The Actin-binding Protein Comitin (p24) Is a Component of the Golgi Apparatus

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Abstract. Comitin (p24) was first identified in Dictyostelium discoideum as a membrane-associated protein which binds in gel overlay assays to G and F actin. To analyze its actin-binding properties we used purified, bacterially expressed comitin and found that it binds to F actin in spin down experiments and increases the viscosity of F actin solutions even under high-salt conditions. Immunofluorescence studies, cell fractionation experiments and EM studies of vesicles precipitated with comitin-specific monoclonal antibodies showed that comitin was present in D. discoideum on: (a) a perinuclear structure with tubular or fibrillar extensions; and (b) on vesicles distributed throughout the cell. In immunofluorescence experiments using comitin antibodies NIH 3T3 fibroblasts showed a similar staining pattern as D. discoideum cells. Using bona fide Golgi markers the perinuclear structure was identified as the Golgi apparatus. The results were supported by an electron microscopic study using cryosections. Based on these data we propose that also in Dictyostelium the stained perinuclear structure is the Golgi apparatus. In vivo the perinuclear structure was found to be attached to the actin and the microtubule network. Alteration of the actin network or depolymerization of the microtubules led to its dispersal into vesicles distributed throughout the cell. These results suggest that the Golgi apparatus in D. discoideum is connected to the actin network by comitin. This protein seems also to be present in mammalian cells.

Microtubules play a central role in rapid organelle movement in animal cells (Allen et al., 1982; 1985; Weiss et al., 1988), in vesicle-mediated transport from Golgi to ER (Lippincott-Schwartz et al., 1990) and in intercompartmental transport. In addition, microtubules are involved in maintaining the Golgi structure since disruption of the microtubule network during mitosis or drug-induced disassembly leads to fragmentation and scattering of Golgi fragments throughout the cell (for an overview see Kreis, 1990). Reassembly of the microtubule network results in reaggregation of the Golgi cisternae at the microtubule organizing center (MTOC). During this process the Golgi elements move along microtubules; neither intermediate filaments nor microfilaments appear to be involved in the reassembly (Ho et al., 1989).

There are, however, reports that the actin-based microfilament system is involved in intracellular vesicle transport. For example, microinjection of actin-binding proteins, such as DNase I, gelsolin, and synapsin I into the axonal cytoplasm inhibits the fast axonal transport (Goldberg et al., 1980; Isenberg et al., 1980; Brady et al., 1984; McGuinness et al., 1989). Furthermore, latex beads coated with myosin move on actin bundles of the alga Chara (Shimmen and Yano, 1984) and on reconstituted actin filaments (Spudich et al., 1985). Kuznetsov et al. (1992) studied the direct movement of vesicles in the squid axoplasm by AVEC-DIC microscopy and demonstrated that vesicles move on microtubules, detach, and continue moving on actin tracks. The observed actin-based movement was found to be much slower than the microtubule-based movement and only unidirectional. These observations indicate that actin- and microtubule-based transport systems function in parallel.

In contrast to the mammalian Golgi apparatus, very little is known about the Golgi structure and the vesicle-mediated transport in Dictyostelium discoideum. In this thin section EM Roos (1982) identified membrane cisternae surrounding the MTOC and Schwarz (1973) could show that the perinuclear Dictyostelium Golgi apparatus contains one to three dictyosomes, consisting of three to nine cisternae approximately 1.2 μm in length.

In a search for proteins that might be able to connect vesicles with cytoskeletal components, we studied p24, a membrane-associated actin-binding protein in D. discoideum (Stratford and Brown, 1985; Noegel et al., 1990). This protein consists of two domains, an NH2-terminal core domain, comprising 144 amino acids and a COOH-terminal...
domain of 41 amino acids characterized by six repeats of a
GYPPQG motif in a tandem array. A similar motif is present
in synexin (Döring et al., 1991; Greenwood and Tsang,
1991), synaptophysin, Octopus rhodopsin, and a number of
other proteins (Noegel et al., 1990; Matsushima et al.,
1990). Based on the absence of hydrophobic stretches long
enough to span a lipid bilayer as well as the absence of a sig-
nal sequence, p24 is not expected to be a transmembrane
protein (Noegel et al., 1990).

In the present study we have prepared mAbs directed
against the unique core domain and show that p24 is present
on the surface of vesicles and on a perinuclear structure
which appears to be the Dictyostelium Golgi apparatus. As
judged from the labeling of the Golgi apparatus and vesicles
exclusion chromatography column (Sephacryl-S200, Pharmacia, Freiburg,
buffer (MES; pH 6.25, 150 mM KCl, 2 mM EDTA, 1 mM DTT).
lar, 1990). The reaction mix (150/d) contained usual-ly
~shear viscometry was carried out after a 20-min incubation of comitin
expression was induced by a heat shock for 30 rain at 42°C and the bacterial
cells. Fixed cells were washed twice for 5 min each in PBS, twice for 15
min each in PBG (0.5% BSA, 0.2% gelatine in PBS) incubated overnight
with anti-comitin antibody, anti-sea urchin tubulin antibody, ESA3 (Dudun,
et al., 1991), polyclonal anti-p58 serum (Lahitten et al., 1992), CRTR433
(Jassin et al., 1989), anti-MPR300 (Kornfeld and Mellman, 1989) at 50
mM KCl, ACI-M11 (Robinson, 1987), 173-185-1 (Schopohl et al., 1992)
at 50
mM KCl, washed six times with PBG and incubated for 1 h with 1:200 diluted
FITC (Cappel Laboratories, Cochranville, PA) or 1:2,000 diluted Cy3
(Jackson Immunoresearch Laboratories Inc., Avonduie, PA) conjugated,
affinity-purified goat anti-mouse IgG or anti-rabbit IgG antibodies and 4,6-
diamidino-2-phenylindole at 1:1000 (DAPI stock; 1 mg/ml in 70% ethanol;
Sigma). After washing twice with PBG and PBS, the cells were embedded
in gelvatol (Langanger et al., 1983) and examined with a Zeiss Axiopt
fluorescence microscope (Carl Zeiss, Oberkochen, Germany). Labeling
of NIH 3T3 fibroblast endocytic compartments was performed according to
Swanson (1989) using fluorescein-labeled ovalbumin and transferrin (Mo-
ular Probes, Eugene, OR; 1 mg/ml in DME, 1-h incubation) except that
the cells were preincubated for 30 min with unlabeled ovalbumin (1 mg/ml).
Incubation of NIH 3T3-fibroblasts in brefeldin A (Sigma) (5 /tg/ml in DME)
or nocodazole (Sigma) (50 /tg/ml in DME) was performed for 60 min at 37°C.

Cryomicrotomy

NRK cells were removed with 20 mg/ml proteinase K, centrifuged, and fixed
with 8% paraformaldehyde overnight. Cryosections were labeled with either
anti-comitin or with an irrelevant primary antibody (anti-BSV-G) fol-
lowed by protein A gold. The method is as described by Griffiths et al.
(1984) and Griffiths (1993).

Cell Fractionation and Vesicle Isolation

Cell fractionation was performed according to Hohmann et al. (1985). D.
discoideum cells were grown at 21°C to ~5 x 10^6 cells/ml in liquid nutri-
tient medium, harvested, and washed in 17 mM Soerensen phosphate buffer
(pH 7.0). The cells were resuspended to a density of 3.5 x 10^7 cells/ml in
Hepes-DTT buffer (10 mM Hepes/NaOH, pH 7.4, 1 mM DTT, 0.5 mM
PMRF, 2 mM benzamidine) and lysed in a Parr bomb by nitrogen cavitation
every 10 min for 1000 psi. Microscopic examination re-
vealed that no intact cells in the lysate existed. The lysate was centrifuged
for 20 min at 10,000 g and the comitin-containing pellet homogenized in
Hepes-DTT buffer. For centrifugation in a discontinuous sucrose gradient,
8 ml of the homogenized pellet (equivalent of 2.5 x 10^7 cells) was layered
onto a sucrose gradient consisting from bottom to top of five 6 ml layers of
1.45, 1.32, 1.17, 1.02, 0.88 M sucrose in Hepes-DTT buffer. After cen-
trifugation for 18 h at 25,000 rpm in a rotor (TST-26; Kontron Analytical,
Redwood City, CA), 11 fractions were collected from the top to the bottom

Low-shear Viscometry and Low-speed Sedimentation Assays

Low-shear viscometry was carried out after a 20-min incubation of comitin
and actin at 25°C in a falling ball viscometer (MacLean-Fletcher and Pol-
land, 1980). The reaction mix (150 µl) contained usually 6 mM rabbit mus-

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Figure 1. Binding of comitin to F-actin. Skeletal muscle actin (6 μmol) was polymerized with increasing amounts of purified, recombinant comitin, and analyzed by low speed sedimentation assays (A) and low-shear viscometry (B). For low-speed sedimentation assays the mixtures were centrifuged at 16,000 g. Supernatants and pellets were resuspended to the original volume, separated by SDS-PAGE (15% acrylamide), and analyzed by densitometry. The amount of pelleted actin relative to the total actin content (100%) is shown in A. Standard deviations are given.

Figure 2. Effect of high salt or H+ concentration on binding of comitin to F-actin. Skeletal muscle actin (6 μmol) was polymerized in the absence (−) or presence (+) of 0.58 nmol (A) or 0.84 nmol (B) purified recombinant comitin. The effect of high salt (A) or pH (B) was analyzed by adding 1 M KCl or 100 mM imidazol (pH 6, 7, or 8) until a KCl concentration of 120, 200 mM or a pH of 6, 7, and 8 was reached. The mixtures were centrifuged at 16,000 g. Supernatants and pellets were resuspended to the original volume, separated by SDS-PAGE (15% acrylamide), and analyzed by densitometry. The amount of pelleted actin relative to the total actin content (100%) is shown. Standard deviations are given.

Cytoskeleton Analysis

Cytoskeleton analysis was essentially performed according to Payrastre et al. (1991). All steps were carried out at 4°C. Cytoskeletons were prepared by resuspending 5 × 10⁷ cells in 10 ml lysis buffer (1% Triton X-100, Pierce Chemical Co., Rockford, IL, 10 mM imidazole/NaOH, pH 7.0, 10 mM KCl, 10 mM EGTA, 2 mM NaN₃, 0.5 mM PMSF, 2 mM benzamidine, 100 μM sodium orthovanadate). After 20 min gentle shaking the cytoskeletons were sedimented at 20,000 g for 20 min and washed two times with 10 ml lysis buffer without Triton. Washed cytoskeletons were solubilized in 1 ml actin depolymerization buffer (0.6 M...
Figure 3. Localization of comitin in D. discoideum strain AX2. Axenically grown AX2 cells were stained with mouse mAb 190-340-8 against comitin (red, A-D). (A) Shows the phase contrast image, (B) comitin labeling, (C) double labeling with DAPI (blue) and (D) double labeling with rabbit antiserum against sea urchin tubulin (green) indicating the colocalization of the perinuclear comitin staining zone and the MTOC. Bar, 5 μm.

Kl, 100 mM Pipes, pH 6.5, 0.5 mM PMSF, 2 mM benzamidine, 100 μM sodium orthovanadate) for 20 min under gentle shaking and centrifuged for 20 min at 20,000 g. The actin-depleted pellet was incubated again with 1 ml actin depolymerization buffer and centrifuged. Both supernatants were pooled, dialyzed for 15 h against actin polymerization buffer (10 mM Pipes, pH 6.8, 1 mM EGTA, 2 mM MgCl2, 0.5 mM PMSF, 2 mM benzamidine, 100 μM sodium orthovanadate), and centrifuged for 20 min at 20,000 g. The actin pellet thus obtained was washed with actin polymerization buffer and, like all other pellets and supernatants adjusted with TEDABP to a final volume of 10 ml. For comparison, the first 20,000 g supernatant was centrifuged at 100,000 g for 1 h and the obtained pellet homogenized in 10 ml TEDABP. Equal volumes of all supernatant and pellet fractions were analyzed by SDS-PAGE or Western blot analysis using comitin- and cap34-specific mAbs (Hartmann et al., 1989).

For immunochemistry studies the cells were placed on a coverslip, allowed to adhere for 20 min and incubated for 20 min with lysis buffer at room temperature or 4°C. The attached cytoskeletons were either unfixed or fixed with methanol and processed as described for immunofluorescence studies.

Golgi Fragmentation in D. discoideum by DMSO and Cold Treatment

D. discoideum cells were placed on a coverslip and allowed to adhere for 20 min. For disruption of the actin network the attached cells were incubated for 0, 5, 10, 20, 45 min in 5% DMSO in Soerensen phosphate buffer (pH 6.0) at room temperature (Yumura and Fukui, 1983). For disruption of the microtubule network the attached cells were transferred to a cold chamber where the cells were incubated at a temperature between 0-0.3°C for 1 h in phosphate buffer. All cells were immediately fixed in methanol and analyzed by immunofluorescence.

Miscellaneous Methods

SDS-PAGE was performed according to Laemmli (1970), and immunoblotting using the method of Towbin et al. (1979). Protein samples were prepared by incubation in SDS-PAGE sample buffer at 20°C. Protein was determined according to the method of Bradford (1976) using BSA as a standard.

Results

Interaction of Purified Recombinant Comitin with Actin

The comitin cDNA (Noegel et al., 1990) was cloned into the expression vector pT7-7 such that the protein was synthesized without any additional amino acids. The recombinant protein was purified in 6 M guanidinium chloride and the protein carefully renatured by stepwise dialysis against comitin.
reassembly buffer. This material was used to determine the interaction of comitin with actin.

The binding of comitin to F actin filaments was assayed by mixing increasing amounts of comitin with a constant amount of actin and analyzing the mixtures by low-speed sedimentation assays and low-shear viscometry. In low-speed sedimentation assays addition of increasing amounts of comitin led to an almost complete sedimentation of actin whereas in the absence of comitin only ~4% of total F actin was pelleted (Fig. 1 A). Addition of comitin increased the viscosity of F actin solutions which was due to an increase in the total protein concentration (Fig. 1 B). Both experiments showed that comitin bound to F actin and indicated that it has a bundling or cross-linking activity. The binding was not due to an unspecific ionic interaction between the basic comitin (pI 9.5) and actin, because increasing salt concentrations and different pH values had no effect on comitin–actin binding in spin-down assays. The binding of comitin to F actin was reduced under high salt conditions but was still significantly higher than in the control samples; an alteration of the pH between 6 and 8 had almost no effect (Fig. 2).

Cellular Distribution of Comitin and Presence in Mammalian Cells

In an attempt to elucidate the cellular distribution of comitin, mAbs were prepared against the core region of comitin. Previously isolated antibodies against the complete protein could not be used for immunofluorescence localizations of comitin because they were all directed against the GYPPQ tail of comitin and recognized several proteins in D. discoideum containing similar or related GYPPQ motifs, like the one in synexin (Döring et al., 1991).

Distribution of Comitin in D. discoideum

Immunofluorescence studies using comitin core-specific mAbs gave a distinct staining pattern in D. discoideum cells (Fig. 3). A spotlike or vesicular staining was observed which was distributed all over the cell. In addition, the antibodies recognized a structure close to the nucleus. The shape of this
central body varied between round, oval or Y shaped and had lateral extensions. Double-staining experiments using comitin-specific antibodies and DAPI, a fluorescent dye which binds to DNA, showed that the central body was located in the perinuclear region (Fig. 3 C). In the case of Y-shaped structures the stained material formed a cap around the nucleus. In uninucleate cells one perinuclear body was seen, in multinucleate cells each nucleus carried one perinuclear body. These bodies were sometimes interconnected by long, thin fiber- or tubule-like extensions. In double-staining experiments with anti-comitin and anti-tubulin antibodies the MTOC was localized within the much larger, comitin-stained perinuclear structure in a peripheral position (Fig. 3 D). The comitin-stained vesicles were not lysosomes, since immunofluorescence studies using a lysosome-specific mAb showed that lysosomes were different in size and had a different distribution within the cells (Schopohl et al., 1992). Double-staining with Golgi markers could not be performed since there are no such markers available for D. discoideum.

The comitin distribution was also investigated by cell fractionation experiments. Cells were lysed by nitrogen cavitation in a Parr bomb and centrifuged at 10,000 g. The pellet fraction, which contained all the comitin, was either layered onto a discontinuous sucrose gradient or applied to a sucrose flotation gradient. In both gradients comitin was present in two distinct fractions (data not shown). The LD comitin fraction was in the 0.95–1.32 M sucrose fractions which contained nuclei, nuclei attached structures, and ER membranes (Hohmann et al., 1985; Cardelli, 1987). The presence of comitin in Golgi-containing sucrose fractions, the perinuclear location of the central comitin stained body, its localization close to the MTOC and its morphology strongly indicated that comitin is a component of the Golgi apparatus.

Presence of Comitin on Vesicular Structures

Comitin was initially identified as a membrane-associated protein. Its presence on the surface of D. discoideum membranes was confirmed by transmission EM on native comitin harboring structures. To label and isolate the target structures from comitin-containing sucrose gradient fractions in one step, magnetic iron beads coupled to comitin-specific mAb were sedimented by using a standard ceramic magnet. The antibody-coated beads were decorated with vesicles of diameters between 100 to 500 nm (Fig. 4 B). These structures were not observed in control experiments when beads without antibody were used. The presence of comitin on the vesicles was confirmed by Western blot analysis (Fig. 4 A) and immunogold labeling where up to three gold particles were found on vesicles (Fig. 4 B).

Localization in Mammalian Cells

All antibodies directed towards comitin including a previously isolated one (Noegel et al., 1990) recognized mainly a 17-kD protein in immunoblots of mouse NIH 3T3-fibro-
Figure 7. Colocalization of comitin with Golgi elements in NIH 3T3-fibroblasts. NIH 3T3 cells were double labeled by simultaneous incubations with monoclonal anti-comitin antibodies 190-68-1 (A and D) and a rabbit anti-p58 serum directed against pre- and cis-Golgi elements (B–D). In C the location of the nucleus is indicated by DAPI staining. Anti-p58 was detected with FITC anti-rabbit IgG (green), anti-comitin antibody with TRITC anti-mouse IgG (red). A double exposure is shown in D. The region of overlap is given by the yellow staining. Bar, 10 μm.

blasts (Fig. 5). In immunofluorescence studies a vesicular, spotlike staining distributed all over the cell and a caplike perinuclear structure were observed in 3T3 cells similar to the staining obtained in D. discoideum (Fig. 6, A and B). In some cells a punctate staining of plasma membrane structures was seen. To identify the comitin decorated structures in NIH 3T3 cells the morphology and the cellular distribution of various compartments were determined in immunofluorescence studies with CTR433, an antibody specific for the medium compartment of Golgi apparatus (Jasmin et al., 1989), β-COP-specific E5A3 antibody (Duden et al., 1991), α-adaptin-specific AC1-M11 antibody (Robinson, 1987) and an anti-MPR 300 antibody (Kornfeld and Mellman, 1989) (Fig. 6, C–F). The Golgi apparatus as seen with CTR433 had the same structure and location within the cell as the perinuclear structure stained with the comitin antibody. An overlap between pre- and cis-Golgi elements and the comitin-stained perinuclear structure could be shown in a double-staining experiment with anti-p58 antibodies (Lahtinen et al., 1992) (Fig. 7). This overlap is confined to a distinct region and both antibodies exhibit also a staining of no overlap. More detailed information was obtained by an immunogold EM investigation where the antibody was consistently found to label the cisternae and associated elements of the Golgi apparatus (Fig. 8). Variable amounts of labeling was also found on the cytoplasmic side of rough ER membranes as well as early and late endosomes (results not shown).

Sensitivity of the Comitin-stained Structure to Brefeldin A and Nocodazol

The fungal metabolite brefeldin A is known to lead to a redistribution of resident and itinerant Golgi proteins into the ER and finally to the morphological disappearance of the Golgi complex (for an overview see Klausner et al., 1992). Brefeldin A treatment of NIH 3T3 cells led to reversible disappearance of the comitin-stained perinuclear structure, the spotlike staining was still observed (Fig. 9). The microtubule depolymerizing agent nocodazol causes fragmentation of the Golgi apparatus into distinct elements dispersed throughout the cell (for an overview see Kreis et al., 1990). Similar to BFA, in nocodazol-treated fibroblasts the comitin-stained perinuclear structure disappeared and new spotlike structures appeared with a size larger than the one of stained vesicles in untreated cells (Fig. 9). The p58 distribution was also altered in response to drug treatment. It differed in both instances from the comitin distribution. Double staining however showed that the two antigens co-localized also in the same structure (data not shown).

The Comitin-carrying Structures are Associated with the Cytoskeleton

The linkage of comitin-decorated vesicles and of the Golgi apparatus to actin filaments was investigated by analyzing isolated cytoskeletons, and by determining in vivo the effect of actin disassembly on these structures. The cytoskeleton
analysis was essentially done according to Payrastre et al. (1991) who used this technique to identify microfilament-associated kinases. Cells of *D. discoideum* were incubated for 20 min at 4°C with 1% Triton X-100 (McRobbie and Newell, 1984) to extract lipids and soluble proteins. The remaining material contained actin, actin-associated proteins, remnants of microtubules, and nuclei. Comitin was equally distributed between the insoluble and supernatant fractions (Fig. 10 A). The comitin in the supernatant fraction could be sedimented at 100,000 g, indicating that it was particulate. Immunofluorescence labeling of native or fixed cytoskeletons revealed the cytoskeleton-attached fraction of comitin as spotlike structures (Fig. 10 B).

The nature of the cytoskeleton to which comitin-decorated structures were linked was determined by solubilization of isolated cytoskeletons with potassium iodide (KI). This treatment caused rapid depolymerization of actin filaments. After centrifugation the pellet contained the nuclear fraction, whereas the supernatant was strongly enriched in actin, actin-binding proteins, and comitin as shown by SDS-PAGE and Western blot analysis. Dialysis of the supernatant against actin polymerization buffer resulted in reassembly of actin filaments which could be sedimented again with comitin. This result showed that comitin is an actin-associated protein which links vesicles to microfilaments. Since KI treatment did not dissolve comitin polymers (Weiner, O. H., B. Leiting, M. Schleicher, and A. A. Noegel, manuscript in preparation) the possibility is excluded that the presence of comitin in the actin fractions was due to solubilization and aggregation of comitin in parallel but independent of actin.

**In Vivo Association of the *D. discoideum* Golgi Apparatus with the Actin Network**

Disruption of the actin network in vivo has an effect on the Golgi apparatus. Yumura and Fukui (1983) using transmission EM have observed that treatment of *D. discoideum* cells with 5% DMSO leads to a rapid dissociation of the class I actin network from the plasma membrane and within 5–15 min to the formation of circumferential actin bundles inside...
Figure 9. Localization of comitin in 3T3 fibroblasts after BFA or nocodazol treatment. NIH 3T3 cells were grown on coverslips, treated with DME (A), BFA (B; 5 μg/ml in DME), or nocodazol (C; 50 μM in DME) for 60 min at 37°C. The cells were immediately fixed and labeled with mAb 190-68-1 directed against comitin. Bar, 10 μm.

Figure 10. Association of comitin decorated vesicles with the actin network. (A) Cytoskeletons from D. discoideum AX2 cells were prepared by lysing the cells with 1% Triton X-100 followed by centrifugation (20,000 g). The supernatant was again centrifuged at 100,000 g leading to a supernatant (L-S/S) and pellet (L-S/P) fraction. Washed cytoskeleton pellets (L-P) from centrifugation at 20,000 g were extracted with a 0.6 M KI containing actin depolymerization buffer and again centrifuged (20,000 g) leading to a supernatant fraction (K-I-S) which contained actin, actin-binding proteins and a pellet fraction (K-I-P). Actin was re-polymerized and sedimented at 20,000 g (supernatant, D-S; pellet, D-P). Equal amounts were analyzed by SDS-PAGE (15% acrylamide) and immunoblotting (arrow, comitin; asterisk, cross-reacting proteins). (B) D. discoideum cells were attached to coverslips, incubated for 0–45 min with 5% DMSO and analyzed by immunofluorescence studies using anti-comitin and, as a control, anti-tubulin antibodies (Fig. 11). The DMSO-induced alteration of the microfilament system led to fragmentation of the comitin-associated perinuclear structure within 5 min. After 10 min the structure was completely destroyed and the fragments generated were distributed all over the cell. Then the fragments started to reassemble in the perinuclear region. This reassembly process continued, and after 30 min the comitin-associated perinuclear structure had been completely restored in its original size and location. In the microtubule network no alterations were observed during DMSO treatment.

Discussion

Microtubules are the only cytoskeletal elements for which a relationship to the Golgi complex has been clearly estab-
Figure 11. Disassembly of actin and microtubule networks leads to reversible fragmentation of the *D. discoideum* Golgi apparatus. *D. discoideum* AX2 cells were attached to coverslips, incubated for 0, 5, 10, 20, and 45 min in 5% DMSO, and analyzed by staining with mAb 190-340-8. The last picture in the lower panel (0°) shows cells which were incubated for 1 h at 0°C. Cold treatment led to microtubule disassembly which caused a fragmentation of the Golgi apparatus into vesicles. Bar, 10 μm.

Established. Depolymerization of microtubules by drugs or during mitosis leads to a reversible fragmentation of Golgi stacks and redistribution of Golgi elements throughout the cytoplasm. This argues that microtubules and microtubule-associated proteins play a role in maintaining the organization and location of the Golgi complex. Microtubules are also involved in many trafficking processes, like vesicular transport in the secretory pathway, in endocytosis and in the transport of vesicles between Golgi and ER. The involvement of microtubules in the secretory pathway is however not absolute, since upon depolymerization of microtubules proteins are still secreted but the selective targeting is lost (for review see Kelly, 1990).

Involvement of the microfilament network in the last steps of the secretory cycle in acinar cells of the exocrine pancreas has already been suggested by Bauduin et al. (1975). The observation that vesicle traffic from the TGN network to the basolateral membrane in MDCK and Caco-2 cells is unaffected by microtubule disruption (for an overview see Nelson, 1992) strengthens the hypothesis that the actin system may be involved in this process. The involvement of the actin cytoskeleton in exocytosis appears to be generally accepted (Kelly, 1991). Myosin I molecules can move vesicles along actin cables (Adams and Pollard, 1989), and may also in vivo mediate vesicular transport by the microfilament system (Coudrier et al., 1992).

We have begun to investigate the cellular role of the actin-binding protein comitin (p24) which was isolated in an attempt to identify membrane proteins that bind filamentous actin. Binding to actin has been demonstrated in a gel overlay assay with 125I-labeled cross-linked F actin as a probe and in a two-phase binding assay. In this assay F and G actin partition between dextran and polyethylene glycol into the p24-containing interphase (Stratford and Brown, 1985). Our results show that it is associated with Triton-insoluble cytoskeletons. Binding in vitro to F actin has been confirmed by low-speed sedimentation assays. Low-shear viscometry showed that comitin has a bundling or cross-linking activity. Furthermore the cellular distribution of comitin in *Dictyostelium* cells was altered by DMSO treatment. DMSO has long been known to lead to a reversible rearrangement of actin filaments and has been used to assess the role of microfilaments in mediating motility and maintenance of cell structure (Pollack and Rifkin, 1976; Fukui, 1978; Osborn and Weber, 1980). In *Dictyostelium*, DMSO leads to a dislocation of the cortical microfilaments and their separation from the plasma membrane. These filaments have been designated as class I microfilaments. Class II microfilaments are resistant to dislocation by DMSO and remain at the plasma membrane (Yumura and Fukui, 1983). Redistribution of comitin upon a DMSO treatment indicates its association with the class I F actin pool.

By biochemical and immunofluorescence experiments comitin was identified not only as a microfilament associated protein but also as a Golgi component. The cDNA sequence indicates that comitin has no leader sequence and does not traverse the membrane. It should therefore be present on the cytoplasmic surface of the vesicle or Golgi membranes. It could therefore form a stabilizing scaffold on the cytoplasmic Golgi surface thus linking these structures to the mi-
coat could form a structural framework that ensures the integrity of cellular compartments (Mellman and Simons, 1992). It could also be responsible for maintaining the correct location of the Golgi apparatus (Kreis, 1990).

In addition, the possibility may be discussed that comitin has a role in the regulation of transport processes. Comitin could form a cage or coat as has been observed for clathrin (Pearse, 1975), the COP proteins (Serafini et al., 1991; Dudenh et al., 1991; Waters et al., 1991) and caveolin (Rothberg et al., 1992). Clathrin, the COP proteins and caveolin function as vesicle coat proteins and are presumed to play a crucial role in vesicular transport of membranes and proteins between compartments. These coat proteins mentioned have specific functions and are involved in different transport processes: clathrin-coated vesicles mediate the endocytic uptake from the plasma membrane, COP-coated vesicles mediate the transport between ER and Golgi and between Golgi compartments, and caveolin was found to be present on endocytic vesicles of an alternate uptake pathway for bulk phase and membrane molecules. A prerequisite for vesicle-mediated transport is the regulation of coat formation. Accordingly, clathrin and the components of COP-coated vesicles exist in a membrane bound and a soluble form (Pryer et al., 1992). A soluble form of comitin has not been observed. Nevertheless, this protein may be involved in the regulation of transport. Preliminary results indicate that comitin is phosphorylated both on serine and tyrosine residues.

We are very grateful to Dr. Günther Gerisch for stimulating discussions and critical reading of the manuscript. We further thank Dr. Gerard Marriot, Ulrike Haus, and Dr. Paul Fisher for help with the manuscript and discussions, Regina Brokamp and Daniela Rieger for excellent help during the production of the mAbs, Dr. Michael Meyer for providing mammalian cell lines, Drs. Douglas B. Murphy, John V. Kilmartin, Jaakko Saraste, Thomas Kreis, Georg Batting, Michel Bornens, Kurt von Figura, Margaret S. Robinson, and Dorothy Schopp for providing antibodies, Dr. Wieland B. Huttner for helpful suggestions, and Dr. Murray Stewart for help with the immunogold labeling.

The work was supported by grants from the European Community and the Deutsche Forschungsgemeinschaft to M. Schleicher and A. A. Noegel.

Received for publication 18 February 1993 and in revised form 9 June 1993.

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