Insulin Receptor Substrate (IRS)-1 and IRS-2 Are Tyrosine-phosphorylated and Associated with Phosphatidylinositol 3-Kinase in Response to Brain-derived Neurotrophic Factor in Cultured Cerebral Cortical Neurons*

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Masashi Yamada‡§, Hiroshi Ohnishi‡, Shin-ichiro Nakatani‡, Atsushi Nakatani‡, Toshihiko Ikeuchi‡, and Hiroshi Hatanaka‡

From the 1Institute for Protein Research, Osaka University, 3–2 Yamadaoka, Suita, Osaka 565, Japan and the 2Mitsubishi Kasei Institute of Life Science, 11 Minamiosoyo, Machida, Tokyo 194, Japan

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophins, promotes differentiation and survival of various types of neurons in the central nervous system. BDNF binds to and activates the tyrosine kinase receptor, TrkB, initiating intracellular signaling and exerting its effects. Phosphatidylinositol 3-kinase (PI3-K), which has been implicated in promotion of neuronal survival by neurotrophic factors, is a component in the signaling pathway of BDNF. We examined how BDNF activates PI3-K in cultured cerebral cortical neurons. We found that insulin receptor substrate (IRS)-1 and -2 are involved in the BDNF signaling pathway that activates PI3-K. IRS-1 and -2 were tyrosine-phosphorylated and bound to PI3-K in response to BDNF. This BDNF-stimulated signaling via IRS-1 and -2 was inhibited by K-252a, an inhibitor of Trk tyrosine kinase. In addition, signaling via IRS-1 and -2 was markedly sustained as well as the BDNF-induced tyrosine phosphorylation of TrkB. On the other hand, we observed no association of PI3-K with TrkB in response to BDNF. These results indicate that the activation of TrkB by BDNF induces the activation of PI3-K via IRS-1 and -2 rather than by a direct interaction of TrkB with PI3-K in cultured cortical neurons.

Neurotrophins, including nerve growth factor, brain-derived neurotrophic factor (BDNF),¹ neurotrophin (NT)-3, and NT-4/5, are important for regulation of differentiation, survival, and plasticity of various types of neurons (1–9). Neurotrophins bind to and activate the Trk family of receptor tyrosine kinases to exert their effects (10, 11). NGF primarily binds to TrkA, BDNF and NT-4/5 bind to TrkB, and NT-3 binds to TrkC. Trks form homodimers following ligand binding, resulting in auto-phosphorylation on tyrosine residues, which is required for both catalytic and signaling activities (12–16).

Phosphatidylinositol 3-kinase (PI3-K) is activated by the neurotrophins as well as by various growth factors, including epidermal growth factor, platelet-derived growth factor, and hepatocyte growth factor (17–19). In addition, several studies using PC12 cells and cultured cerebellar granule neurons have indicated that the activation of PI3-K is important for the survival-promoting effects of neurotrophic factors (20, 21, 64). PI3-K is a heterodimer composed of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110) (22–24). PI3-K is activated by association of p85 with phosphotyrosine residues on target proteins via SH2 domains of p85 (25). PI3-K is activated by platelet-derived growth factor and hepatocyte growth factor through its binding to specific phosphotyrosine residues on autophosphorylated platelet-derived growth factor and hepatocyte growth factor receptors, respectively (26–29). However, the mechanism by which neurotrophins activate PI3-K remains unclear. There have been contradictory reports regarding whether NGF activates PI3-K via a direct association of PI3-K with the autophosphorylated TrkA or via an indirect interaction of PI3-K with the activated TrkA (30–32).

Insulin receptor substrate (IRS)-1 is well known as a binding protein to PI3-K (33–35). IRS-1 is a major substrate of the insulin receptor that possesses tyrosine kinase activity. PI3-K binds to specific phosphotyrosine residues on IRS-1, resulting in its activation. Recently, IRS-2 has been purified as a protein that associates with SH2 domains of PI3-K and has been shown to have sequence homology with IRS-1 (35–37). IRS-2 is also tyrosine-phosphorylated in response to insulin and binds to and activates PI3-K. IRS-1 and -2 are utilized in intracellular signaling pathways of insulin-like growth factor-1 (IGF-1); growth hormone; interleukin-2, -4, and -7; interferon α and γ, and leukemia inhibitory factor as well as in the insulin-activated pathway (38–42).

Here, we found that BDNF stimulates tyrosine phosphorylation of IRS-1 and -2 in cultured cerebral cortical neurons. In addition, the tyrosine-phosphorylated IRS-1 and -2 are associated with the protein and activity of PI3-K. These results suggest that IRS-1 and -2 are components in the signaling pathway of BDNF and are involved in the activation of PI3-K in cultured cortical neurons.

EXPERIMENTAL PROCEDURES

Cell Culture—Primary cultures of dissociated cerebral cortical neurons were prepared from the brains of embryonic day 18 (E18) rats (Wistar ST, both sexes) as described previously (43). The cells were cultured in a medium consisting of 5% precolostrum newborn calf serum (Mitsubishi Kasei), 5% heat-inactivated horse serum (56 °C, 30 min; Life Technologies, Inc.) and 90% minimum essential medium (Life Technologies, Inc.) containing 3.5 mg/ml glucose, 30 nM selenium, and...
0.5 mg/ml sodium bicarbonate, at a final cell density of 5 × 10⁶ cells/cm² on a polyethyleneimine-coated surface in Costar six-well plates (9.5 cm² of culture surface area). After culturing for 3 days, the medium was changed to serum-containing minimum essential medium plus 1 μM cytosine arabinoside (Sigma), and then the cells were cultured for 6 days. The medium was replaced with serum-free minimum essential medium, and then the assays described below were performed after overnight incubation.

**Immunoprecipitation and Immunoblotting**—Cells were washed once with ice-cold TBS and lysed in a buffer containing 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 2 mM Na₂VO₃, 20 mM Tris-HCl, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 0.7 mM leupeptin, and 1 μg/ml antipain. Lysates were centrifuged at 10,000 × g at 4 °C for 30 min, and then the protein concentration of the clarified lysate was determined by the BCA protein assay. The immunoprecipitation was performed using lysates prepared from cells in 1–2 wells of a six-well plate. Antibody was added to the lysates in excess amounts (2 μl of anti-Trk antiseraum, 1 μg of anti-IRS-1 antibody, 2 μg of anti-IRS-2 antibody, or 1 μg of anti-P13-K (p85 regulatory subunit) antibody and was incubated at 4 °C for 1–3 h. Protein G-Sepharose (10–40 μl gel) was then added and rotated at 4 °C for 30–60 min. The immune complexes were pelleted by centrifugation at 10,000 × g at 4 °C for 1 min and then washed three times with lysis buffer. The immune complexes were eluted with sample buffer (0.125 mM Tris-HCl, pH 6.8, 20% (v/v) glycerol, 4% (v/v) SDS, and 10% (v/v) β-mercaptoethanol) boiled, and then recovered by centrifugation for 1 min in a Microfuge. The eluates were resolved by electrophoresis on 4–20% gradient SDS-polyacrylamide gels according to Laemmli (44). Proteins were transferred onto polyvinylidene fluoride membranes (Millipore Corp.) in 0.1 mM Tris base, 0.192 mM glycine, and 20% (v/v) methanol using a semidyed electrophoretic transfer system. The membranes were blocked with 0.1% (w/v) Tween 20 (T-TBS) containing 1% (w/v) BSA (for detection of phosphotyrosine and P13-K) or 10% (w/v) nonfat dried milk (for detection of IRS-1 and -2) at room temperature for at least 1 h and then incubated with 0.25 μg/ml anti-phosphotyrosine antibody 4G10, 0.5 μg/ml anti-P13-K (p85 regulatory subunit) monoclonal antibody, 0.5 μg/ml anti-IRS-1 antibody, or 1 μg/ml anti-IRS-2 antibody in T-TBS containing 0.1% (w/v) BSA (for detection of phosphotyrosine and P13-K) or 10% (w/v) nonfat dried milk (for detection of IRS-1 and -2) at room temperature for 1 h. After three washes with T-TBS, the membranes were incubated with peroxidase-coupled goat anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc.) (for detection of phosphotyrosine and P13-K), peroxidase-coupled goat anti-rabbit IgG secondary antibody (Zymed) (for detection of IRS-1), or peroxidase-coupled donkey anti-goat IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc.) (for detection of IRS-2) diluted to 1:2,000 with T-TBS at room temperature for 1 h. The membranes were then washed four times with T-TBS and visualized using the ECL chemiluminescence system (Amersham Corp.).

In Vitro P13-K Kinase Assay—P13-K kinase activity was measured as described previously with some modifications (32, 45, 46). Briefly, cells were cultured and washed once with ice-cold TBS and lysed in a buffer containing 1% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 2 mM Na₂VO₃, 20 mM Tris-HCl, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 0.7 mM leupeptin, and 1 μg/ml antipain. Immunoprecipitation of IRS-1 and -2 and Trks was performed as described above. The immunoprecipitation was performed using lysates prepared from cells in one well of a six-well plate. The immunoprecipitates were washed three times with 1% Nonidet P-40, 150 mM NaCl, 0.1 mM Na₂VO₃, 20 mM Tris-HCl, pH 7.5; twice with 0.5 mM LiCl, 0.1 mM Na₂VO₃, and 0.1 mM Tris-HCl, pH 7.5; twice with 100 mM NaCl, 1 mM EDTA, 0.1 mM Na₂VO₃, and 10 mM Tris-HCl, pH 7.5; and twice with a reaction buffer containing 100 mM NaCl, 0.5 mM EGTA, 5 mM MgCl₂, and 20 mM Tris-HCl, pH 7.5. Then an in vitro kinase reaction was carried out with 10 μg of phosphatidylinositol as a substrate and the precipitates in 50 μl of reaction buffer plus 10 μCi of [γ-32P]ATP (10 μM ATP) at 25 °C for 10 min. The reaction was stopped by the addition of methanol:chloroform:HCl (5:10:1, v/v/v) solution. The lower phase was removed and extracted again with chloroform. The extracted solution was spotted on thin layer chromatography (TLC) plates pretreated with 10% (v/v) potassium oxalate:meanol (1:1, by volume), which were developed with chloroform:acetone:methanol:acetic acid:water (40:15:13:12:7, by volume). The P13-K activity was quantified using a Fuji Bio-imaging Analyzer (Fuji Film Co., Ltd.), and then the TLC plates were exposed to x-ray film (Eastman Kodak Co.).

**Results**

Tyrosine-phosphorylated Proteins Associated with P13-K in Response to Neurotrophins—P13-K is activated by binding to tyrosine-phosphorylated proteins via SH2 domains of the p85 subunit (25). To examine what kind of protein binds to and activates P13-K in signaling pathways of neurotrophins in neurons, immunoprecipitates with the anti-p85 antibody from lysates of cultured cerebral cortical neurons treated for 5 min with FGF (as a control), NGF, BDNF, or NT-3 were subjected to Western blotting analysis using an anti-phosphotyrosine antibody. TrkB and TrkC are expressed in cerebral cortical neurons, but TrkA is not (50, 51). In the cultured cortical neurons, we observed that BDNF and NT-3 induced the tyrosine phosphorylation of Trks, thought to be TrkB and TrkC, respectively, but BSA and NGF did not (data not shown). The anti-p85 immunoprecipitates contained at least four proteins (180, 120, 95, and 70 kDa), which showed increased tyrosine phosphorylation in response to BDNF and NT-3 but not in response to BSA or NGF (Fig. 1A). However, tyrosine-phosphorylated Trks with a molecular mass of 145 kDa could not be detected in the immunoprecipitates. When the immunoprecipitates were further immunoblotted with the anti-TrkB-specific monoclonal antibody, we could not detect the TrkB protein (Fig. 1B). Therefore, we consider that P13-K is activated through association...
with the tyrosine-phosphorylated proteins observed in the anti-p85 immunoprecipitates but not through direct association with the activated Trks.

**Neurotrophin-induced Tyrosine Phosphorylation of IRS-1 and -2 and Their Association with p85**—Next, we attempted to identify the tyrosine-phosphorylated proteins in the anti-p85 immunoprecipitates. We found a 180-kDa tyrosine-phosphorylated protein that was thought to be IRS-1 and/or IRS-2. IRS-1 and -2 are 185- and 190-kDa proteins, respectively, based on their migration on SDS-PAGE (33–37). Both IRS-1 and -2 are expressed in the brain, and their tyrosine-phosphorylated forms can bind to p85. To examine whether BDNF induces the tyrosine phosphorylation of IRS-1 and -2 and whether BDNF induces their association with p85, immunoprecipitates with the anti-IRS-1 or -2 antibody from lysates of cultured cerebral cortical neurons were immunoprecipitated with anti-IRS-1 and -2 antibodies, respectively, from 185 μg of protein of the lysates. The immunoprecipitates were analyzed by Western blotting with anti-phosphotyrosine antibody (upper panels), anti-P13-K monoclonal antibody (middle panels), and anti-IRS-1 and -2 antibodies (lower panels in A and B, respectively). The positions of tyrosine-phosphorylated IRS-1 and -2 are indicated by the arrowheads on the right (upper panels in A and B, respectively). The molecular masses are shown to the left of the immunoblots.

**Activity of PI3-K**—We found that BDNF and NT-3 induced the formation of complexes between p85 and IRS-1 or -2. To examine whether these complexes contained the p110 catalytic subunit, PI3-K activity was measured in the anti-IRS-1 and -2 immunoprecipitates of lysates prepared from cultured cerebral cortical neurons treated for 5 min with BSA (as a control), NGF, BDNF, or NT-3. BDNF and NT-3 induced the increase in tyrosine phosphorylation of both IRS-1 and -2, but BSA and NGF did not (Fig. 2). Consistent with the tyrosine phosphorylation of IRS-1 and -2, the amount of p85 protein associated with IRS-1 and -2 increased in response to BDNF and NT-3 but not in response to BSA or NGF (Fig. 2). We could not detect any proteins that showed the increase in tyrosine phosphorylation other than IRS-1 or -2. Therefore, we consider that the PI3-K associated with IRS-1 and -2 is not tyrosine-phosphorylated. These results indicate that PI3-K could be activated by binding of its p85 subunit to tyrosine-phosphorylated IRS-1 and -2 in the signaling pathways of BDNF and NT-3.

**Neurotrophin-induced Association of IRS-1 and -2 with the activated Trks.** The complex forms can bind to p85. To examine whether BDNF induces the tyrosine phosphorylation of IRS-1 and -2 and whether BDNF induces their association with p85, immunoprecipitates of lysates prepared from cultured cerebral cortical neurons treated for 5 min with BSA (as a control), NGF, BDNF, or NT-3 for 5 min and lysed. IRS-1 and -2 and Trks were immunoprecipitated with anti-IRS-1 and -2 antibodies, respectively, from 340 μg of protein of the lysates. The PI3-K activity in the immunoprecipitates was measured as described under "Experimental Procedures." The arrows on the right indicate the origin (Ori.) and the position of phosphatidylinositol 4-monophosphate as a standard (A). The PI3-K activity was quantified using a FUJIX Bio-imaging Analyzer (B–D). The values represent the means ± S.D. of three individual experiments.
Inhibitory Effect of K-252a on BDNF-stimulated Signaling via IRS-1 and -2—To examine the effect of K-252a, an inhibitor of Trk tyrosine kinase (52), on the BDNF-induced tyrosine phosphorylation of IRS-1 and -2 and their association with p85, immunoprecipitates with anti-IRS-1 or -2 antibody from lysates of cultured cortical neurons treated for 5 min with BSA (as a control), NGF, BDNF, or NT-3 in the presence or absence of 1 μM K-252a were subjected to Western blotting analysis using anti-phosphotyrosine and anti-p85 antibodies. K-252a inhibited the BDNF-induced increase in tyrosine phosphorylation of IRS-1 and -2 (Fig. 4, A and B). The BDNF-stimulated increase of the amount of p85 protein associated with IRS-1 or -2 was also suppressed to the level in untreated cells. The higher molecular weight band seemed to be tyrosine-phosphorylated and to show the mobility shift in response to BDNF. When the anti-IRS-2 immunoprecipitates were immunoblotted with anti-IRS-2 antibody, we observed two bands with apparent molecular mass of 170 and 180 kDa in the untreated cells. The higher molecular weight band seemed to be tyrosine-phosphorylated and to show the mobility shift in response to BDNF (Fig. 5B). We consider that the lower molecular weight band is a phosphorylation-unsusceptible IRS-2 protein or a nonspecific band. The shift of the higher molecular weight band was small at 5 min after the addition of BDNF, and then it became larger and was maintained until 5 h. In addition, the amounts of the higher molecular weight protein increased during the period 1.5–5 h after the BDNF addition. In the case of IRS-1, we observed only a very slight mobility shift and no increase in its protein amounts (Fig. 5A).

DISCUSSION

Our results using cultured cerebral cortical neurons provided evidence for the presence of a novel signaling pathway of neurotrophins, i.e. IRS-1 and -2 are components of the neurotrophin-induced signaling pathway. IRS-1 and -2 are known to be phosphorylated on tyrosine residues by the receptors for insulin and IGF-1. No receptor tyrosine kinases except for the insulin and IGF-1 receptors have been reported to utilize IRS-1 or -2. The Trk receptor recognizes IRS-1 as a substrate through the interaction between the phosphotyrosine-binding domain on IRS-1 and the tyrosine-phosphorylated NPXY motif in the juxtamembrane domain of the insulin receptor (55, 56). IRS-2 also has a phosphotyrosine-binding domain, and it has been reported to be recognized by the insulin receptor in a similar way to IRS-1 (57). Trks also have the NPXY motif in their juxtamembrane domain. In addition, Trks belong to the insulin receptor subfamily according to sequence homology of their tyrosine kinase domains (58). Therefore, we consider that
Trks as well as the insulin receptor directly phosphorylate IRS-1 and -2. On the other hand, we observed neither NGF- nor BDNF-induced tyrosine phosphorylation of IRS-1 and -2 in PC12 cells stably expressing TrkB (data not shown). The cultured cortical neurons expressed over 8-fold more TrkB protein than the TrkB-expressing PC12 cells (data not shown). However, the amounts of TrkA and TrkB proteins in the TrkB-expressing PC12 cells were thought to be almost the same as judged from the levels of their neurotrophin-induced tyrosine phosphorylation (data not shown). Therefore, the differences in amounts of Trk proteins between the cortical neurons and the PC12 cells may have caused the distinct responses in the neurotrophin-induced tyrosine phosphorylation of IRS-1 and -2. Alternatively, the neurotrophin-stimulated tyrosine phosphorylation of IRS-1 and -2 in cultured cortical neurons may require a signaling molecule that is not expressed in PC12 cells.

The insulin-induced tyrosine-phosphorylated forms of IRS-1 and -2 bind to the protein-tyrosine phosphatase SHP-2 and the small adaptor protein GRB-2 as well as to PI3-K (35, 59, 60). However, SHP-2 and GRB-2 were not coprecipitated by the anti-IRS-1 or -2 antibodies in response to neurotrophins in cultured cortical neurons (data not shown). The anti-IRS-1 antibody is raised against a synthetic peptide corresponding to a carboxyl-terminal amino acid sequence very close to the SHP-2-binding tyrosine residue on IRS-1, and the anti-IRS-2 antibody is raised against that including the SHP-2-binding tyrosine residue on IRS-2. The anti-IRS-1 and -2 antibodies might not precipitate the complex between SHP-2 and IRS-1 or -2 because of steric interference by SHP-2 in the association of IRS-1 or -2 with the antibodies or because of exclusion of SHP-2 binding to IRS-1 and -2 by the antibodies. On the other hand, we could not detect the 180-kDa tyrosine-phosphorylated protein, which was observed in the anti-Pi3-K immunoprecipitates and was thought to be IRS-1 and/or -2, in the anti-SHP-2 immunoprecipitates in response to neurotrophins in cultured cortical neurons (data not shown). The Trk tyrosine kinases might have different specificity for tyrosine residues on IRS-1 and -2 from other tyrosine kinases including the insulin receptor. In support of the different specificities among the tyrosine kinases, it has been reported that the insulin and IGF-1 receptors have varying specificities for individual tyrosine residues on IRS-1 in vitro (61). IRS-1 and -2 might show a variety of tyrosine-phosphorylated forms in response to different factors, utilizing different subsets of signaling molecules.

Neurotrophic factors induce the sustained activation of the mitogen-activated protein (MAP) kinase pathway (53, 62, 63). The sustained activation of MAP kinases is thought to be a feature of their neurotrophic action. We also observed that the BDNF-induced activation of MAP kinases in the cultured cortical neurons was sustained for at least 5 h after the addition of BDNF (data not shown). Here, we showed that the BDNF-stimulated signaling via IRS-1 and -2 was sustained in the cultured cortical neurons (Fig. 5). To exert neurotrophic effects, BDNF may not only induce the sustained activation of MAP kinases but also stimulate sustained signaling via IRS-1 and -2, which probably leads to sustained activation of PI3-K. Interestingly, the tyrosine phosphorylation of IRS-2 and its association with PI3-K increased again at 5 h after the addition of BDNF, although the phosphorylation and association of IRS-1 gradually declined after the increase at 5 min and did not rise again. The tyrosine phosphorylation of TrkB in response to BDNF also displayed a similar increase to that of IRS-1 (data not shown). Therefore, it is thought that the second increase in IRS-2 signaling was not due to the tyrosine kinase activity of TrkB. The second increase in the tyrosine phosphorylation of IRS-2 might require a tyrosine kinase other than TrkB, the activity of which shows a delayed increase. The expression of this tyrosine kinase and/or a regulatory protein of this kinase may be up-regulated by BDNF. Additionally, we observed that the amount of the IRS-2 protein with the higher molecular weight increased. Therefore, the biphasic response of IRS-2 might be due to the up-regulation of expression of the IRS-2 protein. In addition, IRS-2 displayed mobility shifts, which became larger after the peak of its tyrosine phosphorylation levels at 5 min. This delayed mobility shift of IRS-2 might have resulted from its phosphorylation on serine and/or threonine residues, but not on tyrosine residues, by a kinase that shows delayed activation in response to BDNF. Since the biphasic response of IRS-2 was reproducible, we suggest that some interesting regulatory mechanisms in the neurotrophin-induced signaling is implicated in this phenomenon.

We observed that the anti-Pi3-K antibody coprecipitated several tyrosine-phosphorylated proteins (180, 120, 95, and 70 kDa) in response to BDNF in cultured cerebral cortical neurons (Fig. 1). We assumed that these tyrosine-phosphorylated proteins bind to the SH2 domains of p85 regulatory subunit and activate PI3-K. From the present results, the 180-kDa protein is thought to be IRS-1 and/or -2. We could not detect any tyrosine-phosphorylated proteins other than IRS-1 or -2 in the anti-IRS-1 or -2 immunoprecipitates. Therefore, the tyrosine-phosphorylated proteins with apparent molecular masses of 120, 95, and 70 kDa in the PI3-K precipitates might activate PI3-K independently of IRS-1 or -2. It is assumed that BDNF-induced signaling in the cultured cortical neurons has redundant pathways to activate PI3-K. In this study, we showed that the pathway via IRS-1 or -2 is one of the PI3-K-activating pathways in BDNF-induced signaling in cultured cortical neurons.

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REFERENCES

1. Abiru, Y., Nishio, C., and Hatanaka, H. (1996) Dev. Brain Res. 91, 260–267
2. Akaneya, Y., Tsunoto, T., and Hatanaka, H. (1996) J. Neurophysiol. 76, 4196–4201
3. Kubo, T., Nonomura, T., Enokido, Y., and Hatanaka, H. (1995) Dev. Brain Res. 85, 249–258
4. Nonomura, T., Nishio, C., Lindsay, R. M., and Hatanaka, H. (1995) Brain Res. 683, 129–139
5. Nonomura, T., Kubo, T., Oka, T., Shimokoe, K., Yamada, M., Enokido, Y., and Hatanaka, H. (1996) Dev. Brain Res. 97, 42–50
6. Takei, N., Sasaoka, K., Inoue, K., Takahashi, M., Endo, Y., and Hatanaka, H. (1997) J. Neurochem. 68, 379–385
7. Takei, N., Sasaoka, K., Hayashi, H., Endo, Y., and Hatanaka, H. (1996) Mol. Brain Res. 37, 283–289
8. Lewin, G. R., and Barde, Y. A. (1996) Annu. Rev. Neurosci. 19, 289–317
9. Thoenen, H. (1990) Science 247, 593–598
10. Barabac, M. (1995) Curr. Opin. Cell Biol. 7, 148–155
11. Bothwell, M. (1995) Annu. Rev. Neurosci. 18, 223–253
12. Greene, L. A., and Kaplan, D. R. (1995) Curr. Opin. Neurobiol. 5, 579–587
13. Kaplan, D. R., and Stephens, R. M. (1984) J. Neurobiol. 15, 1404–1417
14. Lemmon, M. A., and Schlessinger, J. (1994) Trends Biochem. Sci. 19, 459–463
15. Segal, R. A., and Greenberg, M. E. (1996) Annu. Rev. Neurosci. 19, 463–489
16. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Annu. Rev. Cell Biol. 10, 251–337
17. Cochet, C., Filhol, O., Payrastre, B., Hunter, T., and Gill, G. N. (1991) J. Biol. Chem. 266, 637–644
18. Graziani, A., Gramaglia, D., Cantley, L. C., and Comoglio, P. M. (1991) J. Biol. Chem. 266, 22087–22090
19. Raffiﬁon, S., and Bradshaw, R. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 90, 9121–9125
20. Yao, R., and Cooper, G. M. (1995) Science 267, 2003–2006
21. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. (1997) Science 275,
22. Escobedo, J. A., Navankasatatus, S., Kavanaugh, W. M., Milfay, D., Fried, V. A., and Williams, L. T. (1993) Cell 65, 75–82
23. Hiles, I. D., Otou, M., Inokawa, J., Matsumoto, S., and Ito, H. (1992) J. Biol. Chem. 267, 20692–20699
24. Ruiz-Larrea, F., Vicendo, P., Vaish, P., End, D., and Panayotou, G. (1993) J. Biol. Chem. 268, 1451–1459
25. Carpenter, C. L., Auger, K. R., Chanudhuri, M., Yoakim, M., and Schaffhausen, B. (1993) Mol. Cell Biol. 13, 999–1010
26. Cooper, J. A., and Kashishian, A. (1993) Mol. Cell Biol. 13, 999–1010
27. Klippel, A., Escobedo, J. A., Fantl, W. J., and Williams, L. T. (1992) Mol. Cell Biol. 12, 5649–5657
28. Ponzetto, C., Bardelli, A., Maina, F., dalla Zonca, P., Giordano, S., Obermeier, A., Lammers, R., Wiesmuller, K. H., Jung, G., Schlessinger, J., and Marshall, C. J. (1994) EMBO J. 13, 3015–3022
29. Ponzetto, C., Bardelli, A., Maina, F., dalla Zonca, P., Giordano, S., Graziani, A., Panayotou, G., and Comoglio, P. M. (1994) Cell 77, 261–271
30. Obermeier, A., Lammers, R., Wiesmuller, K. H., Jung, G., Schlessinger, J., and Ulrich, A. (1993) J. Biol. Chem. 268, 22963–22966
31. Ohmichi, M., Decker, S. J., and Saltiel, A. R. (1992) Neuron 9, 769–777
32. Soltoff, S. P., Rabin, S. L., Cattley, L. C., and Kaplan, D. R. (1992) J. Biol. Chem. 267, 17472–17477
33. Backer, J. M., Myers, M. G., Jr., Shoelson, S. E., Chin, D. J., Sun, X. J., and White, M. F. (1992) EMBO J. 11, 3439–3479
34. Keller, S. R., and Lienhard, G. E. (1994) Trends Cell Biol. 4, 115–119
35. Waters, S. B., and Pessin, J. E. (1996) Trends Cell Biol. 6, 1–4
36. Petti, M.-E., Sun, X.-J., Bruening, J. C., Araki, E., Lipes, M. A., White, M. F., and Kahn, C. R. (1995) J. Biol. Chem. 270, 24670–24673
37. Sun, X.-J., Wang, L.-M., Zhang, Y. T., Yenush, L., Myers, M. G., Jr., Glasheen, E., Lane, W. S., Pierce, J. H., and White, M. F. (1995) J. Biol. Chem. 270, 27183–27186
38. Argetsinger, L. S., Liu, H., Myers, M. G., Jr., Billestrup, N., White, M. F., and Carter-Su, C. (1996) J. Biol. Chem. 271, 29401–29404
39. Uddin, S., Verich, L., Sun, X.-J., Sweet, M. E., White, M. F., and Platanias, L. C. (1995) J. Biol. Chem. 270, 15938–15941
40. Johnston, J. A., Wang, L.-M., Hansson, E. P., Sun, X.-J., White, M. F., Oakes, S. A., Pierce, J. H., and O’Shea, J. J. (1995) J. Biol. Chem. 270, 28527–28530
41. Platanias, L. C., Uddin, S., Yetter, A., Sun, X.-J., and White, M. F. (1996) J. Biol. Chem. 271, 278–282
42. Enokido, Y., Akaneya, Y., Niinobe, M., Mikoshiba, K., and Hatanaka, H. (1992) Brain Res. 599, 261–271
43. Laemmli, U. K. (1970) Nature 227, 680–685
44. Bocchini, V., and Angeletti, P. U. (1969) Proc. Natl. Acad. Sci. U. S. A. 64, 787–794
45. Conlon, C. M., and Bronner-Fraser, M. (1992) Development 115, 119–126
46. Argetsinger, L. S., Hsu, G. W., Myers, M. G., Jr., Billestrup, N., White, M. F., and Carter-Su, C. (1995) J. Biol. Chem. 270, 24670–24673
47. Steininger, T. L., Wainer, B. H., Klein, R., Barbacid, M., and Palfrey, H. C. (1993) Brain Res. 612, 330–335
48. Bocchini, V., and Angeletti, P. U. (1969) Proc. Natl. Acad. Sci. U. S. A. 64, 787–794
49. Suda, K., Barde, Y. A., and Thoenen, H. (1978) J. Neurochem. 31, 450–458
50. Merlio, J. P., Ernfors, P., Jaber, M., and Persson, H. (1992) Neuroscience 51, 513–532
51. Olmsted, D. J., and White, M. F. (1995) J. Biol. Chem. 270, 27183–27186
52. Qui, M. S., and Green, S. H. (1992) J. Neurochem. 59, 19–37
53. Steininger, T. L., Wainer, B. H., Klein, R., Barbacid, M., and Palfrey, H. C. (1993) Brain Res. 612, 330–335
54. Bocchini, V., and Angeletti, P. U. (1969) Proc. Natl. Acad. Sci. U. S. A. 64, 787–794
55. Marshall, C. J. (1995) Cell 80, 179–185
56. Steiner, G., and Marik, A. (1994) Mol. Cell. Biol. 14, 6433–6442
57. Szewczuk, M., and Marik, A. (1994) Mol. Cell. Biol. 14, 6433–6442
58. Sawka-Verhelle, D., Tartare-Deckert, S., White, M. F., and Van Obberghen, E. (1996) J. Biol. Chem. 271, 5980–5983
59. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42–52
60. Sugimoto, S., Wandless, T. J., Shoelson, S. E., Neel, B. G., and Walsh, C. T. (1994) J. Biol. Chem. 269, 13614–13622
61. Ward, C. W., Gough, K. H., Raschke, M., Wan, S. S., Tribbick, G., and Wang, J. (1996) J. Biol. Chem. 271, 5603–5609
62. Uddin, S., Yenush, L., Myers, M. G., Jr., Shoelson, S. E., and White, M. F. (1995) J. Biol. Chem. 270, 24670–24673
63. Shelden, D. J., and White, M. F. (1995) J. Biol. Chem. 270, 24670–24673
64. Shimokawa, H., Kudo, T., Numakawa, T., Abiru, Y., Enokido, Y., Takei, N., Ikeuchi, T., and Hatanaka, H. (1997) Dev. Brain Res. 101, 197–206