Acknowledgements
The author is funded by the Swedish Natural Science Research Council.

29 Warrant, E. J. (1988) *Proceedings of the IEEE International Conference on Systems, Man and Cybernetics* pp. 1107–1110, Pergamon Press
30 Stavenga, D. G. and Schwemer, J. (1984) in *Photoreception and Vision in Invertebrates* (Ali, M. A., ed.), pp. 11–61, Plenum
31 Messenger, J. B. (1981) in *Handbook of Sensory Physiology, Vol. 7, Pt 6C* (Autrum, H., ed.), pp. 93–200, Springer
32 Marshall, N. J. (1988) *Nature* 333, 499–500
33 Cronin, T. W. and Marshall, N. J. (1989) *Nature* 339, 137–140
34 Bernard, D. G. and Miller, W. H. (1968) *Invest. Ophthalmol.* 7, 416–434
35 Rossel, S. (1989) in *Facets of Vision* (Stavenga, D. G. and Hardie, R. C., eds), pp. 298–316, Springer
36 Snyder, A. W., Stavenga, D. G. and Laughlin, S. B. (1977) *J. Comp. Physiol.* 116, 183–207
37 Hardie, R. C. (1986) *Trends Neurosci.* 9, 419–423
38 Land, M. F. (1985) in *Neurobiology of Arachnids* (Barth, F. G., ed.), pp. 53–78, Springer
39 Land, M. F. (1987) in *Comparative Physiology: Life in Water and on Land* (Dejours, P., Bolis, L., Taylor, C. R. and Weibel, E. R., eds), pp. 289–302, Liviana Press
40 Pumphrey, R. J. (1961) in *The Cell and the Organism* (Ramsay, J. A. and Wigglesworth, V. B., eds), pp. 193–208, Cambridge University Press
41 Land, M. F. (1979) *Nature* 280, 396–397
42 Kirschfeld, K. (1967) *Exp. Brain Res.* 3, 248–270
43 Exner, S. (1891) *Die Physiologie der facettirten Augen von Krebsen und Insecten* (English transl. by Hardie, R. C., 1988), Springer
44 Vogt, K. (1975) *Z. Naturforsch.* 30, 691
45 Land, M. F. (1976) *Nature* 263, 764–765

Watching G proteins at work

Rainer Uhl, Rafael Wagner and Nicholas Ryba

Rainer Uhl, Rafael Wagner and Nicholas Ryba are at the Max-Planck-Institut für Biophysikalische Chemie, Am Fassberg, D-3400 Göttingen. Rainer Uhl is currently at the Department of Physiology and Biophysics, University of Washington, SJ-40, Seattle, WA 98195, USA and Nicholas Ryba is currently at the Department of Biochemistry, University of Leeds, Leeds LS2 9JT, UK.

It has been known for over a century that rod photoreceptors in the living retina contract and swell in response to light[1]. Although it is still not known whether this structural light-response is of any functional significance, it has recently been possible to correlate the underlying molecular processes with the activation and deactivation of the photoreceptor G protein, transducin. The technique of light-scattering allows the monitoring of minute changes in cell dimensions, and using this non-invasive experimental approach it can be shown that certain properties of the coupling between transducin and rhodopsin are different in a structurally well-preserved system as compared with rod material used for conventional biochemical studies. Thus, not unlike a psychiatrist, who often learns more about a patient's 'interiors' by observing the body language than by direct interrogation, a biochemist, studying the 'body language' of a cell, may extract information about delicate 'cell interior processes' that would be perturbed by more direct experimental approaches.

Structure and function in biological systems are like Siamese twins: rarely can they be separated without serious detrimental effects, yet this is what biochemists often have to do, destroying the cell structure to render the cell interior accessible for examination. An amazingly high number of intracellular processes seem unaffected by such treatment, but others, like the processes involved in the delicate balance between activation and deactivation of the visual G protein, transducin, are seriously perturbed. As a consequence, certain regulatory processes do not occur at all and others are orders of magnitude slower than *in vivo*. This article demonstrates a method whereby this dilemma may be overcome by allowing the enzymatic cascade of visual transduction to proceed in a more natural structural environment, and describes how, by monitoring properties of this intact structural environment itself, processes occurring within and adjacent to these structures can be studied. The close coupling of functional components is not only preserved, but also a prerequisite for the measurements we describe.

G proteins have been shown to play a key role in the stimulus–response coupling of many important biological systems[2]. The details of the structural interactions of G proteins with their reaction partners, and also the mechanism by which the interaction takes place, are unknown. Most models suggest that the dissociation of the G protein subunits occurs during activation, and that the active species is the dissociated GTP-binding α-subunit[2]. In general the isolated study of a G protein-dependent transduction system is impossible without total disruption of the cellular structure. The visual transduction system is unique in that it can be isolated as a structurally intact entity, the functionally intact rod outer segment (ROS). The G protein system working in its interior has an unusually high gain and speed and a dynamic gain control, which is achieved by balancing activation and deactivation kinetics[3,4]. Moreover, the transduction machinery can be activated by a defined stimulus (the energy, duration and timing of an actinic light pulse are easily controlled), providing a unique opportunity to watch G proteins working *in situ*.

The G protein of the photoreceptor cell, transducin, mediates the communication between photoexcited rhodopsin and a cGMP-specific phosphodiesterase[3,4], as schematically depicted in Fig. 1. The cGMP concentration, in turn, modulates the conducting properties of the plasma membrane and hence the current entering the photoreceptor[5,6]. *In vivo* a large number of G proteins is activated by a single, bleached rhodopsin (with concomitant GDP/GTP exchange), and the activation of the whole transducin pool may take less than 1 s[7]. Deactivation of transducin is accomplished by hydrolysis of the bound GTP, and its reactivation is prevented by quenching the active form of rhodopsin, presumably by phosphorylation and subsequent binding of a 48 kDa protein ('arrestin')[8–10]. From the time course of the electrophysiological light-response it is clear that the deactivation processes must also occur in about 1 s, yet in the *in vitro* systems studied by conventional biochemical methods, deactivation is 1–2 orders of magnitude slower (reviewed in Ref. 11).

0166 - 2236/90/$02.00

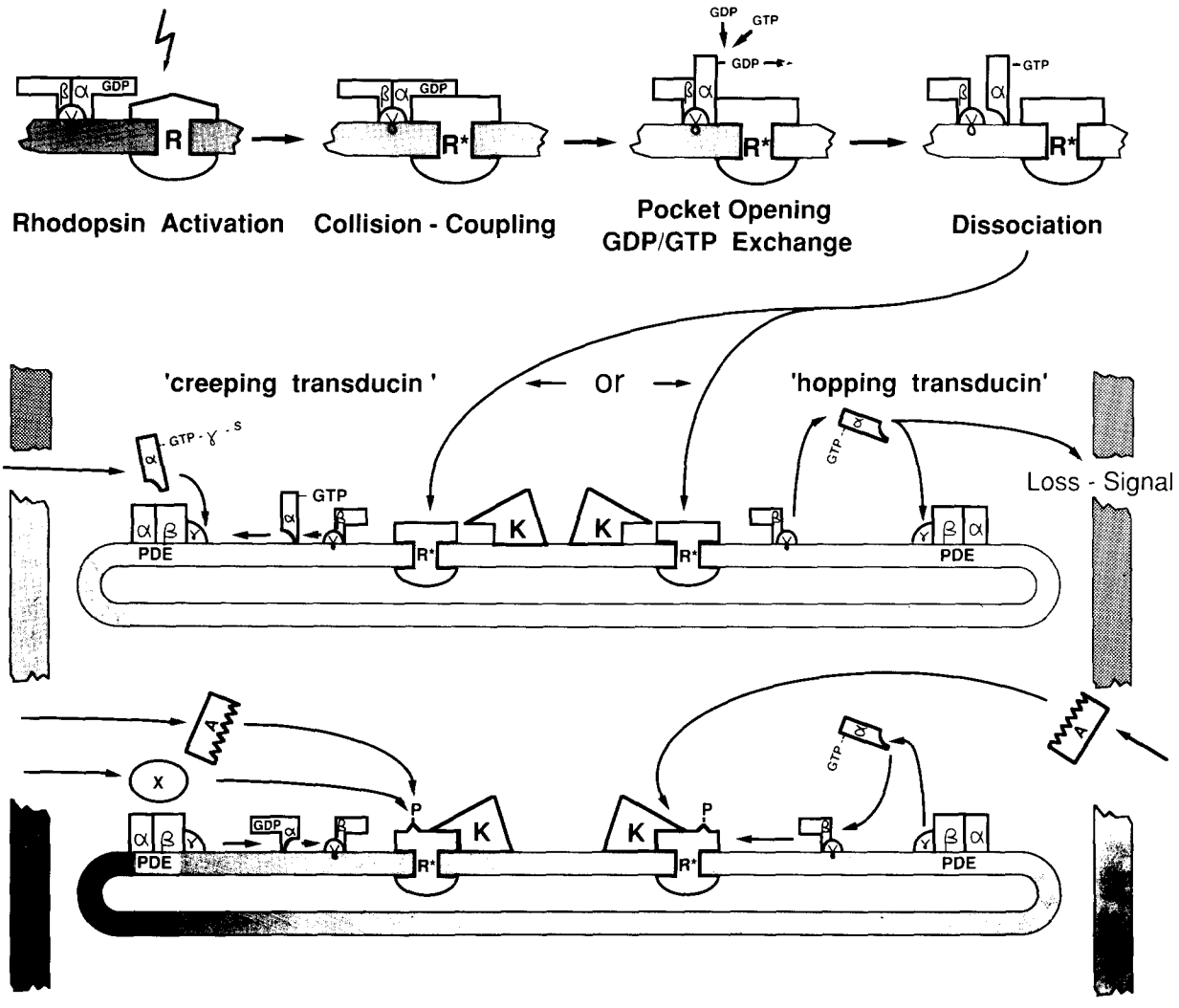


Fig. 1.
Schematic diagram of the light-induced enzyme cascade in visual transduction. Abbreviations: R, rhodopsin; R, activated rhodopsin; K, kinase; A, arrestin; X, unidentified helper protein. The T-shaped symbol represents the transducin heterotrimer. The communication between rhodopsin and phosphodiesterase is mediated by the active form of transducin, the GTP-binding T_α, and it may take place either along the surface of the disc membrane (two-dimensional diffusion, 'creeping transducin') or through the interdiscal space (three-dimensional diffusion, 'hopping transducin'). Deactivation of T_α occurs much faster on the disc surface, and therefore reassociation of the freely diffusing T_α with the membrane is likely to precede GTP hydrolysis and recombination with its βγ-subunit.*

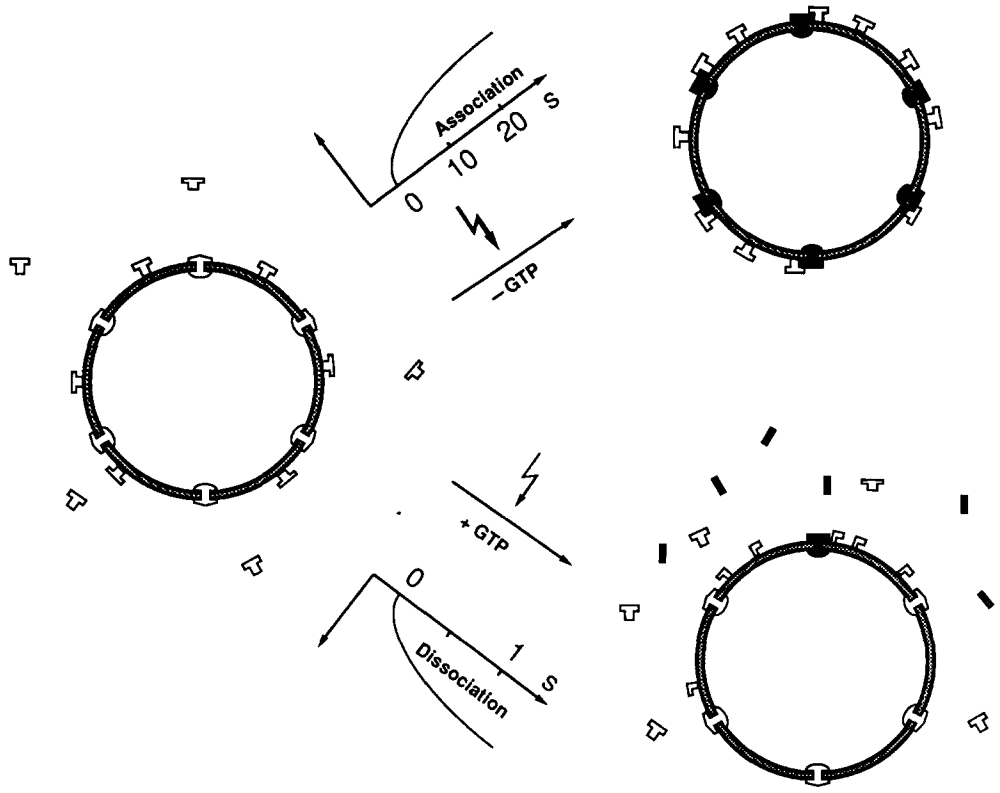
A real-time assay for the activation and deactivation of transducin

Most of our knowledge on the kinetics of G protein activation and deactivation is based on data from light-scattering experiments (reviewed in Refs 12–16). It is beyond the scope of this article to give a critical review of the numerous reports on light-scattering signals from photoreceptor cells, but a few words are necessary to characterize and justify the particular real-time assay on which all the results given here are based.

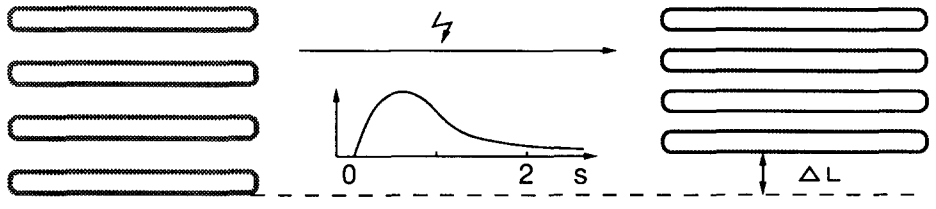
Two classes of light-induced, transducin-related mass redistributions, leading to changes in the light-scattering behaviour of photoreceptor ROS membranes, have been documented, and three classes of ROS preparations have been used in previous light-scattering studies.

Light-scattering changes. (1) An isotropic change in scattering power (Fig. 2A), accompanying changes in the partitioning of transducin between the disc membrane surface and the free aqueous space[12–14]. *Association* occurs when disc membranes are illuminated in the absence of GTP, i.e. under conditions where each bleached rhodopsin binds a single copy of transducin. Consequently, the equilibrium between bound and free transducin is disturbed and previously free transducin is adsorbed by the membrane. It is this relatively slow process, and *not* the fast binding of transducin to activated rhodopsin (R^*), that leads to an enhanced contrast and hence to an increased total scattering[13]. *Dissociation*, on the other hand, occurs in the presence of GTP, where the bleaching of one molecule of rhodopsin can catalyse a highly amplified GDP/GTP exchange. Upon dissociation from rhodopsin and its $T_{\beta\gamma}$-subunit, activated T_α becomes soluble (for exceptions, see below). As a result, all previously membrane-bound T_α is sequentially released into the surrounding medium, leading to a concomitant decrease in the total scattering power of the membrane suspension[13]. Note that the dissociation process is more than an order of magnitude faster than the slow membrane association of previously free transducin (see time scale differences in Fig. 2A).

(2) A non-isotropic change in scattering (Fig. 2B) with a distinct angular profile[15–17], probably reflecting an axial contraction of the disc stack[12,14]. It is brought about by a screening of surface charges on the disc surface during an early stage of transducin activation, probably associated with structural changes around the GDP/GTP-binding pocket in T_α. This was concluded from the similarity of light-scattering signals (same maximal amplitude, same angular dependence) observed in the absence of GTP, where one molecule of R* binds to one molecule of transducin, leading to the opening of one transducin pocket, and in the presence of GTP, where one R* causes the opening of hundreds or thousands of pockets[17,18]. If the signal merely reflected the tight binding of transducin to R*, as previously suggested[12], it would not persist in the presence of GTP where dissociation of the complex



A Isotropic change in scattering due to change in contrast



B Non-isotropic change in scattering due to disc stack contraction

Fig. 2. *Two classes of structural change are associated with light-induced transducin-related phenomena.* **(A)** *Changes in the partitioning of transducin between the disc membrane and the free aqueous space. Binding of previously free transducin leads to an increase in total scattering, whereas the release of activated T_α from the membrane causes the scattering to decrease. The T-shaped symbol represents the transducin heterodimer.* **(B)** *The opening of the nucleotide-binding pocket within T_α causes a change in surface charge, leading to a transient shrinkage of the disc stack (length-change, ΔL). It is not known at present if the re-swelling occurs as soon as the bound GTP has been hydrolysed, or if reassociation with the $\beta\gamma$-subunit is necessary.*

occurs. If, on the other hand, it reflected the subsequent GDP/GTP exchange and/or the dissociation process, it would not be observed in the absence of GTP. Consistent with this, it was found that in the presence of GTP the signal is much more light-sensitive, saturating at very low bleaches, than in the absence of GTP. The latter case reflects the 1:1 stoichiometric interaction, i.e. complete saturation requires the number of rhodopsin molecules bleached to be equivalent to the number of molecules in the transducin pool[17–19]. Since one molecule of R* has to interact only with one of transducin the signal is much faster.

ROS preparations. (1) From sonicated ROS membranes or reconstituted systems only the first category of light-scattering signals is observed. The only exception is when highly concentrated vesicle suspensions are used where the average distance between vesicles is so small that changes in surface charge lead to rapid changes in the aggregation or disaggregation pattern of the vesicles. This also gives rise to characteristic light-scattering changes which have been used to study transducin-related phenomena[11,20].

(2) In disc-stack preparations used for previous studies, i.e. in ROS with perforated plasma membranes, both classes of light-scattering changes can be observed simultaneously. The inactive form of transducin, $T_{\alpha\beta\gamma}$, partitions between the membrane and the free aqueous space[14], and since the latter is about 2000–5000 times bigger in the light-scattering experiment than in the sealed ROS, a substantial fraction of the total transducin is in the unbound state before the flash, i.e. it cannot interact with rhodopsin in less than 1 s[14,15]. Upon illumination, the membrane-associated fraction is rapidly bound to rhodopsin and the nucleotide-binding pocket opens (Fig. 1), at which point the disc stack contracts. If GTP is present, GDP/GTP exchange occurs, followed by the dissociation of the $T_{\alpha\beta\gamma}$ complex. T_α, which in type (2) preparations is released into the aqueous phase, is consequently lost through the holes in the plasma membrane into the surrounding medium (Fig. 1, right). Because the surface charge screening effect of transducin disappears with the loss of T_α from the interdiscal space, the disc stack contraction is transient. It is superceded by the so-called *loss signal*, the decrease in isotropic scattering due to the loss of refractile material[12,14]. This is analogous to the *dissociation signals* described above.

(3) There are ROS disc stacks that only exhibit the second class of light-scattering changes. Their isolation requires the use of *in vitro* dark-adapted cattle eyes[21] and a very gentle preparation procedure, yielding mostly intact ROS[18]. These are rendered permeable for nucleotides and proteins by a quick freeze/thaw cycle in liquid nitrogen[18] in the presence of 100 mM sucrose as a cryoprotectant. When these precautions are taken and the permeabilization is carried out immediately before experiment, the resulting disc stacks differ from type (2) ROS in some major aspects. First, there is essentially no partitioning of $T_{\alpha\beta\gamma}$ between the disc membrane and the surrounding medium[18]. Second, flash illumination in the presence of physiological concentrations of GTP and ATP leads to a contraction of the disc–disc lattice which is not only rapid and transient (Fig. 3A), but also repeatable and highly reproducible (Fig. 3B). The

amplitude of the signal is much higher than in previous studies with type (2) ROS, probably because the fraction of transducin molecules associated with the membrane before the flash (and hence available for rapid activation) is much higher. Biochemically, the signal correlates with known aspects of transducin activation[17,22]: the signal is fully reversible in the presence of GTP and ATP and it becomes irreversible when GTP is replaced by GTP-γ-*S*. It saturates at very low bleaches – a single photoisomerization per disc can lead to a signal representing the activation of the total transducin pool – and it is unaffected by the preactivation of the phosphodiesterase (PDE) with exogenous, permanently activated transducin (T_α-GTP-γ-*S*). This last characteristic would not be observed if the signal were due to PDE rather than transducin activation[23,24].

The correlation of the falling phase of the signal with transducin deactivation rests on the observation that the signals exhibit full recovery and can subsequently be repeated over and over again (Fig. 3B). The angular dependence of the signal recovery matches that of the preceding rising phase, i.e. the signal arises from a reproducible contraction and subsequent relaxation of the disc stack. When a second flash is applied before the signal has completely recovered, a correspondingly smaller signal results, indicating that a certain maximal amplitude, determined by the total amount of transducin that can be activated, cannot be exceeded. This, in turn, implies that once deactivated, as manifest by light-scattering, transducin is immediately available for reactivation.

The rate of the rising phase increases with the number of rhodopsin molecules bleached per flash (see below), and at non-physiologically high bleaches, where one rhodopsin molecule activates, on average, only one transducin molecule, its rise-time approaches the kinetics of the metarhodopsin I/II transition, e.g. the rise-time of the appearance of the active form of rhodopsin[25]. As the structural assay can follow even this rapid process, it is presumed that at physiological bleaches, where the activation process is highly amplified and hence much slower, the structural changes do not lag significantly behind the processes they reflect, thus making the signal an accurate kinetic representation of the actual transducin activation process. The transducin activation–deactivation cycle studied using class (2) light-scattering changes obtained from category (3) disc stacks differs in some specific aspects from the more conventionally derived model, and these differences are discussed below.

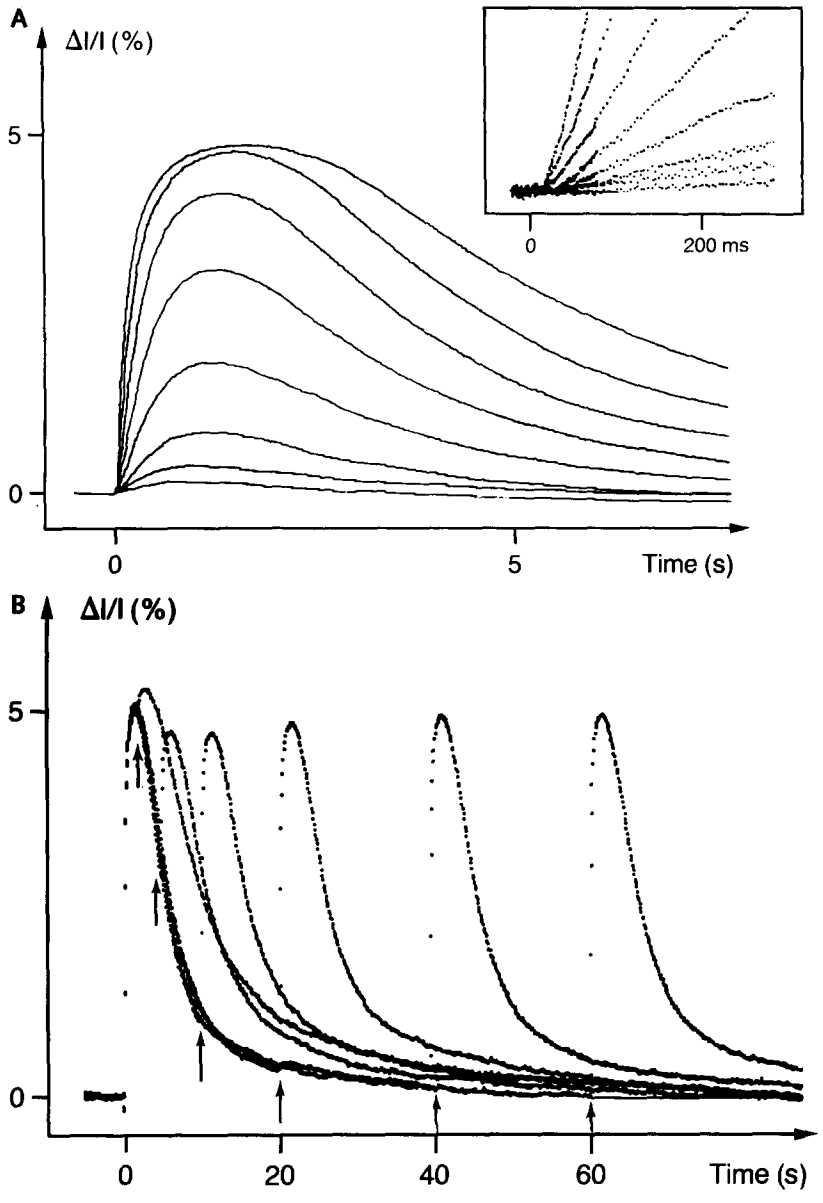


Fig. 3. *The transient contraction of the disc stack leads to a concomitant increase in light scattering, here measured at a scattering angle of 24°.* **(A)** *Light-scattering responses to a series of short flashes (20 ms). Flash energy is increased stepwise by a factor of two, starting with a fractional bleach of 8.5 × 10^{-6}. The response is graded with flash energy and saturates at a bleach of about 2 × 10^{-3}. The saturating response amplitude ($\Delta I/I = 5 \times 10^{-2}$) corresponds to a change in length of the disc stack of about 5%*[15]*. This means that the aqueous space between two adjacent discs drops from 150 Å to about 142.5 Å.* Insert*: time window immediately after the flash.* **(B)** *The disc stack contraction recovers and can be evoked repeatedly. The trace is a superposition of six double-flash experiments, carried out sequentially with a single disc stack preparation. The first flash (95% saturating) of each sequence was always applied at t = 0, whereas the second flashes, marked by arrows, followed after a variable delay of 2, 5, 10, 20, 40, or 60 s. The signal amplitudes depend solely on the degree of recovery that has occurred before the second flash.*

Activated transducin never leaves the disc surface

The widely accepted model for PDE activation, the 'hopping mechanism', suggests that transducin bridges the gap between rhodopsin and PDE by diffusion in the free aqueous phase between adjacent discs[26,27]. The hopping mechanism, schematically depicted on the right of Fig. 1, was based mainly on two observations, namely that T_α is soluble in isotonic salt when in its active, GTP-binding form[28], and on the appearance of the loss signal (see above), a light-scattering signal reflecting the loss of activated transducin from broken ROS into the surrounding medium[12,27]. However, loss signals do not occur in the *close-to-intact system* and changes in light scattering are strictly reproducible and can be repeatedly evoked[17]. While supernatants taken from ROS bleached in the presence of GTP or even GTP-γ-*S* show no appreciable T_α fraction[18], the plasma membrane remains permeable to exogenously added fluorescent dyes[18], T_α-GTP-γ-*S*[17] and arrestin[29] (see also below) throughout the experiment. As free T_α-GTP-γ-*S* can enter the stack, it should also be able to

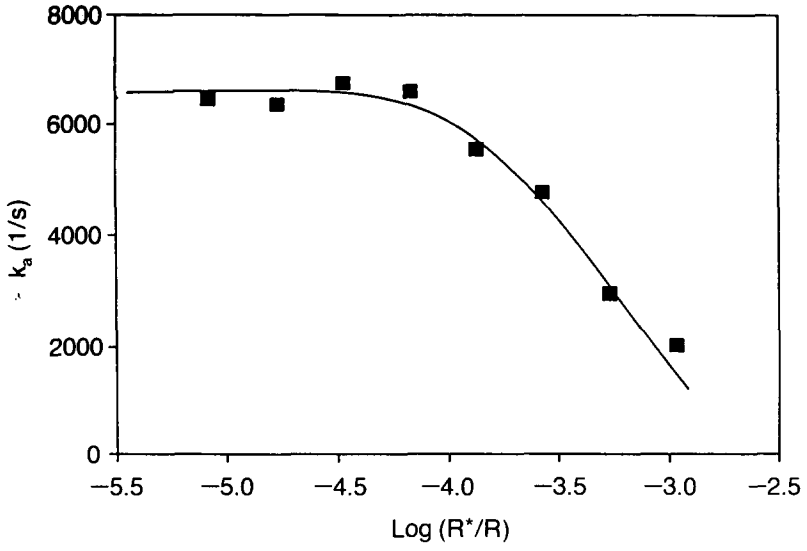


Fig. 4. *Transducin activation rate is a function of the fractional bleach. An activation constant k_a of 6500/s implies that the total transducin pool could be turned over 6500 times per second if all rhodopsin molecules were activated, corresponding to a turn-over time of 1 ms.*

leave it, and because it does not, it must remain membrane-associated. Moreover, in the presence of physiological concentrations of ATP and GTP, the state of T_α or of the membrane, which is responsible for the membrane association, is stable. Quite remarkably, 100 saturating flashes can be applied over a period of more than an hour without deterioration of the full reversibility of the signals. This, and the fact that the irreversible signals described previously could be reproduced simply by perturbing the structure of the disc stack before the experiment, makes us confident that the membrane-associated T_α observed throughout activation and deactivation reflects a physiological state of the membrane and its associated proteins.

Further support for this view comes from the observation that neither the kinetics of the rising phase of the signals in Fig. 3, nor their recovery, depends in any way on the concentration of the ROS[19,22]. Recently it has been observed that even in broken ROS (type 2 preparations) there is a small fraction (5–10%) of the total transducin that cannot be washed off the membrane. It accounts for almost all of the PDE-activating power of the preparation[30].

The theoretical argument[26,27] that activated transducin must diffuse in the free aqueous phase because diffusion is two orders of magnitude faster there than at the disc surface does not seem very strong; diffusion is certainly faster in the aqueous space, but the probability of a favourable steric interaction between T_α and PDE is definitely much higher in the case of two-dimensional versus three-dimensional diffusion because of a dramatically reduced rotational freedom. Although theoretically impossible to assess the contributions of the two factors[3,7], experimentally it is clear that membrane-bound T_α is a much more powerful activator of PDE than soluble T_α[30].

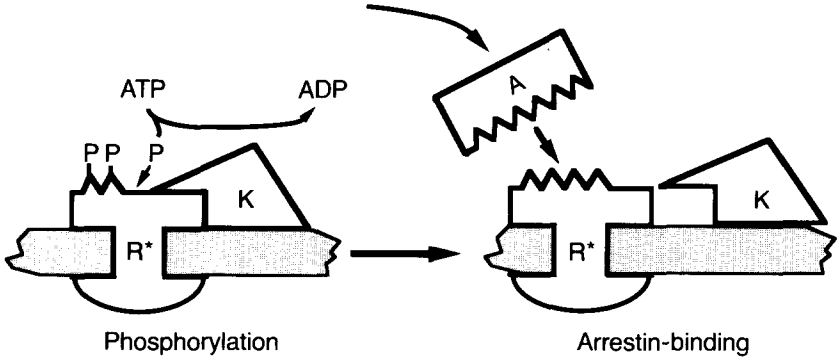


Fig. 5. *Schematic diagram of the rhodopsin deactivation process, thought to occur by means of rhodopsin phosphorylation and subsequent stoichiometric 1:1 interaction with arrestin, the 48 kDa protein of the ROS. Abbreviations: R*, activated rhodopsin; P, phosphorus; K, kinase; A, arrestin.*

The rate-limiting step of transducin activation is diffusion-controlled

The rate of transducin activation increases with increasing flash energy (Fig. 3A, inset). However, it is not significantly accelerated when the temperature is raised from 21°C to 37°C. Such a low temperature dependence (i.e. activation energy) is found only where the rate-limiting step is diffusion-controlled, so the rate-limiting step in transducin activation must be the diffusion of the partners involved in collision coupling.

The sigmoidal starting phase of the activation process (Fig. 3A, inset) implies that there must be at least two diffusion-controlled steps, and therefore at least three reactants involved in collision coupling. This is borne out by the independence of the sigmoidicity from temperature between 21°C and 37°C. Since the kinetics are independent of the GTP concentration above 50 μM GTP, the third partner cannot be GTP. It has previously been suggested that two or more transducin molecules may interact cooperatively during activation[31], and oligomeric complexes of G protein have been found in other systems[32,33], so it is possible that such cooperation could be responsible for the observed sigmoidal rising phase.

Under optimal conditions transducin activation occurs in about 1 ms

The rates of transducin activation in Fig. 3A can be used to calculate the time it takes a rhodopsin molecule to activate a single transducin. Since the maximal signal amplitude (A_{max}) corresponds to a completely activated transducin pool, the signal slope divided by A_{max} yields a value for the fraction of the total transducin pool that is activated per second. When divided by the fraction of the rhodopsin pool bleached to achieve a given slope, an activation constant, k_a, is derived. This constant corresponds with the number of times the total transducin pool could be turned over per second by a full complement of bleached rhodopsins. By multiplying this value with the molar ratio between transducin and rhodopsin (0.15; Ref. 18), one obtains the number of transducin molecules turned over per R* molecule per second. At low bleaches, a k_a of 6500/s is found, indicating a turnover of 1000 transducin molecules per R* molecule per second (Fig. 4)*. At higher bleaches

* A transducin activation time of about 1 ms has been reported previously[34]. However, in this study, only a fraction of the transducin molecules was membrane-associated before the flash and could contribute to the light-scattering signal used for the kinetic analysis. As a consequence, the turn-over number was seriously overestimated.

(more than three to four rhodopsin molecules bleached per disc surface), this value decreases, reflecting competition between rival rhodopsin molecules on a particular disc surface for a given transducin molecule.

Transducin deactivation through GTPase occurs in less than 1 s

Transducin deactivation requires the hydrolysis of the bound GTP. *In vitro* GTPase activity rates of 1–2 min^{-1} have been found, much slower than the recovery of the *in vivo* system (reviewed in Refs 11,26). In a system where activated T_α stays membrane-associated, however, GTPase is much faster, as can be seen from the fast signal recovery and re-excitability in Fig. 3. Even faster recoveries of light sensitivity have been observed in the presence of hydroxylamine[22], a reagent that causes rapid inactivation of R* by forming a Schiff-base linkage with the retinal chromophore of metarhodopsin II. The resulting free opsin can no longer reactivate transducin molecules, and when the artificial rhodopsin inactivation process is made fast enough, GTPase activity becomes the rate-limiting step in the process of transducin deactivation[7]. Under such conditions we found a GTPase turnover number of 1–2 s^{-1}, independent of the concentration of the rod membrane material. This indicates that unlike in less well-preserved structures, where GTPase is much slower and becomes accelerated when the ROS concentration is increased[11], all necessary protein components are membrane-associated[22]. The crucial factor responsible for a physiological rate of GTPase activity may either be the membrane association, or a catalytic stimulation of the process by $T_{\beta\gamma}$ or even PDE (Fig. 1).

Rapid deactivation of rhodopsin requires phosphorylation, a 48 kDa protein and additional, soluble proteins

The rapid transducin deactivation in Fig. 3, which occurs in the absence of hydroxylamine, requires the addition of soluble ROS proteins. Due to the ruptured plasma membrane, the endogenous soluble proteins are diluted by more than 1/4000 during the light-scattering experiment. Without the addition of soluble proteins, the deactivation process becomes much slower and biphasic, with a complete recovery after a saturating flash taking over 2 min[29]. In agreement with previous reports[8,10], this (slow) deactivation occurs even in the complete absence of soluble ROS proteins (including the 48 kDa protein) and must therefore reflect rhodopsin phosphorylation.

Arrestin, the 48 kDa protein of the ROS, has been suggested to bind to phosphorylated rhodopsin with a 1:1 stoichiometry, thus 'capping' rhodopsin and further decreasing its activating power[9]. However, this simple mechanistic picture (Fig. 5) seems to be incorrect, since arrestin requires other soluble proteins in order to exert its effect[28]; ROS supplemented with purified arrestin alone show even slower deactivation, and a crude mixture of soluble ROS proteins extracted in the dark, containing very little arrestin is also without effect. A soluble ROS fraction enriched in arrestin by light-adapting the retina before extraction (light adaptation leads to a migration of arrestin from the inner to the outer segment[35]) causes a small, but reproducible acceleration of recovery, and a mixture of soluble proteins and purified arrestin has a very pronounced effect (see Fig. 6). The signals shown in Fig. 3 were recorded in the presence of such a mixture. We are currently trying to identify the soluble protein component (named 'X' in Fig. 1) responsible for the synergistic action, and also to understand the underlying mechanism.

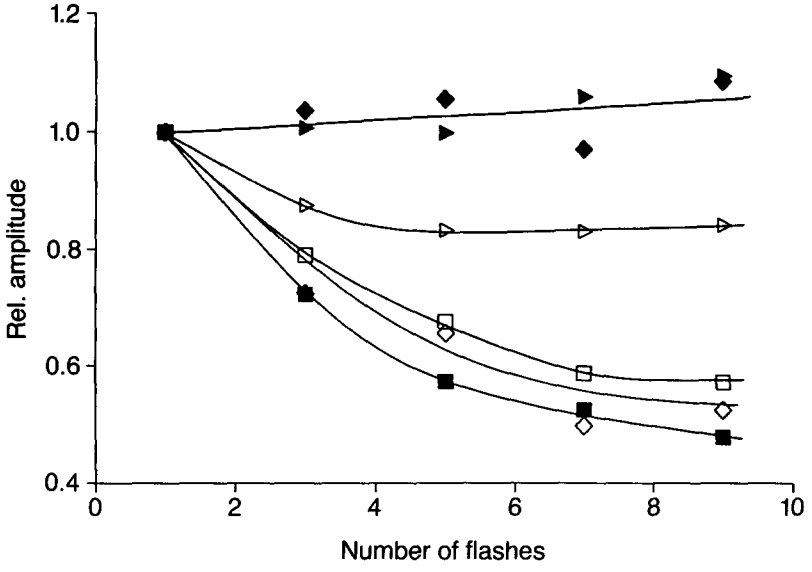


Fig. 6. *Loss in responsiveness upon repetitive stimulation in the presence of different fractions of soluble ROS proteins. Saturating flashes (as in Fig. 3B) were applied every minute and the decline of the response amplitude with time was determined. An incomplete recovery between flashes is observed in the absence of soluble proteins, in the presence of purified arrestin, and in the presence of a dark extract of soluble proteins low in arrestin. A light extract, with the same amount of other soluble proteins, but more arrestin, causes some improvement, and the admixture of a soluble fraction and extra arrestin has a dramatic effect. Symbols: □, control; ■, 48 kDa protein; ◇, dark extract; ▷, light extract; ◆, dark extract + 48 kDa protein; ▶, light extract + 48 kDa protein.*

One single bleached rhodopsin can activate all transducin molecules on a particular disc surface

It has previously been shown that the bleaching of a single rhodopsin can lead to the activation of all PDE molecules on a particular disc surface. This was derived from a plot of PDE activity versus fractional bleach, which could best be described by Poisson statistics based on an active rhodopsin pool equivalent to the number of rhodopsin molecules per disc surface[7]. Plotting the amplitude of the disc stack contraction in the same way yields a very similar curve, but only under conditions where the rate of transducin deactivation is low (when Ca^{2+} concentration is high, see below, or in the presence of GTP-γ-S)[19]. The active rhodopsin pool of 26000 derived from this analysis is close to the number of rhodopsin molecules on a bovine disc surface. If transducin could diffuse freely between the discs, such a value would be highly fortuitous. However, as neither R* nor T_α diffuses through aqueous space, the activating action of a single bleached rhodopsin molecule is confined to the disc surface it resides in, meaning that a single rhodopsin bleached per disc can fully activate transducin[19]. With a molar ratio of transducin:rhodopsin of 0.15 in the ROS[18], the gain of the primary amplification step is about 4000.

Acknowledgements
The authors gratefully acknowledge the financial support from the DFG (research grant to RU UH30/7). RU was a Heisenberg fellow and NR was supported by a European Science Exchange Fellowship.

The rate of rhodopsin deactivation and hence the sensitivity of the primary amplification step in visual transduction is calcium-dependent

A gain of 4000 in the primary amplification stage requires that the deactivation process be slow compared with activation. This only occurs at unphysiologically high Ca^{2+} concentrations (1 mM)[19]. At lower Ca^{2+} concentrations the rate of rhodopsin deactivation is accelerated, the time to peak shortened, and the gain of the primary amplification stage consequently reduced[19]. This effect, which closely resembles light adaptation, suggests that the rate of rhodopsin deactivation is Ca^{2+}-dependent, since it is no longer observed when rhodopsin is quenched by other means, e.g. in the presence of hydroxylamine, where GTPase is rate-limiting for deactivation of T_α[19]. The steepest change occurs when Ca^{2+} concentration is varied between 1 and 0.1 μM, i.e. within the physiological range. Since the Ca^{2+} concentration has been shown to drop in the light[36], part of the process of adaptation may occur at the primary amplification step. From the fact that rods still function in ambient light photolysing hundreds of rhodopsins per disc per second, one has to postulate the existence of gain regulation at every stage, otherwise saturation at the level of a single stage would render the total transduction chain irresponsive.

Concluding remarks

From the above it has become clear how important it can be to use non-invasive techniques that leave the cell structure relatively unaltered, when studying delicate regulatory processes. While the experiments reviewed in this article clearly constitute a step towards a less invasive approach, they still require permeabilization of the ROS in order to supply them with nucleotides. Unfortunately, soluble proteins are lost through the same holes that the nucleotides get in, and some deactivation processes do only occur with (almost) physiological speed when these proteins are replaced. This is rather cumbersome, since even with the material from over 60 cattle eyes, the concentration of, for instance, arrestin, is still far below the physiological level (8 μM as opposed to over 100 μM). It may well be that with higher arrestin concentrations an even faster recovery of transducin activity is achieved and that the Ca^{2+} regulation of the sensitivity becomes more pronounced. A way out of this dilemma would be to apply the non-invasive light-scattering technique to single cells that are supplied with nucleotides through a patch pipette. Such experiments are currently being prepared.

Finally, the increasing evidence that G proteins similar to transducin are involved in a wide range of processes in cellular control makes this discussion highly significant. In particular, the higher degree of association implied here may explain how distinct G protein-linked receptor and effector systems function in a single cell without the danger of cross-reactivity. The increased rate of deactivation through GTPase activity significantly simplifies the understanding of this aspect of signal transduction. While the cautionary note that disruption of a system may alter G protein properties is disturbing, the roles of the extremely highly conserved β-subunit and small γ-subunit of G proteins, which have never been satisfactorily explained, may emerge from studies of more intact preparations.

Selected references

1 Engelmann, T. W. (1885) *Pflügers Arch. Ges. Physiol.* 35, 498–508
2 Gilman, A. G. (1987) *Annu. Rev. Biochem.* 56, 615–649
3 Liebman, P. A., Parker, K. R. and Dratz, E. A. (1987) *Annu. Rev. Physiol.* 49, 765–791
4 Stryer, L. (1986) *Annu. Rev. Neurosci.* 9, 81–119
5 Fesenko, E. E., Kolesnikow, S. S. and Lyubarsky, A. L. (1985) *Nature* 313, 310–313
6 Yau, K-W. and Nakatani, K. (1985) *Nature* 317, 252–255
7 Liebman, P. A. and Sitaramayya, A. (1984) *Adv. Cyclic Nucleotide Protein Phosphoryl. Res.* 17, 215–225
8 Sitaramayya, A. (1986) *Biochemistry* 25, 5460–5468
9 Wilden, U., Hall, S. W. and Kühn, H. (1986) *Proc. Natl Acad. Sci. USA* 83, 1174–1178
10 Bennet, N. and Sitaramayya, A. (1988) *Biochemistry* 27, 1710–1715
11 Dratz, E. A., Lewis, J. W., Schaechter, L. E., Parker, K. R. and Kliger, D. S. (1987) *Biochem. Biophys. Res. Commun.* 146, 379–386
12 Chabre, M. (1985) *Annu. Rev. Biophys. Biophys. Chem.* 14, 331–360
13 Schleicher, A. and Hofmann, K. P. (1987) *J. Membr. Biol.* 95, 271–281
14 Bruckert, F., Vuong, T. M. and Chabre, M. (1988) *Eur. Biophys. J.* 16, 207–218
15 Hofmann, K. P., Schleicher, A., Emeis, D. and Reichert, J. (1981) *Biophys. Struct. Mech.* 8, 67–93
16 Uhl, R., Desel, H. and Wagner, R. (1985) *J. Biochem. Biophys. Methods* 11, 31–43
17 Wagner, R., Ryba, N. J. P. and Uhl, R. (1987) *FEBS Lett.* 221, 253–259
18 Uhl, R., Desel, H., Ryba, N. J. P. and Wagner, R. (1987) *J. Biochem. Biophys. Methods* 14, 127–138
19 Wagner, R., Ryba, N. J. P. and Uhl, R. (1989) *FEBS Lett.* 242, 249–254
20 Caretta, A. and Stein, P. J. (1986) *Biochemistry* 25, 2335–2341
21 Ryba, N. J. P. and Uhl, R. (1989) *Exp. Brain Res.* 74, 618–624
22 Wagner, R., Ryba, N. J. P. and Uhl, R. (1988) *FEBS Lett.* 234, 44–48
23 Kamps, K. M. P., Reichert, J. and Hofmann, K. P. (1985) *FEBS Lett.* 188, 15–20
24 Pepperberg, D. R., Kahlert, M., Krause, A. and Hofmann, K. P. (1988) *Proc. Natl Acad. Sci. USA* 85, 5531–5535
25 Uhl, R., Hofmann, K. P. and Kreutz, W. (1978) *Biochemistry* 17, 5347–5352
26 Chabre, M. (1987) *Trends Biochem. Sci.* 12, 213–215
27 Chabre, M. and Deterre, P. (1989) *Eur. J. Biochem.* 179, 255–266
28 Kühn, H. (1981) *Curr. Top. Membr. Transp.* 15, 171–201
29 Wagner, R., Ryba, N. J. P. and Uhl, R. (1988) *FEBS Lett.* 235, 103–108
30 Wensel, T. G. and Stryer, L. (1988) in *Enzyme Dynamics and Regulation* (Boon Chock, P. *et al.*, eds), pp. 102–112, Springer-Verlag
31 Wessling-Resnick, M., Kelleher, D. J., Weiss, E. R. and Johnson, G. L. (1987) *Trends Biochem. Sci.* 12, 473–477
32 Rodbell, M. (1980) *Nature* 284, 17–21
33 Santos, E., Nebreda, A. R., Bryan, T. and Kempner, E. S. (1988) *J. Biol. Chem.* 20, 9853–9858
34 Vuong, T. M., Chabre, M. and Stryer, L. (1984) *Nature* 311, 659–661
35 Mangini, N. and Pepperberg, D. R. (1988) *Invest. Ophthalmol. Vis. Sci.* 29, 1221–1234
36 Yau, K-W. and Nakatani, K. (1985) *Nature* 313, 579–582