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Recognition of a 170 kD protein in mammalian Golgi complexes by an antibody against malarial intraerythrocytic lamellae

Wenlu Li*†, Gilbert-André Keller†, and Kasturi Haldar*

Abstract. Human erythrocytes infected with the malarial parasite *Plasmodium falciparum* contain flattened membrane lamellae. It has been suggested that the lamellae may be involved in the sorting of malarial proteins to the cytoplasm and the cell membrane of the host erythrocyte. We have previously shown that the lamellae accumulate sphingolipids by virtue of their lipid composition in a manner similar to the *trans*-Golgi and the *trans*-Golgi network in mammalian cells. In this paper, we show by immunofluorescence microscopy that a monoclonal antibody to the lamellae labeled a perinuclear organelle that colocalized with WGA and the mannose-6-phosphate receptor in cultured mammalian cells. Immunoelectron microscopy experiments revealed that LWLI labels cisternae of the *trans*-face and the *trans*-Golgi network. Western blot analysis of subcellular fractions using LWLI detected a 170 kD protein which is associated with the luminal side of Golgi membranes of rat liver and is conserved in all cell lines studied. Our results indicate that (i) the 170 kD protein is a novel marker of the mammalian *trans*-Golgi and the *trans*-Golgi network and (ii) in addition to similarities in their morphological and lipid characteristics, the lamellae induced by *P. falciparum* in erythrocytes share proteinaceous determinants with the Golgi apparatus of mammalian cells.

Keywords: *P. falciparum*-infected erythrocyte, malaria lamellae, Golgi apparatus, *trans*-Golgi network

Introduction

The Golgi apparatus plays a pivotal role in the biosynthetic processing and sorting of newly synthesized proteins and lipids (reviewed by Pfeffer and Rothman, 1987; Mellman and Simons, 1992). In animal cells, the Golgi apparatus is a polarized organelle composed of membrane cisternae which are functionally divided into several distinct, juxtaposed and biochemically heterogeneous subcompartments (*cis, medial, trans*, and *trans*-Golgi network) (reviewed by Farquhar and Palade, 1981; Dunphy and Rothman, 1985; Mellman and Simons, 1992). Distinct morphologic features characterize the different subcompartments of the apparatus. The *cis*-side Golgi elements usually consist of ovoid membranous profiles interconnected by fenestrated electron dense bridges that can be selectively impregnated with osmium-tetroxide (Rambourg et al., 1974, 1989; Rambourg and Clermont, 1990). The *medial* and *trans*-elements are composed of narrow, elongated cisternae. The last subcompartment, the *trans*-Golgi network (TGN), appears as a broad network of membranous tubules and vesicular structures. Newly synthesized proteins are sorted and posttranslationally modified while they are moved via vesicular transport through the Golgi apparatus, from the *cis*- to the *trans*-side of the stacks (Rothman and Orci, 1992). In mammalian cells,

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many of the resident Golgi enzymes that catalyze steps in the synthesis of macromolecules have been identified, characterized and used as markers to distinguish the different subcompartments of the Golgi apparatus (Goldberg and Kornfeld, 1983; Dunphy et al., 1985; Roth et al., 1985; Niehrs and Huttner, 1990; Futerman et al., 1990; Jeckel et al., 1990). The mature mammalian erythrocyte is devoid of subcellular organelles and lacks biosynthetic activities. However, when human erythrocytes are infected by the malarial parasite *P. falciparum*, the transport activities in the human erythrocyte are dramatically altered. Membranous structures, including flattened membrane lamellae, membrane circles and electron-dense structures are induced by the parasite in the cytoplasm of infected erythrocytes. Electron microscopy analysis shows that these structures morphologically resemble the organelles of the mammalian secretory pathway (Aikawa et al., 1986; Atkinson et al., 1987). Little is known of the biogenesis of these structures or the mechanisms by which proteins synthesized in the parasite are sorted and transported to the cytoplasm and the cell membrane of the infected erythrocytes. However, since the ultrastructural appearance of the intraerythocytic lamella (originally referred to as Maurer's cleft) resembles a single Golgi cisterna of mammalian stacks (Langreth et al., 1978), it has been proposed that lamellae may function as a transport apparatus for membrane and protein traffic between the parasite and the host cell (reviewed by Barnwell, 1990). Additional evidence that the lammellae may play a role corresponding to that of the Golgi apparatus in mammalian cells may be found in the fact that a fluorescent derivative of C6-NBD-ceramide that accumulates in the *trans*-Golgi apparatus of mammalian cells (Lipsy and Pagano, 1985) also accumulates in the lamellae (Haldar et al., 1991).

Monoclonal antibodies that react with different subsets of these parasite-induced structures have been raised by several laboratories (Kara et al., 1988; Hui and Siddiqui, 1988; Etzion and Perkins, 1989; Stanley et al., 1989; Stenzel and Kara, 1989; Li et al., 1991). We previously raised a battery of monoclonal antibodies against malarial proteins and identified a monoclonal antibody (LWLI) which specifically binds to the flattened lamellae in *P. falciparum*-infected erythrocytes (Li et al., 1991). The antibody recognized a 45/50 kD doublet in Western blots and immunoprecipitation of *P. falciparum* lysates of infected erythrocytes. Cell fractionation studies indicate that LWLI reacts exclusively with parasite-induced structures localized in the erythrocyte cytoplasm and not with the parasite itself (Das et al., 1994). We now extend these results to show that LWLI also reacts with the Golgi apparatus in a variety of mammalian cell types. LWLI recognizes a conserved 170 kD protein which is localized on the luminal side of Golgi membranes in immunoblot experiments. We also determined by immunoelectron microscopy that the 170 kD protein is located exclusively in the *trans*-Golgi cisternae and the TGN.

Materials and methods

Cells

MDBK and CHO cells were grown in RPMI 1640 containing 10% fetal bovine serum, HFF and MDCK cells were grown in DME containing 10% fetal bovine serum and MKN7 cells were grown in F12/DMEM containing 10% fetal bovine serum at 37°C, 5% CO2.

Production of LWLI and indirect immunofluorescence microscopy

LWLI was produced and characterized as described (Li et al., 1991). Cells grown on coverslips were washed free of serum, fixed and permeabilized in acetone, or with 3% formaldehyde and 1% Triton X-100 in phosphate buffered saline (PBS) and probed with the monoclonal antibody LWLI (dilution 1:200) followed with fluorescein isothiocyanate (FITC)-goat anti mouse Ig G, A and M antibodies (Sigma), as described (Li et al., 1991). In co-staining assays, MDBK cells were incubated with 5 μg/ml Sh-rhodamine-WGA (Sigma) or rabbit anti mannose-6-phosphate receptor antibody (a gift from S. Kornfeld) used at a concentration of 10 μg/ml and subsequently LWLI. Cells on coverslips were treated with 2 μM monensin (Sigma) in RPMI 1640 for 30 min at 37°C (Lipsky and Pagano, 1985) and processed for fluorescence microscopy, or subsequently washed free of monensin and allowed to recover in culture medium at 37°C for 2 h, prior to the fluorescence microscopy. Cells were examined in an Olympus IMT2 fluorescence microscope fitted with a photomicrographic adapter.

Immunoelectron microscopy

Cells grown to confluence in 35 mm Petri dishes were washed in serum free medium, fixed and processed as described by McLean and Nakane (1974). Briefly, the cells were fixed in 2% formaldehyde/lysine/phosphate buffer, permeabilized with 0.5% saponin-PBS, subsequently probed with LWLI and then peroxidase conjugated anti-mouse IgM (Boehringer Mannhein Co.) in the presence of 0.5% saponin. Post-fixation was carried out in 1% reduced osmium, dehydrated through graded ethanols and propylene oxide, and embedded in Eponate 12 epoxy monomer resin. Ultrathin sections were cut and mounted on 200-mesh thin bar hexagonal copper grids, counterstained with ethanolic uranyl acetate and lead citrate and examined in a Philip CM12 transmission electron microscope.

Isolation of subcellular fractionation and immunoblot analysis

Rat livers were homogenized, and fractionated on two sucrose gradients (Leelavathi et al., 1970; Tabas and

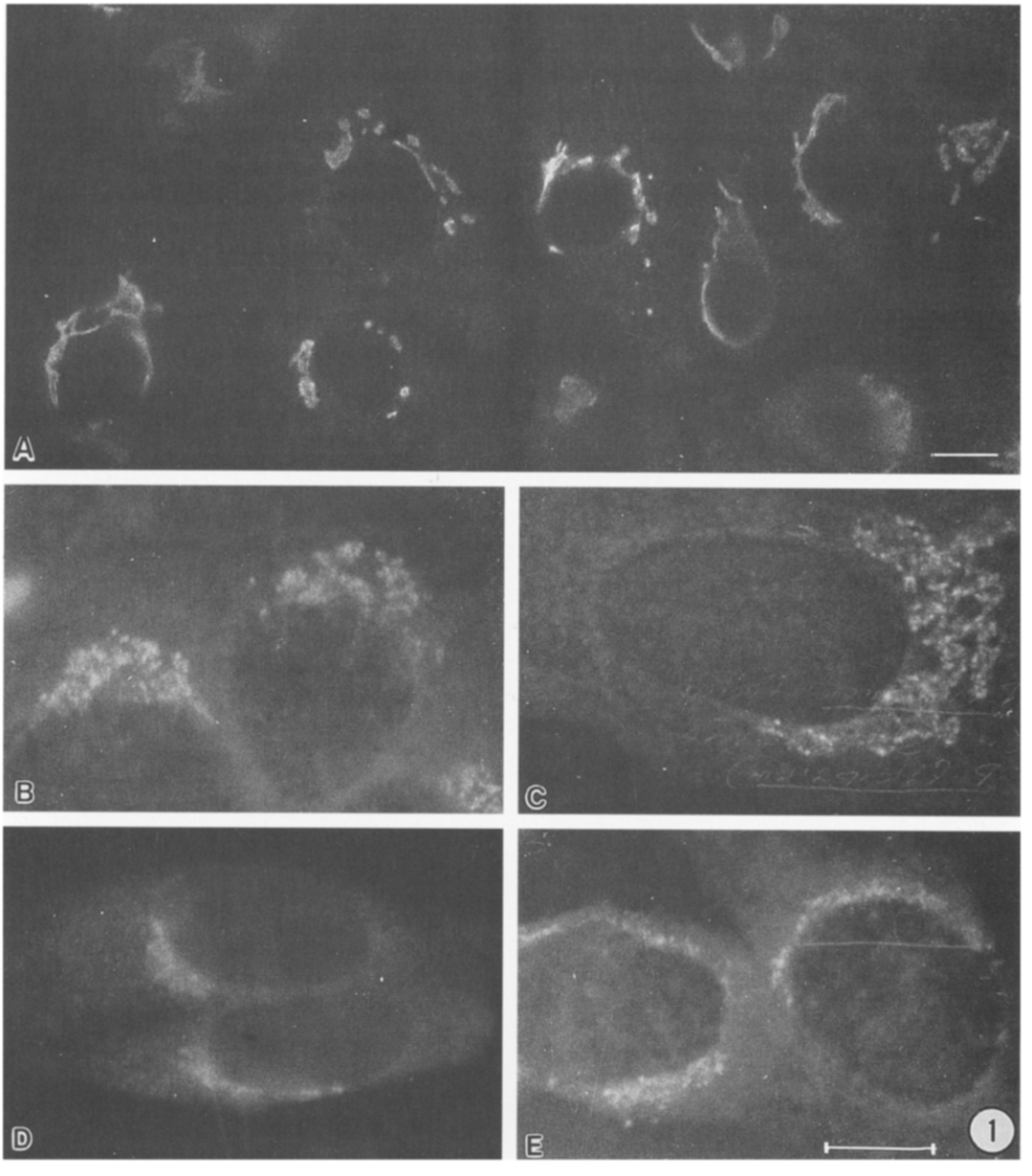


Fig. 1 Immunofluorescence microscopy with LWLI in established mammalian cell lines (MKN7 (A), MDBK (B), HFF (C), CHO (D) and MDCK (E)). A bright perinuclear fluorescence can be seen in all the cells visible in the field (A). Higher magnifications show the reticulated structure of the organelle (B, E). Bars = 10 μm.

Kornfeld, 1970). PNS, all interface fractions and sucrose layers were collected and analyzed. The top 0.5 M sucrose layer in the second gradient contained negligible levels of protein or LWLI reaction (not shown) and was therefore not included. 70% of the galactosyl-transferase activity (Balch et al., 1984; Dunphy and Rothman, 1983; Briles et al., 1977) was recovered at 0.5M/1.0 M sucrose with about 200-fold purification relative to PNS. 100% of the activity of the enzyme was resistant to trypsin digestion, indicating that the Golgi were intact. 5′nucleotidase and glucose-6-phosphatase (Futerman et al., 1990; Touster et al., 1970; Aronson and Touster, 1974) were used as markers for the plasma membrane and ER and found to be enriched in the 1.1/1.25 M sucrose interface. Cytosol in Figure 6A, lane 3 was prepared by collecting the supernatant after centrifugation of PNS at 100 000 × *g* (Leelavathi et al., 1970). In Figure 6 A-C, 66 μg of protein from each fraction was used. ^{125}I-goat anti-mouse IgM, was the secondary antibody. The distribution of proteins in the different fractions was calculated from densitometric scans of the resulting autoradiograms. Chinese hamster ovary (CHO) cells were homogenized and fractionated as described (Balch et al., 1984). Two interfaces were obtained: one at 0.8/1.2 M sucrose and a second at 1.2/1.4 M sucrose. In a typical preparation, galactosyl-transferase was enriched 20-fold in the 0.8/1.2 M sucrose interface, 80% of which was protease protected.

Protease protection and carbonate wash

Protease protection was carried out as described by Balch et al. (1984). Briefly, 0.1 mg/ml of trypsin or proteinase K was added into the Golgi fractions in the absence or presence of Triton X-100 and incubated at 37°C for 30 min. The reaction was stopped with soybean trypsin inhibitor (Sigma) or 5% TCA. Golgi fractions were treated with 0.1 M sodium carbonate, centrifuged at 100 000 *g* (Fujiki et al., 1982). 66 μg of proteins per lane were electrophoresed, transferred to nitrocellulose, and incubated with LWLI as described above.

Membrane preparations from various cell lines

Mammalian cells were grown to confluence, harvested by trypsin (0.25% in PBS) digestion. The cells were washed free of serum, resuspended in a Tris-sucrose buffer (Balch et al., 1984) and homogenized by 30 syringe passages through a 27 gauge needle. PNS was diluted 10-fold into sodium carbonate (Fujiki et al., 1982) and the samples were subjected to centrifugation at 100 000 × *g*. Pellets were prepared for SDS-PAGE and immunoblots as described above.

Results

LWLI labels the Golgi complex of cultured mammalian cells

Our previous work demonstrated that the monoclonal antibody LWLI recognized proteins in intraerythrocyte cisternae in P. Falciparum-infected erythrocytes. Because the intraerythrocyte lamella morphologically resembles an individual cisterna of the eucaryotic Golgi apparatus, it was of interest to determine whether LWLI would also react with this organelle in mammalian cells. Madin-Darby bovine kidney (MDBK), a human gastric cell line (MKN7), CHO, human foreskin fibroblasts (HFF) and Madin-darby canine kidney (MDCK) cells were immunolabeled with LWLI. Figure 1A is a low magnification of MKN7 cells that represents the characteristic immunolabeling pattern obtained with LWLI antibody. The immunofluorescence was concentrated in the perinuclear region where Golgi complexes are located. At higher magnifications, the fluorescence was visualized as an anastomic network of tubules in MDBK and HFF cells (Fig. 1 B, C), whereas in CHO and MDCK LWLI appeared to label a more compact structure (Fig. 1 D, E). To determine unambiguously that the organelle labeled by LWLI was the Golgi apparatus, we simultaneously labeled cells with LWLI and wheat germ agglutinin (WGA), a lectin that preferentially labels the Golgi apparatus (Virtanen et al., 1980). Figure 2 A and B shows that the labeling pattern for LWLI and for WGA was superimposable and clearly co-localized within the same organelle, although subtle discrepancies could be observed. In addition, both the immunofluorescence patterns for LWLI and WGA were disrupted and dispersed throughout the cytoplasm in cells treated with monensin, a drug that perturbs the Golgi structure (Tartakoff, 1983), (Fig. 2 C, D, vs A, B respectively). Two hours after removal of monensin, both the LWLI and WGA resumed their original perinuclear fluorescence patterns (not shown). In most cell types, the 215 kD mannose-6-phosphate receptors (MPRs) are localized primarily in the TGN, and in the endosomal compartment (Geuze et al., 1985; Griffiths et al., 1988). We therefore simultaneously immunolabeled MDBK cells with LWLI and with a rabbit antibody against the MPR. The labeling pattern obtained with LWLI clearly matched the localization of the MPRs although it was not totally superimposable and additional staining for MPRs could be detected in vesicles close to the Golgi apparatus that probably correspond to endosomes (Fig. 3A, B). However, some segments of the network labeled by LWLI were superimposable with the immunolabeling pattern for MPR. In a series of control experiments (not shown), the pattern obtained with LWLI was unequivocally distinct from that obtained with antibodies to coated vesicles (α adaptin or clathrin; Robinson, 1987; Brodsky, 1985), and different from the patterns observed after incubating cells with a compound that binds the endoplasmic reticulum (3, 3′-dihexyloxacarbocyanine iodide, DioC6; Teresaki et al., 1984), or that of one pinocytic marker (lucifer yellow-dextran) which concentrates in the endolysosomal compartment (Ferris et al., 1987; Storrie et al., 1984). Taken together with the co-localization studies shown in Figure 2 and Figure 3, these results indicate that LWLI recognized an antigen(s) that was present in the elements of the Golgi apparatus in mammalian cells.

LWLI labels elements of the *trans*-face of the Golgi apparatus and the TGN

Since the Golgi apparatus consists of heterogeneous sets of subcompartments, immunoelectron microscopy was carried out to determine whether the antigen(s) recognized by LWLI was present in all the elements of the Golgi apparatus or confined to one sub-compartment of the organelle. Preliminary gold immunolabeling experiments established that LWLI had only limited access to the antigen on thin cryosections. This steric hindrance resulted in very low immunolabeling density. Consequently, MDBK, MKN7, and pituitary cells were fixed and processed for immunoperoxidase cytochemistry. This technique is highly sensitive because the immunocytochemical signal is enzymatically amplified by the DAB reaction. Although the morphological preservation of the organelles was not always optimal due to the weak fixation regimen (glutaraldehyde as low concentration as 0.02% totally abolished immunoreactivity), the DAB reaction product was observed only in the Golgi apparatus with the exception of all other organelles. The reaction product was consistently

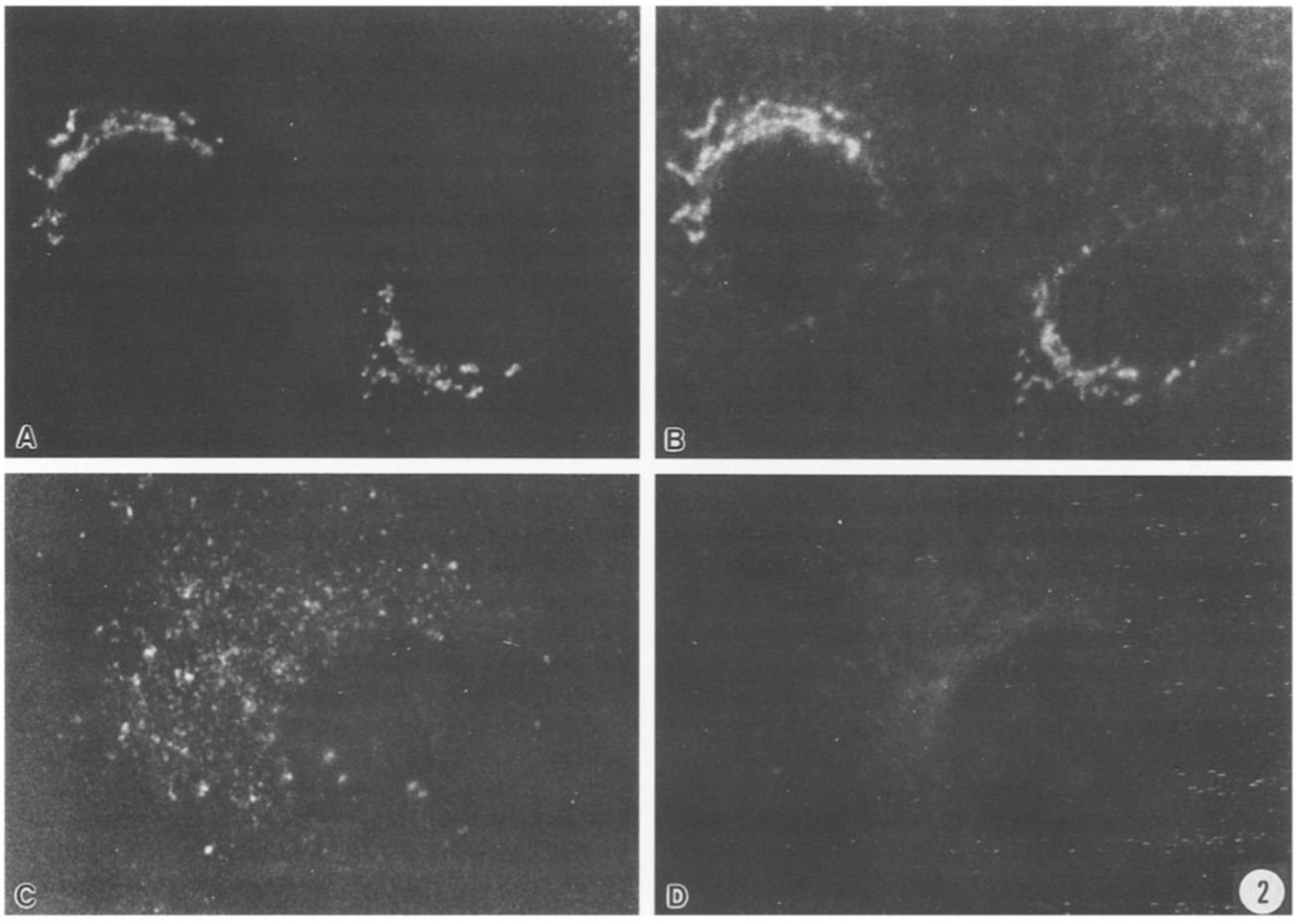


Fig. 2 Colocalization of LWLI fluorescence (A, C) with WGA (B, D), a lectin with high affinity for the Golgi apparatus. In normal MDBK cells (A, B) the immunofluorescence pattern obtained with LWLI, which completely coincides with the lectin-stained Golgi apparatus, indicates that LWLI stains a structure that corresponds to the Golgi apparatus. The perinuclear fluorescence is greatly reduced, becomes vesiculated and occupies a more diffuse pattern in the cytoplasm of MDBK cells treated with monensin (C, D).

localized in cisternae situated at one side of the Golgi stacks, and most often, was observed over the luminal side of short membrane segments (Fig. 4B, C, D, and 5). However, in a few instances, LWLI labeled the membranes of an entire cisterna (Fig. 5D). In addition to the labeling of the luminal side of the membranes (Fig. 4 B, C, D), electron opaque reaction product could be seen inside the cisternae (Figs 4, 5). We believe that this labeling is not specific and represents an artifact caused by the diffusion of oxidized DAB reaction product from the membrane (Courtoy et al., 1983) rather than the localization of an antigen of the cisternal content. Occasionally, the reaction product was observed in the lumen of the cisternae but still seemed closely apposed to the membranes of the cisternae (Fig. 4B).

Since the spatial configuration of the Golgi stacks varies not only from one cell type to the other but also within the same cell, it is occasionally difficult to determine the *cis*-from the *trans*-face of the organelle. We therefore immunolabeled pituitary cells in which, even in the absence of cytochemical markers, the *trans*-side can be unequivocally identified by the presence of budding secretory granules. As shown in Figures 4 and 5, LWLI immunolabeled the membranes of one or two cisternae situated at the *trans*-face of the organelle (Fig. 4C, D) and the TGN (Fig. 5). We were unable to detect reaction product in *cis*- and *medial* cisternae of the Golgi apparatus or in the other cellular compartments. These ultrastructural immunolabeling experiments clearly indicated that LWLI reacted with a luminal determinant situated in the *trans*-cisternae and the TGN. The fact that no DAB reaction product was detected in mature secretory granules or in the plasma membrane indicated that the antigen recognized by the antibody does not correspond to a protein transported through the Golgi apparatus.

LWLI recognizes a 170 kD membrane protein specific to Golgi membranes

To identify the luminal antigen(s) recognized by LWLI in the mammalian Golgi apparatus, Golgi and other membrane compartments from rat liver cells were fractionated using standard procedures (Leelavathi et al.,

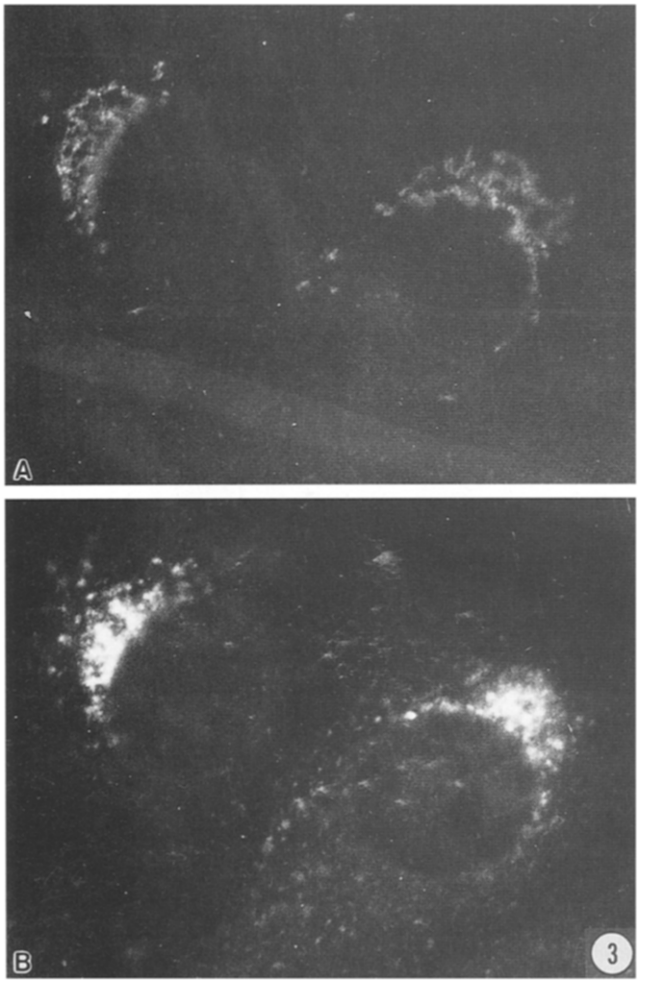


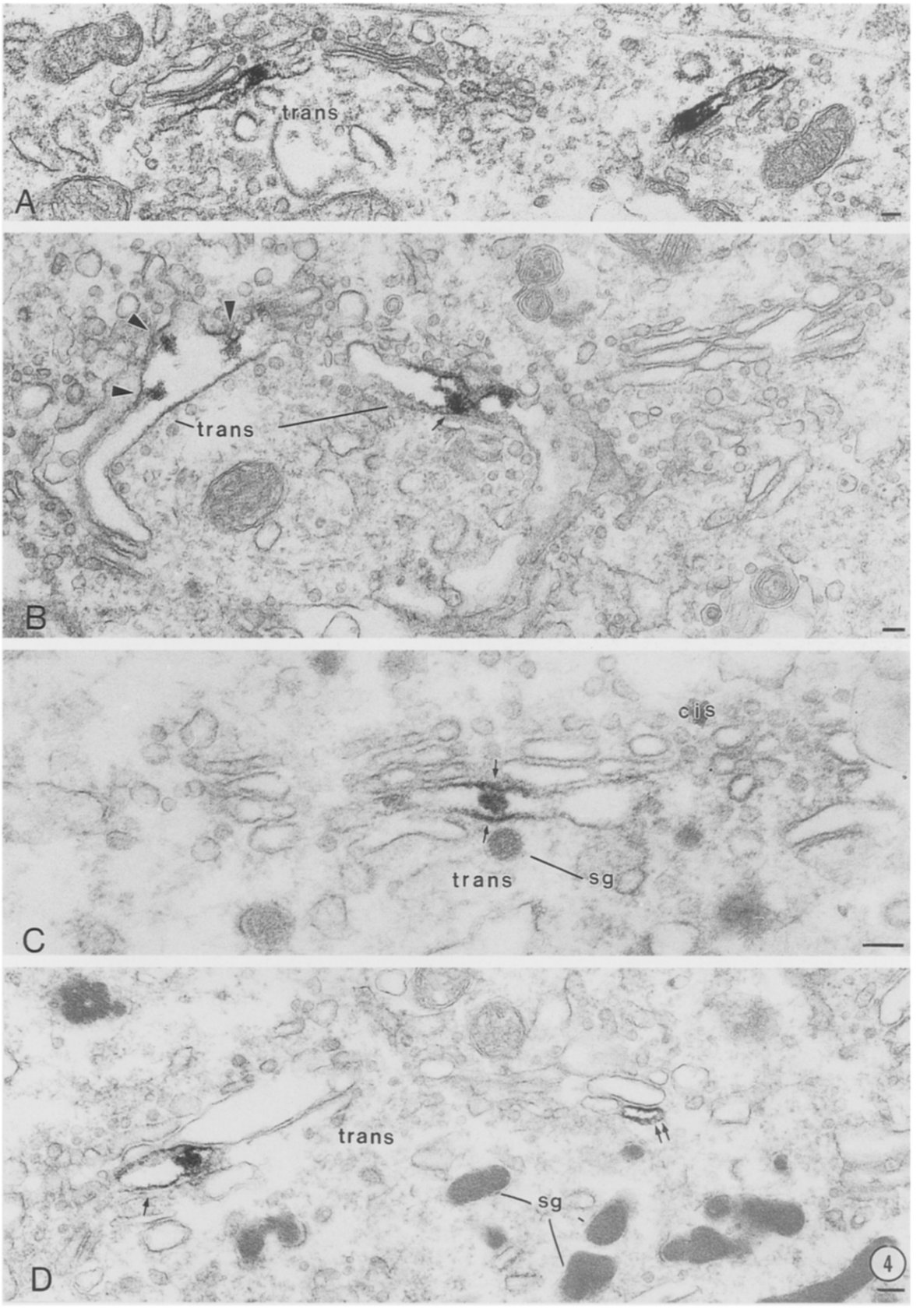
Fig. 3 Double indirect immunolabeling using LWLI (A) and a rabbit antibody to the 215 kD MPR (B) in MDBK cells. The labeling obtained with LWLI is not totally superimposable with the typical Golgi pattern for the MPRs. This is probably due to the presence of MPRs positive early endosomes in the Golgi region.

1979; Tabas and Kornfeld, 1979). Enzyme assays showed that the activity of the Golgi marker galactosyl-transferase was enriched about 200-fold in rat liver Golgi fraction compared to the post-nuclear supernatant (PNS). The proteins in the Golgi fraction were TCA precipitated and used in Western blot analysis with LWLI. The results presented in Figure 6A lane 1 indicate that LWLI recognized one protein band that corresponded to a 170 kD protein in molecular weight in rat liver Golgi fraction. Densitometry analysis showed that more than 85% of the amount of the 170 kD protein was enriched in the Golgi fraction. The distribution of this protein closely coincided with the peak of the galactosyl-transferase activity (Fig. 7). It should be noted that the 170 kD protein was not detected or below the level of detection in all other non-Golgi fractions, including PNS (Fig. 6A lane 2). Surprisingly, LWLI also recognized a protein band corresponding to 38 kD MW in non-Golgi fractions. This 38 kD protein was recovered in the supernatant after $100\,000 \times g$ centrifugation of the PNS (Fig. 6A lane 3). Therefore, the 170 kD protein appears to be the only Golgi protein recognized by LWLI in rat liver. Two lines of evidence strongly suggest that the 170 kD protein is associated with the Golgi cisternae and may be a transmembrane protein that protrudes into the lumen of the cisternae. Firstly, the 170 kD protein remained with the pellet after carbonate wash which yields Golgi membrane sheets (Fig. 6B) (Fujiki et al., 1982). Secondly, the 170 kD protein was resistant to degradation when the Golgi fractions were subjected to exogenous trypsin (Fig. 6C). Since LWLI immunolabeled the Golgi apparatus of various cell lines, we examined whether the 170 kD protein was also present in CHO cells by treating Golgi fractions of these cells with proteinase K (Fig. 8). Although several additional bands appeared to react with the antibody, the 170 kD protein was the only band protected from protease degradation. The 170 kD from CHO Golgi protein also remained with the membrane after carbonate wash indicating that the protein is tightly associated with the Golgi membranes (Fig. 9, lane 1). The 170 kD is different from mannosidase II as it was not recognized by a specific antibody to this *medial-* and *trans*-Golgi resident enzyme (Velasco et al., 1993) (data not shown).

The 170 kD Golgi protein is conserved in mammalian cells

The morphological observation both at the light and the ultrastructural levels and the immunoblot analysis of the cell fractionation results suggested that the protein may be conserved between mammalian species. To examine this possibility, crude membrane preparations were prepared by treating the PNS of different cells with a carbonate solution and subsequently high speed centrifugation. The 170 kD protein was detected with LWLI in total membrane fractions from CHO, MDBK, MDCK, and HFF cells (Fig. 9, lanes 1, 3, 5 and 7, respectively). Although additional bands could be seen, the 170 kD protein was the only protein conserved in all cells we tested.

Figs. 4 and 5 Immunolocalization of the antigen recognized by LWLI in elements of the *trans*-Golgi region (Fig. 4) and the TGN (Fig. 5). In these cultured cells, the Golgi apparatus consists of stacks of 3 to 5 cisternae. In all Figures, the immunoreactive protein is detected by the presence of the DAB electronopaque reaction product in cisternae of the *trans*-face of the Golgi apparatus. The labeling is facing the luminal side of the *trans*-cisternae (A). The reaction products is not always homogeneously distributed on the membranes of the *trans*-Golgi but appears to label discreet regions of the membrane (B, arrowheads). The labeling is restricted to a small portion of the membranes (C, arrows). DAB reaction product is either contained within the cisternal lumen (A, B, C, D) or associated with the membrane of the cisterna (D, double arrows). The apparent labeling seen within the lumen is probably due to the diffusion of DAB during the processing of the sample. MDBK (4, A, B), and pituitary cells (4, C, D).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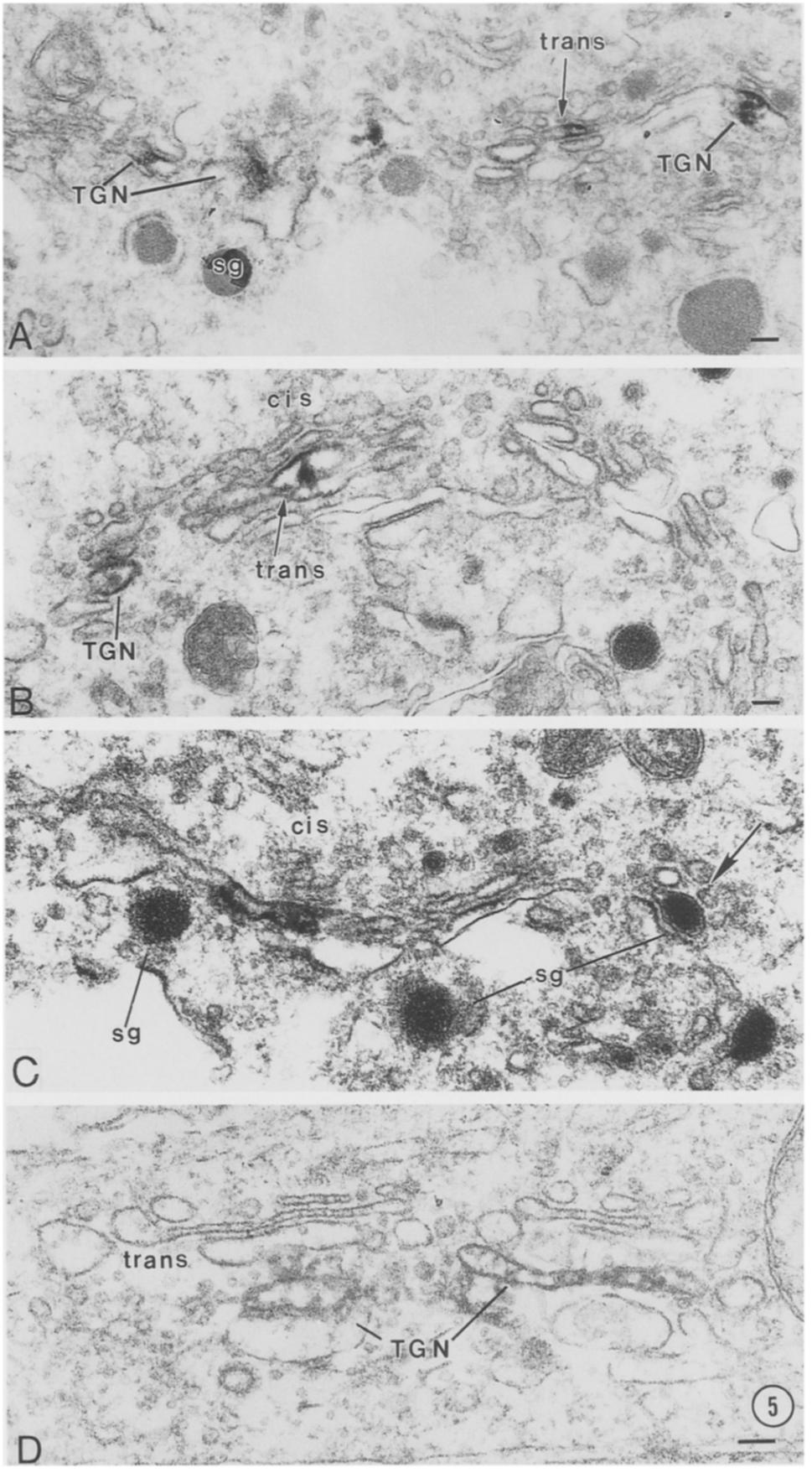

Fig. 5 Immunolabeling of the *trans*-Golgi and TGN. Figure 5 A, B, C, pituitary cells 5D, MKN7 cells. DAB reaction product can be seen in the TGN. In Figure 5C the arrow points at a budding secretory granule. Bars = 0.1 μm.

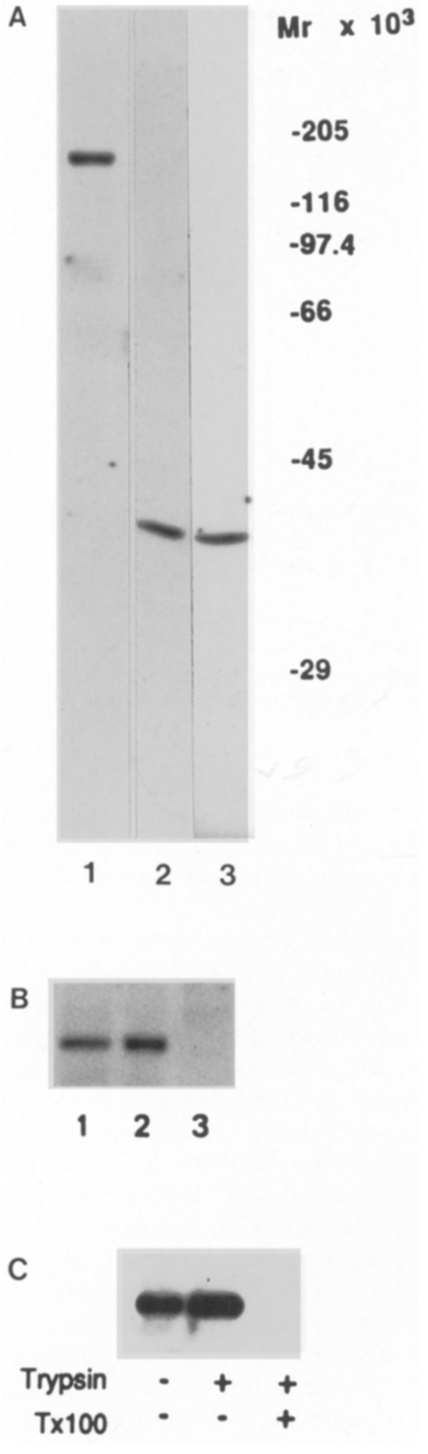

Fig. 6 Rat liver proteins recognized by LWLI in immunoblot analysis of cell fractions. (A) Proteins in Golgi fraction (lane 1), PNS (lane 2) and cytosol (lane 3) probed with LWLI. The 170 kD protein is the only protein recognized in the Golgi fraction. In the PNS and cytosol fractions, the antibody appears to react with a protein with a MW of 38 kD. (B) immunoblot analysis of 170 kD protein in isolated Golgi after carbonate wash show that the 170 kD protein is associated with Golgi membranes. Untreated Golgi fraction (lane 1), pellet (lane 2) and supernatant (lane 3) (66 μg of proteins per lane) (C). The 170 kD is facing the lumenal side of Golgi membranes as determined by protease protection. Isolated Golgi (lane 1), Golgi + 0.1 mg/ml trypsin (lane 2), and Golgi + 0.1 mg/ml trypsin and 1% Triton X-100 (lane 3).

Discussion

The intraerythrocyte lamellae (also known as the clefts or cisternae) contained in the cytoplasm of erythrocytes infected with *P. faliciparum* have been proposed to play a role similar to that of the Golgi apparatus in processing or sorting of parasitic proteins to the cytoplasm and the membrane of the host cells (Howard et al., 1987). It has now been shown that the lamellae are part of a network of tubulo-vesicular membranes (TVM) to which the parasite export the Golgi marker, sphingomyelin synthase (Haldar et al., 1991; Elmendorf and Haldar, 1994). Considering that organelle structure, biochemical activities as well as fundamental functions may have been conserved among evolutionary divergent organisms, we reasoned that antibodies to the lamella could cross react with components of the mammalian Golgi apparatus. We first established by immunofluorescence that the antibody to the lamellae, LWLI labels a perinuclear organelle that co-localizes with WGA and the MPRs. LWLI does neither react with any marker enzymes of the Golgi apparatus such as mannosidase II (124 kD) nor with any other proteins known to be involved in protein transport, such as clathrin (180 kD). Secondly, we determined by immunoelectron microscopy that the antigen is not homogeneously distributed within the different Golgi subcompartments but is localized in the *trans*-cisternae and the TGN. Thirdly, we demonstrated by Western blot analysis of enriched Golgi fractions that the antibody consistently recognized a single luminal protein of 170 kD in molecular weight. Golgi proteins of molecular weight close to 170 kD have been characterized. For example, one of them, α-COP is a membrane protein thought to be involved in the formation and budding of coated vesicles from the Golgi apparatus (Serafini et al., 1991; Rothman and Orci, 1992). The 170 kD protein recognized by LWLI is likely not to be α-COP as proteinase K digestion and immunoelectron microscopy have shown that α-COP is localized on the external surface of vesicles isolated from Golgi fractions. A protein with a molecular weight of 170 kD is associated with the membranes enriched in MPR isolated from calf liver (Messner et al., 1989). This protein is also probably not the 170 kD protein identified by LWLI because MPR membranes, which are not enriched in galactosyl-transferase and sialyl-transferase, are unlikely to be derived from the Golgi apparatus. Taken together, the 170 kD protein recognized by LWLI appears to be a novel resident protein of the mammalian Golgi apparatus.

The immunolabeling pattern for the 170 kD protein overlaps with those of several Golgi markers. The TGN harbors carbohydrate-modifying enzymes, such as α2,6-sialyltransferase which are, in addition, localized to the Golgi stacks (Roth et al., 1985). The TGN also contains integral membrane proteins that can transit to distant compartments of the secretory pathway. At

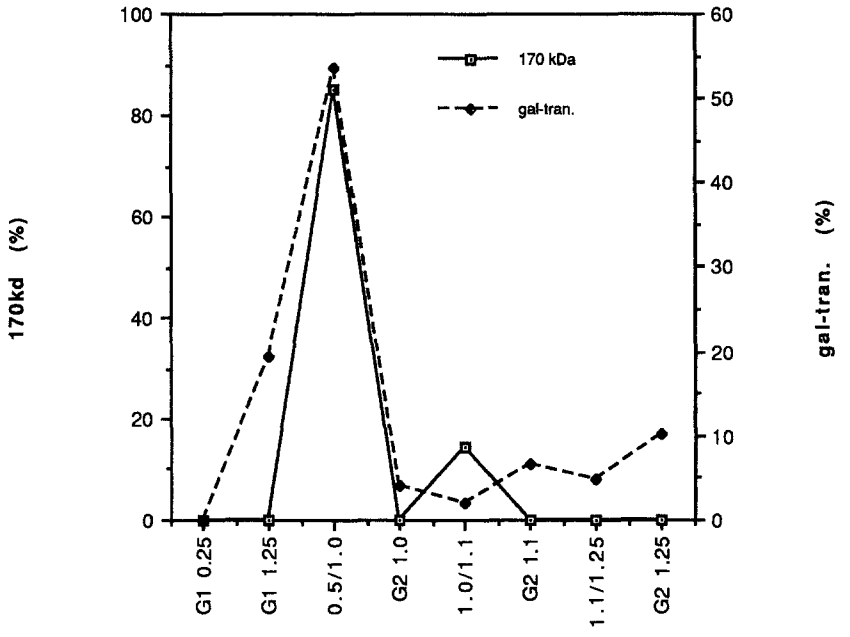

Fig. 7 Distribution of the 170 kD protein compared to the activity of the Golgi galactosyl-transferase in subcellular fractions of rat liver. The presence of the 170 kD protein corresponds to the major peak of gal-tr. activity. G1, first gradient; G2, second gradient; sucrose concentrations of each fraction are as indicated. Enzyme activity was calculated as nmol/mg/h.

steady state both TGN38/41 and furin are concentrated not only within the tubules and vesicles of the network (Bosshart et al., 1994) but also in endosomal vesicles and the plasma membrane (Ladinsky and Howell, 1992). As we have been unable to localize the 170 kD protein to any other organelles, the 170 kD protein appears to be a *trans*-cisternae and TGN resident protein. The *trans*-Golgi and the TGN are now considered as two different entities that are morphologically and functionally distinct. Recent studies have determined that at least one group of signals involved in the retention of integral Golgi proteins in the *cis*- and *medial*-Golgi are localized in the membrane spanning domains (Swift and Machamer, 1991). Since the spanning domains of different proteins that are retained in these Golgi subcompartments have shown no obvious homology, it is believed that the retention process probably depends on physical properties. The retention of membrane proteins to the TGN is mediated by a recognition signal as a segment of 11 amino acids in the cytoplasmic tail of TGN38/41 can confer TGN localization to an otherwise plasma membrane protein (Humphrey et al., 1993). Similarly, the retention of furin in the TGN is also mediated by a signal within its cytoplasmic domain (Bosshart et al., 1994). The sequence or the configuration that localizes the 170 kD protein to the *trans*-cisternae and the TGN will have to await sequencing of the protein. Our data suggest, however, that the mechanisms that target proteins to the membranes of *trans*-Golgi may also act to sort proteins to the TGN. Clearly, our understanding of the compartmentalization and the interactions between the different elements of the Golgi complex are still evolving. Further investigations using the 170 kD protein as a marker may help clarify some aspects of the organization of the *trans*-Golgi apparatus and the TGN.

The immunoblot analysis showing that LWLI recognized protein bands such as the 38 kD band in cytosolic fractions from rat liver seem in disagreement with the morphological observations. There are several conceivable explanations for this apparent discrepancy. It can be also argued that the bands seen in the immunoblots of the cytosolic fractions correspond to authentic cytosolic proteins. Our inability to detect them in the immunofluorescence and electron microscopy preparations may be due to the fact that they were released and lost during the permeabilization. Alternatively, it is well documented that proteins or protein fragment can leak from membrane compartments during homogenization and sediment in other cell fractions (Leighton et al., 1968; Alexon et al., 1985). An example of this is HMG CoA reductase, a 97 kD transmembrane glycoprotein of the endoplasmic reticulum (Liscum et al., 1983). The COOH-terminus of the protein is hydrophilic and protrudes in the cytoplasm. The latter domain is readily released from the cytoplasmic face of the microsomes and yields a soluble fragment of about 55 kD upon purification while the spanning domains remain inserted in the membrane. Similarly, the low molecular weight bands may be fragments of the 170 kD membrane protein released from the Golgi membranes during fractionation and purification.

In *P. falciparum*-infected cells, parasite-encoded proteins that are destined to the cytoplasm or the plasma

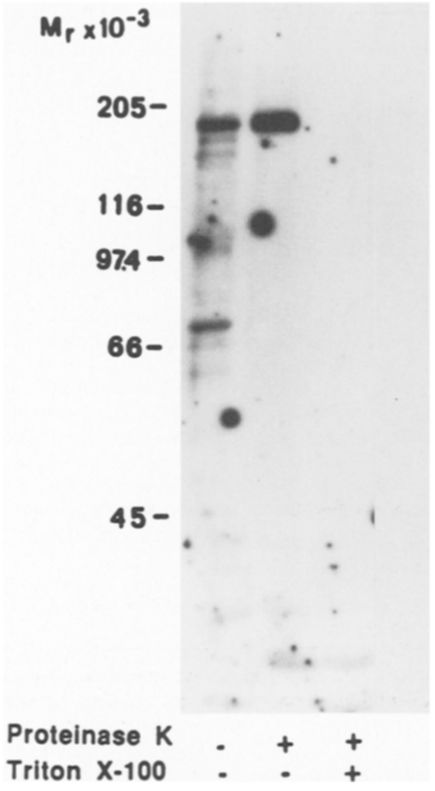

Fig. 8 The 170 kD protein recognized by LWLI in CHO Golgi fraction is protected from proteinase K digestion. Golgi fractions were treated with 100 μg/ml proteinase K in the absence (lane 2) or presence of Triton X-100 (lane 3). Untreated sample (lane 1).

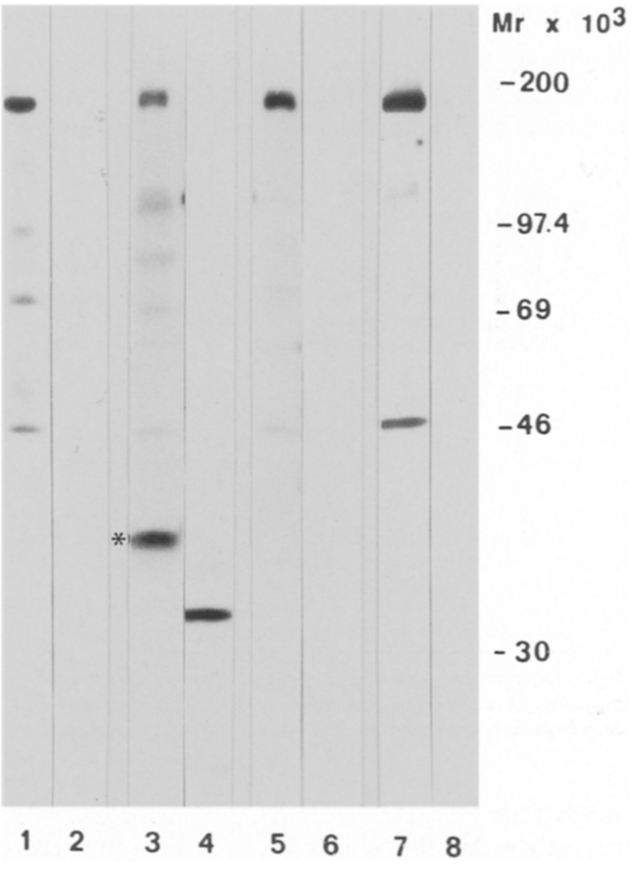

Fig. 9 A conserved 170 kD integral membrane protein in mammalian cells. Membrane fractions from CHO (lanes 1, 2), MDBK (lanes 3, 4) MDCK (lanes 5, 6) and HFF cells (lanes 7, 8) were probed in immunoblots with LWLI (lanes 1, 3, 5, 7) or MOPC 104E (a control antibody against α1-3 linked glucose) (lanes 2,4,6,8). *This protein band is not reliably detected in membranes isolated from MDBK cells and may reflect a proteolytic fragment or a contaminating supernatant protein in this preparation.

membrane of the erythrocyte have to cross the parasitophorous vacuole that separates the parasite from the cytoplasm of the host cell (reviewed by Howard, 1987; Barnwell, 1990). Most plasmodial proteins that have been transported into the erythrocyte cytoplasm are contained in the intraerythrocytic membranes (Aikawa et al., 1986; Stanley et al., 1989). Models for vesicular transport in malaria-infected erythrocytes have been proposed. They postulate that the transport of polypeptides from the parasite to the erythrocyte cytoplasm and plasma membrane is carried out via vesicles in budding and fusing events similar to those described in eucaryotic cells (Barnwell 1990; Gunther et al. 1991; Elmendorf and Haldar, 1993). Some characteristic features of the mammalian secretory pathway have also been identified in *P. falciparum*. For example, the transport of newly synthesized proteins can be disrupted by Brefeldin A or by reducing the temperature to 15 or 20°C (Crary and Haldar, 1992; Elmendorf et al., 1992; Elmendorf and Haldar, 1993A); the sequencing of proteins that are exported by *Plasmodium* indicates the presence of the cleavable N-terminal hydrophobic signal sequence required for recognition and the translocation of secretory protein into the endoplasmic reticulum (Ragge et al., 1990); and a plasmodial BiP homologue ending in SDEL has been identified (Kumar et al., 1991; Kumar and Zheng, 1992). Furthermore, the presence of a parasite homologue of ERD2 (Elmendorf and Haldar, 1993B) as well as the activity of sphingomyelin synthase (Haldar et al., 1991), and thiamine pyrophosphatase (Haldar, in preparation), enzymes of the *cis*- and *trans*-Golgi respectively, have been demonstrated in infected erythrocytes. Interestingly, ERD2 is concentrated within the parasite while the sphingomyelin synthase and the thiamin pyrophosphatase are associated, at least in part, with the intraerythrocytic membranes (Elmendorf and Haldar, 1993B and 1994). These observations indicate that some of the Golgi activities can be exported from the parasite into its host cell. While the significance of this remains unclear, our results provide evidence that plasmodium-induced lamellae share some compositional features with the mammalian Golgi apparatus and support the hypothesis that the lamellae may play a role similar to the Golgi elements in the infected erythrocytes.

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