RdgBα reciprocally transfers PA and PI at ER-PM contact sites to maintain $PI(4,5)P_2$ homeostasis during phospholipase C signalling in *Drosophila* photoreceptors.

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Abbreviations:
PLC, phospholipase C;
PI, phosphatidylinositol;
PI4P, phosphatidylinositol 4-phosphate;
$PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate;
DAG, diacylglycerol;
PA, phosphatidic acid;
CDP-DAG, cytidine diphosphate-diacylglycerol;
CDS, CDP-DAG synthase;
PIS, phosphatidylinositol synthase;
$I(1,4,5)P_3$, inositol 1,4,5-trisphosphate;
PITP, phosphatidylinositol transfer protein;
PITP-d, PITP domain;
PC, phosphatidylcholine;
RdgB, Retinal degeneration Type B;
ER, endoplasmic reticulum;
PM, plasma membrane;
LPAAT, Lyso-PA acyl transferase;
GPAT, Glycero-phosphate acyl transferase;
PI4K; PI 4-kinase;
PI4P5K, PI4P 5-kinase;
DGK, diacylglycerol kinase;
PLD, phospholipase D;
G-3-P, Glycero-3-phosphate;
FFAT, two phenylalanines in an acidic tract
PA-PLA1, PA-preferring phospholipase A1;
ATRAP, Angiotensin II receptor-associated protein
SRC, subrhabdomeric cisternae

Abstract
Phosphatidylinositol (PI) is the precursor lipid for the synthesis of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) at the plasma membrane (PM) and is sequentially phosphorylated by the lipid kinases, PI 4-kinase and PI4P-5-kinase. Receptor-mediated hydrolysis of $PI(4,5)P_2$ takes PM but PI resynthesis occurs at the endoplasmic reticulum (ER). Thus $PI(4,5)P_2$ resynthesis requires the reciprocal transport of two key intermediates, phosphatidic acid (PA) and PI between the ER and the PM. Phosphatidylinositol transfer proteins (PITPs), defined by the presence of the PITP domain, can facilitate lipid transfer between membranes; the PITP domain comprises a hydrophobic cavity with dual specificity but accommodates a single phospholipid molecule. The Class II PITP, RdgBα is a multi-domain protein and its PITP domain can bind and transfer PI and PA. In *Drosophila* photoreceptors, a well-defined G-protein-coupled phospholipase Cβ signalling pathway, phototransduction defects resulting from loss of *RdgBα* can be rescued by expression of the PITP domain provided it is competent for both PI and PA transfer. We propose that RdgBα proteins maintain $PI(4,5)P_2$ homeostasis after PLC activation by facilitating the reciprocal transport of PA and PI at ER-PM membrane contact sites.

Introduction
Phospholipase C activation by cell surface receptors mediates the hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to inositol 1,4,5-trisphosphate ($I(1,4,5)P_3$) and diacylglycerol (DAG). Depletion of $PI(4,5)P_2$ at the plasma membrane impacts several processes including cytoskeletal dynamics, ion channel regulation and endocytosis, and therefore rapid replenishment of $PI(4,5)P_2$ is essential to maintain cellular function. To resynthesise $PI(4,5)P_2$, DAG is first phosphorylated to phosphatidic acid (PA) at the plasma membrane. PA is the substrate for CDP-DAG synthase (CDS) to produce CDP-DAG which is converted into phosphatidylinositol (PI) by PI synthase (PIS) at the endoplasmic reticulum [1] (see Fig. 1). At the plasma membrane, PI can be sequentially phosphorylated by the resident PI 4-kinaseα and PI4P-5-kinase to $PI(4,5)P_2$ [2]. The topological arrangement of these enzymes requires the transfer of PI and PA in opposite directions between two membrane compartments, the PM and the ER (Fig. 1). Whilst the enzymes involved in these steps of lipid metabolism are well characterised, the major question yet to be resolved is the mechanism of PI and PA transfer between the two membrane compartments. This question was raised in 1975 by Michell when the 'PI cycle' was suggested to be important in cellular function and predicted that lipid transporters would move the lipids between the membranes [3]. A lipid transport protein capable of transporting PI had just been identified in 1974 supporting this concept [4].

In 1993, we identified a soluble 35kDa protein, phosphatidylinositol transfer protein (PITPα) as capable of supporting $I(1,4,5)P_3$ production in permeabilised cell preparations [5-7]. PITPα has dual specificity; it can accommodate either a PI or a phosphatidylcholine (PC) molecule within its hydrophobic cavity [8;9]. The issue of PA transport has remained unresolved until recently. A previous study, again in permeabilised cells, indicated that PA transport from the ER was not mediated by soluble proteins or by vesicular transport and we suggested that it could be protein-mediated at sites of close membrane contact by membrane proteins [10]. In 2012, we identified Class II PITPs as PA and PI transport proteins [11], unlike PITPα and PITPβ which transport PI and PC. In yeast, a PA transporter that functions in mitochondria, Ups1 in complex with Mdm35, was also identified in 2012; Ups1 and PITPα share structural homology but not sequence homology [12-14]. In this review, we

focus on members of the Class II PITP family that can reciprocally transfer PI in exchange for PA at membrane contact sites during PLC signalling (Fig. 2).

Introduction to the PITP family

The PITP family has five members in the mammalian genome subdivided into two Classes based on sequence analysis (Fig. 2). Analysis of the lipid transfer and binding activities of the various PITP domains establish that Class I PITP are PI and PC transfer proteins whilst Class II PITPs are PI and PA transfer proteins (Fig. 3). Class I comprises the single domain PITPs (α and β) and Class II comprises the RdgB proteins. The RdgB family is so named as the founding member of this class was first identified as the Retinal degeneration B mutant in *Drosophila* [15]. The PITPs are defined by the presence of the PITP domain (Pfam: IPtrans (PF02121)); this domain can bind and facilitate the exchange of PI between membrane compartments without a requirement for ATP. The hallmark of this domain is four amino acid residues present on two beta strands. These residues (T59, K61, E86 and N90 using mouse numbering) are conserved in the majority of the PITP sequences found in the sequence database across different species. Single point mutations in any of these residues renders them inactive for PI binding or transfer [9]. There are three RdgB proteins in the mammalian genome, two of which are large multi-domain proteins containing the DDHD and LNS2 domain. The DDHD domain is 195 amino acids and is also present in other proteins including DDHD1 and DDHD2 (KIAA0725p) that possess PA-PLA1 activity and p125 (Sec23-interacting protein). This domain has been shown to bind PI4P *in vitro* [16;17]. The LNS2 domain is 130 amino acids and is also found in lipins (PA phosphatase; although the motif required for phosphatase activity is not conserved) and has been shown to bind PA [18]. These proteins possess six hydrophobic regions and thus endogenous proteins, both in mammals and flies, are membrane-associated [19-21]. The proteins can be solubilised at pH 10 or high salt but are not solubilised with Triton X100 suggesting that the proteins are peripheral and are potentially associated with the cytoskeleton. The third RdgB protein, RdgBβ, consists of the PITP domain followed by an unstructured eighty amino acid N-terminal extension which is phosphorylated at two sites to form a 14-3-3 binding site [22]. In addition to binding 14-3-3, the PITP domain binds an integral adaptor protein, ATRAP (angiotensin II receptor-associated protein) [23].

Expression pattern of PITPs

In order to understand the function of the PITPs, establishing their cellular expression pattern and localisation is vital. In mammals, PITPα is highly enriched in the brain but is found in virtually all tissues [24]. Likewise, PITPβ is enriched in liver, lung and muscle but is present at the protein level in most tissues examined. When cytosols from different tissues have been fractionated by size exclusion chromatography, a single peak of PI transfer activity is found which is due to the combined presence of PITPα and PITPβ [25]. RdgBβ, on the other hand, is enriched in the heart and in the brain but when cytosols prepared from these tissues are analysed for PI transfer activity, a peak of activity associated with RdgBβ is not observed. This suggests that RdgBβ functions locally at membrane contact sites and is probably recruited to membranes by ATRAP [22]. The endogenous localisation of the two multi-domain proteins, RdgBαI and RdgBαII has been analysed in only a few cases. RdgBαI (also known as PITPNM1) is found in inner hair cells of the cochlea, the retina and in specific areas of the brain [26;27]. RdgBαII expression is highly restricted; it is mainly found in the retina and the dentate granular cells in the hippocampus [27;28]. In the retina, it is found discretely in the GABAergic amacrine cells and the ganglion cell layer [29].

The *Drosophila* and *C.elegans* genomes only contain a single RdgBα protein. In *Drosophila*, the protein is enriched in photoreceptors but also other specific regions of the brain [30]. *C.elegans* RdgBα also shows specific expression in the nervous tissue [31]. It is present in a subset of neurons including ASER and AWC. (*C.elegans* possess 302 neurons and all the neurons have been named and their positions mapped [32].)

Analysis of *Drosophila* RdgBα in phototransduction

Sensory transduction in *Drosophila* photoreceptors relies on G-protein coupled phospholipase C activation. Rhodopsin, together with Gq and PLCβ (NorpA) are concentrated in the microvillar membranes whilst *Drosophila* RdgBα (Dm-RdgBα) localises to the subrhabdomeric cisternae (SRC) [30;33]. Light induces robust activation of PLCβ resulting in the consumption of $PI(4,5)P_2$ [34]. In the absence of Dm-RdgBα, photoreceptors show a defective electrical response to light along with retinal degeneration. The retinal degeneration phenotype of the *rdgB* mutant requires ongoing light-activated PLC-mediated hydrolysis of $PI(4,5)P_2$. Thus rearing flies in the dark or *norpA* mutants that lack a functional PLCβ is protective [35]. In contrast, retinal degeneration is accelerated in flies that express constitutively-active *dGq*. Retinas of *rdgB;dGq* double mutants degenerate even in the dark and is PLC-dependent [36]. Phosphatidylinositol synthase (PIS) is required for a key step during $PI(4,5)P_2$ regeneration and overexpression of PIS is reported to suppresses the retinal degeneration of *rdgB* and *cds* mutants [37]. The *rdgB* mutants show reduced amounts of $PI(4,5)P_2$ in the rhabdomeres even before they are exposed to any light. Additionally, the time-course of $PI(4,5)P_2$ resynthesis after light exposure is delayed and is accompanied by an increase in PA levels [21].

Expression of a PITP domain competent to transfer both PI and PA is sufficient to rescue both retinal degeneration and the electrophysiological light response [21]. Class I PITPs, either mammalian or *Drosophila*, which transfer PI and PC but not PA, are unable to facilitate rescue. Mutation of the residues required for binding and transferring PI (T59, K61, N90) in either the PITP domain or the full length protein are also unable to rescue function. Docking of the Class I PITPs to membranes requires two tryptophan residues and mutation of these residues disrupts membrane docking although their lipid binding capacity is unimpaired [9;25]. In Class II PITPs, this motif is YW and mutation of this motif in the full length RdgBα protein is unable to rescue retinal degeneration and the electrical response. Together these data provide compelling genetic evidence to support the concept that the *rdgB* mutant phenotype is due to the inability to replenish $PI(4,5)P_2$ levels following stimulation by light and the mechanism of RdgBα function in the restoration of $PI(4,5)P_2$ levels depends on PI and PA transfer. Thus reciprocal transfer of PA and PI from the PM to SRC and vice versa by the PITP domain of RdgBα provides an elegant solution to the maintenance of $PI(4,5)P_2$ homeostasis during PLC signaling (Fig. 4).

Analysis of RdgBα function in *C.elegans*

In the worm, the ortholog of RdgBα**,** Ce-RdgBα (also referred to as PITP-1) is required for sensory transduction in specific neurons including ASER, AWC and ASH that are involved in the gustation, olfaction and osmo-sensation respectively. This recapitulates the enrichment of Dm-RdgBα in sensory organs of the adult head. Individual phenotypes resulting from loss of Ce-RdgBα can be restored when Ce-RdgBα is re-expressed in specific neurons [31]; as in *Drosophila*, reconstitution of the Ce-RdgBα mutant with the PITP domain alone is sufficient to rescue mutant phenotypes supporting the notion that the PITP domain contains an important functional activity of this protein.

Ce-RdgBα is localised in the axons of the sensory neuron, ASER, involved in salt attraction. The reduced salt attraction seen in Ce-RdgBα mutants could be suppressed by mutations in DAG kinase, that causes accumulation of DAG indicating that DAG elevation is required for synaptic transmission. These results suggest that Ce-RdgBα maintains $PI(4,5)P_2$ levels required for the production of DAG via PLC. The PLC in question has been identified as PLC-epsilon, as a mutation in this enzyme also alters salt preference [38]. Collectively these findings imply that the function of Ce-RdgBα occurs in the context of PLC signalling and supports lipid turnover during the $PI(4,5)P_2$ cycle.

Although Ce-RdgBα is essential for a number of sensory behaviours, locomotion and egg-laying are unaffected indicating a highly specific requirement. Both locomotion and egg laying are defective in *egl-8* PLCβ mutants and *egl–30* Gqα mutants indicating that these processes are dependent on PLC signalling [39] but not Ce-RdgBα. There are two other PITPs in *C.elegans* and could potentially participate in these PLC-dependent processes. Their lipid transfer activities have not been examined.

RdgBα is present at membrane contact sites

In *Drosophila* photoreceptors, Dm-RdgBα is found at the membranes of the subrhabdomeric cisternae (SRC) which lies underneath the rhabdomeric plasma membrane at the bases of the photoreceptive microvilli [30;33]; the SRC is equivalent to the ER and lies 10nm from the microvillar plasma membrane. The N-terminal PITP domain is followed by a disordered region of 120 amino acids before the 'FFAT' motif (that would anchor the protein to the ER by binding to Dm-VAP) (Fig. 4). A fully extended amino acid is 3.3 angstroms and therefore a disordered region of 120 a.a could in principle span 40nm, more than sufficient for the PITP domain to make contact with the plasma membrane. Thus the PITP domain of RdgBα would be able to reach out to the plasma membrane (Fig. 4). The orientation of the LNS2 and DDHD domains is not known however.

Recent studies in mammalian cultured cell lines also suggest that mammalian RdgBαI (also known as PITPNM1/Nir2) and mRdgBαII (also known as PITPNM2/Nir3) are present at ER-plasma membrane contact sites [40-43]. These studies suggest that over-expressed mRdgBα is recruited from the cytosol to the PM by the LNS2 domain binding to PA generated by agonist-activated PLC. To replenish the $PI(4,5)P_2$ pool, the PITP domain was also required. Although the conclusions from the studies in *Drosophila*, *C.elegans* and the cultured cell lines are broadly in agreement, there are differences.

Firstly, there is conflicting data concerning RdgBαI localisation in cultured cell lines. Since some of these studies relied on over-expression, this may affect the localisation. In one study, RdgBαI was localised to the Golgi whilst two other studies found it to be cytosolic [18;41;42]. In the study by Kim et al, both endogenous and myc-tagged RdgBα was Golgi-localised and upon stimulation with EGF, RdgBα translocated to the plasma membrane [18]. The PITP domain determined Golgi-localisation, whilst translocation to the plasma membrane required the LNS2 domain. The LNS2 domain was found to bind PA *in vitro* and it was suggested that the translocation was due to a rise in plasma membrane PA. In contrast, Liou and colleagues have reported that elevation of cytosol Ca^{2+} triggers the translocation of E-Syt to ER-PM junctions and this subsequently recruits cytosolic RdgBαI to the junctions. (E-Syt1 is a transmembrane protein localised to the ER which contains an SMP domain and five C2 domains. One of the C2 domains can bind to plasma membrane $PI(4,5)P_2$ in a Ca^{2+}-

dependent manner [44]). Recruitment to ER-PM junctions was independent of PI binding to the PITP domain but PI binding was required for $PI(4,5)P_2$ replenishment.

The study by Kim and Balla have also examined the role of RdgBαI in maintaining $PI(4,5)P_2$ levels after PLC activation [42]. Their study was conducted in HEK293 cells stably expressing the angiotensin II receptor. They also report that RdgBαI is mainly cytosolic with some localisation at the ER. No Golgi localisation was observed. In RdgBαI knockdown cells, they report less agonist–stimulated DAG, but observe a larger increase in PA at the PM. A decrease in the synthesis of the intermediate CDP-DAG was also seen. They also observe that RdgBαI localises to ER-plasma membrane contact sites upon stimulation and is dependent on the FFAT motif binding to VAP proteins. Using an experimental protocol that monitored the removal of PA from the plasma membrane, they found that upon over-expression of RdgBαI, PA removal was faster compared to the controls. Removal of PA was dependent on the PITP domain. Most interestingly, mutation of T59 to either E or A prevented the stimulatory effect on PA clearance. Although not shown for human RdgBαI, it has been previously shown that mutation of T59 to A or E in either Class I or Class II inhibits PI transfer but not PA transfer activity of Dm-RdgBα PITP domain [9;21]. It is therefore puzzling why mutation of T59 disrupts PA clearance. It would be interesting to examine PA transfer activity of these mutants. For RdgBαI to associate with membranes, they suggest that the region that connects the LNS2 domain with the DDHD domain contains a short sequence that bears resemblance to the DAG binding part of the C1 domain. This domain together with the LNS2 was required for plasma membrane association to DAG.

Concluding remarks

Studies from both model organisms, *Drosophila* and *C.elegans*, clearly indicate that RdgBα is part of the PLC signal transduction cascade where the PITP domain is central to its function and is required for the replenishment of $PI(4,5)P_2$ after its degradation by PLC. However, in these model organisms, RdgBα is required in highly-specific situations. In the case of *Drosophila*, it is required for phototransduction whilst in *C.elegans* it is obligatory for sensory transduction in a set of defined neurons. In mice, the requirement for RdgBαII also appears to be highly-specific. RdgBαII mutants display defects in circadian photoentrainment and the pupillary light response but only in dim light and not in bright light [29]. In bright light, the intrinsically photosensitive retinal ganglion cells (ipRGC) use melanopsin to activate PLCβ4 but RdgBαII is not required. (It is not clear if RdgBαI is expressed in these cells.) Phospholipase C signalling in mammals is ubiquitous and there are 13 PLCs in the human/mouse genome. Gene knockouts of individual PLCs provide a variety of strong phenotypes including epilepsy, embryonic lethality etc. [45]. In contrast, mice null for RdgBαI and RdgBαII show only very subtle phenotypes; mice are grossly normal and fertile suggesting that either RdgBα functions in specific neuronal circuits such as sensory transduction, learning and memory [26;29] or other PITPs can compensate. (It has been previously reported that RdgBαI-null mice could not be generated [28] but a more recent study report otherwise [26]). There are five PITP proteins in the mammalian genome and while both Class I and II PITPs can transfer PI, only Class II PITPs can transfer PA. Class I PITPs are ubiquitously expressed but this leaves open the question of PA transfer from the PM to ER. Is it possible that not all PLC signalling cascades need a PITP, or alternatively Class I PITP can also supply PI for $PI(4,5)P_2$ homeostasis? Maybe, replenishment of PI at the PM from the much larger pool of the ER is more critical than PA removal. This question can be addressed by deletion of all the PITP proteins.

Phospholipase C activation by cell surface receptors mediates the hydrolysis of $PI(4,5)P_2$ to $I(1,4,5)P_3$ and DAG. Although the initial focus of the PLC pathway was on the generation of the second messengers, $I(1,4,5)P_3$ and DAG, PLC activation can also acutely regulate the levels of $PI(4,5)P_2$. In some cases, $PI(4,5)P_2$ depletion is more relevant than the second messengers.
For example, both phototransduction in *Drosophila* and inhibition of KCNQ2/3 potassium channels by activation of muscarinic cholinergic receptors requires depletion of $PI(4,5)P_2$. In the case of phototransduction, depletion of $PI(4,5)P_2$ regulates Ca^{2+} entry through Trp channels and in the case of KCNQ2/3, depletion of $PI(4,5)P_2$ is required for closure of the potassium channels. This demands intense hydrolysis but also requires rapid replenishment to avoid any impact on endocytosis or on the actin cytoskeleton which are sensitive to $PI(4,5)P_2$ levels.

The level of $PI(4,5)P_2$ depletion that occurs during GPCR signalling is dependent on the number of receptors activated [46-48]. If $I(1,4,5)P_3$/DAG production is the required response, the number of receptors that need to be activated can be small; and $PI(4,5)P_2$ depletion will be minimal. The amount of $I(1,4,5)P_3$ required to maximally release cytosol Ca^{2+} can be saturated with minimal depletion of $PI(4,5)P_2$. On the other hand, if $PI(4,5)P_2$ depletion is required for regulation of ion channels as discussed above, then a higher density of receptor activation is required [47;48]. Thus full amplitude calcium responses can be elicited with minimal $PI(4,5)P_2$ depletion. Alternatively, if inhibition of potassium channels is required, then substantial $PI(4,5)P_2$ hydrolysis is required.

In addition to GPCRs, receptor tyrosine kinases also activate PLC, in this case, the PLCγ family. Again, EGF receptors can also induce significant $PI(4,5)P_2$ hydrolysis, in this case, to release the actin binding protein, cofilin. Cofilin is restrained at the plasma membrane by being bound to $PI(4,5)P_2$. Upon $PI(4,5)P_2$ hydrolysis, cofilin is released and can now bind and sever F-actin for restructuring the actin cytoskeletal network [49]. These examples demonstrate the versatility of $PI(4,5)P_2$, not only as a provider of second messengers, but $PI(4,5)P_2$ functioning as a regulator as an intact lipid.

The difference in $PI(4,5)P_2$ hydrolysis depending on the required output raises the important question: are there special mechanisms in place when $PI(4,5)P_2$ depletion is the required response (as opposed to the production of $I(1,4,5)P_3$/DAG) for replenishing the $PI(4,5)P_2$ levels? Do PITP proteins become essential only when the amount of $PI(4,5)P_2$ hydrolysed is high as this places a burden on the cell and rapid replenishment is required ? When PLC stimulation which occurs at the physiological level for increasing Ca^{2+}, $PI(4,5)P_2$ hydrolysis will be limited and therefore replenishment of $PI(4,5)P_2$ may not require specialised mechanisms. Thus we would propose that PITP proteins become essential mainly during intense PLC signalling.

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Legend to Figures

Figure 1 Topological organization of the 'PI Cycle'
Agonist-dependent activation of phospholipase C (PC) at the plasma membrane hydrolyses PI(4.5)P_2 to DAG which is rapidly converted to PA. PA can also be generated by phospholipase D upon receptor activation. To resynthesise PI, PA has to be transported to the ER and after its synthesis, PI has to be transported to the plasma membrane where the two lipid kinases work in sequence to replenish the PI(4,5)P_2 levels. The molecular machinery that moves the PA and PI between the ER and the plasma membrane is suggested to be a member of the PITP family of proteins. Abbreviations used: PI, phosphatidylinositol; DAG, diacylglycerol; DGK, diacylglycerol kinase; PC, phosphatidylcholine; PLD, phospholipase D; PIS, PI synthase; CDP-DAG, cytidine diphosphate-diacylglycerol; CDS, CDP-DAG synthase; LPAAT, Lyso-PA acyl transferase; GPAT, Glycero-phosphate acyl transferase; PI4Kα; PI 4-kinase; PI4P5K, PI4P 5-kinase; PA, phosphatidic acid; LPA, lyso-PA;

Figure 2 Proteins with a PITP domain in humans, *Drosophila* and *C.elegans*
The PITP domain is ~260 a.a. Class I PITPs comprise the PITP domain only whilst RdgBα proteins are multi-domain proteins. The 'FFAT' motif (EFFDAxE) binds to endoplasmic reticulum-localised VAP proteins [50]. Mammals including humans contain 5 proteins with a PITP domain whilst *Drosophila* and *C.elegans* contain 3 proteins. The proteins are grouped into Class I and Class II based on sequence; Class I PITPs bind and transfer PI and PC whilst Class II PITPs bind and transfer PI and PA.

Figure 3
Lipid transfer activities of the PITP domains of Class I and Class II RdgB proteins
Human PITPα (black circles), human RdgBβ (green triangles) and the PITP domain of human RdgBαI (yellow triangles) and *Drosophila* (red circles) were examined for PI, PC and PA transfer activity. PITPα transfer PI and PC whilst RdgB proteins transfer PI and PA. The y-axis represents the amount of labelled lipid transferred to an acceptor compartment as a percentage of the total input present in the donor compartment.

Figure 4
Reciprocal PI and PA transfer by RdgBα
RdgBα is localised at the ER membrane and the PITP domain of RdgBα can transfer PI and PA between the ER and plasma membrane at sites of close membrane contact. In *Drosophila* photoreceptors the gap between the rhabdomere (PM) and the subrhabdomeric cisternae (SRC) is estimated to be 10nm. The FFAT motif in RdgBα can bind to the integral VAP protein present at the SRC.

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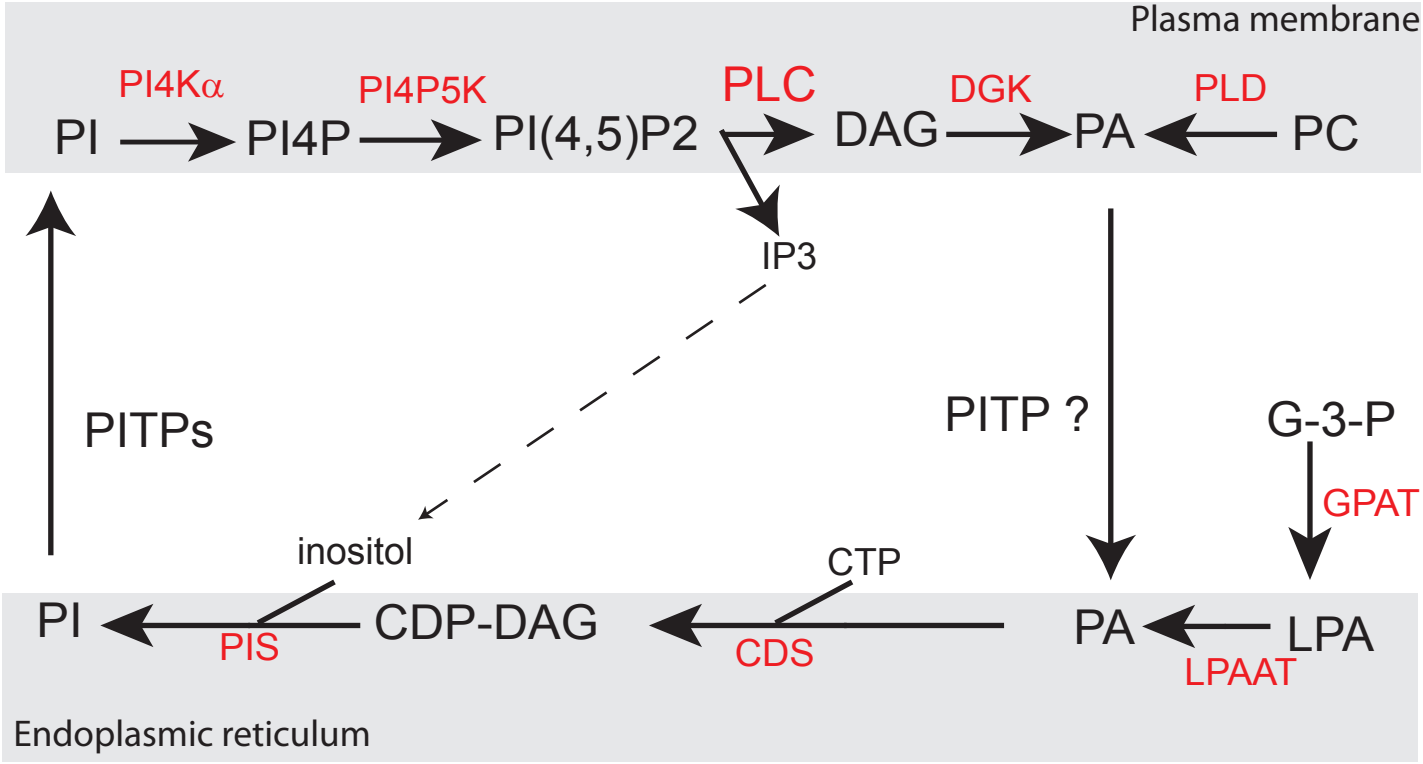
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PLD
PI
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PI(4,5)P2
DAG
PA
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IP3
PITPs
PITP ?
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PI
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CDS
PA
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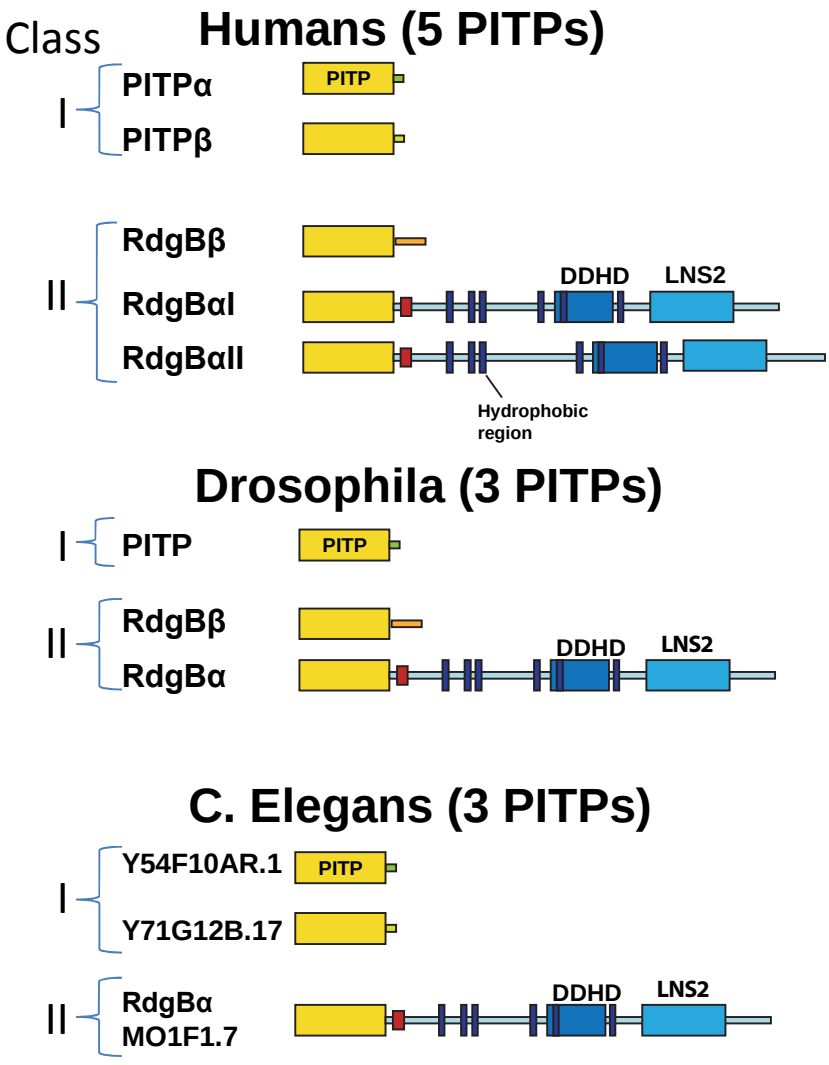
LPAAT
Endoplasmic reticulum

Fig. 1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PITPβ
PITP
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RdgBαII
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Hydrophobic region
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I
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PITP
II
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C. Elegans (3 PITPs)
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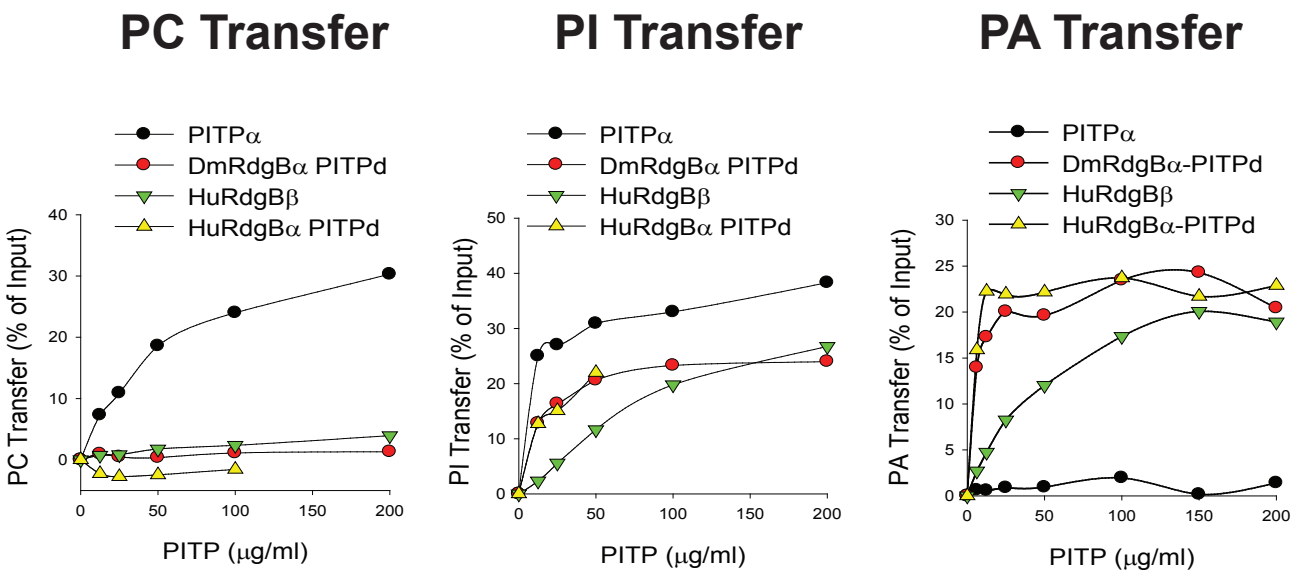
Fig. 2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PA Transfer
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HuRdgBα-PITPd
PC Transfer (% of Input)
PI Transfer (% of Input)
PA Transfer (% of Input)
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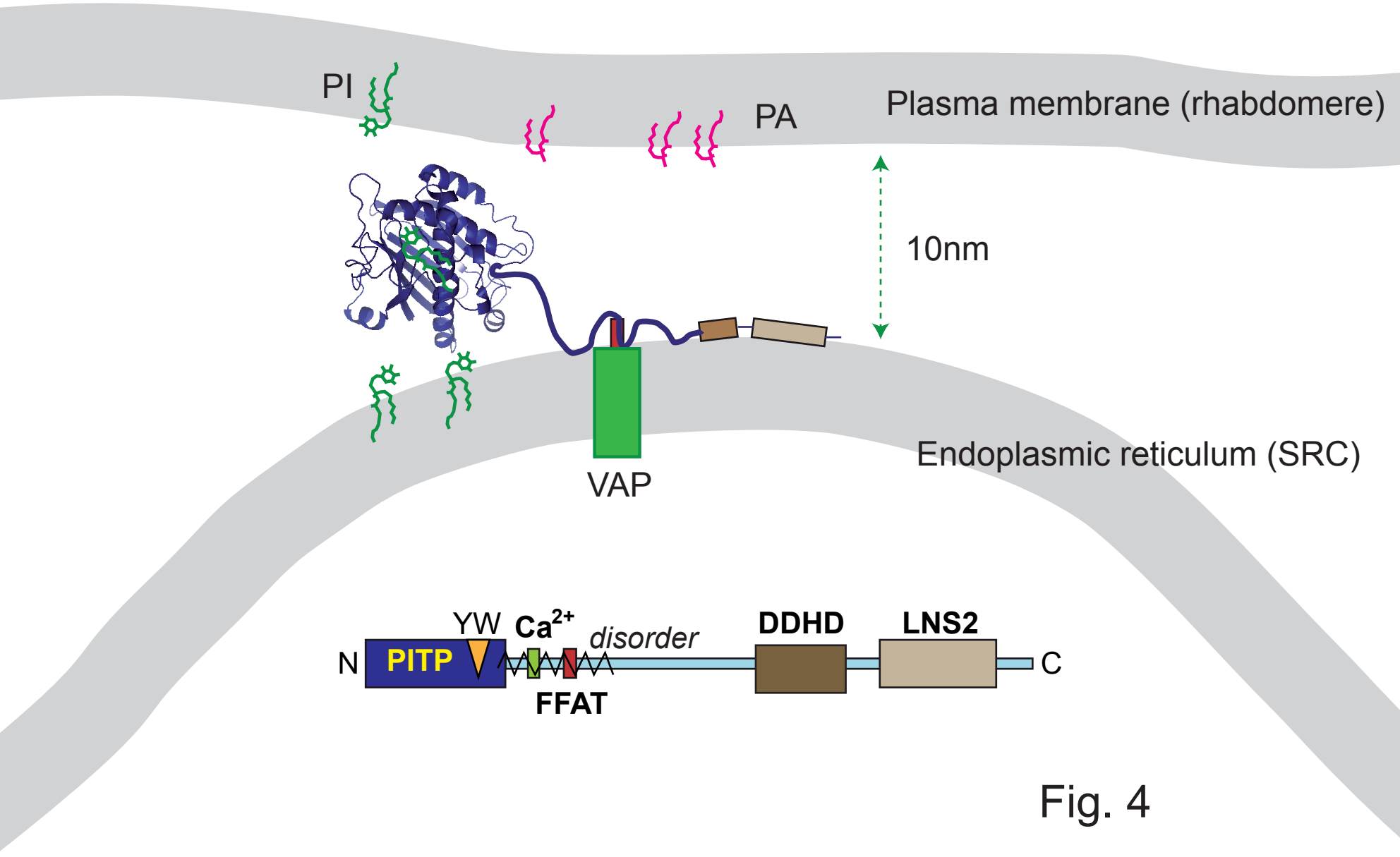
Fig. 3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VAP
Endoplasmic reticulum (SRC)
YW
Ca2+
disorder
DDHD
LNS2
N
PITP
C
FFAT

Fig. 4