Cloning and Characterization of Dfak56, a Homolog of Focal Adhesion Kinase, in Drosophila melanogaster*

(Received for publication, April 22, 1999, and in revised form, June 17, 1999)

Jiro Fujimoto‡, Kazunobu Sawamoto§, Masataka Okabe¶, Yasumitsu Takagi**†, Tohru Tezuka‡, Shingo Yoshikawa††, Haruko Ryo§§, Hideyuki Okano¶¶, and Tadashi Yamamoto‡‡¶¶

From the ‡Department of Oncology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan, the §Division of Neuroanatomy, Department of Neuroscience, Biomedical Research Center, Osaka University Graduate School of Medicine and Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Osaka 565-0871, Japan, the ¶¶Division of Radiation Biology and Medical Genetics, Osaka University Graduate School of Medicine, Osaka 565-0871, Japan, the †Hirohashi Cell Configuration Project, Exploratory Research for Advanced Technology, Japan Science and Technology Corporation, Tsukuba 300-2635, Japan, and the ‡‡Department of Molecular Neurobiology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba 305-0006, Japan

The focal adhesion kinase (FAK) protein-tyrosine kinase plays important roles in cell adhesion in vertebrates. Using polymerase chain reaction-based cloning strategy, we cloned a Drosophila gene that is homologous to the vertebrate FAK family of protein-tyrosine kinases. We designated this gene Dfak56 and characterized its gene product. The overall protein structure and deduced amino acid sequence of Dfak56 show significant similarity to those of FAK and PYK2. Dfak56 has in vitro autophosphorylation activity at tyrosine residues. Expression of the Dfak56 mRNA and the protein was observed in the central nervous system and the muscle-epidermis attachment site in the embryo, where Drosophila position-specific integrins are localized. The results suggest that like FAK in vertebrates, Dfak56 functions downstream of integrins. Dfak56 was tyrosine-phosphorylated upon integrin-dependent attachment of the cell to the extracellular matrix. We conclude that the Dfak56 tyrosine kinase is involved in integrin-mediated cell adhesion signaling and thus is a functional homolog of vertebrate FAK.

Focal adhesion kinase (FAK)1 is a member of a growing family of non-receptor protein-tyrosine kinases (1) and was originally identified as a putative substrate for the oncogenic protein-tyrosine kinase pp60v-src (2). Accumulating data, however, show that FAK tyrosine phosphorylation is induced upon adhesion of cells to the extracellular matrix through the surface integrin (3) and upon stimulation of cells by a variety of other extracellular factors including those for receptor tyrosine kinases and for G-protein-coupled receptors (4–6). FAK associates with multiple cellular components including other focal adhesion-associated proteins and signaling molecules (7–11).

© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.

The costs of publication of this article were defrayed in part by the payment from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment from the Ministry of Education, Science, and Culture of Japan.

ACKNOWLEDGMENTS

This work was supported by a grant for advanced cancer research from the Ministry of Education, Science, and Culture of Japan. 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.

EXPERIMENTAL PROCEDURES

Isolation of cDNA and Genomic DNA Clones—Polymerase chain reaction was performed using fully degenerate oligonucleotides corresponding to the amino acid sequences HRDIAAR (for the forward primer) and DVWAFG (for the reverse primer) as primers and 108 primer. The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBankTM/EBI Data Bank with accession number D88898.

This paper is available on line at http://www.jbc.org

29196
template. The amino acid sequences were located in conserved motifs of the kinase domain and are unique for FAK and PYK2. Amplified cDNAs (~200 base pairs) were subcloned into the pBluescript vector (Stratagene) and sequenced with Thermosequenase (Amersham Pharmacia Biotech). Duplicate filters from a Drosophila embryo cDNA library were prepared for filters at 65 °C in 7% SDS and 0.5 mM sodium phosphate-buffer (pH 8.0) containing 100 µg/ml salmon sperm DNA (Sigma) and then hybridized to the 32P-labeled cDNA fragment for 16 h at 65 °C. After hybridization, the filters were washed with 0.2× SSC and 0.1% SDS. Positive clones were isolated and sequenced on both strands. Other molecular biological techniques were performed as described previously (24). DNA and protein sequence analyses were performed using Genetics Computer Group software packages and ClustalW and BLAST programs employing the DDBJ/GenBank[TM]/EBI Data Bank running at the Human Genome Center of the University of Tokyo (Tokyo, Japan).

**Southern and Northern Blotting**—Genomic DNA from wild-type (Oregon-R strain) Drosophila melanogaster (1 µg/sample) digested with suitable restriction enzymes was electrophoresed on 1% agarose gel. mRNA samples from cultured cells and Drosophila tissues were electrophoresed on formaldehyde-containing 1.2% agarose gel. The fractionated RNAs were then transferred to nylon membranes (Hybond, Amersham Pharmacia Biotech) and subjected to hybridization using full-length Dfak56 cDNA and the 5’-portion (SacII fragment, corresponding to nucleotides 1-2743) of the Dfak56 cDNA as probes. Hybridizations were performed employing the same procedure as that used for cDNA screening.

**Cells and Antibodies**—Drosophila Schneider cells (S2) and derivatives of their integrin-expressing cells were a gift from D. L. Brower (Arizona University). BG2-c6 and 7E10 cells were described previously (25, 26). The cells were cultured at 27 °C under normal atmospheric conditions in Shields and Sang M3 medium (Sigma) supplemented with 10% of fetal calf serum. Simian COS cells were cultured at 37 °C under 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum.

To generate rabbit anti-Dfak56 polyclonal antibodies, glutathione S-transferase (GST)-Dfak56 was constructed by inserting the cDNA fragment encoding amino acid residues 1–280 of the Dfak56 protein into the pGEX-2T expression vector (Amersham Pharmacia Biotech). Expression of the GST fusion proteins in Escherichia coli BL21 was induced with isopropyl-β-D-thiogalactopyranoside, and the proteins were purified with glutathione-Sepharose (Amersham Pharmacia Biotech) as described previously (27). A New Zealand White rabbit was immunized with the purified GST-Dfak56 fusion protein. Antiserum raised against Dfak56 were purified using GST and the GST-Dfak56 protein bound to a HiTrap NHS-activated affinity column (Amersham Pharmacia Biotech). Anti-phosphotyrosine monoclonal antibody 4G10 was purchased from Transduction Laboratories, Inc.

**In Situ Hybridization and Immunohistochemistry**—Whole-mount in situ hybridization was conducted using digoxigenin-labeled RNA probes as described previously (28). The digoxigenin-labeled RNA probes (sense and antisense) were prepared by a standard procedure with a digoxigenin-RNA labeling kit (Roche Molecular Biochemicals) and full-length Dfak56 cDNA as a template.

Whole-mount immunohistochemistry was performed as described (29) using rabbit anti-Dfak56 antibodies at 1:100 dilution. The secondary antibody used was horseradish peroxidase-conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc.) at 1:500 dilution. Immunohistochemistry with cultured cells was performed as described previously (30).

**Western Blotting and Immunocomplex Kinase Assay**—Cells were lysed in TNNE buffer (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM Nonidet P-40, and 2 mM EDTA) containing 1 mM sodium orthovanadate, 50 units/ml aprotinin, 0.1 mM phenylmethylsulfonyl fluoride, and 10 mM NaF. Insoluble materials were removed by centrifugation. The lysates were separated by 7.5% SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Trans-Blot, Bio-Rad). The proteins reacted with anti-Dfak56 antibodies were visualized with a chemiluminescence detection kit (Renaissance, NEN Life Science Products). For immunoprecipitation, the cell lysates were precleared with Protein A-Sepharose 4B (Amersham Pharmacia Biotech) for 1 h at 4 °C. After preclearing, the bead slurry was incubated for 2 h at 4 °C with anti-Dfak56 antibodies and protein-A Sepharose. The immunocomplex was washed several times with TNNE buffer and then with kinase buffer (20 mM HEPES (pH 7.4), 10 mM MgCl2, and 10 mM MnCl2). Following addition of [γ-32P]ATP (370 kBq, 110 TBq/mmol), the immunoprecipitates were incubated for 30 min at 30 °C and then separated by 7.5% SDS-polyacrylamide gel electrophoresis. The gels were dried and subjected to autoradiography. Phosphoamino acid analysis of phosphorylated proteins and two-dimensional analysis of tryptic phosphopeptides were performed as described previously (31).

**RESULTS**

**cDNA Cloning and Sequence Analysis of a Drosophila Homolog of Focal Adhesion Kinase**—To identify a Drosophila FAK homolog of protein-tyrosine kinase, we used polymerase chain reaction with degenerate oligonucleotide primers to amplify cDNAs from a Drosophila embryo cDNA library. The sequences of the degenerate primers corresponded to the amino acid sequences conserved in both FAK and PYK2. Amplified cDNA fragments were subcloned into plasmids and sequenced. We searched the DDBJ/GenBank[TM]/EBI Data Bank against these sequences. A cDNA fragment was revealed to contain a novel sequence with homology to FAK and PYK2. The cDNA fragment was subsequently used to screen the same library. Four cDNA clones of varying sizes were isolated, and the one containing the longest insert (4.3 kilobase pairs) was sequenced on both strands. The complete nucleotide sequence of the cDNA predicted an open reading frame of 1198 amino acids (Fig. 1A). The calculated molecular mass of the predicted protein was 130 kDa. Since the cDNA hybridized at 56A or 56B on the second chromosome, which was determined by in situ hybridization with polytene chromosomes (data not shown), we named the gene Dfak56.

The deduced amino acid sequence of the putative product of the Dfak56 gene showed the presence of a protein kinase domain. Like FAK and PYK2, Dfak56 has large N- and C-terminal sequences flanking the kinase domain. Comparison of the amino acid sequence of the Dfak56 kinase domain with the protein sequence data bases revealed that the kinase domain of Dfak56 is most similar to that of FAK (59% identity to human FAK) and PYK2 (52% identity to human PYK2) (Fig. 1B). The sequences of the N- and C-terminal regions are relatively divergent. The amino acid sequence alignment of the kinase domains of these proteins is shown in Fig. 1C. Unlike the vertebrate counterparts, Dfak56 contains an additional 24-amino acid sequence near the ATP-binding site of the kinase. In the C-terminal regions, FAK and PYK2 have a conserved sequence of −150 amino acid residues. The sequence is called the focal adhesion targeting sequence because it is essential for localization of FAK to focal adhesions (32). Dfak56 also carries a focal adhesion targeting-like sequence (~40% identity to human FAK) in the C-terminal region (Fig. 1B), suggesting that the focal adhesion targeting sequence is conserved as a functional domain.

There are tyrosine phosphorylation sites within FAK that are conserved in PYK2. A major autophosphorylation site (Y397AEl for human FAK and Y405AEI for human PYK2) that mediates binding to the SH2 domain of Src-like protein-tyrosine kinases is well conserved in Dfak56 (Y430AEI). Another tyrosine phosphorylation site (Y925ENV for FAK and Y954CAT) serves for binding of the GRB2 SH2 domain. Tyrosine 954 in Dfak56 is likely to correspond to this phosphorylation site although the sequence is slightly divergent (Y954CAT). The C-terminal region of FAK has two PPXY motifs, P172PKPSPRP and P178PKPKPRP, which can bind to the SH3 domain. These sequences are highly conserved in PYK2, and only one of them (P172PKPSPR corresponding to P172PKPSPR of FAK) is conserved in Dfak56. We conclude that Dfak56 is a Drosophila homolog of vertebrate FAK family protein-tyrosine kinases. The sequence data did not reveal which kinase, FAK or PYK2, is the counterpart of Dfak56.

Southern hybridization analysis strongly suggested that Dfak56 is a single-copy gene of Drosophila (Fig. 2A). Moreover, under less stringent washing conditions, no bands other than
those for Dfak56 were found (data not shown). Therefore, we tentatively concluded that Drosophila contains no close relatives of Dfak56.

Temporal and Spatial Expression of Dfak56—Northern hybridization analysis of Drosophila poly(A)1-selected RNAs from various developmental stages (embryo, larva, pupa, and adult) and cell lines showed that two mRNA species of 5 and 6 kb hybridized with the Dfak56 cDNA probe (Fig. 2B). A short transcript of ~1.5 kb was also detected in embryonic and larval RNA samples. This short transcript did not hybridize to the cDNA probe corresponding to the 5'-portion (including the coding region N-terminal to the kinase domain) of the Dfak56 mRNA, suggesting that this transcript corresponds to the 3'-portion of the Dfak56 mRNA. The expression of a short transcript was also reported in the case of the avian FAK gene (33).

To determine the spatial expression of the Dfak56 gene, we performed whole-mount in situ hybridization with the embryos. In stage 16 embryos, strong expression of the Dfak56 mRNA was detected in the central nervous system and the junction of muscle and epidermis (Fig. 3A and B). Immunohistochemical analysis also showed expression of the Dfak56 protein in the muscle attachment (Fig. 3C). A cross-sectioned
Drosophila embryos. Whole-mount RNA in situ hybridization (A and B) and immunohistochemistry (C and D) were performed with stage 16 embryos. A shows whole embryos, and B and C show magnified views of the surface of the embryo. D shows a cross-section of the embryo. e, epidermis; m, muscle cells. Dfak56 is expressed at the muscle attachment sites (arrowheads).

view of the immunostained image suggests that Dfak56 is expressed predominantly in the epidermal cells, but less or not at all in the muscular cells (Fig. 3D).

**Subcellular Localization of Dfak56**—We examined the subcellular localization of the Dfak56 protein using the *Drosophila* neuronal cell line BG2-c6 (25). The BG2-c6 cells expressed the Dfak56 mRNA (Fig. 2B). They also expressed integrins, and formation of integrin clusters was observed in cells with several focal adhesion proteins, including p21-activated kinase and α-actinin (30). Immunofluorescent staining of the BG2-c6 cells with anti-Dfak56 antibodies produced a focal adhesion-like pattern (Fig. 4A). The nucleus was also stained with these antibodies. In some populations, the edges of the cells were also stained. Staining with anti-phosphotyrosine antibody produced a pattern that overlapped with that of anti-Dfak56 antibodies, except for the nucleus (Fig. 4B). Staining with anti-phosphotyrosine and anti-βps integrin antibodies (Fig. 4C) showed colocalization of tyrosine-phosphorylated proteins with integrin. These data suggested that Dfak56 is a component of focal adhesion in *Drosophila*.

**Kinase Activity of Dfak56**—We tested for protein kinase activity and phosphorylation of Dfak56 with mutational analysis. By site-directed mutagenesis, an amino acid substitution at residue 430 (tyrosine to phenylalanine) was introduced (Y430F mutant). This residue corresponds to the major autophosphorylation site. This was confirmed by two-dimensional phosphopeptide mapping of the wild-type and Y430F mutant proteins (Fig. 5C). A major spot in the peptide map of wild-type Dfak56 (arrow) was not observed in that of the Y430F mutant.

Phosphorylation of Dfak56 upon Cell Attachment to the Extracellular Matrix—*Drosophila* S2 cells and stable transfecants (34) expressing *Drosophila* PS integrin chains of αps1 and βps (called PS1 cells) or αps2 and βps (called PS2 cells) were cultured on plastic dishes. Among them, only PS2 cells could attach to a plastic dish in serum-containing medium because of the cross-reactivity of αps2βps integrin to vertebrate vitronectin and fibronectin (34). Using these cell lines, the levels of phosphorytrosine in the Dfak56 protein were examined following integrin-dependent cell attachment to the extracellular matrix. As shown in Fig. 6, the Dfak56 protein was tyrosine-phosphorylated in PS2 cells that attached to the surface of the dish. This indicated that Dfak56 is tyrosine-phosphorylated upon integrin-dependent cell attachment to the extracellular matrix. Note that tyrosine-phosphorylated proteins of 50–60 kDa were associated with the Dfak56 protein (Fig. 6). These phosphoproteins were also present in the anti-Dfak56 precipitates of PS1 cells or suspended PS2 cells, but not in precipitates of parental S2 cells. This suggests that the interaction between Dfak56 and these phosphoproteins requires the presence of integrins and is enhanced by cell attachment to the extracellular matrix.

**DISCUSSION**

We have identified a *Drosophila* homolog of vertebrate focal adhesion kinase and termed it Dfak56. Dfak56 shares both
sequence and structural homology with the vertebrate FAK family of protein-tyrosine kinases (FAK and PYK2). We demonstrated that Dfak56 is tyrosine-phosphorylated following integrin-dependent cell attachment to the extracellular matrix. This property is the unique functional feature that distinguishes FAK from PYK2. Thus, we conclude that Dfak56 is a functional homolog of vertebrate FAK rather than PYK2. However, tyrosine phosphorylation of Dfak56 and PYK2 occurs in response to a number of stimuli, and some of them overlap (stimuli for the G-protein-coupled receptor, e.g. bradykinin) (35–37), suggesting that the biochemical function of FAK and PYK2 is partly interchangeable. Furthermore, the similarity of the kinase domains between FAK and PYK2 (50–60% amino acid identity) is almost the same as that between Dfak56 and FAK or PYK2. Therefore, we strongly suspect that Drosophila has only one FAK-related gene and that the ancestor gene duplicated and diverged in vertebrates during evolution.

In chicken embryonic fibroblasts, the FAK gene is alternatively transcribed to generate another short mRNA that encodes a C-terminal portion of the FAK protein named FAK-related non-kinase (33). Ectopic expression of FAK-related non-kinase in fibroblasts blocks the formation of focal adhesions on fibronectin, acting as an inhibitor of FAK (38). We observed a relatively short, 1.5-kb transcript corresponding to the 3’-portion of the Dfak56 mRNA only in embryo and larva (Fig. 2B). This mRNA might encode a FAK-related non-kinase-like protein. If so, Dfak56-mediated cell adhesion processes may be regulated by the expression of this FAK-related non-kinase-like protein in embryogenesis.

Dfak56 expression is mainly observed in the embryonic central nervous system and the muscle attachment process. In muscle attachment, PS integrins and tiggrin, the ligand for PS2 integrin, are expressed. Mutants defective for these proteins show improper formation of muscle attachment, resulting in embryonic lethality (39, 40). In vertebrate neurons, FAK is highly expressed and localized to a growth cone (41, 42), suggesting that FAK plays a role in neural development and axon guidance. Recently, the role of integrins in axon guidance in Drosophila was determined (43). Null mutations in either the αPS1 or αPS2 subunit gene caused widespread axon pathfinding errors that could be rescued by supplying the wild-type integrin subunit to the mutant nervous system. Another novel α integrin subunit gene, Volado, was identified to mediate olfactory learning of Drosophila (44). These data suggest that the integrin-mediated signal transduction pathway is important for the function of the central nervous system. Given the similar expression patterns and the close functional relation between integrins and FAK in vertebrates, Dfak56 may also participate in neural functions of Drosophila.

One of the most effective strategies for the study of signal transduction pathways is to identify novel upstream or downstream factors of the known players in the signaling cascade. The genetics of Drosophila are suited for the large-scale screen-
ing to isolate the suppressor or enhancer if a visible mutant of the interested gene is available. Using this system, a number of the novel molecules implicated in the receptor protein-tyrosine kinase-mediated signal transduction pathways have been identified (45–47). In the locus of the Dfak56 gene, polytene band 56A to 56B, we could not find mutant alleles for the Dfak56 gene in the FlyBase data base. However, if we could isolate the mutants of Dfak56, the genetics of Drosophila would allow analysis of the cell adhesion signaling, and the findings would relate back to studies of higher organisms.

In conclusion, we cloned Dfak56, a Drosophila homolog of the FAK gene, and characterized its product by determining its expression, kinase activity, and signaling through integrin-mediated cell attachment. Future genetic studies would facilitate the analysis of the roles of FAK family proteins in vivo.

Acknowledgments—We thank T. Todo for the cDNA library and D. L. Brower for the integrin-expressing cell lines.

REFERENCES

1. Hanks, S. K., and Polte, T. R. (1997) Bioessays 19, 137–145
2. Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B., and Parsons, J. T. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5192–5196
3. Guan, J. L. (1997) Int. J. Biochem. Cell Biol. 29, 1085–1096
4. Zachary, I., Sinnett-Smith, J., and Rozengurt, E. (1992) J. Biol. Chem. 267, 19931–19934
5. Tippmer, S., Bossenmaier, B., and Haring, H. (1996) Eur. J. Biochem. 236, 953–959
6. Haimovich, B., Regan, C., DiFazio, L., Ginalis, E., Ji, P., Purohit, U., Rowley, R. B., Bolen, J., and Greco, R. (1996) J. Biol. Chem. 271, 16332–16337
7. Hildebrand, J. D., Schaller, M. D., and Parsons, J. T. (1995) Mol. Biol. Cell 6, 637–647
8. Tachibana, K., Sato, T., D’Avirro, N., and Morimoto, C. (1995) J. Exp. Med. 182, 1089–1099
9. Schlaepfer, D. D., Hanks, S. K., Hunter, T., and van der Geer, P. (1994) Nature 372, 786–791
10. Schlaepfer, D. D., Broome, M. A., and Hunter, T. (1997) Mol. Cell. Biol. 17, 1702–1713
11. Tremblay, L., Hauck, W., Aprikian, A. G., Begin, L. R., Chapdelaine, A., and Chevalier, S. (1996) Int. J. Cancer 66, 164–171
12. Lew, S., Moreno, H., Martinez, R., Canoll, P., Peles, E., Musacchio, J. M., Plowman, G. D., Rady, B., and Schlessinger, J. (1995) Nature 376, 737–745
13. Sasaki, H., Nagura, K., Ishino, M., Tobokiwa, H., Kotani, K., and Sasaki, T. (1995) J. Biol. Chem. 270, 21206–21219
14. Yu, H., Li, X., Marchetto, G. S., Dy, R., Hunter, D., Calvo, B., Dawson, T. L., Wilm, M., Anderegg, R. J., Graves, L. M., and Éarp, H. S. (1996) J. Biol. Chem. 271, 29993–29998
15. Avraham, S., London, R., Fu, Y., Ota, S., Higegowdara, D., Li, J., Jiang, S., Pasztor, L. M., White, R. A., Groisman, J. E., and Avraham, H. (1995) J. Biol. Chem. 270, 27742–27753
16. Ilic, D., Furuta, Y., Kanazawa, S., Takada, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., and Yamamoto, T. (1995) Nature 377, 539–544
17. Fearon, D. Y., Ilic, D., Kanazawa, S., Takeda, N., Yamamoto, T., and Aizawa, S. (1995) Oncogene 11, 1889–1995
18. Stephens, L. E., Sutherland, A. E., Klimanskaya, I. V., Andrieux, A., Meneses, J., Pedersen, R. A., and Damsky, C. H. (1995) Genes Dev. 9, 1883–1895
19. Bogart, T., Brown, N., and Wilcox, M. (1987) Cell 51, 925–940
20. MacKrell, A. J., Blumberg, B., Haynes, S. R., and Fessler, J. H. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2633–2637
21. Wehrli, M., DiAntonio, A., Fearnley, I. M., Smith, R. J., and Wilcox, M. (1993) Mech. Dev. 43, 21–36
22. Brower, D. L., Brabant, M. C., and Bunch, T. A. (1995) ImmunoL Cell Biol. 73, 558–564
23. Prount, M., Damania, Z., Soong, J., Fristrom, D., and Fristrom, J. W. (1997) Genetics 146, 275–285
24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Uo, K., Nishihara, S., Sakuma, M., Togashi, S., Ueda, R., Miyata, Y., and Miyake, T. (1994) In Vitro Cell. Dev. Biol. Anim. 30, 209–216
26. Fessler, L. I., Nelson, R. E., and Fessler, J. H. (1994) Methods Enzymol. 245, 271–294
27. Smith, D. B., and Johnson, K. S. (1988) Gene (Amst.) 67, 31–40
28. Tautz, D., and Pfeifle, C. (1989) Chromosoma (Berl.) 98, 81–85
29. Patel, N. H. (1994) Methods Cell Biol. 44, 445–467
30. Takagi, T., U. K., Miyake, T., and Hirohashi, S. (1998) Neurosci. Lett. 244, 149–152
31. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–149
32. Schlaepfer, J. D., Schaller, M. D., and Parsons, J. T. (1993) J. Cell Biol. 123, 993–1005
33. Schaller, M. D., Borgman, C. A., and Parsons, J. T. (1993) Mol. Cell. Biol. 13, 795–791
34. Bunch, T. A., and Brower, D. L. (1992) Development (Camb.) 116, 239–247
35. Tippmer, S., Bossenmaier, B., and Haring, H. (1996) Eur. J. Biochem. 236, 953–959
36. Dikic, I., Takiwa, G., Lev, S., Courtneidge, S. A., and Schlessinger, J. (1996) Nature 383, 547–550
37. Slack, B. E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7281–7286
38. Richardson, A., and Parsons, T. (1996) Nature 380, 538–540
39. Leptin, M., Bogaert, T., Lehmann, R., and Wilcox, M. (1989) Cell 56, 401–408
40. Bunch, T. A., Graner, M. W., Fessler, L. I., Fessler, J. H., Schneider, K. D., Kerschen, A., Choy, L. P., Burgess, B. W., and Brower, D. L. (1998) Development (Camb.) 125, 1679–1689
41. Burgaya, F., Menegon, A., Menegoz, M., Valtorta, F., and Girault, J. A. (1995) Eur. J. Neurosci. 7, 1810–1821
42. Stevens, G. R., Zhang, C., Berg, M. M., Lambert, M. P., Barber, K., Cantalopes, I., Rottenberg, A., and Klein, W. L. (1996) J. Neurosci. Res. 44, 445–455
43. Hoang, B., and Chiba, A. (1998) J. Neurosci. 18, 7847–7855
44. Grotewiel, M. S., Beck, C. D., Wu, K. H., Zhou, X. R., and Davis, R. L. (1998) J. Neurosci. 18, 445–450
45. Perrimon, N. (1994) Curr. Opin. Cell Biol. 6, 260–266
46. Raabe, T., Riesgo, E. J., Liu, X., Bausenwein, B. S., Deak, P., Maroy, P., and Hafen, E. (1996) Cell 85, 911–920
47. Therrien, M., Chang, H. C., Solomon, N. M., Karim, F. D., Wassarman, D. A., and Rubin, G. M. (1995) Cell 83, 879–888