A set of proteins in bovine rod outer segments is specifically methylated by S-adenosyl-L-methionine. The reaction can be demonstrated in the intact retina as well as in fragmented preparations of isolated rod outer segments. The apparent molecular weights of these proteins are 88,000, 61,000, and a subset between 21,000 and 26,000. The $M_r = 88,000$ protein is shown to be the $\alpha$ subunit of the rod outer segment cGMP phosphodiesterase by peptide mapping, two-dimensional gel electrophoresis, the ionic strength dependence of its interaction with the membrane, and immunoprecipitation by antiserum raised against purified phosphodiesterase. For each of these proteins, the incorporated methyl groups are hydrolyzed in alkaline yield methanol, indicating that the proteins are carboxymethylated.

In recent years, several proteins in addition to the visual pigment rhodopsin have been identified and shown to participate in producing a primary visual response in photoreceptor cells (for review see Ref. 1). The molecular mechanisms by which these components create a light-triggered response, restore the cell to the untriggered state, and regulate the photosensitivity are little understood. In seeking mechanisms to biochemically explain some of these processes, we noted several general analogies between eucaryotic phototransduction and procaryotic (or eucaryotic) chemotactic sensory systems. Both systems initiate signals via receptors for an appropriate stimulus (2, 3), both somehow involve cGMP (4-6) and calcium (7, 8) in processing the information, and both regulate the photosensitivity to light in producing a primary visual response in photoreceptor cells. We report here the finding of specific methyl-accepting proteins in bovine rod outer segments with apparent molecular weights of 88,000, 61,000, and a set of polypeptides between 21,000 and 26,000. We have shown the $M_r = 88,000$ protein to be the $\alpha$ subunit of the cGMP phosphodiesterase present in rod outer segments and demonstrated that the methylation occurs by esterification of carboxyl groups in these proteins.

**Experimental Procedures**

**Materials**—Bovine eyes were obtained from a local slaughterhouse immediately postmortem, transported on ice in a light-tight container, and dissected in the dark to obtain the retinas. S-Adenosyl-L-[methyl-$^3$H]methionine, L-[35S]methionine, cyclic [3',5']-guanosine monophosphate, and 14C-labeled protein molecular weight standards were purchased from Amersham Corp. and L-[3H]methionine from New England Nuclear. Sinefungin was purchased from Calbiochem-Behring and trypsin and Staphylococcus aureus aureus V8 protease were from Miles Laboratories, Inc. Trypsin/chymotrypsin inhibitors was purchased from Mobay Chemical Corp. Protein A-Sepharose 6 MB was from Pharmacia Fine Chemicals. Materials for gel electrophoresis were from Bio-Rad and Amphotoline from LKB Instruments, Inc. All other biochemicals were purchased from Sigma.

**Rod Outer Segment Preparation**—Rod outer segments were isolated from fresh bovine retinas by the method of Papermaster and Dreyer (12) as modified by Baehr et al. (13) and stored in small aliquots at $-80^\circ C$ until used. The concentration of rhodopsin in the membranes was determined from the absorbance of an aliquot solubilized in 1% Ammonyx LO as described previously (13). The $A_{370}/A_{880}$ ratio was typically 2.5-2.6.

**Phosphodiesterase Isolation and Assay**—Phosphodiesterase was isolated, and the basal activity was assayed as described by Baehr et al. (13, 14). In order to assay phosphodiesterase activity in 100 ml ROS extracts (which contained very low levels of the enzyme), the following procedure was employed. Twenty ml of assay mixture were lyophilized in an Eppendorf tube. Twenty ml of extract were then added and incubated at 37°C for 45-60 min. All other procedures were as described.

**Electrophoresis and Fluorography**—SDS-PAGE was carried out as described (14). SDS-PAGE was run in either 15% Laemmli gels (15% acrylamide, 0.4% bisacrylamide) (15) or in low cross-linked gels (15% acrylamide, 0.08% bisacrylamide) (13). Two-dimensional gels were run as described by O'Farrell (16) and Baehr et al. (13). Radioactive proteins were visualized by fluorography as described (17).

**Methylation of Proteins in Isolated ROS**—Methylation was carried out under fluorescent room lights for 45 min at 37°C in 400 ml of 50 mM Hepes, pH 7.0, 100 mM NaCl, 5 mM MgCl2, 0.1 mM dithiothreitol, 100 mM phenylmethylsulfonyl fluoride, 80 Kallikrein-inactivating units of Trasylol, ROS containing 50 mcg of rhodopsin, and 10 mcg of L-[3H]AdoMet (67 Ci/mmol, 3.7 x 10$^{-2}$ mCi).

**Radioactive Labeling and Methylation of Proteins in the Intact Retina**—Retinal proteins were labeled according to the protocol of O'Brien et al. (18) as described in further detail by Baehr et al. (14). One retina was incubated with 1 mcg of either L-[3H]methionine (200 mcC/microml, 3.1 x 10$^{-4}$ m) or L-[35S]methionine (1170 Ci/mmol, 6.5 x 10$^{-4}$ m) for 6 hours, in 10 ml of Dulbecco's modified Eagle's medium (+Gln, -Met). In experiments in which protein synthesis was inhibited, the retina was preincubated for 1 h with 10 $\mu$g/ml of puromycin before adding the methionine to the culture medium. After incorporation of the label, ROS were isolated from the retina as described (14).

**Protein Elation from ROS Membranes**—The protocol used was based on that described by Baehr et al. (13) adapted from the observations of Kuhn (19). Soluble proteins were extracted three times in moderate ionic strength buffer (10 mM Tris, pH 6.8, 100 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, 100 $\mu$M phenylmethylsulfonyl fluoride). Periodically bound membrane proteins were then eluted with low ionic strength buffer (10 mM Tris, pH 6.8, 1 mM dithiothreitol, 100 $\mu$M phenylmethylsulfonyl fluoride, ±100 $\mu$M GTP). Each extraction was carried out in a volume of 100 ml, the membranes were

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The abbreviations used are: ROS, rod outer segments; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; AdoMet, S-adenosyl-L-methionine; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate.

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recovered by sedimentation at 18,000 × g for 15 min, and the material was applied directly to a SDS gel for analysis.

**Immunoprecipitation**—Rabbit antiserum, raised against purified bovine rod outer segment phosphodiesterase, was a generous gift from Dr. Dorothy Roof (Department of Biological Sciences, Purdue University). Three low ionic strength extracts of ROS were pooled and made 150 mM in NaCl and 0.1 mM in EDTA. In competition experiments, 10 μg of pure phosphodiesterase were then added. Antiserum (or control serum) was added at a 1/77 dilution, and the solution was incubated at 37 °C for 15 min and then 4 °C overnight. After warming to room temperature, 50 μl of a suspension of Protein A-Sepharose (containing ~10 μg of Protein A in buffer A (10 mM Heps, pH 7.0, 0.1 mM EDTA, 150 mM NaCl)) were added and rocked for 1 h. The beads were pelleted, resuspended in buffer A, and washed twice by pelleting through 0.5 ml of 1 M sucrose, 0.1% bovine serum albumin in the same buffer. After resuspension in electrophoresis sample buffer, the beads and sample buffer were boiled and applied to a gel for electrophoresis and fluorography as described above.

**One-dimensional Partial Peptide Mapping**—Rod outer segment proteins were methylated as described above. Retinal proteins were labeled in situ with [35S]methionine, and rod outer segments were isolated as described above. Gel bands from these ROS preparations were then subjected to peptide mapping by a modification of the method of Cleveland et al. (20). ([35S]Met-labeled proteins were used only to enhance the sensitivity of the technique. Identical results were obtained when pure unlabeled phosphodiesterase was used and detected by silver staining.) Outer segment proteins were separated by electrophoresis in 15% acrylamide, 0.08% bisacrylamide gels. The gel was stained for 5 min, destained 15, and dried without heat. The appropriate band in each lane was excised from the dried gel, swollen in 0.1 M Tris, pH 6.8, 2% SDS, 1 mM dithiothreitol, 1 mM EDTA, 10% glycerol for 60 min at room temperature and stored at −80 °C in the same buffer until used. The swollen gel pieces were loaded into the wells of a 15% acrylamide, 0.4% bisacrylamide gel, overlaid with the indicated amount of protease (S. aureus V8 or chymotrypsin), and run into the stacking gel. Electrophoresis was interrupted for 1 h when the proteins were in the middle of the stacking gel to achieve partial proteolysis and then resumed.

**Carboxymethylation Assay**—Proteins were assayed for esterified methyl groups by the microdistillation procedure of Stock and Koshland (21).

**Identification of [3H]Methanol**—Radioactive methanol was identified by reaction with 3,5-dinitrobenzoyl chloride by the method of Neish (22). The fragmentation pattern of the distillate was recorded on an AEI MS9 mass spectrometer at 70 eV.

**RESULTS**

**Incorporation of Methyl Groups into Bovine Photoreceptor Proteins**—Incubation of a fragmented subcellular preparation of bovine rod outer segments with the methyl group donor S-adenosyl-L-[methyl-3H]methionine results in radioactive labeling of discrete polypeptides. These can be resolved by electrophoresis of the ROS in the presence of sodium dodecyl sulfate. In contrast to the many Coomassie blue-stained proteins of ROS (Fig. 1a), only two distinct proteins and a set of five to six smaller polypeptides are labeled (Fig. 1b, lane 1). These methylated compounds are sensitive to proteolytic digestion by trypsin (Fig. 1b, lane 2) but are stable to phos-
phospholipase C (Fig. 1b, lane 3). Thermolysin and papain also digest the bands, but phospholipase D, RNase A, and DNase I have no effect (data not shown). The transfer of the methyl group can be inhibited by heating the membrane preparation to 100 °C before incubating it with AdoMet (Fig. 1b, lane 4) or by the addition of known inhibitors of methylation, Sinefungin or S-adenosyl-L-homocysteine (23), to the incubation mixture (Fig. 1b, lanes 5 and 6). The addition of cGMP to the reaction mixture increases the level of methylation of the M, = 88,000 protein (data not shown). The susceptibility of the methylated compounds to proteases and their insensitivity to other classes of degradative enzymes imply that they are, in fact, proteins and not phospholipids (24) or nucleic acids (25, 26) (both of which have been shown to be methylated in other systems). That the reaction is enzymatic is suggested by its specificity and thermal sensitivity and the inhibitory action of Sinefungin and S-adenosyl-L-homocysteine.

In vivo, AdoMet is synthesized by the reaction of ATP with L-methionine (27). No methyl transfer is observed in isolated ROS when L-[methyl-3H]methionine with or without 1 mM ATP replaces the [3H]AdoMet in the incubation mixture (data not shown). The inability of the system to utilize methionine as a source of methyl groups implies that AdoMet is not synthesized in this isolated membrane preparation under these conditions. Methionine, however, does serve as a source of methyl groups for ROS proteins in the intact retina (Fig. 1c). Fresh bovine retinas were incubated with [3H]Met in tissue culture medium. After 6 h of incubation, ROS were isolated from the retina and the proteins were separated by SDS-PAGE. The methionine is incorporated into proteins during translation, and fluorography shows the many bands typical of ROS (Fig. 1c, lane 1) (14). The methionine, however, is also incorporated into AdoMet and thereby serves as a methyl group donor as well. The presence of these methyl groups can be detected after alkaline hydrolysis by the microdistillation assay of Stock and Koshland (21), in which there is no interference from label incorporated as methionine into proteins (see below). Alternatively, protein synthesis can be inhibited by incubation of the retina with 10 µg/ml of puromycin. In control experiments, [35S]Met was shown not to be incorporated into protein in the presence of puromycin (data not shown). When [3H]Met labeled in the terminal methyl group is used, however, [3H]AdoMet is still formed, and even in the presence of puromycin a set of discrete polypeptides is labeled (Fig. 1c, lane 2). The M, = 88,000 methylated protein is easily visualized. The M, = 61,000 and 21,000–26,000 proteins are still methylated but are only faintly visible on the original exposures. The two low molecular weight bands, running at or near the dye front, are as yet unidentified. The labeling studies of intact retinal tissue strongly suggest that enzymatic transfer of methyl groups from AdoMet to a set of specific protein side chains is a metabolic function inherent to photoreceptor cells.

Identification of the M, = 88,000 Polypeptide As the α Subunit of Phosphodiesterase—The M, = 88,000 methylated protein has a mobility identical with that of the large subunit of the bovine retinal cGMP phosphodiesterase. This enzyme has been isolated and characterized as a peripheral membrane-bound component of rod discs and shown to consist of three subunits of M, = 88,000, 84,000 and 13,000 (13). To establish that the M, = 88,000 methylated protein corresponds to the large subunit of phosphodiesterase (α), several experiments were carried out. Bovine phosphodiesterase is stably bound to disc membranes at moderate ionic strength, but eluted at low ionic strength (13). In the same manner, none of the methylated polypeptides are eluted when extracted with buffer of moderate ionic strength (Fig. 2a, lanes 1.1–1.3). The M, = 88,000 methylated protein, however, is eluted upon extracting with low ionic strength buffer (Fig. 2a, lanes 2.1–3.1). Small volumes for extraction were used here so the sample could be immediately applied to a gel for analysis to avoid further processing of the labile material. This results in incomplete extraction of the methylated protein (Fig. 2a, lane B) and the phosphodiesterase activity (see below). Quantitative extraction of both is effected with larger volumes (data not shown). The relative amounts of the M, = 88,000 [3H] protein and phosphodiesterase activity in each extract are compared in Fig. 2b. The elution of the methylated protein
correlates with a significantly increased level of elutable phosphodiesterase activity. A quantitative comparison of membrane-bound phosphodiesterase and extracted soluble enzyme can not be made because the specific activity of the enzyme is different in these two states (13). Assay of the extracted methylated proteins (Fig. 2a, lane B) revealed, however, that they contained ~25% residual phosphodiesterase activity and ~35% residual counts/min incorporated into the M, = 88,000. In addition, the interaction of several ROS polypeptides (but not phosphodiesterase) with the disc membranes has been shown to be influenced by GTP (19). The elution of the methylated proteins is unaffected by the addition of GTP (Fig. 2a, lane 3). Thus, the elution behavior of the M, = 88,000 protein is similar to that of phosphodiesterase, one of whose subunits is M, = 88,000. The decrease in the lower molecular weight methylated proteins remaining after the extractions (Fig. 2a, lane B) is apparently due to membrane losses during the procedure through nonspecific adsorption to pipettes since similar amounts of rhodopsin are also lost (data not shown). These methylated proteins have never been seen in any extract. The inability to extract the other methylated proteins suggests that they may be integral membrane proteins.

The low ionic strength extract of methylated ROS proteins was subjected to two-dimensional electrophoresis (Fig. 3). Separation was in a nondenaturing polyacrylamide gel followed by SDS-PAGE which has been used to characterize the phosphodiesterase (13). The profile of radioactivity in the first dimension gel (Fig. 3a) correlates with that of the phosphodiesterase activity (Fig. 3a) and the stained phosphodiesterase bands (Fig. 3b), i.e. the M, = 88,000 methylated protein has the same mobility as the M, = 185,000 phosphodiesterase under nondenaturing conditions. Fluorography of the second dimension SDS gel shows only one band (Fig. 3c) that aligns exactly with the position of the stained phosphodiesterase α band. The addition of unlabeled pure phosphodiesterase to the extract enhances (co-migrates with) the Coomassie blue-stained bands (data not shown). Two-dimensional gels in which the separation is based on isoelectric focusing and SDS-PAGE (16) also show that the radioactivity co-migrates with the stained phosphodiesterase α band. In our hands, the phosphodiesterase subunits streak in the isoelectric focusing gels and the methylated protein behaves in the same manner (data not shown).

Rabbit antiserum, raised against purified rod outer segment phosphodiesterase, was used to demonstrate that the M, = 88,000 methylated protein and phosphodiesterase share common antigenic determinants. Immunoprecipitation was carried out with low ionic strength extracts of ROS (Fig. 4). The antiserum has been shown to bind purified phosphodiesterase on protein blots. Fig. 4a demonstrates that the serum will specifically precipitate the three subunits of phosphodiesterase from ROS extracts. The M, = 88,000 methylated protein is also immunoprecipitated by this antiserum (Fig. 4b, lane 3). In addition, pure phosphodiesterase will compete for the binding of the methylated protein to the antibody (Fig. 4b, lane 4). The M, = 88,000 methylated protein is, therefore, antigenically related to phosphodiesterase.

Partial peptide mapping was carried out to further examine the relationship between the methylated M, = 88,000 protein and the two large subunits of phosphodiesterase. Maps generated with S. aureus V8 protease were compared using phosphodiesterase subunits labeled by in situ translation in the presence of [35S]methionine and the M, = 88,000 protein labeled by methylation of ROS proteins by addition of [3H]AdoMet to isolated ROS membrane fragments (Fig. 5). As expected, only a small number of peptides are visible in the M, = 88,000 protein digest since only those fragments that contain [3H]methyl groups are observed. The phosphodiesterase subunits are uniformly labeled by methionine and therefore give rise to a larger number of bands. All labeled peptides generated from the M, = 88,000 methylated protein have corresponding fragments in the phosphodiesterase α pattern,
Methylation of ROS Proteins

Fig. 4. Immunoprecipitation of the $M_r = 88,000$ methylated protein by antiserum raised against pure rod outer segment phosphodiesterase (PDE). Immunoprecipitations were carried out on low ionic strength ROS extracts as described under “Experimental Procedures.” a, immunoprecipitation of $^{35}$S[Met ROS proteins to demonstrate the specificity of the antiserum. Rod outer segment proteins labeled with $^{35}$S[Met (as described under “Experimental Procedures”) were used to enhance the sensitivity of the technique. Lane 1, pooled low ionic strength extracts of $^{35}$S[Met-labeled ROS proteins; lane 2, immunoprecipitated proteins from the low ionic strength extract using anti-phosphodiesterase serum; lane 3, immunoprecipitated proteins from the low ionic strength extract using control serum. b, immunoprecipitation of the $M_r = 88,000$ methylated protein. Lane 1, methylated ROS proteins as in Fig. 1b; lane 2, low ionic strength extract of methylated ROS proteins as in Fig. 2a; lane 3, immunoprecipitated proteins from the low ionic strength extract using anti-phosphodiesterase serum; lane 4, immunoprecipitated proteins from a low ionic strength extract that contained 10 µg of additional pure phosphodiesterase using anti-phosphodiesterase serum; lane 5, immunoprecipitated proteins from the low ionic strength extract using control serum. a is a 1-day exposure, and b is a 3-day exposure of Kodak XAR-5 film. 92.5 K, for example, $M_r = 92,500$.

Fig. 5. One-dimensional partial peptide maps of $^{35}$S[Met-phosphodiesterase $\alpha$, $^{35}$S[Met-phosphodiesterase $\beta$, and $^3$H]methyl- $M_r = 88,000$ (88 K) methyl-accepting protein. Proteins were labeled and peptides generated with S. aureus V8 protease as described under “Experimental Procedures.” To achieve equivalent band intensity, $\sim 4$ µg of protein were used in lanes with protease, compared to $1$ µg in lanes without protease. After electrophoresis, the gel was fluorographed and exposed to Kodak XAR-5 film for 7 days. Note: the high molecular weight band present in each lane is an artifact due to aggregated protein that barely enters the running gel. Phosphodiesterase (PDE) seems particularly susceptible to aggregation upon drying the gel pieces.

but not in the phosphodiesterase $\beta$ pattern; the pattern of fragments generated from the digestion of phosphodiesterase $\alpha$ is clearly distinct from that of phosphodiesterase $\beta$ and analogous to that of the methylated protein. Similar results are seen by digestion of these proteins with chymotrypsin (data not shown). In addition, the $\alpha$ and $\beta$ subunits of phosphodiesterase can be distinctly separated by electrophoresis in long (~30 cm) gels. The methylated protein clearly co-migrates with phosphodiesterase $\alpha$ and not phosphodiesterase $\beta$ in such gels (data not shown). Thus, the elution behavior, mobility in a nondenaturating gel, and antigenicity suggest that one of the proteins methylated in rod outer segments is the cGMP phosphodiesterase. The mobility in a denaturing gel as well as partial peptide mapping indicate that it is the $\alpha$ subunit of phosphodiesterase.

Photoreceptor Proteins Are Carboxymethylated—Proteins
have been shown to be methylated on several different amino acid residues including lysine, arginine, histidine, glutamic acid, and aspartic acid (27). To determine whether the ROS proteins undergo N- or O-methylation, the methylated proteins were assayed for alkaline labile radioactivity. Methanol is released during the alkaline hydrolysis of methyl esters, and it can be recovered and quantitated by distillation and liquid scintillation counting (21). Fig. 6 shows the results of such experiments on ROS proteins methylated in a fragmented ROS preparation by addition of [3H]AdoMet. The radioactivity in each band detected by fluorography is alkaline labile and distillable. Similar results are obtained with proteins methylated by incubating intact retinal tissue with [3H]Met (data not shown).

Two independent techniques were employed to show that the distillable radioactivity was, in fact, methanol. The mass spectrum of the distillate from the $M_r = 21,000$ [3H]methyl protein (which had sufficient label for these studies) showed molecular ion peaks at $m/e = 34$ and 32 corresponding to tritiated and carrier methanol, respectively. Alternatively, 3,5-dinitrobenzoyl chloride was added to the distillates of the $M_r = 21,000$ and 88,000 proteins, and the radioactivity was recovered as the methyl ester of 3,5-dinitrobenzoic acid (m.p. = 106.5–108 °C) by the method of Neish (22). The results are therefore consistent with carboxymethylation of glutamate or aspartate residues of ROS proteins.

**DISCUSSION**

Incubation of rod outer segments with AdoMet results in the carboxymethylation of a set of specific proteins of photoreceptor cells. The molecular weights of the labeled bands are unique and (perhaps with exception of the $M_r = 61,000$ band) differ from methylated proteins from mammalian (rat) thymus, liver, and neural tissue (31). The photoreceptor phosphodiesterase is known to be localized in the rod outer segment (32, 33), and we feel the other methylated proteins are probably also outer segment components. This supposition is supported by the facts that methylation is carried out with isolated ROS fragments, that the intensity of labeling is roughly equivalent for these bands and reproducible from preparation to preparation, and that the proteins, when methylated in situ, co-migrate with the outer segments during purification of the ROS on sucrose gradients. Preliminary results also show the presence of a similar set of bands, excepting the $M_r = 61,000$ protein, in preparations of frog ROS; the appropriate band for phosphodiesterase $\alpha$ is also present which has a molecular weight of 95,000 in our gel system. Moreover, we observe that isolation of frog ROS results in a preparation more cleanly separated from inner segments than that of bovine ROS. We cannot at this time, however, rule out the possibility that the $M_r = 61,000$ or set of $M_r = 21,000$–26,000 proteins may be contaminants, e.g. from rod inner segments.

Although there are minor $M_r = 61,000$ and 21,000–26,000 proteins in gels of ROS, no known function has yet been assigned to them. It is, however, possible that the set of proteins with molecular weights between 21,000 and 26,000 is only one polypeptide resolved into a pattern of bands, such as that produced by the methylation of the receptor proteins in the chemotactic systems. Multiple methylation of methyl-accepting chemotaxis proteins has been shown to result in several regularly spaced bands on a gel; as a methyl-accepting chemotaxis protein is increasingly methylated, its mobility in SDS gels also increases (28–30). We hope to identify the $M_r = 21,000$–26,000 set of bands in ROS in the near future and show whether they arise by the same mechanism.

The molecular function of methylation of these ROS proteins must now be established to enable us to integrate this process into the molecular mechanisms for photoreception and transduction. Possible roles for the methylation include post-translational modification to specify the enzyme's functional state or conformation (34) or to serve as a signal for the transport or metabolic processing (35) of the protein; alternatively, the covalent modification may serve a more dynamic reversible role, perhaps in regulating the cell's response to light. That we observe O-methylation, which is a reversible modification in other systems (36, 37), instead of N-methylation is consistent with a modulatory role.

The function of the outer segment phosphodiesterase is quite well established. It has been shown to be a light-activated enzyme that functions to decrease the cytoplasmic concentration of cGMP when the cell is exposed to light (4, 5, 38). Two reports have suggested that cGMP may control the activity of protein kinases) which reversibly phosphorylate ROS proteins (39, 40), and models in which cGMP (thus phosphodiesterase) has a role in transduction have been discussed (41). It is therefore intriguing that one of the subunits of phosphodiesterase can be methylated. The methylation could influence the activity of the phosphodiesterase or might be a silent modification, i.e. one that does not directly influence the enzyme's activity. Silent phosphorylations of several enzymes have been described and functions have been pos-

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1. W. Baehr, unpublished results.
Methylation of ROS Proteins

Our attempts to demonstrate light-dark control of methylation of this protein have not been consistently reproducible (43). The level of methylation of phosphodiesterase in vitro is, however, influenced by the cGMP concentration. Using the broken ROS preparation, the number of methyl groups incorporated increases as the concentration of cGMP increases. Experiments are in progress to determine whether or not this modification is also controlled by cGMP concentrations in vivo.

Establishing methylation of proteins in photoreceptor cells increases the generalized analogies between photosensory transduction and chemotactic sensory transduction. In both cases, the presence of the appropriate stimulus, light for photoreceptors and a repellent (or attractant decrease) for chemotactic bacteria, leads to a decrease in intracellular cGMP (4, 6). In the bacterial system, this decrease is correlated with decreases in the steady state level of methylation of methyl-accepting chemotaxis proteins and a slow return of the cGMP concentration to that of the unstimulated state (6). As discussed above, the level of phosphodiesterase methylation in vitro is also influenced by the cGMP concentration. There are, however, striking differences between these two systems. In chemotaxis, it is the receptor that is modified (10). In the outer segment, there is no evidence for carboxymethylation of rhodopsin; it is the phosphodiesterase (a set of other proteins) that is methylated. Thus, how far these analogies may ultimately be pushed for explaining two very different responses from two decisively different stimuli in the photoreception and the chemotactical systems remains to be determined.

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