Regulation of Cyclic Nucleotide Concentrations in Photoreceptors: An ATP-Dependent Stimulation of Cyclic Nucleotide Phosphodiesterase by Light

(cyclic AMP/cyclic GMP/adenylate cyclase/rhodopsin/retinal rods)

NAOMASA MIKI, JAMES J. KEIRNS, FREDERICK R. MARCUS, JENNY FREEMAN, AND MARK W. BITENSKY

Department of Pathology, Yale University School of Medicine, 310 Cedar Street, New Haven, Connecticut 06510

Communicated by Lewis Thomas, August 80, 1973

ABSTRACT Regulation of cyclic nucleotide concentrations in rod outer segments (Rana pipiens) has been further examined. The present studies show that illumination markedly diminishes the concentration of cyclic nucleotides in suspensions of photoreceptor membranes, but the locus of regulation is cyclic nucleotide phosphodiesterase (EC 3.1.4.-) (light-stimulated) and not adenylate cyclase. There is a marked disproportionality between bleaching of rhodopsin and stimulation of phosphodiesterase. Bleaching only 0.6% of the rhodopsin produces half the stimulation produced by bleaching 100% of the rhodopsin. The process of activation of phosphodiesterase by light is in two steps, a light-dependent step followed by an ATP-dependent step. Illumination (in the absence of ATP) produces a trypsin-resistant, heat-labile, macromolecular stimulator. In the presence of 0.75 mM ATP (GTP or ITP) this stimulator produces a greater than 5-fold increase in the V_max of photoreceptor phosphodiesterase without changing the K_m. At physiological substrate concentrations (10^-7 M) the rate of hydrolysis of cyclic GMP is 23 times greater than that of cyclic AMP. The light-produced stimulator appears unique to the photoreceptor membranes and does not activate phosphodiesterase in other tissues.

The role of cyclic nucleotides in visual excitation has been a subject of investigation since 1970. Earlier data had suggested that light regulates cyclic nucleotide concentrations in photoreceptor outer-segment membranes (1-5). The present studies (6) confirm our earlier findings but provide a new locus for the effects of illumination. Although light regulates the amounts of retrievable newly synthesized cyclic nucleotides in suspensions of photoreceptor membranes, the mechanism of this regulation is a stimulation of cyclic nucleotide phosphodiesterase (EC 3.1.4.c) by light rather than an inhibition of adenylate cyclase. In our discussion of these earlier findings, the term “light-mediated adenylate cyclase inactivation” should more appropriately be replaced by “light-mediated phosphodiesterase activation.”

Earlier studies failed to detect light activation of phosphodiesterase because this activation depends on an unanticipated requirement for the presence of ATP. The absence of ATP from earlier phosphodiesterase reaction mixtures reflects the fact that it is not required for phosphodiesterase assay and, in fact, can competitively inhibit phosphodiesterase. We now find that ATP renders photoreceptor phosphodiesterase sensitive to a greater than 5-fold stimulation by light. This fact accounts entirely for changes in cyclic AMP (cAMP) concentrations, which we have previously recorded in photoreceptor organelles as a function of illumination. The sensitivity of phosphodiesterase to an ATP-dependent light-generated activator appears unique to the photoreceptor system. The present paper describes some properties of this light-sensitive phosphodiesterase and explains properties previously attributed to photoreceptor cyclase. Furthermore, it examines some possible mechanisms of phosphodiesterase activation by light and ATP, including the involvement of calcium or a protein kinase.

MATERIALS AND METHODS

All dark procedures (dissection, dark assay, etc.) were done under near-infrared light with a Metascope No. 9902E infrared image converter (Varo, Inc., Garland, Texas). Infrared sources were constructed from tungsten lamps and CS7/56 filters (Corning Glass Co., Corning, N.Y.). Rana pipiens were dark-adapted for 12 hr and killed; the eyes were dissected. Rod outer segments were obtained by flotation on 47% sucrose (6).

The photoreceptor membranes prepared in this way and examined by electron microscopy immediately after flotation consist of intact outer segments (judged to be more than 95% of the membranes). After the harvesting of these outer segments and their dispersion in a hypotonic medium, the predominant morphological element is a vesicle smaller than the intact disc.

The phosphodiesterase activity was assayed by incubating tritiated cAMP with the photoreceptor membranes (20-40 μg of protein per tube) at pH 7.4 and 32°C, using a reaction volume of 20 μl and an incubation time of 1 min. The buffer for phosphodiesterase assay is 50 mM Tris-HCl, 3 mM MgCl2, and 5 mM [γ-3H]cAMP (diluted to 3.5 Ci/mol, Schwartz-Mann). The reaction was stopped by boiling. Adenylate cyclase was assayed with 100-150 μg of protein per tube and an incubation time of 5 min in a reaction mixture containing 50 mM Tris-HCl buffer, 3 mM MgCl2, 0.1 mM papaverine hydrochloride, 1 mM cAMP, 0.5 mM [γ-3H]ATP (diluted to 500 Ci/mol, New England Nuclear Corp.), 2.5 mM theophylline, and a regenerating system (2 mg/ml of creatine phosphokinase and 35 mM phosphocreatine, Tris salt). Labeled cAMP was isolated by sequential chromatography in two solvents on polyethyleneimine-cellulose thin layers (6, 7). Phosphodiesterase activity with cGMP as substrate was measured in exactly the same way except for the substitution of cGMP for cAMP. After development with the first solvent, cGMP (Rf = 0.35) was easily separated from GTP, GDP, and GMP (Rf all less than 0.08). In the second solvent, cyclic GMP moved less than 0.5 cm while gua-
Table 1. Apparent inhibition of adenylate cyclase by light and recovery of cyclic AMP in adenylate cyclase assay

<table>
<thead>
<tr>
<th></th>
<th>Dark</th>
<th>Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP formed per 10 min per mg of protein</td>
<td>168 pmol</td>
<td>24 pmol</td>
</tr>
<tr>
<td>Recovery of cyclic AMP (1 mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenylate cyclase reaction mixture excluding ATP</td>
<td>85%</td>
<td>86%</td>
</tr>
<tr>
<td>Adenylate cyclase reaction mixture</td>
<td>85%</td>
<td>10%</td>
</tr>
<tr>
<td>Phosphodiesterase reaction mixture</td>
<td>30%</td>
<td>32%</td>
</tr>
<tr>
<td>Phosphodiesterase reaction mixture + 0.5 mM ATP</td>
<td>29%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Adenylate cyclase was assayed as described in the text. The cyclic AMP was purified by thin-layer chromatography and counted (6, 7). For recovery, 1 mM cyclic [3H]AMP (3 Ci/mol) was incubated for 0 or 5 min in the buffers for cyclase or phosphodiesterase assay. The labeled cAMP was purified and counted (6, 7). Percent recovery is (cAMP counts after 5 min)/cAMP counts after 0 min) × 100. The table shows the means for six determinations under each condition. The range of values for a given entry was no more than 7%.

nine, guanosine, and xanthosine all moved to a final position more than 10 cm ahead of GMP.

Protein concentrations were determined by the method of Lowry (8), and ATP concentrations by the luciferase method (9). Rhodopsin spectra were measured after dilution of photoreceptor materials 5–10 times with 1% cetyltrimethylammonium bromide. Protein kinase was assayed by the method of Kuo and Greengard (10). SQ20009 was a gift from Dr. Sidney Hess (Squibb). Purified (11) bovine-heart protein kinase was kindly provided by Dr. Paul Greengard. Purified (12) rabbit-thyroid protein kinase was a gift from Dr. Steve Spaulding. Trypsin, soy trypsin inhibitor, and phospholipase-C were obtained from Sigma Chemical Co. 1-Methyl-3-isobutylxanthine was from Aldrich Chemical Co.

Results

Evidence for Increased cAMP Destruction in the Presence of ATP and Illuminated Photoreceptor Membranes. Our previous interpretation of the data arose from the impression that recovery of cyclic nucleotides in adenylate cyclase assay of photoreceptor membrane preparations was adequate. Measurements of phosphodiesterase activity in illuminated membranes using either a phosphodiesterase reaction mixture or an adenylate cyclase reaction mixture from which ATP had been excluded, showed that recovery of labeled cAMP was better than 85%. Under these conditions the apparent light-dark difference in cyclase activity was about 7:1 (Table 1). However, using an adenylate cyclase reaction mixture which included unlabeled ATP, we have now observed the following: the recovery of cAMP in the dark is better than 85%. However, under that unique condition where ATP and light are present together, we observe a marked stimulation of phosphodiesterase activity so that only 5–10% of newly synthesized or added cAMP was recovered in spite of the presence of papaverine, theophylline, and 1 mM unlabeled cAMP (Table 1).

Nucleoside Triphosphate Requirements for the Activation of Phosphodiesterase by Light. We then examined the requirements for nucleoside triphosphate in the light-dependent stimulation of phosphodiesterase. Phosphodiesterase activity in light and dark is shown as a function of ATP concentration in Fig. 1. Half-maximal activation is seen at an ATP concentration of 0.2 mM. Inhibition (probably competitive) of phosphodiesterase activity is seen at an ATP concentration of 2.5 mM. GTP or ITP (0.75 mM) are as effective as ATP in the light-dependent phosphodiesterase activation reaction. ADP and AMP are without effect.

An aliquot of photoreceptor material was illuminated in the absence of ATP and then added (in the ratio of 2% illuminated to 98% unilluminated) to unilluminated material. If no ATP was present during assay the phosphodiesterase was not activated. If ATP was added to the unilluminated material (98% by volume) the illuminated material (2% by volume) produced full activation. If, on the other hand, 0.3 mM ATP was added to unilluminated photoreceptor material which was then bleached and added to the unilluminated

![Fig. 1. Requirement for nucleoside triphosphate in light stimulation of phosphodiesterase. Phosphodiesterase activity of illuminated (○) or unilluminated (●) rod outer-segment membranes as a function of ATP concentration. The values shown are the means of three determinations. The maximum range of values for a given point was 6%. A unit of phosphodiesterase activity is 1 μmole of cAMP hydrolyzed per min per mg of protein. The experiment was repeated three times with the same result.](image-url)

Table 2. Light-dependent and ATP-dependent steps in phosphodiesterase activation

<table>
<thead>
<tr>
<th></th>
<th>Phosphodiesterase activity (μmol of cAMP destroyed per min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ATP present</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>0.3 mM ATP present but diluted to 0.006 mM before assay</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>0.3 mM ATP present only during assay</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>0.3 mM ATP present during both bleaching and assay</td>
<td>4.2 ± 0.2</td>
</tr>
</tbody>
</table>

Phosphodiesterase activity is measured in a mixture of 2% illuminated-98% unilluminated photoreceptor membranes. The specific activity of illuminated material was 4.6 ± 0.2; that of the unilluminated material was 0.8 ± 0.1. The table shows the mean and standard error for three determinations. The experiment was repeated four times with the same results.
TABLE 3. Effect of phosphodiesterase inhibitors on the photoreceptor enzyme

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Phosphodiesterase activity (% of light or dark value without inhibitors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 100</td>
</tr>
<tr>
<td>Theophylline (0.05 mM)</td>
<td>85 95</td>
</tr>
<tr>
<td>Isobutylmethylxanthine (0.05 mM)</td>
<td>35 35</td>
</tr>
<tr>
<td>Dipiridamol (Persantine) (0.05 mM)</td>
<td>65 52</td>
</tr>
<tr>
<td>Squibb 20009</td>
<td>35 45</td>
</tr>
<tr>
<td>Papaverine</td>
<td>56 56</td>
</tr>
</tbody>
</table>

Phosphodiesterase activity was assayed by using the reaction mixture in the test plus 0.75 mM ATP. With no inhibitor specific activity in the dark is 0.6 μmol of cAMP destroyed per min per mg of protein, and in the light it is 3.5 μmol of cAMP/min per mg of protein. The percentages shown are the means for three determinations. The maximum range of a given value was 7%. The experiment was repeated twice with similar results.

Material in the usual 2.98 ratio (thus effectively diluting the ATP to below 10 μM), no phosphodiesterase activation was achieved unless additional ATP was added (Table 2).

Relationship Between Phosphodiesterase Activation and Rhodopsin Bleaching. The effects of illumination on phosphodiesterase were quantitated with mixing experiments, which had been useful in attempting to establish a relationship between rhodopsin bleaching and cyclase activity (6). A portion of the photoreceptor membranes was maximally bleached with room fluorescent light. Phosphodiesterase activity in various mixtures of illuminated and unilluminated materials was then measured. We found that half-maximal stimulation of phosphodiesterase was achieved with a mixture of only 0.6% bleached material to 99.4% unbleached (Fig. 2). If the relationship between bleaching and phosphodiesterase activity is studied by bleaching for various time intervals and then ascertaining the percent rhodopsin bleached, similar results are obtained. Light exposures that bleach about 5, 20, 50, or 100% of the rhodopsin all gave maximal phosphodiesterase activation.

Steady-State Kinetic Parameters and Substrate Preference for Phosphodiesterase. Phosphodiesterase activity as a function of cAMP concentration was examined in both light and dark. These experiments revealed that the K_m for cAMP in both light and dark was 8 mM. However the V_max was in...

TABLE 4. Effects of homogenization and p-mercuribenzoic acid on light- and ATP-dependent stimulation of phosphodiesterase

<table>
<thead>
<tr>
<th>Treatment of photoreceptor material</th>
<th>Phosphodiesterase activity (μmol of cAMP hydrolyzed per min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark with 0.75 mM ATP</td>
</tr>
<tr>
<td>None</td>
<td>0.7 0.7 3.5</td>
</tr>
<tr>
<td>p-Mercuribenzoic acid (0.1 mM)</td>
<td>0.3 0.3 0.3</td>
</tr>
<tr>
<td>Homogenization</td>
<td>3.4 3.3 3.5</td>
</tr>
<tr>
<td>EGTA (1 mM)</td>
<td>0.7 0.7 3.4</td>
</tr>
<tr>
<td>CaCl (1 mM)</td>
<td>0.7 0.6 3.4</td>
</tr>
</tbody>
</table>

Each entry represents the mean of three determinations. The variation from the mean was less than 6%.
TABLE 5. Sedimentation of rod outer-segment phosphodiesterase

<table>
<thead>
<tr>
<th>Centrifugal fraction</th>
<th>Phosphodiesterase activity (% of light or dark activity in un-fractionated material)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td>Unfractionated</td>
<td>100a</td>
</tr>
<tr>
<td>Pellet (60,000 × g, 30 min)</td>
<td>60</td>
</tr>
<tr>
<td>Pellet (60,000 × g, 30 min) resuspended and pelleted twice</td>
<td>1</td>
</tr>
<tr>
<td>Pellet (200,000 × g, 60 min)</td>
<td>83</td>
</tr>
<tr>
<td>Supernatant (200,000 × g, 60 min)</td>
<td>1</td>
</tr>
</tbody>
</table>

Phosphodiesterase activity was assayed with the reaction mixture described in the text plus 0.75 mM ATP. Specific activity, 0.64 µmol/min per mg of protein. Specific activity, 3.2 µmol/min per mg of protein. The percentages shown are the means of four experiments. The maximum range of values for a given entry was 6%.

creased more than 5-fold as a result of illumination (Fig. 3A). Phosphodiesterase activity with cGMP as a substrate has also been examined. Light increased the Vₘₐₓ for the hydrolysis of cGMP by phosphodiesterase in a manner comparable to that of cAMP. The Kₘ for cGMP is 0.16 mM both in light and dark (Fig. 3B). At physiological substrate concentrations (10⁻⁷ M) (13), the ratio of activities for the substrates (cGMP/cAMP) is 23/1.

Inhibitors of Phosphodiesterase Activity. The photoreceptor phosphodiesterase was examined for its sensitivity to these phosphodiesterase inhibitors: isobutylmethylxanthine, Squibb 20009, papaverine, dipyridamol, and theophylline. All of the inhibitors were examined at a concentration of 0.05 mM. Isobutylmethylxanthine was most effective (Table 3).

Effects of Aging, Homogenization, p-Mercuribenzoic Acid, and Ca⁺⁺ on Photoreceptor Phosphodiesterase. At 4° the photoreceptor membrane phosphodiesterase remained stable for about 3 days, after which it exhibited a gradual decrease (Fig. 4). In addition, the ability of the material to be activated by light significantly diminished by the fourth day (Fig. 4). The material was stable and light sensitive for at least 1 month at −20°. The effects of mechanical trauma on photoreceptor phosphodiesterase were examined by homogenizing the material (microhomogenizer, glass on glass, 30 strokes by hand) in the dark and then examining activity in the dark and in the light in the presence and absence of ATP. These experiments revealed that homogenization obviated the requirement for light and ATP, producing a fully activated phosphodiesterase (Table 4). Significantly, addition of 1 mM calcium or 0.5 mM EGTA to the light or dark photoreceptor material did not influence phosphodiesterase activity. Addition of 1 mM p-mercuribenzoic acid before illumination blocked the stimulation of phosphodiesterase activity by ATP and light (Table 4).

Effects of Washing or Sedimentation. The photoreceptor membranes were washed by sedimentation at 60,000 × g and resuspension in buffer twice. After this procedure, we found that the phosphodiesterase was absent from the 60,000 × g pellet. If the photoreceptor material is fractionated by a se-

TABLE 6. Some properties of the phosphodiesterase stimulator

<table>
<thead>
<tr>
<th>Treatment of illuminated photoreceptor material before mixing</th>
<th>Phosphodiesterase activity (µmol of cAMP destroyed per min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.1</td>
</tr>
<tr>
<td>Trypsin digestion</td>
<td>3.3</td>
</tr>
<tr>
<td>Phospholipase-C digestion</td>
<td>3.0</td>
</tr>
<tr>
<td>Heating (90°, 10 min)</td>
<td>0.7</td>
</tr>
<tr>
<td>Dialysis against 0.5 mM EGTA</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Phosphodiesterase activity is measured in a mixture of 2% illuminated/98% unilluminated photoreceptor membranes in the reaction mixture described in the text plus 0.75 mM ATP. The specific activity of unilluminated membranes is 0.7; that of illuminated membranes is 3.7. The values shown are the means of three determinations. The maximum range of values for a given entry was 8%.

Sequential centrifugation at 60,000 and 200,000 × g, most of the phosphodiesterase is in the 60,000 × g pellet and essentially none in the 200,000 × g supernatant (Table 5).

Characteristics of the Light-Produced Stimulator of Photoreceptor Phosphodiesterase. Stimulator was prepared by illumination of rod outer segments and assayed by addition to dark photoreceptor membranes (2% light, 98% dark). Heating of the illuminated material to 90° for 10 min resulted in destruction of the light-generated activator. However, treatment of the material with trypsin (0.12 mg/ml) for 1 hr at 25° followed by addition of soy bean inhibitor (0.24 mg/ml), or treatment with phospholipase-C (0.12 mg/ml) for 1 hr followed by dilution, had no effect on the stimulator. Extensive dialysis of the illuminated photoreceptor material against 0.5 mM EGTA did not affect its ability to stimulate phosphodiesterase (Table 6).

Effects of Protein Kinase on the Activation of Photoreceptor Phosphodiesterase. Two different protein kinases (from bovine myocardium and from rabbit thyroid) were evaluated for

![Fig. 4. Effects of storage at 4° on phosphodiesterase activity. Unilluminated material was stored at 4° in the dark and assayed with 0.75 mM ATP either before (•) or after (○) illumination. Values shown are the means of three determinations (maximum range 10%). A unit of phosphodiesterase activity is 1 µmol of cAMP hydrolyzed per min per mg of protein.](attachment:fig4.png)

Effects of Storage at 4° on Phosphodiesterase Activity. Unilluminated material was stored at 4° in the dark and assayed with 0.75 mM ATP either before (•) or after (○) illumination. Values shown are the means of three determinations (maximum range 10%). A unit of phosphodiesterase activity is 1 µmol of cAMP hydrolyzed per min per mg of protein.
their ability to mimic light in the activation of photoreceptor phosphodiesterase. Neither of the kinases was able to mimic the effects of illumination when added to the unilluminated photoreceptor material. Neither kinase stimulated phosphodiesterase in lighted photoreceptor material, and neither kinase exhibited measurable phosphodiesterase activity by itself.

Effects of Phosphodiesterase Stimulator on Other Tissues. The effect of the stimulator generated by illumination of photoreceptor membranes on phosphodiesterase in rat liver, myocardium, and brain was studied. In no instance was there a stimulation of these phosphodiesterases with or without ATP. The phosphodiesterase activity of the mixture was the sum of the phosphodiesterase activities of the photoreceptor membranes and the other tissue.

DISCUSSION

These data demonstrate that light regulates the amount of cyclic nucleotides retrieved from photoreceptor membranes by controlling the rate of hydrolysis rather than the rate of synthesis. All previous conditions that have been established for the preparation of light-sensitive photoreceptor cyclase are actually necessary for the preparation of light-stimulated phosphodiesterase (6). The observation that washing removed light-inactivation of cyclase is actually explained by the fact that washing removes phosphodiesterase. Similarly, ATP concentration requirements, the effects of light leaks, the effects of homogenization, and the effects of aging (6) are all explained in terms of a light-activated phosphodiesterase.

The properties that had previously been attributed to an inhibitor of cyclase now appear to reside in an activator of phosphodiesterase. These include resistance to trypsin and phospholipase-C, heat lability, nondialyzability, and inactivation by p-mercuribenzoic acid. In view of the fact that small amounts of activator can transform large quantities of phosphodiesterase to a fully active form, the process is probably enzymic. Furthermore, the sensitivity of this process to minute quantities of light favors its physiological significance.

The stimulation sequence can be divided into two steps. The first is light-sensitive and ATP-independent; the second involves an interaction between ATP, phosphodiesterase, and the activator generated in step one. The necessity to regard the activation sequence as a two-step process derives from the observation that nucleotide triphosphates need not be present during illumination. Their ultimate addition is necessary, however, in the absence of visible light.

The work of Yoshikami and Hagins (14) has suggested that calcium is involved in the regulation of sodium conductance in the photoreceptor system. Neufeld et al. (15) have shown that ATP can bind the calcium to bovine disc membranes. Szuts (18) has found that illumination can lower the calcium content of photoreceptor disc membranes. We have been unable, however, to show any connection between calcium and the light stimulation of phosphodiesterase in photoreceptor membranes. The photoreceptor stimulator differs in several respects from the brain phosphodiesterase activator described by Cheung (16) and by Kakiuchi (17). The brain phosphodiesterase stimulator is calcium- rather than ATP-dependent as well as trypsin-sensitive and heat-labile. It should be noted that the stimulator of photoreceptor phosphodiesterase does not stimulate the phosphodiesterase in other tissues.

The presence of a light-sensitive, ATP-dependent phosphodiesterase stimulation is a unique marker for the photoreceptor membrane system. The lower K_m for the cGMP hydrolysis and its 23-fold greater velocity at physiological cyclic nucleotide concentrations suggest that cGMP participates in the regulation of photoreceptor function at least in Rana pipiens. Our data do not permit us to distinguish whether we have been dealing with two or one phosphodiesterase, but the data clearly show that photoreceptor phosphodiesterase is light-activated with either cAMP or cGMP as substrate. Although extrinsic protein kinases do not activate photoreceptor phosphodiesterase, a role for an intrinsic kinase in the activation sequence is not excluded.

Three questions remain in the elucidation of the role of cyclic nucleotides in photoreceptor physiology. The first is the identity of the link between photon capture and the generation of the activator; the second is the biochemical identity of the activator; and the third is its mode of action. The answers to these questions will depend on the purification of photoreceptor phosphodiesterase and on isolation of the activator.

The importance of cyclic nucleotides in the regulation of photoreceptor function is supported by these findings, which confirm the earlier demonstration that light regulates their concentrations in the rod outer segment. On the basis of these data, the locus of this regulation is the phosphodiesterase whose activation by light is dependent on the presence of nucleotide triphosphate and whose preferred substrate (in Rana pipiens) is cGMP.

This work was supported by USPHS Grants AM15016 and CA13444, by American Cancer Society Grant BC-106C and by the grant from the Jane Coffin Childs Memorial Fund for Medical Research. J.J.K. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.