In recent years, the combination of classical electrophysiological techniques and targeted gene manipulations have provided for novel, powerful ways to address key questions in visual transduction. Electrophysiological techniques allow precise characterization of the electrical and chemical events that underlie phototransduction while gene manipulations allow specific modifications of the molecules of the transduction cascade. A particularly elegant example of how these techniques can complement one another is reported in the article by Sakurai et al. on p. 21 of this issue. To investigate the contribution of the different and unique properties of rod and cone visual pigments, Sakurai et al. generated mice in which the mouse green cone opsin gene was “knocked into” the rhodopsin gene locus, so that the mouse green cone pigment was expressed in rods, either alone (mG) or coexpressed with rhodopsin (Rh/mG). These manipulations left the other participants in the rod transduction cascade unchanged, allowing evaluation of the distinct contributions of each of the two visual pigments to the overall physiological properties of the photoreceptor.

Vertebrate visual transduction is perhaps the most studied of all of the G protein–coupled receptor signaling cascades. A host of physiological and biochemical experiments have been performed over the past 30 years to investigate the details of this cascade. The application of methods in molecular genetics has complemented these classical approaches, and has allowed mechanistic questions that were beyond the reach of the classical methods to be addressed.

To appreciate the utility of the molecular genetic approach in dissecting the contributions of the different actors in the visual transduction cascade it is useful to consider a schematic of the process (Fig. 1).

Transduction starts with the absorption of light quanta by visual pigments located in membrane structures within the outer segments of rod and cone photoreceptors. Both rod and cone pigments are opsins, members of the superfamily of seven transmembrane helix G protein–coupled receptors (for review see Nathans, 1987). The ligand and chromophore, 11-cis retinal (in land-based vertebrates), is covalently bound to opsin via a protonated Schiff base bond to the ε-amino group of a conserved lysine in the seventh transmembrane segment of opsin. Photon absorption triggers a rapid photoisomerization of the chromophore from the 11-cis to the all-trans form to produce an activated form, Meta II (Hubbard and Kropf, 1958). Meta II then catalyzes the exchange of GDP for GTP on the α-subunit of the G-protein transducin to produce a second activated form G*. Each activated transducin can then bind to the γ-subunit of a phosphodiesterase to produce G*-PDE. This complex catalyzes the enzymatic destruction of cGMP, which results in the closure of cation channels in the photoreceptor outer segment membrane. Channel closure causes membrane hyperpolarization and a decrease of transmitter release by synaptic processes connected to secondary retinal neurons (Koutalos and Yau, 1993; Yau, 1994; Ebrey and Koutalos, 2001; Fain et al., 2001; Burns and Arshavsky, 2005).

Just as important as the activation is the deactivation of the excited forms of Meta II, transducin, and PDE (McBee et al., 2001; Lamb and Pugh, 2004). Without timely termination of each of these steps, it would be impossible for the retina to sense the complex and rapidly changing visual information generated by images moving across the retina. Deactivation starts with the quenching of activated Meta II, which in rods in intact retina has a spectroscopically measured lifetime on the order of minutes to tens of minutes (Baumann, 1972; Brin and Ripps, 1977; Kibbelbek et al., 1991). However, physiological measurements show that its effective lifetime is no longer than a few seconds and possibly much shorter (Pepperberg et al., 1992; Lyubarsky and Pugh, 1996; Matthews, 1996; Murnick and Lamb, 1996). This short effective lifetime of Meta II in rods is due to the rapid phosphorylation of Meta II by rhodopsin kinase (GRK1) and subsequent binding to arrestin (Arr1), a capping protein that effectively terminates activation (Hofmann et al., 1992; Arnis et al., 1994).

The decay of Meta II in cones is considerably more rapid than in rods (Imai et al., 1995, 1997; Ala-Laurila et al., 2006), but the factors that regulate the lifetime of Meta II in cones, and its effects on photoresponses, are less clear. In cone photoreceptors, Meta II is phosphorylated by two different but related kinases (GRK1 and
concentration of rhodopsin in rod membranes in order to demonstrate by measurements of photoresponses of S- and M-cone photoreceptors in Ndt<sup>−/−</sup>/Grk<sub>1</sub><sup>−/−</sup> mice, which showed that the recovery of flash responses was considerably slowed compared with responses measured in wild-type mice (Nikonov et al., 2005).

Another important step is the deactivation of activated transducin. This occurs as the GTP bound to the activated transducin-phosphodiesterase complex (G<sup>α</sup><sub>E*</sub> complex) is hydrolyzed to GDP. Intrinsic GTPase activity is accelerated by transducin’s binding to a GTPase activating protein complex (GAP) that includes a regulator of protein signaling, RGS9, together with its obligatory subunit, Gβ5 (for review see Burns and Arshavsky, 2005). In rods, RGS9 regulated GTPase deactivation of the G<sup>α</sup><sub>E*</sub> complex is rate limiting for inactivation (Krispel et al., 2006). RGS9 is expressed at high levels in bovine cone photoreceptors (Gowan et al., 1998), and it is required for normal inactivation of mouse cone phototransduction (Lyubarsky et al., 2001), but it is not yet known whether this step is rate limiting for the recovery of cones.

The light-activated hydrolysis of cytosolic cGMP by the activated G<sup>α</sup>-PDE<sup>α</sup> complex results in a closure of cGMP-gated ion channels leading to a decreased Na<sup>+</sup> and Ca<sup>2+</sup> influx, which in conjunction with the continued Ca<sup>2+</sup> efflux from the outer segment via the Na<sup>+</sup>-Ca<sup>2+</sup> antiporter, causes cytosolic [Ca<sup>2+</sup>] to decrease. This decrease triggers a number of important adaptive changes (for review see Fain et al., 2001), including an increase in the guanylyl cyclase rate resulting in increased cyclic GMP synthesis, an increase in phosphorylation of rhodopsin due to a decrease in calcium-bound recoverin, and an increase in the sensitivity of the cGMP channel via the calcium binding protein, calmodulin. All of these mechanisms appear to be important for regulating response recovery in rods as well as cones (for review see Burns and Arshavsky, 2005).

As the field of molecular genetics has expanded, the number of tools that can be used to investigate the specific elements and mechanisms in this complex scheme also has expanded. As applied together with electrophysiological investigations, the methods that have been used can be divided into distinct classes. One approach is to knock out a gene responsible for a product that is important to the function of the cascade, but not critical to its overall operation. One can then test for perturbations in physiological responses that result. For example, knockouts of the genes for rhodopsin kinase (GRK1) (Chen et al., 1999), arrestin (Xu et al., 1997), and RGS9 (Chen et al., 2000) have been expressed in mouse retina and the physiological effects measured. In all cases, responses were slower to recover, as expected. In knockout experiments of a slightly different type, a hemizygous knockout of rhodopsin was used to decrease the concentration of rhodopsin in rod membranes in order to demonstrate that membrane protein diffusion sets the speed of rod phototransduction (Calvert et al., 2001). An additional use of the knockout technology was that of knocking out rod transducin in mice in order to isolate cone photoreceptors for electrical recording (Nikonov et al., 2006). Without rod transducin, rods were no longer functional, thus isolating cone photoreceptors for electrophysiological studies. Similarly, knockouts of most of the visual retinoid cycle proteins have been created, including RH11, IRBP, LRAT, RPE65, and RDH2, though a summary of the effects is beyond the scope of this commentary.

Although the knockout approach has been very effective in addressing the physiological roles of many players in the visual transduction cascade, its utility is limited in the study of the central players, such as the visual pigment or the G protein. Elimination of these proteins results in complete elimination of the light response. Here, the gene knockin approach is an effective alternative. This method has the advantage that the wild-type protein can be totally eliminated by substitution of another form of the same protein or sometimes to good effect, the wild type and another form of the protein can be co-expressed and studied within the same cells. The report by Sakurai et al. is an outstanding example of the power of this latter approach. This study combines single-cell recording and membrane noise analysis with molecular biological techniques, to which the authors added an arsenal of other biochemical techniques—including measurements of the relative and absolute expression levels of the pigment, spectroscopy of the expressed visual pigments, histology of the mutant mouse retinae, biochemical measurements of the levels of expression of other transduction proteins (α-transducin, phosphodiesterase [α and β], and rhodopsin kinase [GRK1]), and binding assays to assess the levels of transducin activation. The concerted use of all of these techniques sets a new benchmark for multidisciplinary studies to examine retinal function at the cellular level.

There are two principal conclusions from the experiments in which the green cone pigment was substituted for rhodopsin in rods. The first is that the amplitude of the single-photon response (the reduction in the receptor current produced by the absorption of a single photon by the green cone pigment) is one third of that produced by a photon captured by rhodopsin. The second is that the rate of thermal activation of the mouse green pigment in situ in darkness is 1.7 × 10<sup>−7</sup> s<sup>−1</sup>. This rate is ~860-fold higher than that of rhodopsin. Both of these differences can be attributed solely to the visual pigment, as all other players in the cascade were unperturbed in their expression. These conclusions were reached on the basis of two experimental variants. In the first, physiological and biochemical parameters were compared between wild-type rods and rods that expressed only mouse green pigment (mG/mG). The
second model used a heterozygous expression of a wild type–like mutant rhodopsin, E122Q, and mouse green pigment (RhE122Q/mG) in the same rods. E122Q rhodopsin previously has been shown by members of this same group to have characteristics similar to wild-type rhodopsin (similar amplitude of the quantal response and similar pigment stability), but the absorption spectrum is shifted to the blue by ~23 nm compared with mouse green (Imai et al., 2007). One caveat is that the expression level of mouse green opsin in rods is quite low, only ~11% of rhodopsin in wild-type retina. This low expression level may reflect that the promoter region in the construct was unchanged, and that the mechanisms involved in trafficking cone opsin to the nascent discs is not fully compatible with mouse rods. The authors controlled for complications due to this low level of expression by examining expression in Rh/mG heterozygotes.

Thus, incorporation of both pigments into the same rod allowed the comparison of response properties and noise characteristics with all other participants in the transduction cascade unchanged. The conclusions reached using each approach were identical.

Sakurai and coworkers attributed the differences in single-photon amplitude to differences in the intrinsic properties of rhodopsin and mouse green visual pigments. They hypothesize that these intrinsic differences may arise from differences in the temperature-dependent equilibrium between Meta I and Meta II in rhodopsin and mouse green pigment. Alternatively, these differences may arise from differences in the decay rate of Meta II. Their result is surprising in the light of earlier results of Kefalov et al. (2003), who reported that the single-photon response generated by human or salamander red cone pigment was similar to that of human rhodopsin, when expressed in the same amphibian rods. The reason(s) for this difference is not understood.
The second important result is that the rod pigment is >800-fold more stable than the cone pigment, and, as such, generates less photon-like quantal noise in darkness. This transduction noise, also known as “dark light,” is due to the cone pigment activating the cascade just as real light does (Barlow, 1956) and produces desensitization of the cone, much as does background light in the rod. Thus, the reliability of signaling by photoreceptors in dim light is a function not only of the size of the single quantum response, but also of the noise generated by false positive signals. The unique features of the visual pigments that stabilize rhodopsin compared with cone opsin and that cause single quantum responses triggered by rhodopsin to be larger than cone opsin–triggered responses thus underlie the high signal-to-noise ratio in wild-type rods. These features are critical factors in setting the limits of signal reliability in rods and cones. Thus, these two key factors are fundamentally important in setting the detection limit of each cell type.

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