J. Biochem. **124**, 1-7 (1998)

Downstream of Phosphatidylinositol-3 Kinase, a Multifunctional Signaling Molecule, and Its Regulation in Cell Responses

Yasuhisa Fukui,[1] Sayoko Ihara, and Satoshi Nagata

Laboratory of Biological Chemistry, Division of Applied Biological Chemistry, Graduate School of Agriculture and Life Science, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657

Received for publication, February 25, 1998

Phosphatidylinositol (PI)-3 kinase is an enzyme that phosphorylates the D-3 position of PI and its derivatives. It is activated immediately after growth factor or differentiation factor stimulation, suggesting that PI-3 kinase is involved in signal transduction of the stimulation. PI-3 kinase appears to play various important roles including signaling to the nucleus, vesicle transport, and rearrangement of the cytoskeleton since many cell responses which require these events are affected by inhibition or activation of PI-3 kinase. To understand how PI-3 kinase can act in such multiple ways, it is important to identify the factors downstream of PI-3 kinase. In this review, we discuss the factors downstream of PI-3 kinase and the methods used to identify them. Recent studies revealed that some proteins involved in vesicle transport or in rearrangement of the cytoskeleton are regulated by the phospholipids generated by PI-3 kinase, implying the mechanism by which PI-3 kinase regulates these cell responses.

Key words: cell growth, cytoskeleton, differentiation, phosphatidylinositol-3 kinase, vesicle transport.

We have seen a great break through in the field of regulation of cell growth and differentiation in recent years. One finding was that growth factors or growth factor receptors are homologues of oncogene products. This combined the cell biology and oncology fields. With the combination of the accumulating knowledge in the two fields, we became able to build up an idea of the way how cell growth and differentiation are controlled. The signals of growth factors outside cells are transduced from the plasma membrane to the nucleus as well as to other parts of the cells. We know that many signaling molecules are involved in these processes. Analysis of the cellular counterparts of oncogenes revealed that their products were components of the signal transduction pathways: some activating and others forming the cascades. As expected, most of the oncogene products turned out to be constitutively active mutants of the cellular signaling molecules, which keep on stimulating cell growth. Having wild type and constitutively active mutant genes, studies in this field have advanced greatly in the past few years. The second finding was the formation of signaling complexes of growth factor receptors and phosphotyrosine-containing molecules after growth factor stimulation. The first idea of signal transduction was that an activated growth factor receptor generated a specific second messenger, which in turn would be transferred to the nucleus. The finding that many signaling molecules congregate at the growth factor receptors changed this notion. Now, we know that multiple signals are generated to induce multiple reactions in parallel after a single growth factor stimulation. The signals will reach not only the nucleus but also other parts of the cells. This leads to the activation of the whole cells. The cell responses are not simple enough to be regulated by a single signal. For instance, cell growth comprises not only replication of the chromosomal DNA but also that of all parts of the cells, including proteins, lipids and sugars. A whole cell must prepare for cell division after growth factor stimulation. Therefore, it is quite acceptable that cells need the activation of multiple signaling cascades in parallel to regulate the cell responses. Many growth factors induce the activation of similar signaling pathways, including MAP kinase, phospholipase C, and PI-3 kinase pathways. However, the combination of the pathways and the duration of activation of the pathways differ with the growth factor receptors. For instance, PDGF can activate all three pathways as well as some other signaling molecules, whereas CSF-1 cannot activate phospholipase C in some cells (*1*). This difference in the combination of the signals may be responsible for the difference in the cell responses caused by the growth factors. In the case of signal transduction through tyrosine kinase type growth factor receptors, the formation of the signaling complexes is mainly performed through binding of the autophosphorylation sites of the growth factor receptors to the src homology 2 (SH2) domains of the signaling molecules. The signaling molecules may be activated in the complexes and transduce the signals into the cytoplasm or to the nucleus. After this idea had been established, a huge number of signaling molecules containing SH2 domains have been discovered, indicating that there may be fine networks of the signaling pathways to

[1] To whom correspondence should be addressed. Phone: +81-3-3812-2111 (Ext. 5110), Fax: +81-3-3812-0544, E-mail:ayfukui@hongo.ecc.u-tokyo.ac.jp

© 1998 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012

regulate various cell responses. However, recent studies implied that these cascades are much more complicated. For instance, Ras, a small G protein involved in the control of cell growth, can activate not only its direct target, Raf, a serine threonine kinase, but also Rac, another G protein involved in the regulation of cytoskeletal rearrangement, PI-3 kinase (see below), and probably some others. This may not necessarily mean that Ras always activates all the downstream molecules. If the activation of Ras always activates all of these factors, cell responses may not be correctly regulated. When Rac needs to be activated, the signal may reach Rac through Ras, but when it is not needed, cells somehow appear to stop activating Rac even when Ras is activated. There must be strict selection of the downstream factors to be activated depending on the situation of the cells. How this regulation is performed is a mystery. Likewise, PI-3 kinase also appears to have its own unique downstream factors. In this review, we survey the factors downstream of PI-3 kinase and discuss how a variety of these downstream factors might be regulated.

PI-3 kinase in signal transduction

PI-3 kinase is the enzyme that phosphorylates the D-3 position of the inositol ring of PI (*2*). The term, PI-3 kinase, includes all the enzymes that phosphorylate the D3 position of PI and its derivatives. There are several types of PI-3 kinases; some of them phosphorylate PI only, whereas others can phosphorylate phosphoinositides as well (*3*). The PI-specific PI-3 kinase was originally found in yeast as a gene that is involved in protein sorting (*4*). Mammalian cells have homologues of this type of PI-3 kinase, suggesting that a similar mechanism of protein sorting may be present in mammalian cells (*3*). It is suggested that there is another type of PI-3 kinase capable of phosphorylating PI and PI-4-P (*3*). These PI-3 kinases could produce PI-3,4-P_2 mainly. The PI-3 kinases discussed here are the ones that are activated by growth factor stimulation and generate PI-3,4,5-P_3 from PI-4,5-P_2. These enzymes can phosphorylate PI and PI-4-P as well as PI-4,5-P_2 *in vitro* (*5*). However, PI-3 kinase appears to select PI-4,5-P_2 only in intact cells to generate PI-3,4,5-P_3 mainly (see below), although the level of PI is about two magnitudes higher than that of PI-4-P or PI-4,5-P_2. This suggests that there may be strict regulation in the selection of the substrate *in vivo*, which is absent *in vitro*. The mechanism underlying this is not known at all at present. The level of PI-3,4,5-P_3 increases immediately after growth factor stimulation and then usually decreases rapidly (*5–8*). The level of PI-3,4-P_2 is also elevated but the peak appears a little later than that of PI-3,4,5-P_3. The level of PI-3-P does not change dramatically. Both PI-3,4-P_2 and PI-3,4,5-P_3 can serve as second messengers (see below). The fact that the level of PI-3,4,5-P_3 increases first and PI-3,4-P_2 accumulates afterwards suggests that PI-3,4,5-P_3 is generated first and that it can then be dephosphorylated to yield PI-3,4-P_2. There may be no enzymes that hydrolyse PI-3,4,5-P_3 to release the head group from the backbone of the lipid (*9*). The dephosphorylation reaction may be carried out by specific phosphatases. Synaptojanin, a protein abundant in the nerve terminus, is one of the candidates (*10*). This molecule removes the phosphate group at the 5 positions of inositol (I)-1,3,4,5-P_4 and PI-3,4,5-P_3. SHIP, another I-1,3,4,5-P_4 and PI-3,4,5-P_3 phosphatase, is also an important candidate. This phosphatase contains a SH2 domain and becomes phosphorylated on B cell signalling (*11–13*). These phosphatases dephosphorylate not only PI-3,4,5-P_3 but also other inositol phosphates. In addition to these phosphatases, we and others have reported the presence of PI-3,4,5-P_3 specific phosphatases (*14*). Although the importance of the phosphatases is well recognized, which molecule is responsible for this reaction remains unclear.

The PI-3 kinases discussed here, which are able to phosphorylate PI-4,5-P_2, may be classified into two groups (*3, 15*). One group comprises these activated by the tyrosine kinases. This type of PI-3 kinase consists of two subunits, p110 and p85. P110 may be the catalytic subunit and p85 a regulatory adaptor subunit. P85 is named after its molecular size, however, some of the newly found p85s have different molecular sizes. P85s usually have two SH2 domains, a *src* homology 3 (SH3) domain, which has been shown to bind to proline-rich sequences of proteins, proline-rich sequences, and other regions which might be used as binding sites for other proteins. P110 requires p85 for its full activity, suggesting that p85 may be necessary for maintenance of the conformation of p110. The PI-3 kinases comprising another group may be regulated by the β,γ subunits of trimeric G proteins. These PI-3 kinases may also form heterodimers. P110γ exhibiting the catalytic activity binds to p101, which may be an adaptor molecule.

Activation of PI-3 kinase

The p110-p85 heterodimer type PI-3 kinases are activated in various ways. One type of activation comprises binding to the autophosphorylation sites of receptor tyrosine kinases. For instance, PI-3 kinase binds to the PDGF receptor through the SH2 domain of p85. The binding of a polypeptide phosphorylated at tyrosine corresponding to the binding site of PI-3 kinase increases the specific activity of PI-3 kinase (*7, 8, 16*). There are some other receptor tyrosine kinases, such as c-kit, CSF-1 receptor and c-met, which may activate PI-3 kinase. In contrast, PI-3 kinase does not bind to the insulin receptor even after stimulation with insulin. Instead, PI-3 kinase binds to insulin receptor substrate 1 (IRS1), which is heavily phosphorylated by the insulin receptor. This binding also increases the specific activity of PI-3 kinase (*17, 18*). There are many receptors which exhibit this activation mechanism. The IGF-1 receptor, NGF receptor, and c-Ros are examples (*19*). These receptors phosphorylate unique proteins to activate PI-3 kinase. These two mechanisms are similar because the binding of phosphotyrosine-containing proteins activates PI-3 kinase. However, the difference between the two is that growth factor receptor (such as PDGF receptor)-PI-3 kinase complexes are found in the membrane fraction whereas tyrosine-phosphorylated receptor substrate (such as IRS1)-PI-3 kinase complexes are fractionated into the cytosolic fraction. This difference in localization of the PI-3 kinase complexes may be important for consideration of the multiple functions of PI-3 kinase. PI-3 kinase can also be activated by the Ras oncogene product (*20*). The activated form of Ras (Ras-GTP) can bind to p110, and co-expression of the activated Ras in mammalian cells results in the activation of the PI-3 kinase pathway, suggesting that this signaling pathway is present in cells. In addition to these mechanisms, PI-3 kinase can be activated by translocation to the membrane. It has been shown that targeting of PI-3

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012

kinases to the membrane on addition of the fatty acid groups results in activation of some downstream factors of the pathway (*21*). These are artificial proteins, however, it is possible that the native PI-3 kinase is activated by targeting to the membrane. Why are there so many activation mechanisms present? The answer to this question may be that PI-3 kinase is multifunctional. PI-3 kinase has been suggested to be involved in at least signal transduction to the nucleus, cytoskeletal rearrangement, and membrane trafficking. PI-3 kinase must act in different locations in each case. Therefore, there must be mechanisms that target PI-3 kinase to the right place. Here, we propose a hypothesis: the role of PI-3 kinase differ with where PI-3 kinase is targeted, and this targeting depends on how PI-3 kinase is activated (Fig. 1).

Downstream of PI-3 kinase

It is of interest to determine the location where PI-3 kinase is activated. However, this is quite difficult at present, because of the absence of good probes for PI-3,4-P_2 or PI-3,4,5-P_3. One method is to search for downstream factors and detect their locations. Factors downstream of PI-3 kinase have been searched for mainly by three methods. The finding of selective inhibitors of PI-3 kinase facilitated these studies. The cell responses blocked by these inhibitors were considered to be downstream of PI-3 kinases. Growth factor receptors lacking the binding sites for PI-3 kinase were also useful. Finally, dominant negative mutants of PI-3 kinase were used to block the pathway. By these methods, some molecules were identified as factors downstream of PI-3 kinase. Akt, a serine threonine kinase bearing a pleckstrin homology (PH) domain, is one of the proteins identified in this way (*22-24*). Activation of Akt was inhibited by wortmannin, a PI-3 kinase inhibitor, and by the dominant negative allele of PI-3 kinase. The PDGF receptor with a point mutation at the binding site of PI-3 kinase was not able to activate Akt whereas the wild type PDGF receptor was. Activation of Akt was abolished when the PH domain of Akt was removed, suggesting that the PH domain is responsible for the interaction with the message produced by PI-3 kinase. Both PI-3,4-P_2 and PI-3,4,5-P_3 bound to the PH domain of Akt, however, only PI-3,4-P_2 was effective in triggering of Akt protein kinase activity (*25-27*). In this case, not PI-3,4,5-P_3 but PI-3,4-P_2 appears to be the second messenger that transduces the signal. Akt is activated by stress as well as the growth factor stimulation. Activation of Akt by stress such as peroxide is sensitive to wortmannin, whereas that induced by heat shock is not (*28*). After the stress, elevation of the PI-3,4,5-P_3 level is observed in both cases, however, the accumulation of PI-3,4-P_2 is observed only after treatment with peroxide, *i.e.* not after heat shock (Konishi *et al.*, submitted). These results suggest that there are PI-3 kinase dependent and independent pathways that activate Akt, and strongly support the idea that it is PI-3,4-P_2 that activates Akt and that elevation of PI-3,4,5-P_3 is not enough for activation of Akt *in vivo*. Activation of Akt is not simply controlled by phospholipids. It is also controlled by phosphorylation, which has been suggested to be carried out by other kinase PI-3,4,5-P_3-dependent protein kinases (PDKs) (*29, 30*). These kinases are directly activated by PI-3,4-P_2 or PI-3,4,5-P_3. In addition to these kinases, an

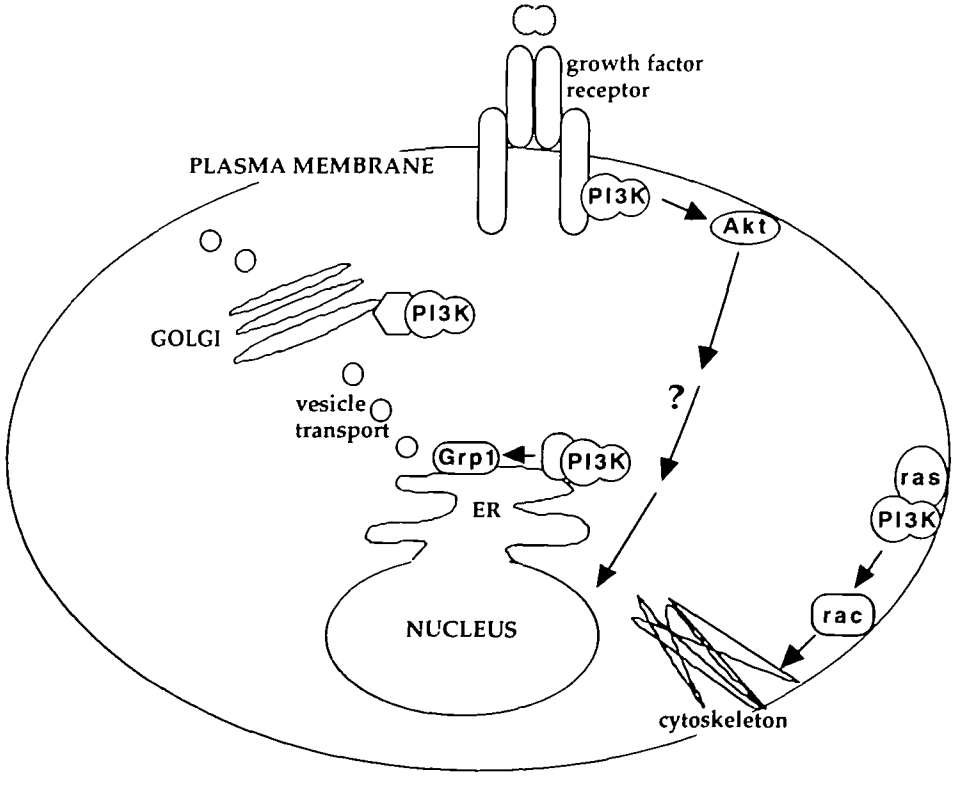

Fig. 1. **Activation of PI-3 kinase at various locations.** Adaptor molecules may target PI-3 kinase to the right location to generate the second messenger at the right place.

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012

additional kinase which depends on PI-3 kinase activity is predicted. Therefore, it appears that a whole cascade of these protein kinases are regulated by the phospholipids which are the products of PI-3 kinase. Why this mechanism is convenient for organisms is not known. P70 S6 kinase and glycogen synthase kinase have been identified in a similar way. Initially, these kinases were thought to be downstream of Akt, however, recent studies suggested that the mechanism by which these kinases are activated is not so simple. P70 S6 kinase seems to be activated by PDK1, but how PI-3 kinase is involved in the system is not clear (*31*).

Since the PH domain of Akt is responsible for the binding of PI-3,4-P_2 and PI-3,4,5-P_3, the PH domain-containing proteins have been surveyed as to the binding to 3′-phosphorylated phosphoinositides. Among the proteins capable of binding to polyphosphoinositides, Btk (Bruton's tyrosine kinase), a nonreceptor tyrosine kinase, bound to PI-3,4,5-P_3 and I-1,3,4,5-P_4 with much higher affinity than to PI-3,4-P_2 or PI-4,5-P_2 (*32*). Btk is a member of the Tec tyrosine kinase family with a large regulatory domain in the amino terminal portion. It is involved in B-cell development, and mutations in the PH domain cause X-linked immunodeficiency in mice and X-linked gammaglobulinaemia in humans (*33, 34*). Therefore, it is likely that the binding of PI-3,4,5-P_3 to Btk is important for the regulation of the cell responses *in vivo*. It was shown that the specific activity of Btk increases upon binding to PI-3,4,5-P_3, suggesting that Btk is downstream of PI-3 kinase. Other members of the Tec tyrosine kinase family also bind to PI-3,4,5-P_3, implying that the activities of these tyrosine kinases are regulated by PI-3 kinase in a similar way (*35, 36*). Phospholipase C was also shown to bind to PI-3,4,5-P_3 through its PH domain. It is targeted to the membrane after stimulation by PDGF and thus becomes activated (*37*).

Novel protein kinase Cs were also identified as the targets of PI-3 kinase (*38-43*). This was shown by the direct addition of PI-3,4-P_2 or PI-3,4,5-P_3 for the *in vitro* reaction of the purified C kinases or by *in vivo* experiments. PKCζ, ε, η, δ, and λ are examples. These PKCs contain incomplete diacylglycerol (DAG) binding domains. cPKCs such as α and β harboring a complete DAG binding domain also reacted with PI-3,4,5-P_3. PKCs without any DAG binding domain at all were not activated by PI-3,4,5-P_3. These results suggest that a portion of the DAG binding domain is required for the activation. The involvement of PI-3 kinase in the activation of these PKCs was also shown in intact cells. For example, the phosphorylation of pleckstrin, a substrate of PKC, is inhibited when platelets are treated with wortmannin, and the addition of PI-3,4-P_2 or PI-3,4,5-P_3 to permeabilized cells induces the phosphorylation of pleckstrin. Therefore, pathways from PI-3 kinases to PKCs appear to be present *in vivo*.

It has been suggested that the action of Rac may be controlled by PI-3 kinase activity. Inhibition of PI-3 kinase activity results in the loss of the membrane ruffling reaction after growth factor treatment or cell motility which may be responsible for the invasiveness of the transformed cells. Consistent with this, expression of the constitutively active PI-3 kinase in the cells enhances these cell responses. Since these important cell responses are suggested to be regulated by Rac, many investigators have been studying how Rac is regulated by PI-3 kinase (*44-47*). Several Rac-GEFs have been identified. They all contain Dbl homology (DH) domains, the catalytic centers of GEF activity, and PH domains. Vav is one of the guanine nucleotide exchanging factors (GEF) of Rac, which are responsible for the activation of Rac (*48*). Since some PH domains have been shown to interact with PI-3,4,5-P_3, the effects of PI-3,4,5-P_3 on

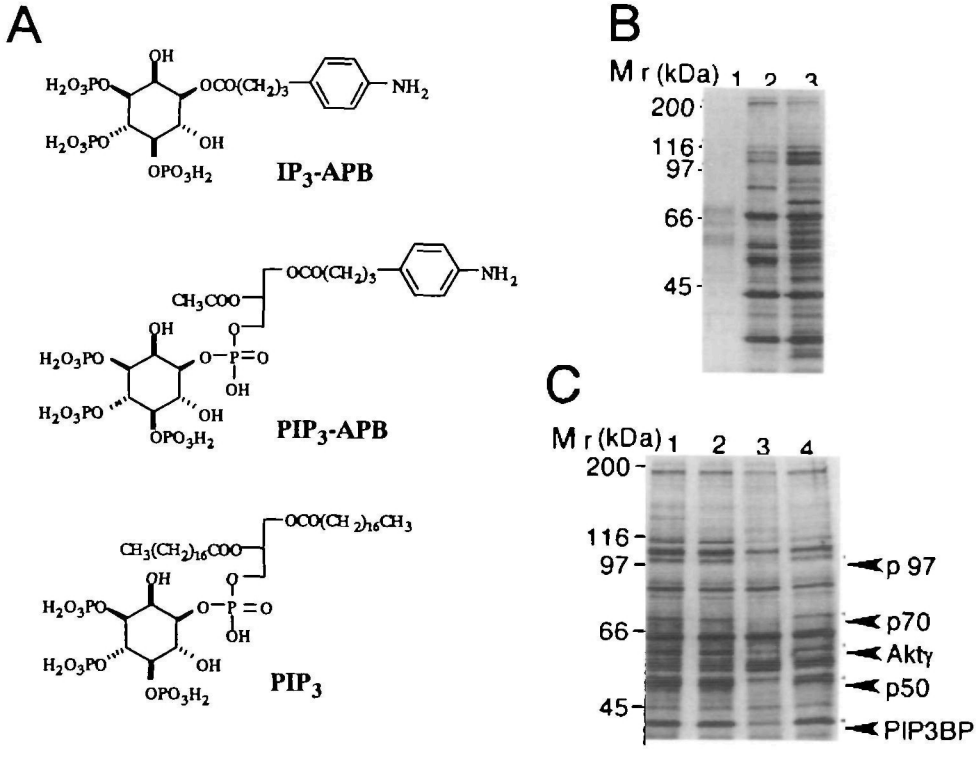

Fig. 2. **Selection of PI-3,4,5-P_3 binding proteins with affinity beads.** A: The structures of PI-3,4,5-P_3 analogues. B: Cell lysates from bovine brain were mixed with control beads (lane 1), IP3-APB (lane 2), or PIP3-APB (lane 3), and then the proteins bound to the beads were analyzed by SDS-polyacrylamide gel electrophoresis. C: The cell lysates were preincubated with 4 mM PI-4,5-P_2 (lane 2), 4 mM PI-3,4,5-P_3 (lane 3), or 0.4 mM PI-3,4,5-P_3 (lane 4), and then mixed with the PIP$_3$-APB beads. The proteins that bound to the beads were analyzed. Lane 1, control experiment without any competitor. Akt and PIP3BP have been identified. Other bands are unknown, but are probably novel PI-3,4,5-P_3 binding proteins.

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012

the reactions of Rac-GEFs have been examined. Tyrosine-phosphorylation of Vav enhances its GEF activity. PI-3,4,5-P_3 stimulated the tyrosine phosphorylation of Vav by Lyn *in vitro*, active Vav being formed (*49*). This suggests that PI-3,4,5-P_3 can interact with the PH domain of Vav to change its conformation.

One approach for identifying the target of PI-3 kinase is to use polyphosphoinositides as probes for the screening of an expression library. Grp1 (general receptor of phosphoinositides) was identified by this method. To search for general receptors for 3′-phosphorylated phosphoinositides, lipids from bovine brain phosphorylated by PI-3 kinase were used as a probe. This probe mixture contained PI-3-P, PI-3,4-P_2, and PI-3,4,5-P_3 for the detection of binding proteins for all of these phosphoinositides. Surprisingly, the clone in this experiment coded for a peptide which bound to PI-3,4,5-P_3 specifically. The peptide, named Grp1, although it binds to PI-3,4,5-P_3 and I-1,3,4,5-P_4, but not to PI-3-P or PI-3,4-P_2, contained a PH domain and a Sec7 homology domain, which is shared by cytohesin-1 and ARNO, which are guanine nucleotide exchange factors (GEF) of ARF1, a small G-protein involved in vesicle transport. Grp1 also exhibited GEF activity toward ARF1 but not toward ARF5 or ARF6, other types of ARF. Importantly, this GEF activity was enhanced by PI-3,4,5-P_3, linking PI-3 kinase and membrane trafficking (*50*).

PI-3,4,5-P_3 affinity columns are also available. The scientists studying I-3,4,5-P_4 binding proteins have been using this method. I-3,4,5-P_4, which is identical to the head group of PI-3,4,5-P_3, was conjugated to beads. The beads were useful for identifying the I-3,4,5-P_4 binding proteins. The use of PI-3,4,5-P_3 showed that some of the I-3,4,5-P_4 binding proteins bound to PI-3,4,5-P_3 even better than to I-3,4,5-P_4. Centaurin α is one example (*51*). It bound best to PI-3,4,5-P_3. Another protein, PIP3BP, which turned out to be highly homologous to centaurin α, was identified using a different analogue (*52*). The two proteins contain a zinc finger motif which is most homologous to that of ARF-GTPase activating protein, and bind to PI-3,4,5-P_3 but not to PI-3,4-P_2 or PI-3-P. The two proteins are also homologous in other parts. PIP3BP has two PH domains, both of which are responsible for the binding to PI-3,4,5-P_3. The domain of centaurin α corresponding to the two PH domains of PIP3BP is called the ankyrin repeat. Some of the key amino acid residues in centaurin α may be closer to ankyrin repeats than to PH domains, however, the structures of the two domains may be similar. It is interesting that Grp1 is homologous to ARF-guanine nucleotide exchanging factor and the two proteins mentioned here are homologous to ARF-GTPase activating proteins. However, no GTPase-activating activities toward ARF have been detected in these two proteins so far.

The analogues used here contained no glycerol group or fatty acids. A better analogue of PI-3,4,5-P_3 was designed to produce a column which specifically detects PI-3,4,5-P_3. The new analogue contains a glycerol group as well as fatty acids (Fig. 2A). The structures of the fatty acids are important for the reactivity of the lipids to the enzymes. Usually, natural PI contains unsaturated fatty acids at the second position of the glycerol group. PI-3,4,5-P_3, with a long saturated fatty acid at the second position of the glycerol group, cannot be dephosphorylated by the PI-3,4,5-P_3 phosphatase, but a shorter fatty acid at the same place allows the reaction (unpublished results). Likewise, PI-3 kinase does not utilize PI with a long fatty acid at the second position as a substrate but utilizes PI with a shorter fatty acid (unpublished results). These results suggest that the fatty acid at the second position should be an unsaturated one or a short one for correct recognition. The analogue with a glycerol group and fatty acids was designed according to this information. Compared with an analogue without a glycerol group, the new analogue bound to many additional proteins (Fig. 2B) (*36*). The binding of these proteins appeared to be PI-3,4,5-P_3 specific, since the presence of free PI-3,4,5-P_3 blocked the binding (Fig. 2C). Some of them were identified as Aktγ, GAP1m, and Tec. These proteins were not detected until the new analogue was synthesized. They all contained PH domains, which were shown to be the binding domains for PI-3,4,5-P_3. These results suggest that the PI-3,4,5-P_3 binding proteins recognize not only the head group but also other parts of PI-3,4,5-P_3. Therefore, there may be a distinct group of proteins which do not bind to I-1,3,4,5-P_4 but to PI-3,4,5-P_3. Many unknown bands are detected with this method, implying the presence of many novel PI-3,4,5-P_3 binding proteins.

Increasing evidence suggests that PI-3 kinases are multifunctional and play many important roles in cell responses. The products, PI-3,4-P_2 and PI-3,4,5-P_3, may be responsible for the signaling. There appear to be many binding proteins for these lipids, which may be able to receive the signals from these phospholipids. If these phospholipids are generated everywhere in cells, all of these binding proteins may be activated and cell behavior will not be correctly controlled. However, we did not see such confusion in the cells, suggesting that the signals from these phospholipids are correctly directed to the right place. This could be performed by the targeting of PI-3 kinases to the right places. PI-3 kinases can be activated in various ways. The well studied mechanism is the binding of phosphotyrosine-containing proteins such as IRS-1/2. Many tyrosine kinase type receptors phosphorylate unique proteins which are able to bind to PI-3 kinase after phosphorylation at tyrosine. Although these phosphotyrosine-containing proteins have not been characterized well, these molecules can act as adopter molecules for PI-3 kinase to be activated at the right position. It is, therefore, critical to determine where these polyphosphoinositides are generated to understand the roles of PI-3 kinase. Since there have been no good probes for detecting these polyphosphoinositides in intact cells, the only way to determine the location where PI-3 kinase is activated is to identify the PI-3,4-P_2 and PI-3,4,5-P_3 binding proteins and determine their locations. Thanks to the efforts of many laboratories, we have found a number of target molecules of PI-3 kinases. However, it is clear that not enough molecules have been found yet to know the whole picture of the functions of these lipids. Further searches for the target molecules of the 3′-phosphorylated polyphosphoinositides as well as the detection of activation of PI-3 kinase in intact cells is required to understand the mechanisms of action of these phospholipids.

REFERENCES

1. Cantley, L.C., Auger, K.R., Carpenter, C., Duckworth, B.,

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012

Graziani, A., Kapeller, R., and Soltoff, S. (1991) Oncogenes and signal transduction. *Cell* **64**, 281-302
2. Whitman, M., Downes, C.P., Keeler, M., Keller, T., and Cantley, L. (1988) Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* **332**, 644-646
3. Domin, J. and Waterfield, M.D. (1997) Using structure to define the function of phosphoinositide 3-kinase family members. *FEBS Lett.* **410**, 91-95
4. Schu, P.V., Takegawa, K., Fry, M.J., Stack, J.H., Waterfield, M.D., and Emr, S.D. (1993) Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* **260**, 88-91
5. Auger, K.R., Serunian, L.A., Soltoff, S.P., Libby, P., and Cantley, L.C. (1989) PDGF-dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. *Cell* **57**, 167-175
6. Carter, A.N. and Downes, C.P. (1992) Phosphatidylinositol 3-kinase is activated by nerve growth factor and epidermal growth factor in PC12 cells [published erratum appears in *J. Biol. Chem.* 1992 Nov. 15; **267** (32): 23434]. *J. Biol. Chem.* **267**, 14563-14567
7. Downes, C.P. and Carter, A.N. (1991) Phosphoinositide 3-kinase: a new effector in signal transduction? *Cell. Signalling* **3**, 501-513
8. Parker, P.J. and Waterfield, M.D. (1992) Phosphatidylinositol 3-kinase: a novel effector. *Cell Growth Differ.* **3**, 747-752
9. Serunian, L.A., Haber, M.T., Fukui, T., Kim, J.W., Rhee, S.G., Lowenstein, J.M., and Cantley, L.C. (1989) Polyphosphoinositides produced by phosphatidylinositol 3-kinase are poor substrates for phospholipases C from rat liver and bovine brain. *J. Biol. Chem.* **264**, 17809-17815
10. McPherson, P.S., Garcia, E.P., Slepnev, V.I., David, C., Zhang, X., Grabs, D., Sossin, W.S., Bauerfeind, R., Nemoto, Y., and De Camilli, P. (1996) A presynaptic inositol-5-phosphatase. *Nature* **379**, 353-357
11. Damen, J.E., Liu, L., Rosten, P., Humphries, R.K., Jefferson, A.B., Majerus, P.W., and Krystal, G. (1996) The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase. *Proc. Nat. Acad. Sci. USA* **93**, 1689-1693
12. Kavanaugh, W.M., Pot, D.A., Chin, S.M., Deuter-Reinhard, M., Jefferson, A.B., Norris, F.A., Masiarz, F.R., Cousens, L.S., Majerus, P.W., and Williams, L.T. (1996) Multiple forms of an inositol polyphosphate 5-phosphatase form signaling complexes with Shc and Grb2. *Curr. Biol.* **6**, 438-445
13. Lioubin, M.N., Algate, P.A., Tsai, S., Carlberg, K., Aebersold, A., and Rohrschneider, L.R. (1996) p150Ship, a signal transduction molecule with inositol polyphosphate-5-phosphatase activity. *Genes Dev.* **10**, 1084-1095
14. Kabuyama, Y., Nakatsu, N., Homma, Y., and Fukui, Y. (1996) Purification and characterization of the phosphatidylinositol-3,4,5-trisphosphate phosphatase in bovine thymus. *Eur. J. Biochem.* **238**, 350-356
15. Carpenter, C.L. and Cantley, L.C. (1996) Phosphoinositide 3-kinase and the regulation of cell growth. *Biochim. Biophys. Acta* **1288**, M11-M16
16. Carter, A.N. and Downes, C.P. (1995) Molecular mechanisms of regulation of phosphoinositide 3-kinase by insulin, insulin-like growth factor 1 and nerve growth factor in PC12 cells. *Biochem. Soc. Transact.* **23**, 148-152
17. Backer, J.M., Myers, M.G., Jr., Shoelson, S.E., Chin, D.J., Sun, X.J., Miralpeix, M., Hu, P., Margolis, B., Skolnik, E.Y., Schlessinger, J. *et al.* (1992) Phosphatidylinositol 3′-kinase is activated by association with IRS-1 during insulin stimulation. *EMBO J.* **11**, 3469-3479
18. Yonezawa, K., Yokono, K., Shii, K., Ogawa, W., Ando, A., Hara, K., Baba, S., Kaburagi, Y., Yamamoto-Honda, R., Momomura, K., *et al.* (1992) In vitro association of phosphatidylinositol 3-kinase activity with the activated insulin receptor tyrosine kinase. *J. Biol. Chem.* **267**, 440-446
19. Ohmichi, M., Decker, S.J., and Saltiel, A.R. (1992) Activation of phosphatidylinositol-3 kinase by nerve growth factor involves indirect coupling of the trk proto-oncogene with src homology 2 domain. *Neuron* **9**, 769-777
20. Rodriguez-Viciana, P., Warne, P.H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M.J., Waterfield, M.D., and Downward, J. (1994) Phosphatidylinositol-3-OH kinase as a direct target of Ras [see comments]. *Nature* **370**, 527-532
21. Klippel, A., Reinhard, C., Kavanaugh, W.M., Apell, G., Escobedo, M.A., and Williams, L.T. (1996) Membrane localization of phosphatidylinositol 3-kinase is sufficient to activate multiple signal-transducing kinase pathways. *Mol. Cell. Biol.* **16**, 4117-4127
22. Bellacosa, A., Testa, J.R., Staal, S.P., and Tsichlis, P.N. (1991) A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. *Science* **254**, 274-277
23. Burgering, B.M. and Coffer, P.J. (1995) Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* **376**, 599-602
24. Franke, T.F., Yang, S.I., Chan, T.O., Datta, K., Kazlauskas, A., Morrison, D.K., Kaplan, D.R., and Tsichlis, P.N. (1995) The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* **81**, 727-736
25. Frech, M., Andjelkovic, M., Ingley, E., Reddy, K.K., Falck, J.R., and Hemmings, B.A. (1997) High affinity binding of inositol phosphates and phosphoinositides to the pleckstrin homology domain of RAC/protein kinase B and their influence on kinase activity. *J. Biol. Chem.* **272**, 8474-8481
26. Klippel, A., Kavanaugh, W.M., Pot, D., and Williams, L.T. (1997) A specific product of phosphatidylinositol 3-kinase directly activates the protein kinase Akt through its pleckstrin homology domain. *Mol. Cell. Biol.* **17**, 338-344
27. Franke, T.F., Kaplan, D.R., Cantley, L.C., and Toker, A. (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science* **275**, 665-668
28. Konishi, H., Matsuzaki, H., Tanaka, M., Ono, Y., Tokunaga, C., Kuroda, S., and Kikkawa, U. (1996) Activation of RAC-protein kinase by heat shock and hyperosmolarity stress through a pathway independent of phosphatidylinositol 3-kinase. *Proc. Nat. Acad. Sci. USA* **93**, 7639-7643
29. Alessi, D.R., Deak, M., Casamayor, A., Caudwell, F.B., Morrice, N., Norman, D.G., Gaffney, P., Reese, C.B., MacDougall, C.N., Harbison, D., Ashworth, A., and Bownes, M. (1997) 3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. *Curr. Biol.* **7**, 776-789
30. Stephens, L., Anderson, K., Stokoe, D., Erdjument-Bromage, H., Painter, G.F., Holmes, A.B., Gaffney, P.R.J., Reese, C.B., McCormick, F., Tempst, P., Coadwell, J., and Hawkins, P.T. (1998) Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science* **279**, 710-714
31. Pullen, N., Dennis, P.B., Andjelkovic, M., Dufner, A., Kozma, S.C., Hemmings, B.A., and Thomas, G. (1998) Phosphorylation and activation of p70s6k by PDK1. *Science* **279**, 707-710
32. Rameh, L.E., Arvidsson, A.K., Carraway, K.L.R., Couvillon, A.D., Rathbun, G., Crompton, A., VanRenterghem, B., Czech, M.P., Ravichandran, K.S., Burakoff, S.J., Wang, D.S., Chen, C.S., and Cantley, L.C. (1997) A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. *J. Biol. Chem.* **272**, 22059-22066
33. Desiderio, S. (1997) Role of Btk in B cell development and signaling. *Curr. Opin. Immunol.* **9**, 534-540
34. Kurosaki, T. (1997) Molecular mechanisms in B cell antigen receptor signaling. *Curr. Opin. Immunol.* **9**, 309-318
35. August, A., Sadra, A., Dupont, B., and Hanafusa, H. (1997) Src-induced activation of inducible T cell kinase (ITK) requires phosphatidylinositol 3-kinase activity and the pleckstrin homology domain of inducible T cell kinase. *Proc. Nat. Acad. Sci. USA* **94**, 11227-11232
36. Shirai, T., Tanaka, K., Terada, Y., Sawada, T., Shirai, R., Hashimoto, Y., Nagata, S., Iwamatsu, A., Okawa, K., Li, S.,

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012

Hattori, S., Mano, H., and Fukui, Y. (1998) Specific detection of phosphatidylinositol 3,4,5-trisphosphate binding proteins by the PIP3 analogue beads: An application for rapid purification of the PIP3 binding proteins. *Biochim. Biophys. Acta* **1402**, 292-302
37. Falasca, M., Logan, S.K., Lehto, V.P., Baccante, G., Lemmon, M.A., and Schlessinger, J. (1998) Activation of phospholipase C gamma by PI 3-kinase-induced PH domain-mediated membrane targeting. *EMBO J.* **17**, 414-422
38. Nakanishi, H., Brewer, K.A., and Exton, J.H. (1993) Activation of the zeta isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **268**, 13-16
39. Toker, A., Meyer, M., Reddy, K.K., Falck, J.R., Aneja, R., Aneja, S., Parra, A., Burns, D.J., Ballas, L.M., and Cantley, L.C. (1994) Activation of protein kinase C family members by the novel polyphosphoinositides PtdIns-3,4-P-2 and PtdIns-3,4,5-P-3. *J. Biol. Chem.* **269**, 32358-32367
40. Toker, A., Bachelot, C., Chen, C.S., Falck, J.R., Hartwig, J.H., Cantley, L.C., and Kovacsovics, T.J. (1995) Phosphorylation of the platelet p47 phosphoprotein is mediated by the lipid products of phosphoinositide 3-kinase. *J. Biol. Chem.* **270**, 29525-29531
41. Zhang, J., Falck, J.R., Reddy, K.K., Abrams, C.S., Zhao, W., and Rittenhouse, S.E. (1995) Phosphatidylinositol (3,4,5)-trisphosphate stimulates phosphorylation of pleckstrin in human platelets. *J. Biol. Chem.* **270**, 22807-22810
42. Moriya, S., Kazlauskas, A., Akimoto, K., Hirai, S., Mizuno, K., Takenawa, T., Fukui, Y., Watanabe, Y., Ozaki, S., and Ohno, S. (1996) Platelet-derived growth factor activates protein kinase C epsilon through redundant and independent signaling pathways involving phospholipase C gamma or phosphatidylinositol 3-kinase. *Proc. Nat. Acad. Sci. USA* **93**, 151-155
43. Akimoto, K., Takahashi, R., Moriya, S., Nishioka, N., Takayanagi, J., Kimura, K., Fukui, Y., Osada, S., Mizuno, K., Hirai, S., Kazlauskas, A., and Ohno, S. (1996) EGF or PDGF receptors activate atypical PKC lambda through phosphatidylinositol 3-kinase. *EMBO J.* **15**, 788-798
44. Shaw, L.M., Rabinovitz, I., Wang, H.H., Toker, A., and Mercurio, A.M. (1997) Activation of phosphoinositide 3-OH kinase by the alpha 6 beta 4 integrin promotes carcinoma invasion. *Cell* **91**, 949-960
45. Keely, P.J., Westwick, J.K., Whitehead, I.P., Der, C.J., and Parise, L.V. (1997) Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. *Nature* **390**, 632-636
46. Hordijk, P.L., ten Klooster, J.P., van der Kammen, R.A., Michiels, F., Oomen, L.C., and Collard, J.G. (1997) Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. *Science* **278**, 1464-1466
47. Nimnual, A.S., Yatsula, B.A., and Bar-Sagi, D. (1998) Coupling of Ras and Rac guanosine triphosphatases through the Ras exchanger Sos. *Science* **279**, 560-563
48. Crespo, P., Schuebel, K.E., Ostrom, A.A., Gutkind, J.S., and Bustelo, X.R. (1997) Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature* **385**, 169-172
49. Han, J., Luby-Phelps, K., Das, B., Shu, X., Xia, Y., Mosteller, R.D., Krishna, U.M., Falck, J.R., White, M.A., and Broek, D. (1998) Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav. *Science* **279**, 558-560
50. Klarlund, J.K., Rameh, L.E., Cantley, L.C., Buxton, J.M., Holik, J.J., Sakelis, C., Patki, V., Corvera, S., and MP, C. (1998) Regulation of GRP1-catalyzed ADP ribosylation factor guanine nucleotide exchange by phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **273**, 1859-1862
51. Hammonds-Odie, L.P., Jackson, T.R., Profit, A.A., Blader, I.J., Turck, C.W., Prestwich, G.D., and Theibert, A.B. (1996) Identification and cloning of centaurin-alpha—A novel phosphatidylinositol 3,4,5-trisphosphate-binding protein from rat brain. *J. Biol. Chem.* **271**, 18859-18868
52. Tanaka, K., Imajoh-Ohmi, S., Sawada, T., Shirai, R., Hashimoto, Y., Iwasaki, S., Kaibuchi, K., Kanaho, Y., Shirai, T., Terada, Y., Kimura, K., Nagata, S., and Fukui, Y. (1997) A target of phosphatidylinositol 3,4,5-trisphosphate with a zinc finger motif similar to that of the ADP-ribosylation-factor GTPase-activating protein and two pleckstrin homology domains. *Eur. J. Biochem.* **245**, 512-519

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on October 1, 2012