In retinal photoreceptors, highly polarized organization of the light-sensitive organelle, the rod outer segment, is maintained by the sorting of rhodopsin and its associated proteins into distinct post-Golgi vesicles that bud from the trans-Golgi network (TGN) and by their vectorial transport toward the rod outer segment. We have developed an assay that reconstitutes the formation of these vesicles in a retinal cell-free system. Vesicle formation in this cell-free assay is ATP-, GTP-, and cytosol-dependent. In frog retinas vesicle budding also proceeds at 0°C, both in vivo and in vitro. Vesicles formed in vitro are indistinguishable from the vesicles formed in vivo by their buoyant density, protein composition, topology, and morphology. In addition to the previously identified G-proteins, these vesicles also contain rab11. Concurrently with vesicle budding, resident proteins are retained in the TGN. Collectively these data suggest that rhodopsin and its associated proteins are sorted upon exit from the TGN in this cell-free system. Removal of membrane-bound GTP-binding proteins of the rab family by rab GDP dissociation inhibitor completely abolishes formation of these vesicles and results in the retention of rhodopsin in the Golgi. A monoclonal antibody to the cytoplasmic (carboxyl-terminal) domain of rhodopsin and its Fab fragments strongly inhibit vesicle formation and arrest newly synthesized rhodopsin in the TGN rather than the Golgi. Therefore rhodopsin sorting at the exit from the TGN is mediated by the interaction of its cytoplasmic domain with the intracellular sorting machinery.

Reconstitution of intracellular sorting events in cell-free systems has significantly contributed to our understanding of the molecular mechanisms that underlie membrane trafficking. Together with genetic studies in yeast and studies of regulated exocytosis of synaptic vesicles, in vitro assays reconstituting intra-Golgi transport have yielded invaluable information about common mechanisms involved in membrane budding and fusion from yeast to mammals (for review see Rothman and Warren (1994) and Rothman (1994)). Several cell-free assays have been employed to reconstitute early steps in exocytosis (endoplasmic reticulum to Golgi and intra-Golgi transport) and endocytosis. A limited number of studies have established in vitro conditions for cell-free post-Golgi vesicle formation from the TGN (de Curtis and Simons, 1989; Tooze and Huttner, 1990; Salamero et al., 1990; Ohashi and Huttner, 1994). However, these events have not been studied in the primary tissues of neuronal origin.

Photoreceptors are excellent models for cell biological studies because continuous ROS membrane renewal by polarized sorting of rhodopsin and its associated proteins on post-Golgi vesicles results in the addition of up to 3 μm/min of ROS membranes (reviewed in Besharse (1986), Simons and Zerial (1993), and Deretic and Papermaster (1995)). Rhodopsin-bearing post-Golgi vesicles isolated from frog retinas can be distinguished from all other subcellular compartments by several criteria, including kinetics of formation, buoyant density, and protein composition (Deretic and Papermaster, 1991). Several proteins of the small G-protein rab family and α-crystallins are vesicle-associated proteins and potential regulators of vesicle budding and sorting (Deretic and Papermaster, 1993a, 1995; Deretic et al., 1994, 1995). However, the mechanism of action of these associated proteins is still elusive in the absence of an assay that allows access to the intracellular compartments involved in vesicle formation.

Inherited retinal diseases in humans and in animal models suggest that some of the mutations in the rhodopsin gene may lead to transport defects that ultimately result in retinal degeneration and blindness (Dryja et al., 1990; Olsson et al., 1992; Roof et al., 1994; Sung et al., 1991, 1994). The rhodopsin residues that are mutated in inherited retinal degenerations are conserved in the frog rhodopsin sequence, consistent with their important contribution to the stability of rhodopsin structure and its function in the photoreceptor cell (Pittler et al., 1992).

In addition, retinal cells also appear to be very sensitive to the defects in isoprenylation of small G-proteins and rab proteins in particular (Seabra et al., 1993; Pittler et al., 1995). Therefore, identification of the proteins that interact with rhodopsin and guide it on its journey to the ROS, as well as the establishment of the role of rab proteins in these processes are of essential importance for the understanding of the establishment and maintenance of photoreceptor polarity and health.

We have developed a cell-free assay that reconstitutes not only rhodopsin transport through the Golgi but also post-Golgi vesicle budding. Due to the complexity of the retinal tissue, biosynthetic organelles of the rod inner segments are accessible only upon removal of the ROS and the neural retina. Using the fractionation technique described before (Deretic and Papermaster, 1995), we have obtained a cell-free assay for primary rhodopsin transport. This assay allows access to the intracellular compartments involved in vesicle formation.
master, 1991), we have generated a frog retinal PNS that is highly enriched in the photoreceptor cell’s biosynthetic membranes. When supplemented with ATP and an exogenous ATP-regenerating system, this PNS is able to support rhodopsin transport and sorting in vitro. We used this cell-free assay to study the factors involved in the biogenesis of rhodopsin-bearing post-Golgi vesicles. We find that vesicle formation depends on the access of cytoplasmic proteins to the carboxy-terminal domain of rhodopsin in the TGN membranes and on the presence of membrane-bound rab proteins.

**MATERIALS AND METHODS**

**Southern leopard frogs**, Rana berlandieri (100–250 g) purchased from the Rana Co. (Brownsville, TX) were maintained in a 12-h light/dark cycle and fed live crickets. [35S]-Express protein labeling mixture (25 and Papermaster 1991, 1993b). Seven retinas were incubated with all experiments were conducted under dim red light.

**Electrophoresis, Immunoblotting, and Detection of the GTP-binding Proteins—SDS-polyacrylamide and two-dimensional gel electrophoresis were performed as described previously (Deretic and Papermaster, 1993a; Deretic et al., 1994, 1995). To quantitate [35S]-labeled rhodopsin in retinal subcellular fractions, dried SDS gels were autoradiographed for various times at −70 °C using Kodak BioMax MR film with intensifying screens. Dried gels were also exposed to storage phosphor screens, and the intensity of luminescence associated with the rhodopsin band was measured and analyzed by a PhosphorImager densitometer (Molecular Dynamics).

**Immunoblotting** was performed as described (Deretic et al., 1995) using the ECL Western blotting Detection System (Amersham Corp.). Rab 11 and rab 2GTP-binding proteins were detected by [32P]GTP overlays as described (Deretic and Papermaster, 1993a). Blots were autoradiographed at 70°C using Kodak BioMax MR film with intensifying screens. Autoradiograms and Hyperfilm ECL were scanned using the Image processing and analysis program (Wayne Rasband, NIH).

**Miscellaneous Procedures—**To determine the distribution of the TGN membranes sialyltransferase was assayed as described (Deretic and Papermaster, 1993a). Post-Golgi vesicle membranes were digested with thermolysin according to Deretic and Papermaster (1991). Electron Microscopy—Membranes from the sucrose gradient fractions were pelleted for 1 h at 50,000 rpm in a Beckman SW 50.1 rotor with adaptors to obtain a small pellet. Pellets were fixed with 2% glutaraldehyde in 120 mM cacodylate, pH 7.4, containing 3% sucrose for 30 min at 4°C, postfixed with 1% OsO4, stained with uranyl acetate, and embedded in 2% agarose as described (Deretic and Papermaster, 1991). Blocks of membranes in agarose were dehydrated in ethanol and embedded in Epon. Thin sections along the axis of sedimentation were examined in a Phillips 301 electron microscope.

**RESULTS**

**Post-Golgi Vesicles Carrying Newly Synthesized Rhodopsin Are Formed in a Retinal Cell-Free System in the Presence of ATP—**Newly synthesized rhodopsin is incorporated by photoreceptor cells into post-Golgi vesicles when isolated frog retinas are pulse-labeled for 60 min and chased for 2 h (Fig. 1, top panel). Post-Golgi vesicle membranes have buoyant density of 1.09 g/ml and sediment in fractions 4–6 (maximum fraction 5) on the shallow linear equilibrium sucrose density gradients, which allow their separation away from the TGN as well as the Golgi, plasma membrane, synaptic vesicles, and endosomes (Deretic and Papermaster, 1991, 1993a; Deretic et al., 1995). Immunosolubilization using specific antibodies has shown that fractions 4–6 are highly enriched (~85%) in rhodopsin-bearing post-Golgi vesicles (Deretic and Papermaster, 1991).

To study the role of intrinsic and associated components that regulate vesicle formation, we have reconstituted this process in a cell-free system. For this purpose we modified the assay described by Tootle and Huttner (1990, 1992). Retinal proteins are pulse-labeled for 60 min in the isolated frog retinas, the rod outer segments are removed, and the remains of the retinas are homogenized. After low speed centrifugation to remove neural retina and nuclei, retinal PNS is supplemented with ATP and an ATP-regenerating system or with an ATP-depleting system (Davey et al., 1985) and incubated at 22 °C for an additional 2 h. Figure 1 shows that in PNS supplied with ATP, radiolabeled rhodopsin appears in vesicle-enriched fractions 4–6 of the gradient. By contrast, ATP depletion arrests rho-
Frog Retinal PNS Also Proceeds at Low Temperature

that which is initially formed

compared with 15% in the absence of ATP. The amount of the

fractions 4–6 contain

that all the components necessary for this process are present

rhodopsin is arrested infractions 7–10 that contain TGN membranes. The identity of these compartments was previ-

ously demonstrated by their galactosyltransferase and sialyl-

transferase activity, respectively (Deretic and Papermaster

1991, 1993a); see also Fig. 3). The distribution of newly syn-

thesized rhodopsin in the heavy fractions (9–12) enriched in Golgi and TGN membranes. The identity of these compart-ments was previously demonstrated by their galactosyltransferase and sialyltransferase activity, respectively (Deretic and Papermaster 1991, 1993a); see also Fig. 3). The distribution of newly synthesized rhodopsin in the gradient is different; whereas at 22°C rhodopsin predominantly accumulates in the Golgi-enriched fractions 11 and 12 (identified by their galactosyltransferase activity), at 0°C these fractions are partially depleted, and rhodopsin is arrested in fractions 7–10 that contain TGN membranes (Fig. 3). This suggests that rhodopsin transport proceeds until ATP is depleted by the frog photoreceptor ATPases rather than by the exogenous ATP-depleting system that is apparently inefficient at low temperature. Vesicle formation also proceeds in vivo in isolated frog retinas incubated at 0°C (Fig. 4).

Resident Membrane Proteins of the TGN Are Retained during Cell-free Vesicle Budding—We wanted to verify that our in vitro assay reconstitutes the physiological process of vesicle formation rather than the fragmentation of the TGN membranes, especially because this process does not appear to be slowed down at low temperature. Fig. 3 shows that the distribution of sialyltransferase, a resident membrane protein of the TGN, is not affected by vesicle budding in the cell-free assay at 22 or 0°C, and it parallels the distribution of the enzyme after in vivo vesicle formation. The activity of sialyltransferase in fractions 4–6 is negligible. This suggests that our assay indeed monitors vesicle budding and not the fragmentation of the TGN.

Fractions 7–10 are enriched in sialyltransferase activity contributed by the TGN membranes. These are also the fractions that accumulate radiolabeled rhodopsin during the chase on ice in the absence of added ATP (see Fig. 2).

Cytosol and G-proteins Are Necessary for Vesicle Formation—To test the role of cytosolic proteins in vesicle formation, pulse-labeled retinal PNS is further centrifuged through a 0.5 M sucrose cushion, and membranes that enter the cushion are separated from the soluble proteins. After this fractionation step, which separates a significant proportion of cytosolic proteins from the membranes, vesicle formation, as measured by the accumulation of radiolabeled rhodopsin in pooled fractions 4–6, is diminished to ~25% of control and is completely restored if the cytosol is added back (Fig. 4). Similarly, 1 μM
GTPγS inhibits vesicle formation by ~50% after 30 min of preincubation. These data are consistent with the existence of a pool of membrane-bound cytosolic proteins and GTP-activated G-proteins that are interacting at the onset of incubation and appear to sustain initial rounds of vesicle budding. In addition, the observed inhibitory effect of GTPγS is a consequence of its pleiotropic effect on heterotrimeric and small G-proteins.

Small GTP-binding Proteins Are Sorted to the Vesicles Formed in Vitro—We have compared the signature subset of membrane-associated small G-proteins in intact retinal photoreceptors (data not shown). The subcellular distribution of rab11 parallels that of rab6 in retinal subcellular fractions 4–6, which are enriched in these fractions 4–6. After the completion of an in vitro or an in vivo chase, each subcellular fraction is assayed for sialyltransferase activity, and the distribution of the enzyme is expressed as a percentage of total activity recovered in retinal subcellular fractions.

The distribution of sialyltransferase, a resident membrane protein of the TGN, is not affected by vesicle budding in a cell-free assay at 22 or 0 °C and resembles its distribution after in vivo vesicle formation (control). The bulk of sialyltransferase is associated with fractions 7–11, and very little is found in vesicle-enriched fractions 4–6. After the completion of an in vitro or an in vivo chase at both 22 °C and 0°C, there is no difference from the in vivo control as observed after in vivo vesicle formation. This suggests that the post-Golgi vesicle budding in the retinal cell-free system is selective because only specific rab proteins, previously identified in retinal photoreceptors, are properly sorted to the vesicles formed in vitro, and no additional GTP-binding proteins are found associated with their membranes.

GTP-binding proteins of post-Golgi vesicles formed in vivo (Deretic and Papermaster, 1993a; Deretic et al., 1995) with the content of vesicles that are formed in vitro by high resolution two-dimensional gel electrophoresis and [32P]GTP overlays. Fig. 5 shows that the GTP-binding protein composition of the vesicles formed in vivo or in vitro at 22 or 0 °C are indistinguishable; they contain previously identified proteins rab6, rab8, and rab3, as well as another TGN and post-Golgi vesicle-specific protein rab11, now identified using the rab11 antiserum (Urbe et al., 1993). After vesicle formation, Golgi-enriched fractions retain a more complex small G-protein pattern, as observed after in vivo vesicle formation. This suggests that the post-Golgi vesicle budding in the retinal cell-free system is selective because only specific rab proteins, previously identified from retinal cultures, are properly sorted to the vesicles formed in vitro, and no additional GTP-binding proteins are found associated with their membranes.

High Resolution Two-dimensional Gel Electrophoresis Reveals the Greatly Simplified Protein Pattern of Post-Golgi Vesicles Formed in Vitro Compared with the Golgi-enriched Membranes—We have now extended our previous analysis of vesicle-associated GTP-binding proteins by two-dimensional gel electrophoresis to include all vesicle proteins. Silver-stained gels of post-Golgi vesicles formed in vitro at 22 °C and its corresponding Golgi enriched fraction are shown in Fig. 6. The
COOH-terminus of Rhodopsin Is Involved in Sorting

protein composition of the vesicles formed in vivo (not shown) is identical to that of the vesicles formed in vitro, in keeping with already established identity of their GTP-binding proteins (shown in Fig. 5). Although the high resolution and very sensitive staining procedure reveal more vesicle-associated proteins than previously recognized, the protein composition of the vesicles is still simple when compared with the Golgi-enriched fraction. Anomalous isoelectric focusing of rhodopsin in these gels (probably due to its unusual carbohydrate chains) does not permit its identification as a well focused spot. Immunoblotting with anti-rhodopsin antibodies and the autoradiograms of the 35S-labeled vesicles reveal a smear in the 35-kDa region (data not shown), which is not apparent by silver staining. Other previously characterized vesicle proteins are readily identified by this technique: the α subunit of transducin, αA and αB crystallin, and membrane-bound actin (Deretic and Papermaster, 1991; Deretic et al., 1994, 1995). The number of membrane-associated and membrane proteins of the vesicles is significantly reduced compared with the Golgi-enriched fraction, whereas certain proteins like α-transducin and αA and αB crystallin are enriched in the vesicle fraction. Taken together these data indicate that selective budding of the vesicles results in retention of the TGN proteins and in vitro sorting of post-Golgi vesicle proteins away from the TGN.

In Vitro Formed Vesicles Are Morphologically Identical to the Vesicles Formed in Vivo and Have the Same Topology—Morphological analysis of the vesicles formed in a cell-free assay by electron microscopy shows profiles indistinguishable from the vesicles formed in vivo (Fig. 7A). We have previously shown that fraction 5 contains small vesicles (~300 nm diameter) without a visible coat and very little contamination from other morphologically distinguishable subcellular organelles (Deretic and Papermaster, 1991). This is also true for the vesicles formed in vitro, as shown in Fig. 7A. By contrast, the Golgi-enriched fraction 12 is very heterogeneous (Fig. 7B), but occasional profiles that may represent cross-sections of Golgi cisternae can be seen after in vitro vesicle budding.

Rhodopsin-bearing post-Golgi vesicles formed in vivo in retinal cultures contain rhodopsin in the membrane with its carboxyl-terminal domain exposed to the cytoplasm and amino-terminal domain inside the vesicle (Deretic and Papermaster, 1991). We have probed the orientation of rhodopsin in the vesicles that are formed in vitro at 22 or 0°C using mAb 11D5 to the carboxyl-terminal domain of rhodopsin and found that they contain rhodopsin inserted in the membrane with this domain exposed at the cytoplasmic side and susceptible to limited proteolysis with thermolysin (Fig. 8). This suggests that in vitro formed vesicles are topologically identical to the vesicles formed in vivo.

rab Proteins Are Essential Regulators of Rhodopsin Transport—To test the role of rab proteins in the transport of newly synthesized rhodopsin, we removed the membrane-associated rab proteins by incubating retinal PNS with purified rab GDI. This cytosolic protein regulates rab protein function by preventing GDP dissociation and subsequent nucleotide exchange onto rab proteins (Sasaki et al., 1990). We used recombinant bovine His6-tagged rab GDI, which has the ability to interact with wide range of rab proteins and extract GDP-bound rab proteins from the membrane (Ulrich et al., 1993, 1994). We preincubated pulse-labeled retinal PNS with 200 μM GDP and 2 μM recombinant rab GDI and followed the appearance of newly synthesized rhodopsin in the post-Golgi vesicle fractions 4–6 after the addition of ATP. Fig. 9A shows that under these conditions the TGN proteins are not seen.
conditions the amount of membrane bound rab6 and rab8 in the vesicle fraction is reduced to <40% of the original content. The amount of soluble GTP-binding proteins, and rab6 in particular, increases 3-fold (Fig. 9B). Extraction of membrane-bound rab proteins with 2 μM rab GDI results in the accumulation of more slowly migrating (untrimmed) forms of rhodopsin in the Golgi (fractions 11 and 12) and complete inhibition of post-Golgi vesicle formation (Fig. 9C). This GDI effect is concentration-dependent (IC50 = 0.3 μM), and the reduction in the radiolabeled rhodopsin content in vesicle fractions 4–6 is accompanied by an equivalent increase in Golgi fractions 11 and 12 (Fig. 10).

The Cytoplasmic Domain of Rhodopsin Is Involved in Vesicle Budding from the TGN—Finally, we wanted to test if the cytoplasmic domain of rhodopsin plays a role in the budding of post-Golgi vesicles from the TGN and in intracellular sorting. We preincubated pulse-labeled retinal PNS for 30 min in the presence of 100 μg of anti-rhodopsin COOH-terminal antibody mAb 11D5, previously used to immunoprecipitate rhodopsin-bearing post-Golgi membranes (Deretic and Papermaster, 1991). Binding of mAb 11D5 to the cytoplasmic domain of rhodopsin results in the profound (>85%) inhibition of vesicle formation during the cell-free chase, whereas control antibody of the same IgG subclass has no effect (Fig. 11). Completely processed radiolabeled rhodopsin accumulates in the TGN-enriched fractions 7–10. Golgi-enriched fractions 11 and 12 have a similar content of newly synthesized rhodopsin as the control. The distribution of other vesicle-associated radiolabeled proteins, as exemplified by 45-kDa protein, resembles the distribution of rhodopsin. Anti-rhodopsin antibody arrests post-Golgi vesicle formation to nearly the same extent as removal of ATP (Figs. 1 and 4) or rab proteins (Fig. 10).

To test if potential antibody cross-linking changes the sedimentation of the vesicles rather than inhibiting their budding, we also tested the mAb 11D5 Fab fragments. 11D5 Fab fragments inhibit vesicle formation in the same fashion as the whole antibody (Fig. 11B), whereas control Fab fragments have no effect (data not shown). Because Fab fragments cannot cause membrane cross-linking, this implies that mAb 11D5 inhibits rhodopsin transport. The presence of the antibody in the cell-free assay does not appear to interfere with the intra-Golgi transport because radiolabeled rhodopsin is found in lighter fractions (7–10) compared with its arrest in fractions 11 and 12 by GDI. We conclude that mAb 11D5 exhibits its inhibitory effect on the vesicle formation from the TGN. This suggests that the intracellular sorting machinery recognizes the cytoplasmic domain of rhodopsin upon its appearance in the TGN, where it is sorted to the appropriate vesicular carriers.

**DISCUSSION**

Establishment of a cell-free system that reconstitutes rhodopsin-bearing post-Golgi vesicle formation represents the first step toward understanding the molecular mechanisms of polarized sorting in rod retinal photoreceptor cells. Studies of intracellular trafficking of newly synthesized rhodopsin may also provide a more general insight, because rhodopsin is a member of the large family of seven-helix transmembrane receptors. We find that rhodopsin-bearing post-Golgi vesicles can form in the frog retinal cell-free system under physiological conditions and that vesicle budding is accompanied by the retention of resident membrane proteins within the TGN. Similar conditions for cell-free post-Golgi vesicle formation in other cells have been reported previously (Tooze and Huttner, 1990; Tooze et al., 1990; Salamero et al., 1990; Jones et al., 1993). However, in contrast to the cells or cell-lines that originate from the warm blooded animals, frog-derived tissues also appear to be able to maintain membrane transport at very low temperatures.

Sustained intracellular transport at 0°C may be due to the preservation of membrane fluidity at low temperatures in frog
photoreceptors. We have recently found that the polyunsaturated docosahexaenoic acid, a major contributor to the exceptionally high ROS membrane fluidity, incorporates into transport membrane phospholipids at the site of vesicle formation at 22 °C. It will be of interest to determine the temperature dependence of this process to define the role of lipid remodeling in intracellular transport and the post-Golgi vesicle budding.

A potential chaperone activity of αA and αB-crystallins may also contribute to the stability of post-Golgi vesicle formation at low temperature. These heat shock proteins associate uniquely with rhodopsin-bearing vesicles and may provide protection against temperature-induced stress (Deretic et al., 1994).

The presence of excess (2 μM) rab GDIs in the retinal cell-free assay probably inhibits intra-Golgi transport by depleting a rab protein pool present on the Golgi membranes and inhibiting recycling and recruitment of soluble rabs. Similar effects of rab GDIs on the intra-Golgi transport in other cells have been reported (Elazar et al., 1994; Peter et al., 1994). The pattern and the distribution of newly synthesized rhodopsin upon removal of rab proteins in retinal cells greatly resembles the effect of short (30 min) exposure to brefeldin A which results in the disruption of the Golgi and inhibition of rhodopsin processing (Deretic and Papermaster, 1991). The similarity between GDI- and brefeldin A-induced arrest of transport suggests that removal of rab proteins interferes with the glycoprotein processing in the Golgi (and, by inference, possibly its structure as well), which ultimately leads to reduced vesicle formation. rab6 is extracted from the photoreceptor membranes upon treatment with GDI (Fig. 9). Because rab6, as well as rab1, appears to be critical for the integrity of the Golgi complex (Martínez et al., 1994; Peter et al., 1994), their collective removal from the photoreceptor Golgi by rab GDI may contribute to the arrest of transport there. Additional experiments will be necessary to determine the role of rab6 and other post-Golgi vesicle-associated rab proteins in the budding and targeting of rhodopsin-bearing post-Golgi vesicles.

All rhodopsin-bearing vesicle-associated rab proteins are properly sorted to the post-Golgi vesicles formed in vitro. How-

![Graph](http://www.jbc.org/)

**Fig. 10.** Rab GDIs affects rhodopsin transport in a concentration-dependent manner. Retinal PNS was preincubated with 200 μM GDP for 1 h at 22 °C followed by 30 min at 30 °C with increasing concentrations of GDI. After SDS-PAGE the distribution of radiolabeled rhodopsin content of the vesicular pool in fractions 4–6 (IC50 ~ 0.3 μM GDI) is accompanied by an equivalent increase in the Golgi-enriched fractions 11 and 12.

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2 E. Rodríguez de Turco, D. Deretic, N. Bazan, and D. S. Papermaster, manuscript in preparation.
not only regulates its polarized sorting but also the sorting of ROS proteins transducin and cGMP phosphodiesterase, its partners in signal transduction (Deretic and Papermaster, 1991). Moreover, additional evidence for the important regulatory role of rhodopsin has been provided by Roof et al. (1994), who showed that in transgenic mice mis-sorting of mutant rhodopsin also leads to delocalization of other nonmutant ROS proteins.

Sorting into specific post-Golgi vesicles in photoreceptors may be accompanied by the binding of the cytosolic coat proteins that are presumed to be essential to deform the membrane and drive the budding process. This coat could be assembled at or near the cytosolic surface of rhodopsin, or perhaps rhodopsin molecules may bind integral TGN membrane protein(s) responsible for the recruitment of coat proteins and/or regulation of sorting to the appropriate vesicular carriers. In either case, antibody bound to the cytosolic surface of rhodopsin could prevent coat binding to its membrane receptor(s).

Preliminary experiments show that protein p200, a candidate for a coat protein that regulates vesicle budding from the TGN (Narula et al., 1992; Ladinsky et al., 1994) associates with photoreceptor TGN membranes but not the post-Golgi vesicles and could participate in vesicle budding.

It is possible that some of the coat proteins are photoreceptor-specific. This is consistent with reports of nonpolarized expression of rhodopsin in polarized kidney-derived cell lines, which suggest that these cells lack appropriate machinery to sort rhodopsin to one domain of the plasma membrane (Oprian et al., 1987; Nathans et al., 1989). Although we find that proteins that regulate transport to the basolateral plasma membrane in epithelial cells, such as rab8, may be involved in rhodopsin transport in rods, (Deretic et al., 1995), it is possible that photoreceptors use additional molecular information to sort rhodopsin.

It is difficult to assess which part of the cytoplasmic domain of rhodopsin is responsible for the sorting function because bound mAb 11D5 or its Fab fragments probably render the entire surface unavailable for interactions with other proteins. However, it is possible that the extreme COOH-terminal that binds the antibody also contains the sorting signal. The five COOH-terminal amino acids QVA(S)PA are highly conserved among different species. This domain of rhodopsin is responsible for the sorting function because photoreceptors use additional molecular information to sort rhodopsin.

Our data indicate that the cytoplasmic domain of rhodopsin interacts with the intracellular sorting machinery at the exit from the TGN. We are currently attempting to map the region of rhodopsin responsible for this interaction using synthetic peptides derived from the cytoplasmic domain of bovine and frog rhodopsin. Our future efforts will also be focused on identification of the membrane and/or coat proteins that interact with rhodopsin upon exit from the TGN. These factors should further define the mechanism of development of photoreceptor polarity, which may involve the modification of the sorting machinery common to other polarized cells, or the development of additional sorting stations to fulfill the maintenance of three highly polarized domains in this specialized neuron.

Acknowledgments—We are grateful to Dr. David Papermaster for continuous support and for critical reading of the manuscript and to Dr. Kai Simons for valuable comments and advice. We thank Nancy Ranney for support electron microscopy and Drs. Bruno Goud, Lukas Huber, Jean Gruenberg, Rob Parton, Kathryn Howell, Martin Latterich, and Paul Hargrave for helpful discussions. We also thank Drs. Bruno Goud and Rob Parton for their gifts of antibodies and reagents and Dr. Kathryn Howell for p200 immunoblottings.

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Cytoplasmic Domain of Rhodopsin Is Essential for Post-Golgi Vesicle Formation in a Retinal Cell-free System
Dusanka Deretic, Belen Puleo-Scheppke and Claudia Trippe

J. Biol. Chem. 1996, 271:2279-2286.
doi: 10.1074/jbc.271.4.2279

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