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.

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Letter to the Editor

Identification of an ER Retrieval Signal in a Retroviral Glycoprotein

Soluble or membrane-spanning proteins that are resident in the endoplasmic reticulum (ER) possess short amino acid sequence motifs that result in their localization in the ER by retrieval, retention, or both. One such protein targeting signal, the carboxy-terminal tetrapeptide KDEL (Munro and Pelham, 1987), retrieves soluble proteins from the Golgi complex to the ER lumen by interacting with the KDEL receptor (Lewis and Pelham, 1992). A second consensus motif, consisting of two lysines located at positions −3 and either −4 or −5 from the cytoplasmic carboxyl terminus, was identified in 14 of 15 ER resident type 1 membrane proteins (Jackson et al., 1990) (Table 1). Proteins with the dilysine signal are retrieved from post-ER compartments to the ER (Jackson et al., 1993) by cytosolic coat proteins (COPs) controlling retrograde vesicular transport (Cosson and Letourneur, 1994; Letourneur et al., 1994). Type 2 membrane proteins that reside in the ER possess an analogous double-arginine ER localization signal at their cytoplasmic amino termini (Schutze et al., 1994).

Enveloped viruses bud through the cellular membrane to which their glycoproteins sort (reviewed by Stephens and Compans, 1988). Some viral glycoproteins sort to the specialized apical or basolateral plasma membrane domains of polarized epithelial cells and direct virion budding across those membranes. For example, gp160 of human immunodeficiency virus type 1 (HIV-1) sorts to the basolateral domain (Owens et al., 1991; Lodge et al., 1994), while hemagglutinin of influenza virus sorts to the apical domain (Roth et al., 1983). To mediate budding of infectious viral particles through intracellular membranes, viral glycoproteins must fulfill two requirements: a signal for sorting to an intracellular compartment and an interaction with the core proteins of the virus. For example, membrane-spanning domains of the M protein of coronaviruses (Machamer and Rose, 1987; Swift and Machamer, 1991) or the G1 protein of bunyaviruses (Matsuoka et al., 1994) specify glycoprotein accumulation at the Golgi complex. The adenovirus glycoprotein E19 possesses a dilysine signal that localizes it to the ER (Jackson et al., 1990) where it binds class I molecules to diminish recognition of adenovirus-infected cells by cytotoxic T lymphocytes (Cox et al., 1991).

The foamy viruses are a genus of retroviruses that infect a wide variety of mammalian hosts; e.g., they are ubiquitous in nonhuman primates, bovines, and felines and occasionally infect humans and other mammals (Hooks and Gibbs, 1975). However, foamy viruses have yet to be associated definitively with any disease process (Weiss, 1988; Mergia and Luciw, 1991; Neumann-Haefelin et al., 1993). Similar to certain classic oncoviruses, the foamy viruses utilize a B/D-type virus assembly strategy by which immature viral capsids assemble in the cytoplasm in advance of virion budding (Achong et al., 1971; Hooks and Gibbs, 1975). As demonstrated by electron microscopy, the characteristic foamy cytopathic effect is due to syncytium formation and a proliferation of swollen, intracytoplasmic membrane-bound structures. Furthermore, foamy viruses bud intracellularly into these cytoplasmic structures and also from the plasma membrane (Hooks and Gibbs, 1975).

What mechanisms cause the foamy viruses to bud at intracellular membranes, a site of maturation that is unusual among infectious retroviruses? We hypothesized that foamy virus glycoproteins must possess a specific signal for sorting to an intracellular compartment of the secretory pathway. We then searched foamy virus glycoprotein sequences for the presence of known intracellular compartment retention/retrieval motifs. Viral sequences were obtained from the original publications or from the National Center for Biotechnology Information database. Remarkably, we identified the dilysine ER retrieval signal at the cytoplasmic carboxyl termini of four of the five available foamy virus glycoprotein sequences (Table 2). The bovine syncytial virus (BSV) possessed a lysine in postion −3 and arginines in positions −4, −5, and −6, similar to the rabbit 53 kDa sarcoplasmic reticulum protein (SER 53Kd; see Table 1). It is likely that the arginines in positions −4, −5, and −6 of BSV compensate for the lack of lysine in positions −4 or −5, as was suggested for SER 53Kd (Jackson et al., 1990). The conservation of this well-characterized ER localization signal within the glycoproteins of foamy viruses of human, chimpanzee, rhesus macaque, African green monkey, or bovine origin suggested that it fulfills a critical function in foamy virus biology. The dilysine motif was not present in the glycoproteins of lentiviruses, oncoviruses, or an intracisternal A particle (data not shown).

This finding allowed the formulation of a working mecha-

Table 1. The Dilysine Motif in ER Resident Type 1 Membrane Proteins

Protein	Amino Acid Sequence	Reference
E19 protein of adenovirus serotype 3	RPRQSNEE**KEK**MP	Jackson et al., 1990
Human UDP-glucuronosyltransferase	KGRVKKAH**KSK**TH	Jackson et al., 1990
Human high mobility group coenzyme A reductase	LQDLQGTC**TKK**SA	Jackson et al., 1990
SER 53Kd	TGCGTPKN**RYK**KH	Jackson et al., 1990

The carboxy-terminal amino acid sequences of E19 protein of adenovirus serotype 3 and three other ER resident type 1 membrane proteins are shown. The lysines at position −3, −4, or −5 (shown in bold) have been experimentally determined to be essential for localization of these proteins to the ER (Jackson et al., 1990).

Table 2. The Dilysine Motif in Glycoproteins of Five Foamy Viruses

Protein	Amino Acid Sequence	Reference
Human foamy virus	K I VSW I PT**KKK**NQ	Flugel et al., 1987
Simian foamy virus of chimpanzee origin	K I VSW I PT**KKK**SQ	Herchenroeder et al., 1994
Simian foamy virus of rhesus macaque origin	K I I SWLPG**K**LKKN	Mergia et al., 1990
Simian foamy virus of African green monkey origin	K I VSWLPG**KKK**RN	Renne et al., 1992
BSV	RWLAVE**RRRK**QE	Renshaw and Casey, 1994

The carboxy-terminal cytoplasmic domains of glycoproteins of the five foamy viruses for which glycoprotein sequences are available. The lysines at positions −3 and either −4 or −5 (shown in bold) represent the dilysine motif.

nistic model for foamy virus assembly. First, following translation, protein folding, and oligomerization, foamy virus glycoproteins follow the secretory pathway out of the ER. Second, in early post-ER compartments, the glycoproteins meet one of two possible fates—budding or retrieval—mediated by their cytoplasmically exposed glycoprotein tails. If the glycoprotein cytoplasmic tails interact with preassembled viral capsids, which may localize to the same intracellular site, coordinated budding of glycoprotein-bearing infectious virus particles into an early post-ER compartment results. If the glycoprotein dilysine signal interacts with the cytoplasmic coatomer complex, retrieval of the glycoprotein from the Golgi to the ER by retrograde vesicular transport results. Viral glycoproteins reaching the Golgi are thereby recycled and may complete a budding interaction on their next transit through the early post-ER compartment. Third, the prominent cytoplasmic vacuolization that is the hallmark of foamy virus infection is a cytopathic effect of this virus on the secretory pathway. Fourth, since tissue cultures infected with foamy viruses form syncytia and since virus budding from the plasma membrane occurs in some cell types (Gelderblom and Frank, 1987), foamy virus glycoproteins must also escape through the Golgi stacks by the default secretory pathway. This implies either that the interactions of glycoprotein cytoplasmic tails with viral capsids or COPs are saturable or that competitive interactions with other proteins (or viral cytopathic effects) limit the efficiency of budding or retrieval and create the third possible fate for the glycoproteins—escape to the plasma membrane. The post-ER intermediate compartment protein ERGIC-53, which also possesses a dilysine signal, was reported to saturate its pre-Golgi retention machinery when overexpressed, leading to cell surface expression of ERGIC-53 (Kappeler et al., 1994).

Teleologically, foamy viruses appear to have evolved a novel assembly strategy to ensure survival. The role of the ER retrieval signal in the biology of these superior symbiotic viruses can be established with biochemical and genetic experimental approaches. Furthermore, the foamy viruses may be useful probes of the newly appreciated role of coatomer (reviewed by Pelham, 1994) in selective retrograde transport of membrane proteins from the Golgi complex to the ER.

P. A. Goepfert,* G. Wang,† and M. J. Mulligan*†
*Department of Medicine
†Department of Microbiology
The University of Alabama at Birmingham
Birmingham, Alabama 35294-2170

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