Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 356 (2007) 255–259
BBRC
www.elsevier.com/locate/ybbrc

Biochemical characterization of the type I inositol polyphosphate 4-phosphatase C2 domain

Colin T. Shearn *, F. Anderson Norris [1]

Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011, USA

Received 12 February 2007
Available online 28 February 2007

Abstract

Inositol polyphosphate 4 phosphatases (IP4Ps) are enzymes involved in the regulation of phosphoinositide 3-kinase lipid signaling. They catalyze the hydrolysis of the 4-position phosphate from phosphatidylinositol 3,4-bisphosphate to phosphatidylinositol 3-phosphate. In this paper we have characterized a lipid binding C2 domain located on the N-terminus of type I IP4Ps. Mutational analysis of the lipid binding loops suggests that Asp61, Asp120, Asp123, Lys122, Arg124 are involved in lipid binding in vitro. In addition, we show that this C2 domain binds calcium but calcium is not involved in lipid binding. This paper provides insight into the mechanism of membrane interaction of IP4Ps.

Keywords: C2 domain; Calcium; PtdIns 3-kinase; Phosphatidylinositol 3,4-bisphosphate

Inositol polyphosphate 4-phosphatases (IP4Ps) are Mg^{2+}-independent phosphatases that catalyze the hydrolysis of the D-4 position phosphoester of the lipid second messenger, phosphatidylinositol 3,4-bisphosphate ($PtdIns(3,4)P_2$) to produce PtdIns(3)P [1]. IP4P Iα has been implicated in the regulation of phosphatidylinositol 3-kinase (PI3K) signaling in platelets and in the regulation of the oxidative burst in human neutrophils [2,3]. A mouse containing a catalytically inactive form of type I IP4P has been characterized. The ''Weeble'' mouse contains a stop mutation in the IP4P Iα gene and presents a phenotype of neurological dysfunction and death early in life [4]. In *Caenorhabditis elegans*, PI3K second messengers are involved downstream of the insulin like receptor DAF-2. Recent RNAi evidence suggests that IP4Ps are involved in PI3K signaling downstream of the DAF-2 receptor (J. Walker, K. Foreman, C.T. Shearn, K. Christina, D. Mampreian, J.A. Powell-Coffman, F.A. Norris, unpublished work). Although IP4Ps were originally purified from soluble fractions in mouse brains, the possible role of these phosphatases in PI3K signaling suggests that they must interact with membranes to have access to the lipid substrate. The basis of this mechanism is currently not known.

C2 domains are lipid and protein binding domains first discovered in protein kinase C that are approximately 130 amino acids in length. They consist of a conserved β sandwich containing of four antiparallel β strands that form two β-sheets. There are two major types of C2 domains, topology I and topology II. The overall conformation of the two topologies is very similar. Structural studies comparing 109 α-carbons between the two topologies have shown that the overall structures differ by only 1.4 Å [5–7]. Some examples of topology I and topology II C2 domains include the synaptotagmin C2A (Syt C2A) domain and the phospholipase Cδ C2 (PLCδ C2) domain. Both of these proteins contain calcium-dependent C2 domains that interact with lipids and calcium via the calcium binding regions (CBR) connecting the β-strands [8–12]. In addition to calcium-dependent C2 domains there

* Corresponding author. Present address: Department of Pharmacy, University of Colorado at Denver and Health Sciences Center, 4200 East Ninth Avenue, C424, Denver, CO 80262, USA. Fax: +1 303 315 6281.
E-mail address: colin.shearn@uchsc.edu (C.T. Shearn).
[1] Present address: Lander University, Greenwood, SC 29649, USA.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.02.115

are C2 domains that bind lipids via a Ca^{2+} independent mechanism. Calcium independent C2 domains interact with lipids primarily via positively charged residues within the C2 domain loops [13–17].

Previous studies and sequence analysis of IP4P Iα show the existence of a C2 domain located in the N-terminal region of this protein [18,19]. Cellular localization studies suggest that the IP4P C2 domain plays a role in localization of IP4P to endosomal membranes [18]. In this paper we use biochemical techniques to determine the lipid and calcium binding characteristics of the human type I IP4P C2 domain.

Materials and methods

Reagents. D(+)-sn-1,2-di-*O*-hexadecanoylglyceryl, 3-*O*-phospho linked phosphatidylinositol (3,5)-bisphosphate (PtdIns(3,5)P_2) was from Echelon Biosciences Inc. Porcine brain L-α-phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P_2), porcine brain L-α-phosphatidylinositol (4)-phosphate (PtdIns(4)P), L-α-phosphatidylserine, liver L-α-phosphatidylinositol (PI), L-α-phosphatidylcholine (PC) and L-α-phosphatidic acid (PA) were from Avanti Polar Lipids, Lissamine Rhodamine B 1,2 dihexadecanoyl sn-glycero-3-phosphatidylethanolaminetriethylammonium salt (Rh-PE) and Fluo 5N was from Molecular Probes. The protease inhibitor cocktail and Fatty acid free BSA were from Sigma. Bacterial Protein Extraction Reagent (B-PER) was from Pierce. Glutathione Sepharose 4B was from Amersham Biosciences.

Plasmids. All vectors were produced using standard procedures and verified by sequencing. Full length IP4P Iα3 was cloned into the baculoviral vector pAcGHLT-A (BD Biosciences Pharmingen). Using the Quick Change Site Directed Mutagenesis kit (SDM) (Stratagene) an Nde-I site was introduced at the 5′ end of pCDNA3 Iα3. The oligonucleotides were sense 5′-GCTTGATATCGCTAGCCATATGCACAGCAAGAGC ACAGCCC-3′ antisense 5′-GGGCTGTGCTCTTGCTGTGCATATGG CTAGCGATATCAAGC-3′. Nde-PCDNA3 Iα3 was subsequently cut with Nde-I, Not I and ligated into pAcGHLT-A. This vector was subsequently used as a template for all other constructs. All other constructs were produced using SDM. The initial C2 domain was created by producing a stop codon in pAcGHLT-A-Iα3 with the following oligos: sense-5′-GGAGGAGAAGTCAGACTAACGGCCCCCTGTGACC-3′, antisense-5′-GGTCACAGGGGGCCGTTAGTCTGACTTCTCCTCC-3′. All other mutants were created from pAcGHLT-A-type I IP4P C2 by SDM using the following oligonucleotides: (R62Q, K63N)-sense-5′-CTGCATACTCCATCGCTAGATCAGAACCCAAATAGTTTTGTTG CG-3′, antisense-5′-CGCAACAAAACTATTTGGGTTCTGATCTAGC GATGGAGTATGCAG-3′, (K123N, R125) sense-5′-CTCTCCGTGTA TGATGTCAACGATCAATCTCAGGGAACAATG-3′, antisense-5′-C ATTGTTCCCTGAGATTGATCGTTGACATCATACACGGAGAG-3′, (D121A, D124A) sense-5′-CTCTCCGTGTATGCTGTCAAAGCTAGA TCTCAGGG-3′, antisense-5′-CCCTGAGATCTAGCTTTGACAGCAT ACACGGAGAG-3′, 9D62A), sense-5′-GCATACTCCATCGCTAGCT CGAAAGCCAAATAG-3′, antisense-5′-CTATTTGGCTTTCGAGCTA GCGATGGAGTATGC-3′. All constructs were verified by sequencing at the Iowa State Sequencing Center. All constructs were transfected into *Sf-9* cells using Effectene transfection reagent (Qiagen) with 0.1 μg of linearized baculoviral DNA (BD-Pharmingen) for every 0.3 μg of plasmid. The virus was amplified as per manufacturers instructions.

Preparation of lipids. Fluorescent lipids were prepared as follows: Unless otherwise stated, 20% PtdIns(4,5)P_2, 79% (PC) and 1% Rh-PE were dried using a Savant SC110 Speed Vacuum, the lipids were subsequently resuspended in 10 mM Hepes, 100 mM NaCl pH 7.4, sonicated briefly and kept on ice until use.

Fluorescent lipid pull-down assay. Recombinant Syt C2A was purified by Glutathione Sepharose chromatography. The samples were subsequently mixed at 4 °C for 4 h, washed three times in 10 mM Hepes, 1% Triton X-100, 500 mM NaCl, 2 mM EDTA (pH 7.4), three times in 10 mM Hepes, 1% Triton X-100, 100 mM NaCl, 2 mM EDTA (pH 7.4) followed by three times in 10 mM Hepes, 100 mM NaCl, 2 mM EDTA (pH 7.4). Proteins were then quantified using a Coomassie blue stained 10% SDS–PAGE gel. All type I IP4 P C2 domain proteins were expressed in Sf-9 cells as described previously [20]. Flasks were infected for 3 days and subsequently lysed in 10 mM Hepes, 1% triton, 100 mM NaCl, 2 mM EDTA (pH 7.4) plus protease inhibitor cocktail. Following lysis, the protein was quantified on the beads and the beads were used in the following assay. For the lipid pull-down assay equal amounts of protein beads were incubated in 300 μl of lipid binding buffer (10 mM Hepes, 100 mM NaCl pH 7.4) plus the appropriate lipid. Each lipid mixture was 20% lipid, 79% PC and 1% Rh-PE unless otherwise stated. The beads were allowed to shake at 37 °C for 1.5 h. The beads were washed three times in lipid binding buffer and read on a Titertek fluoroscan II (EFLAB, Finland) microtiter plate reader Ex-544, Em-584. All calcium concentrations were determined using Fluo5 N and the Max Chelator program (http://www.stanford.edu/~cpatton/maxc.html). All graphs were created using Sigma Plot.

Ca^{2+} overlay assay. For each protein 2 μg was spotted on a nitrocellulose membrane pre-wet with Buffer A (50 mM Tris, 100 mM NaCl, 0.1 mM EDTA pH 7.4) using a Bio-Dot microfiltration apparatus (Bio-Rad). The membrane was incubated for 20 min with 70 nM $^{45}Ca^{2+}$ (Specific Activity 16 mCi/mg). Following incubation the membrane was washed briefly twice in Buffer A without EDTA, dried, and subjected to autoradiography overnight on Kodak X-ray film.

Results

Sequence analysis of the human type I IP4P C2 domain

Taking the data from the RPS Blast search and previously published reports, a manual alignment was constructed using known C2 domain alignments (Supplemental data) [7,18,21]. Based on this homology, we hypothesized that there is sufficient sequence similarity between conserved hydrophobic residues located in the β-strands and the overall alignment of conserved residues within the calcium binding regions (CBR) to suggest this domain is a C2 domain. The absence of homology with β-strand 1 in Syt C2A and the presence of homology with β-strand 8 in PLCδ1 indicate that the IP4P C2 domain is topology II.

Type I IP4P C2 contains some of the Asp residues (D61, D120) located in CBR1 and CBR3 that are known to coordinate calcium in other C2 domains such as synaptotagmin [11]. In addition, Asp123 is shifted one position from the Syt C2A Asp residue. Surrounding these Asp residues are positively charged Lys and Arg residues (R62, K63, K123, R125). In Syt C2A and PLCδ C2 the positively charged residues are involved in lipid binding [8,9]. The fact that not all of the Asp residues are conserved suggests that the mechanism of calcium binding of type I IP4P C2 will be different than Syt C2A and the IP4P C2 domain will also interact with lipids by a mechanism that is not similar to typical calcium binding C2 domains.

Calcium binding of type I IP4P C2

While many C2 domains such as Syt C2A and PLCδ C2 bind calcium, there are, however calcium-independent C2

domains [8,9,12,13,16]. To examine the ability of the type I IP4P C2-like domain to bind calcium we performed calcium overlay assays (Fig. 1A). A nitrocellulose membrane was spotted with type I IP4P C2, Syt C2A (positive control) and GST (negative control). Type I IP4P C2 binding with $^{45}Ca^{2+}$ was similar to that of Syt C2A. The mechanism of calcium binding was further explored by mutating D61A within CBR1 (Fig. 1B). In a calcium overlay assay the D61A mutant did not bind calcium supporting the role of negatively charged amino acids in CBR1 binding calcium. This data combined with previously published data showing that the type I IP4P C2 domain binds lipids both in vitro and in vivo supports the hypothesis that the type I IP4P C2 domain is a calcium-dependent lipid binding C2 domain [18].

Effects of calcium on IP4P type I C2 lipid binding

In order to evaluate the effect of calcium on lipid binding, we performed a lipid pull-down in the presence of EDTA or increasing amounts of calcium (Supplemental data). Calcium concentrations were determined using Fluo5N and the Max Chelator program. Surprisingly, chelation of calcium using 0.2 mM EDTA did not affect lipid binding. Moreover, as calcium concentrations were increased up to 1.0 mM, there was a significant decrease in lipid binding. This decrease however did not occur at physiologically relevant concentrations and is probably due to non-specific ionic interference. We also examined the effects of EDTA compared to EGTA and saw no difference in lipid binding (data not shown). The Syt C2A domain was used as a positive control. Lipid binding of Syt C2A was strongly inhibited by EDTA and was not inhibited by increasing concentrations of calcium consistent with data previously reported [22]. Thus, although the type I IP4P C2 domain will bind calcium, calcium is not involved in lipid binding.

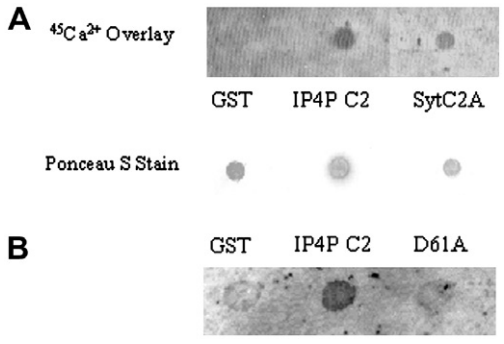

Fig. 1. Calcium binding of type I IP4P C2. (A) Overlay assay of $^{45}Ca^{2+}$ binding of GST-IP4P C2 compared to GST and GST-synaptotagmin C2A. Two micrograms of each protein was spotted on a nitrocellulose membrane and incubated with $^{45}Ca^{2+}$. Following autoradiography exposure, the membrane was stained with Ponceau Red. (B) Overlay assay of $^{45}Ca^{2+}$ binding of CBR1 mutant GST, GST-IP4P C2 and GST-D61A. Two micrograms of each protein was spotted on a nitrocellulose membrane and incubated with $^{45}Ca^{2+}$.

To further test the role of the CBRs in lipid binding, mutations were constructed to examine the role of the conserved Asp residues located in CBR1 and CBR3 (Fig. 2). In the lipid pull-down assay, the mutant D61A affecting CBR1 increased lipid binding by approximately 1.5-fold of the WT C2 domain. A double mutant D121A, D124A located in the predicted CBR3, increased lipid binding by approximately sixfold when compared to wild type. Thus, mutations that abolish negatively charged residues located within loops I and III enhance lipid binding.

Characterization of the mechanism of electrostatic lipid interaction of type I IP4P C2 domains

Previous evidence has shown that C2 domains can interact with lipid bilayers via several different mechanisms. These include electrostatic interactions involving basic residues, calcium-dependent electrostatic interactions and hydrophobic interactions [5,22]. In order to clarify the role of electrostatic interactions, we performed a lipid pull-down assay with increasing concentrations NaCl (Fig. 3). Binding of type I IP4P C2 to PtdIns(4,5)P_2 was decreased by approximately $50 \pm 18\%$ when the concentration of NaCl increased to 1 M. This result indicates lipid binding is partially through electrostatic interactions. Other calcium-dependent anionic lipid binding C2 domains such as Syt C2A and Piccolo exhibit primarily electrostatic lipid interactions [22,23].

In order to further characterize the electrostatic interaction, we examined the role of positively charged residues present in the CBRs. Lipid binding properties of the double

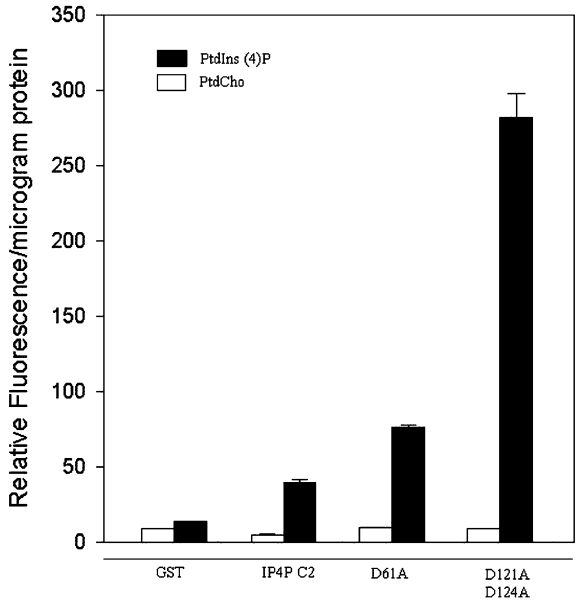

Fig. 2. Mutational analysis of type I IP4P C2 lipid binding/microgram of protein of CBR1 and CBR3 Asp mutants. Five micrograms of (IP4P C2 WT, (D61A), (D120A, D123A), or GST control beads were used for each assay. PC binding is in gray and PtdIns(4)P binding is in black. The data are mean with SE using three independent experiments.

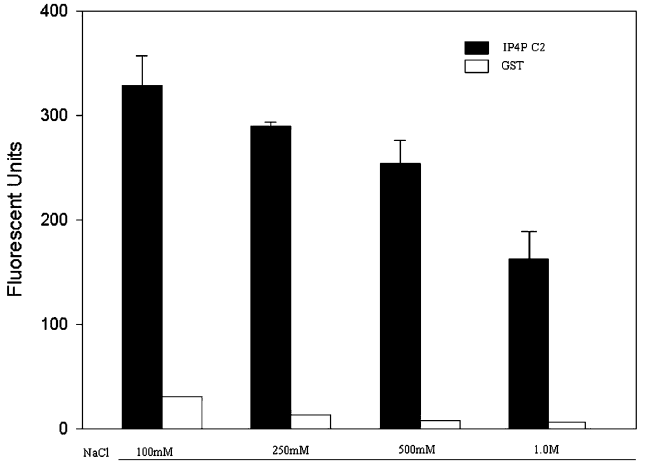

Fig. 3. Type I IP4P C2 binding to phospholipid vesicles, effects of NaCl. Effects of NaCl concentration on GST IP4P C2 beads lipid binding using 20% PtdIns(4,5)P_2 vesicles compared to GST beads. Lipid vesicles were prepared as described previously. For each assay, 5 μg of protein were used. IP4P C2 binding is shown in black bars, GST binding is shown in white.

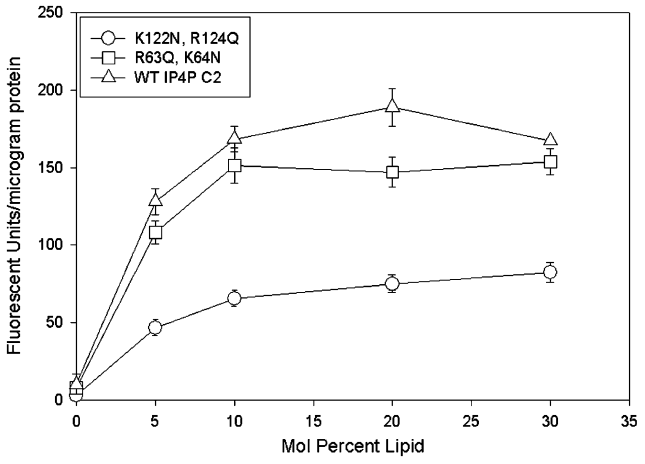

Fig. 4. Lipid binding/microgram of protein of CBR1 and CBR3 mutants. Five micrograms of IP4P C2, (R62N, K63Q), (K123N, R125Q) beads were used for each assay. The data are means with SE using three independent experiments.

mutant R62N, K63Q (CBR1) and the double mutant K123N, R125Q (CBR3) were examined in the presence of increasing mol percent lipids (Fig. 4). The CBR1 double mutant had very little effect but the CBR3 double mutant showed a decreased ability to bind lipids by approximately $50 \pm 12\%$. This is in agreement with the 50% decrease seen in Fig. 3 when examining the effect of increasing salt concentrations. This data provides evidence of the role of K123 and R125 in lipid binding.

Discussion

The majority of C2 domains bind lipids in a calcium-dependent mechanism [6]. However, there are examples of C2 domains such as those found in PKCδ, PKCε and the lipid phosphatase PTEN that do not require calcium to bind lipids [13–17]. In this report we describe a novel C2 domain that will bind calcium but does not require calcium for lipid interactions.

Analysis of the sequence of the type I IP4P C2 domain suggests that it is a topology II C2 domain. Although this domain only contains three out of five Asp residues frequently involved in calcium binding, we show that it binds calcium and that calcium binding can be abolished by a mutation of Asp residues within the predicted CBRs. This is consistent of a mechanism that is similar to other calcium binding C2 domains such as Syt C2A and PLCδ1 [5,7].

The addition of 1 M NaCl to the C2A domain of synaptotagmin I and other calcium-dependent C2 domains has been shown to bind lipids primarily through electrostatic interactions as the addition of NaCl has been shown to block lipid binding [5,11,22,23]. Lipid binding in the presence 1 M NaCl reduced binding by only 50% suggesting that the mechanism of lipid interaction is both electrostatic combined with non-electrostatic interactions. To evaluate which amino acids are involved in the lipid-binding interface, we mutated positively charged amino acids located in CBR1 and CBR3. Mutation of these residues in calcium-dependent and calcium-independent C2 domains reduces the ability to bind lipid [5,13]. When we mutated R62N, K63Q (CBR1) and K123N, R125Q (CBR3), lipid binding was only decreased in the CBR3 mutation. This data demonstrates that not all of the positively charged residues located in the loops are involved in lipid binding; however, positive residues located in CBR3 are important for forming a positively charged electrostatic surface for lipid interaction.

Cumulatively, these results suggest that lipid binding of the type I IP4P C2 domain does not require calcium. To gain additional insight concerning the role of calcium binding, mutations of Asp residues located within CBR1 and CBR3 were constructed. Analogous mutations of Asp residues have been shown to abolish lipid binding in other C2 domains [8]. When the lipid binding characteristics of D61A (CBR1) and the D121A, D124A (CBR3) double mutant were examined, enhanced lipid binding was observed. This suggests that lipid binding might be regulated by the elimination of the negative charges provided by the Asp residues in loops I and III. The greatest effect was seen in the CBR3 mutation indicating that it plays a major role in lipid interactions consistent with the earlier result of K123N, R125Q (CBR3) exhibiting decreased lipid binding.

Calcium has been shown to negatively regulate IP4P stability and phosphatase activity in the regulation of IP4P has previously been reported in platelets [24]. This regulation is performed by the calpain family of calcium-dependent proteases, which cleave type I IP4P following thrombin stimulation [24,25]. This study shows that the type I IP4P C2 domain binds calcium but that this binding

is not involved in lipid association. It does not, however, rule out calcium having a role in other regulatory mechanisms in type I 4-phosphatases.

In summary, based on sequence analysis in combination with biochemical analyses we have identified a calcium independent electrostatic mechanism for type I IP4P association with lipid membranes. We have also identified lysine and arginine residues involved in this association. These data provides insight on the mechanism of membrane targeting of type I IP4P. Further studies will examine the ability of these mutants to affect localization of type I IP4P to endosomal membranes.

Acknowledgments

We would like to thank Phytodyne Inc. for the use of the Titertek fluorimeter, Dr. Marit Nilsen-Hamilton for graciously allowing us the use of the Dot-blot, Dr. Yeon-Kyun Shin for the GST-SytC2A and Dr. David Senchina for providing the use of Sigma Plot.

This work was supported by Roy G. Carver Foundation Grant (02-99).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.02.115.

References

[1] F.A. Norris, V. Auethavekiat, P.W. Majerus, The isolation and characterization of cDNA encoding human and rat brain inositol polyphosphate 4-phosphatase, J. Biol. Chem. 270 (1995) 16128–16133.

[2] F.A. Norris, R.C. Atkins, P.W. Majerus, Inositol polyphosphate 4-phosphatase is inactivated by calpain-mediated proteolysis in stimulated human platelets, J. Biol. Chem. 272 (1997) 10987–10989.

[3] C.D. Ellson, S. Gobert-Gosse, K.E. Anderson, K. Davidson, H. Erdjument-Bromage, P. Tempst, J.W. Thuring, M.A. Cooper, Z.Y. Lim, A.B. Holmes, P.R. Gaffney, J. Coadwell, E.R. Chilvers, P.T. Hawkins, L.R. Stephens, PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40(phox), Nat. Cell Biol. 7 (2001) 679–682.

[4] A. Nystuen, M.E. Legare, L.D. Shultz, W.N. Frankel, A null mutation in inositol polyphosphate 4-phosphatase type I causes selective neuronal loss in weeble mutant mice, Neuron 32 (2001) 203–212.

[5] T.C. Sudhof, J. Rizo, C2-Domains in Ca^{2+} Signaling, Elsevier Academic Press, San Diego, 2003.

[6] J. Rizo, T.C. Sudhof, C2-domains, structure and function of a universal Ca^{2+} binding domain, J. Biol. Chem. 273 (1998) 15879–15882.

[7] E.A. Nalefski, J.J. Falke, The C2 domain calcium-binding motif: structural and functional diversity, Protein Sci. 5 (1996) 2375–2390.

[8] X. Zhang, J. Rizo, T.C. Sudhof, Mechanism of phospholipid binding by the C2A-domain of synaptotagmin I, Biochemistry 37 (1998) 12395–12403.

[9] O. Perisic, H.F. Paterson, G. Mosedale, S. Lara-Gonzalez, R.L. Williams, Mapping the phospholipid-binding surface and translocation determinants of the C2 domain from cytosolic phospholipase A2, J. Biol. Chem. 274 (1999) 14979–14987.

[10] B.A. Davletov, T.C. Sudhof, A single C2 domain from synaptotagmin I is sufficient for high affinity Ca^{2+}/phospholipid binding, J. Biol. Chem. 268 (1993) 26386–26390.

[11] R. Fernandez-Chacon, O.H. Shin, A. Konigstorfer, M.F. Matos, A.C. Meyer, J. Garcia, S.H. Gerber, J. Rizo, T.C. Sudhof, C. Rosenmund, Structure/function analysis of Ca^{2+} binding to the C2A domain of synaptotagmin 1, J. Neurosci. 22 (2002) 8438–8446.

[12] B. Davletov, O. Perisic, R.L. Williams, Calcium-dependent membrane penetration is a hallmark of the C2 domain of cytosolic phospholipase A2 whereas the C2A domain of synaptotagmin binds membranes electrostatically, J. Biol. Chem. 273 (1998) 19093–19096.

[13] S. Corbalan-Garcia, S. Sanchez-Carrillo, J. Garcia-Garcia, J.C. Gomez-Fernandez, Characterization of the membrane binding mode of the C2 domain of PKC epsilon, Biochemistry 42 (2003) 11661–11668.

[14] W.F. Ochoa, J. Garcia-Garcia, I. Fita, S. Corbalan-Garcia, N. Verdaguer, J.C. Gomez-Fernandez, Structure of the C2 domain from novel protein kinase Cepsilon. A membrane binding model for Ca(2+)-independent C2 domains, J. Mol. Biol. 311 (2001) 837–849.

[15] H. Pappa, J. Murray-Rust, L.V. Dekker, P.J. Parker, N.Q. McDonald, Crystal structure of the C2 domain from protein kinase C-delta, Structure 6 (1998) 885–894.

[16] J.O. Lee, H. Yang, M.M. Georgescu, A. Di Cristofano, T. Maehama, Y. Shi, J.E. Dixon, P. Pandolfi, N.P. Pavletich, Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association, Cell 99 (1999) 323–334.

[17] M.M. Georgescu, K.H. Kirsch, P. Kaloudis, H. Yang, N.P. Pavletich, H. Hanafusa, Stabilization and productive positioning roles of the C2 domain of PTEN tumor suppressor, Cancer Res. 60 (2000) 7033–7038.

[18] I. Ivetac, A.D. Munday, M.V. Kisseleva, X.M. Zhang, S. Luff, T. Tiganis, J.C. Whisstock, T. Rowe, P.W. Majerus, C.A. Mitchell, The type Ialpha inositol polyphosphate 4-phosphatase generates and terminates phosphoinositide 3-kinase signals on endosomes and the plasma membrane, Mol. Biol. Cell 16 (2005) 2218–2233.

[19] C.T. Shearn, J. Walker, F.A. Norris, Identification of a novel spliceoform of inositol polyphosphate 4-phosphatase type I alpha expressed in human platelets: structure of human inositol polyphosphate 4-phosphatase type I gene, Biochem. Biophys. Res. Commun. 286 (2001) 119–125.

[20] F.A. Norris, R.C. Atkins, P.W. Majerus, The cDNA cloning and characterization of inositol polyphosphate 4-phosphatase type II. Evidence for conserved alternative splicing in the 4-phosphatase family, J. Biol. Chem. 272 (1997) 23859–23864.

[21] A. Marchler-Bauer, J.B. Anderson, C. DeWeese-Scott, N.D. Fedorova, L.Y. Geer, S. He, D.I. Hurwitz, J.D. Jackson, A.R. Jacobs, C.J. Lanczycki, C.A. Liebert, C. Liu, T. Madej, G.H. Marchler, R. Mazumder, A.N. Nikolskaya, A.R. Panchenko, B.S. Rao, B.A. Shoemaker, V. Simonyan, J.S. Song, P.A. Thiessen, S. Vasudevan, Y. Wang, R.A. Yamashita, J.J. Yin, S.H. Bryant, CDD: a curated Entrez database of conserved domain alignments, Nucleic Acids Res. 31 (2003) 383–387.

[22] S.H. Gerber, J. Rizo, T.C. Sudhof, Role of electrostatic and hydrophobic interactions in a Ca2+ dependent phospholipid binding by the C2A domain from synaptotagmin I, Diabetes 51 (2002) S12–S18.

[23] J. Garcia, S.H. Gerber, S. Sugita, T.C. Sudhof, J. Rizo, A conformational switch in the Piccolo C2A domain regulated by alternative splicing, Nat. Struct. Mol. Biol. 11 (2004) 45–53.

[24] F.A. Norris, R.C. Atkins, P.W. Majerus, Inositol polyphosphate 4-phosphatase is inactivated by calpain-mediated proteolysis in stimulated human platelets, J. Biol. Chem. 272 (1997) 10987–10989.

[25] D.E. Goll, V.F. Thompson, H. Li, W. Wei, J. Cong, The Calpain system, Physiol. Rev. 83 (2003) 731–801.