Molecular Motors Are Differentially Distributed on Golgi Membranes from Polarized Epithelial Cells

Karl R. Fath, Gina M. Trimbur, and David R. Burgess

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Abstract. Microtubules (MT) are required for the efficient transport of membranes from the *trans*-Golgi and for transcytosis of vesicles from the basolateral membrane to the apical cytoplasm in polarized epithelia. MTs in these cells are primarily oriented with their plus ends basally near the Golgi and their minus-ends in the apical cytoplasm. Here we report that isolated Golgi and Golgi-enriched membranes from intestinal epithelial cells possess the actin based motor myosin-I, the MT minus-end–directed motor cytoplasmic dynein and its in vitro motility activator dynactin (p150/*Glued*). The Golgi can be separated into stacks, possessing features of the Golgi cisternae, and small membranes enriched in the *trans*-Golgi network marker TGN 38/41. Whereas myosin-I is present on all membranes in the Golgi fraction, dynein is present only on the small membrane fraction. Dynein, like myosin-I, is associated with membranes as a cytoplasmic peripheral membrane protein. Dynein and myosin-I coassociate with membranes that bind to MTs and cross-link actin filaments and MTs in a nucleotide-dependent manner. We propose that cytoplasmic dynein moves Golgi membranes along MTs to the cell cortex where myosin-I provides local delivery through the actin-rich cytoskeleton to the apical membrane.

POLARIZED epithelial cells contain an apical and a basolateral plasma membrane domain that is separated by cell–cell junctional complexes. The protein and lipid components of the plasma membrane and the organization of the underlying cytoskeleton are distinct for these domains. Membrane proteins destined for the apical surface of polarized intestinal epithelial cells, like those of other polarized epithelial cells, reach this surface via two routes: a direct pathway or via an indirect or transcytotic (via the basolateral membrane) pathway (Louvard et al., 1992; Mays, et al. 1994). Pharmacological studies suggest that although not absolutely required, the efficient transport of apically directed membranes in polarized epithelia requires intact microtubules (MTs)[1] (Bennett et al., 1984; Hugon et al., 1987; Achler et al., 1989; Parczyk et al., 1989; Breitfeld et al., 1990; Gilbert et al., 1991). When polarized epithelia are treated with MT-disruptive drugs, there is a delay in delivery of materials to the apical plasma membrane, although 50–80% of the materials still reach the apical domain. The remaining materials are missorted to the basolateral domain. The most recent and complete pharmacological study on the role of MTs on both the direct and transcytotic pathway for apical membrane protein delivery in intestinal epithelial cells clearly shows that MTs are important for both the direct and the indirect pathways (Gilbert et al., 1991). This study complements the analysis of Bomsel et al. (1990), who suggest that late endosomal fusions appear to require MTs and other work (Achler et al., 1989; Breitfeld et al., 1990; Hunziker et al., 1990) which shows that MTs are necessary for normal movements of transcytotic vesicles to the apical cytoplasm. Therefore, for either the direct or the indirect pathway, MTs play a crucial role in facilitating transport of apically targeted membrane protein transport vesicles to the apical surface.

Observations in vitro (Coffe and Raymond, 1990; van der Sluijs et al., 1990) and in vivo (Cooper et al., 1990) suggest that Golgi and Golgi-derived membranes can bind to MTs, possibly via motor proteins. Work on organelle motility in axons has led to the principle that kinesin is the motor for anterograde or Golgi to cell surface movement, whereas dynein is the motor for retrograde movement (see reviews by Schroer and Sheetz, 1991; Skoufias and Scholey, 1993). This idea has been extended as a generalization for Golgi trafficking in all cells (Bloom, 1992; Swanson et al., 1992; Lippincott-Schwartz, 1993). A widely accepted general model for Golgi to plasma membrane movements is that kinesin is the motor because MTs are polarized with their minus-ends at the nucleus where the Golgi resides. While this simplified model is likely appropriate for fibroblasts and neurons where the microtubule organizing center (MTOC) is adjacent to the nucleus and Golgi, it is incorrect for polarized epithelial cells. In contrast to their organization in fibroblasts, MTs in polarized epithelia are arranged with their minus ends in the

Address all correspondence to David R. Burgess, 246 Crawford Hall, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

1. *Abbreviations used in this paper*: BB, brush border; MT, microtubule; MTOC, MT organizing center; PNS, postnuclear supernatant; WGA, wheat germ agglutinin.

© The Rockefeller University Press, 0021-9525/94/08/661/15 $2.00

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

apical cytoplasm, not near the supranuclear Golgi apparatus (Sandoz et al., 1985; Drenckhahn and Dermietzel, 1988; Achler et al., 1989; Gilbert et al., 1991). MT bundles extend to the basal cytoplasm with their plus ends near the cell base and Golgi with a collection of MTs running transversely in the apical cytoplasm (Sandoz et al., 1985; Bacallao et al., 1989). Although centrioles and γ-tubulin are subjacent to the apical actin cytoskeleton, there is no apparent association with the ends of MTs, nor is there pericentriolar material (Sandoz et al., 1985; Drenckhahn and Dermeitzel, 1988; Bacallao et al., 1989; Gilbert et al., 1991; Rizzolo and Joshi, 1993).

A role for motors in the translocation of vesicles in vivo is supported by studies showing that the fusion of apically and basolaterally derived endosomes or Golgi translocation requires MTs and the motor proteins dynein or kinesin (Bomsel et al., 1990; Corthésy-Theulaz et al., 1992). Likewise, in the fibroblast BHK cell line where the MTOC, and therefore the MT minus-end, is adjacent to the nucleus and the Golgi, cytoplasmic dynein and MTs are required for in vitro fusion of endosomal carrier vesicles from early endosomes with late endosomes (Aniento et al., 1993). Furthermore, there are many other reports that dynein is involved in organellar movement towards the MT minus-end (Paschal et al., 1987; Euteneur et al., 1988; Schnapp and Reese, 1989; Schroer et al., 1989; Hirokawa et al., 1990; Lacey and Haimo, 1992; Lin and Collins, 1992).

MTs in polarized epithelial cells rarely, if at all, extend through the cell cortex to the plasma membrane (Sandoz et al., 1985; Drenckhahn and Dermietzel, 1988). The cell cortex, especially that of the apical brush border (BB), is rich in actin and is likely absent of MT-based movements (Burgess, 1987; Louvard, 1989; Fath et al., 1993). The basolateral cortex of epithelial cells is also MT poor and actin rich, although not as organized as the BB. The basolateral membrane, beneath the zonula adherens actin circumferential ring, is composed of an anastomosing actin meshwork containing fodrin, ankyrin, and myosin-I and -II among other cytoskeletal proteins (Mooseker, 1985; Burgess, 1987). Therefore, the final 0.5 to 1 μm to the epithelial cell apical or basolateral plasma membrane must be traversed by a vesicle in the absence of MTs and through an actin meshwork. Thus, MT-based motors may not be sufficient to translocate a vesicle from the Golgi to the cell surface, no matter whether it is taking the direct or the indirect path. Another motor, perhaps attached to the same vesicle translocated first along MTs, may be required to move vesicles through the actin-rich terminal web to reach the plasma membrane.

A good candidate for a motor functioning in the epithelial cell cortex is the actin-based motor myosin-I (Sandoz et al., 1985; Achler et al., 1989; Fath et al., 1990; Fath and Burgess, 1993). The myosin-Is are a class of actin-activated mechanochemical motors directed toward the membrane-associated ends of actin filaments (Pollard et al., 1991; Cheney and Mooseker, 1992) that have been identified in many cell types and are likely associated with both intracellular and plasma membranes (reviewed by Cheney and Mooseker, 1992; Titus, 1993; Fath and Burgess, 1994). Based largely on conjecture developed from its in vitro properties, on the polarity of actin filaments within microvilli and on the morphology of microvillus growth, myosin-I in the intestinal epithelium has been proposed to move membranous vesicles transporting proteins and lipids from the Golgi or from basolateral endosomes to their sites of incorporation into the apical plasma membrane (Conzelman and Mooseker, 1987; Shibayama et al., 1987; Collins et al., 1990; Fath et al., 1990). Moreover, since the basolateral membrane is also underlain by a rich actin meshwork it is equally likely that transport through or from the basolateral cortex is accomplished using an unconventional myosin attached to a transport vesicle. Electron microscopic immunolocalization of myosin-I on vesicles in the apical cytoplasm of enterocytes (Drenckhahn and Dermietzel, 1988) is consistent with such a membrane translocation role. We previously showed that myosin-I was potentially involved in such movements (Fath and Burgess, 1993) by isolating a mixed population of Golgi-enriched cytoplasmic membranes from isolated intestinal epithelial cells and found that a population of membranes, which bind actin filaments in vitro in an ATP-sensitive manner, possessed myosin-I as a cytoplasmically oriented peripheral membrane protein.

To determine if cytoplasmic membranes in epithelial cells potentially utilize both a MT- and actin-based motility we have begun looking for membranes expressing several motors. We show in the present study that myosin-I, cytoplasmic dynein and its activator dynactin (p150/*Glued*) are associated with Golgi membranes that were isolated from enterocytes by two different methods, while kinesin, although present in the cells, was absent from these membranes. Because myosin-I cosedimented with dynein-containing Golgi membranes that were bound to MTs, we propose that some membranes contain both myosin-I and dynein. Therefore, organelles may possess both types of motors which may be activated or differentially regulated in different cell regions. We show that some membranes possess only myosin-I and lack dynein which suggests that there must be some precise regulatory mechanism placing or maintaining specific motors on subpopulations of membranes within cells. Because of the orientation of MTs in polarized epithelia, these results suggest a hypothesis that cytoplasmic dynein moves apically targeted membranes to the apical cytoplasm where myosin-I provides local delivery to the apical membrane.

Materials and Methods

Isolation of Golgi Stacks, Golgi-enriched Membranes and Subcellular Fractionation

Polarized intestinal epithelial cells (enterocytes) were isolated from chicken intestinal villus and crypts as previously described (Fath and Burgess, 1993). A Golgi fraction was prepared from villus enterocytes using the method of Leelavathi (Leelavathi et al., 1970). All steps were performed at 4°C. 4 ml of packed cells were homogenized in 6 ml of 0.5 M sucrose PKM buffer (100 mM potassium phosphate, pH 6.5, 3 mM $MgCl_2$, and 3 mM KCl) using a hand held tissue grinder (Tissue-Tearor; Biospec Products, Inc.) at setting 2 for 1 min on ice. The nuclei and any intact cells were pelleted at 600 *g* for 10 min and the postnuclear supernatant (PNS) layered over a 5-ml pad of 1.3 M sucrose–PKM and centrifuged at 105,000 g_{max} (SW41; Beckman Instrs., Inc., Fullerton, CA) for 60 min. The membranes concentrating on the 1.3 M sucrose pad were collected, adjusted to 1.1 M sucrose–PKM at a final volume of 3 ml. A step gradient of 1.4 M sucrose, 1.3 M sucrose, 1.25 M sucrose, 1.1 M sucrose (membrane fraction), and 0.5 M sucrose was centrifuged in a SW41 rotor at 90,000 g_{max} for 90 min. The 0.5/1.1 M sucrose interface was enriched in Golgi stacks and after washing was termed the Leelavathi Golgi fraction. To wash membranes of any soluble material, all fractions were collected, diluted with PKM buffer, cen-

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

trifuged for 30 min at 150,000 g_{max}, and resuspended in PEMS buffer (10 mM Pipes, 1 mM EGTA, 2 mM $MgCl_2$, and 0.25 M sucrose, pH 7.0).

In order to subfractionate Leelavathi Golgi further, in some experiments the 0.5/1.1 M sucrose interface was diluted with an equal volume of 0.25 M sucrose-PKM and centrifuged at 10,000 g (SS-34 rotor, Du Pont Co., Wilmington, DE) for 15 min. The resulting pellet is referred to as stacks. The small membranes remaining in the supernatant were collected by centrifugation at 266,000 g_{max} in a TLA 100.4 rotor (Beckman Instrs., Inc.) for 30 min. An unfractionated sample was also centrifuged at 266,000 g_{max} to collect total membranes. All pellets were resuspended in an equal volume of 0.25 M sucrose-PKM buffer.

Golgi-enriched membranes were also isolated from chicken intestinal epithelial cells by the method of Weiser as previously described (Fath and Burgess, 1993). In addition to isolation by sedimentation gradients, and to ensure that protein-lipid aggregates containing myosin-I or dynein were not artifactually cosedimenting with these Weiser Golgi membranes, in some experiments, membranes were isolated using isopycnic flotation gradients. The previously described sucrose step gradient was underlayed by membranes that were brought to 1.38 M sucrose (40% wt/wt) by addition of 2.55 M sucrose.

For subcellular fractionation, nuclei, BB, and demembranated BB were fractionated from isolated intestinal epithelial cells as described by Matsudaira and Burgess (1982). The supernatant remaining after BB isolation (largely cleared of BB), was sequentially centrifuged at 20,000 g_{max} for 20 min, 80,000 g_{max} for 1 h, and 150,000 g_{max} for 3 h.

Immunological Techniques

The following antibodies were used: cytoplasmic dynein heavy chain (mAb 440.4, Steuer et al., 1990); cytoplasmic dynein intermediate chain (mAb 70.1, Steuer et al., 1990; mAb 74.1, Holzbaur et al., 1991); dynactin (mAb 150.1, Gill et al., 1991); kinesin heavy chain (mAb SUK-4, Ingold et al., 1988); β-COP (M3A5, Allan and Kreis, 1986); TGN-38/41 (Luzio et al., 1990; Jones et al., 1993); α-mannosidase-II (Velasco et al., 1993); α-tubulin (mAb AA4.3, Walsh, 1984); γ-tubulin (Joshi, 1993); and our affinity-purified myosin-I polyclonal Ab (UPT-8). The myosin-I antibody was elicited using purified BB myosin-I and was affinity purified using BB myosin-I bound to Immobilon-P membranes (Millipore Corp., Bedford, MA) according to the method of Olmstead (1981). The antibody was monospecific for myosin-I on immunoblots of BB proteins (data not shown). The antibody to alkaline phosphatase was purchased from Chemicon Intl. Inc. (Temecula, CA).

Indirect immunofluorescent light microscopy using 5-μm-thick frozen sections of adult chicken duodena were performed as described previously (Fath et al., 1990). In some experiments, intestinal pieces were chilled for several minutes on ice to allow cold-labile MT depolymerization, then frozen in liquid nitrogen and cryosectioned before fixation. Antibody staining was specific as determined by incubation with labeled secondary antibodies alone (images not shown). Polymerized actin was localized with 22 nM rhodamine-phalloidin (Molecular Probes, Inc., Eugene, OR) in Tris-buffered saline. Nuclei were localized with the DNA stain DAPI (4′,6-diamidino-2-phenylindole; Sigma Chem. Co., St. Louis, MO) that was used at 0.25 μg/ml in Tris-buffered saline. Golgi membranes were labeled with TRITC-wheat germ agglutinin (WGA; Sigma Chem. Co.) at 1 μg/ml (Tartakoff and Vassalli, 1983). The inclusion of 0.1 M *N*-Acetyl-D-Glucosamine in the blocking and staining solutions uniformly decreased TRITC-WGA labeling intensity at least 10-fold as determined by photometry (data not shown). Fluorescently labeled sections were mounted in 90% glycerol, 10% Na_2CO_3, pH 9.6, containing 100 mg/ml 1,4-Diazabicyclo[2.2.2]octane (Aldrich Chem. Co., Milwaukee, WI) as a photobleaching retardant. Photographs were taken with either TMAX 400 or TechPan 2415 (Eastman-Kodak Co., Rochester, NY) films.

For immunoblotting, samples were electrophoresed on 10% SDS-PAGE microslab gels (Matsudaira and Burgess, 1978) or 5–17% gradient SDS-PAGE gels (for dynein heavy chain blot only, Fig. 8 *B*) and electrophoretically transferred to 45 μm Immobilon-P membranes (Millipore Corp.). The 10% gels were electrophoretically transferred in either Towbin (Towbin et al., 1979) or CAPS buffer (LeGendre and Matsudaira, 1989), and the 5–17% gels were transferred in Towbin buffer containing 0.1% SDS. Membranes were immunoprobed as previously described (Fath and Burgess, 1993). Bound primary antibodies were detected using peroxidase-labeled secondary antibodies and chemiluminescence detection kits (Amersham Corp., Arlington Heights, IL or DuPont NEN) on X-OMAT AR film (Eastman-Kodak) and developed with GBX (Eastman-Kodak). To quantitate immunoblots, autoradiographs were scanned on an Ultroscan densitometer (LKB Instruments Inc., Bromma, Sweden). Molecular mass markers were prestained proteins in the SDS-7B kit from Sigma Chem. Co.

Cytoskeletal Protein-Membrane Binding

Isolated Weiser membranes (0.15 mg/ml) were incubated at room temperature for 30 min in either PEMS alone, PEMS containing 0.5 mg/ml taxol-stabilized MTs, or PEMS containing 0.5 mg/ml taxol-stabilized MTs plus 10 mM ATP. Taxol was included in all solutions at 20 μM. Incubated samples were then layered onto a 1.22 M sucrose pad and centrifuged at 259,000 g_{max} for 30 min at room temperature in a TLS-55 (Beckman Instrs. Inc.) rotor. The pellets and supernatants were quantitatively recovered and immunoblotted for cytoplasmic dynein intermediate chains, then stripped of bound antibody (as outlined in Amersham ECL kit booklet) and reprobed with affinity-purified myosin-I antibody. The tubulin was phosphocellulose purified and did not contain cytoplasmic dynein or kinesin as determined by immunoblotting (data not shown) and was polymerized in PIPES, GTP, and 20 μM taxol. In some experiments the pelleted membranes were negatively stained (see below).

MT membrane-binding experiments were also done with Leelavathi small membranes essentially as described above with minor modifications. Small membranes were collected by centrifugation onto a 0.9 M sucrose in PKM cushion in a TLS55 rotor at 259,000 g_{max} for 30 min at room temperature, incubated with taxol-stabilized MTs in the presence or absence of 10 mM ATP. The mixture was layered over a 0.9 M sucrose-PKM cushion and centrifuged as above to pellet membranes bound to MTs. The density of the sucrose pad was reduced to 0.9 M because MT-bound Leelavathi small membrants floated on 1.2 M sucrose. Supernatants and pellets were collected and analyzed by immunoblotting.

To determine morphologically if single small Golgi membranes could bind to both actin filaments and MTs, we took advantage of the fact that actin filaments and MTs that were bound to membranes floated on 1.1 M sucrose when centrifuged. The unbound filaments and MTs pelleted. Therefore, membranes were incubated with a MT/actin filament mixture ± 10 mM ATP in the same conditions as described above for the Weiser membranes. After 25 min at room temperature, the samples were layered over 1.1 M sucrose pad and centrifuged at 259,000 g_{max} for 30 min at room temperature in a TLS-55 (Beckman Instrs., Inc.) rotor. The supernatants and pellets were collected and negatively stained for EM.

Extraction of Cytoplasmic Dynein from Membranes

5 μg of Weiser membranes in PEMS were incubated with either 0.6 M KI, 1.0 M NaCl, or PEMS buffer alone for 30 min at 4°C. For the alkali stripping, 5 μg of vesicle protein was diluted 20-fold with 100 mM Na_2CO_3 (pH 11.5) and incubated for 30 min at 4°C (Fujiki et al., 1982). The salt and alkali-treated membranes were then pelleted at 315,000 g_{max} for 30 min at 4°C in a TLA 100 rotor (Beckman Instrs., Inc.). Supernatants were aspirated, precipitated with 10% (vol/vol) TCA on ice, washed with 100% ethanol, then resuspended in 20 μl SDS sample buffer. Pelleted membranes were solubilized directly with 20 μl SDS sample buffer. Samples were immunoblotted to determine the relative distribution of dynein intermediate chains in the supernatants and pellets.

UV Photolysis of Membrane-bound Cytoplasmic Dynein

UV photocleavage was performed as previously described (Schroer et al., 1989) and samples immunoblotted with cytoplasmic dynein heavy chain mAb 440.4.

Electron Microscopy

Golgi stacks and membranes were fixed in suspension with an equal volume of 4% glutaraldehyde-PKM buffer on ice overnight. The fixed membranes were pelleted at 266,000 g_{max} for 30 min and osmicated with 2% OsO_4 for 2 h on ice in the dark. The pellets were dehydrated through graded ethanols into propylene oxide and embedded in resin (Fath and Burgess, 1992). Sections were stained with uranyl acetate and lead citrate and viewed in a Zeiss electron microscope at 50 kV. For negative staining, carbon-over-Formvar-coated grids were floated on a drop of Golgi stacks or Golgi-enriched membranes for several minutes. The grids were rinsed by quickly touching the surface of two drops of PEMS, then stained with 1% aqueous uranyl acetate.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

Miscellaneous

Protein concentrations were determined with either the Bicinchoninic Acid Assay (Pierce Chemical Co., Rockford, IL) or Bio-Rad Protein Assay (Bio Rad Labs., Hercules, CA) using BSA or Bovine plasma γ-globulin as standards. Galactosyltransferase assays were done as described by Fath and Burgess (1993) using the method of Aoki (1990). All sucrose concentrations were confirmed with an Abbe refractometer (American Optical, Buffalo, NY).

Results

Organization of the Actin- and MT-based Cytoskeleton in Polarized Intestinal Epithelia

The MTs in polarized intestinal epithelia, as in many postmitotic epithelia (Joshi, 1993) are oriented with their slow-

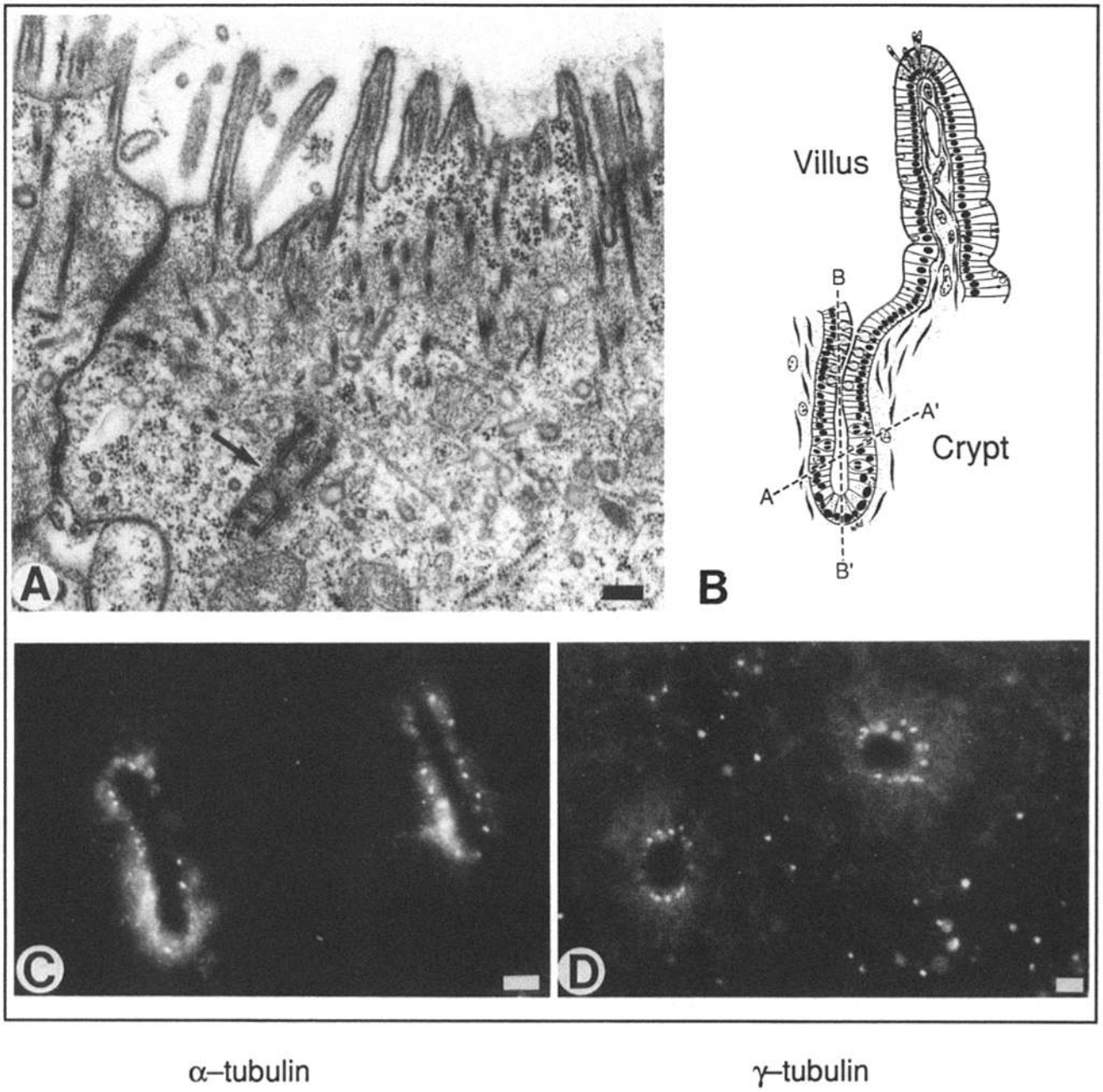

Figure 1. The centrioles and minus-ends of microtubules in intestinal epithelial cells lie in the apical cytoplasm. Identification of centrioles in the apical cytoplasm by EM and by immunolabeling with α-and γ-tubulin antibodies. (*A*) Electron micrograph showing the apical positioning of centrioles (arrow) in a crypt cell. (*B*) This drawing illustrates the relationship of the Crypt of Lieberkühn with the intestinal villus. The dashed lines indicate the planes of sections used in the immunofluorescent micrographs. (*C* and *D*) fluorescent light micrographs of frozen sections (plane of section A→A') of intestinal enterocytes in which the cold-labile MTs were allowed to depolymerize before fixation and immunolabeling with either α-tubulin (*C*) or γ–tubulin (*D*) mAbs. Note the punctate distribution of both α- and γ-tubulin in the pericentriolar region. Bars: (*A*) 0.2 μm; (*C* and *D*) 5 μm.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

growing or minus-ends in the apical cytoplasm near the centrioles (Achler et al., 1989; Gilbert et al., 1991). The distribution of centrioles in intestinal crypts was easily seen by immunofluorescence or EM. When the majority of cold-labile MTs were depolymerized before fixation (Fig. 1 *C*), immunofluorescent detection of MTs revealed bright spots, likely centrioles and cold-stable MTs, near the luminal surface of each cell. As an aid in orientation, we have included a schematic drawing that illustrates the planes of sections used in our immunofluorescence micrographs (Fig. 1 *B*). The fluorescent sections on this plate were cut with orientation A→A′; therefore, crypts appear as doughnuts with the lumen towards the center. γ-tubulin, a protein found near the MTOCs of many cells (Joshi, 1993), was also concentrated in distinct spots near the luminal surface (Fig. 1 *D*). The γ-tubulin puncta in the lamina propria surrounding the crypts likely corresponds to MTOCs of immunocompetent cells. Thin section electron micrographs confirmed the apical positioning of the centrioles (Fig. 1 *A*). The clustering of cold stable MTs and centrioles in the apical cytoplasm subjacent to the brush border (Fig. 1 *C*), where colchicine-resistant MTs are found (Hugon et al., 1987; Achler et al., 1989; Gilbert et al., 1991), and the immunolocalization of γ-tubulin to the apical cytoplasm (Fig. 1 *D*) are all consistent with the electron microscopic demonstration of MT minus-ends being apical (Bacallao et al., 1989).

The distribution of cytoplasmic dynein, dynactin, kinesin, and myosin-I in polarizing intestinal epithelial cells was determined by immunolocalization on frozen sections. The distribution of these proteins relative to the actin-rich BB was shown by double labeling the sections with rhodamine-phalloidin. Dynein (Fig. 2 *A*; Fig. 3 *A*) and dynactin (p150/*Glued*) (Fig. 2 *E*), a major component of a protein complex that activates in vitro vesicle motility mediated by cytoplasmic dynein (Gill et al., 1991), were found throughout the cytoplasm in a diffuse, punctate pattern. Dynein and dynactin (p150/*Glued*) were concentrated in the apical cytoplasm and BB, but, unlike myosin-I (Fig. 2 *G*), were excluded from the microvilli. Kinesin (Fig. 2 *C*) was localized in a punctate manner both above and below the nucleus, and basally and apically in a row of spots with a similar distribution as centrioles (Fig. 1). Myosin-I was diffusely distributed throughout the apical cytoplasm in addition to being concentrated in the BB and microvilli (Fig. 2 *G*).

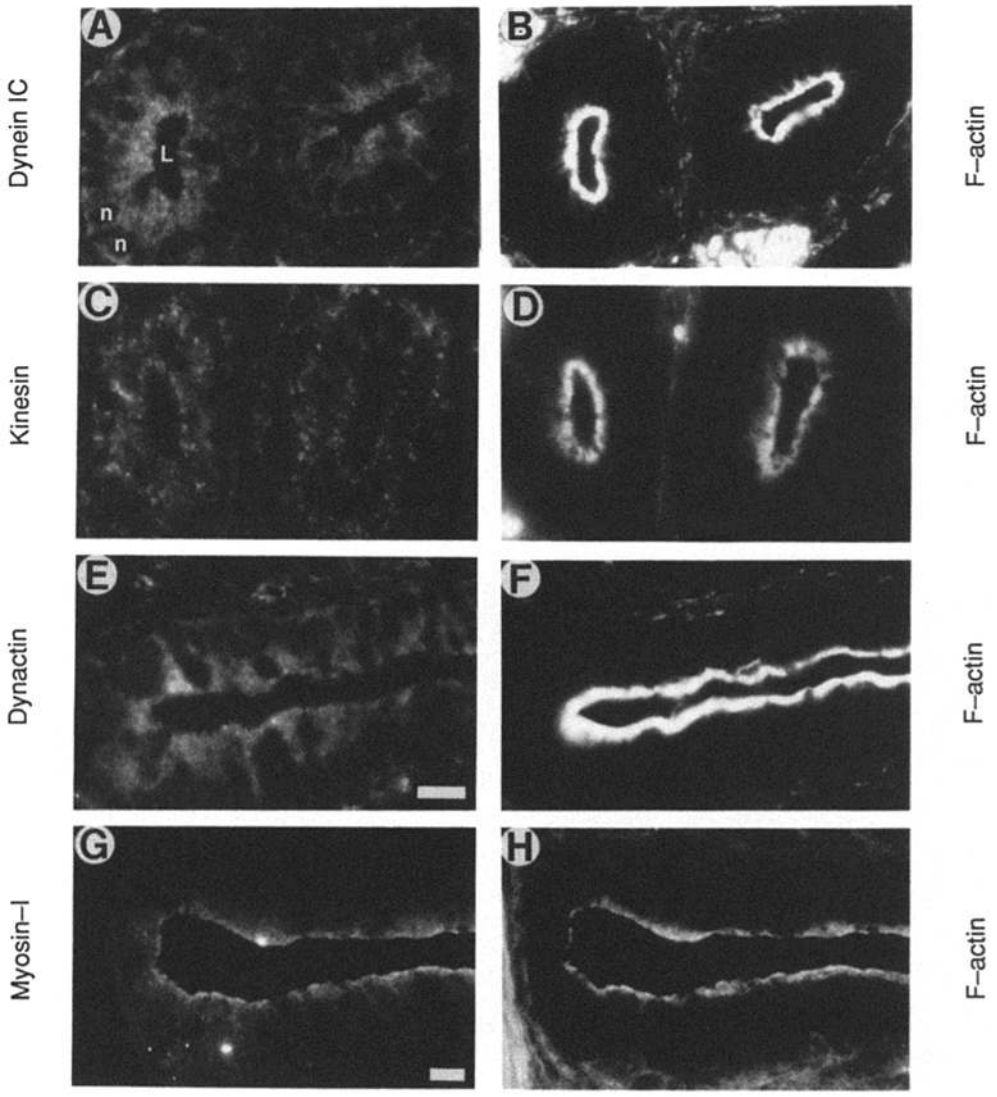

Figure 2. Distribution of cytoplasmic dynein, dynactin (p150/*Glued*), kinesin and myosin-I in frozen sections of chicken intestinal crypts. Fluorescent light micrograph pairs of intestinal crypts cut in cross-section (A–D; A→A′ plane of section in Fig. 1) or longitudinal-section (E-H; B→B′ plane of section in Fig. 1). *A*, *C*, *E*, and *G* are labeled by indirect immunofluorescence and *B*, *D*, *F*, and *H* are labeled with rhodamine-phalloidin for polymerized actin. Cytoplasmic dynein (*A*; mAb 70.1, intermediate chains) and dynactin (p150/*Glued*) (*E*) are found in a punctate pattern throughout the cell, but are concentrated in the apical cytoplasm. The same sections stained for F-actin (*B* and *F*) shows that they are not associated with microvilli. As an orientation aid, the position of two nuclei in *A* are labeled *n* and the lumen of the same crypt is labeled with an *L*. Kinesin (*C*) is found in a punctate pattern with a concentration above and below the nucleus, basally and just subjacent to the BB in foci that may correspond to the centriolar region. Myosin-I is diffusely distributed throughout the apical cytoplasm, but is more concentrated in the actin-rich BB than is cytoplasmic dynein or kinesin. Bars: (*A–F*) 10 μm; (*G* and *H*) 10 μm.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

Some of the immunofluorescent images suggested that a portion of cytoplasmic dynein may be near the Golgi complex. To morphologically determine if there was an association of dynein with the Golgi apparatus as has been suggested for CHO cells (Corthésy-Theulaz et al., 1991), frozen sections were double labeled for dynein and for the Golgi with an antibody to the Golgi protein α-mannosidase-II (Fig. 3 *B*) or with fluorescent WGA (Fig. 3 *E*). Although we observed occasional coincidences of dynein with the Golgi markers immediately adjacent and apical to the nucleus (Fig. 3, *arrowheads*), the majority of dynein was more widely distributed in a punctate manner. Note that α-mannosidase-II was also found at the apical surface as has been shown previously (Velasco et al., 1993) in addition to cell-cell junctional complexes (Fig. 3 *B*; more evident in higher magnification images not shown). Although WGA can also label apical surface proteins, as it did in the differentiated cells on the villus (not shown), the apical surface of crypt cells did not label with WGA. It is unlikely that the observed coincidence of dynein with WGA or α-mannosidase-II is on endosomes because WGA did not label the crypt apical membrane. Moreover, since endocytosis is minimal from the apical surface in the adult duodenum (Louvard et al., 1992), it is likely that the WGA and α-mannosidase-II staining is on the Golgi and not endosomes containing apical plasma membrane proteins.

Cytoplasmic Dynein Is Membrane Associated in the BB and in Subcellular Fractions of Isolated Intestinal Epithelial Cells

The punctate distribution of cytoplasmic dynein in intestinal epithelial cells suggests, as has been shown in other cells (Hirokawa et al., 1990; Goltz et al., 1992; Lacey and Haimo, 1992; Lin and Collins, 1992), that dynein may be associated with cytoplasmic membranes. To biochemically assess if cytoplasmic dynein was associated with membranes, we determined its distribution on immunoblots of subcellular fractions of isolated enterocytes (Fig. 4). Dynein was associated with membrane-intact BB, which possess cytoplasmic vesicles (Burgess and Prum, 1982), as well as with large organelles (20,000- and 80,000-*g* pellets) and microsomal (150,000-*g* pellet) membranes. The identity of an immunoreactive band of slower mobility in the BB, which was infrequently observed in this and other fractions, is unknown. Dynein was absent from the nuclear pellet and the demembranated BB cytoskeleton. On the same fractions, kinesin was concentrated in the 80,000-*g* pellet (data not

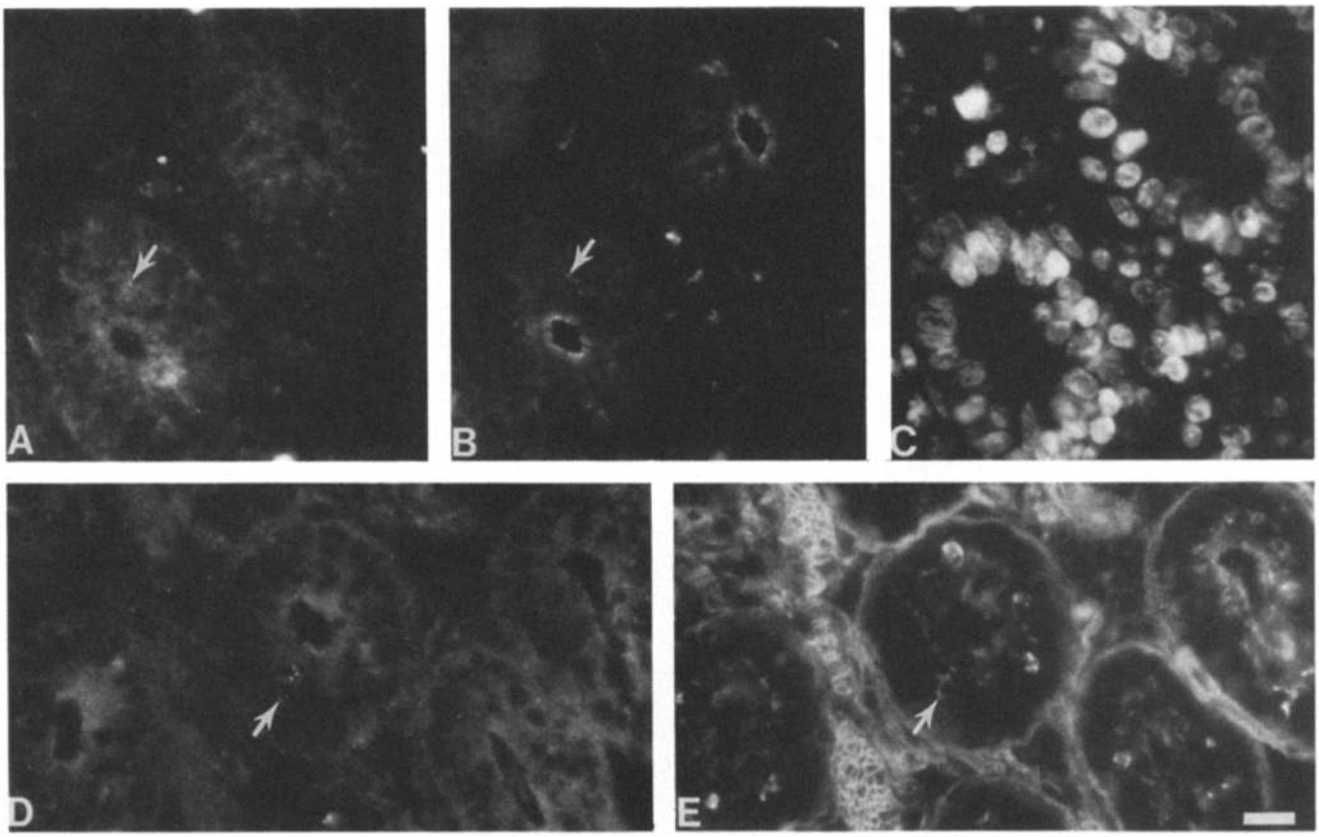

Figure 3. Dynein is present, although not concentrated in the Golgi apparatus. Fluorescent light micrograph of intestinal crypts cut in cross-section (A→A′ plane of section in Fig. 1). To determine if dynein was concentrated in the Golgi apparatus, frozen sections were triple labeled for dynein intermediate chains (*A*), for the Golgi apparatus with α-mannoisidase-II (*B*) and for nuclei with DAPI (C; 4′,6-diamidino-2-phenylindole). Sections were also double labeled for dynein heavy chain (*D*) and for the Golgi with TRITC-WGA (*E*; Tartakoff and Vassalli, 1983). Although there is occasional codistribution of dynein with supranuclear Golgi markers (*arrowheads*), dynein has a punctate, largely apical distribution that is not exclusively concentrated in the Golgi. Bar, 10 μm.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

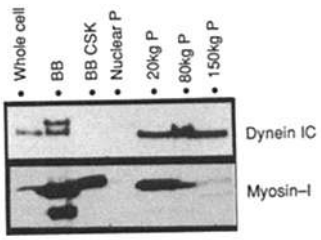

Figure 4. Cytoplasmic dynein is associated with intracellular membranes. Isolated chicken intestinal epithelial cells were fractionated into intact BB (*BB*); demembranated BB (*BB CSK*); nuclei (*Nuclear P*); 20,000, 80,000, and 150,000 *g* pellets. Each lane was loaded containing cell equivalencies of protein except for whole cell which is underloaded 20-fold. Dynein is shown by immunoblotting to be mainly associated with large organelles and microsomes, but not with nuclei or demembranated BB. Myosin-I was less concentrated in the microsomal membranes, but was associated with the BB cytoskeleton. The faster migrating myosin-I immunoreactive band in the BB corresponds to a common proteolytic fragment of myosin-I.

shown). These results suggest that cytoplasmic dynein and kinesin are associated with membranes within the BB, as well as with other light membranes, and not attached directly to the BB cytoskeleton. These subcellular distributions are consistent with the immunolocalization images that show a punctate distribution of MT motors that include a distribution in the BB. Although the actin-based motor myosin-I shared a similar distribution with cytoplasmic dynein (Fig. 4), it differed mainly in that myosin-I was enriched in the BB cytoskeleton where it links microvillus actin filaments to the membrane (Matsudaira and Burgess, 1979) and was less concentrated in the microsomal pellets. A common proteolytic fragment of myosin-I is also seen that is most prevalent in the BB. These results extend our previous reports of myosin-I associated with Golgi-enriched membranes (Fath and Burgess, 1993); furthermore, they suggest that cytoplasmic dynein and kinesin are associated with intracellular membranes but are not directly associated with the BB cytoskeleton.

Association of Myosin-I and Cytoplasmic Dynein, but Not Kinesin, with Isolated Golgi and Golgi-enriched Membranes

We next wished to determine if a portion of the enterocyte membrane-associated dynein or kinesin identified by subcellular fractionation was associated with Golgi membranes. A Golgi fraction was isolated from enterocytes using conventional methods that were developed for rat liver Golgi studies (Leelavathi et al., 1970). These membranes, banding at the 0.5/1.1 M sucrose interface, were identified as Golgi enriched by three criteria. First, large numbers of Golgi stacks were present as determined by EM (Fig. 5, *A* and *B*). Second, the membranes were enriched for the Golgi markers β-COP (Allan and Kreis, 1986), TGN-38/41 (Luzio et al., 1990; Jones et al., 1993), and α-mannosidase-II (Velasco et al., 1993) (Table I). Third, the membranes were 6.2 ± 1.9-fold (SD; $n = 3$) enriched in specific activity for the Golgi enzyme galactosyltransferase relative to its activity in the PNS (Fig. 5 *D*, lane *4*). Immunoblot analysis of this Golgi fraction with antibodies against myosin-I and dynein (Fig. 5 *C*) showed that both motors were present, while kinesin, although present in the total cell homogenate, was absent (data not shown).

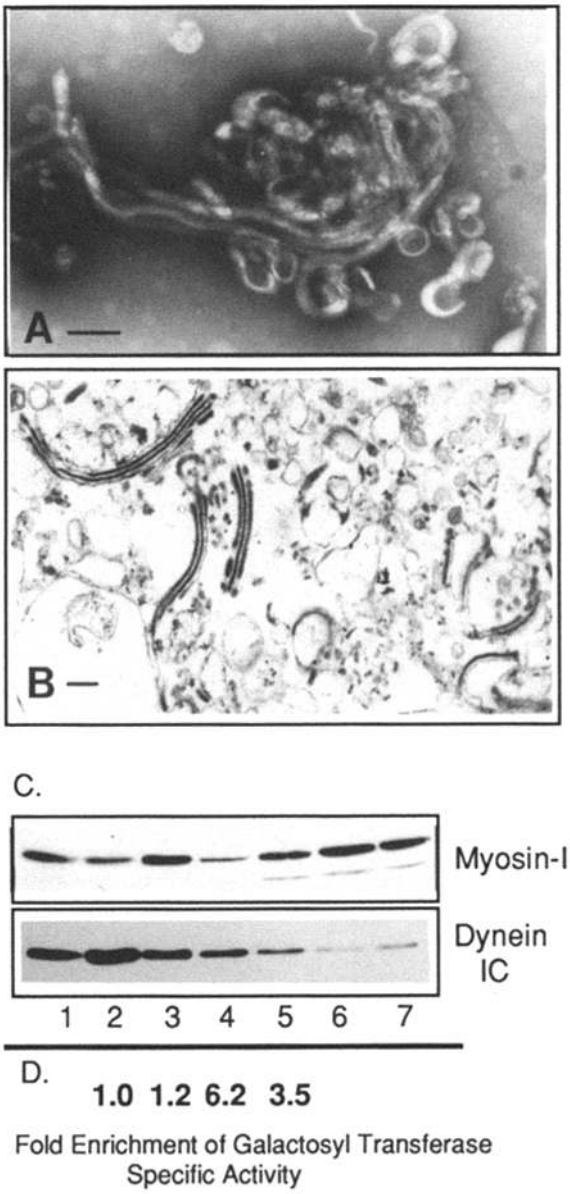

Figure 5. Characterization of the Leelavathi Golgi fraction. Membranes banding at the 0.5/1.1 M sucrose interface contained Golgi stacks as seen by negative staining (*A*) and thin section EM (*B*). (*C*) Distribution of myosin-I (≈110 kD) and dynein intermediate chains (≈70 kD) in various fractions generated during Leelavathi Golgi preparation. Lane *1*, total enterocyte homogenate; lane *2*, PNS; lane *3*, membranes concentrating above the 1.3 M sucrose pad; lane *4*, 0.5/1.1 M sucrose interface; lane *5*, 1.1/1.25 M sucrose interface; lane *6*, 1.25/1.3 M sucrose interface; lane *7*, 1.3/1.4 M sucrose interface. These blots show that the Golgi fraction (lane *4*) contains both dynein and myosin-I. (*D*) The fold enrichment of the *trans*-Golgi marker enzyme galactosyltransferase in the fractions corresponding to lanes *2–5*. The average specific activity in the PNS (0.135 nmol/min/mg) was set at 1.0. Note the ≈sixfold enrichment for galactosyltransferase in the Golgi stack fraction. Bar, 0.2 μm.

Although both dynein and myosin-I were found on Golgi membranes, quantitation of immunoblots showed that neither was enriched in the Golgi (Fig. 5 *C*, Table I). Only a small amount of PNS myosin-I (0.007%) or dynein (0.002%) was present in the Golgi fraction (Table II). Although 62% of the PNS myosin-I was membrane associated, the majority

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

Table I. Fold Enrichment of Motor Proteins and Golgi Markers in Membrane Fractions*

	Membrane Fraction Banding at Sucrose Interface			
	0.5/1.1 M‡	1.1/1.25 M	1.25/1.3 M	1.3/1.4 M
β-COP	1.6	0.8	0.6	2
TGN 38/41	11.3	10.6	10.4	13
α-mann II	5.4	1.7	2.8	1.0
Myosin-I	0.7	2.4	3.4	2.9
Dynein	0.23	0.08	0.01	0.03

* Fold enrichment was determined by densitometric analysis of immunoblots of SDS-polyacrylamide gels loaded with equivalent amounts of protein and is expressed relative to its level in the PNS. Values given are from a representative preparation containing membranes from four chickens.
‡ The 0.5/1.1 M sucrose interface is the Golgi fraction.

was found in denser fractions (Fig. 5 *C*, lanes *6* and *7*) that include fragmented microvilli (not shown). We have previously shown that greater than 50% of total myosin-I is associated with the brush border (Fath and Burgess, 1993). Dynein was associated with lighter membranes than was myosin-I, and the majority (69%) of dynein was soluble (Table II). In contrast, only ~10% of the PNS myosin-I or 5% of the total myosin-I was soluble. The small fraction of both myosin-I and dynein found associated with Golgi membranes is consistent with our immunofluorescent observations showing no concentration of these motors with Golgi, but rather, a diffuse punctate distribution in the apical cytoplasm.

Dynein Is Found on a Subpopulation of Golgi Membranes

The Leelavathi Golgi fraction (Fig. 6 *A*) was further subfractionated by differential centrifugation. As analyzed by transmission EM, low speed centrifugation (10,000 *g*) pelleted intact Golgi stacks (Fig. 6 *B*). Material that remained in the supernatant was collected at 266,000 g_{max} (Fig. 6 *C*) and contained a homogeneous collection of small membranes which ranged in diameter from 50–400 nm (Fig. 6 *C*). Immunoblots of these fractions generated by differential centrifugation showed that the membrane stacks possessed myosin-I, α-mannosidase-II, β-COP, and alkaline phosphatase, but not dynein or TGN38/41 (Fig. 7; Table III). The small membranes contained myosin-I, dynein, β-COP, TGN38/41, and alkaline phosphatase, but very small amounts of α-mannosidase-II (Fig. 7; Table III). Approximately one-half the amount of myosin-I and three times the amount of β-COP was present on the stacks as was on the small membranes. The membrane morphology and distribution of the markers

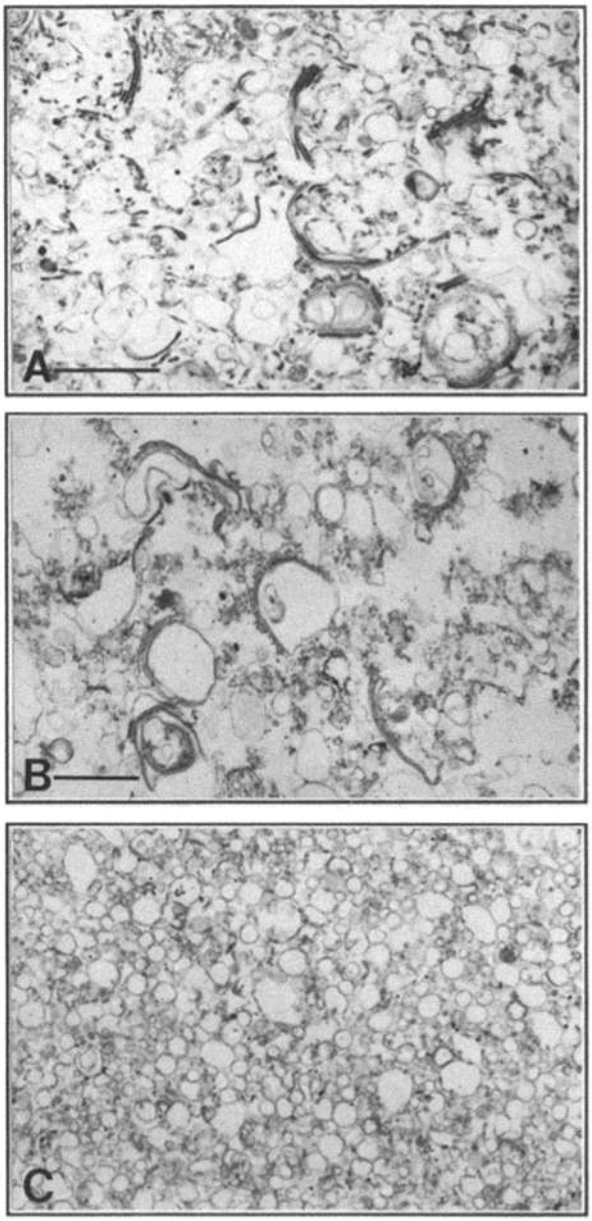

Figure 6. Thin section electron microscopy of subfractionated Golgi membranes. Total Leelavathi Golgi membranes (*A*), were subfractionated as described in the text. EM of these fractions showed that the 10,000 *g* pellet contains Golgi stacks (*B*), while the supernatant (*C*), is a fairly homogeneous fraction of small membranes. *B* and *C* are at the same magnification while *A* is at a slightly higher magnification. Bar, 1 μm.

Table II. Comparison of the Amount of Myosin-I and Dynein Found in Fractions from a Golgi Preparation Relative to PNS

Fraction	Contents	% Protein*	% Myosin-I‡	% Dynein‡
600 *g* supernatant	PNS	100	100	100
105,000 *g* supernatant	Cytosol	63	10.7	69
105,000 *g* pad	Membranes	39§	62	0.05
0.5/1.1 M interface	Golgi fraction	0.01	0.007	0.002

* The percentages were calculated relative to the amount of protein in the PNS and are from a representative preparation containing membranes from four chickens.
‡ The percentages of the individual proteins were determined by scanning densitometry of immunoblots of each fraction relative to the PNS.
§ This is a slight overestimation due to cytosolic contamination of the membrane pad.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

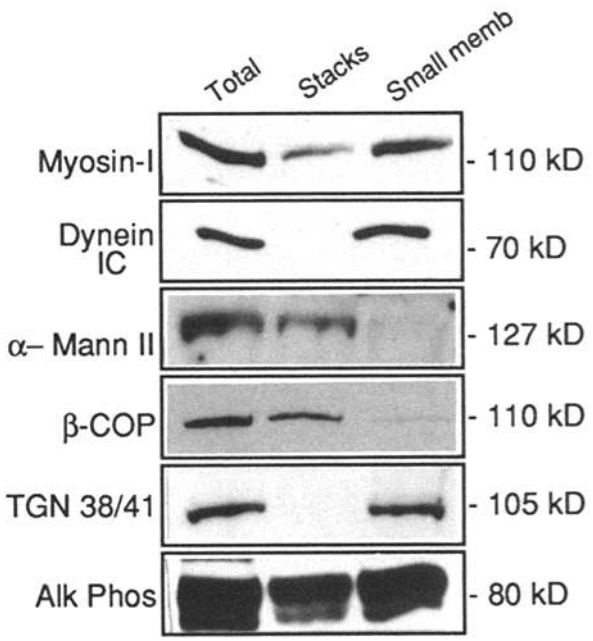

Figure 7. Immunoblot analysis of subfractionated Golgi membranes. Leelavathi Golgi membranes were subfractionated by centrifugation at 10,000 *g* for 15 min. The resulting pellet was termed stacks. The membranes remaining in the supernatant were collected by centrifugation at 266,000 g_{max} for 30 min and termed small membranes. An unfractionated sample of Leelavathi Golgi membranes was also centrifuged at 266,000 g_{max} for 30 min to pellet all membranes (*Total*). Each fraction was resuspended in an equal volume of buffer, fractionated by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies. The mobilities of the molecular weight markers and their mass (kD) are indicated.

suggests that the 10,000-*g*-pelleted membranes correspond to Golgi stacks, whereas the small membranes have some features of the *trans*-Golgi network (e.g., contain TGN38/41). We wished to determine if the small membrane fraction contained only single membranes or a network of membranes that was not apparent in thin sections. Negative stain EM of unpelleted small membranes contained only single vesicles and not elongate membranes or networks (data not shown).

Table III. Characterization of Golgi Membranes

	Leelavathi Golgi ρ = 1.0635–1.1415 g/cm³			Weiser Golgi ρ = 1.0592–1.1175 g/cm³
	Total	Stacks	Small membranes	
GalTase*	6.2	9.3	3.6	2.9
Alk. phos.	++	+	+	+
β-COP	++++	+++	+	–
TGN-38/41	+	–	+	+
α-mann-II	+	+	–	+
Myosin-I	+++	+	++	+
Dynein	+	–	+	+
Kinesin	–	–	–	–‡
Dynactin	+	+	+	+

Alk. phos., alkaline phosphatase; α-mann-II, α-mannosidase-II; GalTase, galactosyltransferase.
* Enrichment of GalTase specific activity relative to its activity in the PNS.
‡ Kinesin was present on denser membranes (ρ = 1.1562–1.1765 g/cm³).

Characterization of Weiser Golgi Membranes

Because the conventional Leelavathi Golgi isolation protocol induced BB microvilli fragmentation due to the lack of Ca^{2+} chelators (see Burgess and Prum, 1982), and in order to prepare smaller membranes for biochemical studies, we also isolated Golgi-enriched membranes from intestinal epithelial cells by another method (Fath and Burgess, 1993) that was originally developed to isolate enterocyte Golgi membranes (Weiser et al., 1978). We found that these Golgi-enriched membranes, which were 50–150 nm in diameter (Fath and Burgess, 1993), were 2.9 ± 0.76-fold (SD; n = 3) enriched in specific activity for the Golgi marker galactosyltransferase relative to its activity in the PNS. These Weiser membranes possessed alkaline phosphatase as measured by activity (Fath and Burgess, 1993) and possessed α-mannosidase-II and TGN 38/41, but lacked β-COP, and kinesin (data not shown) as determined by immunoblotting. Immunoblots of membranes showed that as with the Leelavathi Golgi stacks, Weiser membranes contain cytoplasmic dynein as well as myosin-I (Fig. 8 *A*). On the same membranes, an antibody recognizing the cytoplasmic dynein heavy chain recognized a polypeptide (Fig. 8 *B*) that had the same electrophoretic mobility as sea urchin sperm axonemal dynein heavy chain (data not shown). In some experiments, to determine if protein–lipid aggregates were cosedimenting with Golgi membranes, Weiser membranes were also gradient purified using a flotation step. As with experiments in which the gradients were overlayed with sample, we found by immunoblotting that both dynein (mAb 70.1) and myosin-I banded with Golgi membranes on flotation gradients (data not shown). The dynactin (p150/*Glued*) complex of proteins is required for the cytoplasmic dynein-based trans-

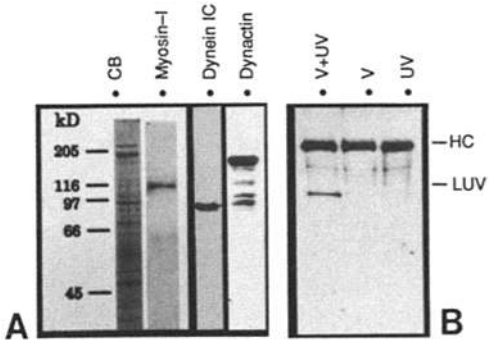

Figure 8. Cytoplasmic dynein and dynactin (p150/*Glued*) are associated with Weiser Golgi-enriched membranes: immunoblotting and UV photocleavage. (*A*) Golgi-enriched membranes were isolated from chicken intestinal crypts by the method of Weiser (Fath and Burgess, 1993) and analyzed by immunoblotting. Coomassie blue (*CB*) profile of Golgi vesicle proteins and immunoblots showing that these membranes possess myosin-I, cytoplasmic dynein intermediate chains (IC, mAb 70.1) and dynactin (mAb 150.1). A portion of this figure was adapted from Fath and Burgess (1993). (*B*) When membranes were irradiated with UV light in the presence of monomeric vanadate (*V* + *UV*), the cytoplasmic dynein heavy chain was photocleaved to a mAb 440.4-immunoreactive band at ≈200 kD that corresponds to the LUV fragment of other dyneins (Gibbons et al., 1987). When membranes were incubated with vanadate in the dark (*V*), or in the absence of vanadate, but with UV irradiation (*UV*), dynein was not photocleaved.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

port of vesicles along MTs in vitro (Gill et al., 1991). We found by immunoblotting that dynactin, a major protein of this complex, was also present on isolated Weiser Golgi-enriched membranes (Fig. 8 *A*) and was also present on Leelavathi Golgi stacks (data not shown). A summary of the features of membranes isolated according to Weiser and the Leelavathi Golgi preparation is presented in Table III. Analysis of a more dense membrane fraction (ρ = 1.1562–1.1765 g/cm^3) from the Weiser protocol gradient identified a population of membranes which possessed β-COP, myosin-I, dynein, and kinesin (data not shown).

To confirm the association of cytoplasmic dynein with membranes, Weiser membranes were UV irradiated at 366 nm in the presence of monomeric vanadate and Mg-ATP, conditions that cleave dynein heavy chain at the V_1 site into characteristic 228- and 200-kD major fragments (Gibbons et al., 1987). On immunoblots of irradiated samples, mAb 440.4 recognized an ≈200-kD immunoreactive band that corresponds with the lower molecular mass fragment (LUV) of dynein (Fig. 8 *B*). This band appeared in the presence of both UV and vanadate (Fig. 8 *B*, lane *V+UV*), but was absent when either the UV irradiation (Fig. 8 *B*, lane *V*) or vanadate (Fig. 8 *B*, lane *UV*) were omitted. mAb 440.4 does not recognize the larger HUV fragment of dynein (Steuer et al., 1990). The immunorecognition of cytoplasmic dynein heavy and intermediate chains, in addition to the UV photocleavage of the heavy chain, provide strong evidence of the association of cytoplasmic dynein with Golgi-enriched membranes.

Cytoplasmic Dynein Is a Vesicle Peripheral Membrane Protein

To assess the nature of the association between cytoplasmic dynein and Golgi-enriched membranes, Weiser membranes were stripped of peripheral membrane proteins by high salt or alkaline pH. Membranes were incubated in 1.0 M NaCl, 0.6 M KI, or 100 mM sodium carbonate (pH 11.5), then centrifuged to pellet the membranes away from the released proteins. We followed the distribution of cytoplasmic dynein in these fractions on immunoblots using mAb 70.1 because of the more quantitative electrophoretic transfer of the lower molecular mass intermediate chains than the larger heavy chain. In contrast to what we found previously for myosin-I where ~50% was extractable by high salt or pH (Fath and Burgess, 1993), nearly all dynein was released from the membrane by high salt or pH (Fig. 9). These results suggest that cytoplasmic dynein is a vesicle peripheral membrane protein that is bound directly or indirectly to the membrane by electrostatic interactions.

Golgi-enriched Membranes Bind to Microtubules in an ATP-sensitive Fashion

A dynein-mediated vesicle motility on MTs requires that dynein be on the membrane "cytoplasmic" surface and that it binds to and releases from MTs in a nucleotide-sensitive manner. Isolated Weiser membranes, previously shown to be intact and right-side-out (Fath and Burgess, 1993), were incubated with taxol-stabilized MTs in the presence or absence of ATP, then centrifuged over a 1.22 M sucrose cushion, a concentration allowing MTs but not membranes to pellet. The resultant supernatants and pellets were immunoblotted

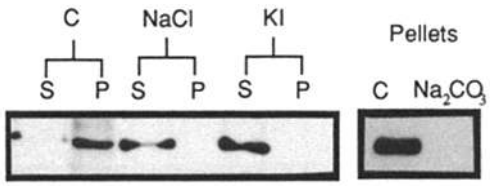

Figure 9. Cytoplasmic dynein is a Golgi vesicle peripheral membrane protein. To determine the positioning of cytoplasmic dynein in the Golgi vesicle membrane, isolated membranes were incubated with high salt or alkaline buffers, pelleted and supernatants and pellets immunoblotted. Following incubation in PEMS buffer alone (*C*; *control*), 1.0 M NaCl or 0.6 M KI, membranes were pelleted and resultant supernatants (*S*) and pellets (*P*) blotted for cytoplasmic dynein intermediate chains. Dynein behaves as a peripheral membrane protein since it pellets with membranes incubated in buffer alone, but is released from the membranes into the supernatant when membranes are incubated in NaCl or KI. In another experiment, pellets were examined after incubation in PEMS (*C*) or 0.1 M sodium carbonate (pH 11.5). Cytoplasmic dynein is stripped from the pelleted membranes incubated at alkaline pH.

for cytoplasmic dynein intermediate chains and a portion of each was negative stained to verify MT-vesicle binding. All membranes (not shown) and dynein (Fig. 10) were found in the supernatant in experiments in which membranes were centrifuged in the absence of MTs. In contrast, when membranes were preincubated with MTs in the absence of ATP and then centrifuged, nearly all dynein was detected in the pellet (Fig. 10). Negative stain EM confirmed that the pellet contained membranes that were bound to MTs (Fig. 10). MT binding was sensitive to ATP; when membranes were incubated and centrifuged with MTs in the presence of 10 mM ATP, neither dynein (Fig. 10) nor membranes (not shown) pelleted with MTs. Although not shown, in similar binding assays, Leelavathi small membranes also bound to MTs in an ATP-sensitive manner. These MT-binding assays suggest that dynein is on the membrane cytoplasmic surface and that membranes bind to and release from MTs in an ATP-sensitive manner. We have previously demonstrated a similar nucleotide-dependent binding of these Golgi-enriched membranes to actin filaments (Fath and Burgess, 1993).

Myosin-I and Dynein Coexist on a Subset of Golgi-enriched Membranes

Recent morphological and genetic studies suggesting that individual cytoplasmic vesicles may possess both a MT- and an actin filament-based motility (Kuznetsov et al., 1992; Lillie and Brown, 1992; Bearer et al., 1993) led us to explore if individual Golgi-derived membranes expressed both cytoplasmic dynein and myosin-I. Therefore, we wished to determine if myosin-I partitioned with dynein-associated membranes in the MT-pelleting assays. That is to say, if we used MTs to affinity-isolate those membranes expressing dynein, would myosin-I (which does not bind to MTs) partition with these membranes? In these experiments, myosin-I remained in the supernatant with either Weiser or Leelavathi small membranes when membranes were incubated in buffer alone (Fig. 10, *V*) or when incubated with MTs plus ATP (Fig. 10, *V+MT+ATP*). When membranes were incubated with MTs

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

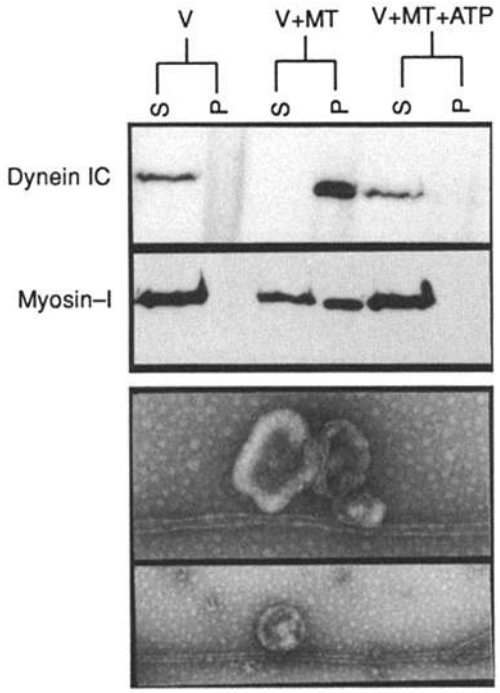

Figure 10. Isolated Weiser Golgi membranes contain myosin-I and cytoplasmic dynein: coisolation in MT pelleting assays. Golgi-enriched membranes were incubated with taxol-stabilized MTs and pelleted through a 1.22 M sucrose pad. Supernatants (*S*) and pellets (*P*) were immunoblotted for cytoplasmic dynein intermediate chains and myosin-I. In PEMS buffer alone (*V*), all the myosin-I and dynein remained in the supernatant. When incubated with MTs in the absence of ATP (*V+MT*), all of the dynein and ~50% of the myosin-I pelleted. Negative stain EM confirms that pelleted membranes are bound to MTs as the micrographs show. When membranes were incubated with MT+ATP (*V+MT+ATP*), all dynein and myosin-I remained in the supernatant and membranes were not seen in the pellet by negative staining (data not shown). MT width, 25 nm.

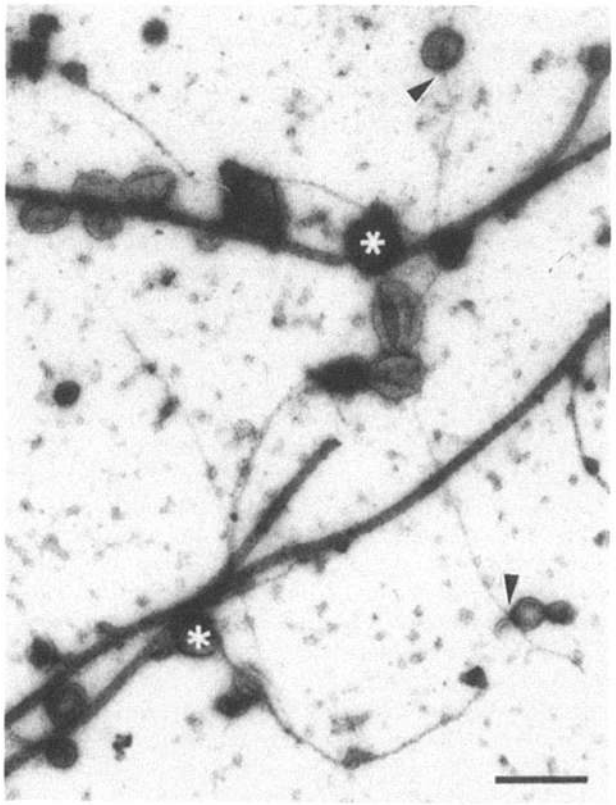

Figure 11. Isolated small membranes bind both actin and MTs. Small Golgi membranes were incubated with actin filaments and MTs and centrifuged over a sucrose pad in conditions in which membranes caused bound cytoskeletal elements to remain in the supernatant (see Materials and Methods). This negative stain EM micrograph shows that single membranes bind to MTs or to actin filaments. Several actin filaments with attached membranes are indicated by arrowheads. A smaller number of membranes (*asterisks*) appear to cross-link both actin filaments and MTs. Bar, 0.2 μm.

in the absence of ATP, ~50% of the myosin-I pelleted with membranes (Fig. 10, *V+MT*). By contrast, nearly all of the cytoplasmic dynein pelleted under the same conditions.

We repeatedly attempted to immunoisolate Golgi membranes with several antibodies to dynein (mAb 70.1, mAb 440.4, and mAb 74.1) and several antibodies to myosin-I (data not shown). Although the antibodies could immunoprecipitate their respective antigens from solution, they all failed to bring down the membrane-associated motors in a repeatable fashion. Therefore, in a double-blind experiment, Leelavathi small membranes were mixed with actin filaments and MTs in the presence or absence of ATP, centrifuged over a 1.1 M sucrose pad to separate membrane-bound cytoskeletal elements (which float) from unbound actin and MTs and negative stain EM of the supernatants generated. The absence of ATP increased by sevenfold the number of membrane-bound cytoskeletal elements (Fig. 11). In the absence of ATP, we observed single membranes binding to actin filaments, others binding to MTs, and a smaller number appearing to cross-link both cytoskeletal elements. In this experiment, in the microtubule-binding experiment shown in Fig. 10, and in the actin binding experiment shown in Fath and Burgess (1993), only rarely were membrane aggregates observed (where membranes were bound to one another and not bound to cytoskeleton or where one membrane was bound to cytoskeleton and at the same time bound to another membrane). Therefore, it is unlikely that the myosin-I–containing membranes found in the pellet in Fig. 10 sediment solely due to aggregation with dynein-containing membranes. These results suggest that a population of cytoplasmic dynein-containing membranes possess myosin-I and further, that another population of membranes possess myosin-I, but not dynein. It is also possible that some membranes possess only dynein.

Discussion

Here we report the distribution of the molecular motors myosin-I and dynein in polarized intestinal epithelial cells. Both immunofluorescence and biochemical methods show that neither myosin-I nor dynein is enriched in Golgi membranes. We find that cytoplasmic dynein and its motility activator dynactin (*p150/Glued*) are concentrated in the apical cytoplasm in a diffuse and punctate pattern and are also present in the region of the Golgi apparatus. By subcellular fractionation we find that a significant amount of enterocyte

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

dynein is bound to intracellular membranes and a small amount associated with isolated Golgi membranes. The concentration of dynein in punctate spots in the apical cytoplasm, near the minus-ends of MTs where a minus-end–directed motor would tend to accumulate, is consistent with dynein's role in moving membranes along MTs. Negative staining and immunoblotting of pelleting assays show that Golgi membranes previously shown to be right-side-out and intact (Fath and Burgess, 1993), possess cytoplasmic dynein and bind MTs in an ATP-dependent manner. We subfractionated the traditional Leelavathi Golgi preparation by differential centrifugation into Golgi stacks and smaller membranes with some characteristics of the *trans*-Golgi network. We find that while myosin-I is present on both fractions, dynein is present only on the small membranes. We identify membrane-bound cytoplasmic dynein by: (*a*), immunorecognition of heavy chain and comigration with similar chains from sea urchin axonemes; (*b*), vanadate-sensitive UV photocleavage of heavy chain; (*c*), immunorecognition of intermediate chains; (*d*), and an ATP-sensitive release from MTs. These membranes also possess dynactin (p150/*Glued*) but lack kinesin which has also been shown to be absent from Golgi membranes isolated from bovine brain (Leopold et al., 1992). We have previously shown that these membranes have the actin-based motor myosin-I bound to the cytoplasmic surface via electrostatic interactions (Fath and Burgess, 1993). Membranes are now shown also to possess both motors because MT-dependent pelleting of membranes sediment both cytoplasmic dynein and myosin-I, suggesting that they are on the same vesicle.

In this report we have isolated Golgi membranes using methods developed for intestinal epithelial cells by Weiser (Weiser et al., 1978) and intact Golgi by the method of Leelavathi that was developed for use in liver (Leelavathi et al., 1970). A comparison of the properties of the membranes isolated by the two methods is shown in Table III. Although both methods isolate membranes of similar density on sucrose gradients, the membranes from the Weiser method were isolated in buffers containing EGTA and are more stringently triturated by homogenization. The inclusion of EGTA in the buffers used in the Weiser method decreased the possibility of contamination from vesiculated microvilli that contain myosin-I (Burgess and Prum, 1982). Microvillar vesicles, however, band in a denser fraction than Golgi stacks (data not shown). The Dounce homogenization used in the Weiser method generated smaller and more uniform membranes that were useful in MT-binding studies. By subfractionating the traditional Leelavathi membranes by a low speed centrifugation (15 min at 10,000 *g*) we separated morphological stacks and Golgi cisternal markers (β-COP, galactosyltransferase, α-mannosidase-II) from smaller membranes that possessed the *trans*-Golgi network marker TGN38/41. The Golgi marker profile of the Weiser membranes suggests that they most closely correspond to the small Leelavathi membranes. Both the Weiser membranes and small Leelavathi membranes contain dynein and myosin-I, while Golgi stacks contained only myosin-I.

The understanding of compartment-specific marker proteins for the Golgi apparatus and carrier vesicles is not as advanced in enterocytes by comparison with the well characterized Golgi in NRK cells or liver (see Velasco et al., 1993 for discussion and references). We have used α-mannosidase-II as a Golgi marker protein; however, we found by immunofluorescence that this enzyme, like galactosyltransferase (Roth et al., 1985), while enriched in the Golgi, is also found on the plasma membrane of intestinal epithelial cells (Velasco et al., 1993). Despite the lack of enterocyte specific Golgi markers, the isolation of morphologically identifiable Golgi stacks at the correct density, which possess the enzymes galactosyltransferase, α-mannosidase-II, and the Golgi markers TGN38/41 and β-COP, suggests that the membranes isolated in our study represent Golgi membranes. Furthermore, our study now provides a thorough characterization of enterocyte Golgi membranes.

Dynein is bound to Golgi membranes via electrostatic interactions. It is probably attached to the membrane outside of the head region because the MT and ATP (and vanadate) binding sites are accessible. As has been found for other isolated cytoplasmic vesicles (Lacey and Haimo, 1992; Leopold et al., 1992), enterocyte Golgi membranes contain tubulin as shown by immunoblots with an α-tubulin antibody (data not shown). We propose that dynein is not bound to Golgi membranes via intact microtubules for the following reasons: (*a*), endogenous MTs were never observed in negative stained preparations of membranes, while short exogenous MTs are easily detected; (*b*), contaminating MTs would have to be both cold- and dilution-stable during vesicle isolation in the absence of taxol; (*c*), Golgi membranes band at ≈0.91 M sucrose while membranes bound to exogenous MTs are denser (*d*), exogenous MTs induce pelleting of all dynein suggesting that at least some dynein on each vesicle has unoccupied MT-binding sites and therefore must be bound to the membrane by a different mechanism. Although a dynein membrane-binding protein has not been identified, a putative kinesin-binding protein has been identified (Toyoshima et al., 1992; Yu et al., 1992) and the binding of cytoplasmic dynein to brain microsomes requires a protease-sensitive surface protein (Yu et al., 1992). This does not rule out the possibility that unpolymerized membrane tubulin binds to dynein as has been suggested for kinesin (Skoufias et al., 1994). Because cytoplasmic dynein is not found on Golgi stacks which possess myosin-I but is present on the small membranes, it is likely that there are specific dynein binding proteins in a subpopulation of enterocyte Golgi membranes.

We have determined that a small amount of cytoplasmic dynein (0.002%) or myosin-I (0.007%) is associated with enterocyte Golgi. While this number is most likely an underestimate because not all of the Golgi is found at the 0.5/1.1 M sucrose interface (Table I) and some motors are likely released from the membrane during cell fractionation, these motors are undoubtably also associated with other cellular organelles. Most notably, more than 50% of the myosin-I is associated with the BB (Fath and Burgess, 1993). The low percentage of motors that we found associated with cellular organelles is consistent with observations that isolated bovine brain synaptic vesicles contain ~1 kinesin molecule for every 16 isolated bovine brain synaptic vesicles (Leopold et al., 1992) or 1 kinesin per 100-nm chick brain microsomes (Toyoshima et al., 1992). This low density of motors per membrane may also explain our inability to consistently immunoisolate Golgi membranes with dynein or myosin-I antibodies. Moreover, in vitro motility studies have shown that only 1 kinesin molecule is sufficient to translocate a MT several micrometers (Howard et al., 1989). Thus, only a few at-

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

tached motors per vesicle is probably sufficient for intracellular trafficking.

Although dynein and membranes bind to MTs in an ATP-dependent manner, and kinesin was not detected on Leelavathi and Weiser Golgi membrane immunoblots, it is formally possible that another unidentified MT-based motor or MT-binding protein is present on the membranes. A Golgi MT-binding protein, which is not reported to bind MTs in an ATP-sensitive manner, has been identified in rat liver (Bloom and Brashear, 1989), however, its presence has not been analyzed in the intestinal epithelial cell Golgi. The binding of endocytic vesicles to MTs independent of motor proteins has also been shown (Scheel and Kreis, 1991; Pierre et al., 1992). Methods are not available to directly show that the membrane-bound dynein is responsible for the ATP-sensitive MT binding we observed. For example, there are no dynein antibodies that inhibit the interaction of dynein with MTs. Furthermore, it is unclear what effects UV-vanadate photocleavage has on organellar dynein-MT binding, especially in the presence of other potential motors (Schnapp and Reese, 1989; Schroer et al., 1989). However, preliminary evidence suggests that Weiser Golgi-enriched membranes move on MTs in a minus-end direction in in vitro motility assays (Fath and Burgess, unpublished results). Such a minus-end–directed motility would support a role for dynein in Golgi dynamics although minus-end–directed kinesins such as the *Drosophila* ncd protein have been discovered (Walker et al., 1990).

It has been proposed that one role of dynein in some cells may be to maintain the Golgi apparatus near the minus-ends of MTs and centrosomes (Corthésy-Theulaz et al., 1992). The maintenance of the Golgi apparatus at its supranuclear position requires intact MTs in rat intestinal epithelial cells (Achler et al., 1989). Because the Golgi is found nearer the plus-ends of MTs in enterocytes, as in other simple epithelial cells, by extension it could be suggested that kinesin may serve this role in these cells. However, using the mAb SUK-4 we did not detect kinesin associated with Golgi stacks or Golgi-enriched membranes, nor was kinesin detected on Golgi membranes isolated from bovine brain (Leopold et al., 1992). Kinesin was detected on another, as of yet not well characterized, denser population of membranes from intestinal epithelial cells. It is also possible that a unique kinesin or another MT plus-end–directed protein that is not recognized by SUK-4 antibody is present on Golgi membranes.

As presented in the Introduction, the slowing but incomplete inhibition of apical membrane protein delivery from the Golgi in the apparent absence of MTs and the lack of MTs in the actin-rich cell cortex suggests that a MT-based movement alone cannot account for a movement of membranes from the Golgi apparatus to the plasma membrane. Moreover, since the average pore size of cytoplasm is smaller than vesicles (Luby-Phelps et al., 1987; Provance et al., 1993) passive diffusion cannot account for significant movement of vesicles from the ends of MTs to the apical plasma membrane (Schroer and Sheetz, 1991); some active mechanism must be required. The final 0.5–1 μm to the epithelial cell apical or basolateral plasma membrane must be traversed in the absence of MTs and through an actin meshwork. This meshwork is so highly cross-linked that when the terminal web forms, even organelles as small as ribosomes (~25 nm) are excluded away from the membrane. It has been proposed that an actin-based motor, such as myosin-I, is required to move vesicles through this meshwork to the membrane (Sandoz et al., 1985; Achler et al., 1989; Fath et al., 1990; Fath and Burgess, 1993). We have previously shown that a population of Weiser Golgi-enriched membranes isolated from intestinal epithelial cells binds actin filaments in an ATP-sensitive manner and possesses myosin-I as a cytoplasmically-oriented peripheral membrane protein (Fath and Burgess, 1993) and now show that myosin-I is present throughout the Golgi. Because actin filaments are not abundant in the inner cytoplasm leading from the Golgi complex to the cell apex, we proposed that perhaps individual vesicles possess both a MT-based and an actin filament-based motor and utilize both to traverse from the *trans*-Golgi to the apical plasma membrane (Fath and Burgess, 1993). Thus, our isolation of membranes possessing both cytoplasmic dynein and myosin-I and the finding of membranes that bind both actin filaments and MTs is consistent with this proposal.

Several other reports suggest that Golgi-derived vesicle movement occurs via both MT- and actin-based motors working in series. Kuznetsov et al. (1992) using video enhanced and fluorescence microscopy, demonstrated that single axonal organelles that were translocating along MTs also moved along what were likely actin filaments. In yeast, cells with a mutant Myo2p (an unconventional myosin) do not form buds, have a disrupted actin cytoskeleton, and have a buildup of cytoplasmic vesicles (Johnston et al., 1991). The overexpression of the kinesin-like presumed motor Smylp can suppress Myo2p defects (Lillie and Brown, 1992). Therefore, in yeast, there is the possibility of a functional redundancy in use of both microtubule and actin based motors. Likewise, in polarized epithelial cells the decreased rate, but incomplete inhibition of membrane delivery to the apical surface in the presence of MT-disruptive drugs, suggests that either some stable MTs remain intact, or that MTs are necessary only to provide directionality while another transport system is utilized. Alternatively, the MT disruption studies may reveal a functionally redundant alternative mechanism, which is normally hidden or little used in the cell interior. Another motor, perhaps attached to the same vesicle may serve as a backup to MT-based movements in the inner cytoplasm. Thus, unconventional myosin's normal role in polarized epithelia would be the final stages of membrane translocation through the actin-rich cell cortex which would be hierarchically arranged in function with a MT motor. Our results confirm and extend these reports by providing the initial biochemical observation that Golgi membranes express both myosin-I and cytoplasmic dynein. These data provide a foundation for further experiments designed to identify the in vivo role of these motors and how attachment of motors to membranes is regulated. Further, these results suggest that the type of mechanochemical motor placed on membrane organelles, or motor regulation in different areas of the cell, may provide a mechanism for polarized delivery of membranes in cells as diverse as neurons and epithelial cells.

We thank Dr. David Hackney (Carnegie-Mellon University, Pittsburgh, PA) for providing purified tubulin and taxol; Drs. M. Sheetz (Duke University, Durham, NC) and T. Schroer (Johns Hopkins University, Baltimore, MD) for cytoplasmic dynein and dynactin mAbs; Dr. K. Pfister (University of Virginia, Charlottesville, VA) for dynein antibody mAb 74.1; Dr. C.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

Walsh (University of Pittsburgh, Pittsburgh, PA) for α-tubulin mAb, Dr. K. Howell (University of Colorado, Boulder, CO) for TGN3841 antisera; Dr. M. Farquhar (University of California, San Diego, CA) for α-mannosidase-II antisera; Dr. H. Joshi (Emory University, Atlanta, GA) for γ-tubulin antisera; and Dr. T. Kreis (University of Geneva, Geneva, Switzerland) for the β-COP antisera (mAb M3A5). The kinesin mAb was from Dr. J. Scholey (University of California, Davis, CA) and the Developmental Studies Hybridoma Bank (under contract N01-HD-6-2915 from the National Institute of Child Health and Human Development). We also thank Ms. Lisa Lemmler and Ms. Vicki Malley for excellent technical support; Salim Mamajiwalla for subcellular fractionation and Tom Harper for assistance with EM.

This work was supported in part by National Institutes of Health grant DK 31643.

Received for publication 11 March 1994 and in revised form 24 May 1994.

References

Achler, C., D. Filmer, C. Merte, and D. Drenckhahn. 1989. Role of microtubules in polarized delivery of apical membrane proteins to the brush border of the intestinal epithelium. *J. Cell Biol.* 109:179–189.

Allan, V. J., and T. E. Kreis. 1986. A microtubule-binding protein associated with membranes of the Golgi apparatus. *J. Cell Biol.* 103:2229–2239.

Aniento, F., N. Emans, G. Griffiths, and J. Gruenberg. 1993. Cytoplasmic dynein-dependent vesicular transport from early to late endosomes. *J. Cell Biol.* 123:1373–1387.

Aoki, D., H. E. Appert, D. Johnson, S. S. Wong, and M. N. Fukuda. 1990. Analysis of the substrate binding sites of human galactosyltransferase by protein engineering. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3171–3178.

Bacallao, R., C. Antony, C. Dotti, E. Karsenti, E. H. K. Stelzer, and K. Simons. 1989. The subcellular organization of Madin-Darby Canine Kidney cells during the formation of a polarized epithelium. *J. Cell Biol.* 109: 2817–2832.

Bearer, E. L., J. A. DeGiorgis, R. A. Bodner, A. W. Kao, and T. S. Reese. 1993. Evidence for myosin motors on organelles in squid axoplasm. *Proc. Natl. Acad. Sci. USA.* 90:11252–11256.

Bennett, G., E. Carlet, G. Wild, and S. Parsons. 1984. Influence of colchicine and vinblastine on the intracellular migration of secretory and membrane glycoproteins: III. Inhibition of intracellular migration of membrane glycoproteins in rat intestinal columnar cells and hepatocytes as visualized by light and electron-microscope radioautography after ^{3}H-fucose injection. *Am. J. Anat.* 170:545–566.

Bloom, G. S. 1992. Motor proteins for cytoplasmic microtubules. *Curr. Opin. Cell Biol.* 4:66–73.

Bloom, G. S., and T. A. Brashear. 1989. A novel 58-kDa protein associates with the Golgi apparatus and microtubules. *J. Biol. Chem.* 264:16083–16092.

Bomsel, M., R. Parton, S. A. Kuznetsov, T. A. Schroer, and J. Gruenberg. 1990. Microtubule- and motor dependent fusion in vitro between apical and basolateral endocytic vesicles from MDCK cells. *Cell.* 62:719–731.

Breitfeld, P. P., W. C. McKinnon, and K. E. Mostov. 1990. Effect of nocodazole on vesicular traffic to the apical and basolateral surfaces of polarized MDCK cells. *J. Cell Biol.* 111:2365–2373.

Burgess, D. R. 1987. The brush border: a model for structure, biochemistry, motility, and assembly of the cytoskeleton. *In* Advances in Cell Biology. Kenneth Miller, editor. Jai Press, Greenwich, CT. 31–58.

Burgess, D. R., and B. E. Prum. 1982. Re-evaluation of brush border motility: calcium induces core filament solation and microvillar vesiculation. *J. Cell Biol.* 94:97–107.

Cheney, R. E., and M. S. Mooseker, 1992. Unconventional myosins. *Curr. Opin. Cell Biol.* 4:27–35.

Coffe, G., and M. Raymond. 1990. Association between microtubules and Golgi vesicles isolated from rat parotid glands. *Biol. Cell (Paris).* 70: 143–152.

Collins, K., J. R. Sellers, and P. Matsudaira. 1990. Calmodulin dissociation regulates brush border myosin I (110-kD-calmodulin) mechanochemical activity in vitro. *J. Cell Biol.* 110:1137–1147.

Conzelman, K. A., and M. S. Mooseker. 1987. The 110-kD protein-calmodulin complex of the intestinal microvillus is an actin-activated MgATPase. *J. Cell Biol.* 105:313–324.

Cooper, M. S., A. H. Cornell-Bell, A. Chernjavsky, J. W. Dani, and S. J. Smith. 1990. Tubulovesicular processes emerge from *trans*-Golgi cisternae, extend along microtubules, and interlink adjacent *trans*-Golgi elements into a reticulum. *Cell.* 61:135–145.

Corthésy-Theulaz, I., A. Pauloin, and S. Pfeffer. 1992. Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. *J. Cell Biol.* 118:1333–1345.

Drenckhahn, D., and R. Dermietzel. 1988. Organization of the actin filament cytoskeleton in the intestinal brush border: a quantitative and qualitative immunoelectron microscope study. *J. Cell Biol.* 107:1037–1048.

Euteneur, U., M. P. Koonce, K. K. Pfister, and M. Schliwa. 1988. An ATPase with properties expected for the organelle motor of the giant amoeba, *Reticulomyxa. Nature (Lond.).* 332:176–178.

Fath, K. R., and D. R. Burgess. 1993. Golgi-derived vesicles from developing epithelial cells bind actin filaments and possess myosin-I as a cytoplasmically oriented peripheral membrane protein. *J. Cell Biol.* 120:117–127.

Fath, K. R., and D. R. Burgess. 1994. Membrane motility mediated by unconventional myosin. *Curr. Opin. Cell Biol.* 6:131–135.

Fath, K. R., S. D. Obenauf, and D. R. Burgess. 1990. Cytoskeletal protein and mRNA accumulation during brush border formation in adult chicken enterocytes. *Development (Camb.).* 109:449–459.

Fath, K. R., S. N. Mamajiwalla, and D. R. Burgess. 1993. The cytoskeleton in development of epithelial cell polarity. *J. Cell Sci. Suppl.* 17:65–73.

Fujiki, Y., A. L. Hubbard, S. Fowler, and P. B. Lazarow. 1982. Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. *J. Cell Biol.* 93:97–102.

Gibbons, I. R., A. Lee-Eiford, G. Mocz, C. A. Phillipson, W.-J. Y. Tang, and B. H. Gibbons. 1987. Photosensitized cleavage of dynein heavy chains. *J. Biol. Chem.* 262:2780–2786.

Gilbert, T., A. Le Bivic, A. Quaroni, and E. Rodriguez-Boulan. 1991. Microtubular organization and its involvement in the biogenetic pathways of plasma membrane proteins in Caco-2 intestinal epithelial cells. *J. Cell Biol.* 113:275–288.

Gill, S. R., T. A. Schroer, I. Szilak, E. R. Steuer, M. P. Sheetz, and D. W. Cleveland. 1991. Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J. Cell Biol.* 115:1639–1650.

Goltz, J. S., A. W. Wolkoff, P. M. Novikoff, R. J. Stockert, and P. Satir. 1992. A role for microtubules in sorting endocytic vesicles in rat hepatocytes. *Proc. Natl. Acad. Sci. USA.* 89:7026–7030.

Hirokawa, N., R. Sato-Yoshitake, T. Yoshida, and T. Kawashima. 1990. Brain dynein (MAP1C) localizes on both anterogradely and retrogradely transported membranous organelles in vivo. *J. Cell Biol.* 111:1027–1037.

Holzbaur, E. L. F., J. A. Hammarback, B. M. Paschal, N. G. Kravit, K. K. Pfister, and R. B. Vallee. 1991. Homology of a 150K cytoplasmic dynein-associated polypeptide with the *Drosophila* gene *Glued. Nature (Lond.).* 351:579–583.

Howard, J., A. J. Hudspeth, and R. D. Vale. 1989. Movement of microtubules by single kinesin molecules. *Nature (Lond.).* 342:154–158.

Hugon, J. S., G. Bennett, P. Pothier, and Z. Ngoma. 1987. Loss of microtubules and alteration of glycoprotein migration in organ cultures of mouse intestine exposed to nocadazole or colchicine. *Cell Tissue Res.* 248:653–662.

Hunziker, W., P. Male, and I. Mellman. 1990. Differential microtubule requirements for transcytosis in MDCK cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3515–3525.

Ingold, A. L., S. A. Cohn, and J. M. Scholey. 1988. Inhibition of kinesin-driven microtubule motility by monoclonal antibodies to kinesin heavy chains. *J. Cell Biol.* 107:2657–2667.

Johnston, G. C., J. A. Prendergast, and R. A. Singer. 1991. The *Saccharomyces cerevisiae* MYO2 gene encodes an essential myosin for vectorial transport of vesicles. *J. Cell Biol.* 113:539–551.

Jones, S. M., J. R. Crosby, J. Salamero, and K. E. Howell. 1993. A cytosolic complex of p62 and rab6 associates with TGN38/41 and is involved in budding of exocytic vesicles from the *trans*-Golgi network. *J. Cell Biol.* 122:775–788.

Joshi, H. C. 1993. γ-tubulin: the hub of cellular microtubule assemblies. *Bioessays.* 15:637–643.

Kuznetsov, S. A., G. M. Langford, and D. G. Weiss, 1992. Actin-dependent organelle movement in squid axoplasm. *Nature (Lond.).* 356:722–725.

Lacey, M. L., and L. T. Haimo. 1992. Cytoplasmic dynein is a vesicle protein. *J. Biol. Chem.* 267:4793–4798.

Leelavathi, D. E., L. W. Estes, D. S. Feingold, and B. Lombardi. 1970. Isolation of a Golgi-rich fraction from rat liver. *Biochim. Biophys. Acta.* 211:124–138.

LeGendre, N., and P. T. Matsudaira. 1989. Purification of proteins and peptides by SDS-PAGE. *In* A practical guide to protein and peptide purification for microsequencing. P. T. Matsudaira, editor. Academic Press, Inc., San Diego, CA. 49–69.

Leopold, P. L., A. W. McDowall, K. K. Pfister, G. S. Bloom, and S. T. Brady. 1992. Association of kinesin with characterized membrane-bounded organelles. *Cell Motil. Cytoskeleton.* 23:19–33.

Lillie, S. H., and S. S. Brown. 1992. Suppression of a myosin defect by a kinesin-related gene. *Nature (Lond.).* 356:358–361.

Lin, S. X. H., and C. A. Collins. 1992. Immunolocalization of cytoplasmic dynein to lysosomes in cultured cells. *J. Cell Sci.* 101:125–137.

Lippincott-Schwartz, J. 1993. Bidirectional membrane traffic between the endoplasmic reticulum and Golgi apparatus. *Trends Cell Biol.* 3:81–88.

Louvard, D. 1989. The function of the major cytoskeletal components of the brush border. *Curr. Opin. Cell Biol.* 1:51–57.

Louvard, D., M. Kedinger, and H. P. Hauri. 1992. The differentiating intestinal epithelial cell: establishment and maintenance of functions through interactions between cellular structures. *In* Annual Review of Cell Biology. G. Palade, editor. Annual Reviews, Inc., Palo Alto, CA. 157–195.

Luby-Phelps, K., P. E. Castle, L. Taylor, and F. Lanni. 1987. Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. *Proc.*

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022

Natl. Acad. Sci. USA. 84:4910–4913.
Luzio, J. P., B. Brake, G. Banting, K. E. Howell, P. Braghetta, and K. K. Stanley. 1990. TGN 38: identification, sequencing and expression of an integral membrane protein of the trans-Golgi network. *Biochem. J.* 270:97–102.
Matsudaira, P. T., and D. R. Burgess. 1978. SDS microslab linear gradient polyacrylamide gel electrophoresis. *Anal. Biochem.* 87:386–396.
Matsudaira, P. T., and D. R. Burgess. 1979. Identification and organization of the components in the isolated microvillus cytoskeleton. *J. Cell Biol.* 83:667–673.
Matsudaira, P. T., and D. R. Burgess. 1982. Organization of the cross-filaments in intestinal microvilli. *J. Cell Biol.* 92:657–664.
Mays, R. W., Beck, K. A., and Nelson, J. W. 1994. Organization and function of the cytoskeleton in polarized epithelial cells: a component of the protein sorting machinery. *Curr. Opin. Cell Biol.* 6:16–24.
Mooseker, M. S. 1985. Organization, chemistry and assembly of the cytoskeletal apparatus of the intestinal brush border. *Annu. Rev. Cell Biol.* 1:209–241.
Olmstead, J. B. 1981. Affinity purification of antibodies from diazotized paper blots of heterogenous protein samples. *J. Biol. Chem.* 256:11955–11957.
Parczyk, K., W. Haase, and C. Kondor-Koch. 1989. Microtubules are involved in the secretion of proteins at the apical cell surface of the polarized epithelial cell, Madin-Darby Kidney. *J. Biol. Chem.* 264:16837–16846.
Paschal, B. M., H. S. Shpetner, and R. B. Vallee. 1987. Map 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties. *Nature (Lond.).* 105:1273–1282.
Pierre, P., J. Scheel, J. E. Rickard, and T. E. Kreis. 1992. CLIP-170 links endocytic vesicles to microtubules. *Cell.* 70:887–900.
Pollard, T. D., S. K. Doberstein, and H. G. Zot. 1991. Myosin-I. *In* Annual Review of Physiology. J. F. Hoffman, editor. Annual Reviews Inc., Palo Alto, CA. 653–681.
Provance, D. W., A. McDowall, M. Marko, and K. Luby-Phelps. 1993. Cytoarchitecture of size-excluding compartments in living cells. *J. Cell Sci.* 106:565–578.
Rizzolo, L. J., and H. C. Joshi. 1993. Apical orientation of the microtubule organizing center and associated γ-tubulin during the polarization of the retinal pigment epithelium *in vivo*. *Dev. Biol.* 157:147–156.
Roth, J., M. J. Lentze, and E. G. Berger. 1985. Immunocytochemical demonstration of ecto-galactosyltransferase in absorptive intestinal cells. *J. Cell Biol.* 100:118–125.
Sandoz, D., M.-C. Lainé, and G. Nicolas. 1985. Distribution of microtubules within the intestinal terminal web as revealed by quick-freezing and cryosubstitution. *Eur. J. Cell Biol.* 39:481–484.
Scheel, J., and T. E. Kreis. 1991. Motor protein independent binding of endocytic carrier vesicles to microtubules *in vitro*. *J. Biol. Chem.* 266:18141–18148.
Schnapp, B. J., and T. S. Reese. 1989. Dynein is the motor for retrograde axonal transport of organelles. *Proc. Natl. Acad. Sci. USA.* 86:1548–1552.
Schroer, T. A., and M. P. Sheetz. 1991. Functions of microtubule-based motors. *In* Annu. Rev. Physiol. Joseph F. Hoffman, editor. Annual Reviews, Inc., 629–652.
Schroer, T. A., E. R. Steuer, and M. P. Sheetz. 1989. Cytoplasmic dynein is a minus end-directed motor for membranous organelles. *Cell.* 56:937–946.
Shibayama, T., J. M. Carboni, and M. S. Mooseker. 1987. Assembly of the intestinal brush border: appearance and redistribution of microvillar core proteins in developing chick enterocytes. *J. Cell Biol.* 105:335–344.
Skoufias, D. A., and J. M. Scholey. 1993. Cytoplasmic microtubule-based motor proteins. *Curr. Opin. Cell Biol.* 5:95–104.
Skoufias, D. A., D. G. Cole, K. P. Wedaman, and J. M. Scholey. 1994. The carboxyl-terminal domain of kinesin heavy chain is important for membrane binding. *J. Biol. Chem.* 269:1477–1485.
Steuer, E. R., L. Wordeman, T. A. Schroer, and M. P. Sheetz. 1990. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature (Lond.).* 345:266–268.
Swanson, J. A., A. Locke, P. Ansel, and P. J. Hollenbeck. 1992. Radial movement of lysosomes along microtubules in permeabilized macrophages. *J. Cell Sci.* 103:201–209.
Tartakoff, A. M., and P. Vassalli. 1983. Lectin-binding sites as markers of Golgi subcompartments: proximal-to-distal maturation of oligosaccharides. *J. Cell Biol.* 97:1243–1248.
Titus, M. A. 1993. Myosins. *Curr. Opin. Cell Biol.* 5:77–81.
Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.
Toyoshima, I., H. Yu, E. R. Steuer, and M. P. Sheetz. 1992. Kinectin, a major kinesin-binding protein on ER. *J. Cell Biol.* 118:1121–1131.
van der Sluijs, P., M. K. Bennett, C. Antony, K. Simons, and T. E. Kreis. 1990. Binding of exocytic vesicles from MDCK cells to microtubules *in vitro*. *J. Cell Sci.* 95:545–553.
Velasco, A., L. Hendricks, K. W. Moremen, D. R. P. Tulsiani, O. Touster, and M. G. Farquhar. 1993. Cell type-dependent variations in the subcellular distribution of α-mannosidase I and II. *J. Cell Biol.* 122:39–51.
Walker, R. A., E. D. Salmon, and S. A. Endow. 1990. The *Drosophila claret* segregation protein is a minus-end directed motor molecule. *Nature (Lond.).* 347:780–782.
Walsh, C. 1984. Synthesis and assembly of the cytoskeleton of *Naegleria gruberi* flagellates. *J. Cell Biol.* 98:449–456.
Weiser, M. M., M. M. Neumeier, A. Quaroni, and K. Kirsch. 1978. Synthesis of plasmalemmal glycoproteins in intestinal epithelial cells. Separation of Golgi membranes from villus and crypt cell surface membranes; glycosyltransferase activity of surface membrane. *J. Cell Biol.* 77:722–734.
Yu, H., I. Toyoshima, E. R. Steuer, and M. P. Sheetz. 1992. Kinesin and cytoplasmic dynein binding to brain microsomes. *J. Biol. Chem.* 267:20457–20464.

Downloaded from http://rupress.org/jcb/article-pdf/126/3/661/1261603/661.pdf by guest on 25 August 2022