Minireview

Using structure to define the function of phosphoinositide 3-kinase family members

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1. Introduction

It is now clear that lipid phosphorylation and hydrolysis plays a pivotal role in the transmission of extracellular stimuli across the cytoplasm [1,2]. Consequently, great effort has been applied to characterize the enzymes responsible for these signalling events [3–5]. This article will present an overview of one family of lipid kinases; namely the phosphoinositide 3-kinases (PI3-kinases) which are found in species ranging from slime mold to man and catalyse the addition of phosphate to the 3′ position on the inositol ring of phosphoinositides.

We offer a classification of the rapidly expanding number of PI3-kinase family members based principally on their structure and discuss our understanding of their regulation and downstream targets. In this way we hope to provide a framework within which the nomenclature of PI3-kinase family members can be standardised.

2. The early years

In the mid 1980's immunoprecipitates of $pp60^{v-src}$, polyoma middle T/$pp60^{c-src}$ complexes and $p68^{v-ros}$, the transforming protein of the avian sarcoma virus UR2 were found to contain a novel lipid kinase activity which phosphorylated phosphatidylinositol (PtdIns) on the 3′ position of its inositol ring (reviewed in [6]). A similar PI3-kinase activity was later found associated with activated platelet derived growth factor receptors where PtdIns(4)P and PtdIns(4,5)P_2 were used as substrate [7]. None of these 3′ phosphorylated lipids served as substrates for phospholipase C or D suggesting that they could fulfil an independent second messenger function [8]. Such findings stimulated great excitement since they suggested that pharmaceutical attenuation of this novel lipid kinase activity could inhibit mitogenesis and consequently control oncogenesis. Numerous studies have subsequently demonstrated a much broader role for this lipid kinase and its products in cell physiology/ pathophysiology [4].

At the time it was unclear as to how one enzyme could mediate such a multiplicity of functions. A clue as to how such specificity could be achieved was however, already evident from those early experiments. Ligand stimulation acutely elevated levels of both PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 compared to resting cells (reviewed in [9]). In contrast, the constitutively elevated level of PtdIns(3)P remained largely unaltered. Such results suggested that PtdIns(3)P plays a more central, metabolic role whilst the more highly phosphorylated phosphoinositides function as the ligand responsive second messengers.

3. Characterization of the enzyme

PI3-kinase was first identified as an 85-kDa phosphoprotein whose appearance correlated with a PI3-kinase activity in immunoprecipitates from a number of polyoma middleT mutants [10]. These studies facilitated the purification and subsequent cloning of a heterodimeric complex comprised of an 85-kDa adaptor (p85) and a 110-kDa catalytic subunit (p110) [11,12]. Cloning of the PI3-kinase catalytic subunit revealed a sequence similarity within its catalytic domain to yeast Vps34p a protein involved in vesicular trafficking later shown to also possess PI3-kinase activity [13]. Such sequence alignment within the kinase domains allowed the use of polymerase chain reaction based strategies to isolate and characterize novel PI3-kinase family members. Over the last five years we have witnessed an explosion in the number of PI3-kinase catalytic and regulatory subunits described in the literature [5]. Consequently, the wealth of information generated has proved confusing for many unfamiliar with this area of study. Fortunately, as we learn more about this family of signalling molecules, a basis for their classification has emerged.

4. Three classes of PI3-kinase

The classification of PI3-kinase family members we propose is based on their structure and probable mechanism of regulation. In mammals, we currently know of 7 forms of the catalytic subunit and 5 forms of associated binding partner / adaptor subunit. These catalytic and adaptor subunits can be divided into three main classes (Fig. 1):

4.1. Class I PI3-kinases

These enzymes all form a heterodimeric complex with an adaptor protein which renders them responsive to ligand stimulation. In vitro, they can utilise PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 as substrate but in the cell, they appear to preferentially phosphorylate PtdIns(4,5)P_2 [9]. The reason for this disparity is presently unclear although it is most likely to be a consequence of substrate presentation (see below).

This class of PI3-kinase is subdivided based on the form of adaptor subunit with which the catalytic subunit associates.

4.2. Class I_A PI3-kinases

These form a heterodimeric complex consisting of a 110–120-kDa catalytic subunit and an adaptor protein which contains two src homology 2 (SH2) domains. Mammalian class

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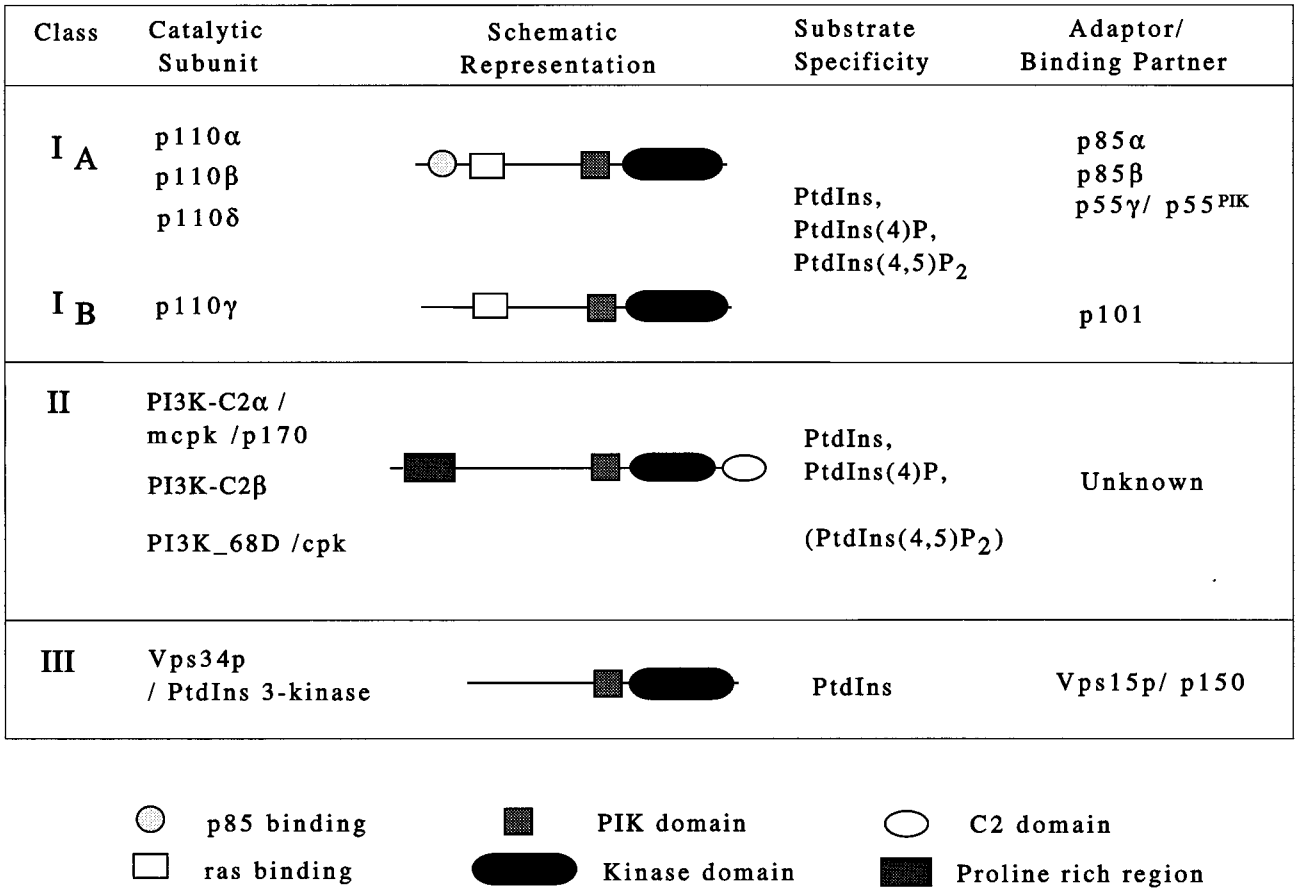

Fig. 1. The classification of PI3-kinase family members. The assignment of catalytic subunits to a particular class is based on sequence homology within the catalytic domain [5]. To date, PI3K-C2α is the only class II member which has been shown to phosphorylate PtdIns(4,5)P_2.

IA catalytic subunits include p110α, p110β and p110δ [14–16]. They all contain a domain which binds p85, a region for ras association [17] a PI kinase (PIK) domain and a C-terminal catalytic domain. The PIK domain is a region conserved amongst all PI3 and PI4-kinases and although its role is presently unclear, it is likely to be involved in substrate presentation [18]. Although both p110α and p110β demonstrate a ubiquitous tissue distribution, the expression of the recently identified p110δ is restricted to leukocytes thereby implying a specificity of function [16]. All members of this class bind the p85 adaptor subunit through which they can be recruited to receptor signalling complexes upon activation of tyrosine kinase activity [19].

The first adaptors to be identified for the class I_A catalytic subunits were p85α and p85β [20]. Both proteins contain two SH2 domains separated by an inter SH2 domain through which the adaptor interacts with p110 [21]. At the N terminus there is a src homology 3 (SH3) domain [22] and a breakpoint cluster region homology (BH) domain which has homology with the GAP (GTPase activating protein) domain of the BCR gene product [23] Although BH domains have been identified in a number of proteins which display GAP activity towards the Rho family GTPases, no equivalent biochemical activity has, as yet, been found for the BH domain of p85. Two proline rich regions flank the BH domain raising the possibility that the p85 SH3 domain may interact with either of these polyproline motifs to confer an allosteric change in the adaptor [24]. Such conformational change, perhaps upon receptor binding, might modulate the kinase activity of the catalytic subunit.

A number of smaller adaptor proteins have been identified which also bind the class I_A catalytic subunits. These molecules are homologous to p85 but lack its SH3 domain, one of the two proline rich regions and the BH domain. Although some are generated by alternate splicing of the p85α gene, mouse $p55^{PIK}$ and its bovine homologue p55γ (p85γ) are encoded by independent genes [25,26]. The significance of such adaptor diversity is unclear since they do not display selectivity in binding the catalytic subunit. Since they show a tissue specific distribution, it is possible that such structural modifications may be of regulatory significance. Nature has thus provided a number of deletion mutants with which comparative biochemical analysis may be performed to reveal the functional role of the p85 SH3 and BH domains.

In addition to lipid kinase activity, class I_A PI3-kinases also possess an intrinsic ser/thr protein kinase activity [27,28]. This serves to phosphorylate the associated adaptor within the inter-SH2 domain of p85 in the case of p110α and p110β [28], or facilitates autophosphorylation as with p110γ [16]. The insulin receptor protein tyrosine kinase substrate IRS-1 is the only exogenous protein reportedly phosphorylated by p110α [29]. Phosphorylation of p85α Ser 608 which is located within its inter-SH2 domain has been proposed to regulate the lipid kinase activity of the catalytic subunit [28]. It may be of some

functional significance however that some of the smaller adaptor subunits lack a residue equivalent to Ser 608 in this region.

4.3. Class I_B PI3-kinases

Studies in platelets and neutrophils, have identified a form of PI3-kinase acting downstream of receptors which signal through heterotrimetric G-proteins [30,31]. Fractionation of neutrophil and U937 cell lysates resulted in the isolation of a PtdIns(4,5)P_2 selective kinase activity which was chromatographically distinct from the class IA enzymes [32]. This PI3-kinase complex was activated by heterodimeric GTPase $\beta\gamma$ subunits, possessed a native molecular mass of 220 kDa and did not contain a p85 like adaptor subunit. The cloning of p110γ defined a G-protein activated PI3-kinase catalytic subunit but its associated adaptor remained elusive [33]. With an effort not seen in the PI3-kinase field for many years, Stephens et al recently purified and cloned a porcine protein of 120 kDa, highly related to p110γ and a 97-kDa adaptor termed p101 based on its electrophoretic mobility [34]. Binding of p110γ to the p101 adaptor rendered it considerably more sensitive to activation by $\beta\gamma$ subunit. Although the p101/p110γ complex cannot be stimulated by tyrosine phosphorylated peptides, p110γ does contain a functional ras binding domain [35,36].

5. Class II PI3-kinases

These catalytic subunits are the largest (170–220 kDa) and the most recently identified form of PI3-kinase. Distinctively, they all contain a C-terminal C2 domain (Fig. 1). The C2 domain was originally defined as the second of four conserved regions within mammalian protein kinase C (PKC) where it conferred a Ca^{2+} sensitive phospholipid binding [37]. This property is not however, displayed by all C2 domains since only the first (C2A) but not the second (C2B) of two C2 domains within the synaptic vesicle protein synaptotagmin behaves in this manner [38]. In contrast, the synaptotagmin C2B domain mediates both a Ca^{2+}-dependent dimerization [39] and a Ca^{2+} independent association with the clathrin-AP2 complex [40].

The description of *Drosophila* PI3K 68_D [41] was followed shortly by the two murine sequences m-cpk and p170 [42,43], human PI3K-C2α [44] and an independent confirmation of the 68_D sequence (cpk) [42]. The two mouse proteins are likely to be products of the same gene and are the murine homologue of human PI3K-C2α. When lipid substrate specificity was examined, both PI3K_68D and p170 were shown to phosphorylate only PtdIns and PtdIns(4)P [41,43]. This offered the attractive proposition that structural differences between members of each class of PI3-kinase might reflect the type of 3′ phosphorylated phosphoinositide they produce. This hypothesis was revised when the lipid substrate specificity of human PI3K-C2α was determined. In the presence of PtdSer, PI3K-C2α also phosphorylated PtdIns(4,5)P_2 [44]. Although it is unclear whether this is a property displayed by other class II members it demonstrates the importance of lipid presentation during the assignment of in vitro activity and may explain the discrepancy between the in vitro findings and apparent in vivo specificity of p110α. Additionally, PI3K-C2α and murine p170, but not *Drosophila* 68_D/cpk, are refractory to both wortmannin and LY294002, two widely used inhibitors of PI3 kinase activity.

Like the synaptotagmin C2B domain, the PI3K 68_D C2 domain binds phospholipids in a Ca^{2+}-independent manner but its role within the class II PI3-kinase family members remains unclear [41]. Currently there is no indication that the C2 domain confers a Ca^{2+} sensitivity to this class of enzyme. The recent identification of a second, human class II PI3-kinase suggests the potential for differential regulation of enzyme activity as observed amongst the class I members [45].

6. Class III PI3-kinases

Vps34p and its human homologue PtdIns 3-kinase can only phosphorylate PtdIns [46]. Since Vps34p is the only PI3-kinase present in yeast, members of this class are considered to be the primordial form of PI3-kinase. Consequently, yeast provides an ideal model system within which to study the consequence of PtdIns(3)P production. Mutational analysis has identified a role for this lipid kinase in vesicular trafficking, osmoregulation and enodocytosis (reviewed in [47]). Vps34p binds the ser/thr kinase Vps15p which recruits it to membranes and activates lipid kinase activity. Similarly, PtdIns 3-kinase binds p150 a homologue of Vps15p [48]. We know little about the functional domains of PtdIns 3 kinase, aside from its catalytic and PIK domains and an as yet uncharacterized motif which binds p150.

In contrast, the study of the Vps15/p150 protein is more advanced. It has an N-terminal myristylation site, a ser/thr kinase domain, a series a HEAT repeats [49] and C-terminal WD motifs [50]. Although these domains provide a focus for study, their significance is presently unclear. Our studies indicate that mammalian p150 serves to target PtdIns 3-kinase to a perinuclear localization and using the yeast Vps34p/Vps15p model as a paradigm it is likely that the human homologues will fulfil an equivalent biological role in mediating vesicular trafficking. The possibility that PtdIns(3,5)P_2 mediates the downstream effects of the class III PI3-kinases requires further investigation [51].

7. PI3-kinase related proteins

These are large proteins (>270 kDa) which contain a region homologous to the PI3-kinase catalytic domain. They include the ataxia telangiectesia-mutated gene product, the targets of rapamycin (TOR) and the DNA-dependent protein kinase (reviewed in [52]). To date, none of these proteins have been found to display lipid kinase activity.

8. Regulation of PI3-kinase activity

Most of what we know about PI3-kinase activation and regulation has been gained from the study of the class I molecules. The reason for this is principally chronological, nevertheless, a greater understanding of class II and III enzymes is being generated through their identification and the development of appropriate reagents. Such studies will allow us to better understand how the different family members can fulfil their physiological function in a concerted manner.

Class I PI3-kinases have been demonstrated to play a role in almost every receptor mediated signalling event. Ligand stimulation results in either the activation of intrinsic receptor tyrosine kinase activity, the recruitment of an associated tyrosine kinase or the activation of heterotrimeric G-proteins.

When tyrosine residues become phosphorylated and are presented within a pTyr-X-X-Met consensus sequence they serve as docking sites for the class IA adaptor subunit SH2 domains [53]. Phosphopeptide binding to p85 SH2 domains results in an activation of lipid kinase activity [35]. Translocation to the plasma membrane not only brings the catalytic subunit into close proximity with its lipid substrate but it also facilitates the interaction with GTP bound ras thereby increasing PI3-kinase activity [17]. This places the class IA PI3-kinases on a pathway parallel to the raf ser/thr kinase [54]. The degree to which PI3-kinase activity is dependent upon ras is currently unclear [55].

Little is known about how heterotrimeric G-proteins activate the class IB PI3-kinase. Whilst Gβγ can directly stimulate p110γ lipid kinase activity this stimulation is considerably enhanced in the presence of the p101 adaptor [34].

9. Downstream targets of PI3 kinase

The hunt to identify major downstream targets of PI3-kinase has been fraught with difficulty. The identification of lipid binding domains such as the pleckstrin homology (PH) domain [56] and the C2 domain supports the view that downstream targets are likely to be proteins. Some have even considered if the protein kinase activity intrinsic to the catalytic subunit could mediate its downstream response [57]. Potential downstream targets of PI3-kinase activity include:

9.1. $p70^{S6}$ kinase

$p70^{S6}$ kinase phosphorylates the 40S ribosomal protein S6 in response to mitogenic stimuli and plays a key role in G1 to S phase transition through the cell cycle [58]. Although activation of $p70^{S6}$ kinase is achieved by a ser/thr protein kinase activity independent of the raf/MAPK pathway [59], evidence suggest that $p70^{S6}$ kinase is an important downstream target of PI3-kinase. Overexpression of receptor mutants which fail to bind the p85 adaptor and the use of PI3 kinase inhibitors both block ligand induced activation of $p70^{S6}$ kinase [60]. Furthermore, constitutive PI3-kinase activity results in the phosphorylation of $p70^{S6}$ kinase and antisera which target $p70^{S6}$ kinase block the mitogenic activity of PI3-kinase [61,62]. Mammalian TOR is another important regulator of $p70^{S6}$ kinase but neither p110 nor mammalian TOR phosphorylate $p70^{S6}$ kinase directly. Since N-terminal truncations of $p70^{S6}$ kinase render it sensitive to wortmannin but not rapamycin, it is thought that PI3-kinase and mammalian TOR activate $p70^{S6}$ kinase independently [63,64].

9.2. Akt kinase

The Akt ser/thr kinase is the cellular homologue of the retroviral oncogene v-akt and is activated upon ligand stimulation of receptor tyrosine kinases [65,66]. The Akt protein contains an N-terminal PH domain [56] in addition to the protein kinase domain. As with $p70^{S6}$ kinase, expression of receptor mutants which do not bind p85 and inhibitors of PI3 kinase block ligand induced Akt activation [66,67]. Although a direct and specific activation of Akt kinase activity by $PI(3,4)P_2$ has been reported [68,69] the significance of these findings has been questioned by the isolation of an Akt kinase termed PDK1 [70]. PDK1 is activated by both $PtdIns(3,4)P_2$ and $PtdIns(3,4,5)P_3$. Downstream of Akt lies GSK3, a constitutively active protein kinase whose activity is attenuated by Akt [71]. GSK3 appears to act as a negative inhibitor of both transcription and translation factors such as c-jun and eIF-2B in addition to metabolic enzymes such as glycogen synthase [72]. Consequently, activation of Akt by PI3-kinase leads to the phosphorylation and inhibition of GSK3 which, in turn, activates a number of key regulatory proteins.

9.3. Rac

The ras related GTP binding protein rac has been suggested as a downstream target of PI3-kinase [73,74]. Rac is thought to activate the JNK/stress activated protein kinase which phosphorylates c-jun [75].

9.4. Protein kinase C and related kinases

Calcium-independent isoforms of protein kinase C and protein kinase C related kinases have been found to be activated in vitro by 3′ phosphoinositides [76,77]. A similar mechanism has been proposed to act in whole cells [78]. Unfortunately, the specificity of these effects remains a contentious issue.

10. Conclusion

The pace at which novel PI3-kinase family members have been identified has surpassed the rate at which we have learnt about their biology. We are currently on the threshold of completing our catalogue of the players and through structural analysis we will soon learn much about their mechanisms of activation. By comparison, our understanding of how the activity of different family members is orchestrated within the cell and how the 3′ phosphorylated lipids achieve their spectrum of cellular effects remains rudimentary. The challenge ahead is to answer such questions and learn how best to exploit this knowledge for therapeutic benefit.

11. Apologies

We apologise to the authors of original papers whose reference could not be cited due to space limitations.

References

[1] Liscovitch, M. and Cantley, L.C. (1994) Cell 77, 329–334.
[2] Divecha, N. and Irvine, R.F. (1995) Cell 80, 269–278.
[3] Nishizuka, Y. (1992) Science 258, 607–614.
[4] Carpenter, C.L. and Cantley, L.C. (1996) Curr. Opin. Cell. Biol. 8, 153–158.
[5] Zvelebil, M.J. et al. (1996) Phil. Trans. R. Soc. Lond. B 351, 217–223.
[6] Cantley, L.C. et al. (1991) Cell 64, 281–302.
[7] Auger, K.R. et al. (1989) Cell 57, 167–175.
[8] Serunian, L.A. (1989) J. Biol. Chem. 264, 17809–17815.
[9] Irvine, R.F. (1992) Curr. Opin. Cell. Biol. 4, 212–219.
[10] Courtneidge, S.A. and Heber, A. (1987) Cell 50, 1031–1037.
[11] Carpenter, C.L. (1990) J. Biol. Chem. 265, 19704–19711.
[12] Morgan, S.J., Smith, A.D. and Parker, P.J. (1990) Eur. J. Biochem. 191, 761–767.
[13] Schu, P.V. et al. (1993) Science 260, 88–92.
[14] Hiles, I.D. et al. (1992) Cell 70, 419–429.
[15] Hu, P., Mondino, A., Skolnik, E.Y. and Schlessinger, J. (1993) Mol. Cell. Biol. 13, (12) 7677–7688.
[16] Vanhaesebroeck, B. et al. (1997) Proc. Natl. Acad. Sci. (in press)
[17] Rodriguez-Viciana, P. et al. (1996) EMBO J. 15, 2442–2451.
[18] Flanagan, C.A. et al. (1993) Science 262, (5138) 1444–1448.
[19] Domin, J., Dhand, R. and Waterfield, M.D. (1996) J. Biol. Chem. 271, (35) 21614–21621.
[20] Otsu, M. et al. (1991) Cell 65, 91–104.

[21] Dhand, R. et al. (1994) EMBO J. 13, 511–521.
[22] Gout, I. et al. (1993) Cell 75, 25–36.
[23] Musacchio, A., Cantley, L.C. and Harrison, S.C. (1996) Proc. Natl. Acad. Sci. 93, 14373–14378.
[24] Kapeller, R. et al. (1994) J. Biol. Chem. 269, (3) 1927–1933.
[25] Antonetti, D.A., Algenstaedt, P. and Kahn, C.R. (1996) Mol. Cell. Biol. 16, 2195–2203.
[26] Pons, S. et al. (1995) Mol. Cell. Biol. 15, 4453–4465.
[27] Carpenter, C.L. et al. (1993) Mol. Cell. Biol. 13, (3) 1657–1665.
[28] Dhand, R. et al. (1994) EMBO J. 13, 522–533.
[29] Lam, K. et al. (1994) J. Biol. Chem. 269, 20648–20652.
[30] Kucera, G.L. and Rittenhouse, S.E. (1990) J. Biol. Chem. 265, 5345–5348.
[31] Stephens, L. et al. (1993) EMBO J. 12, 2265–2273.
[32] Stephens, L. et al. (1994) Cell 77, (1) 83–93.
[33] Stoyanov, B. et al. (1995) Science 269, 690–693.
[34] Stephens, L.R. et al. (1997) Cell 89, 105–114.
[35] Rordorf-Nikolic, T. et al. (1995) J. Biol. Chem. 270, 3662–3666.
[36] Rubio, I., Buckle, P., Trutnau, H. and Wetzker, R. (1997) Biotechniques 22, 269–271.
[37] Kaibuchi, K. et al. (1989) J. Biol. Chem. 264, (23) 13489–13496.
[38] Li, C., Davletov, B.A. and Sudhof, T.C. (1995) J. Biol. Chem 270, (42) 24898–24902.
[39] Chapman, E.R., An, S., Edwardson, M. and Jahn, R. (1996) J. Biol. Chem. 271, (10) 5844–5849.
[40] Zhang, J.Z., Davletov, B.A., Sudhof, T.C. and Anderson, R.G. (1994) Cell 78, (5) 751–760.
[41] MacDougall, L.K., Domin, J. and Waterfield, M.D. (1995) Curr. Biol. 5, 1404–1415.
[42] Molz, L., Chen, Y.W., Hirano, M. and Williams, L.T. (1996) J. Biol. Chem. 271, (23) 13892–13899.
[43] Virbasius, J.V., Guilherme, A. and Czech, M.P. (1996) J. Biol. Chem. 271, (23) 13304–13307.
[44] Domin, J. et al. (1997) Biochem. J. (in press)
[45] Volinia, S. et al. (1997) Abstract 164 in: Keystone Symposium on Molecular and Cellular Biology.
[46] Volinia, S. et al. (1995) EMBO J. 14, (14) 3339–3348.
[47] De Camilli, P., Emr, S.D., McPherson, P.S. and Novick, P. (1996) Science 271, 1533–1539.
[48] Panaretou, C., Domin, J., Cockcroft, S. and Waterfield, M.D. (1997) J. Biol. Chem. 272, 2477–2485.
[49] Andrade, M.A. and Bork, P. (1995) Nat. Genet. 11, (2) 115–116.
[50] Neer, E.J., Schmidt, C.J., Nambudripad, R. and Smith, T.F. (1994) Nature 371, 297–300.
[51] Whiteford, C.C., Brearley, C.A. and Ulug, E.T. (1997) Biochem. J. 323, 597–601.
[52] Keith, C.T. and Schreiber, S.L. (1995) Science 270, 50–51.
[53] Kapeller, R. and Cantley, L.C. (1994) BioEssays 16, (8) 565–576.
[54] Marshall, C.J. (1995) Cell 80, 179–185.
[55] Valius, M. and Kazlauskas, A. (1993) Cell 73, (2) 321–334.
[56] Lemmon, M.A., Ferguson, K.M. and Schlessinger, J. (1996) Cell 85, 621–624.
[57] Hunter, T. (1995) Cell 83, 1–4.
[58] Lane, H.A., Fernandez, A., Lamb, N.J.C. and Thomas, G. (1993) Nature 363, 170–172.
[59] Ferrari, S. et al. (1992) Proc. Natl. Acad. Sci. USA. 89, 7282–7286.
[60] Chung, J. et al. (1994) Nature 370, 71–75.
[61] Weng, Q.P. et al. (1995) Proc. Natl. Acad. Sci. USA. 92, 5744–5748.
[62] Mc Ilroy, J. et al. (1997) Mol. Cell. Biol. 17, 248–255.
[63] Cheatham, L., Monfar, M., Chou, M.M. and Blenis, J. (1995) Proc. Natl. Acad. Sci. USA. 92, 11696–11700.
[64] Weng, Q.P. et al. (1995) Mol. Cell. Biol. 15, 2333–2340.
[65] Belacossa, A., Testa, J.R., Staal, S.P. and Tsichlis, P.N. (1991) Science 254, 244–247.
[66] Burgering, B.M.T. and Coffer, P.J. (1995) Nature 376, 599–602.
[67] Franke, T.F. et al. (1995) Cell 81, 727–736.
[68] Klippel, A., Kavanaugh, W.M., Pot, D. and Williams, L.T. (1997) Mol. Cell. Biol. 17, 338–344.
[69] Franke, T.F., Kaplan, D.R., Cantley, L.C. and Toker, A. (1997) Science 275, 665–668.
[70] Alessi, D.R. et al. (1997) Curr. Biol. 7, 261–269.
[71] Cross, D.A.E. et al. (1995) Nature 378, 785–789.
[72] Welsh, G., Wilson, C. and Proud, C.G. (1996) Trends Cell. Biol. 6, 274–279.
[73] Hawkins, P.T. et al. (1995) Curr. Biol. 5, 393–403.
[74] Klippel, A. et al. (1996) Mol. Cell. Biol. 16, 4117–4127.
[75] Minden, A. et al. (1995) Cell 81, 1147–1157.
[76] Toker, A. et al. (1994) J. Biol. Chem. 269, (51) 32358–32367.
[77] Palmer, R.H. et al. (1995) J. Biol. Chem. 270, (38) 22412–22416.
[78] Zhang, J. et al. (1995) J. Biol. Chem. 270, 22807–7777822810.