The PDZ protein TIP-1 interacts with the Rho effector rhotekin and is involved in Rho signaling to the serum response element
Caroline Reynaud, Stéphane Fabre, Pierre Jalinot

To cite this version:
Caroline Reynaud, Stéphane Fabre, Pierre Jalinot. The PDZ protein TIP-1 interacts with the Rho effector rhotekin and is involved in Rho signaling to the serum response element. Journal of Biological Chemistry, American Society for Biochemistry and Molecular Biology, 2000, 275 (43), pp.33962-33968. 10.1074/jbc.M000465200 . hal-02693940

HAL Id: hal-02693940
https://hal.inrae.fr/hal-02693940
Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
The PDZ Protein TIP-1 Interacts with the Rho Effector Rhotekin and Is Involved in Rho Signaling to the Serum Response Element*

Received for publication, January 21, 2000, and in revised form, July 31, 2000
Published, JBC Papers in Press, August 11, 2000, DOI 10.1074/jbc.M000465200

Caroline Reynaud, Stéphane Fabre‡, and Pierre Jalinot§

From the Laboratoire de Biologie Moléculaire et Cellulaire, Unité Mixte de Recherche 5665, CNRS-Ecole Normale Supérieure de Lyon, 46, Allée d’Italie, 69364 Lyon Cedex 07, France

A wealth of studies has established that proteins encoded by various types of viruses establish specific contacts with key regulatory cellular proteins. Recently, a novel class of cellular proteins characterized by a specific protein motif, the PDZ domain, has been shown to be targeted by viral transforming proteins. The PDZ domain, the name of which corresponds to the first letter of PSD-95 (a post-synaptic density protein), Discs-large (a Drosophila tumor suppressor), and ZO-1 (a tight junction protein), is known to be present in a rapidly increasing number of proteins exhibiting diverse functions (1, 2). The proteins E4 open reading frame 1 of adenovirus type 9, E6 of human papilloma virus type 16 or 18, and Tax of human T-cell lymphotrophic virus, type 1 Tax protein can interact via its C terminus with various proteins including a PDZ domain. In this work, one of them, TIP-1, is characterized as a cytoplasmic 14-kDa protein mainly corresponding to one PDZ domain. A two-hybrid screen performed with TIP-1 as bait showed that it interacts with the human homologue of rhotekin that was previously identified in mice as a Rho effector. Both human and mouse rhotekins exhibit at their C termini the sequence QSPV-COOH that matches the X(S/T)-XV-COOH consensus known for proteins recognizing PDZ domains. Mutation of the serine and valine residues to alanine impairs interaction of rhotekin with TIP-1. Transient expression experiments with a reporter construct including the c-Fos serum response element (SRE) showed that coexpression of TIP-1 with the constitutively active RhoA.V14 mutant and human rhotekin caused a strong activation of the SRE. A negative mutant of Rho, RhoA.N19, was unable to cooperate with TIP-1 and rhotekin. The positive effect of TIP-1 was also lost when the C terminus of rhotekin was mutated. These data show that the complex of active Rho with its effector rhotekin bound to TIP-1 produces in the cytoplasm a signal that triggers strong activation of the SRE.

The human T-cell lymphotrophic virus, type 1 Tax protein has also been shown to interact with a series of cellular proteins characterized by the presence of a PDZ domain (6). Two-hybrid studies have shown that the PDZ domain recognizes the X(S/T)-XV-COOH motif, X being any amino acid, at the C-terminal ends of proteins (7). This model of interaction stems from study of the interaction of PSD-95 with subunits of the glutamate receptor, as well as with shaker type potassium channels (8, 9). These original observations have been extensively confirmed with various PDZ proteins and have also received support from resolution of the structure of the third domain of PSD-95 in complex with a 9-aminoc acid peptide (10). This showed that the peptide forms an antiparallel β-sheet with strand β-3 of the PDZ domain that includes six β strands interrupted by two α helices (10). The C-terminal amino acids of the peptide exchange several hydrogen bonds with a loop between strands β-2 and β-3. A systematic study of peptides able to interact with particular PDZ domains has shown that many variations were possible on the basis of the X(S/T)-XV-COOH consensus (11). The C-terminal valine can be substituted with other hydrophobic or aromatic residues in the case of particular PDZ domains. This suggests that a specific relationship exists between a given PDZ domain and the C terminus of the associated proteins. If a number of PDZ proteins are cytoplasmic or localized at the inner side of the membrane, where they can interact with transmembrane receptors, playing an important role in receptor clustering and signal transduction (12, 13), this is not a general feature. Indeed PDZ domains have been identified in nuclear proteins (14), as in Bridge-1, which acts as a coactivator for the E12 basic helix-loop-helix factor (15). From what is currently known about PDZ proteins, it is clear that the presence of a PDZ domain correlates with association with a protein partner, but not with a specific function.

In a previous study, several PDZ proteins were identified as able to bind to the C-terminal end of the HTLV-1 Tax protein (6). Here we report cloning of the complete cDNA of one of them, TIP-1. The protein corresponding to this cDNA has been characterized, and a two-hybrid study showed that TIP-1 binds to human rhotekin (h-rhotekin), an effector of the Rho GTPases. An effect of RhoA, h-rhotekin, and TIP-1 on activation of the c-Fos serum response element is characterized.

EXPERIMENTAL PROCEDURES

Plasmids—Full-length TIP-1 cDNA was obtained by rapid amplification of cDNA ends-PCR using the Human Leukocyte Marathon-Ready cDNA library (CLONTECH). To generate pGB-TIP-1, TIP-1 cDNA was amplified using Pfu polymerase, digested with SalI restriction enzyme, and inserted between the SmaI and SalI restriction sites of pGBGly, which is a derivative of pGB-T9 (CLONTECH). The pTL-TIP-1 plasmid was made by subcloning the TIP-1 cDNA into the EcoRI

kinase 1; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; BS, binding site.

* This work was supported by grants from the Agence Nationale de Recherches sur le SIDA and from the Association pour la Recherche sur le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Present address: Unité de Physiologie de la Reproduction et des Comportements, INRA, 37380 Nouzilly, France.
§ To whom correspondence should be addressed. Tel.: 33-4-72728563; Fax: 33-4-72729674; E-mail: pjalinot@ens-lyon.fr.

The abbreviations used are: HTLV-1, human T-cell lymphotrophic virus, type 1; SRE, serum response element; FCS, fetal calf serum; h, human; m, mouse; GEF, guanine nucleotide exchange factor; LIMK-1, LIM
Activation of SRE by Rho, Rhotekin, and PDZ Protein TIP-1

Sequence of TIP-1 protein. Acid sequence alignment of human, mouse, and C. elegans (C459.7) TIP-1 is shown. These sequences were aligned using the ClustalW program (24) available on the Pole Bio-informatique Lyonnais web site. Alignment gaps are shown by dashes. Perfect matches are indicated by asterisks below the alignment. Conservative substitutions of high similarity and low similarity are marked by two dots and one dot, respectively. The six β sheets (white bars, βA–βF) and the two α helices (black bars, aA and aB) present in the structure of the PDZ3 motif of PSD95 (6) are indicated as they can be deduced from alignment of TIP-1 with different PDZ domains including PDZ3 of PSD95 (6). The GenBank accession numbers of the proteins are as follows: TIP-1 Hs, AF028823; TIP-1 Mm, W59126, TIP-1 Ce (C459.7), U2132.

Cell Culture, Immunoblot, and Immunoprecipitation—COS7 and HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (FCS) for regular culture and 5% FCS and were positive for β-galactosidase expression. Plasmids from these cultures were recovered and transformed in Escherichia coli XL1-blue by electroporation as described previously (22).

Generation of TIP-1-specific Antibodies—TIP-1 protein was produced in E. coli from the pET1 expression vector. In induced bacteria TIP-1 protein was present within inclusion bodies. After lysis by sonication, the protein was present within inclusion bodies. After lysis by sonication, the protein was solubilized in SDS loading buffer, TIP-1 was purified by electroelution from an SDS-polyacrylamide gel. The 14-kDa band corresponding to TIP-1 was lyophilized and, after mixing with adjuvant, injected into a rabbit.

Two-hybrid Screen in Yeast—Two-hybrid screening was carried out using the Matchmaker II kit (CLONTECH), according to manufacturer’s instructions. HF7c yeast strain including pGB-TIP-1 was further transformed with a cDNA library of Epstein-Barr virus-transformed human lymphocytes (21). Yeast cells were plated on SD medium complemented with Ura, Lys, and Ade, along with 5 mM aminotrizole. These subcellular fractions were prepared as described under "Experimental Procedures". After migration through a 14% polyacrylamide gel, an immunoblot was performed with the antibody to TIP-1 (1:500 dilution). Positions of the bands of a molecular size marker run in parallel along with that of TIP-1 are indicated.
adjusted to 7.5 μg, and the amount of SV40 promoter containing expression vectors was adjusted to 4.5 μg with pSV5.

For COS7 cells, the precipitate was washed 16 h after its addition and cells were further incubated in Dulbecco’s modified Eagle’s medium plus 5% FCS for 36 h before lysis in radioimmunoprecipitation assay buffer (23). Lysates were centrifuged for 10 min at 12,000 rpm, and supernatants were used for immunoblot or for immunoprecipitation, which were performed as described (23). All antibodies were diluted as described (23). All antibodies were diluted 1:250 for immunoprecipitation and 1:500 for immunoblotting. After transfection, HeLa cells were grown 16 h in medium with 5% FCS, and 24 h in medium containing 0.5% FCS. Induction by serum was made by incubating cells for 8 h in medium supplemented with 15% FCS. CAT enzyme-linked immunosorbent assays were performed according to the manufacturer’s instructions (Roche). Secreted protein, acidic, rich in cysteine (SPARC) was harvested and washed twice in PBS. The cellular pellet was resuspended in 0.3 M NaCl adjusted to pH 8.0, and supernatant was kept (solution 1) and the pellet was resuspended in 0.3 M NaCl adjusted to 7.5 M NaCl. Total volume was measured and NaCl was added to a final concentration of 300 mM. After incubation for 30 min on ice and centrifugation for 20 min at 20,000 rpm, the supernatant containing the nuclear fraction was snap-frozen in dry ice and stored at −80 °C.

RESULTS

Characterization of TIP-1 Protein—Screening by the two-hybrid method with HTLV-1 Tax protein as bait led to isolation of six different proteins, including one or several PDZ domains (6). Three of them were related to previously characterized proteins, PSD-95, β1-syntrophin, and lin-7. As a first step to characterize TIP-1 encoded by one of the clones previously identified, its full-length cDNA was isolated from a leukocyte library (see “Experimental Procedures”). The cDNA included an in-frame stop codon 25 codons upstream from an AUG, which hence was considered as the initiation codon. The reading frame starting at this position encodes a 124-amino acid protein, which mainly corresponds to a PDZ domain flanked by 14 and 12 amino acids at the N-terminal and C-terminal ends, respectively. The calculated molecular mass is 13.7 kDa. Data bank searches identified expressed sequence tags encoding a murine homologue and analysis of the Caenorhabitis elegans genome showed the existence of a homologue in this distant organism. In these three species, the number of amino acids is conserved and an alignment with the ClustalW program (24) revealed that the part is likely to confer some specificity to the PDZ domain of TIP-1.

In order to characterize the product of the human cDNA, a rabbit polyclonal antibody was raised against TIP-1 protein produced in bacteria. Immunoblot analysis of a COS7 cells extract with this antibody gave some background, but a band was clearly revealed at 14 kDa (Fig. 2 A, lane 3). Transfection of the COS7 cells with a mammalian expression vector including the entire coding sequence increased the intensity of this 14-kDa band (Fig. 2 A, lane 1). These observations confirmed the proposed reading frame for the cDNA. Expression of TIP-1 was detected in various cell lines as COS7 (Fig. 2 A, lane 2), HeLa (Fig. 2 A, lane 3), CV1 (Fig. 2 A, lane 4), and BHK21 (Fig. 2 A, lane 5). However, by comparison with these cell lines, TIP-1 appeared weakly expressed in PBLs as well as in T-cell lines expressing Tax.

To determine the subcellular localization of the protein, fractionation experiments were performed. HeLa and NIH 3T3 cells were fractionated giving membrane, cytoplasmic, and nu-

Fig. 3. Sequence of h-rhotekin. The amino acid sequence of human rhotekin translated from the longest cDNA isolated by two-hybrid screening with TIP-1 as bait, was aligned with that of mouse rhotekin as described in legend to Fig. 1. Symbols used to mark alignment gaps, perfect matches, and high and low similarity are described in the legend to Fig. 1. Amino acids of the Rho binding domain are in italics in the m-rhotekin sequence. The GenBank accession numbers of m-rhotekin and h-rhotekin are U54838 and AP299512, respectively.
clear extracts (see “Experimental Procedures”). Immunoblot analysis of these extracts unambiguously showed that the endogenous protein is in the cytoplasmic soluble fraction (Fig. 1B), its presence being only detected in this fraction.

**TIP-1 Interacts with Rhotekin**—It is now well established that PDZ domains bind to the C-terminal end of their partner proteins. In order to identify such partner proteins, a two-hybrid screen was performed with a bait corresponding to the entire TIP-1 coding sequence fused to that encoding the GAL4 DNA binding domain. A total of 7.4 million transformants were analyzed. Analysis of 265 positive clones showed that many corresponded to the GAL4 DNA binding domain associated out of frame with a cDNA, the resulting fusion protein ending by a motif matching the consensus X:(S/T):X-V-COOH known for proteins able to bind PDZ domains.2 Besides these artificial constructs, two different clones corresponded to a cDNA encoding the human homologue of rhotekin, which was originally identified as a protein preferentially associating with a mutated form of RhoC that mimicked the GTP-bound form of this small GTPase (25). The authors showed that rhotekin can associate with the activated form of RhoA and RhoC, but not with that of Rac or Cdc42, and they described the existence of three different splice variants of rhotekin (25). The two cDNAs obtained in the two-hybrid screen performed with TIP-1 correspond to a different form, one that lacks part of the sequence at the N-terminal end. Alignment of the human and mouse forms showed a very good conservation between these two species, in particular in the Rho binding domain (Fig. 3). This latter domain is likely to fold into an antiparallel coiled-coil finger, which binds to Rho, as was recently shown for the homologous Rho binding domain of PKN (26). Interestingly, a 6-amino acid sequence at the C-terminal end, WLQSPV-COOH, is perfectly conserved and fits with the X:(S/T):X-V-COOH consensus. This strongly suggested that rhotekin binds to the PDZ domain of TIP-1 by its C-terminal end.

To further validate the interaction between TIP-1 and h-rhotekin, immunoprecipitation experiments were performed. COS7 cells were transfected with vectors expressing TIP-1 and h-rhotekin fused at its N-terminal end to the Flag epitope. Proteins were immunoprecipitated with an anti-Flag antibody and analyzed with the antibody to TIP-1. This experiment showed that h-rhotekin associates with TIP-1 in these cells (Fig. 4A, lane 3). To establish that this interaction indeed relies on the C terminus of rhotekin, a double point mutation was introduced to change the natural sequence QSPV-COOH to QAPA-COOH. This C-terminal mutant of Rhotekin was unable to precipitate TIP-1 (Fig. 4A, lane 4), which was, however, clearly present in the extract (Fig. 4A, lane 2). Analysis of the blot with the anti-Flag antibody showed that equal amounts of wild type and mutated h-rhotekin were precipitated in this experiment.2 It was also verified that h-rhotekin indeed binds to the active form of Rho. COS7 cells were transfected with vectors expressing the Flag-tagged h-rhotekin and the active RhoA.V14 mutant fused to the Myc epitope. Immunoblot analysis with the antibody to Flag of proteins immunoprecipitated with the antibody to the Myc epitope clearly revealed presence of h-rhotekin (Fig. 4B). Collectively, these results show that h-rhotekin binds to the activated form of RhoA and to TIP-1. The former interaction involves the Rho binding domain at the N-terminal end of h-rhotekin (25), and the latter the C-terminal extremity of this protein as demonstrated here. Given the small size of Rho and TIP-1 proteins and that the domains involved are completely different, these interactions are likely not to be mutually exclusive.

**Activation of the SRE Enhancer Activity by h-Rhotekin and TIP-1**—Rho GTPases are involved in many important cellular functions such as cytoskeleton reorganization, cell growth control, development, and transcriptional regulation (27). Various effectors binding to the GTP-bound active form of Rho have been identified (25, 28). In agreement with previous observations (29), a detailed analysis relying on mutants in the RhoA effector loop has clearly established the involvement of the ROCK kinase in stress fiber induction as well as cell transformation by Rho (30). By contrast, the effectors linking active Rho to activation of the SRE/CArG box are still uncharacterized. Therefore, the effect of h-rhotekin and TIP-1 in this pathway was investigated. HeLa cells were transfected with a CAT reporter construct including the wild type c-Fos SRE upstream of the TK promoter (19). After transfection, cells were cultured in low amounts (0.5%) of fetal calf serum. Under these conditions, treatment of the cells by serum led to a clear activation of transcription (Fig. 5, lane 1). Expression of RhoA.V14 in cells kept in low serum also stimulated transcription (Fig. 5, lane 2). Transfection of the cells with the vector expressing h-rhotekin did not cause a significant activation, nor did it modify the
Activation of SRE by Rho, Rhotekin, and PDZ Protein TIP-1

**FIG. 5.** RhoA.V14, F-h-rhotekin, and TIP-1 proteins activate the SRE. HeLa Cells were cotransfected with the SRE reporter plasmid and expression vectors EXV-RhoA.V14, pSGF-h-rhotekin, and pTL-TIP-1, as indicated. The total amount of expression vector including the SV40 early promoter was adjusted to a constant amount using the pSG5 parental vector. CAT enzyme concentrations were measured by enzyme-linked immunosorbent assay (Roche) according to manufacturer’s instructions. -Fold induction was determined with respect to activity of the reporter construct alone. Each point of transfection was performed in duplicate. The mean of two independent experiments is represented. Error bars indicate standard deviation.

**DISCUSSION**

The Rho small GTPases have multiple functions and affect key regulatory cellular processes (27, 28). In agreement with...
these pleiotropic effects, numerous effectors able to bind these GTPases in their active state have been identified. Some of these effectors exhibit kinase activity, whereas the others have no known enzymatic activity. Previous studies have implicated the kinase ROCK in induction of stress fibers and also cell transformation (29, 30). Our results show that h-rhotekin and TIP-1 markedly increase transactivation of the c-Fos SRE by RhoA.V14. The PDZ protein TIP-1 can by itself produce a significant activation of the same intensity as that exerted by RhoA.V14. h-rhotekin alone showed no or weak effects. This indicates that TIP-1 is probably downstream of h-rhotekin in the pathway leading to SRE activation. However, it is clear that TIP-1 alone cannot produce the strong activation seen with all three components. This indicates that the complex of all three proteins is necessary to trigger maximal activation.

The results of fractionation experiments show that TIP-1 is cytoplasmic. This observation is in agreement with immunofluorescence analyses, which showed that overexpressed RhoA.V14, h-rhotekin, and TIP-1 are co-localized in the cytoplasm.3 The nature of the signal emitted by the association of RhoA.V14, h-rhotekin, and TIP1 that causes activation of the SRE within the nucleus remains to be understood.

Interestingly, Sotiropoulos et al. (31) have shown recently that LIM kinase-1 (LIMK-1) is able to activate SRF by acting on the actin dynamics. This kinase would exert its effect by increasing the amount of monomeric actin versus polymerized actin. How the increased monomeric actin modifies the activity of SRF is presently unknown. Interestingly, LIMK-1 includes a PDZ domain. One can wonder whether a specific effector might bridge activated Rho and LIMK-1 by interacting both with GTP-bound Rho and LIMK-1 via the PDZ domain. Rhotekin might exert such a function. When the C-terminal ends of the various Rho effectors are compared, it appears that rhotekin is not the only protein exhibiting a PDZ domain binding site (PDZ-BS). The C-terminal end of Citron also fits with the PDZ-BS consensus (Table I). In agreement with this observation, an interaction of Citron with PSD-95 in GABAergic neurons from the hippocampus has been reported (32). Rhophilin exhibits a threonine at position –2, but the C-terminal residue is a proline. In the study by Songyang et al. (31), a proline was never observed as C-terminal residue, raising doubts about the notion that this Rho effector C-terminal end can represent a functional PDZ-BS. The other kinase Rho effectors do not include a PDZ-BS. Additional studies will be required to determine whether or not rhotekin and Citron can bridge activated Rho with LIMK-1.

A number of PDZ proteins are known to interact with the

---

3 S. Fabre, unpublished data.

---

**TABLE I**

Comparison of the sequences of the C-terminal ends of Rho effectors

| Protein      | Sequence                        | PDZ-BS Consensus |
|--------------|---------------------------------|------------------|
| Rhotekin Mm  | ...FSKSOLGPDVLQSV-c              |                  |
| Citron Mm    | ...TLPSQVKNVQDSV-c               |                  |
| Rhophilin Mm | ...SHDPCTNRNCVCTC-c              |                  |
| PKN Hs       | ...EQAAFLDFDFVAGGC-c             |                  |
| Rock-1 Mm    | ...QSFKVKMTGKTS-c                |                  |
| Rock-2 Mm    | ...SRRPQRLAPKPS-c                |                  |
| P140mDia Mm  | ...PTILEEAKLGRAS-c               |                  |

**FIG. 7.** Activation of the SRE by F-h-rhotekin and TIP-1 requires the active form of Rho. Transfection in HeLa cells was performed with the SRE-APm reporter construct and vectors expressing F-h-rhotekin, TIP-1, and either RhoA.V14 or the dominant negative mutant RhoA.N19 as indicated. Data are represented as described in legend to Fig. 5.

**FIG. 8.** Activation of the SRE by F-h-rhotekin and TIP-1 depends on the serine and valine residues at the C terminus of h-rhotekin. Transfection in HeLa cells was performed with the SRE-APm reporter construct and vectors expressing RhoA.V14, TIP-1, and F-h-rhotekin either wild type or bearing a double point mutation changing the QSV-PV-COOH wild type C-terminal end into QAPA-COOH as indicated. Data are represented as described in legend to Fig. 5.
cytoskeleton (33–35). Despite its small size, it is possible that TIP-1 has this property. Such a possibility could be related to an effect of TIP-1 on actin polymerization. It could also allow the anchoring of Rho-containing protein complexes at specific sites. A way to explain the multiplicity of the effects of Rho, as well as the high number of its effectors, could be that it is present at different specialized subcellular sites. A PDZ protein as TIP-1 could allow anchoring of the effector and, as a consequence, of active Rho at a particular position, leading to a given effect. A scaffold role for proteins with multiple PDZ domains is now well established (36–38). Precise studies of the subcellular localization of the Rho effectors and of the corresponding PDZ proteins, when appropriate, should allow the testing of such an hypothesis.

Our results establish that PDZ proteins could be important molecules in the Rho pathways, in particular activation of the Rho pathways, in particular activation of the Rho effectors. Such a domain is indeed present in KIAA0380, a class of molecules participating in the regulation of the Rho effectors, both upstream and downstream of Rho and such PDZ proteins. Interestingly, another PDZ domains is present at different specialized subcellular sites. A way to explain the multiplicity of the effects of Rho, as well as the high number of its effectors, could be that it is the anchoring of Rho-containing protein complexes at specific sites. A PDZ protein such as TIP-1 could allow anchoring of the effector and, as a consequence, of active Rho at a particular position, leading to a given effect. A scaffold role for proteins with multiple PDZ domains is now well established (36–38). Precise studies of the subcellular localization of the Rho effectors and of the corresponding PDZ proteins, when appropriate, should allow the testing of such an hypothesis.

Acknowledgments—We are very grateful to Olivier Geneste from Richard Treisman’s laboratory, and to Silvio Gutkind, Marc Billaud, and Bohdan Wasylyk for the kind gift of plasmids. We thank Janet Maryanski for critical reading of this manuscript.

REFERENCES

1. Ponting, C. P., and Phillips, C. (1995) Trends Biochem. Sci. 20, 102–103
2. Ponting, C. P. (1997) Protein Sci. 6, 464–468
3. Lee, S. S., Weiss, R. S., and Javier, R. T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6670–6675
4. Kiyono, T., Hiraiwa, A., Fujita, M., Hayashi, Y., Akiyama, T., and Ishibashi, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11612–11616
5. Suzuki, T., Ohsumi, Y., Uchida-Toita, M., Akiyama, T., and Yoshida, M. (1999) Oncogene 18, 5967–5972
6. Rouset, R., Fabre, S., Desbois, C., Bantignies, F., and Jaliniot, P. (1998) Oncogene 16, 643–654
7. Saraz, J., and Heldin, C. H. (1996) Trends Biochem. Sci. 21, 455–458
8. Kim, E., Niethammer, M., Rothschild, A., Jan, Y. N., and Sheng, M. (1995) Nature 378, 85–88
9. Kornau, H. C., Schenker, L. T., Kennedy, M. B., and Seeburg, P. H. (1995) Science 269, 1737–1740
10. Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M., and MacKinnon, R. (1996) Cell 85, 1067–1076
11. Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chishti, A. H., Crompton, A., Chan, A. C., Anderson, J. M., and Cantley, L. C. (1997) Science 275, 73–77
12. Fanning, A. S., and Anderson, J. M. (1998) Curr. Top. Microbiol. Immunol. 228, 269–293
13. Fanning, A. S., and Anderson, J. M. (1999) J. Clin. Invest. 103, 767–772
14. Poulat, F., Barbera, P. S., Desclozeaux, M., Soulier, S., Moniot, B., Bonneau, N., Buizet, B., and Berta, P. (1997) J. Biol. Chem. 272, 7167–7172
15. Thomas, M. K., Yao, K. M., Tenser, M. S., Wong, G. G., and Habener, J. F. (1999) Mol. Cell. Biol. 19, 8492–8504
16. Green, S., Iseman, I., and Sheer, E. (1988) Nucleic Acids Res. 16, 369
17. Rouset, R., Desbois, C., Bantignies, F., and Jaliniot, P. (1996) Nature 381, 328–331
18. Hill, C. S., Wynn, J., and Treisman, R. (1995) Cell 81, 1159–1170
19. Giovane, A., Pintzas, A., Maira, S. M., Schiesczan, P., and Wasylyk, B. (1994) Genes Dev. 8, 1592–1513
20. Wallis, O. C., and Wallis, M. (1990) J. Mol. Endocrinol. 4, 61–69
21. Durfee, T., Becherer, K., Chen, P.-L., Yeh, S.-H., Yang, Y., Kilburn, A. E., Lee, W.-H., and Elledge, S. J. (1993) Genes Dev. 7, 555–569
22. Hoffman, C. S., and Winston, F. (1987) Gene (Amst.) 57, 267–272
23. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 447–470, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Higgins, D. G., Thompson, J. D., and Gibson, T. J. (1996) Methods Enzymol. 238, 383–402
25. Reed, T., Furuyashiki, T., Ishizaki, T., Watanabe, G., Watanabe, N., Fujisawa, K., Mori, N., Madalpe, P., and Narumiya, S. (1996) J. Biol. Chem. 271, 13556–13560
26. Maekaki, R., Ikara, K., Shimizu, T., Kuroda, S., Kaibuchi, K., and Hoshkima, T. (1999) Mol. Cell. Biol. 19, 793–803
27. Van Aelst, L., and D’Souza-Schoeny, C. (1997) Genes Dev. 11, 2351–2362
28. Hall, A. (1998) Science 279, 509–514
29. Leung, T., Chen, X. Q., Manse, E., and Lim, L. (1996) Mol. Cell. Biol. 16, 5313–5323
30. Sahai, E., Alberts, A. S., and Treisman, R. (1998) EMBO J. 17, 1350–1361
31. Sotiropoulos, A., Gineitis, D., Copeland, J., and Treisman, R. (1999) Cell 99, 159–169
32. Zhang, W., Vazquez, L., Apperson, M., and Kennedy, M. B. (1999) J. Neurosci. 19, 96–108
33. Hildebrand, J. D., and Soriano, P. (1999) Cell 99, 485–497
34. Satoh, A., Nakaniishi, H., Ohashi, H., Wada, M., Takahashi, K., Satoh, K., Hirao, K., Nishioka, H., Hata, Y., Mizoguchi, A., and Takai, Y. (1998) J. Biol. Chem. 273, 3470–3475
35. Mandai, K., Nakaniishi, H., Satoh, A., Ohashi, H., Wada, M., Nishioka, H., Itoh, M., Mizoguchi, A., Aoki, T., Fujimoto, T., Matsuuda, Y., Tsukita, S., and Takai, Y. (1997) J. Cell Biol. 139, 517–528
36. Scott, K., and Zucke, C. S. (1998) Nature 395, 805–808
37. Tsunoda, S., Sierralta, J., Sun, Y., Boddner, R., Suzuki, E., Becker, A., Socolich, M., and Zucke, C. S. (1997) Nature 388, 243–249
38. Ranganathan, R., and Ross, E. M. (1997) Curr. Biol. 7, R770–R773
39. Rumenapp, U., Blouquin, A., Schwerer, G., Schabowski, H., Poema, A., and Jakobs, K. H. (1999) FEBS Lett. 459, 315–318
40. Chan, A. M., Takai, S., Yamada, K., and Miki, T. (1996) Oncogene 12, 1259–1266

4 S. Fabre and C. Reynaud, unpublished data.