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Review

Phosphoinositide signalling in *Drosophila*☆

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ABSTRACT

Phosphoinositides (PtdInsPs) are lipids that mediate a range of conserved cellular processes in eukaryotes. These include the transduction of ligand binding to cell surface receptors, vesicular transport and cytoskeletal function. The nature and functions of PtdInsPs were initially elucidated through biochemical experiments in mammalian cells. However, over the years, genetic and cell biological analysis in a range of model organisms including *S. cerevisiae*, *D. melanogaster* and *C. elegans* have contributed to an understanding of the involvement of PtdInsPs in these cellular events. The fruit fly *Drosophila* is an excellent genetic model for the analysis of cell and developmental biology as well as physiological processes, particularly analysis of the complex relationship between the cell types of a metazoan in mediating animal physiology. PtdInsP signalling pathways are underpinned by enzymes that synthesise and degrade these molecules and also by proteins that bind to these lipids in cells. In this review we provide an overview of the current understanding of PtdInsP signalling in *Drosophila*. We provide a comparative genomic analysis of the PtdInsP signalling toolkit between *Drosophila* and mammalian systems. We also review some areas of cell and developmental biology where analysis in *Drosophila* might provide insights into the role of this lipid-signalling pathway in metazoan biology. This article is part of a Special Issue entitled Phosphoinositides.

1. Introduction

Research using *Drosophila* has been influential in driving forward discovery in a number of areas of biology. These include insights into fundamental principles of genetics such as the chromosomal theory of heredity, the finding that ionising radiation causes mutations and uncovering the underlying principles fundamental to the organisation of body plan in a metazoan (reviewed in [1]). In addition, work using *Drosophila* as a model laid the foundation of the field of neurogenetics; i.e., linking behaviour or brain function with the activity of individual gene products [2] and most recently, the molecular basis of innate immunity [3].

The influence of *Drosophila* as a model organism in biological research has been driven by a number of factors. These include its short life cycle and unparalleled tractability to genetic manipulation driven by over one hundred years of technological development. Moreover, work over many years has shown that key biochemical pathways and cellular processes are conserved between *Drosophila* and vertebrate systems. The *Drosophila* genome was amongst the first to be fully sequenced [4] and a comparison of its content with the genomes of other organisms revealed the conservation of a core set of proteins between all species, including mammals. Remarkably, although the human genome is about 17 times the size of the *Drosophila* genome, there are only about twice as many genes in the human genome as compared to the fly genome [85]; in many cases this is accounted for by the presence of two separate genes encoding a specific type of protein. Given this compact genome along with advanced tools for genetic manipulation, *Drosophila* offers a tractable system for the analysis of fundamental, conserved cellular processes in eukaryotes, particularly metazoans. Finally, the fly genome contains orthologues for almost 75% of genes implicated in human diseases [5], thus offering a potential model system for the relatively rapid discovery of cellular disease mechanisms.

In this review we present a view of PtdInsP signalling focussing on studies in *Drosophila*. There are multiple areas of modern biology that have been informed by *Drosophila* research and many of these are covered in other chapters in this issue that focus on specific biological phenomena. We provide a curation of the known components of the PtdInsP signalling system as encoded in the *Drosophila* genome. In addition we cover some areas of research that are not specifically covered elsewhere in this issue; activation of G-protein coupled receptors (GPCRs) by PtdInsP signalling, tissue patterning, neurobiology and the regulation of growth.

2. Historical aspects

The history of research into PtdInsP signalling using *Drosophila* as a model dates back to the work of Yoshiki Hotta and his colleagues, who analyzed the basis of defective phototransduction in mutants isolated in a forward genetic screen [6,7]. The analysis of visually defective mutants isolated in these screens involved the application of biochemical

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approaches to understanding the molecular basis of defective phototransduction and resulted in the identification of a phospholipase C (PLC) and diacylglycerol kinase (DGK) activity in *Drosophila* heads [8]. These studies were instrumental in demonstrating the importance of PtdInsP metabolism for a signalling pathway *in vivo*; these were the first demonstration of the importance of the hydrolysis of phosphatidylinositol-4,5-bis phosphate [$PI(4,5)P_2$] in mediating a physiological process in an intact organism.

3. Biochemistry of PI signalling in *Drosophila*

Phosphatidylinositol (PI) is a glycerophospholipid with a *myo*-inositol head group (a cyclic alcohol with six hydroxyl groups). In PI the hydroxyl group at position 1 of *myo*-inositol is esterified to the sn-3 hydroxyl group of phosphatidic acid (PA) through a phosphodiester bond. Typically, individual classes of membrane phospholipids include families of molecular species that differ in the acyl chain length of the constituent fatty acids but with a conserved head group [9]. Among the major phospholipid classes, PI is unusual in that this class shows a highly restricted distribution of molecular species with respect to acyl chain length; in mammalian tissues, a large fraction (>85%) of the PI is composed of a single molecular species, C38:4, consisting of stearoyl (C18:0) at sn-1 and arachidonyl (C20:4) at sn-2. A recent study has shown that as a consequence, most of the PIP, PIP_2 and PIP_3 in mouse tissues also have the acyl chain composition of C38:4 [10]. In *Drosophila* tissues as well, acyl chain length diversity is recapitulated across multiple phospholipids classes and many tissues [11,12]. However, in the case of *Drosophila* tissues there appear to be two differences: (i) The longest acyl chain lengths seen are typically no longer than C18 [13,14] (ii) the acyl chain composition of PI does not shown the same bias towards a single species (C38:4) as seen in mammalian cells. Phospholipids are synthesized *de novo* from diacylglycerol (DAG) or PA; it is thought that subsequently the fatty acid esterified at sn-2 is remodelled using a specific deacylation/reacylation pathway known as the "Lands" cycle [15]. The key enzymes involved in this pathway are phospholipase A_2 that cleaves the fatty acid at position 2 and a lysophospholipid acyltransferase (LPAT). The *Drosophila* genome encodes three genes homologous to LPAT. One of these, *farjavit,* was able to incorporate 20:4 acyl chains into yeast mutants that lacked LPAT activity [16]. However, LC-MS analysis of mutants of the *Drosophila* LPAT genes did not show any major change in the fatty acyl chain composition of PI at the level of the whole animal [16].

4. Phosphoinositide signalling toolkit

Conceptually PtdInsP signalling can be considered to be dependent on enzymes that generate PI and its derivatives, enzymes that degrade them, as well as binding proteins that bind specifically to one of these molecules. The word 'toolkit" as used in the context of this review refers to that group of molecular components encoded in the *Drosophila* genome through which PtdInsP signalling is effected.

5. Synthesis of phosphoinositides

5.1. Phosphatidylinositol synthase

The *Drosophila* genome encodes a single phosphatidylinositol synthase (*dPis*) [17] (Table 2). Loss of *dPis* function results in both organismal and cell lethality, thus formally demonstrating the essential requirement of PI in maintaining metazoan cell function. It is widely accepted that the synthesis of PI occurs at the endoplasmic reticulum (ER) where the enzyme dPIS is localised; yet the lipid kinases that use PI as a substrate to generate its phosphorylated derivatives are not localised at this membrane and neither are the products of these lipid kinases found at the ER.

5.2. PI transfer proteins

Given that PI is a lipid that cannot diffuse freely across the cytosol from the ER, it is likely that the supply of PI from the ER is mediated by two processes (i) vesicular transport (ii) activity of PI transfer proteins (PITPs) [18,19]. PITPs are able to transfer PI between two membrane compartments in an energy independent manner. Based on sequence similarity, three subtypes of PITP have been defined: Class I, Class IIA and Class IIB. The *Drosophila* genome contains a single gene encoding each of these classes (Table 2). Indeed, the founding member of Class II PITPs in *Drosophila* is encoded by *rdgB* (*r*etinal *d*egeneration *B*). This gene was identified in the context of visual transduction where it is required to support GPCR mediated $PI(4,5)P_2$ turnover (reviewed in [20]). Class I PITP, encoded by *vib*/*gio* (vibrator) has been implicated in spermatogenesis and neuroblast development [21,22]. These studies showed that cytokinesis, a key event during spermatogenesis, is defective in *vib*/*gio* mutants. Interestingly, two independent studies have shown that $PI(4,5)P_2$ at the cleavage furrow is important for normal cytokinesis [23]. The function of Class IIB PITP in *Drosophila* (rdgBβ) is presently unclear.

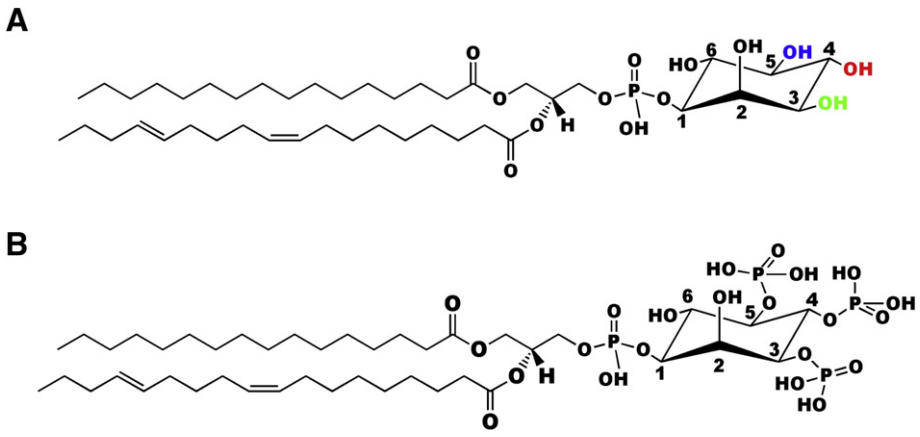


Fig. 1. Chemical structure of phosphatidylinositol (A) and phosphatidylinositol-(3,4,5)- trisphosphate (B). The fatty acids shown esterified at *sn*-1 and *sn*-2 position are 16:0 and 18:2 respectively and represent the likely acyl chain composition in *Drosophila* tissues. The hydroxyl groups at positions 3, 4 and 5 that are subject to phosphorylation by phosphoinositide kinases are shown in green, red and blue respectively (A) and the phosphate groups at positions 3, 4 and 5 of phosphatidylinositol-(3,4,5)-trisphosphate are illustrated (B).

5.3. Phosphoinositide kinases

The ability of PtdInsPs to act as signalling molecules is based on the generation of metabolic derivatives that are phosphorylated on the inositol head group. To date, phosphorylation of positions 3, 4 and 5 of the inositol ring have been identified *in vivo* (Fig. 1). These hydroxyl groups can be phosphorylated individually and in combinations. As a consequence, seven PtdInsPs can be generated; three monophosphates [PI3P, PI4P and PI5P], three bisphosphates [PI(4,5)P_2, PI(3,4)P_2 and PI(3,5)P_2] and one trisphosphate PI(3,4,5)P_3 (Fig. 2). These phosphorylations are catalysed by an evolutionarily conserved group of enzymes, the phosphoinositide kinases that have two properties: (a) They are specific with respect to the substrate they utilise. These can include PI or specific mono or bisphosphate derivatives of PI. (b) They show specificity for the position on the inositol ring at which they catalyse the addition of a phosphate group. All of the known lipid kinases that catalyse phosphorylation of the inositol head group in mammalian systems are represented by an orthologue in the *Drosophila* genome (Table 1); in most cases the fly gene has been identified by homology to a previously known mammalian counterpart. Although phosphoinositide kinases are conserved in all eukaryotic genomes from yeast to human, Class I and Class II phosphoinositide 3-kinases as well as type II phosphatidylinositol phosphate kinases are present only in metazoan genomes; consistent with this observation, genes encoding these enzymes are seen in the genome of *Drosophila*. In general, for a given class/type of lipid kinase activity, the *Drosophila* genome encodes one gene compared to several in the case of a typical mammalian genome. There is, however, one exception to this rule; PIP5K activity that generates PI(4,5)P_2 using PI4P as a substrate is encoded in the fly genome by two distinct genes – *sktl* and *CG3682*. Since the *Drosophila* genome is smaller than the human genome, it is possible that alternative splicing of single genes in the fly may generate multiple isoforms that perform the functions of the separate genes encoding a given activity in the mammalian genome. These alternate splice variants may have differential tissue distribution or may have distinct biochemical properties providing the full repertoire of biochemical functions encoded in a mammalian genome.

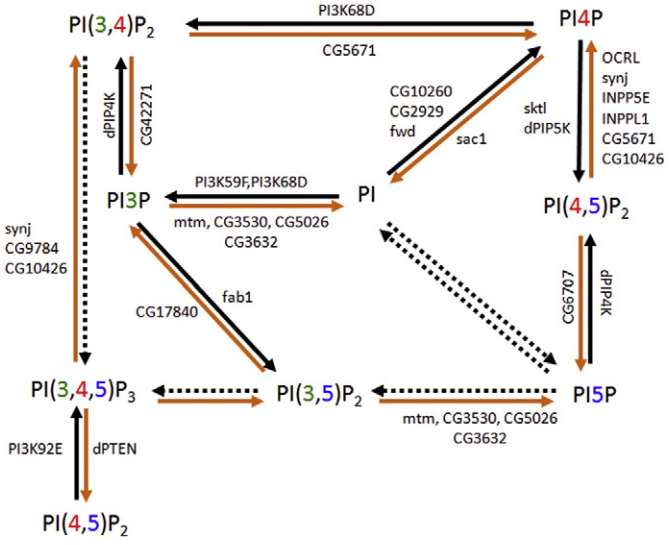


Fig. 2. Schematic representation of the metabolic pathways implicated in the interconversion of phosphoinositides in eukaryotic cells. Kinase reactions are marked by black arrows and phosphatase reactions by orange ones. Metabolic conversions which have not been described or whose enzymatic basis is uncertain are marked using arrows with dotted lines. *Drosophila* genes encoding enzymes that catalyse specific reactions are marked alongside the arrows. Phosphate groups at positions 3,4 and 5 are shown in green, red and blue respectively.

6. Metabolism of phosphoinositides

6.1. Phospholipases

The hydrolysis of PI(4,5)P_2 by receptor activated phospholipase C (PLC) enzymes was amongst the earliest described examples of the requirement for PtdInsPs in cellular signalling. PLC enzymes hydrolyse the phosphodiester bond between the inositol head group and the glycerol backbone of PI(4,5)P_2 to generate inositol-1,4,5-trisphosphate (IP_3) and DAG. In mammalian systems five classes of PLC have been described, PLC β, γ, δ, ε and ζ. While all of these classes of PLC hydrolyse PI(4,5)P_2, the mechanism by which they are activated during cellular signalling varies. While PLCβ is activated by heterotrimeric G-protein subunits following GPCR-ligand binding, PLCγ is recruited via its SH2 domain to activated receptor tyrosine kinases at the plasma membrane. PLCδ is activated by high levels of intracellular Ca^{2+}; PLCζ is activated following fertilization in mammalian embryos but the mechanism of activation remains unclear. The *Drosophila* genome encodes three PLC genes; two highly related to the PLCβ4 subfamily of mammalian enzymes and a single PLCγ (*sl*) (Table 2). Remarkably the single PLCγ does not appear to be essential; null mutants in *sl* are viable as adults although loss of function mutants in murine PLCγ are lethal during embryonic development.

6.2. Phosphoinositide phosphatases

PtdInsPs can be degraded by the action of lipid phosphatases that are enzymes capable of catalysing the dephosphorylation of phosphate groups from position three, four or five of the inositol head group. Based on their specificity for the position of phosphate present on the inositol ring, these enzymes are broadly grouped into three categories – namely 3, 4 and 5 phosphatases (Table 1). Within each of these categories, enzymes are classified again based on the specific substrate on which they act to remove the phosphate from a specific position. Thus there are three broad groups of 5-phosphatases that remove the 5-phosphate from PI(4,5)P_2, PI(3,4,5)P_3 or PI(3,5)P_2 (Table 1). All of these categories of enzymes are represented by orthologues in the *Drosophila* genome with each category of enzyme activity being represented by fewer numbers of genes than in a typical mammalian genome.

7. Phosphoinositide binding proteins

One of the major biochemical properties of PtdInsPs is their ability to bind to and modulate the activity of target proteins; indeed a recent study has suggested that there may be up to four hundred proteins that bind PtdInsPs in human cells [24]. A number of approaches have been used to identify such PtdInsP binding proteins. In many cases the PtdInsP binding properties of a protein have been discovered while studying the functions of that specific protein. However, there have also been biochemical approaches that have been used to discover PtdInsP binding proteins using high-throughput approaches; these have included lipid overlay assays with large numbers of recombinant protein domains [25], screens for proteins from a given tissue that can bind to beads with a specific PtdInsP immobilized on it [26,27] and more recently, a SILAC based quantitative mass spectrometry approach [24]. These approaches have identified two types of PtdInsP binding proteins, those that bind a particular PtdInsP in a highly selective manner (Tables 3, 4, 5) and those that show more degenerate binding specificity (Table 6), being able to bind more than one PtdInsP. Binding to PtdInsP may occur through clearly identifiable domains such as a PH or FYVE domain or it may occur via positively charged amino acids that do not occur in the context of a well-defined protein domain, as in the case of Kir channels.

In general most proteins identified as PtdInsP binders in mammalian cells have clear orthologues in *Drosophila* (Tables 3–6). There are a limited number of examples in which the orthologue of a known PtdInsP binding protein is not represented in the *Drosophila* genome or it is

Table 1
Drosophila phosphatidylinositol kinases and phosphatases.

Name of Enzyme	Reaction catalyzed	Mammalian Genes	*Drosophila* ortholog(s)	Human disease association
Phosphoinositide kinases				
Phosphatidylinositol 4 kinase				
Type II	PI – > PI4P	PI4KIIA	CG2929 [108]	
Type III	PI – > PI4P	PI4KA/PIK4CA	CG10260 [43,109]	Schizophrenia; Bipolar disorder*
		PIK4CB	CG7004/*fwd* [110,111]	
Phosphatidylinositol phosphate kinase				
Type I	PI4P – > PI(4,5)P_2	PIP5K1A	CG9985/*sktl* [39,112]	
			CG3682/*PIP5K59B*	
			/*dPIP5K*	
		PIP5K1B		
		PIP5K1C		Lethal contracture syndrome Type 3#
Type II	PI5P – > PI(4,5)P_2	PIP4KIIA	CG17471/*dPIP4K* [81]	Schizophrenia; Bipolar disorder*
		PIP4KIIB		
		PIP4KIIC		
Type III	PI3P – > PI(3,5)P_2	PIP5K3	CG6355/*fab1* [104]	Francois-Neetens Mouchetée Fleck Corneal Dystrophy#
Phosphatidylinositol 3 kinase				
Class IA	PI(4,5)P_2 – > PI(3,4,5)P_3	PIK3CA	CG4141/PI3K92E [113,114]	Somatic activating mutations in tumors#
Class IB				
		PIK3CB		
		PIK3CD		
		PIK3CG		
Class II	PI(4,5)P_2 – > PI(3,4,5)P_3	PIK3C2	CG11621/PI3K68D [113,115]	Schizophrenia; Bipolar disorder*
		PIK3C2		
		PIK3C2		
Class III	PI – > PI3P	PIK3C3	CG5373/PIK359F/*vps34* [116]	
Adaptor subunits	-	p85, p85, p55, p55, p101, p84, p150	CG2699/PI3K21B [117,118]	
Phosphoinositide phosphatases				
3 phosphatase	PI3P – > PI	MTM, MTMR1, MTMR2	CG9115/*mtm* [106]	Myotubular myopathy#;
	PI(3,5)P_2 – > PI5P			Type 4B Charcot- Marie Tooth disease#
		MTMR3, MTMR4	CG3632	
		MTMR6, MTMR7	CG3530	
		MTMR8/9	CG5026	
	PI(3,4,5)P_3 – > PI(4,5)P_2	PTEN, TPTE2	CG5671/*dPTEN* [71,105]	Mutated in many tumors#; Cowden's disease#
4 phosphatase	PI(4,5)P_2 – > PI5P	TMEM55A, TMEM55B	CG6707	
	PI(3,4)P_2 – > PI3P	INPP4A, INPP4B	CG42271	
	PI4P – > PI	SAC1ML	CG9128/ *sac1* [119]	
5 phosphatase	PI(3,5)P_2 – > PI3P	FIG4	CG17840	Amyotrophic lateral sclerosis 11#, Charcot-Marie-Tooth disease, type 4 J#, Yunis-Varon syndrome#
	PI(4,5)P_2 – > PI4P	OCRL	CG3573/*ocrl* [120]	Lowe's syndrome
	PI(4,5)P_2 – > PI4P	SYNJ1, SYNJ2, INPP5B,	CG6562/*synj* [93]	Early onset Parkinson's disease 20#, Schizophrenia; Bipolar disorder*
	PI(3,4,5)P_3 – > PI(3,4)P_2	INPP5D (SHIP1)		
	PI(3,4,5)P_3 – > PI(3,4)P_2	INPP5K (SKIP)	CG9784	
	PI(3,4,5)P_3 – > PI(3,4)P_2	INPPL1 (SHIP2)	CG10426	Type 2 Diabetes*, Hypertension*, Opsismodysplasia#
	PI(3,4,5)P_3 – > PI(3,4)P_2	INPP5F (SAC2)	CG7956	
	PI(3,4,5)P_3 – > PI(3,4)P_2	INPP5E		

*Polymorphisms/Association studies; # Monogenic disorders.
Two independent approaches were used to identify the *Drosophila* orthologues of known phosphoinositide binding proteins (1) Mammalian phosphoinositide binding proteins were identified through literature survey, selecting those examples where phosphoinositide binding has been experimentally demonstrated. Each mammalian protein sequence was queried against the *D. melanogaster* protein database using BLASTp software (http://blast.ncbi.nlm.nih.gov) (default parameters unchanged) (2) Each protein was also subjected to bioinformatic analysis using the Consurf server (http://consurf.tau.ac.il) that identifies orthologues for a given mammalian protein from multiple species represented in UNIPROT. An MSA thus generated validates the conservation of amino acid resides across the entire length of the protein. A true orthologue is defined as one with minimal percentage identity set to 35%, using the MAFFT-L-INS-i alignment algorithm.
Drosophila genes represented in this review as orthologues satisfy both of the above criteria.

present without the PtdInsP binding domain itself being conserved. Proteins that bind PtdInsP are largely conserved and in some cases, experimental analysis has shown that their regulation by PtdInsP occurs in an equivalent manner [28–30].

8. Biological functions of PtdInsPs in *Drosophila*

8.1. Transduction of signals by GPCRs

Historically, the oldest known function of PtdInsPs in cellular signalling is the role of PI(4,5)P_2 as a substrate for PLC, generating the second messengers IP_3 and DAG. However, more recently it has become clear that many PtdInsPs, including PI(4,5)P_2, are able to bind proteins in cells, regulate their activity and hence influence ongoing cellular events. Proteins whose activity is influenced by PtdInsPs include those that regulate cell membrane functions (e.g. channels and transporters), vesicular transport and cytoskeletal function. In *Drosophila* these principles are broadly conserved. In the fly, hydrolysis of PI(4,5)P_2 by PLC generates IP_3 that releases Ca^{2+} from intracellular stores through an IP_3 receptor [31] and activation of protein kinase C by DAG. To date, most work on the regulation of GPCR signalling via PtdInsPs in *Drosophila* has been performed on adult *Drosophila* photoreceptors, which have been an

Table 2
Drosophila phospholipases and phosphatidylinositol transfer proteins.

Name of Molecule	Mammalian Genes	*Drosophila* ortholog	Disease association
Phosphatidylinositol synthase		CG9245/*dPIS*	
Phosphoinositide specific Phospholipase C			
Phospholipase Cβ	PLCB1, PLCB2, PLCB3, PLCB4	CG4574/*Plc21C* [121] CG3620/*norpA* [122]	Auriculocondylar syndrome 2[@]
Phospholipase Cγ	PLCG1, PLCG2	CG4200/*sl* [123]	
Phospholipase Cδ	PLCD1, PLCD2, PLCD3, PLCD4	-	Hereditary Leukonychia[@]
Phospholipase Cζ	PLCZ1	-	
Phospholipase Cε	PLCE1	-	
Phosphatidylinositol transfer protein			
Class I			
PITPα	PITPNA	CG5269/*vib*/*gio* [21,22]	
PITPβ	PITPNB		
Class IIA			
RdgBαI (Nir2)	PITPNM1	CG11111/*rdgB* [124]	
RdgBαII (Nir3)	PITPNM2		
RdgBαIII (Nir1)	PITPNM3		
Class IIB			
RdgBβ	PITPNC1	CG17818	

@ Monogeneic disorders.
Two independent approaches were used to identify the *Drosophila* orthologues of known phosphoinositide binding proteins (1) Mammalian phosphoinositide binding proteins were identified through literature survey, selecting those examples where phosphoinositide binding has been experimentally demonstrated. Each mammalian protein sequence was queried against the *D. melanogaster* protein database using BLASTp software (http://blast.ncbi.nlm.nih.gov) (default parameters unchanged) (2) Each protein was also subjected to bioinformatic analysis using the Consurf server (http://consurf.tau.ac.il) that identifies orthologues for a given mammalian protein from multiple species represented in UNIPROT. An MSA thus generated validates the conservation of amino acid resides across the entire length of the protein. A true orthologue is defined as one with minimal percentage identity set to 35%, using the MAFFT-L-INS-i alignment algorithm.
Drosophila genes represented in this review as orthologues satisfy both of the above criteria.

Table 3
Phosphatidylinositol monophosphate binding proteins.

Name of Protein	Mammalian Gene	Binding Domain	*Drosophila* Orthologue
PI3P			
Alfy	WDFY3	FYVE	CG14001/Blue cheese
EEA-1	EEA1	FYVE	CG4030/Rabaptin-5
Endofin	ZFYVE16	FYVE	CG15667/SARA
SARA	ZFYVE9		
Hrs	HGS	FYVE	CG2903/Hrs
IRAS	NISCH	PX	CG11807
MTMR4	MTMR4	PH-GRAM	CG3632
ORP2	OSBPL2	PH	CG3860
Phafin-1	PLEKHF1	FYVE	CG14782/Rush hour [30]
Phafin-2	PLEKHF2		
PIKFyve	PIKFYVE	FYVE	CG6355/Fab1
Rabankyrin-5	ANKFY1	FYVE	CG41099
Rabenosyn-5	ZFYVE20	FYVE	CG8506/Rabenosyn-5
Snx16	SNX16	PX	CG6410/Snx16
Snx17	SNX17	PX	CG5734
Snx27	SNX27	PX	CG32758
Snx3	SNX3	PX	CG6359/Snx3
UVRAG	UVRAG	C2	CG6116/UVRAG
WDFY1	WDFY1	FYVE	CG5168
WIPI-1α*	WIPI-1	FYVE	CG7986/Atg18
WIPI-2	WIPI-2		
NADPH oxidase	NOX1	p^{40} PHOX	CG34399/dNox – Phox domain not conserved
DFCP-1	ZFYVE1	FYVE	No *Drosophila* orthologue
FYCO1	FYCO1	FYVE	No *Drosophila* orthologue
RUFY1	RUFY1	FYVE	No *Drosophila* orthologue
PI4P			
AP-1	AP1B1, AP1M1, AP1M2, AP1S1, AP1S2, AP1S3		CG9113/AP-1γ
CERT	COL4A3BP	GPBP PH	CG7207/CERT
EpsinR	CLINT1	ENTH	CG42250/LqfR
GGA1	GGA1	VHS/GAT	CG3002/Gga
GOLPH3	GOLPH3	GPP34	CG7085/Rotini
MyD88	MYD88	CTE	CG2078/dMyD88
OSBP	OSBP, OSBP2	PH	CG6708/OSBP
Orp4			
FAPP-1	PLEKHA3	PH	No *Drosophila* orthologue
FAPP-2	PLEKHA8	PH	No *Drosophila* orthologue
PI5P			
Dok5	DOK5	PH	CG13398
UHRF1	UHRF1	CTD	No *Drosophila* orthologue

* Also shows weak binding to $PI(3,5)P_2$.
Two independent approaches were used to identify the *Drosophila* orthologues of known phosphoinositide binding proteins (1) Mammalian phosphoinositide binding proteins were identified through literature survey, selecting those examples where phosphoinositide binding has been experimentally demonstrated. Each mammalian protein sequence was queried against the *D.melanogaster* protein database using BLASTp software (http://blast.ncbi.nlm.nih.gov) (default parameters unchanged) (2) Each protein was also subjected to bioinformatic analysis using the Consurf server (http://consurf.tau.ac.il) that identifies orthologues for a given mammalian protein from multiple species represented in UNIPROT. An MSA thus generated validates the conservation of amino acid resides across the entire length of the protein. A true orthologue is defined as one with minimal percentage identity set to 35%, using the MAFFT-L-INS-i alignment algorithm.
Drosophila genes represented in this review as orthologues satisfy both of the above criteria.

influential model for the analysis of G-protein coupled $PI(4,5)P_2$ turnover [32]. In these cells photoisomerisation of the GPCR rhodopsin results in the activation of PLCβ and the hydrolysis of $PI(4,5)P_2$. The sequence of reactions thus triggered that lead to the resynthesis of $PI(4,5)P_2$ have been studied in this system using a combination of approaches. A detailed review of this topic has been recently published [33] and Fig. 3 illustrates the molecular components identified in this pathway and their spatial organization in the *Drosophila* photoreceptor. In addition a recent genetic screen has identified a set of novel GPCRs whose activity is transduced by the Gq/PLC signalling pathway and triggers IP_3 induced calcium release; these receptors appear to be required for the development and function of neurons required to maintain flight in *Drosophila* [107].

8.2. Cell and developmental biology

The evolution of the metazoan body plan brought with it the challenge of patterning cells to form tissues as well as coordinating cell growth across the entire organism. The process of tissue formation is underpinned by several events that include cell adhesion as well as the activity of morphogens that influence the differentiation and collective behaviour of cells [34]. Morphogens are molecules that by their graded distribution modulate gene expression resulting in cell fate assignment and maintenance, thereby affecting development. Metazoan model organisms, particularly *Drosophila,* have been key to unravelling the mechanisms underlying morphogen signalling activity. The signalling capacity of morphogens is dependent on cellular processes such as secretion, directional transport and endocytosis of the morphogen as well as cell surface receptors through which it acts and given the role of PtdInsPs in these basic cellular processes, there is emerging interest in their role in regulating tissue morphogenesis.

8.2.1. Cell polarity

The generation of asymmetry in plasma membrane components, both proteins and lipids, is a prerequisite for the differentiation and function of polarised cells. In the context of metazoans, polarised cells are essential for responding to and transducing developmental cues and triggering tissue morphogenesis. The molecular mechanisms that underpin the development of apical and basolateral membranes with unique compositions are complex and are reviewed elsewhere [35].

The cell biology of polarised cells has been extensively studied in a mammalian cell line – MDCK cells – but also in an organismal context using *Drosophila* and *C. elegans* as genetic models.

Table 4
$PI(4,5)P_2$ binding proteins.

Name of Protein	Mammalian Gene	Binding Domain	*Drosophila* Orthologue
Amphiphysin	AMPH	BAR	CG8604/Amphiphysin
Annexin-11	ANXA11		CG9968/Annexin-B11
Annexin-A2	ANXA2		CG9579/Annexin-B10 (X)
AP2 complex – subunit μ	AP2M1		CG7057/AP-50
AP180	SNAP91	ANTH	CG2520/Like-AP180
Brag2	IQSEC1	PH	CG32434/Schizo
c-Abl	ABL1		CG4032/Abl tyrosine kinase
Capping protein heterodimer (α/β)	CAPZA1, CAPZB		CG10540, CG17158/Capping protein heterodimer (α/β)
CAPS	CAPS	PH	CG33653/CAPS
Clathrin Heavy Chain	CLTC		CG9012/Clathrin Heavy Chain
Clathrin Light Chain A	CLTA		CG6948/Clathrin Light Chain
Cofilin	CFL1		CG4254/Twinstar
Coronin-1A	CORO1A		CG9446/Coro
Dynamin2	DNM2	PH	CG18102/Shibire
Efa6	PSD	PH	CG31158/Efa6
Endophilin A1	SH3GL2	BAR	CG14296/Endophilin A [125]
Epsin1	Epn1	ENTH	CG8532/Liquid facets
Exo70	EXOC7		CG7127/Exo70
Ezrin*	EZR	FERM	*CG10701/Moesin [126]
Moesin*	MSN		
Radixin*	RDX		
FBP-17	FNBP1	F-BAR	CG15015/Cip4
IP3R	ITPR1		CG1063/IP3R
Kir1.1^	KCNJ1		^CG44159/Irk1
Kir2.1^	KCNJ2		
Kir3.1^	KCNJ3		
Kir3.2^	KCNJ6		
Kir6.1^	KCNJ8		
Kir6.2^	KCNJ11		
Kv7.1@	KCNQ1		@CG33135/KCNQ
Kv7.2@	KCNQ2		
Kv7.3@	KCNQ3		
m-Calpain	CAPN2		CG8107/Calpain-B [127]
Metastasis suppressor 1	MTSS1	I-BAR	CG33558/Missing-in-metastasis (MIM) [128]
N-WASP	WASL		CG1520/WASp
NCX	TLX2		CG5685/Calx
NHE1	SLC9A1		CG9256/NHE2
Nox5	NOX5		CG34399/Nox
Plasma gelsolin+	GSN	PH	+CG1106/Gelsolin
Scinderin+	SCIN		
Villin+	VIL1		
PMCA	ATP2B1		CG42314/PMCA
Rabphilin-3A	RPH3A		CG11556/Rabphilin
Septin7	SEPT7		CG8705/Peanut
Son-of-sevenless 1	SOS1	PH	CG7793/Son-of-sevenless
Synaptotagmin 1	SYT1		CG3139/dSyt-1
Synaptotagmin-Like 4 (Granuphilin)	SYTL4		CG44012/Bitesize
Talin	TLN1	FERM	CG6831/Rhea
Tiam1	TIAM1	PH	CG34418/Still Life
TRPM7	TRPM7		CG44240/TRPM
Tubby	TUB	CTD	CG9398/King tubby
Twinfilin	TWF1		CG3172/Twinfilin
Vinculin	VCL		CG3299/Vinculin
α-Actinin	ACTN1	PH	CG4376/α-actinin
α1-Syntrophin	SNTA1	Split PH	CG7152/Syntrophin-like 1
α2-Spectrin	SPTAN1	PH	CG1977/α-Spectrin
β2-Spectrin	SPTBN2	PH	CG5870/β-spectrin [129]
hERG	KCNH2		CG3182/seizure – PIP_2 binding region not conserved
AKAP79	AKAP5		No *Drosophila* orthologue
Disabled-2	DAB2	PTB	No *Drosophila* orthologue
GAP43	GAP43		No *Drosophila* orthologue
NHE3	SLC9A3		No *Drosophila* orthologue
TRPM4	TRPM4		No *Drosophila* orthologue
TRPM8	TRPM8		No *Drosophila* orthologue

Table 5
$PI(3,4,5)P_3$, $PI(3,4)P_2$ and $PI(3,5)P_2$ binding proteins.

Name of the protein	Mammalian gene	Binding Domain	Drosophila orthologue
$PI(3,4,5)P_3$ binders			
P-Rex1	PREX1	PH	-
Cytohesin3/GRP1	CYTH3	PH	CG11628/ Steppke
Cytohesin2/ARNO	CYTH2	PH	CG11628/ Steppke
Cytohesin1/B2-1	CYTH1	PH	CG11628/ Steppke
GAP1m/RASA2	RASA2	PH	-
Pleckstrin homology-like domain family B member 2/LL5B	PHLDB2	PH	-
Unconventional Myosin X	MYO10	PH	-
SYNIP/Syntaxin-binding protein 4	STXBP4	WW	-
ArfGAP with dual PH domains 1/Centaurin1	ADAP1	PH	-
Arf-GAP with dual PH domain-containing protein 2/Centaurin alpha 2	ADAP2	PH	-
Tyrosine-protein kinase BTK[EC = 2.7.10.2]	BTK	PH	-
$PI(3,4)P_2$ binders			
TAPP1/Pleckstrin homology domain-containing family A member 1	PLEKHA1	PH	-
TAPP2/Pleckstrin homology domain-containing family A member 2	PLEKHA2	PH	-
$PI(3,5)P_2$ binders			
Clavesin1	CLVS1	Sec14	-
Raptor/Regulatory-associated protein of mTOR	RPTOR	WD	-
TRPM-L1/Mucolipin-1	MCOLN1		-
TRPML2/Mucolipin2	MCOLN2		
TRPML3/Mucolipin3	MCOLN3		CG8743/ TRPML [130]
TPC2/Two pore calcium channel protein 2	TPCN2		-

Two independent approaches were used to identify the *Drosophila* orthologues of known phosphoinositide binding proteins (1) Mammalian phosphoinositide binding proteins were identified through literature survey, selecting those examples where phosphoinositide binding has been experimentally demonstrated. Each mammalian protein sequence was queried against the *D. melanogaster* protein database using BLASTp software (http://blast.ncbi.nlm.nih.gov) (default parameters unchanged) (2) Each protein was also subjected to bioinformatic analysis using the Consurf server (http://consurf.tau.ac.il) that identifies orthologues for a given mammalian protein from multiple species represented in UNIPROT. An MSA thus generated validates the conservation of amino acid resides across the entire length of the protein. A true orthologue is defined as one with minimal percentage identity set to 35%, using the MAFFT-L-INS-i alignment algorithm.
Drosophila genes represented in this review as orthologues satisfy both of the above criteria.

In the context of this discussion, it has been suggested that the asymmetric distribution of PtdInsPs, i.e., $PI(4,5)P_2$ being enriched at the apical membrane and $PI(3,4,5)P_3$ at the basolateral membrane, is a

Notes to Table 4: *, ^, @ and + denote multiple mammalian proteins with a single Drosophila orthologue. Two independent approaches were used to identify the *Drosophila* orthologues of known phosphoinositide binding proteins (1) Mammalian phosphoinositide binding proteins were identified through literature survey, selecting those examples where phosphoinositide binding has been experimentally demonstrated. Each mammalian protein sequence was queried against the *D.melanogaster* protein database using BLASTp software (http://blast.ncbi.nlm.nih.gov) (default parameters unchanged) (2) Each protein was also subjected to bioinformatic analysis using the Consurf server (http://consurf.tau.ac.il) that identifies orthologues for a given mammalian protein from multiple species represented in UNIPROT. An MSA thus generated validates the conservation of amino acid resides across the entire length of the protein. A true orthologue is defined as one with minimal percentage identity set to 35%, using the MAFFT-L-INS-i alignment algorithm.
Drosophila genes represented in this review as orthologues satisfy both of the above criteria.

Table 6
Phosphoinositide binding proteins with degenerate specificity.

Mammalian Protein	Mammalian gene	Binding domain	Drosophila orthologue
PI3P, PI4P, PI5P			
Vacuolar fusion protein MON1 homolog A/SAND1	MON1A		CG11926
PX-RICS/Rho GTPase-activating protein 32	ARHGAP32	PX	Cd-GAPr/CG10538
p125A(Sec23 interacting protein)	SEC23IP	SAM-DDHD	CG8552
Myotubularin-related protein 3[EC = 3.1.3.48]	MTMR3	PH-GRAM	CG3632
Peroxisome biogenesis factor 1	PEX1	ND	Pex-1/ CG6760
PI3P, PI4P			
Willin	FRMD6	FERM	Expanded/CG4114
PI4P, PI5P			
EpsinR/ Clathrin interactor 1	CLINT1	ENTH	Liquid-facets related/CG42250
PI3P, PI(4,5)P_2			
Numb	NUMB	PTB	Numb/CG3779
PI4P, PI(4,5)P_2			
Shc/ SHC-transforming protein 1	SHC1	PTB	Shc/CG3715
Mint-1, Mint-2*		PTB	X11lb/CG32677
PI3P, PI4P, PI5P, PI(4,5)P_2			
ORP11*/ Oxysterol-binding protein-related protein 11	OSBPL11	PH	CG1513
PI4P, PI5P, PI(3, 5)P_2			
ORP9*/ Oxysterol-binding protein-related protein 9	OSBPL9	PH	CG1513
PI3P, PI4P, PI5P, PI(3, 5)P_2			
TRPC5/ transient receptor potential cation channel, subfamily C, member 5	TRPC5		-
PI(4,5)P_2, PI(3,4,5)P_3			
SeptinH5	SEPT5		Septin4/CG9699
PI3P, PI4P, PI5P, PI(3,5)P_2, PI(3,4,5)P_3			
TRPC1			TRPM/CG44240
PI3P, PI(3,5)P_2			
Sorting nexin 2	SNX2	PX	Snx1/CG2774
sorting nexin 1	SNX1	PX	
PI (3,4) P_2, PI(4, 5)P_2			
Sorting nexin-18*	SNX18	PX	SH3PX1/CG6757
Sorting nexin-5	SNX5	PX	Snx6/ CG8282
PI3P, PI(3,4)P_2, PI(3, 4, 5)P_3			
Kif16B	KIF16B	PX	Klp98A/ CG5658
JFC1/Synaptotagmin like protein	SYTL1	C2A	-
PI(3, 4)P_2, PI(3, 4, 5)P_3			
DAPP1/Dual adaptor for phosphotyrosine and 3-phosphoinositides 1	DAPP1	PH	-
ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3/Centaurin delta 3	ARAP3	PH	-
AKT1/PKB alpha	AKT1	PH	-
AKT2/PKBB	AKT2	PH	AKT1/CG4006 [131]
AKT3/PKBG	AKT3	Ph	-
PDK1/3-phosphoinositide dependent protein kinase 1	PDPK1	PH	PDK1/CG1210
SBF1/SET binding factor 1	SBF1	PH	-
Kindlin-2/fermitin family member 2#	FERMT2	PH domain within FERM	Fermitin1/CG14991 Fermitin2/Cg7729
PI3P, PI4P, PI (3, 4)P_2, PI (3, 5) P_2, PI (4, 5)P_2			
SGIP1/SH3-domain GRB2-like (endophilin) interacting protein 1	SGIP1		CG8176
PI3P, PI4P, PI5P, PI(3,4)P_2, PI(3,5)P_2, PI(4,5)P_2, PI(3,4,5)P_3			
Neurofibromin 2/Merlin	NF2	FERM	Merlin/CG14228 [132]
PI5P, PI(3,4)P_2, PI(4,5)P_2, PI(3,4,5)P_3			
Anilin/Scraps	ANLN	PH	Scraps/CG2092 [133]
PI(3,4)P_2, PI(4,5)P_2, PI(3,5)P_2, PI(3,4,5)P_3			
WISP*/Sorting nexin 9	SNX9		SH3PX1/CG6757
PI(3,4,5)P_3, PI (3,4)P_2, PI(4,5)P_2			
TIAM1/T-lymphoma invasion and metastasis-inducing protein 1	TIAM1	PH	-
ArhGAP9/ Rho GTPase activating protein 9	ARHGAP9	PH	-

Represents entries where multiple mammalian proteins have a single fly orthologue.
Represents mammalian entries having multiple fly orthologue.
Two independent approaches were used to identify the *Drosophila* orthologues of known phosphoinositide binding proteins (1) Mammalian phosphoinositide binding proteins were identified through literature survey, selecting those examples where phosphoinositide binding has been experimentally demonstrated. Each mammalian protein sequence was queried against the *D. melanogaster* protein database using BLASTp software (http://blast.ncbi.nlm.nih.gov) (default parameters unchanged) (2) Each protein was also subjected to bioinformatic analysis using the Consurf server (http://consurf.tau.ac.il) that identifies orthologues for a given mammalian protein from multiple species represented in UNIPROT. An MSA thus generated validates the conservation of amino acid resides across the entire length of the protein. A true orthologue is defined as one with minimal percentage identity set to 35%, using the MAFFT-L-INS-i alignment algorithm.
Drosophila genes represented in this review as orthologues satisfy both of the above criteria.

determinant of polarity [36], a phenomenon that is also reported in *Drosophila* tracheal cells [37]. A role for dPTEN, the 3-phosphatase that degrades PI(3,4,5)P_3 to generate PI(4,5)P_2, has been suggested for the maintenance of the apical membrane in *Drosophila* photoreceptors [38]. A number of studies have also looked at the function of a PI4P 5-kinase – *sktl* – [produces PI(4,5)P_2] in the context of cell polarity. *sktl* is expressed ubiquitously during development and loss of function mutants in this gene cause cell lethality in a number of tissues (Fig. 4D) [39]. Partial loss-of-function alleles in *sktl* or tissue specific overexpression of this gene have been used to analyse its function. Analysis of oogenesis in *sktl* mutants has shown that polarised maternal RNAs (e.g.: *bicoid*, *oskar*) are mislocalised , as is the oocyte nucleus (Fig. 4A) [40]. Reduced *sktl* function also results in a defect in spermatid maturation and elongation of the flagellum (Fig. 4B) [41]. In somatic tissues, overexpression of *sktl* in developing photoreceptors during early pupal metamorphosis results in a block in rhabdomere (apical plasma

membrane domain) biogenesis, an effect that is dependent on the kinase activity of the enzyme (Fig. 4E) [11]. Given the multiple effects of *sktl* in polarised cell development in *Drosophila,* some studies have attempted to understand the biochemical consequence of the loss of *sktl* activity that impacts polarised cell function, i.e., PI4P accumulation or depletion of $PI(4,5)P_2$. One approach has been to ectopically express the bacterial $PI(4,5)P_2$-4 phosphatase *SigD* in *Drosophila* cells; this should result in the depletion of $PI(4,5)P_2$ and generation of PI5P, an approach that might help to distinguish between effects due to loss of $PI(4,5)P_2$ versus accumulation of PI4P, both of which could have occurred in *sktl* loss of function; such studies have suggested a role for the loss of $PI(4,5)P_2$ underlying these phenotypes [41,42]. In these developmental settings, $PI(4,5)P_2$ is thought to exert its effects via $PI(4,5)P_2$ binding proteins, thereby regulating vesicular transport and cytoskeletal function. For example, Sec8, a component of the exocyst machinery, is known to bind $PI(4,5)P_2$ as does the actin binding protein moesin that is activated by $PI(4,5)P_2$ binding. Indeed, a large number of $PI(4,5)P_2$ binding proteins that are known in eukaryotic cells could participate in these processes. A recent study using *Drosophila* oogenesis as a model has shown that disruption of PI4KIIIα also results in defects of oocyte polarity [43]. PI4KIIIα is the enzyme predicted to produce PI4P that will be used by *sktl* as the substrate to generate $PI(4,5)P_2$; given the similarity of phenotypes seen by disruption of PI4KIIIα and *sktl,* it is presently not possible to attribute the defects to functions of PI4P itself or those of $PI(4,5)P_2$.

8.2.2. Morphogen signalling

Conceptually PtdInsP could influence morphogen signalling by a number of distinct mechanisms. In the case of the well studied EGF signalling, it has been reported that $PI(4,5)P_2$ influences the activity of the EGF receptor [44,45] and is also the substrate for PLCγ, a key element of EGFR mediated transduction. Further, a recent study in mammalian cells has suggested that an isoform of PIP5K, the enzyme that generates $PI(4,5)P_2$ may regulate endosome to lysosome trafficking of EGFR [46]. While many of these studies have been informed by analysis in cell culture models, the significance of such regulation *in vivo* is unclear and studies in genetically tractable metazoans such as *Drosophila* may provide an insight into these processes. In *Drosophila,* adult body appendages are formed from sacs of equivalent epithelial cells named imaginal discs that are subject to morphogen mediated patterning to give rise to the adult structures. Although this process has been studied extensively, there have been limited studies on the role of PtdInsPs in this process. Using wing disc patterning as a genetic model, Macdougall *et al.* have suggested that the lipid kinase activity of *Drosophila* Class II PI3K affects vein patterning in the context of EGF signalling as well as Notch signalling pathways [47]; Class II PI3K has also been implicated in the regulation of the actin cytoskeleton, endolysosomal system and cell shape in *Drosophila*. Although it has been suggested that Class II PI3K regulates PI3P levels in *Drosophila*, the biochemical activity relevant to Class II PI3K function *in vivo* remains unresolved. The link between the effects of Class II PI3K at the level of single cells on PI3P levels, its effects on the actin cytoskeleton and the endosomal system and how this translates into tissue patterning is unclear. Analysis of myotubularin (*mtm*) that encodes a 3-phosphatase has revealed a role for this gene in regulating cell shape in haemocytes; loss-of-function mutants in *mtm* result in reduced cell protrusions whereas overexpression of *mtm* results in excessive protrusions in these cells [106]. *mtm* mutants are also reported to have altered levels of PI3P in the endolysosomal compartment and defects in the cortical actin cytoskeleton and endolysosomal compartment. These defects were associated with an enhanced distribution of GFP tagged protein probes for PI3P, suggesting that PI3P is one of the substrates for *mtm*. This study suggests that the activity of Class II PI3K and myotubularin may regulate PI3P dynamics in the endosomal system, thus regulating both vesicular transport and the actin cytoskeleton.

A recent study has suggested a functional relationship between PI4KIIIα, Sac1 and the activity of the Hedgehog (Hh) receptor Smoothened (Smo). The balance of PI4KIIIα and Sac1 function appears to impact Smo activation and consequent activation of the Hh pathway. It is reported that Patched (Ptc), the receptor-inhibitor of morphogen Hh, regulates the levels of PI4P by modulating the activity of PI4KIIIα in the context of development in *Drosophila.* The observations made are based on genetic interactions and the mechanism by which Ptc could achieve such a regulation of PI4KIIIα is yet to be elucidated (8). In summary, while there are strong indications that PtdInsPs can impact morphogen signalling, the detailed mechanisms by which they do so remains to be discovered.

8.2.3. Cell migration

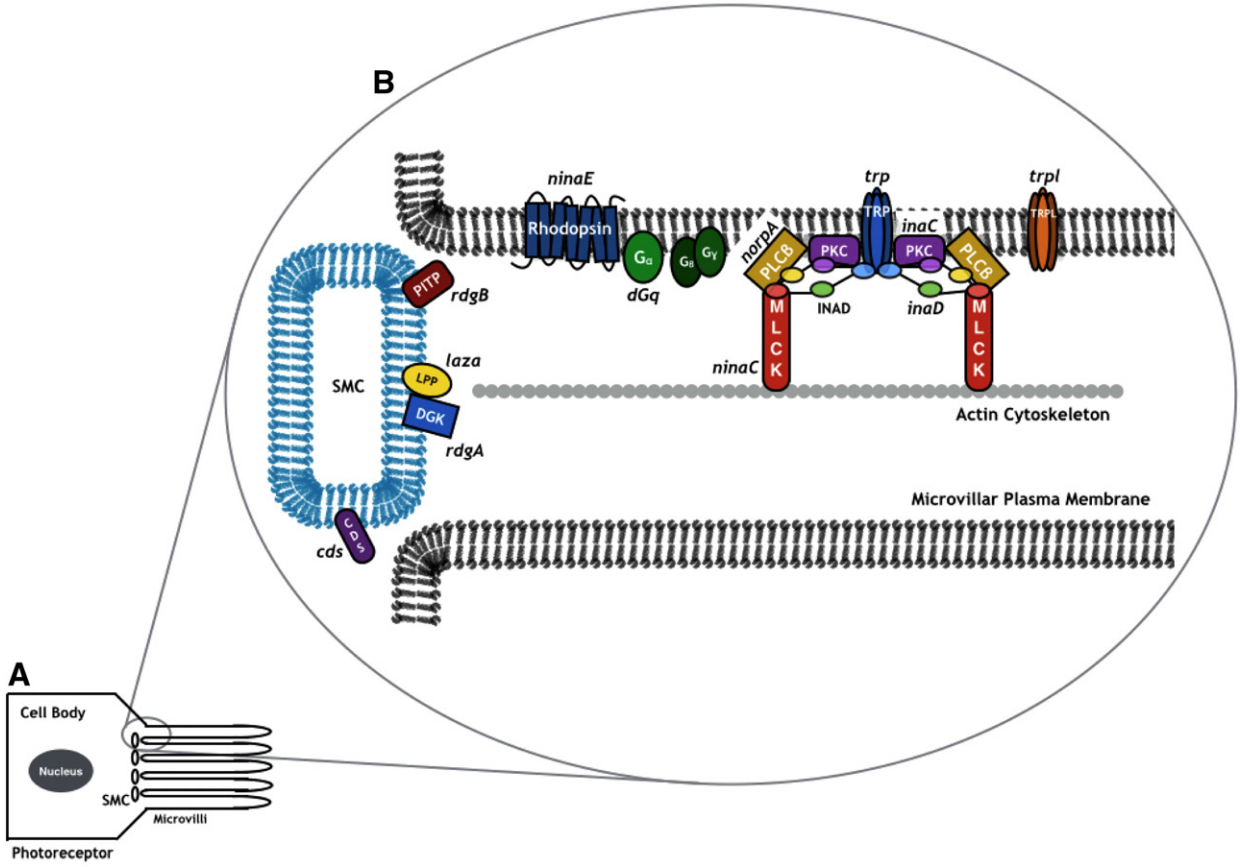
Migration of cells is a universal phenomenon observed during embryonic development in both vertebrates and invertebrates, in wound healing and in actively mobile cell types such as haemocytes. A number of molecular players have been implicated in cell migration including cytoskeleton regulating proteins, small GTPases such as Cdc42 and regulators thereof, Wnt signalling and lipids; the specific lipids that have been implicated in this process are $PI(4,5)P_2$ and $PI(3,4,5)P_3$ (reviewed in [48]). Imaging studies in a number of systems has shown the accumulation of $PI(3,4,5)P_3$ at the leading edge of migrating cells and Class I PI3K as well as PTEN, two key regulators of $PI(3,4,5)P_3$ levels, have been shown to be redistributed in a polarised manner in migrating cells. However, an essential role for this lipid in polarised migration remains to be established as it has been seen that *Dictyostelium* lacking enzymes that generate $PI(3,4,5)P_3$ [49] as well as neutrophils lacking PI3K [50] are able to sense and move along a concentration gradient of chemo-attractant.

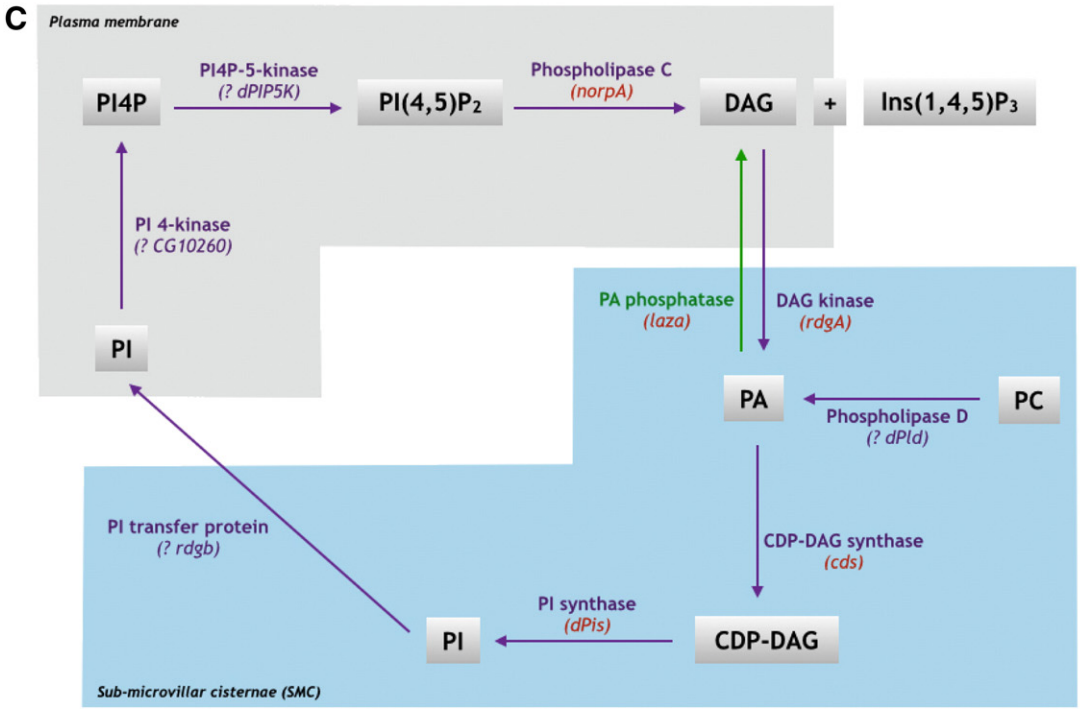
Studies on chemotaxis in *Drosophila* have focussed on studying the migration of cells in the context of intact tissues. The migration of a number of cell types has been studied; most prominently that of haemocytes (reviewed in [51]) , but also that of epithelial cells during wound healing, dorsal closure during embryogenesis and the migration of mesodermal cells. Expression of a dominant negative version of Class I PI3K and the use of a chemical inhibitor for this enzyme suggested a role for Class I PI3K in regulating haemocyte migration to wound sites in embryos [52]. A separate study looked at the accumulation of $PI(3,4,5)P_3$ and localisation of PTEN in the context of dorsal closure and wound healing in *Drosophila* embryos and found that $PI(3,4,5)P_3$ enrichment correlated with actin cytoskeleton remodelling at the apical surface of migrating cells [53]; the polarised accumulation of $PI(3,4,5)P_3$ so observed was due to removal of the $PI(3,4,5)P_3$ phosphatase PTEN from apical junctions. Analysis of the Rho GEF encoded by *pebble* has implicated $PI(4,5)P_2$ in the regulation of cell migration. The migration defect seen in embryonic mesodermal cells from *pebble* mutants could be suppressed by reducing the activity of PIP5K (*sktl* and *dPIP5K*). These enzymes generate most of the $PI(4,5)P_2$ in *Drosophila* and hence this result implies a role of $PI(4,5)P_2$ itself in cell migration. In support of this idea, imaging using fluorescent probes found $PI(4,5)P_2$ rather than $PI(3,4,5)P_3$ to be enriched at the front of the migrating cells [54].

9. Growth and development

The regulation of growth in a metazoan is complex and involves multiple elements including nutrient availability, sensing, assimilation and absorption. Multiple cell types co-operate to facilitate nutrient assimilation into macromolecular biosynthesis. Invertebrate models, particularly insects were among the first to be used to investigate the relation between nutrition during larval development and growth control [55]. Such experiments, initially rooted in physiology, have now branched into those using the myriad genetic tools available in *Drosophila*, leading to an understanding of the biochemical nature of growth regulation [56].

A critical element of growth is the ability to sense nutrients and trigger signalling pathways that facilitate assimilation of these, resulting in the synthesis of biomolecules. A common element of this process that is conserved across all eukaryotes is the Ser/Thr kinase mTOR [57]. In mammalian cells Vps34 activity is required to transduce amino acid stimulation into the TORC1 output, pS6K [58,59]. *vps34* encodes Class III PI3K, implying a role for its product PI3P in this process (reviewed in [60]). mTOR activity is required to mediate amino acid sensing into





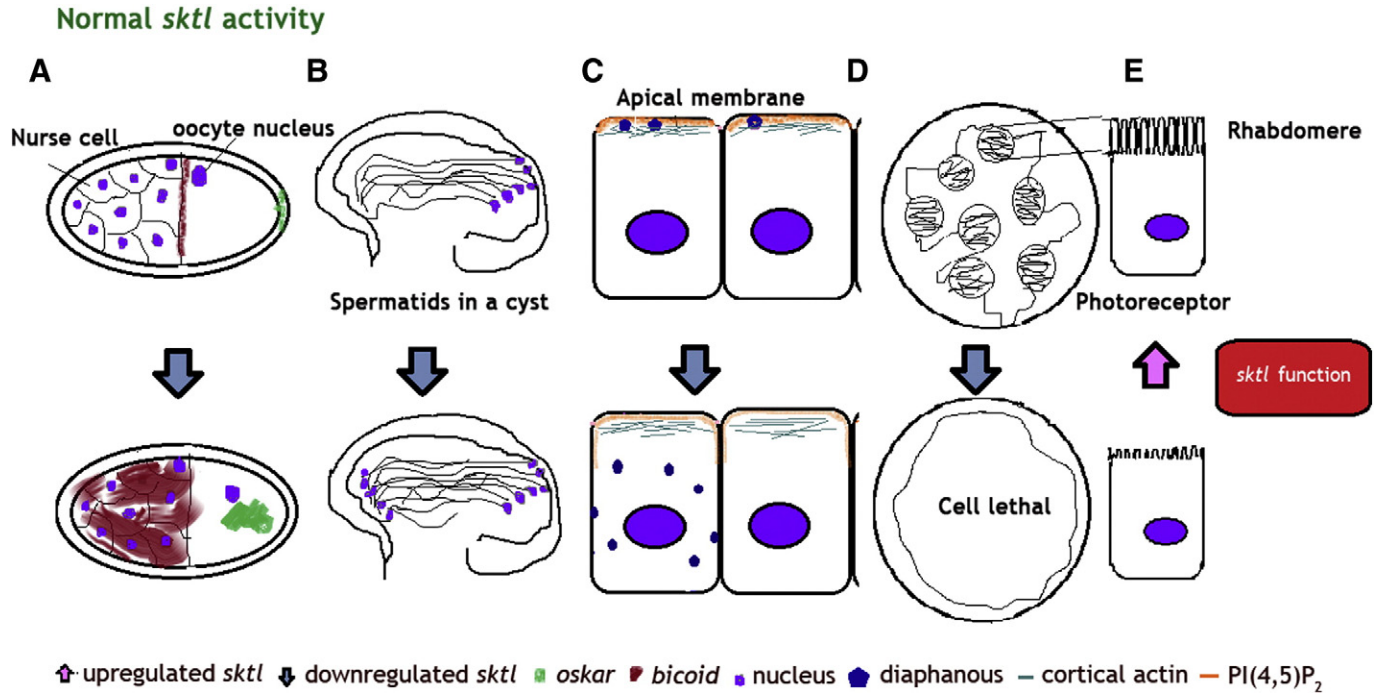


Fig. 4. Effect of *sktl* and PI(4,5)P_2 in regulating *Drosophila* cell polarity. Representative images of four different cell types in *Drosophila* where manipulating *sktl* acitvity or PI(4,5)P_2 levels affect cell polarity in terms of localisation of cellular components or structures. A) Mislocalisation of oocyte nucleus and maternal m-RNAs in stage 8–10 oocyte of *sktl* hypomorph. B) Developing spermatids in a testicular cyst. Spermatid nuclei are mislocalised at both edges of the cyst. C) Polarised epitelium having mislocalisation of apical protein (represented here is diaphanous, an actin binding formin) in case of reduction of apical PI(4,5)P_2. D) Photoreceptors of *Drosophila*, loss of *sktl* results in lethality of these photorecptor cells. Transverse section of the photoreceptor is represented. E) Individual photoreceptor, showing reduction of rhabdomere (apically invaginated plasma membrane) on overexpression of *sktl* during early development of the eye.

growth, a feature that is also conserved in *Drosophila* [61]. However, surprisingly and in contrast to cultured mammalian cells, null mutants in *Drosophila vps34* do not show a defect in TORC1 activation although formation of early autophagosomes and fluid phase endocytosis are affected [62]. The significance of this difference remains unresolved; experiments equivalent to that performed in *Drosophila* are yet to be performed in a mouse knockout of *vps34*.

A second element of nutrient sensing, unique to metazoans, is an endocrine one that involves the communication of glucose levels in the circulation to the individual cells of a metazoan so that they can tune themselves to enhance glucose uptake and modulate macromolecular biosynthesis. In mammals this response is underpinned by the release of insulin by the β-cells of the endocrine pancreas in response to rising levels of blood glucose that occurs following nutrient ingestion. The cells of a metazoan respond to circulating insulin when it binds to the insulin receptor, a receptor tyrosine kinase that is conserved across metazoans. When stimulated by agonists, one of the key outcomes of insulin receptor activation is the recruitment of Class I PI3K to the plasma membrane, where it is catalytically active, phosphorylating PI(4,5)P_2 to generate PI(3,4,5)P_3. The PI(3,4,5)P_3 thus generated binds to and facilitates the activation of two separate kinases, phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B (PKB/AKT). The resulting signalling cascade leads to many downstream effects including glucose uptake as well as a transcriptional program whose end result is cell growth [63].

In *Drosophila*, the insulin receptor is encoded by the *InR* gene [64], loss of function mutants for which are embryonic lethal. However, some heteroallelic combinations survive to adulthood and these mutants are significantly reduced in size when compared to wild-type flies [64,65]. This phenotype is recapitulated by mutants in the fly homologue of insulin receptor substrate IRS (*dIRS/chico*) implying a key role for insulin signalling in growth and development. The effects of this mutation are cell autonomous and the reduction in organism size is achieved through a simultaneous, but not equivalent, reduction in both cell size and cell number. The ligand for InR in *Drosophila* is a set of insulin like peptides (dILPs) that are released principally but not exclusively by a set of neurosecretory cells in the brain in response to nutrients [66].

In mammals, ligand binding to InR triggers phosphorylation of IRS following which both the mitogen activated protein kinase (MAPK) and PI3K pathways are activated. The essential molecular details of Class I PI3K activation and signalling downstream of InR are conserved between the fly and mammalian systems and will not be covered here [67]. Studies on the role of Class I PI3K in growth regulation in the fly have principally been done with a view to understanding growth control in a metazoan context. When the catalytic subunit of the enzyme in *Drosophila* (Dp110) was expressed ectopically in wing and eye imaginal discs (larval tissue that eventually gives rise to adult organs), the resulting adults had enlarged wings and eyes compared to wild-type flies. Conversely, flies expressing catalytically dead Dp110 showed

Fig. 3. A) Representation of *Drosophila* photoreceptor showing the spatial organisation of the rhabdomere microvilli with respect to the sub-microvillar cisternae (SMC) and the cell body. B) Expanded view of a microvillus and associated SMC. Known phototransduction components are shown, along with gene names in italics. G_{α}, G_{β}, G_{γ} are the subunits of heterotrimeric G-protein; PLCβ – phospholipase C β; PKC – protein kinase C; TRP – transient receptor potential channel; TRPL – transient receptor potential like channel; MLCK – myosin light chain kinase; INAD – inactivation no afterpotential D (scaffolding protein); PITP – phosphatidylinositol transfer protein; LPP – lipid phosphate phosphohydrolase; DGK – diacylglycerol kinase; CDS – CDP-DAG synthase. C) Representation of key lipid intermediates of the phototransduction cycle. Enzymes responsible for the reactions are shown along the arrows, with gene names in italics. Purple italics represent putative genes involved; while the involvement of rdgB in the cascade is accepted, the molecular nature of its function remains unknown. The cellular compartments/membranes at which these reactions occur have been represented. PI(4,5)P_2 – phosphatidylinositol-4,5-bisphosphate; DAG – diacylglycerol; Ins(1,4,5)P_3 – inositol-1,4,5-trisphosphate; PA – phosphatidic acid; PC – phosphatidyl choline; CDP-DAG – cytidine diphosphate diacylglycerol; PI – phosphatidylinositol; PI4P – phosphatidylinositol-4-phosphate.

reduced wing and eye sizes when compared to wild-type flies. Mutants for the adaptor/regulatory subunit p60 also showed similar phenotypes, establishing it as a player in the signalling pathway regulating growth control [68–70]. The function of Class I PI3K is antagonized by the action of a 3-phosphatase PTEN, which dephosphorylates $PI(3,4,5)P_3$ to generate $PI(4,5)P_2$. The *Drosophila* orthologue, *dPTEN*, has been shown to be involved in growth control; *dPTEN* mutants exhibit an overgrowth phenotype, which is cell autonomous and results from changes in cell size and proliferation rates [71,105] Overexpression of dPTEN results in reduced organ sizes, reflecting the phenotypes seen in *Dp110* and *chico* mutants. Genetic interaction studies show that *dPTEN* overexpression suppresses PI3K overexpression phenotypes, whereas opposite effect occurs in the background of *chico* mutation. The *dPTEN* mutant overgrowth phenotype also suppresses the reduced growth phenotype of *chico* mutants [71,72]. Thus, in the *in vivo* context, PTEN is a negative regulator of Class I PI3K.

Analysis of further downstream elements of the Class I PI3K signalling pathway has also been performed. Overexpression of dPDK phenocopies the overexpression of Dp110 and the simultaneous overexpression of both proteins results in exaggerated phenotypes. Likewise, overexpression of dAkt results in increased cell size while mutant clones are smaller than the wild-type cells. Co-overexpression of dPDK1 and dAkt results in highly enhanced overgrowth phenotypes, with the interaction between the two proteins confirmed by biochemical experiments showing that dAkt is activated in the presence of dPDK1 [73–75]. Similarly, analysis of *Drosophila* ribosomal protein S6 kinase (dS6K) [76] and d4E-BP [77,78] have demonstrated an essentially conserved pathway between flies and mammals in growth control. In summary, genetic analysis in *Drosophila* has demonstrated the conserved nature of the insulin signalling pathway between the vertebrate and invertebrate systems at a cell autonomous level (Fig. 5).

Class I PI3K signalling also appears to also have a non cell-autonomous role in the regulation of growth in *Drosophila*. Growth control in flies is mediated by the activity of cells from multiple tissues that includes the neurosecretory cells that secrete dILPs, the ring gland that regulates the secretion of ecdysone and juvenile hormone as well as the fat body that appears to generate a non-cell autonomous signal to mediate organismal growth control [79]. Colombani *et al.* found that enhancing Class I PI3K activity specifically in the cells of the ring gland, a key element in this network, resulted in inhibition of organismal growth; conversely, reduction of Class I PI3K activity in the ring gland resulted in an enhancement of body size [80]. This study suggests that Class I PI3K activity in the ring gland acts to regulate ecdysone signalling, thereby impacting developmental time and hence growth. Conversely it is suggested that ecdysone signalling itself regulates the activity of the transcription factor dFOXO – a key element of the insulin signalling cascade. The mechanism underlying the regulation of ecdysone synthesis and release by Class I PI3K signalling remains to be elucidated.

A recent study has also implicated a novel member of PtdInsP signalling in the regulation of *Drosophila* growth. Loss of function mutants in *Drosophila* PIP4K (dPIP4K), the enzyme that catalyses the

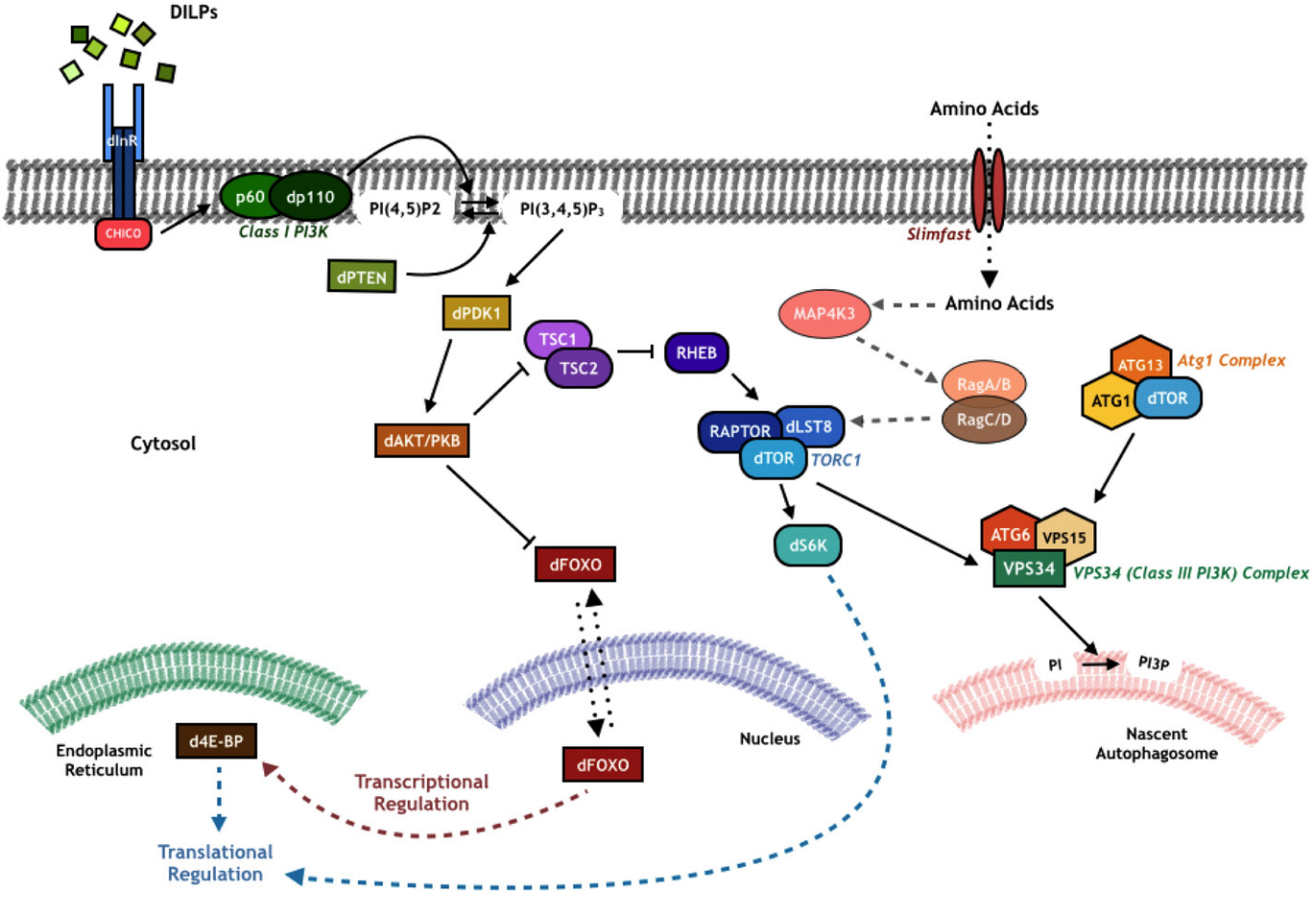


Fig. 5. Representation of the dependence of insulin/insulin-like signalling (IIS), TOR signalling and nutrient-sensing pathways on the different PI3K classes and their lipid products (PIP_3 and PI3P). Dashed grey lines represent interactions whose nature is still unclear. dInR – Insulin receptor; CHICO – insulin receptor substrate; p60/dp110 – subunits of class I PI3K; dPTEN – phosphatase and tension homolog deleted on chromosome ten; dPDK1 – phosphoinositide dependent kinase 1; dAKT – protein kinase B; dFOXO – forkhead box protein; d4E-BP – eIF4E binding protein; TSC – tuberous sclerosis complex; RHEB – Ras homolog enriched in brain; RAPTOR – regularly associated protein of mTOR; dLST8 – lethal with Sec thirteen 8; dTOR – target of rapamycin; dS6K – S6 kinase; MAP4K3 – mitogen activated protein kinase kinase kinase kinase 3; Rag A/B/C/D – Ras-related GTP binding protein; ATG1 – autophagy related protein 1; ATG13 – autophagy related protein 13; ATG6 – autophagy related protein 6; VPS15 – vacuolar protein sorting-associated protein 15; VPS34 – class III PI3K.

phosphorylation of PI5P at D-4 position to form PI(4,5)P_2, results in slower growth and development of larvae and a reduction in cell size [81]. This growth defect is associated with a defect in TOR signalling and could be rescued by the overexpression of the TORC1 regulator Rheb. However, the signalling inputs into TORC1 activity that require dPIP4K for transduction remain to be deciphered. *dPIP4K* mutants show no reduction in PI(4,5)P_2 levels but show elevation in the level of PI5P suggesting that PI5P may be a novel regulator of growth [81]; the mechanism by which it may do so remains unclear.

Another aspect of PtdInsP signalling involves the generation of PI(3,5)P_2 from PI3P through the action of a PI3P 5-kinase, known as *Fab1* in *Drosophila*. Fab1 and its lipid product PI(3,5)P_2 have been implicated in late endosomal dynamics in yeast. [103] Rusten *et al.* have reported that in mutants of *Fab1*, PI(3,5)P_2 was undetectable using GFP tagged Atg8/SVP as a probe for this lipid. In larval garland cells of *Fab1* mutants, it was found that endocytic flux was impaired and cells were populated with enlarged endosomes and multi-vesicular bodies. At a molecular level multiple receptors and ligands (such as Wg and Notch) were seen to accumulate in the endosomal compartment. At the cellular and organismal level, this study reported that multiple larval cells as well as the animal itself were increased in size indicating that this enzyme negatively regulates growth [104]. The mechanism underlying this increased growth remains unknown.

10. Autophagy

Autophagy is an important cellular process which involves both selective and non-selective degradation of cellular components. Autophagy has been implicated in regulating both developmental processes such as tissue sculpting and well as in the evolution of diseased states such as neurodegeneration. The process of autophagy is under strict cellular control and several protein complexes such as the autophagy related gene-1 (Atg1) complex, Vps34 complex, Atg5-Atg12-Atg16 complex and Atg2-Atg9 complex have been implicated in the regulation of autophagy. These complexes are largely conserved across eukaryotes. In *Drosophila*, it is known that Atg1 and Atg13 form part of the Atg1 complex, along with dTOR [98,99]. The Vps34 complex has been shown, so far, to comprise of Vps34, Atg6 and Vps15, a regulator of Vps34 [86,87]. Vps34 is the Class III PI3K that produces PI3P from PI, a process that is essential for the formation of nascent autophagosomes in both yeast and mammalian cells [60] . In mammalian cells, it is known that Vps34 mediates TOR-dependent nutrient signalling pathways and thereby regulates cellular growth. In *Drosophila*, however, it is reported that Vps34 is dispensable for such function as null mutants for the kinase do not show defects in cellular proliferation, nor do they exacerbate the growth phenotypes seen in mutants for the TOR pathway. This apparent difference between an otherwise conserved set of processes between mammalian and *Drosophila* systems remains to be understood. However, TOR-mediated nutrient sensing appears to be important for correct mobilisation of Vps34 to autophagosomes, as seen by the regulatory link between TOR and Atg1 [62].

In summary the insulin/insulin-like signalling (IIS) pathway, nutrient sensing pathway and autophagy are all closely linked and impact growth (Fig. 5).

11. Ageing

The identification of the *C. elegans* mutant *age-1* as one that extends lifespan elicited interest in the role of signalling pathways associated with the regulation of ageing [82]. *age-1* encodes a Class I PI3K [83] and together with the finding that mutants in the *C. elegans* orthologues of INR (*daf-6*) and FOXO (*daf-16*) also impact lifespan in the worm, the idea that the insulin receptor signalling pathway impacts lifespan in metazoans arose. The impact of this pathway in the fly has been studied in *Drosophila* as well, principally using mutants in *dInR*, *chico* and *dFOXO*. Mutations in *chico*/*dIRS* extend lifespan in adult flies in a dose-dependent manner as do heteroallelic, viable mutants of dInR. These studies have been reviewed in [84]. However, the role of the Class I PI3K signalling pathway in the context of ageing in *Drosophila* has not been studied extensively and remains an area for future study.

11.1. Regulation of Neuronal function

PtdInsPs and their turnover have been implicated in a number of aspects of neuronal function. Historically the oldest link between PtdInsP metabolism and neuronal function was the observation that Li^{3+}, a compound that is effective in the treatment of manic depressive psychoses is an inhibitor of inositol 5-phosphatase and inositol monophosphatase, enzymes that are essential for the recycling of IP_3 that is generated following the hydrolysis of PI(4,5)P_2 by receptor activated PLC [100]. This led to the lithium hypothesis which states that the failure to recycle inositol phosphates to PI(4,5)P_2 following PLC activation due to the inhibitory activity of Li^{3+} leads to reduced neuronal excitability. While this model had been tested in mammalian cell culture systems, analysis of mutants in *Drosophila* inositol polyphosphate 1-phosphatase (*ipp*) has provided *in vivo* evidence to support the lithium hypotheses. Mutants in *ipp* show reduced synaptic transmission when measured at the larval neuromuscular junction and the application of Li^{3+} to wild type synapses phenocopies the *ipp* mutant phenotype [97]. Likewise mutants in DAG kinase or PA phosphatase that reduce the level of PA generated following PI(4,5)P_2 hydrolysis in *Drosophila* photoreceptors results in a defect in PI and presumably PI(4,5)P_2 resynthesis [101,102].

The synapse is a fundamental unit of neuronal function and studies in *Drosophila* have tested the role of PtdInsPs in regulating synaptic morphology as well as synaptic vesicle turnover. Mutants in *Drosophila* synaptojanin (*synj*) [PI(4,5)P_2 5-phosphatase] have been isolated and show defects in synaptic transmission in both adult photoreceptors as well as the larval neuromuscular junction [93]. These were accompanied by endocytic defects as measured by FM1-43 uptake as well as depletion of synaptic vesicles as detected by electron micrography. It is reported that *synj* interacts with endophilin and that the overexpression of endophilin partially rescues the phenotypes resulting from loss of *synj* function. The phosphoinositide kinase that generates PI(4,5)P_2 to support the synaptic vesicle cycle in *Drosophila* remains to be identified. Verstreken *et al.* have identified an evolutionarily conserved protein Tweek, which when mutated results in synaptic vesicle recycling defects, altered distribution of PI(4,5)P_2 at synapses as well as mislocalization of PI(4,5)P_2 binding proteins at the synapse [94]. These defects can be rescued by removing one copy of *synj*, indicating that PI(4,5)P_2 levels are important for such processes. PI(4,5)P_2 may execute its function by remodelling the actin cytoskeleton through PI(4,5)P_2 binding proteins like WASP, as shown in Khuong et al. [95].

The role of PtdInsPs in synaptic development and plasticity is a relatively new area. It has been shown that the activity of Class I PI3K has the ability to induce the formation of new synapses even in adult *Drosophila* [88]. Functionally, Class I PI3K activity modulates neuronal excitation through the action of FOXO, as studied in the neuromuscular junctions of *Drosophila* larvae [89]. Studies using genetic epistasis experiments have shown that CaMKII functions upstream of this enzyme, activating it for an mGluR (metabotropic glutamate receptor) mediated response [92]. Such functional modulations might be mediated by PI(3,4,5)P_3 punctae in the pre-synaptic region, which in turn regulates exocytosis by affecting the localisation of SNARE proteins like syntaxin-1A to the active zones [91]. Such regulated release of neurotransmitters alters the post-synaptic anatomy (bouton number and size) of neurons through the controlled clustering of DmGluRs [90]

Although a number of nervous system diseases that involve elements of the phosphoinositide toolkit have been described, there have been relatively limited studies of these using *Drosophila* models. In a *Drosophila* model for amyotrophic lateral sclerosis (ALS), loss of function of dVAP and consequently, SacI (a PI4P 4-phosphatase) results in

increased levels of PI4P. VAPB is known to be a causal agent for ALS in mammals. Forrest *et al.* report a physical association between the *Drosophila* orthologue dVAP and SacI. Downregulation of SacI phenocopies the neuronal defects seen by downregulation of dVAP. Notably, it is reported that reduced levels of either protein result in increased levels of PI4P, a phenomenon that can be rescued by reducing levels of PI4P through the depletion of PI 4-kinase. The phosphatase activity of SacI appears to be dependent on its association with dVAP [96]. These observations need to be studied in greater detail. However, the development of novel *Drosophila* models of neuronal diseases remains an exciting area for future development.

12. Concluding Remarks

Despite enormous progress in understanding the biology of PtdInsPs in eukaryotes, many questions remain as exciting challenges for the future. Although we understand how PtdInsPs regulate basic subcellular processes at the level of individual cells, the manner in which they impact overall cellular output is less clear: for example, how widely does PI4P influence the secretome of a cell or indeed what is the overall impact of PI3P on remodelling the plasma membrane through its regulation of endocytosis. This question will require analysis in an *in vivo* context where genetic analysis in model organisms such as *Drosophila* will likely be influential.

Through their cell-autonomous effects on basic cellular events such as membrane transport, PtdInsPs are likely to impact the generation and function of molecules such as morphogens and endocrine signals that play key roles in tissue organization and homeostasis; these are vital to the organization of the metazoan body plan and function. There has been limited analysis of such non cell-autonomous effects of PtdInsP function and the field is likely to benefit from analysis in *Drosophila* where molecular genetic approaches for uncovering mechanisms in cell and developmental biology processes is particularly well developed. The molecular toolkit used to effect PtdInsP metabolism and function in metazoans seems conserved between the invertebrate (e.g.: *Drosophila*) and mammalian systems, yet rendered in a simplified format from the genomic point of view. This is likely to facilitate genetic analysis of these processes in *Drosophila*.

Although a large number of PtdInsP binding proteins have been identified by biochemical approaches, the analysis of their functions is a relatively new area. Each PtdInsP species in itself appears to have specific functions; the significant number of proteins with non-overlaping PtdInsP binding specificity supports this idea. Additionally, the seven PtdInsPs could collectively generate a code that endows a given cellular membrane with a distinct molecular identity. Unravelling this PtdInsP code, particularly using genetic approaches is likely to benefit from studies in *Drosophila*; the development and application of new genome editing technologies such as TALEN and CRISPR to *Drosophila* research will facilitate rapid progress in this area.

In conclusion, the principles of PtdInsP signalling seem largely conserved between *Drosophila* and other metazoan models. With a fully sequenced, relatively compact genome and sophisticated molecular genetic technology, studies in *Drosophila* will be influential in understanding the organising principles of metazoan biology.

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