Communication

The Tyrosine-phosphorylated Hepatocyte Growth Factor/Scatter Factor Receptor Associates with Phosphatidylinositol 3-Kinase*

Andrea Graziani‡, Daniela Gramaglia‡, Lewis C. Cantley§, and Paolo M. Comoglio‡¶

From the ‡Department of Biomedical Sciences and Oncology, University of Torino Medical School, 10126 Torino, Italy and the §Department of Physiology, Tufts University Medical School, Boston, Massachusetts 02111

The receptor for hepatocyte growth factor, also known as scatter factor (HGF/SF), has recently been identified as the 190-kDa heterodimeric tyrosine kinase encoded by the MET proto-oncogene (p190^{MET}). The signaling pathway(s) triggered by HGF/SF are unknown. In A549 cells, a lung epithelial cell line, nanomolar concentrations of HGF/SF induced tyrosine phosphorylation of the p190^{MET} receptor. The autophosphorylated receptor coprecipitated with phosphatidylinositol 3-kinase (PI 3-kinase) activity. In GTL16 cells, a cell line derived from a gastric carcinoma, the p190^{MET} receptor, overexpressed and constitutively phosphorylated on tyrosine, coprecipitated with PI 3-kinase activity and with the 85-kDa PI 3-kinase subunit. In these cells activation of protein kinase C or the increase of intracellular [Ca^{2+}] inhibits tyrosine phosphorylation of the p190^{MET} receptor as well as the association with both PI 3-kinase activity and the 85-kDa subunit of the enzyme. In an in vitro assay, tyrosine phosphorylation of the immobilized p190^{MET} receptor was required for binding of PI 3-kinase from cell lysates. These data strongly suggest that the signaling pathway activated by the HGF/SF receptor includes generation of D-3-phosphorylated inositol phospholipids.

Hepatocyte growth factor was isolated as a powerful mitogen for hepatocytes and other epithelial cells (1–3). Scatter factor was independently isolated as a chemotactic factor promoting epithelial cell motility and tumor invasion (4, 5). Recent work has shown that the two factors are identical proteins encoded by the same gene (6, 7). The different biological effects of hepatocyte growth factor/scatter factor (HGF/SF)[1] are triggered by binding to the same receptor, the protein encoded by the MET proto-oncogene (7). The Met protein had already been identified as the receptor for hepatocyte growth factor (8, 9).

The MET-encoded receptor is a 190-kDa heterodimeric transmembrane protein (p190^{MET}) made of a 50-kDa α subunit disulfide-linked to a 145-kDa β subunit (p145$^{\beta}$) (10, 11). The α subunit and the NH-terminal portion of p145$^{\beta}$ are exposed at the cell surface (12). The carboxyl-terminal portion is cytoplasmic and includes a tyrosine kinase domain (13–15), and phosphorylation sites are involved in the regulation of its activity (16). The kinase activity is positively regulated by autophosphorylation on tyrosine (17), and it is negatively regulated by protein kinase C activation (18) or a transient increase of intracellular Ca^{2+} concentration (19). Stimulation of p145$^{\beta}$ subunit tyrosine phosphorylation after exposure to HGF/SF was observed both in intact cells (7–9) and in vitro with partially purified Met protein (9).

The signaling pathway(s) triggered by HGF/SF have not yet been investigated. Similarly, the substrates of the p190^{MET} receptor kinase are unknown. In this work we show that the tyrosine-phosphorylated form of p190^{MET} receptor associates with phosphatidylinositol 3-kinase, a key enzyme involved in the transduction of signals generated by tyrosine kinase receptors (20, 21).

EXPERIMENTAL PROCEDURES

Reagents, Cell Lines, and Antibodies—All reagents, unless specified, were purchased from Sigma. Protein A covalently coupled to Sepharose was purchased from Pharmacia LKB Biotechnology Inc. Reagents for SDS-PAGE were from Bio-Rad. ^{14}C-Methylated molecular mass standards were from Amersham Corp.

A549 lung carcinoma cells were obtained from American Type Cell Catalogue. GTL16 is a clonal cell line derived from a poorly differentiated gastric carcinoma (12). Cells were cultured in RPMI 1640 medium (Seromed) containing 10% fetal calf serum (Seromed) and maintained at 37 °C in a humidified atmosphere with 5% CO$_2$.

HGF/SF was purified from the supernatant of MRC5 human fibroblasts by heparin-Sepharose column chromatography (Pharmacia) eluted by a linear gradient of 0.5–1.8 M NaCl as described (5).

Phosphotyrosine antibodies were raised as previously described (22). Monoclonal antibodies against the anti-extracellular domain of the Met protein were obtained as described elsewhere (48). Polyclonal antibodies against the 85-kDa subunit of PI 3-kinase were raised in chickens immunized with a synthetic peptide as described elsewhere.[2]

Preparation of Cell Lysates, Immunoprecipitation, and Western Blotting—Subconfluent A549 cells were starved for 2 days in serum-free medium, stimulated by HGF/SF as indicated, and then lysed in buffer A (25 mM Hepes buffer, pH 8, 10% glycerol, 1% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 2 mM EGTA, 0.5 mM dithiothreitol, 0.2 mM

[1] The abbreviations used are: HGF/SF, hepatocyte growth factor/scatter factor; PI 3-kinase, phosphatidylinositol 3-kinase; PI 4-kinase, phosphatidylinositol 4-kinase; PI, phosphatidylinositol; PIP, PI(3)P, PI(4)P, phosphatidylinositol monophosphate; PIP$_2$, PI(3,4)P$_2$, PI(4,5)P$_2$, phosphatidylinositol bisphosphate; PIP$_3$, PI(3,4,5)P$_3$, phosphatidylinositol trisphosphate; PDGF, platelet-derived growth factor; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; HPLC, high pressure liquid chromatography; TPA, 12-O-tetradecanoylphorbol-13-acetate.

[2] R. Kapeller and L. Cantley, manuscript in preparation.

phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, 1 µg/ml aprotinin, and 1 µg/ml soybean trypsin inhibitors) supplemented with 0.5 mM sodium vanadate, 0.1 mM ammonium molybdate, 10 µM phenylarsine oxide, and 1 mM $ZnCl_2$. Subconfluent monolayers of GTL16 cells were serum-starved for 2 days and, unless otherwise indicated, lysed in buffer A containing 0.1 mM vanadate. Lysates were spun at 15,000 × g for 15 min, and the supernatants were immunoprecipitated after 2 h of incubation with monoclonal anti-Met antibodies cross-linked to rabbit anti-mouse Ig-Protein A-Sepharose. The immunoprecipitates were washed as previously described (23). Western blotting from both whole cell lysate or immunoprecipitates was performed as described before (17, 19).

PI 3-Kinase Assay—PI 3-kinase assay was performed directly on the beads as described by Auger *et al.* (23). Adenosine (0.2 mM) was added to the reaction mixture to inhibit residual PI(4) kinase activity (33). For definitive identification, PIP, PIP_2, and PIP_3 separated by TLC were eluted, deacylated, and the respective glycerophosphoinositol phosphate derivatives separated on HPLC by a Partisil Sax column (Whatman) as described (23, 24).

Binding of PI 3-Kinase to $p190^{MET}$ in Vitro—Confluent GTL16 cells were lysed in buffer A, and $p190^{MET}$ was purified by immunoprecipitation as described above. The Protein A-Sepharose-immobilized $p190^{MET}$ was dephosphorylated by alkaline phosphatase (Promega) treatment for 20 min at 15 °C. The immunocomplexes were washed as in the immunoprecipitation protocol. When indicated samples were rephosphorylated by incubation for 15 min at room temperature with 0.1 mM ATP, 10 mM $MnCl_2$. The immunocomplexes were extensively washed as above. A549 cells were made quiescent by 48-h serum starvation and lysed with buffer A supplemented with 0.5 mM sodium vanadate, 0.1 mM ammonium molybdate, and 1 mM $ZnCl_2$. The lysates were then incubated for 1 h at 4 °C with the immobilized $p190^{MET}$. The complexes were washed as described in the immunoprecipitation protocol and probed as indicated.

RESULTS

HGF/SF Induces Tyrosine Phosphorylation of $p190^{MET}$ Receptor and Association with PI 3-Kinase in A549 Cells— Stimulation of A549 cells for 15 min with 50 ng/ml purified HGF/SF induced tyrosine phosphorylation of the $p190^{MET}$ receptor. This was observed by probing the anti-Met immunoprecipitates with anti-phosphotyrosine antibodies. The $p145^\beta$ subunit was heavily phosphorylated on tyrosine after 15 min of stimulation with HGF/SF (Fig. 1, *A* and *B*). Notably, two additional proteins of molecular mass close to 85 and 60 kDa were also co-precipitated by anti-*MET* antibodies and were found to contain phosphotyrosine. 85 kDa is the molecular mass of the putative regulatory subunit of PI 3-kinase (26–28, 37), which was found to be phosphorylated on tyrosine when present in a complex with several tyrosine-phosphorylated proteins (reviewed in 20). PI 3-kinase activity, assayed in the presence of adenosine to inhibit residual non-specifically bound PI 4-kinase (33), was present in the immunocomplexes from HGF/SF-stimulated cells. Fig. 1C shows the TLC separation of the D-3-phosphorylated inositol lipids generated *in vitro* by incubation of the anti-Met immunocomplex in the presence of sonicated lipid substrates and [γ-^{32}P] ATP. The identities of PIP, PIP_2, and PIP_3 generated *in vitro* were confirmed by HPLC analysis of the deacylated derivatives to be PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3 (not shown). No PI 3-kinase activity was detectable in immunocomplexes precipitated from unstimulated cells.

Tyrosine Phosphorylation of $p190^{MET}$ Receptor Regulates the Physical Association with PI 3-Kinase in Intact GTL16 Cells— In GTL16 cells, due to the amplification of the *MET* gene, the $p190^{MET}$ receptor is overexpressed and constitutively phosphorylated on tyrosine (10). Moreover, we have previously shown that in these cells it is possible to negatively regulate the kinase activity of $p190^{MET}$ by activation of protein kinase C or by increasing the intracellular Ca^{2+} concentration (18, 19). We therefore took advantage of this model system to further investigate if tyrosine phosphorylation of $p190^{MET}$
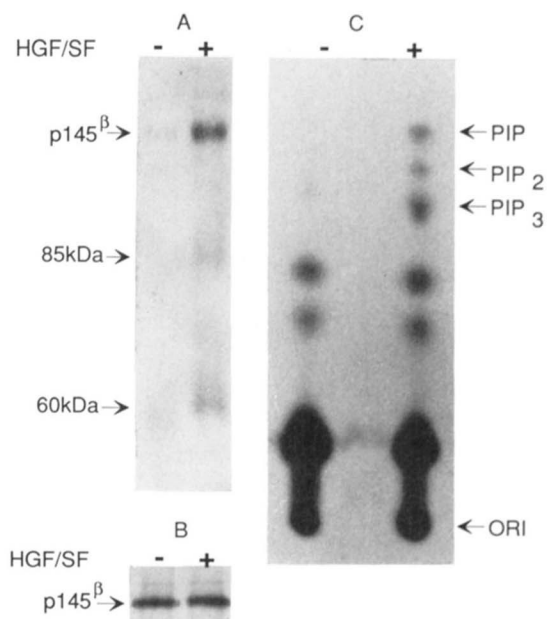

FIG. 1. **HGF/SF triggers tyrosine phosphorylation of the $p145^\beta$ receptor subunit and promotes its association with PI 3-kinase.** Control (−) or HGF/SF-stimulated (+) A549 cells were lysed and immunoprecipitated with anti-Met antibodies. Proteins in the immunocomplexes were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-phosphotyrosine antibodies (*panel A*) or anti-Met antibodies (*panel B*). The immunocomplexes were assayed for PI 3-kinase activity in the presence of a mixture of PI, PI(4)P, PI(4,5)P_2, phosphatidylserine, and [τ-^{32}P]ATP. *Panel C* shows the autoradiogram of a thin layer chromatography separation of the reaction products extracted by chloroform/methanol. The migration of PIP, PIP_2, and PIP_3 is indicated by *arrows*. The radioactivity at the origin (*ORI*) is due to [τ-^{32}P]ATP and other water-soluble ^{32}P-labeled materials that were not completely extracted from the chloroform layer.
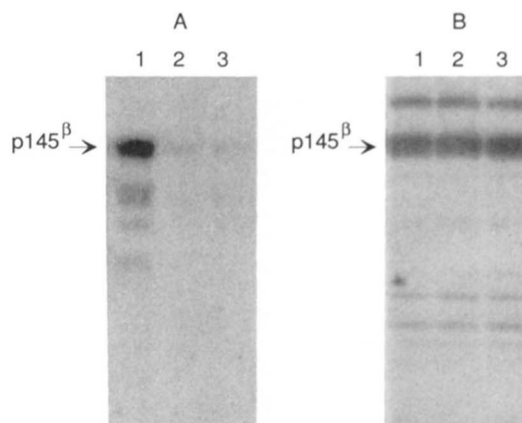

FIG. 2. **Tyrosine phosphorylation of the HGF/SF receptor $p145^\beta$ subunit is negatively regulated by protein kinase C activation and increase of intracellular (Ca^{2+}).** Proteins solubilized from GTL16 cells were separated in SDS-PAGE transferred to nitrocellulose and probed with anti-phosphotyrosine antibodies (*panel A*) or anti-Met antibodies (*panel B*). *Lane 1*, control cells; *lane 2*, cells treated with 160 nM TPA for 1 h; *lane 3*, cells treated with the 10 µM Ca^{2+} ionophore A23187 for 5 min.

regulates its association with PI 3-kinase. Subconfluent cultures of GTL16 cells were starved for 3 days in serum-free medium and treated either with TPA (160 nM for 1 h) or with the Ca^{2+} ionophore A23187 (10 µM for 5 min). $p190^{MET}$ tyrosine phosphorylation was assessed by probing the blotted whole cell lysates with anti-phosphotyrosine and with anti-
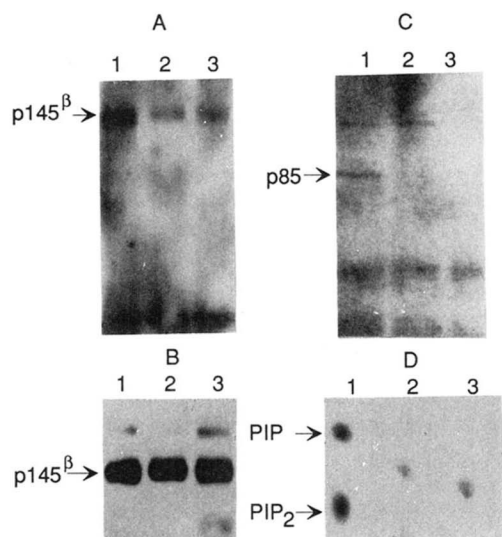
FIG. 3. **Tyrosine phosphorylation of the HGF/SF receptor p145$^\beta$ subunit regulates association with PI 3-kinase in intact cells.** Proteins were solubilized from GTL16 cells treated as indicated below and immunoprecipitated by anti-Met antibodies. The immunoprecipitates were separated in SDS-PAGE, blotted to nitrocellulose paper, and probed with anti-phosphotyrosine antibodies (*panel A*), anti-Met antibodies (*panel B*), or with an antiserum raised against a synthetic peptide derived from the sequence of the 85-kDa subunit of PI 3-kinase (*panel C*). The immunocomplexes were also assayed for PI 3-kinase activity as described, except PI(4,5)P$_2$ was not used as a substrate. (*panel D*). *Lane 1*, control cells; *lane 2*, cells treated with 160 nM TPA for 1 h; *lane 3*, cells treated with the 10 μM Ca^{2+} ionophore A23187 for 5 min. The unequal chromatographic separation of radiolabeled species in *lanes 2* and *3* resulted from the solvent front running unevenly. The 85-kDa subunit and the PI 3-kinase activity are associated with the phosphorylated but not with the unphosphorylated p145$^\beta$ subunit.

Met antibodies (Fig. 2). The p190^{MET} receptor was then immunoprecipitated from control and TPA or A23187 Ca^{2+} ionophore-treated cells; immunoprecipitates were blotted and probed with either anti-phosphotyrosine or anti-Met antibodies. The amount of p190^{MET} tyrosine phosphorylated in the immunoprecipitates from untreated cells was severalfold higher (Fig. 3, *A* and *B*). The PI 3-kinase activity, measured as D-3-phosphoinositides generated *in vitro*, co-precipitated with tyrosine phosphorylated p190^{MET} from control cells but could not be detected in the immunocomplexes from TPA and Ca^{2+} ionophore-treated cells (Fig. 3D).

To demonstrate the physical association of the 85-kDa subunit of PI 3-kinase with tyrosine-phosphorylated p190^{MET}, immunoprecipitates from GTL16 cells, either control or treated with TPA or the Ca^{2+} ionophore, were blotted and probed with chicken antibodies directed against the 85-kDa putative regulatory subunit of PI 3-kinase (p85). The 85-kDa subunit of PI 3-kinase was indeed found to associate only with the tyrosine-phosphorylated form of p190^{MET} (Fig. 3C).

Tyrosine Phosphorylation of p190^{MET} Receptor Is Required for Binding of PI 3-Kinase in Vitro—In order to formally prove that p190^{MET} tyrosine phosphorylation is a switch for association of PI 3-kinase, we developed an *in vitro* association assay. Sepharose-immobilized p190^{MET} receptors were dephosphorylated by alkaline phosphatase treatment and extensively washed to remove any associated PI 3-kinase activity. In half of the samples the receptors were re-phosphorylated on tyrosine as described under "Experimental Procedures." The unphosphorylated and the re-phosphorylated immobilized receptors were incubated for 1 h at 4 °C with a lysate prepared from A549 cells or with the lysis buffer alone. The
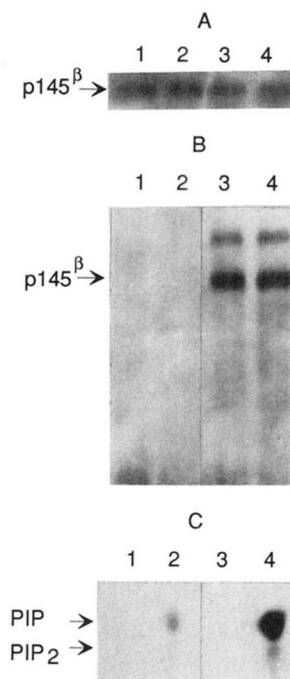

FIG. 4. ***In vitro* association of PI 3-kinase requires tyrosine phosphorylation of the HGF/SF receptor p145$^\beta$ subunit.** p190^{MET} HGF/SF receptor was immunopurified, immobilized to protein-A Sepharose beads, and dephosphorylated. Part of the samples were left as controls (*lanes 1* and *2*); part were re-phosphorylated on tyrosine *in vitro* (*lanes 3* and *4*). A lysate of A549 cell was incubated for 1 h at 4 °C with the samples shown in *lanes 2* and *4*. The samples in *lanes 1* and *3* were incubated with control lysis buffer. *Panel A,* amount of receptor detectable by Western blot with anti-Met antibodies is identical in all samples. *Panel B,* tyrosine phosphorylation state of the receptor (detected by Western blot with phosphotyrosine antibodies) remained unchanged during the incubation with the lysate. *Panel C,* autoradiogram of the PI 3-kinase assay (PI(4,5)P$_2$ was not used as substrate) showing that the tyrosine-phosphorylated p190^{MET} receptor associated with the PI 3-kinase.

cell lysates did not contain any anti-phosphotyrosine immunoprecipitable PI 3-kinase activity, suggesting that the enzyme neither is phosphorylated on tyrosine nor is associated with tyrosine-phosphorylated proteins (data not shown). However, PI 3-kinase activity from the cell lysate did bind to the tyrosine-phosphorylated p190^{MET} receptor. No PI 3-kinase activity associated with the unphosphorylated receptor, and no residual activity was found in samples incubated with buffer alone (Fig. 4C). The amount of p190^{MET} bound to the beads and its state of phosphorylation were not affected by the incubation with A549 cell lysate; as shown by probing with anti-Met and anti-phosphotyrosine antibodies the immunocomplexes incubated with either cell lysates or buffer alone (Fig. 4, *A* and *B*). This experiment demonstrates that tyrosine phosphorylation of p190^{MET} is a switch for association with PI 3-kinase *in vitro*.

DISCUSSION

In this paper we present the first evidence that tyrosine phosphorylation of the HGF/SF receptor is a switch for association of PI 3-kinase. This enzyme plays a key role in the signal transduction pathway by generating D-3-phosphoinositides (23, 24), a novel class of putative intracellular second messengers (20, 26). The role in signal transduction of PI(3)P, PI(3,4)P$_2$, and PI(3,4,5)P$_3$ is not yet understood, although their cellular levels *in vivo* are elevated in cells entering the mitotic cycle or transformed by activated oncogenes (23, 34–36). PI 3-kinase, recently purified to homogeneity, is a het-

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erodimeric protein consisting of two subunits of 110 and 85 kDa (26). The cDNA encoding the 85-kDa subunit has recently been cloned (27, 28, 37). The 85-kDa subunit, lacking enzymatic activity, features two SH-2 domains and directly associates with tyrosine-phosphorylated proteins (26–28, 32, 37). Moreover, upon binding to tyrosine-phosphorylated protein, the 85-kDa subunit becomes phosphorylated on tyrosine (32, 38, 39). This molecule, acting as a regulatory subunit for the catalytic 110-kDa subunit of PI 3-kinase may be regulated by tyrosine phosphorylation (26, 27).

So far PI 3-kinase has been found to associate with and to be activated by a number of tyrosine kinase receptors, including the receptors for PDGF (33), colony-stimulating factor-1 (40), insulin (41, 42), epidermal growth factor (reviewed in 20), and stem cell growth factor (44). The association is ligand-dependent and mediated by tyrosine phosphorylation of the receptor cytoplasmic domain.

It has been shown that phosphorylation of a specific tyrosine (Tyr^{751}) in the kinase insert domain of the human β-PDGF receptor is critical for association with PI 3-kinase (45). Similarly, *in vitro* binding of PI 3-kinase to the mouse β-PDGF receptor is inhibited by competition with synthetic peptides containing the homologous Tyr^{739} in the phosphorylated state (39). This tyrosine is included in a sequence highly conserved in other receptor kinases which associate with PI 3-kinase. From these homologous sequences a putative consensus (-YXXM-) has been deduced (20).

We have shown that PI 3-kinase associates with the $p190^{MET}$ HGF/SF receptor. The association is mediated by tyrosine phosphorylation of the $p145^{\beta}$ subunit. The intracellular domain of $p145^{\beta}$ is itself a tyrosine kinase, whose activity is controlled by binding to the specific ligand. $P145^{\beta}$ also contains multiple tyrosine autophosphorylation sites (16) and a consensus sequence for binding to PI 3-kinase (-$\underline{Y}^{1313}$EV$\underline{M}$-).[3] It has been suggested that the recruitment of PI 3-kinase to tyrosine-phosphorylated receptors is a necessary step for activation *in vivo* (20). Furthermore the 85-kDa subunit of PI 3-kinase was found to be phosphorylated on tyrosine only when associated to the membrane (31). These data are compatible with the hypothesis that the 85-kDa regulatory subunit upon association to the HGF/SF-activated receptor is a target for its tyrosine kinase activity.

It is conceivable that the D-3-polyphosphoinositides generated by PI 3-kinase play a role in the signaling pathway triggered by HGF/SF. This factor induces different biological responses in epithelial cells, including mitogenesis and stimulation of cell motility. Interestingly, D-3-polyphosphoinositides are generated in response to other extracellular signals inducing either cell growth (23, 40, 41, 42) or chemotaxis (47). PI 3-kinase may not be the only signal transducer activated by HGF/SF. Other proteins may associate with the $p190^{MET}$ receptor and may be the target of its tyrosine kinase activity. Consistent with this hypothesis we detected other low abundance proteins that are tyrosine-phosphorylated upon stimulation or constitutive activation of $p190^{MET}$. The identification of these molecules and the analysis of their physiological significance are in progress.

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[3] The $p190^{MET}$ sequence numbering refers to the cDNA sequence revised by Ponzetto *et al.* (46). The sequence has been filed on the EMBL Data Bank under the code number X54559.

REFERENCES

1. Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K., and Shimizu, S. (1989) *Nature* **342**, 440–443
2. Miyazawa, K., Tsubouchi, H., Naka, D., Takahashi, K., Okigaki, M., Arakaki, N., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Gohda, E., Daikuhara, Y., and Kitamura, N. (1989) *Biochem. Biophys. Res. Commun.* **163**, 967–973
3. Deleted in proof
4. Stoker, M., Gherardi, E., Perryman, M., and Gray, J. (1987) *Nature* **327**, 239–242
5. Weidner, K. M., Behrens, J., Vanderckove, J., and Birchmeier, W. (1990) *J. Cell Biol.* **111**, 2097–2108
6. Weidner, K. M., Arakaki, N., Vandekerchove, J., Weingart, S., Hartmann, G., Rieder, H., Fonatsch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y., and Birchmeier, N (1991) *Proc. Natl. Acad. Sci. U. S. A.*, **88**, 7001–7005
7. Naldini, L., Weidner, M., Vigna, E., Gaudino G., Bardelli, A., Ponzetto, C., Narsimhan, R., Hartmann, G., Zarnegar, R., Michalopoulos, G., Birchmeier, W., and Comoglio, P. M. (1991) *EMBO J.* **10**, 2867–2878
8. Bottaro, D. P., Rubin, J. S., Faletto, D. L., Chan, A. M.-L., Kmiecick, T. E., Vande Woude, G. F., and Aaronson, S. A. (1991) *Science* **251**, 802–804
9. Naldini, L., Vigna, E., Narshiman, R., Gaudino, G., Zarnegar, R., Michalopoulos, G., and Comoglio, P. M. (1991) *Oncogene* **6**, 501–504
10. Giordano, S., Ponzetto, C., Di Renzo, M. F., Cooper, C. S., and Comoglio, P. M. (1989) *Nature* **339**, 155–156
11. Giordano, S., Di Renzo, M. F., Narsimhan, R., Cooper, C. S., Rosa, C., and Comoglio, P. M. (1989) *Oncogene* **4**, 1383–1388
12. Giordano, S., Di Renzo, M. F., Ferracini, R., Chiado'Piat, L., and Comoglio, P. M. (1988) *Mol. Cell. Biol.* **8**, 3510–3517
13. Park, M., Dean, M., Kaul, K., Braun, M. J., Gonda, M. A., and Vande Woude, G. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 6379–6383
14. Deleted in proof
15. Deleted in proof
16. Ferracini, R., Longati, P., Naldini, L., Vigna, E., and Comoglio, P. M. (1991) *J. Biol. Chem.* **266**, 19558–19564
17. Naldini, L., Vigna, E., Ferracini, R., Longati, P., Gandino, L., Prat, M., and Comoglio, P. M. (1991) *Mol. Cell. Biol.* **4**, 1793–1803
18. Gandino, L., Di Renzo, M. F., Giordano, S., Bussolino, F., and Comoglio, P. M. (1990) *Oncogene* **5**, 721–725
19. Gandino, L., Munaron, L. M., Naldini, L., Ferracini, R., Magni, M., and Comoglio, P. M. (1991) *J. Biol. Chem.* **266**, 16098–16104
20. Cantley, L. C., Auger, K., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., and Soltoff, S. (1991) *Cell* **64**, 281–302
21. Deleted in proof
22. Comoglio, P. M., Di Renzo, M. F., Tarone, G., Giancotti, F., Naldini, L., and Marchisio, P. C. (1984) *EMBO J.* **3**, 483–489
23. Auger, K. R., Serunian, L. A., Soltoff, S. P., Libby, P., and Cantley, L. C. (1989) *Cell* **57**, 167–175
24. Deleted in proof
25. Deleted in proof
26. Carpenter, C. L., Duckworth, B. C., Auger, K. R., Cohen, B., Schafflausen, B. S., and Cantley, L. C. (1990) *J. Biol. Chem.* **265**, 19704–19711
27. Otsu, M., Hiles, I., Gout, I., Fry, M., Ruiz-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A., Morgan, S., Courtneidge, S., Parker, P., and Waterfield, M. (1991) *Cell* **65**, 91–104
28. Escobedo, J., Navankasattusa, S., Kavanaugh, M., Milfay, D., Fried, V., and Williams, L. (1991) *Cell* **65**, 75–82
29. Kaplan, D. R., Whitman, M., Schaffhausen, B., Pallas, D., White, M., Cantley, L., and Roberts, T. (1987) *Cell* **50**, 1021–1029
30. Deleted in proof
31. Cohen, B., Liu, Y., Druker, B., Roberts, B., and Schaffhausen, B. (1990) *Mol. Cell. Biol.* **10**, 2909–2915
32. Cohen, B., Yoakim, M., Pwnica-Worms, H., Roberts, T., and Schaffhausen, B. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4458–4462
33. Whitman, M., Kaplan, D., Roberts, T., and Cantley, L. (1987) *Biochem. J.* **247**, 165–174
34. Deleted in proof
35. Deleted in proof
36. Vartikovski, L., Daley, G., Jakson, P., Baltimore, B., and Cantley, L. (1991) *Mol. Cell. Biol.* **11**, in press
37. Skolnik, A., Margolis, B., Mohammadi, M., Lowenstein, E., Fisher, R., Drepps, A., Ullrich, A., and Schlessinger, J. (1991) *Cell* **65**, 83–90
38. Kazlauskas, A., and Cooper, J. A. (1990) *EMBO J.* **9**, 3279–3286
39. Escobedo, J., Kaplan, D., Kavanaugh, M., Turck, C., and Williams, L. A. (1991) *Mol. Cell. Biol.* **11**, 1125–1132
40. Deleted in proof
41. Deleted in proof
42. Deleted in proof
43. Deleted in proof
44. Lev, S., Givol, D., and Yarden, Y. (1991) *EMBO J.* **10**, 647–654
45. Kazlauskas, A., and Cooper, J. A. (1989) *Cell* **58**, 1121–1133
46. Ponzetto, C., Giordano, S., Peverali, F., Della Valle, G., Abate, M., Vaula, G., and Comoglio, P. M. (1991) *Oncogene* **6**, 553–559
47. Traynor-Kaplan, A. E., Thompson, B. L., Harris, A. L., Taylor, P., Omann, G. M., and Sklar, L. A. (1989) *J. Biol. Chem.* **264**, 15668–15673
48. Prat, M. P., Crepaldi, T., Gandino, L., Giordano, S., Longati, P., and Comoglio, P. M. (1991) *Mol. Cell. Biol.*, in press