Association of the Multisubstrate Docking Protein Gab1 with the Hepatocyte Growth Factor Receptor Requires a Functional Grb2 Binding Site Involving Tyrosine 1356*

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Hepatocyte growth factor/scatter factor (HGF) is a multifunctional factor that induces mitogenesis, motility, invasiveness, and branching tubulogenesis of several epithelial and endothelial cell lines in culture. The receptor for hepatocyte growth factor has been identified as the Met tyrosine kinase. Upon stimulation with hepatocyte growth factor, the Met β subunit becomes highly phosphorylated on tyrosine residues, one of which, tyrosine 1356 within the carboxyl terminus, is crucial for dissociation, motility, and branching tubule formation in Madin-Darby canine kidney epithelial cells. Tyrosine 1356 forms a multisubstrate binding site for the Grb2 and Shc binding domain; PI 3-kinase, phosphatidylinositol 3-transferase; an oncogene, Tpr-Met (11). The Met receptor is synthesized as a 170-kDa precursor that undergoes glycosylation, proteolytic cleavage, and disulfide bond formation to yield a mature 190-kDa heterodimeric molecule consisting of a 40-kDa α and a 145-kDa β subunit (12). The β subunit spans the plasma membrane, and its cytoplasmic portion contains a catalytic kinase domain as well as several potential sites of tyrosine phosphorylation (13, 14). Using receptor chimeras, we and others have demonstrated that the Met receptor cytoplasmic domain is sufficient to mediate the pleiotropic biological responses to HGF in epithelial cells (15–17) and that these events require Met-dependent protein tyrosine phosphorylation (18, 19).

Phosphorylated tyrosine residues in the non-catalytic cytoplasmic domains of receptor-tyrosine kinases act as specific binding sites for Src homology 2 (SH2) and phosphotyrosine binding (PTB) domain-containing proteins, which in turn transmit intracellular signals (reviewed in Ref. 20). Although signaling pathways downstream from receptor-tyrosine kinases that are involved in a mitogenic response have been characterized in detail, little is known of the signaling pathways involved in cell dissociation, motility, and morphogenesis.

Upon stimulation with HGF, the Met β subunit becomes highly phosphorylated on tyrosine residues (10, 21), and from structure-function analyses, tyrosine residues within the carboxyl terminus are crucial for biological activity. The Met carboxyl terminus contains 3 tyrosine residues (Tyr-1349, Tyr-1356, and Tyr-1365) (22), two of which (Tyr-1349 and Tyr-1356) are highly conserved between other members of the Met receptor family, Sea and Ron (23). Tyrosine 1356 within the carboxyl terminus is crucial for biological activity. The Met carboxyl terminus contains 3 tyrosine residues (Tyr-1349, Tyr-1356, and Tyr-1365) (22), two of which (Tyr-1349 and Tyr-1356) are highly conserved between other members of the Met receptor-tyrosine kinase gene family, Sea and Ron (23). Tyrosine 1356 within the carboxyl terminus is crucial for biological activity. 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quency downstream from tyrosine 1356 is VNR, which re-

presents a consensus binding site for several SH2 domain-containing substrates (24, 25). Consistent with this, tyrosine 1356 forms a multisubstrate binding site for the Grb2 adaptor pro-

tein, the p85 subunit of PI 3-kinase, phospholipase Cγ, and 

SHP2 (18, 23, 26, 27), and together with tyrosine 1349 is 

required for association and/or phosphorylation of the Shc 

adaptor protein (28, 29). Cells expressing a Met receptor mu-

tant that fails to associate with the Grb2 adaptor protein 

(N1358H), yet retains the ability to interact with other sub-

strates, scatter in response to HGF but fail to form branch-

ing tubules, suggesting that Grb2-dependent signaling pathways may be involved in the morphogenic activities of HGF (27).

To investigate the signaling molecules that are activated by 

the Met receptor, we have identified HGF-induced phosphoproteins in MDCK cells. We have established that proteins of 100–130 kDa are highly phosphorylated following HGF stim-

ulation of MDCK cells and that one of these is the Grb2-

associated binding protein Gab1, a possible insulin receptor 

substrate-1 (IRS-1)-like signal transducer. We show that Gab1 is the major substrate for the Met kinase and that efficient 

association of Gab1 requires a functional Grb2 binding site 

involving Tyr-1356 in the Met carboxyl terminus. Met receptor 

mutants that fail to induce branching morphogenesis are im-

paired in their ability to interact with Gab1 suggesting that 

Gab1 may play a role in these processes.

**EXPERIMENTAL PROCEDURES**

*Antibodies*—Monoclonal anti-phosphotyrosine PY20 and RC20H were obtained from Transduction Laboratories (Lexington, KY), and 4G10 was obtained from Upstate Biotechnology Inc. (Lake Placid, NY). Rabbit polyclonal antibodies which recognize the human Met receptor or Tpr-Met protein were generated against a carboxyl-terminal peptide of the human Met protein as described previously (Ab 143) (14).

*Cell Culture*—MDCK cells were obtained from Dr. Michael Stoker, COS-1 cells from Dr. Gordon Wong, and all other cell lines were obtained from the American Type Tissue Culture Collection. All cell lines were maintained in Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum. MDCK cells overexpressing human HGF receptor were generated by retroviral infection.

**HGF Stimulation of MDCK Cells**—MDCK cells were seeded at 1 million per 100-mm dish. The next day, cells were washed with DMEM and serum-starved for 24 h in 6 ml of DMEM containing 0.02% FBS. HGF, purified as described elsewhere (15), was then added at 100 units/ml for the time indicated. Cells were immediately lysed in 1 ml of cold lysis buffer (see below) and whole cell lysates were prepared as described below.

**Whole Cell Extracts**—Cells were harvested in lysis buffer A containing 50 mM Hepes, pH 7.4, 0.15 mM NaCl, 10% glycerol, 0.1% Triton X-100, 1 mM phenylmethylsulfon fluoride, 1 mM benzamidine, 2 μg/ml apro-

tin, 2 μg/ml leupeptin, 1 mM Na3VO4, 1 mM NaF. Alternatively, tissues were homogenized with a Polytron in buffer A. Lysates were obtained by an extraction of solubilized proteins on ice for 30 min, followed by centrifugation at 12,000 × g for 15 min. Protein concentrations were determined by the method of Bradford (47).

**Transient Transfections**—Generation of the wild-type and mutant Tpr-Met or colony-stimulating factor-Met cDNAs has been described elsewhere (15, 20, 26, 30). Briefly, Tpr-Met or colony-stimulating factor-Met cDNAs have been described (15, 20, 26, 30). 6

**Immunoprecipitation and Western Blotting**—Cell lysates (0.1–0.5 

ml) were immunoprecipitated with 1.5 μl of anti-Met sera (anti-144), or antibodies to Gab1. The immune complexes were collected and washed with 50 ml of buffer containing 50 mM Hepes, pH 7.4, 10 mM MnCl2, 1 mM phenyl-

methylsulfon fluoride, 1 mM benzamidine, 2 μg/ml apro-

tin, 2 μg/ml leupeptin, and 50 μM ATP for 30 min at room temperature. Tpr-Met and 

associated complexes were washed three times with buffer A and lysates (1 mg of protein) from various serum-starved cell lines were added for 3 h at 4 °C with gentle agitation. Complexes were washed three times with buffer A, once with kinase buffer, and phosphorylation of bound proteins was performed in kinase buffer containing 10–15 μCi of γ[32P]ATP with or without 50 mM PEP and ATP. Incorporated radio-

labeled proteins were then resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

**Glutathione S-Transferase Fusion Proteins**—The plasmids contain-

ing the cDNAs encoding different mutant GST-Grb2 fusion proteins were transfected into the DH5α Escherichia coli strain. Fusion pro-

teins were expressed and induced with isopropyl-1-thio-

galactopyranoside and affinity-purified with glutathione-Sepharose beads (Phar-

macia) as described (31). GST fusion proteins (1–3 μg) immobilized on Sepharose were incubated with 0.5 ml of MDCK cell lysates (1 mg) for 3 h at 4 °C with rocking. Bound proteins were washed three times with buffer A and eluted twice with 250 μl of 10 mM glutathione in buffer A for 15 min at 4 °C. The combined eluates were then used in the in vitro association assay as described above.

**Immunoprecipitation and Western Blotting**—Cell lysates (0.1–0.5 

ml) were incubated with appropriate antibodies for 1 h at 4 °C with gentle agitation. Volumes were adjusted to 0.5 ml with buffer A. The immune complexes were collected and washed with buffer A, and resolved by PAGE. The proteins were transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, NH), blocked for 1 h with TBST (10 mM Tris-CI, pH 7.4, 2.5 mM EDTA, 150 mM NaCl, 0.1% Tween 20) containing 3% bovine serum albumin at room temperature and incubated with the appropriate antibody for 1 h at room temper-

ature. Bound antibodies were revealed with horseradish peroxidase-linked protein A, and the signals were visualized with an enhanced chemiluminescence (ECL) detection system (Amersham).

**Far Western Analysis**—For Far Western analysis, wild-type and mutant Tpr-Met proteins obtained from transient transfection of COS-1 cells (1/5 plate) were immunoprecipitated with anti-Met sera (anti-144), separated by SDS-PAGE, and transferred to nitrocellulose. GST fusion proteins generated as described above were purified on glutathione-Sepharose. Far Western blots were incubated with 2.5 μg ml−1 GST fusion protein for 1 h at 4 °C. Bound proteins were detected by anti-GST (Santa Cruz Biotechnology) and then secondary antibody (goat anti-mouse IgG). Bound antibodies were revealed with horseradish peroxidase-linked protein A, and the signals were visualized with an ECL detection system (Amersham).

**Phosphoamino Acid Analysis**—Radiolabeled phosphoproteins were visualized by autoradiography, excised from the polyacrylamide gel, and subjected to hydrolysis in 6 N HCl for 90 min at 110 °C. Liberated phosphoamino acids were then resolved by two-dimensional thin layer electrophoresis (32) and identified by the staining of internal phospho-

tyrosine/serine/threonine standards with ninhydrin.

**V8 Protease Digestion**—Radiolabeled phosphoproteins were resolved on a 10% polyacrylamide gel, located by autoradiography, excised, and subjected to in situ Staphylococcus aureus V8 protease digestion by the method of Cleveland et al. (33). Briefly, 2 μl of V8 protease was added per well, and the digestion was carried out for 30 min in the stacking gel at room temperature. Phosphopeptides were separated in a resolving 10% polyacrylamide gel and visualized by autoradiography.

**RESULTS**

**HGF-induced Tyrosine Phosphorylation of Proteins in MDCK Cells**—To identify signaling pathways that mediate the pleio-

tropic actions of HGF in epithelial cells, we have characterized HGF-induced tyrosine phosphorylation of proteins in MDCK cells. MDCK cells dissociate and scatter in response to HGF and form branching tubules when suspended in a three-dimen-

sional matrix. Total cellular proteins from cell lysates prepared from untreated and HGF-stimulated MDCK cells were immu-

noprecipitated and immunoblotted with monoclonal anti-

phosphotyrosine antibodies (PY20). Following stimulation of MDCK cells with HGF for 2 min, multiple tyrosine-phosphorylated proteins were detected (Fig. 1A). A protein of 145 kDa corre-

sponding in size to the Met receptor, was phosphorylated at low levels, whereas the predominant tyrosine-phosphorylated proteins migrated with molecular mass of 110–130 kDa (Fig. 1A). The level of phosphorylation of these proteins remained high for at least 15 min. To establish whether the 145-kDa protein was the Met receptor, MDCK cells overexpressing the Met
MDCK cell lysates were found to associate signaling pathways as the full-length receptor (18, 26, 27, 29). The Tpr-Met receptor (Tpr-Met) is a constitutively activated kinase that is difficult to detect using metabolic labeling of tissue culture cells but can be detected by tyrosine kinase to detect protein-protein interactions that are association assays have been used in studies of other receptor-protein complexes. A lysate from a single plate was immunoprecipitated with monoclonal antiphosphotyrosine antibody (4G10), resolved on an 8% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with antiphosphotyrosine (4G10) or polyclonal anti-Met receptor antibodies (Ab 143). Molecular mass markers and the position of the 145-kDa β-chain of the Met receptor are indicated.

receptor were stimulated with HGF, and proteins were immunoprecipitated with monoclonal anti-phosphotyrosine antibodies (4G10) and immunoblotted with either anti-phosphotyrosine (4G10) or anti-Met sera (Ab 143) (12) (Fig. 1B). Following stimulation with HGF, an increase in tyrosine phosphorylation of a protein of 145 kDa was detected (Fig. 1B, lanes 1 and 2) that corresponded to the β subunit of the Met receptor (Fig. 1B, lanes 3 and 4).

In Vitro Association Assays Identify Met Substrates of 100 and 130 kDa in MDCK Cells—To identify whether any of the phosphoproteins in MDCK cells associate with the Met receptor kinase we have used an in vitro association assay. In vitro association assays have been used in studies of other receptor-tyrosine kinases to detect protein-protein interactions that are difficult to detect using metabolic labeling of tissue culture cells (34). We have shown that the oncogenic counterpart of the Met receptor (Tpr-Met) is a constitutively activated kinase that trans-autophosphorylates on the same tyrosine residues as those found in the Met receptor (18, 30). Consistent with this, Tpr-Met associates with and activates the same substrates and signaling pathways as the full-length receptor (18, 26, 27, 29). Using this approach, two proteins of 100 and 130 kDa from MDCK cell lysates were found to associate with Tpr-Met or an activated Met receptor (data not shown) and become phosphorylated in the presence of [γ-32P]ATP (Fig. 2A).

We thus took advantage of the ability of Tpr-Met to be activated in an HGF-independent manner to use in vitro association assays. The interaction between Tpr-Met or Met (data not shown) and the p100 and p130 proteins was dependent on an active Met kinase, since a Tpr-Met mutant (K1108A) that contains a lysine to alanine substitution in the phosphotransfer domain is kinase-inactive and failed to phosphorylate and/or associate with both the p100 and p130 proteins (Fig. 2A, lanes 2 and 3). The intensity of phosphorylation of the p100 and p130 was unaltered following alkaline treatment which preferentially hydrolyzes phosphoserine and phosphothreonine residues (35), consistent with these proteins being phosphorylated on tyrosine residues (data not shown). To investigate this possibility, phosphoamino acid analysis was performed on the p100/130 proteins isolated from the polyacrylamide gel in Fig. 2A (lane 2). This analysis showed only the presence of phosphotyrosine (Fig. 2B) demonstrating that the p100/130 proteins are not phosphorylated by a serine/threonine kinase, but directly by a tyrosine kinase in the in vitro assays. Thus, since Tpr-Met is the only kinase activity detected in these assays by autophosphorylation, we concluded that the p100/130 proteins are substrates for the Met kinase.

The p100 and p130 Phosphoproteins Are Related—To determine if the p100 and p130 proteins were unique to MDCK epithelial cells we investigated whether similar proteins were detected using the in vitro association kinase assay in cell lysates prepared from other cell lines and tissues. Phosphorylated proteins of molecular masses ranging from 100 to 120 kDa were identified in extracts from human placenta, a human carcinoma cell line (HeLa), and a monkey kidney cell line (COS-1) as well as from mouse kidney, lung, and whole embryo tissues (Fig. 3A). To establish whether these proteins were related, radiolabeled phosphoproteins of 100–130 kDa in mass were excised from the gel and subjected to partial V8 protease mapping. A similar pattern of V8 protease-digested phosphopeptides was obtained from the p100 and p130 proteins expressed in MDCK cells, suggesting that they are related proteins (Fig. 3B, lanes 4 and 5). Moreover, the patterns of phosphopeptides obtained for the 100–120-kDa proteins expressed in human placenta, HeLa (Fig. 3B, lanes 1 and 6), and COS-1 cells (data not shown) were similar to that obtained from the p100 and p130 proteins from MDCK cells. Although p100/130 proteins from murine tissues (kidney and lung) gave a pattern distinct from that of MDCK cell proteins, interspecies differences at the primary amino acid level make V8 protease mapping limited in the comparison of proteins between different species. Nevertheless, under identical conditions of digestion, our results...
suggest that the 100–130-kDa phosphoproteins identified in the in vitro association assay from MDCK, HeLa, and COS cells, as well as human placental tissue bear structural homology.

A Functional Grb2 Binding Site in the Met Receptor Is Required for Association with p100/130—The carboxyl terminus of the Met receptor contains three tyrosine residues, Tyr-1349, Tyr-1356, and Tyr-1365, two of which (Tyr-1349 and Tyr-1356) are highly conserved between members of the Met receptor subfamily, Sea and Ron (23). To determine if the interaction and phosphorylation of the p110/130 proteins was dependent on specific tyrosine residue(s) in Met we have used mutant Tpr-Met proteins containing substitutions of phenylalanine for tyrosine at positions 1349, 1356, 1365, or 1349/1356 in the Met receptor carboxyl terminus. When compared with the wild-type Tpr-Met protein, the Y1356F and Y1349F mutants showed slightly reduced levels of association/phosphorylation with the p100/130 proteins (55 and 65%, respectively) (Fig. 4, A and D, lanes 1, 3, and 7). In contrast, the Y1356F mutant showed a significant reduction in association/phosphorylation of the p100/130 proteins to 10% that of the wild-type levels, and the double mutant Y1349F/Y1356F failed to associate with or phosphorylate the p100/130 proteins (Fig. 4, A and D, lanes 1, 4, and 6). The ability of the p100/130 proteins to associate with or be phosphorylated by the Y1356F or Y1349F/Y1356F mutant proteins showed no correlation with their in vitro kinase activity which was 80 and 72% of wild type, respectively (Fig. 4D). This suggested that Tyr-1356 was critical for the efficient association of Tpr-Met with the p100/130 proteins. Moreover, the absence of association with the Y1349F/Y1356F mutant suggests that Tyr-1349 in addition to Tyr-1356 is required for full association with the p100/130 proteins.

The amino acid sequence downstream from Tyr-1356 (VNV) represents a consensus binding site for multiple SH2 domain-containing substrates including the Grb2 adaptor protein (24, 25). In addition to binding Grb2, Tyr-1356 together with Tyr-1349 forms a multisubstrate binding site for the p85 subunit of PI 3-kinase, SHP2, phospholipase Cγ, and the Shc adaptor protein (23, 29). Significantly, a Met mutant (N1358H), which has selectively lost the ability to bind Grb2 (Fig. 4B, lane 5) while retaining wild type levels of in vitro kinase activity (Fig. 4D) and the ability to bind Shc (Fig. 4C, lane 5) PI 3-kinase, SHP2, and phospholipase Cγ in the in vitro association assay (27, 29) showed a decreased ability to associate with the p100/130 proteins, similar to that of the Y1356F mutant (Fig. 4A, lanes 4 and 5). These data suggest that since both the Y1356F and N1358H mutants fail to associate with Grb2, the majority of the p100/130 proteins may be recruited to Met via the Grb2 adaptor protein. Alternatively, the asparagine two amino acids downstream from Tyr-1356 may be required for the direct binding of the p100/130 proteins.

The Grb2 Adaptor Protein Mediates Association of p100/130 with Met—To establish if the p100/130 proteins interacted with Grb2, we used a GST-Grb2 fusion protein to identify Grb2-associating proteins from MDCK lysates. GST or GST-Grb2 fusion proteins were first adsorbed to glutathione-Sepharose beads, then incubated with lysates prepared from serum-starved MDCK cells. The GST or GST-Grb2 and associated proteins were eluted from the Sepharose with glutathione and subsequently assayed in “pull down” assays containing GST-Grb2 (Fig. 5A, lanes 3 and 4) but not in those containing GST alone (Fig. 5A, lane 2).

These data suggest that Grb2 may act as an adaptor protein to recruit the p100/130 proteins to the Met kinase where they are subsequently phosphorylated directly by Tpr-Met. To determine the domain(s) of Grb2 required for the interaction with the p100/130 proteins, GST fusion proteins containing the SH2 domain of Grb2 with the amino- or carboxyl-terminal SH3 domains of Grb2 were used in pull down assays to isolate proteins from MDCK cells. Proteins that associated with the full-length Grb2 or fusion proteins containing the SH2, NH2-SH3-SH2, or SH2-SH3-COOH domains of Grb2 were subjected to phosphorylation by Tpr-Met as described above (Fig. 5B). The Grb2 carboxyl-terminal SH3 was required for association...
with the p100/130 proteins and subsequent phosphorylation by Tpr-Met. Fusion proteins containing either the Grb2 SH2 domain or the amino-terminal SH3-SH2 domain were insufficient (Fig. 5B, lanes 3 and 4). These results are consistent with a model where the carboxyl-terminal SH3 domain of Grb2 associates with the p100/130 proteins and the Grb2 SH2 domain recruits these proteins to a phosphorylated Tyr-1356 in the carboxyl terminus of the Met receptor.

**The p100/130 in MDCK Cells Are Immunologically Related to Gab1**—A novel Grb2-binding protein of 115 kDa, Gab1 (Grb2-associated binder-1), was recently isolated by screening a cDNA expression library prepared from a human glial tumor cell line with a GST-Grb2 fusion protein (36). Gab1 is phosphorylated following stimulation of cells with EGF or insulin and is thought to function as a multisubstrate docking protein, with the ability to couple to other signaling pathways in a manner similar to IRS-1 (36). To establish whether the p100/130 proteins in MDCK cells corresponded to Gab1, cell lysates prepared from MDCK cells were immunodepleted with anti-Gab1 antibodies prior to the in vitro association assay. No p100/130 proteins were detected in the in vitro association assay in the MDCK cell lysate depleted of Gab1 (Fig. 6B, lanes 2 and 4), whereas abundant p100/130 proteins were detected in the protein A-Sepharose-treated lysate (Fig. 6B, lanes 1 and 3) providing evidence that the p100/130 proteins from MDCK cells that associate with and are phosphorylated by Tpr-Met corresponded to Gab1. To determine if the level and specificity of binding of the p95 and p115 Gab1 immunoreactive proteins in MDCK cells (Fig. 6A) correlated with the association and phosphorylation of the p100/130 proteins detected in MDCK cells and HeLa cells, respectively (Fig. 3).}

**Fig. 4.** The p100/130 proteins require tyrosine 1349 and 1356 for association with Met. A, wild-type Met (lane 1) or various Met mutant proteins (lanes 2–7) from transiently transfected COS-1 cells were immunoprecipitated with anti-Met sera (Ab 143) and activated by phosphorylation with ATP. Extracts (1 mg of protein) from serum-starved MDCK cells were then added. To visualize proteins associated with activated Met proteins, complexes were washed several times with buffer A and then incubated in kinase buffer with [γ-32P]ATP. Complexes were resolved by an 8% SDS-PAGE and visualized by autoradiography. B and C, the ability of each Met mutant in A to associate with the Grb2 or Shc adaptor proteins was detected by immunoblot analysis with anti-Grb2 serum (B) or with anti-Shc serum (C). D, the percentage phosphorylation of the p100/130 proteins by Tpr-Met mutants and the kinase activity of the various Tpr-Met mutants is indicated as a histogram. Values are an average of three independent experiments.
Association of Gab1 with the Met/HGF Receptor

**FIG. 5.** The p100/130 proteins interact with the carboxyl-terminal SH3 domain of Grb2. A, GST or a GST-Grb2 fusion protein (1–3 μg) was immobilized on Sepharose beads and incubated with lysates (1 mg of protein) prepared from serum-starved MDCK cells. Bound proteins were washed with lysis buffer A and eluted in the same buffer containing 10 mM glutathione. MDCK cell proteins eluted with GST or GST-Grb2 fusion proteins were then associated with an activated Tpr-Met protein. Complexes were washed, and associated proteins were phosphorylated in the presence of [γ-32P]ATP, resolved on an 8% SDS-PAGE, and visualized by autoradiography. B, lysates from serum-starved MDCK cells were associated with GST (lane 1), GST-Grb2 (lane 2), or GST fusion proteins containing the Grb2 SH2 (lane 3), Grb2 amino-terminal (N)-SH3-SH2 (lane 4), or Grb2 SH2-SH3-(C) carboxy-terminal (lane 5) domains. GST fusion and associated proteins were eluted with glutathione and subjected to an association/kinase assay with activated Tpr-Met as described in A. Proteins were resolved on an 8% SDS-PAGE and visualized by autoradiography.

wild-type Tpr-Met and Y1349F mutant proteins (Fig. 6C, lanes 1 and 3), whereas less Gab1 associated with the Y1356F and N1358H mutants, and neither protein was associated with the Y1349F/Y1356F double mutant (Fig. 6C, lanes 4–6). These results suggest that the majority of Gab1 associated with Met either directly through the Grb2 adaptor protein or required an asparagine residue two amino acids downstream from Tyr-1356. Recently an interaction between Gab1 and Met was detected using the yeast two-hybrid system suggesting that Gab1 can bind Met directly (37). To examine whether the direct association of Gab1 with Met requires the asparagine residue downstream from Tyr-1356 in a manner similar to the Grb2 adaptor protein, wild-type Met and mutant proteins were transferred to nitrocellulose and blotted with either a GST-Gab1 fusion protein (amino acids 203–689) or a GST-Grb2 fusion protein. The GST-Grb2 fusion protein which associates with Tyr-1356 in Met (26) bound to both the wild-type and the Y1349F mutant Met protein (Fig. 6D, lanes 1 and 3), whereas the GST-Gab1 fusion protein bound only to immobilized wild-type Met protein and not with any of the mutant proteins (Fig. 6D, lane 1), even though similar levels of Met proteins are present.

**Gab1 Is a Physiological Substrate for the Met Receptor—**To examine if Gab1 is a physiological substrate for the Met recep-
tor-tyrosine kinase, phosphotyrosine-containing proteins were immunoprecipitated from HGF-stimulated MDCK cell lysates with anti-phosphotyrosine antibodies and immunoblotted with anti-Gab1 sera. While no Gab1 was detected prior to HGF stimulation (Fig. 7A, lane 1), a smear of Gab1 immunoreactive proteins of 100–130 kDa were detected by 2 min following stimulation of MDCK cells with HGF (Fig. 7A, lanes 2–5). To determine if Gab1 associates with the Met receptor in vivo, hemagglutinin-tagged Gab1 was transiently coexpressed with wild-type or mutant Met receptors, which have a high basal level of kinase activity when overexpressed in 293T cells (23). Immunoprecipitation of Gab1 with anti-hemagglutinin antibody followed by immunoblotting with anti-Met sera revealed that similar levels of wild-type and Y1349F mutant Met proteins coimmunoprecipitated with Gab1, whereas little Y1356F or N1358H and no Y1349F/Y1356F mutant proteins coimmunoprecipitated with Gab1 (Fig. 7B). Thus association of Gab1 with Met in vivo paralleled that of Gab1 association with Met in vitro.

**DISCUSSION**

HGF is a multifunctional factor that stimulates growth, scatter, and branching tubulogenesis of epithelial cells in culture. These responses are mediated through the Met receptor tyrosine kinase. To identify novel signaling pathways that are activated by HGF, we examined the increase in tyrosine phosphorylation of proteins following stimulation of epithelial MDCK cells with HGF. We have established that proteins of 100–130 kDa are highly phosphorylated following stimulation of epithelial cells and that one of these is the Grb2-associated binding protein, Gab1.

Gab1 shows the greatest sequence similarity with IRS-1, a major substrate of the insulin receptor (38). IRS-1 and related members IRS-2 (39) and DOS (daughter of sevenless) (40, 41) constitute a new family of multisubstrate docking proteins. These proteins have in common with Gab1 an amino-terminal pleckstrin homology domain that may play a role in subcellular localization or substrate recognition (42), in addition to multiple tyrosine residues that may act to recruit SH2 or PTB domains. Gab1 is a tyrosine-phosphorylated following stimulation of cells with insulin or EGF (36). Thus Gab1 may be a target for several protein-tyrosine kinases and in a manner similar to IRS-1, function as a multisubstrate docking protein. However, unlike IRS-1, Gab1 does not contain an SH2 or PTB binding domain, and the mechanism by which Gab1 interacts with the EGF or insulin receptor-tyrosine kinases is unknown.

In an attempt to identify substrates from epithelial MDCK cells that associated with and were phosphorylated by the Met receptor, we have used an in vitro association assay (34). Using this assay, we showed that two proteins of 100 and 130 kDa are the predominant substrates from epithelial MDCK cells (Fig. 1, A and B) that associated with and are phosphorylated by the Met receptor kinase (Fig. 2A). The association of the p100/130 proteins with the Met receptor required Met kinase activity (Fig. 2A) and was primarily dependent on phosphorylation of tyrosine 1356 and to a lesser extent tyrosine 1349 (Fig. 4A). This is consistent with previous data showing that tyrosine 1356 in the carboxyl terminus of the Met receptor acts as a multisubstrate binding site and is critical for the association of Grb2, the p85 subunit of PI 3-kinase, phospholipase Cγ, and SHP2 and together with tyrosine 1349 is required for phosphorylation of the Shc adaptor protein (23, 26, 29). Significantly, a mutant Met protein (N1358H) that abolishes only Grb2 binding, yet retains the ability to associate with the p85 subunit of PI 3-kinase, phospholipase Cγ, SHP2, and Shc (27) showed a similar large reduction in association/phosphorylation of the
p100/130 proteins to 10% that of the wild type level (Fig. 4, A and D). This suggested that the majority of the p100/130 proteins associated with Met either indirectly through the Grb2 adaptor protein or required an asparagine residue two amino acids downstream from Tyr-1356. Consistent with the former, the p100/130 proteins from serum-starved MDCK cells associated with a GST-Grb2 fusion protein containing the carboxyl-terminal SH3 domain of Grb2, permitting the SH2 domain of Grb2 to couple this complex to Tyr-1356 in the Met receptor (Fig. 5 A).

Recently, Gab1 was identified as a novel Grb2-associated protein. We show that Gab1 immunoreactive proteins of 95 and 115 kDa from serum-starved MDCK cells or of 110 kDa from serum-starved HeLa cells associated with an activated Met kinase in vitro (Fig. 6 A). In a manner similar to the p100/130 proteins, the Gab1 proteins in MDCK cells required Tyr-1349 in addition to a functional Grb2 binding site downstream from Tyr-1356 for efficient association with the Met receptor (compare Figs. 4A and 6C). Moreover, phosphorylation of Gab1 in the in vitro kinase assay produced a shift in mobility from proteins of 95 and 115 kDa in MDCK cells, to a diffuse 100–130-kDa species, and of the 110-kDa protein in HeLa cells to a 120-kDa species, demonstrating that Gab1 is a direct substrate for the Met kinase (Fig. 6 A). Significantly, when an MDCK cell lysate is depleted of Gab1, with anti-Gab1 sera, no p100/130 proteins were detected in the in vitro association kinase assay, demonstrating that the p100/130 proteins from MDCK cells are Gab1 (Fig. 6B). Consistent with Gab1 being a downstream target in the Met signaling pathway, Gab1 was rapidly tyrosine-phosphorylated and associated with tyrosine-phosphorylated proteins following stimulation of MDCK cells with HGF (Fig. 7 A) and coimmunoprecipitated with the Met receptor in vivo (Fig. 7B).

We show here that the Grb2 adaptor protein couples Gab1 with the Met receptor and in the absence of Grb2 or a Grb2 binding site, little Gab1 can associate with Met (Figs. 4A, 5A,
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FIG. 7. Gab1 associates with Met in vivo and is phosphorylated following HGF stimulation of MDCK cells. A, MDCK cells were serum-starved in DMEM supplemented with 0.02% FBS for 24 h and subsequently stimulated with HGF (100 units/ml) for the times indicated. Cells were solubilized in lysis buffer A (see “Experimental Procedures”) by scraping. Proteins from whole cell lysates from one plate were immunoprecipitated with monoclonal antiphosphotyrosine antibodies (4G10). Proteins in immune complexes were resolved by 8% SDS-PAGE and immunoblotted with anti-Gab1 sera. B, lysates from 293T cells transiently transfected with expression plasmids encoding either wild-type or mutant forms of the Met receptor, and hemagglutinin-tagged Gab1 were immunoprecipitated with anti-hemagglutinin antibody, resolved by 8% SDS-PAGE, and immunoblotted with anti-Met antibody or anti-Gab1 antibody.

6C, 7B, and 8). However, only upon loss of Grb2 binding in conjunction with a Y1349F mutation did Gab1 fail to associate with Met (Figs. 4A, 6C, and 7B). Thus in addition to a functional Grb2 binding site downstream from Tyr-1356, full association of Gab1 with the Met receptor required Tyr-1349. While this paper was in preparation an interaction between Gab1 and Met was detected using the yeast two-hybrid system (37), suggesting that Gab1 is a Met-dependent substrate that contributes to morphogenesis (37). Moreover, the overexpression of a fusion protein containing the Met binding domain of Gab1 blocked HGF-induced cell dissociation (37).

We have previously shown that Met receptor mutants that fail to induce branching morphogenesis fail to interact with Grb2 yet still activate Ras-dependent pathways, presumably mediated through a Shc-Grb2-SOS complex (27). Thus, Grb2-dependent pathways that are distinct from Ras are implicated in the formation of branching tubules in response to HGF. Interestingly, overexpression of Gab1 in MDCK cells promotes the formation of branching tubules in matrix culture in the absence of HGF, demonstrating that Gab1 is a Met-dependent adaptor protein SH2 domain to a consensus binding sequence down-stream from Tyr-1356 (YVNV). The associated complex may be stabilized through an additional Gab1-mediated interaction with a phosphorylated Tyr-1349 residue. C, a Y1349F mutant retains high levels of association with Gab1 (55% of wild type) mediated through a Tyr-1356-Grb2-Gab1 interaction. D, a Y1356F mutant associates at a low level with Gab1 (10% of wild type) through a possible direct association of Gab1 with a phosphorylated Tyr-1349.

FIG. 8. Model for association of Gab1 with Met. A and B, after activation of the Met receptor-tyrosine kinase, Tyr-1356 is phosphorylated and acts to recruit a Grb2-Gab1 complex via binding of the Grb2 adaptor protein SH2 domain to a consensus binding sequence downstream from Tyr-1356 (YVNV). The associated complex may be stabilized through an additional Gab1-mediated interaction with a phosphorylated Tyr-1349 residue. C, a Y1349F mutant retains high levels of association with Gab1 (55% of wild type) mediated through a Tyr-1356-Grb2-Gab1 interaction. D, a Y1356F mutant associates at a low level with Gab1 (10% of wild type) through a possible direct association of Gab1 with a phosphorylated Tyr-1349.

Gab1 is a multisubstrate docking protein that, following stimulation of cells with EGF, associates with PI 3-kinase, SHP2, and phospholipase Cγ and thus may act to amplify or coordinate signals downstream from receptor-tyrosine kinases (36). PI 3-kinase activity is required for dissociation, motility, and branching morphogenesis of epithelial cells in matrix culture following stimulation with HGF (43, 44) and thus may be of greater physiological significance for branching morphogenesis.

Gab1 is a substrate for the EGF receptor (36), EGF does not stimulate branching morphogenesis in MDCK cells which have abundant EGF receptors (45, 46). Thus, it is critical to evaluate which Met-regulated Gab1-mediated signals are required for branching morphogenesis of epithelial cells and if these are modulated in distinct spatial or temporal manners downstream from receptor-tyrosine kinases resulting in different biological responses.

REFERENCES

1. Rosen, E. M., Nigam, S. K., and Goldberg, I. D. (1994) J. Cell Biol. 127, 1783–1787
2. Grant, D. S., Kleinman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., Kinseilla, J. L., Pulverini, P., and Rosen, E. M. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 127, 1783–1787
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Sci. U. S. A. 90, 1937–1941
3. Matsumoto, K., and Nakamura, T. (1993) in Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the c-Met Receptor (Goldberg, I. D., and Rosen, E. M., eds) pp. 226–248, Birkauser Verlag, Basel
4. Rong, S., Bodescot, M., Blair, D., Dunn, J., Nakamura, T., Mizuno, K., Park, M., Chan, A., Aaronson, S., and Vande Woude, G. F. (1992) Mol. Cell. Biol. 12, 5152–5158
5. Schmidt, C., Bladt, F., Goedecke, S., Brinkmann, V., Zschiesche, W., Harpe, M., Gherardi, E., and Birchmeier, C. (1995) Nature 373, 699–702
6. Uehara, Y., Minowa, O., Mori, C., Shiota, K., Kuno, J., Noda, T., and Kimura, N. (1995) Nature 373, 702–705
7. Lokker, N. A., Mark, M. R., Luis, E. A., Benneth, G. L., Robbins, K. A., Baker, M. T., and Williams, L. T. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2261–2265
8. Naldini, L., Vigna, E., Bardelli, A., Ponzetto, C., Pelicci, P. G., and Comoglio, P. M. (1996) J. Biol. Chem. 271, 22211–22217
9. Naldini, L., Weidner, K. M., Vigna, E., Gaudino, G., Bardelli, A., Ponzetto, C., Narasimhan, R. P., Hartmann, G., Zarnegar, R., Michalopoulos, G. A., Birchmeier, W., and Comoglio, P. M. (1991) EMBO J. 10, 2667–2678
10. Bottaro, D. P., Rubin, J. S., Pailet, D. L., Chan, A. L., Kmiecik, T. E., Vande Woude, G. F., and Aaronson, S. A. (1991) Science 251, 802–804
11. Park, M., Dean, M., Cooper, C. S., Schmidt, M., O’Brien, S. J., Blair, D. G., and Vande Woude, G. (1986) Cell 45, 859–864
12. Rodrigues, G. A., Naujokas, M. A., and Park, M. (1991) Mol. Cell. Biol. 11, 2962–2970
13. Gonziatti-Haces, M., Seth, A., Park, M., Copeland, T., Oroszlan, S., and Vande Woude, G. F. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 21–25
14. Naldini, L., Vigna, E., Ferrarini, R., Longati, P., Gaudino, L., Prat, L., and Comoglio, P. M. (1991) Mol. Cell. Biol. 11, 1793–1803
15. Zhu, X., Naujokas, M. A., and Park, M. (1996) Cell Growth Differ. 7, 359–366
16. Weidner, K. M., Sachs, M., and Birchmeier, W. (1994) J. Cell Biol. 121, 145–154
17. Komada, M., and Kitamura, N. (1993) Oncogene 8, 2381–2390
18. Zhu, X., Naujokas, M. A., Fixman, E. D., Torossian, K., and Park, M. (1994) J. Biol. Chem. 269, 29943–29948
19. Weidner, K. M., Sachs, M., Riethacker, D., and Birchmeier, W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2587–2601
20. Pawson, T. (1995) Nature 373, 573–580
21. Naldini, L., Vigna, E., Narasimhan, R. P., Gaudino, G., Zarnegar, R., Michalopoulos, G. A., and Comoglio, P. M. (1991) Oncogene 6, 501–504
22. 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
23. Ponzetto, C., Bardelli, A., Zhen, Z., Maina, F., dalla Zanca, P., Giordano, S., Graziani, A., Panayotou, G., and Comoglio, P. M. (1994) Cell 77, 261–271
24. Songyang, Z., Shoelson, S. E., McGlade, J., Olivier, P., Pawson, T., Bustelo, X. R., Barbacid, M., Sabe, H., Hanafusa, H., Y. T., Ren, R., Baltimore, D., Ratnofsky, S., Feldman, B. A., and Cantley, L. C. (1994) Mol. Cell. Biol. 14, 2777–2785
25. Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechfelder, R. J., Neel, B. G., Birge, R. B., Fujardo, J. E., Chou, M. M., Hanafusa, H., Schwaffhausen, B., and Cantley, L. C. (1993) Cell 72, 767–778
26. Fixman, E. D., Naujokas, M. A., Rodrigues, G. A., Moran, M. F., and Park, M. (1995) Oncogene 10, 237–249
27. Fournier, T. M., Kamikura, D., Teng, K., and Park, M. (1996) J. Biol. Chem. 271, 22211–22217
28. Pelicci, G., Giordano, S., Zhen, S., Salcini, A. E., Langfrancon, L., Bardelli, A., Panayotou, G., Waterfield, M. D., Ponzetto, C., Pelici, P. G., and Comoglio, P. M. (1995) Oncogene 10, 1631–1638
29. Fixman, E. D., Fournier, T. M., Kamikura, D. M., Naujokas, M. A., and Park, M. (1996) J. Biol. Chem. 271, 13116–13122
30. Kamikura, D. M., Naujokas, M. A., and Park, M. (1996) Biochemistry 35, 1010–1017
31. Smith, D. R. and Johnson, K. S. (1988) Gene 67, 31–40
32. Cooper, J. A., Sefton, B. M., and Hunter, T. (1983) Methods Enzymol. 99, 387–402
33. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106
34. Morrison, D. K., Kaplan, D. R., Escobedo, J. A., Rapp Ulf, R., Roberts, T. M., and Williams, L. T. (1989) Cell 58, 649–657
35. Cooper, J. A., and Hunter, T. (1981) Mol. Cell. Biol. 1, 165–178
36. Holgado-Madruga, M., Emlet, D. B., Moscletello, D. K., Godwin, A. K., and Wang, A. J. (1996) Nature 379, 560–564
37. Weidner, K. M., Dicesare, S., Sachs, M., Brinkmann, V., Behrens, J., and Birchmeier, W. (1996) Nature 384, 173–176
38. Sun, X. J., Rothenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Cahill, D. A., Goldstein, B. J., and White, M. F. (1991) Nature 352, 73–77
39. Sun, X. J., Wang, L. M., Zhang, Y., Yenush, L., Myers, M. G., Jr., Glashen, E., Lane, W. S., Pierre, J. H., and White, M. F. (1995) Nature 377, 173–177
40. Raabe, T., Rieger-Ecovar, J., Liu, X., Bausenwein, B. S., Dierk, P., Maroy, P., and Hafen, E. (1996) Cell 85, 911–920
41. Herbst, R., Carroll, P. M., Allard, J. D., Schilling, J., Raabe, T., and Simon, M. A. (1996) Cell 85, 899–909
42. Lemmon, M. A., Ferguson, K. M., and Schlessinger, J. (1996) Cell 85, 621–624
43. Derman, M. P., Cuha, M. J., Barros, E. J., Nigam, S. K., and Cantley, L. C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2777–2781
44. Royal, I., and Park, M. (1995) J. Biol. Chem. 270, 27780–27787
45. Haugel-DeMouzon, S., Csermly, P., Zoppini, G., and Kahn, C. R. (1993) J. Cell Physiol. 150, 180–187
46. Montesano, R., Schaller, G., and Orci, L. (1991) Cell 66, 697–711
47. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
Association of the Multisubstrate Docking Protein Gab1 with the Hepatocyte Growth Factor Receptor Requires a Functional Grb2 Binding Site Involving Tyrosine 1356

Linh Nguyen, Marina Holgado-Madruga, Christiane Maroun, Elizabeth D. Fixman, Darren Kamikura, Tanya Fournier, Alain Charest, Michel L. Tremblay, Albert J. Wong and Morag Park

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