The *Met* Receptor Tyrosine Kinase Transduces Motility, Proliferation, and Morphogenic Signals of Scatter Factor/ Hepatocyte Growth Factor in Epithelial Cells

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Abstract. Depending on the target cells and culture conditions, scatter factor/hepatocyte growth factor (SF/HGF) mediates several distinct activities, i.e., cell motility, proliferation, invasiveness, tubular morphogenesis, angiogenesis, or cytotoxicity. A small isoform of SF/HGF encoded by a natural splice variant, which consists of the NH_2-terminal hairpin structure and the first two kringle domains but not the protease homology region, induces cell motility but not mitogenesis. Two types of SF/HGF receptors have recently been discovered in epithelial cells, the high affinity c-*Met* receptor tyrosine kinase, and low affinity/high capacity binding sites, which are probably located on heparan sulfate proteoglycans. In the present study, we have addressed the question whether the various biological activities of SF/HGF are transduced into cells by a single type of receptor. We have here examined MDCK epithelial cells transfected with a hybrid cDNA encoding the ligand binding domain of the nerve growth factor (NGF) receptor and the membrane-spanning and tyrosine kinase domains of the *Met* receptor. We demonstrate that all biological effects of SF/HGF upon epithelial cells such as the induction of cell motility, proliferation, invasiveness, and tubular morphogenesis can now be triggered by the addition of NGF. Thus, it is likely that all known biological signals of SF/HGF are transduced through the receptor tyrosine kinase encoded by the c-*Met* protooncogene.

SCATTER factor/hepatocyte growth factor (SF/HGF)[1] has several distinct effects on cells in culture: (*a*) it is a strong mitogen for primary hepatocytes as well as other cell lines (Nakamura et al., 1987; Gohda et al., 1988; Zarnegar and Michalopoulos, 1989; Rubin et al., 1991); (*b*) it was independently identified as a motility and invasion-inducing factor for epithelial and endothelial cells (Stoker et al., 1987; Gherardi et al., 1989; Rosen et al., 1990; Weidner et al., 1990, 1991; Bussolino et al., 1992); (*c*) it has cytotoxic and cytostatic effects on certain other cell lines (Higashio et al., 1990; Tajima et al., 1991; Shiota et al., 1992); and (*d*) it was found to be an inducer of epithelial tubulogenesis (Montesano et al., 1991*a*,*b*). In vivo, SF/HGF thus could play a role in tissue regeneration, tumor progression, and embryological processes, which generally require both cell motility and cell proliferation.

The identity between SF and HGF was demonstrated by amino acid sequencing, by immunological methods, and by comparison of the biological activities (Gherardi and Stoker, 1990; Weidner et al., 1990, 1991; Furlong et al., 1991; Konishi et al., 1991) as well as by cDNA cloning, by receptor binding studies, and by the fact that a single gene exists in the human genome (Weidner et al., 1991; Naldini et al., 1991*a*). The SF/HGF cDNA, recently isolated from several sources such as placenta, liver, leukocytes, or fibroblasts (Miyazawa et al., 1989; Nakamura et al., 1989; Seki et al., 1990; Rubin et al., 1991; Weidner et al., 1991), encodes a 90-kD protein with sequence and overall structure similar to plasminogen and other enzymes involved in blood clotting and fibrinolysis. The primary translation product is an inactive precursor (Hartmann et al., 1992; Lokker et al., 1992) which is proteolytically processed into the active, disulfide-linked heterodimer. The heavy chain consists of a hairpin structure and four kringle domains, the light chain exhibits sequence similarity to serine proteases. Due to the absence of important catalytic amino acid residues (e.g., the serine of the active site), this light chain has probably no protease activity.

A high affinity receptor of SF/HGF ($K_d \sim 0.2$ nM) has recently been discovered in epithelial cells, the c-*Met* protooncogene product (Bottaro et al., 1991; Naldini et al., 1991*a*,*b*). *Met* is a transmembrane receptor tyrosine kinase (Cooper et al., 1984; Park et al., 1987) made of a 145-kD β- and a 50-kD α-subunit, which are synthesized as a single chain precursor (Giordano et al., 1989*a*,*b*). The α-chain and the NH_2-terminal portion of the β-chain are exposed on the cell surface, the COOH-terminal portion of the β-chain is located in the cytoplasm and contains the tyrosine kinase domain and phosphorylation sites involved in the regulation of

1. *Abbreviations used in this paper*: NGF, nerve growth factor; SF/HGF, scatter factor/hepatocyte growth factor.

its activity and in signal transduction (Ferracini et al., 1991; Naldini et al., 1991*c*). Isoforms of the *Met* receptor lacking the tyrosine kinase domain have recently also been described (Prat et al., 1991; Komoda et al., 1992). SF/HGF binds specifically to the *Met* receptor, as demonstrated by coprecipitation in immuncomplexes and by chemical crosslinking to the β-subunit of the *Met* protein, and it induces rapid autophosphorylation of *Met* on tyrosine residues (Bottaro et al., 1991; Naldini et al., 1991*a*,*b*; Hartmann et al., 1992). In addition, low affinity/high capacity binding sites of SF/HGF (K_d 2–7 nM) are detected in binding experiments (Naldini et al., 1991*a*; Komada et al., 1992). Since it is known that SF/HGF binds to heparin (Nakamura et al., 1987; Gohda et al., 1988; Zarnegar and Michalopoulos, 1989; Rosen et al., 1989; Weidner et al., 1990), it is likely that heparan sulfate proteoglycans of the extracellular matrix and the cell surface provide these additional binding sites. However, it was previously not known which of the pleiotropic signals of SF/HGF are mediated into the cells by which of these receptors.

A further interesting aspect of the action of SF/HGF results from the analysis of mutant and variant SF/HGF isoforms. We could recently show that the separately expressed heavy chain of SF/HGF as well as an isoform consisting of the NH_2-terminal hairpin structure and the first two kringle domains (Miyazawa et al., 1991; Chan et al., 1991), are sufficient to induce the motility but not the mitogenicity response (Hartmann et al., 1992).

In the present investigation we have addressed the question which of the SF/HGF receptors are responsible for the transmission of the diverse biological responses. For this purpose, we have introduced into epithelial cells a cDNA expression vector encoding a hybrid tyrosine kinase receptor which consists of the membrane spanning and cytoplasmic domains of *Met* fused to the extracellular domain of the nerve growth factor (NGF) receptor (*trk*). It has previously been shown that the extracellular domains of such hybrid receptors provide the ligand specificity, whereas the tyrosine kinase domain is responsible for the signalling specificity (Riedel et al., 1989; Berhanu et al., 1990). We then asked which of the biological activities normally induced by SF/HGF could now be observed in response to NGF.

Materials and Methods

Cytokines and Cell Lines

Recombinant SF/HGF was produced by cloning the SF/HGF cDNA (Weidner et al., 1991) into the pBlue Bac vector followed by expression in Sf9 insect cells using the Maxbac baculovirus system (Invitrogen, San Diego, CA). A one-step purification on Heparin Sepharose resulted in pure SF/HGF with a specific activity of 5 scattering units per nanogram protein (cf., Weidner et al., 1990). Purified NGF (2.5S) was purchased from Boehringer Mannheim GmbH (Mannheim, Germany) and ^{125}I-NGF from Amersham Buchler GmbH (Braunschweig, Germany). The origin and culture of the MDCK epithelial cell line (MDCK-2) is described in Behrens et al. (1989).

Construction and Expression of the NGF Receptor–Met Hybrid cDNA

The *trk* cDNA (encoding the human NGF receptor, plasmid LM6) was a kind gift of Dr. L. Parada (Frederick, MD). The mouse *Met* cDNA was isolated from a mouse liver cDNA library (ML 1035b; Clontech, Palo Alto, CA) using human *Met* cDNA as a probe (a kind gift of Dr. G. Vande Woude, Frederick, MD). Sequencing of our isolate revealed identity to the previously characterized mouse cDNA (Iyer et al., 1990; Chan et al., 1988). The cDNA fragments for the construction of the hybrid receptor were generated by restriction digest and PCR amplification using standard techniques (Sambrook et al., 1989): Fragment I encoding the extracellular portion of *trk* by EcoRI–BamHI digest (nucleotides −120 to 681); fragment II encoding the extracellular portion of *trk* by PCR resulting in a BamHI–XbaI fragment (nucleotides 682 to 1220); fragment III encoding the membrane-spanning and cytoplasmic portion of *Met* by AlwI–NotI digest (nucleotides 2787 to 4452). Nucleotides are numbered from the translation start site (Martin-Zanca, 1989; Iyer et al., 1990). The PCR reaction to generate fragment II was performed on plasmid LM6 using the primers 5′-GGTCCAGGTGCCCAATGCCTCGG and 5′-AGCTGCTCTAGATCGTCCTTCTTCTCCACCGG and Vent DNA polymerase (Biolabs, Beverly, MA). After ligation of fragment I and II with T4 DNA ligase (Biolabs), fragment III was introduced after removing the 5′ protruding ends of the XbaI and AlwI sites by Mung Bean nuclease (Stratagene, La Jolla, CA). The correct sequence of fragment II and the resulting hybrid cDNA was confirmed by sequencing using T7 DNA polymerase (Pharmacia Fine Chemicals, Piscataway, NJ). The hybrid receptor cDNA was introduced into the SalI site of the pBAT expression vector (Nagafuchi and Takeichi, 1988). Transfection of the hybrid receptor into MDCK epithelial cells was carried out using the calcium phosphate coprecipitation method of Graham and van der Eb (1973). In brief, 5×10^5 MDCK cells were transfected with 10 μg *trk–Met* construct and 1 μg pSV2-neo. After culture in neomycin selective medium (0.8 mg/ml G418/DMEM/10% FCS), resistant colonies were analyzed for their sensitivity towards NGF and expression of the introduced cDNA.

RNA Preparation, Northern Blotting, and Binding Assays

RNA was prepared according to Chomczynski and Sacchi (1987). 12 μg total RNA was glyoxylated and separated on a 1% agarose gel, transferred to Hybond-N membranes (Amersham Buchler GmbH) and hybridized with random-primed ^{32}P-labeled cDNA probes as described (Weidner et al., 1991). The last two washing steps were with 0.5× standard saline citrate/0.1% SDS at 62°C for 15-min each. Filters were subjected to autoradiography overnight at −70°C.

Specific cell surface binding of ^{125}I-NGF (2.5S, 2,000 Ci/mmol) was analyzed according to Klein et al. (1991) and Cordon-Cardo et al. (1991). In brief, 10^5 cells were incubated for 2 h at 4°C with 5 ng/ml ^{125}I-NGF in DME, 0.1% BSA in the presence or absence of a 100-fold excess of unlabeled NGF. Cells were washed five times in DME, 0.1% BSA, lysed in 1 M NaOH, and radioactivity was measured in a γ-counter.

Assays for Cell Motility, Matrix Invasion, Morphogenesis, and Growth Stimulation

Dissociation and cell motility was examined in the MDCK colony dissociation assay (Stoker et al., 1987; Weidner et al., 1990) in the presence or absence of the indicated concentrations of SF/HGF or NGF. Matrix invasion was examined as described previously (Behrens et al., 1989; Weidner et al., 1990), except that collagen was purchased from Seromed (Collagen G; Heidelberg, Germany). Invasive cells were counted after 3 d of culture by light microscopy. Tubule formation of MDCK cells was analyzed as described by Montesano et al. (1991*a*). In brief, MDCK cells were cultured within collagen gels until cyst formation occurred, and then exposed to SF/HGF or NGF for different time periods. Growth stimulation by SF/HGF and NGF was investigated on MDCK cells cultured between two layers of collagen matrices. 5×10^4 MDCK cells were plated on collagen gels in six-well plates (Nunc, Roskilde, Denmark) and overlayed with a second layer of collagen matrix. After incubation for 1 d in DME-10% FCS, the cells were further cultured in the presence or absence of SF/HGF or NGF. Growth was measured by counting cells in representative areas.

Metabolic Labeling, Immunoprecipitation, and Western Blotting

MDCK cells were labeled overnight with 0.2 mCi/ml [^{35}S]cysteine (Amersham Buchler GmbH) in cysteine-free DME-10% FCS, washed with PBS, and extracted for 20 min at 4°C with RIPA-kinase lysis buffer (50 mM Hepes, pH 7.2, 10 mM EDTA, 0.1% SDS, 1% NP-40, 0.5% deoxycholate, 50 mM Na-pyrophosphate, 100 mM Na-fluoride, 2 mM Na-orthovanadate, 30 mM phenylphosphate, 1 mM Zn-chloride, 50 μM ammonium molybdate, 35 μM phenyl arsine oxide, 1.25 mM PMSF, 10 μg/ml aprotinin, 50 μg/ml leupeptin). After clearing by ultracentrifugation at 100,000 *g*, immu-

noprecipitation was carried out using a 1:200 dilution of a polyclonal antiserum directed against the COOH terminus of mouse *Met* (Iyer et al., 1990; a generous gift of Dr. G. Vande Woude, Frederick) and protein A-Sepharose (Pharmacia Fine Chemicals). For competition, the immunizing peptide sp260 (10 μg/ml) was used. After washing in RIPA-kinase lysis buffer and high-salt buffer (50 mM Hepes, pH 7.2, 0.5 M Li-chloride, 10 mM EDTA, 1.25 mM PMSF, 10 μg/ml aprotinin, 2 mM orthovanadate), the immunoprecipitates were solubilized in boiling SDS-sample buffer and electrophoresed under non-reducing conditions on a 7.5% SDS-polyacrylamide gel, followed by fluorography at −70°C (cf. Iyer et al., 1990; Naldini et al., 1991*a*).

For examining tyrosine autophosphorylation of the NGF receptor-*Met* hybrid, MDCK transfectants were incubated for 30 min at 37°C in the presence or absence of 100 ng/ml NGF in DME, 0.5% BSA, 7 μM phenyl arsine oxide. After lysis of the cells, immunoprecipitation with anti-*Met* antibody, and SDS gel electrophoresis as described above, the proteins were transferred onto Nitrocellulose membranes (Millipore Corp., Bedford, MA), probed with an anti-phosphotyrosine mAb (UBI, Lake Placid, NY), and stained using the enhanced chemiluminescence system (ECL, Amersham, Braunschweig).

Results

Construction and Expression of a NGF Receptor (trk)-Met Hybrid Tyrosine Kinase

The cDNA encoding the extracellular domain of the human high affinity NGF receptor (*trk*; Martin-Zanca et al., 1989; Kaplan et al., 1991) was fused to the sequence coding for the transmembrane and cytoplasmic regions of mouse *Met* (Fig. 1). The chosen fusion point is located exactly at the outside border of the transmembrane domain. The resulting hybrid receptor (NGFR-*Met*) thus consists of the ligand-binding domain of the NGF receptor containing characteristically spaced cysteinyl residues (Martin-Zanca et al., 1989). The cytoplasmic portion of the hybrid receptor consists of the tyrosine kinase domain of *Met* which should confer *Met*-specific downstream signalling. The hybrid receptor cDNA in pBAT was cotransfected with pSV2-neo into MDCK epithelial cells, and subclones which have integrated the foreign DNA were selected for by G418.

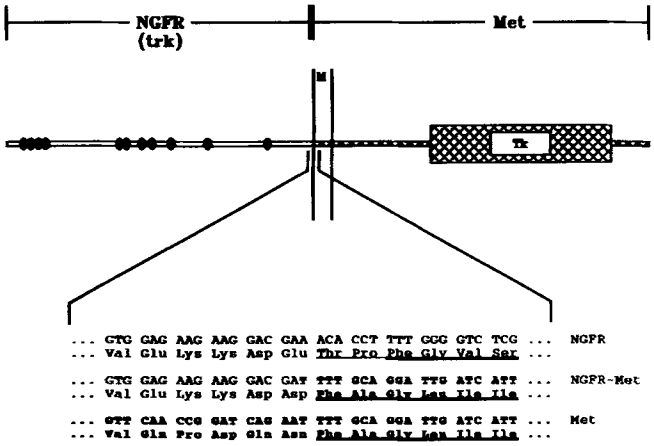

Figure 1. Schematic structure of the NGF receptor-*Met* hybrid. The extracellular domain of the human NGF receptor (*trk*) is fused to the transmembrane and cytoplasmic domains of the mouse *Met* (marked are the cysteinyl residues of *trk*; *Tk*, tyrosine kinase domain of *Met*). Nucleotide and amino acid sequences of the fusion point at the outside border of the transmembrane region are shown below (*underlined*, sequences of the transmembrane region; *NGFR*, human NGF receptor; *Met*, mouse *Met*; NGFR-*Met*, receptor hybrid). The cloning strategy resulted in one conserved amino acid change at the last position of the NGF receptor sequence, i.e., Glu to Asp.

The NGF Receptor-Met Hybrid Tyrosine Kinase Confers NGF-mediated Dissociation and Motility Response in MDCK Epithelial Cells

G418-resistant MDCK colonies were expanded and tested functionally for their ability to respond with cell dissociation toward the new ligand NGF. In fact, five out of 24 clones dissociated at NGF concentrations between 10 and 30 ng/ml (see example in Fig. 2, *b* and *c*; compare to control without NGF in *a*). Parental MDCK cells and control cells containing only pSV2-neo showed no dissociation or morphological shape changes caused by NGF, even at high NGF concentrations (Fig. 2 *d*).

The NGF Receptor-Met Hybrid Tyrosine Kinase Is Expressed and Properly Located in the Plasma Membrane of the MDCK Transfectants

RNA from the NGF-responding subclones was analyzed by Northern blotting for the expression of the *trk-Met* hybrid. In all transfectants which were sensitive to NGF in the dissociation assay, we detected a new mRNA species with the expected size of 4.5 kb, which hybridized with both a human *trk* probe coding for the extracellular portion of the NGF receptor (Fig. 3, left filter, lanes *a–d*) and a mouse *Met* probe coding for the membrane-spanning and cytoplasmic region (Fig. 3, right filter, lanes *a–d*). Control MDCK cells did not express NGF receptor transcripts (Fig. 3, left filter, lane *e* and *f*). However, we could detect endogenous *Met* in all MDCK clones tested (Fig. 3, right filter, lanes *a–f*). Approximately equal amounts of hybrid receptor and endogenous *Met* mRNA were observed in the NGF-sensitive MDCK clones. The proper cellular location of the NGF receptor-*Met* hybrid in the transfected MDCK cells was examined by cell surface binding experiments using ^{125}I-NGF. Specific binding to the NGF receptor-*Met* transfectants could in fact be measured (Fig. 4), which was competed for by an excess of unlabeled ligand. No specific binding was seen on control MDCK cells containing only the pSV2-neo plasmid. These combined results indicate that the introduced NGF receptor-*Met* hybrid is properly expressed and inserted into the plasma membrane of MDCK cells and forms a functional transmembrane receptor.

The trk-Met Hybrid Receptor is Phosphorylated on Tyrosine Residues in Response to NGF

An antibody directed against the COOH-terminus of *Met* was used to immunoprecipitate the *trk-Met* hybrid receptor. A new protein with a mol wt of 140 kD was in fact detected in metabolically labeled, NGF-sensitive MDCK cells (Fig. 5 *A*, lanes *a* and *b*, see arrow). Control MDCK cells expressed only endogenous *Met* with a mol wt of 190 kD (*arrowhead* in Fig. 5 *A*, lane *c*). Immunoprecipitation of both the hybrid receptor and *Met* was prevented in the presence of competing peptide (Fig. 5 *A*, lanes *d–f*, see also Materials and Methods). Tyrosine autophosphorylation of the hybrid receptor was examined in transfected MDCK cells which were treated with and without NGF. The addition of the new

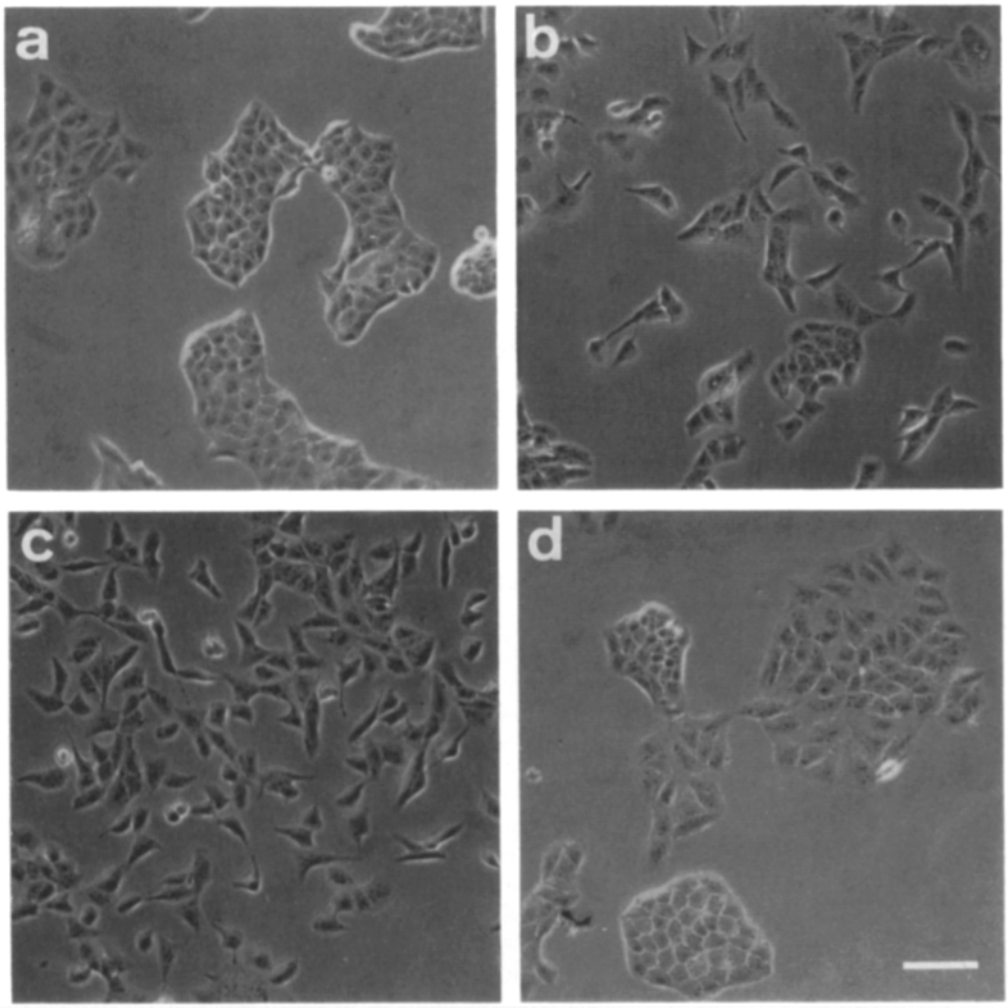

Figure 2. NGF receptor–*Met* transfected MDCK cells are dissociated in a dose-dependent manner by NGF. (*a*) No factor, (*b*) 10 ng/ml NGF, and (*c*) 30 ng/ml NGF. pSV2-neo control transfectants are not affected by NGF even at high concentrations (*d*, 100 ng/ml NGF). Bar, 100 μm.

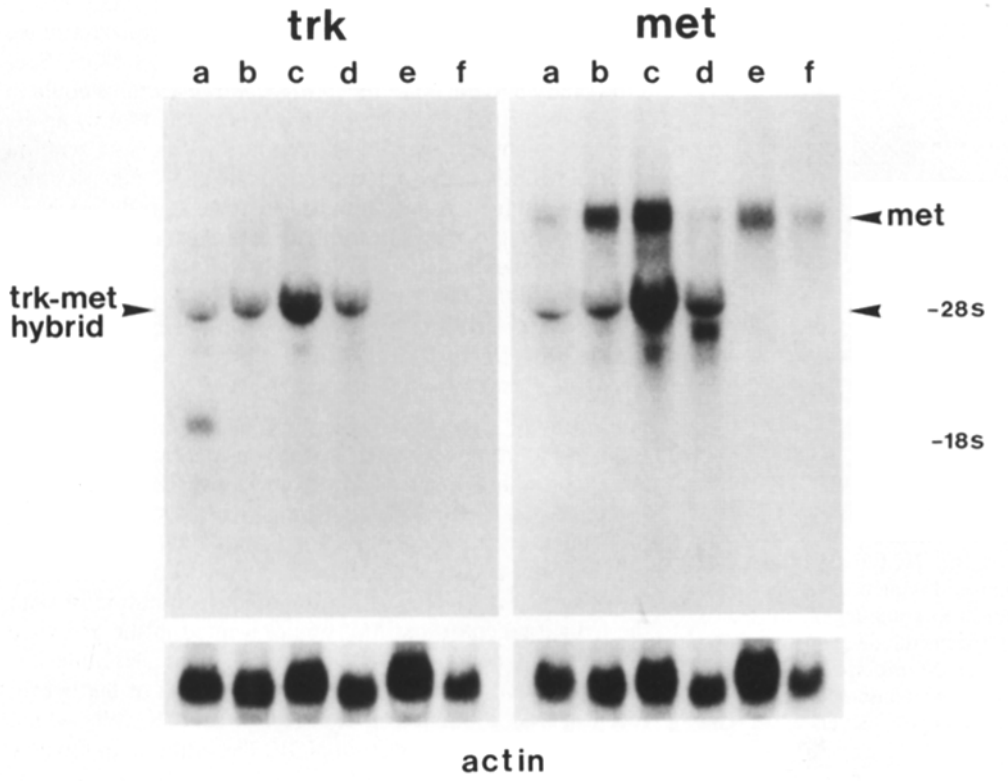

Figure 3. Detection of NGF receptor (*trk*)–*Met* hybrid mRNA in MDCK transfectants by Northern blotting. (*a–d*) The specific *trk-Met* hybrid transcript (4.5 kb) was detected with a *trk* probe (left filter) and a *Met* probe (right filter) in the NGF-sensitive *trk–Met* transfectants 7, 2, 18, and 3; (*e* and *f*) represent neo control transfectants 1 and 2. The *Met* probe also detected endogenous *Met* in all MDCK cell clones. RNA loading was verified by hybridization of the filters with a ^{32}P-labeled actin probe.

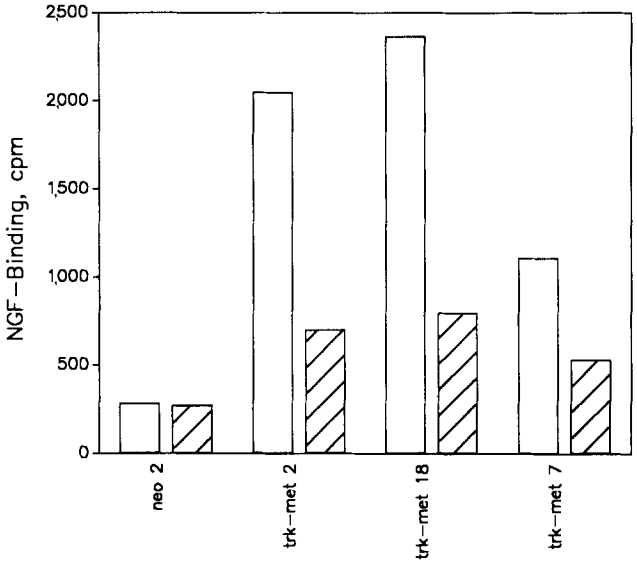

Figure 4. Expression of *trk-Met* hybrid receptor on the surface of MDCK cell clones confers specific NGF binding. Binding of 5 ng/ml ^{125}I-NGF was measured in the absence (□) or presence (▨) of 500 ng/ml unlabeled NGF. No specific binding was seen on control cells (*neo 2*). Mean values of triplicate samples of two independent experiments are given.

ligand strongly increased tyrosine phosphorylation of the 140-kD hybrid protein, as revealed by Western blotting of immunoprecipitates using anti-phosphotyrosine antibodies (arrow in Fig. 5 *B*, lanes *a* and *c*, compare with the control in the absence of NGF, lanes *b* and *d*). Control transfectants did not show any specific labeling of the 140-kD protein (Fig. 5 *B*, lanes *e* and *f*). The endogenous 190-kD *Met* was not transphosphorylated in response to NGF (Fig. 5 *B*); vice versa, stimulation of the *trk-Met* transfected MDCK cells with SF/HGF resulted in strong autophosphorylation of the 190- but not the 140-kD protein (data not shown, cf., also Hartmann et al., 1992). Thus, binding of the NGF to the extracellular portion of the *trk–Met* hybrid receptor leads to the activation of the chimeric *Met* tyrosine kinase.

The trk–Met *Hybrid Mediates NGF-dependent Invasiveness and Tubulogenesis of MDCK Epithelial Cells in Collagen Gels*

When MDCK epithelial cells are plated on top of collagen gels, SF/HGF induces invasiveness of single cells into the gel matrix (Weidner et al., 1990, 1991). Depending on the plating density, up to 10% of the cells can thus be forced to enter the gel within 2–3 d. We show here that MDCK clones expressing the NGF receptor–*Met* hybrid invade collagen gels in response to NGF (Fig. 6). The overall sensitivity of these cells toward NGF and SF/HGF was similar. The control clones containing pSV2-neo were only sensitive to SF/HGF but not to NGF.

When SF/HGF is added to MDCK epithelial cells pregrown as cysts in collagen gels, the factor induces the outgrowth of tubules within a few days (Montesano et al., 1991*a*,*b*). Similar outgrowth of tubular networks is produced by NGF in MDCK cells expressing the NGF receptor–*Met* hybrid. This process appears to occur in two steps; within the first 1–2 d, mainly single cells and small cell groups move away from the cysts (Fig. 7 *c*; compare to the control without NGF in *a*. After 2–5 d, however, the invading cells form cords of tubular structures (Fig. 7 *e*). The responsiveness of the transfected cells toward NGF and SF/HGF was similar (Fig. 7, *d* and *f*); control clones containing pSV2-neo were insensitive toward NGF (Fig. 7 *b*).

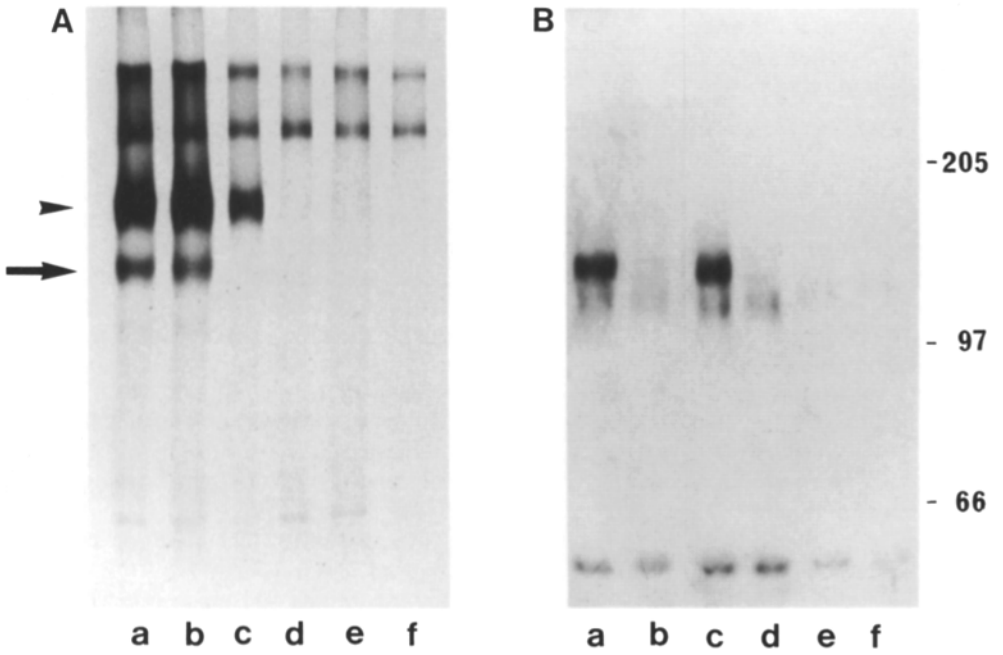

Figure 5. The 140 kD *trk–Met* hybrid receptor is phosphorylated on tyrosine residues in response to NGF. (*A*) [^{35}S]-cysteine-labeled MDCK clones expressing *trk-Met* (*a* and *d*, *trk-Met* 2; *b* and *e*, *trk-Met* 18) and a clone expressing only the neo resistance gene (*c* and *f*) were lysed and immunoprecipitated by an anti-*Met* polyclonal antibody in the absence (*a–c*) and presence (*d–f*) of competing peptide. The arrow marks the hybrid receptor at 140 kD, the arrowhead indicates the endogenous *Met* at 190 kD. (*B*) MDCK clones expressing the *trk-Met* hybrid (*a* and *b*, *trk-Met* 2; *c* and *d*, *trk-Met* 18) and a clone containing pSV2-neo only (*e* and *f*) were stimulated in the presence (*a*, *c* and *e*) and absence (*b*, *d*, and *f*) of NGF (100 ng/ml). The hybrid receptor was immunoprecipitated with anti-*Met* antibody followed by Western blotting using an anti-phosphotyrosine antibody. The arrow marks the tyrosine-phosphorylated 140-kD hybrid receptor.

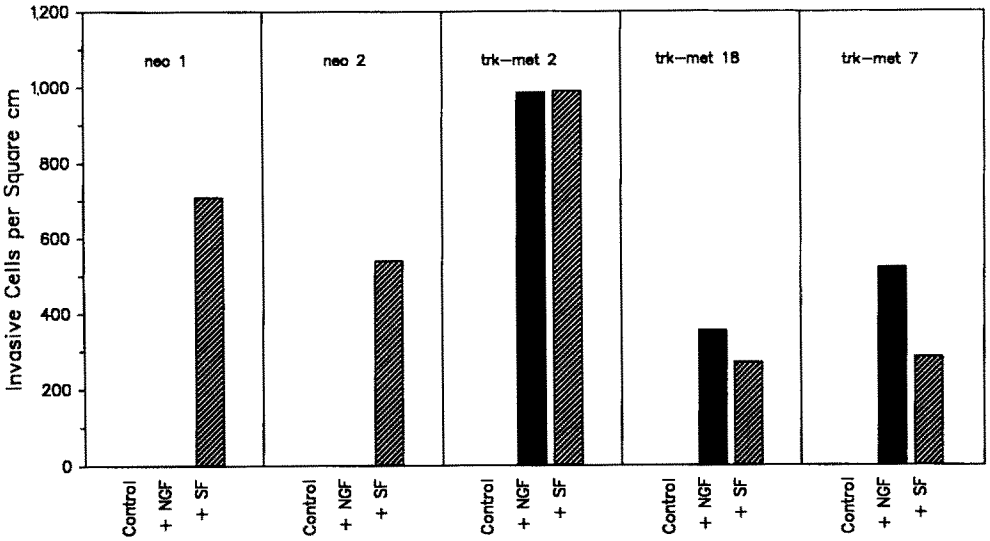

Figure 6. NGF induces invasiveness of *trk–Met* transfected MDCK cells into collagen matrices. NGF (100 ng/ml, black bars) and SF/HGF (100 ng/ml, hatched bars) strongly induced invasion of the *trk–Met*-expressing MDCK clones, whereas pSV2-neo clones only responded to SF/HGF. Without addition of factors no invasion was measured (*control*).

The NGF Receptor–Met Hybrid Mediates NGF-dependent Growth Stimulation

In the course of this study we observed that parent MDCK cells proliferate slowly when cultured within collagen matrices (Fig. 8, and data not shown); in the presence of SF/HGF, however, we found strong growth promotion. A similar growth-stimulatory effect is also inducible by the addition of NGF to transfectants expressing the NGF receptor–*Met* hybrid (Fig. 8), whereas control clones were not sensitive to NGF.

These combined results using MDCK epithelial cells expressing the hybrid NGF receptor–*Met* tyrosine kinase clearly demonstrate that *Met* is the key component to transmit the SF/HGF signals into epithelial cells and to elicit the pleiotropic biological effects. The responses mediated by *Met* thus include cell dissociation and shape change toward a fibroblastoid morphology, the motility response, the invasiveness into extracellular matrices, and the morphogenic (tubulogenic) action as well as the growth-promoting activity in collagen gels.

Discussion

The motility/growth factor SF/HGF induces a variety of biological responses in different cell types (cf., Nakamura et al., 1989; Rosen et al., 1990; Weidner et al., 1990, 1991; Rubin et al., 1991; Montesano et al., 1991*b*; Shiota et al., 1992; Bussolino et al., 1992), and there exist at least two types of SF/HGF receptors in epithelial cells (Naldini et al., 1991*a*): the high affinity *Met* receptor and low affinity/high capacity binding sites. Since SF/HGF is known to bind to heparin, these low affinity binding sites are likely confined to heparan sulfate proteoglycans. Although it has been demonstrated that binding of SF/HGF to epithelial cells results in the autophosphorylation of tyrosine residues of the *Met* receptor (Bottaro et al., 1991; Naldini et al., 1991*a*,*b*; Hartmann et al., 1992), it was previously not known which of the biological signals, if any, are mediated by which of the known SF/HGF receptors. Furthermore, heavy chain isoforms of SF/HGF produced only the motility but not the mitogenicity response (Hartmann et al., 1992), suggesting the participation of different types of receptors in motogenic and mitogenic signal transduction. In the present investigation we could clearly show, by examining a hybrid NGF receptor–*Met* tyrosine kinase in MDCK epithelial cells, that cell dissociation and motility response, induction of invasiveness and tubulogenesis, and growth stimulatory effects are mediated specifically through the hybrid *Met* tyrosine kinase. Thus, it is likely that all known biological responses of SF/HGF are actually transduced by the *Met* receptor, while the role of the low affinity binding sites is presently unknown.

The question whether signal transduction through the *Met* receptor is sufficient to transduce the pleiotropic effects of SF/HGF could here not be addressed by simply expressing the *Met* cDNA in *Met*-negative cells. For instance, fibroblasts which do not express significant levels of *Met*, often produce the ligand and they are motile without stimulation: epithelial cells which are main targets for SF/HGF, express both high and low affinity receptors. In contrast, the chosen approach of expressing a hybrid NGF receptor–*Met* kinase in epithelial cells was ideally suited to address our question: (*a*) High affinity NGF receptors (the *trk*'s) are not expressed in epithelial cells (Klein et al., 1990; Martin-Zanca et al., 1990); (*b*) Low affinity NGF receptors (p75; Johnson et al., 1986; Radecke et al., 1987) could also not be detected on MDCK cells; and (*c*) In contrast to SF/HGF, NGF does not bind to heparin. Thus, in the used experimental system neither heparan sulfate proteoglycan binding sites nor other endogenous receptors interfere with the action of NGF via the hybrid receptor. Furthermore, MDCK epithelial cells could here be used for the transfection with the hybrid receptor cDNA, and these cells show a wide variety of biological responses to SF/HGF.

The MDCK cells transfected with the NGF receptor–*Met* hybrid cDNA expressed a novel 4.5-kb mRNA species, which hybridized to both *trk* and *Met* sequences. The newly generated 140-kD hybrid protein could be immunoprecipitated with anti-*Met* antibodies and was found to be properly located in the plasma membrane, as shown by NGF surface binding. Furthermore, the 140-kD hybrid receptor was au-

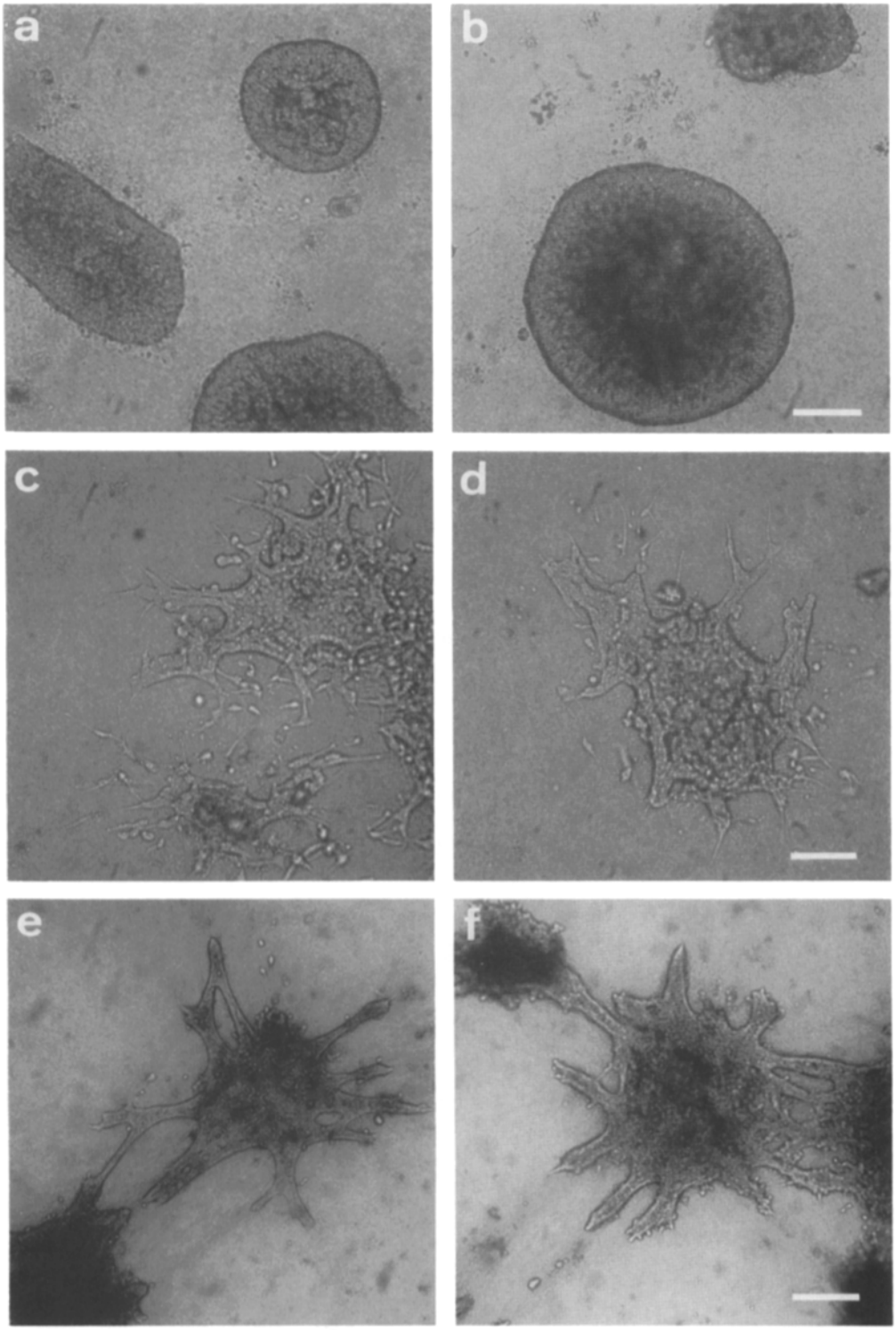

Figure 7. NGF acts as an epithelial morphogen for *trk-Met* transfected MDCK cells. MDCK cells expressing the hybrid receptor and control MDCK cells were grown for two weeks in collagen gels until the formation of large cysts was observed. The addition of NGF (200 ng/ml) for one (*c*) or 5 d (*e*), or SF/HGF (200 ng/ml) for one (*d*) or 5 d (*f*), resulted in the outgrowth of single cells and the formation of a tubular network. (*a*) No factor added, (*b*) neo control clone, 5 d after the addition of 200 ng/ml NGF. Bars: (*a–d*) 100 μm; (*e* and *f*) 250 μm.

tophosphorylated on tyrosine residues in response to NGF. Thus, the MDCK cells expressing the *trk-Met* hybrid receptor met all formal requirements for mediating NGF signals via the *Met* tyrosine kinase. Remarkably, the MDCK cell clones which were initially selected to show the NGF response in the dissociation assay, were also sensitive, at the expected concentrations of ligand, in the invasion and tubulogenesis assays as well as in the newly developed growth assay. This indicates that one type of hybrid receptor population, for which we have selected by one particular assay, also transmits the other biological signals.

We have previously found that SF/HGF induces invasiveness of various epithelial cells when these are plated on the top of collagen gels (Weidner et al., 1990, 1991). It was suggested that this process resembles the progression of carcinoma cells to a more malignant stage in vivo. Montesano et

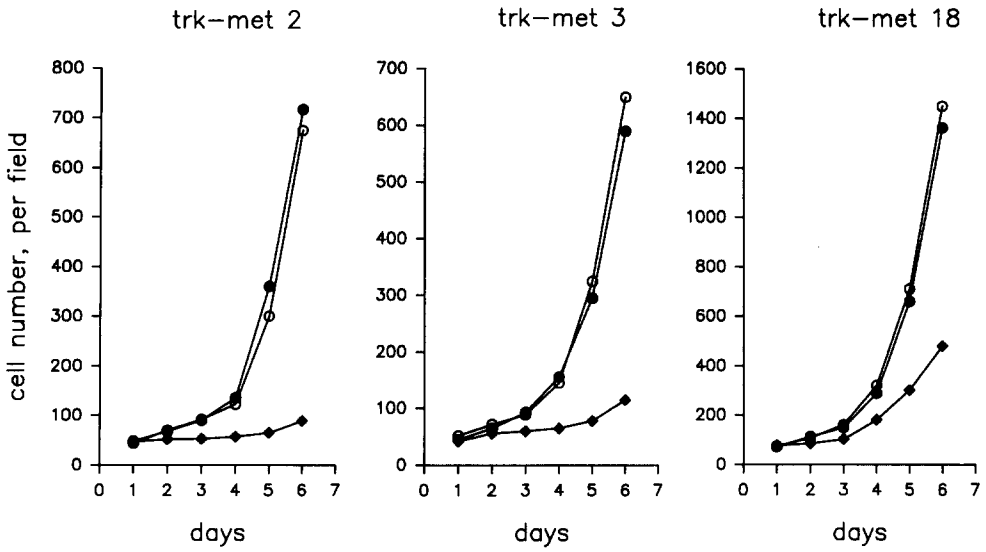

Figure 8. NGF stimulates proliferation of *trk-Met* expressing MDCK cells. The cells were plated between two layers of collagen gels and were further cultured in 200 ng/ml NGF (○), 200 ng/ml SF/HGF (●), or no factor (◆). Cell numbers were determined by counting five representative fields of 1.4 mm^2. Mean values of two independent experiments are given.

al. (1991*a*,*b*) have recently reported that precultured cysts of MDCK cells in collagen gels form tubular structures following the addition of SF/HGF. It was suggested that this process resembles the outgrowth of kidney tubules during embryonal morphogenesis. How can the invasiveness of (mainly single) cells be reconciled with the outgrowth of tubules? When we here examined MDCK cells expressing the NGF receptor–*Met* hybrid in collagen gels, we could clearly identify two stages of outgrowth (Fig. 7). In a first wave, mainly single cells migrated away from the cysts, while at later stages the formation and outgrowth of tubules was observed. This two-stage behavior was also seen when the outgrowth was induced by SF/HGF. Thus, our data suggest that invasiveness and tubulogenesis (at least in its initial phase) occur by a similar mechanism.

Previously, we did not assay for proliferation signals in the case of MDCK cells, since these cells are difficult to growth-arrest in conventional tissue culture. However, when MDCK cells were here cultured between sheets of collagen gels, we observed clear inhibition of proliferation, which could be overcome by the addition of SF/HGF. Proliferation could also be triggered with NGF in the MDCK cells expressing the NGF receptor–*Met* tyrosine kinase. Similarly, growth of MDCK cells in soft agar was stimulated by SF/HGF (Uehara and Kitamura, 1992). This clearly indicates that motility, morphogenic, and growth responses of MDCK cells toward SF/HGF are transduced by the activation of a single receptor, *Met*. It is obvious that the cytotoxic and angiogenic effects of SF/HGF (as seen in other cell types) could not be tested with our transfected MDCK cells and thus, the question whether these biological responses to SF/HGF are also transduced through *Met* will require further studies.

The motility/growth factor SF/HGF has a unique structure which has not been observed in other motility or growth factors (for the case of thrombin see Vu et al., 1991). SF/HGF is similar in its amino acid sequence to proteases involved in fibrinolysis or blood coagulation. In these proteins, the kringle domains and other modules contained within one subunit bind to specific protein sites, whereas the second subunit, the serine protease, then cleaves selected substrates (Van Zonneveld et al., 1986; Roldan et al., 1990). We have recently shown that a naturally occurring isoform of SF/HGF consisting of only the NH_2-terminal region and the first two kringle domains of the heavy chain is able to bind the *Met* receptor and to trigger the motility but not the mitogenic response (Hartmann et al., 1992). The function of the light chain of SF/HGF, which does not seem to exert proteolytic activity, is presently unknown. However, since the whole heterodimeric molecule seems to be required for the generation of a full mitogenic response, the light chain may participate in this activity. Our present data now indicate that the various isoforms of SF/HGF mediate motility and/or growth signals through a single receptor, *Met*. The detailed mechanisms of signalling leading to the different types of cellular responses are not yet known. It is interesting, however, that in the case of the related FGF receptor, a point mutation in the tyrosine kinase domain abolished phosphatidylinositol turnover but not mitogenesis (Peters et al., 1992; Mohammadi et al., 1992). The present data also suggest that the low affinity/high capacity binding sites of SF/HGF on epithelial cells are not directly involved in signal transduction. These low affinity binding sites might still play an important role in the storage of the factor in the extracellular matrix or in its presentation to the *Met* receptor. Such a role for heparan sulfate proteoglycans has recently been demonstrated for the FGF receptor system (Yayon et al., 1991; Rapraeger et al., 1991).

Coordinated movement, growth, and differentiation of cells are essential prerequisites for embryonal development and tissue regeneration in vivo. A reoccurring picture in development is that certain groups of cells influence motility, growth, and differentiation of other cells in their vicinity. In particular, exchange of signals between epithelial and mesenchymal cell compartments are major driving forces in development (cf., Ekblom, 1989; Gumbiner, 1992, for reviews). Recently, several receptor tyrosine kinases expressed in epithelia and their mesenchymally derived ligands have been implicated in these mesenchymal-epithelial interactions (cf., Stern et al., 1990; Montesano et al., 1991*a*,*b*; Sonnenberg et al., 1991; Sonnenberg et al., 1993; Wen et al., 1992;

Holmes et al., 1992). The best characterized of these receptor/ligand systems possibly involved in mesenchymal-epithelial interactions is SF/HGF/c-*Met*. Studies of SF/HGF and *Met* expression in cell culture show that the ligand is generally not produced by the same cells which respond to it and which express the receptor (Stoker et al., 1987; Weidner et al., 1991; Naldini et al., 1991*a*). Furthermore, during mouse embryogenesis the cells expressing SF/ HGF are also different but often in close vicinity to cells which transcribe the c-*Met* gene, suggesting a paracrine mode of action in vivo as well. For example, during kidney development c-*Met* is expressed in the epithelia of the ureter buds and of the proximal and distal tubules; transcripts for SF/HGF are found in the surrounding nephrogenic mesenchyme (Sonnenberg et al., 1993). Our present data suggest that the paracrine effects of SF/HGF in vivo leading to changes in cell motility, migration, differentiation, and proliferation, are also mediated through the protooncogene product c-*Met*.

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