PROSPECTS
Journal of Cellular Biochemistry 112:2203–2209 (2011)

Journal of Cellular Biochemistry

SHIP2 Multiple Functions: A Balance Between a Negative Control of PtdIns(3,4,5)P_3 Level, a Positive Control of PtdIns(3,4)P_2 Production, and Intrinsic Docking Properties

Christophe Erneux,* William's Elong Edimo, Laurence Deneubourg, and Isabelle Pirson

Institut de Recherche Interdisciplinaire (IRIBHM), Université Libre de Bruxelles, Campus Erasme, Bldg. C, 808 Route de Lennik, 1070 Brussels, Belgium

ABSTRACT

The SH2 domain containing inositol 5-phosphatase 2 (SHIP2) belongs to the family of the mammalian inositol polyphosphate 5-phosphatases. The two closely related isoenzymes SHIP1 (or SHIP) and SHIP2 contain a N-terminal SH2 domain, a catalytic domain, potential PTB domain-binding sites (NPXY), and C-terminal proline-rich regions with consensus sites for SH3 domain interactions. In addition, SHIP2 contains a unique sterile alpha motif (SAM) domain that could be involved in SAM–SAM domain interactions with other proteins or receptors. SHIP2 also shows the presence of an ubiquitin interacting motif at the C-terminal end. SHIP2 is essentially a PI(3,4,5)P_3 5-phosphatase that negatively controls PI(3,4,5)P_3 levels in intact cells and produce PI(3,4)P_2. Depending on the cells and stimuli, PI(3,4)P_2 could accumulate at important levels and be a "second messenger" by its own. It could interact with a very large number of target proteins such as PKB or TAPP1 and 2 that control insulin sensitivity. In addition to its catalytic activity, SHIP2 is also a docking protein for a large number of proteins: Cytoskeletal, focal adhesion proteins, scaffold proteins, adaptors, protein phosphatases, and tyrosine kinase associated receptors. These interactions could play a role in the control of cell adhesion, migration, or endocytosis of some receptors. SHIP2 could be acting independently of its phosphatase activity being part of a protein network of some receptors, e.g., the EGF receptor or BCR/ABL. These non-catalytic properties associated to a PI phosphatase have also been reported for other enzymes of the metabolism of myo-inositol such as Ins(1,4,5)P_3 3-kinases, inositol phosphate multikinase (IPMK), or PTEN. J. Cell. Biochem. 112: 2203–2209, 2011.

KEY WORDS: SHIP2; 5-PHOSPHATASE; PHOSPHATIDYLINOSITOL METABOLISM; SIGNAL TRANSDUCTION

Phosphatidylinositol, a membrane phospholipid, can be reversibly phosphorylated at the 3, 4, and 5 positions of myo-inositol to generate phosphoinositides (PIs). Seven are currently known in mammalian cells. Phosphoinositides play a fundamental role in cell physiology, signaling, and physiopathology [Blero et al., 2007; McCrea and De Camilli, 2009; Ooms et al., 2009]. Actually, the seven phosphoinositides display independent specific second messenger functions and therefore their production via PI kinases and removal via PI phosphatases are fundamental issues in cell signaling. PI phosphatases can act at the 3-, 4-, and 5-position of the inositol ring and a large number of enzymes, spliced and truncated isoforms that catalyze these reaction(s) have been reported in mammalian cells.

The most characterized product of PI 3-kinases is PI(3,4,5)P_3, a product of class I subclass of PI 3-kinases. It is a critical lipid second messenger that recruits key enzymes such as PDK1 or PKB which are directly involved in the control of growth, proliferation, and cell survival [Wong et al., 2010]. PI(3,4,5)P_3 is also critical in oncogenic transformation. Two dephosphorylation pathways of PI(3,4,5)P_3 occur in mammalian cells: Dephosphorylation at the 3-position of the inositol ring catalyzed by PTEN and at the 5-position catalyzed by the inositol polyphosphate 5-phosphatases. The role of PTEN as a tumor suppressor gene is well established [Chalhoub and Baker, 2009]. The inositol polyphosphate 5-phosphatases (10 isoenzymes in human cells) are also implicated in numerous human diseases (discussed in McCrea and De Camilli [2009]). PTEN and inositol 5-phosphatases are therefore attractive targets for therapeutic interventions.

The SH2 domain containing inositol 5-phosphatase SHIP1 (or SHIP) and SHIP2 contain a N-terminal SH2 domain, a catalytic domain, potential PTB domain-binding sites (NPXY), and C-terminal proline-rich regions with consensus sites for SH3 domain

Grant sponsor: Fonds de la Recherche Scientifique Médicale; Grant sponsor: Interuniversity Attraction Poles Programme (P6/28)—Belgium Sate—Belgian Science Policy.

*Correspondence to: Christophe Erneux, Institut de Recherche Interdisciplinaire (IRIBHM), Université Libre de Bruxelles, Campus Erasme, Bldg. C, 808 Route de Lennik, 1070 Brussels, Belgium. E-mail: cerneux@ulb.ac.be

Received 5 April 2011; Accepted 7 April 2011 • DOI 10.1002/jcb.23146 • © 2011 Wiley-Liss, Inc.
Published online 18 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

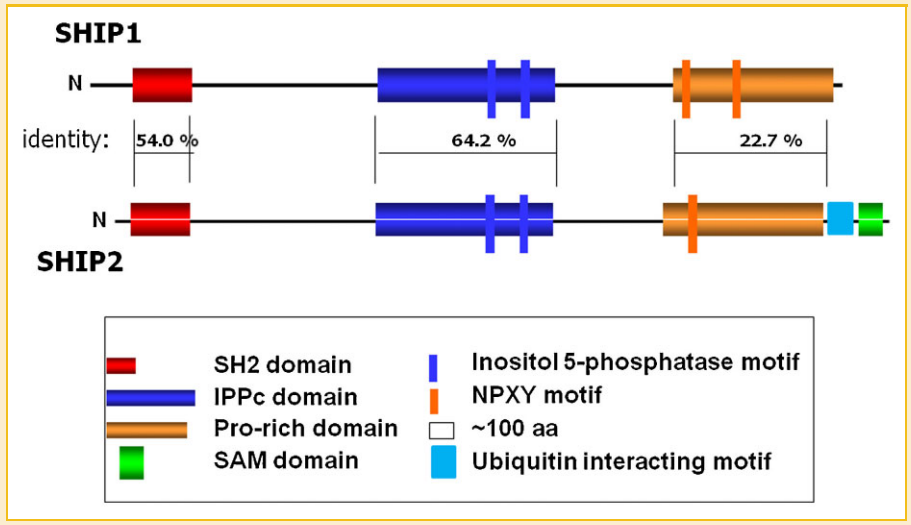

Fig. 1. The structures of human SHIP1 and SHIP2 and interacting domains.

interactions as well as N-terminal proline rich sequences (Fig. 1) [Backers et al., 2003]. The catalytic domain of each enzyme is relatively well conserved with the other isoforms of the inositol 5-phosphatase family. In addition, SHIP2 also contains a unique sterile alpha motif (SAM) domain that could be involved in SAM–SAM domain interactions particularly with the SAM domain of Arf-GAP, Rho-GAP, Ankyrin repeat and PH domain 3 (ARAP3), a GTPase activating protein [Raaijmakers et al., 2007], or that of the Ephrin A2 receptor [Zhuang et al., 2006]. Finally, an ubiquitin interacting motif at the C-terminal end of SHIP2 has been reported that confers ubiquitin binding capacity of the protein [De Schutter et al., 2009]. Both SHIP1/2 also show the presence of sequence areas with unusually high concentrations of proline, glutamic/aspartic acid, serine, and threonine, i.e., PEST sequences that might be target for calpain cleavage [Damen et al., 1998] or direct SHIPs to proteosomal degradation [Schnupf et al., 2006]. The substrate of SHIP2 is $PI(3,4,5)P_3$ and the reaction product is $PI(3,4)P_2$. Purified enzyme can also use $Ins(1,3,4,5)P_4$ (the product of $Ins(1,4,5)P_3$ 3-kinases and inositol phosphate multikinase (IPMK)) as substrate but with a very much reduced specific activity as compared to the ubiquitous type I $Ins(1,4,5)P_3$/$Ins(1,3,4,5)P_4$ 5-phosphatase. $PI(4,5)P_2$ was also identified as a substrate of SHIP2 and in one study, SHIP2 negatively regulates $PI(4,5)P_2$ levels in intact COS-7 cells [Nakatsu et al., 2010]. Based on many data in different models [Taylor et al., 2000], we and others have proposed that SHIPs are essentially $PI(3,4,5)P_3$ 5-phosphatases that negatively control $PI(3,4,5)P_3$ levels in intact cells and produce $PI(3,4)P_2$ [Blero et al., 2007].

SHIP1/2 are very much studied due to their implication in immune response, myeloid cell survival for SHIP1 and insulin signaling and the control of obesity for SHIP2 [Huber et al., 1999; Liu et al., 1999; Clement et al., 2001]. This was concluded from in vivo studies of knockout mice for the two genes. Recent studies also provided evidence suggesting that SHIP1 may act as a tumor suppressor during leukemogenesis and lymphomagenesis [Hamilton et al., 2011]. The SHIP2 function in insulin signaling in mice is probably not unique. SHIP2-null mice showed a distinctive truncated facial profile resulting from an abnormality in skeletal growth in that region [Sleeman et al., 2005]. Loss of *SHIP2* in zebrafish leads to an increased and expanded expression of outputs of FGF-mediated signaling [Jurynec and Grunwald, 2010]. A series of proteomic studies provided evidence of a role of SHIP2 in the signaling pathways of growth factors such as EGF or FGF [Olsen et al., 2006]. Together these three last reports suggest that SHIP2 is involved in many aspects of cell signaling in different cell models and human diseases such as diabetes, cancer, and artherosclerosis (for a review see Suwa et al. [2010]).

How do cells exert specific control of $PI(3,4,5)P_3$ levels via SHIP2 as compared to PTEN? Knowing that a large number of SHIP2 phospho sites have been identified, what is the role of SHIP2 phosphorylation, particularly on tyrosine? Is SHIP2 used as a mechanism to produce $PI(3,4)P_2$ (and related target proteins and associated second messenger function(s))? Is SHIP2 used as a docking protein at the vicinity of receptors, phosphatases or cytoskeletal proteins? Does this imply a role of SHIP2 unrelated to its phosphatase activity? Those questions will be discussed here.

SHIP2 TYROSINE PHOSPHORYLATION

SHIP2 tyrosine phosphorylation has been reported in response to growth factors (EGF, PDGF), insulin, immunoreceptors, cell attachment, and spreading in HeLa cells [Habib et al., 1998; Pesesse et al., 2001; Prasad et al., 2001]. It occurs essentially at two sites Tyr-986 and Tyr-1162 in human SHIP2 [Olsen et al., 2006]. This event has been associated to four different properties: (i) *Change in inositol phosphatase activity*: It was proposed that SHIP2 tyrosine phosphorylation in response to EGF or to vanadate may further activate SHIP2 phosphatase activity [Batty et al., 2007]. This remains controversed in many reports, e.g., in neuroblastoma cells where no change in activity occurred following SHIP2 tyrosine phosphoryla-

tion [Taylor et al., 2000]. It is possible that change in activity could be linked to a particularly large increase in tyrosine phosphorylated SHIP2 in vanadate treated cells, a situation which is not observed in insulin or EGF stimulated cells (WEE and CE, unpublished data); (ii) *change in subcellular localization*: In most cells, SHIP2 localization in serum starved cells is perinuclear [Deneubourg et al., 2009]: Short-term cell stimulation by EGF or serum (5 min) often results in the translocation of SHIP2 to the plasma membrane, i.e., at the site of PI(3,4,5)P_3 production. This was observed, e.g., in HeLa cells and could be correlated with SHIP2 phosphorylation on tyrosine (Fig. 2). Another possibility is that membrane translocation of SHIP2 is necessary for its tyrosine phosphorylation by Src kinases as shown in transformed myeloid cells. Also in this case, tyrosine phosphorylation of SHIP2 serves as a correlate of SHIP2 activation; (iii) *change in protein–protein interaction*: Tyrosine phosphorylation has also been found responsible for the recruitment of adaptors such as Shc to SHIP2 in p210(BCR/ABL)-expressing hematopoietic cells [Wisniewski et al., 1999]. This may not be general while much more cell-specific; (iv) *change in SHIP2 mono-ubiquitination*: SHIP2 mono-ubiquitination has been reported. In COS-7 cells, it was maximal at 30 min stimulation by EGF and no longer observed after transfection of a mutant of SHIP2 that could not be phosphorylated on Y986. The data suggest that SHIP2 Y986 phosphorylation may trigger a conformational change that promotes SHIP2 mono-ubiquitination in COS-7 cells [De Schutter et al., 2009].

Finally, it is worth mentioning that SHIP2 serine and threonine phosphorylation have been reported in large scale proteomic studies (see PhosphositePlus). Whether this could have an impact on SHIP2 function is currently not known.

PtdIns(3,4,5)P_3 PHOSPHATASE ACTIVITY AND PtdIns(3,4)P_2 FORMATION

A major impact of SHIP2 in cells is to negatively control the PtdIns(3,4,5)P_3/PKB pathway and therefore to promote cell survival [Yu et al., 2008]. In this context, evidence has been provided in mouse embryonic fibroblasts (MEFs) and other cells that SHIP2 acts on agonist-provoked stimulated levels of PI(3,4,5)P_3, whereas PTEN acts at basal level and is still active after long-term stimulation [Blero et al., 2007]. In addition to this negative control, SHIP2 is also producing PtdIns(3,4)P_2 (particularly in response to PDGF, vanadate, or H_2O_2) [Batty et al., 2007]. SHIP2 is, therefore, an enzymatic pathway for PtdIns(3,4)P_2 and further metabolism to PI(3)P that function as second messengers [Ivetac et al., 2005]. Inositol polyphosphate 4-phosphatase-II (INPP4B) an enzyme that acts on PtdIns(3,4)P_2 as substrate is a tumor suppressor in epithelial carcinomas and is lost in some human basal-like breast cancers [Fedele et al., 2010]. This indicates that PtdIns(3,4)P_2 is tightly regulated and may accumulate at different levels depending on the cells. This could explain the observation of pro-oncogenic and anti-cancer effects of SHIP2 in different cell models as PtdIns(3,4)P_2 is also an activator of PKB [Suwa et al., 2010]. The importance of PtdIns(3,4)P_2 and second messenger function is also underscored by the recent genetic evidence of a role of TAPP1 (tandem pleckstrin homology domain containing protein-1) and TAPP2 adaptors binding to PtdIns(3,4)P_2 in regulating whole body insulin sensitivity in mice [Wullschleger et al., 2011]. In this case, PtdIns(3,4,5)P_3 is controlled at two steps: directly via SHIP2 phosphatase activity and indirectly via PtdIns(3,4)P_2 production and a negative feedback loop

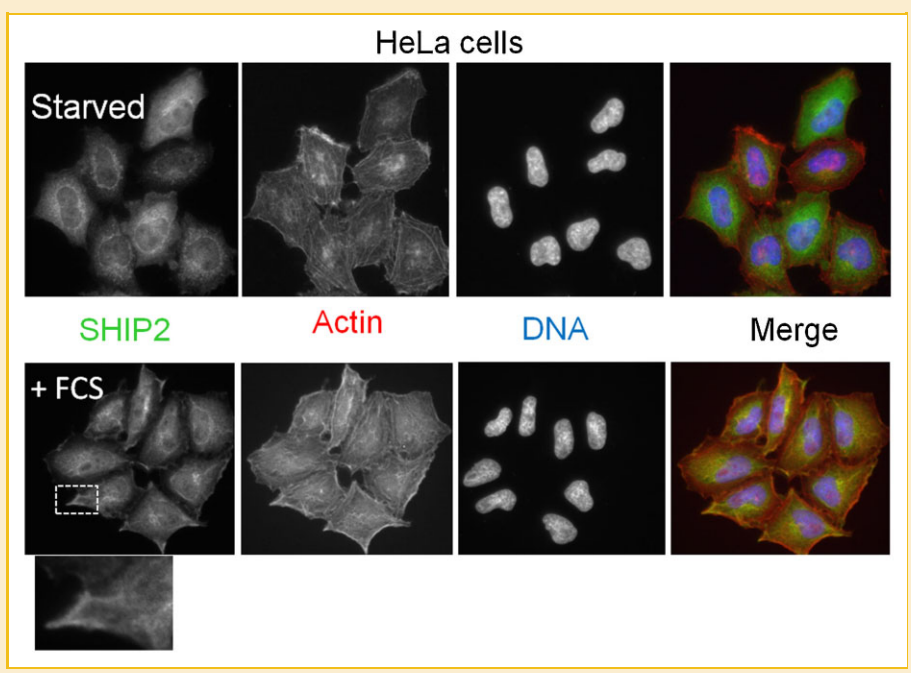

Fig. 2. SHIP2 immunostaining in HeLa cells in starved and after serum (FCS) stimulation (5 min). SHIP2 (green) was stained with the Abcam SHIP2 antibody and a secondary DyLight488 anti rabbit antibody. Actin cytoskeleton was stained with phalloidin–Alexa Fluor594 (red). Nuclei were stained with Hoechst in blue.

that down-regulates the insulin and PI 3-kinase network. Finally, catalytic activity of SHIP2 is important for efficient lamellipodia formation and cell spreading in HeLa cells [Prasad et al., 2001]. It is possible here that PtdIns(3,4)P_2 interacts with specific proteins in this model.

SHIP2 INTERACTION IN A NETWORK OF CYTOSKELETAL PROTEINS, PROTEIN PHOSPHATASES, AND TYROSINE KINASE RECEPTORS (Table I)

An interaction between SHIP1 and the actin cytoskeleton was initially observed in human platelets stimulated by thrombin: Tyrosine phosphorylated SHIP1 was relocated to the cytoskeleton upon activation in an aggregation and integrin engagement-dependent manner. The same relocation event was observed with SHIP2, except that SHIP2 exhibited a higher affinity for the cytoskeleton network, even in resting platelets [Giuriato et al., 2003]. The interaction between SHIP2 cytoskeletal and focal adhesion proteins has been widely reported in many different cells: Filamin, the c-Cbl-associated protein (CAP), $p130^{Cas}$, c-Cbl, vinexin, or ARAP3 [Dyson et al., 2001; Prasad et al., 2001; Vandenbroere et al., 2003; Paternotte et al., 2005; Raaijmakers et al., 2007]. Other interactors involve adaptors (DOK1), APS, immunoreceptors, and some growth factor receptors particularly the EGF receptor (Table I). Finally, two reports have pointed out an interaction between SHIP2 and protein phosphatases either directly or indirectly: (i) In vitro substrate trapping identified eight proteins bound to the active site of protein-tyrosine phosphatase 1B (*PTP1B*): SHIP2 was one of the identified substrate [Mertins et al., 2008] and (ii) mass spec analysis identified PR130/B''α (PR130) regulatory B-type subunit of protein phosphatase 2A (PP2A) as another interaction partner of SHIP2 [Zwaenepoel et al., 2010]. Together, the data suggest possible regulation of Tyr, Ser, or Thr phosphorylation of SHIP2 particularly in response to EGF.

An interesting concept was recently proposed in the study of the SHIP2 interactor intersectin 1. This scaffold protein was initially identified by yeast two hybrid-based study and at endogenous level (e.g., in C2C12 cells) by reciprocal co-immunoprecipitation between SHIP2 and intersectin [Xie et al., 2008b]. It was subsequently confirmed in rat brain in pull down experiments using a construct of the proline rich region of SHIP2 stretching from amino acid 1022–1078 [Nakatsu et al., 2010]. SHIP2 was localized at endocytic clathrin coated pits an effect which is, at least in part, due to the interaction of intersectin and specific amino acids at SHIP2 C-terminal proline rich sequences (amino acids 1050–1054). Mutation of this motif in SHIP2 prevented the interaction and this SHIP2 mutant shows a diffuse localization with some accumulation at focal adhesions [Nakatsu et al., 2010]. This result is interesting as it illustrates the impact of SHIP2 protein partner(s) to specific cell localization. The presence or absence of an established protein partner (e.g., filamin, vinexin, or intersectin) may explain some specificity of SHIP2 localization in a given cell. In the same context, data have been reported that the identity of SHIP2 interactors, identified by co-immunoprecipitation, does vary according to the cell context: e.g., SHIP2 does not associate with Shc in IgE-activated RBL mast cells but it does in A20 B cells [Leung and Bolland, 2007]. If generalized to the SHIP2 interactome, this would provide specificity in SHIP2 localization and function depending on the cell model.

TABLE I. Identified SHIP2 Interacting Proteins

SHIP2 protein interactors	Method used for identification
Receptors	
EGF	co-immunoprecipitation [Pesesse et al., 2001]
PDGF	co-immunoprecipitation [Taylor et al., 2000]
FcγRIIb (ITIM)	co-immunoprecipitation [Muraille et al., 2000]
FcγRIIA (ITAM)	co-immunoprecipitation [Pengal et al., 2003]
CSF	mass spec [Wisniewski et al., 1999]
cMET	yeast two hybrid [Koch et al., 2005]
Abl	mass spec [Brehme et al., 2009]
Ephrin A2	yeast two hybrid [Zhuang et al., 2006]
Adaptors/regulators	
Shc	co-immunoprecipitation [Habib et al., 1998]
$p130^{Cas}$	co-immunoprecipitation [Prasad et al., 2001]
p85α	mass spec [Brehme et al., 2009]
APS	yeast two hybrid [Onnockx et al., 2008]
DOK1	mass spec [Cunningham et al., 2010]
JIP1	yeast two hybrid [Xie et al., 2008a]
Cytoskeletal proteins and/or regulators of endocytosis	
c-Cbl	yeast two hybrid [Vandenbroere et al., 2003]
vinexin	yeast two hybrid [Paternotte et al., 2005]
filamin	yeast two hybrid [Dyson et al., 2001]
intersectin 1	yeast two hybrid [Xie et al., 2008b]
ARAP-3	yeast two hybrid [Raaijmakers et al., 2007]
CIN-85	mass spec [Havrylov et al., 2009]
Protein phosphatases and subunits	
PTP1B	mass spec [Mertins et al., 2008]
PR130	mass spec [Zwaenepoel et al., 2010]

The identification of interactors is largely depending on the cell model and may not be general. It also refers to specific methods for identification as indicated.

SHIP2 IN A NETWORK OF NON-CATALYTIC MECHANISMS

SHIP2 silencing in HeLa cells leads to enhanced EGF receptor degradation, ubiquitination, and increased association of the EGF receptor with c-Cbl [Prasad and Decker, 2005]. In another cell model, in MDA-231 breast cancer cells, lowering endogenous SHIP2 expression decreases ligand-induced endocytosis of the Ephrin A2 receptor [Prasad, 2009]. In this model, SHIP2 promotes cell migration and this effect is associated to sustain EGF receptor–PKB signaling. Cytoskeletal abnormalities were also seen in SHIP2 knock down of HeLa cells. The correct localization of SHIP2 could lead to the generation of local pools of PtdIns(3,4)P_2, thereby regulating the activity of guanine nucleotide exchange factors specific for Rho family of proteins [Raftopoulou and Hall, 2004]. Another possibility is that SHIP2 is acting as a scaffold for its interaction to cytoskeletal proteins. To give two examples, in macrophages incubated with IgG-coated SRBCs, SHIP2 can be recruited to the site of phagocytosis (the phagocytic cup) and colocalize with F-actin [Ai et al., 2006]. In K562 cells, an interaction proteome screen of BCR/ABL revealed only seven major core components including SHIP2. SHIP2 association to BCR/ABL may thereby provide a pathway for actin remodeling [Brehme et al., 2009].

The c-Jun NH2-terminal kinase (JNK)-interacting protein 1 (JIP1) was identified as a protein partner of SHIP2 in a yeast two hybrid screening [Xie et al., 2008a]. In transfection studies in COS-7 cells, it was shown that SHIP2 could modulate JIP-1 mediated JNK pathway, particularly JIP-1 tyrosine phosphorylation. The effect was still observed by the use of a catalytic mutant of SHIP2 instead of wild type SHIP2. These data are consistent with a role of SHIP2 docking properties independently of its catalytic activity provided that the levels of $PI(3,4,5)P_3$ are sufficient to be recognized (and used) as substrate for SHIP2. Finally, SHIP2 is a negative regulator of clathrin coated pits growth [Nakatsu et al., 2010]. Again, although local pools of phosphoinositides could participate in the mechanism, it is possible that docking properties of SHIP2 are also involved here, particularly with the scaffold protein intersectin.

It is of interest that the same concepts also apply for SHIP1: SHIP1 appeared to be required for platelet contractility and thrombus organization. In this model, SHIP1 may act on the actin cytoskeleton organization both via its docking properties and via its $PI(3,4,5)P_3$ 5-phosphatase activity [Severin et al., 2007].

GENERALIZATION TO OTHER SIGNALLING ENZYMES ACTING ON INOSITOL PHOSPHATES OR PI

Inositol phosphate kinases are involved at multiple steps of the myo-inositol cycle to phosphorylate inositol phosphates starting from $Ins(1,4,5)P_3$ up to InsP5, InsP6, InsP7, and InsP8. Inositol trisphosphate 3-kinase A (Itpka), one of the isoenzyme in the inositol trisphosphate 3-kinase family (specific for the 3-position), acts on $Ins(1,4,5)P_3$ as substrate and catalyzes the production of $Ins(1,3,4,5)P_4$. This enzyme shows a high affinity for F-actin at its N-terminal end and is very much enriched in dendritic spines in the hippocampus. Overexpression of Itpka in the lung carcinoma cell line H1299 induces the formation of actin-based cell protrusions and increases migration. This indicates that the cells lose their ability to form actin stress fibers and show increased invasive migration properties [Windhorst et al., 2008]. These effects are mediated by non-enzymatic activities of Itpka. Inositol phosphate multikinase (IPMK) can also act on $Ins(1,4,5)P_3$ as substrate to form $Ins(1,4,5,6)P_4$ and subsequently $Ins(1,3,4,5,6)P_5$. IPMK appears to be a physiological mTOR cofactor, by controlling mTORC1 stability and amino acid-induced mTOR signaling. IPMK stabilizes mTOR-raptor binding in the mTORC1 complex. Data obtained in IPMK-depleted MEFs indicated that the action of IPMK on mTOR is independent of its catalytic activity [Kim et al., 2011]. SHIP1 inhibits CD95/APO-1/Fas-induced apoptosis in T lymphocytes unexpectedly, by promoting CD95 N-glycosylation independently of its phosphatase activity [Charlier et al., 2010]. Finally, phosphatase-independent activity of nuclear PTEN regulates the anaphase-promoting complex (APC)-CDH1 also known as a tumor-suppressive complex. This observation provides an explanation for the tumor-suppressive activity that can be seen with catalytically inactive PTEN [Song et al., 2011]. Together, these four examples provide evidence that both inositol phosphatases and kinases of the metabolism of PI have additional important roles unrelated to their enzymatic activities but in relation to docking properties to particular domains of the protein.

CONCLUSIONS

Considering the important role of tyrosine phosphorylation in cell signaling, we have discussed the consequences of SHIP2 tyrosine phosphorylation. It is often assumed (not always demonstrated) that SHIP1 and SHIP2 tyrosine phosphorylation could be an index of phosphatase activation [Batty et al., 2007]. Alternatively, two major mechanisms could be triggered by tyrosine phosphorylation: Firstly, the interaction between the phosphatase and a protein interactor that could be modulated by phosphorylation. This was already proposed for the PI 5-phosphatase OCRL and the Rab5 effector APPL1 phosphorylated by cAMP dependent protein kinase [Erdmann et al., 2007]. Secondly, tyrosine phosphorylation could serve as a signal for adjusting SHIP2 level. SHIP1 tyrosine phosphorylation promotes its polyubiquitination thereby adjusting SHIP1 level. The same mechanism may apply for SHIP2: in macrophages, IL-4 triggers the tyrosine phosphorylation and proteasomal degradation of both SHIP1 and SHIP2 [Ruschmann et al., 2010].

We have shown that, as a result of its structure, SHIP2 is clearly more complex and integrates multiple activities. SHIP2 function in intact cells recapitulates at least two major control mechanisms: Post-translational modifications (e.g., phosphorylation or ubiquitination) and interaction with specific protein interactors. Those identified interactors are cytoskeletal proteins, regulators of endocytosis, tyrosine kinase receptors, adaptors, and protein phosphatases therefore providing a link between a PI phosphatase and many aspects of cell signaling.

A possible structural model of SHIP2 involves three conformational states: a silent perinuclear state, an active state due to the presence of protein interactors, phosphorylation events, ubiquitination or enzyme–phospholipid interactions, and a third state that includes SHIP2 docking properties only (Fig. 3). Those properties of SHIP2 could influence localization of the enzyme as well as the ratio between individual phosphoinositides ($PI(3,4,5)P_3$ and $PI(3,4)P_2$) that cooperate in PKB activation. We have stressed the importance of potential SHIP2 non-catalytical mechanisms, their implication in

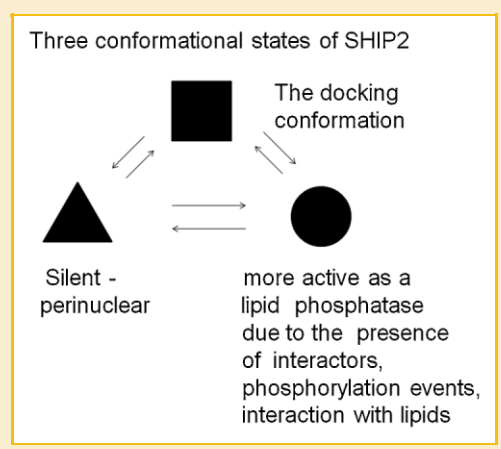

Fig. 3. Hypothetical model of SHIP2 in intact cells. The existence of three conformational states of SHIP2 is proposed as indicated.

cell migration and spreading. Those non-catalytic mechanisms of SHIP2 could be generalized to other enzymes of the inositol cycle such as the IPMK, SHIP1, and PTEN. It could be speculated that more examples of such activities will be reported in the future. These non-catalytic functions may prove to be fundamental in cell signaling.

ACKNOWLEDGMENTS

This work was supported by a grant from the "Fonds de la Recherche Scientifique Médicale" (FRSM) and by a grant of the Interuniversity Attraction Poles Programme (P6/28)—Belgium Sate—Belgian Science Policy to CE. LD and WE are supported by a Télévie fellowship, the Fonds Van Buuren or Hoguet. The authors would like to thank Drs. Bernard Payrastre, Stéphane Schurmans, Jean-Marie Vanderwinden, and Geoffrey Gloire for many helpful discussions. In efforts to adhere to the journal's policy the authors had to limit the number of references. They apologize for this.

REFERENCES

Ai J, Maturu A, Johnson W, Wang Y, Marsh CB, Tridandapani S. 2006. The inositol phosphatase SHIP-2 down-regulates FcgammaR-mediated phagocytosis in murine macrophages independently of SHIP-1. Blood 107:813–820.

Backers K, Blero D, Paternotte N, Zhang J, Erneux C. 2003. The termination of PI3K signalling by SHIP1 and SHIP2 inositol 5-phosphatases. Adv Enzyme Regul 43:15–28.

Batty IH, Van der KJ, Gray A, Telfer JF, Dixon MJ, Downes CP. 2007. The control of phosphatidylinositol 3,4-bisphosphate concentrations by activation of the Src homology 2 domain containing inositol polyphosphate 5-phosphatase 2, SHIP2. Biochem J 407:255–266.

Blero D, Payrastre B, Schurmans S, Erneux C. 2007. Phosphoinositide phosphatases in a network of signalling reactions. Pflugers Arch 455:31–44.

Brehme M, Hantschel O, Colinge J, Kaupe I, Planyavsky M, Köcher T, Mechtler K, Bennett KL, Superti-Furga G. 2009. Charting the molecular network of the drug target Bcr-Abl. Proc Natl Acad Sci USA 106:7414–7419.

Chalhoub N, Baker SJ. 2009. PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol 4:127–150.

Charlier E, Conde C, Zhang J, Deneubourg L, Di VE, Rahmouni S, Chariot A, Agostinis P, Pang PC, Haslam SM, Dell A, Penninger J, Erneux C, Piette J, Gloire G. 2010. SHIP-1 inhibits CD95/APO-1/Fas-induced apoptosis in primary T lymphocytes and T leukemic cells by promoting CD95 glycosylation independently of its phosphatase activity. Leukemia 24:821–832.

Clement S, Krause U, Desmedt F, Tanti JF, Behrends J, Pesesse X, Sasaki T, Penninger J, Doherty M, Malaisse W, Dumont JE, Marchand-Brustel Y, Erneux C, Hue L, Schurmans S. 2001. The lipid phosphatase SHIP2 controls insulin sensitivity. Nature 409:92–97.

Cunningham DL, Sweet SM, Cooper HJ, Heath JK. 2010. Differential phosphoproteomics of fibroblast growth factor signaling: Identification of Src family kinase-mediated phosphorylation events. J Proteome Res 9:2317–2328.

Damen JE, Liu L, Ware MD, Ermolaeva M, Majerus PW, Krystal G. 1998. Multiple forms of the SH2-containing inositol phosphatase, SHIP, are generated by C-terminal truncation. Blood 92:1199–1205.

De Schutter J, Guillabert A, Imbault V, Degraef C, Erneux C, Communi D, Pirson I. 2009. SHIP2 (SH2 domain-containing inositol phosphatase 2) SH2 domain negatively controls SHIP2 monoubiquitination in response to epidermal growth factor. J Biol Chem 284:36062–36076.

Deneubourg L, Vanderwinden JM, Erneux C. 2009. Regulation of SHIP2 function through plasma membrane interaction. Adv Enzyme Regul 50:262–271.

Dyson JM, O'Malley CJ, Becanovic J, Munday AD, Berndt MC, Coghill ID, Nandurkar HH, Ooms LM, Mitchell CA. 2001. The SH2-containing inositol polyphosphate 5-phosphatase, SHIP-2, binds filamin and regulates submembraneous actin. J Cell Biol 155:1065–1079.

Erdmann KS, Mao Y, McCrea HJ, Zoncu R, Lee S, Paradise S, Modregger J, Biemesderfer D, Toomre D, De CP. 2007. A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway. Dev Cell 13:377–390.

Fedele CG, Ooms LM, Ho M, Vieusseux J, O'Toole SA, Millar EK, Lopez-Knowles E, Sriratana A, Gurung R, Baglietto L, Giles GG, Bailey CG, Rasko JE, Shields BJ, Price JT, Majerus PW, Sutherland RL, Tiganis T, McLean CA, Mitchell CA. 2010. Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. Proc Natl Acad Sci USA 107:22231–22236.

Giuriato S, Pesesse X, Bodin S, Sasaki T, Viala C, Marion E, Penninger J, Schurmans S, Erneux C, Payrastre B. 2003. SH2-containing inositol 5-phosphatases 1 and 2 in blood platelets: Their interactions and roles in the control of phosphatidylinositol 3,4,5-trisphosphate levels. Biochem J 376:199–207.

Habib T, Hejna JA, Moses RE, Decker SJ. 1998. Growth factors and insulin stimulate tyrosine phosphorylation of the 51C/SHIP2 protein. J Biol Chem 273:18605–18609.

Hamilton MJ, Ho VW, Kuroda E, Ruschmann J, Antignano F, Lam V, Krystal G. 2011. Role of SHIP in cancer. Exp Hematol 39:2–13.

Havrylov S, Rzhepetskyy Y, Malinowska A, Drobot L, Redowicz MJ. 2009. Proteins recruited by SH3 domains of Ruk/CIN85 adaptor identified by LC-MS/MS. Proteome Sci 7:21.

Huber M, Helgason CD, Damen JE, Scheid M, Duronio V, Liu L, Ware MD, Humphries RK, Krystal G. 1999. The role of SHIP in growth factor induced signalling. Prog Biophys Mol Biol 71:423–434.

Ivetac I, Munday AD, Kisseleva MV, Zhang XM, Luff S, Tiganis T, Whisstock JC, Rowe T, Majerus PW, Mitchell CA. 2005. The type Ialpha inositol polyphosphate 4-phosphatase generates and terminates phosphoinositide 3-kinase signals on endosomes and the plasma membrane. Mol Biol Cell 16:2218–2233.

Jurynec MJ, Grunwald DJ. 2010. SHIP2, a factor associated with diet-induced obesity and insulin sensitivity, attenuates FGF signaling in vivo. Dis Model Mech 3:733–742.

Kim S, Kim SF, Maag D, Maxwell MJ, Resnick AC, Juluri KR, Chakraborty A, Koldobskiy MA, Cha SH, Barrow R, Snowman AM, Snyder SH. 2011. Amino acid signaling to mTOR mediated by inositol polyphosphate multikinase. Cell Metab 13:215–221.

Koch A, Mancini A, El BO, Tamura T. 2005. The SH2-domian-containing inositol 5-phosphatase (SHIP)-2 binds to c-Met directly via tyrosine residue 1356 and involves hepatocyte growth factor (HGF)-induced lamellipodium formation, cell scattering and cell spreading. Oncogene 24:3436–3447.

Leung WH, Bolland S. 2007. The inositol 5'-phosphatase SHIP-2 negatively regulates IgE-induced mast cell degranulation and cytokine production. J Immunol 179:95–102.

Liu QR, Sasaki T, Kozieradzki I, Wakeham A, Itie A, Dumont DJ, Penninger JM. 1999. SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. Gene Dev 13:786–791.

McCrea HJ, De Camilli P. 2009. Mutations in phosphoinositide metabolizing enzymes and human disease. Physiology (Bethesda) 24:8–16.

Mertins P, Eberl HC, Renkawitz J, Olsen JV, Tremblay ML, Mann M, Ullrich A, Daub H. 2008. Investigation of protein-tyrosine phosphatase 1B function by quantitative proteomics. Mol Cell Proteom 7:1763–1777.

Muraille E, Bruhns P, Pesesse X, Daeron M, Erneux C. 2000. The SH2 domain containing inositol 5-phosphatase SHIP2 associates to the immunoreceptor tyrosine-based inhibition motif of Fc gammaRIIB in B cells under negative signaling. Immunol Lett 72:7–15.

Nakatsu F, Perera RM, Lucast L, Zoncu R, Domin J, Gertler FB, Toomre D, De CP. 2010. The inositol 5-phosphatase SHIP2 regulates endocytic clathrin-coated pit dynamics. J Cell Biol 190:307–315.

Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M. 2006. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 127:635–648.

Onnockx S, De Schutter J, Blockmans M, Xie JW, Jacobs C, Vanderwinden JM, Erneux C, Pirson I. 2008. The association between the SH2-Containing inositol polyphosphate 5-phosphatase 2 (SHIP2) and the adaptor protein APS has an impact on biochemical properties of both partners. J Cell Physiol 214:260–272.

Ooms LM, Horan KA, Rahman P, Seaton G, Gurung R, Kethesparan DS, Mitchell CA. 2009. The role of the inositol polyphosphate 5-phosphatases in cellular function and human disease. Biochem J 419:29–49.

Paternotte N, Zhang J, Vandenbroere I, Backers K, Blero D, Kioka N, Vanderwinden JM, Pirson I, Erneux C. 2005. SHIP2 interaction with the cytoskeletal protein Vinexin. FEBS J 272:6052–6066.

Pengal RA, Ganesan LP, Fang HQ, Marsh CB, Anderson CL, Tridandapani S. 2003. SHIP-2 inositol phosphatase is inducibly expressed in human monocytes and serves to regulate Fc gamma receptor-mediated signaling. J Biol Chem 278:22657–22663.

Pesesse X, Dewaste V, De Smedt F, Laffargue M, Giuriato S, Moreau C, Payrastre B, Erneux C. 2001. The Src homology 2 domain containing inositol 5-phosphatase SHIP2 is recruited to the epidermal growth factor (EGF) receptor and dephosphorylates phosphatidylinositol 3,4,5-trisphosphate in EGF-stimulated COS-7 cells. J Biol Chem 276:28348–28355.

Prasad N, Topping RS, Decker SJ. 2001. SH2-containing inositol 5'-phosphatase SHIP2 associates with the p130(Cas) adapter protein and regulates cellular adhesion and spreading. Mol Cell Biol 21:1416–1428.

Prasad NK. 2009. SHIP2 phosphoinositol phosphatase positively regulates EGFR-Akt pathway, CXCR4 expression, and cell migration in MDA-MB-231 breast cancer cells. Int J Oncol 34:97–105.

Prasad NK, Decker SJ. 2005. SH2-containing 5'-inositol phosphatase, SHIP2, regulates cytoskeleton organization and ligand-dependent down-regulation of the epidermal growth factor receptor. J Biol Chem 280:13129–13136.

Raaijmakers JH, Deneubourg L, Rehmann H, de KJ, Zhang Z, Krugmann S, Erneux C, Bos JL. 2007. The PI3K effector Arap3 interacts with the PI(3,4,5)P3 phosphatase SHIP2 in a SAM domain-dependent manner. Cell Signal 19:1249–1257.

Raftopoulou M, Hall A. 2004. Cell migration: Rho GTPases lead the way. Dev Biol 265:23–32.

Ruschmann J, Ho V, Antignano F, Kuroda E, Lam V, Ibaraki M, Snyder K, Kim C, Flavell RA, Kawakami T, Sly L, Turhan AG, Krystal G. 2010. Tyrosine phosphorylation of SHIP promotes its proteasomal degradation. Exp Hematol 38:392–402.

Schnupf P, Portnoy DA, Decatur AL. 2006. Phosphorylation, ubiquitination and degradation of listeriolysin O in mammalian cells: Role of the PEST-like sequence. Cell Microbiol 8:353–364.

Severin S, Gratacap MP, Lenain N, Alvarez L, Hollande E, Penninger JM, Gachet C, Plantavid M, Payrastre B. 2007. Deficiency of Src homology 2 domain-containing inositol 5-phosphatase 1 affects platelet responses and thrombus growth. J Clin Invest 117:944–952.

Sleeman MW, Wortley KE, Lai KM, Gowen LC, Kintner J, Kline WO, Garcia K, Stitt TN, Yancopoulos GD, Wiegand SJ, Glass DJ. 2005. Absence of the lipid phosphatase SHIP2 confers resistance to diary obesity. Nat Med 11:199–205.

Song MS, Carracedo A, Salmena L, Song SJ, Egia A, Malumbres M, Pandolfi PP. 2011. Nuclear PTEN regulates the APC-CDH1 tumor-suppressive complex in a phosphatase-independent manner. Cell 144:187–199.

Suwa A, Kurama T, Shimokawa T. 2010. SHIP2 and its involvement in various diseases. Expert Opin Ther Targets 14:727–737.

Taylor V, Wong M, Brandts C, Reilly L, Dean NM, Cowsert LM, Moodie S, Stokoe D. 2000. 5' phospholipid phosphatase SHIP-2 causes protein kinase B inactivation and cell cycle arrest in glioblastoma cells. Mol Cell Biol 20: 6860–6871.

Vandenbroere I, Paternotte N, Dumont JE, Erneux C, Pirson I. 2003. The c-Cbl-associated protein and c-Cbl are two new partners of the SH2-containing inositol polyphosphate 5-phosphatase SHIP2. Biochem Biophys Res Commun 300:494–500.

Windhorst S, Blechner C, Lin HY, Elling C, Nalaskowski M, Kirchberger T, Guse AH, Mayr GW. 2008. Ins(1,4,5)P3 3-kinase-A overexpression induces cytoskeletal reorganization via a kinase-independent mechanism. Biochem J 414:407–417.

Wisniewski D, Strife A, Swendeman S, Erdjument-Bromage H, Geromanos S, Kavanaugh WM, Tempst P, Clarkson B. 1999. A novel SH2-containing phosphatidylinositol 3,4,5-trisphosphate 5- phosphatase (SHIP2) is constitutively tyrosine phosphorylated and associated with src homologous and collagen gene (SHC) in chronic myelogenous leukemia progenitor cells. Blood 93:2707–2720.

Wong KK, Engelman JA, Cantley LC. 2010. Targeting the PI3K signaling pathway in cancer. Curr Opin Genet Dev 20:87–90.

Wullschleger S, Wasserman DH, Gray A, Sakamoto K, Alessi DR. 2011. Role of TAPP1 and TAPP2 adaptors binding to PtdIns(3,4)P2 in regulating insulin sensitivity defined by knock-in analysis. Biochem J 434:265–274.

Xie J, Onnockx S, Vandenbroere I, Degraef C, Erneux C, Pirson I. 2008a. The docking properties of SHIP2 influence both JIP1 tyrosine phosphorylation and JNK activity. Cell Signal 20:1432–1441.

Xie J, Vandenbroere I, Pirson I. 2008b. SHIP2 associates with intersectin and recruits it to the plasma membrane in response to EGF. FEBS Lett 582:3011–3017.

Yu J, Ryan DG, Getsios S, Oliveira-Fernandes M, Fatima A, Lavker RM. 2008. MicroRNA-184 antagonizes microRNA-205 to maintain SHIP2 levels in epithelia. Proc Natl Acad Sci USA 105:19300–19305.

Zhuang G, Hunter S, Hwang Y, Chen J. 2006. Regulation of EphA2 receptor endocytosis by SHIP2 lipid phosphatase via PI3 kinase-dependent Rac1 activation. J Biol Chem 282:2683–2694.

Zwaenepoel K, Goris J, Erneux C, Parker PJ, Janssens V. 2010. Protein phosphatase 2A PR130/B''alpha1 subunit binds to the SH2 domain-containing inositol polyphosphate 5-phosphatase 2 and prevents epidermal growth factor (EGF)-induced EGF receptor degradation sustaining EGF-mediated signaling. FASEB J 24:538–547.