Review

PLC regulation: emerging pictures for molecular mechanisms

Tom D. Bunney and Matilda Katan

The Institute of Cancer Research, Section for Cell and Molecular Biology, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, UK

Phosphoinositide-specific phospholipase C (PLC) enzymes are common signalling components linked to the activation of most cellular receptors. All PLC families are complex, modular, multi-domain proteins and together cover a broad spectrum of regulatory interactions, including direct binding to G protein subunits, small GTPases from Rho and Ras families, receptor and non-receptor tyrosine kinases and lipid components of cellular membranes. Recent structural determinations of PLC components and their complexes with regulatory proteins and direct mechanistic studies, together with earlier work, have provided the foundation to propose molecular mechanisms that stringently regulate PLC activity.

Functional and signalling diversity of PLC enzymes

PLC enzymes found in eukaryotes comprise a related group of proteins that cleave the polar head group from phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) [1,2]. The best documented consequence of this reaction, and a major cell signalling response, is the generation of two second messengers: inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3), a universal calcium-mobilizing second messenger, and diacylglycerol (DAG), an activator of several types of effector proteins including protein kinase C isoforms. These second messengers provide a common link from highly specific receptors for hormones, neurotransmitters, antigens, components of the extracellular matrix and growth factors to downstream, intracellular targets. In this way, they contribute to the regulation of a variety of biological functions as diverse as cell motility, fertilisation and sensory transduction. In addition to its role as a PLC substrate, PtdIns(4,5)P_2 has other functions and changes in the concentration of this and other phosphoinositides affect cellular processes. One important role of different phosphoinositide species is in protein targeting to specific subcellular compartments with particular importance for the control of membrane trafficking and cell movement [3–5].

There are six families of mammalian PLC enzymes (PLCβ, γ, δ, ε, ζ and η) consisting of 13 isoforms in humans (Figure 1). The regulatory interactions of PLCβ and PLCγ isoforms have been characterised more extensively than other families [1,2]. Early work established that regulation of PLCγ isoforms through receptor and non-receptor tyrosine kinases distinguished this family from the regulation of PLCβ isoforms by Gα and Gβγ subunits of heterotrimeric G proteins. However, studies of the regulation of other PLC families, notably of PLCε, revealed a regulatory link that could be unique for this PLC and a complexity and possible interplay between different families and their regulatory networks [6]. These studies suggest that uniquely among PLC families, PLCε is regulated directly by small GTPases from the Ras family; however, PLCε enzyme activity can be stimulated by subunits of heterotrimeric G proteins and small GTPases from the Rho family [7,8]. Based on these findings, it is unlikely that, in different cellular systems, the stimulation of PtdIns(4,5)P_2 hydrolysis through tyrosine kinase-linked receptors would be mediated solely by PLCγ isoforms or that the activation of G protein-coupled receptors (GPCRs) would in turn activate only PLCβ isoforms. Because Ras GTPases can be activated through both receptor types, PLCε might also participate in the PtdIns(4,5)P_2 hydrolysis triggered by GPCRs and tyrosine kinase receptors. Furthermore, some direct upstream signalling components, such as heterotrimeric G protein subunits and Rho GTPases, could be common to isoforms from several families. In particular, recent evidence indicates that Rac GTPases can stimulate PLCβ2 and PLCγ2 isoforms directly, whereas another member of the Rho-family, RhoA, can interact with and stimulate PLCε [8].

Recently, in addition to a more comprehensive understanding of PLC signalling networks, new insights into the regulatory mechanisms of PLC enzymes at a molecular level have been obtained. Interestingly, despite the signalling diversity, some general principles of PLC regulation at the molecular level have been proposed. In this review, we discuss these emerging models, based on structural determinations of PLC components and complexes with regulatory proteins and direct mechanistic studies.

Structural elements of PLC enzymes: adding new pieces to the jigsaw

PLC families share a conserved core architecture containing an N-terminal pleckstrin homology (PH) domain followed by a series of EF-hands, a catalytic TIM barrel and a C-terminal C2 domain (Figure 1a) [9–11]. The catalytic TIM barrel domain is the most conserved domain among the PLC isoforms both structurally and functionally (Box 1). Other domains within the core structure have well conserved general folds; however, their ligand binding properties can vary. For example, the PH domain from PLCδ1 binds PtdIns(4,5)P_2 with high affinity, whereas the PH domain from PLCβ2 mediates protein–protein

Corresponding author: Katan, M. (Matilda.Katan@icr.ac.uk)

 0968-0004/$ – see front matter doi:10.1016/j.tibs.2010.08.003 Trends in Biochemical Sciences, February 2011, Vol. 36, No. 2

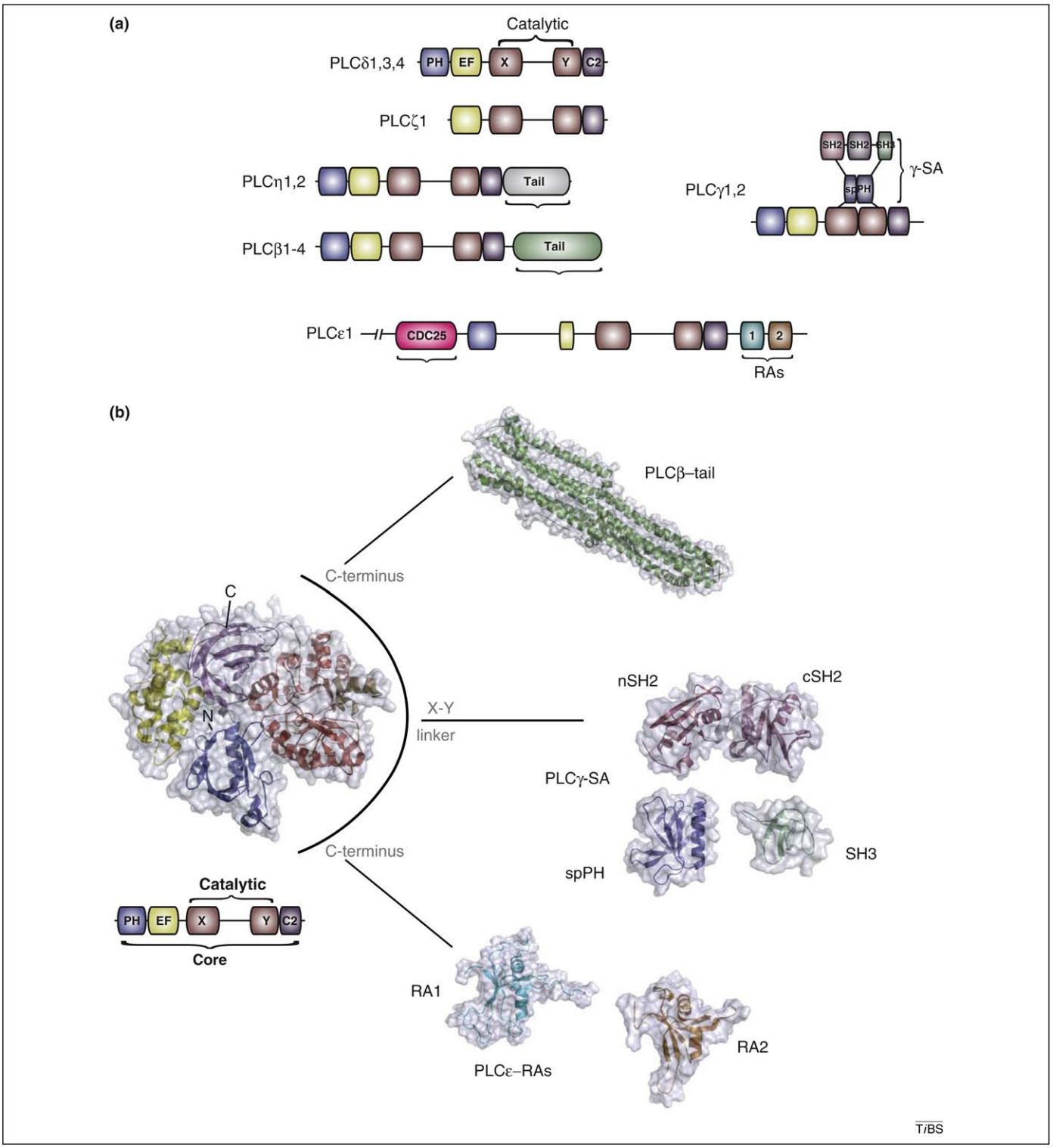

Figure 1. Phosphoinositide-specific PLC families: domain organization and structural insights. (**a**) Domain organization of PLCδ (δ1, δ3 and δ4), PLCζ (ζ1), PLCη (η1, 2), PLCβ (β1-4), PLCγ (γ1, 2) and PLCε (ε1) enzymes highlights their common and unique features. The four domains that comprise PLCδ enzymes (the PH domain; blue), EF-hands (yellow), the catalytic TIM barrel domain (brown) incorporating regions of high sequence similarity X and Y, and the C2 domain (purple) are found (or predicted to be present) in all other PLC families with the exception of PLCζ, which lacks the N-terminal PH domain. The unique regions in PLCη and PLCβ represent a C-terminal extension (tail; green) to the common, four-domain core. In PLCγ, the unique array of domains, the PLCγ-specific array (γSA), is linked to the common core though a linker between the two halves of the catalytic TIM barrel. γSA comprises a ''split'' PH domain (spPH; blue), two SH2 domains (nSH2 and cSH2; shades of purple) and the SH3 domain (green). PLCε is also a complex multi-domain protein with unique domains present at the N-terminus (the CDC25 domain (pink) with another enzymatic, GEF function) and at the C-terminus (two Ras-association or RA domains, RA1 and RA2; teal and brown). Sites of regulatory interactions are either imbedded within the core domains (for example the N-terminal PH domain of PLCβ2 involved in Rac binding) or present within regions specific to PLC families; examples of the latter include the nSH2 domain of PLCγ1 as a site of interaction with growth factor receptors, the spPH domain of PLCγ2 that binds Rac and the RA2 domain from PLCε that binds Ras. (**b**) A number of structures of isolated domains and groups of domains from PLC enzymes have been solved; the PDB ID is given in parentheses. The structure of the PLC core from PLCβ2 (**2FJU**) (left) is similar to the corresponding structure of PLCδ1 (**2ISD**), except that the N-terminal PH domain from PLCδ1 (**2MAI**) is linked flexibly to the remainder of the core. Structures from the regions specific to PLC families include the C-terminal extension from PLCβ (tail region from an avian enzyme) (**1JAD**; top, right). In the case of γSA (middle, right) the structures of the isolated domains from PLCγ1 and PLCγ2 have been reported for the spPH domain (**2FJL**, **2K2J**, **2W2W** and **2W2X**, represented here by **2FJL**), the SH3 domain (**1HSQ**, **1YWO**, **1Y0M** and **2EQI**, represented here by **1Y0M**) and nSH2 and cSH2 domains, either separately (**2DX0**, **2PLD** and **2EOB**) or, as shown here, as a tandem (**3GQI**). The structures of both RA domains from the C-terminal extension of PLCε (bottom, right) have been solved; RA1 (**2BYE**) and RA2 (**2BYF**).

Box 1. Substrate recognition and hydrolysis

Among eukaryotic PLC isoforms, the catalytic domain is the most highly conserved part of the protein. A number of proteins share the same distorted eight-stranded $(\beta\alpha)_8$-barrel (TIM barrel) fold, but the active site topology is unique to PLCs [11]. The N-terminal half (X-box) is the more conserved and contains all catalytic residues. The C-terminal half (Y-box) has an important role in substrate recognition, and particular amino acid residues here account for the reported substrate preference of eukaryotic PLCs (PtdIns(4,5)P_2 > PtdIns4P > PtdIns) [11]. Thus far, PLCδ1 is the only PLC isoform for which a crystal structure is available with Ins(1,4,5)P_3 bound to the active site [9]. This information allows detailed analysis of amino acid residues needed to coordinate the substrate and those important for catalysis. Upon binding, ~50% of the Ins(1,4,5)P_3 becomes buried. The molecule is stereospecifically recognised by polar and charged amino acid side chains that recognize the hydroxyl and phosphate moieties of Ins(1,4,5)P_3. A Ca^{2+} cofactor is coordinated at the active site through predominantly negatively charged amino acids, and through the 2-OH of the Ins(1,4,5)P_3 molecule. This Ca^{2+} plays an essential role in the catalytic mechanism. One notable feature of the active site of PLC is that few of the active site residues move upon substrate binding. This is due to the network of hydrogen bonding and electrostatic interactions that stabilize the positioning of the side chains. This apparent structural rigidity of the PLC catalytic domain has further implications for the type of regulatory mechanisms that can control PLC activity and for the development of pharmacological inhibitors.

The catalytic mechanism has been derived from a combination of structural insights and enzyme kinetic data from wild type and mutant enzymes. The mechanism occurs in two steps: initially, a stable cyclic phosphodiester molecule is formed by phosphotransfer. Subsequently, a phosphohydrolysis event forms the acyclic inositol phosphate (Figure I). An acid and base mechanism is utilised by the enzyme for the hydrolysis of phospholipids. Amino acid residues important for catalysis include His356, His311 and Glu341 (PLCδ1 numbering) [11]. These residues are conserved in all mammalian PLC isoforms.

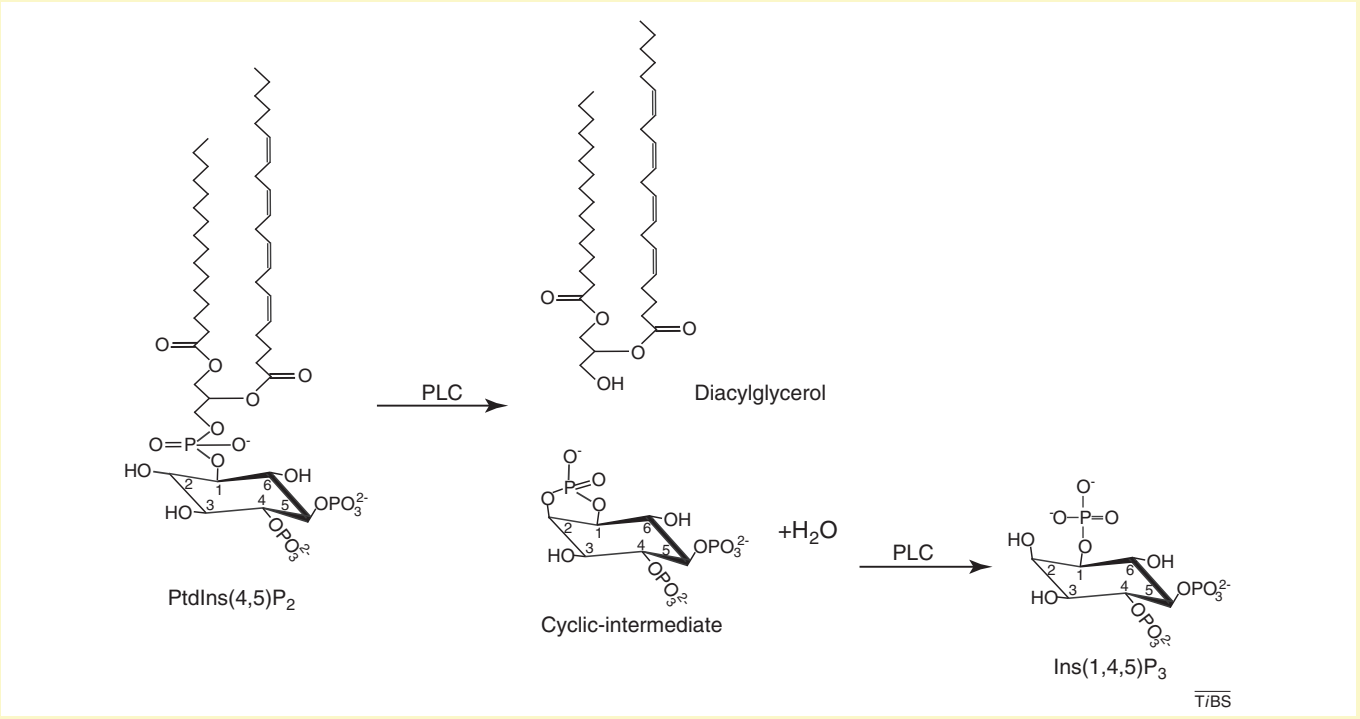

Figure I. Mechanism of substrate hydrolysis.

interactions through binding the GTP-bound form of Rac and Cdc42.

The first structure of the core from PLCδ1, comprising the EF-hands, catalytic domain and C2 domain, revealed that there are extensive inter-domain interactions; the central C2 domain interacts with both EF-hands and the catalytic TIM barrel [9–11]. Importantly, a more recent structure of the PLCβ2 core shows that these inter-domain interactions between the EF-hands, TIM barrel and C2 domain are conserved [12,13]. Furthermore, the structure of the PLCβ2 core also included its PH domain (Figure 1b). Although studies of PLCδ1 suggest that the PH domain is flexibly positioned relative to the other three domains, in PLCβ2 the PH domain is packed tightly between the EF-hands and TIM barrel resulting in a more compact, globular core. Based on these structural insights and domain organization of other PLC enzymes, the EF-hands–C2 domain–TIM barrel unit probably will be structurally highly similar in all PLCs with the N-terminal PH domain attached either loosely or making different interactions with other domains. PLCζ (as well as PLCs from plants) is completely lacking this PH domain.

In the PLCβ, γ, ε, and η families, the common core unit is further elaborated to provide additional, unique modes of regulation. In PLCβ and PLCη, the unique regions are linked to the C-terminus of the core structure. The structure of this C-terminal extension ("tail") from PLCβ has been solved, revealing a novel fold composed almost entirely of three long helices forming a coiled-coil (Figure 1b) [14]. This structure contains elements involved in dimerisation and an electrostatically positive surface initially proposed to be the major site of interaction with Gαq, a known activator of PLCβ enzymes. This, however, needs further experimental support. The C-terminal extension

from PLCη has not been defined either functionally or structurally.

The C-terminal extension in another PLC family, PLCε, contains two ubiquitin-like (Ub) folds (Figure 1b). There are currently many Ub-folds in databases, and only a subset of these domains is likely to form a productive interface with the small GTPase Ras, as shown for several effector proteins where the Ub fold was designated as an RB (Ras binding) or RA (RalGDS/AF-6, Ras-associating) domain [15]. Although initially designated as RA1 and RA2 domains, only the PLCε RA2 domain can bind Ras GTPases owing to overall positive charge and specific residues involved in Ras-binding, both absent from RA1. These two RA domains most likely resulted from DNA duplication, and based on NMR analysis within the tandem pair, behave as loosely tethered, but otherwise independent, domains [16]. PLCε also contains an N-terminal cell division control 25 (CDC25) guanine-nucleotide exchange factor (GEF) domain, predicted to be structurally similar to son of sevenless (SOS), which confers function to this protein as an upstream activator of small GTPases [6]. However, neither the GTPase selectivity nor the physiological significance of this domain has been fully established.

A specific insertion in PLCγ enzymes is located between the two halves (X and Y) of the catalytic TIM barrel (Figure 1b). Whereas relatively short X-Y linkers are probably largely unstructured in other PLC families, the PLCγ insertion (PLCγ-specific array; γSA) is a highly structured region. The γSA includes a second, 'split' PH (spPH) domain. Although this PH domain is split in primary sequence by two Src homology 2 (SH2) domains and an SH3 domain, the most likely 3D organization is that the two parts of the spPH domain form a stable, single domain with a loop incorporating SH domains. Indeed, the NMR and crystal structures of the spPH domains from both PLCγ enzymes show an expected PH domain fold [17–19]. A similar example is provided by the fungal Vps36 (a component of the endosomal sorting complex required for transport (ESCRT)-II machinery) with a typical PH domain fold incorporating a loop insertion with two zinc-finger domains [20]. There are a number of structures of SH2 and SH3 domains from PLCγ enzymes and, very recently, a crystal structure of the nSH2–cSH2 tandem [21]. Within this construct, the two SH2 domains appear not to interact; however, their behaviour within the context of the γSA could be different.

A variety of interactions bring PLCs to the membrane proximity

Together, different PLC enzymes cover a broad spectrum of interactions that contribute to membrane recruitment including binding to inositol-lipids, interaction with small GTPases from Ras and Rho families or heterotrimeric G protein subunits and recognition of specific sites in tyrosine kinase receptors. Structural insights into interactions between PLC binding domains and their targets demonstrated specific features of these interactions and expanded our understanding of general concepts and versatility of interactions at the cellular membrane and its proximity.

Specific and high-affinity binding of the PLCδ1 PH domain to PtdIns(4,5)P_2 provided the first example of a PH domain–phosphoinositide interaction (Figure 2a) [22]. The data resulting from structural determination and functional studies point to the importance of the PtdIns(4,5)P_2 head-group recognition for tethering this enzyme to the plasma membrane, possibly to the regions rich in PtdIns(4,5)P_2, which is also the substrate for the catalytic domain of PLC [11]. The structural requirements for the recognition of polyphosphoinositides by PH domains is now well characterised and as it is the case for the PLCδ1 PH domain, mainly involves amino acid residues in the β1- and β2-strands and the β3-β4-loop [22]. Although N-terminal PH domains from other PLC isoforms might also bind inositol-lipids, firm supporting evidence and structural insights are lacking.

A comprehensive analysis of PLCβ enzymes identified another ligand for the PH domain and another mode of membrane interaction; namely, its role in binding to the membrane-associated small GTPases Rac and Cdc42 in their active, GTP-bound forms (Figure 2b) [8,12]. Furthermore, the PH domain fold within the γSA of PLCγ2 also interacts with Rac (Figure 2c) [18,19]. Interestingly, the Rac binding interface of the PLCγ2 spPH domain resides in β-strand 5 and the α-helix region of the split PH domain. This interface differs notably from the Rac binding surface of the PH domain of PLCβ2, which involves primarily β-strand 1 and loop regions in structural proximity to this strand. These findings extended our understanding of PH domains as versatile interaction surfaces in protein–protein interactions where the surface involved in protein recognition is highly variable and can involve nearly any interface of the PH fold [23]. Importantly, the findings that different structural elements from the PH domain are involved in binding of Rac in PLCγ2 and PLCβ2 further illustrate diversity observed in earlier studies of other effectors, such as the p21-activated kinase (PAK) binding domain (with a Cdc42/Rac interactive binding (CRIB) motif) and Par6 (named for partitioning deficient) binding domain [24]. Based on these studies, it became clear that the interacting domains in different effectors contain different folds that can make important contacts with switch I and switch II regions and with other sites on Rac/Cdc42 GTPases. The PH domains from PLCγ2 and PLCβ2, however, have similar types of interactions that centre only on the switch regions (Figure 2b and c). The strengths of interactions of the two PLC enzymes with Rac are also similar (K_d in the low micromolar range) [18].

The structure of the PLCε RA2 domain–Ras complex illustrates a similar mode in which the common Ub-fold from several well characterised effectors binds membrane-associated Ras GTPases: the binding involves mainly two antiparallel β-sheets of the Ub-fold and the Ras domain, respectively, as well as the first helix of the Ub-domain (Figure 2d) [15]. The interaction surface can include the switch I region from Ras or both, switch I and II, as in the case of the PLCε RA2 domain. However, although the overall structures are similar, the interaction surfaces are distinct, showing significant differences in the specific side chain interactions and electrostatic charge distribution at the interface. Importantly, it is the details of the interaction that determine the binding affinity and kinetic properties. For PLCε RA2, the strength of interaction (K_d

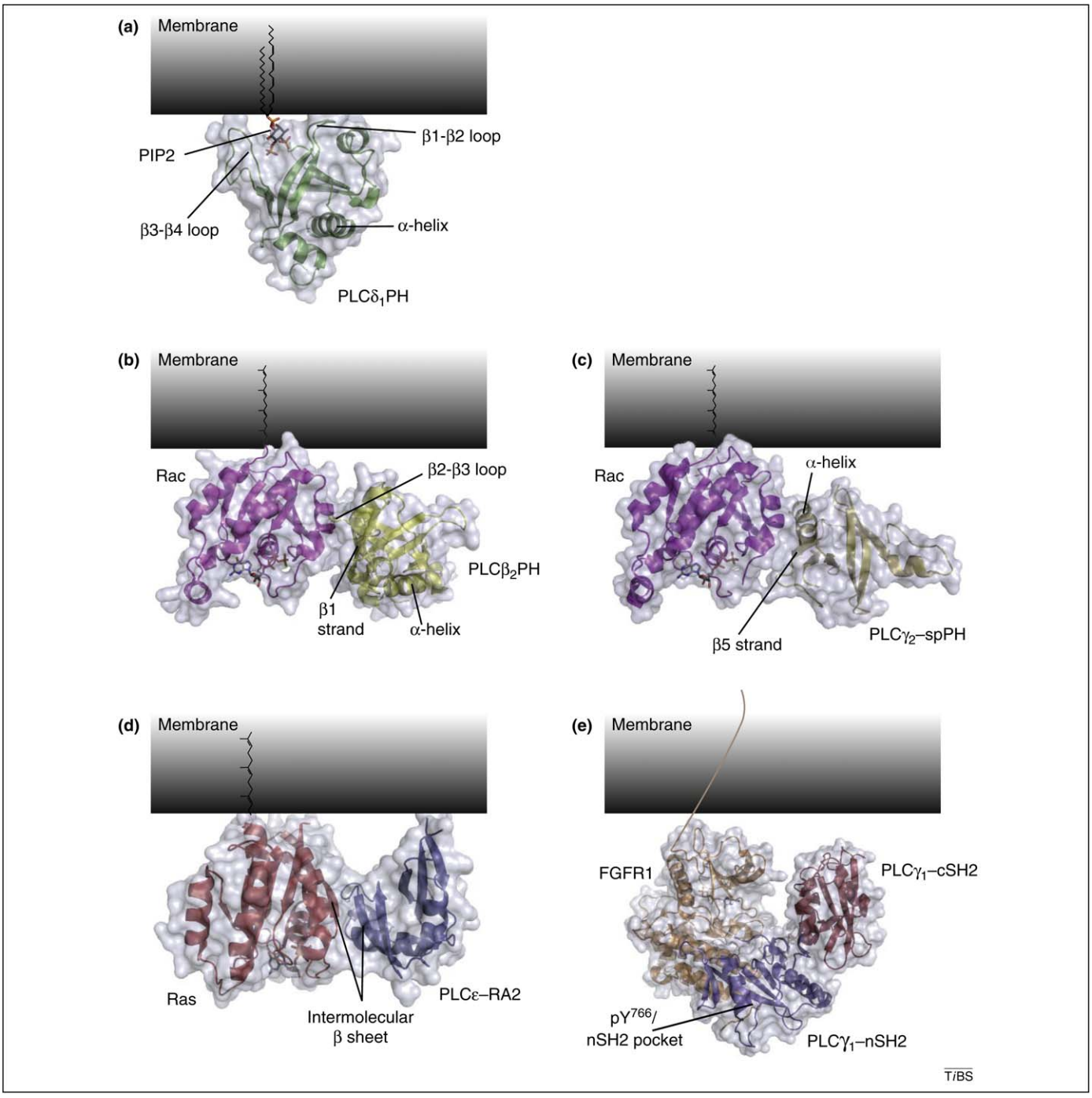

Figure 2. Structural insights into interactions between PLC binding domains and their targets that bring PLCs in proximity to the membrane. Membrane interaction by direct binding of a phosphoinositide head-group has been best documented (both structurally and functionally) for (**a**) the N-terminal PH domain from PLCδ1 (PDB ID **2ISD**) that binds PtdIns(4,5)P_2. Other interactions involving the PH domains that result in bringing PLCs to the membrane are mediated by binding to the membrane-anchored small GTPase Rac; this has been illustrated by structures of complexes of the (**b**) N-terminal PH domain from PLCβ2 with Rac1 (**2FJU**) and (**c**) the PLCγ2 spPH domain with Rac2 (**2W2X**). (**d**) The second RA domain, RA2, from PLCε interacts with Ras (**2C5L**). (**e**) Of the two SH2 domains, nSH2, from PLCγ1 binds to the FGFR1 kinase domain, making interaction with pY766 and an additional site (**3GQI**). Structural elements in the binding domains from PLCs that are involved in binding their targets are indicated. For clarification purposes, in (**a**) and (**b**) the α-helix is indicated to show relative orientations of the PH domains in (**a**), (**b**) and (**c**)

~2.5 μM) and relatively low on- and off-rates are similar to the RalGDS interaction properties; these differ from the much stronger binding by the Ub-fold from Raf (Raf-RBD), which is characterised by a high association rate [15]. It is becoming apparent that both kinetic and dynamic properties of these interactions, in the context of a network topology, have important biological implications for Ras-effector signalling [25,26].

Another fundamental signalling interaction, a selective recognition of phosphotyrosine (pY) signals by SH2 domains [27], also provides a mode of membrane recruitment for PLCγ and other cytosolic proteins that in this way bind to integral or membrane-associated proteins such as receptor tyrosine kinases and protein adapters. The recent crystal structure of a complex of the two PLCγ1 SH2 domains with a fragment from the active fibroblast growth

factor receptor 1 (FGFR1), including the highly conserved C-terminal tail residue Y766, revealed new insights into the molecular basis for recognition and specificity [21]. Interestingly, this structure showed that the nSH2 domain is bound to the pY^{766}LDL sequence within the FGFR1 C-terminal tail, whereas the cSH2 binding site is unoccupied and solvent-exposed (Figure 2e). Furthermore, the nSH2 possesses a secondary binding site located distantly from the nSH2 pY site, interacting with the C-terminal kinase lobe. This interaction covers 533 Å^2, a surface area that is comparable with the area covered by the primary pY interaction site. The presence of this secondary binding site in nSH2 also increases the binding strength significantly; the nSH2 binds 10–40-fold more tightly to the kinase domain of activated FGFR1 than to pY peptides, lowering the dissociation constant to ~30 nM. In contrast to the pY binding sites, residues located in the secondary binding site of nSH2 that mediate interaction with FGFR1 are highly divergent from residues in corresponding positions in cSH2. The presence of the secondary nSH2 binding site explains some previous observations; notably, despite similar strength of binding for pY peptides from FGFR1 and other tyrosine-kinase receptors, only nSH2 appears to be important for the membrane recruitment in response to cell stimulation by some of the growth factors [28–30]. More generally, these studies suggest that the SH2 domain specificity can be mediated by secondary binding sites located outside the phosphotyrosine binding pocket. Indeed, the sequence specificity of the phosphotyrosine binding pockets, determined by directed phosphopeptide library screening, also revealed that a multitude of the sequences can be recognised by several SH2 domains with similar affinity *in vitro* [31]. The observations of additional interaction surfaces open an interesting area for future structural and functional studies that will elucidate whether, and to what extent, the concept of two-site interaction determines the high degree of specificity of other SH2 domain-mediated signalling events *in vivo*.

These diverse modes of membrane recruitment are all tightly regulated events, depending on ligand concentrations (e.g. PtdIns(4,5)P_2), activation of small GTPases and heterotrimeric G proteins and phosphorylation of specific residues by activated tyrosine kinases. In many systems there is also strong experimental evidence that the key residues in the PLC binding domains involved in these interactions are required for the increase in PtdIns(4,5)P_2 hydrolysis seen after cell stimulation [2,29,32]. Based on these findings, the recruitment to the cellular membrane constitutes a crucial step in PLC signalling regulation.

From structures to molecular models of signalling

Despite a large number of studies that relied mainly on the analysis of PLC variants with deletions and point mutations, the advances in understanding molecular mechanisms have been linked closely to an increase in structural information. Additional insights were obtained from studies in the context of lipid membranes and dynamics of protein–membrane interactions (Box 2).

The first mechanistic model of PLC regulation was suggested for the PLC δ1 isoform. This model was based on observations from structural insights and supporting biochemical experimentation (Figure 3b) [9]. According to this model, described as 'tether-and-fix', there are two steps of membrane interactions: initially, the PH domain tethers the enzyme to the membrane by specific binding to PtdIns(4,5)P_2; secondly, interactions in the EF-hands–C2 domain–TIM barrel unit fix the catalytic domain in a productive orientation relative to the membrane. It has been suggested that the PLCδ1 C2 domain could provide this important, second site for membrane binding. Unlike the flexibly linked PH domain, the C2 domain makes an extensive and rigid interface with the catalytic domain, and therefore could orient the catalytic domain in correct apposition to the membrane for catalytic activity. The putative calcium-dependent, ligand binding site in the PLCδ1 C2 domain is on the same side of the enzyme as the active site and the ridge region around its opening, which would become inserted into the membrane in a 'fixed' orientation. Each of the two steps could potentially be regulated. For PLCδ1, a member of the simplest

Box 2. Membrane environment and PLC regulation

It is generally accepted that studies of signalling events that occur in the proximity of the membrane should take into account the influence of the membrane surface environments [42,43]. For PLC enzymes, this need is reinforced by the fact that in addition to regulatory protein–protein interactions in the membrane vicinity, shared with a number of signalling molecules, PLCs recognize and hydrolyse PtdIns(4,5)P_2, a phospholipid that is an integral membrane component. Indeed, PLC enzymes make indirect and multiple direct interactions with the cellular membrane that involve the catalytic, PH and C2 domains in some enzymes. However, placing signalling mechanisms in the context of the cellular membrane remains challenging owing to the fact that membranes are difficult to study *in vitro* and that detailed quantitative information is difficult to obtain *in vivo*. A number of strategies, including more recent methods to image, synthesize and control membranes (*in vitro*, *in vivo* and through computational modelling) have started to generate interesting, new insights.

Conditions that allow analysis of PLC activation by different GTPases and reflect *in vivo* observations have been developed empirically for the study of PLC regulation *in vitro* [44,45]. Further development of this reconstitution assay should facilitate testing of how changes in membrane properties can affect PLC signalling. Furthermore, initial insights into PLC regulation and catalysis in living cells are beginning to emerge. One important aspect of these studies is related to the complex cellular roles of PtdIns(4,5)P_2, including as a precursor of second messengers and as an interacting partner to many intracellular proteins in the regulation of endocytosis, exocytosis, ion channels and cell motility. On the basis of measurements of the diffusion constant of PtdIns(4,5)P_2 in the inner leaflet, it appears that about two-thirds of PtdIns(4,5)P_2 might be reversibly bound to proteins [46]. The use of different fluorescence recovery after photobleaching techniques indicated that protein domains that bind inositol-lipids, such as the PLCδ1-PH domain, retain relatively rapid diffusion in the membrane for the duration of the lipid–protein complex [47]. Similar methodology pointed to different modes of membrane recruitment and diffusion of PLCβ2, depending on its upstream regulator (Gαq, Gβγ or Rac) [35]. Another study of PLCβ in living cells, based on fluorescence resonance energy transfer, successfully determined kinetic parameters of signal transduction from GPCRs, via PLCβ, to ion channels [48,49]. Techniques such as quantum-dot-based optical tracking of single molecules, which recently revealed the dimerization dynamics of individual epidermal growth factor receptors (EGFRs) on living cells [50], could be very important for studies of signalling *in situ*. In addition, single-molecule tracking of PLCγ2 determined the dynamics of transient, short-lived recruitment of PLCγ2 to clusters of CD59, a glycosylphosphatidylinositol-anchored receptor [51].

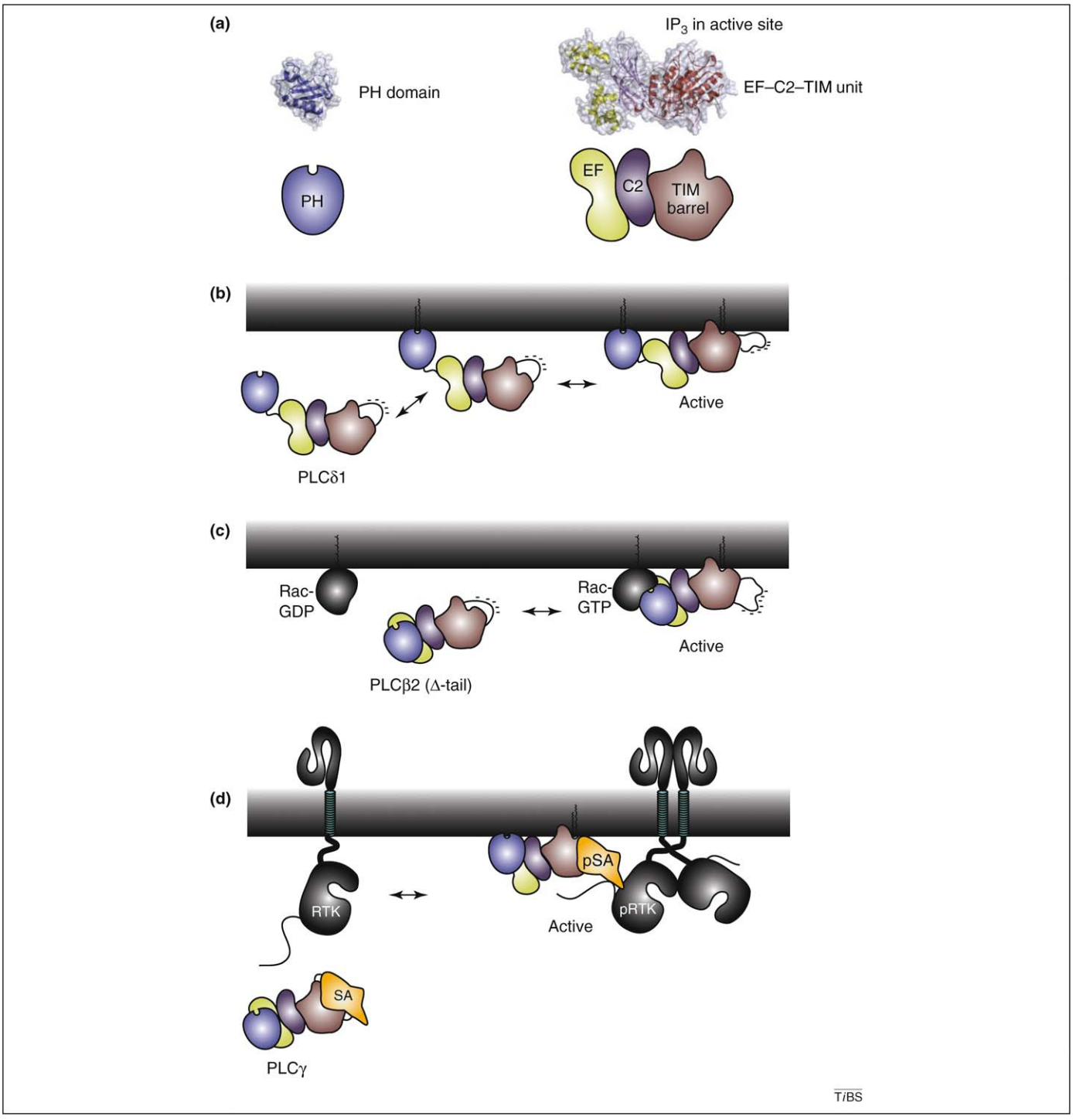

Figure 3. Molecular models for PLC regulation. Determination of structures of PLC domains and groups of domains (Figure 1) and of their complexes with the regulatory molecules (Figure 2) provided the basis for further mechanistic studies. (**a**) Structures comprising the common core can be represented schematically by the PH domain (blue) and the EF-hands–C2 domain–catalytic TIM barrel unit (yellow, purple and brown, respectively). (**b**) The model for regulation of PLCδ1 'tether-and-fix' depicts a two-step membrane interaction mediated by the PH domain binding to PtdIns(4,5)P_2 (tether) and subsequent interactions that bring the EF-hands–C2 domain–catalytic TIM barrel unit to the membrane surface (fix) resulting in higher rate of substrate hydrolysis. (**c**) Another structure-based model, 'fix-and-unmask', has been proposed for the regulation of PLCβ2 by Rac, but could be extended to G protein regulation of PLCβ enzymes and possibly some other PLC families and their activators. According to this model, Rac binding to the PH domain brings the entire enzyme to the membrane proximity in a fixed orientation, resulting in unmasking of the active site by re-positioning the loop known as X-Y linker, rich in negatively charged residues. (**d**) Regulation of PLCγ enzymes by tyrosine kinase receptors probably also involves unmasking of the active site, possibly brought about by conformational changes within γSA resulting from intra-molecular interactions between pY783 and the cSH2 domain.

mammalian PLC family, changes in PtdIns(4,5)P_2 and concentrations of calcium could have such regulatory roles.

A number of studies [2], including a more recent structure-based study of PLCβ2 regulation [13] and identification of gain-of-function mutations in *Plcg2* [33,34], consolidated a concept that PLC enzymes are auto-inhibited under basal, unstimulated conditions. This suggests that a simple model implying that accumulation of the PLC enzyme at the cellular membrane, leading solely to an increase in access to the substrate, could not fully explain

its activation mechanism. Furthermore, important questions arising from the concept of auto-inhibition are related to the identity of structural elements in PLCs involved in auto-inhibition and the mechanism of release of these intra-molecular inhibitory constraints.

Significant insights towards answering such questions have been obtained from structures of a complex of Rac and the PLCβ2-core and this truncated PLCβ2 in the absence of its Rac activator [12,13]. These structural studies suggest that activated Rac induces no conformational change in PLCβ2 and that auto-inhibition of the enzyme occurs owing to occlusion of the active site by the X-Y linker. Indeed, the deletion of the entire linker, including a structured region that makes direct contacts with the catalytic domain and a larger unstructured portion not visible in the crystal structure, leads to a markedly activated enzyme in the basal state [13]. A model that could be described as 'fix-and-unmask' is one possible interpretation of these findings (Figure 3c). According to the model, the binding of Rac to the PH domain, which is more extensively linked to the other core domains in this enzyme, recruits and places the core in a fixed orientation relative to the plasma membrane without causing a conformational change. Given that long stretches of acidic amino acids exist in the X-Y linker, these areas of dense negative charge could be repelled from the negatively charged membrane upon binding of PLCβ2 to membrane-bound Rac. This repulsion, together with steric constraints, presumably removes the X-Y linker from near the active site of PLCβ2 to relieve auto-inhibition, resulting in interfacial activation. More generally, such interfacial activation where PLC activity is restricted to sites of membrane interaction is consistent with the requirements for their stringent regulation. Recent studies of PLCβ2, for example, suggest that depending on the type of activating protein, PLCβ2 can be recruited transiently either to confined regions or undergo fast, surfing-like diffusion along a larger area of the plasma membrane thus tightly controlling PtdIns(4,5)P_2 hydrolysis in time and space [35].

The model proposed for PLCβ2 regulation by Rac could be extended to regulation of PLCβ enzymes by other membrane-resident G protein activators and might, to some extent, apply to other PLCs with similar X-Y linkers. Interestingly, the removal of the X-Y linker from PLCδ1, a flexible region not visible in the crystal structure, similarly enhances its basal activity [13]. In the context of the original model for PLCδ1 (Figure 3b), this finding would suggest that fixing the enzyme in a particular orientation at the membrane is more important for the release of auto-inhibition rather than for a relative positioning of the active site and the substrate as initially proposed. Studies of PLCε suggest that Ras-mediated regulation of this enzyme probably is more complex than that proposed for PLCβ2–Rac interactions. Although a deletion of the X-Y linker resulted in an increase of basal activity [13], the removal of RA domains had a similar effect [16], suggesting a still to be defined interplay between these two regions of PLCε in the regulation of enzyme function.

PLCγ enzymes represent a separate branch from all other PLC families and do not possess stretches of charged residues in their X-Y linkers [1]. Their specific domains, spPH, SH2 and SH3, are shared with a large number of other signalling proteins [27]. Recent structural studies of non-receptor tyrosine kinases illustrate different roles of regions with SH2 and SH3 domains in regulation of their catalytic activity [36]. The SH2 domains of these tyrosine kinases can have two general types of regulatory effects. In the kinase-active state, the SH2 domain can direct subcellular localisation and substrate recruitment and promote an active conformation of the adjacent catalytic domain. The Fes kinase appears to be optimised for this positive mode of regulation [37]. Conversely, the SH2 domain of kinases such as Src, Abl, and ZAP70 can act in conjunction with an additional SH2 or SH3 domains to maintain an inactive state through intramolecular interactions with the catalytic domain [36].

Although some of the regulatory modes described for non-receptor tyrosine kinases might operate in PLCγ, it is unlikely that the γSA, containing the spPH, SH2 and SH3 domains, regulates the catalytic domain (TIM barrel) allosterically, which appears to be the case in many of these kinases. The TIM barrel structure is more rigid and neither substrate binding [9] or binding of the Rac activator (in the case of PLCβ2) causes a conformational change [13]. More generally, an occlusion of the active site and, consequently, an inhibitory impact appears to be the more likely regulatory type of interaction for TIM barrel enzymes [38]. Indeed, there is experimental evidence that the γSA contains elements of auto-inhibition, although they remain to be fully characterised [39]. Furthermore, previous cellular and biochemical studies [30] and recent structural insights into a complex of the tandem nSH2–cSH2 from PLCγ1 and FGFR1 [21] are consistent with the possibility that conformational changes within the γSA contribute to activation. Based on these observations, the conformational change could result from intra-molecular interactions between pY783 and cSH2, which is the SH2 domain not involved in FGF–receptor binding [21,30]. It is well established that phosphorylation of Y783 in the nSH2–cSH2 linker, by FGF-receptor and several other tyrosine kinases, is crucial for the activation process [29,32]. It has been reported that the isolated cSH2 domain can undergo conformational changes following ligand binding [40]. An emerging, speculative model therefore includes the following steps: receptor binding by nSH2, phosphorylation of Y783 by the receptor tyrosine kinase, the pY783–cSH2 interaction and subsequent conformational change to unmask the PLC active site (Figure 3d). However, it is already emerging that different interactions might be taking place between PLCγ1 and other tyrosine kinases implicated in its activation. Indeed, a recent study of PLCγ1 activation by interleukin 2-inducible T-cell kinase (ITK) suggests an interaction between the kinase domain and a surface of the cSH2 outside the pY-binding pocket [41]; in this case, it is possible that the cSH2 could mediate both inter- and intra-molecular interactions.

Concluding remarks and future perspectives

The emerging molecular models for PLC regulation have highlighted some important aspects that characterize control of this enzymatic activity. Studies of several enzymes from different families support the concept that under basal conditions PLC proteins exist in an inactive state owing to

intra-molecular inhibitory constraints that mask substrate access to the active site. To overcome this auto-inhibition, interactions with regulatory proteins could influence PLC conformation directly or, as suggested by recent studies, bring PLC molecules within proximity of the membrane where the local environment could lead to interfacial activation. Further understanding of these regulatory mechanisms at the molecular level is critically dependent on further, more complete determination of PLC structures and regulatory complexes, application of new methodologies to study conformational changes in PLC proteins directly, and development of methodologies to study PLC signalling in the context of cellular membranes.

References

1 Katan, M. (2005) New insights into the families of PLC enzymes: looking back and going forward. *Biochem. J.* 391, e7–e9
2 Suh, P.G. *et al.* (2008) Multiple roles of phosphoinositide-specific phospholipase C isozymes. *BMB Rep.* 41, 415–434
3 Di Paolo, G. and De Camilli, P. (2006) Phosphoinositides in cell regulation and membrane dynamics. *Nature* 443, 651–657
4 Vicinanza, M. *et al.* (2008) Function and dysfunction of the PI system in membrane trafficking. *EMBO J.* 27, 2457–2470
5 Bunney, T.D. and Katan, M. (2010) Phosphoinositide signalling in cancer: beyond PI3K and PTEN. *Nat. Rev. Cancer* 10, 342–352
6 Bunney, T.D. and Katan, M. (2006) Phospholipase C epsilon: linking second messengers and small GTPases. *Trends Cell Biol.* 16, 640–648
7 Harden, T.K. and Sondek, J. (2006) Regulation of phospholipase C isozymes by ras superfamily GTPases. *Annu. Rev. Pharmacol. Toxicol.* 46, 355–379
8 Harden, T.K. *et al.* (2009) Phospholipase C isozymes as effectors of Ras superfamily GTPases. *J. Lipid Res.* 50, S243–S248
9 Essen, L.O. *et al.* (1996) Crystal structure of a mammalian phosphoinositide-specific phospholipase C delta. *Nature* 380, 595–602
10 Williams, R.L. and Katan, M. (1996) Structural views of phosphoinositide-specific phospholipase C: signalling the way ahead. *Structure* 4, 1387–1394
11 Williams, R.L. (1999) Mammalian phosphoinositide-specific phospholipase C. *Biochim. Biophys. Acta* 1441, 255–267
12 Jezyk, M.R. *et al.* (2006) Crystal structure of Rac1 bound to its effector phospholipase C-β2. *Nat. Struct. Mol. Biol.* 13, 1135–1140
13 Hicks, S.N. *et al.* (2008) General and versatile autoinhibition of PLC isozymes. *Mol. Cell* 31, 383–394
14 Singer, A.U. *et al.* (2002) A unique fold of phospholipase C-β mediates dimerization and interaction with G αq. *Nat. Struct. Biol.* 9, 32–36
15 Kiel, C. *et al.* (2008) Analyzing protein interaction networks using structural information. *Annu. Rev. Biochem.* 77, 415–441
16 Bunney, T.D. *et al.* (2006) Structural and mechanistic insights into ras association domains of phospholipase C epsilon. *Mol. Cell* 21, 495–507
17 Wen, W. *et al.* (2006) Structural characterization of the split pleckstrin homology domain in phospholipase C-γ1 and its interaction with TRPC3. *J. Biol. Chem.* 281, 12060–12068
18 Walliser, C. *et al.* (2008) rac regulates its effector phospholipase Cγ2 through interaction with a split pleckstrin homology domain. *J. Biol. Chem.* 283, 30351–30362
19 Bunney, T.D. *et al.* (2009) Structural insights into formation of an active signaling complex between Rac and phospholipase C γ2. *Mol. Cell* 34, 223–233
20 Williams, R.L. and Urbe, S. (2007) The emerging shape of the ESCRT machinery. *Nat. Rev. Mol. Cell Biol.* 8, 355–368
21 Bae, J.H. *et al.* (2009) The selectivity of receptor tyrosine kinase signaling is controlled by a secondary SH2 domain binding site. *Cell* 138, 514–524
22 Lemmon, M.A. (2008) Membrane recognition by phospholipid-binding domains. *Nat. Rev. Mol. Cell Biol.* 9, 99–111
23 Lemmon, M.A. (2004) Pleckstrin homology domains: not just for phosphoinositides. *Biochem. Soc. Trans.* 32, 707–711
24 Dvorsky, R. and Ahmadian, M.R. (2004) Always look on the bright site of Rho: structural implications for a conserved intermolecular interface. *EMBO Rep.* 5, 1130–1136
25 Kiel, C. *et al.* (2008) Association rate constants of ras-effector interactions are evolutionarily conserved. *PLoS Comput. Biol.* 4, e1000245
26 Kiel, C. and Serrano, L. (2009) Cell type-specific importance of ras-c-raf complex association rate constants for MAPK signaling. *Sci. Signal.* 2, ra38
27 Liu, B.A. *et al.* (2006) The human and mouse complement of SH2 domain proteins-establishing the boundaries of phosphotyrosine signaling. *Mol. Cell* 22, 851–868
28 Ji, Q.S. *et al.* (1999) Physiological requirement for both SH2 domains for phospholipase C-γ1 function and interaction with platelet-derived growth factor receptors. *Mol. Cell Biol.* 19, 4961–4970
29 Rhee, S.G. (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu. Rev. Biochem.* 70, 281–312
30 Poulin, B. *et al.* (2005) Intramolecular interaction between phosphorylated tyrosine-783 and the C-terminal Src homology 2 domain activates phospholipase C-γ1. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4276–4281
31 Songyang, Z. *et al.* (1993) SH2 domains recognize specific phosphopeptide sequences. *Cell* 72, 767–778
32 Rebecchi, M.J. and Pentyala, S.N. (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol. Rev.* 80, 1291–1335
33 Yu, P. *et al.* (2005) Autoimmunity and inflammation due to a gain-of-function mutation in phospholipase Cγ2 that specifically increases external Ca^{2+} entry. *Immunity* 22, 451–465
34 Everett, K.L. *et al.* (2009) Characterization of phospholipase C γ enzymes with gain-of-function mutations. *J. Biol. Chem.* 284, 23083–23093
35 Gutman, O. *et al.* (2010) Differential regulation of phospholipase C-β2 activity and membrane interaction by Gαq, Gβ1γ2, and Rac2. *J. Biol. Chem.* 285, 3905–3915
36 Filippakopoulos, P. *et al.* (2009) SH2 domains: modulators of nonreceptor tyrosine kinase activity. *Curr. Opin. Struct. Biol.* 19, 643–649
37 Filippakopoulos, P. *et al.* (2008) Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. *Cell* 134, 793–803
38 Wierenga, R.K. (2001) The TIM-barrel fold: a versatile framework for efficient enzymes. *FEBS Lett.* 492, 193–198
39 Katan, M. *et al.* (2003) Structural and mechanistic aspects of phospholipase Cγ regulation. *Adv. Enzyme Regul.* 43, 77–85
40 Groesch, T.D. *et al.* (2006) Structural basis for the requirement of two phosphotyrosine residues in signaling mediated by Syk tyrosine kinase. *J. Mol. Biol.* 356, 1222–1236
41 Min, L. *et al.* (2009) Itk tyrosine kinase substrate docking is mediated by a nonclassical SH2 domain surface of PLCgamma1. *Proc. Natl. Acad. Sci. U. S. A.* 106, 21143–21148
42 Groves, J.T. and Kuriyan, J. (2010) Molecular mechanisms in signal transduction at the membrane. *Nat. Struct. Mol. Biol.* 17, 659–665
43 Phillips, R. *et al.* (2009) Emerging roles for lipids in shaping membrane-protein function. *Nature* 459, 379–385
44 Illenberger, D. *et al.* (2000) Stimulation of phospholipase C-β2 by Rho GTPases. *Methods Enzymol.* 325, 167–177
45 Gandarillas, N.L. *et al.* (2009) In vitro reconstitution of activation of PLCepsilon by Ras and Rho GTPases. *Methods Mol. Biol.* 462, 379–389
46 Golebiewska, U. *et al.* (2008) Diffusion coefficient of fluorescent phosphatidylinositol 4,5-bisphosphate in the plasma membrane of cells. *Mol. Biol. Cell* 19, 1663–1669
47 Hammond, G.R. *et al.* (2009) Reversible binding and rapid diffusion of proteins in complex with inositol lipids serves to coordinate free movement with spatial information. *J. Cell Biol.* 184, 297–308
48 Falkenburger, B.H. *et al.* (2010) Kinetics of PIP2 metabolism and KCNQ2/3 channel regulation studied with a voltage-sensitive phosphatase in living cells. *J. Gen. Physiol.* 135, 99–114
49 Falkenburger, B.H. *et al.* (2010) Kinetics of M1 muscarinic receptor and G protein signaling to phospholipase C in living cells. *J. Gen. Physiol.* 135, 81–97
50 Chung, I. *et al.* (2010) Spatial control of EGF receptor activation by reversible dimerization on living cells. *Nature* 464, 783–787
51 Suzuki, K.G. *et al.* (2007) Dynamic recruitment of phospholipase Cγ at transiently immobilized GPI-anchored receptor clusters induces IP3-Ca^{2+} signaling: single-molecule tracking study 2. *J. Cell Biol.* 177, 731–742