Dissection of the Asynchronous Transport of Intestinal Microvillar Hydrolases to the Cell Surface

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Abstract. Novel subcellular fractionation procedures and pulse-chase techniques were used to study the intracellular transport of the microvillar membrane hydrolases sucrase-isomaltase and dipeptidylpeptidase IV in the differentiated colon adenocarcinoma cell line Caco-2. The overall rate of transport to the cell surface was two fold faster for dipeptidylpeptidase IV than for sucrase-isomaltase, while no significant differences were observed in transport rates from the site of complex glycosylation to the brush border. The delayed arrival of sucrase-isomaltase in the compartment where complex glycosylation occurs was only in part due to exit from the endoplasmic reticulum. A major slow-down could be ascribed to maturation in and transit of this enzyme through the Golgi apparatus. These results suggest that the observed asynchronism is due to more than one rate-limiting step along the rough endoplasmic reticulum to trans-Golgi pathway.

Materials and Methods

Cell Culture and Labeling with [35S]Methionine

Caco-2 cells were grown in Optilux petri dishes (Falcon Labware, Oxnard, CA) as described (14) or on Millipore filters (HATF0025) in mini-Marbrook chambers (11). The cells were subcultured weekly using the tryp...
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...n/EDTA method (26). Labeling with [35S]methionine was carried out with cells grown on filters 5-15 d after confluence (14). In all experiments, a pulse time of 15 min was used except for the experiments with carboxyl cyclohexylaminomethyl hydroxybenzamide (CCH). For pulse-chase experiments the presence of CCCP were performed essentially according to Fries and Rothman (9) with the following modifications. The filtration chambers were disassembled and the cells were pulse labeled with 250 μCi [35S]methionine for 5 min after a preincubation in methionine-free medium for 15 min. The filters were washed twice with 5 ml of ice-cold PBS containing 0.1 g/liter CaCl2 and 0.059 g/liter MgSO4. The cells were then incubated in 5 ml of the same buffer containing 10 or 100 μM CCCP. 10 min later the cells were transferred to fresh buffer containing CCCP and returned to the 37°C incubator for 45 min before harvesting.

**Isolation of Pre-Golgi Membrane Fraction**

This fraction was isolated from the same Percoll gradient as used for the isolation of the Golgi fraction (see Fig. 5). 1 ml at the bottom of the Percoll gradient (fraction V) was collected and the corresponding volume plus 1 ml (usually 10 ml in total) was discarded. The next 8 ml were pooled and processed for two gradients as follows. 4 g of the fraction was mixed with 2 g 60% ([wt/wt] in 1 mM EDTA, 10 mM triethanolamine-acetic acid, pH 6.5) Metrizamide in a centrifuge tube and overlayed with 2.5 ml each of 17.5 and 11.5% ([wt/wt] same buffer) Metrizamid and, finally, with buffer B. The gradient was run for 7 h at 23,000 rpm (70,600 g) at 8°C in a TST 41.14 rotor (Kontron Elektronik GmbH, Zürich). The 17.5:11.5% interphase was enriched in Golgi-derived membranes. This fraction, if necessary, was diluted with the buffer needed for the subsequent experiment and centrifuged for 1 h at 38,000 rpm (99,800 g) at 4°C in a TST 75.15 rotor (Kontron Elektronik GmbH). For the pulse-chase experiments with filter-grown cells, unlabeled Caco-2 cells grown on petri dishes were used as carriers as in the isolation procedure for brush border membranes.

**Enzyme Assays**

All measurements were performed at 37°C. Alkaline phosphatase (measured according to reference 37) and sucrase (measured according to reference 4) were used as marker enzymes for the brush border membrane. K+-stimulated p-nitrophenylphosphatase (measured according to reference 37; using buffers I and III) and KCN-resistant NADH oxidoreductase (determined as in reference 35) were used as markers for the basolateral membrane and the endoplasmic reticulum, respectively. With the exception of sucrase, all of these enzymes were measured using an LKB reaction rate analyzer. 2086 Mark II. Glucosaminidase (measured according to reference 31) was used as a marker for lysosomes and galactosyltransferase (measured according to reference 40) with 0.5% (wt/vol) Trion X-100 and ovomucoid (as acceptor protein) was used to detect Golgi apparatus-derived membranes. Protein was determined with the Bio-Rad protein assay kit using protein standard I (Bio-Rad Laboratories, Cambridge, MA).

**Results**

Polarized Expression of SI and DPP IV in the Microvillus Membrane of Caco-2 Cells

Before undertaking biosynthetic studies, it was essential to establish the domain-specific location of DPP IV and SI in...
Caco-2 cells grown on Millipore filters. In Fig. 1, immunofluorescent labeling of Caco-2 cryosections with enzyme-specific mAbs clearly showed that SI and DPPIV were detectable exclusively in the brush border membrane while the Na⁺/K⁺-ATPase α-subunit was associated only with the basolateral membrane. We conclude that the surface membrane of Caco-2 cells is polarized with respect to these three enzymes.

**Transport of SI and DPPIV from the Golgi Apparatus to the Cell Surface**

Previous studies on the biogenesis of microvillar enzymes were performed with Caco-2 cells grown on petri dishes (14). Since surface polarity is more developed in cells grown on Millipore filters than on solid supports (I, II), we applied the former system to Caco-2 cells. However, growth of the cells on Millipore filters might alter the biogenesis of the investigated membrane proteins. Therefore, it was important to establish that the previously observed asynchronism of protein transport also occurred in Millipore-grown cells. Fig. 2 shows the time course of conversion of the high-mannose to the complex-glycosylated forms of the two hydrolases in cell homogenates, an event that is mediated by the Golgi apparatus. Half maximal appearance of complex-glycosylated DPPIV and SI occurred at ~15-20 min and 130-140 min, respectively. These results are similar to those of our previous study and therefore suggest that growth of the Caco-2 cell cultures on Millipore filters does not alter the asynchronism of protein transport.

**Figure 1.** Immunofluorescent labeling of 1-μm cryosections of Caco-2 cell monolayers with mAb HBB 3/7542 against DPPIV (a), mAb HBB 2/61488 against sucrase-isomaltase (c), and mAb C62.4 against Na⁺/K⁺-ATPase (e). (b, d, and f) Corresponding phase-contrast micrographs. Note that the hydrolases are restricted to the apical cell surface, while Na⁺/K⁺-ATPase is confined to the lateral aspect of the cells. The lack of immunofluorescence for the latter enzyme of the basal cell surface may be due to the loss of this domain during scraping of the cells. Bar, 24 μm.

**Figure 2.** Conversion of the high-mannose to complex-glycosylated forms of DPPIV (X) and SI (o) in homogenates of Caco-2 cells. Shown are results from pulse-chase experiments with [35S]methionine (pulse, 15 min). After various time intervals of chase, the enzymes were immunoprecipitated and separated by SDS-PAGE. Gel slices corresponding to high-mannose or complex-glycosylated forms of the enzymes were cut out of dried gels, solubilized, and radioactivity was measured in a β-counter.
cells on filters rather than on petri dishes does not significantly alter the overall maturation pattern of the two enzymes under investigation. To determine whether transport of the two enzymes from the site of complex glycosylation in the Golgi apparatus to the cell surface is also asynchronous, we developed a method for the isolation of the brush border membrane from Caco-2 cells. Although seemingly similar to previously published methods for the isolation of brush border membranes from intact tissue, it is worth noting that homogenization conditions and the order of the precipitation steps (i.e., Mg\(^{++}\) before Ca\(^{++}\)) were found to be critical for obtaining highly purified brush border membranes from Caco-2 cells. Tables I and II show that the membrane fraction isolated using this method was enriched in brush border marker enzymes SI and alkaline phosphatase, while contamination by intracellular membranes was minimal. Due to the small number of labeled cells it was advantageous to use carrier dishes (unlabeled Caco-2 cells grown in petri dishes) for the pulse–chase experiment. The clearly asynchronous appearance of DPPIV and SI in the brush border fraction is illustrated in Fig. 3. It is also notable that the autoradiograms lacked any band in the region of the high-mannose form of these proteins, providing additional evidence for the purity of the membrane fraction. The kinetics of the appearance of DPPIV and SI in the brush border membrane of the Caco-2 cells is shown in Fig. 4. The difference between the time of half-maximal appearance in the brush border membrane and the half-maximal appearance of the complex-glycosylated forms in the homogenate was used to calculate a transport rate of 40–60 min for SI and 60–75 min for DPPIV. Thus the transport kinetics from the site of complex glycosylation in the Golgi apparatus to the brush border membrane were similar for the two hydrolases.

| Table I. Specific Activities and Enrichment Factors of Marker Enzymes in Brush Border Membrane Vesicles from Caco-2 Cells |
|---------------------------------------------------------------|
| **Homogenate** | **Isolated membrane** | **Enrichment factor** |
|----------------|----------------------|----------------------|
| Alkaline phosphatase | 53.3 ± 19.5 | 1,103.4 ± 382.3 | 20.9 ± 2.8 \(n = 8\) |
| Sucrase | 3.6 ± 0.5 | 68.3 ± 17.3 | 19.5 ± 3.5 \(n = 5\) |
| K\(^{+}\)-stimulated \(p\)-nitrophenyl phosphatase | 4.8 ± 0.8 | 11.5 ± 8.9 | 2.5 ± 2.1 \(n = 5\) |
| KCN-resistant NADH-oxidoreductase | 174.4 ± 22.3 | 62.4 ± 29.5 | 0.4 ± 0.1 \(n = 8\) |
| Glucosaminidase | 58.5 ± 30.9 | 61.2 ± 41.7 | 1.0 ± 0.3 \(n = 7\) |
| Galactosyltransferase | 0.18 ± 0.01 | 0.08 ± 0.04 | 0.4 ± 0.2 \(n = 5\) |

* Mean ± 1 SD.
\(n\), number of experiments.

| Table II. Yield and Recovery of Marker Enzymes in Isolated Brush Border Membranes from Caco-2 Cells* |
|---------------------------------------------------------------|
| **P4** | **Total** |
|----------------|------|
| Protein | 0.33 ± 0.03 | 82 ± 10 \(n = 6\) |
| Alkaline phosphatase | 6.8 ± 1.0 | 97 ± 15 \(n = 6\) |
| Sucrase | 6.7 ± 1.4 | 76 ± 10 \(n = 4\) |
| K\(^{+}\)-stimulated \(p\)-nitrophenyl phosphatase | 0.88 ± 0.71 | 75 ± 13 \(n = 5\) |
| KCN-resistant NADH-oxidoreductase | 0.10 ± 0.03 | 88 ± 4 \(n = 6\) |
| Glucosaminidase | 0.33 ± 0.03 | 84 ± 7 \(n = 6\) |
| Galactosyltransferase | 0.14 ± 0.06 | 85 ± 10 \(n = 5\) |

* The values are given as percentage of the amount determined in the homogenate and represent means ± 1 SD.
\(\dagger\) Sum of recoveries determined in each fraction.
\(\ddagger\) \(n\), number of experiments.

Figure 3. Appearance of newly synthesized DPPIV and SI in the brush border membrane fraction of Caco-2 cells. The cells were pulse labeled with \([^{35}\text{S}]\)methionine and the hydrolases were immunoprecipitated from the subcellular fraction after various times of chase with a mixture of mAbs against SI and DPPIV.
Appearance and Maturation of DPPIV and SI in the Golgi Apparatus

To further dissect the asynchronous transport of SI and DPPIV, we developed a method for the isolation of a fraction enriched in Golgi-derived membranes (Fig. 5). Such a preparation, in conjunction with the pulse-chase technique, allows one to differentiate between pre-Golgi and intra-Golgi events. Caco-2 cells were homogenized under conditions that left the cisternae of the Golgi apparatus intact while most other membrane compartments vesiculated enabling us to isolate the Golgi elements on gradients. Fig. 6 shows a representative electron micrograph of the final fraction containing the expected drumstick profiles characteristic for the Golgi apparatus. The enzymatic characterization of this fraction, designated “Golgi fraction,” is given in Tables III and IV. The fraction was enriched in galactosyltransferase activity, a marker enzyme for the Golgi apparatus, while enzyme activities for other cellular membranes were not enriched.

A pulse-chase protocol was used to study arrival at and transit through the Golgi fraction of the two hydrolases. A typical autoradiogram of such an experiment is given in Fig. 7. The flow kinetics are drawn in Fig. 8, a and b. The time required for half-maximal labeling of the high-mannose forms of the enzymes in the Golgi fraction was defined as the rate of their transport from the endoplasmic reticulum to the cis side of the Golgi apparatus. This rate, designated “apparent transport rate” (see Discussion), was found to be <15 min for DPPIV and ~45 min for SI. Thus, arrival of SIh (the high-mannose form of SI) in this fraction was delayed. However, this delay only in part accounted for the asynchronism of maturation to Slc (the complex-glycosylated form of SI) in the homogenate (Fig. 2). Fig. 8 b shows that the conversion of SIh to Slc was also substantially delayed in the Golgi fraction when compared to that of DPPIV (Fig. 8 a). In the Golgi fraction a much higher percentage of total SI exists as high-mannose forms than is true for total DPPIV. At the same time this high-mannose SI appears in the Golgi fraction more slowly than the high-mannose DPPIV. Half-maximal appearance of Slc in the Golgi fraction was observed after ~100–110 min. The Slc in the Golgi fraction was maximally labeled after ~180 min at which time the radioactivity of this enzyme in the brush border fraction was half-maximal. When the enzyme approached maximal levels in the brush border fraction (after 300 min) the Slc in the Golgi fraction had decreased by only ~40%, suggesting the existence of a Slc pool that is not immediately exported from the Golgi apparatus. The kinetic behavior of DPPIV is strikingly different from that of SI. Conversion of DPPIVh (the high-mannose form of DPPIV) to DPPIVc (the complex form of DPPIV) was rapid taking ~15 min only. The disappearance of DPPIVc from this fraction was biphasic. A rapid disappearance was observed up to 120 min at which time appearance of DPPIVc in the brush border fraction was close to maximal. This rapid disappearance is therefore most likely due to delivery of DPPIVc to the brush border. At later time points, the radioactivity in DPPIVc disappeared more slowly. Overall these results suggest that the intracellular transport of SI is delayed at a pre-Golgi as well as at an intra-Golgi stage.

Pre-Golgi Events

The above conclusion that the Golgi apparatus significantly contributes to the asynchronous protein transport critically depends on the purity of the Golgi fraction. For example, a copurification of a late endoplasmic reticulum compartment (i.e., transitional elements) with the Golgi fraction could lead to the above results even if exit from the endoplasmic...
Table III. Specific Activities and Enrichment Factors of Marker Enzymes in a Fraction Enriched in Golgi-derived Membranes (FII) and in an Early Biosynthetic Fraction (Ell)

| Enzyme                         | Specific Activity | Enrichment Factor |
|--------------------------------|-------------------|-------------------|
|                                | mU/mg             |                   |
| Galactosyltransferase          | 0.281 ± 0.009     | 8.183 ± 0.811     |
| K⁺-Stimulated p-nitrophenyl   | 11.0 ± 2.3        | 1.9 ± 3.2         |
| Phosphatase                    | 208.0 ± 31.8      | 115.8 ± 87.9      |
| CN-resistant NADH-            | 46.3 ± 7.6        | 45.7 ± 31.0       |
| Oxidoreductase                | 188.6 ± 24.5      | 200.8 ± 44.5      |

The numbers indicate means ± 1 SD of four independent experiments.

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newly synthesized enzymes in this intracellular compartment.

Conclusions drawn from subcellular fractionation studies critically depend on the extent of cross contamination by membranes of other organelles. For instance, contamination of our brush border fraction with Golgi membranes would affect the observed transport rates. The high enrichment factor of marker enzymes for brush border membranes and the low enrichment factor for galactosyltransferase provide strong evidence that cross contamination by Golgi membranes in our preparations is negligible. It has recently been shown that the brush border of the human small intestine can be labeled with polyclonal antibodies raised against galactosyltransferase (28). However, it was not determined in that study whether or not the immunoreacting material had galactosyltransferase activity. It is important to note that we were unable to measure significant galactosyltransferase activity levels in Caco-2 brush border membranes (Table I) while in the galactosyltransferase-enriched Golgi fraction the brush border enzyme activities were low. We therefore believe that galactosyltransferase is still valuable as a marker enzyme for detecting Golgi-derived membranes (see also reference 3). The transport rate of SI to the brush border has most recently been confirmed by pulse-chase experiments in conjunction with a novel surface protease assay (Eilers, U., and H.-P. Hauri, manuscript in preparation).

Our suggestion that the Golgi apparatus significantly contributes to the asynchronous transport is only valid if the Golgi fraction is not significantly contaminated by elements of the endoplasmic reticulum that are involved in protein biosynthesis and transport. This was indeed the case as shown in experiments in which protein exit from the endoplasmic reticulum was blocked by CCCP. Under these conditions no newly synthesized DPPIV was detectable in the Golgi fraction. This shows that the Golgi fraction was sufficiently pure to study the arrival of brush border enzymes in the Golgi apparatus.

Figure 8. Arrival and maturation of newly synthesized DPPIV (A) and SI (B) in the Golgi fraction of Caco-2 cells deduced from pulse-chase experiments (quantification from fluorograms). The highest amount of radioactivity (high-mannose plus complex forms) in either DPPIV or SI in the Golgi fraction relative to the amount of radioactivity in the enzymes in the corresponding homogenate was set to 100%. (×) High-mannose forms; (○) complex forms.

Table V. Apparent Intracellular Transport Rates of Newly Synthesized Microvillar Hydrolases as Deduced from Pulse-Chase Experiments

| Enzyme | ER to brush border* | ER to Golgi‡ | ER to site of complex glycosylation§ | Site of complex glycosylation to brush border‖ |
|--------|---------------------|-------------|----------------------------------|--------------------------------------------|
| SI     | 180–190 min         | 45 min      | 130–140 min                      | 40–60 min                                  |
| DPPIV  | 80–90 min           | <15 min     | 15–20 min                        | 60–75 min                                  |

* Time for half-maximal appearance of mature enzyme in the brush border membrane fraction (Fig. 4).
‡ Time for half-maximal appearance of the high-mannose form in the Golgi fraction (Fig. 8).
§ Time for half-maximal appearance of the complex-glycosylated form in the homogenate (Fig. 2).
‖ Transport rate from ER to brush border minus transport rate from ER to site of complex glycosylation.

Figure 9. Appearance of newly synthesized high-mannose DPPIV (Δ, ▲) and SI (○, ●) in the pre-Golgi fraction (—) as compared to the Golgi fraction (— — —). For purposes of clarity the maximal relative amount of radioactivity in the high-mannose forms was set to 100%.
A much higher percentage of the total SI exists as high-mannose precursor forms than is true for total DPPIV. This high-mannose SI appears in the Golgi complex more slowly than the high-mannose DPPIV. These results clearly indicate an intragolgi rate-limiting step of the conversion of high-mannose to complex SI.

An unexpected but interesting observation is the long apparent residence time of part of the complex-glycosylated hydrolases in the Golgi fraction. This is not likely due to cross contamination by brush border membranes since the turnover of brush border enzymes in these cells is slow (Hauri, H.-P., unpublished data), this suggests that part of the newly synthesized enzymes of this type (including DPPIV, aminopeptidase N, angiotensin I-converting enzyme, and PABA-peptide hydrolase) are synthesized as single-chain, two-active-site polypeptides (33). At least one of them, SI, but probably all three, appear to have evolved by partial duplication of ancestors of one-active-site enzymes (16). Gene duplication may have interfered, to some extent, with the efficient maturation of the enzymes by making protein folding and/or glycosylation more complicated. This speculation is supported by results from studies on patients suffering from hereditary sucrase-isomaltase deficiency. Minor alterations in sucrase-isomaltase (probably point mutations) that are not detectable by SDS-PAGE were found to lead to an inhibition of transport at the level of the Golgi apparatus (13). The rapidly transported peptidases, on the other hand, in particular, the radioactivity corresponding to DPPIV in this fraction continues to decrease at a time when there is no further concomitant increase in the brush border membrane. Since the turnover of brush border enzymes in cell culture is slow (Hauri, H.-P., unpublished data), this suggests that part of the newly synthesized enzymes of this pool never reaches the cell surface but is either degraded in the Golgi apparatus or transported to other intracellular organelles like the lysosomes. Fransen et al. (8) have demonstrated by immunoelectron-microscopy that in human small intestinal biopsies a mAb against SI could label lysosomes in addition to organelles of the biosynthetic pathway. However, it is not known whether this lysosomal SI was directly imported from the Golgi apparatus and hence bypassed the cell surface or whether it originated from endocytosis.

Some of the present data are in line with the suggestions of Danielsen and Cowell (5) who postulated that SI and aminopeptidase N in human intestinal organs are synchronously transported from the site of complex glycosylation to the brush border membrane. However, we disagree with the conclusions of these authors that a pre-Golgi event is the only rate-limiting step for the efficient transport of the hydrolases to the cell surface. Our results strongly suggest that the asynchronous transport of these hydrolases is also due to intra-Golgi events. In the study of Danielsen and Cowell (5), the rate of conversion from transient (high mannose) to mature (complex-glycosylated) form was assumed to be a measure for transport to the Golgi apparatus. However, this event clearly is a medial- to trans-Golgi function (27) and therefore does not reflect initial arrival at the Golgi apparatus.

It is currently unknown to what extent the present observations on the role of the Golgi apparatus in the asynchronous transport can be generalized for endogenous membrane proteins. To our knowledge there is only one previous study dealing with the asynchronous migration to the cell surface of endogenous membrane glycoproteins (44). Although the authors of that study concluded that the different transport rates of two closely related histocompatibility antigens are due to an event associated with the endoplasmic reticulum, their subcellular fractionation data do not strictly rule out the contribution of an intra-Golgi event. It is important to note that for secretory proteins, the endoplasmic reticulum rather than the Golgi apparatus was found to be the rate-limiting step for migration (10, 21, 25, 32). Thus, it is likely that fundamental differences exist between the transport of secretory and membrane proteins.

The molecular basis for asynchronous protein transport is unknown. In intestinal epithelial cells, slowly transported microvillar glycoproteins like SI (14), lactase–phlorizin hydrolase (23), or malase–glucoamylase (22) share a number of common properties that are distinct from the rapidly migrating peptidases. These three enzymes are disaccharidases that are synthesized as single-chain, two–active-site polypeptides (33). At least one of them, SI, but probably all three, appear to have evolved by partial duplication of ancestor genes coding for one–active-site enzymes (16). Gene duplication may have interfered, to some extent, with the efficient maturation of the enzymes by making protein folding and/or glycosylation more complicated. This speculation is supported by results from studies on patients suffering from hereditary sucrase–isomaltase deficiency. Minor alterations in sucrose–isomaltase (probably point mutations) that are not detectable by SDS-PAGE were found to lead to an inhibition of transport at the level of the Golgi apparatus (13). The rapidly transported peptidases, on the other hand, including DPPIV, aminopeptidase N, angiotensin I-converting enzyme, and PABA-peptide hydrolase are synthesized as single-chain, one–active-site polypeptides (12, 33, Sterchi, E., H. Naim, and H.-P. Hauri, unpublished data). Furthermore, they are in general smaller (up to twofold) than the major disaccharidases.

In conclusion, the present study suggests that transit through the Golgi apparatus in addition to exit from the endoplasmic reticulum is rate limiting in the migration of two microvillar hydrolases to the cell surface. The molecular basis of the asynchronous enzyme transport remains to be elucidated. Furthermore, the Golgi apparatus may play an im-
portant role in regulating the surface expression of these enzymes at a posttranslational level.

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References

1. Balcarova-Ständer, J., S. E. Pfeiffer, and K. Simons. 1984. Development of cell surface polarity in the epithelial Madin-Darby canine kidney (MDCK) cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2687–2694.

2. Balch, W. E., and J. E. Rothman. 1985. Characterization of protein transport between successive compartments of the Golgi apparatus: asymmetric distribution of donor and acceptor activities in a cell-free system. *Arch. Biochem. Biophys.* 240:413–425.

3. Boyle, F., S. Snape, P. Duane, N. Cook, and T. Peters. 1986. Galactosyltransferase activity is not localized to the brush border membrane of human small intestine. *Biochim. Biophys. Acta.* 876:171–175.

4. Dahlqvist, A. 1968. Assay of intestinal disaccharidases. *Arch. Biochem. Biophys.* 123:27–33.

5. Danielsen, E. M., and G. M. Cowell. 1985. Biosynthesis of intestinal microvillar proteins. The intracellular transport of aminopeptidase N and acceptor activities in a cell-free system. *Arch. Biochem. Biophys.* 240:413–425.

6. Dunphy, W. G., and J. E. Rothman. 1985. Compartmental organization of the Golgi stack. *Cell.* 42:13–21.

7. Fitting, T., and D. Kabat. 1982. Evidence for a glycoprotein "signal" involved in transport between subcellular organelles. Two membrane glycoproteins encoded by murine leukemia virus reach the cell surface at different rates. *J. Biol. Chem.* 257:14011–14017.

8. Fransen, J. A. M., L. A. Ginsel, H.-P. Hauri, E. Sterchi, and J. Blok. 1985. Immuno-electronmicroscopical localization of a microvillus membrane disaccharidase in the human small-intestinal epithelium with monoclonal antibodies. *Eur. J. Cell Biol.* 38:6–15.

9. Fries, E., and J. E. Rothman. 1980. Transport of vesicular stomatitis virus glycoprotein in a cell-free extract. *Proc. Natl. Acad. Sci. USA.* 77:3870–3874.

10. Fries, E., J. Gostafsson, and P. A. Peterson. 1984. Four secretory protein synthetized by hepatocytes are transported from endoplasmic reticulum to Golgi complex at different rates. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:147–152.

11. Fuller, S., C.-H. von Bonsdorff, and K. Simons. 1984. Vesicular stomatitis virus infects and matures only through the basolateral surface of the polarized epithelial cell line, MDCK. *Cell.* 38:65–77.

12. Hauri, H. P. 1988. Biogenesis and intracellular transport of intestinal brush border membrane hydrolases: use of antibody probes and tissue culture. *Subcell. Biochem.* In press.

13. Hauri, H.-P., J. Roth, E. Sterchi, and M. J. Lentze. 1985. Transport to cell surface of intestinal sucrase-isomaltase is blocked in the Golgi apparatus in a patient with congenital sucrase-isomaltase deficiency. *Proc. Natl. Acad. Sci. USA.* 82:4423–4427.

14. Hauri, H.-P., E. Sterchi, D. Bientz, J. A. M. Fransen, and A. Marxer. 1985. Expression and intracellular transport of microvillus membrane hydrolases in human intestinal epithelial cells. *J. Cell Biol.* 101:838–851.

15. Dethlefsen, K. 1986. The sucrase-isomaltase complex: primary structure, membrane-orientation, and evolution of a stalked, intrinsic brush border protein. *Cell.* 46:227–234.

16. Khashgarian, M., D. Biemond, M. Caplan, and B. Forbes. 1985. Monoclonal antibody to Na,K-ATPase: immunocytochemical localization along nephron segments. *Kidney Int.* 28:899–913.

17. Kenny, A. J., and S. Maroux. 1982. Topology of microvillus membrane hydrolases of kidney and intestine. *Physiol. Rev.* 62:91–128.

18. Kornfeld, R., and S. St. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631–664.

19. Leveque, J. E. 1982. Expression of a differentiated transport function in apical membrane vesicles isolated from an established epithelial cell line. *So-