A Peptide Representing the Carboxyl-terminal Tail of the Met Receptor Inhibits Kinase Activity and Invasive Growth*

(Received for publication, January 4, 1999, and in revised form, May 18, 1999)

Alberto Bardelli‡, Paola Longati, Tracy A. Williams, Silvia Benvenuti, and Paolo M. Comoglio

From the Institute for Cancer Research and Treatment (IRCC), University of Torino, School of Medicine, 10060, Candiolo, Italy

Interaction of the hepatocyte growth factor (HGF) with its receptor, the Met tyrosine kinase, results in invasive growth, a genetic program essential to embryonic development and implicated in tumor metastasis. Met-mediated invasive growth requires autophosphorylation of the receptor on tyrosines located in the kinase activation loop (Tyr1234-Tyr1235) and in the carboxyl-terminal tail (Tyr1349-Tyr1356). We report that peptides derived from the Met receptor tail, but not from the activation loop, bind the receptor and inhibit the kinase activity in vitro. Cell delivery of the tail receptor peptide impairs HGF-dependent Met phosphorylation and downstream signaling. In normal and transformed epithelial cells, the tail receptor peptide inhibits HGF-mediated invasive growth, as measured by cell migration, invasiveness, and branched morphogenesis. The Met tail peptide inhibits the closely related Ron receptor but does not significantly affect the epidermal growth factor, platelet-derived growth factor, or vascular endothelial growth factor receptor activities. These experiments show that carboxy-terminal sequences impair the catalytic properties of the Met receptor, thus suggesting that in the resting state the nonphosphorylated tail acts as an intramolecular modulator. Furthermore, they provide a strategy to selectively target the MET proto-oncogene by using small, cell-permeable, peptide derivatives.

The Met tyrosine kinase is a high affinity receptor for hepatocyte growth factor (HGF/scatter factor)1 (1, 2). Both Met and HGF are expressed in numerous tissues, although their expression is confined predominantly to cells of epithelial and mesenchymal origin, respectively. Signaling via this ligand-receptor pair triggers a unique biological program in target cells leading to “invasive cell growth.” The latter results from the integration of multiple biological responses to HGF such as cell proliferation, survival, motility, invasion of extracellular matrices, and formation of tubular structures (branched morphogenesis) (3–6). During mouse development the coordinated control of the invasive growth program by the HGF/Met pair is essential, since knock-out experiments involving either the ligand or the receptor result in embryonic lethality due to defects in migration of myoblasts, implantation of placenta, and liver development (7–9).

The Met tyrosine kinase is a high affinity receptor for hepatocyte growth factor (HGF/scatter factor)1 (1, 2). Both Met and HGF are expressed in numerous tissues, although their expression is confined predominantly to cells of epithelial and mesenchymal origin, respectively. Signaling via this ligand-receptor pair triggers a unique biological program in target cells leading to “invasive cell growth.” The latter results from the integration of multiple biological responses to HGF such as cell proliferation, survival, motility, invasion of extracellular matrices, and formation of tubular structures (branched morphogenesis) (3–6). During mouse development the coordinated control of the invasive growth program by the HGF/Met pair is essential, since knock-out experiments involving either the ligand or the receptor result in embryonic lethality due to defects in migration of myoblasts, implantation of placenta, and liver development (7–9).

C-MET was originally identified as the cellular counterpart of a transforming gene, TPR-MET, resulting from a chromosomal rearrangement (10, 11). A direct genetic link between MET and human cancer has been established by the identification of activating mutations in the c-MET gene in hereditary papillary renal carcinomas (12). Deregulated activation of the invasive growth phenotype by the MET oncogene confers invasive and metastatic properties to cancer cells (13, 14).

Binding of growth factors has been shown to induce receptor dimerization and is associated with autophosphorylation on tyrosine residues both within and outside the catalytic domain in the receptor dimer (15–17). Whereas the former are required for catalytic activity (catalytic tyrosines), the latter can serve as high affinity binding sites (docking tyrosines) for effector or adaptor molecules that recruit signal transducers to the receptor (18–20).

As for other tyrosine kinase receptors, activation of Met results in autophosphorylation of both “catalytic” and “docking” tyrosines. The major Met phosphorylation site is represented by tyrosines Tyr1234 and Tyr1235 (21). These are located within the activation loop of the kinase domain and are part of a three-tyrosine motif (Tyr1230, Tyr1234, and Tyr1235) conserved in other kinase receptors. Both Tyr1234 and Tyr1235 are essential for full activation of the enzyme (22). Upon phosphorylation of these residues, the enzymatic activity of the Met kinase is up-regulated in an autocatalytic fashion (21, 23).

When the Met receptor is activated, in addition to phosphorylation of the catalytic tyrosines, two other tyrosines (Tyr1349-Tyr1356) located in the carboxy-terminal tail of the receptor become phosphorylated (24–26). These tyrosine residues mediate coupling of the receptor with several SH2-containing effectors, including the Grb2/SoS complex (25, 26), the p85 regulatory subunit of phosphatidylinositol 3-kinase (24), Stat-3 (27), and the multidaptor protein Gab1 (28–30). Tyr1349 and Tyr1356 are strictly required for Met-mediated invasive growth. Substitution of both tyrosines with phenylalanine does not affect the receptor kinase activity but completely abolishes proliferation, motility, invasion, and tubulogenesis (26, 31–33).

Selective inhibition of tyrosine kinase receptors can be useful to study their activation mechanisms, to dissect their signaling pathways, and to interfere with their biological effects. A number of receptors (fibroblast growth factor receptor, Ret, epidermal growth factor receptor, Kit/Steel, Met) are directly involved in human diseases including cancer, skeletal, and other developmental disorders (34–37). Therefore, the development of molecules capable of selective inhibition of tyrosine kinase receptors has a number of potential applications.

* This work was supported by research grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC) and from the Giovanni Armenise-Harvard Foundation for Advanced Scientific Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address. To whom correspondence should be addressed: The Johns Hopkins University-School of Medicine Oncology Center, 424 N. Bond St., Baltimore, MD 21231.

1 The abbreviations used are: HGF, hepatocyte growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; PAGE, polyacrylamide gel electrophoresis; MAP, mitogen-activated protein; PRC, papillary renal carcinomas.
In the current study we sought to identify peptides capable of blocking both the kinase activity and the biochemical properties of the Met receptor. Previous kinetic and crystallographic studies suggest that receptor tyrosine kinases (i.e., IR, fibroblast growth factor receptor) can be inhibited by sequences corresponding to autophosphorylation sites located in the kinase activation loop (38–40). In view of these observations we exploited the use of sequences derived from the activation loop to interfere with the biochemical and biological properties of the Met receptor. Unexpectedly, we found that activation loop peptides do not act as Met inhibitors. We reasoned that sequences containing autophosphorylation sites of the carboxyl-terminal tail could be an alternative approach to modulate receptor activity. Accordingly, we show that a tail peptide inhibited Met kinase activity in vitro, blocked ligand-dependent phosphorylation and signal transduction, and impaired Met-induced invasive growth in transformed epithelial cells. The Met tail peptide does not significantly affect the EGF, PDGF, or VEGF receptor activities, demonstrating that the inhibitory mechanism is selective. These data provide evidence that peptides containing carboxyl-terminal sequences can efficiently work as inhibitors of the Met tyrosine kinase, and suggest that in the resting state the carboxyl-terminal domain may act as an intramolecular modulator of this receptor.

EXPERIMENTAL PROCEDURES
Reagents, Peptides, Antibodies, and Cell Culture—All reagents used were from Fluka (FlukaChemie AG) and Sigma. Reagents for SDS-PAGE were from Bio-Rad. HGF and macrophage-stimulating protein were obtained as described (52, 53). EGF and PDGF were from Sigma. VEGF was provided by Dr. Bussolino. Cell-permeable peptides derived from Met sequences in tandem with the Antennapedia sequence were obtained from Genosys Biotechnologies. Anti-phosphotyrosine, anti-Grb2, and anti-EGF receptor antibodies were purchased from Upstate Biotechnology, Inc. Anti-Active MAP kinase antibody was obtained from Promega, anti-PDGFR receptor was from Transduction Laboratories. Anti-VEGF was from Santa Cruz Biotechnology. Anti-Met and anti-Ron antibodies were obtained as (53, 54). Anti-fluorescein antibody was from Amersham Pharmacia Biotech. A549 cells were from ATCC. G4T16 and MLP-29 (mouse liver progenitor cells) have been previously described (47). Human endothelial cells were obtained from Dr. Bussolino. Cultures of mammalian cells were maintained in Dulbecco's modified Eagle's medium or RPMI supplemented with 10% serum (Sigma) (or 20% in the case of human endothelial cells) in a humidified atmosphere of 5% CO2, air.

Cell Delivery of Peptides—200 μg of each peptide were incubated in 0.01 M NH4HCO3, pH 9.0, at a final concentration of 200 μM in the presence of 100 μg/ml fluoresceinated isocyanate (Sigma) for 3 h at room temperature. Efficiency of fluorescence was verified by 22% SDS-PAGE followed by Western blotting with anti-fluorescein antibodies. To assess cell permeability of peptides, fluoresceinated peptides were added to culture medium at a final concentration of 20 μM, and after 2 h, cells were fixed and examined by fluorescence microscopy. To synthesize the intracellular concentration of Antennapedia peptides, cells were incubated with the Ant-Tyr1234–1235 and Ant-Tyr1346–1356 fluoresceinated peptides for 1 h, washed twice with phosphate-buffered saline, and lysed. Total lysates were analyzed by 22% SDS-PAGE. The presence of the fluoresceinated peptide in the lysate was revealed directly using the Fluorimag system (Molecular Dynamics). The concentration of the peptide was evaluated by comparison with lysates containing known amounts of fluoresceinated peptide.

In Vivo Auto- and Substrate Phosphorylation—The Met or the Trp-Met tyrosine kinase were immunoprecipitated with anti-Met antibodies from G4T16 or from transfected COS cells lysates in the absence of sodium orthovanadate to allow dephosphorylation. After extensive washing, immunoprecipitates were subjected to autophosphorylation in kinase buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 12.5 mM MgCl2, 1 mM dithiothreitol, 20 μM peptide, 10 μM cold ATP, and [γ-32P]ATP (5 μCi/sample) for 20 min at 4°C. The reaction was stopped by adding Laemmli buffer, and samples were analyzed by 8% SDS-PAGE. Gels were dried and exposed for autoradiography. In kinetic experiments, 5 μM myelin basic protein (Sigma) was also included in the reaction as a substrate together with increasing concentrations of peptides, 40 μM cold ATP, and [γ-32P]ATP (5 μCi/sample). The reaction was performed at 4°C for 25 min and stopped by adding Laemmli buffer. Samples were separated by 8–12% SDS-PAGE followed by analysis with the PhosphorImager STORM (Molecular Dynamics). The intensity of bands corresponding to phosphorylated Met and myelin basic protein was quantitated using the program ImageQuant (Molecular Dynamics).

RESULTS
Design of Cell-permeable Peptides Containing Met Autophosphorylation Sites—Activation of the Met receptor results in autophosphorylation of specific tyrosine residues located both in the kinase activation loop (Y1234–Y1235) and in the boxyl-terminal tail (Y1349–Y1356) (21, 25). A number of peptides were synthesized corresponding to the Met autophosphorylation sites (Fig. 1). All peptides contained the Antennapedia internalization domain at the amino terminus (41). This sequence has been shown to freely translocate across biological
membranes with minimal cell toxicity (42). An unphosphorylable version of the Tyr1349–Tyr1356 peptide was obtained by substituting the tyrosines with phenylalanine residues (Ant-Phe1349–1356). Two additional peptides were synthesized including the Antennapedia control peptide (Ant), a shorter version of the Tyr1349–Tyr1356 peptide lacking tyrosine 1356 (Ant-Tyr1349), and a scrambled Tyr1349–Tyr1356 peptide (Ant-scrambled).

**Peptides Containing Met Carboxyl-terminal Sequences Inhibit the Kinase Activity**—We investigated the possibility that peptides derived from the activation loop and the carboxyl-terminal tail could be used as inhibitors of the Met receptor. Peptides including Tyr1234–Tyr1235 and Tyr1349–Tyr1356 were synthesized with an amino-terminal internalization sequence derived from the Antennapedia protein. Peptides corresponding to the Met autophosphorylation sites. Peptides were synthesized with an amino-terminal internalization sequence derived from the Antennapedia protein. Peptides containing Met autophosphorylation sites. A, schematic representation of the functional domains of the Met tyrosine kinase receptor. The tyrosine kinase domain (KD) is indicated by a gray box. Tyr1234 and Tyr1235 are the catalytic tyrosines located in the activation loop (AL) of the kinase domain. Tyr1349–Tyr1356 are located in the carboxyl-terminal tail (CT) of the receptor and upon phosphorylation, generate docking sites for signal transducers. B, list of peptides corresponding to the Met autophosphorylation sites. Peptides were synthesized with an amino-terminal internalization sequence derived from the Antennapedia protein.

**FIG. 1.** Cell-permeable peptides containing Met autophosphorylation sites. A, schematic representation of the functional domains of the Met tyrosine kinase receptor. The tyrosine kinase domain (KD) is indicated by a gray box. Tyr1234 and Tyr1235 are the catalytic tyrosines located in the activation loop (AL) of the kinase domain. Tyr1349–Tyr1356 are located in the carboxyl-terminal tail (CT) of the receptor and upon phosphorylation, generate docking sites for signal transducers. B, list of peptides corresponding to the Met autophosphorylation sites. Peptides were synthesized with an amino-terminal internalization sequence derived from the Antennapedia protein.

**FIG. 2.** Tail peptides inhibit Met kinase activity. A, Met receptor was immunoprecipitated from GTL16 cells, and immunocomplexes were subjected to in vitro kinase assay in the presence of the indicated peptides and [γ-32P]ATP under conditions described under “Experimental Procedures.” Autophosphorylated receptors were separated by SDS-PAGE and analyzed by autoradiography. B, immunoprecipitated Met receptors were subjected to kinase assays in the presence of the indicated peptides and analyzed as in panel A. The inhibitory effect of the peptides was evaluated by measuring either receptor autophosphorylation and phosphorylation of the exogenous substrate myelin basic protein (MBP). After densitometric analysis, values were expressed as percentage of inhibition. •, Ant-Tyr1349–1356; □, Ant-Phe1349–1356; △, Ant-Tyr1234–1235; ○, Ant-scramble; ×, Ant.
of the 145-kDa Met receptor by Western blotting with anti-Met antibodies. GTL-16 cells. The amount of associated receptor was determined by agarose affinity support (Affi-Gel) (11, 12). Immobilized peptides were coupled to heparin-Sepharose and incubated with lysate of Sf9 cells expressing the isolated Met kinase domain (p34Met-KD). The amount of associated Met-KD protein was determined by Western blotting with anti-phosphotyrosine antibodies.

Dimerization causes constitutive activation of the Tpr-Met kinase domain. Met-PRC1228H and Met-PRC1250T are Met mutants (10, 11, 14, 44). Met-PRC variants identified in papillary renal carcinomas (PRC) in juxtamembrane domain and the carboxyl-terminal tail docking sites. The isolated Met kinase domain, containing a polyhistidine tag, is catalytically active and constitutively tyrosine-phosphorylated when expressed in Sf9 cells (data not shown). Immobilized tail peptides were incubated with lysates containing the catalytic domain. The associated kinase domain protein was visualized by immunoblotting with anti-Met antibodies (Fig. 3, panel C). To further characterize the interaction mechanism, we performed the same experiment with tyrosine-phosphorylated peptides. The phosphorylated tail peptide interacts with the Met catalytic domain with a slightly increased efficiency when compared with its unphosphorylated counterpart (data not shown).

**Carboxyl-terminal Peptides Inhibit the Kinase Activity of Oncogenic Met Mutants—**Oncogenic forms of the Met receptor have been described including Tpr-Met and Met-PRC mutants. In Tpr-Met the extracellular domain of Met is replaced by Tpr sequences, which provide two strong dimerization motifs. Dimerization causes constitutive activation of the Tpr-Met kinase, which acquires transforming and metastatic properties (10, 11, 14, 44). Met-PRC1228H and Met-PRC1250T are Met variants identified in papillary renal carcinomas (PRC) in which critical residues located in the kinase domain are mutated (12). Tpr-Met and Met-PRC mutants were expressed in COS cells, and association experiments with immobilized tail peptides were performed as described in the previous paragraph. Both the Tpr-Met and Met-PRC mutants were found to interact with the immobilized peptides (data not shown). To evaluate the inhibitory potential of tail peptides on the mutant forms of Met, the corresponding proteins were immunopurified from transfected COS cells with anti-Met-specific antibodies. Peptides were then compared for their ability to inhibit the kinase activity in autophosphorylation assays performed in vitro in the presence of the indicated peptide (20 μM). We observed that the tail peptides inhibit the kinase activity of Met mutants (Fig. 4). These data suggest that the tail peptides could be used as inhibitors of the Met oncogenic potential.

**Efficient Cell Delivery of Met-derived Peptides Containing the Antennapedia Internalization Sequence—**To verify that the Antennapedia-containing peptides could efficiently translocate across the plasma membrane, peptides were labeled with fluorescein (see “Experimental Procedures”). When added to cell cultures, peptides were recovered in the intracellular compartment of epithelial cells after as little as 15 min of incubation (Fig. 5). The amount of internalized peptides increased with its concentration in the culture medium with saturation at 25 μM (data not shown). Peptides were detectable intracellularly for up to 16 h. No significant differences were detected among the internalization efficiency of the different peptides. To evaluate the delivery efficiency, two different peptides were added to the culture medium (final concentration 20 μM), and the intracellular peptide concentrations were calculated (see “Experimental Procedures”). The cytosolic peptide concentration was found to be in the micromolar range (10–20 μM), which is sufficient to achieve inhibition of the Met receptor kinase activity (see Fig. 2). The relatively high intracellular peptide concentration is similar to that of the peptides originally added to the culture medium. This can be explained considering that translocation of the peptides across the plasma-membrane occurs in the absence of a receptor (42). In this situation the peptides can diffuse freely across the membrane, and equilibrium can be reached.

**Carboxyl-terminal Peptides Impair HGF-dependent Met Autophosphorylation in Intact Cells—**The ability of the tail peptides to inhibit Met autophosphorylation in vitro suggests that they could also be used to impair ligand-dependent activation of the receptor in intact cells. To verify this hypothesis, Met-expressing epithelial cells were serum-starved and treated with the peptides before ligand stimulation. Cells were incu-
bated with recombinant HGF, and the level of Met tyrosine phosphorylation was evaluated by immunoblotting with anti-phosphotyrosine antibodies (Fig. 6, panel A). In agreement with the data obtained in vitro, both the Tyr and Phe versions of tail peptides efficiently blocked ligand-dependent Met autophosphorylation, whereas the activation loop peptide was ineffective. In addition, the scrambled and the truncated tail peptides had little effect on ligand-dependent Met phosphorylation, confirming the specificity of the inhibitory effect (Fig. 6, panel A). Dose-response experiments showed that the inhibitory potential was dependent on peptide concentration with almost complete inhibition observed at 20 μM (Fig. 6, panel B). The effect of the peptides was reversible as shown by recovery of HGF-dependent Met phosphorylation 16 h after treatment (Fig. 6, panel C).

**Met Tail Peptides Inhibit the Closely Related Ron Receptor but Do Not Significantly Affect the EGF, PDGF, or VEGF Receptor Activities**—The inhibitory effect of the Met tail peptide on ligand stimulation of other receptor tyrosine kinases was evaluated. We tested a highly homologous receptor such as Ron and other distant Met relatives such as the EGF, PDGF, and VEGF receptors. Cells expressing the receptor of interest were serum-starved, treated with the Met or control peptides, and stimulated with the appropriate ligand. The receptors were immunoprecipitated, and tyrosine phosphorylation was measured by immunoblotting with antibody directed against phosphotyrosine (Fig. 7). Interestingly, the Met carboxyl-terminal peptides significantly impaired ligand stimulation of the Ron receptor, whereas they had either no or a minor effect on activation of the EGF, PDGF, and VEGF receptors. These data show that the Met peptide acts through a mechanism that is similar for Met and Ron; furthermore they indicate that the inhibitory activity of the Met peptide is selective.

**Tail Peptides Inhibit Met-dependent Downstream Signaling**—The HGF/Met pair triggers invasive growth by activation of a cascade of downstream signaling events. After ligand phosphorylation Met binds and phosphorylates the multiadaptor protein Gab1, which in turn recruits and activates a number of SH2-containing effectors (28–30). The signal is then transmitted to the nucleus via activation of various pathways including the MAP kinase cascade (25). We assessed whether the peptides that impair Met kinase activity also inhibited receptor signaling. Panel A of Fig. 8 shows that in cells treated with the Tyr or the Phe version of the tail peptide, Met-dependent Gab1 phosphorylation is impaired. This is also the case for activation of the p42 MAP kinase as evaluated using activation specific antibodies (Fig. 8, panel B). These data show that in addition to blocking the Met kinase activity, tail peptides also interrupt downstream signaling initiated by the HGF/Met interaction.

**Tail Peptides Inhibit HGF-mediated Cell Migration, Invasiveness, and Branched Morphogenesis**—HGF/Met promote a highly integrated biological program leading to "invasive

**Fig. 6. Inhibition of ligand dependent Met phosphorylation by tail peptides.** A, effect of peptides derived from the activation loop or the carboxyl-terminal tail on ligand-dependent Met phosphorylation. A549 cells were serum-starved for 3 days and treated with the indicated peptides at a final concentration of 20 μM. Two hours later cells were treated with recombinant HGF for 15 min and then lysed. Met phosphorylation was evaluated by immunoprecipitation (IP) of the receptor with anti-Met antibodies. The identity of the phosphorylated protein was confirmed by reprobing the same blot with an anti-Met antibody. Ctr, control. B, dose-response activity of the Ant-Tyr1349-1356 peptide on ligand-dependent Met phosphorylation. Serum-starved A549 cells were treated with the tail peptide at the indicated concentrations and stimulated with HGF. Met phosphorylation was assessed as described in A. C, time course activity of the Ant-Tyr1349-1356 peptide on ligand-dependent Met phosphorylation. Serum-starved A549 cells were treated for 2 h with the tail peptide (20 μM). At the indicated time points, cells were stimulated with HGF, and Met phosphorylation was assessed as above. The arrows indicate the position of the Met receptor β-chain (p145Met).
mediated invasiveness, whereas it results in partial inhibition of cell motility. To measure the effects of the peptides on morphogenesis, MLP-29 cells were grown in a tridimensional type-I collagen matrix for 3 days. By this time spherical aggregates of cells were observed that, after HGF stimulation, differentiated and formed tubular-like structures (Fig. 9, panel D). In the presence of the tail peptides this response was dramatically impaired. As in the invasion assay, the activation loop peptide had no effect. We also found that the Phe version of the tail peptide inhibited morphogenesis more efficiently than its Tyr counterpart. This data correlates with the relative activities of the two peptides in blocking the Met kinase activity in vitro (cf. Fig. 2, panel B).

**DISCUSSION**

Tyrosine kinase receptors are involved in human diseases including cancer, metabolic disorders, and developmental defects (34–37). On the basis of their mechanism of action, there are at least two possible strategies to inhibit receptor tyrosine kinases. On one side the catalytic process can be targeted by developing inhibitors of the enzymatic activity. On the other, receptor coupling to signal transducers can be blocked using molecules that bind the SH2 domain of the effector proteins. The finding that multiple receptors are coupled to overlapping arrays of SH2 effectors makes it difficult to interfere with signaling by a single receptor without simultaneously affecting others.

In this work we sought to target the biochemical and biological properties of the Met receptor by interfering with the mechanism of receptor autophosphorylation. On the basis of previous crystallographic studies we initially used peptides containing autophosphorylation sites located in the activation loop (39, 40). Unexpectedly, the activation loop peptide did not block Met autophosphorylation, although it interfered with substrate phosphorylation at high concentrations. Molecular
modeling of the Met cytoplasmic domain suggested that the tail could actually get in contact with the catalytic pocket (data not shown). We therefore exploited the possibility that tail sequences could modulate the kinase activity of the Met receptor. A tail-derived peptide blocked both auto and substrate phosphorylation of the Met receptor. Receptor tyrosine kinases preferentially phosphorylate tyrosine residues followed by a hydrophobic residue in the 13 position (48). The tyrosines located in the Met activation loop do not match the optimal consensus for phosphorylation. Conversely, tyrosines located in the carboxyl-terminal tail are predicted to be optimal substrates of the Met receptor. In agreement with this, we reported recently that Met preferentially phosphorylates peptides derived from the carboxyl-terminal tail compared with peptides derived from the activation loop (49). Substrate selectivity may therefore account for the differential effect on the Met kinase activity displayed by activation loop and tail peptides.

The inhibitory potential of the tail peptide requires the presence of a unique amino acid sequence (Ile-Gly-Glu-His-Tyr1349-Val-His-Val-Asn-Ala-Thr-Tyr1356) because either a peptide truncated at tyrosine 1356 or a scramble version were inactive. The finding that a Phe1349-Phe1356 nonphosphorylable analogue also blocked the Met kinase activity suggested that the inhibitory mechanism relies on peptide binding to the active site rather than on ATP depletion. This was confirmed by experiments demonstrating the interaction of the immobilized tail peptide with the Met receptor.

The crystal structures of the insulin and fibroblast growth factor receptors indicate that, in the inactive state, the activation loop blocks the access of the substrate peptide to the catalytic site loop (39, 40). This situation may be different in Met. The inhibitory activity of peptides containing sequences from the Met carboxyl-terminal tail suggest that this region modulates the function of the receptor. One possibility is that, in the inactive receptor state, the tail interferes with access of the substrate to the catalytic pocket of the enzyme. Ligand-induced dimerization may unleash the kinase activity by releasing this autoinhibitory mechanism. Alternatively, the tail could impair receptor phosphorylation by interacting with another moiety of the catalytic domain. We are currently performing crystallographic studies to verify this hypothesis.

Interestingly the Met tail peptide also inhibited Ron, a Met-related receptor, but not the EGF, VEGF, and PDGF receptors. Among the receptor tyrosine kinases, Ron has the highest sequence homology with Met. In particular, the amino acid sequence surrounding the tail phosphorylation sites (Y-
The Carboxyl-terminal Tail Inhibits the Met Receptor

hydrophobic-X-hydrophobic-X_H3Y-hydrophobic-X-hydrophobic) is conserved between Met and Ron (25). The finding that the inhibitory activity was restricted to receptors of the Met family implies a common mechanism. These experiments further indicate that intramolecular peptide sequences can be utilized to selectively target the catalytic properties of tyrosine kinase receptors.

The membrane internalization properties of the Antennapedia homeodomain were used to transduce the Met inhibitory peptides into epithelial cells. Once internalized, the tail peptide blocked both ligand-dependent autophosphorylation and downstream Met signaling. In particular, the peptides impaired Gab1 phosphorylation and MAP kinase activation. The effects were dose-dependent and reversible, confirming the specificity of the inhibitory process.

A number of biological assays were used to study whether peptides derived from the Met tail could interfere with HGF-induced invasive growth as measured by cell motility, invasion, and branched morphogenesis. Met-mediated invasion and tubulogenesis were severely impaired by the tail receptor peptide. Interestingly, motility was only partially affected, suggesting that a low level of receptor activation is sufficient to induce cell flattening and dissociation. This is in agreement with previous data showing that Met receptor mutants unable to promote invasion and tubulogenesis are still competent in inducing motility (31, 50, 51). Met-triggered invasive growth is required for embryonic development, whereas its inappropriate activation confers to cancer cells invasive and metastatic properties. Selective inhibitors of this process could be useful in understanding the HGF/Met biology and in targeting the invasive metastatic potential of Met-expressing cells. By demonstrating that tail sequences act as inhibitors of the Met tyrosine kinase, this study provides an approach to interfere with the biological effects triggered by the MET proto-oncogene.

Acknowledgments—We are indebted to S. Toniol for the peptide inhibition experiments, L. Pugliese for molecular modeling the Met cytoplasmic domain, E. Wright for editing the manuscript, and A. Cignetto for the excellent secretarial help. We are grateful to E. Medico and P. Gual for critical reading of the manuscript. The excellent technical assistance of G. Petruccelli and R. Albano is acknowledged.

REFERENCES
1. Bottaro, D. P., Rubin, J. S., Faletto, D. L., Chan, A. M., Kmiecik, T. E., Vande Woude, G. F. & Aaronson, S. A. (1991) Science 251, 802–804
2. Naldini, L., Weidner, K. M., Vigna, E., Gaudino, G., Bardelli, A., Ponzetto, C., Narsimhan, R. P., Hartmann, G., Zanetti, P., Miccolopoulou, G. K., Bircher, W., & Comoglio, P. M. (1991) EMBO J 10, 2867–2876
3. Stoker, M., Gherardi, E., Perryman, M. G. & Gray, J. (1987) Nature 327, 228–242
4. Montesano, R., Matsumoto, K., Nakamura, T., & Ori, L. (1991) Cell 67, 901–908
5. Nakamura, T., Tsumoto, H. & Ichihara, A. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 6498–6502
6. Michalopoulou, G., Houch, K. A., Dolan, M. L. & Leutteke, N. C. (1984) Cancer Res. 44, 4414–4419
7. Blasé, F., Bredt, D., Jentsch, S., Gaudio, G., Bardelli, A., Ponzetto, C., Narsimhan, R. P., Hartmann, G., Zanetti, P., Miccolopoulou, G. K., Bircher, W., & Comoglio, P. M. (1991) EMBO J 10, 2867–2876
8. Stoker, M., Gherardi, E., Perryman, M. G. & Gray, J. (1987) Nature 327, 228–242
9. Montesano, R., Matsumoto, K., Nakamura, T., & Ori, L. (1991) Cell 67, 901–908
10. Schmidt, L., Duh, P. F., Chen, F., Kishita, T., Glenn, C., Choyke, P., Scherer, S. W., Zhuang, Z., Lubensky, I., Dean, M., Allikmets, R., Chadhabaram, A., Beckmann, U. R., Feltis, J. T., Cassadavall, C., Zaman, A., Berns, M. S., Lips, C. J., Walther, M. M., Tsai, L. C., Gei, L., Orrell, C. M., Stackhouse, T. & Zbar, B. (1997) Nat. Genet. 16, 68–73
11. Rong, S., Segal, S., Anver, M., Resau, J. H. & Vande Woude, G. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4731–4735
12. Giordano, S., Bardelli, A., Zhen, Z., Menard, S., Ponzetto, C. & Comoglio, P. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13868–13872
13. Ullrich, A. & Schlessinger, J. (1990) Cell 61, 203–212
14. Johnson, L. N., Noble, M. E. & Owen, D. J. (1996) Cell 85, 149–158
15. Hubbard, S. R., Momand, M. & Schlessinger, J. (1998) J. Biol. Chem. 273, 11877–11890
16. Cannatella, L. C., Auger, K. R., Carpenter, C., Dworkh, R., Graziani, A., Kapeller, R. & Soltoff, S. (1991) Cell 64, 281–302
17. Kavanagh, M. W. & Williams, L. T. (1994) Science 266, 1862–1865
18. Weidner, K. M., Sachs, M. & Birchmeier, W. (1996) Nature 384, 173–176
19. Bardelli, A., Longati, P., Ponzetto, C. & Comoglio, P. M. (1996) J. Biol. Chem. 271, 20811–20819
20. Ponzetto, C., Zhen, Z., Audero, E., Maina, F., Bardelli, A., Basile, M. L., Girard, V., Cipolloni, F., Naldini, L. & Comoglio, P. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 577–587
21. Moley, J. F. (1997) Proc. Acad. Natl. Acad. Sci. U. S. A. 94, 577–587
22. Pasini, B., Ceccherini, I. & Romeo, G. (1996) J. Biol. Chem. 271, 1115–1125