Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Targeting of viral glycoproteins to the Golgi complex

Tom C. Hobman

The study of enveloped viruses has proved to be invaluable in illuminating how eukaryotic cells contend with the problem of transporting and sorting proteins within the exocytic pathway. Owing to the simplistic nature of these viruses, most rely entirely on the host cell to synthesize, transport and sort their protein components to the appropriate locations. Consequently, viral proteins follow the same pathways as endogenous host proteins. The exterior of the viruses consists of a host-derived lipid envelope containing one or more membrane-spanning glycoproteins. The interior of the virion contains RNA or DNA, usually complexed with capsid proteins to form a nucleocapsid. Viral glycoproteins are synthesized on the endoplasmic reticulum (ER) and are then transported vectorially through the Golgi to the cell surface. Most enveloped viruses assemble at the plasma membrane; however, a limited number of viruses are known to bud into intracellular membranes of the ER, intermediate compartment, Golgi and inner nuclear envelope (for detailed reviews, see Refs 1, 2). This review focuses on viruses that bud into Golgi and Golgi-associated membranes.

Exactly why some viruses have evolved to assemble on membranes other than the plasma membrane is unclear. Perhaps by limiting the amount of viral antigen on the cell surface, the virus reduces the chances of complement-mediated lysis of the host cell. For viruses that replicate or assemble relatively slowly this would be advantageous. All the simple RNA viruses that are known to bud into the Golgi are capable of establishing persistent infections. In vivo, establishing and maintaining a persistent infection would certainly be facilitated by limiting the amount of viral protein exposed to the host immune system.

The Golgi complex

In mammalian cells, the Golgi complex is located in the perinuclear region and consists of a variable number of flattened cisternae with dilated rims, arranged parallel to each other. Current models of the Golgi invoke three main compartments, each with different biochemical functions and distinct morphology (for recent reviews on the Golgi complex, see Refs 3–5). Proximal to the ER is the cis-Golgi network (CGN), followed by the medial Golgi stacks and then the trans-Golgi network (TGN). The CGN has been proposed to include the vacuolar elements of the intermediate or salvage compartment as well as the osmophilic fenestrated cisternae of the cis-Golgi. This region is thought to function mainly as the entry site of the Golgi and in recycling of proteins and lipids between the ER and Golgi (for a recent review, see Ref. 6). During transport to the cell surface, the density of viral glycoproteins in Golgi membranes is approximately eightfold greater than in membranes of the ER and it is likely that the concentration step occurs during transport from the ER to the Golgi.

Although it remains to be determined exactly where within the Golgi complex the trimming of mannose residues from asparagine-linked (N-linked) glycans first occurs (i.e. by the action of α-mannosidase I), it is now widely believed that most processing of N-linked carbohydrates occurs in the medial Golgi stacks. Accordingly, enzymes such as α-mannosidase II, N-acetylglucosaminyltransferases and fucosyltransferase reside within the central Golgi stacks. The action of these enzymes is highly sequential and substrate-specific, and it is probably not necessary that each enzyme be vectorially confined to a specific cisterna. In support of this notion, cisternal overlap of previously termed medial and trans-Golgi enzymes has recently been documented.

Terminal glycosylation (addition of galactose and sialic acid) and, for some glycoproteins, sulfation and/or site-specific endoproteolytic cleavages occur in the trans-Golgi and/or TGN. The TGN is also the exit site of the Golgi, where proteins destined for endocytic compartments (and secretory granules in cells involved in regulated secretion) are sorted into clathrin-coated vesicles, while those destined for the plasma membrane exit the Golgi in non-clathrin-coated transport vesicles.

Virus assembly on Golgi membranes

Budding results from interactions between the cytoplasmic domains of viral membrane glycoproteins.
and the nucleoprotein complexes that form the core of the virion. The latter consist of the viral genomic RNA or DNA complexed with capsid proteins. In the case of negative-stranded RNA viruses, a transcriptase or replicase is a minor component of the viral core. The budding mechanisms are poorly understood, but the process ultimately results in envelopment of the viral core by a host-derived membrane studded with viral membrane proteins. Newly formed virions are released into the extracellular space or into the lumen of an intracellular compartment. In the latter case, virions must then traverse the exocytic pathway and be extruded from the cell much like a secretory protein.

Assembly on Golgi membranes is presumably mediated by the targeting of one or more of the structural proteins to this organelle (Fig. 1). However, the capability of a virus to bud into Golgi membranes is not necessarily dependent on the intrinsic targeting information contained within its structural proteins. Rather, the residency time and consequently concentration of viral membrane proteins within a particular intracellular membrane is likely to be a key determinant. For example, Semliki Forest virus (SFV), which normally buds at the cell surface, can be induced to assemble on Golgi membranes when infected cells are treated with monensin. Monensin is a carboxylic ionophore that causes the pH of the Golgi to increase, which often results in a block in protein transport through this organelle. Consequently, SFV spike glycoproteins (which normally assemble on these membranes) into Golgi cisternae but does not interfere with binding of nucleocapsids to membranes. However, blocking transport of bunyavirus glycoproteins to the Golgi with brefeldin A allows virion assembly in the ER. In contrast, monensin inhibits budding of bunyaviruses (which normally assemble on these membranes) into Golgi cisternae but does not interfere with budding of nucleocapsids to membranes. However, blocking transport of bunyavirus glycoproteins to the Golgi with brefeldin A allows virion assembly in the ER.

Of the limited number of viruses that assemble on Golgi membranes, the best studied by far are the coronaviruses and bunyaviruses. Rubella virus, although not as widely studied, has also been observed to bud into Golgi membranes in certain cell types. All these viruses are enveloped RNA viruses, and although they mature on or near Golgi membranes, they have different genome structures, replication strategies, and structural protein expression patterns. Poxviruses have also been suggested to acquire their envelopes from Golgi or associated membranes, but they will not be discussed in this review (see Refs 1, 2).

**Coronaviruses**

Coronaviruses are positive-stranded RNA viruses whose genomes consist of a single (positive polarity) RNA molecule (approximately 27 kb), which associates in the cytoplasm with N protein (a phosphoprotein) to form a helical nucleocapsid. Virus assembly takes place on smooth membrane pre-Golgi elements variously referred to as the intermediate compartment or CGN. However, depending on the particular virus, cell type and time post-infection, virions are also seen to bud into Golgi cisternae and rough ER. Virion size is relatively uniform for murine coronaviruses (90 nm) but the avian types vary considerably (70–120 nm). Viral proteins are encoded by a set of nested subgenomic mRNAs that share a common 3′ end while differing in their 5′ ends.

The viral membrane contains two species of protein: E1 or M (membrane) protein, and E2 or S (spike) protein. S protein (180 kDa) is the larger of the two, and is a type I membrane glycoprotein consisting of a large luminal amino-terminal domain (preceded by a cleavable signal sequence), followed by a single membrane-spanning region and a small carboxy-terminal cytoplasmic domain. S forms a homotrimer and is transported to the plasma membrane. Its functions include cell attachment and fusion activity; however, it is not required for virion formation.

M protein (25 kDa) spans the membrane three times and contains a short glycosylated amino-terminal domain that is luminally disposed, while the larger carboxy-terminal region is exposed to the cytoplasm and is thought to bind to nucleocapsids. In M protein from the prototypical murine hepatitis virus strain A59 (MHV A59), each membrane-spanning domain functions independently as an orientation-specific signal or anchor domain. Unlike S, the distribution of M protein is restricted to intracellular membranes and was therefore previously thought to dictate the site of virus assembly. Expression of M protein from cDNA clones indicates that in the absence of other viral glycoproteins it is targeted distal to the virus budding site, i.e. the Golgi complex. M protein from avian infectious bronchitis virus (IBV) is retained in the cis-Golgi and remains predominantly sensitive to endo H (which cleaves unprocessed asparagine-linked glycans). In contrast, the analogous glycoprotein from MHV A59 (which contains only O-linked sugars) is transported as far as the TGN.

Retention of the avian and murine M glycoproteins in the Golgi appears to involve different domains and possibly different mechanisms. Machamer and colleagues have demonstrated that the Golgi retention signal of IBV M is contained within the first membrane-spanning domain of the protein. This was the first demonstration that Golgi targeting information resides within a transmembrane domain. In contrast, deletion of the various transmembrane domains from the MHV A59 M glycoproteins results in retention in the ER (domains 2 and 3) or transport to lysosomes (domains 1 and 2). Deletion of the carboxy-terminal 18 amino acids from MHV A59 M
In each case, the viral glycoprotein spike consists of two membrane glycoproteins represented by black and white bars. Synthesis of the viral glycoproteins takes place on membranes of the ER. (a) In the absence of heterodimer formation, most unassembled glycoprotein subunits accumulate in the ER whereas properly assembled subunits are transported to and retained in the Golgi complex where viral assembly occurs. The Golgi retention domain could be located on one or both subunits in this case. This scenario is analogous to those of HTV and RV. In the latter case, however, unassembled E1 subunits accumulate in a smooth membrane compartment distinct from but connected to, the rough ER. (b) Here, the Golgi retention signal is located on the white subunit since unassembled black subunits are transported to the plasma membrane. Monomeric white subunits are retained in the ER until they complex with black subunits. The heterodimer is retained at the budding site by the retention signal in the white subunit. (c) Monomeric black subunits are transported to the plasma membrane, whereas white subunits are transported only as far as the Golgi complex. Heterodimers are also retained in the Golgi complex, because the Golgi retention signal is in the white subunit. This situation is analogous to PTV, where G2 (which is transported to the plasma membrane in the absence of G1) is thought to be retained at the budding site via its interaction with G1. (d) Unassembled black subunits do not leave the ER whereas white subunits are transported to the Golgi complex. Again, the signal for Golgi retention is only on one subunit (white). Black subunits are transported out of the ER to the budding site by complexing with white subunits. This situation may be analogous to UUK and possibly PTV. (e) This pathway may be used by coronaviruses. Unassembled M subunits (white) are transported to the Golgi (TGN for MHV A59 or cis-Golgi for IBV); black subunits (S) are slowly transported to the plasma membrane in the absence of heterodimer formation. Heterodimer accumulation in the CGN or intermediate compartment may be facilitated by the slow rate of transport of the larger black subunits.

Protein results in its transport to the cell surface; however, this peptide region is not sufficient to retain the cell surface protein Thy-1 in the Golgi region. After fusion of Thy-1 to the entire M protein molecule, the chimeric protein appears to be retained in the Golgi. The apparent discrepancies between the mechanisms of Golgi retention of the avian and murine coronavirus M glycoproteins remains to be resolved.

To date, endogenous membrane proteins that have been localized to the Golgi membranes are type II membrane proteins (i.e. mannosidases and various...
glycosyltransferases) and the information for Golgi retention is invariably contained within the transmembrane domain and in some cases the flanking cytoplasmic and luminal domains (see Ref. 26). Studies using brefeldin A suggest that the TGN may be a distinct and separate entity from the rest of the Golgi27-29. These experiments indicate that, unlike resident proteins of the Golgi stacks, proteins in the TGN do not seem to redistribute back to the ER during brefeldin A treatment. It is therefore quite possible that proteins may be retained in the TGN via a different mechanism. Accordingly, IBV and MHV A59 M proteins may be retained in the Golgi by different means.

Assembly of coronaviruses in the CGN would presumably require targeting of M protein to this region, since it is required for budding. This would necessitate preventing the bulk of M protein from being transported to the Golgi, since this is distal to the budding site. Two ways in which this could be achieved are: (1) binding of M protein to other viral proteins, to prevent or delay its entry into the Golgi (Fig. 1e), or (2) retrieval of M protein from the Golgi stacks to the budding site. The two possible mechanisms need not be mutually exclusive; however, the former seems more likely in light of recent work by Rottier and colleagues. This group now has evidence that heteroligomerization between M and S proteins occurs in infected cells3. Although S is transported to the plasma membrane, the rate of transport is extremely slow. The rate-limiting step in transport appears to be transfer of the glycoprotein from the ER to the Golgi (t1/2 for acquisition of endo H resistance is 60 min in infected cells and 180 min when S protein is expressed via cDNA37). From this study it has been proposed that the prolonged transport of S from the ER to the Golgi may be instrumental in virus budding in this region. It is also possible that binding of nucleocapsids to the cytoplasmic domain of M protein may modulate its intracellular transport.

Bunyaviruses

The Bunyaviridae is a family of negative-stranded RNA viruses containing upwards of 300 members (for extensive reviews on bunyaviruses, see Refs 30, 31). Virions are spherical (90-100 nm) and contain three major protein species (G1, G2 and N), and a minor protein component (L), which is an RNA-dependent RNA polymerase. N protein associates with the tripartite negative-strand genome to form helical nucleocapsids. Assembly on Golgi membranes is directed by the targeting of the two envelope glycoproteins G1 (70-120 kDa) and G2 (30-63 kDa) to this organelle. G1 and G2 are both type I membrane glycoproteins that are derived via proteolytic cleavages from a common polyprotein precursor encoded by the M RNA segment. The amino termini of G1 and G2 from the precursor. In infected cells, or in cells expressing G1 and G2 together from cDNA, the glycoproteins are localized almost exclusively to the Golgi; very little antigen is detectable on the cell surface. The proteins form a heterodimer in the ER and are presumably retained as such in the Golgi.

Evidence is now increasing that for at least Uukunenim (UUK) and Punta Toro viruses (PTV), G2 is maintained in the Golgi via its interaction with G1.

Although G1 and G2 can be targeted independently to the ER, the ability of the unassembled subunits to exit the ER varies between viruses. For example, with PTV (Fig. 1c) G2 is transported to the cell surface in the absence of G1, while G1 expressed alone accumulates in the Golgi region of the cell32. This situation is different from that of the UUK virus (Fig. 1d) where in the absence of G1, G2 is retained in the ER, whereas G1 accumulates in Golgi-like structures33. Recently, two conflicting studies regarding the fate of individually expressed Hantaan virus (HTV) glycoproteins have been published. Pensiero and Hay concluded that G1 was transported to the Golgi in the absence of G2, while the latter was retained in the ER when expressed alone34. In contrast, Ruusala et al. found that both G1 and G2 were retained in the rough ER when expressed alone from cDNA (Fig. 1a). The opposing results are difficult to reconcile since the same HTV cDNAs were used in both studies, although different cell lines and expression methodologies were used. Ruusala et al.35 based their conclusions upon data from indirect immunofluorescence, cell fractionation and biosynthetic labeling experiments (together with endo H digestion), while Pensiero and Hay provided only indirect immunofluorescence data.

Compared to glycoproteins from viruses that assemble at the plasma membrane (e.g. VSV G protein, influenza hemagglutinin), transport of bunyavirus glycoproteins to the Golgi, as measured by acquisition of endo H resistance, is three to four times slower (i.e. t1/2 = 45-90 min). By monitoring the oxidation of cysteine residues on newly synthesized G1 and G2, it has been found that G1 acquires its tertiary structure much faster than G2 (10 min versus 60 min)36. These results suggest that the relatively slow transport of the G1-G2 heterodimer from the ER to the Golgi may be regulated by the slow folding of G2.

Morphological studies and analysis of asparagine-linked oligosaccharides suggest that transport of bunyavirus heterodimers is arrested proximal to the TGN (i.e. glycoproteins become partially or completely endo H resistant, depending on the virus, but for the most part are not sialylated). Accumulation of the viral glycoproteins reportedly causes a progressive vacuolization of Golgi cisternae even in the absence of virus budding. The viral assembly process is preceded by alignment of nucleocapsids (N protein and RNA) along Golgi membranes containing G1 and G2. Accordingly, N protein has been detected in this region by indirect immunofluorescence and by immunoelectron microscopy. Binding of nucleocapsids to Golgi membranes is believed to occur via
Fig. 2. Ultrathin cryosections of CHO cells stably transfected with genes encoding RV glycoproteins were incubated with monoclonal antibodies to RV E1 glycoprotein followed by rabbit anti-mouse IgG (used as a bridging antibody) and then goat anti-rabbit IgG coupled to 5 nm gold particles. Notice how only the Golgi cisternae (Gc) are labeled while the lumen of the rough ER (er) is not. Similar results were obtained using a monoclonal to E2. This indicates that the RV glycoproteins are efficiently transported to the Golgi. The scale bar represents 1.0 µm. Reproduced from Ref. 49, with permission.

Rubella virus

Rubella virus (RV) is the sole member of the genus Rubivirus within the family Togaviridae. Virions are spherical and approximately 60 nm in diameter; they contain two type I membrane glycoproteins, E2 and E1, and a cytosolic capsid (C) protein that associates with the positive-strand genomic RNA. The three structural proteins are proteolytically derived from a polypeptide precursor encoded by a subgenomic RNA that corresponds to the 3' one-third of the genomic RNA. Both E2 (42-47 kDa) and E1 (57-65 kDa) contain independently functioning cleavable signal peptides at their amino termini that direct translocation into the ER. Experimental evidence suggests that two signal peptidase cleavages may be the only proteolytic events necessary to generate C, E2 and E1 from the polyprotein precursor (Refs 46, 47; T.C. Hobman and S. Gillam, unpublished). Depending on the cell type, RV has been reported to bud from both cell surface (Vero cells) and intracellular membranes (BHK-21 cells). Expression of E2 and E1 from cDNA (M33 strain) indicates that the glycoproteins are targeted to the Golgi complex of both these cell types and others (Fig. 2), with very little antigen found on the cell surface. One potentially important factor that could explain the discrepancy is that different strains of RV were used in the two studies. This phenomenon of differential budding in different cell types is relatively unusual and very intriguing, and can only be resolved by cloning the genes for the structural proteins of the 'Bat' strain of RV (Ref. 48) and expressing the cDNA in various cell types. Unfortunately, this work has not yet been followed up.

E2 and E1 form a heterodimer in the ER shortly after synthesis and are transported to the Golgi as such. Retention of the heterodimer in the Golgi thus directs the process of virus budding into this organelle. Capsid protein also accumulates in the Golgi region by a process that depends on E2 and E1 (Ref. 30). In the absence of E2, E1 is able to exit the rough ER and accumulates in a pre-Golgi compartment that consists of a network of branched tubular membranes. Without E1, the majority of E2 is not able to exit the rough ER although, depending on the level of expression, it can be detected in membranes of the Golgi and to a lesser extent at the cell surface. Therefore, both RV glycoprotein subunits are necessary for efficient transport from the site of synthesis to the budding site (Fig. 1a).

E2 and E1 become resistant to endo H and are partially sialylated, suggesting that they are transported beyond the medial Golgi. While E2 appears to contain only N-linked carbohydrates, E2 contains both N-linked and O-linked sugars. Mature E2 is heterogeneous in size (42-47 kDa) because of the differential processing of the carbohydrate structures that make up to 35% of its apparent mass. E1 appears to fold more slowly than E2, and this process
may modulate heterodimer formation and subsequent transport to the Golgi. Accumulation of RV glycoproteins in the Golgi does not prevent transport of other viral glycoproteins through this organelle. Similar results have been reported for UUK virus glycoproteins.

Mechanisms of Golgi retention At this point, the mechanisms for retention of proteins in the Golgi are purely speculative. Currently, the most favored model suggests that Golgi proteins form large oligomeric structures whose size prevents them from entering transport vesicles. Work by Swift and Machamer is certainly consistent with this model. Their study shows that a chimeric protein retained in the Golgi region (VSV G protein in which the transmembrane domain is replaced by the first membrane-spanning domain from IBV M) forms large aggregates shortly after synthesis. However, in transfected COS cells the chimeric glycoprotein remains sensitive to endo H, and since its Golgi localization has not been verified by electron microscopy, it cannot be concluded unequivocally that this protein is targeted to the Golgi. For example, RV E1 glycoprotein accumulates in pre-Golgi structures located in the Golgi region, which by light microscopy are indistinguishable from bona fide Golgi elements in COS cells. Assuming that Golgi membrane proteins are assembled into large oligomeric complexes, formation of the aggregates is presumably facilitated by interactions between the transmembrane domains and possibly stabilized by flanking luminal and cytoplasmic regions. It has been shown that deletion of the transmembrane and cytoplasmic regions of Golgi proteins results in secretion of the luminal domain into the extracellular space. Conceivably, the Golgi membrane proteins may form a network or lattice structure that consists of more than one protein species. This may be particularly true for the carbohydrate-processing enzymes of the Golgi. In contrast, viral membrane glycoproteins would be expected to form a homogeneous lattice or patch since cellular membrane proteins are excluded from viral envelopes during the budding process. Interestingly, VSV G protein, which is blocked in the TGN by incubation at 20°C, forms regular arrays in the Golgi membranes. It is tempting to speculate that such arrays or lattices are formed by resident Golgi proteins. To test this, antibodies to the cytoplasmic domains of Golgi-specific viral glycoproteins (and endogenous Golgi proteins) could be used to study the lateral mobility of proteins in isolated Golgi preparations. If large oligomers are formed, resident Golgi proteins would be expected to be less mobile and therefore exhibit less lateral diffusion in Golgi membranes than proteins in transit through this organelle.

Retention of membrane proteins in the TGN may involve a different mechanism than in the Golgi stacks. TGN 38 is a type I membrane protein that resides primarily in the TGN. Localization of this protein to the TGN is mediated by its carboxy-terminal cytoplasmic tail, which contains a critical 11 amino acid tyrosine-based motif. MHV M is at least partially dependent upon its cytoplasmically exposed carboxyl terminus for retention in the TGN; however, unlike the analogous segment in TGN 38, this domain is not sufficient to retain a reporter protein at this location. Recently, it was demonstrated that in contrast to the results obtained with membrane proteins of the Golgi stack, overexpression of TGN 38 abolishes its intracellular localization and instead results in its appearance at the plasma membrane. These results strongly suggest that TGN 38 and possibly other TGN membrane proteins are maintained at their locale by a receptor-mediated retrieval process.

Where next? The study of viruses that assemble at the Golgi complex has greatly furthered our understanding of this important organelle. A Golgi retention signal was first localized in the transmembrane domain of a virus glycoprotein, which in turn expedited the discovery of other retention signals and in and around the transmembrane domains of other Golgi proteins. Intracellularly retained viral glycoproteins are proving to be useful as model Golgi membrane proteins, and will undoubtedly shed light onto the mechanism(s) of retention of resident proteins in the Golgi. Studies are well under way to characterize the Golgi targeting signals in the glycoproteins of PTV, UUK and RV by the construction of deletion mutants and by transposing domains from these viral glycoproteins into cell surface proteins to verify that the retention signals function in a trans dominant manner. In fact, peptide segments that include the carboxy-terminal transmembrane regions of PTV G1 and RV E2 glycoproteins appear to contain Golgi targeting information. Fusion of these segments from PTV G1 and RV E2 to the ectodomains of glycoproteins that are normally transported to the cell surface results in retention of the chimeras in the Golgi (S.-Y. Chen et al., submitted; T.C. Hobman and M.G. Farquhar, submitted). In addition, recent results suggest that UUK G1 glycoprotein may contain a Golgi retention signal (R. Persson et al., submitted). As yet, no obvious consensus sequence within the various Golgi retention domains has been identified.

It will also be important to verify the Golgi localization of these and other apparently Golgi-retained proteins by immunoelectron microscopy, since in some cases pre-Golgi compartments cannot be distinguished from Golgi at the light microscopic level. Because the various viral membrane proteins seem to be retained in different regions of the Golgi, they will undoubtedly prove useful in addressing the intriguing problem of intra-Golgi localization. Again, these studies immunoelectron microscopy will be indispensable.

Machamer and colleagues are attempting to investigate the problem of Golgi retention from a genetic angle by screening for conditional mutants of yeast that are unable to retain a Golgi reporter protein. This approach has been enormously successful in identifying genes that encode proteins that operate
along the exocytic pathway. More often than not, the yeast proteins have homologous counterparts that perform identical functions in mammalian cells (which in general are not amenable to the same genetic manipulations as yeast).

In addition to determining how viral proteins are retained at the budding site, other questions about the assembly process remain to be answered. What drives the budding reaction? UUK and RV glycoproteins require considerable time in the ER for heterodimer formation (and possibly assembly into even higher order structures), so do nucleocapsids first bind to the endodomain of these glycoproteins before they are transported to the Golgi? If yes, then what normally prevents virus assembly in the ER? An important clue comes from the work of Liu and Brown, who suggest that viral spike complexes can undergo dramatic rearrangements between the Golgi and the ER lumen, whereas when the mature glycoprotein (and its partner E1) reach the cell surface, this domain is now cytoplasmically disposed and presumably able to bind to nucleocapsid complexes. It seems unlikely that this would be a general mechanism used by all viruses that assemble at the Golgi since brefeldin A treatment prevents virus assembly in the ER? An important clue that, at least for this particular virus, transport to the Golgi is not a prerequisite for nucleocapsid binding.

Therefore a multitude of other factors, including host cell type and time post-infection, are likely to influence where a particular virus assembles within a cell. Solutions to these and related problems are now within our grasp.

Acknowledgements
I thank Peter Rottier, Ralf Pettersson and Richard Compan for providing me with material prior to publication, and Rina Sacco for help with Fig. 1.

References
1 Pettersson, R.F. (1991) *Curr. Top. Microbiol. Immunol.* 170, 67–106
2 Griffiths, G. and Rottier, P. (1992) *Semin. Cell Biol.* 3, 367–381
3 Duden, R., Allan, V. and Kreis, T. (1991) *Trends Cell Biol.* 1, 14–19
4 Farquhar, M.G. (1991) *In Intracellular Trafficking of Proteins* (Steele, G. and Hanover, J., eds), pp. 431–471, Cambridge University Press
5 Mellman, I. and Simons, K. (1992) *Cell* 68, 829–840
6 Saraste, J. and Kuismanen, E. (1992) *Semin. Cell Biol.* 3, 343–355
7 Quinn, P., Griffiths, G. and Warren, G. (1984) *J. Cell Biol.* 98, 2142–2147
8 Plumer, H. et al. (1992) *J. Cell Biol.* 119, 1097–1116
9 Nilsson, T. et al. (1993) *J. Cell Biol.* 120, 5–13
10 Griffiths, G., Quinn, P. and Warren, G. (1983) *J. Cell Biol.* 96, 835–850
11 Kuismanen, E., Saraste, J. and Pettersson, R.F. (1985) *J. Virol.* 55, 813–822
12 Chen, S-Y., Matsuoka, Y. and Compan, R.W. (1991) *J. Virol.* 65, 1427–1439
13 Griffiths, G. et al. (1985) *J. Cell Biol.* 101, 949–964
14 Tooze, J., Tooze, S. and Warren, G. (1984) *Eur. J. Cell Biol.* 33, 281–293
15 Tooze, S.A., Tooze, J. and Warren, G. (1988) *J. Cell Biol.* 106, 1475–1487
16 Delmas, B. and Laude, H. (1992) *J. Virol.* 66, 5367–5375
17 Vennema, H. et al. (1990) *J. Virol.* 64, 339–346
18 Krinsje Locker, J. et al. (1992) *J. Biol. Chem.* 267, 21911–21918
19 Machamer, C.E. and Rose, J.K. (1987) *J. Cell Biol.* 105, 1203–1214
20 Rottier, P.J.M. and Rose, J.K. (1987) *J. Virol.* 61, 2042–2045
21 Machamer, C.E. et al. (1990) *Proc. Natl Acad. Sci. USA* 87, 6944–6948
22 Niemann, H. et al. (1982) *EMBO J.* 1, 1499–1504
23 Krinsje Locker, J. et al. (1992) *J. Biol. Chem.* 267, 14094–14101
24 Armstrong, J., Patel, S. and Riddle, P. (1990) *J. Cell Sci.* 95, 191–197
25 Armstrong, J. and Patel, S. (1991) *J. Cell Sci.* 98, 567–575
26 Machamer, C.E. (1991) *Trends Cell Biol.* 1, 141–144
27 Chege, N.W. and Pfeffer, S.R. (1990) *J. Cell Biol.* 111, 893–900
28 Reaves, B. and Banting, G. (1992) *J. Cell Biol.* 116, 85–94
29 Ladinsky, M.S. and Howell, K.E. (1992) *Eur. J. Cell Biol.* 59, 92–105
30 Pettersson, R.F. et al. (1988) *Mod. Cell Biol.* 6, 65–96
31 Elliott, R.M. (1990) *J. Gen. Virol.* 71, 501–522
32 Chen, S-Y., Matsuoka, Y. and Compan, R.W. (1991) *Virology* 183, 351–365
33 Ronnholm, R. (1992) *J. Virol.* 66, 4525–4531
34 Pensiero, M.N. and Has, J. (1992) *J. Virol.* 66, 1907–1914
35 Riusals, A. et al. (1992) *Virology* 186, 53–64
36 Persson, R. and Pettersson, R.F. (1991) *J. Cell Biol.* 112, 257–266
37 Kuismanen, E. et al. (1982) *Mol. Cell. Biol.* 2, 1444–1458
38 Pesonen, M., Kuismanen, E. and Pettersson, R.F. (1982) *J. Virol.* 41, 390–400
39 Chen, S-Y. and Compan, R.W. (1991) *J. Virol.* 65, 5902–5909
40 Kuismanen, E. (1984) *J. Virol.* 51, 806–812
41 Gahmberg, N. et al. (1986) *J. Virol.* 57, 899–906
42 Oker-Blom, C. et al. (1983) *J. Virol.* 46, 964–973
43 Oker-Blom, C. et al. (1984) *J. Virol.* 49, 403–408
44 Hobman, T.C., Shukin, R. and Gillam, S. (1991) *J. Virol.* 62, 4259–4264
45 Hobman, T.C. and Gillam, S. (1989) *Virology* 173, 241–250
46 Suomalainen, M., Caroff, H. and Barren, M.D. (1990) *J. Virol.* 64, 5500–5509
47 Baron, M.D. and Forsell, K. (1991) *Virology* 185, 811–819
48 Bardelti, G., Tektoff, J. and Gautheron, D. (1979) *Interivirology* 11, 97–103
49 Hobman, T.C., Woodward, L. and Farquhar, M.G. (1993) *J. Cell Biol.* 121, 269–281
50 Hobman, T.C., Lundstrom, M.L. and Gillam, S. (1990) *Virology* 178, 122–123
51 Hobman, T.C., Woodward, L. and Farquhar, M.G. (1992) *J. Cell Biol.* 118, 795–811
52 Lundstrom, M.L., Mauracher, C.A. and Tingle, A.J. (1991) *J. Gen. Virol.* 72, 843–850
53 Gahmberg, N., Pettersson, R.F. and Kaariainen, L. (1986) *EMBO J.* 5, 3111–3118
54 Swift, A.M. and Machamer, C.E. (1991) *J. Cell Biol.* 115, 19–30
55 Colley, K.J. et al. (1989) *J. Biol. Chem.* 264, 17619–17622
56 Luzio, J.P. et al. (1990) *Biochem. J.* 270, 97–102
57 Humphrey, J.S. et al. (1993) *J. Cell Biol.* 120, 1123–1135
58 Liu, N. and Brown, D.T. (1993) *J. Cell Biol.* 120, 877–883