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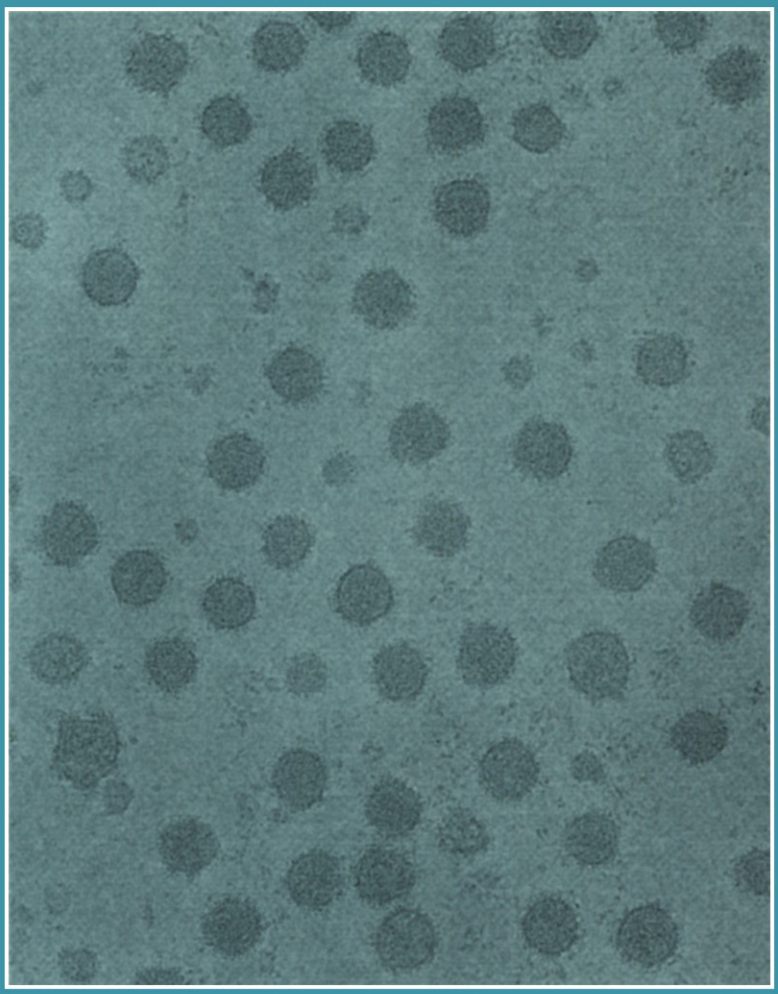
169

Bunyaviridae

Edited by D. Kolakofsky



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Preface

The Bunyaviridae represent an extremely diverse family of viruses, which until recently were relatively poorly understood. These viruses have become increasingly important in both human and veterinary medicine, and, most recently, bunyaviruses have been recognized as plant pathogens as well. This book attempts to treat all aspects of their biology, including their natural history, genetics, virion structure, unusual pathway of intracellular assembly, gene structure and the mechanisms of its expression, antigenicity, and pathogenesis, in a single volume. As such, it fills a void in the virology literature. This volume is also timely, as the molecular description of this family is now almost complete. The reader can expect to find the present state of the art on how bunyaviruses are maintained in nature, and how they replicate and sometimes cause disease.

I would like to thank my co-contributors for the time and effort they have invested to make their chapters as complete as possible, and not least of all, for by and large respecting the deadline. I am also grateful to Dick Compans for suggesting this volume and helping to design its contents, and for the many discussions during his stay in Geneva.

DANIEL KOLAKOFSKY

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The Bunyaviridae and Their Genetics— An Overview

C. R. PRINGLE

1 Introduction

This chapter reviews briefly the special features that distinguish members of the family Bunyaviridae from other viruses and surveys current knowledge of the genetic properties of these viruses, the factors determining their pathogenic potential for vertebrates, and their transmissibility by arthropod vectors. Detailed descriptions of the individual members of the Bunyaviridae and their attributes together with comprehensive referencing can be found in the ensuing chapters and elsewhere (ELLIOTT 1990; GONZALEZ-SCARANO and NATHANSON 1989; BISHOP 1986). Only the genetics of the Bunyaviridae and factors affecting the variability of these viruses are accorded more than superficial attention here.

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1.1 The Distinctive Features and Diversity of Bunyaviruses

Perhaps the single most distinctive feature of the family Bunyaviridae is the remarkable number of named viruses included in this famly (KARABATSOS 1985; BISHOP 1986; CALISHER and KARABATSOS 1988). Almost half (47.4%) of the 530 viruses registered with the American Committee on Arthropod-borne Viruses by the end of 1987 were considered to be bunyaviruses or bunya-like viruses.

The number of confirmed or putative members of the family Bunyaviridae continues to increase, and a more recent survey lists some 342 animal bunyaviruses or bunya-like viruses, now supplemented by a solitary plant bunyavirus (GONZALEZ-SCARANO and NATHANSON 1989). Molecular characterization of the few viruses examined in detail so far suggests that this profusion of names is indeed a true reflection of the genetic diversity of the family. The abundance of names does not reflect an unusual prevalence of bunyaviruses, although in endemic situations bunyaviruses may predominate among arthropod-transmitted viruses in actual frequency. In a survey of arthropod-borne viruses in coastal Ecuador spanning the years 1974–1978, 328 of the 379 viruses isolated from mosquitoes and sentinel hamsters were identified as bunyaviruses, and one bunyavirus alone—the otherwise little renowned Maguari virus—accounted for 64% of the isolations (CALISHER et al. 1983).

The names attributed to bunyaviruses range from the whimsical or enigmatic (Facey's Paddock, Main Drain, Bimbo) to the starkly descriptive (Crimean haemorrhagic fever, Nairobi sheep disease, Rift Valley fever), reflecting the extent to which these individual viruses have impinged on human consciousness and welfare. Apart from their multifarious and sometimes outlandish names the features which distinguish bunyaviruses are the following:

1. Tripartite structure of the genome and the negative sense of the linear single-stranded virion RNA
2. Subdivision of the family predominantly on the basis of coding strategy into six distinct genera (*Bunyavirus, Hantavirus, Nairovirus, Phlebovirus, Uukuvirus,* and *Tospovirus*), which embrace subgeneric groupings of viruses with varying degrees of serological relationship (serogroups)
3. Ambisense encoding of a putative non-structural protein gene in the S genome segment in viruses of the *Phlebovirus*, *Uukuvirus* and *Tospovirus* genera
4. Enveloped pleomorphic particles with four virus-specified proteins: the putative polymerase protein (L), cysteine-rich precursor-derived envelope glycoproteins (G1 and G2, plus G3 in the nairoviruses), and the nucleoprotein (N)
5. Three circular nucleocapsids, comprising in viruses of the *Bunyavirus* genus approximately 2100 molecules of N and 25 molecules of L per particle, all enveloped by a lipid envelope containing approximately 600 molecules of glycoproteins G1 and G2

6. Absence of a matrix protein
7. Maturation by budding from membranes of the Golgi apparatus
8. Close association with specific vertebrate hosts and specific arthropod vectors, with the exception of the phytobunyavirus tomato spotted wilt virus (TSWV), which is transmitted by thrips, and the hantaviruses which do not appear to be arthropod-borne
9. Temporal separation of internal and envelope protein synthesis
10. Priming of synthesis by a cap transfer mechanism analogous to that of influenza virus, except that it occurs in the cytoplasm, and absence of 3′-polyadenylation of mRNA
11. Insensitivity to actinomycin-D and alpha-amanitin, and variable dependence on the presence of an intact functional nucleus
12. Limitation of gene exchange to reassortment of genome sub-units between closely related viruses within serogroups.

1.2 Taxonomic Status

The viruses grouped together as the family Bunyaviridae do not fit easily into the current scheme of classification of RNA viruses, which is based on the form and sense of the genomic RNA sequestered in the extracellular virion. The viruses included in the genera *Bunyavirus, Hantavirus* and *Nairovirus*, at least as far as they have been characterized, can be regarded as conventional negative-strand RNA viruses such as the unsegmented filoviruses, paramyxoviruses and rhabdoviruses, and the 7/8 segmented orthomyxoviruses. In contradistinction the viruses belonging to the *Phlebovirus* and *Uukuvirus* genera and the newly designated *Tospovirus* genus (DE HAAN et al. 1990) resemble the arenaviruses in that the small genome segment (the S RNA) is a linear single-stranded structure of covalently linked negative- and positive-sense half molecules from which separate mRNAs are transcribed. In the Bunyaviridae ambisense encoding of genetic information is restricted to the S RNA and present in three of the six genera only, whereas in the Arenaviridae both sub-units of the genome incorporate ambisense encoded information (SALVATO 1989; SALVATO and SHIMOMAYE 1989; IAPALUCCI et al. 1989). The functional significance of the ambisense coding strategy in the S RNA of bunyaviruses is less obvious than in the arenaviruses where the differential encoding of the membrane glycoprotein and nucleocapsid protein genes in opposite senses in the S RNA may provide temporal separation of the synthesis of internal and external proteins (see Fig. 2). The ambisense S RNA of the bunyaviruses encodes the nucleocapsid protein gene and a putative non-structural protein gene of unknown function. A further distinction may be the differential encapsidation of strands: Full length molecules of both polarities of the S RNA of Uukuniemi virus have been reported to be encapsidated in the ratio of 10:1, whereas only negative-strand molecules of the middle-sized (M) RNA were found in virions (SIMONS et al. 1990).

The features which unite these diverse viruses within a single family are a common morphology, the tripartite genome structure, the absence of a matrix

protein, and budding into intracytoplasmic vesicles from the internal membranes of the Golgi apparatus (BISHOP et al. 1980; SCHMALJOHN and DALRYMPLE 1983; DE HANN et al. 1989; ELLIOTT 1990). The taxonomy of the bunyaviruses below the family level has not yet stabilised. The *Bunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus* and *Uukuvirus* genera have been distinguished by the molecular size and coding properties of their genomes (recently reviewed in ELLIOTT 1990) and the possession of common CF antigens and variable neutralization antigens (or vice versa in the case of the phleboviruses). These genera are also distinguished biologically by their modes of transmission; the bunyaviruses are predominantly mosquito borne, the nairoviruses and uukuviruses are tick borne, and the phleboviruses are sandfly and gnat borne. The phytobunyavirus TSWV is transmitted by thrips and is the only segmented genome plant virus with a lipid envelope. The members of the genus *Hantavirus* are exceptional in that they are transmitted by rodents and have no arthropod vector.

Curiously, although the hantaviruses came to prominence because of their association with epidemic haemorrhagic disease in non-Korean troops serving in Korea in the early 1950s, transmission is always directly from rodents, and person-to-person spread of virus has not been reported. Many bunyaviruses are uncharacterized and remain outside the existing taxonomy; it is not yet clear whether they can be accommodated within the existing generic structure, or whether new genera will be required (ZELLER et al. 1989). The recent recognition of the ambisense nature of the S RNA of Uukuniemi virus may signal the end of the ascendancy of the splitters in Bunyaviridae taxonomy and the emergence of the lumpers, since it has recently been proposed that the phleboviruses and the uukuviruses should be subsumed within the one genus (SIMONS et al. 1990).

1.3 Genome Coding Potential and Gene Products

In common with all other negative-stranded RNA viruses the coding potential of the genome is utilized efficiently. In the genome of Bunyamwera virus, the prototype of the family and the only bunyavirus completely sequenced so far, 95.3% of the RNA encodes polypeptides (ELLIOTT 1989a, b; LEES et al. 1986). Figure 1 illustrates diagrammatically the mean sizes of the RNA subunits of the six genera and their coding properties (data from ELLIOTT 1990). Despite the considerable difference in relative size of the corresponding sub-units in different genera, the coding properties are similar. The large-sized (L) RNA of Bunyamwera virus has a single open reading frame (ORF) encoding a protein of 2238 amino acids (M_r 259 000). A short ORF is present in the negative-sense strand, but no gene product has been identified (ELLIOTT 1989b). No sequence similarity or other homology to any other viral RNA polymerase has been discerned, suggesting that the bunyaviruses are not a recently evolved group. The L protein of viruses of other genera are comparable in size to that of Bunyamwera virus, and it can be presumed that the L RNA of the hantaviruses,

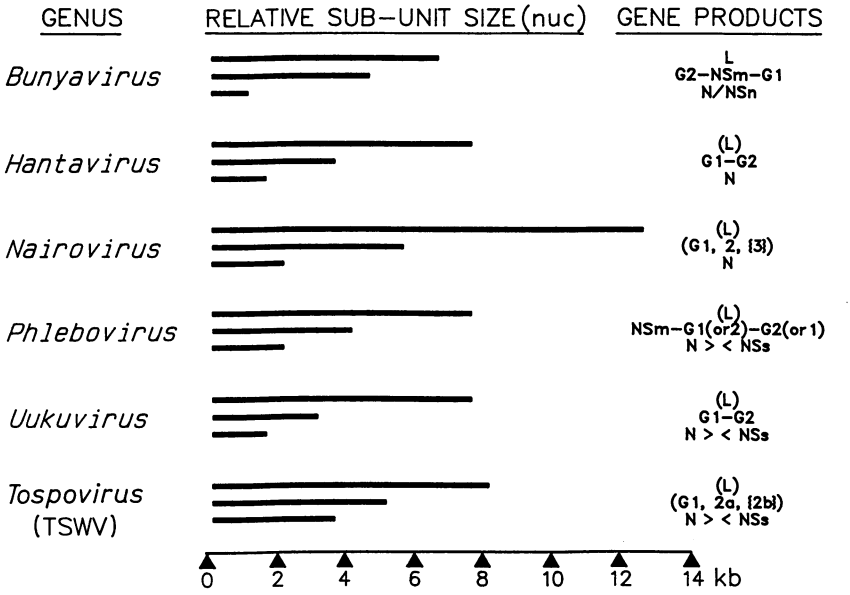

Fig. 1. The tripartite structure of the genome of the Bunyaviridae. Relative sizes of the L, M, and S RNA sub-units and their coding specificities. The sizes of the large (*L*), middle (*M*) and small (*S*) RNAs are represented approximately to scale. The known gene products of each genome sub-unit are listed to the right. From the L RNA: *L*, large (putative polymerase) protein. (The single open reading frame has been confirmed in the *Bunyavirus* genus only). From the M RNA: G1, G2a, G2b, and G3, glycosylated envelope proteins; NSm, a non-structural protein, derived from a single open reading frame, in the order indicated by dashes where known; the internal brackets ({ }) indicate suspected gene products. From the S RNA: N, nucleoprotein; NSs, a non-structural protein; > < indicates ambisense encoding

phleboviruses, uukuviruses and TSWV encode only an L protein. The L RNA of the nairoviruses is considerably larger, and it remains to be established whether additional polypeptides are encoded in this segment.

The M RNA encodes the two (or three in some nairoviruses) envelope glycoproteins of the virion, and in the *Bunyavirus* and *Phlebovirus* genera a non-structural protein in addition. Only one ORF is present in the M RNA of the nine different viruses belonging to the genera *Bunyavirus*, *Hantavirus*, *Phlebovirus* and *Uukuvirus* which have been sequenced so far. The individual polypeptides are thought to be derived by nascent cleavage during translation. Post-translational cleavage of a precursor polypeptide has been reported only for Uukuniemi virus in an in vitro system (ULMANEN et al. 1981). The NSm polypeptide is located terminally in the phleboviruses and internally in the bunyaviruses. As is discussed below the M RNA segment appears to be the major determinant of the pathogenicity and tissue tropism of bunyaviruses.

The S RNA encodes the nucleoprotein and in four of the six genera also a small non-structural protein. The NSs protein is encoded in distinct modes. In the genus *Bunyavirus* the NSs protein is encoded in an overlapping reading frame,

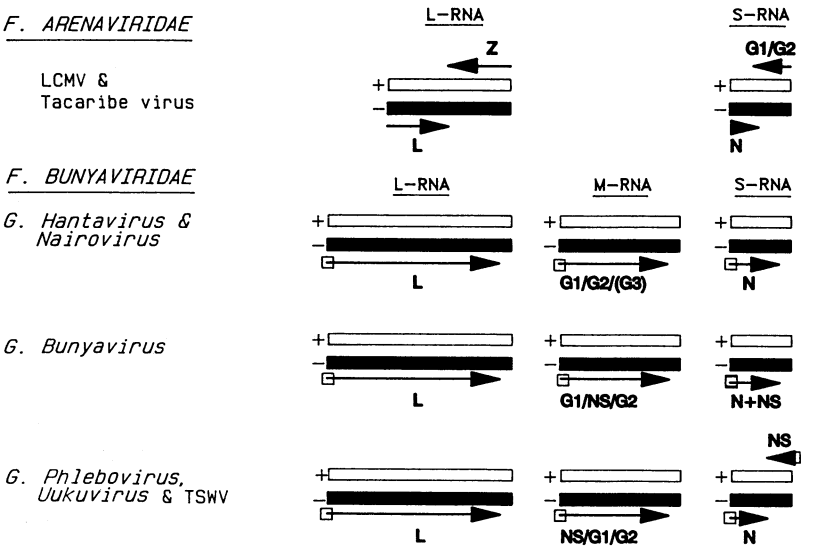

Fig. 2. Comparison of coding strategies in the Bunyaviridae and the Arenaviridae. The negative and complementary positive strands of the genomic sub-units are not drawn to scale. *Arrows*: messenger RNA orientated in the 5′–3′ direction by the *arrowhead*; the encoded gene products are indicated by *letters*. Ambisense encoding of genetic information in non-overlapping reading frames is characteristic of both sub-units of the genome of arenaviruses. The larger sub-unit codes in the negative strand for the L (putative polymerase) protein and in the opposite strand for the Z (putative RNA binding) protein. The smaller sub-unit codes in the negative strand for the N (nucleoprotein), and in the opposite strand for the G1/G2 (envelope) glycoproteins. The bunyaviruses can be separated into three groups; ambisense encoding of genetic information is observed only in the S RNA sub-unit of viruses of the genera *Phlebovirus* and *Uukuvirus* and in TSWV. These viruses are also distinguished from the others by the order of encoding of gene products in the single open reading frame of the M RNA. Viruses in the genus *Bunyavirus* encode a non-structural protein in an overlapping reading frame in the S RNA, and another non-structural protein in a non-overlapping reading frame in the M RNA, whereas viruses in the genera *Hantavirus* and *Nairovirus* encode neither. The bunyavirus mRNAs are capped (□); the symbol "/" indicates derivation from a common precursor; the symbol "+" indicates encoding in overlapping reading frames

and it is now accepted that the N and NSs polypeptides are translated from the same mRNA by separate initiation. A third ORF in the same frame as NSs is present in some (Germiston virus and Maguari virus) but not all members of the genus *Bunyavirus*. However, no gene product has been detected. In the phleboviruses, the uukuviruses and TSWV, however, the N protein is encoded in the complementary (positive-sense) strand as in the other viruses, but the NSs protein is encoded in the negative-sense strand. The N coding sequence is located in the 3′ half of the genome and the NSs coding sequence in the 5′ half, as illustrated diagrammatically in Fig. 2, and the N and NSs proteins are translated from separate subgenomic RNAs. The NSs protein of Rift Valley fever phlebovirus has been reported to be phosphorylated and that of Punta Toro phlebovirus associated with the nucleocapsid, suggesting an involvement in

replication or transcription. The NSs protein of Rift Valley fever phlebovirus is unusual in that it has been detected in the nucleus of infected cells. Only one ORF corresponding to the N protein is present in the S RNA of the hantaviruses and the nairoviruses.

1.4 Genome Structure and Replication

The 3′-terminal sequences of the three RNA sub-units are conserved within each genus. Furthermore, the terminal sequences of the genome sub-units of the phleboviruses and uukuviruses are identical, which together with the ambisense nature of their S RNAs emphasizes the close relationship of these two genera.

The 3′ and 5′ ends of each RNA sub-unit exhibit complementarity. At the 3′ terminus residues 1–11, with the exception of 9, are conserved in the L, M and S RNA of Bunyamwera virus and complementary to the corresponding residues at the 5′ terminus. The next 20 or more residues show segment-specific complementarity. A mismatched residue has been found at position 9 in the terminal sequences of all viruses of the genus *Bunyavirus*, but not in those of viruses of other genera, except for a mismatch at position 12 in Hantaan virus.

The circularity of the nucleocapsids is probably a consequence of the complementarity of the termini, and it has been confirmed that the ends of full-length encapsidated strands were in fact base paired (RAJU and KOLAKOFSKY 1989). Only full-length strands of both negative and positive polarity were encapsidated, whereas mRNAs which were capped and truncated at the 3′ end were not. The evidence favours location of the nucleocapsid recognition signal at the 5′ end (RAJU and KOLAKOFSKY 1987).

As in all other negative-strand RNA viruses, primary transcription of the negative-sense genome is mediated by a virion-associated RNA polymerase. This virion-associated enzyme is also endowed with an endonuclease activity and may be involved in mediating the process of mRNA capping in the cytoplasm. However, although in vitro enzyme activity has been demonstrated, primary transcription in the absence of protein synthesis has been difficult to detect in many instances. BELLOCQ et al. (1987) have attempted to resolve this anomaly by proposing that in the absence of ribosome binding and protein synthesis the nascent mRNA and its template interact, preventing progression of the polymerase. This hypothesis was based on the observation that the presence of rabbit reticulocyte lysate in the in vitro polymerase reaction stimulated production of complete transcripts of S RNA in place of the incomplete transcripts normally observed. Concurrent protein synthesis appeared to be necessary to prevent premature termination during synthesis of mRNA on the S RNA template. RAJU et al. (1989) have shown that this requirement was host cell-dependent, and that it could be mimicked in vitro. In mammalian cells translational competence was required to avoid premature termination at

nucleotide 175, whereas in C6/36 mosquito cells read-through was independent of concurrent translation. Reconstitution experiments suggested that this translational requirement was mediated by host cell factors present in BHK-21 cells which were absent in C6/36 cells.

Maturation occurs predominantly at smooth membranes in the Golgi complex and consequently is inhibited by monensin. Experiments with vaccinia virus recombinants have shown that targeting to the Golgi apparatus is a property of the glycoproteins alone (MATSUOKA et al. 1988; PENSIERO et al. 1988). The glycoproteins are anchored in the membrane with their amino-termini located externally and their carboxy-termini internally. Virus particles are formed by budding into vesicles which are transported to the cell surface, eventually releasing their contents to the exterior. Characteristically bunyavirus-infected cells retain their gross morphology until late in the infectious cycle, long after most of their contents have been lost.

2 The Genetics of the Bunyaviridae

2.1 Sequence Homologies

Only limited sequence information is available at present, but it is clear that the pattern of sequence relationships closely reflects taxonomic status. In general, viruses classified in different genera show little or no sequence similarity. However, significant homology has been detected between the N proteins (33%–35%) and the G proteins (15%–25%) of the prototype uukuvirus Uukuniemi virus, and the phleboviruses Punta Toro virus and Sicilian sandfly virus (SIMONS et al. 1990). This together with their common adoption of ambisense coding and possession of identical sub-unit terminal sequences indicates that the two genera are more closely related than the others and may not merit separate status as genera. The proteins of TSWV, the other virus exhibiting ambisense encoding in the S RNA, have no discernible similarity with these viruses. A separate genus is justified for TSMV for this reason and on account of its unique host and vector specificity.

Viruses classified within the same genus may exhibit limited sequence homology, and viruses belonging to the same serogroup generally show close similarity. For example, the overall homology of the G2 proteins of four members of the *Bunyavirus* genus is about 66%, that of the NSm proteins about 50%, and that of the G1 proteins about 40%. Comparison of the N proteins of six members of the *Bunyavirus* genus belonging to three serogroups revealed 40% overall similarity, whereas the similarity was 80% for members of the same serogroup (ELLIOTT 1989b). The degree of sequence divergence observed even among viruses of the same serogroup is sufficient to justify the designation of these viruses by individual names.

2.2 Temperature-Sensitive Mutants and Homologous Recombination

Temperature-sensitive (ts) mutations have been employed to investigate gene function, to define the genetic basis of virulence and to delineate the extent of reassortment and the limits to gene exchange. The majority of ts mutants were obtained by mutagenization using either 5-fluorouracil, 5-azacytidine or *N*-methyl-*N'*-nitrosoguanidine. The data of OZDEN and HANNOUN (1978) suggested that 5-fluorouracil was a slightly more effective mutagen than NTG. GENTSCH et al. (1977) isolated spontaneous mutants from snowshoe hare bunyavirus at a frequency of 1.7% and from La Crosse virus at a frequency of 1.0%. However, the frequency of spontaneous mutants in a stock of Maguari virus was higher and estimated at 2.7% (IROEGBU 1981).

Some 200 ts mutants have been isolated from 11 viruses; 10 of these viruses belong to two different serogroups of the genus *Bunyavirus* and the remaining one to the genus *Uukuvirus* (Table 1). Homologous recombination between individual pairs of mutants, subsequently shown to be mediated by sub-unit reassortment (GENTSCH et al. 1977, 1979; IROEGBU and PRINGLE 1981a), has been observed in each virus. The majority of the mutants have been assigned to reassortant groups; i.e. non-ts virus is present only in the progeny from mixed infections with parental viruses classified in different reassortant groups. A significant feature was the consistent recovery of mutants assignable to two reassortant groups rather than the three expected from the tripartite structure of the genome.

Table 1. Bunyavirus temperature-sensitive mutants

Genus	Serogroup	Virus	No. of ts Mutants	Reassortment groups[a]				References[b]
				I	II	III	Unclass	
Bunyavirus	Bunyamwera	Batai	5	1	4	0	0	
		Bunyamwera	8	5	3	0	0	1–5
		Maguari	46	12	31	1	2	
		Germiston	8	2	6	0	0	6
		Guaroa	4	2	2	0	0	7
			9	3	5	1[c]	0	8
	California	La Crosse	20	6	14	0	0	
		Snowshoe hare	48	26	20	0	2	
		Tahyna	20	1	17	0	2	8–12
		Trivittatus	12	4	6	0	2	
		Lumbo	9	2	7	0	0	
			8	1	7	0	0	13
Uukuvirus		Uukuniemi	13	3	2	0	8	14

[a] The homology of the reassortment groups does not extend across serogroup boundaries
[b] [1] IROEGBU and PRINGLE 1981a; [2] IROEGBU and PRINGLE 1981b; [3] PRINGLE and IROEGBU 1982; [4] ELLIOTT et al. 1984; [5] IROEGBU 1981; [6] OZDEN and HANNOUN 1980; [7] PRINGLE, unpublished data; [8] BISHOP 1979; [9] GENTSCH and BISHOP 1976; [10] GENTSCH et al. 1977; [11] GENTSCH et al. 1979; [12] GENTSCH et al. 1980; [13] OZDEN and HANNOUN 1978; [14] GAHMBERG 1984
[c] Tentative

Within the Bunyamwera and California serogroups the equivalence of the reassortant groups has been established by mixed infections with heterologous parental viruses. However reassortment has never been observed between viruses belonging to different serogroups, although the combinations of viruses tested has been rather limited (see below). Consequently the homologies of the reassortant groups listed in Table 1, at least as defined by genetic experiments, does not extend beyond the boundaries of serogroups.

Maguari virus of the Bunyamwera serogroup is the exception: One mutant of the 46 ts mutants isolated from mutagenized wild type Maguari virus, mutant tsMAG23(III), recombined with all members of panels of mutants representing reassortant groups I and II and two putative double I + II mutants, thus identifying the missing third reassortant group (PRINGLE and IROEGBU 1982). Mutant tsMAG23(III) exhibited a prounced host restriction which may explain the dearth of group III mutants. The plaque-forming ability of tsMAG23(III) on BS-C-1 cells was at least a 100-fold lower than on BHK-21 cells and yields were a 1000-fold less, whereas group I and II mutants of Maguari virus and several other viruses did not show such marked host cell-dependent differences.

2.3 Complementation

GENTSCH and BISHOP (1976) demonstrated that mutants in different reassortment groups of snowshoe hare virus were able to complement one another, and likewise OZDEN and HANNOUN (1978) detected intergenic complementation between recombining ts mutants of Lumbo virus. However intergenic complementation was difficult to discriminate from reassortment on account of the high frequency and early occurrence of the latter in the multiplication cycle.

IROEGBU and PRINGLE (1981a, b) detected intragenic complementation between individual pairs of ts mutants of Maguari virus belonging to reassortment group I following mixed infection of BHK-21 cells at high multiplicity of infection. These observations are compatible with the assignment of group I mutants to the S RNA with its three ORFs. Complementation was confined to specific pairs of mutants, however, and the group I mutants could not be grouped on this basis. No intragenic complementation was detected in the case of group II mutants, which suggests in view of the presumptive nascent cleavage of the membrane glycoprotein precursor that the assembly and maturation of bunyavirus virions is strictly compartmentalized.

2.4 Heterologous Reassortment and Gene Assignment

Heterologous crosses of different viruses within the same serogroup have been employed to equate reassortment groups and genome sub-units. RNA fingerprinting and dot hybridization with sub-unit specific cDNA probes, in conjunction with phenotypic analyses by SDS/PAGE of radiolabelled polypeptides