary S mRNA sequences (Bishop *et al.*, 1982). These proteins are the viral N protein ( $26 \times 10^3$  Da) and a nonstructural protein (NS<sub>S</sub>,  $10 \times 10^3$  Da). Protein and genetic analyses have confirmed these coding assignments as well as the existence of the S-coded N and NS<sub>S</sub> proteins (Fuller and Bishop, 1982; Fuller *et al.*, 1983; Gentsch and Bishop, 1978; Gentsch *et al.*, 1977b). It has been shown (Bishop *et al.*, 1983) that the SSH S mRNA species have 12- to 15-nucleotide-long, heterogeneous, 5' nonviral sequences that extend beyond the 3' end of the viral RNA (i.e., they probably represent host-derived, presumably capped RNA primers that are used by the viral transcriptase for S mRNA synthesis). Even taking into account these additional 5' sequences, size analyses of SSH and LAC S mRNA species have shown that they are some 85 nucleotides shorter than the viral S RNA (i.e., mRNA transcription terminates before the end of the template RNA is reached; Eshita *et al.*, 1985; Patterson and Kolakofsky, 1984). Only a single SSH S mRNA species has been demonstrated by Northern hybridization, or by viral-sense oligonucleotide-directed reverse transcription and cloning analyses (Bishop *et al.*, 1983; Eshita *et al.*, 1985). It has been concluded therefore from these and *in vitro* translation of purified S mRNA preparations that there is only one S mRNA species that serves for the synthesis of both SSH N and NS<sub>S</sub> proteins (Eshita *et al.*, 1985; Fuller *et al.*, 1983). Previously Patterson and associates (1983) reported the probable existence of multiple LAC S mRNA species from an analysis using viral-sense oligonucleotide-directed reverse transcription and dideoxyribonucleotide incorporation, a result we have not been able to confirm using high levels of triphosphate and similar protocols (unpublished data). Subsequent studies by Patterson and associates (1984) identified a primer-stimulated RNA polymerase and methylated cap-dependent viral endonuclease, in agreement with the postulate that the LAC S mRNA has 5' nonviral primers like those

identified for SSH mRNA species (Bishop *et al.*, 1983; Eshita *et al.*, 1985). Recent studies have demonstrated the synthesis of SSH S mRNA species in virus-infected cells grown in the presence of protein synthesis inhibitors (puromycin or cycloheximide; Eshita *et al.*, 1985) in agreement with the idea that bunyaviruses possess a virion transcriptase (Bouloy and Hannoun, 1976a; Bouloy *et al.*, 1975).

The results of cloning the M RNA species of SSH virus have indicated that its size is  $1.5 \times 10^6$  Da (Eshita and Bishop, 1984). From the DNA cloning analyses, the SSH M RNA has been deduced to code for a precursor to the viral glycoproteins ( $162 \times 10^3$  Da) in a viral-complementary mRNA sequence (Eshita and Bishop, 1984). Genetic and molecular analyses have demonstrated that the bunyavirus M RNA codes for both G1 ( $115 \times 10^3$  Da), G2 ( $38 \times 10^3$  Da), and a second nonstructural protein, NS<sub>M</sub>, that has been estimated to be of the order of  $15 \times 10^3$  Da (Fuller and Bishop, 1982; Gentsch and Bishop, 1979). Presumably these proteins come from the glycoprotein precursor, although their order and mode of derivation from that polypeptide are not known. The M-coded gene products elicit neutralizing antibodies (as expected for external glycoproteins) and are major determinants of the virulence and vector transmission potentials of bunyaviruses (Beaty *et al.*, 1981a,b, 1982; Gentsch *et al.*, 1980; Shope *et al.*, 1981, 1982). Heterogeneous, nonviral, 12- to 15-nucleotide-long extensions to the 5' ends of the M mRNA species have been identified for SSH virus, similar to those reported for the S mRNA species (Eshita *et al.*, 1985).

The size of the SSH (or other bunyavirus) L RNA has not been determined by DNA cloning, but by gel electrophoresis it has been estimated to be of the order of  $3 \times 10^6$  Da (Bishop and Shope, 1979). The SSH L RNA is believed to code for the  $180\text{--}200 \times 10^3$  Da L protein that has been identified in virus preparations (the putative transcriptase–replicase), although formal proof of that postulate has not been reported.

As judged by gel electrophoresis, the sizes of the viral RNA and protein species of some 30–40 other bunyaviruses that have been analyzed are essentially similar to those of SSH virus (Figs. 8 and 9; Bishop and Shope, 1979; El Said *et al.*, 1979; Klimas *et al.*, 1981b; Ushijima *et al.*, 1981).

In summary, bunyaviruses, which are mostly transmitted by mosquito or *Culicoides* species, have RNA sizes of around  $3.3 \times 10^5$  Da (S),  $1.5 \times 10^6$  Da (M), and (estimated)  $3 \times 10^6$  Da (L). These RNA species code for the  $20\text{--}25 \times 10^3$  Da N and  $10 \times 10^3$  Da NS<sub>S</sub> proteins (S RNA), the  $110\text{--}120 \times 10^3$  Da G1,  $30\text{--}40 \times 10^3$  Da G2, and  $15 \times 10^3$  Da NS<sub>M</sub> proteins (M RNA) and, presumably, the  $180\text{--}200 \times 10^3$  Da L protein (L RNA).

### 3. *Nairoviruses*

Thirty-one virus serotypes, subtypes, and varieties are classified to the *Nairovirus* genus of the family Bunyaviridae (Table III). Six serogroups of viruses are recognized, representing the perceived antigenic relationships of viruses in the genus (Casals and Tignor, 1980). The viruses have been isolated from a variety of ticks and animals collected from many different ecological niches (Berge, 1975; Berge *et al.*, 1970, 1971; Bishop and Shope, 1979; Johnson *et al.*, 1979; Karabatosos, 1978; Nuttall *et al.*, 1984). Members representing the six recognized nairovirus serogroups have comparable RNA and protein sizes that are distinct by comparison with those of representatives of the other Bunyaviridae genera. None of the RNA species has been cloned into DNA and sequenced. However, the estimated sizes of the nairovirus L, M, and S RNA species are  $4-5 \times 10^6$  Da (L),  $1.5-1.9 \times 10^6$  Da (M), and  $0.6-0.7 \times 10^6$  Da (S) (Fig. 8; Clerx and Bishop, 1981; Clerx *et al.*, 1981). An example of the differences in the sizes of the viral RNA species of Qalyub (QYB) nairovirus, SSH, and Jerry Slough (JS) bunyaviruses and that of vesicular stomatitis (VSV) rhabdovirus is provided in Fig. 10. Sequence analyses have demonstrated that the 3' end sequences of nairoviruses are different from those of bunyaviruses; phleboviruses, uukuviruses, and hantaviruses (Clerx-van Haaster and Bishop, 1980; Clerx-van Haaster *et al.*, 1982a,b).

The estimated sizes of the major proteins of nairoviruses are  $72-84 \times 10^3$  Da (G1),  $30-40 \times 10^3$  Da (G2), and  $48-54 \times 10^3$  Da (N) (Fig. 9; David-West, 1974; Clerx and Bishop, 1980; Clerx *et al.*, 1981; Foulke *et al.*, 1981). Whether viral-induced nonstructural proteins and a virion L protein are coded by the viruses, as reported for bunyaviruses, is not known. The coding assignments of the RNA species of nairoviruses have not been reported.

### 4. *Uukuviruses*

Nine viruses have been assigned to the *Uukuvirus* genus (Table IV; Begum *et al.*, 1970; Bishop and Shope, 1979; Bishop *et al.*, 1980; Gaidamovich *et al.*, 1971a,b, 1973; Hannoun *et al.*, 1970; Karabatsos, 1978; Lvov *et al.*, 1973a,b; Oker-Blom *et al.*, 1964; Nuttall *et al.*, 1981; Yunker, 1975). No delegation into serogroups has been made, so that currently the uukuviruses are considered to be a single group (Table IV). The viruses have been isolated from a variety of tick and vertebrate sources (Bishop and Shope, 1979).

The sizes of the three viral RNA species of uukuviruses have been estimated to be of the order of  $2.4 \times 10^6$  (L),  $1.1 \times 10^6$  (M), and  $0.5 \times 10^6$  Da (S) (Fig. 8; Pettersson *et al.*, 1977). They have been shown to exhibit a circular configuration that is deduced to be due to end-hydrogen bond-

TABLE III  
 PROPOSED SEROLOGICAL CLASSIFICATION OF  
 VIRUSES OF FAMILY BUNYAVIRIDAE,  
 GENUS *Nairovirus*<sup>a</sup>

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|                                       |
|---------------------------------------|
| Crimean–Congo hemorrhagic fever group |
| Crimean hemorrhagic fever (Congo)     |
| Hazara                                |
| Dera Ghazi Khan group                 |
| Dera Ghazi Khan                       |
| Abu Hammad                            |
| Abu Mina <sup>b</sup>                 |
| Kao Shuan                             |
| Pathum Thani                          |
| Pretoria                              |
| Hughes group                          |
| Hughes                                |
| Farallon <sup>b</sup>                 |
| Raza <sup>b</sup>                     |
| Fraser Point <sup>b</sup>             |
| Great Saltee <sup>b</sup>             |
| Puffin Island <sup>b</sup>            |
| Punta Salinas                         |
| Sapphire <sup>b</sup>                 |
| Soldado                               |
| Zirqa                                 |
| Nairobi sheep disease group           |
| Nairobi sheep disease (Ganjam)        |
| Dugbe                                 |
| Qalyub group                          |
| Qalyub                                |
| Bandia                                |
| Omo                                   |
| Sakhalin group                        |
| Sakhalin (Tillamook <sup>b</sup> )    |
| Clo Mor                               |
| Taggart                               |
| Avalon                                |
| Paramushir                            |

---

<sup>a</sup>Viruses are classified in three steps indicated by degrees of indentation—complex, virus, and subtype; viruses in parentheses are considered varieties.

<sup>b</sup>These viruses are not in the published or working *International Catalogue of Arboviruses* (Berge, 1975; Karabatsos, 1978).

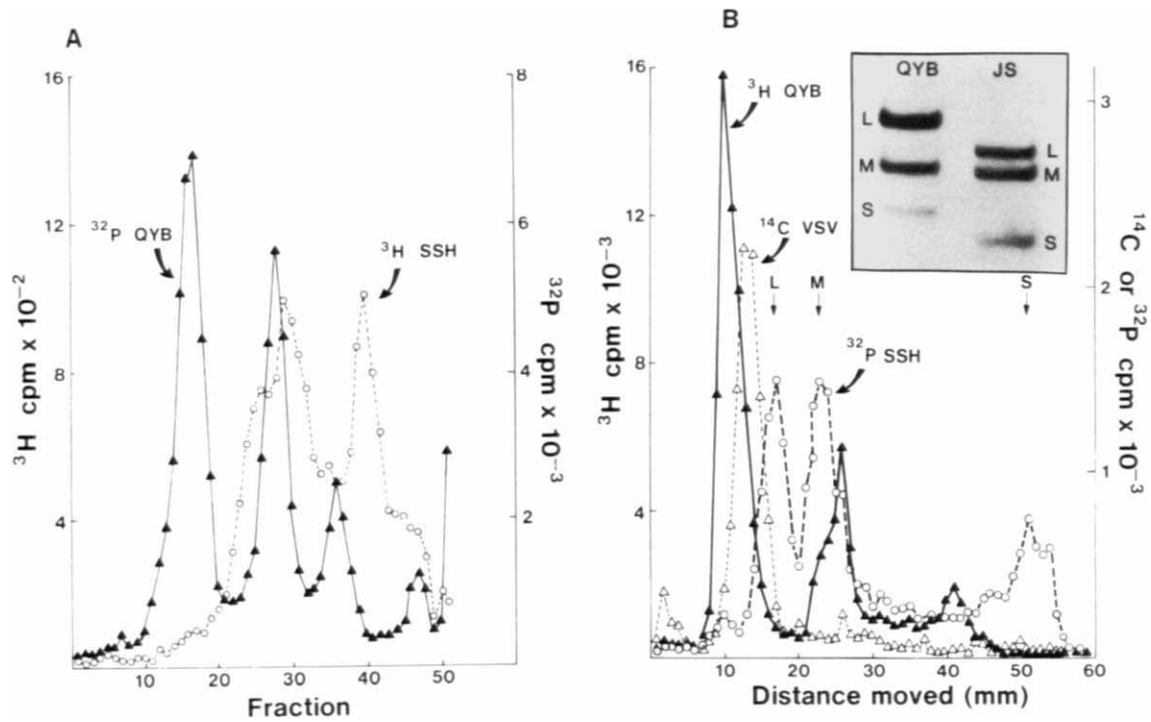


FIG. 10. Resolution of the viral RNA species of aairovirus (Qalyub, QYB) and a bunyavirus (SSH) by sucrose gradient centrifugation (A) and of QYB, SSH, and the rhabdovirus vesicular stomatitis virus (VSV) by polyacrylamide gel electrophoresis. (B) The resolution using agarose gel electrophoresis of the viral RNA of QYB nairovirus and Jerry Slough bunyavirus.

TABLE IV  
 PROPOSED SEROLOGICAL  
 CLASSIFICATION OF VIRUSES OF  
 FAMILY BUNYAVIRIDAE, GENUS  
*Uukuvirus*<sup>a</sup>

---

|                             |
|-----------------------------|
| Uukuniemi group             |
| Uukuniemi                   |
| Oceanside <sup>b</sup>      |
| EgAn1825-61 <sup>b</sup>    |
| Grand Arbaud                |
| Manawa                      |
| Ponteves                    |
| St. Abb's Head <sup>b</sup> |
| Sumakh <sup>b</sup>         |
| Zaliv Terpeniya             |

---

<sup>a</sup>Viruses are classified in three steps indicated by degrees of indentation—complex, virus, and subtype.

<sup>b</sup>These viruses are not in the published or working *International Catalogue of Arboviruses* (Berge, 1975; Karabatsos, 1978).

ing (Pettersson *et al.*, 1977). The RNA species have 3' end sequences (Parker and Hewlett, 1981) that are unlike those of bunyaviruses and nairoviruses, but are similar in sequence to those of phleboviruses (Clerx-van Haaster *et al.*, 1982b). The structural polypeptides include two glycoproteins (G1,  $75 \times 10^3$  Da; G2,  $65 \times 10^3$  Da), an N protein ( $25 \times 10^3$  Da), and an L protein ( $170 \times 10^3$  Da) (Fig. 9; Pettersson *et al.*, 1971, 1977; von Bonsdorff and Pettersson, 1975).

In addition to L and M size RNA, two discrete small RNA species have been observed in Uukuniemi virus-infected cell extracts (Pettersson *et al.*, 1981; Ulmanen *et al.*, 1981). The M size RNA was deduced by *in vitro* translation in the presence of dog pancreas microsomes (plus immune precipitation of the products) to include an mRNA that directed the synthesis of a  $110 \times 10^3$  Da glycoprotein precursor. The larger of the two small RNA species (corresponding in size to the viral S RNA) did not appear to direct the specific synthesis of viral protein during *in vitro* translation analyses (Pettersson *et al.*, 1981; Ulmanen *et al.*, 1981). The smaller species directed the synthesis of two proteins; one was deduced to be a  $30 \times 10^3$  Da nonstructural protein and the other was shown to correspond in size to the  $25 \times 10^3$  Da viral N protein. The N protein was immune precipitated with virus-

specific antiserum (Pettersson *et al.*, 1981). From these data it was concluded that uukuviruses code for discrete, subgenomic mRNA species.

### 5. *Phleboviruses*

Thirty-eight viruses have been assigned to the *Phlebovirus* genus (Table V; Bishop, 1985; Bishop *et al.*, 1980; Tesh *et al.*, 1975, 1982; Travassos *et al.*, 1983). Many of the viruses are transmitted by sandfly species (phlebotomines); some may be transmitted by mosquito or *Culicoides* species (Berge, 1975; Berge *et al.*, 1970, 1971; Karabatsos, 1978). Structurally the viruses resemble uukuviruses in terms of their morphologies, as well as in terms of their RNA and proteins sizes (Figs. 8 and 9). The size of the viral L RNA is estimated to be of the

TABLE V  
PROPOSED SEROLOGICAL  
CLASSIFICATION OF VIRUSES OF  
FAMILY BUNYAVIRIDAE, GENUS  
*Phlebovirus*<sup>a</sup>

---

Sandfly fever Naples group

Sandfly fever Naples  
Tehran  
Toscana  
Karimabad

Bujaru group

Bujaru  
Munguba<sup>b</sup>  
Aguacate

Candiru group

Candiru  
Itaituba  
Nique  
Turuna  
Oriximina<sup>b</sup>  
Punta Toro  
Buenaventura  
Alenquer

Chilibre group

Chilibre  
Cacao

Frijoles group

Frijoles  
Joa<sup>b</sup>

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TABLE V (continued)

---

|                              |
|------------------------------|
| Rift Valley fever group      |
| Rift Valley fever (Zinga)    |
| Belterra <sup>b</sup>        |
| Icoaraci                     |
| Salehabad group              |
| Salehabad                    |
| Arbia <sup>b</sup>           |
| Sandfly fever Sicilian group |
| Sandfly fever sicilian       |
| PaAr814 <sup>b</sup>         |
| Unassigned viruses           |
| Anhanga                      |
| Arumowot                     |
| Caimito                      |
| Chagres                      |
| Gabek forest <sup>b</sup>    |
| Gordil                       |
| Itaporanga                   |
| Pacui                        |
| Rio Grande                   |
| Saint-Floris                 |
| Urucuri                      |

---

<sup>a</sup>Viruses are classified in three steps indicated by degrees of indentation—complex, virus, and subtype; viruses in parentheses are varieties.

<sup>b</sup>These viruses are not in the published or working *International Catalogue of Arboviruses* (Berge, 1975; Karabatsos, 1978).

order of  $2.5\text{--}3 \times 10^6$  Da (Robeson *et al.*, 1979; unpublished data). From DNA cloning and sequencing studies, the M RNA of Punta Toro (PT) phlebovirus has a calculated size of  $1.5 \times 10^6$  Da and the S RNA has a size of  $6 \times 10^5$  Da (Ihara *et al.*, 1984, 1985a). The structural proteins of phleboviruses include small quantities of a  $170\text{--}200 \times 10^3$  Da protein (L protein) that may be a transcriptase component, two glycoproteins (G1,  $60\text{--}70 \times 10^3$  Da; G2,  $50\text{--}60 \times 10^3$  Da), and N protein ( $20\text{--}30 \times 10^3$  Da) (Bishop *et al.*, 1980; Robeson *et al.*, 1979; unpublished data). The DNA cloning and sequencing analyses that have been reported for the M RNA of PT virus have shown that the glycoproteins are made from a  $146 \times 10^3$  Da precursor (Ihara *et al.*, 1985b). Similar results



were obtained for Rift Valley fever virus (Collett *et al.*, 1985). It has been deduced from these studies that in addition to the structural G1 and G2 proteins, the glycoprotein precursor includes an amino-terminal portion (up to  $20 \times 10^3$  Da in the case of PT virus) that is nonstructural (NS<sub>M</sub>). The function of the NS<sub>M</sub> protein remains to be elucidated.

DNA cloning analyses of the S RNA of PT virus have established that it codes for the  $27 \times 10^3$  Da viral N protein in a subgenomic mRNA species and a  $20 \times 10^3$  Da nonstructural protein (NS<sub>S</sub>) that is coded in a subgenomic viral-sense mRNA species (Ihara *et al.*, 1984). No sequence analyses have been reported for the L RNA. Presumably it codes for the viral L protein although whether it codes for other proteins is not known.

Analyses of the mRNA transcription processes of PT virus have indicated that, like bunyaviruses, the S- and M-coded mRNA species have 5' terminal, heterogeneous, presumably nonviral, 12- to 17-nucleotide-long extensions (Ihara *et al.*, 1985a). The PT primary transcription process involves the synthesis of the N mRNA species as evidenced by its accumulation in infected cells grown in the presence of protein synthesis inhibitors such as puromycin or cycloheximide. Apparently no S-coded NS mRNA is synthesized during primary transcription, in agreement with the postulate that NS is coded in a viral-sense mRNA made from an intermediate in RNA replication (the full-length vcRNA) that is not synthesized until viral proteins are available (Ihara *et al.*, 1985a).

### 6. Other Viruses Including Hantaviruses

Shown in Table VI are four serogroups of viruses, as well as 14 unassigned viruses, that are considered to be probable members of the Bunyaviridae on the basis of common morphological, and in some cases biochemical, data. Although no serological results have been reported to substantiate their inclusion in one of the established Bunyaviridae genera, it is possible that some of these viruses may have properties in common with members of those genera. Other than the hantaviruses, almost no information is available on the biochemical and coding properties of the viruses listed in Table VI.

The etiologic agents for hemorrhagic fever with renal syndrome (otherwise described as Korean hemorrhagic fever, epidemic hemorrhagic fever, and nephropathia epidemica—diseases that are endemic in parts of Korea, China, and Europe plus Scandinavia, respectively) are viruses that are related to Hantaan virus, an isolate originally obtained from the Korean striped field mouse, *Apodemus agrarius corea*, and subsequently from other sources including man and laboratory rats (Gajdusek *et al.*, 1982; Kitamura *et al.*, 1983; LeDuc *et al.*, 1982; Lee *et al.*, 1978, 1982; Lee and Johnson, 1982; McCormick *et al.*,

TABLE VI  
 PROPOSED SEROLOGICAL  
 CLASSIFICATION OF VIRUSES OF  
 FAMILY BUNYAVIRIDAE,  
 UNASSIGNED VIRUSES<sup>a</sup>

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|                                     |
|-------------------------------------|
| Bakau group                         |
| Bakau                               |
| Ketapang                            |
| Kaisodi group                       |
| Kaisodi                             |
| Lanjan                              |
| Silverwater                         |
| Hantaan group <sup>c</sup>          |
| Hantaan                             |
| Nephropathia epidemica <sup>b</sup> |
| Prospect Hill <sup>b</sup>          |
| Tchoupitoulas <sup>b</sup>          |
| Mapputta group                      |
| Mapputta                            |
| Maprik                              |
| Gan Gan                             |
| Trubanaman                          |
| Yogue group                         |
| Yogue                               |
| Kasokero <sup>b</sup>               |
| Unassigned viruses                  |
| Aransas Bay <sup>b</sup>            |
| Belmont                             |
| Bhanja                              |
| Keterah (Issyk-Kul)                 |
| Khasan                              |
| Kowanyama                           |
| Lone Star                           |
| Razdan                              |
| Sunday Canyon                       |
| Tamdy                               |
| Tataguine                           |
| Upolu <sup>b</sup>                  |
| Witwatersrand                       |

---

<sup>a</sup>Viruses are classified in three steps indicated by degrees of indentation—complex, virus, and subtype.

<sup>b</sup>These viruses are not in the published or working *International Catalogue of Arboviruses* (Berge, 1975; Karabatsos, 1978).

<sup>c</sup>Proposed as a fifth genus (*Hantavirus*).

1982; Tsai *et al.*, 1982; White *et al.*, 1982). Characterization of Hantaan virus has revealed that it has a tripartite, single-stranded, negative-sense RNA genome, a virion RNA polymerase, and polypeptide species similar in broad terms to those of other Bunyaviridae members (Schmaljohn and Dalrymple, 1983; Schmaljohn *et al.*, 1983). In view of the observations that have been made, Hantaan virus has been proposed as a prototype virus for a fifth genus, the *Hantavirus* genus. Recent studies support this proposition and indicate that the Hantaan viral nucleoprotein, N, is coded by the virion S RNA, although whether it has an ambisense coding strategy, or a simple negative sense arrangement is not yet known (C. Schmaljohn and J. Dalrymple, personal communication). The lack of any identified arthropod vector involved in Hantaan virus transmission contrasts with the evidence for arthropod transmission of many other members of the Bunyaviridae.

### 7. Genetic Attributes of the Bunyaviridae

Genetic studies have only been reported for a few members of the *Bunyavirus* genus. The analyses have primarily concerned Bunyamwera and California serogroup viruses. For members of the California group, intra- and intertypic virus recombination involving RNA segment reassortment has been demonstrated in studies employing *ts* mutants of SSH, LAC, Tahyna, Lumbo, California encephalitis, and trivittatus viruses (Bishop *et al.*, 1984a; Gentsch and Bishop, 1976; Gentsch *et al.*, 1977b, 1979, 1980; Rozhon *et al.*, 1981; Shope *et al.*, 1981). For each of these California group viruses only L and M RNA mutants have been identified. Recombination has been documented for the Bunyamwera group members Batai, Bunyamwera, and Maguari viruses (Elliott *et al.*, 1984; Iroegbu and Pringle, 1981a,b; Pringle and Iroegbu, 1982). Mutants representing three Maguari RNA species have been identified (Pringle and Iroegbu, 1982) as well as complementing mutants for the RNA that encodes the N protein (presumably the S RNA with *ts* mutants representing the S-coded N and NS<sub>S</sub> proteins) (Iroegbu and Pringle, 1981b). No recombination has been documented between the above-mentioned California or Bunyamwera group viruses and *ts* mutants of Guaroa virus, or between *ts* mutants of the Bunyamwera group and California group viruses (Bishop and Shope, 1979; C. R. Pringle and D. H. L. Bishop unpublished data). Recombination has been reported between *ts* mutants of the Group C Caraparu virus (Bishop *et al.*, 1984a).

As discussed previously, reassortant viruses have been useful in deducing the bunyavirus RNA-protein coding assignments and for investigations into the viral gene products that are important to the biological phenotypes of particular bunyaviruses.

Analyses of reassortant viruses that have been derived from virus parents that differ in their ability to induce disease have shown that in laboratory animals the virulence characteristics of the California group bunyaviruses segregate with the origin of the viral M RNA species (Table VII; Shope *et al.*, 1981, 1982). As evidenced from back-cross analyses using a virulent parent and a derived attenuated virus, it has been shown that mutant viral L RNA gene products can mitigate the virulence capabilities of a bunyavirus (Rozhon *et al.*, 1981). The same may apply for mutant S RNA gene products, although that has not yet been demonstrated. However, the principal determinant for the virulence of bunyaviruses in these laboratory animals appears to be the viral glycoproteins.

The natural relationship of LAC bunyaviruses and *Aedes triseriatus* mosquitoes has been studied in detail. This mosquito species has been shown to be efficient at oral (Watts *et al.*, 1972), transovarial (Pantuwatana *et al.*, 1974; Watts *et al.*, 1973a,b), and venereal transmission of LAC virus (Thompson and Beaty, 1977). In the northern regions of the United States, LAC virus overwinters in diapaused *Ae. triseriatus* eggs (Beaty and Thompson, 1975; Watts *et al.*, 1974). Immunofluorescence techniques have been used to determine the virogenesis of LAC virus in *Ae. triseriatus* and to derive anatomic explanations of the unique vector-virus interactions (transovarial and venereal transmission) that are observed in this system. Thus, it has been shown that subsequent to oral infection, virus antigen can be detected in the pyloric portion of the arthropod midgut (6 days postinfection). By 10 days, the virus was found to have disseminated from the midgut and antigen was present in most secondary organ systems, including ovaries and salivary glands. It was observed that LAC virus infection was virtually pantropic in the arthropod, with most organ systems exhibiting large quantities of virus antigen. Detection of virus in ovarian follicles and in accessory sex gland fluid suggested anatomic explanations for the observed transovarial and venereal transmission of the virus (Beaty and Thompson, 1976, 1977).

Although serologically related, each of the California serogroup bunyaviruses has a distinct epizootiology often involving a select vector species and a particular (but not exclusive) vertebrate host. For example, in the United States trivittatus virus is closely associated with an *Ae. trivittatus*-cottontail rabbit feeding cycle. Keystone virus is associated with an *Ae. atlanticus*-squirrel cycle. SSH virus (which is serologically almost indistinguishable from LAC virus) is associated with an *Ae. canadensis* and *Ae. communis* group-snowshoe hare cycle, while LAC virus in the midwest of the United States (and elsewhere) is associated with an *Ae. triseriatus*-chipmunk-tree squirrel cycle

(LeDuc, 1979; Pantuwatana *et al.*, 1972; Sudia *et al.*, 1971). California serogroup viruses have, however, been isolated on occasion from alternate vectors in nature. For example, LAC virus has been isolated occasionally from *Ae. canadensis* and *Ae. communis* group mosquitoes, as well as from tabanids. SSH virus has been isolated infrequently from *Ae. triseriatus* mosquitoes. In addition, at least six of the California group viruses have been recovered from *Ae. vexans*. These observations suggest that the California group viruses reach other mosquitoes but that in view of the low frequency of isolation from such species, there are probably differences in the efficiencies of bunyavirus infection of the various arthropod species.

Since all of the possible genotype combinations of LAC and SSH virus reassortants have been produced by *in vitro* (cell culture) procedures (Gentsch *et al.*, 1977b, 1979; Rozhon *et al.*, 1981), and because *Ae. triseriatus* mosquitoes are not the normal vectors of SSH virus, the question of the viral determinants for permissive replication of LAC and SSH virus in that arthropod species was investigated using either LAC or SSH virus or LAC-SSH reassortant viruses to infect *Ae. triseriatus* mosquitoes. The results that were obtained involving *per os* infection or intrathoracic inoculation (Beaty *et al.*, 1981a, 1982, 1985) indicated that the LAC viral M RNA gene products were the principal determinants of the efficiency of virus infection of that arthropod. Thus, reassortants containing a LAC M RNA (e.g., L/M/S genotypes such as SSH/LAC/SSH) produced disseminated infections and were transmitted by *Ae. triseriatus* mosquitoes with efficiencies comparable to that of LAC virus. By contrast, viruses with a SSH M RNA (e.g., LAC/SSH/LAC) were essentially as inefficiently transmitted as the parent SSH virus. Although attenuating mutations in other LAC RNA species may affect the LAC M gene property (Rozhon *et al.*, 1981), the major viral determinants of efficient vector transmission appear to be the LAC viral glycoproteins (Beaty *et al.*, 1981a, 1982).

From bunyavirus isolation data it can be concluded that, even though the California group viruses are distinct epizootiologically, many are sympatric throughout much of their respective ranges, theoretically providing ample opportunity for dual virus infection of vector species to occur in nature. Intertypic recombinant virus formation and the transmission of the recombinant viruses to a vertebrate host have been demonstrated in dual virus infections of colonized *Ae. triseriatus* mosquitoes by using intrathoracic inoculation or *per os* ingestion of *ts* mutants of LAC and SSH viruses (Beaty *et al.*, 1981b, 1985). Thus, it has been demonstrated that at least certain intertypic bunyaviruses can be generated in the arthropod host, and this conclusion has been supported by the identification of reassortant viruses among field isolates of bunyaviruses (Klimas *et al.*, 1981a; Ushijima *et al.*, 1981).

In summary, the serological, genetic, and molecular features of members of the Bunyaviridae indicate that at least three of the genera are quite distinct in their characteristics (*Bunyavirus*, *Phlebovirus*, *Nairovirus*) and that in many regards uukuviruses resemble phleboviruses. At present the data for the hantaviruses are limited, although the indications are that hantaviruses differ in many of their properties by comparison with members of the four established genera of the family. In the following sections information concerning the infection and coding strategies of bunyaviruses and phleboviruses (as well as other members of the family) is discussed in relation to the perceived hierarchy of virus relationships.

### *B. The Infection Cycle*

For the arthropod-borne members of the family, transmission in nature involves the replication of virus in a permissive invertebrate host (e.g., mosquitoes, *Culicoides*, ticks, phlebotomines, etc.) and virus introduction into a vertebrate during the course of ingestion of a blood meal. Mechanical transmission of virus by arthropods (i.e., not involving virus replication in the insect species) may also occur on occasion. For viruses that are not transmitted by arthropods (e.g., Hantaan virus) transmission is believed to involve respiratory routes of infection. As discussed above, it appears that there is a restricted group of arthropods that is able to transmit any particular arbovirus member of the family. The molecular basis for this presumed restriction is not known, but it may involve inefficient virus replication so that not enough virus is produced (consequently prejudicing the possibility of transmission), or it may indicate that the virus does not reach the salivary gland (or other intermediary tissues). Alternatively there may be a total lack of cell receptors or host components that are necessary for the virus replication process. The observation that a virus species is sometimes recovered from a different species of arthropod no doubt reflects the fact that several types of insect feed on viremic animals. This occurrence may allow the selection of variant viruses that are suitable for transmission by new arthropod hosts. The amount of virus that is transmitted by arthropods is not known. It seems unlikely to be more than a few infectious virus particles in view of the small amounts of saliva that are transferred.

Once introduced into the bite site, it is presumed that the virus infects and replicates in those cells that have receptors with which the virus can interact and stimulate ingestion. The particular cell types, their receptors, and exactly what is involved at the molecular level in cell targeting, virus adsorption, penetration, and uncoating are not known. The end result, though, is the release into the cell cytoplasm of

the viral nucleocapsids. Again, the processes that follow are not understood in molecular detail. In broad terms it is believed that primary transcription of the viral mRNA species occurs in the cytoplasm of the infected cell. Primary transcription is catalyzed by the virion polymerase and can be demonstrated (at least for the S RNA) by the synthesis of viral-complementary mRNA in infected tissue culture cells grown in the presence of puromycin or cycloheximide to inhibit protein synthesis and virus replication (Eshita *et al.*, 1985). Whether all three viral RNA species are transcribed has not been determined. At least for those viruses that have been studied, the viral mRNA synthesis appears to involve the initial acquisition of (presumably capped) oligonucleotide primers from cellular RNA species (mRNA?) and their subsequent extension into the viral-complementary mRNA transcripts. To accomplish this feat the viral polymerase must recognize a suitable precursor RNA species and possibly scan that RNA molecule to identify and locate the 5' end sequence. It must then excise by an endonucleolytic activity the necessary oligonucleotide primer sequence and hold it in juxtaposition to the viral template RNA in order to catalyze the 3' extension of viral-complementary mRNA species.

Following primary transcription, it is believed that translation of the viral-complementary mRNA species occurs with the resulting synthesis of viral proteins. Whether the various mRNA species are translated with equal efficiency has not been determined. No doubt when enough quantities of the viral nucleoprotein and transcriptase-replicase components are formed, RNA replication ensues. The function of the NS<sub>S</sub> protein in these processes is not known. Amplified rates of mRNA transcription (secondary transcription), translation, and RNA replication follow, with the end result of viral morphogenesis at distended Golgi apparatus membranes. Why the viral glycoproteins reside in the Golgi saccules and are not efficiently transferred to the plasma membrane of the infected cell is a subject for future research. Possibly the NS<sub>M</sub> protein is involved.

The release of virus particles from the primary cellular site of virus replication in an infected animal leads to virus dispersal to other cells and organs, and may trigger signs of infection involving particular syndromes of disease. Elevated levels of infectious virus particles in the vascular system (viremia) permit transmission of virus to a feeding arthropod. For mosquitoes, sandflies, *Culicoides*, and the larval forms of ticks, only small amounts of blood are ingested. The success of transmission must therefore depend on the levels of infectious virus in the blood, the numbers of feeding arthropods, and the predisposition of the vertebrate to allow feeding. The latter may depend on the symp-

toms of disease. In view of these factors, the possibility for clonal transmission of virus in nature is probably high (i.e., the transmission of a single infectious virus particle). The evolution of bunyaviruses involving the accumulation of point mutations, occasional codon deletions and duplications, and RNA reassortment, in concert with these transmission factors, probably accounts for the large number of distinct virus types that have been identified for the Bunyaviridae (Table II–V).

### *C. Coding Strategy of Bunyaviruses*

Shown in Fig. 11 is a schematic diagram of what is known about the transcription, replication, and translation strategies of the S RNA species of bunyaviruses. The RNA codes for two proteins (N, NS<sub>S</sub>) in a single viral-complementary S mRNA species. The proteins are read from overlapping sequences. The S mRNA has a nonviral 5' end sequence (some 12–17 nucleotides in length) that originates from cellular (mRNA?) species. This sequence is apparently used as a primer for mRNA synthesis. Overall, the single S mRNA species is shorter than its viral template due to a transcription termination signal.

The M mRNA species of bunyaviruses has similar characteristics to the S RNA (viral-complementary in sequence, 5' nonviral primer, subgenomic in size). It codes for a single gene product, the precursor to the two viral glycoproteins, and the nonstructural protein, NS<sub>M</sub>. The coding arrangement of the bunyavirus L RNA is not known, other than the fact that it codes for a protein (L protein?) in a viral-complementary sequence.

### *D. Ambisense Coding Strategy of Phleboviruses*

Shown in Fig. 12 is a schematic diagram of what is known about the transcription, translation, and replication strategies of the S RNA of phleboviruses. One protein (N) is coded in a subgenomic, viral-complementary S mRNA species that has a 5' nonviral (putative primer) oligonucleotide extension similar to that of bunyaviruses. A second protein (NS<sub>S</sub>) is coded in a subgenomic, viral-sense mRNA species that also has a 5' primer sequence. Therefore, the phlebovirus S RNA has an ambisense coding arrangement which is unlike the coding strategy of the S RNA of bunyaviruses. It is deduced from these observations that the phlebovirus NS<sub>S</sub> mRNA species cannot be synthesized until after RNA replication has commenced. The synthesis of the NS<sub>S</sub> mRNA species is regulated independent of that of the N mRNA species. The



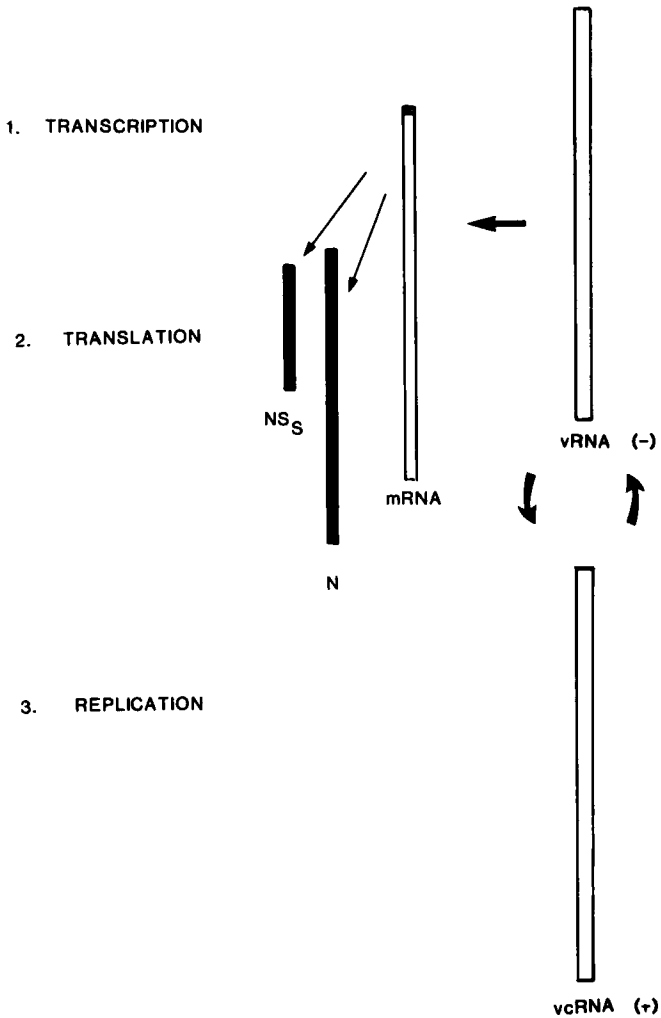


FIG. 11. Coding and replication strategies of bunyavirus S RNA species.

function of NS<sub>S</sub> protein is not known, but presumably it is not involved in the initial stages of viral-complementary RNA replication.

By contrast, the coding strategy of the M RNA of phleboviruses resembles that of bunyaviruses (i.e., there is a single, subgenomic, viral-complementary M mRNA species that has a 5' nonviral primer sequence). The M mRNA codes for the precursor to the two viral glycoproteins and a nonstructural protein, NS<sub>M</sub>. The coding arrangement of the phlebovirus L RNA is not known.

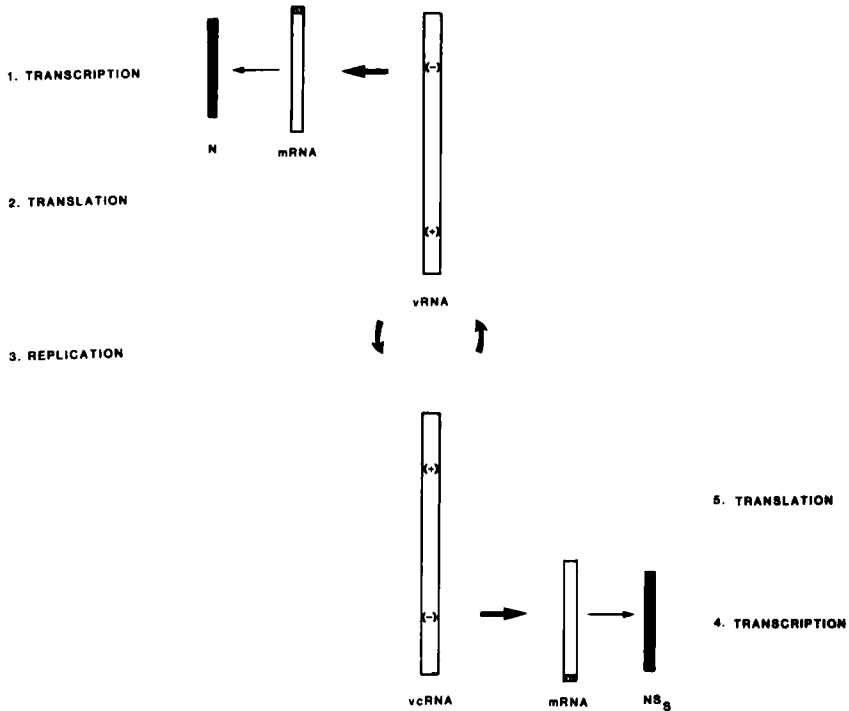


FIG. 12. Coding and replication strategies of phlebovirus S RNA species.

*E. Hierarchy of Relationships among the Bunyaviridae*

Although the coding strategies of each of the RNA species of representative members of all the groups of viruses currently assigned to the Bunyaviridae are not known, a problem is posed by the very distinct strategies of the bunyavirus and phlebovirus S RNA species. What are the evolutionary relationships of these viruses?

Of the different characteristics of viruses that have been recognized, it can be argued that some properties have been difficult to replace during evolution (i.e., they are highly conserved), while others have evolved. Taking into account the variation that is observed among members of other virus families, such highly conserved properties do not include the number of viral coded proteins, nor the numbers of genome or messenger RNA species, nor the sites of virus maturation in a cell, nor the species of animals that are infected, nor the types of disease that are induced. Conserved properties include more basic attributes such as the form of the genetic information (RNA or DNA, single or double stranded) and the transcription and replication strat-

TABLE VII  
THE HIERARCHY OF VIRUS RELATIONSHIPS

|  |                                       |                   |
|--|---------------------------------------|-------------------|
| Family   | Bunyaviridae                          |                   |
| Subfamily  | Bunyavirinae                          |                   |
| Genus  | <i>Bunyavirus</i>                     |                   |
| Serogroup  | Anopheles A group                     | (12) <sup>a</sup> |
|  | Anopheles B group                     | (2)               |
|  | Bunyamwera group                      | (26)              |
|  | Bwamba group                          | (2)               |
|  | C group                               | (16)              |
|  | California group                      | (15)              |
|  | Capim group                           | (10)              |
|  | Gamboia group                         | (7)               |
|  | Guama group                           | (12)              |
|  | Koongol group                         | (2)               |
|  | Minatitlan group                      | (2)               |
|  | Olifantsvlei group                    | (3)               |
|  | Patois group                          | (6)               |
|  | Simbu group                           | (25)              |
|  | Tete group                            | (5)               |
|  | Turlock group                         | (5)               |
| Subfamily  | Phlebovirinae                         |                   |
| Genus  | <i>Phlebovirus</i>                    |                   |
| Serogroup  | Sandfly fever Naples group            | (4)               |
|  | Bujaru group                          | (3)               |
|  | Candiru group                         | (8)               |
|  | Chilibre group                        | (2)               |
|  | Frijoles group                        | (2)               |
|  | Rift Valley fever group               | (4)               |
|  | Salehabad group                       | (2)               |
|  | Sandfly fever Sicilian group          | (2)               |
|  | Unassigned                            | (11)              |
| Genus  | <i>Uukuvirus</i>                      |                   |
| Serogroup  | Uukuniemi group                       | (7)               |
| Subfamily  | Nairovirinae                          |                   |
| Genus  | <i>Nairovirus</i>                     |                   |
| Serogroup  | Crimean-Congo hemorrhagic fever group | (3)               |
|  | Dera Ghazi Khan group                 | (6)               |
|  | Hughes group                          | (8)               |
|  | Nairobi sheep disease group           | (3)               |
|  | Qalyub group                          | (3)               |
|  | Sakhalin group                        | (6)               |
| Other members not assigned to a subfamily or genus |                                       |                   |
| Serogroup  | Bakau group                           | (2)               |
|  | Kaiodi group                          | (3)               |
|  | Hantaan group                         | (4)               |
|  | Maputta group                         | (4)               |
|  | Yogue group                           | (2)               |
|  | Unassigned                            | (12)              |

<sup>a</sup>Numbers in parentheses represent the number of viruses in the group.

egies—for example, the possession and use of a reverse transcriptase to synthesize a DNA intermediate during the replication of retroviruses. For the positive-stranded viruses (caliciviruses, flaviviruses, picornaviruses, togaviruses), a conserved character is the use of the viral RNA species as a messenger RNA. Among the negative-stranded viruses, the ability to synthesize viral-complementary mRNA species is a conserved feature (arenaviruses, bunyaviruses, orthomyxoviruses, paramyxoviruses, and rhabdoviruses). Of the latter viruses, the paramyxoviruses and rhabdoviruses share similar strategies of mRNA transcription. They both initiate mRNA species *de novo* and have similar signals for transcription termination, polyadenylation, and leader RNA synthesis. This can be contrasted to the use of primers to synthesize mRNA species as exemplified by the orthomyxoviruses and members of the Bunyaviridae. Unlike bunyaviruses, orthomyxoviruses produce spliced as well as unspliced messenger RNA species (presumably one of the reasons why transcription of influenza viral mRNA occurs in the nucleus of infected cells). As discussed previously, the transcription strategy of arenaviruses has yet to be resolved.

If it is accepted that the transcription strategy of viruses is a conserved feature, then bunyaviruses and phleboviruses are similar and should be placed together in the same virus family. Other features that they share are the sites of morphogenesis, the coding arrangements of their M RNA species, and their overall structural organization. One difference between them is their S RNA strategies. It has been proposed, therefore, that bunyaviruses and phleboviruses be placed in separate subfamilies to recognize the differences in their S RNA coding strategy. As discussed previously, from the available evidence it appears that the uukuviruses resemble phleboviruses. The reason for subfamily designation is to recognize that the evolution of an ambisense arrangement (or the coding arrangement of the bunyaviruses) must have involved several stages, as discussed previously for arenaviruses. The proposed hierarchy of relationships in the Bunyaviridae is shown in Table VII (Bishop, 1985).

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