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17. O_2 consumption was measured in mTALH cells with the use of a Clark-type electrode (*9*). O_2 consumption was inhibited by ouabain from 220 ± 4 to 153 ± 3 nmol O_2 per 5×10^6 cells per minute and by furosemide from 289 ± 20 to 181 ± 23 nmol O_2 per 5×10^6 cells per minute (mean ± SEM values; $n = 6$), indicating that in mTALH cells a large part of O_2 consumption is driven by ion transport. AA (1 μM) reduced O_2 consumption from 324 ± 22 to 201 ± 19 nmol O_2 per 5×10^6 cells per minute (mean ± SEM values; $n = 7$). Synthetic 20-HETE and 20-COOH-AA standards also decreased O_2 consumption from 254 ± 10 to 160 ± 12 (mean ± SEM values; $n = 11$) and from 260 ± 8 to 190 ± 9 nmol O_2 per 5×10^6 cells per minute (mean ± SEM values; $n = 7$), respectively.
18. Ion content of mTALH cells was measured by flame photometry after treatment with ouabain and furosemide for 15 min or with 20-HETE and 20-COOH-AA for 5 min. Ouabain (1 mM) increased Na^+ by 50 ± 8% and decreased K^+ by 50 ± 7%; whereas furosemide (1 mM) decreased Na^+ and K^+ by 49 ± 7% and 46 ± 5%, respectively (mean ± SEM values; $n = 6$). 20-HETE (1 μM) decreased both Na^+ and K^+ by 58 ± 14% and 24 ± 3%, respectively; 20-COOH-AA (1 μM) decreased both Na^+ and K^+ by 35 ± 6% and 25 ± 17%, respectively (mean ± SEM values; $n = 4$). Changes in the concentration of Na^+ and K^+ were significantly different ($P < 0.05$) from control values by analysis of variance and Newman Keuls' test.
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30 May 1990; accepted 26 November 1990

Identification of the Hepatocyte Growth Factor Receptor as the c-*met* Proto-Oncogene Product

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Hepatocyte growth factor (HGF) is a plasminogen-like protein thought to be a humoral mediator of liver regeneration. A 145-kilodalton tyrosyl phosphoprotein observed in rapid response to HGF treatment of intact target cells was identified by immunoblot analysis as the β subunit of the c-*met* proto-oncogene product, a membrane-spanning tyrosine kinase. Covalent cross-linking of ^{125}I-labeled ligand to cellular proteins of appropriate size that were recognized by antibodies to c-*met* directly established the c-*met* product as the cell-surface receptor for HGF.

HEPATOCYTE GROWTH FACTOR (HGF) was first purified from human and rabbit plasma and rat platelets on the basis of its ability to stimulate mitogenesis of rat hepatocytes (*1–3*). Thus, HGF may act as a humoral factor promoting liver regeneration after partial hepatectomy or liver injury (*4*). The same factor was purified from human fibroblast culture medium and shown to act on melanocytes and a variety of epithelial and endothelial cells (*5*). Together with evidence of HGF expression in several organs (*5–8*), these findings indicate that HGF may also act as a paracrine mediator of proliferation for a broad spectrum of cell types. Molecular cloning of HGF revealed structural similarity to plasminogen and related serine proteases (*5, 9, 10*). Recent evidence that HGF induces rapid tyrosine phosphorylation of proteins in intact target cells suggests that a tyrosine kinase receptor might mediate its mitogenic signal (*5*).

The human mammary epithelial cell line B5/589 is particularly sensitive to the mitogenic effects of HGF (*5*). Intact serum-starved B5/589 cells were treated with HGF (approximately 100 ng/ml) for 10 min at 37°C and solubilized on ice. Phosphotyrosyl proteins were isolated from cell lysates by immunoprecipitation with antibody to phosphotyrosine (anti-pTyr). These proteins were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with the same antibody. Several phosphotyrosyl proteins were detected in untreated cells by this method (Fig. 1A). Treatment of intact cells with HGF induced phosphorylation of a 145-kD protein (p145) (Fig. 1A, center lane). B5/589 cells exposed to epidermal growth factor (EGF) displayed tyrosine phosphorylation of the EGF receptor, but not p145 (Fig 1A, right lane). When lysates from control and HGF-treated cells that had been labeled with [^{32}P]orthophosphate were used for immunoprecipitation with anti-pTyr, phosphorylation of p145 was specifically detected in HGF-treated cells (Fig. 1B). Phosphoamino acid analysis of ^{32}P-labeled p145 confirmed the presence of phosphotyrosine and revealed the presence of phosphoserine as well (Fig. 1C). The HGF-stimulated phosphorylation of p145 on tyrosine and its apparent molecular weight were consistent with the possibility that p145 represented the receptor tyro-

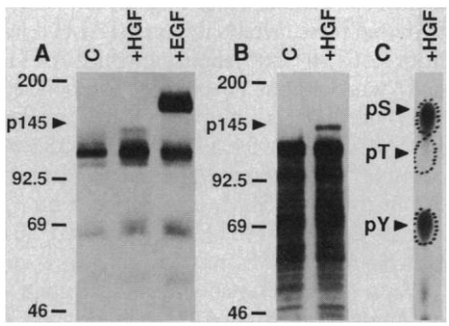

Fig. 1. Tyrosine phosphorylation of p145 in B5/589 human mammary epithelial cells in response to HGF. (**A**) Immunoblot of phosphotyrosyl proteins from untreated control cells (C), treated with HGF, and with EGF (Collaborative Research). HGF was purified as described (*5*). Serum-starved cells were exposed to growth factor (100 ng/ml) for 10 min at 37°C as indicated, detergent-solubilized on ice, and immunoprecipitated with monoclonal antibody to pTyr (Upstate Biotechnology). Immunoprecipitated proteins were resolved by 7.5% SDS-PAGE (*30*) and immunoblotted with the same antibody (*31*). (**B**) Autoradiogram of ^{32}P-labeled phosphoproteins from control (C) and HGF-treated cells. Serum-starved cells were metabolically labeled with [^{32}P]orthophosphate (1.0 mCi/ml) (*32*). The cells were treated with HGF (100 ng/ml) for 10 min at 37°C as indicated and detergent-solubilized on ice. Phosphotyrosyl proteins were immunoprecipitated with anti-pTyr and resolved by 7.5% SDS-PAGE. (**C**) Phosphoamino acid analysis of p145 from lane 2 of (B) was performed as described (*32*). The dotted circles indicate the migration of unlabeled phosphoserine (pS), phosphothreonine (pT), and phosphotyrosine (pY).

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sine kinase for HGF.

A number of receptor-like molecules have been described for which there are as yet no known ligands. One of these is the c-*met* proto-oncogene product, which is a receptor-like tyrosine kinase comprised of disulfide-linked subunits of 50 kD (α) and 145 kD (β) (*11, 12*). In the fully processed c-*met* product, the α subunit is extracellular, and the β subunit has extracellular, transmembrane, and tyrosine kinase domains as well as sites of tyrosine phosphorylation (*12–15*).

To test the hypothesis that p145 might represent the c-*met* protein β subunit, proteins immunoprecipitated by anti-pTyr from control and HGF-treated B5/589 cells were immunoblotted with a monoclonal antibody to the cytoplasmic domain of the human c-*met* product (*16*). The prominent 145-kD protein observed only in HGF-treated cells (Fig. 2A) provided direct evidence that this mitogen induced phosphorylation of the c-*met* protein on tyrosine residues. When whole lysates prepared from identically treated cells were blotted directly with the same antibody to c-*met*, the percentage of c-*met* protein phosphorylated on tyrosine in response to HGF could be quantitated (Fig. 2A). We estimate that at least 10% of the total cellular c-*met* protein content was immunoprecipitable by anti-pTyr after HGF stimulation. Analysis of the time course of HGF action revealed that the c-*met* protein could be recovered by immunoprecipitation with anti-pTyr within 1 min of treatment and that this effect persisted for at least 3 hours (*17*). Comparison of the electrophoretic mobility of p145 under reducing and nonreducing conditions confirmed that it was the β subunit of the c-*met* protein (Fig. 2C). Without reduction, the 50-kD α subunit of the c-*met* protein remains disulfide-linked to the β subunit and substantially retards its migration in SDS-PAGE (*11–15*). Similarly, p145 immunoprecipitated from ^{32}P-labeled B5/589 cells that had been treated with HGF displayed a shift in mobility characteristic of the c-*met* proto-oncogene product when subjected to reducing or nonreducing electrophoretic conditions (Fig. 2C). Together these results identified p145 as the c-*met* protein β subunit and established that HGF stimulated its phosphorylation on tyrosine residues.

The rapidity and extent of c-*met* protein tyrosine phosphorylation in response to HGF supported the possibility that c-*met* protein was the cell-surface receptor for HGF. However, there is evidence that receptor kinases can phosphorylate other receptors (*18, 19*). Thus, conclusive identification of the c-*met* product as the the HGF receptor required a demonstration of their direct interaction. ^{125}I-labeled HGF was unsuitable for covalent affinity cross-linking because it consisted of a mixture of single chain and heterodimeric labeled species (*17*). A smaller form of HGF with similar binding properties (*17*), designated HGFp28, was ^{125}I-labeled and used to characterize the HGF receptor. We compared its cross-linking on B5/589 cells and M426 human fibroblasts, an HGF-insensitive cell line which also lacks detectable amounts of c-*met* protein (Fig. 3A). The ^{125}I-labeled HGFp28 cross-linked to its receptor on B5/589 cells migrated as a 210-kD protein complex under nonreducing conditions (Fig. 3B). Under reducing conditions, a major 170-kD complex was observed (Fig. 3B). These apparent molecular sizes were consistent with a direct interaction between the labeled HGFp28 and the 145-kD β subunit of the c-*met* protein. Under reducing conditions, two minor bands of 190 kD and about 300 kD were also detected (Fig. 3B). Cross-linking of ^{125}I-labeled HGFp28 to the species observed under reducing conditions was blocked by addition of either unlabeled HGFp28 or HGF-neutralizing antisera (*17*). Under identical conditions, ^{125}I-labeled HGFp28 failed to cross-link to any large proteins in M426 cells (Fig. 3B).

To establish that ^{125}I-labeled HGFp28 was physically associated with the c-*met* protein, we immunoprecipitated ^{125}I-labeled HGFp28 cross-linked complexes with a polyclonal antiserum (*15*) specific to the carboxyl-terminal 28 amino acids of the β subunit of the c-*met* protein. The covalently cross-linked major 170-kD and minor 300-kD species detected under reducing conditions were immunoprecipitated by the antibody, and their detection was specifically blocked by competing peptide (Fig. 3C). These results demonstrate a direct molecular interaction between ^{125}I-labeled HGFp28 and the c-*met* β subunit. The composition of

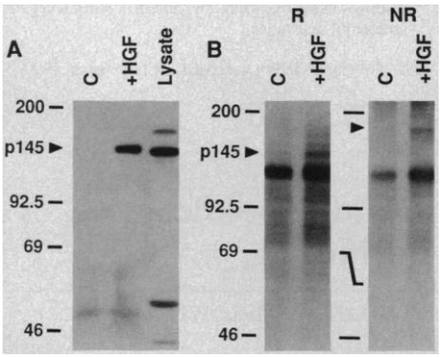

Fig. 2. Identification of p145 as the β subunit of the c-*met* protooncogene product. (**A**) Antibody to c-*met* immunoblot of anti-pTyr immunoprecipitates from control (C) and HGF-treated B5/589 cells. Samples for immunoprecipitation (2 mg of protein) were prepared as described in Fig. 1A, resolved by 7.5% SDS-PAGE, transferred to Immobilon membranes (Millipore), and detected with monoclonal antibody to c-*met* and ^{125}I-labeled protein A. To quantify the percentage of c-*met* protein that was immunoprecipitable with anti-pTyr, 200 μg of B5/589 cell lysate (Lysate) was resolved by SDS-PAGE and immunoblotted directly with monoclonal antibody to c-*met*. (**B**) Autoradiogram of ^{32}P-labeled phosphoproteins from control (C) and HGF-treated B5/589 cells resolved by 7.5% SDS-PAGE under reducing (R) and nonreducing (NR) conditions. Serum-starved cells were metabolically labeled with [^{32}P]orthophosphate, left untreated (C) or treated with HGF, and immunoprecipitated with anti-pTyr as described in Fig. 1B. Samples were reduced with 100 mM β-mercaptoethanol before electrophoresis as indicated.

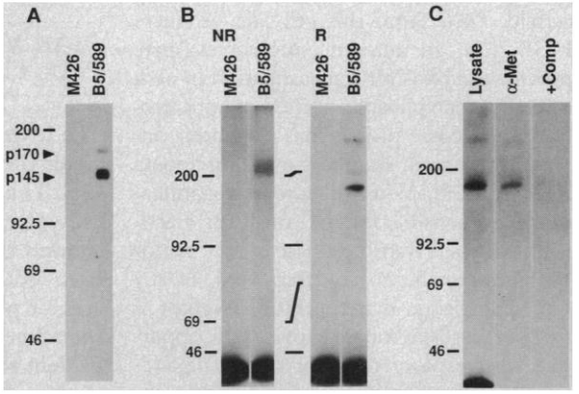

Fig. 3. Covalent affinity cross-linking of ^{125}I-labeled HGFp28 to the c-*met* protein-tyrosine kinase. (**A**) Immunoblot of lysates (200 μg of protein) prepared from M426 human lung fibroblasts and B5/589 cells using monoclonal antibody to the cytoplasmic domain of c-*met* protein. (**B**) Cross-linking of ^{125}I-labeled HGFp28 to M426 and B5/589 cells resolved by 6.5% SDS-PAGE under nonreducing (NR) and reducing (R) conditions. HGFp28 was purified as described (*33*) and labeled with Na^{125}I by the chloramine-T method (*34*). Cells were incubated with Hepes binding buffer (*31*) containing ^{125}I-labeled HGFp28 (5×10^5 cpm) for 45 min at 25°C, washed with cold Hepes-buffered saline (pH 7.4), and treated with disuccinimidyl suberate (*31*). The cells were then solubilized with SDS and boiled for 3 min in the presence of 100 mM β-mercaptoethanol as indicated. ^{125}I-labeled proteins were resolved by 6.5% SDS-PAGE and autoradiography at −70°C. (**C**) Immunoprecipitation of ^{125}I-labeled HGFp28-cross-linked complexes from B5/589 cells with antiserum to the c-*met* peptide (*15*). Sample preparation and cross-linking prior to immunoprecipitation, performed as described in (B), yielded the electrophoretic pattern shown in the left lane (Lysate) under reducing conditions. The adjacent lanes show immunoprecipitation of the cross-linked species with antiserum to the c-*met* peptide (1:100) in the absence (α-MET) or presence (+COMP) of competing peptide (10 μg/ml). Immunoprecipitated proteins were adsorbed to immobilized protein-G (Genex) and eluted with SDS prior to electrophoresis and autoradiography as described in (B).

the minor 300-kD cross-linked species remains to be determined. All of these findings establish that the c-*met* product is the cell surface receptor for HGF.

HGF is structurally related to the family of serine proteases that includes plasminogen, prothrombin, urokinase, and tissue plasminogen activator (*5, 9*). Several proteases, including members of this family, stimulate DNA synthesis presumably through a proteolytic mechanism similar to tryptic activation of the insulin receptor (*20*). Only urokinase has been found to associate with a specific cell-surface receptor, which itself bears no homology to the c-*met* protein or other tyrosine kinase receptors (*21*). HGF, however, lacks two amino acids in the catalytic triad required for proteolytic function (*22*). The direct interaction of HGF with the c-*met* receptor tyrosine kinase suggests a biochemical mechanism of mitogenic signal transduction similar to that of insulin, EGF, and others, and thus represents significant functional divergence from its serine protease homologs.

The *met* oncogene was originally identified in a chemical carcinogen-treated human osteogenic sarcoma cell line by transfection analysis in NIH 3T3 cells (*23*). Its cloning revealed that the oncogene encoded a truncated tyrosine kinase activated by chromosomal rearrangement (*24*). Although the oncogene product is predominantly a cytosolic kinase, the proto-oncogene product is a transmembrane receptor-like protein (*14, 15*), whose transcript is expressed in many tissues (*25, 26*). A high proportion of spontaneous NIH 3T3 transformants overexpress c-*met* (*27*). Moreover, NIH 3T3 transfection analysis has revealed that the murine c-*met* proto-oncogene exhibits transforming activity (*26*). Since this cell line produces HGF (*28*), an autocrine mechanism may provide the basis for transformation in each case. Tyrosine phosphorylation of apparently normal c-*met* protein has also been observed in certain human gastric carcinoma cell lines (*29*). Whether autocrine stimulation is responsible for the constitutive activation of c-*met* tyrosine kinase in such tumor cell lines remains to be determined. In any case, knowledge that the HGF receptor is the c-*met* tyrosine kinase provides the opportunity to explore the role of this ligand-receptor system in normal as well as disease states.

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35. We thank E. Fortney, C. Florio, J. Schindler, J. Artrip, and N. Ellmore for technical assistance, J. Thompson for photography, and N. Lichtenberg for secretarial assistance.

1 November 1990; accepted 28 December 1990

Regulation of Polyphosphoinositide-Specific Phospholipase C Activity by Purified G_q

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The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C yields the second messengers inositol 1,4,5-trisphosphate ($InsP_3$) and 1,2-diacylglycerol. This activity is regulated by a variety of hormones through G protein pathways. However, the specific G protein or proteins involved has not been identified. The α subunit of a newly discovered pertussis toxin–insensitive G protein (G_q) has recently been isolated and is now shown to stimulate the activity of polyphosphoinositide-specific phospholipase C (PI-PLC) from bovine brain. Both the maximal activity and the affinity of PI-PLC for calcium ion were affected. These results identify G_q as a G protein that regulates PI-PLC.

REGULATION OF INTRACELLULAR activities by a wide variety of extracellular signals involves cell surface receptors that interact with GTP (guanosine triphosphate)-dependent regulatory proteins (G proteins) (*1*). The best understood pathways are the regulation of adenylyl cyclase by the G_s proteins and the cGMP (cyclic guanosine monophosphate)-specific phosphodiesterase by the G_t (transducin) proteins. The G_o and G_i subfamilies of G proteins are sensitive to modification by pertussis toxin (PTX) and have been implicated in the inhibition of adenylyl cyclase

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