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Ectoplasm, Ghost in the R cell Machine?

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Abstract

Drosophila photoreceptors (R cells) are an extreme instance of sensory membrane amplification via apical microvilli, a widely deployed and deeply conserved operation of polarized epithelial cells. Developmental rotation of R cell apices aligns rhabdomere microvilli across the optical axis and enables enormous membrane expansion in a new, proximal distal dimension. R cell ectoplasm, the specialized cortical cytoplasm abutting the rhabdomere is likewise enormously amplified. Ectoplasm is dominated by the actin-rich terminal web, a conserved operational domain of the ancient vesicle-transport motor, Myosin V. R cells harness Myosin V to move two distinct cargoes, the biosynthetic traffic that builds the rhabdomere during development and the migration of pigment granules that mediates the adaptive ‘longitudinal pupil’ in adults, using two distinct Rab proteins. Ectoplasm further shapes a distinct cortical endosome compartment, the subrhabdomeral cisterna (SRC), vital to normal cell function. Reticulon, a protein that promotes endomembrane curvature, marks the SRC. R cell visual arrestin 2 (Arr2) is predominantly cytoplasmic in dark-adapted photoreceptors but on illumination it translocates to the rhabdomere, where it quenches ongoing photosignaling by binding to activated metarhodopsin. Arr2 translocation is ‘powered’ by diffusion; a motor is not required to move Arr2 and ectoplasm does not obstruct its rapid diffusion to the rhabdomere.

Keywords

Drosophila; R cell Ectoplasm; SRC; Myosin V; Rab11; Arr2

Introduction

Opsin’s evolution in early metazoa presented animals with the challenge to engage and integrate core cell functions, many already deeply established in the epithelial cell toolkit, to build effective photoreceptors (Plachetzki et al., 2007; Alvarez, 2008; Suga et al., 2008; Lamb et al., 2009). The lineage leading to *Drosophila* met this challenge by integrating a suite of conserved pathways, notably utilizing membrane traffic and the cytoskeleton in a single-minded, extreme amplification of the sensory apical plasma membrane during R cell differentiation. R cell cytoplasm thus greatly magnifies the presentation of these pathways, a useful window into fundamental cell operations. A large body of observation, pioneered by Margaret Perry (Cosens and Perry, 1972), Emiko Suzuki (Matsumoto-Suzuki et al., 1989), Sally Blest and David Stowe (Blest et al., 1982), and Bruce Boschek (Boschek, 1971), reveals fly photoreceptor cytoplasm is regionalized and specialized in service of the photosensory membrane organelle, the rhabdomere. Particularly notable is the microfilament-rich, organelle-poor cytoplasm subjacent to the rhabdomere. First reported as ‘exoplasm’ by Haeckel (Haeckel, 1873), distinct agranular cortical cytoplasm, ectoplasm, was known to classical cell biologists as host to vital cell functions (Just, 1939). This review

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selects from our recent studies to highlight the adaptive organization of *Drosophila* photoreceptor cytoplasm, in particular the emerging role of R cell ectoplasm, a lively venue of conserved functions that animate R cells. Further discussion, including citations to the many reports from numerous labs that inform this review, are found in the original articles, (Satoh et al., 2005; Li et al., 2007; Satoh et al., 2008; Satoh et al., 2010).

The Apical Microvilli Program Dominates R Cell Cytoplasmic Organization

Likely reflecting the evolutionary origin of photoreceptors (R cells) as photosensitive ectodermal cells, *Drosophila* R cells, like their vertebrate rod and cone cell counterparts, are polarized epithelial cells with distinct apical and basolateral plasma membrane domains bounded by a circumferential zonula adherens (z.a.) junctional belt (Fig. 1A). In an elegant morphogenetic trompe l'oeil, rotation of the R cell apical membrane 90° into the plane of the retinal epithelium, introduces a third, proximo-distal, dimension into the planar retinal epithelium that accommodates hyperexpansion the photosensory membrane in the rhabdomere, a columnar stack of apical microvilli projecting across the optical axis (Supporting information animation 1)(Longley and Ready, 1995).

Soon after the four cone cells surrounding each ommatidial R cell cluster are their mutual junctions close like an iris over R cell apices, trapping an apical lumen, the inter-rhabdomeral space (IRS). How this inpocketing is effected is not known but its evolutionary innovation appears a significant advance, allowing enormous sensory membrane expansion and increased sensitivity without increasing the eye's external area and, at the same time, positioning cells which form the lens cells above each cluster (Supporting information animation 2). Ommatidial assembly solely from within the retinal epithelium appears economical and the spectacular success of animals with compound eyes attests the utility of this strategy.

Drosophila R cells are wedge-shaped in cross section and show a stereotyped apico-basal cytoplasmic organization (Fig. 1B). Two distinct domains are apparent in R cell apical membranes, the central rhabdomere and the surrounding and supporting stalk. Primordia of these domains are founded early in R cell morphogenesis with the differentiation of a central, Moesin-organized membrane ringed by a domain rich in Crumbs (Karagiosis and Ready, 2004). The role of Crumbs and cell-cell junctions in retinal development and disease is considered elsewhere (Ready and Tepass, 2004).

Selection of apical microvilli as the morphogenetic program to amplify the sensory membrane fundamentally organizes R cell cytoplasm. Each of the approximately 50,000 microvilli comprising the rhabdomere is ca. 50 nm diameter × 1 μm length and threaded by one or two axial microfilaments (Arikawa et al., 1990) that link to the plasma membrane via the p174 rhabdomere specific isoform of the photoreceptor-specific Myosin III, NINAC (Kumar and Ready, 1995; Hicks et al., 1996). R cells implement microvilli with key variations from enterocyte and kidney brush border and hair cell stereocilia where microvilli project on stout, cross-linked bundles of dozens or hundreds of actin filaments (Nambiar et al., 2010). Active phospho-moesin couples the latter microvilli along their length (Bretscher, 1983), while in fly photoreceptors, active Moesin concentrates at the base of the rhabdomere, a complex interface between the sensory membrane and the specialized cytoplasm of the terminal web below. Phosphorylated Moesin resolves to the rhabdomere primordium in mid pupal R cells (Karagiosis and Ready, 2004). The Ste-20 kinase, Slik, is essential for Moesin phosphorylation and rhabdomere morphogenesis fails in Slik mutants (Hipfner et al., 2004). TRP and TRPL are reported to link moesin to the plasma membrane at the rhabdomere (Chorna-Ornan et al., 2005). In *trp* mutants, reduced Ca²⁺ influx during phototransduction promotes PLC hyperactivity resulting in severe PIP2 reduction in the

rhabdomere (Hardie et al., 2001); in illuminated *trp* mutants, phosphorylated moesin is lost from the rhabdomere base with concomitant rhabdomere terminal web disruption and rhabdomere degeneration (Sengupta, Xia, Ready, Hardie, unpublished). *trp* mutant photoreceptors thus offer an opportunity to characterize the consequences moesin inactivation on cortical actin cytoskeleton organization in real time in living cells.

Terminal webs, specialized, actin-rich submicrovillar cytoskeletons and the ectoplasm they organize, are conserved across eukaryotes and writ large in R cells in the dense, parallel array of actin filaments that projects from the rhabdomere base deep into R cell cytoplasm (Arikawa et al., 1990; Chang and Ready, 2000). Myosin fragment S1 decoration shows rhabdomere terminal web filaments are in 'standard' polarity, with plus ends towards the plasma membrane (Arikawa et al., 1990). Whether rhabdomere terminal web filaments are continuous with microvillar axial filaments or originate in the moesin-organized rhabdomere base, is not known. In enterocyte brush border, microvillar actin bundles extend as distinct 'rootlets' into apical cytoplasm and engage a transverse, stabilizing feltwork of tropomyosin, myosin II, spectrin and intermediate filaments (Benesh et al., 2010). Microvillar rootlets are not apparent in the *Drosophila* photoreceptor rhabdomere terminal web, nor is a transverse filament array ultrastructurally apparent. Immunohistochemistry localizes Myosin II (Baumann, 2004) and spectrin (Baumann, 1998; Lee et al., 2010) to the base of developing rhabdomeres and these may contribute to rhabdomere terminal web organization. Spectrin superfamily member α -actinin immunolocalizes to the rhabdomere terminal web (unpublished), but it appears non-essential since rhabdomeres are normal in homozygous null mutant in R cells (Fyrberg et al., 1998). If rhabdomere terminal web filaments are cross-linked is not known, but the observation, first made by Waddington, that the surface of the R cell nucleus facing the rhabdomere is often strongly indented, may reflect deformation by stiffened cortical cytoplasm, a common attribute of ectoplasm (Waddington, 1962). The substantial rhabdomere terminal web running the length of the R cell may provide a substantial cellular 'backbone'.

R cell ectoplasm is notably organelle-poor, excluding mitochondria, multivesicular bodies and Golgi apparatus and apposition of 'clear' ectoplasm to the base of the optically dense rhabdomere confers wave-guide light propagation on the very elongated rhabdomere, a remarkable advance in R cell efficiency (Kirschfeld and Franceschini, 1969).

The Subrhabdomeric Cisterna Highlights R Cell Cortical ER

Drosophila R cells maintain a large, regionally specialized endoplasmic reticulum (ER), with a distinct cortical domain, the subrhabdomeric cisterna, an elaborate network of smooth ER tubules closely apposed to the rhabdomere base (Matsumoto-Suzuki et al., 1989; Baumann, 1998) (Fig. 2). Ribosome-free smooth ER participates in numerous activities, including lipid biosynthesis and regulation of cytosolic Ca^{2+} (Baumann and Walz, 2001) and the SRC positions membrane associated diacylglycerol kinase (*rdgA*) and phosphatidylinositol transfer protein (*rdgB*), in direct association with the sensory membrane and is implicated in phospholipid and membrane protein transport to the rhabdomere (Suzuki and Hirose, 1991; Vihtelic et al., 1993; Masai et al., 1997). Small membrane tubules reach through rhabdomere terminal web ectoplasm to join rough ER in general R cell cytoplasm. Consistent with a pivotal role for the SRC in rhabdomere maintenance, its dissolution is the first observable phenotype of photoreceptor degeneration in *rdgA* mutants (Masai et al., 1997). Notwithstanding its evident importance to normal photoreceptor function, relatively little is known concerning the development and differentiation of this specialized ER domain

Reticulons are highly conserved ER membrane proteins that promote high-curvature smooth ER tubules at the cell cortex (Shibata et al., 2006). Reticulon-like 1 (Rtnl1) is the sole predicted member of the reticulon protein family in the fly (Wakefield and Tear, 2006). ER deficits were not observed in Rtnl1 null embryo cells, but it may be that phenotypes will be seen in cells like R cells with prominent cortical ER. In preliminary studies, we have found that a reticulon protein exon trap, Rtnl1-GFP (Morin et al., 2001), is strongly expressed in photoreceptors and highlights the SRC and tubular network behind it (Fig. 2). In living cells, Rtnl1 is not present in rhabdomeres. ER can be poorly preserved in cells prepared using standard formaldehyde immunohistochemistry (LaJeunesse et al., 2004), and Rtnl1 distribution is drastically altered in fixed R cells. The cytoplasmic network is lost and Rtnl1 now appears in rhabdomeres. This difference underscores the vital need to incorporate live cell imaging to understand the photoreceptor endomembrane system.

Ectoplasm Is the Realm of Myosin V Powered Vesicle Traffic

In secretory cells, ectoplasm is the gatekeeper of secretory vesicle access to exocytic targets in the plasma membrane; it is a barrier, limiting approach of organelles not destined for exocytosis, and, as the F-actin track along which myosin motors move cargo, a carrier (Giner et al., 2005; Desnos et al., 2007; Trifaro et al., 2008). Differentiating *Drosophila* R cells are highly secretory, with massive biosynthetic membrane traffic delivering rhodopsin and other phototransduction proteins to the growing rhabdomere. During this stage, approximately the second half of pupal life, ectoplasm's carrier role dominates.

Rhodopsin 1 (Rh1), 'outer cell' R1-R6 photopigment, is first detected at about 70% of pupal development as faint, diffuse signals and small puncta spread throughout R cell cytoplasm. Rhabdomere localization follows shortly and continuing massive flux builds the rapidly elongating microvilli with their enormous Rh1 concentration (Kumar and Ready, 1995; Satoh et al., 2005). All stages of the biosynthetic pathway are in fully engaged throughout this building phase and a single confocal immunohistochemistry 'snapshot' catches all simultaneously in play, blurring Rh1 traffic dynamics. A 'blue light' experiment in which a surge of Rh1 pent up in the ER is released into the biosynthetic pathway reveals the timecourse of Rh1 transport. In this experiment, blue light photoisomerization creates the chromophore isomer needed for Rh1 maturation and ER release (Fig. 3A). The surge reaches the rhabdomere by 40 min; delivery is substantial by 60 min; it is largely complete by 180 min. Rh1 transits the Golgi en route to the rhabdomere and is prominent there at 60 min. Golgi is well separated from the SRC and Rh1 transport to the rhabdomere is not via the direct ER>SRC connection. The detailed sequence of events that mediate Rh1 exocytosis at the rhabdomere base and whether the SRC contributes a distinct pre-rhabdomere transit compartment remain to be determined.

Across eukaryotes, ectoplasm is a special operational domain of the conserved MyoV/Rab11/FIP2 vesicle transport complex (Seabra and Coudrier, 2004), and *Drosophila* R cells fully implement this program. Evidenced in blue light experiments, Rab11 co-transport with Rh1 between TGN and the rhabdomere (Fig. 3A). Rab11 and dRip11 colocalize with MyoV at the rhabdomere base, well within rhabdomere terminal web cytoplasm, often appearing as a lumpy and interrupted crescent. In MyoV mutant photoreceptor cells, Rab11 and dRip11 are excluded from the rhabdomere terminal web and stand off from the rhabdomere base.

Loss of function mutations show all three members of the transport trio are essential for Rh1 transport to the rhabdomere. In mosaic eyes containing normal and homozygous null *MyoV^{Q1052s}* R cells, wild type cells show large, Rh1-bright rhabdomeres with little cytoplasmic Rh1, while in mutant cells rhabdomeres are small and Rh1 accumulates in cell

cytoplasm (Fig. 3B). Mutant rhabdomeres contain some Rh1, indicating limited transport still operates, presumably mediated by MyoV persisting after recombination renders cells genetically *MyoV* null. Electron microscopy of mutant cells shows R cell cytoplasm fills with an undelivered cargo in a profusion of pleiomorphic vesicles. Equivalent phenotypes, reduced rhabdomeres and accumulation of cytoplasmic Rh1 are obtained with reduction of Rab11 or dRip11, *Drosophila* FIP2. In summary, we propose MyoV pulls post-Golgi secretory vesicles, marked for rhabdomere delivery by Rab11 and dRip11, through exclusionary ectoplasm along polarized microfilaments leading directly to the exocytic targeting domain at the rhabdomere base (Fig. 3C).

Calcium-Activated Myosin V Closes the *Drosophila* Pupil

A key conserved function of ectoplasmic MyoV is motility and docking of lysosome-derived granules in the cell cortex. In R cells, this function is employed to serve the longitudinal pupil, an automatic gain control in fly photoreceptors; in response to light, small pigment granules migrate to the base of the rhabdomere, where they attenuate waveguide propagation along the rhabdomere and reduce photoreceptor sensitivity approximately an order of magnitude (Kirschfeld and Franceschini, 1969; Franceschini, 1975; Franceschini and Kirschfeld, 1976). At a luminance threshold approximately that of human color vision, 0.3 cd/m² (Franceschini and Kirschfeld, 1976), pigment granules migrate in response to elevations of cytoplasmic free Ca²⁺ from approximately 160 nM in dark-adapted photoreceptors, to greater than 10 mM, with a migration threshold of approximately 1mM (Kirschfeld and Vogt, 1980; Hofstee and Stavenga, 1996; Oberwinkler and Stavenga, 1998). A role for Ca²⁺ in pigment granule migration was presaged by observation that in *Drosophila* mutants later shown to be deficient for light-activated, Ca²⁺-permeable TRP channels, pigment granules did not approach the rhabdomere base but remained in a dark-adapted position (Cosens and Perry, 1972). We assayed photoreceptor pigment-granule migration by observing an optical phenomenon called the deep pseudopupil (DPP), a virtual image that sums rhabdomeres from ca. 20–50 ommatidia with the optics used (Franceschini and Kirschfeld, 1971). Upon illumination, the *Drosophila* DPP typically changes from dark red to bright green with a time constant as small as 2 s (Franceschini, 1972; Franceschini and Kirschfeld, 1976). In dark-adapted Canton-S wild-type eyes, the reflectance of the DPP is low; incident light is effectively channeled into the rhabdomeres and propagates there until it is absorbed by the visual pigment or by the pigment proximal to the rhabdomeres. Within seconds after the light is turned on, DPP reflectance rapidly increases, as pigment granules in the photoreceptor cytoplasm migrate to the rhabdomere base (Fig. 4A, Supporting Information Movie S1). The reflecting pigment granules cause a gold-green crescent in the light-adapted DPP. Note that although the pigment granules do not enter the rhabdomere, some of the light they reflect propagates distally along the rhabdomere, back to the source, seen as a dimmer central glow within the brighter crescent at the rhabdomere base (Franceschini and Kirschfeld, 1976). The pupil remains closed in the presence of continued strong light (Fig. 4A). However, in a strong MyoV loss-of-function mutant, MyoV^{KG04384}, the pupil does not close, even after prolonged, bright illumination (Fig. 4A). Transgenic MyoV expression rescues MyoV^{KG04384} pupil closure (data not shown).

MyoV couples to pigment granules via a Rab-related protein, Lightoid (Satoh et al., 1997; Ma et al., 2004), a seemingly economical choice as Lightoid is a member of the conserved lysosome-related organelle biogenesis pathway that generates pigment granules (Lloyd et al., 1998; Dell'Angelica et al., 2000). R cells thus harness a single motor, MyoV, to power two distinct tasks using two distinct Rab proteins: Rab11 coupling to exocytic cargo during development and Lightoid coupling to pigment granules that mediate adaptation in mature R cells (Fig. 4B).

Arrestin Translocation in R cells

While many eukaryotic cell functions incorporated by R cells long predated opsin's evolution, a vital opsin partner, visual/beta arrestin, emerged together with opsin at the level of Cnidaria and instigated substantial advances in photoreceptor function (Alvarez, 2008). Prominent among these, R cell arrestins, Arr1 and Arr2, quench continuing signaling of photoactivated rhodopsin, Metarhodopsin (M) (Dolph et al., 1993), promoting adaptation over the enormous range of environmental light. Further, fly visual arrestins promote rhodopsin endocytosis and turnover, a matter of R cell life and death (Alloway et al., 2000; Kiselev et al., 2000; Satoh and Ready, 2005; Chinchore et al., 2009).

In order to localize Arr2 in dark-adapted R cells, we reared flies in the dark and dissected and fixed their eyes under infrared illumination. As previously reported, in the dark, ca. 25% of Arr2 immuno-staining localizes to the rhabdomere (Alloway and Dolph, 1999; Lee and Kolodziej, 2002; Satoh and Ready, 2005), constituting a "first-responder" pool for M inactivation (Fig. 5F). The remainder localizes to the cell body cytoplasm, a reserve that can be drawn upon when M out-titrates the rhabdomeric first responders. Arr2 in dark-adapted cytoplasm colocalized strongly, but not exclusively, with the HDEL endomembrane marker (Fig. 5A) and the photoreceptor ER chaperone, NinaA, in a textured cytoplasmic network. Notably, Arr2, HDEL, and NinaA all highlighted the SRC (Matsumoto-Suzuki et al., 1989; Vihtelic et al., 1993). Thin Arr2 immunopositive 'threads', presumably the smooth ER tubules described by Matsumoto-Suzuki et al., 1989 (see Fig. 2), reach across R cell ectoplasm from the SRC to join the extended cytoplasmic endomembrane network. Distinct from vertebrate rod visual arrestin (Nair et al., 2005), Arr2 in dark-adapted R cells did not localize to microtubules. The identity of putative endomembrane binding target(s) remains to be determined; candidates include phosphoinositide components of the lipid bilayer, and/or the NINAC myosin III, both of which have been reported to interact, directly or indirectly, with Arr2 (Lee et al., 2003; Lee and Montell, 2004; Liu et al., 2008).

To track arrestin movements in vivo, we expressed GFP-tagged Arr2 in R1-R6 and monitored Arr2-GFP concentration in the rhabdomeres in real time by imaging rhabdomere fluorescence in intact flies from the DPP (Fig. 5B, Supporting Information Movie S2). The blue excitation light simultaneously served as the stimulus for translocation and was sufficiently bright to convert the majority of R to M within 100 ms. In eyes pre-illuminated with long wavelength (>560 nm) light and then dark-adapted, the DPP showed weak, but obvious GFP fluorescence in the first image captured by a 100 ms blue flash (Fig. 5C and D). In subsequent images repeated at 1 Hz, DPP fluorescence rapidly brightened 2- to 3-fold (2.52 ± 0.10 ; mean $\pm$ SEM, $n = 19$) with an approximately single exponential time course with time constant of 9.1 s in flies dark-adapted for 1 min (Fig. 5D). Upon photoreconversion of M to R, Arr2-GFP returns to R cell cytoplasm with a time constant of 2.4 s. The rapid kinetics of Arr2 translocation into and out of rhabdomeres suggests ectoplasm does not present a significant barrier to arrestin movement.

We used mutants to ask if activation of the phototransduction cascade was required for Arr2 translocation. First, we measured Arr2-GFP translocation in *norpa*^{P24} mutants, which lack the effector enzyme phospholipase C and have no electrophysiological response to light. Strikingly, although an increase in DPP fluorescence, indicative of translocation, was still detected in *norpa* mutants, it was greatly slowed with a distinct delay of 5–10 s (Fig. 5D). We further investigated translocation of endogenous Arr2 in wild-type and mutant photoreceptors using confocal immunofluorescence microscopy. Dark-adapted *norpa* mutant showed endomembrane localization similar to wild-type. By contrast, following 2 min blue light exposure in *norpa* mutants, although Arr2 was enhanced to some extent at the rhabdomere base and stalk, compared with wild-type retinæ, it was now abnormally

retained in cell body cytoplasm. Photoreceptors deficient in other components of the transduction cascade, including the heterotrimeric Gq protein (*dgq1*), TRP channels (*trp³⁰¹*), and double mutants lacking both TRP and TRPL channels (*trpl; trp³⁴³*), showed a similar pattern, with normal dark-adapted Arr2 cytoplasmic localization, but abnormally high levels of residual cytoplasmic Arr2, remaining at 3 min post exposure. Overall, these and other results indicate that light-induced Ca^{2+} influx, while not able to trigger Arr2 translocation without formation of M, greatly accelerates translocation.

Two conflicting models dominate discussion of the mechanism of arrestin translocation: translocation by motor proteins (McGinnis et al., 2002; Peterson et al., 2003; Lee and Montell, 2004; Reidel et al., 2008), and translocation by diffusion (Peet et al., 2004; Nair et al., 2005; Calvert et al., 2006; Slepak and Hurley, 2008). Indeed, in *Drosophila*, it has been proposed that the photoreceptor specific myosin III motor (NINAC) powers Arr2 translocation by delivery of PIP3-enriched vesicles along F-actin tracks to the rhabdomere (Lee et al., 2003; Lee and Montell, 2004), but we find normal Arr2 translocation in *ninaC* null mutants (Satoh and Ready, 2005; Satoh et al., 2010). An alternative hypothesis, initially proposed for vertebrate rods, is that light transforms the photosensory compartment into a “light sink,” a diffusional trap that concentrates Arr2 via high affinity binding to activated metarhodopsin (Mangini et al., 1994; Nair et al., 2005). Such a passive diffusional model predicts that arrestin translocation should be driven by stoichiometric binding to activated M.

To determine if arrestin translocates to rhabdomeres in proportion to M binding targets, we exploited the bistable, photointerconvertible nature of Rh1 that allows the fraction of M to be accurately controlled using spectral illumination (Minke and Kirschfeld, 1979; Belusic et al., 2010). Although 560 nm light evokes a strong photoresponse, Arr2 does not translocate to the rhabdomere; the first responder pool suffices to quench the limited fraction of M produced. With shorter wavelength illumination and increasing M, Arr2 begins to translocate to rhabdomeres until all Arr2 is cleared from cytoplasm with wavelengths shorter than 500 nm (Fig. 5E). More refined measurements using a monochromator to deliver precise spectral illuminations and a sensitive photomultiplier to record Arr2-GFP translocation via DPP fluorescence shows an excellent agreement with a 1:1 stoichiometric relationship between the number of Arr2 molecules translocated and the number of photoisomerized molecules in the rhabdomere (Satoh et al., 2010).

These results suggest a regulated, diffusion driven two-sink model for Arr2 translocation in *Drosophila* R cells (Fig. 5F). Such a two-sink model resembles the interaction-restricted diffusion model proposed to underlie translocation in vertebrate rods, where microtubules constitute the “dark sink” and activated, phosphorylated rhodopsin in outer segments the “light sink” (Nair et al., 2005; Slepak and Hurley, 2008). Our evidence strongly suggests that translocation is normally accelerated by Ca^{2+} influx via the light-sensitive TRP and TRPL channels, and the next challenge will be to identify the underlying mechanism(s). We envisage at least two, not mutually exclusive, possibilities. First, Ca^{2+} might reduce the affinity of Arr2 for its putative endomembrane binding partner(s). Second, Ca^{2+} might “gate” diffusional access of Arr2 to the microvilli, the lumen of which is connected to the rest of the cell body by a narrow (ca. 20–30 nm) neck. Either or both of these novel mechanisms would add to the already rich repertoire of targets regulated by PLC-mediated Ca^{2+} influx pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Cell polarity and topology is conserved between *Drosophila* and mammalian PRCs. (Center) Fly and mammalian retinal neuroepithelium cells are typical polarized epithelial cells; a circumferential zonula adherens (ZA) junctional complex delimits apical from basolateral membrane. Two subdomains develop within photoreceptor apical membranes. (Left) The central domain is amplified either by stacked microvilli to form the *Drosophila* rhabdomere (r), or (Right) by stacked membrane discs to form the mammalian photoreceptor outer segment (os). A supporting membrane domain, the fly stalk (s) or the mammalian inner segment (is) collars the central photosensitive domain. The center/surround boundary of mammalian PRCs is marked by the connecting cilium (cc). Rotation of the *Drosophila* photoreceptor apical membrane aligns rhabdomere microvilli orthogonal to the eye's optical axis. Protrusive growth of outer segment membrane away from the connecting cilium aligns discs orthogonal to the optical axis. (Adapted from Ready and Tepass, 2004). (B). R cell cross-sections show stereotyped cytoplasmic organization. Ectoplasm is dominated by rhabdomere terminal web F-Actin. (Adapted from Li et al., 2007).

Figure 2.

The SRC in live and fixed photoreceptors. (Left) Summary electron microscope reconstruction of the SRC and ER stained using hot osmic acid (Adapted from Matsumoto-Suzuki et al., 1989). Arrows indicate the tubular ER network extending from the SRC. (Right) Confocal z-stack images show Rtnl1-GFP in live and fixed photoreceptors. In live photoreceptors, a delicate network extends from the SRC into and throughout photoreceptor cytoplasm (XY= native acquisition plane). Rtnl1-GFP is absent from live rhabdomeres (r). Note the 'notch' in central staining where R7 SRC ends. The red arrowhead notes cross-section plane. In fixed cells, cytoplasmic Rtnl1-GFP is clumpy, with voids not seen in live cells. Rtnl1-GFP is present in fixed rhabdomeres.

Figure 3.

MyoV, Rab11 and dRip11 tri-protein complex moves rhodopsin to rhabdomere. (A) The time course of Rh1 and Rab11 localizations after blue light irradiation. Arrows and arrowheads indicate colocalization of Rab11 and Rh1 at the rhabdomere base and in cytoplasmic organelles. (Adapted from Satoh et al., 2005). (B) In a large patch covering ~25% of the eye, homozygous *MyoV^{Q1052st}* photoreceptors show cytoplasmic rhodopsin and small rhabdomeres containing modest to moderate staining. Non-mutant cells, marked by GFP (blue), show MyoV staining, large rhodopsin-positive rhabdomeres, and little cytoplasmic rhodopsin. (C) A model shows secretory vesicles are pulled through rhabdomere terminal web cytoplasm by a MyoV-Rab11-dRip11 complex to the exocytic plasma membrane target, the rhabdomere base. (B, C adapted from Li et al., 2007).

Figure 4.

MyoV moves pigment granules to rhabdomere base. (A). Natural reflectance of the DPP under incident white light at 0 s, 10 s, and 1 min after lights on. Canton-S DPPs reflect bright green-gold before 10 s strong illumination (see movie S2). In MyoV^{KG04384}, a MyoV hypomorph, DPP do not reflect, even after 1 min illumination. (B). (Left) Drawing from Kirschfeld and Franceschini, 1969, showing light (bold, arrowed) channeling efficiently along dark-adapted rhabdomeres (Rh). Pigment granules of dark-adapted photoreceptors localize away from the base and promote waveguide propagation. In light-adapted photoreceptors, pigment granules at the rhabdomere base degrade waveguide propagation and rhodopsin exposure; light backscattered by pigment granules (dashed lines) increases DPP reflectance. (Right) A model mechanism for the longitudinal pupil (not to scale). Ltd links MyoV to pigment granules. Both CaM and Mlc-c bind the long, multi-IQ domain MyoV lever arms. Upon illumination, Ca²⁺ elevation promotes MyoV motility and pulls pigment granules to the plus ends (+) of rhabdomere terminal web microfilaments at the base of the rhabdomere. (Adapted from Satoh et al., 2008).

Figure 5.

Light induces arrestin translocates from R cell cytoplasm to the rhabdomere. (A). Arr2 in ommatidia of dark-reared wild-type (*w¹¹¹⁸*) flies colocalized with the endomembrane marker HDEL. Arr2 and HDEL-immunopositive crescents highlight the SRC. Bright rings of HDEL immunofluorescence between ommatidia highlight perinuclear ER of pigment cells, which do not express Arr2. Merged image (right) shows extensive colocalization (yellow). (B). In vivo imaging of Arr2-GFP translocation. Frames at indicated times from a time-lapse movie (100 ms exposure, 1 s^{-1}) of DPP fluorescence in transgenic flies expressing Arr2-GFP in outer photoreceptors, R1-R6 (see Movie S2). The DPP fluorescence increased rapidly in response to blue imaging flashes. (C). Using the DPP as region of

interest, average pixel intensity increased rapidly ~2.5 fold. (D). Averaged time course, normalized ($F_{norm} = (F - F_{min}) / (F_{max} - F_{min})$) Fluorescent intensity increase in wildtype flies fitted with single exponential with time constant 9.1s. While in phototransduction mutant *norpA* flies, the data fitted an exponential with time constant 89.7s. (E). Arr2 localizes to rhabdomeres in proportion to Metarhodopsin content. Dark-adapted wild type flies were illuminated with the indicated wavelength, held for 10 min in the dark, and dissected. Arr2 localization after 560 nm light is indistinguishable from those of dark-adapted photoreceptors. Some Arr2 translocate into the rhabdomeres after 546 nm light illumination. With 520 nm illumination, most Arr2 translocates into rhabdomeres; rhabdomeric arrestins distribute uniformly. Both 470 and 440 illuminations clear Arr2 from the cytoplasm; arrestin accumulates at the sides of the rhabdomeres. Scale bar: 2 μ m. (F). A regulated two-sink model for *Drosophila* Arr2 translocation. Left: Dark-adapted rhabdomeres and adjacent stalk membrane contain R (blue). Cytoplasmic Arr2 (green) associates with ER/endomembrane. Middle: When the fraction of M (fM) lies between ca. 0.08 and 0.33 ($M < Arr2$), high affinity, photoactivated M (red) has bound all first responder Arr2 and recruited additional Arr2 from photoreceptor cytoplasm, initiating translocation and quenching signaling. Right: Stimuli generating M in excess of Arr2 ($fM > 0.33$) result in binding and translocation of virtually all Arr2, but unquenched M remains, generating a prolonged depolarizing afterpotential (PDA). (Adapted from Satoh et al., 2010).