# Not Just Signal Shutoff: The Protective Role of Arrestin-1 in Rod Cells

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## Contents



**Abstract** The retinal rod cell is an exquisitely sensitive single-photon detector that primarily functions in dim light (e.g., moonlight). However, rod cells must routinely survive light intensities more than a billion times greater (e.g., bright daylight). One serious challenge to rod cell survival in daylight is the massive amount of all-*trans*-retinal that is released by Meta II, the light-activated form of the photoreceptor rhodopsin. All-*trans*-retinal is toxic, and its condensation products have been implicated in disease. Our recent work has developed the concept that rod arrestin (arrestin-1), which terminates Meta II signaling, has an additional role in protecting rod cells from the consequences of bright light by limiting free all-*trans*-retinal. In this chapter we will elaborate upon the molecular mechanisms by which arrestin-1 serves as both a single-photon response quencher as well as an instrument of rod cell survival in bright light. This discussion will take place within

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the framework of three distinct functional modules of vision: signal transduction, the retinoid cycle, and protein translocation.

**Keywords** Arrestin • Rhodopsin • Opsin • All-*trans*-retinal • Retinoid cycle • Translocation • Stoichiometry

# 1 Introduction

## *1.1 The Visual System*

The rod cell of the vertebrate retina is able to detect single photons by virtue of its cellular organization, the molecular structure of its photoreceptor rhodopsin, and the efficiency of the visual signal transduction module (Lamb and Pugh 2004; Hofmann et al. 2006, 2009). The rod outer segment (ROS) contains hundreds of flattened membranes (discs) that are densely packed with rhodopsin (Lamb and Pugh 2004). Rhodopsin is a G protein-coupled receptor (GPCR) composed of seven transmembrane helices (Fig. 1a) [see Hofmann et al. (2009)]. Visual signal transduction begins when the covalently linked inverse agonist of rhodopsin, 11-*cis*-retinal, absorbs a photon and isomerizes to all-*trans*-retinal (ATR). This event triggers a series of photo-intermediates that culminates in the active Metarhodopsin II (Meta II, Fig. 1a) (Matthews et al. 1963), which can couple to and activate the heterotrimeric G protein transducin. A single photon elicits a significant cellular response, because a single Meta II can activate hundreds of transducin molecules (Heck and Hofmann 2001), which go on to activate phosphodiesterase enzymes that rapidly hydrolyze intracellular cGMP (Hofmann et al. 2006). Meta II signaling is terminated by a multistep process. Rhodopsin kinase adds multiple phosphates to the C-terminal tail of the receptor (Wilden and Kuhn 1982), which allows the protein arrestin to bind and thereby block further interaction of Meta II with transducin (Wilden et al. 1986).

Meta II decays within minutes, when the Schiff base linking ATR to the protein is hydrolyzed, and Meta II releases a molecule of ATR resulting in the aporeceptor opsin. Arrestin-1 has been observed to modestly slow this process in vitro, but it cannot prevent it (Hofmann et al. 1992; Sommer et al. 2005). After ATR is released from the receptor, it is reduced by retinol dehydrogenase, and the resulting product all-*trans*-retinol diffuses to the nearby retinal pigment epithelium (RPE), where it is converted back to 11-*cis*-retinal by a complex enzymatic reaction sequence (McBee et al. 2001; Lamb and Pugh 2004; Wenzel et al. 2005). This retinoid cycle constitutes a functional module in which photolyzed ATR is re-isomerized to 11-*cis*-retinal in order to regenerate rhodopsin (Hofmann et al. 2006). In contrast to invertebrate rhodopsin, rhodopsin in vertebrates cannot be regenerated with a second photon absorption (Ritter et al. 2008). The complexity of the vertebrate retinoid cycle must confer some benefit by making photoreceptor regeneration

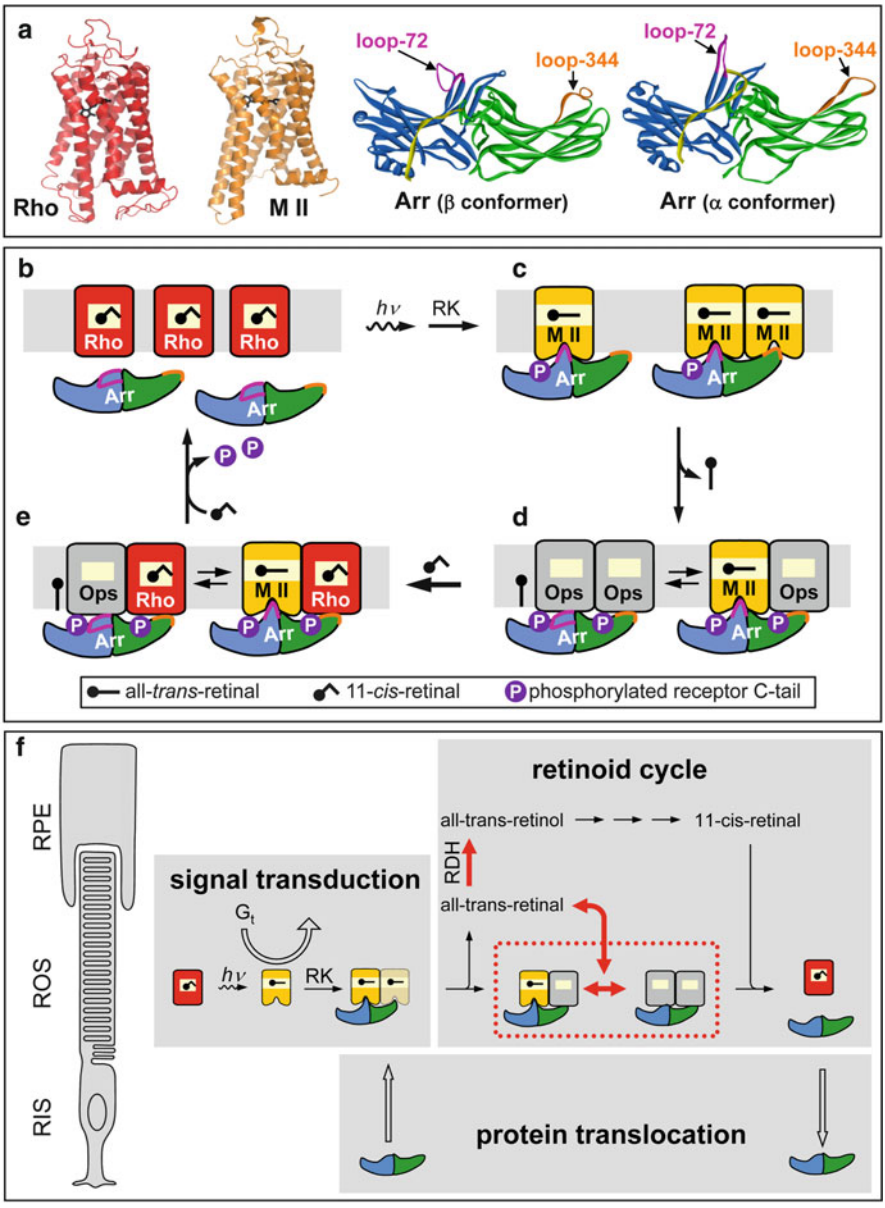


**Fig. 1** Molecular, sub-modular and modular organization of arrestin and rhodopsin in the visual system. (**a**) Crystal structures of rhodopsin (Rho, Protein Data Bank accession 1U19), Meta II (MII, 3PXO) and arrestin (Arr, 1CF1). Two different crystallographic conformers of arrestin (α and β), which differ primarily in the flexible loops of the receptor-binding surface, are shown. For the arrestin models, the N-domain is colored *blue*, the C-domain is colored *green*, the C-tail is *yellow*, loop-72 is *magenta*, and loop-344 is *orange*. (**b**, **c**) Dark-state rhodopsin absorbs light to become Meta II and is phosphorylated by rhodopsin kinase (RK), thereby allowing arrestin to bind at variable stoichiometric ratios (see text for more details). Note that loop-72 (*magenta*) adopts a more extended conformation upon engagement of Meta II-P. (**d**) The arrestin/Meta II-P complex decays

independent of ambient light (Saari 2000) or is necessary to compensate for an unstable Meta II conformation that resulted from evolutionarily advantageous mutations in rhodopsin (Lamb 2009). Whatever the benefit may be, it comes at a high price, since large amounts of ATR are released at higher light levels. ATR is toxic, and its condensation products are linked to age-related macular degeneration (explained in more detail below).

The polarity of the rod cell (that is, having distinct inner and outer segments) is the basis of an additional functional module in vision: protein translocation. In the dark-adapted rod cell, most arrestin-1 is bound to microtubules within the inner segment (Nair et al. 2004). Conversely, most transducin is bound to the disc membranes in the outer segment. Exposure to light causes these two proteins to switch locations. It is still debated whether light-triggered protein translocation takes place solely by passive diffusion, or if energy-consuming molecular motors are involved as well (Nair et al. 2005; Orisme et al. 2010; Satoh et al. 2010). It is also debated whether protein translocation contributes to light adaption (Arshavsky and Burns 2012), serves to save energy, or simply helps the rod cell survive bright light (Gurevich et al. 2011).

## *1.2 Arrestin Structure and Function*

The arrestin molecule consists of two roughly symmetrical clam-shaped lobes, termed the N- and C-domains, and each is composed of a β-sandwich (Fig. 1a) (Granzin et al. 1998; Hirsch et al. 1999). A long C-terminal tail (C-tail) interacts extensively with the N-domain and thereby holds arrestin in an inactive or basal

**Fig. 1** (continued) into an equilibrium in which ATR can re-enter half of the arrestin-bound OpsP. ATR uptake is accompanied by an extension of loop-72. (**e**) 11-*cis*-retinal can enter the other OpsP to regenerate rhodopsin. Removal of ATR is required for complete regeneration which, together with dephosphorylation of OpsP, dissociates arrestin. (**f**) *Signal transduction module:* dark-state rhodopsin (*red*) absorbs light (h$\nu$) to form Meta II (*yellow*), which interacts with transducin ($G_t$) to initiate signaling in the rod cell. Meta II is phosphorylated by rhodopsin kinase (RK) and bound with high affinity by arrestin (*blue*, *green*) at either a one-to-one or one-to-two stoichiometry, thereby blocking signaling. *Retinoid cycle module*: arrestin-bound Meta II-P decays and releases all-*trans*-retinal, leaving OpsP (*gray*). All-*trans*-retinal can re-enter half of the arrestin-bound OpsP population to reform arrestin-bound Meta II-P, thus sequestering toxic all-*trans*-retinal within OpsP [*red dotted box*; for details see (**d**)]. Free all-*trans*-retinal is reduced to all-*trans*-retinol by retinol dehydrogenase (RDH). All-*trans*-retinol diffuses to the retinal pigment epithelium (***RPE***) where it is converted to 11-*cis*-retinal by a multistep enzymatic process. 11-*cis*-retinal is delivered back to the ROS were it recombines with OpsP to regenerate rhodopsin. *Protein translocation module*: arrestin diffuses from the rod inner segment (***RIS***) to the outer segment (***ROS***) upon exposure of the dark-adapted rod cell to light and is retained in the ***ROS*** by its interaction with Meta II-P and OpsP. Regeneration of rhodopsin dissociates arrestin and allows its return to the ***RIS*** during dark adaptation. The cellular and subcellular locations of the reactions are indicated by their placements with respect to the simplified illustration of the rod cell and the ***RPE*** cell on the *left*

conformation. Between the two lobes is a region called the polar core, a buried hydrogen-bond network composed of residues from both domains and the C-tail. Gurevich and colleagues have proposed a mechanism whereby initial engagement of receptor-attached phosphates by Lys14 and Lys15 in the arrestin N-domain destabilizes the local structure and results in release of the C-tail (Vishnivetskiy et al. 2000). C-tail displacement then triggers activating conformational rearrangements in arrestin that eventually promote tight binding to the active receptor (Schröder et al. 2002). Recently, our group gained insight into these activating conformational changes by solving the X-ray crystal structure of a C-terminally truncated splice variant of arrestin called p44 (Kim et al. 2013). Due to the lack of the regulatory C-tail, p44 exists in a preactivated state that is primed to bind the active receptor (Schröder et al. 2002). The activating conformational rearrangements observed in the crystal structure of p44 include a breaking of the polar core, an increase in interdomain flexibility, and a 21° rotation of the domains relative to one another (Kim et al. 2013). In addition, several key loops in the "central crest" region of arrestin, which have been observed experimentally to undergo conformational changes upon receptor binding (Hanson et al. 2006; Sommer et al. 2007; Kim et al. 2012; Vishnivetskiy et al. 2013), show significant displacements in p44 as compared to basal arrestin-1. Notably, strikingly similar conformational changes were observed in the crystal structure of nonvisual arrestin-2 (also called β-arrestin-1) in complex with a fully phosphorylated peptide derived from the V2 vasopressin receptor (Shukla et al. 2013). Together these crystal structures indicate that activation does not entail any large-scale change in the overall structure of arrestin, and changes in flexible loops are key in activating arrestin for receptor binding. In particular, we have identified two loops in either domain of arrestin, loop-72 (Gly68-Ser78, also called the finger loop) in the N-domain and loop-344 (Lue338-Ala348) in the C-domain (Fig. 1a), that play distinct roles in receptor binding (Sommer et al. 2012).

## 2 Arrestin Quenches Meta II Signaling

Arrestin-1 has long been known to play a central role in shutting off the signaling of active Meta II in the rod cell (Wilden et al. 1986; Xu et al. 1997). Since it was shown that arrestin-1 specifically binds and stabilizes phosphorylated Meta II (Meta II-P) at a one-to-one ratio (Schleicher et al. 1989), it was assumed that a single arrestin-1 couples to a single light-activated Meta II-P, as would be generated in a rod cell exposed to dim light. Indeed, arrestin-1 can functionally bind monomeric Meta II-P isolated in nanodiscs (Tsukamoto et al. 2010; Bayburt et al. 2011). However, the bilobed structure and size of arrestin-1 suggest that two receptors could be bound by a single arrestin-1, which would be advantageous when higher percentages of receptors are activated. Our recent work indicates that arrestin-1 can actually bind Meta II at different stoichiometric ratios in the native rod disc membrane, either one-to-one or one-to-two depending on the percentage of activated receptors and the average receptor phosphorylation level (Sommer et al. 2011, 2012). This variability

may arise from the different binding preferences of arrestin's two domains. According to this model, loop-72 (finger loop) within the N-domain specifically engages the active receptor (Sommer et al. 2012) and phosphate sensors within the N-domain bind the phosphorylated receptor C terminus (Fig. 1c). In contrast, loop-344 within the C-domain of arrestin-1 is less specific and can engage either the membrane surface (in the case of one-to-one binding) or a neighboring Meta II (in the case of one-to-two binding) (Fig. 1c). In the case of one-to-two binding, phosphorylation of the second receptor molecule that is engaged by the C-domain is not required (Sommer et al. 2012). This adaptability in binding mode may allow arrestin-1 to be an efficient signal quencher at different light intensities. Arrestin-1 binds monomeric Meta II-P at the low light levels in which the rod cell operates. At higher light intensities, arrestin-1 binding to Meta II-P can also inactivate a neighboring Meta II, even before this second receptor has been phosphorylated, thereby enhancing the signal-quenching efficacy of arrestin-1 and saving the energy required for receptor phosphorylation.

## 3 Arrestin Protects the Rod Cell from the Consequences of Bright Light

Soon after rod arrestin-knockout mice were created (Xu et al. 1997), it was reported that these mice suffer light-dependent retinal degeneration (Chen et al. 1999). Given the known role of arrestin-1, it was assumed that rod cell death occurred due to lack of signal shutoff. Indeed, knocking-out transducin expression protects arrestin-knockout mice from dim light-induced damage, yet unexpectedly does not protect from bright light-induced damage (Hao et al. 2002). This result suggests that retinal damage in bright light is not solely due to excessive signaling. Furthermore, bright light-induced damage is accompanied by induction of the proapoptotic transcription factor AP-1 (Hao et al. 2002; Reme 2005), the expression of which in human RPE cells has been shown to be upregulated by oxidative stress (Kalariya et al. 2008; Chaum et al. 2009). Based on these observations, we hypothesized that arrestin-1 protects the rod cell from toxic levels of ATR (see below). In support of this idea, we previously observed that arrestin-1 interacts with the products of Meta II-P decay, namely, phosphorylated opsin (OpsP) and ATR (Hofmann et al. 1992; Sommer et al. 2005). In the following sections, we discuss the physiological necessity for the limitation of free ATR and our findings regarding how arrestin-1 may accomplish this task.

### *3.1 Bright Light Generates Toxic Levels of ATR in the Rod Cell*

Although necessary for vision, the high concentration of retinal in the rod cell presents a serious challenge to cell survival when light exposure induces the release

of ATR. The toxicity of ATR is apparent in the severe light-induced retinal degeneration suffered by mice that are unable to clear ATR after light exposure because they lack both retinol dehydrogenase (RDH) and the ABCA4 transporter (*Abca4*$^{-/-}$ *Rdh8*$^{-/-}$) (Maeda et al. 2008). RDH reduces ATR to all-*trans*-retinol (Rattner et al. 2000), and the ABCA4 transporter removes ATR from the disc interior (Weng et al. 1999). ATR is in itself toxic (Rozanowska and Sarna 2005; Wielgus et al. 2010) due to its reactive aldehyde component (Sparrow et al. 2010), consistent with the fact that drugs containing primary amines react with retinaldehyde to protect *Abca4*$^{-/-}$ *Rdh8*$^{-/-}$ mice from light-induced retinal damage (Maeda et al. 2011). ATR also photosensitizes cells to ultraviolet or blue light (Harper and Gaillard 2001; Rozanowska and Sarna 2005). In addition to its own toxicity, free ATR generates reactive oxygen species in the rod cell via a cascade of signaling events that stimulates the enzyme NADPH-oxidase (Chen et al. 2012), and cell death occurs from mitochondrial poisoning and caspase activation (Maeda et al. 2009). Furthermore, two molecules of ATR can sequentially react with phosphatidylethanolamine to form a pyridinium bisretinoid, so-called A2E, which collects over time in lipofuscin granules found in the RPE and is correlated with age-related macular degeneration (Sparrow et al. 2010).

In the situation of constant illumination, ATR is continuously generated by the photoactivation and decay of rhodopsin. At the same time, ATR is removed as RDH reduces it to nontoxic all-*trans*-retinol, which in turn diffuses to the RPE to be enzymatically converted back to 11-*cis*-retinal (Lamb and Pugh 2004). Constant illumination thus creates a steady state within the rod disc, meaning that the relative concentrations of rhodopsin, metarhodopsin, and opsin reach a constant level once the rate of photon absorption (i.e., bleaching) equals that of rhodopsin regeneration (Wenzel et al. 2005). This continuous cycle of photoactivation, decay, and regeneration results in a persistent population of ATR, which has been estimated at ~12–30 %[1] of the total amount of rhodopsin in mice kept under normal fluorescent laboratory lighting (Saari et al. 1998; Lee et al. 2010). With an estimated rhodopsin concentration in the mouse retina of ~3–5 mM (Lamb and Pugh 2004; Nickell et al. 2007), total ATR concentration (i.e., free and opsin-bound) would range between 360 μM and 1.5 mM in mice under normal room lighting. If only 10 % of this ATR were free, its steady-state levels would be 40–150 μM. Considering that as little as 100 μM ATR is toxic to cells in culture (Rozanowska and Sarna 2005), and only 25 μM ATR induces oxidative stress in RPE cells (Wielgus et al. 2010), even moderate indoor lighting presents a real risk to the murine retina.

In humans, the level of bleached rhodopsin is lower under a given amount of light as compared to mice, because rhodopsin is regenerated faster in the human retina (Lamb and Pugh 2004). Whereas normal room lighting (~100–200 cd/m$^2$) is sufficient to bleach half of rhodopsin in the mouse retina (Wenzel et al. 2005; Lee et al. 2010), bright daylight (~25,000 cd/m$^2$) is required to bleach half of rhodopsin

[1] The difference might be strain-related (Lamb and Pugh 2004), as the first study utilized one inbred albino strain and the second various pigmented strains.

in humans (Alpern 1971; Rushton and Powell 1972; Kaiser and Boynton 1996). This difference reflects the difference between the nocturnal mouse and the diurnal human. Although steady-state levels of ATR in human subjects have not been reported, it can be safely assumed that bright daylight generates potentially toxic levels of ATR in the human retina.

## 3.2 *Arrestin Stimulates Uptake of ATR by Phosphorylated Opsin*

Twenty years ago our group first reported that ATR stimulates arrestin-1 binding to phosphorylated opsin (OpsP) (Hofmann et al. 1992) and more recently we have determined the mechanism underlying this phenomenon. In the presence of arrestin-1, ATR can enter the binding pocket of OpsP and reform the retinal Schiff base, thereby reforming Meta II-P from OpsP and exogenous ATR (Sommer et al. 2012). Curiously, only half of OpsP receptors in the native rod disc membrane are able to take up ATR in the presence of arrestin-1. This asymmetry of ligand binding is explained by a binding model in which each domain of arrestin-1 functionally engages its own OpsP molecule (Fig. 1d). Loop-72 (finger loop) in the N-domain stabilizes the active conformation of opsin (Ops*) necessary for ATR uptake, whereas loop-344 in the C-domain engages the receptor but does not stimulate ATR uptake.

Considering the dangerous levels of free ATR potentially generated in the rod cell by daylight, arrestin-dependent uptake of free ATR by OpsP represents a valuable protective mechanism that would complement rod cell survival in bright light. Notably, the apparent $K_D$ of ATR for arrestin-bound OpsP (3–5 μM) (Sommer et al. 2012) would be sufficient to reduce free-ATR concentrations to well below those that have been reported to be toxic (Rozanowska and Sarna 2005). Obviously, this proposition depends on the existence of arrestin-bound phosphorylated opsin in vivo. So far, available evidence suggests that highly phosphorylated opsin accumulates in the retina following light exposure (Kennedy et al. 2001; Shi et al. 2005; Lee et al. 2010). In the case of continuous illumination, Lee at al. found that 80 % of receptors were phosphorylated, even though only 55 % of rhodopsin was bleached in steady state (Lee et al. 2010), implying that a significant fraction of dark-state rhodopsin was phosphorylated under these conditions. Furthermore, most receptors incorporate multiple phosphates over time, because dephosphorylation of regenerated rhodopsin was slower than the rate at which a given rhodopsin molecule absorbed another photon (Lee et al. 2010). This finding is especially significant, considering that arrestin's affinity for OpsP scales with the number of phosphates per receptor (Gibson et al. 2000; Vishnivetskiy et al. 2007). These observations also hint at why rhodopsin contains so many phosphorylation sites (seven in bovine, six in mouse and human; see Chap. 4) when only three phosphates are required for high-affinity arrestin-1 binding to light-activated Meta II (Mendez et al. 2000; Vishnivetskiy et al. 2007). The additional phosphorylation

sites might be utilized for arrestin-1 binding to OpsP and arrestin-dependent uptake of ATR. Since arrestin-induced uptake of a single molecule of ATR requires two phosphorylated opsin molecules (Sommer et al. 2012), it can be envisioned that overall receptor phosphorylation level in the rod cell, which increases with relative light level (Lee et al. 2010), switches the rod cell from single photo-detecting to survival mode.

### 3.3 *Arrestin Imposes Asymmetric Ligand Binding Within an Opsin Dimer*

Following arrestin-dependent ATR uptake by OpsP in the rod disc membrane, subsequent uptake of the inverse agonist 11-*cis*-retinal (i.e., rhodopsin regeneration) is blocked in the receptor population that has already taken up agonist but not in the remaining receptor population with empty retinal binding pocket (Fig. 1d, e) (Sommer et al. 2012). This attribute allows each arrestin-bound receptor pair to simultaneously act as a sink for both ATR and 11-*cis*-retinal. Asymmetric ligand binding imposed by arrestin-1 would both protect the rod cell from free ATR and support continuous regeneration with 11-*cis*-retinal.

### 3.4 *Sequestered ATR is Still Accessible to Retinol Dehydrogenase*

Our proposed model of arrestin-mediated protection of the rod cell raises the question of how sequestered ATR eventually enters the retinoid cycle. Stimulation of retinol dehydrogenase (RDH) activity by the addition of its essential cofactor NADPH dissociates the ternary complex of arrestin, OpsP, and ATR (i.e., arrestin/Meta II-P) (Sommer et al. 2012). Several factors contribute to the efficiency of RDH-induced dissociation of the complex. First, with a reported $K_M$ of ~1 μM (Palczewski et al. 1994), RDH binds ATR with higher affinity than arrestin-bound OpsP ($K_D$ ~ 3–5 μM) (Sommer et al. 2012). Second, the ternary complex exists in equilibrium with its dissociated components. In practical terms, ATR is released every minute or two as Meta II decays.[2] Once released, ATR is available for enzymatic reduction by RDH. Since the reduction of ATR is essentially irreversible under cellular conditions, RDH effectively siphons away ATR from the arrestin/OpsP/ATR complex over time.

We hypothesize that arrestin-bound OpsP would serve a sink for ATR that cannot immediately be reduced by RDH (Fig. 1f). Thus, arrestin-dependent

[2] Based on the measured off-rate of ~0.01 $s^{-1}$ for the arrestin-bound Meta II-P complex at physiological temperatures (Sommer et al. 2005).

sequestering would be most vital during those times when the bleaching rate outpaces ATR reduction. Indeed, Blakely et al. have reported that the rate of ATR reduction in isolated mouse rod cells is significantly slower than ATR release from isolated light-activated rhodopsin (Blakeley et al. 2011). In addition, Lee et al. observed in living mice that ATR levels increased faster than the level of its downstream products within the first 10 min after the onset of constant illumination (Lee et al. 2010). Furthermore, ATR reduction following significant bleaches can be impeded by limited NADPH concentration (Miyagishima et al. 2009).

While it is clear that sequestration of ATR cannot stop its entry into the retinoid cycle, the micromolar affinity of ATR for arrestin/OpsP should logically result in arrestin-dependent slowing of ATR reduction. Such an effect has been observed in vitro using isolated rod disc membranes (Hofmann et al. 1992; Palczewski et al. 1994). Interestingly, mouse rods lacking functional arrestin-1 showed no difference in the rate of all-*trans*-retinol formation (Blakeley et al. 2011), and arrestin-1 appeared to have little effect on the rate of 11-*cis*-retinal regeneration in live mice (Palczewski et al. 1999). However, these results do not necessarily contradict our proposed role for arrestin-1, because both studies employed a single bright illumination of short duration and then followed retinoid metabolism in the dark. Considering the majority of arrestin-1 is located in the inner segment in the dark-adapted rod (Strissel et al. 2006; Song et al. 2011), and arrestin-1 translocation to the outer segment takes many tens of minutes (Strissel et al. 2006), such a lighting protocol may minimize the effect of arrestin-1. Further experimentation, which tests the effect of constant illumination, is required to ascertain the influence of arrestin-1 on the enzymatic reduction of ATR in vivo.

## *3.5 Perspectives and Future Directions*

While a lack of functional arrestin-1 in mice leads to significant light-dependent retinal damage (Chen et al. 1999; Hao et al. 2002; Burns et al. 2006), human patients suffering Oguchi disease due to a lack of functional arrestin-1 do not always suffer retinal damage (Paskowitz et al. 2006). It is likely that environmental factors, such as time spent in bright daylight, contribute to whether retinal degeneration accompanies Oguchi disease. In addition, other sinks for ATR may also exist and act in conjunction with arrestin-bound OpsP. These include the side product of Meta II decay, Meta III (Heck et al. 2003a), and secondary binding pockets within opsin (Heck et al. 2003b; Schädel et al. 2003). Even A2E formation has been proposed to protect the rod cell from free ATR (Wielgus et al. 2010), in opposition to the general belief that A2E is a toxic byproduct of vision (Sparrow et al. 2010).

Further work is required to examine how arrestin-1 influences the levels of toxic free-ATR in vivo. Of the many studies that have utilized arrestin-1 knockout mice over the years (Xu et al. 1997; Chen et al. 1999; Palczewski et al. 1999; Hao et al. 2002; Burns et al. 2006; Blakeley et al. 2011), none have examined how

arrestin-1 influences the levels of toxic free-ATR in the living animal exposed to realistic lighting conditions. The approach could be similar to that taken recently by Palczewski and colleagues in their studies of mice lacking RDH and the ABCA4 transporter (Maeda et al. 2009, 2011; Chen et al. 2012). Arrestin-1 knockout mice would be expected to show the same markers for oxidative stress and increased levels of retinal condensation products like A2E when exposed to bright continuous light. Furthermore, drugs containing primary amines would be expected to protect arrestin-1 knockout mice from bright light-induced retinal degeneration [see Maeda et al. (2011)].

## 4 Arrestin Translocation

In mice, arrestin-1 is expressed at 80 % the levels of rhodopsin (molar ratio) (Strissel et al. 2006; Hanson et al. 2007a). The majority of this arrestin-1 (estimated at 80–93 %) is sequestered in the rod inner segment in the dark-adapted rod cell (Strissel et al. 2006; Song et al. 2011). Due to its propensity to dimerize and tetramerize (Schubert et al. 1999; Hanson et al. 2007b), arrestin-1 might be confined to the inner segment because arrestin-1 oligomers cannot diffuse into the narrow spaces between the discs of the outer segment (Najafi et al. 2012). Inner segment confinement of arrestin-1 might be further enhanced by microtubule binding (Nair et al. 2004, 2005). The arrestin-1 present in the dark-adapted outer segment (about one arrestin-1 for every 10 rhodopsins) is monomeric (Hanson et al. 2007b; Najafi et al. 2012) and sufficient to quench signal transduction in dim light. Even lower levels of arrestin-1 (one arrestin-1 for every 200 rhodopsins) are sufficient to support normal photoresponse recovery following light flashes (Cleghorn et al. 2011). Exposure to bright continuous light triggers the translocation of the large pool of arrestin-1 from the rod inner segment to the outer segment over the course of many minutes (Elias et al. 2004; Strissel et al. 2006). Binding to Meta II-P is likely the driving force of translocation [see Slepak and Hurley (2008) and Gurevich et al. (2011)], although other gating and/or transport mechanisms may exist (Orisme et al. 2010). Following translocation, arrestin-1 interaction with phosphorylated receptor species, including Meta II-P and OpsP, maintains arrestin-1 in the outer segment. Importantly, the fact that the rod cell expresses such a large amount of arrestin-1, and this arrestin-1 is mobilized by light levels that by far exceed the operational range of the rod cell (Strissel et al. 2006), supports the idea of arrestin-1 as protector of the rod cell from the effects of brighter light. Evidence suggests that in moderate light, the protective effect of arrestin-1 is based on blocking transducin activation by Meta II and opsin (Hao et al. 2002), since persistent signaling by photoactivated and photo-decayed rhodopsin is deleterious to the rod cell and leads to apoptotis [reviewed in Fain (2006)]. In brighter light, arrestin-mediated protection is based not only on signal quenching (Hao et al. 2002) but probably also the limitation of toxic levels of ATR. In essence, arrestin-bound

OpsP buffers free ATR concentrations, which would be vitally important when the light suddenly intensifies and bleaches more rhodopsin.

Upon the return of the rod cell to darkness, arrestin-1 movement back to the inner segment is slow and follows the time-course of opsin dephosphorylation (Nair et al. 2005).

Moreover, receptor dephosphorylation follows the rate of rhodopsin regeneration (Lee et al. 2010), suggesting that full receptor deactivation by 11-*cis*-retinal uptake is required to dissociate arrestin-1 and return it to the inner segment. Hence the protective effects of arrestin-1 are maintained until the system has fully returned to its dark-adapted state.

## 5 Conclusions

Arrestin-1 exists at the intersection of three functional modules that compose the visual system (Fig. 1f). First to be discovered was the role of arrestin-1 as a terminator of rhodopsin signaling within the visual signal transduction module. Later, arrestin-1 was observed to make dramatic light-dependent migrations between rod cell segments, demonstrating how the protein translocation module facilitates the shift of the rod cell from photon-detection to survival mode. Finally, we describe a role for arrestin-1 in the retinoid cycling module, which helps the rod cell survive bright light by limiting levels of free ATR.

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