Phosphorylation of G-protein-coupled receptors (GPCRs) is a required step in signal deactivation. Rhodopsin, a prototypical GPCR, exhibits high gain phosphorylation in vitro whereby a hundred-fold molar excess of phosphates are incorporated into the rhodopsin pool per molecule of activated rhodopsin. The extent by which high gain phosphorylation occurs in the intact mammalian photoreceptor cell, and the molecular mechanism underlying this reaction in vivo, is not known. Trans-phosphorylation is a mechanism proposed for high gain phosphorylation, whereby rhodopsin kinase, upon phosphorylating the activated receptor, continues to phosphorylate nearby nonactivated rhodopsin. We used two different transgenic mouse models to test whether trans-phosphorylation occurs in the intact photoreceptor cell. The first transgenic model expressed a murine cone pigment, S-opsin, together with the endogenous rhodopsin in the rod cell. We showed that selective stimulation of rhodopsin also led to phosphorylation of S-opsin. The second mouse model expressed the constitutively active human opsin mutant K296E. K296E, in the arrestin background, also led to phosphorylation of endogenous mouse rhodopsin in the dark-adapted retina. Both mouse models provide strong support of trans-phosphorylation as an underlying mechanism of high gain phosphorylation, and provide evidence that a substantial fraction of nonactivated visual pigments becomes phosphorylated through this mechanism. Because activated, phosphorylated receptors exhibit decreased catalytic activity, our results suggest that dephosphorylation would be an important step in the full recovery of visual sensitivity during dark adaptation. These results may also have implications for other GPCR signaling pathways.

Visual pigments, such as rhodopsin and cone opsins, belong to a family of G-protein-coupled receptors (GPCRs) that contain a cluster of Ser/Thr sites at their carboxyl termini. Visual pigments initiate G-protein signaling upon photon absorption. As with other GPCRs, phosphorylation of the carboxyl-terminal Ser/Thr sites, followed by arrestin binding, are required steps in signal deactivation (1). Rhodopsin phosphorylation is catalyzed by rhodopsin kinase (GRK1 or RK), which is activated upon association with light-activated rhodopsin (R*) in the MII conformation (2, 3). In vitro and in vivo evidence shows that phosphorylated rhodopsin exhibits diminished catalytic activity (4–7) and that arrestin binding is required to fully terminate R* signaling (5, 8). Since the discovery of light-activated rhodopsin phosphorylation, a number of studies have reported that, in isolated rod outer segments, several hundred-fold molar excess of phosphates are incorporated into the rhodopsin pool per mol of R* (9–12). Given that each rhodopsin has been observed to incorporate only up to nine phosphates (13), the straightforward interpretation is that nonactivated rhodopsin molecules, which we designate here as R, are phosphorylated as well as R*. This phenomenon has been termed high gain phosphorylation (10). Phosphorylation of nonactivated rhodopsin has also been reported in isolated frog retinas and in living frogs (11). In this system, ~1% of R become phosphorylated following a 3% bleach (i.e. photon absorption by 3% of rhodopsin), and, when living frogs are exposed to prolonged dim light, a higher fraction of R (3%) becomes phosphorylated (11).

The mechanism by which R becomes phosphorylated in vivo is not known. One possibility is that the phosphorylated MII decays to opsin and is reconstituted with the 11-cis-retinal to regenerate a visual pigment prior to dephosphorylation (14). Another possibility is trans-phosphorylation, which was termed to describe a putative mechanism wherein rhodopsin kinase (RK), once activated by associating with R*, phosphorylates a nearby R (15). Evidence in support of this model includes the observation that R*-activated RK is capable of phosphorylating an exogenously added peptide substrate (16–19). In addition, structural studies on the carboxyl terminus of rhodopsin, using site-directed spin labeling, revealed that it is disordered and highly mobile (20). Therefore, given the high density of rhodopsin and its high rotational (21) and lateral (22, 23) diffusion rate on disk membranes, it is plausible that carboxyl termini from neighboring R molecules would be accessible to R*-activated RK.

A major challenge in attempting to demonstrate the presence of trans-phosphorylation is the difficulty in unambiguously distinguishing between phosphorylated R* and phosphorylated R in the same reaction mixture. As a direct test of trans-phosphorylation, Rim et al. (15) designed experimental protocols based on a recombinantly expressed split receptor mutant of rhodopsin that was assembled from two separately expressed fragments. This “split rhodopsin” exhibits light-dependent phosphorylation. More importantly, it can be distinguished from full-length rhodopsin in the same phosphorylation mixture by virtue of its distinct electrophoretic mobility on a denaturing gel. Therefore, the split rhodopsin can be exposed to light and mixed with the nonbleached full-length rhodopsin, or vice versa, to test for the presence of phosphorylated R in the same mixture that contains RK. However, despite numerous attempts using different experimental configur-
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The experiments described above were performed using solubilized receptors and receptors reconstituted into lipid vesicles. However, if the activated kinase needs to be physically associated with R*, or if it can diffuse only a short distance prior to phosphorylating nearby nonactivated R, then trans-phosphorylation may proceed efficiently only on native disk membranes that contain high concentrations of freely diffusible receptor molecules. To test this hypothesis, we generated transgenic mice that expressed a shortwave-sensitive cone pigment, S-opsin, in rod photoreceptors. This system allowed for the preferential activation of rhodopsin by long wavelength light when the two pigments are co-expressed in the native rod disks, and phosphorylation of S-opsin would arise only as a result of trans-phosphorylation. Furthermore, we used the rhodopsin K296E transgenic mouse model, expressing human opsin mutant K296E in mouse rod photoreceptors, to provide additional evidence of the presence of trans-phosphorylation.

EXPERIMENTAL PROCEDURES

S-opsin and K296E Transgenic Mouse Lines—All mice were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research, as well as with University of Southern California IACUC guidelines. S-opsin transgenic mice were mated with rhodopsin knock-out mice (24) to generate S-opsin<sup>41185</sup>−/+ and S-opsin<sup>rho</sup>−/− mice. K296E transgenic mice were bred with arrestin knock-out mice to obtain K296E<sup>arr</sup>−/− mice (8, 25). The S-opsin transgenic mice were born and raised in a 12-h/12-h light/dark cycle. K296E<sup>arr</sup>−/− and arr−/− mice were born and raised in constant darkness to prevent retinal degeneration resulting from constitutive signaling (8).

Standard Peptides—The nonphosphorylated and monophosphorylated peptides corresponding to rhodopsin and S-opsin carboxyl-terminal sequences released by Asp-N cleavage were synthesized by Biomer Technology (Concord, CA). The monophosphorylated S-opsin carboxyl-terminal peptide contained <sup>15</sup>N at the amino-N of residues Val-335 and Val-338. The monophosphorylated rhodopsin carboxyl-terminal peptide had <sup>15</sup>N at the amino-N of Ala-337 and Ala-346 sites. The detection efficiency of each peptide species was determined by integrating the area under each peak on the mass chromatogram, normalized against peptide quantity measured by its A<sub>205 nm</sub> absorbance from the UV-visible chromatogram.

Light Stimulation and Sample Preparation for LC-MS—The light source was a 100-watt quartz tungsten halogen lamp connected to a liquid light guide (Oriel Instruments, Stratford, CT). Light stimulation was controlled by neutral density filters, interference filters (Oriel Instruments), and an electromagnetic shutter (Vincent Associates, Rochester, NY). Light intensity was measured using a calibrated photodiode (United Detector Technology Sensors, Inc., Hawthorne, CA) positioned at an equal distance to the retina. The current was measured using a current amplifier (SR570 current preamplifier; Stanford Research Systems, Sunnyvale, CA). The sample preparation was modified based on published protocol (26). Dissected retinas were either kept in darkness or exposed to calibrated light for 30 s and incubated in darkness for a period of time. The retinas were homogenized in 700 μL of urea buffer (7 M urea, 5 mM EDTA, 20 mM Tris-HCl (pH 7.4)). The retinal membranes were digested with 50 μL of 10 μg/mL Asp-N protease (Roche Applied Science) in 10 mM ammonium bicarbonate (pH 8.0) at room temperature overnight. The peptides were then acidified with formic acid to pH <2 and stored at −20°C.

LC-MS—Twenty-μL peptide samples were loaded onto a C18-capillary column (Thermo Finnigan) in 0.08% heptfluorobutyric acid at a flow rate of 5 μL/min. The peptides were separated by a 5–30% acetonitrile gradient. The eluent was delivered to an LCQ Deca XP mass spectrometer (Thermo Finnigan) to record the full MS and MS/MS spectra. The fragmentation parameters used to break the parent ions by collision-induced dissociation were as follows: activation amplitude of 35%, activation Q of 0.25, and activation time of 50 ms. Rhodopsin or S-opsin carboxyl-terminal peptides were monitored in the mass detector with an isolation width of 1.5 centered on the targeted m/z values of rhodopsin or S-opsin carboxyl-terminal peptides released by Asp-N digestion. The most abundant ion of each peptide was monitored. Identities of ion peaks on the mass chromatogram were confirmed by their MS/MS spectra. After correcting for the detection efficiency of each peptide species, the area under each peak was integrated to quantify amount of peptide.

Isoelectric Focusing—The preparation of retinal sample and IEF gel electrophoresis to separate phosphorylated rhodopsin species was performed as described previously (27). After separation on the IEF gel, the proteins were transferred to a nitrocellulose membrane by capillary forces. Rhodopsin species were detected by Western blot using monoclonal antibody 4D2, which recognizes the amino-terminal domain of rhodopsin (28). Procedures used in separating phosphorylated S-opsin species were similar to those used for rhodopsin, except that the pH gradient of IEF gel used to resolve phosphorylated S-opsins was pH 3–10, instead of pH 3–8, as was the case for rhodopsin. After transferring the proteins to nitrocellulose membrane, phosphorylated S-opsin species were detected by Western blot using an antibody raised against the amino terminus of S-opsin (29).

Mathematical Simulations of Trans-phosphorylation—The simulations were performed on a 200 × 200 square array of rhodopsin. These rhodopsin molecules were arranged as if on a grid and were able to trans-phosphorylate their neighbor to the north, south, east, and west. The array assumed a random distribution composed of 86% rhodopsin and 14% S-opsin (determined by rhodopsin and S-opsin expression level in S-opsin<sup>bmo</sup>−/+ retinas). A small 20 × 20 portion of this grid is illustrated in the top panel of supplemental Fig. S1. We denoted the subset of molecules corresponding to rhodopsin as R. Initially, a random proportion, p, of R, was chosen to become light-activated and phosphorylated. Given the particular value of p used for this iteration, the exact proportion of rhodopsin that would become phosphorylated was chosen by randomly sampling from the entire population of R (supplemental Fig. S1, middle). We denoted this set as R<sub>p</sub>. Each iteration had a different p value chosen at random so that it fell between 0 and 1. Subsequently, we chose a random subset of molecules and labeled them S, corresponding to the proportion of S-opsin. These were chosen so that the final proportion of S equaled 0.14 (supplemental Fig. S1, top and middle). There was no overlap between subsets P and S. Next, we simulated a process wherein each member of P randomly selected just one of its neighboring molecules to become phosphorylated (supplemental Fig. S1, bottom). If the chosen molecule was type S, it is designated as SIP. If the chosen molecule was type R, it became RIP. We recorded the total number of molecules in S that underwent this latter conversion process and became SIP. Simulations were also performed where each member of P was able to cause phosphorylation of two or three neighbors.

We repeated this simulation process 2,500 times for each value of p in order to obtain an accurate estimate of the average number of S molecules that were converted to SIP status. If the mean proportion of such molecules over the course of these 2,500 replications came close to the target proportion of SIPs, denoted as s<sub>p</sub>, which is estimated experimentally, we recorded the value of p that generated this data set. The entire procedure was repeated 5,000 times, which led to a set of accepted p values that are close to the target proportion.
values, and yielded a report on the mean of these p values. Subsequently, we used this value to calculate the number of rhodopsin molecules that became RIP, which includes members of both subset P, and RIP resulted from neighbor selection by P.

RESULTS

We sought to investigate whether RK, once activated by R*, would phosphorylate neighboring unbleached visual pigments in native disk membrane by a trans-phosphorylation mechanism. To address this question, transgenic mice were generated that expressed S-opsin (a mouse cone pigment) in rod photoreceptors expressing endogenous rhodopsin. More importantly, S-opsin is a native substrate for RK in murine cones (30). Characterization of these mice showed that ectopically expressed S-opsin localized exclusively to the rod outer segment (29), produced light responses,3 and was capable of undergoing light-stimulated phosphorylation (see below). Therefore, S-opsin appears to be correctly folded and functional in transgenic rods.

Detection of Rhodopsin and S-opsin Phosphorylation after Activation by 360–420 nm Light—To detect phosphorylation of S-opsin and rhodopsin at their carboxyl termini, we adopted a mass spectrometry-based method described by Kennedy et al. (26). Briefly, treatment of unre-washed retinal membranes with endoproteinase Asp-N released the carboxyl-terminal peptide that contains all (6/6) or most (8/9) of the Ser/Thr phosphorylation sites on rhodopsin and S-opsin, respectively, from the disk membranes. The solubilized peptide mixture, including nonphosphorylated and phosphorylated peptides, was separated by reverse-phase chromatography and detected by a nanospray ionization trap mass spectrometer (Thermo Finnigan DECA XP) at their specific mass-to-charge (m/z) ratios. Synthetic peptides derived from rhodopsin and S-opsin carboxyl-terminal sequences were used to determine their HPLC elution profiles and to calculate the ionization efficiency of each peptide (Fig. 1). Transgenic mice expressing S-opsin were crossed into rhodopsin/R1P — rhodopsin carboxyl-terminal peptide monophosphorylated at site 343; doubly charged; m/z = 974.0; SOP (nonphosphorylated rhodopsin carboxyl-terminal peptide; doubly charged; m/z = 933.1; S1P (S-opsin carboxyl-terminal peptide monophosphorylated at site 328; doubly charged; m/z = 1099.9); and SOP (nonphosphorylated S-opsin carboxyl-terminal peptide; triply charged; m/z = 706.3). RIP synthetic peptide contains 15N isotopes at amino-N of Ala-337 and Ala-346 sites, and S1P synthetic peptide contains 15N isotopes at amino-N of Val-335 and Val-338 sites. Therefore, their m/z values are 1 atomic mass unit greater than those of wild-type peptides obtained from retinal samples (as in Fig. 2 and Fig. 3). ROP and SOP synthetic peptides have the same m/z as wild-type peptides. Of each type of peptide, the most abundant ion with specific charge and m/z was monitored in a narrow mass window of 1.5 Da. The x axis is time course of HPLC elution; the y axis is ion intensity. The area of integrated peak was normalized against a loaded peptide amount measured by its absorption at 205 nm to derive the relative detection efficiency of each peptide. The numbers beside each shaded peak represent the number of ions detected prior to normalization for ionization efficiency. This number is proportional to the quantity of each ion species. When an equal amount of peptide was loaded, the area reflected the sensitivity of the mass spectrometer to each of the peptides. We estimated the relative detection efficiency of each peptide, when normalized to SOP, to be 0.41/0.63/0.34/1 for RIP/ROP/S1P/SOP, respectively. These ratios were used to calculate the amounts of each peptide in the retinal samples.

These data indicate that the ectopically expressed S-opsin is correctly folded and is a substrate for RK in native rod disk membrane.

S-opsin Becomes Trans-Phosphorylated Following Activation of Rhodopsin by Long (515–620 nm) Wavelength Light—Murine S-opsin has maximum absorbance in the ultraviolet range (357 nm (31, 32)). At longer wavelengths, its absorbance falls precipitously, and at λ ⩾ 515 nm, photon absorption by S-opsin is 103-fold less efficient than rhodopsin (31, 32). On the other hand, at the peak absorption of S-opsin, rhodopsin can be readily activated, albeit at ∼25% of its maximum sensitivity at 500 nm (32). We utilized the large separation of spectral sensitivity at long wavelengths to selectively stimulate rhodopsin, and we sought to determine whether S-opsin becomes phosphorylated as a consequence of rhodopsin activation.

S-opsinRho+− retinas were exposed to long wavelength (515–650 nm) light, causing an estimated ∼100% bleach of rhodopsin, and incubated in darkness for 10 min (Fig. 3). As expected, long wavelength light exposure led to efficient phosphorylation of rhodopsin (Fig. 3A). When S-opsin was expressed in the absence of endogenous rhodopsin, it did not become phosphorylated after exposure to 515–620 nm light (Fig. 3B), verifying our expectation that S-opsin would not be efficiently activated by long wavelength light under our experimental settings. Most interestingly, when retinas co-expressing S-opsin and rhodopsin were exposed to long wavelength light, a substantial fraction of the carboxyl terminus of S-opsin became phosphorylated (Fig. 3C), indicating that nonphotolyzed pigments can serve as substrate for activated RK.

3 V. Kefalov, G. W. Shi, K.-W. Yau, and J. Chen, unpublished results.
The extent of S-opsin that becomes indirectly phosphorylated (trans-phosphorylation) was examined at different times after bright light exposure that was sufficient to bleach 100% of rhodopsin (Fig. 4A). Under this condition, the concentration of RK became rate-limiting so that rhodopsin phosphorylation continued to increase as a function of time in dark incubation (26, 33). Fig. 4A illustrates the proportion of phosphorylated peptides normalized to the amount of each pigment population. Although the level of singly phosphorylated rhodopsin reached a steady state at 2 min, the level of S-opsin phosphorylation continued to increase thereafter, and at 10 min, the proportion of monophosphorylated S-opsin reached the same relative level as rhodopsin. The lag of S-opsin phosphorylation behind R* phosphorylation suggests that R* is the preferred substrate for RK; as all available R* is phosphorylated, RK becomes available to phosphorylate neighboring R.

If R* is the preferred substrate, then the relative amount of trans-phosphorylation would likely be higher under low bleach conditions that generate fewer R*. This notion was tested by comparing the levels of S-opsin phosphorylation under different light intensities. As shown in Fig. 4B, the level of S-opsin phosphorylation was minimal shortly following 100% bleach of rhodopsin. This fraction increased 2-fold following a longer period of dark incubation as more R* deactivated. When the retina was exposed to 1.2% bleach, the relative amount of trans-phosphorylation was substantially higher at the earlier time point and remained stable over time (Fig. 4C). In fact, as much as 7% rhodopsin...
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We used mathematical simulations to estimate the fraction of R that becomes trans-phosphorylated. Simulations were based on the experimentally derived value of S-opsin expression level and the amount of S-opsin that became trans-phosphorylated following light stimulation that reached ~1.2% of rhodopsin. By using mass spectrometry, we determined that the amount of trans-phosphorylated S-opsin was ~7.5% of total S-opsin after 1.2% bleach of rhodopsin (Fig. 4C). Based on our simulations, we estimated that 4.9% of R would be trans-phosphorylated, assuming that rhodopsins and S-opsins were equally good trans-phosphorylation substrates. This value was in close agreement with the experimentally assessed value (7.1 ± 2.9%) of the total pool of monophosphorylated rhodopsin; this included directly phosphorylated R* and trans-phosphorylated R (1.2 and 4.9%, respectively). It should be noted that these values underestimated the total number of phosphorylated pigments because only monophosphorylated species were monitored. Nevertheless, they represented an approximation of the underly-
mouse rhodopsin in the dark-adapted K296E/arrestin knock-out retina would be an indication of trans-phosphorylation initiated by the active K296E molecule. The carboxyl-terminal peptide of mouse rhodopsin has an m/z value different from that of human rhodopsin; thus, phosphorylation of human and mouse rhodopsin could be unequivocally determined by LC-MS detection of the respective carboxyl-terminal phosphopeptides. As shown in Fig. 6A, no differences in endogenous mouse rhodopsin phosphorylation were observed when comparing dark-adapted retinas obtained from K296E transgenics and wild-type mice (Fig. 6A). This result is consistent with the observation that K296E is stably inactivated by arrestin binding and, therefore, is incapable of further activating RK (25). To unmask the constitutive activity of K296E, this transgene was crossed into the arrestin background. Indeed, in the absence of arrestin, the constitutive activity of K296E led to readily detectable phosphorylation of endogenous mouse rhodopsin (Fig. 6B). As expected, rhodopsin phosphorylation in the dark was not affected by the absence of arrestin (Fig. 6B). Although trans-phosphorylation was observed only when K296E was placed in the arrestin−/− background, these results nevertheless provide a proof of principle that an activated rhodopsin molecule will lead to trans-phosphorylation of nonactivated visual pigments in vivo.

DISCUSSION

Phosphorylation of nonactivated rhodopsin has been observed in isolated rod outer segment preparations, as well as in living frogs (9–12). However, the mechanism and the extent by which this happens in intact photoreceptor cells of mammals have not been addressed. In this study, we sought to investigate whether unbleached visual pigments become phosphorylated by RK through a trans-phosphorylation mechanism. We utilized the following two transgenic mouse models: one that expressed a cone shortwave pigment, and one that expressed a constitutively active human opsin mutant (K296E) in the rod photoreceptors. These mouse models enabled us to monitor specifically the phosphorylation of unbleached visual pigment, as well as R* molecules, in their native environment, and each model provided independent evidence in strong support of trans-phosphorylation as a mechanism for high gain phosphorylation.

To detect the phosphorylation of visual pigments at their carboxyl termini, a mass spectrometry method was employed. We chose to monitor monophosphorylated peptides in order to report the presence of phosphorylation events because the levels of monophosphorylated species were more readily detectable and quantifiable in our experiments as compared with multiply phosphorylated peptides. Thus, the values we obtained from this method represented an underestimation of the total number of phosphorylated pigment molecules, especially under the conditions after 100% bleach; in such instances, the results from IEF showed that monophosphorylated species arising from phosphorylated R and R* may represent only 1/6 of all of the phosphorylated R*/R molecules. At lower bleach (1.2%), however, monophosphorylated species appeared to be the more abundant species. Therefore, the estimates derived at this light level may be more accurate. The IEF results also provide evidence that trans-phosphorylation produces highly phosphorylated R that, upon photosomerization, will give rise to a diminished response.

Our results suggest that multiple nonactivated rhodopsin molecules become trans-phosphorylated for each R* generated. Given the high diffusion rate of rhodopsin along the lipid bilayer (21–23), one can envision the R*/RK complex making contact with several nonactivated rho-
dopsin molecules and phosphorylating their flexible carboxyl termini. Another possibility is that, once activated by associating with an R*, RK may be able to diffuse some distance away and phosphorylate R. The dependence of the gain of the phosphorylation reaction on the integrity of the rod outer segment preparation as well as the inability of Rim et al. (15) to observe trans-phosphorylation in solubilized proteins are two lines of evidence against freely diffusible, active RK (10). This does not, however, rule out the possibility that active RK may be able to diffuse short distances alone. Future experiments would be required to clearly distinguish between these possibilities.

The highest level of trans-phosphorylation under our experimental conditions, as indicated by S-opsin phosphorylation, was seen after 10 min of dark incubation following exposure to intense light that caused 100% bleach of rhodopsin. In this instance, up to 20% of the entire population of S-opsin became phosphorylated. Although we did not explore the lighting condition that caused the highest level of trans-phosphorylation, our results suggest that a substantial fraction of non-activated visual pigments can be phosphorylated by this mechanism. Besides trans-phosphorylation, another source of light-responsive, phosphorylated rhodopsin may be from regeneration of phosphor-opsin that has not yet been dephosphorylated. This is not unlikely, given the apparent slow rate of dephosphorylation (38). Therefore, it is possible, if not likely, that the level of phosphorylated, nonactive visual pigments per photon absorbed may be higher in vivo where regeneration of rhodopsin is supported. Inasmuch as trans-phosphorylation of S-opsin by R* was assayed using isolated retinas, our system did not report phosphorylated R from this additional pathway.

What may be the physiological consequence of photoactive, phosphorylated visual pigment? It should be noted that, in a fully dark-adapted rod cell, suction electrode recordings have shown that amplitude saturation was reached upon a flash of light that excited ~80 rhodopsin molecules, as well as upon ~400 R*/s under steady background light (39). Under the latter condition, up to 5% R* can accumulate in 10 min given the lag of the phosphatase activity. Therefore, it is plausible that under certain steady state lighting conditions, trans-phosphorylation may have an impact on decreasing transduction gain and thereby extending the range of rod response.

Another process by which trans-phosphorylation may have a profound impact takes place when the retina switches from photopic (cone) vision to scotopic (rod) vision. The switch between photopic and scotopic vision is a process crucial to our ability to detect visual cues within the wide variation of environmental illumination. During the switch from cones to rods, a period of dark adaptation is required for the rod cell to recover maximal sensitivity after a high bleach. Our data indicate that the trans-phosphorylation reaction continues long after light exposure. Because phosphorylated R* catalyzes transducin activation less efficiently, but is deactivated more efficiently, photoisomerization of these phosphorylated Rs is expected to give rise to a response with a slowed rising phase, a decreased amplitude, and a shorter duration and hence, decreased sensitivity during the period of dark adaptation. It is also important to consider that the presence of phosphorylated R would be expected to affect the reproducibility of the single photon response. It is well documented that the invariant shape and size of the elementary light response are salient features of rods that underlie their ability to encode our visual scene under dim light (40). In this situation, dephosphorylation of R is expected to be an important step in fully restoring visual sensitivity during dark adaptation.

Our results indicate that rhodopsin supports trans-phosphorylation because of its high packing density (41, 42) and its ability to encounter other nonactivated visual pigment molecules during its catalytic life.
Light Causes Phosphorylation of Nonactivated Visual Pigments in Intact Mouse Rod Photoreceptor Cells

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