THE EFFECTS OF MONENSIN AND OF PUROMYCIN ON TRANSPORT OF MEMBRANE COMPONENTS IN THE FROG RETINAL PHOTORECEPTOR

II. Electron Microscopic Autoradiography of Proteins and Glycerolipids¹

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Abstract

Monensin converts the Golgi apparatus of rod photoreceptors into distended vacuoles, similar to those seen in other monensin-treated cell types, and leads to the accumulation of [³H]leucine in the distended vacuoles. As evaluated by quantitative, electron microscopic autoradiography, transport of newly made proteins—both to the outer segments and to the presynaptic terminals—is inhibited. These effects suggest that the Golgi apparatus is involved in transport in both principal directions within the highly polarized photoreceptors, a matter of interest since there seems to be only a single, extensive, Golgi apparatus in the cell body. Seemingly there are two distinguishable “sorting” routes, for proteins, out of the Golgi apparatus and, for the terminals, an additional non-Golgi route.

Accumulation of newly made glycerolipids in the outer segments and terminals is less affected by monensin than is accumulation of new proteins, and glycerolipid accumulation is little affected by puromycin, an inhibitor of protein synthesis. These latter findings suggest that the routes or mechanisms of assembly of newly made lipids into membranes in the photoreceptors are at least partially dissociable from those for newly made proteins.

Monensin has been shown to interfere with the transport of a variety of secreted proteins (Tartakoff and Vassalli, 1977) and seems also to affect certain membrane proteins (Johnson and Schlesinger, 1980; Strous and Lodish, 1980; Tartakoff et al., 1981). In the companion paper we have shown that monensin inhibits the insertion of newly made opsin into the rod outer segment of the frog retina (Matheke et al., 1984). The studies described in the present paper continue the investigation of the effects of monensin on transport of membrane components, comparing the effects on the outer segment with the effects on transport to the synaptic terminal. Our interest is in gaining insight into the routes and mechanisms whereby neurons are able to ship different membranes to different cell regions. We also have investigated the effect of monensin on the incorporation of

newly made glycerolipids into the two populations of membranes of interest to us—those of the outer segment and those of the synaptic terminal—since earlier data from our laboratory (Mercurio and Holtzman, 1982b) had suggested that proteins and lipids may be incorporated into pertinent membranes via partially different pathways.

Materials and Methods

Cytochemistry

Experimental animals. Some of the *Rana pipiens* used in the cytochemical experiments were fixed within a few days of arrival from the Lake Champlain Frog Farm (Alberg, VT) and were dark-adapted for 12 hr before the experiment. We have found no differences in pertinent morphology or cytochemistry between such animals and those used routinely for our autoradiographic studies, which were adapted to a 12-hr light/12-hr dark cycle for at least 4 weeks (Matheke et al., 1984) prior to use.

Thiamine pyrophosphatase. Retinas were dissected directly into the fixative solution, 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.4. Fixation was for 1.5 hr on ice, followed by rinsing overnight in cold 0.1 M cacodylate buffer. The retinas were then sectioned at a 25-μ setting

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on a Smith-Farquhar tissue chopper. Sections were incubated for 90 min at 37°C in the following solution: 1.25 gm of sucrose; 25 mg of thiamine pyrophosphatase (co-carboxylase, Sigma Chemical Co., St. Louis, MO); 7 ml of water; 10 ml of 0.2 M Tris maleate buffer, pH 7.2; 5 ml of 0.025 M MnCl_2 ; 3 ml of $\text{Pb}(\text{NO}_3)_2$ (Goldfischer et al., 1964). Postfixation was in 1% OsO_4 in 0.1 M cacodylate buffer. Uranyl acetate staining was done *en bloc* using a solution of 1% uranyl acetate in 0.014 M veronal acetate buffer for 30 min at room temperature. The tissue was dehydrated in graded ethanols followed by propylene oxide and was embedded in Epon.

Thin sections were stained with lead and examined in a Philips 201 electron microscope to localize the reaction product, then unstained semithick sections (0.2 μ) were cut and examined at 100 kV accelerating voltage to ascertain the position and size of the Golgi apparatus.

Impregnation with heavy metals. The method of Droz et al. (1975; also Markov et al., 1976) was employed to visualize the Golgi apparatus by virtue of its relatively high affinity for heavy metals. Retinas fixed for 2 hr on ice in 2.5% glutaraldehyde in 0.1 M cacodylate buffer were cut with a razor blade into approximately 1-mm squares and were impregnated with a 2.5% aqueous solution of uranyl acetate for 1 hr at 37°C. Then they were soaked for 1 hr at 37°C in a solution of 0.2% CuSO_4 , 2.65% $\text{Pb}(\text{NO}_3)_2$, 0.75% sodium citrate, and 0.16 M NaOH. Postfixation was in 1% OsO_4 for 2 hr on ice. After dehydration and embedding in Epon, semithick sections (0.25 μ) were examined at 100 kV in the electron microscope.

Electron microscopic autoradiography

Tissue preparation. Retinas were labeled with [^3H] leucine as in Matheke et al. (1984) or labeled in an identical manner with 2- ^3H glycerol, 50 $\mu\text{Ci}/\text{ml}$ (New England Nuclear, Boston, MA). In brief, the tissue was pulsed for 1 hr in Ringer's containing the labeled precursor with or without 10^{-5} M monensin. The retinas were then rinsed in Ringer's and then fixed or chased in Ringer's with or without monensin for an additional 5 hr before fixation. As in Matheke et al. (1984), the fixation-embedding protocol utilized for both precursors was based upon one we previously had found to minimize extraction of lipids (Mercurio and Holtzman, 1982b): fixation in cold glutaraldehyde and then cold osmium tetroxide, and dehydration in cold acetone followed by embedding in Epon.

Blocks were first evaluated by light microscopy. Blocks with orientation of the photoreceptors that would provide favorable sections of the cells were trimmed, and 100-nm sections of these were placed on parlodion-coated slides according to the method of Bok et al. (1977). Additional incubations with 10 $\mu\text{g}/\text{ml}$ of puromycin (Sigma) present in the Ringer's solution throughout the incubation were carried out and evaluated in the same manner. The slides were developed using a gold latensification procedure (Kopriwa, 1975). The grids were examined and photographed in the electron microscope.

Quantitation. The goal of this quantitation was to evaluate numerically the effect of monensin on the accumulation of label in the rod outer segments and ter-

minals as compared to the cell body. Note that, in line with the requirements for precise localization and quantification of grains, the preparations used were exposed and developed so as to give much lower grain densities than the ones used for Figure 4, *a* and *b*.

Measurements of area were made by computerized image analysis on a Bausch and Lomb Omnicon-3000.

The number of grains per square micron was determined for the region of interest. Because the outer segment leucine label in controls is concentrated at the base of the rod outer segment, the proximal 2.7 μ of the rod outer segment in control and monensin preparations were measured for the outer segment data.

This grain density over the rod outer segment (ROS) was normalized to the grain density over the myoid in order to compensate for the variations in labeling density from preparation to preparation and from section to section due to differences in section thickness, in emulsion density, in exposure time, and the like. For both leucine and glycerol, we did assure ourselves that the control and monensin-treated material showed similar levels of uptake into the photoreceptors, as would have been expected from the companion paper (Matheke et al., 1984). However, given the variations intrinsic to the procedures, the use of relative labeling data based on normalized ratios derived from a given section is essential for accurate detailed comparison. The myoid, referred to for simplicity as RIS (rod inner segment), is the area between the upper boundary of the nucleus and the lower boundary of the "ellipsoid" (the mitochondria-rich area of the cell body). Thus, mitochondrial labeling was eliminated from the quantitation. The large vacuoles into which portions of the Golgi apparatus transform in the monensin-treated tissue showed most of their label associated with the delimiting membranes, plus occasional grains associated with "internal" membranes (many of which actually represent sections through folds and in-pocketings of the surface). This is as expected from the biochemical information on the lipids and proteins being synthesized (Mercurio and Holtzman, 1982b; Matheke et al., 1984) and was readily evident in the examination of the HR circle intersections that forms part of the analyses on which Table I is based (methods of Williams, 1977; the dimensions of HR circles are directly related to the HD distances used to express the spread of grains from a line source.) The vacuoles are expanded, differentially and considerably, in the monensin material. Thus, including the area of their interior, which is almost entirely empty from the viewpoints of interest to us here, in the reference area used for calculating label density leads to the artificial reduction of the apparent relative labeling of the vacuoles in the Williams analysis, noted under "Results." (See the discussion of analogous problems in Mercurio and Holtzman (1982b).) This artifact was avoided in the other quantitations, the principal ones in this paper, by excluding the areas *within* the vacuoles from the reference area and thus treating the vacuole-associated grains as emanating from the membranes. (We did not subcompartmentalize the cytoplasm further because this would require making detailed assumptions about the distribution of label within the compartments and between the compartments that we cannot adequately evaluate. The fact that we did not

eliminate the area within the Golgi sacs of control preparations would not affect the ratios we calculate by more than 4% since this area is a small fraction of the area of the myoid with which we are concerned (Mercurio and Holtzman, 1982b).

The data are expressed as "percentage" of the total grains, that is,

$$\frac{\text{ROS}}{\text{RIS} + \text{ROS}} \times 100$$

where ROS represents grains per square micron over the proximal 2.7 μ of the outer segment, and RIS represents grains per square micron over the myoid. The area of the myoid that we measured is several fold greater than the total area of significant labeling in the outer segment (2- to 5-fold or greater difference in sections of different cells), and the total relevant area of the myoid is considerably greater. The ratios we measured are based on grains per unit area. Therefore, although we cannot directly measure changes in total labeling of the myoid during the chases, within the range of changes we are studying such movement of label would not inordinately affect the ratios or the conclusions we draw. (On "pessimistic" assumptions the effect might lead us to somewhat overestimate the extent of monensin's impact on the terminals.)

Labeling in the synaptic terminals was evaluated using the same logic as that used for the outer segments, substituting grain density over the terminal areas for that of the ROS in the equation.

We cannot reliably correct for the close interweaving of postsynaptic and presynaptic elements at the periphery of the terminals, but this is unlikely to be significant for our data since the label was not concentrated at the periphery.

Results

Thiamine pyrophosphatase (TPPase) activity and heavy metal impregnation in semithick sections

The most striking feature of the Golgi apparatus in the photoreceptor is its length; stacked Golgi sacs are seen along the entire myoid, from the supranuclear region to the base of the ellipsoid, which represents most of the cytoplasmic zone of the cell body (excluding the mitochondria).

In thin sections, stacks of sacs can be seen at every level of the myoid in thin sections. Therefore, it was of interest to know if this represented several discrete Golgi apparatuses located in different regions of the cell, or sections through one large Golgi apparatus. Observations on the Golgi apparatus in thick sections like the ones shown in Figure 1 for TPPase and for heavy metal impregnation in Figure 2 strongly suggest that it is one system spanning the length of the cell. We do not find evidence, for example, for separate apparatuses, one oriented toward the synaptic end and the other toward the outer segment end of the cell.

In any given section, even a thick one, there often are isolated segments of the Golgi apparatus not directly continuous with the rest (Fig. 1), but we know from transverse sections and from serial sections (Mercurio

and Holtzman, 1982a, and unpublished results) that most such isolated Golgi stacks are actually connected to the rest of the apparatus.

Morphological effects of monensin on the photoreceptor

Monensin causes an accumulation of large, distended vacuoles in the myoid (cell body) of the photoreceptor (Fig. 3). These vacuoles closely resemble those characteristic of monensin effects in a variety of other cell types treated with monensin (Tartakoff and Vassalli, 1977) and have been shown to represent distended Golgi sacs and tubules (Griffiths et al., 1983; Quinn et al., 1983; Tougaard et al., 1983).

The change in the morphology of the myoid is evident at the earliest times we have looked, 30 min. Despite the presence of these distended vacuoles, rough endoplasmic reticulum, smooth endoplasmic reticulum, including the subellipsoid smooth endoplasmic reticulum (Mercurio and Holtzman, 1982a), nuclear envelope, and cytosol retain normal appearance. The "condensed" appearance of the mitochondria is characteristic of monensin effects.

As detailed elsewhere (Matheke et al., 1983), the axons and terminals of monensin-treated material show little change in the first hour of our incubations, although by the end of 6 hr of incubation some vacuoles have appeared.

Localization of label by electron microscopic autoradiography of [³H]leucine-labeled tissue

As described under "Materials and Methods," our focus was on relative labeling of outer segments (and terminals) compared to the myoid.

In the outer segment (see Fig. 4) the grains in the control preparations are found principally over the most proximal discs of the photoreceptor and over the plasma membrane (cf. Basinger and Hall, 1973; Mercurio and Holtzman, 1982b), as would have been expected from the light microscopic observations (Matheke et al., 1984). In the terminals, grains were present primarily over regions occupied by synaptic vesicles (cf. Mercurio and Holtzman, 1982a, b).

Our quantitative findings are entirely consistent with the biochemical and light microscopic autoradiographic observations described in the companion paper (Matheke et al., 1984).

Neither with leucine nor with glycerol did we observe significant differences between control and monensin-treated tissue in pulse-only preparations (Fig. 5).

ROS labeling. Both with and without monensin the [³H]leucine is first incorporated into the myoid region of the cell, where the protein synthetic apparatus is located, and at 1 hr transport elsewhere is not yet extensive.

The data from the pulse-chase experiments (Fig. 5) signify that the label enters the ROS in monensin-treated retinas at a much lower rate than in controls. There is a 5-fold difference in the relative labeling of the ROS of monensin-treated retinas compared to controls.

Synaptic terminals (Fig. 6). Mercurio and Holtzman (1982b) observed an increase in relative labeling of the terminals, as compared to the myoid, with time, as expected for transport from the myoid down the short rod axon; this is evident also from comparing the pulse with

Figure 1. A semithick section ($0.2\ \mu$) of a TPPase preparation showing the Golgi apparatus extending from the supranuclear region (nucleus, *N*) to the base of the ellipsoid (*E*). Tissue was stained *en bloc* with uranyl acetate. A small stack of Golgi sacs (arrow) lies adjacent to the large stack. Bar = $1\ \mu$ m.

Figure 2. A semithick section ($0.25\ \mu$) of a heavy metal-impregnated preparation showing the Golgi apparatus extending the length of the myoid, from the nucleus (*N*) to the ellipsoid (*E*). Bar = $1\ \mu$ m.

the pulse-chase data in Figure 6. For our concerns in this study, the most important finding is that with monensin, the terminals in the pulse-chase experiment reach only 50% of the level of relative labeling seen without monensin.

Localization of the grains in the myoid. Analysis using the approaches described in Williams (1977) was performed to determine if the autoradiographic grains in monensin-treated tissue were associated with the vacuoles that represent distended Golgi elements (see Fig. 3) or with another compartment. As can be seen in Table I, the label does seem to be concentrated in the vacuoles. In fact, these data probably underestimate the degree of

concentration, since they are based on the total areas of the compartments, not on relative membrane surface areas (see "Materials and Methods") and Mercurio and Holtzman, 1982b), and the vacuoles are markedly distended as compared with the other compartments.

Electron microscopic autoradiography of [3 H]glycerol-labeled tissue

Through studies based on lipid extraction (Folch et al., 1957) and thin layer chromatography (Hughes and Fraai, 1965) essentially similar to those in our prior work (Mercurio and Holtzman, 1982b), we confirmed our prior report (Mercurio and Holtzman, 1982b) that the glycerol

Figure 3. *a*, Myoid of control photoreceptor showing the Golgi apparatus (G) after 6 hr of incubation. This figure was chosen to illustrate the maximum degree of Golgi swelling observed in our control preparations. Thin section was stained with uranyl acetate and Reynold's lead. *Bar* = 1 μ m. *b*, Myoid of a monensin-treated photoreceptor showing distended vacuoles (V) present in a region extending from the supranuclear zone to the base of the ellipsoid. Note that the smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), and nuclear envelope (NE) are unaffected. *Bar* = 1 μ m.

label principally enters lipids, chiefly phospholipids, and that our dehydration and embedding procedures result in little (less than 2%) extraction of label.

The pattern of labeling of the myoid was as in Mercurio and Holtzman (1982b). Grains were seen over the endoplasmic reticulum, where most of the phospholipids are synthesized (Mercurio and Holtzman, 1982b), and over most other cellular membranes. The grains over the outer segment were distributed more or less uniformly—no bands were evident. Those over the terminals fell mainly over regions rich in synaptic vesicles (cf. Bibb and Young, 1974; Mercurio and Holtzman, 1982b).

Effects of monensin. The labeling pattern with glycerol

differs markedly from that with leucine (Figs. 5 and 6). First, changes in labeling with time of the two compartments of interest to us, especially the ROS, were notably less marked. Second, monensin effects were not striking; some inhibition of the labeling of terminals relative to the total label is seen (the monensin preparation reaches only about 70% of the level of the corresponding pulse-chase control), but there is little inhibition of ROS labeling.

Effects of puromycin (Figs. 5 and 6). Puromycin was seen to have little effect on the incorporation of glycerol into either the outer segments or the synaptic terminals. The concentrations of puromycin used are those reported

Figure 4. *a*, Low power electron micrograph of an autoradiogram of a control retina labeled with [^3H]leucine. The preparation was overexposed relative to those useful for quantitation, so as to reveal clearly the bands of label at the base of the ROS (arrows). Grains are also distributed over the myoid region (M). Bar = 1 μm . *b*, Low power electron micrograph of an "overexposed" autoradiogram of a monensin-treated retina labeled with [^3H]leucine. Note the absence of bands at the base of the ROS and the presence of numerous grains over the distended vacuole-rich region of the myoid (M). Bar = 1 μm .

to reduce incorporation of [^3H]leucine to very low levels (cf. Basinger and Hall, 1973), which we confirmed autoradiographically.

Discussion

The results of this study show that monensin inhibits transport of membrane proteins both to the ROS and the synaptic terminal under conditions where neither protein synthesis nor lipid synthesis is appreciably affected (Matheke et al., 1984). Our findings also suggest

strongly that newly synthesized lipids can travel to the outer segments and the terminals by routes or mechanisms that are partially dissociable from those used for newly made proteins.

Effects of monensin. Monensin is known to suppress the intracellular transport of a variety of secretory proteins (Tartakoff and Vassalli, 1977), although not necessarily their discharge (Tartakoff and Vassalli, 1978). Much less attention has been devoted to membrane proteins. Our results showing the inhibition of transport

Figure 5. *a*, Relative labeling of ROSs with $[^3\text{H}]$ leucine. See "Materials and Methods" for description of measurement and quantitation. The number of blocks used to obtain the data is represented by *n*; approximately 20 cells per block were measured. The data come from three experiments for the pulse-chase experiments and two experiments for the pulse-only preparations. The left-hand column represents the data for pulse, no monensin; the other columns are as labeled. At the termination of the pulse, the relative labeling of the outer segment is approximately equal in both the control and the monensin-treated retinas. At the termination of the chase, however, there is a highly significant ($p < 0.0005$, Student's *t* test) increase, more than 5-fold, in the labeling of the outer segment in the control retina. This change is not seen in the monensin-treated retina. *b*, Relative labeling of ROSs with $[^3\text{H}]$ glycerol. *n* represents the number of blocks examined; for each block approximately 20 cells were measured. The data were obtained from three experiments for the pulse-chase preparations and two for the pulse-only preparations. There is no significant difference between pulse and pulse-chase in labeling of the ROSs in retinas incubated with $[^3\text{H}]$ glycerol. Of special interest is the lack of significant effect of monensin or puromycin on the incorporation of $[^3\text{H}]$ glycerol into the ROSs.

of $[^3\text{H}]$ leucine-labeled membrane proteins to the ROS and the synaptic terminal by monensin are consistent with the few studies by other laboratories showing a suppression of viral and cellular membrane protein transport in non-neuronal cell types (Johnson and Schlesinger, 1980; Kaariainen et al., 1980; Strous and Lodish, 1980; Tartakoff et al., 1981). Alonso and Compan (1981) report that different membrane-delimited viruses, destined to bud from different regions of the cell surface, are affected to different extents by monensin.

The effects of monensin seem to be due to its blocking transit through or out of the Golgi apparatus. The mechanisms underlying this effect are not known but could involve alterations in the distribution of ions such as H^+ across Golgi membranes (Glickman et al., 1983). The vacuoles that accumulate when monensin is present are derived from the Golgi apparatus; this was first suspected from morphological observations but has now been confirmed cytochemically (Griffiths et al., 1983), biochemically (Kitson and Widnell, 1981; Quinn et al., 1983), and immunocytochemically (Tougard et al., 1983). As in our tissue, non-Golgi systems, such as the rough and the smooth endoplasmic reticulum, are affected little if at all.

The inhibition of transport of opsin to the outer segment by monensin is consistent with the evidence invoking a role for the Golgi apparatus in such transport (Young and Droz, 1968; Young, 1974, 1976; Papermaster et al., 1975; Bok et al., 1977; Mercurio and Holtzman, 1982b). Our results seem to indicate that this Golgi role is obligatory for the outer segment membrane assembly. It will be of interest to determine whether monensin affects the pattern of glycosylation of opsin or other proteins; its behavior on gels suggests that the opsin made by monensin-treated rods is similar to native opsin (Matheke et al., 1984), but this by no means rules out differences in terminal steps in construction of the oligosaccharide side chains.

The inhibition of transport of proteins down the photoreceptor's short axon to the synaptic terminal suggests that the Golgi apparatus plays a role in such transport as well. Thus, our findings implicate the Golgi apparatus in transport in both directions within the highly polarized photoreceptor, a finding that will be discussed further below. Hammerschlag et al. (1982) described the inhibition of axonal transport in bullfrog dorsal root ganglia and suggested that passage through the Golgi apparatus is a necessary step prior to fast axonal trans-

Figure 6. See "Materials and Methods" for a description of measurement and quantitation. The data were obtained from the same experiments as were used for Figure 5. The *left-hand column* represents the no-monensin pulse, and the other *columns* represent pulse-chases under the conditions indicated. *a*, Relative labeling of synaptic terminals with [^3H]leucine. The difference in labeling of the terminals between the end of the 1-hr pulse and the end of a 5-hr chase is approximately 2-fold ($p < 0.025$; Student's *t* test). The pulse-chase monensin preparation does not differ significantly from the pulse preparation. *b*, Relative labeling of synaptic terminals with [^3H]glycerol. The difference ($p < 0.025$) between the pulse-chase preparations with and without monensin suggests that there is some inhibition of axonal transport of glycerolipids to the synaptic terminals by monensin. Puromycin has no significant effect on the accumulation of labeled lipids in the terminals.

TABLE I

Williams analysis of grain distribution over the myoid of monensin-treated rods

Analysis, by the method of Williams (1977), of electron microscope autoradiograms obtained from retinas which had been given a "pulse" of [^3H]leucine for 1 hr and "chased" for 5 hr. The number of "area" circles falling over a given compartment was calculated by overlaying the micrographs with a transparent sheet with an array of circles of a radius equivalent to 135 nm, the HR value for Ilford L4 emulsion developed by gold latensification. The "expected" grain number is the number that would be obtained if the cells were uniformly labeled—each compartment having label proportional simply to its relative area. The actual grain number was calculated by overlying each grain in the micrographs with a circle of the same HR value as used for the area determinations and scoring the compartments. The data are expressed as relative specific activity, which is the actual number of silver grains divided by the number of area circle grains normalized to the actual number of silver grains.

The last two categories in the compartment list represent grouped data for the organelles indicated.

Compartment	No. of Area Circles	Actual No. of Silver Grains	Expected No. of Silver Grains	χ^2	% Grains	Relative Specific Activity
Vacuoles	233	84	69.6	2.98	79.2	1.21
Rough endoplasmic reticulum	42	12	12.5	0.02	11.3	0.96
Smooth endoplasmic reticulum	36	4	10.8	4.28	3.8	0.37
Mitochondria/lysosomes/plasma membrane	23	1	6.9	5.04	0.9	0.14
Nucleus/cytoplasm	21	5	6.3	0.27	4.7	0.79
Total	355	106		12.59		
Level of significance				0.025		

port. The mechanisms of axonal transport are complex (reviewed in Lasek and Hoffman, 1976; Grafstein, 1977; Schwartz, 1979), and, although it appears that the monensin arrest is at a pre-axonal stage, the exact cause of the blockage is unknown.

The fact that labeled proteins appear in the terminals rapidly during the pulse plus the fact that the monensin inhibition of the accumulation of labeled protein in photoreceptor terminals relative to the cell body is only

partial most likely reflect the presence of some rough endoplasmic reticulum and of smooth endoplasmic reticulum directly continuous with the rough endoplasmic reticulum in the terminals (Mercurio and Holtzman, 1982a); mitochondria are not present and thus are ruled out as the source of the newly made proteins. In other words, photoreceptors, unlike some other neurons, do seem to have local sources of protein in their axons and terminals. In addition, since the axons are only a few

tens of micrometers long, slow axonal transport, presumably of nonmembranous components (Lasek and Hoffman, 1976; Schwartz, 1979; Grafstein and Forman, 1980) could move some labeled proteins from myoid to terminal during the chase.

Glycerolipids. The methods we have used in our autoradiographic studies of lipids—fixation in the cold to minimize lateral mobility of membrane molecules, and the use of graded, cold acetones (Gould and Dawson, 1976)—were designed to avoid artifactual redistribution of lipids; so far as can be judged, they do so. However, there is no available way to evaluate the extent of lipid redistribution within the plane of membrane systems directly continuous with one another. Thus, our interpretation focused on the pulse-chase experiments so that it concerns systems—the outer segment and synaptic membranes—that are not continuous with the endoplasmic reticulum in the myoid, where the newly made lipids are synthesized (Mercurio and Holtzman, 1982b). We recognize also that, until more adequate methods for dealing with lipids autoradiographically are developed; all interpretations must be tentative.

Previous studies of ROS by others had suggested some independence of the lipid components of the membrane from that of the protein (Bibb and Young, 1974; Anderson et al., 1980). These studies included the demonstration that inhibition of protein synthesis with puromycin did not prevent the continued uptake of choline into the photoreceptor's phosphatidylcholine (Basinger and Hoffman, 1976).

Our previous results had shown that newly made lipids accumulate first in the rough endoplasmic reticulum and in regions of the smooth endoplasmic reticulum that are closely interspersed among the rough cisternae. Subsequently, the lipids move through the myoid in patterns different from those of newly made proteins; there is much less evident accumulation in the Golgi apparatus (Mercurio and Holtzman, 1982b). The present findings extend these observations by showing that when Golgi functioning is blocked, new lipids continue to enter the outer segment. Perhaps the phospholipid exchange proteins known to exist in the retina (Dudley and Anderson, 1978; Papermaster and Schneider, 1982) permit lipid molecules to move from their sites of synthesis to sites at, or on the way to, the outer segment, where the lipids associate with older proteins that have already moved past the Golgi apparatus. We also observed little effect of puromycin on lipid labeling on the outer segment. Since our work employed glycerol, which labels the lipid backbone, as the precursor, it removes the residual uncertainties about the interpretation of the previous puromycin findings with choline, which can enter lipids by exchange reactions involving the head groups. Perhaps the cell incorporates new lipids in its plasma membrane and these lipids can then diffuse into the regions of the cell surface involved directly in the genesis of outer segment membrane.

The literature on lipid transport and synthesis in axons is sparse and complex. There seems to be a distinct tendency for newly made membrane proteins to be accompanied by newly made lipids (Hammerschlag et al., 1982) and some indication of a linkage of lipid transport

and protein synthesis (Grafstein et al., 1975; Schwartz, 1979; Sherbany et al., 1979). But the kinetics of transport of new lipids from perikarya down axons are different from those of new membrane proteins (Grafstein et al., 1975; Grafstein, 1977; Haley et al., 1979; Toews et al., 1979), and, in certain axons at least, some lipid synthetic capabilities have been detected (Gould et al., 1983). Few details of interpretation can be regarded as fixed. Our findings suggest that in the photoreceptor there can be an appreciable dissociation between transport of newly made lipid and that of newly made proteins to the presynaptic terminals. The monensin data do suggest that some of the lipids move via the Golgi apparatus, and, thus, most likely some are transported along with proteins as components of membranes (cf. Schwartz, 1979; Sherbany et al., 1979; Hammerschlag et al., 1982). But the observation that puromycin inhibition of protein synthesis seems not to drastically inhibit the accumulation of labeled glycerolipids in the terminals relative to the myoid suggests that some lipids can associate with previously synthesized proteins, in line with our own prior findings in the myoid region and with the literature cited above (see especially Grafstein et al., 1975; Grafstein, 1977). This lack of effect on the incorporation of [3 H]glycerol in the terminals could depend on local synthetic capacities in the axons or terminals or on the fact that lipids can be moved by a variety of mechanisms (see Holtzman and Mercurio, 1980 for a review) not available to proteins.

Since we have found similar differences from the behavior of proteins using both glycerol and choline as labels (Mercurio and Holtzman, 1982b), it is unlikely that the differences are due principally to the special properties of a particular class of phospholipids, such as the phosphoinositides (see Gould et al., 1978).

Transport to the two poles of the cell. The thick sections of the photoreceptor stained either for TPPase or with heavy metals strongly suggest that the photoreceptor, like many other cell types including conventional neurons (Holtzman and Novikoff, 1984), has only one Golgi apparatus. Clearly, without a complete serial section investigation of many rod cells we cannot be certain that every cell always has one unified network. However, our findings do establish that most of the Golgi sacs are part of a single system running longitudinally in the myoid region, and they rule out "extreme" possibilities. For example, there are not two separate stacks of Golgi sacs, one oriented toward the outer segment and the other toward the presynaptic terminal; this latter arrangement would have been an attractive, simple device for polarized processing and transport.

Since monensin does affect protein transport in both directions, our findings suggest strongly that the cell uses the same Golgi apparatus to process different populations of membranes destined for opposite ends of the cell.

We do not yet fully understand how this comes about. However, our prior morphological and cytochemical studies (see Mercurio and Holtzman, 1982a, especially the Discussion section plus Figs. 11 and 24) seem to indicate that transport of materials destined for the presynaptic terminal depends in part on elongate sacs

that extend away from the Golgi apparatus toward the axon. The present observations may imply that these sacs are produced by the Golgi apparatus, or at least receive crucial components of the materials to be transported from the apparatus. We suspect from the findings discussed by Mercurio and Holtzman (1982a) that these sacs are among the principal sources of membranous structures within the axon, including shorter sacs and some of the synaptic vesicles, which appear to form by budding at sites both near to and distant from the stacked Golgi systems. (As already mentioned, other axonal structures are directly continuous with the endoplasmic reticulum, and we have also reported that a few vesicles and tubules in synaptic terminals show TPPase activity (Evans and Holtzman, 1982) and, thus, may derive relatively directly from the Golgi stacks.) In contrast, production of the vesicles thought (Papermaster et al., 1975, 1979; Kinney and Fisher, 1978; Papermaster and Schneider, 1982) to transport opsin seems to be by direct budding from portions of Golgi-associated sacs that appear to be different from those just discussed (Mercurio and Holtzman, 1982a). In other words, as in polymorphonuclear leukocytes which must also solve the problem of using a single Golgi apparatus to package different products (Bainton and Farquhar, 1968a, b), the photoreceptor may use different portions of the apparatus, possibly different faces, to handle different materials.

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