BRIEF REVIEW

The molecular biology of nairoviruses, an emerging group of tick-borne arboviruses

Lidia Lasecka · Michael D. Baron

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Abstract The nairoviruses are a rapidly emerging group of tick-borne bunyaviruses that includes pathogens of humans (Crimean-Congo hemorrhagic fever virus [CCHFV]) and livestock (Nairobi sheep disease virus [NSDV], also known as Ganjam virus), as well as a large number of viruses for which the normal vertebrate host has not been established. Studies on this group of viruses have been fairly limited, not least because CCHFV is a BSL4 human pathogen, restricting the number of labs able to study the live virus, while NSDV, although highly pathogenic in naive animals, is not seen as a threat in developed countries, making it a low priority. Nevertheless, recent years have seen significant progress in our understanding of the biology of these viruses, particularly that of CCHFV, and this article seeks to draw together our existing knowledge to generate an overall picture of their molecular biology, underlining areas of particular ignorance for future studies.

Introduction

New viral diseases appear with increasing frequency. Just in the last two years we have seen the appearance of a new livestock virus (Schmallenberg) and a new human pathogen (MERS coronavirus). In some cases these viruses appear to be completely new, in others they have ‘emerged’ into our awareness as human use of different habitats changes, leading to increased contact with carriers of disease, whether those carriers are “bush meat” or the many insect and tick species that can act as vectors of disease. One such group of viruses that is rapidly becoming more important is the genus Nairovirus in the family Bunyaviridae. This genus includes a number of human and livestock pathogens, as well as a collection of other viruses about which little is known, not even the host in which they naturally circulate. The nairoviruses show a number of unique features, and the purpose of this article is to summarise our current knowledge of the molecular biology of these viruses and highlight areas where it is to be expected that research will soon bear fruit.

A primary characteristic of the viruses of the genus Nairovirus, distinguishing them from most of the other members of the family Bunyaviridae, is that they are all transmitted by ticks [109]. Based on antibody cross-reactivity, nairoviruses are classified into seven serogroups (Table 1) [32, 39, 190], of which the most important are the Crimean-Congo haemorrhagic fever (CCHF) group, which includes the human pathogen Crimean-Congo haemorrhagic fever virus (CCHFV), and the Nairobi sheep disease (NSD) group, to which belong Nairobi sheep disease virus (NSDV) Dugbe virus (DUGV) and Kupe virus (KUPV). KUPV [43] and Finch Creek virus [102] are the most recently discovered viruses of this genus. CCHFV is arguably the most important from a human perspective, causing haemorrhagic fever in humans, with mortality up to 30 % (reviewed in refs. [58] and [182]). After dengue virus, this is the second most widespread of the arboviruses that are pathogenic to humans; cases of CCHF have been reported in sub-Saharan Africa, the former Soviet Union, Bulgaria, Turkey, China, Pakistan, India, the Arabian Peninsula, northern Greece, Iraq and Iran [26, 53–55, 115, 121–123, 183]. Although CCHFV can infect several mammalian species, it appears to cause disease only in man [124, 158]. Because of its importance as a human pathogen,
most of the available data on nairoviruses come from studies on this virus, though studies on other members of the group have also contributed, notably on NSDV, a virus first identified nearly a hundred years ago as a tick-borne virus that causes severe haemorrhagic gastroenteritis in sheep and goats, with mortality rates of up to 90% in susceptible populations [113, 167, 181]. Interestingly, an Asian virus causing a similar disease, Ganjam virus (GV), was recently identified, based on genetic and serological studies, as being the same virus as that causing NSD in East

Table 1 Viruses of the genus Nairovirus: the serogroup to which they belong and any known association with disease

| Serogroup | Viruses | Known association with disease |
|-----------|---------|--------------------------------|
| Nairobi sheep disease group | Nairobi sheep disease virus (NSDV)=Ganjam virus (GV) | Haemorrhagic gastroenteritis in sheep and goats [106, 113]. Antibodies to NSDV/GV have been reported in humans, but otherwise only laboratory-acquired infections have been seen [13, 45, 133] |
| | Dugbe virus (DUGV) | Frequently isolated from ticks infesting livestock in which DUGV appears to be apathogenic [27, 46, 146]. While one case of human infection has been described [27], the link to DUGV was circumstantial |
| | Kupe virus (KUPV) | |
| Crimean-Congo haemorrhagic fever group | Crimean-Congo haemorrhagic fever virus (CCHFV) | Haemorrhagic fever in man [58, 179, 182] |
| | Hazara virus (HAZV) | |
| | Khazan virus (KHAV) | |
| Hughes group | Farallon virus (FARV) | Humans bitten by infected ticks may experience pruritus (i.e. itching), fever and headache [41, 42, 76] |
| | Fraser Point virus (FPV) | |
| | Great Saltee virus (GRSV) | |
| | Hughes virus (HUGV) | |
| | Puffin Island virus (PIV) | |
| | Punta Salinas virus (PSV) | |
| | Raza virus (RAZAV) | |
| | Sapphire II virus (SAPV) | |
| | Soldado virus (SOLV) | |
| | Zirqa virus (ZIRV) | |
| Dera Ghazi Khan group | Abu Hammad virus (AHV) | |
| | Abu Mina virus (ABMV) | |
| | Dera Ghazi Khan virus (DGKV) | |
| | Kao Shuan virus (KSV) | |
| | Pathum Thani virus (PTHV) | |
| | Pretoria virus (PREV) | |
| Qalyub group | Bakel virus (BAKV) | |
| | Bandia virus (BDAV) | |
| | Omo virus (OMOV) | |
| | Qalyub virus (QYBV) | |
| Sakhalin group [102] | Avalon virus (AVAV) (=Paramushir virus) | |
| | Clor Mor virus | |
| | Finch creek virus | |
| | Kachemak Bay virus (KBV) | |
| | Sakhalin virus (SAKV) | |
| | Taggert virus (TAGV) | |
| | Tillamook virus (TILLV) | |
| Thiafora group | Erve virus (ERVEV) | Erve virus may cause severe headache in man and neurological disorders [165, 184]. Mode of transmission into humans is currently unknown |
| | Thiafora virus (TFAV) | |
Africa [47, 106, 187]. It is not possible to say yet whether GV is a variant of NSDV or vice versa, but it is clear that this virus also has a wide distribution.

Molecular characteristics of nairoviruses

Nairoviruses, like the members of the other genera of the family Bunyaviridae, are enveloped viruses that appear spherical in the electron microscope, with a diameter of approximately 100 nm [21, 46, 142]. As with all bunyaviruses, the genome consists of three segments of negative-sense RNA [39] (reviewed in refs. [109] and [175]). These are termed the small (S) segment, encoding the nucleocapsid (N) protein, which forms a complex with each RNA segment [24, 39]; the medium (M) segment, which encodes a polyprotein that is processed into two mature glycoproteins (Gn and Gc) as well as one or more non-structural proteins [3, 17, 39, 107, 144]; and the large (L) segment, which encodes the viral RNA-dependent RNA polymerase (RdRp) [85, 108].

Viral replication takes place in the cytoplasm, and viral budding occurs in the Golgi [57, 142] (Fig. 1). During viral replication, the genome segments are used as the template for synthesis of both mRNA (transcription) and complementary RNA (cRNA) (replication), where cRNAs are then used as the template for the synthesis of progeny genomic viral RNA (vRNA) [16]. The 5' and 3' untranslated regions (UTRs) of the S, M and L segments contain the minimum cis-acting elements necessary for transcription, replication and packaging [16, 67]. The terminal 5' and 3' non-coding regions of each segment are complementary to each other and highly conserved among different nairoviruses [37]. The first nine nucleotides are usually the same in different segments, which suggests that this sequence is recognised by the viral polymerase to initiate viral transcription/replication [107]. It is not clear whether the complementarity between 5' and 3' ends reflects an interaction between the ends of segments or the requirement for the same 3' sequence in genome and anti-genome RNAs to act as the attachment site for the viral polymerase [14, 66] (reviewed in refs. [109] and [147]). As with other bunyaviruses, the nairoviruses utilise capped primers snatched from host mRNA for initiation of their mRNA transcription [90]. The initiation of cRNA and vRNA synthesis occurs by a different mechanism, which has not yet been fully worked out. The fact that polymerase slippage occurs during mRNA synthesis [90], coupled with the observation that the RNA segments of CCHFV, DUGV and NSDV contain a 5'-terminal pyrimidine [90, 144, 187], while viral RNA polymerases can initiate RNA synthesis only by attaching purines [12], suggests that initiation of RNA replication occurs in a prime-and-realign manner.

Entry and exit

The two membrane glycoproteins (Gn and Gc) are believed to determine cell tropism and the ability of the viruses to infect susceptible cells via recognition and binding of one or more cellular receptors. The specific cellular receptor(s) used by nairoviruses are currently unknown, but the Gc protein is thought to be involved in virus attachment to the target cell receptor, as antibodies against Gc, but not Gn, appear to protect cells from CCHFV infection in plaque reduction neutralisation assays [1, 17]. The ectodomain of CCHFV Gc contains an epitope that is highly conserved among strains [1]. This fragment is probably exposed in the virus, as antibodies against this epitope are neutralising for different strains of CCHFV and protect mice from challenge with CCHFV [1]. Similarly, the Gc of DUGV was also demonstrated to be targeted by neutralising antibodies [107]. Antibodies to the Gn protein were found to be non-neutralising in vitro, though could still be protective in vivo [17].

Based on the finding that an independently folding section of this Gc protein bound to CCHFV-susceptible cells, and using this part of Gc as a probe [185], nucleolin

Fig. 1 Schematic representation of the replication cycle of nairoviruses (family Bunyaviridae). (1) Virus attaches to an unknown cellular receptor (2), after which the virus is internalised into the host cell in an endocytosis-mediated manner. (3) Upon reaching the low-pH environment of the endosome, viral glycoproteins probably undergo a conformational change that releases ribonucleocapsids (RNPs) and viral polymerase (L) into the cytoplasm. (4a) vRNA is transcribed into mRNA, giving rise to synthesis of viral proteins; nucleoprotein (N) and L are synthesised on cytoplasmic ribosomes while (4b) the viral glycoproteins are synthesised as a single polyprotein on ER-associated ribosomes. (4c) Viral RNA (vRNA) is transcribed into complementary RNA (cRNA), which is used as the template for replication of vRNA. (5) The glycoprotein polyprotein is further processed in the ER and Golgi into mature glycoproteins. (6) Once viral proteins and vRNA are synthesised, new virions assemble at the Golgi. (7) The new virus buds into the Golgi and (8) exits the host cell, probably in Golgi-derived vesicles.
has been suggested as a putative CCHFV receptor. Nucleolin is a cellular protein that is abundant in the nucleus but can also be expressed on the cell surface of several cell types [36, 83, 148, 149, 151], including mononuclear phagocytes, endothelial cells and hepatocytes which are known to be targets for CCHFV [28, 185]. Although nucleolin has also been suggested to act as a receptor for several other viruses, such as parainfluenza virus type 3 [22], human immunodeficiency virus (HIV) [118], coxsackie B virus [49] and respiratory syncytial virus (RSV) [162], further studies are required to determine whether nucleolin acts as the primary receptor for CCHFV; in particular, infection studies with wild-type (not cell-culture-adapted) viruses are required to confirm the biological relevance of any potential receptor candidates.

After binding to their receptor, viruses fuse with the plasma membrane or internalise through one of several endocytosis pathways to gain entry into the intracellular environment (reviewed in refs. [40] and [153]). CCHFV entry is dependent on the low pH of the endosomal compartment, which appears to be required in the first steps of the virus entry post-internalisation [74, 156], and the virus has been shown to use clathrin-dependent endocytosis, while caveolin-1 is dispensable for its entry [74, 156]. Studies using dominant negative Rab5 and Rab7 indicate that CCHFV fuses with early, but not late, endosomes to gain access to the cytoplasm [74]. Cholesterol also appears to be important for CCHFV replication, especially at early stages after binding and internalisation; it is possible that depletion of cholesterol from cells traps viral particles in endosomes [88] or interferes with clathrin-mediated endocytosis [140].

The internalisation of CCHFV virions was shown to be dependent on intact microtubules [155]. Further investigation of CCHFV infection showed that disruption of the cell microtubules inhibited viral RNA replication [155], and both intact actin and microtubules are essential for correct distribution of the N protein to the perinuclear area [5, 155]. As nairoviruses enter via endocytosis and bud in the Golgi compartment, the observation of a redistribution of viral proteins and suppression of CCHFV assembly/egress by microtubule modification (depolarisation and stabilisation) [155] is not surprising, and this might be caused by interference with the endogenous secretory pathway, which also occurs along microtubules [101] (reviewed in ref. [87]). Disruption of actin filaments in CCHFV-infected cells also drastically reduced replication of the virus [5]. While the N protein is often located in close proximity to the Golgi apparatus, where generation of new viral particles occurs [16], N does not interact directly with Golgi membranes [5, 134]. The N protein appears to interact with the L protein, even in the absence of viral RNA, as expressed N protein seems to redistribute most of the L protein into the perinuclear area containing N in transfected cells [16].

**The N protein**

The N protein of nairoviruses, at approximately 53 kDa, is almost twice of size of the N proteins of other bunyaviruses, with the exception of those of hantaviruses, which, at approx. 48 kDa, are similar in size [39, 110]. The N protein is the most abundant protein in the virion and encapsidates newly synthesised vRNA and cRNA; this process is necessary for completion of the replication cycle and packaging of the genome into virions [16, 65, 100]. Viruses of different genera in the family Bunyaviridae appear to adopt different mechanisms of RNA encapsidation, e.g. some bunyavirus N proteins recognise generic ssRNA, while others recognise specific structures in the vRNA [110, 120, 135, 137, 150]. There is a need to understand the mechanism by which nairoviruses encapsidate genomic and antigenomic RNA; inhibition of vRNA encapsidation could be a target for potential antiviral drugs, e.g., using an RNA decoy to bind the viral N protein, as has been suggested as an antiviral treatment for hepatitis B virus (HBV) [62], or selecting RNA-binding proteins that target specific packaging sequences in the viral genome [193].

The recently determined crystal structure of the N protein of CCHFV shows that it is built from two major domains: a globular head and an extended stalk [31, 77, 174]. The globular domain is the larger and is formed from both N-terminal and C-terminal helices, while the stalk domain is formed by a group of internal helices (the exact numbering of the helices varies between the several papers publishing the structure of this protein). Three potential RNA-binding regions were identified in the N protein structure. Two positively charged grooves are located at the globular domain: the smaller one is under the stalk domain, and the larger one is in the opposite site of the globular domain. The third positive groove is located at the stalk domain. The positively charged residues forming the RNA-binding site appear to be well conserved across the genus Nairovirus, and several residues were identified as crucial for RNA binding and virus replication, e.g., K132 and Q300 in the smaller groove and K411 and H456 in the larger groove of the head domain [31, 77].

The N protein of CCHFV was also crystallised as a linear oligomer, where three monomeric N subunits were organised in head-to-tail manner, with the stalk domain of one monomer interacting with an oligomeric groove located at the base of the head domain of an adjacent molecule [174]. In addition, the linear oligomers of N were predicted to interact in a dimeric manner to form an antiparallel double superhelix [31, 174]. Further predictions suggest that that there are nine N molecules per turn of the superhelix, which is 210 Å in diameter [174]. Additionally, a positively charged crevice located on the outside of the double superhelix is predicted to serve as an additional RNA binding cleft [174].
Studies performed by Guo and collaborators [77] suggest that the CCHFV N protein, when expressed without RNA, predominantly exists as a monomer, and the nairovirus N protein is only a weak binder of nonspecific RNA in this monomeric state [77, 80, 111, 135]. This suggests that nairovirus N binds RNA only in the oligomeric state and/or that the N recognises specific structures of viral RNA, as was shown for viruses of the genus Hantavirus [110, 120]. However the fact that the nairovirus N protein does not form oligomers without RNA suggests that its oligomerisation is RNA-stabilised, where monomeric N protein requires binding to RNA to form an oligomer, a mechanism that was previously suggested for influenza A virus [160, 161]. In a similar fashion, the N protein of Rift Valley fever virus (RVFV) can form only a short oligomeric form in the absence of RNA [64].

Superimposing the structures obtained for the CCHFV N protein by two different groups, using two CCHFV isolates, revealed the head and stalk with very similar folds, but with a transposition of the stalk domain when comparing these two molecules; results which suggest a flexibility of the stalk domain and the possibility of different conformations of N for different functions or different states of the N-RNA complexes (e.g., transcription vs replication) [31]. Additionally, comparison of the crystal structure of the N protein in the monomeric and oligomeric forms suggests that the stalk domain changes its conformation upon oligomerisation, probably by binding to the oligomerisation groove on the head domain of the adjacent molecule [174]. Such flexibility of the stalk domain has also been shown for the N proteins of RVFV and LASV, which, during binding to an oligomerisation groove on an adjacent N molecule, undergo conformational changes exposing an RNA-binding groove [64, 80]. These structural data have led to suggestions of possible models for the initiation of transcription and replication. Transcription of viral mRNAs by nairoviruses utilises short capped RNA fragments (10-20 nt in length) derived from host mRNAs as primers [90]. Incubation of the CCHFV N protein with primer-length ssRNA resulted in a conformational change of the stalk domain, which resulted in disruption of the oligomeric interactions and release of the monomeric N protein from the antiparallel double superhelix [174]. As discussed by Wang et al., presentation of the capped primers to the ribonucleoprotein (RNP) may initiate conformational changes in the stalk domain, leading to the release of monomeric N and exposing vRNA to the primer and the viral polymerase. Given that head-to-stalk interactions between two adjacent N molecules have also been observed for RVFV, LASV, and Bunyamwera virus (BUNV) [9, 64, 80], it seems likely that the model proposed for CCHFV may be biologically valid.

The N protein of CCHFV has also been shown to have nuclease activity specific for dsDNA and ssDNA (but not for RNA); the importance of this DNase activity and its function in the virus life cycle are unknown [77]. The residues involved in the nuclease activity are located in the globular head domain of the N protein and are conserved in all nairoviruses [77]. In contrast, the N protein of LASV, the head domain of which exhibits high structural homology with that of CCHFV, has RNA-specific nuclease activity [79, 80, 130].

The N protein of nairoviruses also contains a conserved sequence signature specific for catalytic motif II (CMII) of N-6 adenine-specific DNA methylases (Lasecka and Baron, unpublished) (Fig. 2), where the conserved motif NPPW could be involved in substrate binding or in catalytic activity [97, 164]. The motif is located on an exposed loop of the stalk domain, and the function and potential importance of this motif still need to be determined. The methylation of DNA is used for regulation of gene expression, but so far there is no indication that the N protein of nairoviruses travels to the nucleus; the protein does not contain a classical nuclear localisation signal (NLS), nor does it accumulate in the nucleus of infected or transfected cells. N6 methylation is also used as a post-transcriptional modification of mRNA, and RNA methyltransferases appear to contain motifs very similar to the CMI and CMII motifs identified in DNA methyltransferases (reviewed in ref. [119]); further studies are required to determine if the nairovirus N protein can modify its own or host cell RNAs in this way.

Viral glycoproteins

Like those of members of the other genera of the family Bunyaviridae, the M segment of nairoviruses contains a single open reading frame (ORF) encoding a polyprotein that is co- and post-translationally cleaved into the mature viral glycoproteins [39]. The glycoproteins of most nairoviruses are still poorly characterised, and most of the available data come from studies on CCHFV, largely through studies on proteins expressed from plasmids. The processing of the CCHFV M polyprotein to generate the mature glycoproteins appears to be more complex than that of other bunyaviruses, as it involves first the generation of glycoprotein precursors through the action of the signal protease in the endoplasmic reticulum (ER) followed by further cleavages to give rise to the full set of mature glycoproteins, a process that employs other cellular proteases [15, 144, 145, 172] (Fig. 3).

The virions of most of the nairoviruses contain two mature glycoproteins, Gn and Gc [15, 33, 38, 107, 144, 145, 172]; however, two nairoviruses, Hazara virus and Clo
Mor virus, have been shown to contain three structural glycoproteins [68, 175]. The full nairovirus M-encoded polyprotein appears to have six hydrophobic regions (TM0-TM5), which could function as transmembrane helices [3, 144] and act either as classic secretory signal peptides (TM0), membrane anchors (TM1, 3 and 5), or a combination of both (TM2, 4) (Fig. 3). Signal cleavage motifs are found after TM0 (releasing the amino terminus of the Gn precursor, PreGn) and TM4 (releasing the Gc precursor). Sequence inspection revealed a signal cleavage signal immediately after TM2, suggesting that there might be a separate protein released, consisting of the sequence between the distal ends of TM2 and TM4; such a protein has been shown to be produced by CCHFV and has been termed NSM [3]. Further non-structural glycoproteins have been identified in CCHFV-infected cells, referred to as the mucin-like domain (a highly O-glycosylated peptide), GP38, Gn, NSM and Gc indicated. The black bars represent transmembrane helices (TM0-TM5). The initial signal protease (SP) cleavage sites are indicated, and arrows indicate further cleavage sites during polyprotein processing. See text for the details of CCHFV M polyprotein processing. Schematic diagram not drawn to scale and adapted from refs. [4, 161, 162].

Fig. 2 Conserved CMII motif in the N protein of nairoviruses. N protein sequences, either available from NCBI (National Centre for Biotechnology Information) or as sequenced in our laboratory, were aligned using ClustalX2. Viruses and their strain/isolate designation are indicated on the left. CCHFV, Crimean-Congo haemorrhagic fever virus; NSDV, Nairobi sheep disease virus; GV, Ganjam virus; DUGV, Dugbe virus; KUPV, Kupe virus; HAZV, Hazara virus; ERVEV, Erve virus. The numbering at the top indicates the approximate positions in the alignment. The conserved NPPW motif is marked in red. Protein sequence accession numbers are as follows: CCHFV-66/08-Rodopi (ACF93431.2); CCHFV-Afg09-2990 (ADQ57288.1); CCHFV-Ap92 (AAA50177.1); CCHFV-Gujrat-2011 (AFY97403.1); CCHFV-IbAr10200 (F89522.1); CCHFV-Sudan AB1-2009 (AEI70581.1); NSDV-708 (AAM33323.1); GV-40350 (AED88236.1); GV-62873 (AED88237.1); DUGV-NJT130 (ACL68470.2); KUPV-K611 (ABY82500.1); HAZV-JC280 (P2733.2); ERVEV (AFH9034.1)

Fig. 3 Schematic representation of CCHFV M polyprotein processing. A schematic model of the M-encoded polyprotein is shown with the glycoprotein precursors PreGn and PreGc labelled and the approximate positions of the mature proteins mucin-like domain, GP38, Gn, NSM and Gc indicated. The black bars represent transmembrane helices (TM0-TM5). The initial signal protease (SP) cleavage sites are indicated, and arrows indicate further cleavage sites during polyprotein processing. See text for the details of CCHFV M polyprotein processing. Schematic diagram not drawn to scale and adapted from refs. [4, 161, 162].
Gn. This cleavage occurs at a conserved RRLL₁ motif and is effected by the host’s subtilisin kexin isozyme-1/site-1 protease (SKI-1/S1P) [145, 172]. This cleavage has been shown to be critical for virus replication and subsequent infectivity, and lack of the cleavage prevents incorporation of the glycoproteins into viral particles [15]. A similar tetrapeptide (RKPL) is found 41 amino acids downstream of the signalase cleavage site in PreGc, which, although it is not processed also by SKI-1/S1P, is predicted to be utilised by a related subtilisin-like protease in the ER/cis-Golgi to generate the mature Gc (75 kDa) [144, 172]. The mature Gn interacts (directly or indirectly) with the Gc, and both are translocated to the virus-assembly sites in the Golgi [17, 59, 145]. The interaction of mature Gn with Gc is essential for the Gc to travel from ER to Golgi, as Gn, but not Gc, contains a Golgi localisation signal [17]. The ectodomains of Gn and Gc appear to be sufficient for heterodimer formation and transport to the Golgi, indicating that at least partial Golgi-targeting information is located in the Gn ectodomain [17].

The final processing of the glycoproteins takes place in the trans-Golgi, where the mucin-like domain and GP38 are separated from each other by a furin-like protein convertase at the RSKR₁ cleavage site [116, 145]. This cleavage is not required for CCHFV Gn maturation [145], and it appears that not the entire pool of mucin-like domain/GP38 polyprotein is being cleaved, as the non-structural proteins termed GP85 and GP160 contain both the mucin-like domain and GP38 and are resistant to resolution into smaller proteins by denaturation in SDS and urea [145]. Resistance to denaturation also suggests that GP160 is probably not a dimer of GP85 [145]. Interestingly, the mucin-like domain/GP38 and GP38 can fold independently of the rest of Gn and are secreted even when expressed on their own in transfected cells [145]. No specific biological function has been assigned to the mucin-like domain, GP38, GP85 or GP160. The mucin-like domain of the Ebola virus glycoprotein has been shown to play a major role in pathogenesis, including involvement in the observed increase of endothelium permeability [154, 188], and it will be important to see if this is also true of CCHFV, especially given the changes visible in the endothelial cells of CCHFV patients.

The NS₄₅ protein, when expressed in transfected cells on its own, is transported to the Golgi [3]. While the function(s) of the nairovirus NS₄₅ have still to be determined, the fact that Gn requires NS₄₅ for maturation may mean that NS₄₅ is necessary for virus replication [3].

Glycosylation is an important post-translational modification of secreted and membrane proteins that can influence protein folding, transport and function. N-linked glycosylation in particular is known to regulate protein folding, association with chaperones [112], transport, cellular localisation [81, 82], and even virus infectivity (reviewed in ref. [171]). The N-terminal part of the nairovirus M polyprotein, which contains the mucin-like domain, is heavily O-glycosylated, while the adjacent GP38 domain appears to have few O-glycosylation sites [144, 145]. Both domains are also N-glycosylated, the mucin-like domain containing five potential sites and GP38 two [145]. Of two predicted N-glycosylation sites in each of the mature Gn and Gc proteins, only one is functional in Gn (N557), while both sites in Gc (N1054 and N1563) are glycosylated [59]. However, only the glycosylation of the Gn is essential for Gn maturation, correct localisation, and transport of itself and other CCHFV glycoproteins [59]. Given that the Gn glycosylation sites are conserved among CCHFV strains [51], it is likely that correct glycosylation of the CCHFV Gn is critical for virus viability.

Recently, NMR has been used to determine a solution structure for the C-terminal (cytoplasmic) tail of CCHFV Gn, showing that this region contains a dual zinc-finger domain, the sequence of which is highly conserved among nairoviruses [60, 61]. Classical β₂-zinc finger domains have been shown to take part in protein-protein interactions (reviewed in ref. [71]), and it is possible that the dual zinc-finger domain in the C-terminus of the nairovirus Gn protein is involved in interaction with RNP, which would help drive assembly/budding of the virus.

Analysis of the sequences of M segments of other nairoviruses suggests that the general model proposed for processing of the M polyprotein described above for CCHFV holds true for the other nairoviruses; M polyproteins show similar membrane topology, with six transmembrane regions and conserved signalase cleavage sites after the transmembrane domains TM₀, TM₂ and TM₄ (Fig. 3). Glycoprotein maturation from precursor proteins has also been observed for DUGV, where PreGn is around 70 kDa and PreGc around 85 kDa [107]. An exception to this general similarity is Erve virus (ERVEV), which does not contain TM₃ and TM₄, its M polyprotein lacking the entire amino acid sequence between TM₂ and the Gc ectodomain. This suggests that ERVEV lacks an NS₄₅ protein. Most of the nairoviruses for which we have sequence data appear to have a PreGn domain that is about 120 amino acids shorter than that seen in CCHFV, due to a much shorter mucin-like domain, as was previously described for DUGV [144]. Another difference is that other nairoviruses do not contain a furin-cleavage site (RSKR) following the O-glycosylated mucin-like domain. The SKI-1/S1P-like protease cleavage tetrapeptides in many nairoviruses appear to be different to those proposed for CCHFV; however, all appear to fit the consensus subtilisin cleavage sequence (R/K)X₅( hydrophobic)Z₁ (where X is any amino acid and Z is preferably F, K, L or T but not V, P, E, D, C) [56]. This may reflect differences in adaptation
Fig. 4 Schematic representation of the proteins encoded on the L RNA segments of viruses of the family Bunyaviridae. Diagrammatic representation of the L proteins of Rift Valley fever virus (RVFV), Bunyamwera virus (BUNV) and Crimean-Congo haemorrhagic fever virus (CCHFV) in which all proteins were aligned to the polymerase module (i.e., region 3). All proteins contain four (1–4) conserved regions of the RNA-dependent RNA polymerase domain (RdRp), and the conserved motifs (pre-A, A, B, C, D and E) in the polymerase module are also highlighted. However, only the nairovirus L contains the zinc-finger domain and leucine zipper motif. Additionally, the L protein of nairoviruses contains an OTU-like domain (OTU) located at the amino terminus, which contains a topoisomerase-like motif. The approximate size, expressed as the number of amino acids (aa), is indicated for each protein, and the diagram is drawn approximately to scale. See text for details of the various motifs. Adapted from ref. [91]

to the importance of these various stages in the maturation of the glycoproteins for different hosts are clearly required.

The L protein

The L segment of nairoviruses, which contains a single open reading frame (ORF) of approximately 12 kb, encoding a protein of approximately 450 kDa, is almost twice as long as the L proteins of most other bunyaviruses, with the exception of the tospoviruses, in which the L segment is approximately 9 kb in length [48, 85, 94, 108] (Fig. 4). Despite this difference in length and sequence, nairovirus L proteins still show the four conserved functional regions previously described for other bunyavirus L proteins [94, 108, 131, 139, 177]. The bunyavirus L proteins contain the RNA-dependent RNA polymerase (RdRp), and the most conserved region of the L segment among nairoviruses is the region corresponding to the coding sequence for the core catalytic domains of the RdRp [8, 85, 108]. Within this polymerase module (also called region 3) can be distinguished six conserved motifs [94, 108] (Fig. 4). Motifs A through D are conserved among all RNA-dependent polymerases [50, 129]; motif pre-A, upstream of motif A, is present in all RNA-dependent RNA polymerases and reverse transcriptases, and motif E, which is downstream of motif D, is conserved in segmented negative-strand RNA viruses [94]. Motifs A, C and D are predicted to bind nucleoside triphosphates (NTPs) and are therefore likely to be involved in the catalytic functions of the polymerase [50, 114], while motifs B and E are predicted to take part in template and/or primer positioning [114]. Motif pre-A is also predicted to be involved in template positioning [94, 114]. The fact that the inter-motif distances are more or less constant suggests that the polymerase module functions in a structurally dependent manner [94]. Upstream of the polymerase module are regions 1 and 2 (Fig. 4) which are conserved in bunyaviruses and arenaviruses [94, 114], while downstream of the polymerase module is region 4 (Fig. 4), which, although originally suggested to be specific for bunyaviruses [8], appears to be conserved in other segmented negative-strand RNA viruses [94]. Protein sequence analysis shows that the distances between regions appear to be conserved among bunyaviruses, with the exception of the interval between regions 2 and 3, where nairoviruses appear to have much longer amino acid sequences than other bunyaviruses (Fig. 4). Region 1, based on sequence similarity with other viruses, appears to be responsible for capped-snatching endonuclease activity [52, 136, 189]; however, this needs to be confirmed experimentally.

In the case of nairoviruses, the RdRp accounts for only one-third of the entire L protein (Fig. 4), with regions of unidentified function in both the amino (N) and carboxy (C) termini [94]. All bunyaviruses have a significant C-terminal section after the RdRp, although the function of this region is so far unknown in any of the viruses of this family, and there appear to be no cross-genera-conserved motifs [136]. N-terminal to the RdRp motifs, the L proteins of nairoviruses contain several additional domains that are unique to this genus, of which the ovarian-tumour (OTU)-like protease domain is the most studied [2, 29, 85, 89, 94]. This domain belongs to a larger papain-like cysteine protease family also found in other viruses (e.g., blueberry scorch virus (BlScV) of the genus Carlavirus, and equine arteritis virus (EAV) and porcine respiratory and reproductive syndrome virus (PRRSV) of the genus Arterivirus), in Saccharomyces cerevisiae, in Drosophila melanogaster and in mammalian cells [11, 103]. Curiously, the region containing the OTU domain also contains a sequence resembling a topoisomerase-like domain, located at amino acids 85–9, and therefore lying between amino acids that form part of the of the OTU catalytic site – cysteine 40 and histidine 151 [85, 94]. The consensus topoisomerase motif
(SKXYY) is not conserved across nairoviruses, being mostly SLXXY in CCHFV and NSDV/GV, but the active-site tyrosine is conserved across all nairoviruses so far sequenced. Given the structural similarities between topoisomerases and strand-specific recombinases [35], this motif may indicate a role for this region of L in RNA strand manipulation as well as its function as a protease. Alternatively, nairovirus L proteins may include their own topoisomerase activity, rather than having to recruit the host-cell topoisomerase I, as has been shown for at least one non-segmented RNA virus [159].

Downstream of the OTU domain, the L protein of nairoviruses contains a C2H2-type zinc finger domain and a leucine zipper motif [85, 94], both of which are highly conserved among nairoviruses, but the function of which in the viral replication cycle is still to be determined. Interestingly, the zinc-finger domain and leucine zipper motif are located in region 1 and region 2, respectively, of the nairoviral L proteins but do not appear to be present in these regions in the L proteins of other bunyaviruses.

The OTU-domain protease activity is dispensable for virus genome replication [16], and most recent studies have focussed on the effects of this enzymatic activity on host proteins. Mammalian OTU-domain proteins are primarily deubiquitinating enzymes (DUBs), responsible for cleaving the modified peptide bond that links ubiquitin (Ub) to host-cell proteins or to other Ub molecules. Most mammalian DUBs are only able to deubiquitinate and usually have a limited set of targets that they de-conjugate in this way (reviewed in ref. [96]). The OTU domains of nairoviruses, in contrast, are capable of de-conjugating not only Ub but also other ubiquitin-like peptides, notably the interferon-stimulated gene 15 protein (ISG15), removing these peptides from a variety of protein targets [10, 69, 84]. It has been shown that amino acids 1 to 169 of the L proteins of CCHFV, NSDV or DUGV are sufficient for enzymatic activity, and conserved active site residues that are critical for its catalytic activity have been identified [10, 69, 84]. In the last few years, the crystal structures of the OTU domain with and without a ubiquitin molecule have been determined [2, 29, 89]. The CCHFV OTU showed an overall similar structure to yeast Otu1, but with an additional domain formed by two antiparallel β-strands that allow the viral OTUs to bind both Ub and ISG15 [2, 89]. Specific cleavage targets, other than host ubiquitinated or ISGylated proteins, for the viral OTU-like protease have not yet been described. As several potential cysteine-protease-like cleavage sites have been identified in the L protein sequence of nairoviruses [94] and some viral proteins containing an OTU-like protease domain have also been shown to undergo autoproteolytic cleavage to generate multiple mature proteins, e.g., the replicase of BisseV [98], it has been suggested that the L proteins of nairoviruses may also be autoproteolytically cleaved into an active RNA polymerase and protein(s) with additional function [85]. We have raised specific antibodies to the N- and C-termini of the NSDV L protein and shown that such cleavage does not occur in infected cells (Lasecka and Baron, unpublished).

Controlling host innate immune responses

Early studies on nairoviruses showed that, unlike other bunyaviruses, they do not shut off host protein synthesis [33, 176], so the viruses must have a different way of controlling the immediate host cell responses to infection, such as the innate immune response and apoptosis.

CCHFV infection in cultured cells induced apoptosis, albeit at late stages of infection [91, 141]. One of the ways in which nairoviruses might induce apoptosis has been suggested to be via the induction of ER stress [141]. Certainly CCHFV and DUGV have both been shown to induce ER stress [141], and we have observed the same in cells infected with NSDV (Lasecka, unpublished data). During replication, nairoviruses synthesize large amounts of their glycoproteins, which mature in the ER and Golgi [17, 21, 142] and are likely to overload the normal ER protein synthesis machinery. As long as the apoptosis occurs after the virus has completed the assembly and release of progeny virus, apoptosis is not a major problem, but it is unclear as yet whether nairoviruses take active steps to inhibit apoptotic pathways. There is the possibility that apoptosis may be delayed or inhibited in CCHFV infections by the presence of a highly conserved caspase-3 cleavage site (DEVD) in the N protein [31, 91], which may act as a decoy substrate for caspase-3. Although this motif has been suggested to be involved in control of apoptosis [31, 91], the cleavage of N protein by caspase-3 is not required for replication/transcription of a CCHFV minigenome [31]. In addition, this DEVD motif appears to be inaccessible to caspase-3 in the oligomeric form of CCHFV N, suggesting that only N monomers are caspase-3 sensitive [174]. CCHFV also appears to be the only nairovirus that contains the DEVD motif [174]; other nairoviruses, such as NSDV/GV, do not have the motif, yet are still highly pathogenic.

The innate immune response to viral infection has been described in a number of recent reviews [72, 105, 132, 143]. Cells detect viral pathogen-associated molecular patterns (PAMPs) (e.g., double-stranded RNA (dsRNA) or DNA with unmethylated CpG motifs) using pattern recognition receptors (PRRs), of which the most well-known are the Toll-like receptors (TLRs), which scan extra-cytoplasmic spaces including the interior of endosomes and lysosomes, and the cytosolic proteins melanoma-associated
differentiation gene-5 protein (MDA-5) and retinoic acid-inducible gene-I protein (RIG-I), which detect virus-associated PAMPs in the cytoplasm. Recognition of a PAMP leads to activation of the PRR, followed by an intracellular signalling cascade leading to the transcription of interferon β (IFNβ) mRNA. Secreted IFNβ works in an autocrine and paracrine manner by binding to the IFNα/β receptor of both infected and uninfected cells. This in turn activates a signalling pathway, which up-regulates interferon-stimulated genes (ISGs), including IFNα signalling pathway, which up-regulates interferon-stimulated infected and uninfected cells. This delay is related to a delay in translation in response to infection (reviewed in ref. [63]). The delay in ISG56 expression correlates with earlier induction of ISG56, one of the genes whose transcription is significantly reduced the yield of CCHFV [6], while the replication of CCHFV and DUGV is impaired in Vero cells stably expressing the human Mx (MxA) protein; MxA is the product of an ISG and appears to act through sequestration of the N protein of these viruses in a perinuclear region [4, 25].

However, as with other viruses, nairoviruses have developed mechanisms to evade this innate antiviral response. Some of these mechanisms appear to be unique to one virus; others appear to be common to all the viruses in the genus that have been studied. For example, CCHFV can delay IFNα/β production in infected cells; Andersson et al. showed that, in CCHFV-infected cells, an increase in IFNβ mRNA could only be detected 48 h after infection, which leaves the virus a replication window with no antiviral state [7]. This delay is related to a delay in transcription of the transcription factor interferon regulatory factor-3 (IRF-3) to the nucleus [7] and hence a delay in the induction of ISG56, one of the genes whose transcription is regulated by IRF-3. The ISG56 protein is a cytoplasmic protein (p56) that is involved in the global inhibition of translation in response to infection (reviewed in ref. [63]). The delay in ISG56 expression correlates with earlier findings that nairoviruses do not appear to shut off cellular protein synthesis [33, 176]. In contrast to the findings with CCHFV, IFNβ induction was seen in NSDV/GV-infected cells after about 16 hours; however, during those 16 hours, the virus actively blocked induction of IFNβ [84]. NSDV/GV infection also blocked the signalling pathways activated by external IFNα or IFNγ, blocking the activation of transcription from promoters normally activated by one or the other of these cytokines by directly inhibiting the phosphorylation of the transcription factors STAT1 and STAT2 [84].

Of the cytoplasmic PRRs, RIG-I is activated by RNAs with a 5’ triphosphate group, such as viral vRNAs and cRNAs, while MDA-5 is activated by binding dsRNAs [44, 86, 92, 128]. Like other negative-sense RNA viruses, nairoviruses do not produce detectable amounts of dsRNA during replication [178] and hence avoid activation of a number of dsRNA-sensitive PRRs (e.g., MDA-5, TLR3) and dsRNA-dependent enzymes (e.g., dsRNA-activated protein kinase R [PKR] and 2′-5′-oligoadenylate synthetase [2′5′ OAS]). The newly synthesised vRNA and cRNA of nairoviruses, as for other bunyaviruses, is cotranscriptionally encapsidated by the N protein, minimising the exposure of vRNAs and preventing formation of dsRNA intermediates [127, 135, 173]. It is thought that the major sensor for bunyavirus infection is therefore RIG-I. 5′-monophosphate groups are present on RNA molecules generated during viral replication but are absent on cellular RNAs, as cellular mRNA contains a cap structure at the 5′ end, and the transcription products of other cellular RNA polymerases (RNA polymerases I and III) contain a monophosphate at the 5′ end. By utilising capped, short nucleotide sequences snatched from host mRNA to initiate viral mRNA transcription [90], nairoviruses prevent recognition of viral mRNA by PRRs. CCHFV further avoids activation of RIG-I as its vRNA and cRNA have a monophosphate group rather than the triphosphate group found at the 5′ end of most viral RNAs [78], though the mechanism of 5′ monophosphate generation during replication of CCHFV remains unknown. Strategies to generate 5′monophosphates on viral RNA have been shown for other viruses: HTNV utilises a prime-and-realign mechanism to generate 5′ and 3′ complementary ends, where a viral endonuclease (which is probably also involved in cap-snatching) is proposed to remove 5′-terminal extensions, leading to a 5′ monophosphate on the final RNA [73]. Interestingly, this mechanism is not common to all bunyaviruses, as RVFV was shown to contain triphosphate groups at the 5′ ends of its vRNAs, which can activate IFNβ via the RIG-I pathway [78]. It will be interesting to see if other nairoviruses adopt the RNA processing seen for CCHFV. It is possible that bunyaviruses that have developed methods of blocking the RIG-I-activated pathway (e.g., by the activities of a non-structural protein such as the phlebovirus NSs protein) can have triphosphates at the 5′ end of their vRNAs while viruses that do not have an NSs activity must generate monophosphates to avoid activating the RIG-I pathway in the first place [18, 19, 23, 73].

Ubiquitin and ubiquitin-like molecules play important roles in the initiation and maintenance of immune response. For example, they are essential for the action of cytokines such as IFNα/β and tumour necrosis factor alpha (TNFα) (reviewed in ref. [95]). Ubiquitination allows for the activation of nuclear factor kappa B (NF-κB) by targeting the inhibitor of NF-κB (IκB) for degradation [163]. K63-linked ubiquitination activates several molecules of the IFNβ induction pathway, including RIG-I, mitochondrial antiviral
signalling protein (MAVS), TANK-binding kinase-1 (TBK1), IκB kinase-ε (IKK-ε), tumour necrosis factor receptor-associated factor 3 (TRAF3) and TRAF6 [70, 125, 126, 166]. In addition to modulation of the innate immune signalling, ubiquitination also plays an important role in antigen presentation by major histocompatibility complex (MHC) class I and II proteins [152]. ISG15 is an IFN-induced 15-kDa ubiquitin-like molecule that is composed of two-ubiquitin-like domains [20, 117]. Although the precise role of ISG15 in modulation of protein function is unknown, conjugation by ISG15 is also known to modify hundreds of cellular proteins, including several of those involved in the antiviral response, e.g., PKR, MxA, STAT1, RIG-I, Janus kinase 1 (JAK1), and IRF-3 [75, 104, 138, 192].

Both Ub and ISG15 are synthesised as precursors, which are cleaved in order to expose the conjugation sequence (LRLRGGR) by which they are attached to other proteins. The conjugation is mediated by involving activating enzymes (E1), conjugating enzymes (E2) and protein ligases (E3) [157, 180, 191] (reviewed in ref. [169]). Removal of these conjugated proteins is carried out by cellular deubiquitinating enzymes and deISGylating enzymes, which have roles, as expected, in negative feedback regulation of IFN induction and action [93, 99]. As described in the discussion of the nairovirus L protein, the N-terminus of these proteins contains an OTU protease-like domain [85] similar to that often found in mammalian DUBs, and experimental evidence showed that this protease domain in the viral protein indeed deconjugated Ub and ISG15. Global ubiquitination and ISGylation levels were greatly reduced in NSDV/GV-infected cells [84], and overexpression of the amino-terminal end of the L proteins of CCHFV, NSDV or DUGV in cell culture resulted in a similar reduction in ubiquitin- and ISG15-conjugated proteins [10, 30, 69, 84]. The L protein OTU domains of several nairoviruses have been shown to block IFNβ induction and the actions of type I and type II IFNs [10, 69, 84]. Interestingly, at high enough concentrations, even catalytically inactive OTUs are capable of blocking IFNα-induced transcription [10, 69]. This suggests that catalytically inactive OTU domain proteins are still capable of sequestering specific ubiquitinated or ISG15ylated targets by binding to them.

The nsp2 protein of EAV and PRRSV also contain OTU-like domains [69]. Recently, it was shown that both the nsp2 of EAV and the OTU domain of CCHFV L protein are able to deubiquitinate RIG-I and hence block RIG-I-mediated activation of IFNβ [168]. Interestingly, the RNA of neither CCHFV nor EAV appears to activate RIG-I [78, 168], so the fact that these viruses have evolved specific mechanisms to inhibit the RIG-I-mediated induction of IFNβ suggests that RIG-I still has a function in the antiviral response to these viruses. However, unlike CCHFV, the vRNA of NSDV/GV does activate transcription from the IFNβ promoter (Lasecka and Baron, unpublished observations). The ability to directly block the RIG-I pathway would therefore be particularly important for this virus.

Comparison of the OTU domains of different nairoviruses revealed some differences between their affinity for different types of poly-Ub and ISG15 [30]. For instance, CCHFV shows a higher affinity for K63-poly-Ub than K48-poly-Ub, and the CCHFV OTU was more active in the deubiquitination of host proteins than the OTUs of either NSDV or DUGV [10, 30, 84]. On the other hand, while the ERVEV OTU appears to bind any poly-Ub weakly (when compared to those of CCHFV and DUGV), it had higher affinity for ISG15 [30]. This may indicate that different nairoviruses have adopted slightly different ways of utilising their core deubiquitinating and deISGylating activities, which might reflect the wide range of pathogenicity caused by these viruses, or differences in the requirements imposed on these viruses by their arthropod hosts, about which we know very little. ISG15 is not as strongly conserved as ubiquitin among different species, even mammals, so there can be real effects of species preference in ISG15 binding/cleavage, e.g., the CCHFV OTU appears to show a preference for ISG15 of human origin over that of mouse origin, while ERVEV appears to recognise both human and mouse ISG15s equally [30]. The better binding of the murine ISG15 by the ERVEV OTU may be associated with the homology between the ISG15 of mouse and the white-toothed shrew from which ERVEV is commonly isolated [34, 184].

Final remarks

Nairoviruses share many of their features with other bunyaviruses, e.g., replication in the cytoplasm, budding in the Golgi, and their coding and RNA replication strategy. From phylogenetic studies, the members of the genus Nairovirus appear to be most closely related to those of the genus Phlebovirus of all bunyaviruses [108, 131, 139, 177]. However, nairoviruses possess many features not found in other bunyaviruses. Nairoviruses appear to have complex processing of their glycoproteins, which involves the actions of cellular proteases such as SKI-1/S1P-like proteases and furin. The proteins of nairoviruses also contain domains that have not been observed in other bunyaviruses, such as the L protein OTU domain. Nairoviruses express a secreted mucin-like domain, which may play an essential role in the pathogenicity of the virus [154, 188]. Structural similarity between the nairovirus N protein globular domain or the RdRp region of its L protein and equivalent proteins of arenaviruses has been taken to suggest that the nairoviruses are more closely related to arenaviruses than
to members other genera of the family Bunyaviridae [31, 170], with some authors even suggesting that the current classification of the Nairovirus might need re-evaluation in the future [31].

Several areas for future study stand out. Given that the nairoviruses are, in general, tick-borne, while most other bunyaviruses are insect-borne, it is to be expected that the interaction of these viruses with their arthropod hosts will be specific to the virus genus and need specific study. Fortunately, expertise with handling ixodid ticks and tick cell lines is rapidly increasing, and it is to be hoped that our understanding of the replication of these viruses in their tick hosts will catch up with our knowledge of what is happening in mammals. The nairoviruses have been more resistant to the development of successful reverse genetics than e.g. the orthobunyaviruses or the phleboviruses, and development of such a system for nairoviruses will be valuable in helping us understand the roles of various nairovirus-specific domains such as the topoisomerase-like domain, C2H2-zinc finger domain, leucine zipper motif, and OTU domain of the L protein, or the NSM and mucin-like domain from the M segment, both in mammalian and arthropod hosts. The ability to create targeted mutations will also enable us to more rapidly develop stably attenuated viruses that could act as vaccines.

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References

1. Ahmed AA, McFalls JM, Hoffmann C, Filome CN, Stewart SM, Paragas J, Khodjaev S, Shermukhamedova D, Schmaljohn CS, Domns RW, Bertolotti-Ciarlet A (2005) Presence of broadly reactive and group-specific neutralizing epitopes on newly described isolates of Crimean-Congo hemorrhagic fever virus. J Gen Virol 86:3327–3336
2. Akutsu M, Ye Y, Virdee S, Chin JW, Komander D (2011) Molecular basis for ubiquitin and ISG15 cross-reactivity in viral ovarian tumor domains. Proc Natl Acad Sci USA 108:2228–2233
3. Altamura LA, Bertolotti-Ciarlet A, Teigler J, Paragas J, Schmaljohn CS, Domns RW (2007) Identification of a novel C-terminal cleavage of Crimean-Congo hemorrhagic fever virus PreGN that leads to generation of an NSM protein. J Virol 81:6632–6642
4. Andersson I, Bladh L, Mousavi-Jazi M, Magnusson KE, Lundkvist A, Haller O, Mirazimi A (2004) Human MxA protein inhibits the replication of Crimean-Congo hemorrhagic fever virus. J Virol 78:4323–4329
5. Andersson I, Simon M, Lundkvist A, Nilsson M, Holmstrom A, Elgh F, Mirazimi A (2004) Role of actin filaments in targeting of Crimean Congo hemorrhagic fever virus nucleocapsid protein to perinuclear regions of mammalian cells. J Med Virol 72:83–93
6. Andersson I, Lundkvist A, Haller O, Mirazimi A (2006) Type I interferon inhibits Crimean-Congo hemorrhagic fever virus in human target cells. J Med Virol 78:216–222
7. Andersson I, Karlberg H, Mousavi-Jazi M, Martinez-Sobrido L, Weber F, Mirazimi A (2008) Crimean-Congo hemorrhagic fever virus delays activation of the innate immune response. J Med Virol 80:1397–1404
8. Aquino VH, Moreli ML, Moraes Figueiredo LT (2003) Analysis of oropouche virus L protein amino acid sequence showed the presence of an additional conserved region that could harbour an important role for the polymerase activity. Arch Virol 148:19–28
9. Ariza A, Tanner SJ, Walter CT, Dent KC, Shepherd DA, Wu W, Matthews SV, Hiscox JA, Green TJ, Luo M, Elliott RM, Fooks AR, Ashcroft AE, Stonehouse NJ, Ramson NA, Barr RN, Edwards TA (2013) Nucleocapsid protein structures from orthobunyaviruses reveal insight into ribonucleoprotein architecture and RNA polymerization. Nucleic Acids Res 41:5912–5926
10. Bakshi S, Holzer B, Bridgen A, McMullan G, Quinn DG, Baron MD (2013) Dugbe virus ovarian tumour domain interferes with ubiquitin/ISG15-regulated innate immune cell signalling. J Gen Virol 94:298–307
11. Balakirev MY, Tcherniuk SO, Jaquinod M, Chroboczek J (2003) Outbains: a new family of cytochrome proteases in the ubiquitin pathway. EMBO Rep 4:517–522
12. Banerjee AK (1980) 5’-terminal cap structure in eucaryotic messenger ribonucleic acids. Microbiol Rev 44:175–205
13. Banerjee K, Gupta NP, Goverdhun MK (1979) Viral infections in laboratory personnel. Indian J Med Res 69:363–373
14. Barr JN, Werz GW (2005) Role of the conserved nucleotide mismatch within 3’- and 5’-terminal regions of Bunyamwera virus in signaling transcription. J Virol 79:3586–3594
15. Bergeron E, Vincent MJ, Nichol ST (2007) Crimean-Congo hemorrhagic fever virus glycoprotein processing by the endoprotease SKI-1/SIP is critical for virus infectivity. J Virol 81:13271–13276
16. Bergeron E, Albarino CG, Khristova ML, Nichol ST (2010) Crimean-Congo hemorrhagic fever virus-encoded ovarian tumor protease activity is dispensable for virus RNA polymerase function. J Virol 84:216–226
17. Bertolotti-Ciarlet A, Smith J, Streekker K, Paragas J, Altamura LA, McFalls JM, Frias-Staheli N, Garcia-Sastre A, Schmaljohn CS, Domns RW (2005) Cellular localization and antigenic characterization of crimean-congo hemorrhagic fever virus glycoproteins. J Virol 79:6152–6161
18. Billecocq A, Spiegel M, Vialat P, Kohl A, Weber F, Bouloy M, Haller O (2004) NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. J Virol 78:9798–9806
19. Blakqi G, Delbaye S, Habjan M, Blair CD, Sanchez-Vargas I, Olson KE, Attarzadeh-Yazdi G, Frakoudis R, Kohl A, Kalinke U, Weiss S, Michiels T, Staeheli P, Weber F (2007) La Crosse bunyavirus nonstructural protein NSs serves to suppress the I interferon system of mammalian hosts. J Virol 81:4991–4999
20. Blomstrom DC, Fahey D, Kutny R, Korant BD, Knight E Jr (1986) Molecular characterization of the interferon-induced 15-kDa protein. Molecular cloning and nucleotide and amino acid sequence. J Biol Chem 261:8811–8816
21. Booth TF, Gould EA, Nuttall PA (1991) Structure and morphology of Dengue virus (Bunyaviridae, Nairovirus) studied by immunogold electron microscopy of ultrathin cryosections. Virus Res 21:199–212
22. Bose S, Basu M, Banerjee AK (2004) Role of nucleolin in human parainfluenza virus type 3 infection of human lung epithelial cells. J Virol 78:8146–8158
23. Bouloy M, Janzen C, Vialat P, Khun H, Pavlovic J, Huere M, Haller O (2001) Genetic evidence for an interferon-antagonistic function of rift valley fever virus nonstructural protein NSs. J Virol 75:1371–1377
24. Briden A, Dalrymple DA, Elliott RM (2002) Dugbe nairovirus S segment: correction of published sequence and comparison of five isolates. Virology 294:364–371
25. Briden A, Dalrymple DA, Weber F, Elliott RM (2004) Inhibition of Dugbe nairovirus replication by human MxA protein. Virus Res 99:47–50
26. Burney MI, Ghafoor A, Saleem M, Webb PA, Casals J (1980) Nosocomial outbreak of viral hemorrhagic fever caused by Crimean Hemorrhagic fever-Congo virus in Pakistan, January 1976. Am J Trop Med Hyg 29:941–947
27. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R (1996) Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect 116:353–361
28. Burt FJ, Swanepoel R, Shieh WJ, Smith JF, Leman PA, Greer PW, Coffield LM, Rollin PE, Ksiazek TG, Peters CJ, Zaki SR (1997) Immunochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. Arch Pathol Lab Med 121:839–846
29. Capodagli GC, McKercher MA, Baker EA, Masters EM, Brunzelle JS, Pegan SD (2011) Structural analysis of a viral ovarian tumor domain protease from the Crimean-Congo hemorrhagic fever virus in complex with covalently bonded ubiquitin. J Virol 85:3621–3630
30. Capodagli GC, Deaton MK, Baker EA, Lumpkin RJ, Pegan SD (2013) Diversity of ubiquitin and IG515 specificity among nairoviruses’ viral ovarian tumor domain proteases. J Virol 87:3815–3827
31. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron E, Nichol ST, Hiscos JA, Edwards TA, Barr JJ (2012) Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol 86:10914–10923
32. Casals J, Tignor GH (1980) The Nairovirus genus: serological relationships. Intervirology 14:144–147
33. Cash P (1985) Polypeptide synthesis of Dugbe virus, a member of the Nairovirus genus of the Bunyaviridae. J Gen Virol 66(1):141–148
34. Chastel C, Main AJ, Richard P, Le Lay G, Legrand-Quillien MC, Beauchouin JC (1989) Erve virus, a probable member of Bunyaviridae family isolated from shrews (Crocidura russula) in France. Acta Virol 33:270–280
35. Cheng C, Kussie P, Pavletich N, Shuman S (1998) Conservation of structure and mechanism between eukaryotic topoisomerase I and site-specific recombinases. Cell 92:841–850
36. Christian S, Pilch J, Akerman ME, Porkka K, Laakkonen P, Ruusulaiti E (2003) Nucleolin expressed at the cell surface is a marker of endothelial cells in angiogenic blood vessels. J Cell Biol 163:871–878
37. Clerex-van Haaster CM, Clerex JP, Ushijima H, Akashi H, Fuller F, Bishop DH (1982) The 3′ terminal RNA sequences of bunyaviruses and nairoviruses (Bunyaviridae): evidence of end sequence generic differences within the virus family. J Gen Virol 61:289–293
38. Clerx JP, Bishop DH (1981) Qalyub virus, a member of the newly proposed Nairovirus genus (Bunyaviridae). Virology 108:361–372
39. Clerx JP, Casals J, Bishop DH (1981) Structural characteristics of nairoviruses (genus Nairovirus, Bunyaviridae). J Gen Virol 55:165–178
40. Connolly SA, Jackson JO, Jardetzky TS, Longnecker R (2011) Fusing structure and function: a structural view of the herpesvirus entry machinery. Nature Rev Microbiol 9:369–381
41. Converse JD, Hoogstraal H, Moussa MI, Casals J, Kaiser MN (1975) Pretoria virus: a new African agent in the tickborne Dera Ghazi Khan (DGK) group and antigenic relationships within the DGK group. J Med Entomol 12:202–205
42. Converse JD, Hoogstraal H, Moussa MI, Feare CJ, Kaiser MN (1975) Soldado virus (Hughes group) from Ornithodoros (Alectorobius) capensis (Ixodidae: Argasidae) infesting Sooty Tern colonies in the Seychelles, Indian Ocean. Am J Trop Med Hyg 24:1010–1018
43. Crabtree MB, Sang R, Miller BR (2009) Koq virus, a new virus in the family bunyaviridae, genus nairovirus, kenya. Emerg Infect Dis 15:147–154
44. Cui S, Eisenacher K, Kirchhofer A, Brzozka K, Lammens A, Lammens K, Fujita T, Conzelmann KK, Krug A, Hopfner KP (2008) The C-terminal regulatory domain is the RNA 5′-triposphate sensor of RIG-I. Mol Cell 29:169–179
45. Dandawate CN, Work TH, Webb JK, Shah KV (1969) Isolation of Ganjam virus from a human case of febrile illness: a report of a laboratory infection and serological survey of human sera from three different states of India. Indian J Med Res 57:975–982
46. David-West TS, Porterfield JS (1974) Dugbe virus: a tick-borne arbovirus from Nigeria. J Gen Virol 23:297–307
47. Davies FG, Casals J, Jesset DM, Ochieng P (1978) The serological relationships of Nairobi sheep disease virus. J Comp Pathol 88:519–523
48. de Haan P, Kormelink R, de Oliveira Resende R, van Poelwijk F, Peters D, Goldbach R (1991) Tomato spotted wilt virus L RNA encodes a putative RNA polymerase. J Gen Virol 72(9):2207–2216
49. de Verdugo UR, Selinka HC, Huber M, Kramer B, Kellermann J, Hofschnieder PH, Kandolf R (1995) Characterization of a 100-kilodalton binding protein for the six serotypes of coxsackie B viruses. J Virol 69:6751–6757
50. Delarue M, Poch O, Tordo N, Moras D, Argos P (1990) An attempt to unify the structure of polymersases. Protein Eng 3:461–467
51. Deyde VM, Khristolva ML, Rollin PE, Ksiazek TG, Nichol ST (2006) Crimean-Congo hemorrhagic fever virus genomics and global diversity. J Virol 80:8834–8842
52. Dias A, Bouvier D, Crepin T, McCarthy AA, Hart DJ, Baudin F, Cusack S, Ruigrok RW (2009) The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature 458:914–918
53. Dinkins C, Minnack D, Emmich P, Schmitz H, Reinicke T (2002) Crimean-Congo hemorrhagic fever in Kosovo. J Clin Microbiol 40:1122–1123
54. Dunster LM, Ofula V, Beti D, Kazooba-Voskamp F, Burt F, Swanepoel R, DeCock KM (2002) First documentation of human Crimean-Congo hemorrhagic fever, Kenya. Emerg Infect Dis 8:1005–1006
55. el-Azay MY, Scrimgeour EM (1997) Crimean-Congo haemorrhagic fever virus infection in the western province of Saudi Arabia. Trans R Soc Trop Med Hyg 91:275–278
56. Elagoz A, Benjannet S, Mammarbassi A, Wickham L, Seidah NG (2002) Biosynthesis and cellular trafficking of the converse SKI-1/SIP1 ectodomain shedding requires SKI-1 activity. J Biol Chem 277:11265–11275
57. Ellis DS, Southee T, Lloyd G, Platt GS, Jones N, Stamford S, Bowen ET, Simpson DJ (1981) Congo/Crimean haemorrhagic fever virus from Iraq 1979. I. Morphology in BHK21 cells. Arch Virol 70:189–198
58. Ergonul O (2006) Crimean-Congo hemorrhagic fever. Lancet Infect Dis 6:203–214
59. Erickson BR, Deyde V, Sanchez AJ, Vincent MJ, Nichol ST (2007) N-linked glycosylation of Gn (but not Gc) is important for Crimean Congo hemorrhagic fever virus glycoprotein localization and transport. Virology 361:348–355

2 Springer
126

L. Lasecka, M. D. Baron

60. Estrada DF, Boudreault DM, Zhong D, St Jeor SC, De Guzman RN (2009) The Hantavirus Glycoprotein G1 Tail Contains Dual CCHC-type Classical Zinc Fingers. J Biol Chem 284:8654–8660

61. Estrada DF, De Guzman RN (2011) Structural characterization of the Crimean-Congo hemorrhagic fever virus Gn tail provides insight into virus assembly. J Biol Chem 286:21678–21686

62. Feng H, Beck J, Nassal M, Hu KH (2011) A SELEX-screened aptamer of human hepatitis B virus RNA encapsidation signal suppresses viral replication. PLoS One 6:e27862

63. Fensterl V, Sen GC (2011) The ISG56/IFIT1 gene family. J Interferon Cytokine Res 31:71–78

64. Ferron F, Li Z, Danek EI, Luo D, Wong Y, Coutard B, Lantez V, Charrel R, Canard B, Walz T, Lescar J (2011) The hexamer structure of Rift Valley fever virus nucleoprotein suggests a mechanism for its assembly into ribonucleoprotein complexes. PLoS Pathog 7:e1002030

65. Flick R, Pettersson RF (2001) Reverse genetics system for Uukuniemi virus (Bunyaviridae): RNA polymerase I-catalyzed expression of chimeric viral RNAs. J Virol 75:1643–1655

66. Flick R, Elgh F, Pettersson RF (2002) Mutational analysis of the Uukuniemi virus (Bunyaviridae family) promoter reveals two elements of functional importance. J Virol 76:10849–10860

67. Flick R, Kilkki K, Feldmann H, Elgh F (2003) Reverse genetics for crimean-congo hemorrhagic fever virus. J Virol 77:5997–6006

68. Foulke RS, Rosato RR, French GR (1981) Structural polypeptides of Haavara virus. J Gen Virol 53:169–172

69. Frias-Staheli N, Giannakopoulos NV, Kikkert M, Taylor SL, Bridgen A, Paragas J, Rich R, Rowland RR, Schmaljohn CS, Lenschow DJ, Snijder EJ, Garcia-Sastre A, Virgin HWt (2007) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature 446:916–920

70. Fugle RS, Rosato RR, French GR (1981) Structural polypeptides of Haavara virus. J Gen Virol 53:169–172

71. Garcia-Sastre A, Biron CA (2006) Type 1 interferons and the interferon-induced antiviral state. Nature 446:916–920

72. Garcia-Sastre A, Biron CA (2006) Type 1 interferons and the interferon-induced antiviral state. Nature 446:916–920

73. Girardin SE, Nayler G, Tschopp J (2011) PRRs and TRIMs: an alliance against intracellular pathogens. J Biol Chem 286:4538–4542

74. Girardin SE, Nayler G, Tschopp J (2011) PRRs and TRIMs: an alliance against intracellular pathogens. J Biol Chem 286:4538–4542

75. Giannakopoulos NV, Luo JK, Papov V, Zou W, Lenschow DJ, Jacobs BS, Borden EC, Li J, Virgin HW, Zhang DE (2005) Proteomic identification of proteins conjugated to ISG15 in mice and human cells. Biochem Biophys Res Commun 336:496–506

76. Gould EA, Chanas AC, Buckley A, Virma GM (1983) Immunofluorescence studies on the antigenic interrelationships of the Hughes virus serogroup (genus Nairovirus) and identification of a new strain. J Gen Virol 64(3):739–742

77. Guo Y, Wang W, Ji W, Deng M, Sun Y, Zhou H, Yang C, Deng F, Wang H, Hu Z, Lou Z, Rao Z (2012) Crimean-Congo hemorrhagic fever virus nucleoprotein reveals endonuclease activity in bunyaviruses. Proc Natl Acad Sci USA 109:5046–5051

78. Habjan M, Andersson I, Klingstrom I, Schumann M, Martin A, Zimmermann P, Wagner V, Pichlmair A, Schneider U, Mahlberger E, Mirazimi A, Weber F (2008) Processing of genome 5′ termini as a strategy of negative-strand RNA viruses to avoid RIG-I-dependent interferon induction. PLoS One 3:e2032

79. Hastie KM, Kimberlin CR, Zandonatti MA, MacRae IJ, Saphire EO (2011) Structure of the Lassa virus nucleoprotein reveals a dsRNA-specific 3′ to 5′ exonuclease activity essential for immune suppression. Proc Natl Acad Sci USA 108:2396–2401

80. Hastie KM, Liu T, Li S, King LB, Ngo N, Zandonatti MA, Woods VL Jr, de la Torre JC, Saphire EO (2011) Crystal structure of the Lassa virus nucleoprotein-RNA complex reveals a gating mechanism for RNA binding. Proc Natl Acad Sci USA 108:19365–19370

81. Hauri H, Appenzeller C, Kuhn F, Nufer O (2000) Lectins and traffic in the secretory pathway. FEBS Lett 476:32–37

82. Helenius A, Aebl M (2001) Intracellular functions of N-linked glycans. Science 291:2364–2369

83. Hirano K, Miki Y, Hirai Y, Sato R, Itoh T, Hayashi A, Yamakawa M, Eda S, Beppu M (2005) A multifunctional shuttling protein nucleolin is a macrophage receptor for apoptotic cells. J Biol Chem 280:39284–39293

84. Holzer B, Bakshi S, Bridgen A, Baron MD (2011) Inhibition of interferon induction and action by the nairovirus Nairobi sheep disease virus/Ganjam virus. PLoS One 6:e28594

85. Honig JE, Osborne JC, Nichol ST (2004) Crimean-Congo hemorrhagic fever virus genome L RNA segment and encoded protein. Virology 321:29–35

86. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, Pocke H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G (2006) 5′-Triphosphate RNA is the ligand for RIG-I. Science 314:994–997

87. Hunt SD, Stephens DJ (2011) The role of motor proteins in endosomal sorting. Biochem Soc Trans 39:1179–1184

88. Imelli N, Meier O, Boucke K, Hemmi S, Greber UF (2004) Cholesterol is required for endocytosis and endosomal escape of adenovirus type 2. J Virol 78:3089–3098

89. James TW, Frias-Staheli N, Bacik JP, Levingston Macleod JM, Khajehpour M, Garcia-Sastre A, Mark BL (2011) Structural basis for the removal of ubiquitin and interferon-stimulated gene 15 by a viral ovarian tumor domain-containing protease. Proc Natl Acad Sci USA 108:2222–2227

90. Jin H, Elliott RM (1993) Non-viral sequences at the 5′ ends of Uukuniemi virus (Bunyaviridae): RNA polymerase I-catalyzed transcription of the Lassa virus genome L RNA segment and encoded protein. J Gen Virol 74:19365–19370

91. Karlberg H, Tan YJ, Mirazimi A (2011) Induction of caspase activation and cleavage of the viral nucleocapsid protein in different cell types during Crimean-Congo hemorrhagic fever virus infection. J Biol Chem 286:3227–3234

92. Katoh H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441:101–105

93. Kayagaki N, Phung Q, Chan S, Chaudhari R, Quan C, O'Rourke KM, Eby M, Pietras E, Cheng G, Bazan JF, Zhang Z, Arndt D, Dixit VM (2007) DUBA: a deubiquitinase that regulates type I interferon action. Cell 131:1315–1326

94. Kinsella E, Martin SG, Grolla A, Czub M, Feldmann H, Flick R, Zou W, Lenschow DJ, Snijder EJ, Garcia-Sastre A, Virgin HWt (2007) Structure of Rift Valley Fever virus nucleoprotein reveals endonuclease activity and a gating mechanism for RNA binding. Proc Natl Acad Sci USA 108:19365–19370

95. Kirkin V, Dikic I (2007) Role of ubiquitin- and Ubl-binding domains of RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature 446:916–920

96. Komander D, Clague MJ, Urbe S (2009) Breaking the chains: RING finger deubiquitinases. Science 323:1131–1136

97. Kuhn JH, Altamura LA, Kwilas SA, Bavari S, Poeck H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G (2006) 5′-Triphosphate RNA is the ligand for RIG-I. Science 314:994–997

98. Kuo Y, Wang W, Ji W, Deng M, Sun Y, Zhou H, Yang C, Deng F, Wang H, Hu Z, Lou Z, Rao Z (2012) Crimean-Congo hemorrhagic fever virus nucleoprotein reveals endonuclease activity in bunyaviruses. Proc Natl Acad Sci USA 109:5046–5051
97. Labahn J, Granzin J, Schluckebier G, Robinson DP, Jack WE, Schölkraut I, Saenger W (1994) Three-dimensional structure of the adenine-specific DNA methyltransferase M.Taq I in complex with the cofactor S-adenosylmethionine. Proc Natl Acad Sci USA 91:10957–10961

98. Lawrence DM, Rozanov MN, Hillman BI (1995) Autocatalytic processing of the 223-kDa protein of blueberry scorch carlavirus by a papain-like protease. Virology 207:127–135

99. Li S, Zheng H, Mao AP, Zhong B, Li Y, Gao Y, Ran Y, Tien P, Shu HB (2010) Regulation of virus-triggered signaling by OTUB1- and OTUB2-mediated deubiquitination of TRAF3 and TRAF6. J Biol Chem 285:4291–4297

100. Lopez N, Muller R, Prehaud C, Bouloy M (1995) The L protein of Rift Valley fever virus can rescue viral ribonucleoproteins and transcribe synthetic genome-like RNA molecules. J Virol 69:3972–3979

101. Luini A, Mironov AA, Polishchuk EV, Polishchuk RS (2008) Morphogenesis of post-Golgi transport carriers. Histochem Cell Biol 129:153–161

102. Major L, Linn ML, Slade RW, Schroder WA, Hyatt AD, Gardner J, Cowley J, Suhbri A (2009) Ticks associated with macque island penguins carry arboviruses from four genera. PLoS ONE 4:e4375

103. Makarova KS, Aravind L, Koivunen EV (2000) A superfamily of predicted cysteine proteases from eukaryotes, viruses and Chlamydia pneumoniae. Trends Biochem Sci 25:50–52

104. Malakhov MP, Kim KL, Malakhova OA, Jacobs BS, Borden EC, Zhang DE (2003) High-throughput immunoblotting. Ubiquitin-like protein ISG15 modifies key regulators of signal transduction. J Biol Chem 278:16608–16613

105. Malmgard A, Melchjorsen J, Bowie AG, Mogensen SC, Pala- dan SR (2004) Viral activation of macrophages through TLR-dependent and -independent pathways. J Immunol 173:6890–6898

106. Marczinke BI, Nichol ST (2002) Nairobi sheep disease virus, an important tick-borne pathogen of sheep and goats in Africa, is also present in Asia. Virology 303:146–151

107. Marriott AC, Nuttall PA (1996) Large RNA segment of Dugbe Bunyamwera virus. RNA 15:391–399

108. Marriott AC, Nuttall PA (1996) Large RNA segment of Dugbe Bunyamwera virus. In: Elliott EM (ed) The Bunyaviridae. Plenum Press, New York, London, pp 91–104

109. Mir MA, Brown B, Hjelle B, Duran WA, Panganiban AT (2006) Hantavirus N protein exhibits genus-specific recognition of the viral RNA panhandle. J Virol 80:11283–11292

110. Mohl BP, Barr JN (2009) Investigating the specificity and stoichiometry of RNA binding by the nucleocapsid protein of Bunyamwera virus. RNA 15:391–399

111. Molinari M, Helenius A (2000) Chaperone selection during virus countermeasures. J Gen Virol 81:1775–1780

112. Molinari M, Helenius A (2000) Chaperone selection during virus countermeasures. J Gen Virol 81:1775–1780

113. Montgomery RE (1917) On a tick-borne gastroenteritis of sheep and goats occurring in British East Africa. J Comp Pathol 30:28–57

114. Muller R, Poch O, Delarue M, Bishop DH, Bouloy M (1994) Rift Valley fever virus L segment: correction of the sequence and possible functional role of newly identified regions conserved in RNA-dependent polymerases. J Gen Virol 75(Pt 6):1345–1352

115. Nabeth P, Thior M, Faye O, Simon F (2004) Human Crimean-Congo hemorrhagic fever, Senegal. Emerg Infect Dis 10:1881–1882

116. Nakayama K (1997) Furin: a mammalian subtilisin/Kex2p-like endopeptidase involved in processing of a wide variety of precursor proteins. Biochem J 327(Pt 3):625–635

117. Narasimhan J, Wang M, Fu Z, Klein JM, Haas AL, Kim JJ (2005) Crystal structure of the interferon-induced ubiquitin-like protein ISG15. J Biol Chem 280:27356–27365

118. Nisole S, Said EA, Mische C, Prevost MC, Krust B, Bouvet P, Bianco A, Briand JP, Hovavessian AG (2002) The anti-HIV pentameric pseudopeptide HB-19 binds the C-terminal end of nucleolin and prevents anchorage of virus particles in the plasma membrane of target cells. J Biol Chem 277:20877–20886

119. Niu Y, Zhao X, Wu YS, Li MM, Wang XJ, Yang YG (2013) N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinform 11:8–17

120. Osborne JC, Elliott RM (2000) RNA binding properties of bunyaeviruses and nucleocapsid protein and selective binding to an element in the 5′ terminus of the negative-sense S segment. J Virol 74:9946–9952

121. Papa A, Ma B, Kouidou S, Tang Q, Hang C, Antoniadis A (2002) Genetic characterization of the M RNA segment of Crimean Congo hemorrhagic fever virus strains, China. Emerg Infect Dis 8:50–53

122. Papa A, Christova I, Papadimitriou E, Antoniadis A (2004) Crimean-Congo hemorrhagic fever in Bulgaria. Emerg Infect Dis 10:1465–1467

123. Papa A, Dalla V, Papadimitriou E, Kartalis GN, Antoniadis A (2009) Emergence of Crimean-Congo haemorrhagic fever in Greece. Clin Microbiol Infect (Epub ahead of print)

124. Papa A, Velo E, Papadimitriou E, Cahani G, Kota M, Bino S (2009) Ecology of the Crimean-Congo hemorrhagic fever endemic area in Albania. Vector Borne Zoonotic Dis 9:713–716

125. Parvatiyar K, Barber GN, Harhaj EW (2010) TAX1BP1 and A20 inhibit antiviral signaling by targeting TBK1-IKKı kinases. J Biol Chem 285:14999–15009

126. Paz S, Vilasco M, Arguello M, Sun Q, Lacoste J, Nguyen TL, Zhao T, Shestakova EA, Zaari S, Bibeau-Poirier A, Servant MJ, Lin R, Meurs EP, Hiscott J (2009) Ubiquitin-regulated recruitment of IkappaB kinase epsilon to the MAVS interferon signaling adapter. Mol Cell Biol 29:3401–3412

127. Pettersson RF, von Bonsdorff CH (1975) Ribonucleoproteins of Uukuniemi virus are circular. J Virol 15:386–392

128. Pichlmair A, Schultz O, Tan CP, Naslund TL, Liljestrom P, Weber F, Reis e Sousa C (2006) RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science 314:997–1001

129. Poch O, Sauvaget I, Delarue M, Tordo N (1989) Identification of four conserved motifs among the RNA-dependent polymerase encoding elements. EMBO J 8:3867–3874

130. Qi X, Lan S, Wang W, Scheld LM, Dong H, Wallat GD, Ly H, Li MM, Wang XJ, Yang YG (2013) High-throughput immunoblotting. Ubiquitin-like protein ISG15 modifies key regulators of signal transduction. J Biol Chem 285:14999–15009

131. Quinan BR, de Brito Magalhaes CL, Novaes RF, Dos Santos JR, Marquez de Souza F, Genediate AC, Coelho LR, Canini PC, Monteiro PL, Cardoso MR (2008) Ecology of the Crimean-Congo hemorrhagic fever virus genome. Virus Genes 36:435–437

132. Randall RE, Goodburn S (2008) Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol 89:1–47
1264 L. Lasecka, M. D. Baron

135. Raymond DD, Piper ME, Gerrard SR, Smith JL (2010) Structure of the Rift Valley fever virus nucleocapsid protein reveals another architecture for RNA encapsidation. Proc Natl Acad Sci USA 107:11769–11774

136. Regueria J, Weber F, Cusack S (2010) Bunyaviridae RNA polymerases (L-protein) have an N-terminal, influenza-like endonuclease domain, essential for viral cap-dependent transcription. PLoS Pathog 6:e1001101

137. Richmond KE, Chenault K, Sherwood JL, German TL (1998) Characterization of the nucleic acid binding properties of tomato spotted wilt virus nucleocapsid protein. Virology 248:6–11

138. Ritchie KJ, Zhang DE (2004) ISG15: the immunological kin of ubiquitin. Semin Cell Dev Biol 15:237–246

139. Roberts A, Rossier C, Kolakofsky D, Nathanson N, Gonzalez-Scarano F (1995) Completion of the La Crosse virus genome sequence and genetic comparisons of the L proteins of the Bunyaviridae. Virology 206:742–745

140. Rodal SK, Skretting G, Garred O, Vilhardt F, van Deurs B, Roberts A, Rossier C, Kolakofsky D, Nathanson N, Gonzalez-Scarano F (1999) Extraction of cholesterol with methyl-betacyclodextrin perturbs formation of clathrin-coated endocytic vesicles. Mol Biol Cell 10:961–974

141. Rodrigues R, Paranhos-Bacalla G, Vernet G, Peyretete CN (2012) Crimean-Congo hemorrhagic fever virus-infected HepG2 cells induce ER-stress and apoptosis crosstalk. PLoS One 7:e29712

142. Rwambo PM, Shaw MK, Rurangirwa FR, DeMartini JC (1996) Ultrastructural studies on the replication and morphogenesis of Nairobi sheep disease virus, a Nairovirus. Arch Virol 141:1479–1492

143. Samuel CE (2001) Antiviral actions of interferons. Clin Microbiol Rev 14:778–809

144. Sanchez AJ, Vincent MJ, Nichol ST (2002) Characterization of the glycoproteins of Crimean-Congo hemorrhagic fever virus. J Virol 76:7263–7275

145. Sanchez AJ, Vincent MJ, Erickson BR, Nichol ST (2006) Crimean-Congo hemorrhagic fever virus glycoprotein precursor is cleaved by Furin-like and SKI-1 proteases to generate a novel 38-kilodalton glycoprotein. J Virol 80:514–525

146. Sang R, Onyango A, Gachoucha J, Koenigoi S, Ofula V, Dunster L, Okoth F, Coldren R, Tesh R, da Rossa AT, Finkeiner S, Wang D, Crabtree M, Miller B (2006) Tickborne arbovirus surveillance in market livestock, Nairobi, Kenya. Emerg Infect Dis 12:1074–1080

147. Schmaljohn CS, Hooper JW (2001) Bunyaviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology. Philadelphia, pp 1447–1471

148. Seddiki N, Nisole S, Krust B, Cabellebaut C, Guichard G, Muller S, Briand JP, Hovanessian AG (1999) The V3 loop-mimicking pseudopeptide [Kpsi(CH2N)PR]-TASP inhibits HIV infection in primary macrophage cultures. AIDS Res Hum Retroviruses 15:381–390

149. Semenkovich CF, Ostlund RE Jr, Olson MO, Yang JW (1990) A protein partially expressed on the surface of HepG2 cells that binds lipoproteins specifically is nucleolin. Biochemistry (Mosc) 29:9708–9713

150. Severson W, Xu X, Kuhn M, Semutovitch N, Thokala M, Ferron S, Longhi F, Longhi S, Canard B, Jonsson CB (2005) Essential amino acids of the hantaan virus N protein in its interaction with RNA. J Virol 79:10032–10039

151. Shi H, Huang Y, Zhou H, Song X, Yuan S, Fu Y, Luo Y (2007) Nucleolin is a receptor that mediates antiangiogenic and antitumor activity of endostatin. Blood 110:2899–2906

152. Shin JS, Ebersold M, Pypaert M, Delamarre L, Hartley A, Mellman I (2006) Surface expression of MHC class II in dendritic cells is controlled by regulated ubiquitination. Nature 444:115–118

153. Sieczkowski SB, Whittaker GR (2002) Dissecting virus entry via endocytosis. J Gen Virol 83:1535–1545

154. Simmons G, Wool-Lewis RJ, Baribaud F, Netter RC, Bates P (2002) Ebola virus glycoproteins induce global surface protein down-modulation and loss of cell adherence. J Virol 76:2518–2528

155. Simon M, Johansson C, Lundkvist A, Mirazimi A (2009) Microtubule-dependent and microtubule-independent steps in Crimean-Congo hemorrhagic fever virus replication cycle. Virology 385:313–322

156. Simon M, Johansson C, Mirazimi A (2009) Crimean-Congo hemorrhagic fever virus entry and replication is clathrin-, pH- and cholesterol-dependent. J Gen Virol 90:210–215

157. Staub O (2004) Ubiquitylation and isgylation: overlapping enzymatic cascades do the job. Sci STKE 2004:pe43

158. Sun S, Dai X, Aishan M, Wang X, Meng W, Feng C, Zhang F, Hang C, Hu Z, Zhang Y (2009) Epidemiology and phylogenetic analysis of crimean-congo hemorrhagic fever viruses in xinjiang, china. J Clin Microbiol 47:2536–2543

159. Takahashi K, Halfmann P, Oyama M, Kozuka-Hata H, Noda T, Kawaoa Y (2013) DNA Topoisomerase 1 Facilitates the Transcription and Replication of the Ebola Virus Genome. J Virol 87:8862–8869

160. Tarus B, Bakowiez O, Chenasas V, Duchemin L, Estrozi LF, Bourdieu C, Lejal N, Bernard J, Moudouj M, Chevalier C, Delmas B, Ruigrok RW, Di Primo C, Slama-Schwok A (2012) Oligomerization paths of the nucleoprotein of influenza A virus. Biochimie 94:776–785

161. Tarus B, Chevalier C, Richard CA, Delmas B, Di Primo C, Slama-Schwok A (2012) Molecular dynamics studies of the nucleoprotein of influenza A virus: role of the protein flexibility in RNA binding. PLoS One 7:e30038

162. Tayyari F, Marchant D, Moraes TJ, Duan W, Marangelo P, Hegele RG (2011) Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. Nat Med 17:1132–1135

163. Teragoknar V (2006) NFkappaB pathway: a good signaling paradigm and therapeutic target. Int J Biochem Cell Biol 38:1647–1653

164. Timinskas A, Butkus V, Janulaitis A (1995) Sequence motifs characteristic for DNA [cytosine-N4] and DNA [adenine-N6] methyltransferases. Classification of all DNA methyltransferases. Gene 157:3–11

165. Treib J, Kohler G, Haas A, von Blohn W, Strittmatter M, Pindur G, Froesner G, Schimrigk K (1998) Thunderclap headache caused by Ervirus? Neurology 50:509–511

166. Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, Karin M (2010) Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. Nat Immunol 11:70–75

167. Uilenberg G (1997) General review of tick-borne diseases of sheep and goats world-wide. Parasitologia 39:161–165

168. van Kasteren PB, Beugeling C, Ninaber DK, Frias-Staheli N, van Boheemen S, Garcia-Sastre A, Snijder EJ, Kikkert M (2012) Characterization of the nucleic acid binding properties of tomato spotted wilt virus nucleocapsid protein. Virology 318:153–168

169. Vigerust DJ, Shepherd VL (2007) Virus glycosylation: role in virulence and immune interactions. Trends Microbiol 15:211–218

170. Vincent MJ, Sanchez AJ, Erickson BR, Basak A, Chretien M, Seidah NG, Nichol ST (2003) Crimean-Congo hemorrhagic
fever virus glycoprotein proteolytic processing by subtilase SKI-1. J Virol 77:8640–8649
173. von Bonsdorff CH, Saikku P, Oker-Blom N (1969) The inner structure of Uukuniemi and two Bunyamwera supergroup arboviruses. Virology 39:342–344
174. Wang Y, Dutta S, Karlberg H, Devignot S, Weber F, Hao Q, Tan YJ, Mirazimi A, Kotaka M (2012) Structure of Crimean-Congo hemorrhagic fever virus nucleoprotein: superhelical homooligomers and the role of caspase-3 cleavage. J Virol 86:12294–12303
175. Watret GE, Elliott RM (1985) The proteins and RNAs specified by Clo Mor virus, a Scottish Nairovirus. J Gen Virol 66(Pt 11):2523–2516
176. Watret GE, Pringle CR, Elliott RM (1985) Synthesis of bunyavirus-specific proteins in a continuous cell line (XTC-2) derived from Xenopus laevis. J Gen Virol 66(Pt 3):473–482
177. Watts SL, Garcia-Maruniak A, Maruniak JE (2009) Tensaw virus genome sequence and its relation to other Bunyaviridae. Virus Genes 39:309–318
178. Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR (2006) Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J Virol 80:5059–5064
179. Weber F, Mirazimi A (2008) Interferon and cytokine responses to Crimean Congo hemorrhagic fever virus: an emerging and neglected viral zoonosis. Cytokine Growth Factor Rev 19:395–404
180. Welchman RL, Gordon C, Mayer RJ (2005) Ubiquitin and ubiquitin-like proteins as multifunctional signals. Nat Rev Mol Cell Biol 6:599–609
181. White WR (2008) Nairobi Sheep Disease. In: 7th (ed) Foreign Animal Disease. Boca Publications Group, pp 335–342
182. Whitehouse CA (2004) Crimean-Congo hemorrhagic fever. Antiviral Res 64:145–160
183. Williams RJ, Al-Busaidy S, Mehta FR, Maupin GO, Wagoner KD, Al-Awaidy S, Suleiman AJ, Khan AS, Peters CJ, Ksiazek TG (2000) Crimean-congo hemorrhagic fever: a seroepidemiological and tick survey in the Sultanate of Oman. Trop Med Int Health 5:99–106
184. Woessner R, Grauer MT, Langenbach J, Dobler G, Kroeger J, Mielke HG, Mueller P, Haass A, Treib J (2000) The Ebola virus: possible mode of transmission and reservoir. Infection 28:164–166
185. Xiao X, Feng Y, Zhu Z, Dimitrov DS (2011) Identification of a putative Crimean-Congo hemorrhagic fever virus entry factor. Biochem Biophys Res Commun 411:253–258
186. Xiong Y, Eickbush TH (1990) Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J 9:3353–3362
187. Yadav PD, Vincent MJ, Khristova M, Kale C, Nichol ST, Mishra AC, Mourya DT (2011) Genomic analysis reveals Nairobi sheep disease virus to be highly diverse and present in both Africa, and in India in the form of the Ganjam virus variant. Infect Genet Evol 11:1111–1120
188. Yang ZY, Duckers HJ, Sullivan NI, Sanchez A, Nabel EG, Nabel GJ (2000) Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nat Med 6:886–889
189. Yuan P, Bartlam M, Lou Z, Chen S, Zhou J, He X, Lv Z, Ge R, Li X, Deng T, Fodor E, Rao Z, Liu Y (2009) Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. Nature 458:909–913
190. Zeller HG, Karabatsos N, Calisher CH, Digoutte JP, Cropp CB, Murphy FA, Shope RE (1989) Electron microscopic and antigenic studies of uncharacterized viruses. II. Evidence suggesting the placement of viruses in the family Bunyaviridae. Arch Virol 108:211–227
191. Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, Schulman BA, Huibregtse JM, Krug RM (2004) The UbchH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. Proc Natl Acad Sci USA 101:7578–7582
192. Zhao C, Denison C, Huibregtse JM, Gygi S, Krug RM (2005) Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. Proc Natl Acad Sci USA 102:10200–10205
193. Zhu J, Gopinath K, Murali A, Yi G, Hayward SD, Zhu H, Kao C (2007) RNA-binding proteins that inhibit RNA virus infection. Proc Natl Acad Sci USA 104:3129–3134