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Review

A physiological role for the supramolecular organization of rhodopsin and transducin in rod photoreceptors

Daniele Dell'Orco *

Department of Life Sciences and Reproduction, Section of Biological Chemistry, and Centre for Biomedical Computing, University of Verona, Strada le Grazie 8, 37134 Verona, Italy

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ABSTRACT

Vertebrate vision in rod photoreceptors begins when a photon hits the visual pigment rhodopsin (Rh) and triggers the phototransduction cascade. Although the fine biochemical and biophysical details of this paradigmatic signalling pathway have been studied for decades, phototransduction still presents unclear mechanistic aspects. Increasing lines of evidence suggest that the visual pigment rhodopsin (Rh) is natively organized in dimers on the surface of disc membranes, and may form higher order "paracrystalline" assemblies, which are not easy to reconcile with the classical collision-coupling mechanistic scenario evoked to explain the extremely fast molecular processes required in phototransduction. The questioned and criticized existence of paracrystalline Rh rafts can be fully accepted only if it can be explained in functional terms by a solid mechanistic picture. Here we discuss how recent data suggest a physiological role for supramolecular assemblies of Rh and its cognate G protein transducin (G_t), which by forming transient complexes in the dark may ensure rapid activation of the cascade even in a crowded environment that, according to the classical picture, would otherwise stop the cascade.

1. Introduction: toward a revision of the classical model of phototransduction

The phototransduction cascade in vertebrate photoreceptor cells has been deeply studied in its biochemical, biophysical and physiological facets for decades [1–3], rendering it a paradigmatic framework for understanding the molecular basis of many other G-protein-coupled receptor (GPCR) signalling pathways. According to the classical scenario, after the absorption of a photon by its chromophore 11-cis retinal the visual pigment rhodopsin (Rh) undergoes a conformational change becoming photoactivated (R*) and by diffusion in the rod disc membrane it encounters and activates the cognate G protein transducin (G_t), which then propagates the signal to the effector phosphodiesterase 6 (PDE), thus contributing to the extremely high amplification of the cascade. The signal is then transmitted to the other components of the cascade, finally leading to the hyperpolarization of the cell membrane and to the electrical response propagated to downstream retinal neurons (for a comprehensive review see [3]).

It is well established that the molecular events underlying the rapid activation of the cascade require very fast interactions between all the components of the signalling machinery, and based on a series of experiments performed between the 70s and the 90s [4–12] such rapid interactions have been explained by a collisional coupling mechanism driven by the free diffusion of monomeric proteins in the lipid milieu of the rod discs. However, after the first X-ray structure of rhodopsin became available [13], a new era for the structural biology of GPCRs started, which culminated in the high resolution structure of several other GPCRs (for a recent review, see [14]), and a variety of novel structural and functional features of the respective signalling pathways were disclosed. Among the other results, high resolution atomic force microscopy (AFM) and electron microscopy performed on native mouse rod discs led to the surprising result that Rh is organized in highly dense arrays of dimers that overall form paracrystalline rafts of receptors [15–17], a results which was corroborated also for other species by preparative biochemical techniques [18,19] and by high speed microspectrophotometric measurements [20]. The idea that the initiator of the phototransduction cascade could be organized in a way that apparently contradicts the classical scenario, which was quantitatively supported by a number of studies, could not easily be accepted by the community, and not surprisingly some criticism was explicitly raised against the paracrystalline hypothesis [21,22] and even against the dimeric nature of Rh [23].

While increasing evidence started to accumulate for homo- and heterodimerization of GPCRs with functional consequences such as receptor crosstalk and allosteric mechanisms conferring novel pharmaceutical properties (see [24,25] for exemplary reviews),

* Fax: +39 045 802 7170.
E-mail address: daniele.dellorco@univr.it

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despite the homology between Rh and other class-A GPCRs and the extremely high structural and functional similarity between (G_t) and other heterotrimeric G proteins [14,26], the phototransduction cascade and its initiator Rh largely continue to be considered according to the classical view.

In a recent and very interesting study, Govardovskii et al. [20] employed high-speed dichroic microspectrometry to study the diffusion of Rh in photoreceptor membranes of different species (frog, toad, salamander and gecko rods) by measuring the absorption of light by the visual pigment using narrow beams passed sequentially through two sides of the outer segment in order to measure the diffusional exchange of unbleached and bleached pigment between the two halves. Despite the methodology was substantially similar to that employed in the pioneer studies performed in the 70s [7,10], the instruments and the resolution were clearly improved and allowed the authors to discover some artefacts present in prior determinations that did not properly take into account photoproducts of Rh*, especially Meta III, which could distort the interpretation of the early data. Beside measuring a three to five-fold lower lateral diffusion constant for Rh as compared to previous estimates, the study proved that significant amounts of immobile rhodopsin were present in all native discs of the tested species, and the size of the immobile fractions could vary from none to almost 100% of the whole pigment, hence suggesting large areas of paracrystalline assemblies of Rh [20]. The authors speculated that most of the immobile Rh would be virtually excluded from the phototransduction processes, and proposed that a physiologically controlled oligomerization of Rh could instead represent a possible mechanism for adjusting the photoreceptor sensitivity, thus allowing phototransduction. It remains unclear though, how such massive assemblies would dynamically form and dissociate in time frames physiologically relevant for the fast visual processes.

We will now briefly review some other recent lines of evidence that quantitatively support another molecular scenario, in which immobile rafts of Rh receptors in fact would not prevent phototransduction. On the contrary, they may constitute kinetically convenient scaffolds for rapid activation of G_t in the highly crowded environment of rod discs.

2. Rh and G_t may interact at any stage irrespective of light conditions

The interaction between the receptor and the cognate G-protein prior to stimulation was predicted as a possible state in theoretical kinetic analyses of GPCRs [27] and it was experimentally demonstrated by in vivo studies for α2A adrenergic receptors, muscarinic M4 receptors [28] and β2-adrenocepto [29]. Considering the high similarity between Rh and the homolog class A-GPCRs, it is therefore reasonable to hypothesize a similar interaction between Rh and G_t prior to light stimulus. Indeed, evidence that Rh and G_t may interact also in the dark appeared in early biochemical studies [30] and in more recent plasmon resonance-based findings [31,32] and was corroborated in its atomistic details by computational structural analysis [33–35] and molecular dynamics simulations [36].

It can be reasonably argued that specific features of the phototransduction cascade in vertebrates, for example the low concentration of G_t compared to Rh (approximately 1:10) would make it hardly compatible with a purely precoupled Rh–G_t state. In fact, a common objection to the precoupling hypothesis is that if G_t and Rh were precoupled and if this precoupling played any role in signaling, *only* 10% of Rh molecules would be poised to signal and carry on the sequential activation of multiple G_t molecules by the same Rh*. This argument however does not stand, if one assumes that *also* (and not *only*) the 10% of Rh precoupled to G_t can trigger the cascade, as we will see. Precoupling could hence be a sufficient, but by no means necessary condition for triggering the cascade. Clearly, for the necessarily very fast interactions to occur the precoupled Rh–G_t complex, if existing, should be transient and the precoupling phenomenon itself be highly dynamic.

In a very recent study, a surface plasmon resonance-based kinetic analysis was performed focused on different states of the receptor [37]. Bovine Rh solubilised from rod outer segments was immobilized on the surface of a sensor chip, using different coupling strategies developed previously in the same laboratory [38–40], which demonstrated complete functionality of the immobilized receptor. Native purified G_t was flowed on the sensor chip at different concentrations for kinetic studies. Interestingly, very fast associations $\left(k^{on}_{dark} = 4.2 \times 10^5\ M^{-1}\ s^{-1}\right)$ and dissociations $\left(k^{off}_{dark} = 0.148\ s^{-1}\right)$ were observed when the binding experiments were performed in the dark (dotted black line in Fig. 1A), leading to a relatively high 0.36 μM affinity, which is in line with the values reported in earlier equilibrium studies (64 nM–10 μM)[31,32,41]. When the same sample was illuminated for 4 s, sensorgrams showed a significantly higher response amplitude (Fig. 1A, continuous black line) with a slightly slower association $\left(k^{on}_{light} = 2.7 \times 10^5\ M^{-1}\ s^{-1}\right)$ and a significantly slower dissociation $\left(k^{off}_{light} = 4.7 \times 10^{-4}\ s^{-1}\right)$, corresponding to a high affinity (91.8 nM) in agreement with the known high stability of the Rh*–G_t complex [41]. After prolonged bleaching, the chromophore-free form of the receptor opsin was also investigated in its interaction with G_t, leading to greater variability in the kinetics and in the affinity ($K_D \sim 2.7$ nM, see Fig. 1A, grey line). The observed interactions were proven to be protein–protein interactions by a dialysis–delipidation procedure of the immobilized Rh and they were also found to be independent on the immobilization chemistry, thus suggesting that Rh could be dimeric on the surface of the chip as further confirmed by positive controls obtained by flowing Rh over immobilized Rh [37]. This on-chip study represents the first direct evidence of Rh–G_t interaction in the dark, prior to light stimulation. However, it is important to notice that the detected kinetics suggest a highly transient interaction, consistent with very fast association and dissociation of G_t to/from Rh. In particular, the association of G_t with dark Rh appears to be approximately 1.6-fold faster compared with Rh*, whereas the dissociation of dark Rh–G_t complexes is found to be approximately 315-fold faster compared with the photoactivated complexes [37].

Beside the required kinetic information, to properly address the physiological relevance of the dynamic precoupled Rh–G_t state in the dark it would be also important to analyze the interaction in the context of the whole signalling cascade. Nevertheless, the peculiar nature of Rh and specifically its ability to detect even single photons prevents any investigation at the cell level of the interactions between Rh and G_t in the dark by means of spectroscopic techniques such as FRET or BRET, which is instead possible for other GPCRs [28]. On the other hand, a comprehensive dynamic model of phototransduction in rod cells able to describe quantitatively the dynamics of photoresponses starting from the underlying biochemistry was developed previously [42] and it could be used to test the compatibility of the dynamic-precoupling hypothesis within the physiological context involving the whole network.

When the dark Rh–G_t rapid interaction was implemented in the comprehensive model of phototransduction [42] by setting the experimentally determined kinetic constraints, simulations of the photoreceptor in the dark led to very rapid equilibration, consistent with ~25% of the G_t dynamically precoupled to Rh [37]. In summary, it was concluded that photons could hit either: (a) a Rh molecule uncoupled to G_t; (b) a precoupled Rh–G_t complex;

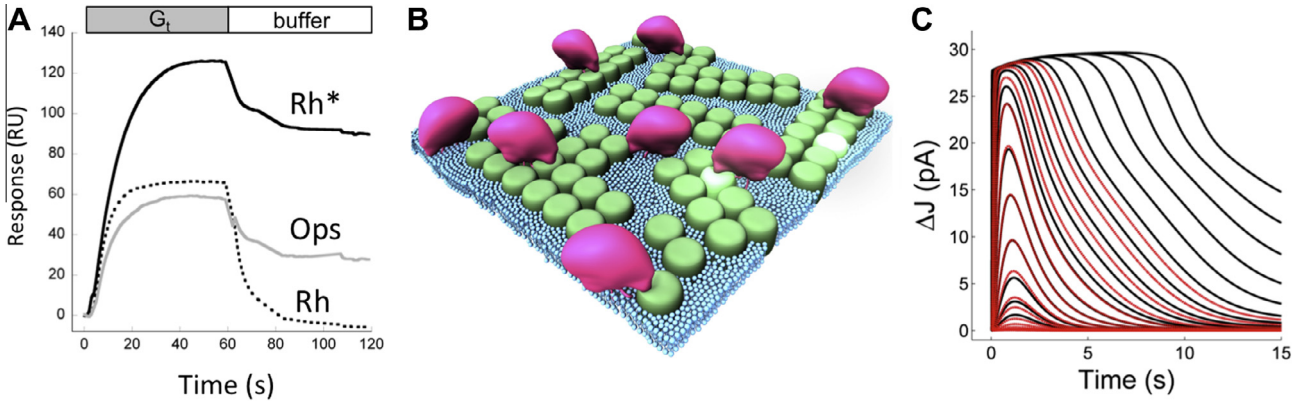

Fig. 1. Results from kinetic analyses and the dynamic scaffolding model. (A) Sensorgrams from surface plasmon resonance binding experiments between 0.33 μM G_t in the mobile phase and 0.22 pmol mm^{-2} Rh immobilized over the sensor chip. The sensorgram relative to dark binding (black dotted line) is compared with that obtained after 4 s illumination of Rh (black continuous line) and that obtained after 40 min bleaching (Ops, gray line). (B) Scheme of the dynamic scaffolding mechanism for Rh–G_t interaction in the dark. A patch of rod disc delimited by the membrane bilayer is represented and proteins other than Rh (green) and G_t (magenta) are omitted for clarity. At equilibrium, approximately 25% of G_t in the disc are dynamically bound to Rh, which is organized in supramolecular architectures such as the rafts of paracrystalline structure shown here, whose most common unit is the Rh dimer. The protein–protein scaffolding is highly dynamic, as a combined result of the diffusion of G_t in the lipid milieu by its farnesyl and acyl modifications and the high rate of dissociation/association from/to dark Rh. When the disc patch is exposed to light, a photon can either hit a preformed Rh–G_t complex or, with higher probability, a G_t-unbound Rh (bright green cylinders represent photoactivated receptors), in both cases triggering the activation of the phototransduction cascade. (C) Simulated photoresponses in an amphibian rod stimulated by 24 ms flashes of increasing intensities, ranging from 1.5 to 52 700 photoisomerizations. The resulting currents, expressing the difference between the dark current and the light-induced photocurrent, are shown for the realistic case in which the light stimulus is proportionally absorbed by both G_t-unbound Rh and pre-formed Rh–G_t complexes (black solid lines) and, ideally, by the preformed complexes only (red dashed lines). In both cases, though with different efficiency, the photoresponse can reach saturation and for all the intensities it shows the typical deactivation kinetics. This research was originally published in Biochemical Journal. Dell'Orco, D. and Koch, K.-W. (2011) A dynamic scaffolding mechanism for rhodopsin and transducin interaction in vertebrate vision. Biochem. J. 440, 263–71 © the Biochemical Society.

(c) both. The probability for the light stimulus to be captured by one of the two forms (Rh or Rh–G_t) depends on their relative abundance at the equilibrium in the dark. If the light stimulus was assumed to be proportionally absorbed by both forms, the shape and quantitative dynamics of the photoresponse resulting from simulations were indistinguishable from those observed experimentally in rod cells (Fig. 1C). If *only* the Rh–G_t preassembled complexes and not free Rh ideally captured the same light stimulus, the effect would be simply a reduction of the overall cascade efficiency, as the shape and the recovery of the photoresponses would both appear normal (Fig. 1C, red lines). Thus, it was quantitatively demonstrated by numerical simulations that no delay or slow-down of any of the reactions building up the phototransduction cascade would occur as a consequence of the transient precoupling. However, it is important to stress that *both forms* of Rh, precoupled with G_t or not, could in fact absorb photons ad trigger the cascade, the only discriminator being the probability of such an event, which depends on the abundance of the preformed complexes relative to the available pigment and could be modified, for instance, in pathologic conditions.

In conclusion, the model proposed by Dell'Orco and Koch [37] based on experimental evidence and results from numeric simulations is that of a "dynamic scaffolding" mechanism (Fig. 1B) for Rh and G_t interaction: with no need for specific scaffolding proteins, the structural organization of the molecular machinery in vertebrate discs may facilitate the interaction in cases of reduced diffusion despite the high concentration of Rh. In fact, it is suggested that besides diffusing in the lipid milieu, G_t has a propensity toward protein-protein interactions with Rh in the dark. These two concerted and opposing diffusion/binding phenomena give rise to a dynamic "hopping" of G_t onto dark-adapted Rh, hence building up a convenient scaffold that maintains a dynamic equilibrium in which approximately 1 out of 4 G_t's are actually bound to Rh at any time independent on the illumination state. This would ensure a sufficient pool of G_t in the case where the nearby photoactivated Rh^* is embedded in a supramolecular cluster of Rh molecules, which according to the classical picture would constitute a diffusional barrier due to molecular crowding.

A similar concept based on dynamic preformed complexes is used to explain the phototransduction cascade in *Drosophila*, [43,44]. This pathway represents one of the fastest known signalling systems, and it was shown to require a scaffolding protein (INAD) that dynamically preassembles parts of the molecular machinery to ensure fast and coordinated visual signaling [43]. Diffusion, evidently, is not the only physical means by which fast interactions may occur.

3. Single photon detection might be influenced by the supramolecular organization of Rh and G_t

Most of the quantitative information available to date as on the response kinetics of rod and cone cells arises to a great extent from electrophysiological recordings. Patch clamp and suction electrode techniques applied to photoreceptors allow one to follow directly the time-course of the membrane potential or photocurrent upon specific light stimulation. Is it possible to identify an electrophysiological correlate of the supramolecular organization of Rh and G_t? Does the plenty of electrophysiological data produced so far show any hint of the presence of Rh assemblies?

In a very recent analysis [45], the role of temperature as a parameter capable of influencing Rh–Rh and Rh–G_t interactions has been considered in the light of electrophysiological evidence. Temperature is known to influence the diffusional properties of Rh [46] and also its interaction with G_t [47] and consistent with the free diffusion model, higher temperatures were shown to significantly enhance (~2.4-fold every 10 °C) the kinetics of flash photoresponses in both lower vertebrates and mammalian rods [48,49]. It is reasonable to conclude that an increase of membrane fluidity leads to faster diffusion of Rh and G_t in the discs and, according to the collision coupling model, more frequent encounters will accelerate the response kinetics [3]. However, a careful analysis of electrophysiological data on single photon responses (SPRs) that have been largely neglected may reveal unexpected incompatibilities with the classical view, as pointed out recently [45]. A study performed by Robinson et al. [50] revealed that the efficiency of photon capture and conversion to an electrical

signal by rat rods increases with decreasing temperature. This phenomenon can be quantified by the concept of effective collecting area A_c (μm^2) which connects the intensity of the light stimulus i (photons/μm^2) to the number of effective photoisomerizations Φ_i, according to the relation: $\Phi_i = A_c i \cdot A_c$ depends on a number of factors, including the geometrical cross section of the rod outer segment accounting for the space distribution of the pigment, the photon absorption characteristics of the ROS and the efficiency of Rh isomerization. Experimentally, A_c is measured by delivering a sequence of dim flashes to estimate the probability of response failure, as shown in Fig. 2A. The product $A_c \cdot i$ is routinely interpreted as the average number of photoisomerizations evoked by a flash, based on the assumption that a Rh* is always able to trigger a photoresponse during its active lifetime. The marked and highly significant increase in A_c (~+40%) observed by Robinson et al. [50] when lowering the temperature of the same rod from body to room levels was similarly observed in mouse rods recorded with the patch clamp technique in a very recent study by Cangiano et al. [51], following a novel technique that allows accurate recordings [52]. While focused studied are certainly necessary to further investigate this interesting phenomenon, these counterintuitive findings pose some questions as on our profound understanding of the molecular mechanisms in phototransduction.

How could the collecting area significantly increase with decreasing temperature? The evidence of no significant Rh translocation between the inner and the outer segment [53] rules out the possibility of a reversible effect of temperature on the number of Rh molecules in the outer segment. Contributions from a potential temperature-dependence of Rh photoisomerization are also to be excluded, since the extinction coefficient and quantum efficiency of Rh only very weakly depend on temperature for wavelengths near its absorption maximum [54]. Therefore, any significant effect of temperature on rod collecting area must emerge downstream of photoisomerization. Clearly, the established view is that any given Rh* binds sequentially to and activates a large number of G_ts, before being inactivated [3]. In this classical framework response failures cannot occur once a Rh* has been produced, and therefore temperature, while modifying reaction kinetics, cannot affect A_c.

While in a free diffusion scenario all Rh*s are made equal in their access to G_t, supramolecular interactions involving both Rh and G_t may strongly depend on temperature (Fig. 3 B) in still unknown ways, and may explain the puzzling finding of A_c dependency on temperature. Moreover, the assumption that *many* G_ts are activated by one Rh* should also be revised. In the last decade, the estimate of the rate of G_t activation by a Rh* [55] and that of the active lifetime of Rh* [56,57] have both seen a striking downward revision. Since the average number of G_ts activated by a Rh* is the

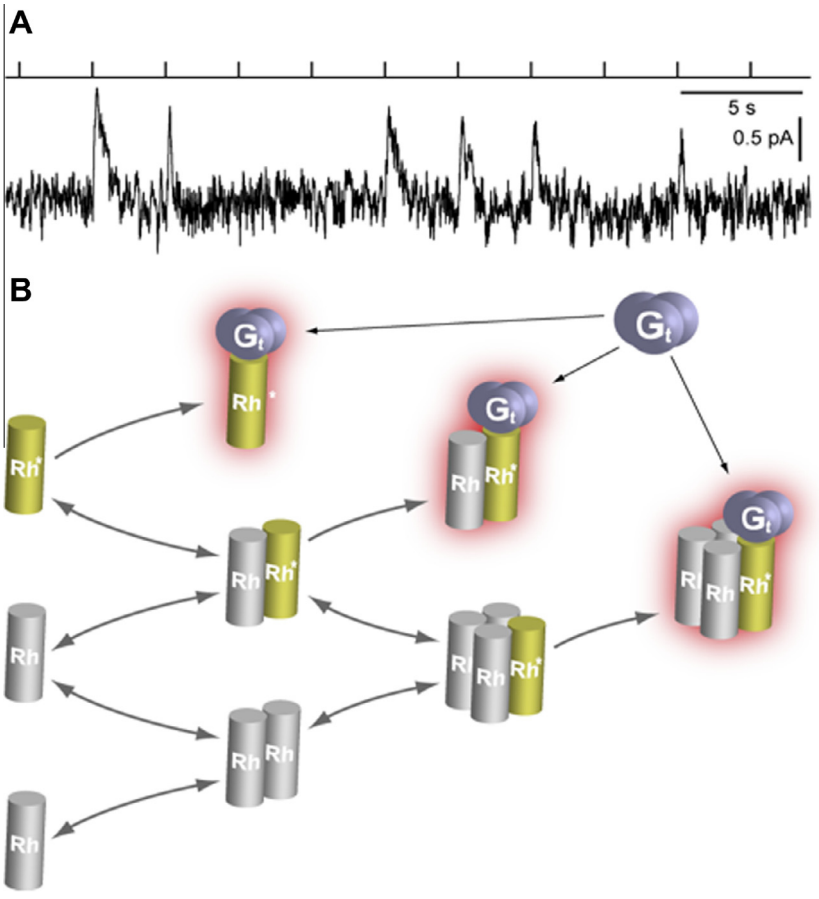

Fig. 2. (A) The photocurrent response of a mouse rod to a sequence of identical dim flashes (0.68 photons/μm^2), recorded with patch clamp at 24 °C. Note that in about half of the deliveries the rod fails to respond. Classically, these are interpreted as cases in which no Rh was photoisomerized. As discussed here, there is evidence in the literature for a possible second contribution to failure, downstream of receptor activation. (B) Schematic representing the supramolecular interactions involved in the early steps of phototransduction, which are potentially affected by temperature. Photoactivated Rh* are marked in gold. Supramolecular complexes highlighted in red are expected to normally trigger the cascade by allowing the activation of G_t, hence catalyzing the GDP to GTP exchange. The binding of G_t to photoactivated Rh is considered an irreversible process. Depending on the steric hindrance due to the presence of other G_t molecules, each inactive Rh (dark-adapted) can transiently bind a G_t molecule in the dark with high affinity ($K_D \approx$ 360 nM), but extremely fast dissociation rate ($k^{off}_{dark} \approx 300\ k^{off}_{light}$; values from [37]). While they might reasonably be affected by temperature, these transient binding processes have been omitted in the figure for clarity. The figure is reproduced from Cangiano, L. and Dell'Orco, D. (2013) Detecting single photons: supramolecular matter? FEBS Lett. 587, 1–4. © FEBS

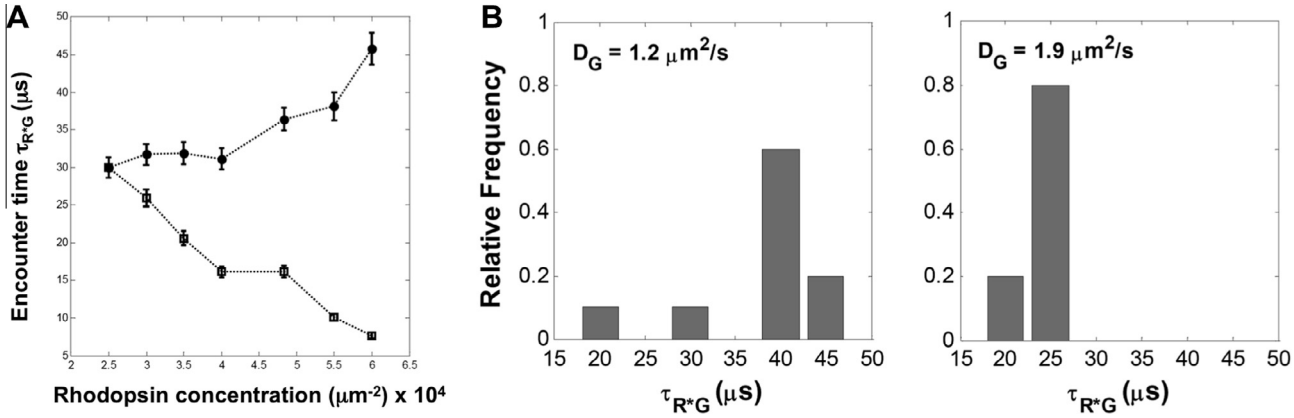

Fig. 3. (A) Results from mesoscopic Monte Carlo simulations of the encounter between a Rh* and a G_t accounting for crowding effects in the classical framework, in which both molecules can diffuse in the lipid milieu with ideal diffusion coefficient set to D_G = 1.2 μm^2 s^{-1} and D_R = 0.7 μm^2 s^{-1}. Data points marked with filled circles represent the case where the concentration of G_t was kept fixed to 2500 μm^{-2} whereas that of Rh was increased up to 60000 μm^{-2}. Data points marked with open squares refer to both concentrations subsequently increased with a fixed concentrations ratio of 10. (B) Histograms reporting the distribution of encounter times τ_{R^*G}'s for two different G_t diffusion coefficients in the framework of randomly sized and shaped rafts of Rh-dimers. In the left panel, G_t diffusion coefficient was set to its "classical" value D_G = 1.2 μm^2 s^{-1}, whereas in the right panel the overall effective diffusion coefficient was attributed to G_t, that is D_G = 1.9 μm^2 s^{-1}. In both cases, Rh-dimers and oligomers were considered immobile, that is D_R = 0 μm^2 s^{-1}. Reprinted with permission from Dell'Orco, D. and Schmidt, H. Mesoscopic Monte Carlo simulations of stochastic encounters between photoactivated rhodopsin and transducin in disc membranes. J. Phys. Chem. B 112, 4419–26. © 2008 American Chemical Society.

product of these two parameters, it will also necessarily undergo a dramatic reduction. Indeed, a G_t activation rate of 240 s^{-1} [56] and a mean active Rh* lifetime of 36 ms [57] perhaps surprisingly would lead to a number of activated G_ts as small as 8–9.

While the possibility that the activation of G_t by Rh* may fail in a fraction of cases has not been carefully considered so far, it appears reasonable that a connection may exist between the number of failures and the supramolecular organization of the molecules involved in the early steps of vision. In future studies it will be important to investigate whether the supramolecular organization of both Rh assemblies, perhaps in paracrystalline rafts, and Rh–G_t transient complexes in the dark are in fact influenced by the temperature, and whether the dynamic scaffolding model (Fig. B) could quantitatively help to understand the temperature dependency of the collecting area, which is apparently at odds with the classical scenario.

4. A putative physiological role for Rh rafts

The physiological role of supramolecular assemblies of Rh remains unclear, although their existence was further suggested by recent unbiased coarse-grained molecular dynamics simulations, which thoroughly explored the self-assembly properties of 64 Rh molecules in a lipid bilayer [58]. The study showed that no energetic barrier accompanies the formation of stable Rh dimers, which in a time frame of 16 μs can assemble to form higher-order structures resembling the controversial rows of dimers observed in AFM experiments [15,17].

The assumption that Rh can spontaneously form dimers and higher-order oligomers in the disc membrane however does not solve the dilemma of the receptor molecules being indeed functional in such supramolecular state, i.e. whether G_t can actually reach a Rh* molecule, thereby being activated and allowing the full occurrence of further cascade steps. It has been suggested that most of the Rh molecules in the oligomeric arrays are virtually excluded from phototransduction [20], but this conclusion would need an explanation for the existence of entire areas of ROS discs found to be occupied by immobile Rhs that may extend to cover up to 100% of the whole pigment. Another model has been proposed that takes into account membrane heterogeneity and diffusion. The diffusion of both G_t and Rh* right after illumination in a bulk lipid phase with relatively low effective viscosity and the subsequent migration of the R*–G_t complex toward microdomains enriched in cholesterol might facilitate the conformational changes required for the signalling steps [59], however this model does not contemplate the existence of Rh oligomers bigger than dimers. Not even the most recent structural determinations by scanning transmission electron microscopy of isolated nucleotide-free Rh*–G_t complexes [60], which provided evidence for two Rh molecules (a Rh dimer) and one G_t heterotrimer as the functional unit initiating the cascade seem able to explain how it is possible that Rh* embedded in the middle of a massive oligomer can effectively bind and activate several G_ts.

The investigation of how the diffusion properties of both Rh and G_t are influenced by specific supramolecular assemblies is not trivial to perform, and to date the only information available arises from mesoscopic stochastic simulations that mimic the first effective encounter between a photoactivated Rh* and G_t[61]. Molecular size, geometric information and protein concentration contributing to increase molecular crowding were all taken into account to assess the effects on the diffusion coefficient of both Rh (D_R) and G_t (D_G), eventually leading to accurate predictions of the time needed for effective encounters to occur (τ_{R^*G}). Simulations were performed in a lattice mimicking a 300 nm × 300 nm patch of disc membrane, in which three different scenarios were compared with respect to their effects on timing, namely: (a) the classical framework, where both G_t and monomeric Rh are allowed to freely diffuse in the ROS disc membrane, (b) an ideal paracrystalline organization of Rh dimers considered as a structural unit, where ordered rows completely cover the disc membrane patch, and (c) the scenario suggested by AFM data [15,17], where Rh dimers organize in differently sized rafts with varying local concentrations. The simulations surprisingly suggested that, at least in the dim light regime, an unexpected favorable effect on the temporal response of early phototransduction reactions may occur when the effective diffusion coefficient is completely attributed to G_t and immobile Rh molecules are packed in highly ordered assemblies rather than building unspecific aggregates [61]. Moreover, setting the diffusion coefficient to their "classical" values, (D_R = 0.7 μm^2 s^{-1} and D_G = 1.2 μm^2 s^{-1}) and increasing the concentration of Rh while keeping that of G_t unaltered leads to apparent crowding effects that significantly slow down the effective

bindings (Fig. 3A). However, if the significantly increased concentration of Rh is achieved by organizing the receptor in paracrystalline rafts of dimers of varying size and shape, modest tuning of D_G exert great effects on τ_{R*G} (Fig. 3B): if the paracrystalline rafts of Rh were made of immobile receptors ($D_R = 0\ \mu m^2\ s^{-1}$) and the overall diffusion coefficient were attributed to G_t ($D_G = 1.9\ \mu m^2\ s^{-1}$), not only would τ_{R*G} decrease significantly becoming consistent with the classical value (about 30 μs), but the distribution of observed encounter frequencies would become much more homogeneous (compare Fig. 3B, left vs. right panel). This is consistent with the concept that, even neglecting the dark precoupling, a pure diffusion-limited framework for Rh–G_t interactions could be kinetically favoured by a supramolecular organization that is intrinsically ordered. Despite receptors could not move in the massive aggregates, the anisotropic and faster diffusion of G_t in those areas, which is fully compatible with the geometric constraints [61], may serve to homogenize the encounter frequency, which could be of high relevance for dim-flashes and single photon responses.

The stochastic simulations of Rh^*–G_t encounters [61] did not include the dynamic precoupling hypothesis, and yet, the results suggest that ordered supramolecular assemblies of Rh instead of being detrimental for the phototransduction cascade and lead to its blocking, could in fact accelerate the effective encounters in the case of highly crowded environments, which is likely the real condition in vertebrate rod discs. It appears therefore rather reasonable to conclude that, if the supramolecular oganization is extended to the dark interaction with G_t according to the dynamic scaffolding hypothesis, the overall kinetic picture would be fully consistent with the one obtained via a classical diffusion-based picture that, however, is becoming deeply questionable.

In conclusion, it has been numerically demonstrated that the kinetics of vertebrate photoresponses obtained upon both dim and bright light stimuli can be fully explained by the dynamic scaffolding mechanisms for Rh^*–G_t interactions [37], and the dark coupling between Rh and G_t could indeed represent a convenient way to keep around a sufficient amount of molecules needed to trigger and propagate the signalling cascade in spite of the crowded environment represented by the supramolecular Rh assemblies. The emerging picture significantly differs from the classical one, and yet it can quantitatively explain at least the early steps in vertebrate vision.

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References

[1] Chen, C.K. (2005) The vertebrate phototransduction cascade: amplificationand termination mechanisms. Rev. Physiol. Biochem. Pharmacol. 154, 101–121.
[2] Lamb, T.D. and Pugh Jr., E.N. (2006) Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. Invest. Ophthalmol. Vis. Sci. 47, 5137–5152.
[3] Pugh Jr., E.N. and Lamb, T.D. (2000). Phototransduction in Vertebrate Rods and Cones: Molecular Mechanisms of AmplificationRecovery and Light Adaptation, Elsevier Science B.V.
[4] Drzymala, R.E., Weiner, H.L., Dearry, C.A. and Liebman, P.A. (1984) A barrier to lateral diffusion of porphyropsin in Necturus rod outer segment disks. Biophys. J. 45, 683–692.
[5] Gupta, B.D. and Williams, T.P. (1990) Lateral diffusion of visual pigments in toad (Bufo marinus) rods and in catfish (Ictalurus punctatus) cones. J. Physiol. 430, 483–496.
[6] Kaplan, M.W. (1984) Rhodopsin lateral diffusion as a function of rod outer segment disk membrane axial position. Biophys. J. 45, 851–853.
[7] Liebman, P.A. and Entine, G. (1974) Lateral diffusion of visual pigment in photorecptor disk membranes. Science 185, 457–459.
[8] Liebman, P.A., Weiner, H.L. and Drzymala, R.E. (1982) Lateral diffusion of visual pigment in rod disk membranes. Methods Enzymol. 81, 660–668.
[9] Poo, M. and Cone, R.A. (1973) Lateral diffusion of rhodopsin in the visual receptor membrane. J. Supramol. Struct. 1, 354.
[10] Poo, M. and Cone, R.A. (1974) Lateral diffusion of rhodopsin in the photoreceptor membrane. Nature 247, 438–441.
[11] Poo, M.M. and Cone, R.A. (1973) Lateral diffusion of phodopsin in Necturusrods. Exp. Eye Res. 17, 503–510.
[12] Wey, C.L., Cone, R.A. and Edidin, M.A. (1981) Lateral diffusion of rhodopsin in photoreceptor cells measured by fluorescence photobleaching and recovery. Biophys. J. 33, 225–232.
[13] Palczewski, K. et al. (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289, 739–745.
[14] Fanelli, F. and De Benedetti, P.G. (2011) Update 1 of: computational modeling approaches to structure–function analysis of G protein-coupled receptors. Chem. Rev. 111, PR438–535.
[15] Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D.A., Engel, A. and Palczewski, K. (2003) Atomic-force microscopy: rhodopsin dimers in native disc membranes. Nature 421, 127–128.
[16] Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D.A., Engel, A. and Palczewski, K. (2004) The G protein-coupled receptor rhodopsin in the native membrane. FEBS Lett. 564, 281–288.
[17] Liang, Y., Fotiadis, D., Filipek, S., Saperstein, D.A., Palczewski, K. and Engel, A. (2003) Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. J. Biol. Chem. 278, 21655–21662.
[18] Shukolyukov, S.A. (2009) Aggregation of frog rhodopsin to oligomers and their dissociation to monomer: application of BN- and SDS–PAGE. Biochemistry (Mosc.) 74, 599–604.
[19] Suda, K., Filipek, S., Palczewski, K., Engel, A. and Fotiadis, D. (2004) The supramolecular structure of the GPCR rhodopsin in solution and native disc membranes. Mol. Membr. Biol. 21, 435–446.
[20] Govardovskii, V.I., Korenyak, D.A., Shukolyukov, S.A. and Zueva, L.V. (2009) Lateral diffusion of rhodopsin in photoreceptor membrane: a reappraisal. Mol. Vis. 15, 1717–1729.
[21] Chabre, M., Cone, R. and Saibil, H. (2003) Biophysics: is rhodopsin dimeric in native retinal rods? Nature 426, 30–31. discussion 31.
[22] Chabre, M. and le Maire, M. (2005) Monomeric G-protein-coupled receptor as a functional unit. Biochemistry 44, 9395–9403.
[23] Chabre, M., Deterre, P. and Antonny, B. (2009) The apparent cooperativity of some GPCRs does not necessarily imply dimerization. Trends Pharmacol. Sci. 30, 182–187.
[24] Birdsall, N.J. (2010) Class A GPCR heterodimers: evidence from binding studies. Trends Pharmacol. Sci. 31, 499–508.
[25] George, S.R., O'Dowd, B.F. and Lee, S.P. (2002) G-protein-coupled receptor oligomerization and its potential for drug discovery. Nat. Rev. Drug Discov. 1, 808–820.
[26] Fanelli, F. and De Benedetti, P.G. (2005) Computational modeling approaches to structure–function analysis of G protein-coupled receptors. Chem. Rev. 105, 3297–3351.
[27] Shea, L. and Linderman, J.J. (1997) Mechanistic model of G-protein signal transduction. Determinants of efficacy and effect of precoupled receptors. Biochem. Pharmacol. 53, 519–530.
[28] Nobles, M., Benians, A. and Tinker, A. (2005) Heterotrimeric G proteins precouple with G protein-coupled receptors in living cells. Proc. Natl. Acad. Sci. USA 102, 18706–18711.
[29] Gales, C., Rebois, R.V., Hogue, M., Trieu, P., Breit, A., Hebert, T.E. and Bouvier, M. (2005) Real-time monitoring of receptor and G-protein interactions in living cells. Nat. Methods 2, 177–184.
[30] Hamm, H.E., Deretic, D., Hofmann, K.P., Schleicher, A. and Kohl, B. (1987) Mechanism of action of monoclonal antibodies that block the light activation of the guanyl nucleotide-binding protein, transducin. J. Biol. Chem. 262, 10831–10838.
[31] Alves, I.D., Salgado, G.F., Salamon, Z., Brown, M.F., Tollin, G. and Hruby, V.J. (2005) Phosphatidylethanolamine enhances rhodopsin photoactivation and transducin binding in a solid supported lipid bilayer as determined using plasmon-waveguide resonance spectroscopy. Biophys. J. 88, 198–210.
[32] Salamon, Z., Wang, Y., Soulages, J.L., Brown, M.F. and Tollin, G. (1996) Surface plasmon resonance spectroscopy studies of membrane proteins: transducin binding and activation by rhodopsin monitored in thin membrane films. Biophys. J. 71, 283–294.
[33] Dell'Orco, D., Seeber, M. and Fanelli, F. (2007) Monomeric dark rhodopsin holds the molecular determinants for transducin recognition: insights from computational analysis. FEBS Lett. 581, 944–948.
[34] Fanelli, F. and Dell'Orco, D. (2005) Rhodopsin activation follows precoupling with transducin: inferences from computational analysis. Biochemistry 44, 14695–14700.
[35] Fanelli, F. and Dell'Orco, D. (2008) Dark and photoactivated rhodopsin share common binding modes to transducin. FEBS Lett. 582, 991–996.
[36] Sgourakis, N.G. and Garcia, A.E. (2010) The membrane complex between transducin and dark-state rhodopsin exhibits large-amplitude interface dynamics on the sub-microsecond timescale: insights from all-atom MD simulations. J. Mol. Biol. 398, 161–173.
[37] Dell'Orco, D. and Koch, K.W. (2011) A dynamic scaffolding mechanism for rhodopsin and transducin interaction in vertebrate vision. Biochem. J. 440, 263–271.
[38] Komolov, K.E., Aguila, M., Toledo, D., Manyosa, J., Garriga, P. and Koch, K.W. (2010) On-chip photoactivation of heterologously expressed rhodopsin allows kinetic analysis of G-protein signaling by surface plasmon resonance spectroscopy. Anal. Bioanal. Chem. 397, 2967–2976.

[39] Komolov, K.E. and Koch, K.W. (2010) Application of surface plasmon resonance spectroscopy to study G-protein coupled receptor signalling. Methods Mol. Biol. 627, 249–260.
[40] Komolov, K.E., Senin, I.I., Philippov, P.P. and Koch, K.W. (2006) Surface plasmon resonance study of g protein/receptor coupling in a lipid bilayer-free system. Anal. Chem. 78, 1228–1234.
[41] Schleicher, A. and Hofmann, K.P. (1987) Kinetic study on the equilibrium between membrane-bound and free photoreceptor G-protein. J. Membr. Biol. 95, 271–281.
[42] Dell'Orco, D., Schmidt, H., Mariani, S. and Fanelli, F. (2009) Network-level analysis of light adaptation in rod cells under normal and altered conditions. Mol. Biosyst. 5, 1232–1246.
[43] Mishra, P., Socolich, M., Wall, M.A., Graves, J., Wang, Z. and Ranganathan, R. (2007) Dynamic scaffolding in a G protein-coupled signaling system. Cell 131, 80–92.
[44] Scott, K. and Zuker, C.S. (1998) Assembly of the Drosophila phototransduction cascade into a signalling complex shapes elementary responses. Nature 395, 805–808.
[45] Cangiano, L. and Dell'Orco, D. (2013) Detecting single photons: a supramolecular matter? FEBS Lett. 587, 1–4.
[46] Coke, M., Restall, C.J., Kemp, CM. and Chapman, D. (1986) Rotational diffusion of rhodopsin in the visual receptor membrane: effects of temperature and bleaching. Biochemistry 25, 513–518.
[47] Kahlert, M. and Hofmann, K.P. (1991) Reaction rate and collisional efficiency of the rhodopsin–transducin system in intact retinal rods. Biophys. J. 59, 375–386.
[48] Lamb, T.D. (1984) Effects of temperature changes on toad rod photocurrents. J. Physiol. 346, 557–578.
[49] Nymark, S., Heikkinen, H., Haldin, C., Donner, K. and Koskelainen, A. (2005) Light responses and light adaptation in rat retinal rods at different temperatures. J. Physiol. 567, 923–938.
[50] Robinson, D.W., Ratto, G.M., Lagnado, L. and McNaughton, P.A. (1993) Temperature dependence of the light response in rat rods. J. Physiol. 462, 465–481.
[51] Cangiano, L., Asteriti, S., Cervetto, L. and Gargini, C. (2012) The photovoltage of rods and cones in the dark-adapted mouse retina. J. Physiol. 590, 3841–3855.
[52] Delia Santina, L., Piano, I., Cangiano, L., Caputo, A., Ludwig, A., Cervetto, L. and Gargini, C. (2012) Processing of retinal signals in normal and HCN deficient mice. PLoS One 7, e29812.
[53] Sokolov, M., Lyubarsky, A.L., Strissel, K.J., Savchenko, A.B., Govardovskii, V.I., Pugh Jr., E.N. and Arshavsky, V.Y. (2002) Massive light-driven translocation of transducin between the two major compartments of rod cells: a novel mechanism of light adaptation. Neuron 34, 95–106.
[54] Hurley, J.B., Ebrey, T.G., Honig, B. and Ottolenghi, M. (1977) Temperature and wavelength effects on the photochemistry of rhodopsin, isorhodopsin, bacteriorhodopsin and their photoproducts. Nature 270, 540–542.
[55] Leskov, I.B. et al. (2000) The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. Neuron 27, 525–537.
[56] Krispel, CM. et al. (2006) RGS expression rate-limits recovery of rod photoresponses. Neuron 51, 409–416.
[57] Gross, O.P. and Burns, M.E. (2010) Control of rhodopsin's active lifetime by arrestin-1 expression in mammalian rods. J. Neurosci. 30, 3450–3457.
[58] Periole, X., Knepp, A.M., Sakmar, T.P., Marrink, S.J. and Huber, T. (2012) Structural determinants of the supramolecular organization of G protein-coupled receptors in bilayers. J. Am. Chem. Soc. 134, 10959–10965.
[59] Wang, Q., Zhang, X., Zhang, L., He, F., Zhang, G., Jamrich, M. and Wensel, T.G. (2008) Activation-dependent hindrance of photoreceptor G protein diffusion by lipid microdomains. J. Biol. Chem. 283, 30015–30024.
[60] Jastrzebska, B., Ringler, P., Lodowski, D.T., Moiseenkova-Bell, V., Golczak, M., Muller, S.A., Palczewski, K. and Engel, A. (2011) Rhodopsin-transducin heteropentamer: three-dimensional structure and biochemical characterization. J. Struct. Biol. 176, 387–394.
[61] Dell'Orco, D. and Schmidt, H. (2008) Mesoscopic Monte Carlo simulations of stochastic encounters between photoactivated rhodopsin and transducin in disc membranes. J. Phys. Chem. B 112, 4419–4426.