Nerve Growth Factor Stimulates a Protein Kinase in PC-12 Cells That Phosphorylates Microtubule-associated Protein-2*

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Some of the effects of nerve growth factor (NGF) may be mediated by changes in protein phosphorylation. We have identified a protein kinase from PC-12 cells that catalyzes the phosphorylation of pig brain microtubule-associated protein (MAP)-2 in vitro. This activity is stimulated 2- to 4-fold in extracts from cells treated with NGF or epidermal growth factor (EGF). The partial purification and characterization of this MAP kinase indicate that it is distinct from previously described NGF-stimulated protein kinases. The NGF-stimulated kinase activity is unaffected by direct addition to the assay of the heat-stable cAMP-dependent kinase peptide inhibitor, staurosporine, or K-252A, slightly stimulated by heparin and is inhibited by sodium fluoride and calcium ions. Treatment of cells with NGF increases the activity of the kinase within 2 min. The activity declines after 10 min, and a second phase of activation is observed at 20-30 min. Comparison of its behavior on gel permeation and sucrose density gradients indicates a molecular mass in the range of 40,000-60,000 daltons. The kinase activity is kinase A with a K_m of 12 μM. Although the pathway of activation of MAP kinase by NGF is unknown, the stimulation can be reversed by treatment of the enzyme with alkaline phosphatase, suggesting that activation involves phosphorylation of the kinase itself. The properties and hormone sensitivity of the PC-12 MAP kinase suggest that it is similar to the previously identified, growth factor-sensitive MAP kinase from 3T3-L1 adipocytes.

Although the molecular mechanisms involved in the action of nerve growth factor (NGF) remain poorly understood, numerous reports indicate a possible role for changes in protein phosphorylation. Enhanced phosphorylation of a number of proteins have been observed in the pheochromocytoma cell line PC-12 in response to NGF, including ribosomal S6 protein (1), tyrosine hydroxylase (2,3), microtubule-associated protein-2 (4-6) and others (7-11). Although the molecular events involved in coupling the NGF receptor to its behavior on gel permeation and sucrose density gradients indicates a molecular mass in the range of 40,000-60,000 daltons. The kinase activity is kinase A with a K_m of 12 μM. Although the pathway of activation of MAP kinase by NGF is unknown, the stimulation can be reversed by treatment of the enzyme with alkaline phosphatase, suggesting that activation involves phosphorylation of the kinase itself. The properties and hormone sensitivity of the PC-12 MAP kinase suggest that it is similar to the previously identified, growth factor-sensitive MAP kinase from 3T3-L1 adipocytes.

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‡The abbreviations used are: NGF, nerve growth factor; EGF, epidermal growth factor; MAP-2, microtubule-associated protein-2; EGTA, ethylenediaminetetraacetic acid; HPLC, high performance liquid chromatography; SDS, sodium dodecyl sulfate.

Experimental Procedures

Materials—All reagents were from Sigma except for tissue culture supplies (GIBCO), 2.5 S NGF (Bioproducts for Science, Indianapolis, IN), and Walsh peptide (generous gift of Dr. Angus Nairn), [γ-32P]ATP (3000 Ci/ mmol) (Amersham Corp.), staurosporine, H7, and K252A (Kamiya Biochemical). The Mono Q HR 5/5 and Superose 12 fast protein liquid chromatography column were from Pharmacia LKB Biotechnology, Inc.

Cell Culture and Preparation of Cell-free Extract—PC-12 cells were adapted to grow on plastic 100-mm tissue culture dishes in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 5% horse serum. Prior to hormonal treatment, the medium was replaced with serum-free medium and incubated for 1 h. Unless otherwise indicated, 4 mM NGF was directly added to the medium and the incubation was continued for the indicated time (2-120 min) at 37°C. After hormonal treatment, the medium was removed, and the cell layer was quickly washed three times with 10 ml of ice-cold Ca2+-free phosphate-buffered saline. Cells were collected by centrifugation at 12,000 × g for 10 min in 200 μl of lysosomal buffer (10 mM Tris, pH 7.4, containing 150 mM NaCl, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 0.1 mM Na3VO4, 2 mM dithiothreitol, 1% Triton X-100, 10 μg/ml leupeptin, and 10 μg/ml aprotinin). The cells were vortexed briefly, kept on ice for 20 min, and centrifuged at 12,000 × g for 5 min. The supernatant was assayed for MAP kinase activity.

Kinase Assays—MAP-2 was purified from pig brain by the method of Vallee (20). 20-μl aliquots of the sample were incubated with 0.2 mg/ml of MAP-2 at 30°C for 10 min in a final volume of 50 μl containing 50 mM Tris, pH 7.4, 2 mM EGTA, 10 mM MgCl2, and 40 μM [γ-32P]ATP (10 μCi). The reaction was stopped by the addition of 10 μl of 5 × Laemmill SDS sample buffer (19) and boiled for 5 min. Phosphorylated MAP-2 was resolved by SDS gel electrophoresis on 7.5% polyacrylamide gels (19). Gels were stained with Coomassie Brilliant Blue R-250. The stained bands containing phospho-MAP-2 were excised from the gels, and incorporated radioactivity was measured by Cerenkov counting. Units of activity are defined as picomoles of 32P incorporated into MAP-2/min. In all assays, the concentration of MAP-2 exceeded that of the kinase.

Partial Purification of the MAP-2 Kinase—Extracts were prepared...
from four 100-mm tissue culture dishes as described above and filtered through a 0.22-μm Millipore filter. An HR 5/5 Mono Q column was equilibrated with buffer A (25 mM Tris, pH 7.4, 2 mM EGTA, 2 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride). Samples were applied at a flow rate of 1 ml/min, washed with 3 ml of buffer A, and eluted with a 30-ml linear gradient of 0-0.5 M NaCl in buffer A. Fractions were immediately assayed for MAP kinase activity. Gel filtration chromatography was carried out using a Superose 12 fast protein liquid chromatography column. The column was equilibrated with buffer A containing 10% glycerol. Cells (5 × 100-mm dishes) were homogenized in 100 μl/plate of the lysis buffer. The resulting protein liquid chromatography column. The column was equilibrated filtration chromatography was carried out using a Superose 12 fast protein liquid chromatography column. The column was equilibrated with buffer A containing 10% glycerol. Cells (5 × 100-mm dishes) were homogenized in 100 μl/plate of the lysis buffer. The resulting supernatants were filtered and 200 μl was applied to the column at a flow rate of 0.5 ml/min.

Sucrose Density Gradient Analysis—Peak fractions from Mono Q chromatography were concentrated by immersible-CX ultrafiltration (Millipore). Aliquots (100 μl) of concentrated fractions were subjected to sedimentation equilibrium analysis in 5-20% sucrose gradients (4 ml) in 25 mM Tris-HCl, pH 7.4, 2 mM dithiothreitol, 2 mM EGTA, and 0.25 M NaCl. After centrifugation at 55,000 rpm for 16 h in an SW 60 rotor, 6 drop fractions were collected and assayed for MAP kinase activity. The migration of bovine serum albumin (M, 66,000) and carbonic anhydrase (M, 29,000) were determined by analysis of parallel gradients.

RESULTS

Identification of an NGF-stimulated MAP Kinase—NGF modulates the phosphorylation state of a number of proteins in PC-12 cells, including certain microtubule associated proteins (5, 6). Such changes in phosphorylation are also observed with epidermal growth factor (EGF) (6). To determine whether some of these changes in protein phosphorylation might be due to the acute activation of a protein kinase, PC-12 cells were treated with or without NGF or EGF. Following exposure to the growth factors, cell lysates were assayed for the activity of a kinase that phosphorylates MAP-2. The incorporation of 32P into MAP-2 was evaluated by SDS-polyacrylamide gel electrophoresis, followed by autoradiography (Fig. 1). Lysates derived from NGF-treated cells consistently exhibited a 2-4-fold increase in this kinase activity compared to control cells (Fig. 1, lanes 1 and 2). Lysates derived from EGF-treated cells exhibited a 1.5-2.5-fold enhanced activity (Fig. 1, lane 3).

Certain activities of NGF can be attenuated by drugs that inhibit protein kinases. K-252A was reported to block the activity of both NGF and EGF-treated cells, but had no effect when added alone (Fig. 1, lanes 6 and 7). To characterize the NGF-stimulated MAP kinase activity in PC-12 cells, the effects of various agents known to modulate protein kinases were added directly to the assay medium (Table I). The cAMP-dependent protein kinase inhibitor peptide (Walsh peptide) had no effect on kinase activity, suggesting that this activity was not due to the cAMP-dependent kinase. Similarly, direct addition of 100 nM staurosporine or 100 nM K-252A did not effect MAP kinase activity in these lysates. These data, in addition to the lack of requirement of the enzyme activity for Ca2+ ions, indicated that this activity was not due to protein kinase C or calmodulin-sensitive kinase. The addition of 500 nM heparin produced a 58% activation of the enzyme activity, indicating that MAP phosphorylation is not due to casein kinase, which is inhibited by low concentration of heparin (24). Finally, the NGF-stimulated activity was reduced to basal levels by the addition of 50 mM NaF. The susceptibility of this NGF-stimulated MAP kinase to these reagents indicates that this activity is similar to that described for the insulin-sensitive enzyme derived from 3T3-L1 adipocytes (17).

Characterization of MAP Kinase Activation by NGF—The concentration dependence of activation of the PC-12 cell MAP kinase by NGF was evaluated. This stimulation was dependent on the concentration of NGF applied to the cells (Fig. 2). The half-maximal concentration for stimulation of the kinase (EC50) by NGF was 0.3 nM, and the effect was maximal at 1 nM. The EC50 for this response is similar to the equilibrium dissociation constant of 0.2 nM, determined by steady state binding analysis at 37 °C (25), suggesting that NGF-receptor interaction is closely linked to MAP kinase activation.

TABLE I

Effects of direct addition of various protein kinase modulators on the activity of NGF-stimulated MAP kinase

<table>
<thead>
<tr>
<th>NGF</th>
<th>Addition</th>
<th>Activity</th>
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<tbody>
<tr>
<td></td>
<td>None</td>
<td>3.50 ± 0.31 (100)</td>
</tr>
<tr>
<td></td>
<td>Heparin, 500 nM</td>
<td>5.54 ± 0.02 (158)</td>
</tr>
<tr>
<td></td>
<td>Staurosporine, 100 nM</td>
<td>3.53 ± 0.11 (101)</td>
</tr>
<tr>
<td></td>
<td>K-252A, 100 nM</td>
<td>3.54 ± 0.02 (101)</td>
</tr>
<tr>
<td></td>
<td>Walsh peptide, 7.4 μM</td>
<td>3.14 ± 0.08 (90)</td>
</tr>
<tr>
<td></td>
<td>NaF, 60 μM</td>
<td>1.13 ± 0.05 (32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00 ± 0.05 (29)</td>
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</tbody>
</table>
NGF Stimulates MAP Kinase

The time course of stimulation of the MAP kinase activity by NGF was explored (Fig. 3). Exposure of cells to NGF resulted in two phases of kinase activation. MAP kinase activity was rapidly increased by NGF, reaching a maximum at 5–10 min, and declined by 20–25 min. This was followed by a second phase of activation at 30 min. Addition to PC-12 cells of the tumor promoting protein kinase C activator phorbol myristate acetate potentiated the second phase of MAP kinase activation, although the basal activity and initial phase of activation remained unchanged. The enzyme activity detected in the second phase of stimulation with NGF alone or NGF plus phorbol myristate acetate exhibited biochemical characteristics and chromatographic behavior identical to the activity detected after 5-min exposure to NGF. Interestingly, the kinetics of MAP kinase activation are similar to the time course observed by Thomas and co-workers (26) for the activation of S6 kinase by EGF in Swiss mouse 3T3 cells. These investigators proposed that the late phase of EGF-induced activation of this kinase might be mediated by protein kinase C.

To determine whether protein kinase C might mediate only the slower activation of MAP kinase by NGF, we examined the effect of various protein kinase C inhibitors on both early and late phase activation. PC-12 cells were preincubated with the protein kinase C inhibitors staurosporine, K-252A, and H7 10 min prior to NGF addition for 5 or 30 min (Table II). As described above, staurosporine inhibited the early phase activation by NGF. Interestingly, the protein kinase inhibitor K-252A had a stimulatory effect on the early phase activation. This effect was observed up to 400 nM K-252A. Neither staurosporine nor K-252A significantly inhibited the late phase activation. The protein kinase inhibitor H7 slightly potentiated both the early and late phase activation. The observation that only staurosporine blocks the activation of MAP kinase by NGF suggests that this activation may require a specific protein kinase that is insensitive to other inhibitors, although it is possible that the specific attenuation of NGF and not EGF stimulation might reflect a mechanism that does not involve a kinase. However, comparison of the effects of the various protein kinase inhibitors provides no evidence for a role of protein kinase C in mediating either the rapid or slower activation of MAP kinase by NGF.

Chromatographic Properties of the NGF-stimulated MAP Kinase—MAP kinase activities residing in lysates from untreated and NGF-treated cells were chromatographed on an HPLC anion exchange column (Fig. 4). The NGF-stimulated MAP kinase activity was retained on a Mono Q column, as detailed under “Experimental Procedures.” 1-ml fractions were collected and assayed for MAP kinase activity.

Physicochemical Properties of the NGF-stimulated MAP Kinase—The apparent molecular weight of the NGF-stimulated MAP kinase was determined by gel filtration chromatography and sucrose density gradient sedimentation. The...
cell-free lysate derived from untreated and NGF-treated PC-12 cells was applied to a Superose 12 fast-protein liquid chromatography column (Fig. 5). MAP kinase activity was detected primarily in a peak eluting just prior to carbonic anhydrase. As described for chromatography on Mono Q, MAP kinase activity was increased 3-4-fold in cells treated with NGF. A Stokes radius of 31 Å was calculated by comparison of the elution profile with standards. Activity that had been partially purified by Mono Q chromatography exhibited identical elution behavior on Superose 12 (not shown).

Peak fractions of the NGF-stimulated MAP kinase activity purified by Mono Q chromatography were concentrated and applied to a 5-20% sucrose density gradient. Gradients were subjected to equilibrium sedimentation, and MAP kinase activity was assayed in collected fractions (Fig. 6). A sedimentation coefficient of 3.6 S was calculated by comparison with standards. Analysis of data from Superose 12 gel filtration and density gradient sedimentation equilibrium yielded an apparent molecular weight of 41,000 (sucrose gradient) or 36,000 (gel filtration). The frictional rates of the protein were calculated to be 1.16, suggesting that MAP kinase is a globular protein that is relatively symmetrical.

**Kinetic Characterization of the NGF-stimulated MAP Kinase**—The time course of MAP kinase activity was evaluated. After purification on Mono Q, the activity of the enzyme was assayed as a function of time. Activity was linear throughout 15 min (data not shown). The dependence of the partially purified MAP kinase on substrate concentration was evaluated (Fig. 7). In assays using MAP-2 (0.2 mg/ml) as substrate in the presence of 10 mM MgCl₂, ATP was the preferred phosphate donor. GTP (up to 0.2 mM) did not reduce ³²P labeling of substrates when added to the standard assay containing 40 μM [γ-³²P]ATP, suggesting that it was a poor phosphorlyl donor for this enzyme (not shown). Evaluation of the dependence of the NGF-stimulated MAP kinase on ATP concentration revealed that the Kₘ and Vₘₐₓ for ATP were 12 μM and 2.9 pmol/min, respectively, under these conditions.

**Effect of Divalent Cations on MAP Kinase Activity**—The effect of Mg²⁺, Mn²⁺, Ca²⁺, and Zn²⁺ on the kinase activity was evaluated (Table III). Both Mg²⁺ and Mn²⁺ supported MAP kinase activity, although Mg²⁺ was the preferred cofactor. In the presence of MgCl₂, addition of 10 mM CaCl₂ to the incubation caused a 60% inhibition of the enzyme activity. ZnCl₂ caused a marked reduction in MAP kinase activity, possibly due to the Zn²⁺-catalyzed reduction of an essential sulfhydryl.

**MAP Kinase Stimulation by NGF Is Reversed by Exposure to Alkaline Phosphatase**—To determine whether protein phosphorylation plays a role in the activation of MAP kinase, extracts from NGF-treated cells were treated with bovine intestinal alkaline phosphatase for 60 min. Following this treatment, control and alkaline phosphatase-treated extracts were chromatographed on Mono Q to separate the phosphatase from the kinase (Fig. 8). Alkaline phosphatase caused a substantial reduction in the MAP kinase activity eluting at 21 min. Interestingly, some of the residual activity eluted earlier from the column, consistent with the loss of a negatively charged group. To insure that the inhibition of MAP kinase activity was due to the direct action of alkaline phosphatase, a preparation of NGF-stimulated MAP kinase was compared for phosphatase activity with and without alkaline phosphatase treatment (not shown).

**Table III**

<table>
<thead>
<tr>
<th>Addition, concentration</th>
<th>MAP-2 phosphorylation</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂, 5 mM</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>MgCl₂, 10 mM</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MnCl₂, 5 mM</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>MnCl₂, 10 mM</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>MgCl₂, 10 mM, CaCl₂, 5 mM</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>MgCl₂, 10 mM, CaCl₂, 10 mM</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>MgCl₂, 10 mM, ZnSO₄, 5 mM</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

**Fig. 5.** Gel filtration chromatography of MAP kinase. Four plates of PC-12 cells were treated with (•) or without (■) 4 nM NGF for 5 min. Cell lysates (5 mg protein/ml) were prepared and chromatographed on Superose 12 as detailed under "Experimental Procedures." 0.5-ml fractions were collected and assayed for MAP kinase activity. Stokes radius was determined by comparison with standards, chymotrypsin (19 Å) and bovine serum albumin (36 Å), according to Siegel and Monty (39). BSA, bovine serum albumin; CA, carbonic anhydrase.

**Fig. 6.** Sucrose density gradient centrifugation of MAP kinase. MAP kinase derived from NGF-treated PC-12 cells was chromatographed in Mono Q HPLC. Peak fractions were concentrated and subject to density gradient centrifugation on a 5-20% sucrose gradient. Six drop fractions were collected from the bottom of the tube and assayed for MAP kinase activity as described under "Experimental Procedures." The sedimentation coefficient of the enzyme was determined by comparison with standards, carbonic anhydrase (CA) (3.2 S), and bovine serum albumin (BSA) (4.3 S), according to Siegel and Monty (39).

**Fig. 7.** Dependence of MAP kinase activity on ATP concentration. MAP kinase derived from NGF-treated cells was purified on Mono Q HPLC. Peak fractions were pooled, and activity was assayed in the presence of increasing concentrations of ATP.
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purified by HPLC and then treated with or without intestinal alkaline phosphatase. As observed in lysates, alkaline phosphatase caused greater than 80% decrease in the activity of the enzyme (not shown). These data suggest that phosphorylation of the enzyme itself may contribute to its catalytic activity, although it remains possible that contaminating proteins are present that inactivate MAP kinase upon addition of alkaline phosphatase.

DISCUSSION

A number of serine protein kinases have been reported to be activated by NGF or EGF in PC-12 cells, including kinase N (9), CAM-dependent kinase (27, 28), S6 kinase (1), and protein kinase C (7, 8). The unique kinetic and physicochemical properties of the NGF-sensitive MAP kinase described here, in addition to its susceptibility to various agents, indicate that it is distinct from previously identified NGF-sensitive kinases. The MAP kinase differed from kinase N in molecular weight, suitability of GTP as substrate, preference for Mg$^{2+}$ as cofactor and chromatographic behavior (9). The MAP kinase also does not appear to be protein kinase C, since it eluted from Mono Q at a higher salt concentration (0.25 M vs. 0.16 M NaCl) (26) and was not inhibited by direct addition of kinase C inhibitors to the assay. Moreover, the inhibition of MAP kinase by calcium rules out kinase C, as well as other Ca$^{2+}$-dependent kinases. The CAM-dependent protein kinase is ruled out by lack of sensitivity to the Walsh peptide (29). Additionally, the MAP kinase differs from both casein kinase and ribosomal S6 kinase on the basis of its chromatographic behavior and sensitivity to heparin (24, 30). Recently, activation of a MAP-2/p70 kinase which phosphorylates MAP-2 in vitro has been described in NGF-treated PC-12 cells (31). This activation was inhibited by K-252A, and this enzyme activity was insensitive to NaF and exhibited an apparent $M_r$ of 100,000. The properties of the NGF-sensitive protein kinase described here, including chromatographic behavior, estimated molecular weight, inhibition by NaF, activation by heparin, and failure of GTP to competitively inhibit the phosphotransferase reaction indicate that it is indistinguishable from the previously identified insulin-stimulated MAP kinase from 3T3-L1 cells (17).

Although the mode of regulation of the insulin-stimulated MAP kinase remains unknown, a mechanism of activation involving phosphorylation has been proposed (14). A growth factor-sensitive phosphotyrosine containing protein was identified that coelutes with the hormone-sensitive MAP kinase activity (17). It has been suggested that MAP kinase is itself a substrate for tyrosine phosphorylation by the growth factor receptor or nonreceptor kinases (18), causing stimulation of its catalytic activity. It is possible that a similar activation scheme is responsible for modulation of the enzyme by NGF. The selective ability of staurosporine to block activation of the enzyme by NGF suggests the involvement of an intermediate protein kinase. Although this putative intermediate kinase is unlikely to be kinase C, since K-252A and H7 were ineffective in blocking the activation of the kinase by NGF, the ability of staurosporine to inhibit tyrosine kinases suggests a possible role for this class of enzyme (32). Interestingly, treatment of the activated MAP kinase (assayed in the lysate or after purification) with alkaline phosphatase dramatically reduced its activity. An NGF-dependent phosphoprotein of $M_r$ 42,000 was identified on SDS-polyacrylamide gel electrophoresis that coeluted with MAP kinase activity on Mono Q and Superose 12 columns. The kinetics of pp42 phosphorylation and activation of MAP kinase in response to both NGF and EGF were similar. It is premature to suggest, however, that the NGF-stimulated MAP kinase is activated by tyrosine phosphorylation. Thus far, we have been unable to adsorb the NGF-stimulated kinase activity to an immobilized anti-phosphotyrosine antibody, nor have we been able to clear enzyme activity by immunoprecipitation with such an antibody.

A number of functional similarities exist between the biological actions of insulin and NGF in PC-12 cells, including maintenance of neuronal viability (33), induction of neurite formation (34), regulation of acidic amino acid, and glucose uptake (35), c-fos induction (36) and stimulation of glycosyl-phosphatidylinositol hydrolysis (32). However, significant differences exist in the basic structures of the NGF and insulin receptors. The insulin receptor has an intrinsic tyrosine kinase activity that is thought to be necessary for receptor function. On the other hand, cDNA cloning of the NGF receptor indicated no evidence for a tyrosine kinase domain in the cytoplasmic region (37), although tyrosine phosphorylation in PC-12 cells has been detected in response to NGF (12). One interesting possibility is that the NGF receptor is noncovalently coupled to a nonreceptor tyrosine kinase that might serve to phosphorylate and activate MAP kinase and other cellular proteins.

The precise role of MAP kinase in NGF action is unknown. The enzyme was similarly activated by both NGF and EGF in these cells. Interestingly, EGF elicits many of the early responses induced by NGF, but does not cause differentiation of PC-12 cells, suggesting perhaps that MAP kinase plays a role in only some of the actions of NGF. Studies in PC-12 cells indicate that MAP-2 undergoes phosphorylation in response to NGF, perhaps leading to changes in cytoskeletal function (38). However, it should be emphasized that the physiological substrates of MAP kinase remain unknown. Sturgill et al. (14) have proposed a role for the insulin-stimulated MAP-2 kinase in the phosphorylation and activation of ribosomal protein S6 kinase II from Xenopus oocytes. The phosphorylation of ribosomal S6 protein is stimulated by a number of growth factors, including NGF. Interestingly, the time course of this response to NGF lags behind that of MAP kinase activation (1). Thus, the rapidity of activation of MAP kinase by NGF indicates that it may be an intermediate in the regulation of a number of proteins involved in the cellular effects of the hormone. The mode of activation of MAP kinase by NGF and other hormones and

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**Fig. 8.** Alkaline phosphatase reduces MAP kinase activity. Four plates of PC-12 cells were treated with 4 nM NGF for 5 min. Cell-free lysates were prepared and treated with (+) or without (□) 62.5 units of bovine intestinal alkaline phosphatase for 90 min. Incubations were stopped by injection of lysates onto a Mono Q column. Fractions were collected and assayed for MAP kinase activity. Intestinal alkaline phosphatase elutes in fraction 14 on this column, clearly resolved from the MAP kinase activity. In separate experiments, alkaline phosphatase similarly inhibited partially purified MAP kinase.

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$^a$T. Miyasaka, M. V. Chao, P. Sherline, and A. R. Saltiel, unpublished results.
the identification of its physiological substrates are under investigation.

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Nerve growth factor stimulates a protein kinase in PC-12 cells that phosphorylates microtubule-associated protein-2.

T Miyasaka, M V Chao, P Sherline and A R Saltiel