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Hypothesis

Detecting single photons: A supramolecular matter?

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ABSTRACT

Rod photoreceptors detect single photons through a tradeoff of light collecting ability, amplification and speed. Key roles are played by rhodopsin (Rh) and transducin (G_t), whose complex supramolecular organization in outer segment disks begs for a functional interpretation. Here we review past and recent evidence of a temperature-dependence of photon detection by mammalian rods, and link this phenomenon with the putative oligomeric organization of Rh and new ideas on the dynamics of Rh–G_t interaction. Identifying an electrophysiological correlate of the supramolecular organization of Rh and G_t may shed light on the evolutionary advantage it confers to night vision.

1. Introduction

Vision in vertebrates begins with the absorption of photons by specialized photoreceptor cells localized in the retina, which convert the information carried by light into an electrical signal relayed to downstream neurons, by means of a complex cascade of biochemical events known as phototransduction [1]. According to the intensity of ambient light, scotopic, mesopic or photopic vision occurs involving a specific mix of the two main photoreceptor types: rods and cones. Evolutionary pressure has shaped the dim-light rod photoreceptor into an exquisitely sensitive device capable of operating at the physical limits imposed by the quantal nature of light. A dark adapted rod transforms the energy of an absorbed photon in a distinct electrical signal called the single photon response (SPR), consisting of a brief membrane potential hyperpolarization that in mammals has a peak amplitude of one to a few mV and time-to-peak of 100–150 ms [2,3]. Despite the presence of some degree of thresholding at downstream synapses, which is important to filter out biological noise [4], a significant fraction of SPRs succeed in modifying the dark discharge rate of retinal ganglion cells and contribute to our perception of light [5]. The *a priori* requirements that must be met by a detector such as the rod are threefold: (i) it must selectively amplify the single photon signal to exceed noise levels by an adequate factor; (ii) the generated signal has to build up quickly to preserve sufficient temporal information about the light source; (iii) it should collect as many photons as possible. The first two requirements conjure towards a dimensionally compact detector and a particularly fast-responding biochemical cascade [6], while the third one implies a high optical density. This is achieved by the stacked disk structure of rod outer segments and a tight packing of the light-activated and G protein-coupled receptor (GPCR) rhodopsin (Rh), present at high concentration (~6 mM) in the disk membranes. In principle a tradeoff exists in fulfilling these requirements, since, as molecular crowding increases, diffusional processes in the membrane slow down. Thus, the organization of the disk membrane proteins in darkness can be expected to strike an optimal, and perhaps delicate balance for single photon signaling.

2. Challenges to the classical view of freely diffusing molecules

In the classical view of phototransduction, the different components of its biochemical machinery have been modeled as freely diffusing molecules (see [7] and references therein), be they membrane bound or cytosolic. Results published in recent years threaten to significantly complicate this picture. Rh, which lies at the start of phototransduction, has been reported to natively dimerize and even oligomerize in paracrystalline rafts within the disk membrane [8–10], or form non-specific aggregates in the central area of the disk, surrounded by a girdle of lipids [11]. Among these, observations in which atomic force microscopy (AFM) was used to image isolated mouse outer segment discs received independent

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support from microspectrophotometric analysis suggesting that a large fraction of Rh may be essentially immobile [12].

At odds with the classical view, in which the receptor and the G protein interact only after receptor activation, several lines of evidence [13–16] suggest that Rh and transducin (G_t) can form complexes already in the dark, although the binding is ineffective toward the activation of the cascade. Recent *in vitro* experiments integrated into a holistic mathematical model concluded that an isolated Rh molecule or a Rh–G_t can both absorb a photon and normally trigger the cascade [7]. This mechanism would be compatible with a dynamic scaffolding, in which ~25% G_t and Rh transiently interact already in the dark, with very fast association/dissociation kinetics that may 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 would constitute a diffusional barrier due to crowding in the classical picture.

The supramolecular organization emerging from all these recent findings hence poses conceptual challenges to the classical model of phototransduction, in which Rh and G_t diffuse freely in the disk surface. In particular, the physiological relevance of some of these data has been questioned [11,17], leading to an ongoing controversy that represents an important facet of a wider debate on the supramolecular organization of GPCRs, and its implications for cellular signaling (for review see [18–20]). The current disagreement over the oligomeric state of Rh and its role, if any, in regulating the efficiency of phototransduction is due, at least in part, to the difficulty of replicating *in vivo* conditions of the rod outer segment in *in vitro* morphological or biochemical studies. It is therefore not surprising that some advance in the quantitative understanding of these physiological processes has been obtained through numerical modeling. Recent mesoscopic simulations of the molecular encounters between photoactivated Rh and G_t suggested that if high-density Rh packing is characterized by a highly ordered structural organization, rather than unspecific aggregation, an unexpected favorable effect on the temporal response of early phototransduction reactions may occur, leading to the inference that responses in line with the classical kinetics could arise from very different microscopic scenarios [21]. The deep implications of this hypothesis become even more profound in the light of very recent coarse-grained molecular dynamics simulations at atomistic resolution, which explored the self-assembly properties of 64 Rh molecules in a lipid bilayer [22]. The substantial absence of energetic barriers in forming Rh dimers was accompanied by a variety of weak and strong Rh-Rh interfaces in unbiased simulations, which overall facilitate the possibility of higher-order structures naturally leading to the rows of dimers observed in the controversial AFM images [8] in a limited time frame of 16 μs [22].

A hypothetical dynamic regulation of Rh oligomerization has been suggested as one possible new mechanism of light adaptation [12]. This proposal must confront with the fact that the classical model of phototransduction has been quite successful at providing a quantitative description of light responses in rods [1]. One may thus ask whether any hint of the presence of supramolecular assemblies can be found in electrophysiological data from intact photoreceptors. One parameter expected to influence Rh–Rh and Rh–G_t interactions and the oligomeric state of Rh is temperature [23,24]. Higher temperatures markedly enhance flash response kinetics in both lower vertebrate and mammalian rods (by roughly 2.4-fold for every 10 °C (Q10); [25,26]), an effect consistent with the free diffusion model through an increase in lipid bilayer fluidity and a corresponding faster diffusion of membrane bound proteins. But if one focuses on the first steps of phototransduction, before the amplifying cascade is set off, intriguing evidence is available from past and recent mammalian photoreceptor recordings that cannot be easily reconciled with the classical model.

3. Neglected electrophysiological data may link form and function

In a thorough but hitherto neglected study in rat, Robinson et al. [27] compared the flash responses of single rods at different temperatures by recording the outer segment photocurrent with the suction electrode technique. They found, among other things, that the efficiency of photon capture and conversion into an electrical signal by rods increases with decreasing temperature. This key rod parameter (see point (iii) in Introduction) has been classically quantified by an effective *collecting area* A_c (μm^2), which links the incident photon density of a light stimulus i (photons/μm^2) to the number of photons evoking a photoisomerization Rh^*, and thus to the number of photoisomerizations Φ_i:

$$\Phi_i = A_c \cdot i$$

A_c depends on the geometrical cross section of the rod outer segment, on its characteristics of photon absorption, and on the efficiency of Rh isomerization. Since the trial-to-trial distribution of photoisomerizations has a Poisson distribution, A_c is conveniently determined by delivering a sequence of dim flashes (Fig. 1A) to estimate the probability of response failure (cf. Fig. 4A in [3]), using the equation:

$$A_c = -\ln(P_{failure}) \cdot i^{-1}$$

Strictly speaking, the A_c obtained with this operational definition measures the efficiency of the rod in converting the available photons into an electrical signal. 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. Robinson and colleagues observed in rat a marked and highly significant increase in A_c (~+40%) when lowering the temperature from body to room levels. This appears to be a robust result, since each rod was tested at different temperatures and both directions of temperature change were tested. An older study on toad rods had also made a more limited attempt to examine the effect of temperature on A_c, without detecting a clear trend [28]. Following the 1993 report by Robinson and colleagues the issue did not appear to have been re-examined, until recently when a similar phenomenon was reported in mouse rods recorded with the patch clamp technique [3]. Here, in contrast to the 1993 study in rat, different rods were recorded at the two temperatures and thus an influence of other confounding factors could not be ruled out. Nonetheless, the striking concordance of the two studies begs the question of how this phenomenon may be explained in terms of our understanding of phototransduction.

A first possibility would be a reversible effect of temperature on the number of Rh molecules in the outer segment, but to explain the large change in A_c an equal change would be required for Rh. While several key proteins involved in phototransduction are known to undergo a massive light-and time-dependent translocation between the inner and outer segments of rods, no such phenomenon occurs with Rh [29]. A significant contribution of a temperature-dependence of Rh photoisomerization should also be ruled out, since the extinction coefficient and quantum efficiency of Rh are very weakly dependent on temperature for wavelengths near its absorption peak [30]. Thus, any significant effect of temperature on rod collecting area must emerge downstream of photoisomerization. Here, the established view is that *any* given Rh^* binds sequentially to and activates a *large number* of G_ts, before being inactivated by the combined action of phosphorylation by rhodopsin kinase and binding by arrestin [1]. In this classical framework response failures cannot occur once a Rh^* has been