Targeting of the SF/HGF Receptor to the Basolateral Domain of Polarized Epithelial Cells

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Abstract. Scatter Factor, also known as Hepatocyte Growth Factor (SF/HGF), has pleiotropic functions including direct control of cell–cell and cell–substrate adhesion in epithelia. The subcellular localization of the SF/HGF receptor is controversial. In this work, the cell surface distribution of the SF/HGF receptor was studied in vivo in epithelial tissues and in vitro in polarized MDCK monolayers. A panel of monoclonal antibodies against the β chain of the SF/HGF receptor stained the basolateral but not the apical surface of epithelia lining the lumen of human organs. Radiolabeled or fluorescent-tagged anti-receptor antibodies selectively bound the basolateral cell surface of MDCK cells, which form a polarized monolayer sealed by intercellular junctions, when grown on polycarbonate filters in a two-chamber culture system. The receptor was concentrated around the cell–cell contact zone, showing a distribution pattern overlapping with that of the cell adhesion molecule E-cadherin. The basolateral localization of the SF/HGF receptor was confirmed by immunoprecipitation after domain selective cell surface biotinylation. When cells were fully polarized the SF/HGF receptor became resistant to non-ionic detergents, indicating interaction with insoluble component(s). In pulse-chase labeling and surface biotinylation experiments, the newly synthesized receptor was found exclusively at the basolateral surface. We conclude that the SF/HGF receptor is selectively exposed at the basolateral plasma membrane domain of polarized epithelial cells and is targeted after synthesis to that surface by direct delivery from the *trans*-Golgi network.

SCATTER Factor (SF)[1] and Hepatocyte Growth Factor (HGF) are identical glycoproteins controlling motility, mitogenesis, and morphogenesis in epithelial cells (Weidner et al., 1991; Naldini et al., 1991a). SF/HGF was originally described as a secretory product of fibroblasts which dissociates epithelial cells, increasing their motility and invasiveness (Stoker et al., 1987; Weidner et al., 1990). The factor was reported to have chemotactic properties (Gherardi et al., 1989) and to promote the progression of carcinoma cells toward malignant invasive phenotypes (Weidner et al., 1990); the factor regulates the level of ECM degradation, by enhancing the synthesis of enzymes involved in ECM proteolysis (Pepper et al., 1992). SF/HGF is a powerful mitogen for hepatocytes in primary cultures (Miyazawa et al., 1989; Nakamura et al., 1989; Zarnegar and Michalo-

poulos, 1989). It also stimulates the growth of other epithelial tissues, such as kidney tubular epithelium and keratinocytes (Kan et al., 1991), melanocytes (Rubin et al., 1991), and endothelial cells (Bussolino et al., 1992; Grant et al., 1993). SF/HGF is a mediator of kidney and liver regeneration (Nagaike et al., 1991; Higuchi and Nakamura, 1991; Michalopoulos, 1990). It acts as a morphogen in the chick embryo (Stern et al., 1990) and induces the three-dimensional organization of MDCK epithelial cells in vitro (Montesano et al., 1991) and blood vessels in vivo (Bussolino et al., 1992; Grant et al., 1993). These features indicate that SF/HGF plays a major role in the differentiation and morphogenetic events which lead to the formation of branching tubules.

The receptor for SF/HGF is the tyrosine kinase encoded by the *MET* proto-oncogene (Bottaro et al., 1991; Naldini et al., 1991a, b). The receptor is a 190-kD heterodimer of a 50-kD α subunit, covalently linked to a 145-kD β subunit (Giordano et al., 1989a). The α subunit is extracellular; the β subunit bears an extracellular portion involved in ligand binding, a membrane-spanning segment, and a cytoplasmic tyrosine kinase domain (Tempest et al., 1986; Gonzatti-Haces et al., 1988). Both subunits originate from glycosylation and

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1. *Abbreviations used in this paper:* CEA, carcinoembryonic antigen; HGF, hepatocyte growth factor; SF, scatter factor.

proteolytic cleavage of a common precursor of 170 kD (Giordano et al., 1989b). The SF/HGF receptor is widely expressed in epithelial tissues lining the lumen of several organs including intestine and kidney (Prat et al., 1991a; Di Renzo et al., 1991). The receptor is expressed in the early stages during development of epithelial organs, while the ligand is expressed in the surrounding mesenchyme (Sonnenberg et al., 1993a, b). SF/HGF and its tyrosine kinase receptor are thought to control mesenchymal/epithelial interactions during development. The mechanisms by which epithelial cells receive and transduce the signal have been analyzed in detail (Bardelli et al., 1992; Graziani et al., 1993; Ponzetto et al., 1993). However, modifications in cell–cell and cell–substrate interactions, leading to dissociation, migration, and remodeling of epithelial monolayers, have remained poorly understood.

The subcellular localization of growth factor receptors expressed by epithelial structures, particularly in polarized layers or tubules, is largely unknown. In a recent report the SF/HGF receptor was found to be associated with the apical microvilli of cells bordering mammary ducts and the intestinal lumen (Tsarfaty et al., 1992). This is somewhat unexpected, because the SF/HGF is present in the circulatory system (Nakamura et al., 1986; Zarnegar and Michalopoulos, 1989) and is stored in the pericellular matrix, mainly as a biologically inactive single-chain precursor (Matsumoto and Yamamoto, 1991; Naldini et al., 1992). In this work, the subcellular distribution of SF/HGF receptor was studied in vivo in epithelial tissues stained by a panel of monoclonal antibodies, and in vitro, in polarized MDCK cell monolayers, by domain selective biotinylation and pulse-chase experiments. The receptor was found to be localized at the basolateral cell surface. Newly synthesized SF/HGF receptor reaches this plasma membrane domain by targeting and direct delivery from the *trans*-Golgi network.

Materials and Methods

Cells and Antibodies

MDCK type II cells were grown in DMEM supplemented with 5% FCS. Cells grown on Transwells (Costar, Cambridge, MA) were seeded at 4.2 × 10^5 cells/cm^2. Cell monolayers were used for experiments on the fourth day of culture. Polarization of the monolayer was assessed by measuring electrical resistance with the Millicell-ERS instrument (Millipore Continental Water Systems, Bedford, MA). In some experiments cells were grown as small colonies, at a final density of 4.2 × 10^3 cells/cm^2. The murine mAbs DO-24 and DN-30, directed against the extracellular domain of the SF/HGF receptor, were obtained after immunization with living cells from the human gastric carcinoma cell line GTL-16 (Prat et al., 1991b). mAb DQ-13, raised against a peptide corresponding to nineteen COOH-terminal amino acids (from Ser^{1372} to Ser^{1390}) of the human *MET* sequence, was also used. Other mAbs and their source were: DECMA-1 to canine E-cadherin (Sigma Chem. Co., St. Louis, MO); 4B4 to the integrin β$_1$ chain (Coulter Immunology, Hialeah, FL). Antiserum against fimbrin was obtained from K. Weber, Max-Planck-Institut fur Biophysikalische Chemie, Gottingen, Germany. Antisera against carcinoembryonic antigen (CEA) and rhodamine-labeled secondary antibodies were purchased from Dakopatts (Glostrup, Denmark).

Immunohistochemical Staining

Normal human tissues were obtained from surgical samples. The tissues were mounted in OCT 4583 embedding compound (Miles Scientific, Naperville, IL) and immediately frozen in liquid nitrogen. Cryostat sections (6-μm thick) cut in a Reichert cryomicrotome were transferred onto microscope slides coated with poly-L-lysine (Sigma Chem. Co.), air-dried, and stored

at room temperature overnight. The samples were fixed in a chloroform-acetone mixture (1:1), air-dried, and incubated for 10 min in PBS supplemented with 1% serum of the same species as the secondary antibody. Sections were overlaid with 50 μl of undiluted supernatant or ascitic fluid at 1:400 dilution at room temperature for 30 min in a moist chamber. After a thorough wash in PBS, the sections were incubated with biotinylated secondary antibodies and processed for the ABC method (avidin-biotin-peroxidase complex) using the Vectastain ABC kit (Vector Labs. Inc., Burlingame, CA). After several washes, 100 μl of substrate were added for 5 min, prepared as follows: 5 mg 3-amino-9-ethylcarbazole (Sigma Chem. Co.) were dissolved in 1 ml N,N-dimethylformamide (Merck, Darmstadt, Germany) supplemented with 9 ml 100 mM sodium acetate pH 5.2 and 100 μl of 12% H$_2$O$_2$. All samples were counterstained with Mayer's haemalum solution, mounted in Kaiser's glycerol gelatin (Merck) and examined with a Zeiss Axiophot photomicroscope equipped with planapochromatic lenses.

For indirect immunofluorescence microscopy, MDCK cells grown on 6.5-mm Transwells were fixed on ice for 5 min with freshly prepared 3% paraformaldehyde, 2% sucrose and incubated at 37°C for 30 min with antibodies. An appropriate dilution of mAbs was added to either the apical or the basolateral compartment of the Transwell unit. Filters were then washed with four changes of PBS-0.2%BSA and incubated with rhodamine-labeled secondary antibodies at 37°C for 30 min. After four washings with PBS-0.2% BSA, filters were cut, mounted on glass slides in a solution of Mowial (Calbiochem-Behring Corp., La Jolla, CA) and viewed with Zeiss epifluorescence optics.

Binding Assays

MDCK cells grown on 6.5-mm Transwells were starved for 30 min in serum-free DMEM and cooled on ice, and 2 μg/ml ^{125}I-labeled DO-24 or DN-30 mAbs (10 × 10^6 cpm/μg, in PBS) was added to the apical or basolateral compartment of the Transwell unit. Binding was carried out for 3 h on ice. Intactness of monolayers was determined by counting the media from both compartments in a gamma counter; generally, less than 1% of the initially added label diffused to the opposite side. Filters were washed with five changes of cold PBS containing 1 mM CaCl$_2$, and 0.5 mM MgCl$_2$, cut, and bound radioactivity counted. Points were performed in duplicate. Nonspecific binding, determined in the presence of a 100-fold excess of cold mAb, was subtracted and represented 15–20% of the total.

Domain-Selective Cell Surface Biotinylation and Immunoprecipitation

MDCK cells grown on 24.5-mm Transwells were washed three times at 4°C with Hank's Balanced Salts Biotinylation Buffer, pH 7.4 (HBB) consisting of 1.3 mM CaCl$_2$, 0.4 mM MgSO$_4$, 5 mM KCl, 138 mM NaCl, 5.6 mM D-glucose and 25 mM Hepes, pH 7.4, and then cooled for 10 min in HBB at 4°C. Sulfosuccinimidobiotin (sulfo-NHS-biotin, Pierce Chem. Co., Rockford, IL) was made 0.5 mg/ml in HBB and applied to either the apical (0.5 ml) or basolateral (1 ml) side of the monolayer with HBB on the opposite surface. After 15 min, the biotin solution was removed and replaced with fresh biotin solution for another 15 min. The reaction was stopped by removing the biotin solution, washing four times quickly and incubating 10 min with Minimal Essential Media containing Hank's Balanced salts, 0.6% BSA, 20 mM Hepes, pH 7.3 (MEM/BSA) at 4°C. Cells were lysed by cutting filters with a scalpel, placing into 0.8 ml of 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% SDS, 1% TX-100, 1% DOC, 5 mM EDTA (RIPA buffer), and inhibitors of proteases (2 mM PMSF [Sigma], 50 μg/ml pepstatin, 50 μg/ml chymostatin and 10 μg/ml antipain [Chemicon, CA]) for 15 min on ice. In some experiments cells on filter were lysed in 5 mM Pipes, pH 7.6, 100 mM NaCl, 5 mM MgCl$_2$, 300 mM sucrose, 5 mM EGTA (DIM buffer), 1% Triton X-100 and inhibitors of proteases. Cell extracts were precleared with protein-A Sepharose and rotated 2.5 h at 4°C with anti-SF/HGF receptor mAbs (DN-30 or DO-24). Immunocomplexes were collected with affinity purified rabbit anti–mouse IgG (Jackson Immuno Research Labs., Inc., West Grove, PA) coupled to Protein-A Sepharose. Beads were washed three times with lysis buffer and samples were eluted by boiling in Laemmli buffer containing 100 mM dithiothreitol. Immunoprecipitates were electrophoresed on 8% SDS-PAGE. Samples were transferred to nitrocellulose, probed with streptavidin-HRP, and visualized on Kodak XOMAT AR film (Rochester, NY) with an enhanced Chemiluminescence kit (ECL, Amersham, UK). Quantification of biotinylated protein was achieved with a laser scanning Personal Densitometer (Molecular Dynamics, Sunnyvale,

CA), using multiple exposures and taking care to scan exposure within the linear range of the film.

Analysis of Protein Targeting to the Cell Surface

MDCK cells grown on 24.5-mm Transwells were starved for 15 min at 37°C in MEM lacking cysteine and methionine and supplemented with 5% dialyzed FCS, then pulse labeled for 15 min in the same medium supplemented with [³⁵S] Translabel (100 μCi/filter, New England Nuclear, Boston, MA). Chases were performed in MEM/BSA at 37°C. Cells were then biotinylated and immunoprecipitated as described above. Immunoprecipitated proteins were eluted from Protein-A beads by boiling 5 min in 10% SDS, diluted in 1 ml of 2.5% Triton Dilution buffer (2.5% Triton X-100, 100 mM NaCl, 5 mM EDTA, 100U/ml Trasylol, 0.02% NaN3, 100 mM triethanolamine-HCl, pH 8.6) and samples were precleared twice with Sepharose CL-2B. Biotinylated proteins were precipitated by rotating overnight with Streptavidin-agarose (Sigma). Beads were washed three times with mixed micelle buffer and one time with final wash buffer before analysis of samples by electrophoresis and either autoradiography or phosphorimaging. A Molecular Dynamics Phosphorimager was used to quantify newly synthesized proteins on the apical or basolateral cell surface.

Results

Immunolocalization of the SF/HGF Receptor in Human Tissues

To analyze the subcellular localization of the SF/HGF receptor, immunoperoxidase staining was performed on human tissue cryostat sections. A panel of monoclonal antibodies (mAbs) directed against the SF/HGF receptor was used: two mAbs recognized different epitopes on the extracellular domain (DO-24 and DN-30) and one mAb reacted against the COOH-terminal tail (DQ-13). In the colon, the whole panel of anti-receptor mAbs selectively stained the basolateral plasma membranes of epithelial cells: staining with DO-24 mAb is depicted in Fig. 1 a. The pattern of reactivity perfectly matches that obtained with the antibody against the β₁ integrin, known to be selectively expressed at basolateral cell sides (Fig. 1 b). The receptor was absent from the apical
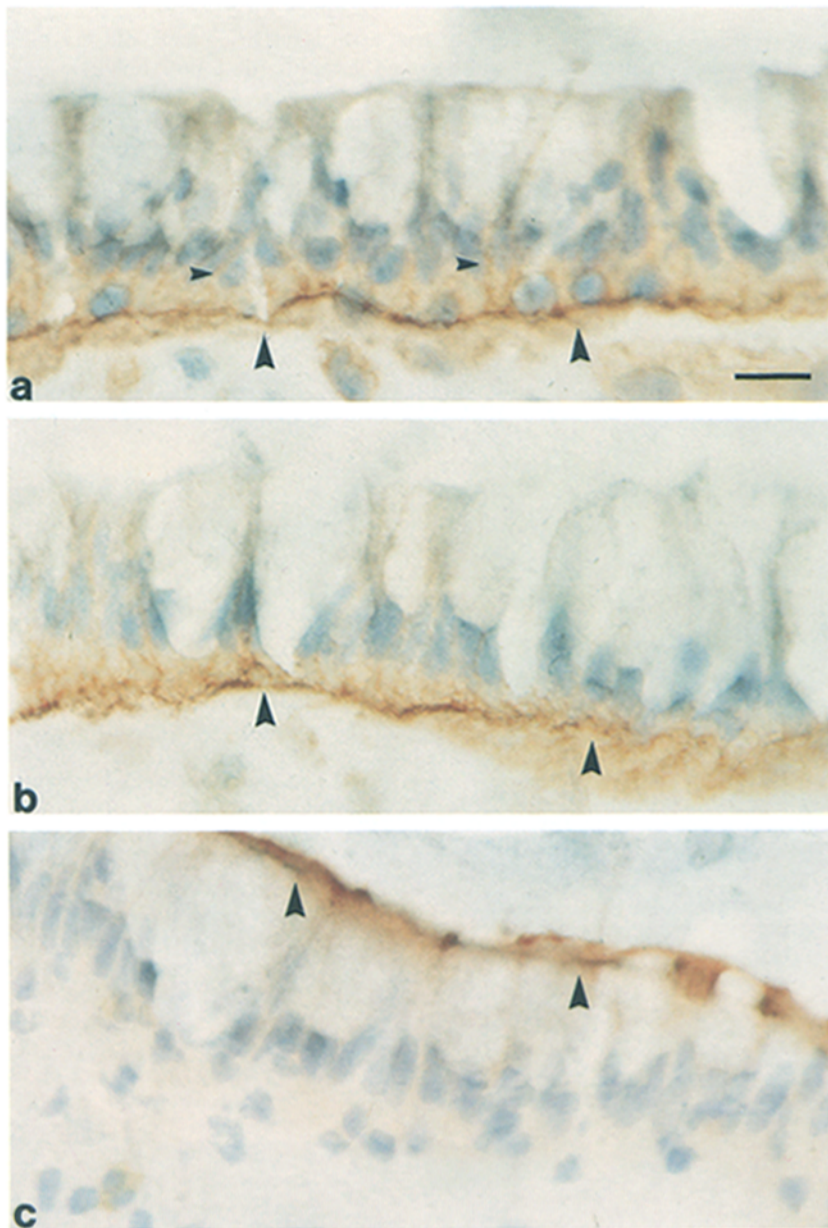
Figure 1. Localization of SF/HGF receptor in human colon epithelium. Cryostat sections were fixed in cold absolute acetone and stained by immunoperoxidase with (a) mAb DO-24 directed against the extracellular domain of the SF/HGF receptor; (b) mAb 4B4 recognizing β₁ integrin; (c) antiserum against fimbrin. The SF/HGF receptor was localized to the basolateral plasma membrane of epithelial cells, with a pattern of staining indistinguishable from that observed for the β₁ integrin. The receptor was absent from the apical side, where fimbrin, a structural marker of the apical brush border, was located. Bar represents 3 μm.

membrane, where fimbrin, a structural marker of the apical brush border, was detected as a positive control (Fig. 1 c). Immunohistochemical analysis on other tubular epithelia, including those lining distinct segments of the gastro-intestinal tract, bile ducts, respiratory tract, mammary gland ducts and kidney tubules, confirmed that the receptor is exposed at the basolateral, but not apical rims (not shown; cfr. Prat et al., 1991a).

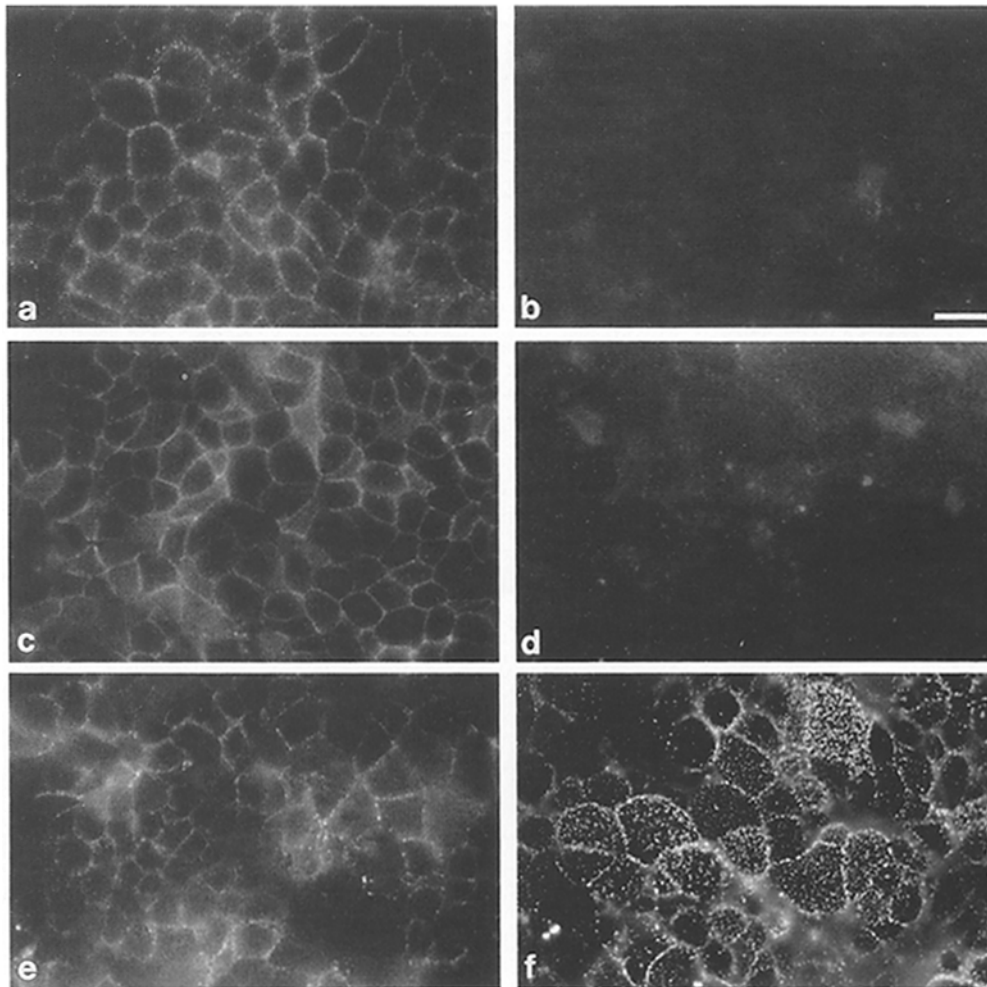## Immunolocalization of the SF/HGF Receptor in MDCK Cells Polarized In Vitro

MDCK cells form epithelial polarized monolayers when grown in culture under appropriate conditions. The apical cell surface is covered with microvilli and faces the culture medium. The basal cell surface adheres to the substratum; the lateral surface is in close contact with adjacent cells in the monolayer. The basolateral surface domain is separated from the apical domain by a junctional complex including tight junctions (Simons and Fuller, 1985; Rodriguez-Boulan and Sabatini, 1978). Monolayers grown on polycarbonate permeable filters (Transwell chambers) expose separately the apical and the basal surfaces to experimental probes (Lisanti et al., 1988). MDCK cells grown on Transwells were fixed without permeabilization and stained with DN-30 or DO-24, two of the above anti-receptor mAbs that are

cross-species reactive. Immunofluorescence staining occurred only when antibodies were presented to the baso-lateral side of MDCK cells (Fig. 2, compare a with b). The antibodies stained the basolateral surface at regions of cell-cell contact, with a pattern indistinguishable from the staining with anti-E-cadherin mAb (Fig. 2, compare a with c). The cell adhesion molecule E-cadherin is known to be selectively localized at the basolateral cell surface domain (Behrens et al., 1985; Le Bivic et al., 1990). In the same experiment, the CEA, analyzed as positive control, was both apical and basolateral, with preferential localization at the apical cell surface covered with microvilli (Fig. 2, compare e with f).

To quantify the expression of the SF/HGF receptor to the different plasma membrane domains, the binding of ^{125}I-labeled monoclonal antibodies presented either to the apical or to the basolateral surfaces of MDCK cells, by addition to the upper or to the lower Transwell chamber, was evaluated. As shown in Fig. 3, more than 90% binding occurred at the basolateral surface. A radiolabeled monoclonal antibody, directed against the intracellular COOH-terminal tail of the SF/HGF receptor, used as a negative control, did not bind either of the cell surface domains (data not shown).

Domain Selective Surface Biotinylation of the SF/HGF Receptor

The presence of SF/HGF receptors on the basal but not api-

Figure 2. Localization of SF/HGF receptor in polarized MDCK cells. MDCK confluent cells were grown on Transwell filters and fixed with 3% paraformaldehyde. Immunofluorescence was performed with anti-SF/HGF receptor mAb (a and b), anti-E-cadherin mAb (c and d), and anti-CEA antiserum (e and f). Antibodies were presented to either the basolateral (a, c, and e) or the apical (b, d, and f) surface of MDCK cells. The SF/HGF receptor is exclusively basolateral and shows a pattern of staining overlapping that of E-cadherin. The CEA antigen is expressed also on the apical surface covered with microvilli. Bar represents 5 μm.
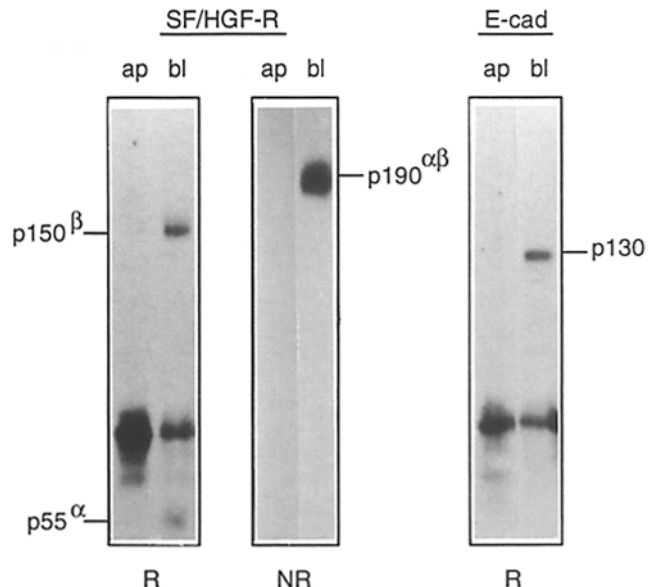
316
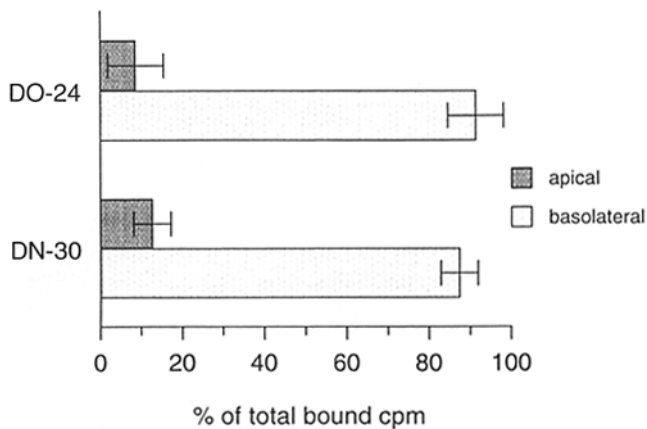
Figure 3. Cell surface distribution of SF/HGF receptor in MDCK cells. The ^{125}I-labeled DO-24 and DN-30 mAbs were presented to either the upper or the lower surface of MDCK cells grown on Transwells. Given are mean values ±1 SD of three independent experiments performed in duplicate. Total values were between 8,000 and 15,000 cpm. More than 90% binding occurred at the basolateral surface.

Figure 4. Steady state distribution of the SF/HGF receptor on the plasma membrane of MDCK cells. MDCK cells, grown on Transwell filters, were biotinylated on either their apical (*ap*) or basolateral (*bl*) surface. Biotinylated SF/HGF receptor and E-cadherin were immunoprecipitated with DO-24 and DECMA-1 mAbs, respectively. Samples were run on SDS-PAGE under reducing (*R*) and non-reducing (*NR*) conditions, transferred to nitrocellulose, and probed with streptavidin-HRP. Apical or basolateral proteins were visualized by ECL. The SF/HGF receptor in MDCK cells is exposed exclusively at the basolateral surface, as a 190-kD heterodimer of a 55-kD α subunit, covalently linked to a 150-kD β subunit.

cal surface of MDCK cells was confirmed by domain selective surface biotinylation and immunoprecipitation. MDCK cells grown on Transwell filters were biotinylated with sulfo-NHS-biotin added either to the apical or to the basolateral surfaces, as described in Materials and Methods. The cells were then lysed, the SF/HGF receptor immunoprecipitated, separated by SDS-PAGE, and transferred to nitrocellulose. The extent of biotinylation was assessed by probing nitrocellulose blots with HRP-conjugated streptavidin and densitometric analysis. More than 95% of the SF/HGF receptor labeled by the biotin was located on the basolateral membrane (Fig. 4, *left panels*). Under identical conditions, ~95% of the cell adhesion molecule E-cadherin, studied as a control, was labeled from the basolateral side (Fig. 4, *right panel*). The additional band of 70 kD associated with immunocomplexes formed by the anti-SF/HGF receptor and anti-E-cadherin antibodies is a non-specific contaminant, likely to be albumin from FCS. Biotin labeled the β (150 kD) and the α (55 kD) chains of the heterodimeric SF/HGF receptor, showing that both are exposed at the basolateral cell surface. The low level of biotinylation of the α chain likely reflects the relative abundance of lysine residues in the α and β chains. The α and β chains of canine SF/HGF receptor displayed a slightly higher molecular weight than the α and β chains of the human receptor (Giordano et al., 1989a). These differences are likely due to species differences in protein glycosylation.

Solubility Properties of SF/HGF Receptor Exposed at the Cell Surface of Polarized or Non-Polarized MDCK Cells

MDCK cells grown on Transwell filters, under conditions of polarization, were biotinylated, and lysed with buffers containing either ionic (SDS) or non-ionic (Triton X-100) detergents. The recovery of surface-labeled receptor was quantified by immunoprecipitation and Western blot with HRP-conjugated streptavidin. The SF/HGF receptor was relatively insoluble in non ionic-detergent (Fig. 5, *left panel*). Similar results were obtained when the solubility of E-cadherin was studied (Fig. 5, *left panel*). It is known that the relative insolubilty in non-ionic detergent of E-cadherin is due to formation of non-covalent complexes with components of the cytoskeleton (Hirano et al., 1987; Nelson et al., 1990; Tsukita et al., 1992). In a parallel experiment the polymeric immunoglobulin receptor, expressed in polarized MDCK cells, was slightly better solubilized in Triton X-100 than SDS (not shown). We then dealt with the question of whether polarization could influence detergent solubility properties of the SF/HGF receptor. Steady-state biotinylation followed by immunoprecipitation and probing with HRP-streptavidin was performed on MDCK cells grown in small colonies (non-polarized). Fig. 5 (*right panel*) shows that, unlike polarized cells, a significant fraction of the receptor exposed at the surface of non-polarized cells was soluble in Triton X-100.

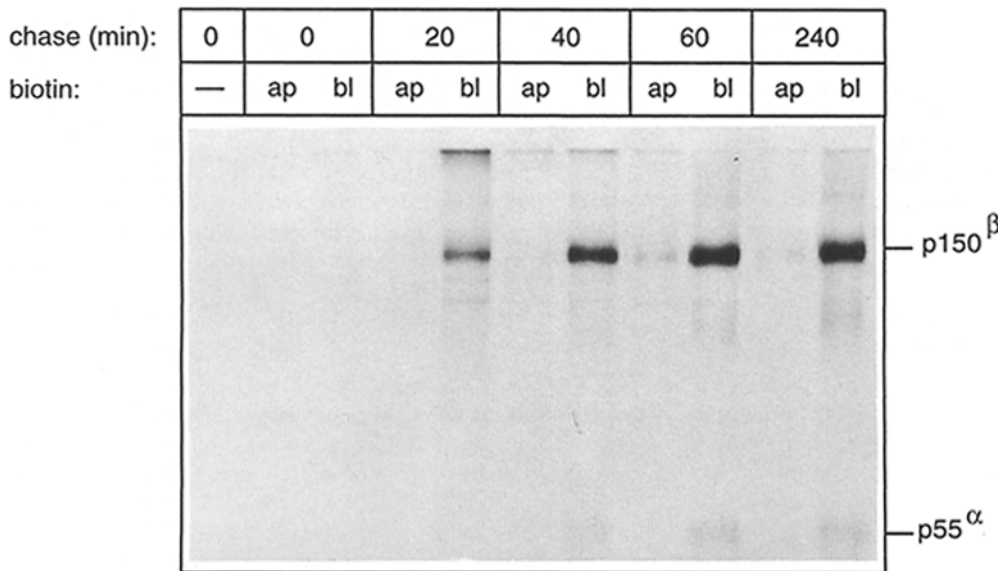### Pulse-Chase Analysis of SF/HGF Receptor Biosynthetic Delivery

The cell-surface delivery of newly synthesized SF/HGF receptor in polarized MDCK cells was examined by the widely used approach of combining pulse-chase and domain-selective biotinylation (Le Bivic et al., 1989). MDCK cells grown on Transwell filters were pulse-labeled with [35S] Translabel for 15 min and biotinylated, after various periods of chase,

POLARIZED CELLS   NON POLARIZED CELLS

SF/HGF-R   E-cad   SF/HGF-R   E-cad

p150 β
p130

S   T   S   T   S   T   S   T
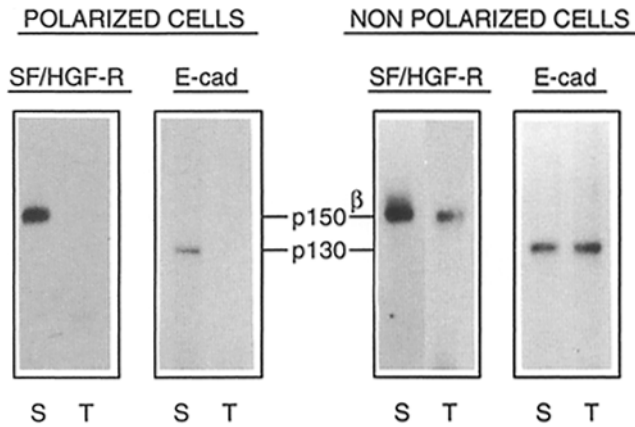
Figure 5. Solubility properties of the SF/HGF receptor in polarized and non-polarized MDCK cells. MDCK cells were grown on Transwell filters at final cell density of 4.2 × 10^5 cells/cm² (polarized) and 4.2 × 10^3 cells/cm² (non-polarized). Biotinylated SF/HGF receptor and E-cadherin were immunoprecipitated from cells extracted with buffers containing SDS (S) or Triton X-100 (T), as detergents. Reduced samples were SDS-gel electrophoresed, transferred to nitrocellulose, and blotted with streptavidin-HRP. If cells are polarized, more than 95% of the receptor exposed at the cell surface is insoluble in non-ionic detergent.

on either the apical or the basolateral surface. The SF/HGF receptor was immunoprecipitated, solubilized, reprecipitated with streptavidin-agarose, and revealed by fluorography after SDS-PAGE (Fig. 6). The amount of SF/HGF receptor on the apical or basolateral surface was quantified by phosphorimaging. The SF/HGF receptor appeared at the plasma membrane surface after 20 min of chase as a mature heterodimer composed of a 150-kD (β) and a 55-kD (α) chains. More than 90% of the newly synthesized receptor exposed at the cell surface was directly delivered to the basolateral membrane domain.

Discussion

In this work we show that the SF/HGF receptor is selectively localized at the basolateral surface of the polarized epithelia in vivo as well as in experimental conditions in vitro. This conclusion is supported by three independent types of evidence: (1) immunohistochemical staining pattern obtained with different monoclonal antibodies and frozen sections; (2) binding of the specific antibodies to the basolateral side of MDCK cells polarized in vitro; (3) selective biotinylation of proteins exposed at the basolateral surface. A similar basolateral distribution of the EGF receptor has been previously demonstrated by measuring the binding of radiolabeled ligand to MDCK cells polarized in Transwell chambers (Maratos-Flier et al., 1987). Previous work has reported the association of the SF/HGF receptor with the apical microvilli of cells bordering mammary ducts and intestinal lumen (Tsarfaty et al., 1992). The basolateral localization of the SF/HGF receptor is consistent with the site of action of the ligand, which is present in the circulatory system (Nakamura et al., 1986; Zarnegar and Michalopoulos, 1989) and is stored in the pericellular matrix (Matsumoto and Yamamoto, 1991; Naldini et al., 1992).

Interestingly, the staining of SF/HGF receptor is intense at the lateral surfaces of the cell in the region of cell–cell contacts, and displays a pattern similar to that elicited by antibodies against the cell adhesion molecule E-cadherin (Vestweber and Kemler, 1985; Behrens et al., 1985; Gumbiner et al., 1988). A junctional localization of the SF/HGF tyrosine kinase receptor is potentially significant. A role of tyrosine kinases in the control of junction formation is suggested for tyrosine kinases of the c-*Src* family: elevated levels of tyrosine phosphorylation induced by the c-*Src* family kinases have been found in isolated adherens junctions (Tsukita et al., 1991; Volberg et al., 1992). Transformation with v-*Src* causes loss of epithelial differentiation and gain of invasiveness, which correlate with tyrosine phosphorylation of the E-cad-

chase (min):	0	0		20		40		60		240	
biotin:	—	ap	bl	ap	bl	ap	bl	ap	bl	ap	bl

— p150 β

— p55 α

Figure 6. Targeting of the SF/HGF receptor to the basolateral plasma membrane of MDCK cells. MDCK cells on Transwell filters were metabolically pulse labeled with [³⁵S] Translabel for 15 min and biotinylated at the apical or basolateral cell surface after various periods of chase. The SF/HGF receptor was immunoprecipitated, solubilized, reprecipitated with streptavidin-agarose, and detected by autoradiography. At the earliest timepoints at which the SF/HGF receptor is detected at the cell surface it is found almost exclusively basolateral. These results are representative of four experiments, using the mAbs DN-30 or DO-24 for immunoprecipitation. All experiments gave similar results.

herin/β-catenin complex (Behrens et al., 1992). SF/HGF, which disrupts cell–cell adhesion, does not appear to influence the steady-state synthesis, the down-modulation and the phosphorylation of E-cadherins (Weidner et al., 1990), while it causes rapid internalization of desmosomal proteins (Bhargava et al., 1991). It has been recently claimed that β-catenin is tyrosine-phosphorylated in response to cell stimulation with SF/HGF (Shibamoto et al., 1994). Whether the SF/HGF receptor phosphorylates β-catenin directly or through other associated tyrosine kinases has yet to be investigated. In this context, it is worth mentioning that activated SF/HGF receptor associates with the c-Src tyrosine kinase (Ponzetto et al., 1994).

The SF/HGF receptor is highly insoluble in non-ionic detergents when cells are fully polarized. The detergent insolubility of the receptor correlates with that of E-cadherin, known to form complexes with insoluble cytoskeletal components upon extensive cell–cell contact (Hirano et al., 1987; Nelson et al., 1990; Ozawa et al., 1990; McNeill et al., 1990; Wollner et al., 1992). On the contrary, the polymeric immunoglobulin receptor, known not to associate with cytoskeleton, is even better solubilized in Triton X-100 (not shown). Polarization in epithelial cells is regulated through different mechanisms, which include selective retention and vectorial delivery at one surface. The first mechanism is mediated by interaction with other proteins, such as the cytoskeleton, extracellular matrix, or adhesion proteins on adjacent cells (for review see Nelson et al., 1992). A number of basolateral proteins form complexes with detergent insoluble cytoskeletal proteins (Nelson and Veshnock, 1987; McNeill et al., 1990; Wollner et al., 1992). In the case of the Na⁺/K⁺ ATPase the basolateral cell surface distribution is determined by stabilization and accumulation on the basolateral membrane (Hammerton et al., 1991). In the case of E-cadherin, both mechanisms are operative (Wollner et al., 1992). The data reported in this paper suggest that the basolateral distribution of the SF/HGF receptor might be mediated by stabilization through association with detergent insoluble intracellular components. However, the hypothesis that the detergent insolubility of the receptor results from its localization in caveolae, where other cellular kinases have been recently found (Sargiacomo et al., 1993), has to be taken into consideration.

Vectorial delivery of proteins to either the apical or the basolateral cell surfaces is another mechanism operating for maintaining polarization in epithelial cells (for review see Mostov et al., 1992). This paper shows that the newly synthesized SF/HGF receptor is directly targeted to the basolateral surface from the *trans*-Golgi network. MDCK cells, that have already established a polarized distribution of membrane proteins, sort newly synthesized proteins in the Golgi complex and deliver them either to the apical or to the basolateral plasma membrane upon recognition of specific sorting signals (Hopkins, 1991). Computer analysis of the SF/HGF receptor amino acid sequence failed to identify any homologies to the signals for basolateral sorting that have been found in the LDL receptor (Matter et al., 1992) and the polymeric IgA receptor (Casanova et al., 1991). Cytoplasmic domains involved in selective sorting have also been found in the transferrin and immunoglobulin FcRII receptors (Hunziker et al., 1991). We should thus assume that the SF/HGF receptor contains a yet unidentified sorting signal

for basolateral targeting. This will be unveiled by mutagenesis studies.

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