Cell Type–dependent Variations in the Subcellular Distribution of α-Mannosidase I and II

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Abstract. α-mannosidases I and II (Man I and II) are resident enzymes of the Golgi complex involved in oligosaccharide processing during N-linked glycoprotein biosynthesis that are widely considered to be markers of the *cis-* and *medial*-Golgi compartments, respectively. We have investigated the distribution of these enzymes in several cell types by immunofluorescence and immunoelectron microscopy. Man II was most commonly found in *medial-* and/or *trans-* cisternae but showed cell type–dependent variations in *intra*-Golgi distribution. It was variously localized to either *medial* (NRK and CHO cells), both *medial* and *trans* (pancreatic acinar cells, enterocytes), or *trans-* (goblet cells) cisternae, or distributed across the entire Golgi stack (hepatocytes and some enterocytes). The distribution of Man I largely coincided with that of Man II in that it was detected primarily in *medial-* and *trans*-cisternae. It also showed cell type dependent variations in its *intra*-Golgi distribution. Man I and Man II were also detected within secretory granules and at the cell surface of some cell types (enterocytes, pancreatic acinar cells, goblet cells). In the case of Man II, cell surface staining was shown not to be due to antibody cross-reactivity with oligosaccharide epitopes. These results indicate that the distribution of Man I and Man II within the Golgi stack of a given cell type overlaps considerably, and their distribution from one cell type to another is more variable and less compartmentalized than previously assumed.

INHERENT to current models of the Golgi complex is that it is both polarized and compartmentalized (11, 15, 16, 20, 22, 39). The evidence for compartmentalization is based on immunocytochemical and biochemical (cell fractionation) findings on the distribution within the Golgi complex of enzymes involved in the synthesis and modification of N-linked glycoproteins (reviewed in 15–17). Three functionally distinct compartments have been envisaged based on their ability to be separated from one another on sucrose density gradients. These consist of a light fraction containing the late acting Golgi enzymes, galactosyl- and sialytransferase, and a denser one containing the earlier acting enzymes α-mannosidase II (Man II)[1] and GlcNAc transferase I (11, 13, 14, 18). These fractions were assumed to correspond to *trans-* and *medial*-Golgi elements, respectively, because

galactosyl- and sialyltransferase have been localized by immunocytochemistry to *trans*-cisternae and the *trans*-Golgi network (TGN) (40, 42), and GlcNAc transferase I has been similarly localized to *medial*-Golgi cisternae (14). A third fraction, heavier than the other two, was found to contain GlcNAc phosphotransferase and GlcNAc phosphodiesterase, the enzymes that generate the mannose-6-phosphate recognition marker on lysosomal enzymes (18). Based on its high density this compartment was believed to correspond to the *cis*-Golgi cisternae. Mannosidase I (Man I) has also been assumed to reside in the *cis*-Golgi, because it acts on oligosaccharides of proteins that have just left the ER (1). However, none of these earlier acting enzymes have yet been localized within the Golgi stack by immunocytochemistry. In fact relatively few Golgi enzymes have been localized to date due to the fact that relatively few antibodies have been generated that are suitable for such purposes (17).

In this paper we have localized by immunoelectron microscopy two Golgi enzymes—Man I and II—that are thought to represent markers for the *cis-* and *medial*-Golgi compartments, respectively. Man I is the enzyme that modifies $Man_{9,8,6}GlcNAc_2$ oligosaccharides that enter the Golgi complex (53, 55, 56) to produce the transient endoglycosidase D-sensitive structure $Man_5GlcNAc_2$ (27).

1. *Abbreviations used in this paper:* endo D, endoglycosidase D; endo H, endoglycosidase H; Man I, α1, 2-mannosidase I; Man II, GlcNAc transferase I-dependent α1,3(α1,6)-mannosidase; MEL, murine erythroleukemia; RBL, rat basophilic leukemia.

Man II modifies the GlcNAcMan$_5$GlcNAc$_2$ intermediate to complete the mannose trimming reactions and gives rise to the endoglycosidase H (endo H)–resistant oligosaccharide, GlcNAcMan$_3$GlcNAc$_2$ (30, 52, 56). At present information on the localization of these enzymes within the Golgi stack is conflicting. Studies in which the glycosylation of VSV G protein has been followed in vitro have supported the idea that both Man I and II are located in the proximal (cis/medial) rather than the more distal (trans, TGN) region of the Golgi (1, 4, 5, 45). In a previous immunocytochemical study it was reported that Man II has a broad distribution across the Golgi stack in hepatocytes (34); however, it was localized to cis- and medial-cisternae in rat basophilic leukemia (RBL) cells (23) and BHK cells (3, 8). It is not clear whether these differences are due to differences in the methods or antibodies used or if they represent true variations from one cell type to the other. Our present results indicate that the distribution of both Man I and II in the Golgi complex is both variable and cell type-specific, but most often the two enzymes are localized together in medial- and/or trans-cisternae. These results are in keeping with other recent immunocytochemical evidence indicating that Golgi compartmentalization is more variable and less rigid than often assumed (6, 33, 36, 39).

Materials and Methods

Materials

Fab fragments of goat anti-rabbit IgG conjugated to HRP were purchased from Biosys (Compiègne, France), F(ab')$_2$ fragments of goat anti-rabbit IgG conjugated to FITC were purchased from TAGO (Burlingame, CA). 5 nm gold, goat anti-rabbit conjugate was obtained from Amersham Corp. (Arlington Heights, IL). Protein A and Sephadex G-25 columns were from Pharmacia Fine Chemicals (Piscataway, NJ). FITC-WGA, saponin, and BSA were obtained from Sigma Immunochemicals (St. Louis, MO). N-glycanase was from Genzyme (Cambridge, MA). Tissue culture reagents were obtained from GIBCO BRL (Gaithersburg, MD). PVDF was purchased from Millipore Continental Water Systems (Bedford, MA).

Antibodies

Anti-Man II. The catalytic domain of Golgi Man II was purified as described (32) and used to immunize a rabbit. The specificity of this antibody is the same as described for a previous polyclonal antibody raised against the intact enzyme (30) which was shown to immunoprecipitate Man II (124,000 mol wt) from rat liver, 3T3 cells and Hela cells, and to be distinct from both lysosomal and cytosolic α-D-mannosidases and Golgi α-Man IA and IB.
Anti-Man I. Rabbit polyclonal antibody against Golgi Man I purified from rat liver has been previously characterized (55). This antibody recognizes a 58-kD doublet on immunoblots of total microsomal proteins from both rat liver and pancreas, and quantitatively precipitates both Man IA and IB activities. It is therefore referred to as Man I antibody. It does not cross-react with other α-D-mannosidases such as cytosolic or lysosomal mannosidases, or Golgi Man II (55). The antibody (IgG fraction) was found to show cross-reactivity to fibronectin and was subsequently affinity depleted of cross-reactivity by passing it through a fibronectin-Sepharose 4B column.

Preparation of Antibodies to Deglycosylated Man II

1 mg of protein A-purified IgG was incubated with 30 μg N-glycanase deglycosylated Man II which had been electrophoretically transferred to a polyvinylidene difluoride membrane. The membrane was washed with 0.5 M NaCl, 10 mM Tris, pH 8.0, 5 mM EDTA buffer, and the bound IgG fraction was eluted with two rinses (0.5 ml, 1 min) of 1 M propionic acid (pH 2.3) containing 0.5% BSA, followed by two rinses (0.5 ml, 1 min) 1.5 M potassium thiocyanate. The eluant was neutralized and desalted by immediate application to a Sephadex G-25 column, concentrated on a Centricon 30 membrane, and dialyzed against PBS containing 50% glycerol.

Cell Culture

Y3-Ag1.2.3 rat myeloma cells were obtained from American Type Culture Collection (Rockville, MD) (CRL 1631) and cultured in high glucose DME with 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin, 10% (vol/vol) FCS. Normal rat kidney (NRK) cells were cultured in this same medium supplemented with 2.5% (vol/vol) FCS, and 2.5% (vol/vol) newborn calf serum. CHO K1 cells were cultured in Ham's F12 medium containing 10% (vol/vol) FCS, 2 mM L-glutamine, and antibiotics.

Immunolabeling

Indirect immunofluorescence was carried out on cultured cells on coverslips or on semithin (1 μm) cryosections cut on a microtome equipped with an FC-4 cryoattachment (Reichert Jung, Vienna). Cultured cells were fixed with 2% paraformaldehyde in phosphate buffer, pH 7.4, and permeabilized with 0.1% Triton X-100. They were then rinsed, blocked with PBS/1% BSA, and incubated with primary antibody (1–2 h at room temperature) and with FITC-conjugated secondary antibodies (1 h). Semithin cryosections were similarly stained, except that some sections were partially deglycosylated by incubation with 2 M NaBH$_4$ in 0.1 M NaOH at 37°C for 6 h. Section deglycosylation was monitored by assessing the disappearance of FITC-wheat germ agglutinin staining.

For immunoperoxidase, cultured cells were fixed in periodate-lysine-paraformaldehyde fixative for 4 h at room temperature (7). Fixed cells were permeabilized with 0.005% saponin in PBS/0.5% BSA and sequentially incubated with primary antibodies and HRP-conjugated secondary antibodies
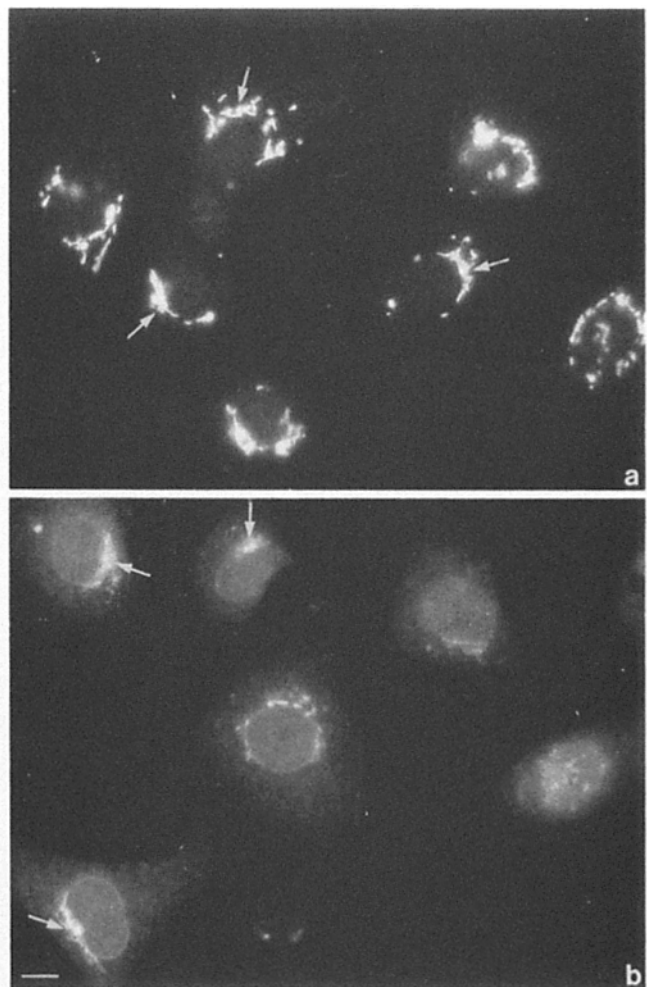

Figure 1. Immunofluorescence localization of Man II (a) and I (b) in NRK cells showing that these enzymes are concentrated in the Golgi region. Cells were grown on coverslips, fixed in paraformaldehyde, permeabilized and processed for immunofluorescence as described in Materials and Methods. Bar, 5 μm.
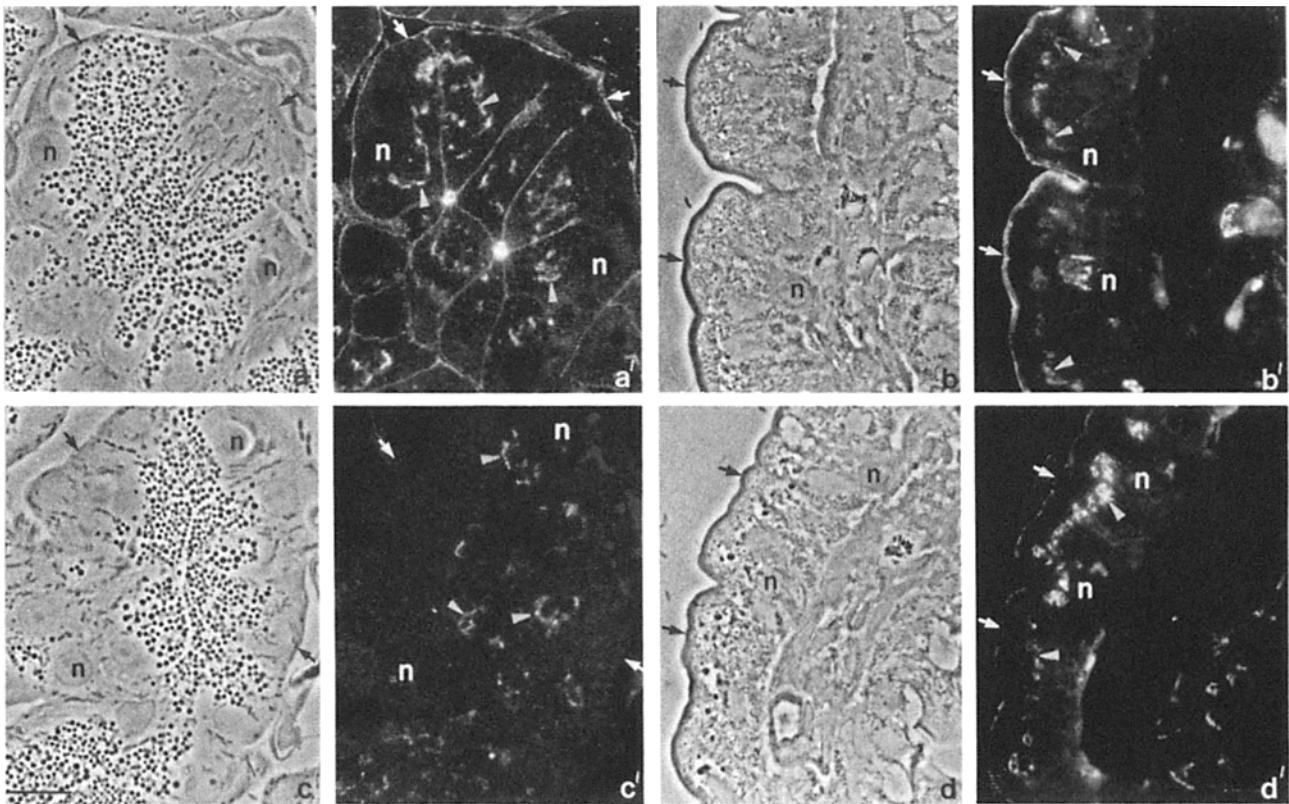
Figure 2. Phase (*a–d*) and immunofluorescence (*a'–d'*) micrographs demonstrating the distribution of Man II in pancreatic acinar cells (*a* and *c*) and intestinal epithelial cells (*b* and *d*) in semithin (0.5–1 μm) cryosections. Staining for Man II is seen in both the Golgi region (*arrowheads*) and at the cell surface (*arrows*). In sections incubated with anti-Man II that had been affinity purified on deglycosylated Man II (*c* and *d*), staining is reduced but not eliminated on the plasma membrane of both exocrine pancreas (compare *a'* with *c'*) and intestinal epithelium (compare *b'* and *d'*). *n*, nucleus. Bar, 10 μm.
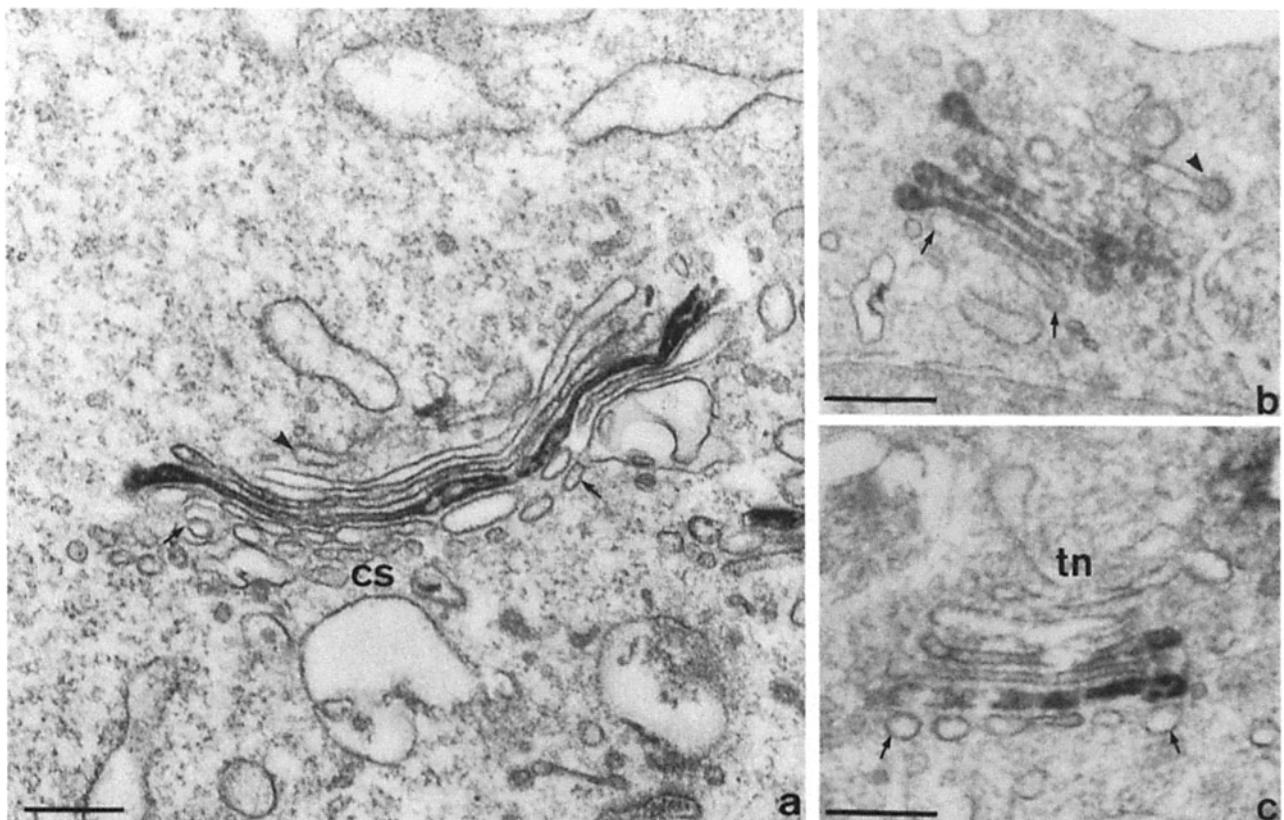
Figure 3. Immunoperoxidase detection of Man II in the Golgi complex of NRK (*a*) and CHO (*b* and *c*) cells. In both cell types Man II is localized in one to four *medial*-cisternae. The first, fenestrated *cis*-cisterna (*arrows*) and the clathrin-coated buds (*arrowheads*) of the TGN (*tn*) are indicated. Bar, 0.25 μm.
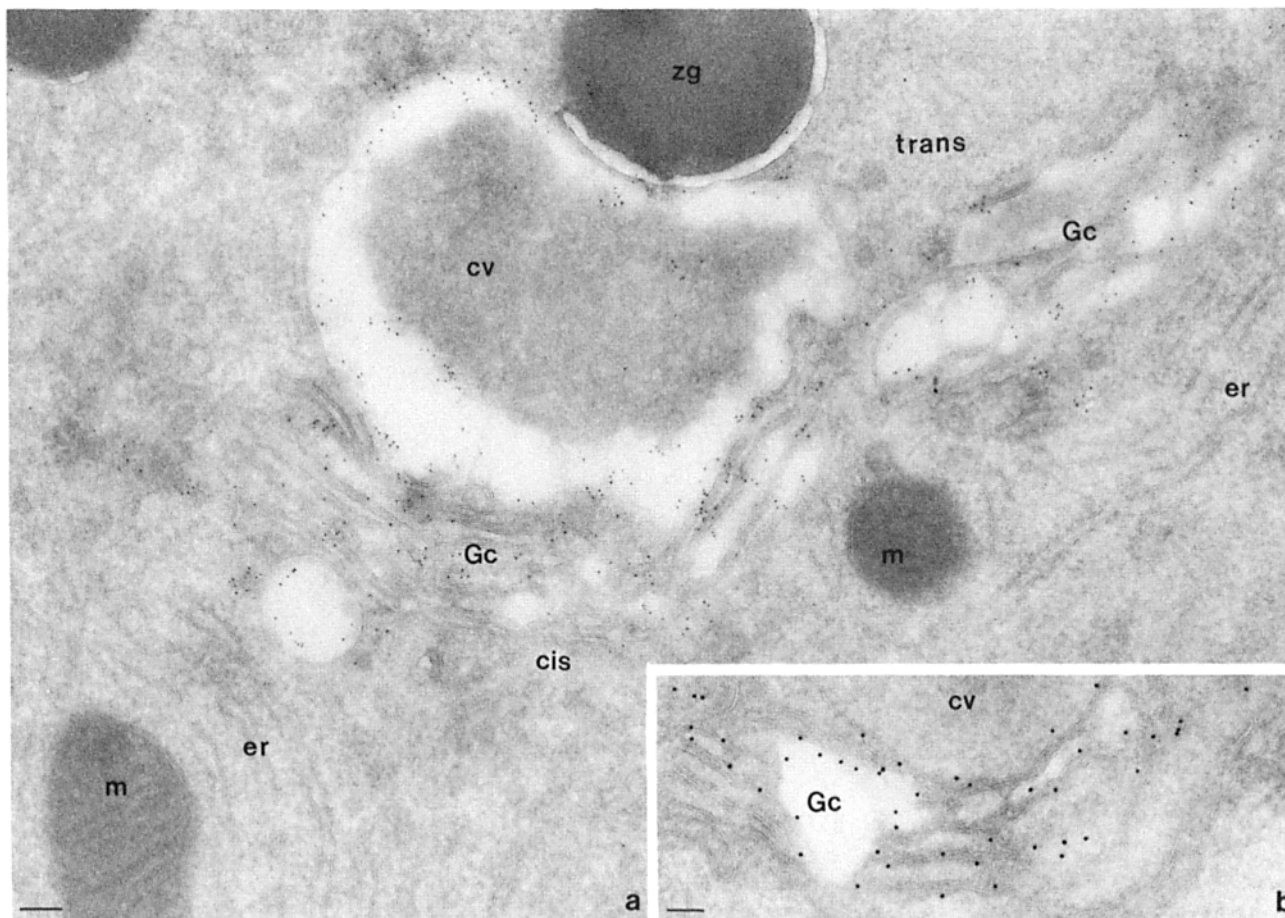
Figure 4. Localization of Man II in the pancreatic acinar cell by immunogold labeling of ultrathin cryosections. (*A*) Gold particles are seen across the Golgi stack (*Gc*) but are most numerous in *medial-* and *trans*-cisternae and a condensing vacuole (*cv*). (*B*) A similar distribution of label is seen with antibody affinity purified on deglycosylated Man II. *er*, endoplasmic reticulum; *m*, mitochondria; *zg*, zymogen granule. Bar, 0.1 μm.

diluted in the same buffer. They were then fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 30 min, and then processed for immunoperoxidase (7).

For immunogold labeling rat tissues were fixed in 3% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% glutaraldehyde in PBS for 1 h at room temperature. After blocking free aldehyde groups with 50 mM NH$_4$Cl for 30 min, the tissue fragments were embedded at −35°C in Lowicryl K4M (41). Alternatively, tissue pieces were fixed for 1 h in 3% formaldehyde, 0.05% glutaraldehyde in 0.1 M cacodylate-HCl buffer, pH 7.4, infiltrated with a mixture of 50% polyvinylpyrrolidone and 2.3 M sucrose, and processed for ultracryomicrotomy (54). Immunogold labeling was carried out in some cases on plastic (Lowicryl)-embedded sections and in others on ultrathin cryosections. Ultrathin sections were cut from Lowicryl-embedded tissue, incubated with primary antibody followed by 8 nm protein A-gold complexes prepared by the tannic acid method (48). Ultrathin cryosections were prepared (54) incubated with primary antibody followed by 5 nm gold, goat anti–rabbit conjugate. Labeled Lowicryl sections were stained with uranyl acetate followed by lead citrate, and labeled ultrathin cryosections were absorption stained with 0.2% methyl cellulose, and 2.2% polyvinyl alcohol (54).

Results

Man I and Man II Are Localized to the Golgi Region in Cultured Cells

By immunofluorescence both Man I and Man II were concentrated in the Golgi region of NRK (Fig. 1) and CHO cells

(not shown). These enzymes were not detected at the cell surface or in any other cell organelle. In the case of Man II a wide variety of cell lines were tested, including COS cells, murine erythroleukemia (MEL) cells, 3T3 cells, rat embryonic fibroblasts, AtT-20 pituitary cells, and GH3 pituitary cells, with the same results. Thus our findings verify the usefulness of Man II as an intracellular Golgi "marker" in cultured cell lines. In specialized cells in situ, Man I and Man II (Fig. 2, *a* and *b*) were found in the Golgi region, but were also present at the cell surface (see below).

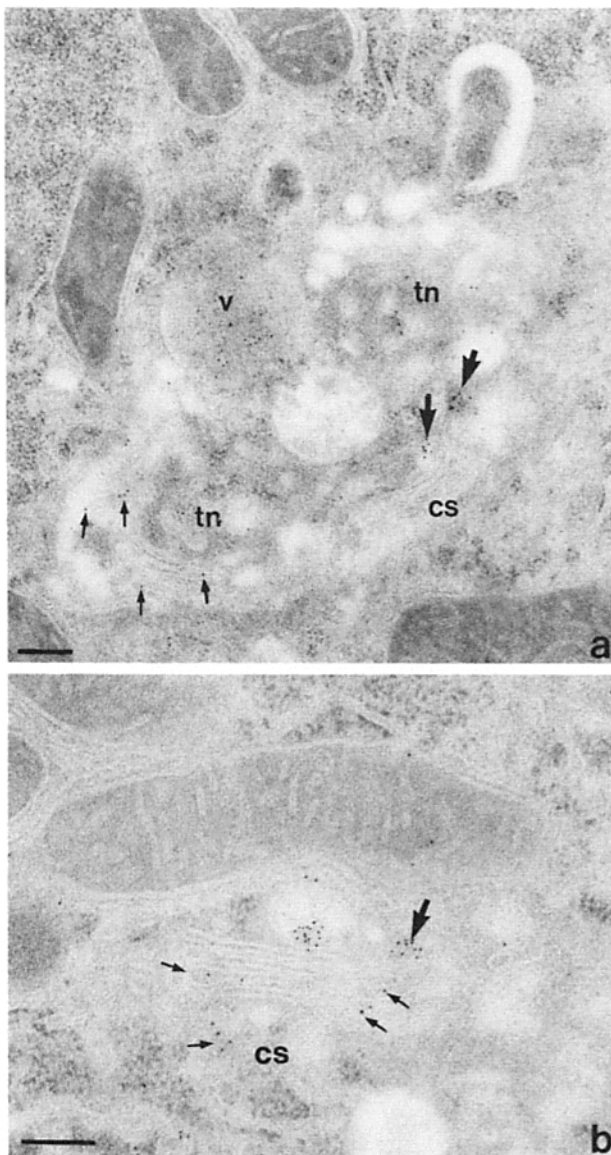### The Distribution of Man II within the Golgi Stack Varies among Different Cell Types

In NRK (Fig. 3 *a*) and CHO cells (Fig. 3, *b* and *c*), Man II was localized in two to three *medial*-Golgi cisternae sandwiched between one to two nonreactive cisternae on each side. In other cell types it had a different distribution: It was detected in both *medial-* and *trans*-cisternae in pancreatic acinar cells (Fig. 4 *a*), exclusively in *trans*-cisternae in goblet cells of both duodenum (Fig. 5 *a*) and colon (Fig. 5 *b*), in *medial-* and *trans*-cisternae and the TGN in duodenal enterocytes (Fig. 5, *c* and *d*), and all across the Golgi stack in hepatocytes (Fig. 6), and some enterocytes (Fig. 5 *e*). It was also detected on the membranes and in some cases
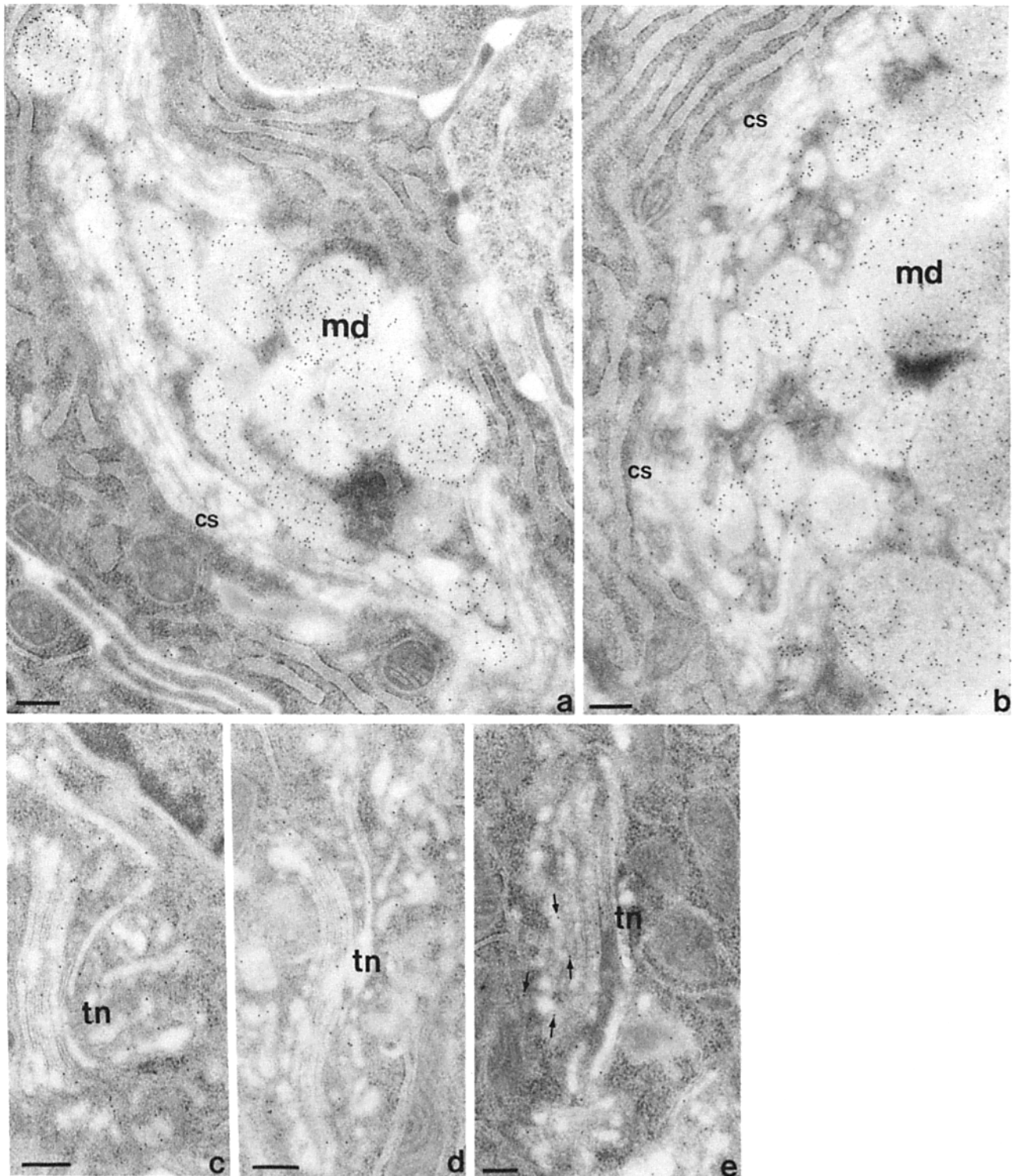
Figure 5. Distribution of Man II across the Golgi complex of intestinal epithelial cells. In goblet cells of both duodenum (*a*) and colon (*b*) Man II is exclusively localized in the *trans*-Golgi cisternae and the lumen of mucin droplets (*md*), especially those newly derived from the *trans* side of the Golgi stack. No labeling of cis (*cs*) cisternae is seen. In enterocytes (*c–e*), Man II is detected in the TGN (*tn*) and two to three cisternae on the *trans* side of the Golgi stack; occasionally, the *cis*-cisternae (*arrows*) are also reactive (*e*). Lowicryl K4M thin sections stained with anti-Man II and protein A-gold. Bar, 0.25 μm.

within the contents of secretory granules/vesicles, i.e., condensing vacuoles and zymogen granules of exocrine pancreas (Fig. 4 *a*), secretory vesicles of hepatocytes (Fig. 6, *a* and *b*), and mucin droplets of goblet cells (Fig. 5, *a* and *b*).

These results indicate that Man II is found predominantly in *medial*- and/or *trans*-cisternae in the majority of cell types, but its precise distribution within the Golgi stack varies from one cell type to another.

Figure 6. Distribution of Man II in the Golgi complex of hepato-cytes. Immunogold labeling is predominantly found at the rims (*large arrows*) of the *trans*-Golgi cisternae, the TGN (*tn*), and secretory vesicles (*v*). It is also detected, although with less intensity, in association with cis and *medial*-Golgi cisternae (*small arrows*). Thin sections were obtained from Lowicryl-embedded rat liver. Bar, 0.25 μm.

The Distribution of Man I within the Golgi Overlaps with That of Man II

The distribution of Man I within the Golgi also varied. It was concentrated in the *trans*-cisternae and the TGN in NRK cells (Fig. 7, *e–g*) and in *medial*- and *trans*-cisternae of enterocytes (Fig. 8, *e* and *f*). Occasionally, the enzyme extended more broadly in the *cis*-direction, i.e., including the *medial*-cisternae of NRK (Fig. 7 *h*) and goblet cells (Fig. 8, *a* and *b*) and *cis*-cisternae of enterocytes (Fig. 8 *g*). It was also found all across the Golgi stack in pancreatic acinar cells (Fig. 9), rat Y3-Ag1.2.3 myeloma cells (Fig. 7, *a–c*), and hepatocytes (not shown), but was most concentrated in *medial*- and *trans*-Golgi cisternae. In all the foregoing with

the exception of myeloma cells (where it was not possible to compare their distributions due to low reactivity of Man II) the localization of Man I clearly overlapped with that of Man II (Table I). As in the case of Man II, labeling for Man I was also present in secretory granules/vesicles of pancreatic acinar cells (Fig. 9) and goblet cells from both duodenum (Fig. 8, *a* and *b*) and colon (Fig. 8, *c* and *d*). It can be concluded that Man I, like Man II, is concentrated primarily in *medial*- and *trans*-Golgi subcompartments where it overlaps and largely coincides with the distribution of Man II.

## Man I and Man II Are Found at the Cell Surface in Some Cell Types

In several specialized cell types, i.e., pancreatic acinar cells and enterocytes, both Man II (Figs. 2 and 10) and Man I (Fig. 11) were found along the plasma membrane as well as associated with Golgi membranes. Both the apical and basolateral plasma membrane domains of enterocytes and pancreatic acinar cells were labeled with both Man I (Fig. 11) and Man II (Fig. 2) antibodies. Labeling was particularly intense at the apical surface with numerous gold particles delineating the membrane of the microvilli in both duodenum (Figs. 10 *a* and 11, *a–c*) and colon (Fig. 11 *d*). There was also significant labeling of the lateral surfaces (Fig. 11 *a*), but the staining was lower on the basal surface in contact with the basement membrane (Fig. 11 *c*).

To rule out that staining was due to reactivity of the antibody with carbohydrate epitopes, Man II antibody that had been affinity purified on deglycosylated Man II (Figs. 2, *c'* and *d'*, and 10) or deglycosylated tissue sections (not shown) were used. Under such circumstances staining of the plasma membrane was reduced but not eliminated. These results indicate that although some of the cell surface staining obtained with antibody raised against Man II may be due to cross-reactivity with carbohydrate epitopes, the majority of the staining is due to reactivity against epitopes present within the polypeptide. It can be concluded that Man I and II are expressed at the cell surface in some cell types.

Discussion

Man I and II Are Not Segregated into Separate and Distinct Golgi Subcompartments

We have used immunofluorescence, immunoperoxidase, and immunogold techniques to localize Man I and II, two key enzymes in the biosynthesis of N-linked glycoproteins, which are widely believed to represent markers for the *cis*- and *medial*-Golgi subcompartments, respectively. Our immunolocalizations in a variety of cell types indicate that Man I and II: (*a*) exhibit cell type-dependent variations in their Golgi distribution; and (*b*) are not rigidly segregated from one another. Rather, the two enzymes overlap considerably, and both enzymes are concentrated in *medial*- and/or *trans*-cisternae of cell types examined. We recognize that while immunocytochemical techniques allow the proteins to be localized in precise Golgi subcompartments, we may detect only the sites of major concentrations of the enzymes and may fail to detect lower concentrations. Also, the sites of maximal enzyme activity may not be precisely coincident with the sites of maximal enzyme concentration identified by immunocytochemistry. It is possible, however, to compare
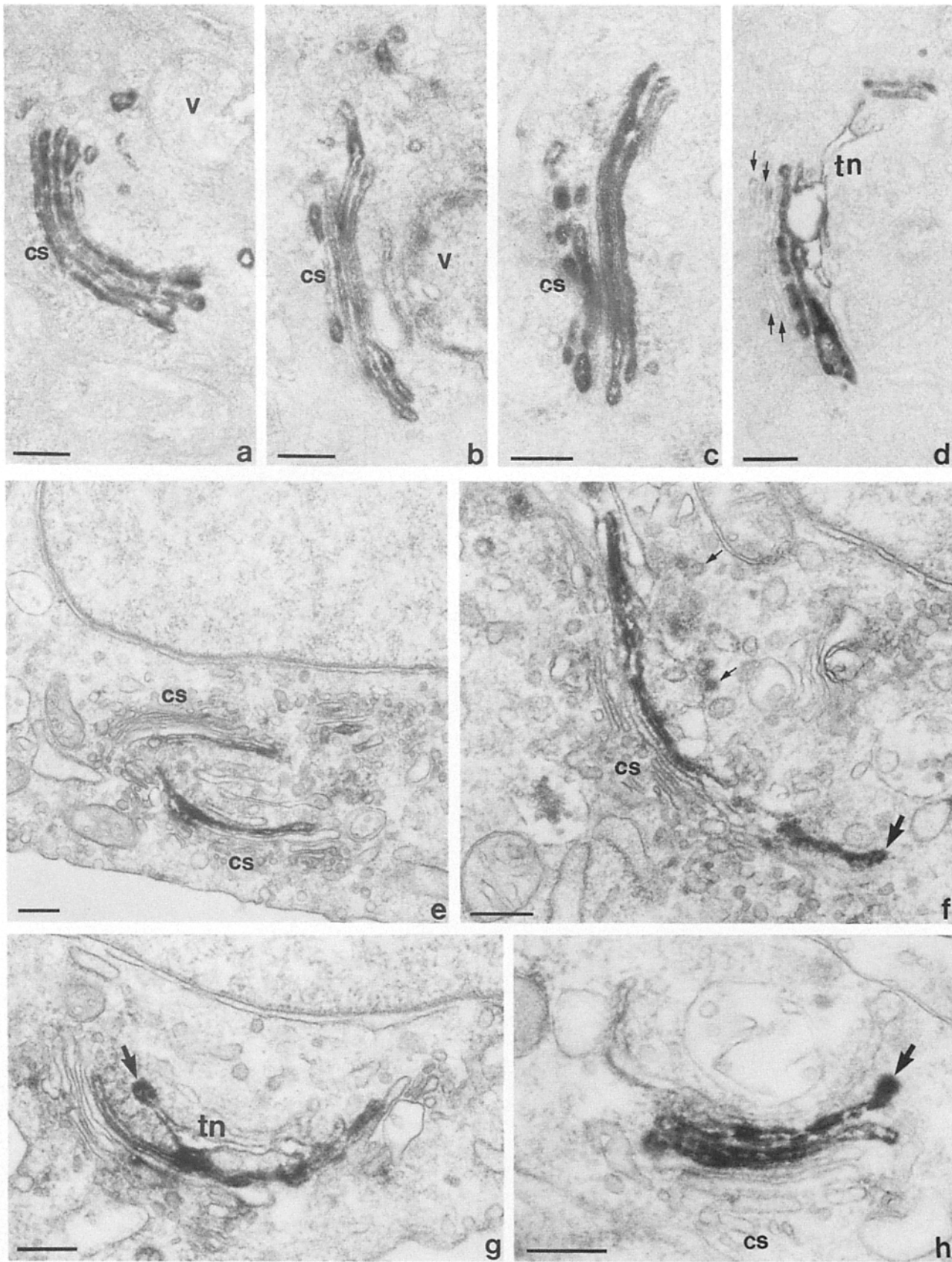
Figure 7. Detection of Man I in the Golgi complex of cultured cells processed by immunoperoxidase. In Y3-Ag1.2.3 rat myeloma cells (*a–c*), Man I is commonly present in all cisternae across the Golgi stack. In some myeloma cells (*d*), however, the cis (*cs*) cisternae (*arrows*) are not reactive. In NRK cells (*e–h*) Man I is localized in the *trans*-cisternae and the TGN (*m*). The latter can be identified by the presence of clathrin coated pits (*large arrows*) and vesicles (*small arrows*) which are also labeled. *v*, secretory vesicles. Bar, 0.25 µm.
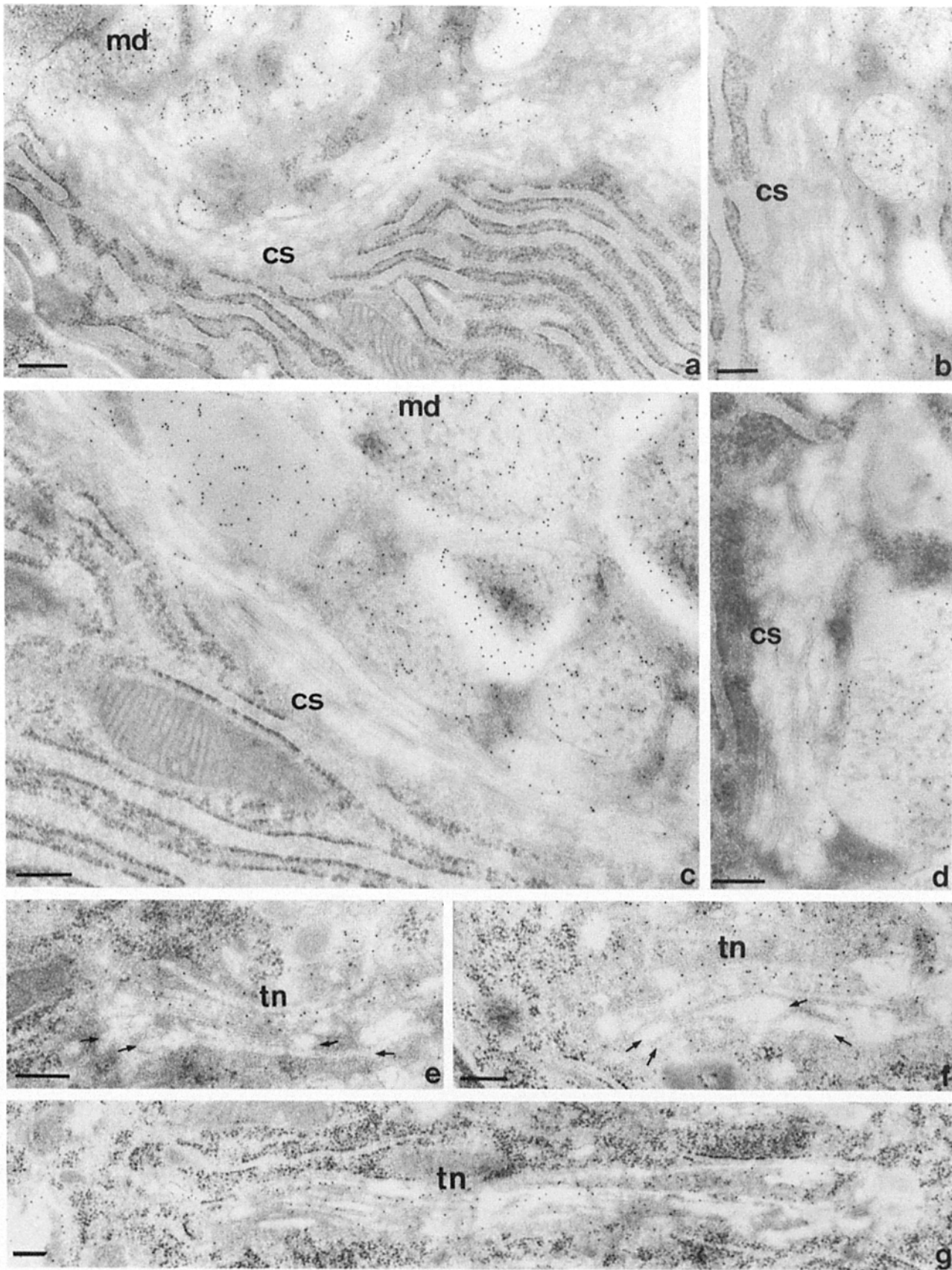
Figure 8. Localization of Man I in the Golgi complex of intestinal epithelial cells. Ultrathin sections of both duodenum (*a, b,* and *e*) and colon (*c, d, f,* and *g*) embedded in Lowicryl K4M and stained with anti-Man I and protein A-gold (8 nm). In goblet cells (*a–d*) Man I is concentrated in one to two *trans*-Golgi cisternae and in the lumen of mucin droplets (*md*). In enterocytes (*e–g*) Man I is localized in the *medial*- and *trans*-Golgi cisternae and the TGN (*tn*). Usually the enzyme is not detected in one to two *cis*-(*cs*) cisternae (*arrows*), except in some enterocytes (*g*) where Man I is distributed all across the Golgi stack. Bar, 0.25 μm.
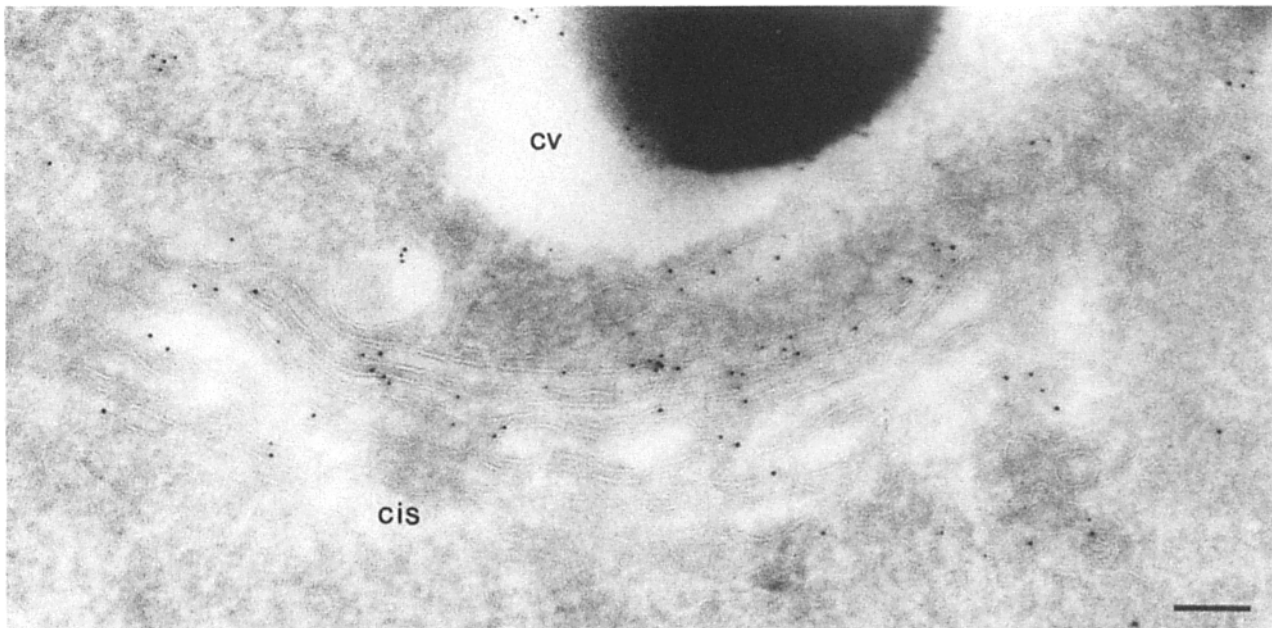
Figure 9. Distribution of Man I in the Golgi complex of a pancreatic acinar cell. The *medial-* and *trans*-Golgi cisternae and a condensing vacuole (*cv*) are labeled. Ultrathin cryosection reacted with anti-Man I and goat anti–rabbit gold (5 nm). Bar, 0.25 μm.

different distribution patterns that may correspond to different Golgi subcompartmentalization of the *N*-glycosylation reactions and to conclude that there is considerable overlap in the distribution of these two enzymes.

Differences in Distribution of Golgi Enzymes among Different Cell Types

We have found considerable variation in the distribution of Man I and Man II from one cell type to another. Cell type–specific differences in Golgi distribution have also been reported for two terminal glycosyltransferases, α2,6 sialyltransferase and blood group A α1,3 *N*-acetylgalactosaminyltransferase. These enzymes and their glycosylation products have been found to be differentially distributed in the Golgi complex of adjacent intestinal goblet cells and enterocytes (43). In addition, there are reports of endogenous proteins in the *cis*- (58) and *medial*- (19) Golgi cisternae that bear terminal N-linked oligosaccharide modifications, suggesting

either that they are exposed to the terminal transferases at their site of residence or that they traverse the stack and acquire terminal modifications in the trans compartments and are then targeted back to earlier Golgi compartments. Our observations support the concepts that: (*a*) the cisternal localization of Golgi enzymes involved in the processing of *N*-glycoproteins differs in different cell types (19, 43); and (*b*) there is considerable overlap in the distribution of glycoprotein processing enzymes among Golgi subcompartments.

Moreover, as indicated by the variable distribution of both Man I and Man II in enterocytes or Man I in rat myeloma and NRK cells, some variations in the distribution of these enzymes in the Golgi complex may occur within the same cell type. This suggests that Golgi compartmentalization of *N*-glycosylation processing enzymes may be influenced by other factors such as the functional state of the cell. For instance, Golgi distribution of certain cytochemical markers is known to be affected by cell differentiation (10, 35, 36) or by hyperstimulation (49).

Table I. Golgi Localization of α-Man I and II

|	Goblet cells	Enterocytes	Pancreatic acinar cells	Hepatocytes	Y3-Ag 1.2.3 myeloma cells	NRK cells	CHO cells
Man I	*trans*-cisternae secretory vesicles	middle/*trans*-cisternae and TGN§	middle/*trans*-cisternae, zymogen granules	all cisternae‡	all cisternae‖	*trans*-cisternae and TGN‖	ND*
Man II	*trans*-cisternae, secretory vesicles	middle/*trans*-cisternae, and TGN§	middle/*trans*-cisternae, zymogen granules	all cisternae‡, secretory vesicles	ND*	middle cisternae	middle cisternae

* ND, not determined because of low level of staining.
‡ Includes *cis*-, middle and *trans*-cisternae plus the TGN.
§ In some cells it was across the Golgi stack.
‖ In some cells it was middle/*trans*-cisternae and TGN.
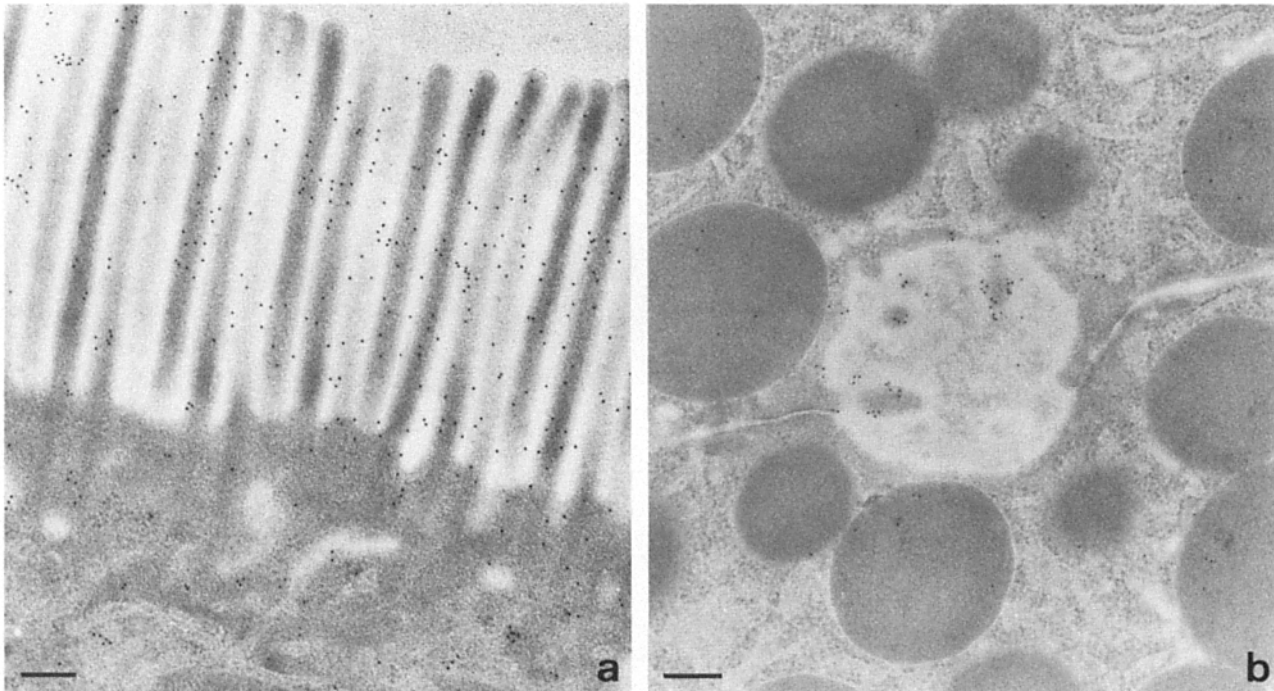
Figure 10. Immunogold labeling of the intestinal brush border (*a*) and a pancreatic acinar cell (*b*) with anti-Man II IgG that had been affinity purified on deglycosylated Man II. Note that in both cases, there is labeling of the apical plasma membrane along the intestinal microvilli and the acinar lumen, respectively. Lowicryl K4M thin sections stained with anti-Man II and protein A-gold (8 nm). Bar, 0.25 μm.

Man I and II Are Concentrated in Medial/Trans Cisternae in Most Cell Types

Man I is the earliest acting of the Golgi enzymes that modify N-linked glycoproteins (52, 55, 56). It cleaves the $Man_{9,8,6}GlcNAc_2$ oligosaccharide to give rise to the endo D–sensitive $Man_5GlcNAc_2$ structure. Although it has not been previously localized by immunoelectron microscopy, it has been assumed by some to be a *cis*-Golgi marker. Furthermore, appearance of endo D–sensitive glycoproteins during biosynthesis has often been taken as indication of transport of the glycoprotein to the *cis*-cisternae of the Golgi complex (1, 4, 5). This is in spite of the fact that: (*a*) Man I and II activities cannot be resolved from one another in an in vitro transport system (45); and (*b*) the two enzymes comigrate on sucrose density gradients (11, 12, 13). We found that in some of the cell types we have studied Man I is concentrated either exclusively in the *trans*-Golgi cisternae (goblet cells) or in both *medial-* and *trans*-Golgi cisternae (enterocytes, pancreatic acinar, and NRK cells) (Table I).

Man II cleaves the $GlcNAcMan_5GlcNAc_2$ oligosaccharide to produce the endo H–resistant structure, $GlcNAc$-$Man_3GlcNAc_2$ (52, 56). Due to the availability of excellent polyclonal (30) and monoclonal (3, 8) anti–Man II antibodies, this enzyme has been widely used as a Golgi marker in immunofluorescence assays (e.g., see references 21, 23, 38). This enzyme was previously localized by immunoelectron microscopy across the Golgi stack of hepatocytes (34), in the *medial*-Golgi cisternae of both BHK and NRK cells (3, 8), and in *cis-* and *medial*-cisternae of RBL cells (23). Based on biochemical findings, it was recently proposed that Man II is also present in the *cis*-Golgi compartment (45). Our immunocytochemical observations indicate that Man II, like Man I, is concentrated in different regions of the Golgi complex depending on the cell type but is predominantly concentrated in *medial-* and *trans*-Golgi subcompartments where its distribution overlaps with that of Man I. Golgi localization of both mannosidases in the same Golgi subcompartments is in agreement with the biochemical findings (11–13, 45) and is not inconsistent with their coordinate action during N-glycoprotein processing (22, 31).

It appears that N-glycoprotein processing by these mannosidases occurs largely in more distal Golgi cisternae in the cell types examined. Alternatively, processing in these cells could be initiated in the *cis*-Golgi cisternae by a different enzyme. For instance, an endomannosidase activity has been demonstrated in the rat liver Golgi that can provide an alternative processing route to Man I for glycoproteins that enter the Golgi complex still bearing glucose residues (24, 25, 29). In the case of the final mannose (Man II) trimming the possibility exists that it is partially or totally carried out in an earlier Golgi compartment by another enzyme such as the $\alpha1,3/\alpha1,6$-mannosidase described in BHK cells (28).

It is also possible that relatively few copies of Man I and Man II, below the level of detection by immunocytochemistry, are present and enzymatically active in earlier (*cis*) Golgi cisternae in the cell types we have studied. In fact, in rat myeloma cells and hepatocytes we have detected Man I and II in all Golgi compartments including the *cis*-cisternae.

Localization of Man I and II on the Cell Surface

Both Man I and II have been immunolocalized on the cell surface of duodenal and colonic enterocytes and pancreatic acinar cells. Although some of the cell surface immunoreactivity with Man II may be due to cross-reactivity of the antibody with carbohydrate epitopes, two lines of evidence indicate that the enzyme is found at the cell surface. First,
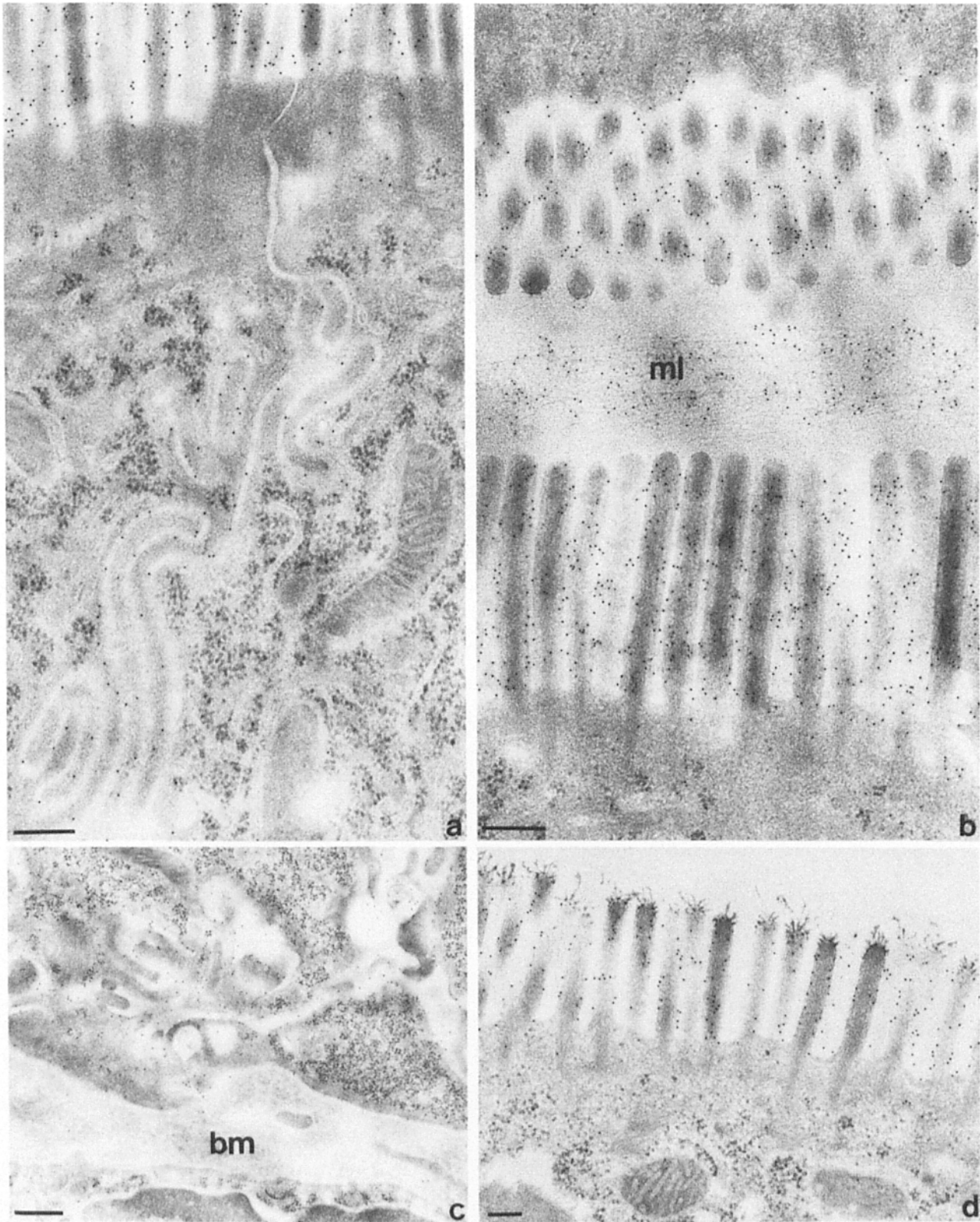
Figure 11. Detection of Man I on the plasma membrane of intestinal epithelial cells. Lowicryl K4M sections of duodenum (*a–c*) and colon (*d*) stained with anti-Man I and protein A-gold (8 nm). The apical (*a, b,* and *d*), lateral (*a*), and basal (*c*) plasma membrane domains and the mucus layer (*ml*) associated with the epithelium are all labeled (*b*). *bm,* basement membrane. Bar, 0.25 μm.

antibody that had been affinity purified on deglycosylated Man II reacts with cell surface epitopes of both enterocytes and pancreatic acinar cells. Second, cell surface staining is detected on chemically deglycosylated sections. Therefore we conclude that Golgi mannosidases are in fact expressed at the plasma membrane of certain cell types.

There are now an increasing number of cases where Golgi enzymes involved in glycoprotein processing have been localized at the plasma membrane of individual cell types, including β1,4-galactosyltransferase (47, 50), α2,6-sialyltransferase (51) and N-acetylgalactosaminyltransferase (2). Furthermore, a fucosyltransferase (37) and an α-D-mannosidase activity have recently been described on the plasma membrane of spermatozoa (57). Usually a role in either cell-substratum and/or cell-to-cell recognition processes has been proposed for these cell surface–associated enzymatic activities. However, before any function can be assigned to Golgi Man I and II at the plasma membrane it must be determined if they are enzymatically active at this location.

General Comments on Markers of Golgi Subcompartments

Thus far the number of Golgi markers that are available are quite limited. Relatively few Golgi-specific antibodies have been generated that are useful for this purpose. The list is limited to a few glycoprotein processing enzymes, galactosyltransferase (40) and sialyltransferase (42) (trans-Golgi and TGN); GlcNAc transferase I (14) (medial-Golgi); and monoclonal and polyclonal antibodies to Golgi proteins such as p53 (46) and p58 (44) (intermediate compartment and cis-Golgi), TGN38 (26) (TGN), a 160-kD protein (medial) (22), and two peripheral Golgi proteins (9) (reviewed in reference 17). Moreover, the use of the available antibody markers has been further limited by factors such as species specificity (e.g., some antibodies to galactosyltransferase recognize the enzyme only in human cells), limited distribution (or detectability) of the antigen in only a few cell types, broader distribution of antigen than Golgi in some cell types.

Among those that are available one of the most widely used for immunofluorescence is Man II for which mouse monoclonal (3, 8) and rabbit polyclonal (30) antibodies have been generated. The rabbit polyclonal antibody we have recently raised gives a strong signal confined to the Golgi region in many cultured cell lines including rat (e.g., NRK, REF52, GH$_3$, RBL cells), mouse (AtT-20, 3T3 cells), hamster (CHO), and human (Hela) cell lines (Farquhar, M. G., unpublished data). Our present immunoelectron microscopic observations confirm that in all cell types examined Man II represents a valid intracellular marker for the Golgi complex for immunofluorescence assays. However, neither it nor Man I can be taken as a marker for any specific Golgi subcompartment since its distribution within the Golgi varies from one cell type to another. Typically both enzymes were found to be most concentrated in middle and trans-Golgi cisternae.

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