Structure-Function Relationships of the Mouse Gap1m

DETERMINATION OF THE INOSITOL 1,3,4,5-TETRAKISPHOSPHATE-BINDING DOMAIN

(Received for publication, April 10, 1996, and in revised form, May 13, 1996)

Mitsunori Fukuda‡§ and Katsuhiko Mikoshiba‡

From the Molecular Neurobiology Laboratory, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki 305, the Department of Molecular Neurobiology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, and the Calciocalcine Net Project, Exploratory Research for Advanced Technology (ERATO), 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

Gap1IP4BP, one of a member of Ras GTPase-activating proteins, has been identified as a specific inositol 1,3,4,5-tetrakisphosphate (IP$_4$)-binding protein (Cullen, P. J. *, Hsuan, J. J., Truong, O., Letcher, A. J., Jackson, T. R., Dawson, A. P., and Irvine, R. F. (1995) Nature 386, 527-530). In this paper we describe Gap1m, which is closely related to Gap1IP4BP, to also be an IP$_4$-binding protein and show that the pleckstrin homology domain (PH) is the central IP$_4$-binding domain by expressing fragments of the mouse Gap1m in Escherichia coli as fusion proteins and examining their activities. However, in addition to the PH domain, an adjacent GAP-related domain and carboxyl terminus are required for high affinity specific IP$_4$ binding. The PH domain is highly conserved in the Gap1 family and also has striking homology to the amino-terminal region of Bruton’s tyrosine kinase. Substitution of Cys for Arg at position 628 in the PH domain corresponding to the mutation of Bruton’s tyrosine kinase observed in X-linked immunodeficiency (Xid) mice results in the central IP$_4$ binding domain of Gap1m similar to that of the wild type Gap1m. Our results suggest that the PH domain of Gap1m functions as a modulatory domain of GAP activity by binding IP$_4$ and phospholipids.

Inositol 1,4,5-trisphosphate (IP$_3$) is one of the well characterized second messengers that trigger IP$_3$ receptor-mediated Ca$^{2+}$ release from intracellular pools (1, 2). IP$_3$ is rapidly metabolized by various phosphatases and kinases. Among the metabolites of IP$_3$, inositol 1,3,4,5-tetrakisphosphate (IP$_4$) is produced by the specific IP$_3$-3 kinase (3), and has been suggested to have a physiological function potentially as a second messenger regulating intracellular Ca$^{2+}$ concentration (4-11). However, the actual function of IP$_4$ is still a matter of debate because the specific receptor protein involving intracellular Ca$^{2+}$ homeostasis has not been identified.

Recently Cullen et al. (12) established that Gap1IP4BP, one of a member of Ras GTPase-activating proteins (GAP), is a specific IP$_4$-binding protein and showed that IP$_4$ specifically restored the Ras GAP activity after inhibition by phospholipids in vitro. To determine the physiological function of IP$_4$ binding to Gap1IP4BP in vivo, it is necessary to determine its binding site. A knowledge of the binding site allows the effects of specific antibodies against or peptides from the binding domain in functioning cells. Using this strategy, the roles of inositol high polyphosphates, including IP$_4$, inositol 1,3,4,5,6-pentakisphosphate (IP$_5$), and inositol 1,2,3,4,5,6-hexakisphosphate (IP$_6$) have been determined in neurotransmitter release (13-19). We have determined that the C2B domain of synaptotagmin II is an inositol high polyphosphate-binding domain by structure-function analysis (13). The two C2 domains of synaptotagmin were shown to have different functions in synaptic vesicle trafficking by injecting the domain-selective antibodies against C2A or C2B domain into the squid giant presynapse (15, 17), superior cervical ganglion cells (18), and chromaffin cells (19). Specifically, these results indicated that the C2A domain functions as a Ca$^{2+}$ sensor in exocytosis, the inositol high polyphosphate blocks synaptic transmission by binding to the C2B domain, and the C2B domain is also involved in endocytosis.

Gap1m was originally identified as a mammalian homologue of Drosophila Gap1 (20, 21), and at least two forms of Gap1 have been shown to be present in mammals (Gap1m, see Ref. 21; and Gap1IP4BP/Gap1l, see Refs. 12 and 22). Both proteins are almost identical in size and share the same structural features, two C2 domains homologous to the C2 regulatory region of protein kinase C, a GAP-related domain (GRD), and a pleckstrin homology (PH) domain (12, 20, 21). The structures of these domains are highly conserved in the Gap1 family from Drosophila to human. However, the functional properties of the C2 domains and the PH domain have not been elucidated.

In the present study, we report studies on bacterially expressed fusion proteins of the mouse Gap1m and show that the central IP$_4$-binding domain is the PH domain, not the C2 domains. The PH domain is an approximately 100-residue protein module that is found in many proteins involved in signal transduction (23-25). Although these domains are divergent, PH domains of the Gap1 family and Bruton’s tyrosine kinase (Btk), a cytoplasmic tyrosine kinase, are highly conserved (21, 26). Btk is crucial for B cell development and a mutation in the PH domain of Btk in the X-linked immunodeficiency (Xid) mice interferes with normal B cell signaling (26). We introduced a mutation into the PH domain of Gap1m similar to that of Btk in Xid mice and found that the mutant Gap1m had a dramatically reduced IP$_4$ binding activity but retained normal Ras GAP activity.
activity. On the basis of these results, we discuss this novel function of the PH domain of Gap1m and Btk in signal transduction.

MATERIALS AND METHODS

Chemicals—Phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) were obtained from Sigma. IP₄, IP₅, and IP₆ were purchased from Boehringer Mannheim. IP₃ was from Calbiochem. All other chemicals were commercial products of reagent grade. Solutions were prepared in deionized water.

cDNA Cloning of Mouse Gap1m—cDNA encoding Gap1m (amino acids 22–276) from mouse cerebellum was amplified by the reverse transcriptase polymerase chain reaction (PCR) (LA-PCR kit; Takara Shuzo, Japan) for 40 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 50°C for 2 min, and extension at 72°C for 3–4.5 min. The extension time was increased by 30 s every 10 cycles. The sense and antisense primers were designed on the basis of the rat Gap1m cDNA (21) as follows: primer 1 (sense, amino acid residues 22–29), 5'-CGGATCCGAAAATGGAACTGTGT-3'; primer 2 (sense, amino acid residues 164–171), 5'-CGGATCCGAAAATGGAACTGTGT-3'; primer 3 (sense, amino acid residues 270–276), 5'-CAAGATCTAGAATTAGGTTCTGTCACAA-3'; primer 4 (sense, amino acid residues 609–616), 5'-CGGATCCGAAAATGGAACTGTGT-3'; primer 5 (antisense, amino acid residues 270–276), 5'-CAAGATCTAGAATTAGGTTCTGTCACAA-3'; primer 6 (antisense, amino acid residues 702–708), 5'-CGAATTCTGTTGTGGTCACCTGCTCA-3'; and primer 7 (antisense, amino acid residues 841–847), 5'-CGAATTCTGTTGTGGTCACCTGCTCA-3'. The PCR products were purified on a 0.7% agarose gel and extracted with a GeneClean kit II (Bio 101). After digestion with BamHI (or BglII) and EcoRI, the cDNA inserts were subcloned into the BamHI-EcoRI site of pGEX-2T (Pharmacia Biotech Inc.) and verified by DNA sequencing. When compared with rat Gap1m (21), 23 amino acid substitutions were found (97% identity): Lys at position 157 was altered to Arg (K157R), T235S, N280S, S283F, P302T, Y318C, A330T, R354Q, D382E, Q389P, S450N, S464N, A465T, R491K, N495H, R556K, K635R, K646R, S665G, M698V, A777V, and V763I.

Characterization of IP₄-Binding Domain of Gap1m

Because they were also found in two independent PCR products of Gap1m, these changes are probably not due to PCR-induced errors because they were also found in two independent PCR products of Gap1m.

Preparation of GST Fusion Proteins—Various domains of mouse Gap1m (Fig. 1A) were expressed as glutathione S-transferase (GST) fusion proteins in Escherichia coli M109 and purified by glutathione-Sepharose 4B chromatography (Pharmacia) according to the manufacturer's recommendation. GST-A (C2A + C2B) coded for amino acids 22–276 of mouse Gap1m; GST-B (C2B), amino acids 164–276; GST-C (C2B + GRD + PH), amino acids 164–708; GST-D (GRD), amino acids 702–708; GST-E (PH), amino acids 609–708; GST-F (GRD + PH + C terminus), amino acids 270–847, and GST-G (PH + C terminus), amino acids 609–847.

Site-directed Mutagenesis—Site-directed mutagenesis of GST-F (R628C) and GST-F (K626Q, K627Q, R628Q) was performed using PCR techniques as follows. In the case of GST-F (R628C), for example, cDNA fragment encoding amino acids 609–638 was amplified from GST-F by primer 4 and mutagenic primer A 5'-TGAGCTCTCTGCTGTCAGGACAGCAGATATTTCTTCGAGA-3'. The PCR fragments encoding the mutant PH domain of Gap1m (R628C) and GST-F (K626Q, K627Q, R628Q) was produced by the same techniques using mutagenic primer B 5'-TGAGCTCTCTGCTGTCAGGACAGCAGATATTTCTTCGAGA-3'.

Measurement of [3H]IP₄ Binding to GST Fusion Proteins—GST fusion proteins (5 µg) were incubated with 9.6 nM [3H]IP₄ (DuPont NEN) in 50 µl of 50 mM HEPES-KOH, pH 7.2, for 10 min at 4°C. The sample was then mixed with 1 µl of 50 mg/ml γ-globulin and 51 µl of a solution containing 30% polyethylene glycol 6000 and 50 mM HEPES-KOH, pH 7.2, and placed on ice for 5 min. The precipitate obtained by centrifugation at 10,000 × g for 5 min was solubilized in 500 µl of Solvable (DuPont NEN) and radioactivity was measured in Aquasol 2 (DuPont NEN) with a liquid scintillation counter (13). Inhibition of specific
[\( ^{3}H \)IP]$_{4}$ binding to GST fusion proteins was also performed in the above reaction mixture containing various inositol phosphates.

Phospholipid Binding Assay—Four kinds of liposomes (PS, PI, PC, and PE) were prepared in 50 mM HEPES-KOH, pH 7.2, and 100 mM NaCl by sonication, collected by centrifugation, and equilibrated with 50 mM HEPES-KOH, pH 7.2, and 100 mM NaCl in the presence of 2 mM EGTA or 0.5 mM Ca$^{2+}$. GST fusion proteins (5-10 \( \mu \)g) were incubated with liposomes corresponding to 160 \( \mu \)g of phospholipid for 15 min at room temperature (27). After centrifugation, 12000 \( \times g \) for 10 min at room temperature, the phospholipid pellets were washed in 500 \( \mu \)l of the above equilibration buffer and then extracted with 300 \( \mu \)l of acetonitrile at -20 °C for 30 min to remove excess lipid. The pellets obtained by centrifugation at 12000 \( \times g \) for 15 min at 4 °C were dissolved in SDS sample buffer. The proteins in the supernatants were precipitated by adding an equal volume of 20% trichloroacetic acid. After a 15-min incubation on ice, the samples were centrifuged at 12000 \( \times g \) for 15 min at 4 °C, and the precipitates were mixed with SDS sample buffer. Equal proportions of the supernatants and pellets were analyzed by 10% SDS-polyacrylamide gel electrophoresis followed by Coomassie Brilliant Blue R-250 staining. The protein concentrations were determined by the Bio-Rad protein assay kit using bovine serum albumin as a reference.

Ras GAP Assay—GAP assays were performed according to Calés et al. (28) with modifications. Ha-Ras (6 \( \mu \)g) was incubated at 37 °C for 5 min with 0.1 \( \mu \)M [\( ^{32}P \)GTP (3000 C/mm; DuPont NEN) in the presence of 50 mM HEPES-KOH, pH 7.2, 3 mM EDTA, and 5 mM dithiothreitol. Free [\( ^{32}P \)GTP was removed by Microcon10 (Amicon). Ha-Ras[\( ^{32}P \)GTP complex (about 300 ng) was incubated with 1 \( \mu \)g of wild or mutant GST-F in 10 \( \mu \)l of a solution containing 50 mM HEPES-KOH, pH 7.2, 5 mM MgCl$_{2}$, and 5 mM dithiothreitol in the presence or absence of 53 \( \mu \)M PS (and IP$_{4}$) for 10 min at 37 °C. After addition of 5 \( \mu \)l of 10 mM EDTA and 0.5% SDS, the samples were incubated for 5 min at 70 °C to release bound nucleotide from Ha-Ras. After centrifugation, the supernatant (0.5 \( \mu \)l) was loaded onto a TLC plate (PE1-cellulose, Merck) and chromatographed in 0.5 M lithium chloride and 1 M formic acid. The amounts of GTP and GDP were determined by a Fuji Biomage Analyzer (Fuji Photo Film Co.) using an imaging plate.

### RESULTS

Determination of the IP$_{4}$-binding Domain of the Mouse Gap1m—To delineate the IP$_{4}$-binding domain of Gap1m, we produced seven GST fusion proteins, which almost covered the entire Gap1m sequence (amino acid residues 22–847) (Fig. 1A). Although the C2B domain of Gap1m was expected to be an IP$_{4}$-binding domain like that of synaptotagmin II (12, 13), the IP$_{4}$ binding activity was mapped to the PH domain as shown in Fig. 1B. However, competition experiments indicated that the PH domain alone is not sufficient for high affinity IP$_{4}$ binding and inositol phosphate binding specificity (Fig. 2). In the case of GST-E, which contained only the PH domain, the order of competitive potencies was IP$_{6} >$ IP$_{4} >$ IP$_{5} >$ IP$_{3}$, and the apparent dissociation constant (K$_{d}$) for IP$_{4}$ was 350 nM (Fig. 2A). Thus the PH domain alone behaves as an inositol high polyphosphate-binding domain like the C2B domain of synaptotagmin II (13). In contrast, GST-F, which contained the GRD, PH domain, and C (carboxyl) terminus, bound IP$_{4}$ with high affinity (K$_{d}$ = 40 nM) and showed an inositol phosphate binding specificity that was similar to that of the purified porcine protease Gap1m-RBIP (IP$_{4} >$ IP$_{5} >$ IP$_{3} >$ IP$_{1}$, see Refs. 29 and 30).

Table I summarizes the inositol phosphate binding properties of GST-C to -G was analyzed by competition experiments as shown in Fig. 2. ND, not determined because of lack of IP$_{4}$ binding activity.

| Name   | Specificity | Affinity for IP$_{4}$ (K$_{d}$) |
|--------|-------------|---------------------------------|
| GST-A  | ND          | ND                              |
| GST-B  | ND          | ND                              |
| GST-C  | IP$_{4}$ > IP$_{3}$ > IP$_{5}$ > IP$_{6}$ | 500 nM |
| GST-D  | IP$_{4}$ > IP$_{3}$ > IP$_{5}$ > IP$_{6}$ | 300 nM |
| GST-E  | IP$_{4}$ > IP$_{3}$ > IP$_{5}$ > IP$_{6}$ | 350 nM |
| GST-F  | IP$_{4}$ > IP$_{3}$ > IP$_{5}$ > IP$_{6}$ | 40 nM |
| GST-G  | IP$_{4}$ > IP$_{3}$ > IP$_{5}$ > IP$_{6}$ | 400 nM |

**Fig. 3.** **Mutational analysis of the IP$_{4}$ binding domain of Gap1m**. A, sequence similarity between the PH domains of human Gap1m (hsGap1m, residues 578–676; K03599), mouse Gap1m (mGap1m, residues 609–708; D30734), Drosofibila Gap1 (dmGap1, residues 768–866; M86655), and mouse Bruton's tyrosine kinase (Btk, residues 9–138; L08697). Residues that are identical in the four sequences are boxed. As compared to Gap1 family, Btk has three insertions in the PH domain (bottom sequences). The Arg residue at position 28 of Btk which is replaced by Cys in Xid mice (see Ref. 26) is indicated by an asterisk. Roman figures at the top indicate the subdomain of the PH (see Ref. 25). B, schematic representation of site-directed mutation of GST-F (R628C) and GST-F (K627Q, K627Q, L628Q). Identical amino acids are shown by vertical bars and mutations are indicated by a box. C, mutant GST-F (5 \( \mu \)g) was analyzed by \([\text{3}H]\)IP$_{4}$ binding assays as described under "Materials and Methods." The data are means ± S.E. of three measurements normalized to 100% for binding to GST-F.
The N-terminal region of the PH domain contained a cluster of conserved positively charged amino acids (K wx x g K/K/K/K/K, subdomain II). These basic residues between subdomains I and II (Fig. 3A) are likely to be essential for IP₄ binding to Gap1m by analogy with the IP₃ binding to β-spectrin (31) and phospholipase C-ε1 (32, 33). To test whether this region is important for the binding of negatively charged IP₄, we introduced mutations into this region and analyzed the mutant GST-F in IP₄ binding assays (Fig. 3B). GST-F (R628C) carried a conversion of Arg into Cys at position 628, which is one of the essential residues for IP₄ binding to Gap1m.

Analysis of Phospholipid-Binding Domain of Gap1m—Maeckawa et al. (34) reported that the Gap1m could be isolated from both the cytoplasmic and particulate fractions of rat brain. To determine the membrane associated domain of Gap1m, we first examined the phospholipid binding capacity of two C2 domains (C2A + C2B) and PH domain (Fig. 4, top left and right). GST-A mostly bound PS liposomes in a Ca²⁺-dependent manner like the C2A domain of synaptotagmins (27, 35, 36) but did not bind PI, PC, and PE liposomes. In contrast, most of the GST-G bound PS liposomes even in the presence of 2 mM EGTA (top right). Both GST-G (R628C) and GST-F (K626Q, K627Q, R628Q), were also tested. Both mutants showed a reduced phospholipid binding capacity (Fig. 4, bottom right). In addition, IP₄ binding to GST-F was inhibited by PS in a dose-dependent manner (data not shown).

These results suggest that the PH domain is involved in association with membranes and that the IP₄-binding site overlaps with the phospholipid-binding site of the PH domain.

Ras GAP Activity of Wild and Mutant Gap1m—To further examine whether the mutant lacking IP₄ binding capacity showed Ras GAP activity, the GAP assay was carried out using Ha-Ras[α-³²P]GTP as a substrate (Fig. 5). GST-F (R628C) also activated the GTPase activity of Ha-Ras but was less potent as an activator than wild type GST-F (Fig. 5). These results are consistent with the fact that the mutant Btk protein (R28C) in Xid mice shows normal kinase activity (26). In the presence of 53 μM PS liposomes corresponding to the concentration in the inner plasma membrane leaflet, the Ras GAP activity of both GST-F and GST-F (R628C) were slightly suppressed. This weak suppression was meaningful because it was restored by 10 μM IP₄ in the case of GST-F but not GST-F (R628C), probably due to the lack of IP₄ binding capacity in this mutant protein.

DISCUSSION

In this study we have demonstrated the structure-function relationships of the mouse Gap1m in terms of Ras GAP activity, IP₄, and phospholipid binding. From a study of deletion mutants of Gap1m, the PH domain was determined to be an effector domain for GAP activity and functions as a central IP₄- and phospholipid (PS)-binding domain.

In a previous study (13, 14), we have reported that the C2B domain did not (Fig. 4, bottom left), the C2A domain alone is sufficient for Ca²⁺/phospholipid binding. In contrast, most of the GST-G bound PS liposomes and interacted weakly with PI liposomes irrespective of the presence of Ca²⁺. The phospholipid binding properties of the mutant PH domain, GST-G (R628C) and GST-F (K626Q, K627Q, R628Q), were also tested. Both mutants showed a reduced phospholipid binding capacity (Fig. 4, bottom right). In addition, IP₄ binding to GST-F was inhibited by PS in a dose-dependent manner (data not shown).
domain of synaptotagmins I, II, and IV are essential for inositol high polyphosphate binding and determined the putative IP$_4$ binding motifs (HLMQGKRLLKKTVVKGNPFEFRKSF; amino acid residues 315–346 of synaptotagmin I). However, it is not easy to predict whether other proteins that have the C2B domain bind IP$_4$ because there are some exceptions. For example, synaptotagmin III has putative IP$_4$ binding sequence in the C2B domain but did not bind IP$_4$, probably due to the steric hindrance of the C-terminal flanking region (14). We infer that the C2B domain of Gap$_{1}^{m}$ is such a case because the peptide from the C2B domain of Gap$_{1}^{m}$ (amino acid residues 194–228; ATVSLVGPMDQKTTKKKTQNSPQNEFYEV), corresponding to the putative IP$_4$-binding domain of synaptotagmin II, bound IP$_4$ ($K_{d} > 1 \mu M$; data not shown).

The PH domain is a new divergent protein module of approximately 100 amino acids found in many proteins involved in signal transduction (23, 24). Although the consensus functional properties have not been elucidated, many PH domains bind to the $\beta y$ subunits of heterotrimeric G proteins (37), and phosphatidylinositol 4,5-bisphosphate (38) and the PH domain of inositol 4,5-bisphosphate) directly affect the GRD of Gap$_{1}^{m}$ like but not the C2B domain of Gap$_{1}^{m}$ showed Ca$^{2+}$-dependent sites, double C2 domains and the PH domain. The C2A domain binds IP$_4$ and phospholipids and/or inositol high polyphosphate. Loss of these properties in the mutant Btk protein (R28C) may cause false activation or inactivation of kinase activity or protein-protein interaction via the SH2 (Src homology domain 2) or the SH3 domain. On the basis of these results, together with our own observations, we propose that some PH domains function as modulatory domains of kinases.

Acknowledgments—We thank Dr. Takanoi Kigawa for helpful advice, Drs. Michio Niinobe, Akihiko Mizutani, and Mikako Shioruzu for critical reading of the manuscript, and Toshio Kojima, Kuniko Takahashi, and Masako Suengae for expert technical assistance. We are grateful to members of the Mikoshiba Laboratory for valuable discussions.

REFERENCES

1. Berridge, M. J. (1993) Nature 361, 315–325
2. Furuchi, T., and Mikoshiba, K. (1995) J. Neurochem. 64, 953–960
3. Irvine, R. F., Letcher, A. J., Heslop, J. P., and Petersen, O. H. (1987) FEMS Lett. 38, 25–40
4. Irvine, R. F., and Moor, R. M. (1987) Biochem. Biophys. Res. Commun. 146, 10708–10712
5. Morris, A. P., Gallacher, D. V., Irvine, R. F., and Petersen, O. H. (1997) Nature 330, 653–655
6. Parès, I., and Miledi, R. (1987) Proc. R. Soc. Lond. Biol. 232, 59–70
7. Snyder, P. M., Krause, K.-H., and Welsh, M. J. (1988) J. Biol. Chem. 263, 11048–11051
8. Hill, T. D., Dean, N. M., and Boynton, A. L. (1988) Science 242, 1176–1178
9. Czegy, L., Gallacher, D. V., Irvine, R. F., and Petersen, O. H. (1988) FEMS Lett. 251, 43–48
10. Ely, J. A., Hunyady, L., Bauk, A. J., and Catt, K. J. (1990) Biochem. J. 268, 333–338
11. Yoo, S. H. (1991) Biochem. J. 278, 381–385
12. Cullen, P. J., Hsu, J. J., Truong, O. L., Letcher, A. J., Jackson, T. R., Dawson, J. P., and Irvine, R. F. (1995) Nature 376, 527–530
13. Fukuda, M., Aruga, J., Niinobe, M., Aimoto, S., and Mikoshiba, K. (1994) J. Biol. Chem. 269, 29206–29211
14. Fukuda, M., Kojima, T., Aruga, J., Niinobe, M., and Mikoshiba, K. (1995) J. Biol. Chem. 270, 26523–26527
15. Fukuda, M., Moreira, J. E., Lewis, F. M. T., Sugimori, M., Niinobe, M., Mikoshiba, K., and Llinás, R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10703–10707
16. Llinás, R., Sugimori, M., Lang, E. J., Morita, M., Fukuda, M., Niinobe, M., and Mikoshiba, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12990–12993
17. Mikoshiba, K., Fukuda, M., Moreira, J. E., Lewis, F. M. T., Sugimori, M., Niinobe, M., and Llinás, R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10703–10707
18. Mochida, S., Fukuda, M., Niinobe, M., Kobayashi, H., and Mikoshiba, K. (1995) Satellite Symposium 15th ISN Meetings, Hamamatsu, Japan, June 27–30 (abstr.).
19. Ohara-Imaizumi, M., Fukuda, M., Niinobe, M., Misonou, H., Ikeda, K., Kumakura, K., and Mikoshiba, K. (1995) Satellite Symposium 15th ISN Meetings, Hamamatsu, Japan, June 27–30, pp. 125–126 (abstr.).
20. Gaul, U., Mardon, G., and Rubin, G. M. (1992) Cell 68, 1007–1019
21. Maekawa, M., Li, S., Iwamatsu, A., Morishita, T., Yokota, K., Imai, Y., Kihoka, S., Nakamara, S., and Hattori, S. (1994) Mol. Cell. Biol. 14, 6879–6885
22. Baba, H., Fuss, B., Urano, J., Poulet, P., Watson, J. B., Tannard, F., and Madsen, W. B. (1995) Naunyn. Schmied. Arch. 416, 846–858
23. Mayer, B. J., Ren, R., Clark, K. L., and Baltimore, D. (1993) Cell 73, 629–630
24. Haselk, R., Kede, H. B., and Hemmings, B. A. (1993) Nature 363, 309–310
25. Mochida, A., Gibson, T. F., Thompson, J. P., and Saraste, M. (1993) Trends Biochem. Sci. 18, 343–348
26. Rawlings, D. J., Saffran, D. C., Tsukada, S., Largespeada, D. A., Grimaldi, J. C., Cohen, L., Mohr, R. N., Bazzan, J. F., Howard, M., Copeland, N. G., Jenkings, N. A., and Witte, D. O. (1993) Science 261, 358–361
27. Fukuda, M., Kojima, T., and Mikoshiba, K. (1996) J. Biol. Chem. 271, 8430–8434
28. Cullen, P. J., Hancock, J. F., Marshall, C. J., and Hall, A. (1988) Nature 332, 548–551
29. Cullen, P. J., Dawson, A. P., and Irvine, R. F. (1995) Biochem. J. 305, 139–143
30. Cullen, P. J., Chung, S.-K., Chang, T.-F., Dawson, A. P., and Irvine, R. F. (1995) FEMS Lett. 358, 246–252
31. Hyvönen, M., Macas, M. J., Nilges, M., Oschinat, H., Saraste, M., and Wilmanns, M. (1995) EMBO J. 14, 4676–4685
32. Yagisawa, H., Hirata, M., Kanamatu, T., Watanabe, Y., Ozaki, S., Sakuma, K., Tanaka, H., Yabuta, N., Kamata, H., Hirata, H., and Nogima, H. (1994) J. Biol. Chem. 269, 20179–20188
33. Hirata, M., Kanamatu, T., Sakuma, K., Noga, T., Watanabe, Y., Ozaki, S., and Yagisawa, H. (1994) Biochem. Biophys. Res. Commun. 205, 1563–1571
34. Mikoshiba, M., Nakamura, S., and Hattori, S. (1993) J. Biol. Chem. 268, 22948–22952
35. Davletov, B. A., and Sudhof, T. C. (1993) J. Biol. Chem. 268, 26386–26390
36. Li, C., Uhilich, B., Zhang, J. Z., Anderson, R. G. W., Brousse, N., and Sudhof, T. C. Nature 385, 594–595
37. Trouhara, K., Inagaki, J., Pitcher, J. A., Shaw, G., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 10217–10220
38. Harlan, E. J., Haiduk, P. J., Yoon, H. S., and Fesik, S. W. (1994) Nature 381, 168–170
39. Bodlaj, G., and McCormick, F. (1991) Nature 351, 576–579