Association of Protein Kinases ERK1 and ERK2 with p75 Nerve Growth Factor Receptors*

(Rceived for publication, April 27, 1993, and in revised form, June 10, 1993)

Cinzia Volonté, James M. Angelastro, and Lloyd A. Greene
From the Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University, New York, New York 10032

Extracellular signal-regulated protein kinases (ERKs) constitute a family of protein serine-threonine kinases implicated in a variety of cell-signaling pathways. In cultured rat pheochromocytoma PC12 cells, ERK1 and ERK2 are activated by nerve growth factor (NGF), which also induces rapid association between ERK1 and the high affinity gp140\textsuperscript{prototk} tyrosine kinase NGF receptor. In the present work, we investigated the possible association between ERKs and the low affinity NGF receptor, p75. Extracts of PC12 cells (before and after NGF treatment) were subjected to immunoprecipitation with anti-p75 antibodies or antisera; the immune complexes were then assessed for the presence of ERK proteins and tyrosine phosphorylation or for ERK activity using a specific substrate peptide. ERK1 and, to a lesser extent, ERK2 were found to be constitutively associated with p75. NGF did not modulate the total amount of ERK proteins co-immunoprecipitated with p75 but did markedly stimulate the level of p75-associated ERK catalytic activity. NGF treatment also enhanced the tyrosine phosphorylation of a p75-associated species that co-migrates with ERK1 in Western blots. Finally, K-252a, a compound that specifically inhibits activation by NGF of gp140\textsuperscript{prototk}, abolished the latter effect. These findings indicate that NGF, via activation of gp140\textsuperscript{prototk}, leads to association of enzymatically active ERKs with p75 and raise the possibility that this interaction may play a role in the NGF mechanism of action.

Nerve growth factor (NGF)\textsuperscript{1} (Levi-Montalcini, 1987; Levi and Alema, 1991) stimulates a variety of responses in target cells, and the signaling pathways that mediate them have been the subject of many recent investigations. Two distinct transmembrane cell surface receptors for NGF have been recognized and characterized (reviewed by Bothwell (1991), Ross (1991), and Meakin and Shooter (1992)). One is gp140\textsuperscript{prototk}, the protein tyrosine kinase product of the \textit{trk} protooncogene, that is activated and autophosphorylated upon NGF binding and that has been identified as a functional receptor for NGF (Kaplan et al., 1991; Klein et al., 1991; Loeb et al., 1991; Cordon-Cardo et al., 1991). The other NGF receptor, designated p75 (Johnson et al., 1986; Radeke et al., 1987), binds NGF and other members of the neurotrophin family and has no known direct signaling activity. The presence or binding of NGF to p75 does not appear to be required for gp140\textsuperscript{prototk}-mediated responses to NGF (Hosang and Shooter, 1985; Radeke and Feinblatt, 1991; Meakin and Shooter, 1991, 1992; Meakin et al., 1992; Drinkwater et al., 1992; Itanez et al., 1992). However, evidence has been presented that indicates that p75 may play a role in high affinity NGF binding and perhaps in regulating specificity of neurotrophin receptors (Hempstead et al., 1989, 1991; Yan et al., 1991; Berg et al., 1991; Ip et al., 1993). Furthermore, mice in which expression of the p75 gene has been selectively interrupted, display an abnormal phenotype, including reduced numbers of sensory neurons (which are targets of neurotrophins) (Lee et al., 1992).

There is ample evidence that growth factor receptors specifically associate with a spectrum of signaling molecules (among which are also protein kinases) that may directly bind to the receptor or may indirectly associate with other receptor-binding proteins (Volonté et al., 1993a, 1993b; Loeb et al., 1992; Vetter et al., 1991, Ulrich and Schlessinger, 1990; Maher, 1988; Veillette et al., 1988). Previous work has shown that several proteins can be specifically co-immunoprecipitated from rat PC12 pheochromocytoma cells (Green and Tischler, 1976) by antibodies directed against p75. These include a serine kinase activity that phosphorylates several species present in the immunocomplex and that does not appear to be NGF-regulated (Ohmichi et al., 1991) and an NGF-regulated kinase activity that phosphorylates mainly threonine residues when myelin basic protein is used as a substrate (Volonté et al., 1993a) that is similar to protein kinase N (Volonté and Greene, 1992b) and is inhibitable by purine analogs. Purine analogs suppress many biological responses of NGF in PC12 cells (Volonté et al., 1989; Volonté and Greene, 1990, 1992a).

Since receptor-associated serine/threonine kinases may play a range of potential roles in growth factor signaling, we have carried out experiments to detect whether additional such enzymes are associated with p75 NGF receptor. One set of protein kinases of particular interest are the ERK/MAP kinases (Ray and Sturgill, 1987; Rossoamando et al., 1989; Boulton et al., 1990, Boulton and Cobb, 1991). These widely expressed serine/threonine kinases are activated in response to a variety of growth factors including the neurotrophins (Boulton et al., 1991b; Tsao and Greene, 1991; Tsao et al., 1990; Miyasaka et al., 1990). ERK activation appears to require phosphorylation of both tyrosine and threonine resi-
ERK1 and ERK2 Associate with p75

21411

dues and to occur via a cascade of signaling events that includes Ras p21 and several additional protein kinases (Boulton et al., 1991a, 1991b; Ahn et al., 1991; Crews et al., 1991; Her et al., 1981). We report here evidence for specific cellular interaction between p75 and ERKs and for regulation by NGF of p75-associated ERK activity.

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]ATP (3000 Ci/mmole) was purchased from Du Pont-New England Nuclear; [γ-32P]Protein A was from ICN; Protein A-Sepharose CL-4B was from Pharmacia LKB Biotechnology Inc.; 2-aminopyridine and ATP were purchased from Sigma; MAP kinase substrate peptide was from Upstate Biotechnology, Inc. (Lake Placid, NY).

Anti-rat p75 NGF receptor mouse monoclonal ascites (192) (Chandler et al., 1984) was a gift from Dr. Alonzio H. Ross (Worcester Foundation for Experimental Biology, Shrewsbury, MA). Anti-phosphotyrosine antibody 4G10 (Kaplan et al., 1984) at 1:200 was a gift from Dr. D. R. Kaplan (Frederick Cancer Research Center, Frederick MD). The rabbit polyclonal antiserum, which recognizes the intracellular portion of p75 NGF receptors (Ohmichi et al., 1991) was a kind gift from Drs. S. J. Decker (Parke Davis/Warner Lambert, Ann Arbor, MI) and M. V. Chao (Cornell University Medical Center, New York, NY).

Cell Culture—PC12 cells were cultured as previously described on collagen-coated culture dishes (150-mm diameter) in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum (Greene and Tischler, 1976). NGF, purified from mouse submaxillary glands (Mobley et al., 1972), was directly added to the cultures for 5 min, unless otherwise specified, at a final concentration of 100 ng/ml. The cultures were washed twice with ice-cold phosphate-buffered saline (PBS) and then quick-frozen in liquid nitrogen and stored at −80°C until use.

Immunoprecipitation of p75 and p75-associated Proteins—Cells were lysed for immunoprecipitation in 750 μl of lysis buffer containing 50 mM Tris (pH 7.4), 10% glycerol, 1% Triton X-100, 150 mM NaCl, 100 mM NaN3, 5 mM Na2VO4, 1 mM Na3VO4, 10 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 200 μl kalirikin-inactivating units/ml aprotinin, and 1 μg/ml leupeptin. After extraction on ice for 10 min, lysates were centrifuged at 100,000 × g for 15 min at 4°C. Supernatants were reserved and normalized for protein concentration. Equal amounts of protein were incubated for 1 h at 4°C with anti-rat p75 NGF receptor mouse monoclonal ascites (192) (Chandler et al., 1984) at a dilution of 1:700. Protein A-Sepharose, prepared as suggested by Upstate Biotechnology, was added to the lysate for 1 h. The immune complex was pelleted at 4°C in a microcentrifuge, and the pellets were washed three times (1 ml/wash) with Tris/G (20 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol).

Assay of p75 NGF Receptor-associated Protein Kinase Activity—After immunoprecipitation, the immune complexes were suspended in 150–250 μl of ice-cold phosphorylation buffer (180 mM MES, pH 6.8, 1 mM EGTA, 0.5 mM MgSO4, 2 mM phenylmethylsulfonyl fluoride, 100 μg kalirikin-inactivating units/ml Trasylol, 5 μg/ml leupeptin), and 20 μl were used for phosphorylation assays. These were performed in phosphorylation buffer (50 μl final volume) in the presence of 10 mM MnCl2, using 2 μl (1 μM) of 55 MAP peptide as substrate and 5 μCi of [γ-32P]ATP (3000 Ci/mmole) in a final ATP concentration of 20 μM. The mixture was incubated for 15 min at 37°C, and phosphate incorporation was quantified as described by Clark et al., 1991. To determine net incorporation into the peptide, parallel assays were performed without substrate, and the values obtained were subtracted from the total incorporation.

SDS-Polyacrylamide Gel Electrophoresis, Western Blotting, and Immunoreactions—Immunoprecipitates or whole cell extracts were boiled for 5 min in Laemmli sample buffer (Laemmli, 1970) and subjected to SDS-polyacrylamide gel electrophoresis (10% acrylamide). Proteins were electroblotted onto nitrocellulose (Schleicher & Shuell) (Towbin et al., 1979). Nonspecific binding was blocked with 3% bovine serum albumin in PBS for 2 h at 25°C. The nitrocellulose was subsequently incubated with primary antibody in 3% bovine serum albumin/PBS overnight at 4°C. Anti-ERK antisera 837 and 692 were used at 1:67 dilution, 691 and C356 were used at 1:200, and anti-phosphotyrosine was used at 1:1000, all in 3% bovine serum albumin/PBS (Boulton and Cobb, 1991). After washes with 0.2% Nonidet P-40 in PBS (1 h, 4 changes), immunoreactive bands were revealed by 1211-Protein A (1 μg/ml) followed by autoradiography using Kodak X-AR film.

Protein Determination—Protein concentrations were determined by the method of Bradford (1976), using reagents and protocols purchased from Bio-Rad Laboratories and with bovine serum albumin as standard.

RESULTS

To investigate whether there is association in PC12 cells between the p75 NGF receptor and ERKs, p75 was immunoprecipitated from extracts of PC12 cells treated with or without NGF (using monoclonal antibody 192 (Chandler et al., 1984) at 1:700 dilution) and the stringently washed immunocomplexes were subjected to Western immunoblotting with various anti-ERK antisera (Boulton and Cobb, 1991). The data in Fig. 1 (lanes a–d) show the immunoblotting achieved with nonimmunoprecipitated whole cell extracts of cells treated with (lanes c and d) or without (lanes a and b) NGF for 5 min. Antiserum 837, which preferentially recognizes ERK1 (lane a and c), and antiserum 692, which equally detects both ERK1 and ERK2 (lane b and d), confirm both the presence of ERK1 and ERK2 in the extracts and the absence of modulation of their expression by NGF. Lanes e–h of Fig. 1 compare the immunoblotting achieved with p75 immunoprecipitates of replicate cultures. Antiserum 837 (lanes e and g) and 692 (lanes f and h) reveal the presence of ERK1 protein in the immunocomplex and show essentially the same level of association before (lanes e and f) and after (lanes g and h) 5 min of NGF treatment. Antiserum 692 also detects the presence of ERK2 in the immunocomplex, but the level relative to that of ERK1 appears to be considerably lower than in cell extracts.

Fig. 2 illustrates the results achieved when p75 immunoprecipitates from control (lanes a–c, g, and h), and NGF-treated (lanes d–f, i, and j) PC12 cells were immunoblotted with two additional anti-ERK antisera, 691 (lanes b and f) and C356 (lanes h and j). Antiserum 691 recognizes ERK1 and ERK2 equally well, whereas C356 preferably recognizes ERK1. The results obtained with anti-ERK antiserum 837 are included for comparison (lanes a, e, g, and i). These data confirm the presence of both ERK1 as well as a relatively lesser amount of ERK2 proteins in the immunocomplex and that NGF treatment does not affect the extent of ERK-p75 interaction.

Several controls were performed to test the specificity of the observed results. First, when the immunoprecipitation protocol was performed in the absence of primary antibody, no ERK proteins were detected that nonspecifically bound to Protein A-Sepharose. Second, no ERK protein was detected by Western blotting of the immunoprecipitate obtained when a nonimmune antiserum was used in place of anti-p75. This rules out the possibility that ERKs nonspecifically bind to immunoglobulins. Third, no signal was obtained when the immunoprecipitates were immunoblotted with a nonimmune antiserum (Fig. 2, lanes c and d). Fourth, immunoblotting did not detect ERK protein in immunoprecipitates obtained with a polyclonal antiserum that recognizes the intracellular domain of p75 receptor (Ohmichi et al., 1991). Because Protein A is not always quantitative in recognizing mouse IgG, immunoprecipitations were also carried out using anti-p75 antibody 192 and rabbit anti-mouse IgG conjugated to Protein A-Sepharose. The p75 antibody was also tested at dilutions as low as 1:200. In both cases, there was no difference in recovery of p75-associated ERK proteins as compared with those achieved with the standard conditions described above. We next investigated whether the p75-associated ERKs might be enzymatically active and whether this activity is modulated.
FIG. 1. Detection of ERK proteins by anti-ERK antisera 837 and 692 in whole cell extracts and p75 NGF receptor immunoprecipitates. For whole cell experiments, extract from control (a, b) and NGF-treated (c, d) PC12 cell cultures were prepared, and, in each case, 150 μg of protein was subjected to Western immunoblotting with anti-ERK antisera 837 (a, c) or 692 (b, d) as described under "Experimental Procedures." For immunoprecipitations, equal amounts of extracted cell protein (4.1 mg) were subjected to immunoprecipitation with antibody 192 prepared against p75 NGF receptors (see "Experimental Procedures"). Aliquots (1/4) of the immunoprecipitated material from control (e, f) and NGF-treated (g, h) cultures were subjected to Western immunoblotting with anti-ERK antisera 837 (e, g) or 692 (f, h). Immunoreactive proteins were revealed by 125I-Protein A. The large arrow indicates the position of ERK1 protein, and the small arrow indicates the position of ERK2 protein. The positions of molecular mass markers \( M \times 10^{-3} \) are indicated on the right. Comparable results were achieved in three independent experiments.

![Western blot image](image-url)

FIG. 2. Detection of ERK proteins by anti-ERK antisera 837, 691, and C356 in p75 NGF receptor immunoprecipitates. A, equal amounts (3.8 mg) of extracted protein from control (a–c) and NGF-treated (d–f) cultures were subjected to immunoprecipitation with antibody 192 prepared against p75 NGF receptors. Aliquots (1/4) of the immunoprecipitated material were subjected to Western immunoblotting with anti-ERK antisera 837 (a, e) or 691 (b, f) or with a nonimmune antiserum (c, d). B, equal amounts (4 mg) of extracted protein from control (g, h) and NGF-treated (i, j) cultures were subjected to immunoprecipitation with antibody 192 prepared against p75 NGF receptors. Aliquots (1/4) of the immunoprecipitated material were subjected to Western immunoblotting with anti-ERK antisera 837 (g, i) or C356 (h, j). Immunoreactive proteins were revealed by 125I-Protein A. The large arrow indicates the position of ERK1 protein, and the small arrow indicates the position of ERK2 protein. The positions of molecular mass markers \( M \times 10^{-3} \) are indicated. Comparable results were achieved in three independent experiments.

![Western blot image](image-url)
by NGF. The major site of myelin basic protein phosphorylation by ERK family members has been identified, and a peptide, designated S5, which includes the amino acids surrounding this site, has been used as specific substrate for these kinases (Sanghera et al., 1990; Erikson et al., 1990; Clark-Lewis et al., 1991). P75 immunoprecipitates derived from PC12 cells treated with or without NGF for 5 min were incubated with the peptide under conditions optimal for ERK kinase activity. As shown in Fig. 3, there was an approximately 4-fold greater level of S5-phosphorylating activity in immunocomplexes obtained from NGF-treated cells. This activity was totally blocked by 10 mM 2-aminopurine, a protein kinase inhibitor known to be effective on ERK activity (Boulton et al., 1991a; Tsao and Greene, 1991; Volonté and Greene, 1992a). In control experiments, the S5-phosphorylating activity was absent in immune complexes prepared in the absence of primary antibody (data not shown).

Cytoplasmic ERKs appear to require both threonine and tyrosine phosphorylation for activation. The experiment shown in Fig. 3 was carried out to further characterize ERKs associated with the p75 receptor and to assess the relationship of p75-associated ERK-like kinase activity with ERK phosphorylation. Whole cell extracts (lanes a–d) or p75 immune complexes (lanes e–h) from cultures exposed to NGF for 0 (lanes a, c, f, and g) or 5 min (lanes b, d, e, and h) were subjected to immunoblotting with an anti-phosphotyrosine antibody. As previously observed in whole cell extracts, NGF induces the tyrosine phosphorylation of proteins that co-migrate with ERK1 and ERK2. Examination of the material from the immunoprecipitates reveals that NGF markedly stimulates the tyrosine phosphorylation of a band that co-migrates with ERK1. This band was absent in samples in which nonimmune antibodies were used (data not shown).

K-252a is a kinase inhibitor previously reported to selectively block responses of PC12 cells to NGF, including activation and tyrosine phosphorylation of soluble ERKs. This inhibitor suppresses activation of gp140 

\[ \text{MAP kinase substrate S5 peptide (Sanghera et al., 1990; Erikson et al., 1990; Clark-Lewis et al., 1991)} \]

As shown in Fig. 4 (lanes c, d, g, and h), pretreatment of the cultures with 200 nM K-252a for 1 h abolished the NGF-stimulated tyrosine phosphorylation of the ERK1 co-migrating band in both the whole cell extract (lane d) and p75 immunocomplex (lane h).

**DISCUSSION**

In the present work, we have used co-immunoprecipitation to provide evidence for association of ERK proteins and ERK catalytic activity with the p75 NGF receptor. Four different anti-ERK antisera and two different anti-p75 antisera/antibodies were employed as well as a variety of controls to rule out nonspecific binding. Furthermore, under our experimental conditions, we were not able to detect the presence of Trk in the p75 immunoprecipitates. Our findings indicate that ERK1 and, to a lesser extent, ERK2 associate with the p75 NGF receptor. Moreover, although the total extent of this association does not appear to be modified by NGF treatment, NGF significantly increases the level of ERK-like catalytic activity associated with p75. This activity is detected with the ERK/MAP kinase substrate S5 peptide (Sanghera et al., 1990; Erikson et al., 1990; Clark-Lewis et al., 1991) and is inhibited by 2-aminopurine, a known inhibitor of ERKs (Tsao and Greene, 1991; Boulton et al., 1991a). Further consistent with

![Fig. 3. ERK proteins immunoprecipitated by anti-p75 NGF receptor antibody are functionally active.](image)

**FIG. 3.** ERK proteins immunoprecipitated by anti-p75 NGF receptor antibody are functionally active. Equal amounts (4.9 mg) of extracted protein from PC12 cells treated with or without NGF were subjected to immunoprecipitation with antibody 192 generated against p75 NGF receptors. In each case, phosphorylation reactions were carried out with aliquots (1/9) of the total immunoprecipitated material using peptide S5 MAP as substrate (all as described under "Experimental Procedures"). Specifically incorporated cpm/ aliquot are shown. Where indicated, the phosphorylation assays were carried out in the presence of 10 mM 2-aminopurine. Values represent averages of duplicate determinations ± range. Comparable results were achieved in two independent experiments.

| S5 MAP phosphorylation (cpm/ aliquot) |
|----------------------------------------|
| No addition                            |
| 10 mM 2-AP                             |

![Fig. 4.](image)

**FIG. 4.** ERK1 immunoprecipitated by anti-p75 NGF receptor antibody is phosphorylated on tyrosine. PC12 cells were pretreated for 1 h without (a, b, c, f) or with (c, d, g, h) K-252a and then for an additional 5 min without (a, c, f, g) or with (b, d, e, h) NGF. a–d, whole cell extracts (140 μg of protein) were subjected to Western immunoblotting with anti-phosphotyrosine antibody. e–h, equal amounts of extracted cell protein (7 mg) were subjected to immunoprecipitation with antibody 192 generated against p75 NGF receptors. Aliquots (1/3) of the immunoprecipitated material were subjected to Western immunoblotting with anti-phosphotyrosine antibody. Immunoreactive proteins were revealed by [γ-32P]-Protein A. The large arrow indicates the position of ERK1 protein, and the small arrow indicates the position of ERK2 protein. At the moment we do not know the identity of the major band present in the immunoprecipitate, which is not modulated by NGF. The positions of molecular mass markers (M, × 10^6) are indicated on the right. Comparable results were achieved in two independent experiments.
this was the observation that NGF induced the association with p75 of a tyrosine-phosphorylated protein that co-migrates with ERK1 on Western immunoblots. Activation of ERK activity occurs via phosphorylation on both a threonine and a tyrosine residue, and NGF stimulates rapid tyrosine phosphorylation of ERK1 and ERK2 in PC12 cells (Ray and Sturgill, 1988; Pavne et al., 1991, Boulton et al., 1991b).

Our observations suggest that association between ERKs and p75 is not dependent on NGF but that NGF enhances p75-associated ERK phosphorylation and activity. This raises the possibility that exposure to NGF promotes the tyrosine phosphorylation and activation of ERK1 that is already bound to p75. However, our data do not permit us to distinguish between this and the alternate possibility that NGF promotes association of active, tyrosine-phosphorylated ERK1 with p75. In the latter case, this could occur either by exchange with nonactive ERKs or, assuming that tyrosine-phosphorylated ERKs constitute only a small proportion of total ERK protein, by bulk addition of active ERKs.

The effects of NGF observed here could either be mediated directly through binding of the factor to p75 or indirectly via its role in NGF receptor-mediated signaling. Our observations support the latter. Thus, K-252a, a potent inhibitor of NGF-promoted Trk kinase activity and of NGF-dependent responses in PC12 cells (Koizumi et al., 1988; Berg et al., 1992), abolished the appearance of the tyrosine-phosphorylated ERK1-like band in p75 immunoprecipitates derived from NGF-treated cells.

Interaction/phosphorylation of receptor or receptor-associated enzymes by serine/threonine kinases has been documented (Northwood et al., 1991; Omichi et al., 1991). Several different kinase activities appear to associate with p75 NGF receptor complexes. Omichi et al. (1991) described a serine kinase activity that phosphorylates several species present in p75 immunocomplexes. In contrast to the ERK-like activity described here, however, that enzyme does not appear to be regulated by NGF. We previously reported that p75 immunoprecipitates contain an additional protein kinase activity that is NGF-regulated (Volonté et al., 1993a). However, unlike ERK activity, that activity was suppressed by low concentrations of the purine analog 6-thioguanine. Thus, our findings indicate the presence of a third protein kinase activity (which we tentatively assign to ERKs) that can associate with p75.

Interaction with ERKs does not appear to be limited to p75. Loeb et al. (1992) demonstrated that NGF promotes the association of ERK1, but not of ERK2, with Trk NGF receptors. As judged by the presence of S5 peptide kinase activity, the Trk-associated ERK1 is also enzymatically active. However, there are several notable differences between the two cases. First, in contrast to the present observations with p75, no ERK association with Trk could be detected in the absence of NGF. Second, although ERK1 protein and ERK-like S5 peptide kinase activity were present in Trk immunocomplexes from NGF-treated cells, unlike the p75 results described here, no tyrosine-phosphorylated ERK1 could be detected in association with Trk.

Our findings raise the issue of the functional significance of the interaction between ERKs and p75 NGF receptors. One possibility is that p75 itself (Taniuchi et al., 1998) is a substrate for ERKs. However, we have been unable to demonstrate NGF-stimulated phosphorylation of p75 in intact PC12 cells. Alternatively, other p75-associated molecules may be ERK substrates or ERKs themselves may be substrates for other p75-associated activities. The biological importance of such effects is presently difficult to assess, in part because the role of p75 in NGF signaling is itself unclear. Although p75 expression is not required for Trk-mediated NGF signaling in at least some systems (cf. Ihanez et al., 1992), several possible roles for p75 NGF receptors in conjunction with Trk have been suggested. These include formation of high affinity NGF receptors and regulation of the specificity of neurotrophin interactions with various Trk receptors (Hempstead et al., 1989; Berg et al., 1991; Yan et al., 1993). That p75 plays at least some critical role in neurotrophin signaling is suggested by the sensory deficits exhibited by mice in which expression of this gene has been compromised (Lee et al., 1992). Irrespective of the activities of p75, it seems reasonable to speculate that such functions could well be regulated by associated ERK activity. Moreover, as we have shown here, since ERKs appear to be activated by NGF in a Trk-dependent manner, association of ERKs with p75 could provide a means for Trk to influence the properties of p75.

Acknowledgments—We thank Dr. D. R. Kaplan for anti-phosphotyrosine antibody 4G10, Dr. A. H. Ross for anti-rat p75 NGF receptor mouse monoclonal ascites (192), Drs. S. J. Decker and M. V. Chao for rabbit polyclonal antisera (which recognizes the intracellular portion of p75 NGF receptor), and Dr. M. H. Cobb for various anti-ERK antisera. The expert technical assistance of Cynthia Nixon is greatly appreciated.

REFERENCES

Ahn, N. G., Seger, R., Bratlien, R. L., Diltz, C. D., Tonks, N. K., and Krebs, E. G. (1991) J. Biol. Chem. 266, 4220-4227.

Berg, M., Sternberg, J., Hemplestad, B., and Chao, M. V. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7106-7110.

Berg, M. J., Sternberg, D. W., Parada, L. F., and Chao, M. V. (1992) J. Biol. Chem. 267, 15-26.

Bothwell, M. (1991) Cell 65, 915-918.

Boulton, T. G., and Cobb, M. H. (1991) Cell Regul. 2, 357-371.

Boulton, T. G., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moosmaw, C., Lai, E., and Cobb, M. H. (1990) Science 249, 64-67.

Boulton, T. G., Gregory, J. S., and Cobb, M. H. (1991a) Biochemistry 30, 278-296.

Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DeFilipps, R. A., Panayotatos, N., Cobb, M. H., and Yancopoulos, G. D. (1991b) Cell 65, 663-675.

Bradford, M. (1976) Anal. Biochem. 72, 248-254.

Chandler, C. E., Parsons, L. M., Hosang, M., and Shooter, E. M. (1984) J. Biol. Chem. 259, 6882-6889.

Clark, L., Sanghera, J. S., and Pelcz, S. L. (1991) J. Biol. Chem. 266, 15180-15184.

Cordon-Cardo, C., Tapley, P., Jing, S., Nanduri, V., O'Rourke, E., Lamballe, F., Pommier, Y., Klein, R., Jones, K. R., Reichardt, L. F., and Barde, M. (1991) Cell 66, 173-183.

Cripps, A. M., Alessandri, F., and Erikson, R. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8845-8849.

Drinkwater, C. C., Suter, U., Angst, C., and Shooter, E. M. (1992) Proc. Soc. Lond. B Biol. Sci. 249, 307-313.

Erikson, A. K., Payne, D. M., Martino, P. A., Rosamond, A. J., Shabanowitz, J., Weber, J. M., Hunt, D. F., and Sturgill, T. W. (1990) J. Biol. Chem. 265, 17228-17236.

Greene, L. A., and Tischler, A. S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 2424-2428.

Hempstead, B. L., Schleifer, L. S., and Chao, M. V. (1989) Science 245, 373-375.

Her, J. H., Wu, J., Rall, T. B., Sturgill, T. W., and Weber, M. J. (1991) Nucleic Acids Res. 19, 2743-2746.

Hosang, M., and Shooter, E. M. (1985) J. Biol. Chem. 260, 655-662.

Ihanetz, C., Ebendal, T., Barbagyi, G., Murray-Rust, J., Bindell, T. L., and Akeson, H. (1992) Cell 69, 1111-1121.

Ip, N. Y., Stitt, T. N., Tapley, P., Klein, R., Glass, D. J., Pandi, J., Greene, L. A., Barbeaud, M., and Yancopoulos, G. D. (1993) Neuron 10, 1-20.

Johnson, D., Lanahan, A., Buck, C. R., Sehgal, A., Morgan, C., Mercer, E., Bothwell, M., and Chao, M. (1989) Cell 47, 545-554.

Kaplan, D. R., Morrison, D. K., Wong, G., McCormick, F., and Williams, L. T. (1990) Cell 61, 125-133.

Kaplan, D. R., Hempstead, B., Barbeaud, M., Chao, M., and Parada, L. F. (1991) Science 252, 554-555.

Klein, R., Jing, S., Nanduri, V., O'Rourke, E., and Barbeaud, M. (1991) Cell 65, 665-675.

Koizumi, H., Martin-Zanca, D., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moosmaw, C., Lai, E., and Cobb, M. H. (1992a) J. Biol. Chem. 267, 13-16.

Koizumi, H., Martin-Zanca, D., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moosmaw, C., Lai, E., and Cobb, M. H. (1992b) J. Biol. Chem. 267, 13-16.

Levi-Montalcini, R. (1987) Science 237, 1114-1116.
Loeb, D. M., Maragos, J., Martin-Zanca, D., Chao, M. V., Parada, L. F., and Greene, L. A. (1991) Cell 66, 961–966
Loeb, D. M., Tsao, H., Cobb, M. H., and Greene, L. A. (1992) Neuron 9, 1053–1065
Maher, P. A. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6788–6791
Meakin, S. O., and Shooter, E. M. (1991) Neuron 6, 153–163
Meakin, S. O., and Shooter, E. M. (1992) Trends Neurosci. 15, 223–230
Meakin, S. O., Suter, U., Drinkwater, C. C., Welcher, A. A., and Shooter, E. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2374–2378
Miyasaka, T., Chao, M. V., Sherline, P., and Saltiel, A. R. (1990) J. Biol. Chem. 265, 4730–4735
Mobley, W. C., Shenker, A., and Shooter, E. M. (1972) Biochem. 15, 5543–5552
Northwood, I. C., Gonzalez, G. A., Wartmann, M., Raden, D. L., and Davis, R. J. (1991) J. Biol. Chem. 266, 15266–15276
Ohmichi, M., Decker, S. J., and Saltiel, A. R. (1991) Cell Regul. 2, 691–697
Pavne, D. M., Rossmanno, A. J., Martino, P., Erickson, A. K., Her, J., Shabanowitz, J., Hunt, D. F., Weber, M. J., and Sturgill, T. W. (1991) EMBO J. 10, 885–892
Radeke, M. J., Misko, T. P., Hau, C., Herzenberg, L. A., and Shooter, E. M. (1987) Nature 325, 593–597
Radeke, M. J., and Feinstein, S. (1991) Neuron 7, 141–150
Ray, B. L., and Sturgill, T. W. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1502–1506
Ray, B. L., and Sturgill, T. W. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3753–3757
Ross, A. H. (1991) Cell Regul. 2, 685–690
Rossmanno, A. J., Payne, D. M., Weber, M. J., and Sturgill, T. W. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6940–6943
Sanghera, J. S., Aebersold, R., Morrison, H. D., Bures, E. J., and Pelech, S. L. (1990) FEBS Lett. 273, 223–226
Tanisichi, M., Johnson, E. M., Jr., Roach, P. J., and Lawrence, J. C., Jr. (1986) J. Biol. Chem. 261, 13342–13349
Towbin, H., Staehlin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
Tsao, H., and Greene, L. A. (1991) J. Biol. Chem. 266, 12981–12988
Tsao, H., Aletta, J. M., and Greene, L. A. (1990) J. Biol. Chem. 265, 15471–15490
Ullrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
Vaillette, A., Bookman, M. A., Horak, E. M., and Bolen, J. B. (1988) Cell 55, 301–308
Vetter, M. L., Martin-Zanca, D., Parada, L. F., Bishop, J. M., and Kaplan, D. R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5650–5654
Volontè, C., and Greene, L. A. (1989) J. Biol. Chem. 264, 11050–11055
Volontè, C., and Greene, L. A. (1992a) J. Neurochem. 58, 700–708
Volontè, C., and Greene, L. A. (1992b) J. Biol. Chem. 267, 21665–21670
Volontè, C., Rokenstein, A., Loeb, D. M., and L. A. Greene. (1989) J. Cell Biol. 109, 2396–2403
Volontè, C., Ross, A. H., and Greene, L. A. (1993a) Mol. Biol. Cell 4, 71–78
Volontè, C., Loeb, D. M., and Greene, L. A. (1993b) J. Neurochem., in press
Yan, H., Schlessinger, J., and Chao, M. V. (1991) Science 252, 561–564