Proof Delivery Form

Meeting number: SA156

BST reference: BST2014/0149

Number of colour figures: Figures 1 and 2

Number of pages (not including this page): 6

Biochemical Society Transactions

Please check your proof carefully to ensure (a) accuracy of the content and (b) that no errors have been introduced during the production process.

- You are responsible for ensuring that any errors contained in this proof are marked for correction before publication. Errors not marked may appear in the published paper.
- Corrections should only be for typographical errors in the text or errors in the artwork; substantial revision of the substance of the text is not permitted.
- Please answer any queries listed below.
- If corrections are required to a figure, please supply a new copy.

Your proof corrections and query answers should be returned as soon as possible (ideally within 48 hours of receipt). You can send proofs by email (editorial@portlandpress.com) or fax (+44 (0)20 7685 2469). Corrections sent by email can be sent as attachments, i.e. annotated PDF files or scanned versions of marked-up proofs

Notes:

1. Please provide the paper's reference number in any correspondence about your article
2. If you have any queries, please contact the publisher by email (editorial@portlandpress.com) or by telephone +44 (0)20 7685 2410

Please return this form with your proof

Queries for author:

Notes to Publisher:

Non-printed material (for Publisher's use only):

Primary: S4

Secondary: S5

Order for Offprints for *Biochemical Society Transactions* Individual Articles

Article number **BST**..... No. of pages .....

Reference number **BST 20**..... **/** ..... Title of paper .....

.....

SECTION 1– INFORMATION

Please return this form with your proof to: *Biochemical Society Transactions* Editorial Office, FAX: +44 (0)20 7685 2469 (Portland Press Limited, Third Floor, Charles Darwin House, 12 Roger Street, London WC1N 2JU, UK).

Offprints must be ordered using this form. If an official order form from your organization is required, it may be sent on later (please quote the article and reference numbers above), but it will be assumed that any such order received is not an additional order. Free offprints are not available and offprints cannot be supplied after publication.

Offprint orders are despatched by surface mail approximately 2 weeks after publication (allow up to 6 months for delivery). If you wish them to be despatched by Airmail please tick the box in section 2 below and add 40% to the indicated price.

***Biochemical Society Transactions* 2011 Offprint Order Charges**

Number of pages	Number required [price in Pounds sterling (£)]				
	50	**100**	**200**	**300**	**400**
1–4	105	145	210	276	332
5–8	132	175	265	360	410
9–12	176	234	370	505	623
13–16	274	347	575	805	1012

Notes

1. For quantities not listed, please ask for a quotation
2. Prices revised October 2007. No price increase for 2011.

SECTION 2– ORDER

☐ I do not wish to order offprints of my article

☐ I wish to purchase..... (minimum 50) offprints of my article at a cost of ..... £/$/€

☐ Please send my offprints by Airmail (add 40% to the price indicated) at an additional cost of ..... £/$/€

☐ Bank charges (if applicable, see section 3) ..... £/$/€

Total cost ..... £/$/€

Name and address to which offprints should be sent (please enter this information clearly):

.....

.....

.....

.....

.....

SECTION 3– PAYMENT

We accept payment:

- in pound sterling cheques drawn on UK banks
- in US dollar cheques drawn on US banks
- in Euro cheques drawn on any European bank
- by Visa, Mastercard and most major debit cards

For payment in $US or Euros, please convert the price shown above at the current rate of exchange and add £15 for bank charges.

Please indicate your preferred payment option:

1 ☐ I enclose a remittance payable to Portland Press Limited

2 ☐ Please send an invoice to the following address (if different from address in section 2)

.....

.....

.....

.....

.....

3 ☐ I wish to pay by Visa/Mastercard/Visa Delta/Switch/Maestro (delete as appropriate)

Card number: .....

Name on card: .....

Start date ..... End date .....

Issue no (Switch only): .....

Full address of cardholder:

.....

.....

.....

.....

Cardholder signature ..... Date .....

Biochemical Society Transactions Editorial Office, Portland Press Limited, Third Floor, Charles Darwin House,12 Roger Street, London WC1N 2JU, UK
Tel: +44 (0)20 7685 2410; fax: +44 (0)20 7685 2469; email: editorial@portlandpress.com; WWW: http://www.portlandpress.com

portlandpresslimited
publishing innovation

Role of calcium in membrane interactions by PI(4,5)P_2-binding proteins

Marina E. Monteiro*, Maria J. Sarmento*[1] and Fábio Fernandes*
*Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Abstract

Ca^{2+} and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) are key agents in membrane-associated signalling events. Their temporal and spatial regulation is crucial for activation or recruitment of proteins in the plasma membrane. In fact, the interaction of several signalling proteins with PI(4,5)P_2 has been shown to be tightly regulated and dependent of the presence of Ca^{2+}, with cooperative binding in some cases. In these proteins, PI(4,5)P_2 and Ca^{2+}-binding typically occurs in different binding sites. In addition, several PI(4,5)P_2-binding proteins are known targets of calmodulin (CaM), which depending on the presence of calcium, can compete with PI(4,5)P_2 for protein interaction, translating Ca^{2+} transient microdomains into variations of PI(4,5)P_2 lateral organization in time and space. The present review highlights different examples of calcium-dependent PI(4,5)P_2-binding proteins and discusses the possible impact of this dual regulation on fine-tuning of protein activity by triggering target membrane binding in the presence of subtle changes in the levels of calcium or PI(4,5)P_2.

Introduction

Living cells have the ability to adapt and respond efficiently to environmental stimuli, mostly due to the presence of messengers whose concentration varies in time and space. In mammalian cells, Ca^{2+} and phosphate ions (e.g. as adenosine phosphates or phospholipids) play a key role in many cell signalling pathways, mainly as a result of localized changes in protein electrostatics. Ca^{2+} binding, as well as phosphorylation, is able to change protein conformation and charge, ultimately affecting their potential interactions and assuring signal transduction [1]. In this context, it is of great importance to better understand the concerted action of Ca^{2+} and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2).

PI(4,5)P_2 is the major polyphosphoinositide present in the inner leaflet of the plasma membrane of mammalian cells. It represents approximately 1 mol% of total plasma membrane phospholipid content, a steady-state level sustained by consecutive phosphorylations and dephosphorylations.

Much of PI(4,5)P_2's functional multiplicity is associated with its capacity to bind several protein domains, targeting proteins to the plasma membrane and controlling their activity in time and space. PI(4,5)P_2-binding domains include pleckstrin homology (PH) domains, Tubby domains and C2 domains, among others [2,3]. Furthermore, electrostatic interactions with PI(4,5)P_2 are also likely to occur through the polybasic region of some proteins [4]. All together, these lipid–protein interactions play a key role in the regulation of numerous vital cell functions, from actin cytoskeleton attachment and reorganization to membrane trafficking. For example, the interaction of PI(4,5)P_2 with ezrin/radixin/moesin (ERM proteins), vinculin and talin, is responsible for the anchoring of actin filaments to the plasma membrane and assembly of focal adhesions [5]. This phospholipid is also involved in membrane ruffle formation and cell motility, through its interaction with the GMC proteins – myristoylated alanine-rich C kinase substrate (MARCKS), growth-associated protein of 43 kDa (GAP-43) and cortical/cytoskeleton-associated protein of 23 kDa (CAP-23) [6,7]. PI(4,5)P2 has also been associated to early stages of endocytosis, interacting with clathrin adaptor proteins such as AP180/CALM during the recruitment of the clathrin coat [8]. Later on in the mechanism, the interaction of PI(4,5)P_2 with dynamin and synaptojanin-1 is related with the fission of endocytic pits [9] and clathrin uncoating [10] respectively.

As well as in endocytosis, PI(4,5)P2 plays different roles throughout exocytosis, interacting with distinct proteins during either vesicle docking, priming or fusion. Some of the more studied examples include synaptotagmin [11] (associated with Ca^{2+}-triggered exocytosis), rabphilin (the effector of rab3 proteins responsible for controlling the SNARE complex formation) [12] and the SNARE protein syntaxin-1 [13].

Several PI(4,5)P_2-binding proteins have been shown to be sensitive to calcium fluctuations, and in several cases PI(4,5)P_2 and calcium binding was shown to be cooperative [11,14]. In the present review, we briefly summarize different examples of calcium-dependent changes in the affinity of PI(4,5)P_2-binding proteins to membranes. The effect of Ca^{2+}

Key words: annexins, C2 domains, calcium, calmodulin (CaM)-binding proteins, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2).

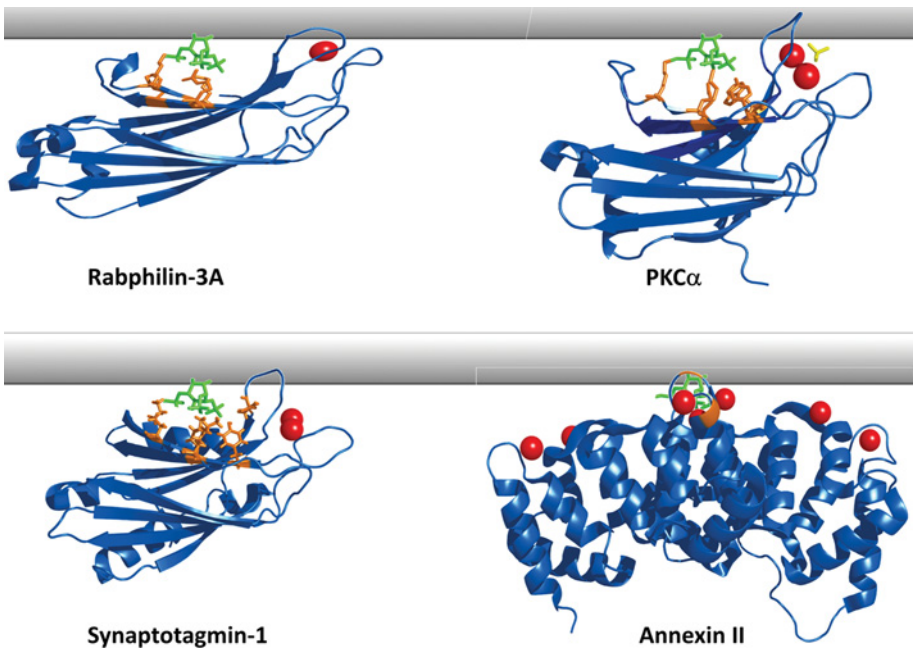
Abbreviations: apoCaM, Ca^{2+}-free CaM; AnxA2, annexin A2; CaM, calmodulin; MARCKS, myristoylated alanine-rich C kinase substrate; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PS, phosphatidylserine.

[1]To whom correspondence should be addressed (email maria.sarmento@tecnico.ulisboa.pt).

Figure 1 | Representative structures of the C2A domain of rabphilin-3A (PDB code 4LT7) [11], the C2 domain of PKCα (PDB code 3GPE) [17], the C2B domain of synaptotagmin-1 (PDB code 1TJX) [18] and annexin II (PDB code 2HYW) [19]

Bound Ca^{2+} ions are shown as red spheres, whereas residues expected to comprise the $PI(4,5)P_2$-binding sites are shown in orange. The likely position of the inositol group of the bound $PI(4,5)P_2$ is shown in green. Images are created with PYMOL (The PyMOL Molecular Graphics System, Version 1.4.1 Schrödinger, LLC.; http://www.pymol.org) and POV-Ray (Persistence of Vision Raytracer; www.povray.org).

COLOUR



on $PI(4,5)P_2$ membrane distribution and its consequences on $PI(4,5)P_2$-protein interactions are also addressed.

Role of calcium in membrane binding by C2 domains

C2 domains are important protein modules in eukaryotic membrane signalling, being involved in signal transduction and membrane trafficking [3]. These domains interact with $PI(4,5)P_2$ and are composed by approximately 130 amino acid residues [3]. The overall structure of C2 domain fold is represented by a Greek key motif composed of a pair of four-stranded β-sheets with flexible surface loops and helices.

C2 domains can interact with lipids using two separate binding sites, one containing Ca^{2+}-binding loops in a lysine-rich cluster [3] and another with a cationic β-groove, which selectivity binds to phosphoinositides in a Ca^{2+}-independent matter [15].

Several signalling proteins containing these C2 domains, such as rabphilin-3A [12], protein kinase C (PKC) [3] or synaptotagmin [16], have specificity for inositol rings in the membrane, especially to $PI(4,5)P_2$ in a Ca^{2+}-dependent manner, as shown in Figure 1. The C2A domain of rabphilin-3A was shown to bind the $PI(4,5)P_2$ headgroup with 16 times higher affinity in the presence of saturating concentrations of Ca^{2+}, whereas its C2B domain binds the $PI(4,5)P_2$ headgroup in a Ca^{2+}-independent fashion with low affinity [12]. On the other hand, the interaction of the C2A domain of rabphilin-3A with the $PI(4,5)P_2$ headgroup increased its affinity to Ca^{2+}, revealing cooperativity between Ca^{2+} and $PI(4,5)P_2$ binding.

Furthermore, binding of the C2 domain of PKCα [17] and the C2B domain of synaptotagmin-1 [16] to $PI(4,5)P_2$ was also shown to be Ca^{2+}-dependent (Figure 1). Synaptotagmin-1, a calcium sensor involved in synaptic vesicle fusion, contains a single transmembrane domain close to the N-terminus, which anchors the protein to synaptic vesicles [16]. This domain is connected by a 61-residue unstructured linker to two C2 domains, C2A and C2B. The C2A domain binds to three Ca^{2+} ions, with affinities ranging from 50 μM to 10 mM, whereas the C2B domain binds two Ca^{2+} ions, both with $\approx$200 μM affinity [11]. As observed for other C2 domains, synaptotagmin-1 C2 domains can bind to anionic phospholipids with low specificity by completing the Ca^{2+}-binding sites with the anionic phospholipid headgroups, whereas interaction with $PI(4,5)P_2$ occurs through the conserved cationic β-groove [15], as represented in Figure 1. The interaction between $PI(4,5)P_2$ and the C2B domain of synaptotagmin was shown to increase the affinity of the C2B domain for Ca^{2+} more than 40-fold. Conversely, Ca^{2+}-binding to C2B domain increases the affinity for $PI(4,5)P_2$

more than 10-fold [16], revealing similar cooperativity between Ca^{2+} and PI(4,5)P_2 binding as the one observed for rabphilin-3A.

The mechanism responsible for this cooperativity is not the coordination of calcium and PI(4,5)P_2 in the binding sites, since the respective binding sites within the C2 domains are located at some distance, but are probably related to protein conformational changes [16].

The dependence on both calcium and PI(4,5)P_2 for binding of PKCα has been defined as a target-activated messenger affinity (TAMA) mechanism [20]. According to this, both the messenger and the target lipid must be present in sufficient concentrations for effective protein binding to the membrane, allowing for protein targeting to PI(4,5)P_2-enriched domains during calcium signalling.

The same mechanism must be operating for rabphilin-3A and synaptotagmin-1, and it is likely to allow for fine-tuning of protein activity and responses for subtle changes in the levels of calcium or PI(4,5)P_2.

Ca^{2+}-dependent PI(4,5)P_2-binding by annexins

Annexins are a conserved family of proteins that share the ability to reversibly bind anionic phospholipids in a Ca^{2+}-dependent fashion [21]. They have been implicated in several vital cell functions, including membrane–cytoskeleton interactions, membrane trafficking, endocytosis, exocytosis, regulation of membrane protein activity, signal transduction and also Ca^{2+} channel activity [22–24].

Each member of the annexin family presents two distinct domains: a highly conserved C-terminal core and a variable N-terminal extension. The annexin core domain is composed of four (eight in annexin A6) homologous α-helical domains of approximately 70 amino acid residues, presenting an overall shape of a slightly curved disc surrounding a central hydrophilic pore. Both Ca^{2+} and phospholipid-binding sites are located on the convex side of this core domain, whereas the concave side interacts with the N-terminal domain. This N-terminus varies in length, amino acid sequence and post-translational modifications along the annexin family, influencing the stability and specific functions of each individual protein [21].

Ca^{2+} usually plays a dual role in the regulation of annexin function. In one hand, Ca^{2+} ions are the mediators of membrane binding, simultaneously coordinating carbonyl and carboxy groups of the protein and phosphoryl moieties of phosphatidylserine (PS) [25]. On the other hand, as shown for annexin A1, Ca^{2+} binding can result in a change in conformation that leads to the exposure of the N-terminal and its interaction with other cytosolic proteins [26].

Despite the similarity of the core domain, annexins present different lipid specificity and are sensitive to different Ca^{2+} concentrations [21], allowing for cytosol-to-membrane translocation to specific sites in the membrane as a response to different Ca^{2+} concentrations. For example, the free Ca^{2+} concentration required for binding to liposomes containing PS may vary from 20 μM to 100 nM for annexin A5 and annexin A2-A100A10 (A2t) heterotetrameric complex respectively [21,27].

Ca^{2+}-dependent PI(4,5)P_2 binding has been demonstrated for different annexins (A2, A4 and A8) [28–30], the more extensively studied being annexin A2 (AnxA2). A2t binds PI(4,5)P_2 with high affinity whereas monomeric AnxA2 exhibits significantly less affinity for inositol binding [31]. The binding site for PI(4,5)P_2 within AnxA2 in the heterotetrameric form was shown to involve Lys^{279} and Lys^{281} [31]. As illustrated in Figure 1, these residues are located within a calcium binding loop, and the carbonyl groups of several residues adjacent to the PI(4,5)P_2-binding residues participate in calcium binding.

In the presence of Ca^{2+}, the interaction of PI(4,5)P_2 with A2t results in the formation of micrometre-size PI(4,5)P_2 clusters whose formation and stability depend on the presence of cholesterol [31]. These PI(4,5)P_2-enriched raft-like domains coincide with the accumulation of the AnxA2-binding partner F-actin, suggesting that AnxA2 may act as an actin nucleator at PI(4,5)P_2 membrane patches, promoting the reorganization of the actin cytoskeleton [28]. Moreover, the formation of these microdomains suggests a wider involvement of AnxA2 in PI(4,5)P_2-dependent processes that are dependent on its lateral clustering, as already demonstrated for Ca^{2+}-triggered granule exocytosis in chromaffin cells [32].

Ca^{2+} modulation of CaM/PI(4,5)P_2-binding proteins

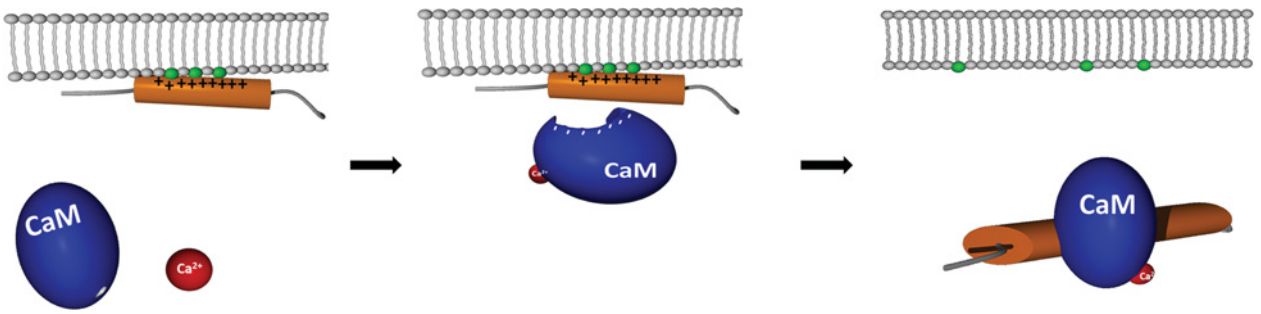
Calmodulin (CaM) is a small, ubiquitous adaptor protein that functions as the major transducer of small and transient Ca^{2+} signals inside cells. Structurally, Ca^{2+}-free CaM (apoCaM) presents a dumbbell-like shape composed of a flexible α-helix flanked by two globular domains, each one consisting of two helix-loop-helix motifs (EF hands) connected by a short antiparallel β-sheet [33,34]. Each domain can bind two Ca^{2+} ions, the C-terminal lobe presenting higher affinity for Ca^{2+} ($K_d \approx 1\,\mu M$) than the N-terminal one ($K_d \approx 12\,\mu M$) [35]. Upon Ca^{2+} binding (Ca/CaM), CaM becomes more elongated and hydrophobic residues in each domain are exposed [35]. These hydrophobic patches can bind to a variety of target proteins, activating its Ca^{2+} sensor activity and triggering its ability to relieve protein autoinhibition, remodel active sites, and dimerize proteins [33,36].

In cells, there are hundreds of proteins that contain CaM recruitment sites and consequently bind CaM. Their heterogeneity in structure and function can be explained by the structural flexibility of the calcium sensor. CaM targets differ in their affinity for apoCaM and Ca/CaM, but can also modulate CaM sensitivity for Ca^{2+}. Taking into account the low level of free CaM inside cells (i.e. $\approx$1 % in cardiac myocytes) [37], these differences in affinity usually dictate which proteins are activated at any given time, especially after Ca^{2+} stimulation [38].

Figure 2 | Example of a PI(4,5)P_2/CaM switching mechanism

Proteins containing the positive effector domain (orange) have the ability to laterally sequester PI(4,5)P_2 (green) in the membrane. In case of higher affinity for Ca/CaM than apoCaM, the protein interacting with PI(4,5)P_2 detaches from the membrane to bind Ca/CaM upon a Ca^{2+} stimulus, reverting the initial PI(4,5)P_2 clustering. Images are created with AC3D (http://www.inivis.com) and POV-Ray (Persistence of Vision Raytracer; www.povray.org).

COLOUR



Among the CaM protein targets, a few were also shown to bind PI(4,5)P_2 and, for that reason, make the bridge between Ca^{2+} signalling and PI(4,5)P_2 regulated cellular functions. These CaM/PI(4,5)P_2-binding proteins include not only the GMC proteins, also called 'pipmodulins' [7], but also the SNARE proteins synaptobrevin-2 and syntaxin-1 [39], among others. Structurally, these proteins present an unstructured cluster of positively charged amino acid residues (effector domain) capable of electrostatically sequester PI(4,5)P_2 to localized patches in the membrane (Figure 2). Since CaM is also negatively charged, it can compete with PI(4,5)P_2 as a response to Ca^{2+} concentration fluctuations, depending on the relative affinity of the protein for apoCaM and Ca/CaM [1,40].

This PI(4,5)P_2/CaM switching mechanism, represented in Figure 2, has been well demonstrated for the 'pipmodulin' MARCKS, a natively unstructured protein anchored to the plasma membrane through an N-terminal myristate. Because it presents a conserved basic effector domain, it also interacts electrostatically with negatively charged phospholipids [40]. Several experiments have shown that 13 basic residues are responsible for laterally sequestering 3 PI(4,5)P_2 molecules in a reversible manner [4,41,42]. MARCKS presents high affinity for Ca/CaM ($\approx$ 4 nM) [4]. As a consequence, when the local Ca^{2+} concentration increases, Ca/CaM competes with PI(4,5)P_2 for the polybasic region of the protein. Because the myristate anchor is not enough to hold the protein in the membrane, MARCKS translocates to the cytosol, releasing the sequestered PI(4,5)P_2 molecules [43,44] and consequently increasing its free concentration in the plasma membrane. In summary, CaM translates the Ca^{2+} transient microdomains into variations of PI(4,5)P_2 lateral organization, contributing for the tight regulation of PI(4,5)P_2-dependent cellular processes.

Direct PI(4,5)P_2/Ca^{2+} interaction

So far we have discussed the effect of Ca^{2+} on PI(4,5)P_2–protein interactions. However, Ca^{2+} may have a direct impact on PI(4,5)P_2 lateral distribution, facilitating protein sorting to the plasma membrane at sites already enriched in PI(4,5)P_2. At non-physiological concentrations of both Ca^{2+} ($\geqslant$1 mM) and/or PI(4,5)P_2 (5–50 %), Ca^{2+} was already shown to promote macroscopic clustering of PI(4,5)P_2 in membrane model systems, either in lipid monolayers [45,46], large unilamellar vesicles (LUVs) [47] and giant unilamellar vesicles (GUVs) [48]. Recently, our group and others have demonstrated that Ca^{2+}-induced PI(4,5)P_2 clustering occurs even at physiological concentrations of PI(4,5)P_2 and Ca^{2+}, in supported lipid monolayers [49] and free-standing bilayers [50].

Moreover, in artificial membranes containing cholesterol, Ca^{2+} seems to favour partition of PI(4,5)P_2 molecules to raft-like domains [45,49]. This suggests that Ca^{2+} may even potentiate protein anchoring in lipid rafts, by increasing PI(4,5)P_2 density in these microdomains.

Altogether, these recent findings seem to indicate that PI(4,5)P_2, besides interacting with Ca^{2+} sensors such as C2-contaning proteins, annexins and CaM, may act as a Ca^{2+} sensor itself, responsive to physiological Ca^{2+} concentrations.

Concluding remarks

The Ca^{2+}-dependent binding of several signalling proteins with PI(4,5)P_2 is well established. This dependence is not based on the simultaneous coordination of Ca^{2+} ions with acidic residues from the protein and phosphate groups from phospholipids as observed with many PS-binding domains, but typically involves two different sets of protein residues (or binding sites). In addition, several CaM/PI(4,5)P_2-binding proteins exhibit Ca^{2+}-dependent binding of PI(4,5)P_2 due to competition of CaM with the phosphoinositol for protein binding. In these conditions, these PI(4,5)P_2-binding proteins operate as integrators of two different signals, Ca^{2+} and PI(4,5)P_2 concentrations. The physiological relevance of these mechanisms is likely to be associated with the possibility of fine-tuning of protein activity by triggering

target membrane binding in the presence of subtle changes in the levels of calcium or $PI(4,5)P_2$.

Funding

M.J.S. and F.F. are recipients of fellowships from Fundação para a Ciência e a Tecnologia (FCT) [grant numbers SFRH/BD/80575/2011 and SFRH/BPD/64320/2009 respectively]. We acknowledge funding by FCT [project references PTDC/QUI-BIQ/112067/2009 and RECI/CTM-POL/0342/2012].

References

1 Clapham, D.E. (2007) Calcium signaling. Cell **131**, 1047–1058 CrossRef PubMed
2 Szentpetery, Z., Balla, A., Kim, Y.J., Lemmon, M.A. and Balla, T. (2009) Live cell imaging with protein domains capable of recognizing phosphatidylinositol 4,5-bisphosphate: a comparative study. BMC Cell Biol. **10**, 67 CrossRef PubMed
3 Guerrero-Valero, M., Marín-Vicente, C., Gómez-Fernández, J.C. and Corbalán-García, S. (2007) The C2 domains of classical PKCs are specific PtdIns(4,5)P_2-sensing domains with different affinities for membrane binding. J. Mol. Biol. **371**, 608–621 CrossRef PubMed
4 Gambhir, A., Hangyás-Mihályné, G., Zaitseva, I., Cafiso, D.S., Wang, J., Murray, D., Pentyala, S.N., Smith, S.O. and McLaughlin, S. (2004) Electrostatic sequestration of PIP_2 on phospholipid membranes by basic/aromatic regions of proteins. Biophys. J. **86**, 2188–2207 CrossRef PubMed
5 Logan, M.R. and Mandato, C.A. (2006) Regulation of the actin cytoskeleton by PIP_2 in cytokinesis. Biol. Cell **98**, 377–388 CrossRef PubMed
6 Sheetz, M.P., Sable, J.E. and Döbereiner, H.G. (2006) Continuous membrane–cytoskeleton adhesion requires continuous accommodation to lipid and cytoskeleton dynamics. Annu. Rev. Biophys. Biomol. Struct. **35**, 417–434 CrossRef PubMed
7 Laux, T., Fukami, K., Thelen, M., Golub, T., Frey, D. and Caroni, P. (2000) GAP43, MARCKS, and CAP23 modulate $PI(4,5)P_2$ at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. J. Cell Biol. **149**, 1455–1472 CrossRef PubMed
8 Ford, M.G., Pearse, B.M., Higgins, M.K., Vallis, Y., Owen, D.J., Gibson, A., Hopkins, C.R., Evans, P.R. and McMahon, H.T. (2001) Simultaneous binding of PtdIns(4,5)P_2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. Science **291**, 1051–1055 CrossRef PubMed
9 Jost, M., Simpson, F., Kavran, J.M., Lemmon, M.A. and Schmid, S.L. (1998) Phosphatidylinositol-4,5-bisphosphate is required for endocytic coated vesicle formation. Curr. Biol. **8**, 1399–1404 CrossRef PubMed
10 Cremona, O., Di Paolo, G., Wenk, M.R., Lüthi, A., Kim, W.T., Takei, K., Daniell, L., Nemoto, Y., Shears, S.B., Flavell, R.A. et al. (1999) Essential role of phosphoinositide metabolism in synaptic vesicle recycling. Cell **99**, 179–188 CrossRef PubMed
11 Guillén, J., Ferrer-Orta, C., Buxaderas, M., Pérez-Sánchez, D., Guerrero-Valero, M., Luengo-Gil, G., Pous, J., Guerra, P., Gómez-Fernández, J.C., Verdaguer, N. and Corbalán-García, S. (2013) Structural insights into the Ca^{2+} and $PI(4,5)P_2$ binding modes of the C2 domains of rabphilin 3A and synaptotagmin 1. Proc. Natl. Acad. Sci. U.S.A. **110**, 20503–20508 CrossRef PubMed
12 Montaville, P., Coudevylle, N., Radhakrishnan, A., Leonov, A., Zweckstetter, M. and Becker, S. (2008) The PIP_2 binding mode of the C2 domains of rabphilin-3A. Protein Sci. **17**, 1025–1034 CrossRef PubMed
13 Van den Bogaart, G., Meyenberg, K., Risselada, H.J., Amin, H., Willig, K.I., Hubrich, B.E., Dier, M., Hell, S.W., Grubmüller, H., Diederichsen, U. and Jahn, R. (2011) Membrane protein sequestering by ionic protein–lipid interactions. Nature **479**, 552–555 CrossRef PubMed
14 Corbalán-García, S. and Gómez-Fernández, J.C. (2010) The C2 domains of classical and novel PKCs as versatile decoders of membrane signals. Biofactors **36**, 1–7 PubMed
15 Cho, W. and Stahelin, R.V. (2006) Membrane binding and subcellular targeting of C2 domains. Biochim. Biophys. Acta **1761**, 838–849 CrossRef PubMed
16 Van den Bogaart, G., Meyenberg, K., Diederichsen, U. and Jahn, R. (2012) Phosphatidylinositol 4,5-bisphosphate increases Ca^{2+} affinity of synaptotagmin-1 by 40-fold. J. Biol. Chem. **287**, 16447–16453 CrossRef PubMed
17 Guerrero-Valero, M., Ferrer-Orta, C., Querol-Audí, J., Marin-Vicente, C., Fita, I., Gómez-Fernández, J.C., Verdaguer, N. and Corbalán-García, S. (2009) Structural and mechanistic insights into the association of PKCα-C2 domain to PtdIns(4,5)P_2. Proc. Natl. Acad. Sci. U.S.A. **106**, 6603–6607 CrossRef PubMed
18 Cheng, Y., Sequeira, S.M., Malinina, L., Tereshko, V., Söllner, T.H. and Patel, D.J. (2004) Crystallographic identification of Ca^{2+} and Sr^{2+} coordination sites in synaptotagmin I C2B domain. Protein Sci. **13**, 2665–2672 CrossRef PubMed
19 Shao, C., Zhang, F., Kemp, M.M., Linhardt, R.J., Waisman, D.M., Head, J.F. and Seaton, B.A. (2006) Crystallographic analysis of calcium-dependent heparin binding to annexin A2. J. Biol. Chem. **281**, 31689–31695 CrossRef PubMed
20 Corbin, J.A., Evans, J.H., Landgraf, K.E. and Falke, J.J. (2007) Mechanism of specific membrane targeting by C2 domains: localized pools of target lipids enhance Ca^{2+} affinity. Biochemistry **46**, 4322–4336 CrossRef PubMed
21 Raynal, P. and Pollard, H.B. (1994) Annexins: the problem of assessing the biological role for a gene family of multifunctional calcium- and phospholipid-binding proteins. Biochim. Biophys. Acta **1197**, 63–93 CrossRef PubMed
22 Hayes, M.J., Shao, D.-M., Grieve, A., Levine, T., Bailly, M. and Moss, S.E. (2009) Annexin A2 at the interface between F-actin and membranes enriched in phosphatidylinositol 4,5-bisphosphate. Biochim. Biophys. Acta **1793**, 1086–1095 CrossRef PubMed
23 Podszywalow-Bartnicka, P., Kosiorek, M., Piwocka, K., Sikora, E., Zablocki, K. and Pikula, S. (2010) Role of annexin A6 isoforms in catecholamine secretion by PC12 cells: distinct influence on calcium response. J. Cell. Biochem. **111**, 168–178 CrossRef PubMed
24 Lizarbe, M.A., Barrasa, J.I., Olmo, N., Gavilanes, F. and Turnay, J. (2013) Annexin–phospholipid interactions: functional implications. Int. J. Mol. Sci. **14**, 2652–2683 CrossRef PubMed
25 Swairjo, M.A., Concha, N.O., Kaetzel, M.A., Dedman, J.R. and Seaton, B.A. (1995) Ca^{2+}-bridging mechanism and phospholipid head group recognition in the membrane-binding protein annexin V. Nat. Struct. Biol. **2**, 968–974 CrossRef PubMed
26 Rosengarth, A. and Luecke, H. (2003) A calcium-driven conformational switch of the N-terminal and core domains of Annexin A1. J. Mol. Biol. **326**, 1317–1325 CrossRef PubMed
27 Schlaepfer, D.D., Mehlman, T., Burgess, W.H. and Haigler, H.T. (1987) Structural and functional characterization of endonexin II, a calcium- and phospholipid-binding protein. Proc. Natl. Acad. Sci. U.S.A. **84**, 6078–6082 CrossRef PubMed
28 Rescher, U., Ruhe, D., Ludwig, C., Zobiack, N. and Gerke, V. (2004) Annexin 2 is a phosphatidylinositol (4,5)-bisphosphate binding protein recruited to actin assembly sites at cellular membranes. J. Cell Sci. **117**, 3473–3480 CrossRef PubMed
29 Junker, M. and Creutz, C.E. (1994) Ca^{2+}-dependent binding of endonexin (annexin IV) to membranes: analysis of the effects of membrane lipid composition and development of a predictive model for the binding interaction. Biochemistry **33**, 8930–8940 CrossRef PubMed
30 Goebeler, V., Ruhe, D., Gerke, V. and Rescher, U. (2006) Annexin A8 displays unique phospholipid and F-actin binding properties. FEBS Lett. **580**, 2430–2434 CrossRef PubMed
31 Gokhale, N.A., Abraham, A., Digman, M.A., Gratton, E. and Cho, W. (2005) Phosphoinositide specificity of and mechanism of lipid domain formation by annexin A2–p11 heterotetramer. J. Biol. Chem. **280**, 42831–42840 CrossRef PubMed
32 Chasserot-Golaz, S., Vitale, N., Umbrecht-Jenck, E., Knight, D., Gerke, V. and Bader, M.F. (2005) Annexin 2 promotes the formation of lipid microdomains required for calcium-regulated exocytosis of dense-core vesicles. Mol. Biol. Cell **16**, 1108–1119 CrossRef PubMed
33 James, P., Vorherr, T. and Carafoli, E. (1995) Calmodulin-binding domains: just two faced or multi-faceted? Trends Biochem. Sci. **20**, 38–42 CrossRef PubMed
34 Chattopadhyaya, R., Meador, W.E., Means, A.R. and Quiocho, F.A. (1992) Calmodulin structure refined at 1.7 Å resolution. J. Mol. Biol. **228**, 1177–1192 CrossRef PubMed
35 Bossuyt, J. and Bers, D.M. (2013) Visualizing CaMKII and CaM activity: a paradigm of compartmentalized signaling. J. Mol. Med. **91**, 907–916 CrossRef PubMed

36 Hoeflich, K.P. and Ikura, M. (2002) Calmodulin in action: diversity in target recognition and activation mechanisms. Cell **108**, 739–742 CrossRef PubMed
37 Wu, X. and Bers, D.M. (2007) Free and bound intracellular calmodulin measurements in cardiac myocytes. Cell Calcium **41**, 353–364 CrossRef PubMed
38 Persechini, A. (2002) Calmodulin is a limiting factor in the cell. Trends Cardiovasc. Med. **12**, 32–37 CrossRef PubMed
39 Di Giovanni, J., Iborra, C., Maulet, Y., Lévêque, C., El Far, O. and Seagar, M. (2010) Calcium-dependent regulation of SNARE-mediated membrane fusion by calmodulin. J. Biol. Chem. **285**, 23665–23675 CrossRef PubMed
40 McLaughlin, S. and Murray, D. (2005) Plasma membrane phosphoinositide organization by protein electrostatics. Nature **438**, 605–611 CrossRef PubMed
41 Rauch, M.E., Ferguson, C.G., Prestwich, G.D. and Cafiso, D.S. (2002) Myristoylated alanine-rich C kinase substrate (MARCKS) sequesters spin-labeled phosphatidylinositol 4,5-bisphosphate in lipid bilayers. J. Biol. Chem. **277**, 14068–14076 CrossRef PubMed
42 Wang, J., Gambhir, A., Hangyás-Mihályné, G., Murray, D., Golebiewska, U. and McLaughlin, S. (2002) Lateral sequestration of phosphatidylinositol 4,5-bisphosphate by the basic effector domain of myristoylated alanine-rich C kinase substrate is due to nonspecific electrostatic interactions. J. Biol. Chem. **277**, 34401–34412 CrossRef PubMed
43 Arbuzova, A., Murray, D. and McLaughlin, S. (1998) MARCKS, membranes, and calmodulin: kinetics of their interaction. Biochim. Biophys. Acta **1376**, 369–379 CrossRef PubMed
44 Kim, J., Shishido, T., Jiang, X., Aderem, A. and McLaughlin, S. (1994) Phosphorylation, high ionic strength, and calmodulin reverse the binding of MARCKS to phospholipid vesicles. J. Biol. Chem. **269**, 28214–28219 PubMed
45 Levental, I., Christian, D.A., Wang, Y.-H., Madara, J.J., Discher, D.E. and Janmey, P.A. (2009) Calcium-dependent lateral organization in phosphatidylinositol 4,5-bisphosphate (PIP_2)- and cholesterol-containing monolayers. Biochemistry **48**, 8241–8248 CrossRef PubMed
46 Ellenbroek, W.G., Wang, Y.-H., Christian, D.A., Discher, D.E., Janmey, P.A. and Liu, A.J. (2011) Divalent cation-dependent formation of electrostatic PIP_2 clusters in lipid monolayers. Biophys. J. **101**, 2178–2184 CrossRef PubMed
47 Summers, S.A., Guebert, B.A. and Shanahan, M.F. (1996) Polyphosphoinositide inclusion in artificial lipid bilayer vesicles promotes divalent cation-dependent membrane fusion. Biophys. J. **71**, 3199–3206 CrossRef PubMed
48 Carvalho, K., Ramos, L., Roy, C. and Picart, C. (2008) Giant unilamellar vesicles containing phosphatidylinositol(4,5)bisphosphate: characterization and functionality. Biophys. J. **95**, 4348–4360 CrossRef PubMed
49 Wang, Y.-H., Collins, A., Guo, L., Smith-Dupont, K.B., Gai, F., Svitkina, T. and Janmey, P.A. (2012) Divalent cation-induced cluster formation by polyphosphoinositides in model membranes. J. Am. Chem. Soc. **134**, 3387–3395 CrossRef PubMed
50 Sarmento, M.J., Coutinho, A., Fedorov, A., Prieto, M. and Fernandes, F. (2014) Ca^{2+} induces $PI(4,5)P_2$ clusters on lipid bilayers at physiological $PI(4,5)P_2$ and Ca^{2+} concentrations. Biochim. Biophys. Acta **1838**, 822–830 CrossRef PubMed

Received 23 May 2014
doi:10.1042/BST20140149