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Biological Implications of Inositol 1,4,5-Trisphosphate Signalling from Genetic Studies in Multicellular Organisms

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Components of Inositol 1,4,5-trisphosphate signalling exist in most metazoan cells and have been implicated in several Ca^{2+} -regulated processes. The effect of these processes on the development and behaviour of the whole organism requires further analysis. In this review I have discussed how mutants of the $InsP_3$ receptor, the G-protein Gαq and the two phospholipases PLCβ and PLCγ, exhibit diverse developmental and behavioural phenotypes in three model organisms viz., Drosophila melanogaster, Caenorhabditis elegans and mouse. The diversity of phenotypes observed suggests that individual components of the $InsP_3$ signalling pathway can also function as components of other cellular signalling pathways. Further understanding of how these components modulate and interact with known and unknown signalling pathways requires concerted molecular genetic and biochemical approaches.

Key Words: **Intracellular Ca^{2+}, $InsP_3$ receptor, Gq mutants, Phospholipase C mutants, Larval moulting, Axon guidance**

Introduction

Biological systems perceive and react to the physical world through the process of signal transduction. Molecules involved in biological signalling function to interpret physical signals from outside into a biochemical language that can be understood, at the first level, by individual cells. Where, signals have to be relayed beyond a single cell, as in multicellular animals, more complex signal transduction pathways have evolved. Release of intracellular Ca^{2+} in response to Inositol 1,4,5-trisphosphate ($InsP_3$) generation in the cell, is one such signalling pathway (figure 1). It is well established that stimulation of specific receptors by extracellular signals such as sensory stimuli (e.g. odorants), neurotransmitters, hormones and growth factors can lead to generation of $InsP_3$ in cells (Berridge 1993). *The primary target of $InsP_3$ within the cell is the $InsP_3$ receptor, which is present on the membranes of intracellular Ca^{2+} stores. The $InsP_3$ receptor functions as a ligand gated ion channel that releases Ca^{2+} from intracellular stores* (Ehrlich & Watras 1988, Ferris *et al.* 1989, Maeda *et al.* 1991, Mignery & Sudhof 1990). *$InsP_3$-mediated Ca^{2+} release has been studied extensively in primary cultured cells, isolated cell lines and tissue slices where it is known to regulate secretion in certain exocrine cells and the excitable properties of neuronal cells* (Emptage *et al.* 1999, Emptage 1999, Nakamura *et al.* 1999, Tse & Tse 1999). *However, less well undertood are the effects of these cellular phenomena on the development or behaviour of an organism. For this purpose it is necessary to analyse the physiological consequences of $InsP_3$ mediated Ca^{2+}-release at the organismal level. The object of this review is to discuss how disrupting components of $InsP_3$ signalling by genetic means effects development and behaviour in specific model organisms. Wherever relevant, the correlation of these phenotypes with characterised cellular phenotypes will also be addressed.*

Abbreviations: *$InsP_3$, Inositol 1,4,5-trisphosphate; Gq, alpha subunit of the heterotrimeric G-protein Gαq; PLC, phospholipase C.*

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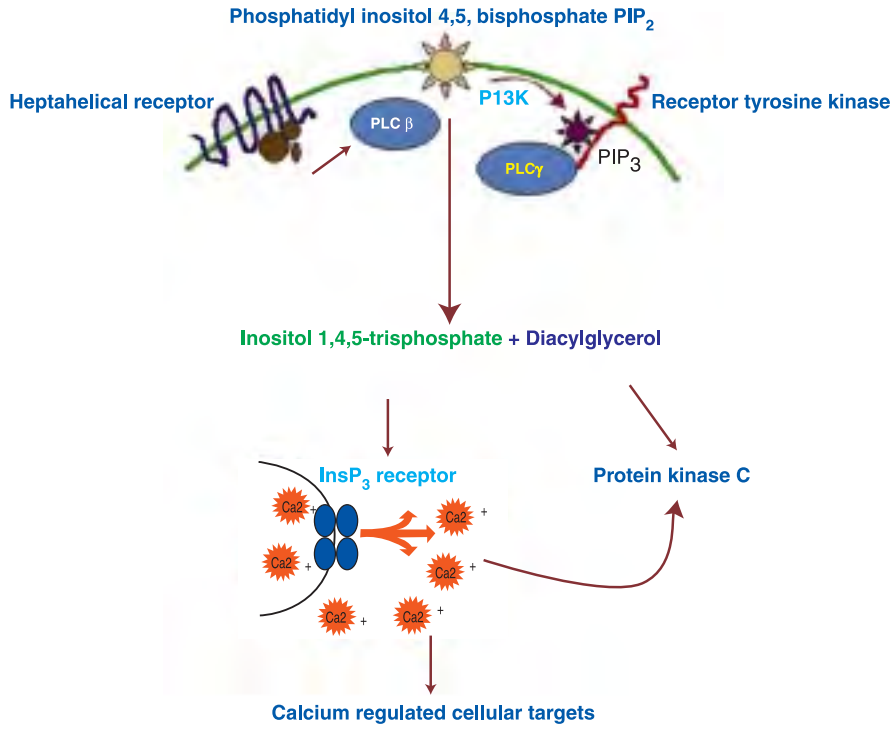

Figure 1 *Known components of $InsP_3$ signalling. An individual cell type may contain either one or both types of receptors shown. PIP_3, phosphatidyl inositol 3,4,5 – trisphosphate; PI3K, Phosphatidyl inositol 3 kinase; InsP3, Inositol 1,4,5-trisphosphate; PLC, phospholipase C.*

The $InsP_3$ Receptor

Drosophila melanogaster

One way to specifically inhibit $InsP_3$ signalling is through disruption of the $InsP_3$ receptor gene or its function. This has been achieved in *Drosophila melanogaster*, *Caenorhabditis elegans*, and mouse where mutants for $InsP_3$ receptor genes have been identified (table 1; Acharya et al. 1997, Clandinin et al. 1998, Dal Santo et al. 1999, Matsumoto et al. 1996, Street et al. 1997, Venkatesh & Hasan 1997). *Drosophila* embryos that are homozygous null for a deficiency of the $InsP_3$ receptor develop and hatch normally as first instar larvae (Acharya et al. 1997, Venkatesh & Hasan 1997). Larval growth and development in *Drosophila* normally proceeds through a series of well-timed moults, followed by a period of metamorphosis, which culminates with the eclosion of an adult fly. In $InsP_3$ receptor deficiency larvae ($itpr^{90B0}$) the moult into second instar larval stage is delayed. Furthermore these larvae die as second instars, with no obvious phenotypic defects. It has been suggested that larval growth is abnormal in *itpr* mutants (Acharya et al. 1997). While *itpr* mutant larvae do appear smaller than their wild-type counterparts, in the weaker alleles this difference is made up with time. Thus while the potential for growth is normal the rate of growth is slow. Weak mutant alleles of the *itpr* gene ($itpr^{1664}$) also exhibit delayed moulting at all larval and pupal transitions. These delays can be rescued by feeding the larvae with the steroid hormone ecdysone, levels of which are known to regulate the timing of larval moulting in insects. $InsP_3$ signalling in *Drosophila* larvae thus regulates the timing of moulting. It appears to do this by either altering the levels of ecdysone directly or the level of genes directly downstream of ecdysone such as the transcription factor E74 (Venkatesh & Hasan 1997). RNA profiles for the E74 gene (levels of which normally follow the pattern of larval ecdysone peaks), are considerably altered in $itpr^{1664}$ organisms. The mechanism by which these molecular changes are brought about and moulting is delayed in *itpr* mutants requires further investigation. From interaction studies between *itpr* mutant alleles and mutants of the cAMP signalling pathway, it is evident that larval

Table 1. *A brief summary of phenotypes associated with mutations of genes that could effect $InsP_3$ signalling (for references see the corresponding section in the text).*

Organism	Gene	Phenotype
Mouse	Gαq	1. ***Defective platelet activation**** (***coupled to the thrombin receptor***)
		2. ***Climbing fibres in the cerebellum do not regress with development***
		3. ***Activated*** Gq ***expression effects heart development***
Mouse	IP_3R ***type*** 1	1. ***Lethal post-natal day*** 23
		2. ***Ataxia and epilepsy***
		3. ***Effects long-term depression in the cerebellum***
Mouse	***PLCß***1	***Epileptic seizures accompanied by sudden death***
Mouse	***PLCß***3	***Chemoattractant and Somatostatin signalling***
Mouse	***PLCß***4	***Motor dis-coordination***
Mouse	***PLCγ***1	***Smaller sized embryos, lethal after embryonic day*** 9
D. melanogaster	Gαq *(dgq)*	1. ***Impaired visual transduction***
		2. ***Gain-of-function transgene effects axon guidance***
D. melanogaster	***PLCß(norpA)***	***Impaired visual transduction***
D. melanogaster	***PLCγ(sl)***	***Wing and eye development***
D. melanogaster	IP_3R *(itpr)*	1. ***Larval or pupal lethal; defect in regulation of larval molting***
		2. ***Adults recover faster from olfactory adaptation***
C. elegans	Gαq *(egl30)*	1. ***Null alleles are lethal; hypomorphs show defects in egg laying and movement***
		2. ***Required for facilitation of synaptic transmission***
C. elegans	***PLCß*** *(egl8)*	1. ***Egg laying defects***
		2. ***Required for facilitation of synaptic transmission***
		3. ***Defecation phenotype***
C. elegans	IP_3R *(itr-1)*	1. ***Gain-of-function allele rescues defects in EGF homologue signalling related to spermathecal dilation;***
		2. ***Hypomorph effects a behavioural rhythm related to defecation***

* ***similar phenotype in human patients showing*** 50% ***reduced*** Gq ***activity***

moulting is under complex control and contains negative feedback loops between ecdysone levels and signalling pathways that regulate these levels (***Venkatesh et al.*** 2001).

Thus, contrary to what might have been expected, given the number of receptor tyrosine kinases and heptahelical receptors that couple to $InsP_3$ signalling in cell lines (***Clapham*** 1995), ***the effect of mutating the $InsP_3$ receptor in*** *Drosophila* ***appears quite conservative during development. It should be noted however that in these studies levels of the $InsP_3$ receptor during embryogenesis are probably unaltered. This is due to the presence of a strong maternal contribution of*** *itpr* ***RNA deposited in the oocyte, because of which homozygous mutant embryos may not exhibit any mutant phenotypes. Nevertheless, in cases where the maternally contributed RNA is derived from mutant*** *itpr* ***alleles, embryonic development is unaffected, since eggs laid from females that carry partially-viable combinations of mutant*** *itpr* ***alleles develop normally. Genetic methods exist by which***

the maternal contribution can be removed completely from embryos and these need to be attempted to understand the role played (if any), of $InsP_3$-mediated Ca^{2+} -release in the embryonic development of *Drosophila*. This is particularily important given the body of evidence from echinoderm and vertebrate eggs where a rise in cytoplasmic Ca^{2+} occurs at fertilisation. Injection of inhibitory agents specific to the $InsP_3$ pathway, such as antibodies against the $InsP_3$ receptor in *Xenopus* (Kume-S et al. 1997) and mouse (Mehlmann et al. 1996, Miyazaki et al. 1992), a dominant negative form of PLCγ in starfish (Carroll et al. 1997) or a dominant negative form of the $InsP_3$ receptor in starfish (Iwasaki et al. 2002), have shown that at least a part of this increase in Ca^{2+} levels is $InsP_3$-mediated. It is thought that the function of this increase in Ca^{2+} is towards egg - activation.

More recently a set of adult viable alleles for the $InsP_3$ receptor have been generated in *Drosophila* and tested for olfactory physiology. Electrophysiological evidence from other organisms suggests that $InsP_3$ and possibly the $InsP_3$ receptor are key players in primary olfactory sensory transduction (Bruch 1996, Cadiou et al. 2000, Kashiwayanagi 1996, Kashiwayanagi et al. 2000, Munger et al. 2000). In *Drosophila itpr* mutants however, the primary olfactory response to several classes of chemicals, as measured by electro-antennograms (EAG), is completely normal. Instead there appears to be a role for $InsP_3$ receptor function in maintenance of adaptation in sensory neurons after exposure to a strong olfactory stimulus. Recovery from adaptation is faster in some mutant heterozygotes and all viable allelic combinations tested (Deshpande et al. 2000). Thus Ca^{2+} release from the $InsP_3$ receptor probably inhibits, directly or indirectly, a component of olfactory transduction in *Drosophila* olfactory neurons.

Caenorhabditis elegans

In *C. elegans* mutants for the $InsP_3$ receptor gene *(itr-1)* were first isolated as suppressors of an ovulation phenotype caused by mutants of the Epidermal Growth Factor gene homolog, *lin-3* (Clandinin et al. 1998). Several of these suppressors appear to be gain-of-function alleles. Genetic and phenotypic analysis of *itr-1* mutants indicates that $InsP_3$ mediated Ca^{2+} release maybe required for spermathecal dilation prior to ovulation. Interestingly, poor fertility is also seen among *itpr* mutant alleles that survive as adults in *Drosophila* (Hasan G unpublished observations). Since oogenesis is normal in these females the defect probably lies in egg-laying. As in *Drosophila*, eggs laid by partially fertile mutant females in *C. elegans*, develop normally, indicating that embryonic development in either organism is not highly sensitive to $InsP_3$ receptor function. However, these studies do not address the effect of stronger mutants, with greater or complete loss-of-function of the $InsP_3$ receptor gene, on embryonic development, in either organism. Studies with expression of dominant negative $InsP_3$ binding fragment during *C. elegans* embryogenesis have suggested a possible role for $InsP_3$ signalling in cytokinesis and gastrulation (Walker et al. 2002). At this stage it is not possible to state conclusively if this signalling is through the $InsP_3$ receptor or another molecule that binds $InsP_3$.

Mutant analysis of another $InsP_3$ receptor mutant allele, (originally referred to as *dec4* and now called *itr-1 [sa73])* in *C. elegans* has defined a role for the $InsP_3$ receptor in establishing a defecation rhythm, the focus of which lies in intestinal cells, and not neurons or muscles as might have been expected (Dal Santo et al. 1999). Thus the contraction of a series of body wall muscles, beginning from the posterior, occurs in response to $InsP_3$ mediated Ca^{2+}-release in intestinal cells. These cells presumably send a rapid signal, the nature of which is still unknown, to the posterior body wall muscles to initiate their contraction. This remarkable observation underscores the importance of $InsP_3$ signalling in co-ordinating the behaviour of a multicellular organism. Since in this organism the $InsP_3$ receptor is expressed in tissues such as the isthmus and terminal bulb of the pharynx, gonadal sheath cells and spermatheca, the authors suggest a generalised role for the $InsP_3$ receptor in fast rhythms, other examples of which are pharyngeal pumping and egg laying (Baylis et al. 1999, Dal Santo et al. 1999, Gower et al. 2001). Pharyngeal pumping rates, in response to food intake, are indeed somewhat lower in the *itr-1 (sa73)* allele. They are also significantly lower when measured from animals expressing a dominant negative form of the $InsP_3$ receptor and animals with RNAi constructs specific for the $InsP_3$ receptor (Walker et al. 2002).

Mouse

Developmental phenotypes of the mouse knockout ***for the*** $InsP_3R$ ***type*** I ***gene have not been analysed in the embryonic stages.*** $InsP_3$ ***receptor type*** 1 ***is the primary neuronal form in mammals.*** Homozygous ***mutant animals mostly die*** *in utero* ***indicating a developmental role for this gene, though a few animals do survive beyond birth*** (***Matsumoto et al.*** 1996). ***These mutant animals exhibit ataxic behaviour and suffer from epileptic fits. Development of the brain and cerebellum,*** where ***maximal expression is observed, appears normal. Since the cerebellum is the "motor co-ordination centre" of the brain it appears likely that signalling through the*** $InsP_3$ ***receptor is required for adult motor learning and co-ordination. This idea is strongly supported by physiological studies on the generation of long-term depression*** (LTD) ***in cerebellar Purkinje neurons obtained from mutant animals.*** LTD ***at the Purkinje cell - Climbing fibre synapse has been proposed as a candidate mechanism for the cellular basis of motor learning and co-ordination. In*** $InsP_3R$ ***type*** 1 knock-***out mice*** LTD ***is not induced in Purkinje cells on appropriate stimulation*** (Inoue ***et al.*** 1998). LTD, ***as well as long-term potentiation*** (LTP), ***also occur in the hippocampal region of the brain*** where ***they are thought to be the underlying cellular basis for learning and memory. Studies on the CA1 and CA3 regions of the hippocampus from*** IP_3R ***type*** 1 ***mutant mice have shown that the*** $InsP_3$ ***receptor functions to reduce levels of*** LTP ***in both regions,*** while ***it is required for induction of*** LTD ***only in the CA3 region*** (***Fujii et al.*** 2000, Itoh ***et al.*** 2001). ***These studies show that the*** $InsP_3$ ***receptor functions to generate the correct level of synaptic plasticity in the nervous system, and interestingly it seems to do this by lowering synaptic activity. In contrast,*** when ***the electrophysiological properties of gastric smooth muscle from*** $InsP_3$ ***receptor type*** 1 knock-***out mice*** were ***studied, they showed a direct dependence on the*** $InsP_3$ ***receptor. Specifically,*** slow ***waves, required for correct initiation of spike potentials, are absent in the mutants. This results in either*** quiescence ***or irregular bursts of spike potentials*** (***Suzuki et al.*** 2000). ***Changes in these physiological properties are likely to have significant biological consequences.***

There also exists a spontaneous mutant allele of the $InsP_3$ ***receptor Type*** 1 ***gene in mouse, referred to as*** *opisthotonos (opt)* . ***In this mutant, levels of the*** $InsP_3$ ***receptor type*** 1 ***are much lower in the cerebellum, and*** 107 ***amino acids are deleted from the coupling domain of the*** $InsP_3$ ***receptor,*** (***Street et al.*** 1997). ***This mutant is viable, but exhibits ataxic behaviour*** with ***age. Single channel properties of the*** $InsP_3$ ***receptor form equivalent to that present in*** *opt* ***mutant animals, has shown that it has a lower conductance and ATP potentiation level, resulting in significantly lower levels of*** Ca^{2+} ***release*** (***Tu et al.*** 2002).

In addition, the mammalian genome codes for two other forms of the $InsP_3$ ***receptor referred to as type***2 ***and type***3 ***which are expressed in a variety of cell types*** (***Taylor et al.*** 1999). ***Mutants for either of these genes are not available. All three types of*** $InsP_3$ ***receptors are expressed in cells of the immune system and several pharmacological studies suggest that*** Ca^{2+} ***release through the*** $InsP_3$ ***receptor has an important role to play in long term activation of both*** B ***and T lymphocytes*** (***Fluckiger et al.*** 1998, ***Jayaraman et al.*** 1995, ***Scharenberg*** & ***Kinet*** 1998).

Other components of $InsP_3$ signalling

$InsP_3$ ***signals in cells are generally thought to be generated by activation of two kinds of receptors*** (***figure*** 1). ***While receptor tyrosine kinases activate phospholipase C-γ, several heptahelical receptors, on ligand activation, switch on the heterotrimeric*** G-***protein subunit,*** Gαq, ***which in turn activates phospholipase C-β. Both phospholipases hydrolyse the membrane lipid phosphatidyl inositol*** 4,5 ***bis-phosphate*** (PIP_2) ***to generate*** $InsP_3$ ***and Diacylglycerol*** (DAG). ***Individual phenotypes observed through mutation of the*** $InsP_3$ ***receptor could thus be a result of signalling through either type of surface receptor. In order to resolve receptor types linked to a specific phenotype of*** $InsP_3$ ***signalling, a comparison of developmental phenotypes observed by mutating*** Gαq ***and*** PLCβ ***on the one hand and*** PLCγ ***on the other, should prove informative.***

Drosophila melanogaster

Gαq and PLCß

In *Drosophila*, ***the*** *Dgq* ***gene encodes a*** Gαq-***like protein. A splice-variant of this gene,*** *Dgqα3*, ***is***

found in *Drosophila* embryos (Ratnaparkhi et al. 2002). By *in situ* localisation of RNA and protein it has been demonstrated that *Dgqα3* is expressed in the embryonic central nervous system (CNS) when post-mitotic neurons begin sending out their axonal projections. In order to test the functional importance of Dgqα3 expression in the developing CNS, a dominant active form of Dgqα3 (*Ac*Gq3) was expressed in post-mitotic neurons. This gain-of-function strategy results in frequent misrouting of axonal projections from the CNS (Ratnaparkhi et al. 2002). This phenotype is modulated by mutations in receptors that are known to affect axonal guidance such as Robo and Frazzled (*Drosophila* homolog of Deleted in Colorectal Carcinoma1 or DCC1). These receptors do not fit into the classical definition of heptahelical class or receptor tyrosine kinases. Since signalling downstream of these axon guidance receptors is not completely understood, molecular interaction of Gαq with these pathways is also unresolved as yet. However, as discussed below, it seems unlikely that the action of Gαq in this process is through activation of PLCß and Ca^{2+}-release from the $InsP_3$ receptor.

Two genes, referred to as *norpA* and *PLCß-21C*, code for PLCß in *Drosophila*. Expression of these genes, by RNA in situ hybridisation in *Drosophila* embryos, demonstrates that while *PLCß-21C* RNA is expressed in the developing CNS, this is not the case for *norpA* (figure 2). Consequently, CNS development was monitored in embryos homozygous for *PLCß-21C* mutant alleles (Weinkove et al. 1999). Surprisingly, these develop as normal embryos with no axon guidance defects, indicating that this gene does not function during CNS development (unpublished observations of S. Banerjee). Thus, an involvement of either PLCß or the $InsP_3$ receptor in axon guidance by *Ac*Gq3 seems unlikely. Possible downstream effector candidates are the Src-family of non-receptor tyrosine kinases, which are known to be activated by Gαq in mammalian cell lines (Ma & Huang 1998).

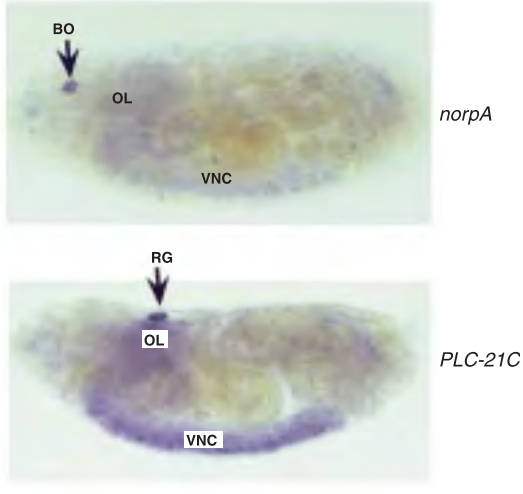

Figure 2 *In situ* RNA expression of the two PLCß genes, *norpA* and *PLC21C*, present in the genome of *Drosophila melanogaster*. Late stage embryos are shown, in which most larval organs, including the nervous system, have formed. *BO*, Bolwigs organ (which is the larval visual sensory organ); *OL*, optic lobes; *VNC*, ventral nerve cord; *RG*, ring gland, a larval neuro-endocrine gland. Embryos are shown dorsal side up and anterior to the left.

Loss-of-function phenotypes of *dgqα3* have not been assessed as yet due to the absence of a mutant allele for this splice variant form. In addition to corroborating the *Ac*Gq3 function, these mutants should prove useful for carrying out interaction studies to help identify other components in Gq signalling during axon guidance. They will also be helpful for studying larval phenotypes of Gαq and comparing these with $InsP_3$ mutant phenotypes. An alternate strategy to study *Dgqα3* loss-of-function phenotypes would be to silence this gene using the newly developed double-stranded RNA technology (Lam & Thummel 2000). In this method a construct expressing dsRNA specific for the gene of interest is cloned under the activity of a tissue-specific inducible promoter such as UAS-GAL4 in *Drosophila*, (Brand et al. 1994). This has the advantage that tissue-specific phenotypes can be analysed in the absence of indirect effects from other regions of the embryo or larva.

A splice variant of the *Dgq* gene, *Dgqα1*, is expressed in the adult eye of *Drosophila*. A mutant allele that disrupts this eye-specific transcript is viable but strongly defective in visual transduction (Scott et al. 1995). Similar defects are seen in adult eyes expressing Activated Gqα1 (Lee et al. 1990), and in mutants for the PLCß gene, *norpA* (McKay et al. 1995). In contrast, adult eyes in which the $InsP_3$ receptor gene has been mutated or completely deleted (through methods of creating tissue mosaics) respond normally to light stimuli (Acharya et al. 1997, Raghu et al. 2000). Thus the light-induced depolarisation of visual receptor cells in *Drosophila* is not induced by $InsP_3$-mediated

Ca^{2+} release. A possible mechanism of Gαq ***and PLCß action might be through depletion of membrane-bound PIP2 levels*** (**Hardie et al.** 2001). ***Protein Kinase C*** (**PKC**), **which is activated by Diacylglycerol** (***figure*** 1), **is also unlikely to be a direct effector since mutants in this gene effect light adaptation but not transduction** (**Smith et al.** 1991). ***These results clearly demonstrate that intracellular Ca^{2+} release from the $InsP_3$ receptor is not required for opening of Transient receptor potential*** (***Trp***) ***channels during*** *Drosophila* ***visual transduction as had been proposed in earlier studies*** (**Hardie & Minke** 1995). ***Thus mammalian cell models of "capacitative-calcium entry" based on the activation of Trp channels in*** *Drosophila*, ***are now being revised*** (**Broad et al.** 2001, **Elliott** 2001, **Minke & Selinger** 1996, **Petersen et al.** 1995).

Receptor Tyrosine Kinases and PLCγ

The other arm of $InsP_3$ signalling, as defined in mammalian cell lines, is through receptor tyrosine kinases and PLCγ (figure 1). ***The*** *Drosophila* ***genome encodes a single PLCγ gene, mutations in which are viable. These mutants have been referred to as*** *small wing (sl)* ***since they exhibit small wings in adults. In addition, they show subtle defects in eye development*** (**Thackeray et al.** 1998). ***Genetic interaction studies indicate that PLCγ phenotypes arise due to over-activity of the Ras/Mitogen Activated Protein Kinase*** (***MAPK***) ***signalling pathways and are thought to be due to lower levels of DAG resulting in lower PKC activity. The phenotypes suggest that there is no direct link with $InsP_3$ signalling. This is further confirmed by looking at the larval development, wing and eye phenotypes of*** *itpr* ***and*** *sl* ***double mutants. These appear to be the same as that seen for individual mutant genotypes indicating that the two genes do not interact*** (***Joshi R D, unpublished observations***), ***and are therefore unlikely to function as part of the same pathway.***

C. elegans

Among other components of $InsP_3$ signalling in *C. elegans*, ***mutations in the gene for*** Gαq *(egl30)* ***and PLCß (egl8) have been obtained.*** *egl30* ***mutants exhibit defects in egg laying, pharyngeal pumping and locomotion with the focus of all these defects apparently in the associated muscles*** (**Brundage et al.** 1996). *egl8* ***mutants also exhibit similar defects in egg laying and locomotion*** (**Lackner et al.** 1999). ***Gαq mutants play a role in neuronal function by facilitation of synaptic transmission. From genetic analysis and interaction studies it seems likely that the downstream effector for these phenotypes is PLCß and atleast in the case of synaptic facilitation, the stimulation of synaptic transmission appears to be through Diacylglycerol*** (***DAG***) ***and not $InsP_3$*** (**Lackner et al.** 1999). ***Thus, a mechanistic link between PLCß and the $InsP_3$ receptor remains to be defined clearly. It is interesting though, that in*** *egl8* ***mutants the posterior body contraction step of the defecation cycle is defective. Further investigation of this phenotype, and its relation to the defecation rhythm phenotype shown by the*** *itr-1(sa73)* ***or the*** *dec4* ***allele, may provide the link between PLCß and the $InsP_3$ receptor.***

Since $InsP_3$ receptor mutants were initially isolated as suppressors of the *lin-3* ***gene, which is a mutant in the epidermal growth factor, it would appear that a part of $InsP_3$ signalling in*** *C. elegans* ***is through PLCγ. The exact mechanism by which the*** *lin-3* ***gene signals to the $InsP_3$ receptor in*** *C. elegans* ***remains to be elucidated.***

Mouse

Gαq and PLCß

Motor dis-coordination defects, seen in mice lacking the $InsP_3$ receptor Type 1 ***gene, are also observed in*** Gαq ***and PLCß4 knock-out mice*** (**Rebecchi & Pentyala** 2000). ***In the*** Gαq ***mutant mice this phenotype has been linked to a failure to cull extra climbing fibres that innervate cerebellar Purkinje cells*** (**Offermanns et al.** 1997). ***Normally, these fibers regress in the third postnatal week of development. An equally rigorous analysis of post-natal cerebellar development needs to be carried out in $InsP_3$ receptor knock-out mice and PLCß4 mutant mice to ascertain if one of the causes of ataxic behaviour observed in these mutants is similar to that seen for*** Gαq ***mutant mice. Interestingly, PLCß4, which is homologous to the*** *norpA* ***gene product, also appears to play a role in the processing of visual information in mouse.*** ***However, unlike*** *Drosophila*, ***it has no direct effect on visual transduction.***

Mutants in genes for other members of the Gq ***subfamily*** (Gα11, Gα14, Gα15 ***and*** Gα16) ***have not been studied so far. All of these*** Gα ***subunits are found to couple to PLCß isozymes, mutants for***

which have been analysed. The distinct phenotypes observed indicate the importance of these enzymes in physiology and development. The absence of PLCß1 leads to sudden death with epileptic seizures, suggesting that the development and/or maintenance of inhibitory brain pathways is altered. In contrast PLCß2 is primarily expressed in cells of the immune system. It has been found to have a role, along with PLCß3, in chemoattractant - induced superoxide production in neutrophils from mice carrying knockouts for one or both genes (Li et al. 2000). Both PLCs also appear to inhibit chemotactic activity in response to certain chemoattractants. As a consequence, PLCß3 mutant mice develop spontaneous multifocal skin ulcers due to hyperinfiltration of leukocytes. The requirement of $InsP_3$ signals in these phenotypes requires investigation. A clear link between PLCβ3 and intracellular Ca^{2+} mobilisation (possibly through $InsP_3$) has been established during the response of smooth muscle cells to somatostatin in PLCß3 knockout mice (Romoser et al. 2001).

In transgenic mice, where activated Gαq is expressed transiently early in heart development, there is an onset of hypertrophy and cardiac myopathy late in development. These phenotypes are observed well after AcGαq expression is undetectable. A brief period of signalling through AcGq thus appears to cause a long term change in regulation of signalling pathways that control heart development (Mende et al. 1998). At least a part of this change in signalling requires activation of calcineurin, which is a Ca^{2+}- calmodulin-dependent protein phosphatase, known to activate the NFAT class of transcription factors (Crabtree 1999). Studies from T cells have shown that calcineurin activation occurs after stimulation of the T cell receptor and PLCγ, suggesting that Ca^{2+} release from the $InsP_3$ receptor is required for calcineurin activation. A prediction from these studies is that $InsP_3$ receptor mutants in mice will have defects in heart development.

Receptor Tyrosine Kinases and PLCγ

Two types of PLCγ genes exist in the mammalian genome. Of these the PLCγ1 product is ubiquitously expressed, while PLCγ2 is more specific to regions of the brain and cells of haematopoietic origin (Rebecchi & Pentyala 2000). When isolated, mammalian PLCγ activity is similar in the tyrosine phosphorylated and non-phosphorylated forms of the enzyme, raising the question as to what causes increase of PLCγ activity *in vivo*. It now appears that the membrane lipid phosphatidylinositol-3,4,5-trisphosphate (PIP_3 - formed by activation of PI3-kinases) is a potent activator of PLCγ (Bae et al. 1998). Thus activation of PLCγ by receptor tyrosine kinases is considerably more complex than suggested earlier. Activation of PLCγ by the PDGF receptor appears to require both tyrosine phosphorylation and PIP_3 formation (Rameh et al. 1998). PLCγ can also be activated by cytokine and Ig receptors that have no intrinsic tyrosine kinase activities. In these cases certain non-receptor tyrosine kinases are recruited for PLCγ activation (Rebecchi & Pentyala 2000).

Transgenic mice in which both copies of the gene for PLCγ1 have been disrupted develop normally till embryonic day 9, and then die. The embryos look normal, but interestingly, are smaller in size (Ji et al. 1997). These data suggest a conditional and stage specific effect on growth, which is essential. Increased level of PLCγ expression in certain tumours supports the idea of it's requirement during growth (Noh et al. 1998, Noh et al. 1994, Park et al. 1994). In *Drosophila*, study of the *sl* mutants has shown that PLCγ functions to down-regulate the Ras signalling pathway (Thackeray et al. 1998). Similar results have been obtained in mammalian cell lines, but here the down-regulatory effect appears to be receptor specific (Rebecchi & Pentyala 2000). Thus, while an interaction between PLCγ and Ras signalling components exists, it is not clear how this interaction leads to the observed effects on growth in mouse and *Drosophila*.

Signalling through *PLC δ and ε*

Mammalian genomes also encode two other types of phospholipase C enzymes referred to as PLCδ (Rebecchi & Pentyala 2000) and PLCε (Kelley et al. 2001, Wing et al. 2001). Of these, homologs of PLCδ exist in both *Drosophila* and *C. elegans*, while a PLCε homolog, first discovered in *C. elegans* (Shibatohge et al. 1998), has to date not been found in the *Drosophila* genome. Mutant phenotypes of these genes have not been characterised so far. Studies with these enzymes in mammalian cell lines

indicate that while PLCδ could be activated by a novel type of G protein referred to as G_h (Kang et al. 2002), PLCε is activated by Gα12, Gβγ subunits and certain small GTPases such as Ras and Rap2B (Evellin et al. 2002, Kelley et al. 2001, Song et al. 2002, Wing et al. 2001). Both types of PLCs can modulate intracellular Ca^{2+} levels in cell lines suggesting that their physiological roles may be of relevance in the context of $InsP_3$ signalling.

Conclusions

Studies on mutants of the $InsP_3$ receptor and other components of $InsP_3$ signalling strongly suggest (and in some cases clearly show) that $InsP_3$ signalling in an identified physiological or developmental context does not necessarily follow the linear pathways shown in figure 1, which were primarily derived from cell line experiments. This complexity is likely to be due to several factors. From an evolutionary perspective, G-proteins and phospholipases evolved before the $InsP_3$ receptor, as evident from the presence of the first two signalling molecules in yeast. A homologous gene for the $InsP_3$ receptor is however, not found in the yeast genome (Goffeau et al. 1996). Thus, not surprisingly, Gαq and Phospholipases are found to have signalling roles independent from $InsP_3$ generation and Ca^{2+}- release. This is exemplified by the finding that mutants of Gαq and PLCß in either C. elegans or Drosophila have phenotypes other than those shown by $InsP_3$ receptor mutants. Some of these phenotypes have been attributed to the formation of Diacylglycerol and subsequent activation of Protein Kinase C. Other phenotypes, such as the effect of AcGq in Drosophila axon guidance, need further analysis for identification of downstream targets.

The converse question, as to whether activation of the $InsP_3$ receptor occurs in the absence of PLC activation, also needs to be addressed. For $InsP_3$ receptor mutant phenotypes obtained in C. elegans and Drosophila, the involvement of PLCγ appears unlikely. Activation of Gq, PLCß and PLCδ need to be studied further before a definitive answer can be obtained. In this context, recent work from vertebrates has shown that the $InsP_3$ receptor can be activated by certain endogenous proteins in the absence of $InsP_3$ (Thrower et al. 2002, Yang et al. 2002). The physiological relevance of this finding is yet to be addressed.

The proposed pleiotropic nature of $InsP_3$ signalling has deterred comprehensive analyses of this pathway's role in vertebrate development, physiology and behaviour. However, with well-defined methods of creating tissue and stage specific gene knockouts available now in mice, it should be possible to address this biologically important issue directly. Such a targeted genetic approach, in combination with siRNA methods (Martinez et al. 2002, Tuschl 2002), and cDNA microarray experiments designed to look at expression of $InsP_3$ regulated genes in specific tissues, should provide better insights into the downstream components of $InsP_3$ signalling.

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