The distinct protein and lipid constituents of the apical and basolateral membranes in polarized cells are sorted by specific signals. O-Glycosylation of a highly polarized intestinal brush-border protein sucrase isomaltase is implicated in its apical sorting through interaction with sphingolipid-cholesterol microdomains. We characterized the structural determinants required for this mechanism by focusing on two major domains in pro-SI, the membrane anchor and the Ser/Thr-rich stalk domain. Deletion mutants lacking either domain, pro-SI_{LAST} (stalk-free) and pro-SI_{AMA} (membrane anchor-free), were constructed and expressed in polarized Madin-Darby canine kidney cells. In the absence of the membrane anchoring domain, pro-SI_{AMA} does not associate with lipid rafts and the mutant is randomly delivered to both membranes. Therefore, the O-glycosylated stalk region is not sufficient per se for the high fidelity of apical sorting of pro-SI. Pro-SI_{LAST} does not associate either with lipid rafts and its targeting behavior is similar to that of pro-SI_{AMA}. Only wild type pro-SI containing both determinants, the stalk region and membrane anchor, associates with lipid microdomains and is targeted correctly to the apical membrane. However, not all sequences in the stalk region are required for apical sorting. Only O-glycosylation of a stretch of 12 amino acids (Ala_{37}-Pro_{48}) juxtapose the membrane anchor is required in conjunction with the membrane anchoring domain for correct targeting of pro-SI to the apical membrane. Other O-glycosylated domains within the stalk (Ala_{49}-Pro_{57}) are not sufficient for apical sorting. We conclude that the recognition signal for apical sorting of pro-SI comprises O-glycosylation of the Ala_{37}-Pro_{48} stretch and requires the presence of the membrane anchoring domain.

Biological membranes of polarized cells contain an asymmetrical distribution of lipid and protein components (1–4). Both kinds of biomolecules are sorted in the trans-Golgi network complex into different types of vesicles for apical or basolateral delivery (5, 6). Whereas all basolateral sorting signals of membrane proteins described to date reside in the cytoplasmic domains (7, 8), apical signals appear to be luminal (7, 9–11). One criterion for apical delivery could be the presence of asparagine-linked carbohydrates, since their removal by tunicamycin treatment or site-directed mutagenesis results in nonpolar secretion (12, 13). Additionally, insertion of novel N-glycosylation sites into the normally randomly secreted rat growth hormone leads to apical secretion (14). Further evidence has accumulated that some apical membrane proteins accumulate in sphingolipid- and cholesterol-rich microdomains (15), which have been termed sphingolipid-cholesterol rafts. Glycophosphatidylinositol-anchored membrane proteins as well as trans-membrane proteins, like the hemagglutinin of influenza virus (16) or intestinal brush-border proteins like sucrase isomaltase and dipeptidyl peptidase (17) are associated with lipid rafts. Rafts can be discriminated from other membraneous components based on their insolubility in nonionic detergents like Triton X-100 or CHAPS at low temperature.

By virtue of its structural features and sorting behavior sucrase isomaltase (SI, EC 3.2.1.48; 10) constitutes an exquisite model protein to identify apical sorting signals and to unravel molecular mechanisms underlying apical targeting. SI is an intestinal transmembrane protein that is apically targeted in a highly polarized manner in association with lipid rafts (17, 18). It is a heavily N- and O-glycosylated protein that is composed of two homologous subunits, sucrase and isomaltase. Inhibition or drastic reduction of O-glycosylation in Caco-2 cells by using benzyl-N-acetylgalactosaminide substantially affects the high sorting fidelity of pro-SI ending with a random delivery of the protein to both membranes (19). Importantly, O-linked glycans mediate apical sorting through association with lipid rafts. Pro-SI contains a stretch of a Ser/Thr-rich domain in immediate proximity of the membrane (20). Similar domains, referred to as stalk regions, have been also described for glycoporphin (21), the neurotrophin receptor (22) or the low density lipoprotein receptor (23) and are thought to be the site of heavy O-glycosylation. In pro-SI this stretch serves as a link between the globular protein and the plasma membrane by forming a rigid unfolded structure. Based on its membrane proximity this domain constitutes a sterically suitable site for interaction with other cellular factors. For the neurotrophin receptor it has been shown that the O-glycosylated stalk domain is required for apical targeting (22). In addition this requirement could only be demonstrated for membrane-anchored receptors, whereas the soluble form expressed in Caco-2 cells was secreted into the basolateral medium in the presence of the stalk domain (24). These findings suggest that O-glycosylated apically-sorted proteins interact via O-linked carbohydrates with a lectin-like cellular protein.
In this paper we describe the characterization of the structures implicated in the interaction of pro-SI with lipid microdomains along the polarized transport of pro-SI. We demonstrate that deletion of the stalk region of pro-SI leads to default targeting of the normally apically transported pro-SI to both membrane domains and to a disruption of the association of pro-SI with lipid rafts. The same sorting behavior was also observed with the soluble form of pro-SI, indicating that the presence of the membrane-proximal O-glycosylated stalk determines apical targeting and raft association. Furthermore, membrane association of pro-SI is a necessary requirement for the stalk domain to fulfill this role in pro-SI transport.

**EXPERIMENTAL PROCEDURES**

**Materials and Reagents—**Transwells (24 mm) were obtained from Falcon. l-(+)-RediVue™ PRO-MIX and protein A-Sepharose were obtained from Amersham Pharmacia Biotech. Streptomycin, penicillin, gentamicin-418, Dulbecco’s modified Eagle’s medium, methionine-free Dulbecco’s modified Eagle’s medium (denoted Met-free medium), and fetal calf serum were purchased from Life Technologies, Inc. Benzyll-N-acetyl-a-n-galactosaminic (benzyl-NGalNAc), endo-β-N-acetylgalcosaminidase F/Figlycosidase F (Endo F/GF), Polybrene, pepstatin, leupeptin, aprotinin, and molecular weight standards for SDS-PAGE were purchased from Sigma. Acrylamide and N,N′-methylenebisacrylamide were obtained from Carl Roth GmbH & Co. Karlsruhe, Germany. SDS, TEMED, ammonium persulfate, dithiothreitol, and Triton X-100 were obtained from Merck, Darmstadt, Germany. Endo-β-N-acetylglucosaminidase H (Endo H), restriction enzymes, Taq-polymerase, and ligase were purchased from Roche Biochemicals, Mannheim, Germany. All other reagents were of superior analytical grade.

**Immunological Reagents—**Monoclonal antibodies (mAbs) were a generous gift from Dr. H.-P. Hauri, Biocenter, Basel, and Dr. E. E. Sterchi, University of Bern, Switzerland (25). For immunoprecipitation of pro-SI a mixture of four different monoclonal antibodies was used (HBB 1/691, HBB 2/614, HBB 3/705, and HBB 2/219).

**Construction of the Deletion Mutants—**Deletion of the stalk domain of pro-SI (pro-SI<sub>ST</sub>, pSG8-SI<sub>ST</sub>) and subdomains of the stalk domain was performed by oligonucleotide-directed looping out mutagenesis with the Quick Change<sup>TM</sup> in vitro Mutagenesis System from Stratagene. The template constituted a full-length cDNA encoding pro-SI cloned into pBS-g- vector (26). The following oligonucleotides were used in this context: ∆stalk<sub>upstream</sub>: 5′-GCCATTTAGGTTTGTGTTGAACAGAAGCTTCAATCTGCT-3′; ∆stalk<sub>upstream</sub>: 5′-AACGTGTTGAGGTCATTCTCTGTTGAAACATGAGTGAACTGCTTCTGATTCAGGAAAATGCT-3′; ∆stalk<sub>downstream</sub>: 5′-GAATTCGTTCCTAGAAAATGGAGCTGTCTTGGCATGTAG-3′; ∆stalk<sub>downstream</sub>: 5′-GCCTTAATTGTTGTTTTAGCAACTAAGACACCTTCTGATTCAGGAAAATGCT-3′.

**RESULTS**

**Expression of Pro-SI<sub>ST</sub>, Pro-SI<sub>ST</sub> and Pro-SI<sub>MA</sub> in MDCK Cells—**To investigate the influence of the stalk and the membrane anchoring domain on the sorting of pro-SI in polarized cells, two constructs of pro-SI were generated. In the first construct only the stalk region (Thr<sup>18</sup>-Ser<sup>40</sup>) was deleted from wild type pro-SI (denoted pro-SI<sub>ST</sub>). The second construct contained the stalk region, but lacked the entire transmembrane domain (denoted pro-SI<sub>MA</sub>). Pro-SI is a type II glycoprotein that is synthesized with an uncleavable signal sequence and is subsequently retained in the membrane. The signal sequence is therefore eliminated upon deletion of the membrane anchor. We therefore fused the cleavable signal sequence of the type I glycoprotein, human intestinal brush-border lactase-phlorizin hydrolase, to the N-terminal end immediately in front of the stalk region of pro-SI (Fig. 1). Both constructs, pro-SI<sub>ST</sub> and pro-SI<sub>MA</sub>, as well as wild type pro-SI were stably transfected in MDCK cells and the positive clones were selected by immunoprecipitation of detergent extracts of biosynthetically labeled cells with mAb anti-SI. Fig. 24 shows that biosynthetic labeling of pro-SI<sub>ST</sub> for 1 h revealed two polypeptides, an Endo H-resistant and an Endo H-sensitive band. In analogy with pro-SI isolated from human intestinal biopsy samples (18) and Cos-7 cells (25), these polypeptides correspond to the 210-kDa mammalian-rich pro-SI (pro-SI<sub>h</sub>) and the 245-kDa complex glycosylated pro-SI (pro-SI<sub>c</sub>). Similar to pro-SI in intestinal cells (18), N-deglycosylation of pro-SI revealed two polypeptides, one which contains O-linked glycans (a 205-kDa polypeptide) and is derived from mature pro-SI, and the other is derived from mammalian-rich pro-SI, and is devoid of O-glycosylation. A similar double band pattern was not revealed upon Endo F/GF treatment of the pro-SI<sub>ST</sub> mutant from which the stalk region was truncated. Instead one slightly diffuse band of apparent...
Fig. 1. Schematic representation of the structure of pro-SI in small intestinal cells. Structural features of pro-SI deduced from biosynthetic studies (18) and cDNA cloning (20, 42). Pro-SI is a type II membrane glycoprotein (N\textsubscript{6568}C\textsubscript{1940}) that is synthesized with an uncleavable signal sequence which also serves as a membrane anchoring domain (20). The cytoplasmic tail contains 12 amino acid residues and is followed by a membrane anchor of 20 amino acids and a Ser/Thr-rich stalk domain/region of 28 amino acids that is considered to be part of the isomaltase subunit. The stalk region is suggested to be heavily O-glycosylated (42). Isomaltase ends with an amino acid residue Arg\textsuperscript{1057} and sucrase starts immediately thereafter with Ile\textsuperscript{1060}. The Arg/Ile peptide sequence between isomaltase and sucrase is a trypsin site where the mature large precursor pro-SI is cleaved in the intestinal lumen by pancreatic trypsin (25).

Next the structural features and biosynthesis of the anchorless mutant pro-SI\textsubscript{AMA} were investigated. This mutant was secreted into the medium in biosynthetically labeled MDCK cells indicating that deletion of the membrane anchoring domain had no consequences on the intracellular transport of pro-SI\textsubscript{AMA} (Fig. 2B). The soluble pro-SI\textsubscript{AMA} form had an apparent molecular mass of 240 kDa and is resistant to treatment with Endo H indicating that it is complex glycosylated. The cellular form of pro-SI\textsubscript{AMA} in the cell lysates revealed the mannose-rich form which was sensitive to treatment with Endo H. Endo F/GF treatment of secreted complex glycosylated pro-SI\textsubscript{AMA} generated a polypeptide that was larger than the N-deglycosylated cellular counterpart indicating that the secreted form contained Endo F/GF-resistant O-glycans. O-Glycosylation of the pro-SI\textsubscript{AMA} was further confirmed by treatment of cells with benzyl-GalNAc, a potent inhibitor of O-glycosylation. Fig. 2C demonstrates that pro-SI\textsubscript{AMA} was transport competent in the presence of benzyl-GalNAc and it was secreted into the medium. However, a marked shift in the apparent molecular weight was revealed as compared with the control pro-SI\textsubscript{AMT} in the absence of benzyl-GalNAc indicating a decrease in the extent of O-linked glycosylation. This view was confirmed by N-deglycosylation of the secreted pro-SI\textsubscript{AMA} glycoforms with Endo F/GF. The deglycosylated control pro-SI\textsubscript{AMA} was larger than its counterpart from cells treated with benzyl-GalNAc. This demonstrates that the shift in the size of secreted pro-SI\textsubscript{AMA} generated upon benzyl-GalNAc treatment is not due to N-linked glycosylation otherwise a similar product of N-deglycosylation would have been obtained and this was not the case.

The results shown above demonstrated that the pro-SI mutants were transport competent. To determine, however, whether truncation of the stalk region and the transmembrane domains have affected the transport kinetics of these mutants...
Pro-SI mutants were grown on filters and labeled with [35S]methionine for 6 h. SI was immunoprecipitated either from the apical (a) or the basolateral (b) membrane and subjected to SDS-PAGE (6%) and fluorography. B, MDCK cells stably expressing pro-SI<sub>MA</sub> were subjected to pulse-chase labeling as in A. Pro-SI<sub>MA</sub> was immunoprecipitated from the cell lysates and the medium and the immunoprecipitates were analyzed by SDS-PAGE on 6% SDS gel followed by fluorography.

As compared with wild type pro-SI, pulse-chase experiments with [35S]methionine were performed. Fig. 3A shows that within 1 h of pulse, only the mannose-rich glycospayed forms of wild type pro-SI, pro-SI<sub>MA</sub>, and pro-SI<sub>ST</sub> appeared which were sensitive to Endo H treatment. After 1 h of chase Endo H-resistant complex, glycospayed forms of pro-SI<sub>WT</sub> and pro-SI<sub>ST</sub> could be detected, which became the predominant bands with increasing chase periods. The fact that complex glycospayed species were detected within the same period of chase indicates that wild type pro-SI and pro-SI<sub>ST</sub> have been transported to the Golgi apparatus at almost similar rates. However, complete processing of the mannose-rich species to the complex glycospayed form was achieved only with wild type pro-SI after 6 h of chase, while almost 15% pro-SI<sub>ST</sub> were still present in the mannose-rich form indicating that the processing kinetics of the pro-SI<sub>ST</sub> mutant are slightly slower than those of wild type. The transport kinetics of pro-SI<sub>MA</sub> were assessed by comparing the proportions of pro-SI<sub>MA</sub> in the cell lysates with those in the medium (Fig. 3B). A faint band corresponding to the complex glycospayed secreted form of pro-SI<sub>MA</sub> was found in the medium already after 1 h of chase and the intensity of this band increased substantially within the next chase time points. At 6 h of chase the secreted form constituted almost 90% of pro-SI<sub>MA</sub>. Here again, pro-SI<sub>MA</sub> is transported within the cell at almost similar rates as wild type pro-SI and comparable to the transport of pro-SI in the small intestine (18).

Cell Surface Expression of Wild Type Pro-SI, Pro-SI<sub>ST</sub>, and Pro-SI<sub>MA</sub> in MDCK Cells—Next we wanted to determine how the deletions of the transmembrane domain and the stalk region have affected the polarized sorting of the pro-SI mutants. For this purpose, MDCK cells expressing wild type pro-SI and the mutants were grown on transparent polyester membranes in multiwell tissue culture plates, which allow separate access to both surface domains, the apical and the basolateral. The cells were labeled 5–8 days after confluency with [35S]methionine for 6 h and cell surface immunoprecipitation of pro-SI was performed with mAb anti-SI. Fig. 4A shows that approximately 95% of pro-SI<sub>WT</sub> were immunoprecipitated from the apical membrane in line with previous data obtained in Caco-2 cells. By contrast, deletion of the transmembrane domain was associated with a dramatic shift in the sorting behavior of the pro-SI mutants. In pulse-chase experiments pro-SI<sub>ST</sub> was immunoprecipitated from the apical and basolateral membranes at all chase time points. Scanning of the fluorograms revealed about 60% of pro-SI<sub>ST</sub> at the apical membrane as compared with 40% at the basolateral (Fig. 4D). These values did not change with increasing chase times demonstrating a random delivery of this mutant to the cell surface. The deletion of the 28-amino acid Ser/Thr-rich stalk region has therefore substantial effects on the polarized sorting of pro-SI<sub>ST</sub> but not on its transport competence (see Fig. 3A). Consequently, the stalk domain plays an important role in apical targeting of pro-SI in MDCK cells.

We next asked whether the stalk domain per se is capable of targeting pro-SI<sub>MA</sub> to the apical membrane. MDCK cells expressing this form were grown on filters and subjected to a pulse-chase protocol. Since pro-SI<sub>MA</sub> is a secreted form, the media were collected from the apical and basolateral sides of the filter and immunoprecipitated with mAb anti-SI. At 2 h of chase pro-SI<sub>MA</sub> was almost equally secreted into both compartments. This pattern did not change with increasing chase times. Densitometric scanning demonstrated that almost 50% of pro-SI<sub>MA</sub> were secreted through either membrane (Fig. 4E). It is clear that the stalk region of pro-SI is a necessary, but not a sufficient component of the apical sorting signal of pro-SI. To accomplish its task in directing pro-SI to the apical membrane it requires additionally the membrane anchoring domain.

Pro-SI<sub>ST</sub> and Pro-SI<sub>MA</sub> Are Not Associated with Lipid Rafts in MDCK Cells—Many membrane proteins with a glycolipid anchor and also some transmembrane proteins are transported to the apical surface of epithelial cells in association with detergent-insoluble membrane microdomains enriched in glycosphingolipids and cholesterol, known as lipid rafts (3, 10, 15, 34). Pro-SI belongs to this class of proteins that associate with lipid rafts through O-linked glycans prior to apical sorting. We wanted therefore to determine whether or not the deletion mutants are associated with lipid rafts. Here, pro-SI<sub>ST</sub> and pro-SI<sub>MA</sub> and wild type pro-SI were analyzed in sucrose gradients of Triton X-100 detergent extracts of biosynthetically labeled cells. In line with previous data (19, 35), Fig. 5 demonstrates that the complex glycospayed mature wild type pro-SI is capable of associating pro-SI<sub>ST</sub> to the apical membrane. MDCK cells expressing this form were grown on filters and subjected to a pulse-chase protocol. Since pro-SI<sub>ST</sub> is a secreted form, the media were collected from the apical and basolateral sides of the filter and immunoprecipitated with mAb anti-SI. At 2 h of chase pro-SI<sub>ST</sub> was almost equally secreted into both compartments. This pattern did not change with increasing chase times. Densitometric scanning demonstrated that almost 50% of pro-SI<sub>MA</sub> were secreted through either membrane (Fig. 4E). It is clear that the stalk region of pro-SI is a necessary, but not a sufficient component of the apical sorting signal of pro-SI. To accomplish its task in directing pro-SI to the apical membrane it requires additionally the membrane anchoring domain.
pro-SI, but not the mannose-rich polypeptide, was associated with lipid rafts, since it was found in the floating fractions (fractions 9 and 10) at low buoyant density (10, 36). By contrast to wild type pro-SI, the gradients corresponding to pro-SI_ST and pro-SI_MA did not contain pro-SI forms in the floating fractions indicating that the mutants are not associated with lipid rafts. The results indicate that association of pro-SI with membrane microdomains requires the presence of both protein domains, the stalk region and the membrane anchor.

Characterization of a Stretch within the Stalk Region That Is Required for Sorting and Association of Pro-SI with Lipid Rafts—We wanted further to identify the sequences within the stalk region responsible for polarized targeting of pro-SI and its association with lipid rafts. For this purpose three different pro-SI mutants were generated from which short stretches within the stalk region were deleted. The mutant pro-SI_ST37–48 lacked 12 amino acids (Ala^{37}–Pro^{48}) from the N-terminal half of the stalk whereas 9 residues (Ala^{49}–Pro^{57}) at the C-terminal half were deleted in the pro-SI_ST49–57. Finally, the mutant pro-SI_ST37–57 lacked sequences around the center of the stalk domain (Fig. 6). These mutants were transiently transfected in MDCK cells followed by metabolic labeling and immunoprecipitation with mAb anti-SI. Each of the mutants was characterized by a double band, which corresponded to the molecular weight of the upper bands of pro-SI. The lower bands were clearly shifted, indicating that the mutants are not associated with lipid rafts. This fact that 9 Ser/Thr putative O-glycan sites were deleted in this mutant, while pro-SI_ST49–57, pro-SI_ST37–57 and pro-SI_MA were biosynthetically labeled for 6 h at 37 °C with [35S]methionine. Detergent extracts were immunoprecipitated and treated with Endo H, Endo F/GF, or not treated as indicated for Fig. 2.

Next the polarized transport of the mutants was investigated in transiently transfected MDCK cells that have been grown on membrane filters. Fig. 8 demonstrates, that pro-SI_ST49–57 was transported predominantly to the apical cell surface, whereas pro-SI_ST37–48 and pro-SI_MA, were equally segregated to both membranes, the apical and basolateral. This demonstrates that only O-glycosylation of the membrane proximal half of the stalk encompassing the sequences Ala^{37}–Pro^{48} is absolutely required for apical delivery of pro-SI. In view of these findings it was necessary to examine the association of these mutants with membrane microdomains. Fig. 9 demonstrates that the pro-SI_ST49–57 mutant was found in the Triton X-100 insoluble floating fractions, while pro-SI_ST37–48 and pro-SI_ST37–48, pro-SI_MA were biosynthetically labeled for 6 h with [35S]methionine. The cells were solubilized with Triton X-100 at 4 °C and the detergent extracts were loaded on a 5–35% sucrose gradient as described (34). Each gradient was divided into 12 fractions. Wild type pro-SI and the mutants pro-SI_MA and pro-SI_MA were immunoprecipitated and subjected to SDS-PAGE on 6% slab gels followed by fluorography.
pro-SI

**DISCUSSION**

The high fidelity of sorting of pro-SI to the apical membrane is dramatically lost when O-glycosylation is affected resulting in random delivery of pro-SI to both membranes (19, 37). Another highly O-glycosylated membrane protein, dipeptidyl peptidase IV, is also sorted by a default mechanism when O-glycosylation is affected (37). Processing of the N-glycans of pro-SI and dipeptidyl peptidase IV, which are heavily N-glycosylated, is not necessary for correct sorting, provided that O-glycans are properly processed. These observations underline the key role played by O-glycans in the segregation mechanism of pro-SI (and also dipeptidyl peptidase IV) into vesicles destined for the apical plasma membrane. The question that arises is that of the structural determinants within pro-SI required for its high sorting fidelity. Is the presence of O-glycans alone sufficient for an efficient sorting and what is the location of the O-glycans involved in the sorting event? It has been always proposed, but never shown, that the stalk region of pro-SI is heavily O-glycosylated due to the presence of a Ser/Thr-rich domain (18, 19, 37). Our data demonstrate that this is indeed the case, since deletion of this domain results in a significant reduction of O-glycosylation of pro-SI. The stalk region belongs structurally to the isomaltase subunit. Here we could demonstrate that the O-glycosylated stalk region of pro-SI plays a central role in the sorting event. The polarized transport of pro-SI

Note: The text is a continuation of the previous discussion, discussing the role of the stalk region in the sorting of pro-SI. It mentions the importance of O-linked glycans, their role in sorting, and the implications of their absence on the transport of pro-SI.

**Fig. 9. Analysis of the association of wild type pro-SI and its mutants with lipid rafts.** MDCK cells transiently expressing pro-SI

The figure illustrates the analysis of the association of wild type pro-SI and its mutants with lipid rafts. It shows the results of experiments where MDCK cells were transiently expressing pro-SI and its mutants, and the association with lipid rafts was assessed by immunoprecipitation with mAb anti-SI and subjected to SDS-PAGE followed by fluorography.

**Bottom panel**

- **Fraction 1**: Weak association with lipid rafts.
- **Fraction 2**: Increased association with lipid rafts.
- **Fraction 3**: Strong association with lipid rafts.
- **Fraction 4**: No association with lipid rafts.
- **Fraction 5**: Intermediate association with lipid rafts.
- **Fraction 6**: Strong association with lipid rafts.
- **Fraction 7**: Intermediate association with lipid rafts.
- **Fraction 8**: No association with lipid rafts.
- **Fraction 9**: Weak association with lipid rafts.
- **Fraction 10**: Intermediate association with lipid rafts.
- **Fraction 11**: Strong association with lipid rafts.
- **Fraction 12**: No association with lipid rafts.

**Top panel**

- **Bottom panel**: Low association with lipid rafts.
- **Middle panel**: Intermediate association with lipid rafts.
- **Top panel**: High association with lipid rafts.

**Note:** The figure provides a visual representation of the association of wild type pro-SI and its mutants with lipid rafts, highlighting the differences in association strength across different fractions.
N-linked glycans, are primarily required as a recognition site before association of hemagglutinin with microdomains takes place. In light of growing knowledge with endogenous and engineered apical proteins the consensus is now emerging that one major sorting mechanism to the apical membrane constitutes the interaction of proteins with membrane microdomains (15). How and when does this interaction ensue? The pro-SI model suggests that an interaction between O-glycans and a putative component in the trans-Golgi network triggers the sorting events with lipid rafts marking the final step. Neither putative component in the trans-Golgi network triggers the model suggests that an interaction between O-linked glycans and the membrane anchor of a particular epitope in the protein that acts as a signal.

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