Motogenic and Morphogenic Activity of Epithelial Receptor Tyrosine Kinases

Martin Sachs,* K. Michael Weidner,* Volker Brinkmann,* Ingrid Walther,* Axel Obermeier,‡ Axel Ullrich,‡ and Walter Birchmeier*

*Max-Delbrück-Center for Molecular Medicine, 13125 Berlin, Germany; and ‡Max-Planck-Institute for Biochemistry, 82152 Martinsried, Germany

Abstract. Receptor tyrosine kinases play essential roles in morphogenesis and differentiation of epithelia. Here we examined various tyrosine kinase receptors, which are preferentially expressed in epithelia (c-met, c-ros, c-neu, and the keratin growth factor [KGF] receptor), for their capacity to induce cell motility and branching morphogenesis of epithelial cells. We exchanged the ligand-binding domain of these receptors by the ectodomain of trkA and could thus control signaling by the new ligand, NGF. We demonstrate here that the tyrosine kinases of c-met, c-ros, c-neu, the KGF receptor, and trkA, but not the insulin receptor, induced scattering and increased motility of kidney epithelial cells in tissue culture. Mutational analysis suggests that SHC binding is essential for scattering and increased cell motility induced by trkA. The induction of motility in epithelial cells is thus an important feature of various receptor tyrosine kinases, which in vivo play a role in embryogenesis and metastasis. In contrast, only the c-met receptor promoted branching morphogenesis of kidney epithelial cells in three-dimensional matrices, which resemble the formation of tubular epithelia in development. Interestingly, the ability of c-met to induce morphogenesis could be transferred to trkA, when in a novel receptor hybrid COOH-terminal sequences of c-met (including Y14 to Y16) were fused to the trkA kinase domain. These data demonstrate that tubulogenesis of epithelia is a restricted activity of tyrosine kinases, as yet only demonstrated for the c-met receptor. We predict the existence of specific substrates that mediate this morphogenesis signal.

In the last decade, a large variety of receptor tyrosine kinases were molecularly characterized (for reviews see Ullrich and Schlessinger, 1990; van der Geer et al., 1994). Several of these receptors were first discovered because of their transforming potential and were therefore associated with mediating mitogenic signals (Cooper et al., 1984; Ullrich et al., 1984; Takahashi et al., 1985; Birchmeier et al., 1986). However, it has recently become evident that tyrosine kinase receptors can also regulate decisive events in development; i.e., these receptors control cell movement, morphogenesis, and differentiation (Montesano et al., 1991*a*,*b*; Peles et al., 1993; Schuchardt et al., 1994; Bladt et al., 1995, Cheng et al., 1995; Drescher et al., 1995; Meyer and Birchmeier, 1995; Werner et al., 1994; Yang et al., 1995; for a recent review see Birchmeier and Birchmeier, 1993).

Previously, we and others have investigated the morphogenic activity of receptor tyrosine kinases on epithelial cells. It is noteworthy that several tyrosine kinase receptors exhibit exclusive or prevalent expression on epithelial cells. These include c-met, c-ret, c-ros, c-neu (c-erbB2), and the keratinocyte growth factor receptor (KGFR)[1] (Miki et al., 1991; Sonnenberg et al., 1991, 1993; Peles et al., 1992; Schuchardt et al., 1994). The corresponding ligands, scatter factor/ hepatocyte growth factor (SF/HGF), keratinocyte growth factor (KGF), and neuregulin, were found to be preferentially synthesized by mesenchymal cells (Stoker et al., 1987; Sonnenberg et al., 1993; Rubin et al., 1989; Orr-Urtreger et al., 1993, Meyer and Birchmeier, 1995). SF/HGF has been described as a pleiotropic factor with various activities on epithelial cells in culture: (*a*) it promotes growth of primary hepatocytes and other epithelial cells (Miyazawa et al., 1989; Nakamura et al., 1989; Zarnegar and Michalopoulos, 1989); (*b*) it can induce motility and invasiveness of epithelial and endothelial cells (Stoker et al., 1987; Weidner et al., 1990; Bussolino et al., 1992;

Address all correspondence to Walter Birchmeier, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13125 Berlin, Germany. Tel.: (49) 30 9406 3800. Fax: (49) 30 9406 2656. e-mail: wbirch @mdc-berlin.de.

1. *Abbreviations used in this paper*: IR, insulin receptor; KGF, keratinocyte growth factor; KGFR, KGF receptor; PI3, phosphatidylinositol 3; PLCγ, phospholipase C γ; SF/HGF, scatter factor/hepatocyte growth factor.

© The Rockefeller University Press, 0021-9525/96/06/1095/13 $2.00

Grant et al., 1993); and (*c*) it has a third and unusual activity on epithelial cells, the ability to induce branching morphogenesis or the formation of crypts in three-dimensional matrices (Montesano et al., 1991*a*,*b*; Berdichevsky et al., 1994; Brinkmann et al., 1995; Soriano et al., 1995). SF/HGF also controls branching morphogenesis of mouse mammary glands in organ culture and is expressed at appropriate times in the mammary gland during development (Yang et al., 1995). The various signals of SF/HGF are mediated by the c-met tyrosine kinase, as has been shown by the use of a hybrid receptor (Weidner et al., 1993; cf. also Bottaro et al., 1991; Naldini et al., 1991; Komada and Kitamura, 1993; Zhu et al., 1994).

KGF was characterized as a growth factor for skin epithelial cells and is a member of the FGF family (Rubin et al., 1989), which reacts with the FGF2-IIb receptor (Miki et al., 1991). The c-neu (c-erbB2) receptor, together with two closely related molecules, c-erbB3 and c-erbB4, mediates the signaling of a recently described factor, neuregulin (Holmes et al., 1992; Wen et al., 1992; Carraway and Cantley, 1994). Neuregulin (also named neu differentiation factor, heregulin, glial growth factor, or ARIA) is produced by mesenchymal and nerve cells (Orr-Urtreger et al., 1993; Meyer and Birchmeier, 1994) and acts on epithelial, muscle, and glial cells (Falls et al., 1993; Marchionni et al., 1993; Meyer and Birchmeier, 1995). Other epithelial receptor tyrosine kinases exist, to which no ligands were assigned yet, such as c-ros or c-ret (Riethmacher et al., 1994; Schuchardt et al., 1994).

The importance of receptor tyrosine kinases and their ligands in mesenchymal-epithelial interactions has recently been supported by genetic experiments. Targeted mutations in the SF/HGF and the c-met genes in mice lead to an identical phenotype, embryonal lethality caused by severe deficiencies in the development of embryonal liver and placenta (Schmidt et al., 1995; Uehara et al., 1995). Furthermore, SF/HGF and c-met $-/-$ animals do not form particular muscles because of a defect in migration of myogenic precursor cells from the somites to these target sites (Bladt et al., 1995). A dominant-negative KGF receptor under the control of the human lung surfactant protein C promoter induced severe defects in lung development (Peters et al., 1994). This transdominant receptor also interfered with the development of the skin when expressed in keratinocytes in vivo and prevented normal wound healing (Werner et al., 1993, 1994). A targeted mutation in the c-ret gene of the mouse induced severe hypoplasia or aplasia of the kidney and defects in development of the enteric nervous system (Schuchardt et al., 1994). Targeted mutation of the neuregulin, c-erbB2, and c-erbB4 genes in transgenic mice leads to embryonal death at day 10 due to severe heart defects. In addition, cranial ganglia are absent, and the Schwann cell population in the trunk is greatly reduced (Meyer and Birchmeier, 1995; Lee et al., 1995; Gassmann et al., 1995). Targeted mutation of c-ros leads to male sterility due to abnormal development of epididymal epithelia (Riethmacher-Sonnenberg et al., 1996).

A major question concerns the specific signal transduction pathways involved in growth, morphogenesis, and differentiation of epithelia that are mediated by tyrosine kinase receptors. Considerable evidence has been accumulated that relates to signal transduction by activated (oncogenic) tyrosine kinases. For instance, one of five COOH-terminal tyrosine residues of neu/erbB2 (Y1253) is responsible for transducing the mitogenic signal, a biochemical pathway that includes ras, mitogen-activated protein kinase, and transactivation of c-jun (Ben-Levy et al., 1994). In c-met, Y1354 (Y15), and to a lesser degree Y1347 (Y14), are involved in mitogenic signaling. These sites bind GRB-2, phospholipase C γ (PLCγ), c-src, and phosphatidylinositol 3 (PI3) kinase and other substrates (Ponzetto et al., 1994; Fixman et al., 1995). A c-ret pathway used in mitogenic signaling has also been characterized, and this activity is changed in germline mutations of MEN2A and MEN2B patients (Santoro et al., 1994; 1995). Ligand-dependent substrate activation of receptor tyrosine kinases with morphogenic signals have also been studied: for instance, the COOH-terminal domain of let-23 (an EGF receptor homologue in *Caenorhabditis elegans*) could be partitioned into subdomains with different cell type–specific functions (Aroian et al., 1994). In the NGF receptor (trkA), mutation of Y490 (an SHC binding site) and a deletion of a conserved sequence in the juxtamembrane domain abolished NGF-induced neurite outgrowth in PC12 cells (Obermeier et al., 1994; Stephens et al., 1994; Peng et al., 1995). Ligand-induced dissociation and branching morphogenesis of c-met in epithelial cells were affected by mutation of Y1354 (Y15) and Y1363 (Y16) (Weidner et al., 1995). In the PDGFβ receptor, the kinase insert contains the structural requirements for the motility response in endothelial cells (Wennström et al., 1994), which are binding sites for PI3 kinase (Fantl et al., 1992; Kashishian et al., 1992). Neurite outgrowth of PC12 cells could also be induced by the PDGFβ receptor; here, PI3 kinase and PLCγ are involved (Vetter and Bishop, 1995).

In the present investigation, we have analyzed the potential of several epithelial receptor tyrosine kinases to induce motility and morphogenic signals in MDCK epithelial cells. We found that the signal for branching morphogenesis of kidney epithelial cells is specific for c-met, whereas dissociation and increased motility can be transmitted by several tyrosine kinase receptors. A morphogenic signal could be created by fusing the COOH-terminal tail of c-met to the kinase domain of the trkA receptor, indicating that Y14 to Y16 of c-met are critical in mediating a signal that induces morphogenesis.

Materials and Methods

Construction and Expression of the trkA Receptor Hybrids

The trkA-cDNA (encoding the human NGF receptor, plasmid pLM6) was obtained from Dr. L. Parada (National Cancer Institute, Frederick, MD). The cDNAs of the human insulin receptor (IR) as well as the trk-Y751F and trk-Y785F hybrids (plasmids pINS3, pCMV-ET-Y751F, and pCMV-ET-Y785F) were as described (Obermeier et al., 1993). The cDNAs of the murine c-ros tyrosine kinase (plasmid pMR1N), the rat c-neu proto-oncogene (pSVneuN), and the mouse keratinocyte growth factor receptor (plasmid KGFR-pCEV27) were provided by Dr. C. Birchmeier (MDC, Berlin, Germany), Dr. M.F. Rajewsky (Institute of Cell Biology, Essen, Germany), and Dr. S.A. Aaronson (National Institutes of Health, Bethesda, MD), respectively. The construction of the trk-met hybrid is described in Weidner et al. (1993).

The cDNA fragments for the construction of the trk-ros hybrid receptor were generated as follows: fragment I encoding the extracellular por-

tion of trkA (pLM6) with an XbaI and an NheI site at the 3′-end was digested with XbaI, blunt ended with Mung bean nuclease, and digested with NheI. This fragment was ligated with fragment II encoding the transmembranous and the cytoplasmic portion of c-ros. Fragment II was made in two steps: (*a*) PCR with the primers 5′-ATAACAGAAACAGAATTCATACTTACTATCATAGTT and 5′-CCTTCAGGAACTCAATCTTCTCC and pMR1N as template was used to generate an internal EcoRI site at the 5′-end of the transmembranous region of c-ros. (*b*) This fragment was fused to the COOH-terminal part of c-ros using an internal AvrII site. For in-frame fusion with fragment I, the EcoRI site in fragment II was partially filled with dATP, blunt ended with Mung bean nuclease, and ligated as NheI fragment.

For construction of the trk-neu hybrid, a COOH-terminal fragment of the extracellular part of trkA and the transmembrane and cytoplasmic part of c-neu was amplified with the primers 5′-GGTCCAGGTGCCCAATGCCTCGG (W3), 5′-AATGATGAATGTTTCGTCCTTCTTCTCCACCG, 5′-CGGTGGAGAAGAAGGACGAAACATTCATCATT, and 5′-GGTGGTGGTGACTAGTTCATACAGGTACATCCAG (S1), respectively. The resulting fragments were purified, mixed, and used as templates for a second PCR with the primers W3 and S1. The generated chimeric fragment was digested with BamHI and SpeI, purified, and ligated into a modified pUC118 vector with MscI and SpeI sites. Ligation with a NH_2-terminal KpnI–BamHI fragment of trkA resulted in the full-length hybrid.

For trk-KGFR, the primers W3, 5′-GCAGTAAATAGCTATTTCGTCCTTCTTCTCCACCG, 5′-AAGAAGGACGAAATAGCTATTTACTGCATAGGG, and 5′-GTGGTGGTGTCTAGATTATGTTTTAACACTGCCG were used with the templates pLM6 and KGFR-pCEV27. The amplified fusion fragment was digested with BamHI and XbaI and ligated with the NH_2-terminal EcoRI–BamHI fragment of trkA. The trk-IR hybrid and the trk-Y490F mutant were made using a similar strategy: for the trk-IR hybrid, the primers W3, 5′-GGGGCCGATGATAATTTCGTCCTTCTTCTCCAC, 5′-AAGAAGGACGAAATTATCATCGGCCCCCTCATCT, and 5′-GTGGTGGTCGACTTAGGAAGGATTGGACCGAGG were used. The amplified trk-IR fusion fragment was digested with BamHI-SalI, purified, and ligated into pUC118. The NH_2-terminal KpnI–BamHI fragment of trkA was ligated into this plasmid and resulted in the full-length trk-IR hybrid. For the trk-Y490F mutant, primers W3, 5′-CACAGGCATCACTGAAGAATTGTGGGTTCT, 5′-ATCGAGAACCCACAATTCTTCAGTGATGCCTGTG (S2), and 5′-CACCAGTCCCTGGCCCACTAGAC were used with pLM6 as template. An internal Bam HI–NheI fragment containing the Y490 codon in trkA was replaced by the fragment containing the Y490F mutation. For constructing trk-Y751F and trk-Y785F, the internal NheI site in the trkA cDNA and the plasmids pCMV-ET751F and pCMV-ET-785F were used to mutate the wild-type codons Y751 and Y785. An NheI site at the 3′-end of the cDNAs of trkA, trk-Y751F, and trk-Y785F was introduced by ligation with an NheI linker. The NheI fragment of the trkA was then replaced by the corresponding fragments of the mutated trkA hybrids. For constructing the two trkA receptors containing the COOH-terminal tail of c-met, the primers S2, 5′-GGAGAAGATTGAGGACAGCCGGGCGTGCACATCC, 5′-TGTGCACGCCCGGCTGTCCTCAATCTTCTCCACG, and 5′-GAGGACACTAGTGCTAGCTCATGTGTTCCCCTCGCCATC with the templates pLM6 and the trk-met hybrid were used. After amplification of the trk-met fusion fragment, the tail of trkA could be replaced by the c-met tail from the corresponding trk-met hybrid.

The correct sequences of the hybrid and mutant receptor cDNAs were confirmed by sequencing using T7 DNA polymerase (Pharmacia, Uppsala, Sweden). The hybrid receptor cDNAs were cloned into the pBAT expression vector (Nagafuchi and Takeichi, 1988) and stably introduced into MDCK epithelial cells by calcium phosphate cotransfection (Graham and van der Eb, 1973) with pSV2neo. G418-resistant cell clones were assayed for NGF-induced cell dissociation, branching morphogenesis, and expression of the various receptors.

Assays for Cell Motility and Morphogenesis

Dissociation and motility of MDCK cells were examined in a 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, (2.5 S; Boehringer Mannheim GmbH, Mannheim, Germany). Recombinant SF/HGF was expressed in Sf9 insect cells and purified as described (Weidner et al., 1993). Photomicrographs of the cells were taken after 24 h with a phase contrast microscope (Zeiss, Oberkochen, Germany). Branching morphogenesis of MDCK cells was analyzed as described (Montesano et al., 1991*b*; Weidner et al., 1993) with modifications: collagen gels were prepared at 4°C as 1:1 solutions of collagen R (2 mg/ml; Serva Feinbiochemica GmbH, Heidelberg, Germany) and collagen G (3 mg/ml; Seromed, Heidelberg, Germany) containing DME, 10% FCS, and 2.2 mg/ml $NaHCO_3$. In earlier studies, we have used only collagen G (cf. Brinkmann et al., 1995). Since the optical quality of new batches of collagen G were recently different, we included collagen R (which is optically clear, but alone does not promote growth and morphogenesis). The mixture was neutralized with 1 N NaOH, and 500 μl were plated per well in a 12-well tissue-culture plate (Nunc, Roskilde, Denmark) and gelized at 37°C for 30 min. Nearly confluent MDCK cells were trypsinized to a stage of small aggregates, and aggregates containing 10^4 cells were plated in a second layer of 500 μl collagen solution. After 30 min at 37°C, the gels were covered with 2 ml cell culture medium and incubated overnight at 37°C and 5% CO_2. The next day, medium was changed, factors were added (50 ng/ml SF/HGF, 400 ng/ml NGF), and the cultures were inspected daily. Photomicrographs were taken with a light microscope (Zeiss) equipped with Nomarski interference optics. For fine structure analysis, collagen cultures were fixed with

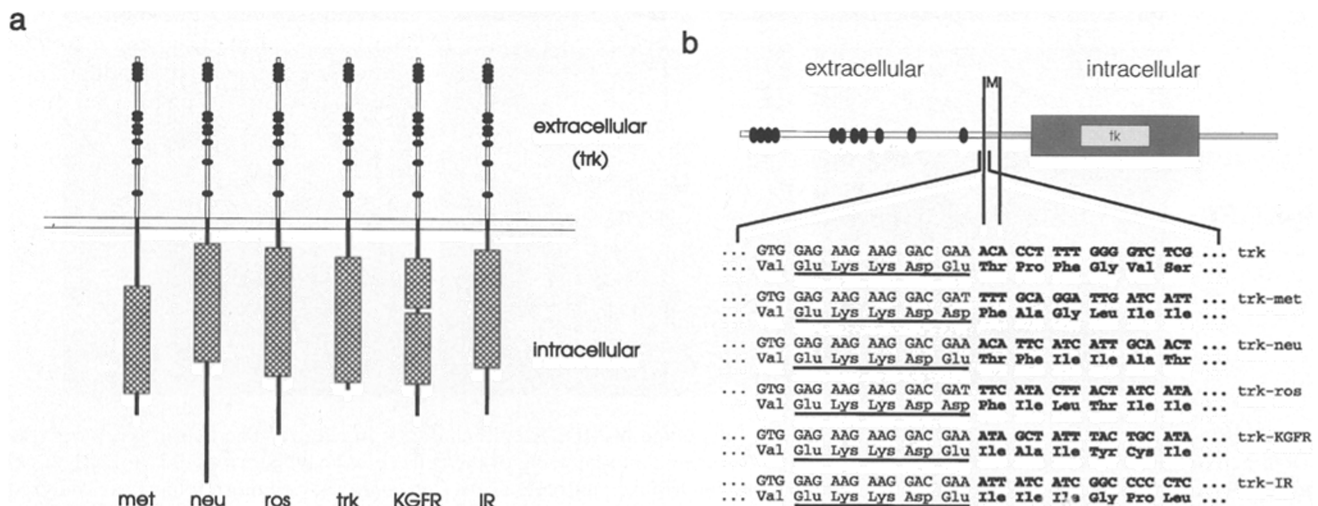

Figure 1. Structure of various hybrid tyrosine kinase receptors. (*a*) The ectodomain of trkA is fused to the transmembrane and cytoplasmic domains of c-met, c-neu, c-ros, the KGFR, and the IR, respectively. Ellipses, cysteine residues in the ectodomain; boxes, location of the tyrosine kinase domains within the cytoplasmic part. (*b*) Nucleotide and amino acid sequences of the fusion points between the extracellular and the transmembrane region (*underlined*, hydrophilic sequence of the trkA ectodomain; *bold type*, adjacent transmembrane region of the various fused receptors). For construction of the receptors, see Materials and Methods.

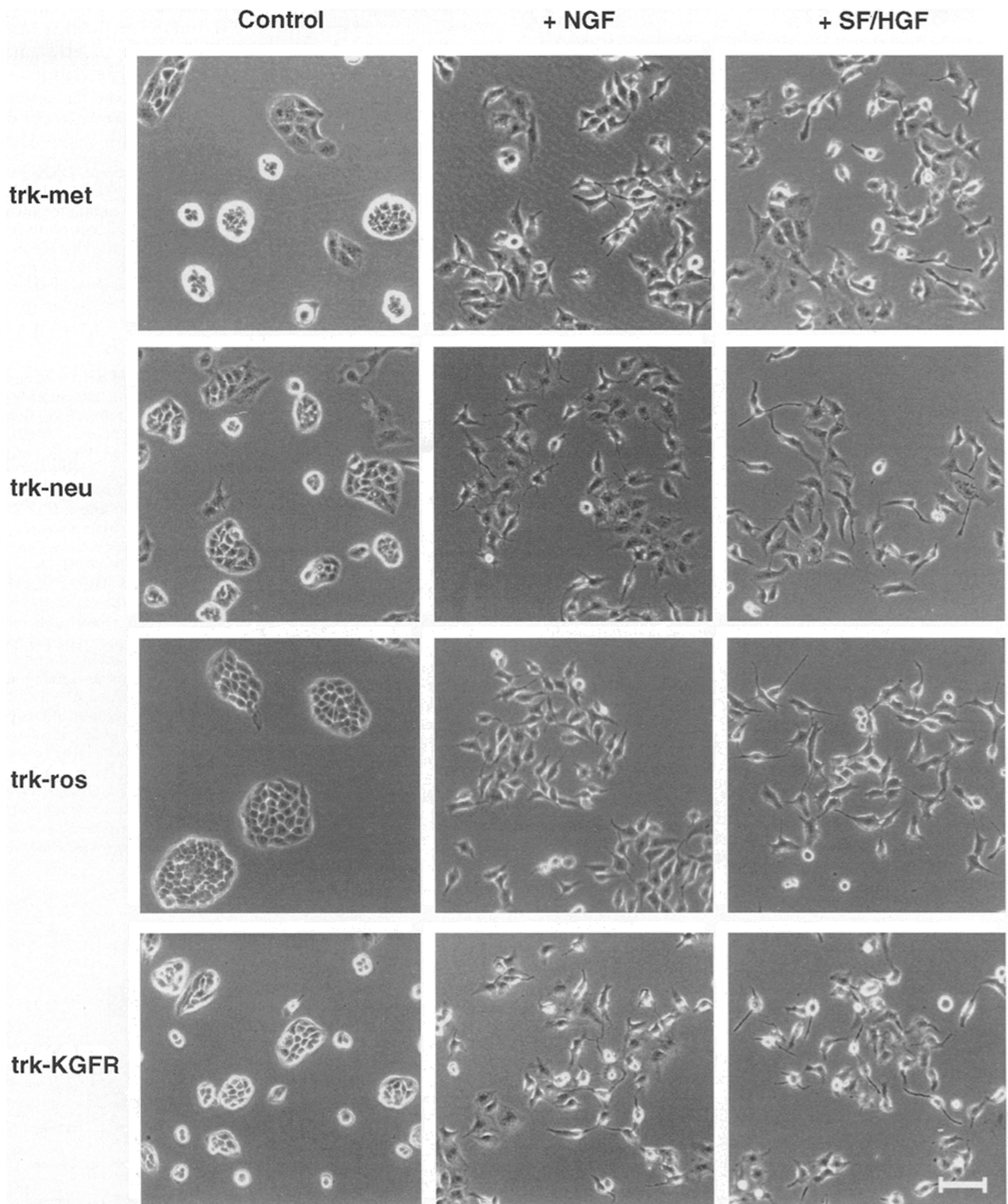

Figure 2. Effect of various hybrid receptors on the cellular phenotype of MDCK epithelial cells in culture. The hybrid receptors trk-met, trk-neu (c-erbB2), trk-ros, and trk-KGFR induced NGF-dependent scattering of the cells (*middle column*), as did SF/HGF (*right column*). The concentrations of NGF and SF/HGF were 50 ng/ml. *Control*, untreated cells (*left column*). Cell morphology was analyzed after 24 h. Bar, 20 μm. For the efficiency of successful transfections, see Table I.

2.5% glutaraldehyde (Sigma, Deisenhofen, Germany), and small blocks containing representative cell aggregates were cut from the gel. After postfixation with OsO_4, the blocks were contrasted with tannic acid and uranyl acetate. The specimens were dehydrated in a graded ethanol series and embedded in Epon 812. Ultrathin sections (50–70 nm) were contrasted with lead citrate and analyzed in an electron microscope (EM 10; Zeiss). Semithin sections (0.5 μm) were stained with toluidine blue and analyzed with a light microscope (Axiophot; Zeiss).

Immunoprecipitation and Western Blotting

For examining tyrosine autophosphorylation of the various tyrosine kinase hybrids, 5 × 10^6 MDCK cells were incubated for 10 min at 37°C in the presence or absence of NGF (50 ng/ml) in DME, 0.5% BSA, 7 μM phenyl arsine oxide, washed with PBS, and extracted for 1 h at 4°C with RIPA-kinase lysis buffer (Weidner et al., 1993). After clearing by ultracentrifugation at 100,000 *g*, immunoprecipitation was carried out using protein A–Sepharose (Pharmacia) and a 1:200 dilution of polyclonal antisera directed against the COOH terminus of mouse c-met, human c-neu, human trkA (Santa Cruz Biotechnology Inc., Santa Cruz, CA), human IR (Transduction Laboratories, Lexington, KY), and mouse c-ros (kindly provided by Dr. C. Birchmeier). After washing with RIPA-kinase lysis buffer and high salt buffer, the immunoprecipitates were separated by 6% SDS-PAGE. The proteins were transferred to nitrocellulose membranes (Amersham Intl., Little Chalfont, UK), probed with antiphosphotyrosine mAb PY-20 (Upstate Biotechnology Inc. [UBI], Lake Placid, NY), and stained using the enhanced chemiluminescence system (ECL; Amersham Intl.). Alternatively, the various receptors were immunoprecipitated with the mAb TTM-9.9.2 directed against the NH_2 terminus of human trkA (kindly provided by Dr. M. Barbacid, Bristol-Meyers-Squibb Princeton, NJ).

Results

Construction and Biological Activities of Chimeras between the NGF Receptor (trkA) and Various Epithelial-specific Tyrosine Kinases

We have previously shown that a chimeric receptor containing the extracellular sequences of the NGF receptor and the transmembrane and cytoplasmic sequences of c-met transmits c-met–specific signals in the presence of NGF (Weidner et al., 1993). Here we have constructed a series of hybrids of trkA and the kinase domains of c-neu (c-erbB2), c-ros, KGFR, and IR (Fig. 1 *a*). In all, the first hydrophobic amino acid of the transmembrane domain of the receptors was fused in frame to the sequences encoding the last (acidic) amino acid of the extracellular sequence of trkA (Fig. 1 *b*). The hybrid cDNAs were cotransfected with a neomycin resistance gene into MDCK cells; for each cDNA transfection 30–70 independent G418-resistant clones were analyzed for biological activities and then for expression of the receptors. Wild-type trkA was also examined.

Table I. Stable Transfectants of MDCK Cells with Hybrid Receptor Tyrosine Kinases as Analyzed for NGF-dependent Scattering

Hybrid receptors	Cell scattering	
	Responding clones/total	*%*
trk-met	17/48	35
trk-neu	12/34	35
trk-ros	16/68	24
trk-KGFR	23/60	38
trk-IR	0/60	0
trkA	17/71	24
trk-Y490F (SHC)	0/44	0
trk-Y751F (PI3K)	18/56	32
trk-Y785F (PLCγ)	9/44	20
trk-Y14-16 of met	24/56	43
trk-Y490F-Y14-16 of met	0/81	0
trk-Y15F of met	8/61	13
trk-Y16F of met	10/72	14
trk-Y14/16F of met	10/62	16
trk-Y14/15F of met	13/68	19

MDCK cell clones that express the various hybrid receptors were first examined for NGF-induced cell dissociation (Fig. 2; Table I and II). We found that the chimeric c-met, c-neu, c-ros, and KGF receptors induced dissociation and scattering of the cells in the presence of NGF (Fig. 2, *middle column*). 20–40% of the G418-resistant clones were responsive to NGF, which is similar as previously reported with trk-met (Table I; cf. also Weidner et al., 1993). The cells transfected with different chimeric receptors were also reactive to SF/HGF (Fig. 2, *right column*). Cells transfected with the trk-IR construct were not dissociated by NGF (Table II). However, our trk-IR hybrid was active in other cells (Isakoff et al., 1995). We should also note that neuregulin, the ligand of c-erbB3 and c-erbB4, which are coreceptors of c-neu (c-erbB2) (Karuagaran et al., 1996; Carraway and Cantley, 1994), does not scatter MDCK epithelial cells. Also, acidic FGF that binds to the KGF receptor (Miki et al., 1991) does not dissociate the cells (data not shown). We have not examined MDCK cells for the expression of these receptors.

Surprisingly, the transfected wild-type trkA receptor also dissociated MDCK epithelial cells in the presence of NGF (Fig. 3). This finding allowed us to examine the substrate requirements for dissociation of MDCK cells since trkA with mutated substrate binding sites have been described: Y490, Y751, and Y785 of trkA are binding sites for SHC, PI3 kinase, and PLCγ, respectively (Obermeier et al., 1994). We found that loss of SHC binding of trkA due to mutation of tyrosine 490 to phenylalanine prevented NGF-induced cell dissociation (Fig. 3; and Table II). The cells were still reactive to SF/HGF. Mutations of the other two tyrosine residues of trkA, Y751 and Y785, had no inhibitory effect. Thus, signals mediated by various receptor ty-

Table II. Biological Effects of Various Hybrid Receptors on MDCK Cells

Hybrid receptors	Dissociation, motility	Branching morphogenesis
trk-met	+	+
trk-neu	+	−
trk-ros	+	−
trk-KGFR	+	−
trk-IR	−	−
trkA	+	−
trk-Y490F (SHC)	−	−
trk-Y751F (PI3K)	+	−
trk-Y785F (PLCγ)	+	−
trk-met	+	+
trk-met Y14F	+	+
trk-met Y15F	+	↓*
trk-met Y16F	+	↓
trk-met Y15F/Y16F	+	↓
trk-met Y14F/Y16F	+	↓
trk-met Y14F/Y15F	↓	−
trk- Y14-16 of met	+	+
trk- Y490F-Y14-16 of met	−	−
trk-Y15F of met	+	−
trk-Y16F of met	+	−
trk-Y14/16F of met	+	−
trk-Y14/15F of met	+	−

*The downward pointing arrow indicates reduced biological activity (cf. Weidner et al., 1995).

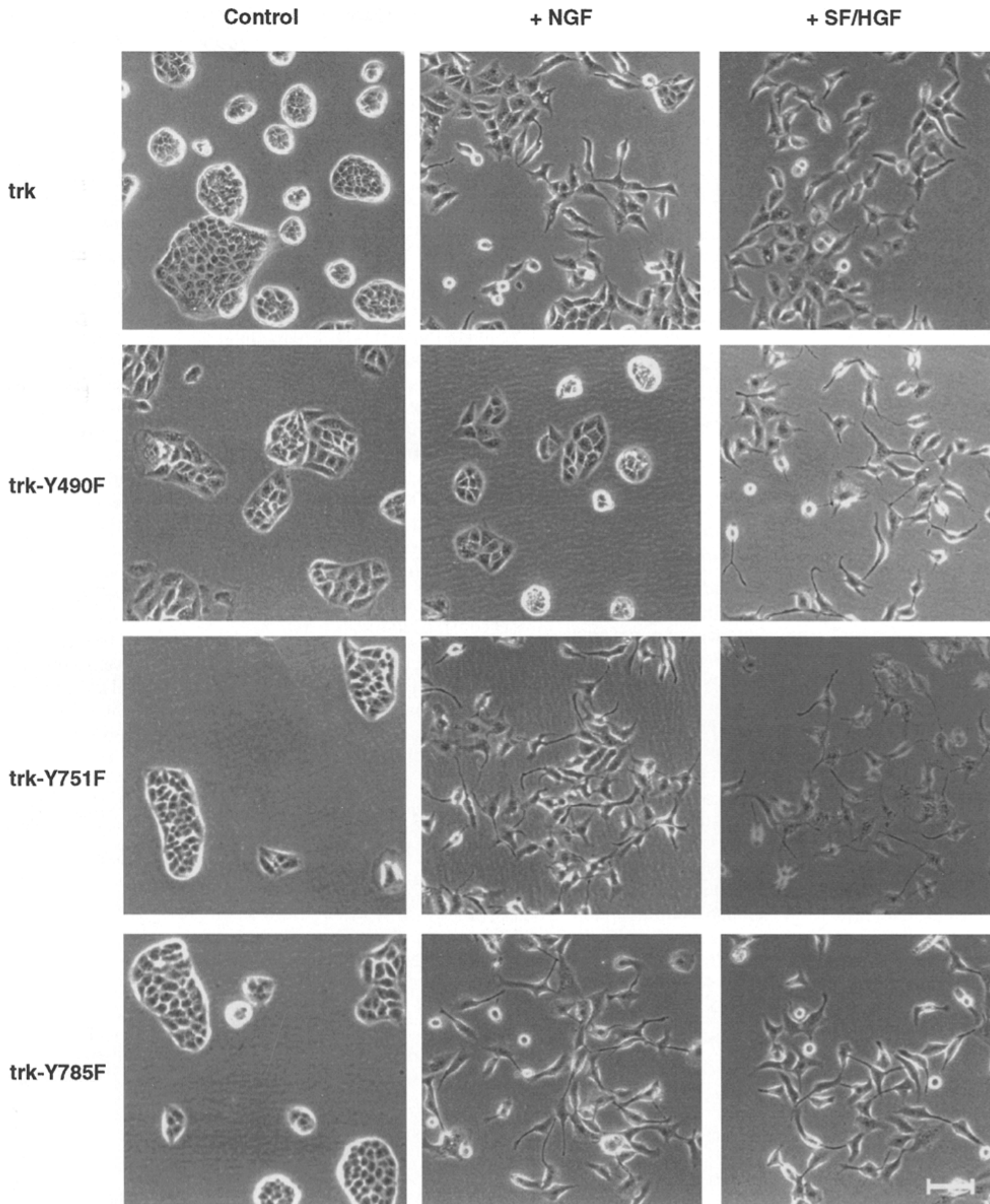

Figure 3. Effect of various Y → F mutants of trkA on the phenotype of MDCK epithelial cells. The Y751F and Y785F mutants of trkA induced NGF-dependent scattering of the cells in a similar manner as the wild-type trkA receptor. In contrast, the Y490F mutation in the trkA receptor abolished NGF-dependent scattering of the cells. MDCK cells were cultured with NGF (50 ng/ml), SF/HGF (50 ng/ml), or without factor (control). Cell morphology was analyzed after 24 h. Bar, 20 μm. For efficiency of successful transfection, see Table I.

rosine kinases can scatter kidney epithelial cells. In addition, binding of a particular substrate, SHC, appears to be necessary for mediating this motility response.

We also examined the expression and autophosphorylation levels of the various hybrid tyrosine kinase receptors in the NGF-responding cell clones (Fig. 4). We found that tyrosine phosphorylation of c-met, c-neu, c-ros, and the IR was clearly dependent on the presence of NGF (cf. *arrows*

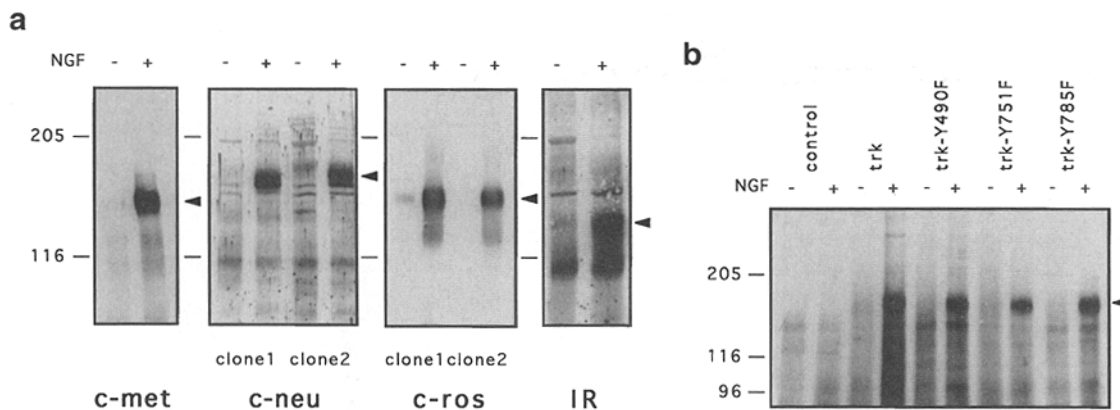

Figure 4. NGF-dependent tyrosine phosphorylation of the various trkA hybrids and mutants. Transfectants of MDCK cells expressing various hybrid receptors (*a*) and trkA mutants (*b*) were analyzed for NGF-dependent tyrosine phosphorylation of the receptors. Receptors were immunoprecipitated with the corresponding antisera directed against the COOH termini of the kinases and Western blotted using an antiphosphotyrosine antibody (cf. Materials and Methods). (*Arrows*) Receptors phosphorylated in response to NGF.

in Fig. 4 *a*). All hybrid receptors had the expected molecular mass, i.e., trk-met, 145 kD; trk-ros, 151 kD; trk-neu, 165 kD; and trk-IR, 144 kD. Wild-type trkA and its mutants showed identical molecular masses of 140 kD (Fig. 4 *b*). In general, the clones transfected with one particular hybrid cDNA and selected for NGF-induced scattering expressed similar amounts of receptor molecules and showed similar levels of receptor phosphorylation (cf. *c-neu* and *c-ros* in Fig. 4 *a*). Immunoprecipitations of the hybrid receptors with an mAb against the NH_2 terminus of trkA also showed similar amounts of reacting protein (data not shown). Wild-type and mutant trkA receptors were also expressed and phosphorylated at similar levels in various cell clones (Fig. 4 *b*). We have also examined other receptor chimeras such as trk-ret and trk-ddr (the discoidin domain receptor) (cf. Johnson et al., 1993; Lai and Lemke, 1994; Alves et al., 1995); these receptors were expressed in MDCK cells, but did not display NGF-dependent tyrosine phosphorylation, and were therefore not used for further experiments.

The Specificity of c-met for Branching Morphogenesis

Branching morphogenesis of the various MDCK cell transfectants, which responded to NGF by cell scattering, was examined by culturing the cells in three-dimensional gels of collagen type I (cf. Materials and Methods). In the absence of ligand, the cells formed slowly growing, hollow cysts (Fig. 5, *left column*). We observed that only the transfected trk-met hybrid, but not trk-ros, trk-neu, and wild-type trkA, induced branching morphogenesis in response to NGF (Fig. 5, *middle column*; cf. also Table II). The trk-met hybrid also clearly promoted proliferation of the cells in collagen; we have here not quantified this activity (cf. Weidner et al., 1993). Again, all transfected cell clones responded with branching morphogenesis in the presence of SF/HGF, indicating that the signaling capacity for branching morphogenesis was intact (Fig. 5, *right column*). The trk-KGFR and trk-IR hybrids did also not induce NGF-dependent branching (Table II).

Previous results of our laboratory have indicated that mutations of Y15 (Y1354), Y16 (Y1363), and, to a lesser degree, Y14 (Y1347) of c-met lead to the disturbance of branching morphogenesis of MDCK cells (Weidner et al., 1995; cf. Table II). Here, we attempted to create a trkA receptor with the ability to induce branching morphogenesis; to do this we fused to it the COOH-terminal sequence of c-met encoding Y14-16 (Fig. 6, c.f. Materials and Methods). This new receptor chimera (trk-Y14-16 of met) indeed induced scattering and, surprisingly, also morphogenesis of MDCK cells in the presence of NGF (Fig. 7; Table II). Detailed inspection indicated that the tubules induced by the chimeric receptor are identical to the ones formed in the presence of SF/HGF (compare Fig. 7, *b* and *c*). Analysis of tissue sections showed that the NGF-induced branches consist of single layers of cells surrounding a central lumen. The cells of the tubules are well polarized, showing basally localized nuclei and apical microvilli (Fig. 7, *d* and *e*). This new trk-met receptor did not induce scattering and morphogenesis when Y490 of trkA was mutated (Table II). Mutation of Y15 (Y1354) or Y16 (Y1363) of the tail of c-met in the new trk-met receptor abolished morphogenesis but not scattering. The data thus demonstrate that the novel combination of the trkA tyrosine kinase domain linked with COOH-terminal sequences of c-met is sufficient to induce a morphogenic response when activated by the ligand.

Discussion

Developmental studies have indicated that various receptor tyrosine kinases can mediate different signals in epithelia. Here we have analyzed various epithelial receptor tyrosine kinases for their potential to induce biological responses in kidney epithelial cells, such as the ability to form branching tubules or to induce cell scattering and increased cell motility. Branching morphogenesis is particularly relevant in the development of many epithelial organs, which occurs in kidney, lung, breast, prostate, or salivary gland. Interestingly, we found that the tyrosine kinase c-met, which is the receptor for SF/HGF, induces tubule formation and branching of kidney epithelial cells exclusively. Various other tyrosine kinase receptors such as c-neu,

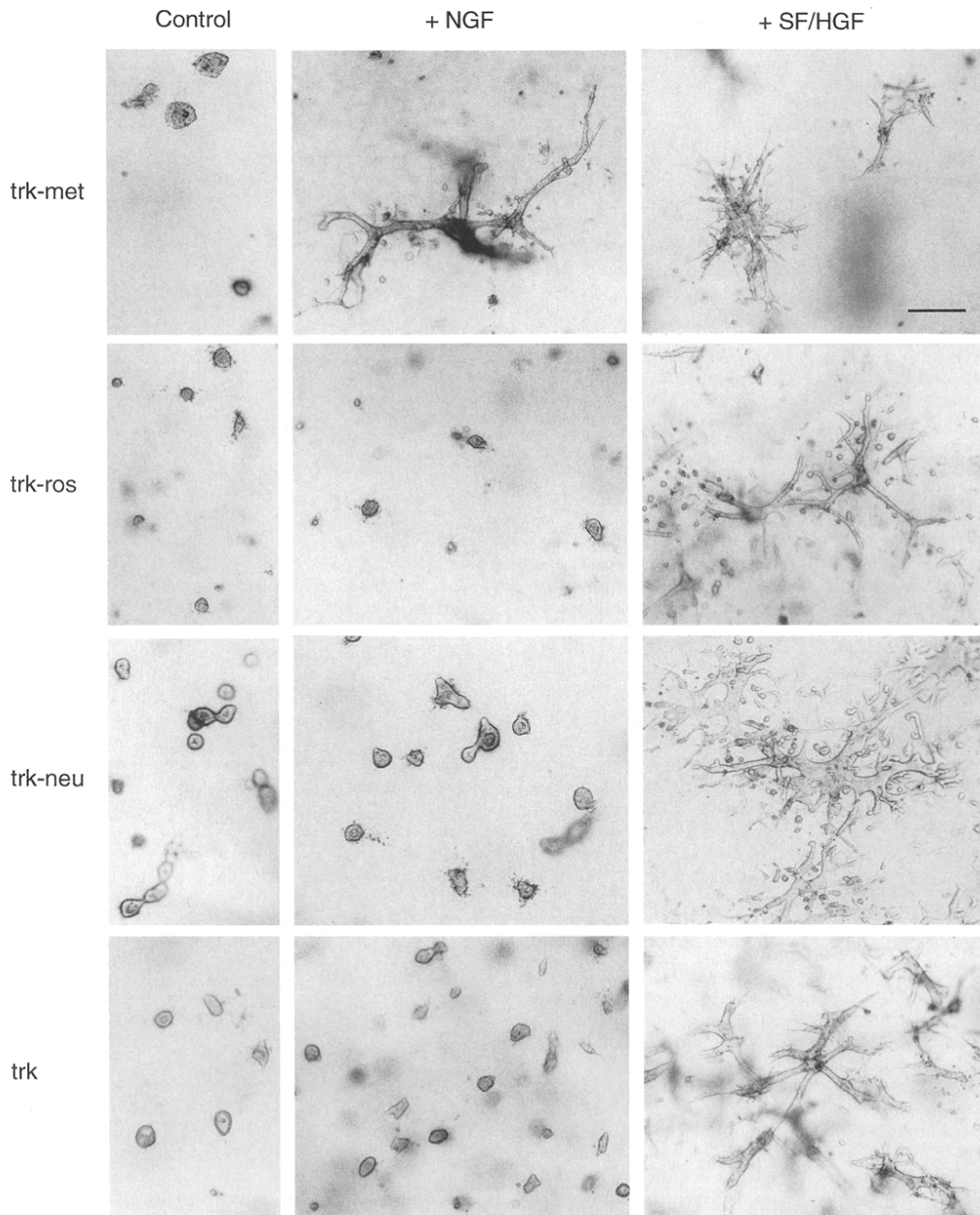

Figure 5. Effect of various hybrid receptors on NGF-induced branching morphogenesis of MDCK cells in collagen gels. Only the trk-met receptor conferred NGF-dependent branching morphogenesis in a similar manner as activation of the endogenous c-met receptor by SF/HGF. MDCK cell clones were precultured in collagen gels (cf. Materials and Methods), incubated with NGF (400 ng/ml), SF/HGF (50 ng/ml), or without factor (control), and analyzed for branching morphogenesis. Bar, 180 μm.

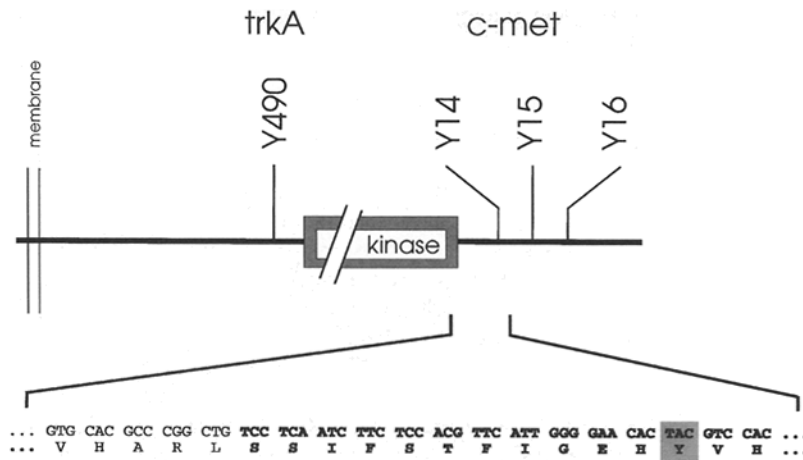

Figure 6. Structure of the trkA hybrid with the COOH terminus of c-met. The region surrounding the fusion point is enlarged below; it shows the COOH-terminal leucine (*L*) of the trkA kinase domain, which is fused to the c-met tail (*bold type*) containing Y14 (Y1347), Y15 (Y1354), and Y16 (Y1363). Y14 (Y1347) of c-met is dashed in the enlargement.

c-ros, the KGFR, or trkA did not display this activity but were able to dissociate and to scatter epithelial cells. This latter is a surprising result, since scattering was thought to be specific for SF/HGF in these cells. Only one other protein, "scatter factor–like factor" has been shown to dissociate MDCK cells (Bellusci et al., 1994*a*). Interestingly, the ability to form branching tubules could be newly created when the COOH terminus of c-met (which contains the major substrate binding sites) was fused to the COOH terminus of the kinase domain of trkA. Thus, the COOH-terminal part of c-met mediates the signals required for the formation of branched tubules in kidney cells and may bind novel, as yet unidentified substrates.

We have previously demonstrated that the two functions, scattering and morphogenesis of epithelial cells, can be induced by separate means: the two-kringle variant of SF/HGF, which is a small splice form containing only the NH_2-terminal hairpin structure and the first two-kringle

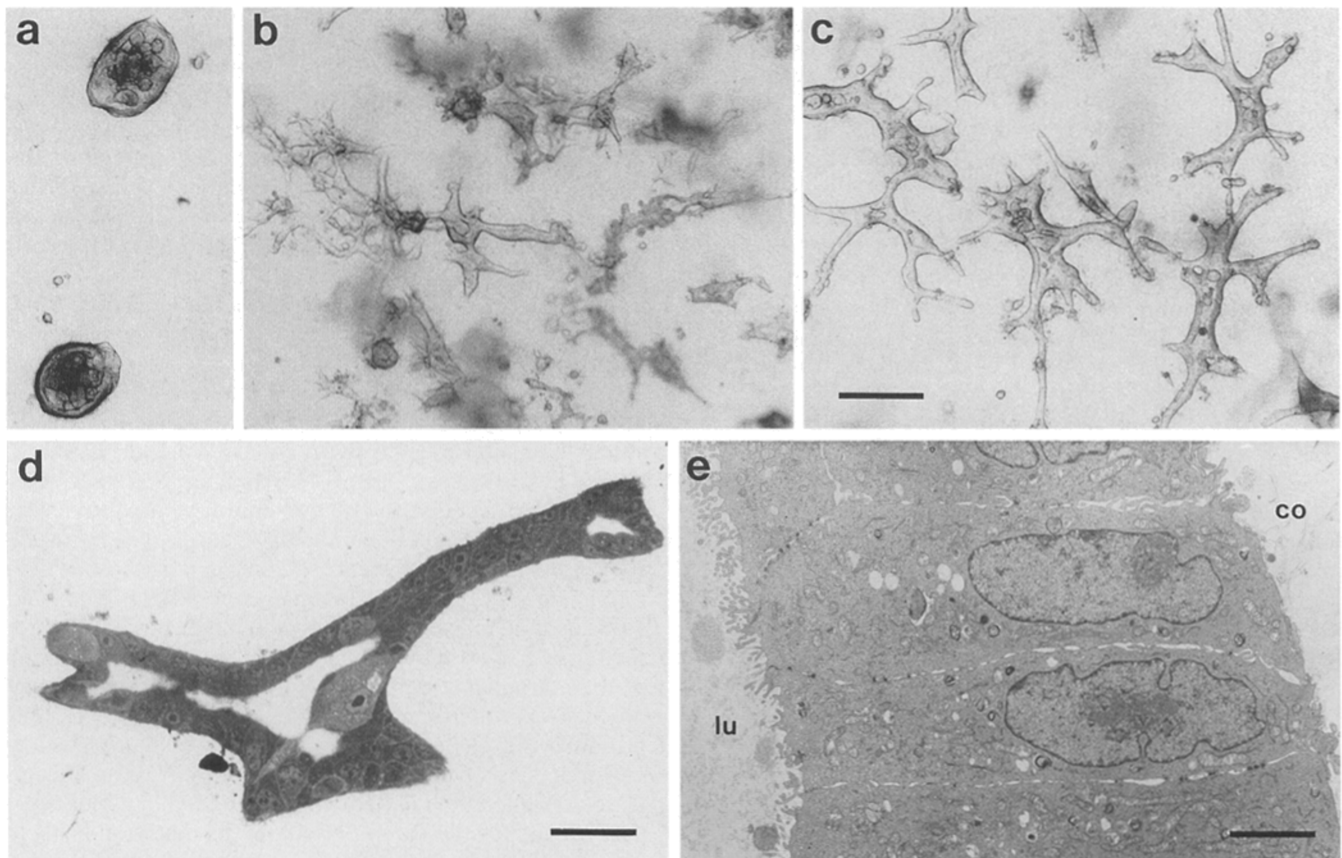

Figure 7. Effect of trkA with the COOH-terminal tail of c-met on NGF-induced branching morphogenesis of MDCK cells in collagen gels. Branching morphogenesis was induced with NGF (*b*; 400 ng/ml) and SF/HGF (*c*; 50 ng/ml); *a* is the control without factors. Branching structures were sectioned and inspected by light (*d*) and electron microscopy (*e*). *lu*, lumen of branched structures; *co*, collagen matrix. Bars: (*a–c*) 184 μm; (*d*) 33 μm; and (*e*) 3 μm.

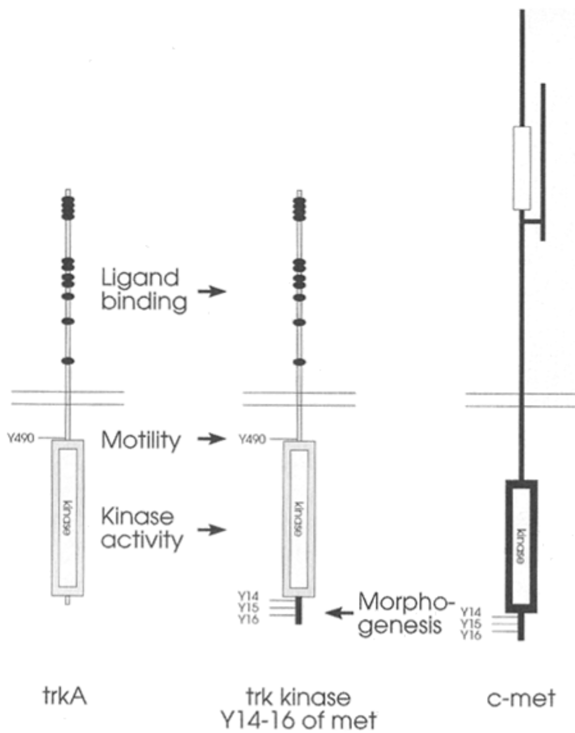

Figure 8. Scheme of the different modules in trkA and c-met required for scattering and branching morphogenesis. In the receptor chimera trk-Y14-16 of met (*middle*), the ligand-binding region, the juxtamembrane region including the SHC binding site for motility (*Y490*), plus the kinase domain originate from trkA. The COOH terminus (*black bar*) containing Y14 (1347), Y15 (Y1354), and Y16 (Y1363) is from c-met and promotes branching morphogenesis.

domains, promoted only dissociation of the cells but not the formation of branched tubules or growth (Hartmann et al., 1992; data not shown). Here we were now able to separate these two distinct pathways on the receptor level (Fig. 8). We found that several receptor tyrosine kinases can scatter epithelial cells, and we have previously reported that v-src (expressed as a temperature-sensitive mutant) also leads to scattering of MDCK cells (Behrens et al., 1993). It appears that the action of the substrate SHC is essential for this response: mutation of the SHC binding site Y490 in trkA, but not mutation of the binding sites for PI3 kinase or PLCγ (Y751 and Y785) (cf. Obermeier et al., 1994), abolished scattering. In c-met, double mutation of the potential SHC binding sites Y14 and Y15 (Y1347 and Y1354) (cf. Ponzetto et al., 1994) also prevented the scattering response (Weidner et al., 1995). Accordingly, overexpression of SHC in fibroblasts increased cell motility (Pelicci et al., 1995). Furthermore, the ras pathway in epithelial cells is required for the motility response induced by SF/HGF, since the dominant-negative ras-N17 mutant interfered (Hartmann et al., 1994; cf. Ridley et al., 1995). Thus, cell dissociation and scattering of epithelial cells appears to require activation of SHC and possibly recruitment of GRB-2 (cf. Rozakis-Adcock et al., 1992, 1993; Egan et al., 1993; Pawson and Schlessinger, 1993) that activate the ras pathway. We must also assume that the motility response of trkA and c-met induced by SHC requires binding through the PTB (PI) and SH2 domains, respectively (Kavanaugh and Williams, 1994; Blaikie et al., 1994; Gustafson et al., 1995; Pelicci et al., 1995; Zhou et al., 1995).

Recently, the importance of the SF/HGF/c-met system in cell motility in vivo was clearly demonstrated by gene ablation experiments in mice (Bladt et al., 1995). Myoblast precursor cells, which derive from epithelioid somites and express c-met, cannot migrate into SF/HGF expressing limb buds and other targets in both SF/HGF and c-met −/− embryos. Cell migration induced by SF/HGF also plays a crucial role in invasion and metastasis (Weidner et al., 1990; Bellusci et al., 1994*b*; Rosen et al., 1994). It has recently been shown that SF/HGF is expressed in human breast carcinomas and that the level of expression correlates with the state of tumor progression (Yamashita et al., 1994).

We also show here that only the c-met receptor, but not other tyrosine kinases, induces tubule formation of kidney epithelial cells. Mutation of Y15 (Y1354) and Y16 (Y1363), and to a lesser degree Y14 (Y1347), reduced this activity (cf. Weidner et al., 1995). The requirement for signals that induce branched tubules was further studied using a newly created hybrid between the kinase domain trkA (which induces only scattering) and the substrate binding sites Y14-16 of c-met (which can confer branching morphogenesis) (Fig. 8). It is known that Y14 and Y15 of c-met represent a tandem binding site for many substrates such as PI3 kinase, PLCγ, rasGAP, c-src, and others (Ponzetto et al., 1994). However, these substrates can also bind to other receptor tyrosine kinases that confer only scattering; for instance, trkA has binding sites for SHC, PI3 kinase, and PLCγ (Obermeier et al., 1994); c-neu (c-erbB2) has putative binding sites for rasGAP, PLCγ, syp, GRB-2, and SHC (Segatto et al., 1993; Carraway and Cantley, 1994; Ricci et al., 1995). It is therefore suggested that tubule formation of epithelial cells induced by c-met requires the activation of additional, as yet unknown substrates. The sequence flanking Y16 (Y1363, VAPYPSLL), which affects morphogenesis (Weidner et al., 1995), has in fact an unusual sequence and might represent a new substrate binding site. We have recently begun a search for new substrates that may mediate the morphogenesis response of c-met by using the yeast two-hybrid system (cf. Gustafson et al., 1995; Waters et al., 1995) and have identified substrates that bind specifically to c-met (Weidner, K.M., M. Sachs, S. DiCesare, and W. Birchmeier, manuscript in preparation). Alternatively, cell motility and branching morphogenesis might be due to differences in signal intensity and/or signal persistence ("shouting vs whispering") (cf. Dikic et al., 1994; Traverse et al., 1994; Marshall, 1995). For instance, strong signaling might be required for scattering followed by reduced activity during reformation of the epithelial structures. Alternatively, morphogenesis might require stronger signaling than scattering. Our present work has demonstrated, however, that branching morphogenesis by c-met does not require Y1001. This tyrosine residue in the juxtamembrane region of c-met negatively regulates the receptor activity; its mutation leads to constitutive scattering and fibroblastoid morphology of epithelial cells (Weidner et al., 1995). In conclusion, we suggest here that scattering of epithelial cells requires the action of the substrate SHC, whereas new, as yet unidentified substrates induce branching morphogenesis.

Interestingly, we could transfer signaling specificity of c-met, i.e., particularly the unique activity of the receptor to induce formation of branching tubules, upon the trkA receptor by transferring COOH-terminal sequences of c-met that are active in substrate binding. Exchanges of sequences in receptor tyrosine kinases were previously reported for the kinase insert domains of PDGFα and β receptors (Arvidsson et al., 1992) and PDGFβ and FGF receptors (Wennström et al., 1994). It was thus possible to transfer the activity of membrane ruffling upon the new receptors. Our exchange involved distantly related tyrosine kinases and an element (the COOH terminus of c-met) that is structurally entirely different from the corresponding element in trkA. This new finding is again a strong indication of a modular arrangement of the elements in tyrosine kinases, i.e., the kinase domain, on the one hand, and various substrate binding regions with multiple functions on the other.

The recent gene ablation experiments of SF/HGF and c-met in mice have revealed an important role of this signaling system in epithelial development (Schmidt et al., 1995; Uehara et al., 1995). Embryonic liver and placenta are affected in these mutants. A function of SF/HGF in the development of epithelia has also been demonstrated by organ culture experiments with kidney and mammary gland explants (Woolf et al., 1995; Yang et al., 1995). We have recently also shown that SF/HGF, besides controling branching of kidney, breast, and prostate epithelia cells, induces further characteristic epithelial organoids such as crypts and alveoli (Brinkmann et al., 1995). Considering the relevance of morphogenesis of epithelia for the development of many eukaryotic organs, the identification of specific substrates that control these processes in vivo will therefore be an important task in the future.

We thank Dr. Mariano Barbacid for the anti-trkA antibody TTM-9.9.2, and Drs. Stuart Aaronson, Carmen Birchmeier, Louis Parada, M.F. Rajewsky, and Vasiliy Pachnis for cDNAs. We also thank Carmen Birchmeier for helpful discussions and critical reading of the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft.

Received for publication 21 December 1995 and in revised form 15 March 1996.

References

Alves, F., W. Vogel, K. Mossie, B. Millauer, H. Hofler, and A. Ullrich. 1995. Distinct structural characteristics of discoidin I subfamily receptor tyrosine kinases and complementary expression in human cancer. *Oncogene.* 10:609–618.

Aroian, R.V., G.M. Lesa, and P.W. Sternberg. 1994. Mutations in the Caenorhabditis elegans let-23 EGFR-like gene define elements important for cell-type specificity and function. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:360–366.

Arvidsson, A.K., C.H. Heldin, and L. Claesson-Welsh. 1992. Transduction of circular membrane ruffling by the platelet-derived growth factor beta-receptor is dependent on its kinase insert. *Cell Growth & Differ.* 3:881–887.

Behrens, J., L. Vakaet, R. Friis, E. Winterhager, F. Van Roy, M.M. Mareel, and W. Birchmeier. 1993. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. *J. Cell Biol.* 120:757–766.

Bellusci, S., G. Moens, J.P. Thiery, and J. Jouanneau. 1994*a*. A scatter factor-like factor is produced by a metastatic variant of a rat bladder carcinoma cell line. *J. Cell Sci.* 107:1277–1287.

Bellusci, S., G. Moens, G. Gaudino, P. Comoglio, T. Nakamura, J.-P. Thiery, and J. Jouanneau. 1994*b*. Creation of an hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity. *Oncogene.* 9:1091–1099.

Ben-Levy, R., H.F. Paterson, C.J. Marshall, and Y. Yarden. 1994. A single autophosphorylation site confers oncogenicity to the Neu/ErbB-2 receptor and enables coupling to the MAP kinase pathway. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:3302–3311.

Berdichevsky, F., D. Alford, B. D'Souza, and J. Taylor-Papadimitriou. 1994. Branching morphogenesis of human mammary epithelial cells in collagen gels. *J. Cell Sci.* 107:3557–3568.

Birchmeier, C., and W. Birchmeier. 1993. Molecular aspects of mesenchymal-epithelial interactions. *Annu. Rev. Cell Biol.* 9:511–540.

Birchmeier, C., D. Birnbaum, G. Waitches, O. Fasano, and M. Wigler. 1986. Characterization of an activated human ros gene. *Mol. Cell. Biol.* 6:3109–3116.

Bladt, F., D. Riethmacher, S. Isenmann, A. Aguzzi, and C. Birchmeier. 1995. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature (Lond.).* 376:768–771.

Blaikie, P., D. Immanuel, J. Wu, N. Li, V. Yajnik, and B. Margolis. 1994. A region in Shc distinct from the SH2 domain can bind tyrosine-phosphorylated growth factor receptors. *J. Biol. Chem.* 269:32031–32034.

Bottaro, D.P., J.S. Rubin, D.L. Faletto, A.M. Chan, T.E. Kmiecik, G.F. Vande Woude, and S.A. Aaronson. 1991. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science (Wash. DC).* 251:802–804.

Brinkmann, V., H. Foroutan, M. Sachs, K.M. Weidner, and W. Birchmeier. 1995. Hepatocyte growth factor/scatter factor induces a variety of tissue-specific morphogenic programs in epithelial cells. *J. Cell Biol.* 131:1573–1586.

Bussolino, F., M.F. Di Renzo, M. Ziche, E. Bocchietto, M. Olivero, L. Naldini, G. Gaudino, L. Tamagnone, A. Coffer, and P.M. Comoglio. 1992. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J. Cell Biol.* 119:629–641.

Carraway, K.L., III, and L.C. Cantley. 1994. A neu acquaintance for erbB3 and erbB4: a role for receptor heterodimerization in growth signaling. *Cell.* 78:5–8.

Cheng, H.J., M. Nakamoto, A.D. Bergemann, and J.G. Flanagan. 1995. Complementary gradients in expression and binding of elf-1 and mek4 in development of the topographic retinotectal projection map. *Cell.* 82:371–381.

Cooper, C.S., M. Park, D.G. Blair, M.A. Tainsky, K. Huebner, C.M. Croce, and G.F. Vande Woude. 1984. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature (Lond.).* 311:29–33.

Dikic, I., J. Schlessinger, and I. Lax. 1994. PC12 cells overexpressing the insulin receptor undergo insulin-dependent neuronal differentiation. *Curr. Biol.* 4:702–708.

Drescher, U., C. Kremoser, C. Handwerker, J. Loschinger, M. Noda, and F. Bonhoeffer. 1995. In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kda tectal protein related to ligands for eph receptor tyrosine kinases. *Cell.* 82:359–370.

Egan, S.E., B.W. Giddings, M.W. Brooks, L. Buday, A.M. Sizeland, and R.A. Weinberg. 1993. Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. *Nature (Lond.).* 363:45–51.

Falls, D.L., K.M. Rosen, G. Corfas, W.S. Lane, and G.D. Fischbach. 1993. ARIA, a protein that stimulates acetylcholine receptor synthesis, is a member of the neu ligand family. *Cell.* 72:801–815.

Fantl, W.J., J.A. Escobedo, G.A. Martin, C.W. Turck, M. del Rosario, F. McCormick, and L.T. Williams. 1992. Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signaling pathways. *Cell.* 69:413–423.

Fixman, E.D., M.A. Naujokas, G.A. Rodrigues, M.F. Moran, and M. Park. 1995. Efficient cell transformation by the Tpr-Met oncoprotein is dependent upon tyrosine 489 in the carboxy-terminus. *Oncogene.* 10:237–249.

Gassmann, M., F. Casagranda, D. Orioli, H. Simon, C. Lai, R. Klein, and G. Lemke. 1995. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature (Lond.).* 378:390–394.

Graham, E.L., and A.J. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology.* 52:456–467.

Grant, D.S., H.K. Kleinman, I.D. Goldberg, M.M. Bhargava, B.J. Nickoloff, J.L. Kinsella, P. Polverini, and E.M. Rosen. 1993. Scatter factor induces blood vessel formation in vivo. *Proc. Natl. Acad. Sci. USA.* 90:1937–1941.

Gustafson, T.A., W.M. He, A. Craparo, C.D. Schaub, and T.J. Oneill. 1995. Phosphotyrosine-dependent interaction of shc and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. *Mol. Cell. Biol.* 15:2500–2508.

Hartmann, G., L. Naldini, K.M. Weidner, M. Sachs, E. Vigna, P.M. Comoglio, and W. Birchmeier. 1992. A functional domain in the heavy chain of scatter factor/hepatocyte growth factor binds the c-Met receptor and induces cell dissociation but not mitogenesis. *Proc. Natl. Acad. Sci. USA.* 89:11574–11578.

Hartmann, G., K.M. Weidner, H. Schwarz, and W. Birchmeier. 1994. The motility signal of scatter factor/hepatocyte growth factor mediated through the receptor tyrosine kinase met requires intracellular action of Ras. *J. Biol. Chem.* 269:21936–21939.

Holmes, W.E., M.X. Sliwkowski, R.W. Akita, W.J. Henzel, J. Lee, J.W. Park, D. Yansura, N. Abadi, H. Raab, G.D. Lewis et al. 1992. Identification of heregulin, a specific activator of $p185^{erbB2}$. *Science (Wash. DC).* 256:1205–1210.

Isakoff, S.J., Y. Yu, Y.-C. Su, P. Blaikie, V. Yajnik, E. Rose, K.M. Weidner, M. Sachs, B. Margolis, and E.Y. Skolnik. 1996. Interaction between the phosphotyrosine binding domain of Shc and the insulin receptor is required for Shc phosphorylation by insulin in vivo. *J. Biol. Chem.* 271:3959–3962.

Johnson, J.D., J.C. Edman, and W.J. Rutter. 1993. A receptor tyrosine kinase found in breast carcinoma cells has an extracellular discoidin I-like domain. *Proc. Natl. Acad. Sci. USA.* 90:5677–5681.

Karuagaran, D., E. Tzahar, R.R. Beerli, X.M. Chen, D. Grausporta, B.J. Ratz-

kin, R. Seger, N.E. Hynes, and Y. Yarden. 1996. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:254–264.

Kashishian, A., A. Kazlauskas, and J.A. Cooper. 1992. Phosphorylation sites in the PDGF receptor with different specificities for binding GAP and PI3 kinase in vivo. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:1373–1382.

Kavanaugh, W.M., and L.T. Williams. 1994. An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. *Science (Wash. DC).* 266:1862–1865.

Komada, M., and N. Kitamura. 1993. The cell dissociation and motility triggered by scatter factor/hepatocyte growth factor are mediated through the cytoplasmic domain of the c-Met receptor. *Oncogene.* 8:2381–2390.

Lai, C., and G. Lemke. 1994. Structure and expression of the Tyro 10 receptor tyrosine kinase. *Oncogene.* 9:877–883.

Lee, K.-L., H. Simon, H. Chen, B. Bates, M.-M. Hung, and C. Hauser. 1995. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature (Lond.).* 378:394–398.

Marchionni, M.A., A.D. Goodearl, M.S. Chen, O.B. McDonogh, C. Kirk, M. Hendricks, F. Danehy, D. Misumi, J. Sudhalter, K. Kobayashi et al. 1993. Glial growth factors are alternatively spliced erbB2 ligands expressed in the nervous system. *Nature (Lond.).* 362:312–318.

Marshall, C.J. 1995. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell.* 80: 179–185.

Meyer, D., and C. Birchmeier. 1994. Distinct isoforms of neuregulin are expressed in mesenchymal and neuronal cells during mouse development. *Proc. Natl. Acad. Sci. USA.* 91:1064–1068.

Meyer, D., and C. Birchmeier. 1995. Multiple essential functions of neuregulin in development. *Nature (Lond.).* 378:386–390.

Miki, T., T.P. Fleming, D.P. Bottaro, J.S. Rubin, D. Ron, and S.A. Aaronson. 1991. Expression cDNA cloning of the KGF receptor by creation of a transforming autocrine loop. *Science (Wash. DC).* 251:72–75.

Miyazawa, K., H. Tsubouchi, D. Naka, K. Takahashi, M. Okigaki, N. Arakaki, H. Nakayama, S. Hirono, O. Sakiyama et al. 1989. Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. *Biochem. Biophys. Res. Commun.* 163:967–973.

Montesano, R., K. Matsumoto, T. Nakamura, and L. Orci. 1991*a*. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell.* 67:901–908.

Montesano, R., G. Schaller, and L. Orci. 1991*b*. Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors. *Cell.* 66:697–711.

Nagafuchi, A., and M. Takeichi. 1988. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 7: 3679–3684.

Nakamura, T., T. Nishizawa, M. Hagiya, T. Seki, M. Shimonishi, A. Sugimura, K. Tashiro, and S. Shimizu. 1989. Molecular cloning and expression of human hepatocyte growth factor. *Nature (Lond.).* 342:440–443.

Naldini, L., K.M. Weidner, E. Vigna, G. Gaudino, A. Bardelli, C. Ponzetto, R.P. Narsimhan, G. Hartmann, R. Zarnegar, G.K. Michalopoulos et al. 1991. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2867–2878.

Obermeier, A., R. Lammers, K.H. Wiesmuller, G. Jung, J. Schlessinger, and A. Ullrich. 1993. Identification of Trk binding sites for SHC and phosphatidylinositol 3′-kinase and formation of a multimeric signaling complex. *J. Biol. Chem.* 268:22963–22966.

Obermeier, A., R.A. Bradshaw, K. Seedorf, A. Choidas, J. Schlessinger, and A. Ullrich. 1994. Neuronal differentiation signals are controlled by nerve growth factor receptor/Trk binding sites for SHC and PLC gamma. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:1585–1590.

Orr-Urtreger, A., L. Trakhtenbrot, R. Ben-Levy, D. Wen, G. Rechavi, P. Lonai, and Y. Yarden. 1993. Neural expression and chromosomal mapping of Neu differentiation factor to 8p12-p21. *Proc. Natl. Acad. Sci. USA.* 90: 1867–1871.

Pawson, T., and J. Schlessinger. 1993. SH2 and SH3 domains. *Curr. Biol.* 3:434–442.

Peles, E., S.S. Bacus, R.A. Koski, H.S. Lu, D. Wen, S.G. Ogden, R. Ben-Levy, and Y. Yarden. 1992. Isolation of the neu/HER-2 stimulatory ligand: a 44 kd glycoprotein that induces differentiation of mammary tumor cells. *Cell.* 69: 205–216.

Peles, E., R. Ben-Levy, E. Tzahar, N. Liu, D. Wen, and Y. Yarden. 1993. Cell-type specific interaction of Neu differentiation factor (NDF/heregulin) with Neu/HER-2 suggests complex ligand-receptor relationships. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:961–971.

Pelicci, G., S. Giordano, Z. Zhen, A.E. Salcini, L. Lanfrancone, A. Bardelli, G. Panayotou, M.D. Waterfield, C. Ponzetto, P.G. Pelicci, and P.M. Comoglio. 1995. The motogenic and mitogenic responses to HGF are amplified by the Shc adaptor protein. *Oncogene.* 10:1631–1638.

Peng, X., L.A. Greene, D.R. Kaplan, and R.M. Stephens. 1995. Deletion of a conserved juxtamembrane sequence in Trk abolishes NGF-promoted neuritogenesis. *Neuron.* 15:395–406.

Peters, K., S. Werner, X. Liao, S. Wert, J. Whitsett, and L. Williams. 1994. Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:3296–3301.

Ponzetto, C., A. Bardelli, Z. Zhen, F. Maina, P. Dallazonca, S. Giordano, A. Graziani, G. Panayotou, and P.M. Comoglio. 1994. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell.* 77:261–271.

Ricci, A., L. Lanfrancone, R. Chiari, G. Belardo, C. Pertica, P. Natali, P. Pelicci, and O. Segatto. 1995. Analysis of protein-protein interactions involved in the activation of the Shc/Grb-2 pathway by the ErbB-2 kinase. *Oncogene.* 11:1519–1529.

Ridley, A.J., P.M. Comoglio, and A. Hall. 1995. Regulation of scatter factor hepatocyte growth factor responses by ras, rac, and rho in MDCK cells. *Mol. Cell. Biol.* 15:1110–1122.

Riethmacher, D., O. Langholz, S. Gödecke, M. Sachs, and C. Birchmeier. 1994. Biochemical and functional characterization of the murine ros protooncogene. *Oncogene.* 9:3617–3626.

Riethmacher-Sonnenberg, E., B. Walter, D. Riethmacher, S. Gödecke, and C. Birchmeier. 1996. The c-ros tyrosine kinase controls regionalization and differentiation of epithelial cells in the epididymis. *Genes & Dev.* In press.

Rosen, E.M., J. Knesel, I.D. Goldberg, L. Jin, M. Bhargava, A. Joseph, R. Zitnik, J. Wines, M. Kelley, and S. Rockwell. 1995. Scatter factor modulates the metastatic phenotype of the EMT6 mouse mammary tumor. *Int. J. Cancer.* 57:706–714.

Rozakis-Adcock, M., J. McGlade, G. Mbamalu, G. Pelicci, R. Daly, W. Li, A. Batzer, S. Thomas, J. Brugge, P.G. Pelicci et al. 1992. Association of the Shc and Grb2/Sem5 SH2-containing proteins is implicated in activation of the Ras pathway by tyrosine kinases. *Nature (Lond.).* 360:689–692.

Rozakis-Adcock, M., R. Fernley, J. Wade, T. Pawson, and D. Bowtell. 1993. The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1. *Nature (Lond.).* 363:83–85.

Rubin, J.S., H. Osada, P.W. Finch, W.G. Taylor, S. Rudikoff, and S.A. Aaronson. 1989. Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proc. Natl. Acad. Sci. USA.* 86:802–806.

Santoro, M., W.T. Wong, P. Aroca, E. Santos, B. Matoskova, M. Grieco, A. Fusco, and P.P. Di Fiore. 1994. An epidermal growth factor receptor/ret chimera generates mitogenic and transforming signals: evidence for a ret-specific signaling pathway. *Mol. Cell. Biol.* 14:663–675.

Santoro, M., F. Carlomagno, A. Romano, D.P. Bottaro, N.A. Dathan, M. Grieco, A. Fusco, G. Vecchio, B. Matoskova, M.H. Kraus et al. 1995. Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. *Science (Wash. DC).* 267:381–383.

Schmidt, C., F. Bladt, S. Gödecke, V. Brinkmann, W. Zschiesche, M. Sharpe, E. Gherardi, and C. Birchmeier. 1995. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature (Lond.).* 373:699–702.

Schuchardt, A., V. D'Agati, L. Larsson Blomberg, F. Costantini, and V. Pachnis. 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature (Lond.).* 367:380–383.

Segatto, O., G. Pelicci, S. Giuli, G. Digiesi, P.P. Di Fiore, J. McGlade, T. Pawson, and P.G. Pelicci. 1993. Shc products are substrates of erbB-2 kinase. *Oncogene.* 8:2105–2112.

Sonnenberg, E., A. Gödecke, B. Walter, F. Bladt, and C. Birchmeier. 1991. Transient and locally restricted expression of the ros1 protooncogene during mouse development. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3693–3702.

Sonnenberg, E., D. Meyer, K.M. Weidner, and C. Birchmeier. 1993. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J. Cell Biol.* 123:223–235.

Soriano, J.V., M.S. Pepper, T. Nakamura, L. Orci, and R. Montesano. 1995. Hepatocyte growth factor stimulates extensive development of branching duct-like structures by cloned mammary gland epithelial cells. *J. Cell Sci.* 108:413–430.

Stephens, R.M., D.M. Loeb, T.D. Copeland, T. Pawson, L.A. Greene, and D.R. Kaplan. 1994. Trk receptors use redundant signal transduction pathways involving SHC and PLC-gamma 1 to mediate NGF responses. *Neuron.* 12:691–705.

Stoker, M., E. Gherardi, M. Perryman, and J. Gray. 1987. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature (Lond.).* 327: 239–242.

Takahashi, M., J. Ritz, and G.M. Cooper. 1985. Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cell.* 42:581–588.

Traverse, S., K. Seedorf, H. Paterson, C.J. Marshall, P. Cohen, and A. Ullrich. 1994. EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor. *Curr. Biol.* 4:694–701.

Uehara, Y., O. Minowa, C. Mori, K. Shiota, J. Kuno, T. Noda, and N. Kitamura. 1995. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature (Lond.).* 373:702–705.

Ullrich, A., and J. Schlessinger. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 61:203–212.

Ullrich, A., L. Coussens, J.S. Hayflick, T.J. Dull, A. Gray, A.W. Tam, J. Lee, Y. Yarden, T.A. Libermann, J. Schlessinger et al. 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature (Lond.).* 309:418–425.

van der Geer, P., T. Hunter, and R.A. Lindberg. 1994. Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu. Rev. Cell Biol.* 10:251–337.

Vetter, M.L., and J.M. Bishop. 1995. Beta PDGF receptor mutants defective for mitogenesis promote neurite outgrowth in PC12 cells. *Curr. Biol.* 5:168–178.

Waters, S.B., K. Yamauchi, and J.E. Pessin. 1995. Insulin-stimulated disassociation of the SOS-Grb2 complex. *Mol. Cell. Biol.* 15:2791–2799.

Weidner, K.M., J. Behrens, J. Vandekerckhove, and W. Birchmeier. 1990. Scat-

ter factor: molecular characteristics and effect on the invasiveness of epithelial cells. *J. Cell Biol.* 111:2097–2108.
Weidner, K.M., M. Sachs, and W. Birchmeier. 1993. The *Met* receptor tyrosine kinase transduces motility, proliferation, and morphogenic signals of scatter factor/hepatocyte growth factor in epithelial cells. *J. Cell Biol.* 121:145–154.
Weidner, K.M., M. Sachs, D. Riethmacher, and W. Birchmeier. 1995. Mutation of juxtamembrane tyrosine residue 1001 suppresses loss-of-function mutations of the met receptor in epithelial cells. *Proc. Natl. Acad. Sci. USA.* 92: 2597–2601.
Wen, D., E. Peles, R. Cupples, S.V. Suggs, S.S. Bacus, Y. Luo, G. Trail, S. Hu, S.M. Silbiger, R. Ben-Levy et al. 1992. Neu differentiation factor: a transmembrane glycoprotein containing an EGF domain and an immunoglobulin homology unit. *Cell.* 69:559–572.
Wennström, S., A. Siegbahn, K. Yokote, A.K. Arvidsson, C.H. Heldin, S. Mori, and L. Claesson-Welsh. 1994. Membrane ruffling and chemotaxis transduced by the PDGF beta-receptor require the binding site for phosphatidylinositol 3′ kinase. *Oncogene.* 9:651–660.
Werner, S., W. Weinberg, X. Liao, K.G. Peters, M. Blessing, S.H. Yuspa, R.L. Weiner, and L.T. Williams. 1993. Targeted expression of a dominant-negative FGF receptor mutant in the epidermis of transgenic mice reveals a role of FGF in keratinocyte organization and differentiation. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:2635–2643.
Werner, S., H. Smola, X. Liao, M.T. Longaker, T. Krieg, P.H. Hofschneider, and L.T. Williams. 1994. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science (Wash. DC).* 266:819–822.
Woolf, A.S., M. Kolatsi-Joannou, P. Hardman, E. Andermarcher, C. Moorby, L.G. Fine, P.S. Jat, M.D. Noble, and E. Gherardi. 1995. Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros. *J. Cell Biol.* 128:171–184.
Yamashita, J., M. Ogawa, S. Yamashita, K. Nomura, M. Kuramoto, T. Saishoji, and S. Shin. 1994. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. *Cancer Res.* 54:1630–1633.
Yang, Y., E. Spitzer, D. Meyer, M. Sachs, C. Niemann, G. Hartmann, K.M. Weidner, C. Birchmeier, and W. Birchmeier. 1995. Sequential requirement of hepatocyte growth factor and neuregulin in the morphogenesis and differentiation of the mammary gland. *J. Cell Biol.* 131:215–226.
Zarnegar, R., and G. Michalopoulos. 1989. Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. *Cancer Res.* 49:3314–3320.
Zhou, M.-M., K.S. Ravichandran, E.T. Olejniczak, A.M. Petros, R.P. Meadows, M. Sattler, J.E. Harlan, W.S. Wade, S.J. Burakoff, and S.W. Fesik. 1995. Structure and ligand recognition of the phosphotyrosine binding domain of Shc. *Nature (Lond.).* 378:584–592.
Zhu, H., M.A. Naujokas, and M. Park. 1994. Receptor chimeras indicate that the met tyrosine kinase mediates the motility and morphogenic responses of hepatocyte growth/scatter factor. *Cell Growth & Differ.* 5:359–366.