# The *Drosophila* photoreceptor as a model system for studying signalling at membrane contact sites

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**Abbreviations:** DAG: Diacylglycerol, DGK: Diacylglycerol kinase, ER: Endoplasmic reticulum, MCS: membrane contact sites, PA: Phosphatidic acid, PC: Phosphatidyl choline, PI: Phosphatidylinositol, $PI(4,5)P_2$: Phosphatidylinositol 4,5 bisphosphate, PM: plasma membrane, PLC: Phospholipase C, SMC: Sub microvillar cisternae, SRC: Sub rhabdomeric cisternae, SSC:Sub surface cisternae

**Abstract**

Several recent studies have demonstrated the existence of membrane contact sites between intracellular organelles in eukaryotic cells. Recent exciting studies have also demonstrated the existence of biomolecular interactions at these contact sites in mediating changes in the membrane composition of the cellular compartments. However, the role of such contact sites in regulating organelle function and physiological processes remains less clear. In this review we discuss the existence of a contact site between the plasma membrane and the endoplasmic reticulum in *Drosophila* photoreceptors. Further, we discuss the role of specific proteins present at this location in regulating phospholipid turnover and its impact in regulating a physiological process, namely phototransduction.

## Introduction

There has been increasing evidence for the involvement of membrane contact sites in a wide variety of sub-cellular events including transfer of lipids and other small molecules between distinct vesicular compartments within cells [1,2] or to provide a platform for enzymes and proteins to act 'in trans' on substrates present on the membranes of a distinct compartment [3]. Several studies have been performed, primarily in yeast to understand molecular interactions occurring at membrane contact sites (MCS) including those between the endoplasmic reticulum (ER) and the other organelles including the plasma membrane (PM), mitochondria, vacuole and endosomes. While there have been several studies in higher eukaryotes on molecular interactions at PM-ER contact sites, clear physiological functions have been ascribed to very few of these. In this review we will discuss *Drosophila* photoreceptors as a model for studying signalling at PM-ER contact sites.

Photoreceptors are primary sensory neurons that detect light and transduce them into electrical signals that are then sent to the brain. Critical to the function of photoreceptors is their ability to maximize photon absorption and this is done through expansion of a domain of the PM that is packed with the receptor for light, rhodopsin. Although ocular photoreceptors show a diverse range of morphology in different organisms, at the cellular level, the organization of these cells falls under two categories ciliary and microvillar [4]. Ciliary photoreceptors (e.g vertebrate rods and cones) are those in which the morphogenesis of the light sensitive membrane is based on a primary cilium whereas in microvillar photoreceptors (e.g insect photoreceptors) the photosensitive membrane is elaborated and organized into finger like projections called microvilli that are supported by a central actin core. Each photoreceptor contains several thousand microvilli that are arranged in a rod like structure called the rhabdomere.

It has been known for some time that in many species, the cell-body of photoreceptors are enriched in internal membrane-bound compartments [reviewed in [5]]. In a subset of these species, such internal membranes, are closely apposed to but distinct from the photosensitive membrane. Such a relationship of internal membranes to PM is a broad feature of many microvillar photoreceptors and is seen in a range of species including planarians, polycheate, annelids, cephalopods and arthropods (Fig. 1A). Occurrence of these membranes has also been reported in teleost cones [6] and amphibian photoreceptors [7]. Interestingly, in some species, such internal membranes are reported to undergo changes in location (e.g. move towards and away from the microvillar membrane) or undergo distention (changes in luminal volume) in response to changes in illumination [[5] and references therein].

## Structure and composition of the internal membranes

The endoplasmic reticulum (ER) is, morphologically speaking, the most diverse organelle in cells, often present in a variety of forms depending on the cell type and its metabolic state. Electron microscopic studies done on photoreceptors have revealed networks of reticular structures present in close proximity to the photosensitive membrane, referred to as subsurface cisternae (SSC). Based on their spatial and morphological appearance within the cell these have been variously named as myeloid, paracrystalline, vesicular, paraboloids in case of vertebrate cone cells, submicrovillar cisternae (SMC) in case of polychaete annelids and as subrhabdomeric cisternae (SRC) in case of arthropods [reviewed in [5]]. Nonetheless, all these represent analogous structures with minor morphological differences. Multiple morphological

classes of internal membranes can all be present in a single photoreceptor in varying proportions and these proportions can change depending on the type of illumination. Specifically and pertinent to this review, several arthropod photoreceptors have been described as possessing sub-surface cisternae (SSC) that are elements of smooth endoplasmic reticulum that are closely apposed to the plasma membrane.

*Drosophila* photoreceptors are polarized cells with the apical domain elaborated into folds of actin-based microvilli that are packed together into a rod shaped structure, the rhabdomere [8] where phototransduction occurs (Fig 1B). The cell body of *Drosophila* photoreceptors contains rough endoplasmic reticulum and shows staining for ER markers such as KDEL [9]. In addition, the region close to the base of the microvilli shows cisternae like specializations of smooth endoplasmic reticulum that are closely apposed to the microvillar PM called sub-microvillar (SMC) or sub-rhabdomeric cisternae [10] (Fig 1C). In electron micrographs, the membranes of these cisternae are separated from the microvillar PM by a cytoplasmic gap of ca. 10 nm. The three dimensional architecture of the SMC in relation to the length of the rhabdomere remains to be determined. It can either be present in the form of discontinuous tubules or a large network of interconnected tubules that would have a large surface to volume ratio. It has recently been reported that the SMC is also marked by reticulon [11]. Reticulons are integral membrane proteins of the ER that are reported to promote formation of high curvature smooth ER at the cell cortex [12].

**Proposed functions of the sub-microvillar cisternae**

One of the earliest physiological functions was proposed by Horridge and Barnard. They proposed that these networks of ER or the so called "palisades" were involved in the regulation of light sensitivity of the locust eye. The movement of the palisades of ER away from the microvillar PM in light-adapted locusts would change the refractive index of the peri-rhabdomeral cytoplasm thus altering sensitivity of the eye [13]. It is well known that the light-sensitive membranes of photoreceptors undergo light-dependent turnover [14]. In this regard, it has been proposed that the internal membranes apposed to the microvilli may play a role in turnover of the light sensitive pigment as well as membrane itself. Hotta and co-workers have noted that in several *Drosophila* mutants that exhibit microvillar degeneration, the earliest defects are seen in the structure of the SMC [15]. They also proposed that the SMC may function in transport of material to and from the rhabdomere [15].

Insect photoreceptors including those from *Drosophila* see large fluxes of intracellular $Ca^{2+}$ during phototransduction. Given that the SMC are located very close to the base of the microvilli it has been proposed that they may act as a store of $Ca^{2+}$ and regulate $Ca^{2+}$ homeostasis. This appears to be the case in honey bee drones where the sacs of ER close to the base of the microvilli accumulate $Ca^{2+}$ as measured by electron probe analysis [16]; however similar studies in *Drosophila* did not show an accumulation of $Ca^{2+}$ in SMC [17].

In microvillar photoreceptors, phototransduction is typically mediated by G-protein coupled phospholipase Cβ (PLC) activation leading to the hydrolysis of the lipid phosphatidylinositol 4,5 bisphosphate [$PI(4,5)P_2$][18]; *Drosophila* photoreceptors are an example of this transduction mechanism. The hydrolysis of $PI(4,5)P_2$ triggers a series of enzymatic reactions [usually referred to as the $PI(4,5)P_2$ cycle] that generate a series of lipid intermediates ultimately leading to $PI(4,5)P_2$ re-synthesis [19]. Importantly, these lipid intermediates are not diffusible and limited to the membrane at which they are generated; they are also distributed across two membrane compartments namely the PM and the SMC (Fig. 2). $PI(4,5)P_2$ is a low

abundance lipid and it is remarkable that despite the high levels of PLC activity that photoreceptors experience its levels remain relatively stable. The question arises as to how the $PI(4,5)P_2$ cycle is tuned such that the consumption of this lipid at the PM is matched by resynthesis. It has been proposed that the transfer of lipid intermediates generated during the $PI(4,5)P_2$ cycle between the PM and the SMC might be facilitated by lipid transfer proteins such as phosphatidylinositol transfer proteins (PITPs)[20]. The founding member of the Class II subfamily of PITPs is *Drosophila* RDGBα (also known in mammals as PITPNM1/ mRdgBα Nir2/). *rdgB* mutants were initially identified as mutants with a defect in phototransduction that showed light-dependent retinal degeneration [reviewed in [21]]. RDGBα is a large multi-domain protein with an N-terminal PITP domain that shows phosphatidylinositol (PI) transfer activity *in vitro* [22]. Its expression is enriched in *Drosophila* photoreceptors [22] and in contrast to the soluble Class I PITPs, it appears to be a membrane-associated protein [23]. Importantly, elegant immunoelectron microscopy studies have shown that RDGBα is localized at SMC in adult photoreceptors [22][24] although the mechanism by which this exquisitely precise sub-cellular localization is achieved remains unknown. *rdgB* mutants show a number of distinctive light-dependent phenotypes including retinal degeneration as well as a reduced electrical response to light [25]. These phenotypes can be rescued by the transgenic expression of PITPNM1, the mammalian ortholog of RDGBα, in *rdgB* mutant photoreceptors [26]. This finding underscores the functional conservation of *Drosophila* RDGB and its mammalian ortholog PITPNM1/mRdgBα.

Biochemical analysis has shown that the PITP domain of Class II PITPs have distinct lipid binding and transfer activities from those of the Class I PITPs. While Class I PITPs are PI/phosphatidylcholine(PC) transfer proteins, the Class II PITP domains have distinct properties [27][28]. In a recent study the PITP domain of RDGB has been shown to bind and transfer both PI and phosphatidic acid (PA) but not PC *in vitro* [23]. *In vivo*, the key phenotypes of *rdgB* can be rescued by reconstitution of *rdgB* mutant with the PITP domain of RDGBα but not the *Drosophila* Class I PITP. This observation underscores the importance of the unique biochemical properties of the PITP domain of RDGBα for its *in vivo* function. This study also demonstrated that RDGBα function was essential to maintain both $PI(4,5)P_2$ and PA homeostasis in photoreceptors during illumination. It is hypothesized that the lipid transfer activity of the PITP domain of RDGBα is essential to transfer PI to the microvillar PM and remove PA from this membrane during G-protein mediated $PI(4,5)P_2$ hydrolysis (Fig 2). Collectively, with other studies [29,30] suggesting a defect in $PI(4,5)P_2$ re-synthesis in *rdgB* mutants, RDGBα is likely to be a key regulator of $PI(4,5)P_2$ levels during PLC activation in *Drosophila* photoreceptors. The evolution of a single protein with dual (PI/PA) transfer activities offers an elegant solution to the need to move two distinct lipids between cellular compartments to maintain the $PI(4,5)P_2$ cycle. Conceptually similar findings were reported in a study performed on the human ortholog of RDGBα, PITPNM1 in mammalian cells [31]. These authors found that intact PITPNM1 function was required to remove PA from the plasma membrane and restore $PI(4,5)P_2$ levels during stimulation of mammalian cells with agonists that stimulate G-protein coupled PLC activity. Although both RDGBα and PITPNM1 seem to function in a similar manner to maintain lipid homeostasis during PLC signalling in *Drosophila* and mammalian cells respectively, there are subtle but important differences in the cell biological mechanisms through which they act. In mammalian cells, PITPNM1 is localized in the ER that has to translocate to position this protein at an ER-PM contact site where it presumably exerts its function. By contrast, in *Drosophila* photoreceptors, the SMC (where RDGBα is localized) are permanently located ca. 10 nm from the base of the microvillar PM. This arrangement may reflect the rapid time scale of the response to photons and consequent PLC

signalling in *Drosophila* photoreceptors that operates on a millisecond timescale [8] thus requiring that the lipid transfer protein be available in place to mediate rapid exchange of lipid metabolites following PLC activation. The importance of the localization of RDGBα at the SMC and the molecular interactions underlying this localization remains to be investigated (Fig 2). One of the possible interaction which might be involved in localizing RDGBα to SMC is the interaction of its FFAT motif with the VAMP associated proteins (VAPs) of ER although additional domains that may mediate localization of the mammalian ortholog Nir2 to the plasma membrane have also been proposed [31–33].

Interestingly, in addition to RDGBα, additional proteins related to lipid metabolism have been reported to localize to the SMC. Using immunogold labeling, Masai et.al found that diacylglycerol kinase (RDGA) also localizes to the SMC [34]. Mutant alleles in which the expressed RDGA protein is mislocalized from the SMC show a near null phenotype. This finding highlights that the localization of RDGA at SMC is essential for normal photoreceptor function. RDGA phosphorylates diacyglycerol (DAG) to generate PA. The substrate of RDGA, namely DAG is generated at the microvillar PM by PLC and the question of how RDGA (present at the SMC) can access a substrate generated at the microvillar PM remains unresolved. Since the distance between the SMC and the microvillar membrane is ca. 10 nm it is interesting to speculate that RDGA might act in trans to access DAG. Like RDGB, RDGA is also a large multi-domain protein of ca. 1200 amino acids and this mechanism of action remains a distinct possibility.

In summary the SMC-PM contact site in *Drosophila* photoreceptors offers several advantages to study the regulation of lipid signalling intermediates. The presence of a relatively stable MCS coupled with the power of *Drosophila* molecular genetics as well as a clear physiological readout will likely prove to be advantageous to understand the *in vivo* regulation of biochemical activity at MCS.

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**Figure legends**

Figure 1: (A) Line illustrations depicting the spatial relationship of the light sensitive plasma membrane and intracellular endoplasmic reticulum in a range of micovillar photoreceptors. Examples from planarians, annelids, cephalopods and arthropods are shown. Photosensitive membrane is marked as P and internal membranes marked as I. (Figure reproduced from ref 5 with permission from John Wiley & Sons). (B) Transmission electron micrograph of a cross-section through a single ommatidium from the compound eye of *Drosophila*. The rhabdomeres of photoreceptors R1 to R7 are indicated in yellow. The basolateral membrane of each cell is indicated in magenta. The nucleus is outlined in green and marked N. (C) High- magnification view of the base of the microvilli from a single *Drosophila* photoreceptor. The line diagram indicates the close apposition of the microvillar plasma membrane (PM) with the sub-microvillar cisternae (SMC).

**Figure 2:** Cartoon indicating the distribution of known molecular components at the plasma membrane and the SMC of *Drosophila* photoreceptors (PIP2: Phosphatidylinositol (4,5) bisphosphate; PI: phosphatidylinositol; PA:phosphatidic acid; PLC: phospholipase C; Gαq and Gβγ: Subunits of G-protein)

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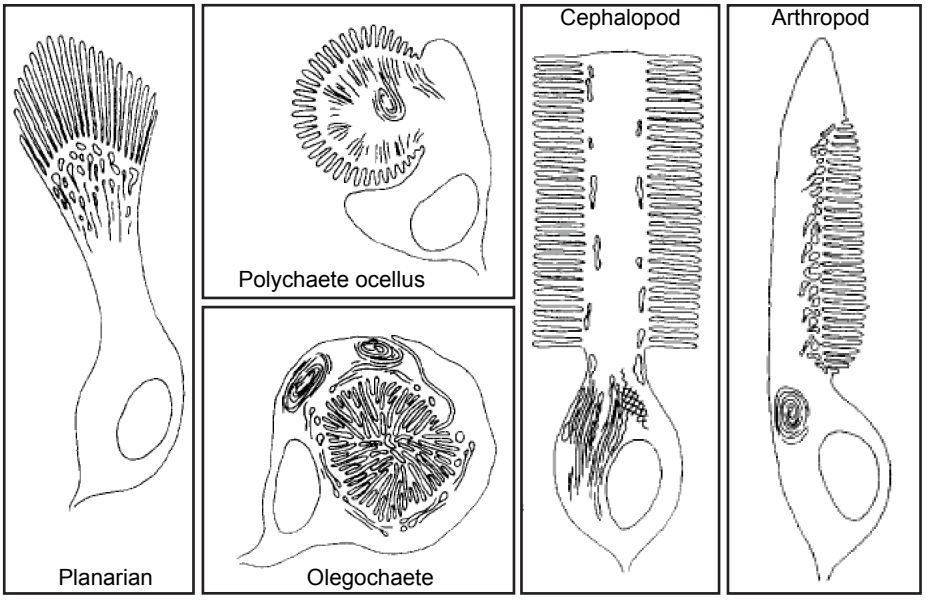
1A

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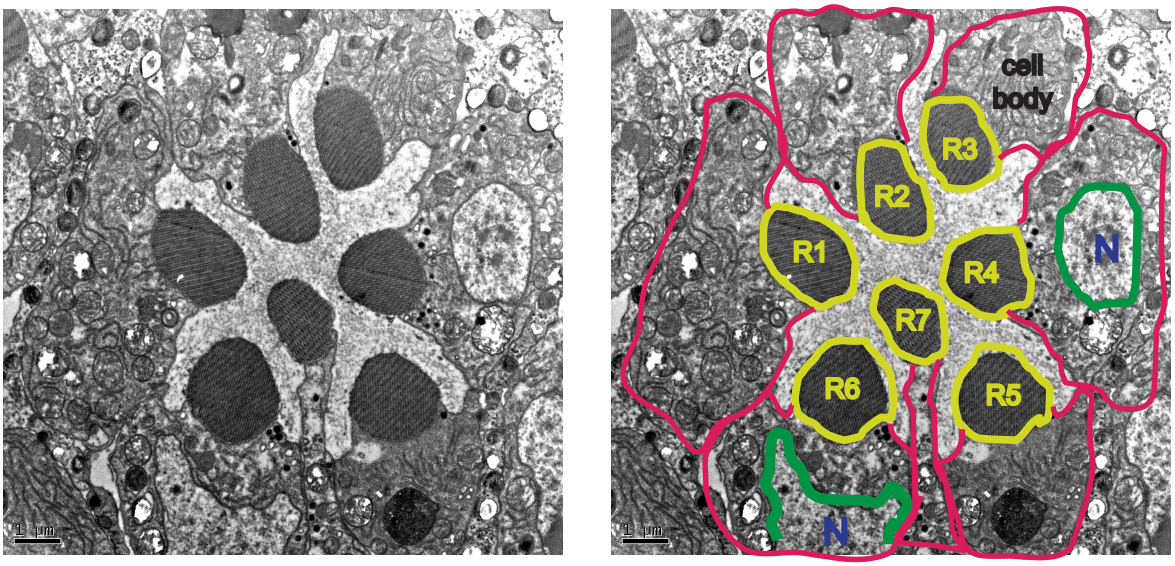


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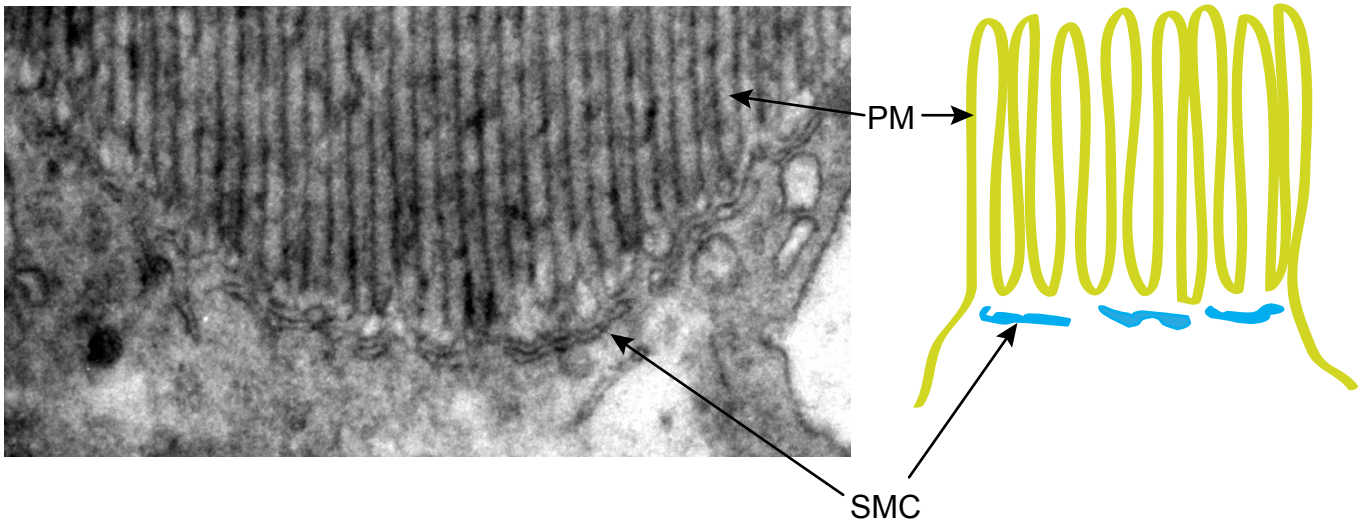


**Figure 1**

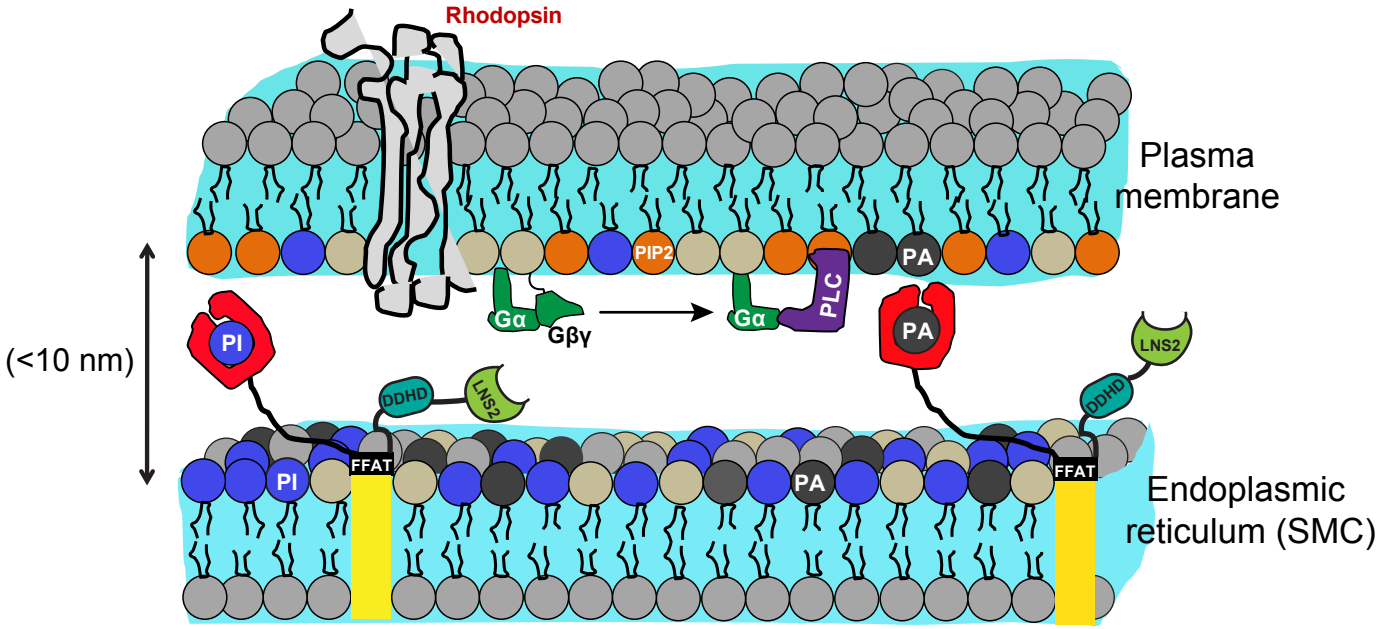
Rhodopsin
Plasma membrane
(<10 nm)
PIP2
PA
PLC
Gα
Gβγ
PI
DDHD
LNS2
FFAT
Endoplasmic reticulum (SMC)

**Figure 2**