Bunyavirus Protein Transport and Assembly*

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1 Introduction

One of the distinctive features of the Bunyaviridae is their intracellular maturation process, which occurs by budding at smooth-surfaced membranes in the Golgi region (MURPHY et al. 1973). Virions bud into the Golgi cisternae and are then transported and released at the cell surface probably by a vesicular transport process utilizing the exocytic pathway. Before assembly of the virus particles, all structural components including proteins as well as viral genome segments must reach the site where virus particles are formed. Recent studies of the envelope glycoproteins have emphasized their role in determining the site of virus maturation. Bunyavirus glycoproteins accumulate at the membranes of the Golgi apparatus prior to virus assembly, indicating that the glycoproteins may serve to direct other structural components to the site of maturation. Recently it has become evident that bunyavirus glycoproteins expressed from cloned cDNAs are retained in the Golgi complex and thus behave similarly to resident proteins of the Golgi complex.

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The present review focuses on the biogenesis of the glycoproteins, including their processing, transport, and possible mechanism of intracellular localization. A series of studies with Uukuniemi virus (UUK) of *Uukuvirus* genus, as well as recent molecular biological approaches using cDNA clones of two phleboviruses, Punta Toro virus (PTV) and Rift Valley fever virus (RVFV), have greatly contributed to understanding the biosynthesis and transport of bunyavirus glycoproteins. The limited information available on the transport of other virus-specific proteins is also summarized.

2 Biogenesis of Proteins Encoded by the M Genome Segment

2.1 Protein Synthesis

Two glycoproteins designated G1 and G2 and, in some genera a nonstructural protein designated NS_M, are encoded by the M genome segment. Sequence analysis (COLLETT et al. 1985; ESHITA and BISHOP 1984; GRADY et al. 1987; FAZAKERLEY et al. 1988; IHARA et al. 1985; LEE et al. 1986; RONNHOLM and PETTERSSON 1987; SCHMALJOHN et al. 1987) as well as the study of mRNA species in virus-infected cells (ESHITA et al. 1985; PARKER et al. 1984) have indicated that these proteins are translated from a single mRNA species as a precursor polyprotein and subsequently cleaved into individual polypeptides. It was also suggested from studies of UUK glycoprotein processing, by synchronized initiation of protein synthesis and short pulse-labeling, that the proteolytic cleavage occurs cotranslationally (KUISMANEN 1984). The designations G1 and G2 have been used for the larger and smaller cleavage products, respectively. However, because of differences in the location of cleavage sites, the gene order of G1 and G2 on the M segment differs among various members of the Bunyaviridae. In the discussion which follows, we therefore sometimes employ the terms "first glycoprotein" and "second glycoprotein" to specify the gene order.

Several hydrophobic regions have been identified in the predicted amino acid sequences of the precursor proteins, as shown in Fig. 1. A hydrophobic region at the amino-terminal end, which precedes the first glycoprotein, is likely to represent a signal peptide sequence. A hydrophobic region at the carboxy-terminal end may function as a membrane-spanning anchor domain of the second glycoprotein. In addition to these terminal hydrophobic sequences, the polyprotein product of the M segment contains several internal hydrophobic regions as well. A hydrophobic region near the carboxy terminus of the first glycoprotein and one preceding the amino terminus of the second glycoprotein could serve as a membrane anchor sequence and as a signal sequence, respectively. The presence of functional internal hydrophobic domains is well

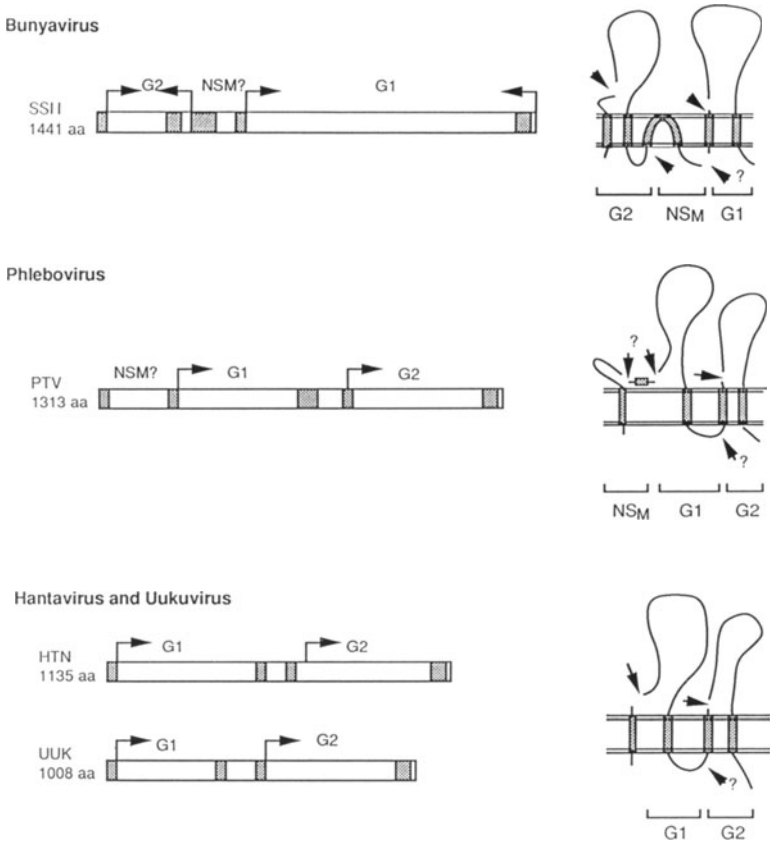

Fig. 1. Gene organization of M genome segments of bunyaviruses and proposed topology of their protein products. The ORF for the precursor protein is shown with hydrophobic regions (■) and known amino termini (┌→) and carboxy termini (←┐) of the G1 and G2 glycoproteins. The predicted transmembrane topology of proteins based on the hydrophobicity profiles is also shown. *Arrows*, polypeptide cleavage sites. References: SSH (ESHITA and BISHOP 1984; FAZAKERLEY et al. 1988), PTV (IHARA et al. 1985), HTN (SCHMALJOHN et al. 1987), UUK (RONNHOLM and PETTERSSON 1987)

documented with the alphavirus precursor polyproteins (HASHIMOTO et al. 1981; RICE and STRAUSS 1981; WIRTH et al. 1977). It is likely that the amino termini of the first glycoprotein are exposed in the lumen of the endoplasmic reticulum (ER) and outside the viral membrane since the N termini follow a typical signal peptide cleavage site and are followed by most of the potential glycosylation sites before another potential transmembrane sequence is found. It was also suggested by a trypsin digestion study of snowshoe hare virus (SSH) that the amino terminus of the second glycoprotein is external (FAZAKERLEY et al. 1988). A model for the transmembrane topology of the glycoproteins of the Bunyaviridae family can be predicted (Fig. 1). Although the exact carboxy terminus of the first glycoprotein for most viruses has not been identified, except for SSH, it is probably located somewhere between the predicted carboxy-terminal anchoring domain of the

first glycoprotein and the signal sequence for the second glycoprotein. The proteolytic cleavage events which generate the two glycoproteins could thus also produce a small peptide and probably a larger nonstructural NS_M molecule for the genus *Bunyavirus*, which may be analogous to the 6-kDa protein found in Semliki Forest virus-infected cells (WELCH and SEFTON 1980). In SSH-infected cells, two small proteins, of 11 and 10 kDa, were identified, and both proteins reacted with antibodies raised against peptides within the predicted NS_M coding region, which is sufficient to code for 19 kDa (FAZAKERLEY et al. 1988). However the exact relationship of these proteins to the NS_M open reading frame (ORF) is not clear.

It was, however, suggested that an alternative translational strategy may exist for the M genome segment of the *Phlebovirus* RVFV. Four polypeptides, 78-kDa, 14-kDa, and the G1 and G2 glycoproteins, were identified as protein products specific for the RVFV M genome segment in RVFV-infected cells as well as in a cell-free transcriptional and translational system (KAKACH et al. 1988, 1989; SUZICH and COLLETT 1988). The biogenesis of these polypeptides was examined by using recombinant vaccinia viruses carrying truncated M genes or M genes with altered potential translational initiation codons (KAKACH et al. 1989; SUZICH et al. 1990). The results suggested that the first and second ATG codons in the same ORF are used for synthesis of the 78-kDa protein and 14-kDa protein, respectively, whereas additional internal in-phase methionine codons as well as the first two ATGs may be utilized for synthesis of the G1 and G2 glycoproteins. Although it is not known whether this translational strategy is used in nature, a similar mechanism which produces multiple protein products from single genes by utilizing internal ATG codons has been also reported for other virus families such as paramyxoviruses (CURRAN and KOLAKOFSKY 1988; MCGINNES et al. 1988) and rhabdoviruses (HERMAN 1986).

2.2 Transport and Glycosylation

All secretory proteins and membrane-bound proteins targeted to various organelles are first synthesized in the rough ER and enter the central vacuolar transport pathway. The organelles involved in this transport system include the rough and smooth ER, the *cis*-, medial, and *trans*-Golgi, the *trans*-Golgi network, secretory vesicles and granules, the endosomal system, lysosomes, and the plasma membrane (KLAUSNER 1989). The envelope glycoproteins of all bunyaviruses so far analyzed possess N-linked oligosaccharide side chains (CASH et al. 1980; PESONEN et al. 1982; SCHMALJOHN et al. 1986). Glycosylation of proteins containing N-linked oligosaccharides is initiated by *en bloc* transfer of preformed oligosaccharide chains from an oligosaccharide pyrophosphoryldolichol intermediate to an asparagine residue in the amino acid sequence Asn-X-Ser/Thr in the newly synthesized polypeptide chain (HUBBARD and IVATT 1981; KLENK and ROTT 1980). Subsequently, sugar residues are modified by enzymes that reside in the ER and the Golgi complex as the glycoproteins are

transported through these organelles (KORNFELD and KORNFELD 1985). Thus, the intracellular transport of the glycoproteins of bunyaviruses as well as other enveloped viruses has been studied by analyzing the oligosaccharides of glycoproteins. Endoglycosidase H (endo H) specifically cleaves high-mannose type oligosaccharide side chains which are the primary sugar residues attached in the rough ER. After removal of glucose and some mannose residues in the ER, the side chains are converted into complex sugar chains by the attachment of the peripheral sugars in the Golgi complex, and these mature forms of sugar chains are no longer susceptible to endo H. Therefore, it is generally considered that the time required for the acquisition of endo H resistance corresponds to the transport time from the ER to the Golgi complex (STROUS and LODISH 1980).

The G1 glycoprotein of La Crosse virus (LAC) and both G1 and G2 glycoproteins of UUK (PESONEN et al. 1982; KUISMANEN 1984), PTV (MATSUOKA et al. 1988), and Hantaan virus (HTN) (SCHMALJOHN et al. 1986) were reported to acquire endo H resistance, but to different extents. In general, the G1 glycoprotein contains complex-type oligosaccharide chains, whereas sugar chains of the G2 glycoproteins are mostly high-mannose type except for HTN in which both glycoproteins seem to contain mostly high-mannose type chains. The G1 and G2 glycoproteins of UUK acquire endo H resistance with a half-life of 45 min and 90–150 min, respectively (KUISMANEN 1984). Similar kinetics were observed for glycoproteins of PTV (CHEN et al. 1990; MATSUOKA et al. 1988) and the G1 glycoprotein of LAC (MADOFF and LENARD 1982). In contrast, the G glycoprotein of vesicular stomatitis virus (VSV) and the influenza virus hemagglutinin (HA) acquire endo H resistance much more rapidly, within 15–20 min after synthesis (COPELAND et al. 1988; DOMS et al. 1987; STROUS and LODISH 1980). Thus, the transport of bunyavirus glycoproteins from the ER to the Golgi complex is a relatively slow process.

After being transported from the ER to the Golgi complex, glycoproteins of bunyaviruses accumulate within the Golgi apparatus where virus assembly takes place. Studies with UUK-infected cells using immunofluorescence and immunoelectron microscopy demonstrated that viral glycoproteins accumulate in the perinuclear region, coinciding with the major binding site for wheat germ hemagglutinin (WGA) which stains predominantly the Golgi membranes (KUISMANEN et al. 1982, 1984). It has been also shown by using a temperature-sensitive mutants ts 12, which is defective for assembly of virus particles at restrictive temperature (GAHMBERG 1984), that the glycoproteins accumulate intracellularly in the absence of virus maturation (GAHMBERG et al. 1986). Recent evidence that glycoprotein molecules are targeted to the Golgi complex in the absence of other viral components (structural or nonstructural) has been obtained from studies of recombinant glycoproteins of PTV (MATSUOKA et al. 1988), HTN (PENSIERO et al. 1988), and RVFV (WASMOEN et al. 1988) which were expressed from cloned cDNA in the recombinant vaccinia expression system. The glycoproteins produced from recombinant viruses were stably retained in the Golgi membranes and were not transported to the cell surface. Thus, early reports of the cell surface expression of glycoproteins in LAC (MADOFF and

LENARD 1982) and UUK (KUISMANEN et al. 1982) virus-infected cells may actually reflect the presence of virus particles transported to the cell surface rather than expression of individual protein molecules.

The precise subcellular localization of the glycoproteins of PTV has been analyzed (CHEN et al. 1991) by using Brefeldin A (BFA), a fungal antibacterial reagent reported to inhibit the transport of proteins out of the ER and cause redistribution of Golgi component to the ER (DOMS et al. 1989; LIPPINCOTT-

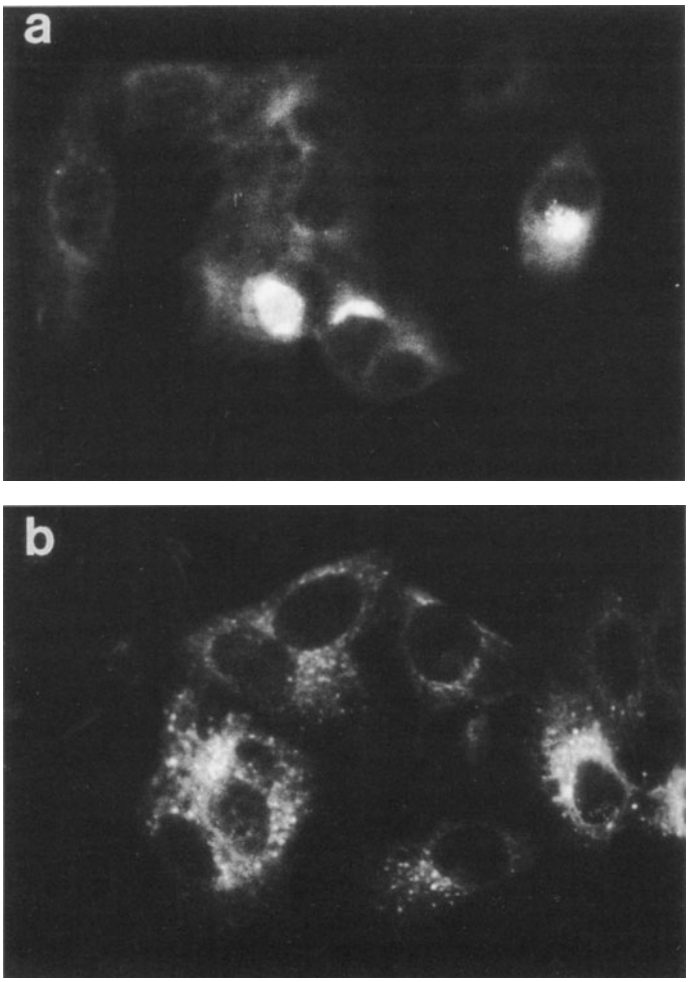

Fig. 2 a, b. Localization of bunyavirus glycoproteins shown by indirect immunofluorescence. **a** PTV glycoproteins expressed from recombinant vaccinia virus showing intracellular retention and accumulation in the Golgi region after 6-h cycloheximide treatment. **b** Effect of BFA treatment showing glycoproteins redistributed throughout the cytoplasm. (From CHEN et al. 1990)

SCHWARTZ et al. 1989; MISUMI et al. 1986; TAKATSUKI and TAMURA 1985). Upon BFA treatment of cells infected with PTV or a recombinant vaccinia virus, PTV glycoproteins which had accumulated in the Golgi region were redistributed throughout the cytoplasm (Fig. 2), whereas little change was observed in the distribution pattern for WGA which has a preferential affinity for the *trans* Golgi network, indicating that PTV glycoproteins specifically localize in the *cis*/medial Golgi membranes (CHEN et al. 1991).

The location and functional roles of other protein products of the M segment are not clear. Two nonstructural proteins are found in SSH-infected cells but do not seem to be glycosylated, and the cellular localization of these proteins has not been reported. The translation products of the predicted NS_M protein coding sequence at the 5′ end of the PTV M segment has not yet been identified either in virions or in infected cells. However, two nonstructural proteins, the glycosylated 78-kDa protein and nonglycosylated 14-kDa protein, are both products from the 5′ end of the coding sequence of the RVFV M genome segment and seem to be localized in the Golgi complex (WASMOEN et al. 1988).

2.3 Possible Mechanisms for Intracellular Transport and Localization

Specific targeting mechanisms must exist for selective transport and localization of proteins in various organelles. The bunyavirus glycoproteins are localized specifically in the membranes of the Golgi complex and thus appear to utilize a similar pathway as that for resident proteins of the Golgi complex. At least two steps which are involved in bunyavirus glycoprotein localization are transport from the ER to the Golgi complex and retention in the Golgi complex. Some proteins remain in the ER as permanent residents, while others are exported to the Golgi complex, most of which are subsequently distributed to various organelles. At least two mechanisms have been demonstrated for retention in the ER. Resident ER proteins may be distinguished from non-resident proteins by the presence of specific signals within the protein sequence. Several soluble proteins including disulfide isomerase, BiP (immunoglobulin heavy-chain binding protein, or grp 78), and others (see review by PELHAM 1989) were found to contain the same C-terminal tetrapeptide sequence, Lys-Asp-Glu-Leu (KDEL). Removal of the KDEL sequence from BiP caused it to be secreted, while addition of this sequence to secretory or lysosomal proteins caused them to accumulate in the ER (MUNRO and PELHAM 1987; PELHAM 1988). Thus, it is evident that the KDEL sequence is responsible for retention of at least some soluble ER specific proteins. Another mechanism to control exit of proteins from the ER has been described as the possession of transport competency. It has become apparent that the correct folding and assembly of proteins is necessary for their efficient transport. A number of mutations intoduced into viral glycoproteins, such as the VSV G protein and influenza HA, were reported to block or slow the export of these proteins from the ER (ROSE and DOMS 1988). Also, for some proteins,

including influenza HA and VSV G protein, assembly into oligomers is required for transport from the ER (COPELAND et al. 1986; DOMS et al. 1988; GETHING et al. 1986; HURTLEY and HELENIUS 1989; KREIS and LODISH 1986). Failure to assemble into an oligomer therefore results in ER retention, without requirement for a specific signal.

In virions, the G1 and G2 glycoproteins of UUK exist as heterodimers (PERSSON et al. 1989). Dimerization seems to occur rapidly after protein synthesis; however, each pair of glycoproteins may not be the cleavage products from the same precursor protein since the transport kinetics for the two glycoproteins were reported to be significantly different; the newly synthesized G1 was incorporated into virions 20 min faster than G2 (PERSSON et al. 1989; WIKSTROM et al. 1989). Similar transport kinetics were also obtained for PTV glycoproteins (CHEN et al. 1991). It was also demonstrated from studies using pulse-chase and subcellular fractionation that UUK G1 and G2 glycoproteins moved rapidly from the ER, and that most of the proteins were shifted to lighter density fractions. Furthermore, the glycoproteins appear to remain in this fraction for 15–20 min before entering the Golgi fraction (WIKSTROM et al. 1989). From these results, it was proposed that the dimerization of UUK glycoproteins occurs at an intermediate compartment between the ER and the Golgi complex. The post-ER compartment which lacks ER markers, such as glucose-6-phosphatase, is also distinct from the Golgi complex. At 15 °C, the membrane proteins of Semliki Forest virus were reported to leave the ER but failed to enter the Golgi cisternae and accumulated in the intermediate vacuolar elements (SARASTE and KUISMANEN 1984). A lysosome protein cathepsin D bearing the ER retention signal (KDEL sequence) accumulated within the ER but appeared to be modified by mannose-6-phosphotransferase, suggesting that the post-ER compartment is the site for the sorting proteins into the Golgi or recycling them back to the ER (PELHAM 1988, 1989). It is possible that bunyavirus glycoproteins accumulate at the same post-ER compartment until they obtain a necessary signal, such as dimerization, for entering the Golgi complex. However, it is still not clear why different populations of G1 and G2 glycoproteins, not derived from the same precursor protein, form dimers. Possibly some conformational change after protein synthesis is necessary for heterodimer formation, and the structural change of the G2 may take longer than that of G1 proteins.

Cell fractionation studies have shown that BiP coprecipitates with UUK glycoproteins from the ER fraction (PERSSON et al. 1989). It was shown that unassembled immunoglobulin heavy chains (BOLE et al. 1986; HAAS and WABL 1983), mutant or unglycosylated forms of influenza HA (COPELAND et al. 1988; GETHING et al. 1986), and immature forms of Sendai virus hemagglutinin neuraminidase (HN) protein (ROUX 1990) all associate with BiP in the ER. However, the significance of the association with BiP for bunyavirus glycoprotein transport is not clear at this point. Protein products from recombinant vaccinia viruses containing only G1 or G2 coding sequence of PTV failed to exit from the ER (CHEN et al. 1991; MATSUOKA, unpublished observation). The recombinant proteins did not seem to associate with BiP since anti-BiP antibody failed to

coprecipitate these proteins. This result may also suggest the importance of an interaction between the two glycoproteins such as dimer formation.

At least one example of a retention signal for a Golgi-specific protein has been defined. The E1 glycoprotein of a coronavirus which also matures at the Golgi membranes contains three membrane-spanning domains. Deletion of the first and second domains resulted the expression of the protein on the cell surface, whereas a deletion of the second and third transmembrane domains resulted in retention in the Golgi complex (MACHAMER and ROSE 1987). These results suggest that the first transmembrane domain contains a Golgi retention signal.

The situation may be more complicated for the bunyavirus glycoproteins, which are derived from a common precursor protein. Do both G1 and G2 glycoproteins contain their own retention signals, or does only one of them possess such a specific signal and interact with the other to halt both in the Golgi? Are extra sequences such as the NS_M coding sequence involved in retention of glycoproteins in the Golgi? A requirement for an NS_M protein in Golgi retention was ruled out at least for phleboviruses. The G1 and G2 glycoproteins expressed from recombinant vaccinia viruses which contain M genome segment sequences of PTV or RVFV with truncated 5′ ends, and thus lack NS_M coding sequence, were synthesized, processed, and transported to the Golgi complex and were stably retained in the Golgi membranes (MATSUOKA et al. 1988; WASMOEN et al. 1988). Therefore, the region preceding the glycoprotein coding sequence of Phleboviruses does not appear to be responsible for the retention of glycoproteins in the Golgi complex.

Based on the results with the E1 glycoprotein of coronavirus, a logical assumption is that one (or more) of the transmembrane regions of the G1 and/or G2 glycoproteins may play an important role for protein retention. Among several hydrophobic regions illustrated in Fig. 1, two hydrophobic sequences near the carboxy terminal of G1 and G2 are predicted to be membrane anchor domains for each protein and might contain a specific retention signal for bunyavirus glycoproteins in the Golgi complex. Construction of molecules with specific deletions, or chimeras between bunyavirus glycoproteins and other glycoproteins which are expressed on cell surfaces, should enable the identification of the sequences which are important for Golgi retention.

Although it has been possible to identify specific amino acid sequences involved as signals for intracellular retention of some viral proteins, no information is available on the mechanisms by which such retention is effected. Some possibilities which may be considered are the following. (a) The sequences could lead to stable interaction with resident cellular molecules; however, the retention of such cellular molecules would then need to be explained. In addition, cellular proteins are not incorporated into budding virus particles, which argues against such a stable interaction. (b) The sorting signals could interact transiently with components of a recycling pathway which retrieves components from other downstream membranes of the exocytic pathway. (c) The sorting signals could mediate lateral interactions between viral glycoproteins leading to

their clustering into localized domains which, because of their curvature or rigidity, may be unable to be incorporated into transport vesicles involved in exit from the Golgi complex.

3 Synthesis and Transport of the Proteins Encoded by the S Genome Segment

The N protein, which is encoded by the S RNA segment, is translated by free ribosomes in the cytoplasm (SCHMALJOHN and PATTERSON 1990). No proteolytic cleavage or posttranslational modification such as phosphorylation has been reported with this protein. The protein is closely associated with the genomic RNAs to form nucleocapsids together with L protein. When and how this interaction occurs is unknown. It was also suggested that N protein controls its own protein synthesis by encapsidating its mRNA in mosquito cells (HACKER et al. 1989). This self-regulation may be related to the establishment of a persistent infection in mosquito cells.

The S segment also encodes a nonstructural polypeptide designated NS_S, although its size and coding strategy varies among the different virus genera (see ELLIOT et al., this volume). It has been reported that the NSs protein of RVFV seems to accumulate in the nucleus in infected cells. High concentrations of NSs were detected in the nuclear fraction from 4 h postinfection (STRUTHERS et al. 1984), and intranuclear fluorescence using immune sheep serum could be observed at 10 h postinfection (STRUTHERS and SWANEPOEL 1982). It is possible that the NSs protein which accumulated in the nucleus may correspond to the intranuclear inclusions in RVFV-infected cells reported previously (SWANEPOEL and BLACKBURN 1977). Neither nuclear inclusions nor intranuclear accumulation of NSs proteins have been reported with other bunyaviruses. The synthesis, processing, and localization of NSs proteins of most bunyaviruses remain to be studied. Monoclonal antibodies against NSs or antibodies raised against NSs-specific polypeptides deduced from known amino acid sequences will be necessary to investigate these processes. The expression of cloned NSs genes, as demonstrated with PTV NSs (OVERTON et al. 1987), will also be a useful for studying the transport and function of this protein.

4 The L Protein

It was shown recently by reassortment that the large (L) segment encodes the L protein (ENDRES et al. 1989). However, little is known about the L protein. Because of its limited quantities both in virions and in infected cells, it is difficult to study

the biogenesis of this protein. L protein is believed to be a viral transcriptase which is present in viral nucleocapsids together with viral genomes and N protein (see KOLAKOFSKY and HACKER, this volume). The nucleocapsids accumulate under the Golgi membranes where viral glycoproteins are concentrated and are incorporated into virions upon budding. Although it is unlikely that the L protein has a role in virus assembly, more data are needed about this protein.

5 Assembly and Release of Bunyavirus

The assembly of the bunyaviruses (Fig. 3) is usually restricted to the Golgi apparatus (KUISMANEN et al. 1982; MURPHY et al. 1973; SMITH and PIFAT 1982) although low levels of budding at the cell surface have also been reported (ANDERSON and SMITH 1987). Although the precise mechanisms of budding of enveloped viruses are not fully understood, it has been suggested that budding involves a transmembrane interaction between membrane glycoproteins and the other components of the virus in the cytoplasm, followed by pinching off from the cell surface (LENARD and COMPANS 1974). Upon budding, virions acquire their lipid bilayer from the host cell membrane, whereas most host cell membrane proteins are excluded from the viral particles. Many viruses, such as orthomyxoviruses, paramyxoviruses, rhabdoviruses, and retroviruses, contain a layer of matrix (M) protein between the lipid bilayer and nucleocapsid. It is evident from studies with VSV that the interaction of the M protein with the nucleocapsid is essential for budding (ONO et al. 1987). However, little is known of the interaction between M protein and viral glycoproteins, although it was suggested that the M protein may function as a bridge between the nucleocapsid and the spike glycoproteins (YOSHIDA et al. 1976). Bunyaviruses, as well as arenaviruses and coronaviruses, do not possess an M protein. For these viruses it was proposed that the binding of the cytoplasmic domain of the glycoprotein to the nucleocapsid was the major driving force for budding (GAROFF and SIMONS 1974; STURMAN et al. 1980).

Electron microscopy studies of sandfly fever viruses (*Phlebovirus*) revealed that viral ribonucleoprotein (RNP) condensed at the cytoplasmic face of the Golgi membranes at areas in which viral envelope glycoproteins accumulated at the luminal face (SMITH and PIFAT 1982). Neither RNP nor glycoproteins were observed on the areas which were not directly involved in budding, suggesting that transmembrane interactions between glycoproteins and RNP are essential for budding (SMITH and PIFAT 1982). It was also indicated by enzymatic digestion that approximately 12% of one or both glycoproteins of Karimabad virus remains at the cytoplasmic membrane face. This portion may thus serve to interact with the RNP complex. The G2 glycoprotein of SSH in which the exact amino and carboxy terminals are defined possesses a long external stretch of amino acids (52) after the hydrophobic region presumed to be a membrane anchoring

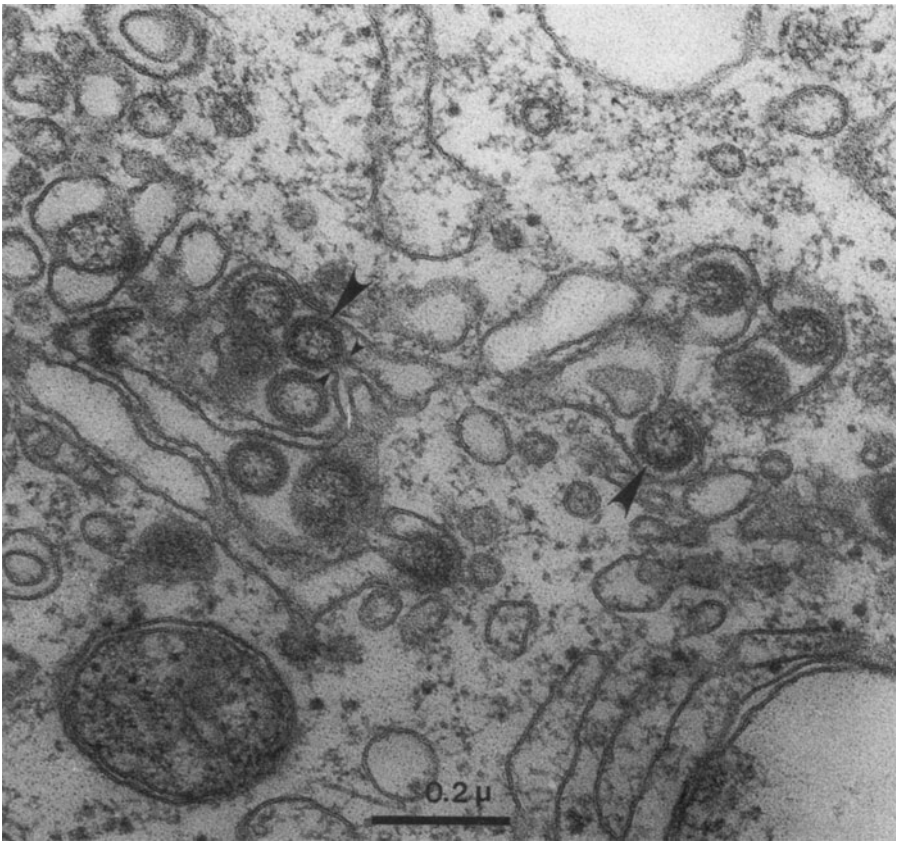

Fig. 3. Assembly of bunyavirus in the Golgi complex. Thin section of PTV-infected Vero cells showing virus particles (arrows) budding from the smooth membrane region of the Golgi apparatus. (From SMITH and PIFAT 1982)

domain, whereas only 20 amino acids are found at the G1 carboxy terminal (FAZAKERLEY et al. 1988). If this 52 amino acid stretch represents the cytoplasmic tail of the G2 glycoprotein of SSH, it may be equivalent to the 12% portion of the Karimabad virus glycoprotein.

Monensin, a carboxylic ionophore which affects the Golgi complex, has been shown to block the transport of membrane as well as secretory proteins from the Golgi complex to the plasma membrane (TARTAKOFF 1983; TARTAKOFF and VASSALLI 1978). Different results have been reported in several studies of the effects of monensin on bunyavirus assembly. In the presence of monensin. Both protein synthesis and virus assembly were inhibited in HTN-infected cells (SCHMALJOHN et al. 1986). In contrast, monensin did not block either virus assembly or the release of SSH bunyavirus particles into culture medium

(ALONSO-CAPLEN et al. 1984). The replication of LAC virus, another member of the *Bunyavirus* genus, was inhibited by monensin, and an accumulation of an incompletely processed form of G1 glycoprotein was observed (CASH 1982; MADOFF and LENARD 1982). This may indicate that proper processing of glycoproteins is required for bunyavirus assembly. The maturation of UUK virus

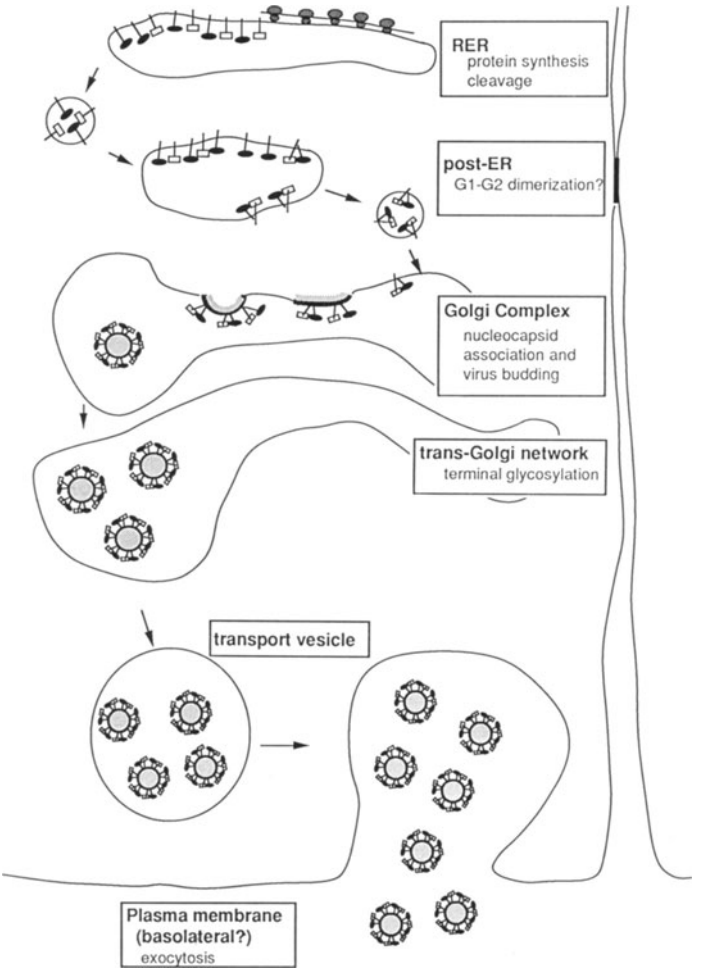

Fig. 4. Model for bunyavirus glycoprotein transport and virus assembly. After translocation into the rough endoplasmic reticulum (*RER*), glycoproteins are transported from the ER to the Golgi complex by acquiring a conformation necessary for the exit from the ER. Virions are assembled by budding at the membranes of the Golgi complex, probably the *cis*/medial Golgi where the glycoproteins accumulate. Virus particles in the Golgi cisternae are further transported through the *trans* Golgi network and released from the cells by exocytosis. In the case of polarized epitherial cells, release appears to occur at the basolateral membranes

was inhibited by monensin at the terminal stage of budding (KUISMANEN et al. 1985). Immunoperoxidase electron microscopy has shown that accumulation of nucleocapsids and viral glycoproteins at the Golgi membranes was not affected by monensin, however, the glycoproteins were assembled into abnormal tubular structures. Surprisingly, monensin did not affect the transport of UUK particles formed before addition of the drug. It is possible that change of the ionic and pH condition of the lumen of the Golgi complex caused by monensin could affect the molecular properties of bunyavirus proteins. In contrast, BFA, which inhibited the transport of proteins out of the ER and caused redistribution of the Golgi-associated proteins, did not affect either protein synthesis or virus maturation in PTV-infected cells (CHEN et al. 1991). During BFA treatment, virions accumulated intracellularly, and as soon as the drug was removed these particles were rapidly transported and released into the medium.

After virus particles bud into the Golgi cisternae, they appear to utilize the exocytic pathway for virus release. The G1 and G2 glycoproteins of mature UUK virions were reported to contain sialic acid (KUISMANEN 1984), indicating that virions assembled in the *cis*/medial Golgi are further transported into the *trans* Golgi where surface glycoproteins are modified by sialyltransferase (KORNFELD and KORNFELD 1985). A schematic pathway of bunyavirus glycoprotein transport and assembly is illustrated in Fig. 4.

The distal side of the *trans* Golgi, designated the *trans* Golgi network, is considered to be a branch point for protein sorting. From here, proteins are sorted into different transport vesicles destined for various organelles (GRIFFITHS and SIMONS 1986). Furthermore, in epithelial cells which maintain apical and basolateral membrane domains separated by tight junctions, it appears that two types of vesicles may exist to transport proteins selectively to the distinct surface domains. Many membrane-associated as well as secretory proteins studied in cultured polarized epithelial cell systems such as Madin-Darby canine kidney cells or human colon carcinoma cells are selectively transported to either or basolateral plasma membrane domains (BURGESS and KELLY 1987; PUGSLEY 1989; SIMONS and FULLER 1985). Assembly of viruses which mature at the plasma membrane is also restricted to the domain to which their glycoproteins are specifically transported; influenza virus and paramyxoviruses are released at apical surfaces and VSV and retroviruses at basolateral surfaces (RODRIGUEZ-BOULAN and SABATINI 1978; ROTH et al. 1983). Bunyavirus particles which mature at the intracellular membranes of the Golgi complex also appear to be transported exclusively to the basolateral domains in polarized epithelial cells after budding. When polarized Vero cells grown on nitrocellulose filters were infected with PTV, the majority of infectious particles were recovered from the basolateral chamber (CHEN et al. 1991). Although the significance of this polarized release is not clear, basolateral release may facilitate spread of the infection to underlying tissues during a systemic infection.

6 Concluding Remarks

The process of virus assembly at intracellular membranes is one of the most interesting features of bunyaviruses. Recent studies have shown that bunyavirus glycoproteins accumulate at the membranes of the *cis*/medial Golgi in the absence of other viral components, clearly indicating their role in determining the intracellular maturation process.

During the past decade, a great deal of information has become available on the molecular biology of bunyaviruses; the sequences of genes, specifically S and M segments, of many bunyaviruses have been determined, and some of the genes have been expressed in various systems. Despite this progress, little is known about certain basic aspects of bunyavirus glycoproteins. Although models for the transmembrane topology of glycoproteins can be predicted from the deduced sequences, direct evidence needs to be obtained concerning the precise configuration of the glycoproteins. In order to determine which portions of the polypeptides are exposed or embedded in the virions (or cell membranes), enzymatic digestion of glycoproteins followed by peptide sequence analysis as well as in vitro analysis of protein synthesis may be necessary. Antibodies against defined peptides of the glycoprotein sequences may also be useful.

The mechanism of intracellular retention and accumulation of bunyavirus glycoproteins is an exciting topic of study. Monoclonal antibodies which can distinguish different maturation stages of the glycoproteins will be powerful tools to study intracellular transport of these proteins. In addition, glycoprotein genes can be dissected at the molecular level by using recombinant DNA approaches, and the exact signals for the retention of these glycoproteins in the Golgi complex may soon be identified. The study of the biogenesis of bunyavirus glycoproteins also provides a useful model system for understanding the mechanisms of biosynthesis and sorting of the Golgi-specific proteins in general.

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References

Alonso-Caplen FV, Matsuoka Y, Compans RW (1984) Transport of viral glycoproteins and its modulation by monensin. In: Compans RW, Bishop DHL (eds) Segmented negative strand viruses. Academic, New York, pp 179–188

Anderson GW Jr, Smith JF (1987) Immunoelectron microscopy of Rift Valley fever viral morphogenesis in primary rat hepatocytes. Virology 161: 91–100

Bole DG, Hendershot LM, Kearney JF (1986) Posttranslational association of immunoglobulin heavy-chain binding-protein with nascent heavy chains in non-secreting and secreting hybridomas. J Cell Biol 102: 1558–1566

Burgess TL, Kelly RB (1987) Constitutive and regulated secretion of proteins. Annu Rev Cell Biol 3: 243–293

Cash P (1982) Inhibition of La Crosse virus replication by monensin, a monovalent ionophore. J Gen Virol 59: 193196

Cash P, Hendershot L, Bishop DHL (1980) The effect of glycosylation inhibitors on the maturation and intracellular polypeptide synthesis induced by snowshoe hare bunyavirus. Virology 103: 235–240

Chen SY, Matsuoka Y, Compans RW (1991) Assembly and polarized release of Punta Toro virus and effect of brefeldin A. J Virol 65: 1427–1439

Collett MS, Purchio AF, Keegan K, Frazier S, Hays W, Anderson DK, Parker MD, Schmaljohn C, Schmidt J, Dalrymple JM (1985) Complete nucleotide sequence of the M RNA segment of Rift Valley fever virus. Virology 144: 228–245

Copeland CS, Doms RW, Bolzau EM, Webster RG, Helenius A (1986) Assembly of influenza hemagglutinin trimers and its role in intracellular transport. J Cell Biol 103: 1179–1191

Copeland CS, Zimmer KP, Wagner KR, Healey GA, Melman I, Helenius A (1988) Folding, trimerization and transport are sequential events in the biogenesis of influenza virus hemagglutinin. Cell 53: 197–209

Curran J, Kolakofsky D (1988) Ribosomal initiation from an ACG codon in the Sendai virus P/C mRNA. EMBO J 7: 245–251

Doms RW, Keller DS, Helenius A, Balch WE (1987) Role for adenosine triphosphate in regulating the assembly and transport of vesicular stomatitis virus G Protein trimers. J Cell Biol 105: 1957–1969

Doms RW, Ruusala A, Machamer C, Helenius A, Rose JK (1988) Differential effects of mutations in three domains on folding, quaternary structure, and intracellular transport of vesicular stomatitis virus G protein. J Cell Biol 107: 89–99

Doms RW, Russ G, Yewdell JW (1989) Brefeldin A redistributes resident and itinerant Golgi proteins to the endoplasmic reticulum. J Cell Biol 109: 61–72

Endres MJ, Jacoby DR, Janssen RS, Gonzalez-Scarano F, Nathanson N (1989) The large viral RNA segment of California serogroup bunyaviruses encodes the large viral protein. J Gen Virol 70: 223–228

Eshita Y, Bishop DHL (1984) The complete sequence of the M RNA of snowshoe hare; bunyavirus reveals the presence of internal hydrophobic domains in the viral glycoprotein. Virology 137: 227–240

Eshita Y, Ericson B, Romanowski V, Bishop DHL (1985) Analysis of the mRNA transcription processes of snowshoe hare bunyavirus S and M RNA species. J Virol 55: 681–689

Fazakerley JK, Gonzalez-Scarano F, Strickler J, Dietzschold B, Karush F, Nathanson N (1988) Organization of the middle RNA segment of snowshoe hare bunyavirus. Virology 167: 422–43

Gahmberg N (1984) Characterization of two recombination-complementation groups of Uukuniemi virus temperature-sensitive mutants. J Gen Virol 65: 1079–1090

Gahmberg N, Kuismanen E, Keranen S, Pettersson RF (1986) Uukuniemi virus glycoproteins accumulate in the cause morphological changes of the Golgi complex in the absence of virus maturation. J Virol 57: 899–906

Garoff H, Simons K (1974) Location of the spike glycoproteins in the Semliki Forest virus membrane. Proc Natl Acad Sci USA 71: 3988–3992

Gething M-J, McCammon K, Sambrook J (1986) Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. Cell 46: 939–950

Grady LJ, Sanders ML, Campbell WP (1987) The sequence of the M RNA of an isolate of La Crosse virus. J Gen Virol 68: 3057–3071

Griffiths G, Simons K (1986) The trans Golgi network: sorting at the exit site of the Golgi complex. Science 234: 438–443

Haas IG, Wabl M (1983) Immunoglobulin heavy chain binding protein. Nature 306: 387–389

Hacker D, Raju R, Kolakofsky D (1989) La Crosse virus nucleopsid protein controls its own synthesis in mosquito cells by encapsidating its mRNA. J Virol 63: 5166–5174

Hashimoto K, Erdei S, Karanen S, Saraste J, Kaarianen L (1981) Evidence for a separate signal sequence for the carboxy-terminal envelope glycoprotein E1 of Semliki Forest virus. J Virol 38: 24–40

Herman RC (1986) Internal initiation of translation of the vesicular stomatitis virus phosphoprotein mRNA yields a second protein. J Virol 58: 797–804

Hubbard SC, Ivatt RJ (1981) Synthesis and processing of asparagine-linked oligosaccharides. Annu Rev Biochem 50: 55–83

Hurtley SM, Helenius A (1989) Control of protein exit from the endoplasmic reticulum. Ann Rev Cell Biol 5: 277–307

Ihara T, Smith J, Dalrymple JM, Bishop DHL (1985) Complete sequence of the glycoproteins and M RNA of Punta Toro phlebovirus compared to those of Rift Valley fever virus. Virology 144: 246–259

Kakach LT, Wasmoen TL, Collett MS (1988) Rift Valley fever virus M segment: use of recombinant vaccinia viruses to study *Phlebovirus* gene expression. J Virol 62: 826–833

Kakach LT, Suzich JA, Colett MS (1989) Rift Valley fever virus M segment: phlebovirus expression strategy and protein glycosylation. Virology 170: 505–510

Klausner RD (1989) Sorting and traffic in the central vacuolar system. Cell 57: 703–706

Klenk HD, Rott R (1980) Cotranslational and posttranslational processing of viral glycoproteins. In: Arber W et al. (eds) Current topics in microbiology and immunology, vol 90. Springer, Berlin Heidelberg New York, pp 19–48

Kornfeld R, Kornfeld S (1985) Assembly of asparagine linked oligosaccharides. Annu Rev Biochem 54: 631–644

Kreis TE, Lodish HF (1986) Oligomerization is essential for transport of vesicular stomatitis virus viral glycoprotein on the cell surface. Cell 46: 929–937

Kuismanen E (1984) Posttranslational processing of Uukuniemi virus glycoproteins G1 and G2. J Virol 51: 806–812

Kuismanen E, Hedman K, Saraste J, Pettersson RF (1982) Uukuniemi virus maturation: accumulation of virus particles and viral antigens in the Golgi complex. Mol Cell Biol 2: 1444–1458

Kuismanen E, Bang B, Hurme M, Pettersson RF (1984) Uukuniemi virus maturation: immunofluorescence microscopy with monoclonal glycoprotein-specific antibodies. J Virol 51: 137–146

Kuismanen E, Saraste J, Pettersson RF (1985) Effect of monensin on the assembly of Uukuniemi virus in the Golgi complex. J Virol 55: 813–822

Lees JF, Pringle CR, Elliott RM (1986) Nucleotide sequence of the bunyamwera virus M RNA segment: conservation of structural feature in the bunyavirus glycoprotein gene product. Virology 148: 1–14

Lenard J, Compans RW (1974) The membrane structure of lipid-containing viruses. Biochim Biophys Acta 334: 51–94

Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD (1989) Rapid redistribution of Golgi proteins into the ER in cells treated with Brefeldin A: evidence for membrane cycling from Golgi to ER. Cell 56: 801–813

Machamer CE, Rose JK (1987) A specific transmembrane domain of a coronavirus E1 glycoprotein is required for its retention in the Golgi region. J Cell Biol 105: 1205–1214

Madoff DH, Lenard J (1982) A membrane glycoprotein that accumulates intracellularly: cellular processing of the large glycoprotein of La Crosse virus. Cell 28: 821–829

Matsuoka Y, Ihara T, Bishop DHL, Compans RW (1988) Intracellular accumulation of Punta Toro virus glycoproteins expressed from cloned cDNA. Virology 167: 251–260

McGinnes L, McQuain C, Morrison T (1988) The P protein and the nonstructural 38 K and 29 K proteins of Newcastle disease virus are derived from the same open reading rame. Virology 164: 256–264

Misumi Y, Misumi, Miki K, Takatsuki A, Tamura G, Ikehara Y (1986) Novel blockage by Brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. J Biol Chem 261: 11398–11403

Munro S, Pelham HRB (1987) A C-terminal signal prevents secretion of luminal ER proteins. Cell 48: 899–907

Murphy FA, Harrison AK, Whitfield SG (1973) Bunyaviridae: morphologic and morphogenetic similarities of bunyamwera serologic supergroup viruses and several other arthropod borne viruses. Intervirology 1: 297–316

Ono K, Dubois-Dalcq ME, Schubert M, Lazzarini RA (1987) A mutated membrane protein of vesicular stomatitis virus has an abnormal distribution within the infected cell and causes defective budding. J Virol 61: 1332–1341

Overton HA, Ihara T, Bishop DHL (1987) Identification of the N and NSs proteins coded by the ambisense S RNA of Punta Toro phlebovirus using monospecific antisera raised to baculovirus expressed N and NSs proteins. Virology 157: 338–350

Parker MD, Smith JF, Dalrymple JM (1984) Rift Valley fever virus intracellular RNA: a functional analysis. In: Compans RW, Bishop DHL (eds) Segmented negative strand viruses. Academic, Orlando, pp 21–28

Pelham HRB (1988) Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment. EMBO J 7: 913–918
Pelham HRB (1989) Control of protein exit from the endoplasmic reticulum. Annu Rev Cell Biol 5: 1–23
Pensiero MN, Jennings GB, Schmaljohn CS, Hay J (1988) Expression of the Hantaan virus M genome segment by using a vaccinia virus recombinant. J Virus 62: 696–702
Persson R, Wikstrom L, Pettersson RF (1989) Dimerization and intracellular transport of two Golgi-specific bunyavirus membrane glycoproteins. In: Compans RW, Helenius A, Oldstone MBA (eds) UCLA symposium on cell biology of virus entry, replication and pathogenesis. Liss, New York, pp 229–242
Pesonen M, Kuismanen E, Pettersson RF (1982) Monosaccharide sequence of protein-bound glycans of Uukuniemi virus. J Virol 41: 390–400
Pugsley AP (1989) Protein targeting. Academic, San Diego
Rice CM, Strauss JH (1981) Nucleotide sequence of the 265 mRNA of Sindbis virus and deduced sequence of the encoded virus structural proteins. Proc Natl Acad Sci USA 78: 2062–2066
Rodriguez-Boulan E, Sabaitni DD (1978) Asymmetric budding of viruses in epithelial monolayers: a model system for study of epithelial polarity. Proc Natl Acad Sci USA 75: 5071–5075
Ronnholm R, Pettersson RE (1987) Complete nucleotide sequence of the M RNA segment of Uukuniemi virus encoding the membrane glycoproteins G1 and G2. Virology 160: 191–202
Rose JK, Doms RW (1988) Regulation of protein export from the endoplasmic reticulum. Annu Rev Cell Biol 4: 257–288
Roth MG, Srinivas RV, Compans (1983) Basolateral maturation of retroviruses in polarized epithelial cells. J Virol 45: 1065–1073
Roux L (1990) Selective and transient association of Sendai virus HN glycoprotein with BiP. Virology 175: 161–166
Saraste J, Kuismanen E (1984) Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. Cell 38: 535–549
Schmaljohn CS, Patterson JL (1990) Bunyaviridae and their replication. In: Fields BN et al. (eds) Virology. Raven, New York, pp 1175–1194
Schmaljohn CS, Hasty SE, Rasmussen L, Dalrymple JM (1986) Hantaan virus replication: effects of monensin, tunicamycin and encoglycosidase on the structural glycoproteins. J Gen Virol 67: 707–717
Schmaljohn CS, Schmaljohn AL, Dalrymple JM (1987) Hantaan virus M RNA: coding strategy, nucleotide sequence, and gene order. Virology 157: 31–39
Simons K, Fuller SD (1985) Cell surface polarity in epithelia. Annu Rev Cell Biol 1: 243–288
Smith JF, Pifat DY (1982) Morphogenesis of sandfly fever viruses (Bunyaviridae family). Virology 121: 61–81
Strous GJAM, Lodish HF (1980) Intracellular transport of secretory and membrane proteins in hepatoma cells infected with vesicular stomatitis virus. Cell 22: 709–717
Struthers JK, Swanepoel R (1982) Identification of a major non-structural protein in the nuclei of Rift Valley fever virus-infected cells. J Gen Virol 60: 381–384
Struthers JK, Swanepoel R, Shepherd SP (1984) Protein synthesis in Rift Valley fever virus-infected cells. Virology 134: 118–124
Sturman LS, Holmes KV, Behnke J (1980) Isolation of coronavirus envelope glycoprotein with the viral nucleocapsid. J Virol 33: 449–462
Suzich JA, Collett MS (1988) Rift Valley fever virus M segment: cell-free transcription and translation of virus complementary RNA. Virology 164: 478–486
Suzich JA, Kakach LT, Collett MS (1990) Expression strategy of a phlebovirus: biogenesis of proteins from the Rift Valley fever M segment. J Virol 64: 1549–1555
Swanepoel R, Blackburn NK (1977) Demonstration of nuclear immunofluorescence in Rift Valley fever infected cells. J Gen Virol 34: 557–561
Takatsuki A, Tamura G (1985) Brefeldin A, a specific inhibitor of intracellular translocation of vesicular stomatitis virus G protein: intracellular accumulation of high-mannose type G protein and inhibition of its cell surface expression. Agric Biol Chem 49: 899–902
Tartakoff AM (1983) Perturbation of vesicular traffic with the carboxylic ionophore monensin. Cell 32: 1026–1028
Tartakoff AM, Vassalli D (1978) Comparative studies of intracellular transport of secretory proteins. J Cell Biol 79: 694–707
Wasmoen TL, Kakach LT, Collett MS (1988) Rift Valley fever virus M segment: cellular localization of M segment-encoded proteins. Virology 166: 275–280

Welch WJ, Sefton BM (1980) Characterization of a small, nonstructural viral polypeptide present late during infection of BHK cells by Semliki Forest virus. J Virol 33: 230–237

Wikstrom L, Persson R, Pettersson RF (1989) Intracellular transport of the G1 and G2 membrane glycoproteins of Uukuniemi virus. In: Kolakofsky D, Mahy B (eds) Genetics and pathogenicity of negative strand virus. Elsevier, New York, pp 33–41

Wirth DF, Katz F, Small B, Lodish HF (1977) How a single Sindbis virus mRNA directs the synthesis of one soluble protein and two integral membrane glycoproteins. Cell 10: 253–263

Yoshida J, Nagai U, Yoshii S, Maeno K, Matsumoto T (1976) Membrane (M) protein of HVJ (Sendai virus): its role in virus assembly. Virology 71: 143–161