Recent advances in the molecular and cellular biology of bunyaviruses

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The family Bunyaviridae of segmented, negative-stranded RNA viruses includes over 350 members that infect a bewildering variety of animals and plants. Many of these bunyaviruses are the causative agents of serious disease in their respective hosts, and are classified as emerging viruses because of their increased incidence in new populations and geographical locations throughout the world. Emerging bunyaviruses, such as Crimean–Congo hemorrhagic fever virus, tomato spotted wilt virus and Rift Valley fever virus, are currently attracting great interest due to migration of their arthropod vectors, a situation possibly linked to climate change. These and other examples of continued emergence suggest that bunyaviruses will probably continue to pose a sustained global threat to agricultural productivity, animal welfare and human health. The threat of emergence is particularly acute in light of the lack of effective preventative or therapeutic treatments for any of these viruses, making their study an important priority. This review presents recent advances in the understanding of the bunyavirus life cycle, including aspects of their molecular, cellular and structural biology. Whilst special emphasis is placed upon the emerging bunyaviruses, we also describe the extensive body of work involving model bunyaviruses, which have been the subject of major contributions to our overall understanding of this important group of viruses.

Introduction

The family Bunyaviridae of segmented, negative-stranded RNA viruses includes over 350 named isolates classified in five genera, namely Orthobunyavirus, Hantavirus, Nairovirus, Phlebovirus and Tospovirus (Fig. 1). Together, these bunyaviruses infect a bewildering variety of animals and plants, and select members have the capacity to cause serious disease in their respective hosts. Four of the five bunyavirus genera include members that are the causative agents of devastating haemorrhagic fevers for which preventative and therapeutic measures are not available, and these viruses are classified as hazard level 3 or 4 pathogens. In addition, many bunyaviruses are classified as emerging pathogens due to their recent increased incidence in new hosts and geographical locations.

With the exception of the hantaviruses, members of the family Bunyaviridae are transmitted primarily via arthropod vectors. Recently, concerns have been raised over the ongoing spread of several of these vectors, notably the *Hyalomma* tick that is associated with Crimean–Congo hemorrhagic fever virus (CCHFV) transmission, and the *Aedes, Culex* and *Anopheles* mosquitoes, which are important vectors for the haemorrhagic fever-causing Rift Valley fever virus (RVFV). These vectors are migrating and establishing a presence in more northerly regions of Europe, possibly due to changes in global climate (Maltezou & Papa, 2010). It is widely accepted that once the vector is established in a new environment, the viruses to which it is susceptible will ultimately follow. Therefore, exposure of new human populations to these serious pathogens seems inevitable.

In addition to these bunyaviruses that threaten human and animal populations directly, there has been a recent resurgence of both existing and newly identified tospoviruses, which threaten diverse global food supplies. In common with human-infecting bunyaviruses, this emergence has been linked to expanding geographical location of the vector, most importantly the western flower thrips *Frankliniella occidentalis* (Pappu et al., 2009). Taken together, these examples of continued emergence indicate that the bunyaviruses will probably continue to pose considerable future global threat to agricultural productivity, animal welfare and human health.

This review presents recent advances in the molecular and cellular biology of bunyaviruses. Emphasis is placed upon the emerging bunyaviruses; however, there is much to be learned about this family from the various model members that allow rapid research without the requirement for high-containment research facilities, and any comprehensive review would be incomplete without their inclusion.

Bunyavirus transmission

The animal- and human-infecting bunyaviruses generally amplify in non-human vertebrates, although humans infected with sandfly fever phlebovirus have been documented to...
sustain sufficient viraemia to also act as amplifying reservoirs during urban epidemics (Guelmino & Jevtic, 1955; Pinheiro et al., 1982). Reports of horizontal human-to-human transmission are rare, the only documented case being for the Andes hantavirus (Padula et al., 1998), although CCHFV has been reported to be transmitted nosocomially, often during surgical procedures on infected individuals (Mardani et al., 2009). Confirmed cases of vertical transmission are also rare (Adam & Karsany, 2008) and so, in general, human infection appears to be a dead-end event.

With the exception of the hantaviruses, which are predominantly spread by rodents and possibly by non-rodent insectivores (Song et al., 2007), bunyaviruses are transmitted by arthropods including mosquitoes, midges, sandflies and ticks. The plant-infecting tospoviruses are also transmitted by arthropods, predominantly thrips. Within these arthropod vectors, bunyaviruses are able to multiply, and are spread both transovarially (vertical transmission) (Tesh & Modi, 1987; Watts et al., 1973) and venereally (horizontal transmission) (Thompson & Beaty, 1977). However, in vivo studies have shown that the outcome of the bunyavirus infectious cycle in the insect vector is markedly different from that in the vertebrate host, being predominantly persistent rather than lytic, and with prolonged shedding of virus over several months following infection.

Hantaviruses maintain a long-term infection within the rodent reservoir that can persist for several years with no overt disease symptoms. Virus is shed in urine, faeces and saliva, and contact with this material can transmit the virus horizontally to other rodents. Human infections are most often associated with contact with aerosolized rodent urine or faeces, and result in a radically different disease outcome: haemorrhagic fever with renal syndrome or hantavirus pulmonary syndrome, the latter resulting in death in approximately 50% of infected individuals (Khan et al., 1996).

### Bunyavirus genome structure

The bunyavirus genome comprises three RNA segments named small (S), medium (M), and large (L), reflecting...
their relative nucleotide length (Figs 2 and 3a). Viruses within each genus share similar overall segment length and a generally common expression strategy for their encoded protein products (Schmaljohn & Nichol, 2006). The genetic organization of the segments is similar across all genera; each template strand possesses non-translated regions (NTRs) located at the 3′ and 5′ termini, which surround a single transcriptional unit (Fig. 3b, c). These NTRs exhibit highly conserved, genus-specific sequences at their extreme termini that comprise cis-acting signals involved in RNA synthesis and segment packaging (Schmaljohn & Nichol, 2006). In addition, the 3′ and 5′ NTRs of each segment display extensive nucleotide complementarity, which is often broken by a single conserved nucleotide mismatch, allowing the possibility that the NTRs may interact through canonical Watson–Crick base pairing. In line with this, there is evidence from both biochemical analyses and direct observation to suggest that bunyavirus RNA segments exist as circular molecules within infected cells and virus particles (Pettersson & von Bonsdorff, 1975; Raju & Kolakofsky, 1989), further suggesting that NTR interaction is required for segment function. The internal regions of the NTRs show considerable variation, both between segments of the same virus and between members of the same genus, and these segment-specific sequences have been shown to play roles in the regulation of RNA synthesis for both Bunyamwera virus (BUNV) and RVFV (Barr et al., 2003; Gauliard et al., 2006). Analysis of recombinant BUNV bearing altered S segments has shown that many of these segment-specific NTR sequences are dispensable for virus multiplication; however, they do make a significant contribution to virus fitness (Lowen & Elliott, 2005).

The possibility that additional signal elements are located outside the segment NTRs cannot be ruled out. For BUNV, most segments in which the cognate pairings of NTR and ORF sequences are rearranged cannot be rescued into viable infectious virus (Lowen et al., 2005), suggesting interplay between the two regions. Only the MLM combination (M NTRs flanking the L ORF) has been recovered, although its accumulation in infected cells is greatly reduced compared with the wild-type L segment, suggesting that the activity of the NTRs has been severely compromised. Coinciding with this, the resulting MLM virus replicates to 100-fold-reduced titres, and furthermore exhibits 100-fold-increased particle:p.f.u. ratios due to reduced incorporation of the MLM segment within virus particles. This finding suggests that the NTR/ORF boundaries may contain sequences involved in segment packaging.

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**Fig. 2.** Schematic representation of genomic RNAs belonging to prototypic members of the five genera classified within the family Bunyaviridae. All bunyaviruses possess three RNA segments named small (S), medium (M) and large (L). Arrows below each segment indicate ORFs expressed using a negative-sense coding strategy, whereas arrows above the segments denote ORFs transcribed as mRNAs from positive-sense, ambisense templates. As described in the text, these schematics are generalizations, and several exceptions to these coding strategies have been identified.
Bunyavirus gene products
Proteins encoded by the S segment

The S segment of all bunyaviruses encodes the nucleocapsid (N) protein, whose primary role is to encapsidate the viral RNA-replication products to form the ribonucleoprotein (RNP) complex. The S segments of most members of the genera Orthobunyavirus, Tospovirus and Phlebovirus also encode a non-structural protein called NSs (Fig. 2), whose primary role is in modulating the host-cell antiviral response through diverse innate-immunity pathways, described in more detail below. The N and NSs proteins of orthobunyaviruses are translated from the same mRNA encoded by the S segment genome (Fig. 3b), whereas the phlebovirus and tospovirus N and NSs proteins are translated from separate mRNAs transcribed from the genomic and antigenomic strands, respectively (Fig. 3c). Whilst these coding strategies are generally conserved and aid in bunyavirus classification, there are exceptions. Viruses within three of the 18 serogroups within the genus Orthobunyavirus do not express an NSs protein, either from an overlapping reading frame or as a separate mRNA (Mohamed et al., 2009). In addition, several serotypes of New World and northern hemisphere hantaviruses also express a functional NSs protein from an overlapping ORF on the N mRNA (Plyusnin, 2002).

As with all negative-stranded RNA viruses, the bunyavirus genome is only able to participate in either RNA synthesis or segment packaging when in the form of the RNP, and consequently the N : RNA interaction is critical for virus viability. Formation of the RNP depends on the association of viral RNA with multiple copies of the N protein such that the RNA becomes encapsidated along its entire length, an activity that also depends on homotypic interactions between adjacent N protein monomers. For BUNV, each N protein monomer is thought to bind 12 nt (Mohl & Barr, 2009), and this stoichiometry is distinct from that of all other negative-stranded RNA viruses studied to date. BUNV N protein oligomerization results in monomers being stacked in ‘head-to-head’ and ‘tail-to-tail’ conformations (Leonard et al., 2005), resulting in a range of different oligomeric states, consistent with its role in RNP formation (Eifan & Elliott, 2009). Furthermore, the BUNV N protein is able to affect replication and transcription activities of assembled RNPs differentially, suggesting that it plays a key role in allowing correct recognition of the bunyavirus template by the RNA-dependent RNA polymerase (RdRp) (Eifan & Elliott, 2009; Walter et al., 2011), although how it achieves this is unclear.

Fig. 3. Schematic representation of a generic bunyavirus virion, and negative-sense or ambisense gene expression strategies. (a) Bunyavirus particles are enveloped and generally spherical, with spikes comprising Gn–Gc heterodimers arranged in genus-specific arrays on the membrane exterior. The bunyavirus genome comprises three RNA segments (S, M and L) wrapped in the viral nucleocapsid (N) protein in a circular conformation. (b) Bunyaviruses that exhibit a negative-sense coding strategy possess genomic RNA segments that contain a single transcriptional unit flanked by NTRs. The corresponding positive-sense antigenome is transcriptionally silent. In the case of the S segment, this transcriptional unit may consist of overlapping ORFs accessed by alternative initiation codons. (c) Bunyaviruses that exhibit an ambisense coding strategy are able to express mRNAs from both genomic and antigenomic RNA strands, with transcription-termination signals positioned in a central intergenic region (IGR).
The hantavirus N protein forms predominantly trimers, and interacting domains have been mapped to both N- and C-terminal regions (Alfadhlì et al., 2001; Kaukinen et al., 2003). In common with the BUNV N protein, homotypic oligomerization is proposed to involve interaction of adjacent monomers in 'head-to-head' and 'tail-to-tail' arrangements, and encapsidation is thought to proceed by successive addition of preformed trimers to the growing RNA strand (Mir & Panganiban, 2004). Nuclear magnetic resonance and X-ray crystallography data show that the hantavirus N-terminal region forms a coiled-coil domain (Boudko et al., 2007; Wang et al., 2008) and show that, by themselves, the N-terminal regions are insufficient for trimerization, implying important involvement of the C termini. The N protein trimer may not be a common assembly intermediate of the bunyavirus RNP, as the RVFV N protein has been shown to predominantly dimerize (Le May et al., 2005), whereas the BUNV N protein forms a variety of N protein multimers, including tetramers (Leonard et al., 2005; Mohl & Barr, 2009). The phlebovirus Uukunenemi virus (UUKV) N protein has been shown to predominantly dimerize, with little evidence of higher-order multimers (Katz et al., 2010). Mutational analysis revealed an important role for N-terminal helical regions in the oligomerization process, and highlighted the importance of aromatic residues in mediating N–N interactions. Multimerization of the tomato spotted wilt virus (TSWV) N protein was proposed to involve a different head-to-tail dimer arrangement, with hydrophobic interacting domains restricted to the extreme terminal regions, although the subsequent identification of additional interacting residues within central regions of the N ORF suggests that dimer formation may be more complex (Kainz et al., 2004; Uhrig et al., 1999).

The only bunyavirus N protein for which complete, high-resolution structural information is available is from RVFV, in both monomer (Raymond et al., 2010) and hexamer (Ferron et al., 2011) forms. The RVFV N protein consists of a globular core comprising two domains, and an extended N-terminal arm that extends away from the core and wraps around the external surface of the adjacent N monomer. Repetition of this arm–core interaction in a directional manner is proposed to drive multimerization, and is consistent with previous mutagenesis data showing that the N terminus is needed for dimerization (Le May et al., 2005). This mechanism of N–N interaction is thought to bestow considerable structural flexibility on the assembled multimers, which is consistent with electron microscopy images of RVFV RNPs that appear more flexible and filamentous in shape than the highly ordered superhelical RNPs of members of the Mononegavirales. Although RNA was not present in the RVFV N structure, a putative RNA-binding cleft was identified, incorporating a positively charged patch containing three arginine and lysine residues. These residues are conserved across all phleboviruses and, when altered through mutagenesis, RNA binding is ablated. The monomer structure also reveals the presence of a hydrophobic pocket at the interface between the two lobes, which is highly conserved among other phleboviruses and is proposed to represent the site of interaction with the viral glycoprotein Gn.

For members of both the genera Hantavirus and Orthobunyavirus, several studies have shown that their respective N proteins exhibit some degree of sequence specificity for the RNAs that they encapsidate and, taken together, they indicate that, whilst there is no obligatory encapsidation sequence, there is evidence of preferred binding for sequences or structural elements represented within the viral genome (Mir & Panganiban, 2005; Osborne & Elliott, 2000). This has been interpreted as playing a contributing role in the exclusive packaging of viral replication products within virus particles, over either viral mRNAs or host RNAs in general.

In addition to its RNA-binding and multimerization abilities, the hantavirus N protein is also reported to possess additional functions relating to viral gene expression and manipulation of the innate immune response within host cells (see below). Remarkably, the Sin Nombre virus (SNV) N protein is thought to be able to functionally replace the entire cellular eIF4F complex, which binds the 5′ cap of cellular mRNAs, delivers the capped mRNA to the 43S pre-initiation complex and acts as a helicase to unwind the mRNA during translation. Consistent with this role, the SNV N protein associates with 5′-capped RNAs in cellular processing (P) bodies, which store cellular mRNAs targeted for degradation (Mir et al., 2008), and furthermore N also binds the 40S rRNA subunit via the ribosomal protein S19. Together, these complexes then facilitate the preferential loading of ribosomes onto capped viral transcripts (Cheng et al., 2011). The advantage to the virus of replacing eIF4F function is thought to be in allowing translation of viral mRNAs to be maintained despite efforts of the cell to shut down the translation machinery. How the hantavirus N protein manages to perform all of these competing roles, in addition to its viral roles of RNA binding, formation of the helical RNP and association with the viral glycoproteins, is a remarkable feat of structural economy, especially given the size of the N protein at just 48 kDa. It is also unknown how the hantavirus N protein manages to coordinate and regulate all of these activities, which must presumably compete for interaction sites at various stages of the virus life cycle, although it has been suggested to depend in part on its oligomeric state (Ontiveros et al., 2010).

Proteins encoded by the M segment

The M segment of all members of the family Bunyaviridae encodes a polyprotein precursor (Fig. 2) that is inserted cotranslationally into the membrane of the endoplasmic reticulum, where it is cleaved into Gn and Gc components by host-cell proteases. These cleaved polypeptides form a disulphide-linked heterodimer, which is transported to, and retained in, the Golgi apparatus. The Gn–Gc heterodimer performs critical roles in mediating virus assembly,
formation of the virus particle and attachment to new target cells (Schmaljohn & Nichol, 2006).

By virtue of a retention signal, the heavily glycosylated Gn–Gc heterodimer is retained in the Golgi, where it associates with RNP s to mediate assembly and budding of mature virus particles. For most bunyavirus Gn–Gc heterodimers, the Golgi-retention signal is located within the Gn component, and for BUNV it has been mapped precisely to within its transmembrane domain (TMD) (Shi & Elliott, 2004). In contrast, the Golgi-retention signal for the phlebovirus UUKV resides within the C-terminal tail (Andersson & Pettersson, 1998), whereas for Punta Toro virus and RVFV, the signal includes residues from both the TMD and the C-terminal tail (Gerrard & Nichol, 2002; Matsuoka et al., 1994). The Golgi-retention signal for the tospovirus TSWV Gn–Gc is also within the Gn cytoplasmic domain and has been mapped to a 10-residue region proximal to, but not including, the TMD (Snippe et al., 2007a). Interestingly, further studies of this TMD revealed a role in transmission, specifically for residue Pro459, which has been shown to be essential for transmission of TSWV from infected thrips to plants (Sin et al., 2005), although mutation of Pro459 does not inhibit virion assembly. In the nairovirus CCHFV, the Golgi-retention signal includes residues localized within the Gn ectodomain (Bertolotti-Ciarlet et al., 2005; Haferkamp et al., 2005). Golgi retention of the Hantaan hantavirus Gn–Gc heterodimer is thought to depend on its correct overall conformation, rather than on the possession of a discrete amino acid sequence (Shi & Elliott, 2002), and similar results have been described for SNV and Andes hantaviruses (Deyde et al., 2005). The Gc protein of Andes virus has recently been shown to act as a membrane-fusion protein with predicted structural similarities to other class 2 fusion proteins. Importantly, this membrane-fusion function is attributed to at least two highly conserved polar, aromatic residues spanning its protein sequence (Cifuentes-Muñoz et al., 2011). Interestingly, almost half of the BUNV Gc ectodomain is dispensable for multiplication in cell culture, and this information has been exploited with the generation of recombinant viruses in which Gn is fused to the fluorescent tags GFP and mCherry (Shi et al., 2010). The resulting fluorescent decoration of the heterodimer allows visualization of individual virus particles and provides a powerful tool for studying many aspects of the bunyavirus life cycle. In a different approach to studying glycoprotein function, virus-like particles of RVFV were assayed for replicative and coating/uncoating functionality in either the presence or the absence of glycoproteins (Piper et al., 2011). The Gn protein was shown to recruit both the RdRp and N protein actively and thus was shown to be essential in the packaging of an authentic virion.

Most members of the genera Orthobunyavirus, Phlebovirus and Tospovirus also encode an NSm protein (Fig. 2), and evidence suggests that CCHFV also generates an NSm protein through novel proteolytic processing (Altamura et al., 2007; Bergeron et al., 2007). The tospovirus NSm protein is translated from a separate mRNA encoded by the antigenome, whereas the orthobunyavirus and phlebovirus NSm is cleaved by cellular proteases from the same polyprotein precursor that yields the Gn and Gc proteins. The BUNV NSm protein is thought to play a role in virus assembly (Shi et al., 2006) and, consistent with this role, NSm is found inside specific Golgi-associated tubular structures within infected cells where virus morphogenesis takes place (Fontana et al., 2008). In contrast, the NSm protein of TSWV is the putative movement protein, involved in inter-cell virus transmission within the infected plant (Kormelink et al., 1994), whereas the phlebovirus NSm has been shown to be non-essential (Gerrard et al., 2007), but may play accessory roles in the regulation of apoptosis (Won et al., 2007).

Proteins encoded by the L segment

For all bunyaviruses, the L segment encodes the viral component of the RdRp, which is the sole protein product of this segment (Fig. 2). As suggested by their name, bunyavirus L proteins are large molecules in excess of 200 kDa that perform several complex functions that together result in the generation of RNA-replication and mRNA-transcription products from their respective viral templates. Comparison and alignment of the RdRps from several groups of negative-stranded RNA viruses reveals that they share the well-characterized polymerase module (region 3), including the defined motifs known as pre-A, A, B, C, and D (Müller et al., 1994; Poch et al., 1990), and these are also present in the PB1 subunit of the influenza A virus (IAV) polymerase.

The La Crosse virus (LACV) L protein-associated endonuclease domain, required for the cap-snatching mechanism of mRNA transcription, is located within its N terminus (Reguera et al., 2010). Alignments with homologues of other members of the family Bunyaviridae indicate that this motif is well-conserved, containing metal-binding and catalytic lysine residues, and shows a high degree of sequence similarity to putative endonuclease motifs of L proteins from other segmented, negative-stranded RNA viruses such as arenaviruses, emaraviruses and tenuiviruses. Furthermore, these motifs are related closely to the structurally characterized endonuclease domain present within the N terminus of the PA subunit (Dias et al., 2009). Comparison between the IAV and LACV endonuclease structures revealed a striking degree of structural similarity, despite essentially no sequence similarity. Taking these findings together with the location of the polymerase module in the IAV PB1, this suggests that a concatemer of all three IAV polymerase subunits in the order PA, PB1, PB2 is topologically equivalent to the single polypeptide of the bunyavirus and arenavirus polymerases. The location of the putative bunyavirus cap-binding domain is currently unclear, although alignment of the known IAV PB2 cap-binding domain within
the PA, PB1, PB2 concatemer indicates that it may reside within the C terminus of the L protein; unfortunately, this region is poorly conserved among bunyaviruses and no obvious motifs are apparent.

The subunit divisions within the IAV polymerase and their alignment described above imply that the bunyavirus polymerase may possess a modular arrangement of functional domains and, consistent with this concept, the BUNV RdRp is able to maintain function following insertion of epitope tags at two locations within its ORF. These altered polymerases can be rescued into infectious viruses with little change in virus growth in BHK-21 cells (Shi & Elliott, 2009), although titres are considerably reduced in both Vero E6 and insect cells.

Sequence alignment shows that the RdRps of members of both the genera *Nairovirus* and *Tospovirus* share the familiar pre-A to E motif structure of the polymerase module, and also include the newly identified endonuclease signature. However, these RdRps are considerably larger than those of members of other bunyavirus genera (Fig. 2), with predicted molecular masses of approximately 330 kDa for the tospoviruses TSWV and *Impatiens* necrotic spot virus, and approximately 450 kDa for the nairoviruses Dugbe virus and CCHFV (de Haan et al., 1991; Honig et al., 2004; Kinsella et al., 2004; Marriott & Nuttall, 1996; van Poelwijk et al., 1997). These additional sequences comprise multiple functional modules, and the best-characterized of these is a cysteine de-ubiquitinating (DUB) motif belonging to the ovarian tumour (OTU) superfamily. Recent work has shown that this domain is able to remove both ubiquitin (Ub) and Ub-like ISG15 modifications from target proteins and, in this respect, is distinct from other known non-viral DUBs, which are monospecific. The structural basis of this novel DUB cross-reactivity has been shown to result from unique OTU-domain topology, permitting the acceptance of the bulky ISG15 substrate (Capodagli et al., 2011; James et al., 2011). The role of the CCHFV OTU domain is probably suppression of the host-cell inflammatory and antiviral responses triggered by Ub and Ub-like modifications, and thus it represents a potent pathogenicity factor that may be a suitable target for antiviral therapies (Frias-Staheli et al., 2007). This OTU domain is relatively small, comprising fewer than 200 residues, and the remainder of the nairovirus RdRp N terminus includes sequences exhibiting homology with known transcription factor, gyrase, helicase and topoisomerase domains (Honig et al., 2004; Kinsella et al., 2004). It has been proposed that the full-length L protein may represent a polypeptide that is processed autoproteolytically by the OTU-like protease domain to yield a polymerase and a range of accessory factors. This suggestion is supported by the finding that the OTU domain is dispensable for RNA-polymerization functions in the context of a model RNA segment (Bergeron et al., 2010).

RdRp of TSWV requires a host-cell factor for replication but not transcription activity in its primary insect vector, the western flower thrips *Frankliniella occidentalis* (de Medeiros et al., 2005). Remarkably, expression of this cell component in normally non-permissive human cells rendered them able to support virus propagation.

**Bunyavirus RNA synthesis**

Each bunyavirus RNA segment acts as the template for two different RNA-synthesis activities: mRNA transcription and RNA replication (Fig. 3). Genomic and antigenomic replication products are full-length complementary copies of each other, whereas transcription products are extended at their 5' ends by a capped oligoribonucleotide sequestered from host-cell mRNAs, and are generally truncated at their 3' ends relative to the genome template (Fig. 3). Each bunyavirus RNA strand incorporates a single transcriptional unit, contrasting with the non-segmented RNA viruses, which possess multiple transcriptional units arranged in a linear order. These fundamental differences are probable consequences of properties of the respective RdRps; the bunyavirus RdRp can only initiate mRNA synthesis at the segment termini, whereas in the non-segmented RNA viruses, initiation can be signalled by specific signals at internal sites throughout the genome. The reason for this is unknown, but may reflect a requirement for the proximity of 3'- and 5'-terminal sequences that together build up the promoter required for transcription initiation. An important functional consequence is that the bunyavirus RdRp cannot reinitiate RNA synthesis following termination of a prior strand. It may be that the ambisense arrangement of ORFs on RNA segments of phlebo-, tosp- and nairoviruses is a means of increasing coding capacity of each segment within the functional confines of an RdRp that cannot reinitiate.

The currently accepted model describing bunyavirus RNA synthesis proposes that, upon entry to an infected cell, the template-associated RdRp performs primary transcription on the input RNA strand, yielding 5'-capped mRNAs. Bunyavirus transcription is distinct from replication in that the mRNAs are not initiated de novo, but instead rely on primers generated from capped host-cell mRNAs in a mechanism that appears similar to that used by IAV, which snatches caps in the nucleus. There is direct evidence to suggest that the capped mRNAs are selected, possibly determined by complementarity of as little as 1 nt between the mRNA and the 3'-template sequence, utilizing a 'prime and realign' mechanism (Garcin et al., 1995).

As primary transcription occurs on input segments prior to the first round of replication, expression of genes on ambisense segments would be predicted to be restricted to the gene in the negative-sense orientation. However, in the case of RVFV, both the ambisense N and NSs genes are transcribed during initial rounds of primary transcription due to incorporation of complementary copies of the three RVFV segments within infecting virus particles (Ikegami et al., 2005).
At some point in time following the onset of primary transcription, the RdRp is able to replicate the input template. The 5' end of the replication product is fundamentally different from that of mRNAs, which implies that they are the products of different pathways that are distinct at their onset. The reason for this apparent change in template activity is poorly characterized, and may reflect a switch in RdRp function through either polymerase modification or association with host-cell components, or may alternatively reflect increased stability of nascent replication products through encapsidation.

**Signals for initiation of transcription and replication**

The sequences responsible for directing the bunyavirus RdRp to transcribe and replicate are located within the segment NTRs, as described above. For BUNV, the transcription and replication promoters have been defined and shown to involve distinct but overlapping sets of nucleotides (Barr & Wertz, 2004, 2005; Barr et al., 2003, 2005; Kohl et al., 2004). Replication depends on inter-terminal complementarity with no apparent sequence specificity, whereas transcription requires the presence of specific nucleotides located at both ends of the RNA template (Barr & Wertz, 2005; Barr et al., 2005; Kohl et al., 2004). These findings strongly implicate a functional requirement for terminal interaction in promoting RNA synthesis, and similar findings have been presented for UUKV (Flick et al., 2004). Whilst the S, M and L segments of each bunyavirus share common conserved nucleotides, they also exhibit segment-specific sequences, and these play important roles in specifying the overall promoter strength of each segment. Taken together, this evidence suggests that bunyavirus RNA-synthesis promoters comprise sequence-independent structural elements formed by inter-terminal base pairing, and also the specific identity of both paired and unpaired nucleotides.

**Transcription termination**

The 3' ends of bunyavirus mRNAs are unusual in that they lack poly(A) tails, although hantaviruses may be an exception to this. Functional analysis of the transcription-termination ability of the BUNV S segment reveals the presence of a 33 nt signal within the 5' NTR that includes a critical heaxanucleotide, 3'-GUCGAC-5' (Barr et al., 2006), and which maps closely to the S mRNA 3' end (Jin & Elliott, 1993). A related sequence is located within the L segment 5' NTR, although not in the M segment, raising the possibility that the M mRNA 3' end is formed by RdRp run-off.

The mRNAs transcribed from ambisense S and M segments of phleboviruses possess 3' ends that map to locations in between the segment ORFs, called intergenic regions. For RVFV, mutagenic analysis of the intergenic region identified a conserved sequence, 3'-C_{13}GUCG-5', involved in transcription termination (Ikegami et al., 2007). This sequence is shared by other phlebovirus ambisense segments and, interestingly, is also related to that identified for BUNV transcription termination. There is some evidence to suggest that the bunyavirus termination mechanism may involve secondary structure in either the template or the nascent strand (Barr, 2007; Bellocq & Kolakofsky, 1987). In support of this possibility, the BUNV termination signal includes a potential stem–loop structure (Barr et al., 2006), and secondary-structure elements within the intergenic regions of related arenavirus segments are required for 3'-end formation of corresponding mRNAs (López & Fränzefernández, 2007; Pinschewer et al., 2005).

**mRNA translation**

The general lack of poly(A) tails on bunyavirus mRNAs would be predicted to impact on viral protein expression within infected cells, given their important role in translation enhancement and mRNA stability. However, there is evidence to suggest that bunyaviruses have adopted alternative strategies to ensure that their own mRNAs are translated efficiently. For SNV (Mir & Panganiban, 2010), preferential translation of viral mRNAs is conferred by a heptanucleotide sequence close to their 5' end, and this enhancement was thought to be mediated by binding of the viral N protein; for Andes virus (Vera-Otarola et al., 2010), sequences within the 3' NTRs of the S mRNA have been shown to provide cap-dependent translational enhancement of model mRNAs, in a mechanism independent of viral proteins; for BUNV, sequences located proximal to the 3' end of newly terminated S mRNAs provide the enhancement role (Blakqori et al., 2009), and mutagenesis indicates that the enhancing element may include a stem–loop structure that is conserved within the corresponding 3' region of orthobunyavirus (Barr et al., 2006) and tospovirus (van Knippenberg et al., 2005) S mRNAs. Whether this structure enhances translation through interaction with a cellular or a viral protein is unknown. Intriguingly, the BUNV 3' stem–loop displays both structural and sequence similarities to the stem–loop present at the 3' ends of histone mRNAs, which are also poly(A)-deficient, raising the possibility that BUNV may have hijacked a cellular poly(A)-independent translation process for its own ends. The strategy of adopting a translation-enhancement mechanism that is distinct from the cellular process may also allow the virus to escape host-cell translation shut-off.

A peculiarity of orthobunyavirus transcription is that it requires concurrent protein synthesis, and this requirement is due to the presence of translocating ribosomes on nascent transcripts, rather than any protein product (Barr, 2007; Bellocq & Kolakofsky, 1987). In support of the involvement of secondary structure in bunyavirus transcription termination (described above), the role of the translocating ribosome is thought to be in preventing the RdRp from recognizing spurious transcription-termination signals within the bunyavirus template, through disruption of secondary structure involving the nascent or template strands. Interestingly, this coupling of
transcription and translation is not required in insect cells (Raju et al., 1989), and this is due to an unidentified host-cell factor found in mammalian cell lysates. In vitro transcription from tospovirus RNPs also shows a requirement for reticulocyte lysates, although these are resistant to inhibitors that prevent ribosome translocation (van Knippenberg et al., 2004), hinting that tospovirus transcription requires a host-cell factor other than translocating 40S subunits. Less is known about the RNA-synthesis activities of RNPs from members of the genera Hantavirus, Nairovirus or Tospovirus, which stems from the lack of efficient reverse-genetics systems for any of their members.

Virus entry, assembly and release

Bunyavirus entry
Details of receptor specificity for bunyaviruses is incomplete, although there is evidence to suggest that hantavirus attachment involves members of the integrin family (Gavrilovskaya et al., 1998) and phlebovirus entry into dendritic cells involves DC-SIGN (Lozach et al., 2011). UUKV infection of A549 cells proceeds via preferential binding to filopodia or dendrites (Lozach et al., 2010), followed by formation of non-coated indentations at the plasma membrane and endocytosis into similarly non-coated vesicles (Fig. 4). In rare instances, UUKV associates with clathrin-coated pits and vesicles and, in support of a possible role for clathrin-mediated endocytosis (CME), UUKV infectivity in clathrin-depleted cells drops by about one-third. Taken together, these findings suggest that, whilst the major route of infectivity is clathrin-independent, CME may also play a role in bunyavirus entry. Co-localization studies using markers for vesicles of the endocytic pathway show that UUKV progresses through both early and late endosomes (LEs) and, at later time points, the majority of UUKV particles were associated with lysosomes. Vacular acidification was critical for UUKV uncoating and infectivity, most probably occurring at the LE step.

Bunyavirus assembly and budding
Bunyaviruses are distinct from most other negative-stranded RNA viruses in that they are generally thought to assemble in tube-like virus factories that are built...
around the Golgi complex (Kikkert et al., 1999; Kuismen et al., 1982; Matsuoka et al., 1991; Murphy et al., 1973; Novoa et al., 2005; Smith & Pitfat, 1982). The assembly site of BUNV is associated with tubular viral factories that are connected to both mitochondria and the rough endoplasmic reticulum, and comprise both cellular and viral components, most notably NSm. These factories are thought to juxtapose virus replication physically to virus assembly, to allow the accumulation of RNPs that can be packaged into virus particles within the lumen of swollen Golgi stacks (Fontana et al., 2008; Novoa et al., 2005; Salanueva et al., 2003) (Fig. 4). For BUNV, the assembly processes exhibited by mammalian and mosquito cells are distinct (Lopez-Montero & Risco, 2011); in mammalian cells, these factories are large and perinuclear and involve the formation of tubular structures that anchor cell organelles to the Golgi compartment. These stacks become deformed and fragmented, resulting in severe disruption of secretory pathways. Viruses subsequently bud into secretory vesicles, in which they are trafficked towards the plasma membrane. In insect cells, the virus factories that form during the acute phase of the infection are less extensive, generally being assembled around a single Golgi stack and being associated with mitochondria, rough endoplasmic reticulum and large vacuoles. Assembled virus particles are not found within the lumen of Golgi stacks, but instead are found within the peripheral Golgi stack, with no evidence of particle accumulation.

The Golgi site of bunyavirus assembly may be driven by the specific retention of the Gn–Gc heterodimer within the Golgi compartment (described above), where the heterodimer accumulates with both Gn and Gc cytoplasmic tails probably protruding through the Golgi membrane. Both tails are probably required for assembly, and have been shown to interact directly with the N protein component of viral RNPs (Hepojoki et al., 2010; Overby et al., 2007; Ribeiro et al., 2009; Shi et al., 2007; Snippe et al., 2007b). Molecular details of this interaction are presently unclear, although recent evidence suggests that it may be mediated by zinc-finger (ZF) domains that are conserved within the Gn cytoplasmic tails in members of four of the five bunyavirus genera. Structural analysis of the Andes hantavirus and CCHFV dual ZFs showed that they both possess the classical ββαα fold but, unconventionally, they are positioned closely such that the ZFs interact with each other (Estrada & De Guzman, 2011; Estrada et al., 2009). Classical ββαα-fold ZFs more typically bind nucleic acids, but can also participate in protein–protein interactions, offering the possibility that these regions may interact with the RNP or indeed the genomic RNA directly.

Details of how the RNPs are transported to the budding compartment are currently obscure, although involvement of cytoskeletal components has been suggested to play a role in the assembly of several bunyaviruses, particularly in transport of the N protein (Andersson et al., 2004a; Fontana et al., 2008; Ramanathan et al., 2007; Ravkov et al., 1998; Scholten et al., 1994). The association between the RNP and the Gn–Gc heterodimer brings together all of the major structural proteins of the virus particle in one compact location within a budding compartment (Fig. 4). This assembly pathway is generally well-accepted for most bunyaviruses, but there are exceptions: for example, the hantavirus Black Creek Canal virus has been visualized budding at the plasma membrane of polarized epithelial cells (Ravkov et al., 1997), and a similar finding was reported for the hantavirus SNV (Goldsmith et al., 1995), raising the possibility that hantaviruses in general may utilize alternative assembly pathways. In addition, the phlebovirus RVFV has been shown to bud from the plasma membrane in primary liver cells, which raises the interesting and important possibility that assembly may be cell type-dependent. The site of nairovirus assembly and budding is poorly characterized, although the retention of CCHFV glycoproteins in the Golgi complex strongly implicates this as the assembly site (Bertolotti-Ciarlet et al., 2005; Haferkamp et al., 2005).

A long-standing question relating to segmented viruses is whether they possess mechanisms that allow selective copackaging of all genome strands into the same particle. The BUNV particle : infectivity ratio has been estimated at between 2.6 and 7.2, which would imply that an efficient and selective mechanism is in operation, rather than random incorporation of segments (Lowen et al., 2005). As described above, recombinant altered viruses have been generated with greatly increased particle : infectivity ratios, hinting that important packaging signals or activities have been disrupted, providing an important route to further characterization of this process. Recent work with RVFV suggests that segment packaging is a coordinated process that results in the efficient and specific co-packaging of the S and M segments (Terasaki et al., 2011). Interestingly, this co-packaging is uncoupled by deletion of a short sequence within the M-segment NTR, which also affects L-segment packaging. Taken together, these findings hint at an important role for this sequence in packaging, and imply that inter-segment interactions may drive the assembly process.

**Bunyavirus morphogenesis**

Bunyavirus morphogenesis has been studied best for BUNV, for which generation of infectious virions in mammalian cells involves the transition of virus particles through three distinct structural stages (Novoa et al., 2005). The initial forms are annular structures that are thought to represent immature precursors, and are named type 1 intracellular viruses. Also detected within infected cells are more dense particles, named type 2 intracellular particles, which possess a more angular and structured envelope. The third form is the infectious extracellular particle, which also has an angular external contour, but also exhibits closely packed surface spike projections that hint at a potentially icosahedral symmetry. Transition between the forms may involve modification to the Gn–Gc...
heterodimer and, specifically, transition between type 1 and type 2 intracellular forms may involve the acquisition of endoglycosidase H-resistant carbohydrate in the trans-Golgi compartment. This modification was suggested to be required for correct heterodimer folding, and also for promoting crucial lateral interactions between adjacent heterodimers within the envelope. The structural change that allows transition of type 2 viruses into the fully infectious extracellular form is less well-characterized, but is thought to occur on exit from the cell.

Structure of the infectious particle

Insight into the detailed three-dimensional structure of infectious bunyaviruses has recently been provided through direct visualization of purified extracellular viruses using cryo-electron tomography (cryo-ET). In the case of the phlebovirus UUKV (Overby et al., 2008), cryo-ET reveals that mature virus particles exhibit a $T=12$ icosahedral symmetry, due to arrangement of the external Gn–Gc glycoprotein spikes. These spikes exhibit pH-dependent conformations, being pointed at pH 7 and more flattened at pH 6. These changes are thought to involve the Gc moiety, and alter the conformation of the heterodimer to facilitate virus attachment. The internal RNP partially interacts with the virus membrane, but otherwise is relatively unorganized, and suggests that lateral contacts between glycoprotein spikes probably play a major role in determining virion morphology. For RVFV, another phlebovirus, the virus particles also exhibit $T=12$ symmetry (Freberg et al., 2008; Sherman et al., 2009), which is also due to the ordered arrangement of glycoprotein heterodimers. Icosahedral averaging reveals that virus particles comprise 12 pentamers and 110 hexamers, each forming a hollow cylinder and probably comprising five and six Gn–Gc heterodimers, respectively.

In contrast, Hantaan and Tula hantavirus particles lack icosahedral symmetry, and single-particle reconstruction techniques reveal that the virion surfaces are instead covered in a lattice of tetragonal spike complexes (Battisti et al., 2011; Huiskonen et al., 2010). Each spike is thought to correspond to four Gn–Gc heterodimers, with density extending into the centre of the particle where it is in close proximity to the internal RNP complexes. In the case of Tula hantavirus (Huiskonen et al., 2010), the tetragonal complexes do not cover the virus particle completely, but instead the spikes are arranged in dense patches interspersed with exposed membrane, with an overall surface coverage of approximately 70%. The interaction between adjacent spike complexes was proposed to confer membrane curvature and, furthermore, the incomplete spike coverage provided a plausible explanation for how the virus particle could be formed using square-shaped tiles. Alignment of the reconstructed spike-complex maps from both Tula and Hantaan hantaviruses indicated that they were in close agreement, suggesting a high structural similarity and a likely common architecture. However, the hantavirus strategy of covering the virus particle with repeating units that are square-shaped rather than pentagonal and hexagonal is fundamentally different from that of the phleboviruses, and in fact any other known enveloped viruses studied to date.

Bunyaviruses and the host-cell antiviral response

A major line of initial host defence against virus infection is the interferon (IFN) system, which is induced by a variety of non-self components via interaction with a range of host cell-encoded pattern-recognition receptors that detect pathogen-associated molecular patterns (PAMPs). Important PAMPs of negative-stranded RNA viruses such as bunyaviruses are either dsRNAs or uncapped 5’ termini of ssRNAs, which are recognized by the RIG-I-like helicases RIG-I and MDA5. The subsequent activation of transcription factors, including nuclear factor $\kappa$B (NF-$\kappa$B) and IFN-regulatory factor 3 (IRF-3), leads to cooperative stimulation of IFN-$\beta$ gene expression and the subsequent expression of many antiviral gene products, including the Mx GTPases and protein kinase R (PKR). The MxA protein has been shown to inhibit the replication of orthobunyaviruses, hantaviruses, phleboviruses and nairoviruses (Bridgen et al., 2004; Frese et al., 1996). This association has been proposed to sequester N away from its intended location, thereby disrupting virus multiplication (Andresson et al., 2004b). In contrast, PKR activation leads to phosphorylation of translation initiation factor eIF-2, and a general downregulation of both viral and host-cell translation.

Suppression of IFN expression

The primary role of the NSs proteins from orthobunyaviruses and phleboviruses is to act as potent inhibitors of the host-cell antiviral response (Blakqori et al., 2007; Bouloy et al., 2001; Bridgen et al., 2001), and more recent work suggests that this may also be the case for NSs proteins expressed from New World hantaviruses (Jääskelainen et al., 2007). In the case of BUNV, NSs-mediated IFN antagonism acts at the stage of IFN transcription. This is achieved by inhibiting the phosphorylation of serine-2 in the heptadecapeptide repeat (YSPTSPS) of the C-terminal domain of the RNA polymerase II (RNP II) RPB1 subunit, which downregulates host-cell mRNA synthesis non-specifically (Thomas et al., 2004). Critical in this inhibition is the interaction of a C-terminal domain of NSs with the MED8 component of the Mediator complex (Léonard et al., 2006), which plays an important role in the regulation of RNP II activity. More recent evidence suggests that an additional N-terminal domain within NSs may also be important for overcoming the host-cell antiviral response, possibly using a different mechanism (van Knippenberg et al., 2010). The NSs protein of LACV is similarly able to block the IFN response and this occurs by targeting RNP II, although it achieves this in a different manner from BUNV (Verbruggen et al., 2011). The LACV NSs causes degradation of the
transcriptionally active hyperphosphorylated form of RNAP II in a proteasome-dependent manner. Whilst the precise mechanism of this degradation is unclear, it shares many similarities with the DNA-damage response (DDR) and raises the possibility that NSs may subvert the DDR pathway to shut off host-cell transcription.

A similar strategy of global host-cell transcription inhibition has also been elucidated for the NSs protein of RVFV; however, the mechanism of action appears quite different. In infected cells, the RVFV NSs protein is found in the nucleus within large filamentous structures that co-localize with the p44 and XPB subunits of TFIIH, preventing formation of the complete functional TFIIH complex. Recently, it has been shown that the NSs of RVFV also binds to the p62 subunit of the TFIIH complex and affects this subunit in a post-translational manner, leading to an overall reduced abundance of p62 and ultimately disrupting host transcription (Kalveram et al., 2011). Also, the RVFV NSs protein has been demonstrated to repress IFN-β expression specifically through association with SAP-30-, YY1- and Sin3A-associated cofactors within the nuclear filaments. Chromatin immunoprecipitation experiments showed that these components are recruited directly to the IFN-β promoter, inhibiting histone modification and thus preventing transcriptional activation (Le May et al., 2008). It has been suggested that the NSs-associated filaments may represent nuclear compartments that lead to suppression of the specific subset of genes whose promoters interact with SAP-30 or YY1. In addition to this global transcriptional repression, the RVFV NSs protein also targets the antiviral response by degrading PKR specifically, and it has been suggested that this additional function may correspond to the enhanced pathogenicity of the wild-type RVFV (Habjan et al., 2009; Ikegami et al., 2009). It is remarkable that these two non-structural proteins have a very similar function, despite sharing virtually no sequence similarity, being disparate in size and being expressed through fundamentally different coding strategies.

An additional strategy to evade innate cellular immunity, demonstrated by members of the genera Hantavirus and Nairovirus, is to trim the 5′-triphosphate moiety from protruding 5′ termini of genomic RNA strands, thus avoiding detection by the RNA helicase RIG-I (Habjan et al., 2008). The mechanism responsible for generating these novel RNA termini is probably ‘prime and realign’ (Garcin et al., 1995), mediated by the viral polymerase during RNA replication. It is interesting to note that all nairoviruses and many of the hantaviruses do not make an NSs protein. Perhaps genome-end modification represents a strategy that contributes to the evasion of cellular innate immunity in the absence of this additional coding capacity.

The role, if any, of NSs during the persistent infection of insect vectors is unknown. Recombinant orthobunyaviruses and phleboviruses with deleted or disabled NSs ORFs grow to similar titres as wild-type viruses in both infected insect cells and inoculated mosquitoes. The levels of host-cell protein expression, greatly reduced in mammalian cells infected with NSs-expressing virus, are unchanged in insect cells. Furthermore, genetic analyses of six orthobunyaviruses in the Anopheles A, Anopheles B and Tete serogroups (five of which have only been associated with insect hosts) revealed the lack of an NSs ORF (Mohamed et al., 2009). These findings point to the role of NSs as an antagonistic, immunosuppressive effector molecule whose activity is either redundant or counter-productive in the context of a persistent infection in an arthropod cell. It is known that the invertebrate-cell environment and the array of immunomodulatory proteins are markedly different from the more dynamic and elaborate mammalian-cell environments that support lytic bunyavirus infection.

RNA interference (RNAi)

The NSs protein of members of the plant-infecting genus Tospovirus is also involved in disabling the host-cell antiviral response, but instead of targeting the expression of IFN, which is expressed by neither plant nor insect cells, the tospovirus NSs protein targets the RNAi mechanism, which represents a powerful hurdle to pathogen infiltration in both plant and insect systems (Garcia et al., 2006; Takeda et al., 2002). Towards elucidating the mechanism of its activity, NSs from several tospoviruses is known to interact specifically with small dsRNAs molecules such as small interfering (si)RNAs (Schnettler et al., 2010), which would more usually be processed by silencing complexes, leading to induction of antiviral pathways. As dsRNA is an effector molecule for RNA-silencing pathways in both insect and plant cells (and also mammalian cells via PKR), sequestration of such molecules would provide a plausible explanation for how the tospovirus NSs counteracts RNAi suppression within multiple host systems.

Whilst mammalian cells have an active RNAi system, it is widely considered that its effect is masked by the more powerful and wide-ranging IFN system. Comparison of NSs sequences of TSWV and LACV shows a considerable degree of amino acid sequence similarity, prompting the hypothesis that the NSs protein of vertebrate-infecting bunyaviruses may also possess anti-RNAi activity. Studies to investigate this possibility appear conflicting, with transient overexpression of LACV NSs protein appearing to disrupt RNAi signalling in mammalian cells (Soldan et al., 2005), whereas in the context of infectious viruses generated by reverse genetics, the LACV NSs protein was shown to possess little or no effect on the RNAi circuit in mammalian or insect cells (Blakqori et al., 2007).

Apoptosis

Several bunyaviruses have been implicated in the induction of apoptosis within a variety of cell types and small-animal models. The first bunyavirus reported to modulate apoptosis was the orthobunyavirus LACV, which was shown to induce apoptosis in some mouse neuronal cell lines and
inhibited the hantavirus N protein as playing a major role in apoptosis signalling. The Hantaan virus N protein causes retention of NF-κB in the cytoplasm of transfected cells (Taylor et al., 2009), possibly through direct interaction, an effect that was reversed in several N protein mutants (Ontiveros et al., 2010). As NF-κB trafficking to the nucleus is one mechanism that leads to transcriptional activation of apoptosis, it was proposed that N protein expression during hantavirus infection is able to down-regulate the induction of apoptosis, and thus represents an important pathogenicity factor. Interaction between the Puumala virus N protein and the Daxx apoptosis enhancer indicates that involvement of the hantavirus N protein in apoptosis signalling may be a widespread phenomenon.

Concluding remarks

For more than 50 years, bunyaviruses have provided scientists with fascinating case studies within the areas of fundamental virology and host-cell biology. This work has both revealed conserved traits that are shared between all bunyaviruses, and identified fundamental genus-specific differences concerning the molecular, cellular and structural biology of the bunyavirus life cycle. Together, this work contributes to a rich picture of the diversity and molecular workings of the family Bunyaviridae as a whole.

An exciting period of bunyavirus research is unfolding: the establishment of infectious virus-rescue systems for members of both the genera Phlebovirus and Orthobunyavirus has revolutionized bunyavirus research, and will undoubtedly pave the way for the eventual development of equivalent rescue systems for members of the remaining three genera. These future landmarks in bunyavirus molecular biology will surely lead to a rapid accumulation of similar knowledge gains. In addition, the recent advances in electron microscopy and application of high-resolution structural techniques to bunyavirus biology will provide a crucial contribution to the further understanding of these important viruses. This understanding will be of critical value in the design of future treatments for bunyavirus-mediated disease, and this task is becoming particularly important in light of both their extreme pathogenicity and their ongoing emergence and spread.

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