

Scatter Factor: Molecular Characteristics and Effect on the Invasiveness of Epithelial Cells

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Abstract. The generation of invasiveness in transformed cells represents an essential step of tumor progression. We have previously shown that MDCK epithelial cells, which are deprived of intercellular adhesion by the addition of anti-Arc-1/uvomorulin antibodies, become invasive for collagen gels and embryonal heart tissue (Behrens, J., M. M. Mareel, F. M. Van Roy, and W. Birchmeier. 1989. *J. Cell Biol.* 108: 2435–2447.). Here we examined whether invasiveness is also induced by scatter factor, which is known to dissociate epithelial cells (Stoker, M., E. Gherardi, M. Perryman, and J. Gray. 1987. *Nature (Lond.)* 327:239–242.). Scatter factor was purified to homogeneity from conditioned medium of human fibroblasts by heparin–Sepharose chromatography, followed by cation exchange chromatography, gel filtration, or preparative SDS gel electrophoresis. We found that scatter factor represents a 92,000 mol wt glycoprotein which, apparently, is converted by limited pro-

teolysis into disulfide-linked 62,000 and 34/32,000 mol wt subunits. Reversed phase HPLC and sequence analysis of tryptic peptides confirmed the suggested molecular structure, and revealed further that scatter factor exhibits sequence similarities to hepatocyte growth factor and to plasminogen. Purified scatter factor in fact induces the invasiveness into collagen matrices of MDCK epithelial cells, and induces or promotes the invasiveness of a number of human carcinoma cell lines. Apparently, the effect on the human cells depends on their respective degree of differentiation, i.e., cell lines with a less pronounced epithelial phenotype were more susceptible to the factor. Scatter factor does not seem to influence synthesis, steady-state level, and phosphorylation of the cell adhesion molecule Arc-1/uvomorulin. Thus, scatter factor represents a clearly defined molecular species which induces, in vitro, the progression of epithelial cells to a more motile, i.e., invasive phenotype.

THE generation of malignant tumors in humans and in experimental animals is a multistep process (Klein and Klein, 1985). An accumulation of somatic mutations results in the loss of growth control of the involved cells, it induces cell invasiveness and the vascularization of tumors, and it finally leads to metastasis to certain target organs. More than 90% of human tumors are carcinomas; transformed epithelial cells proliferate in an uncontrolled fashion, penetrate basement membranes, and invade the underlying mesenchyme. It has been shown that carcinomas can be subdivided by morphological criteria (a) well-differentiated carcinomas exhibit an epithelial tissue structure, show well-developed intercellular junctions, and are weakly or not invasive; and (b) poorly differentiated carcinomas are characterized by an amorphous tissue structure, have fewer junctions, and are more invasive (Weinstein et al., 1976; Gabbert et al., 1985). However, heterogeneous tumors exist in which both differentiated and undifferentiated areas occur simultaneously. This indicates transitions between these two forms, possibly as a result of tumor progression or the action of host factors. In particular, tumor cells at the invasion front can exhibit less differentiated and more motile phenotypes.

The prognosis of carcinomas largely depends on the degree of their invasiveness.

What is presently known about molecular mechanisms that play a role during invasion and metastasis of tumor cells? It has been postulated that certain oncogenes, e.g., *ras*, induce the complete cascade from initial tumor growth to metastasis (Greig et al., 1985; Muschel and Liotta, 1988). However, this interpretation has recently been criticized, since the data are mainly based on experiments with NIH3T3 fibroblasts (note that normal fibroblasts are not even fully transformed by the *ras* oncogene; Land et al., 1983; Van Roy et al., 1986). During the last decade, a series of specific factors possibly involved in invasion and metastasis have been discovered, e.g., proteases (Liotta et al., 1979; Khokha and Denhardt, 1987; Ossowski, 1988), angiogenesis factors (Müller et al., 1987; Ishikawa et al., 1989), motility factors (Liotta et al., 1986; Grey et al., 1989), and cell adhesion molecules (Wewer et al., 1986; Roos and Roosien, 1987; Gehlsen et al., 1988; Behrens et al., 1989). We have previously shown that nontransformed MDCK epithelial cells acquire invasive properties when intercellular adhesion is specifically inhibited by the addition of antibodies against

Arc-1/uvomorulin; the separated cells then assume a fibroblast-like morphology and invade collagen gels and embryonal heart tissue. MDCK cells transformed with Harvey and Moloney sarcoma viruses are constitutively fibroblast-like and invasive, and they are deficient in Arc-1/uvomorulin expression. These data suggest that the loss of adhesive functions of Arc-1/uvomorulin (which is homologous to E-cadherin, cell CAM 120/80, and L-CAM) can be a critical step in the promotion of epithelial cells to a more malignant, i.e., invasive phenotype (Behrens et al., 1985, 1989). A similar correlation between invasiveness, cellular differentiation, and expression of Arc-1/uvomorulin was recently found in human carcinoma cell lines (Frixen, U. H., J. Behrens, G. Eberle, A. Warda, and W. Birchmeier, manuscript submitted for publication).

Recently, several soluble factors affecting the motility of cells (and possibly influencing invasion and metastasis) have been discovered, e.g., autocrine motility factor (Liotta et al., 1986), "slow antigen" (Goodman et al., 1985), motility stimulating factor (Grey et al., 1989), and scatter factor (Stoker et al., 1987). These factors seem to affect cell motility but not cell proliferation. Scatter factor is of particular interest, since it exclusively acts on epithelial cells and it seems to interfere with their differentiation, i.e., MDCK cell colonies are dissociated by the factor and the cells then assume a fibroblast-like morphology. Scatter factor thus appears to exert a similar effect on epithelial cells as the disturbance of intercellular adhesion by anti-Arc-1/uvomorulin antibodies.

In this investigation we have purified scatter factor to homogeneity by affinity chromatography on heparin-Sepharose followed by separation on the basis of charge or molecular weight. Partial internal amino acid sequences generated from electroblotted protein suggests that scatter factor has structural similarities to hepatocyte growth factor and to plasminogen. We show further that scatter factor interferes with the epithelial phenotype of various human carcinoma cell lines and, most importantly, induces MDCK epithelial cells and a subgroup of human carcinoma cells to invade collagen gels.

Materials and Methods

Purification of Scatter Factor

MRC5 human fibroblasts were grown to confluency in cell culture flasks (250 flasks of 225 cm² per preparation; Costar Data Packaging Corp., Cambridge, MA), washed three times with PBS, and further cultured in serum-free DME (40 ml per flask) for 3 d. The conditioned serum-free medium (10 liters) was clarified by centrifugation (10,000 rpm in a JA-10 rotor; Beckman Instruments, Inc., Palo Alto, CA) and filtration (0.45- μ m membranes; Millipore Continental Water Systems, Bedford, MA), pH-stabilized by the addition of 50 mM Tris-Cl, pH 7.5, and loaded at 40 ml/h onto a 4-ml heparin-Sepharose column (Pharmacia Fine Chemicals, Piscataway, NJ) at 4°C. The column was washed with 50 mM Tris-Cl, 0.1 M NaCl, pH 7.5, and then with the same buffer containing 0.5 M NaCl, until absorbance at 280 nm reached the baseline. Scatter factor was eluted with a 100-ml linear gradient from 0.5–1.8 M NaCl at 10 ml/h. Aliquots of the fractions were analyzed by SDS-PAGE (Laemmli, 1970) followed by silver staining (Wray et al., 1981), and by the scatter factor assay on MDCK epithelial cells (Stoker et al., 1987). For this assay, MDCK cells (cell line MDCK-2, compare Behrens et al., 1989) were cultured on microwell plates (400 cells per well; Nunc, Roskilde, Denmark) for 4–8 h. Aliquots of the fractions were added at six different concentrations (threefold serial dilutions) in DME plus 10% FCS and the scattering effect on MDCK cells was monitored by light microscopy after overnight incubation. 1 U of activity

was defined as the lowest amount of scatter factor per ml that clearly dissociates MDCK cells. At this point, scatter factor could be stored at –20°C. Alternatively, serum-free conditioned medium was absorbed on various lectin-affinity columns (Pharmacia Fine Chemicals and P-L Biochemicals, Inc., Milwaukee, WI) and eluted with the corresponding carbohydrate competitors at concentrations of 1 M.

Further purification of scatter factor was performed by cation exchange chromatography on a 0.5-ml Bio-Rex 70 column (Bio-Rad Laboratories, Richmond, CA) in 50 mM Tris-Cl, 0.2% Triton X-100, pH 7.5, at 4°C (one tenth of the heparin-Sepharose pool was usually employed). Before loading, the material was diluted 10-fold with buffer to reduce the salt concentration. After washing, scatter factor activity was eluted with a linear gradient of 0.2–1.2 M NaCl in the same buffer, and the fractions of highest activity (at 0.7–0.8 M salt) were pooled. Alternatively, the heparin-Sepharose eluate (e.g., 200 μ l) was gel filtered on a 0.7 \times 15 cm column of Sephacryl S-400 (Pharmacia Fine Chemicals) in 50 mM Tris-Cl, 0.2% Triton X-100, 0.5 M NaCl, pH 7.5, at 4°C. In the scatter factor assay Triton X-100 did not interfere, since the fractions could be diluted at least 10³-fold. Without Triton X-100, further purification of scatter factor was cumbersome at this stage and resulted in low yields. In other experiments, heparin-Sepharose material was electrophoresed on preparative nonreducing SDS polyacrylamide gels, and the 64-kD band was excised and electroeluted at 4°C in the presence of 0.2% Triton X-100 (compare Müller et al., 1987). Purified scatter factor could be stored at –20°C. Scatter factor was iodinated by the Chloramine T method (Frolik et al., 1984), and treated with O- and N-glycanases (Genzyme, Boston, MA) as suggested by the manufacturer.

Protein Electroblothing and Microsequencing

Highly enriched scatter factor (60 μ g) was subjected to SDS gel electrophoresis under reducing conditions. The separated bands were electroblotted onto polyvinylidene (PVDF) membranes (Millipore Continental Water Systems) as described (Bauw et al., 1987) and visualized by Amido black staining. The membrane pieces with the 92-, 62-, and 34-kD proteins were excised and in situ digested with trypsin (Bauw et al., 1988). Peptides eluting from the membrane were separated by reverse-phase HPLC using a C₄ (0.46 \times 25 cm) column (Vydac; Separations Group, Hesperia, CA). A 0–70% linear gradient of acetonitrile in 0.1% trifluoroacetic acid was applied 5 min after sample loading. The flow rate was 1 ml/min. A Waters-Millipore (Milford, MA) HPLC apparatus, consisting of a gradient controller (model 680), two pumps (model 510), and UV detector (model 411) was used for these analyses. Peptides eluting from the column were recorded at 214 nm and manually collected. Peptides were loaded on polybrene-coated precycled grids and mounted in the reaction chamber of the gas-phase pulsed liquid sequencer (model 120A; Applied Biosystems Inc., Foster City, CA) equipped with an on-line phenylthiohydantoin amino acid analyser (model 120A). The apparatus was run according to the manufacturer's instructions.

Effect of Scatter Factor on the Expression of Arc-1/uvomorulin

MDCK cells (5 \times 10⁵ per 10-cm plate) were cultured in the presence or absence of scatter factor (10 U/ml), and the effect of this treatment on synthesis, steady state level, and phosphorylation of Arc-1/uvomorulin was examined by Western blotting and immunoprecipitation. For Western blot experiments, cells were lysed in 0.5% Triton X-100, L-CAM assay buffer, 1 mM PMSF and centrifuged at 12,000 g for 10 min. Pellets and supernatants were analyzed by immunoblotting with affinity-purified anti-Arc-1/uvomorulin antibodies as described (Behrens et al., 1989). For immunoprecipitations, cells prelabeled for 3 h with 50 μ Ci/ml ³⁵S-methionine (Amersham International, Amersham, UK) or for 4 h with 0.25 mCi/ml ³²Pi (carrier-free; Amersham International) were processed as described (Behrens et al., 1989).

Cell Dissociation and Invasion Assay

For the dissociation assay, MDCK and human carcinoma cells were cultured in 6-well plates (Nunc) and exposed to scatter factor (10 U/ml) in fresh medium overnight. For the invasion assay, 2 \times 10⁵ cells were plated onto collagen type I gels in 6-well plates (Nunc), scatter factor was added after 6 h, and the invasion of cells was usually scored after 3 d by light microscopy (Behrens et al., 1989). New scatter factor was added daily in fresh medium. In some experiments, scatter factor concentration, number of plated cells, and incubation conditions were varied as indicated in the figure legends.

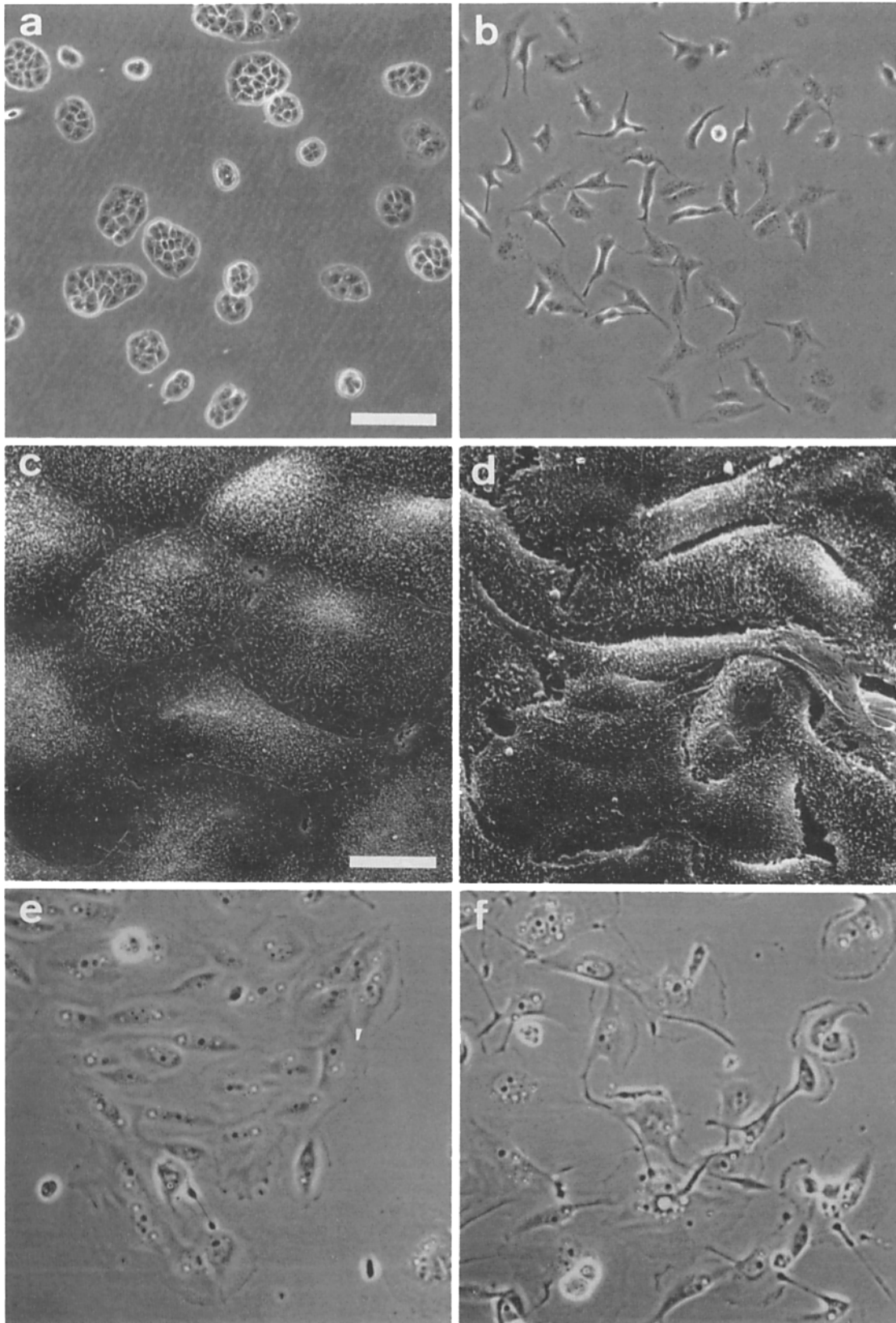


Figure 1. Effect of scatter factor on the appearance of epithelial cell colonies. Purified scatter factor (10 U/ml) was added to MDCK epithelial cells (*b* and *d*) and to A6 epithelial cells from *Xenopus laevis* (*f*) for overnight, and the morphology of the cells was screened by light or scanning EM; (*a*, *c*, and *e*) are the corresponding controls. It can be seen that scatter factor clearly disperses the groups of epithelial cells and that the resulting single cells assume a fibroblastoid morphology. Bars: (*a*) 100 μm ; (*c*) 5 μm .

The human carcinoma cell lines were a generous gift of the tumor cell bank of the German Cancer Research Center (DKFZ), Heidelberg (Dr. H. Löhre). MRC5 fibroblasts were purchased from Flow Laboratories, Inc. (McLean, VA). The experiment with A6 *Xenopus laevis* cells was kindly performed by Dr. P. Hausen (Max-Planck-Institut für Entwicklungsbiologie, Tübingen).

Results

Purification and Molecular Characterization of Scatter Factor

Scatter factor was isolated from serum-free medium conditioned by MRC5 human fibroblasts, which are good producers of the factor (Stoker et al., 1987, see also Materials and Methods). Activity was assayed on MDCK epithelial cells (Behrens et al., 1989), which are clearly dissociated by scatter factor (Fig. 1, *a-d*). Amphibian epithelial cells, cell line A6 of *Xenopus laevis*, are also dissociated by the factor (Fig. 1, *e-f*).

For purification of scatter factor, serum-free conditioned medium was affinity purified on heparin-Sepharose; the activity eluted between 0.9–1.2 M NaCl (Fig. 2 *A*). This step yielded a 500-fold purification with an excellent recovery of the activity (Table I). SDS gel electrophoresis of the active fractions (pools) showed only a few remaining proteins: on reduced gels bands with molecular masses over 200 kD, of 92 kD, 62 kD, and 33 kD; and on nonreduced gels high mol wt material and bands at 64 and 35 kD (Fig. 2, *B* and *C*). Alternatively, scatter factor could be affinity purified on lentil lectin sepharose; however, both yield and purification factor were lower (Table I). Scatter factor also bound to Con A and wheat germ lectin, but not to soybean and peanut lectin; activity could be recovered from the wheat germ lectin but less from Con A (data not shown).

Further purification of scatter factor from the heparin-Sepharose pool was accomplished either by cation exchange chromatography on Bio-Rex 70, electroelution of the 64-kD band from nonreduced preparative SDS gels, or gel filtration on Sephacryl S-400. Bio-Rex 70 chromatography resulted in a 2,000-fold overall purification with a 70% yield (Table I). Comparable yields and purification factors were measured with electroelution and gel filtration (data not shown). Analysis of the material obtained by the three procedures on non-reducing SDS gels revealed a broad band at an apparent mol

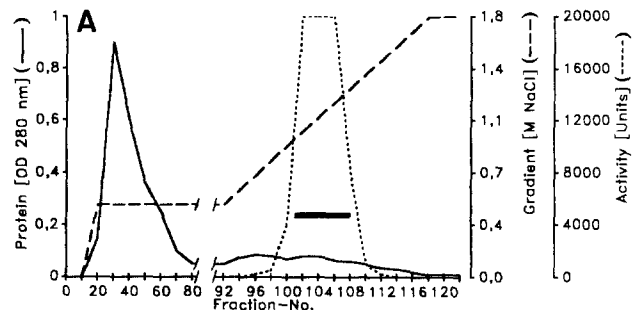


Figure 2. Purification of scatter factor by chromatography on heparin-Sepharose. Serum-free medium conditioned by MRC5 human fibroblasts was affinity purified on a heparin-Sepharose column as described in Materials and Methods. (*A*) Flow diagram: scatter factor activity eluted between 0.9–1.2 M NaCl. The pooled fractions are indicated by a bar. (*B* and *C*) Analysis of the eluted fractions by reducing and nonreducing SDS-PAGE (5–15% linear gradients), respectively. The pooled fractions with scatter factor activity are indicated by a bar. Molecular mass markers are from phosphorylase b (97 kD), serum albumin (66 kD), egg albumin (43 kD), and carbonic anhydrase (31 kD).

wt of 64,000 (Fig. 3, lanes *a,c*, and *f*). Apparently, these purification steps removed the high mol wt material (mainly fibronectin, as shown by reaction with specific antibodies, data not shown) as well as some low mol wt components (compare Fig. 3 *a* with 3 *b*, or 3, *d* and *e* with 3 *f*). The purified scatter factor exhibited an activity of 2 U per nanogram protein.

Upon reduction of scatter factor with 2-mercaptoethanol (which rapidly destroys the biological activity, data not shown) followed by SDS gel electrophoresis, the 64-kD material dissociated into components with apparent mol wt of 92,000 and 62,000, and into a doublet at 34/32,000 (Fig. 4, lanes *a* and *b*). Removal of N-linked carbohydrates from scatter factor with N-glycanase slightly decreased the mol wt of all these components, i.e., the 92-kD was converted to a 86-kD, the 62-kD to a 60-kD, and the 34/32-kD doublet appeared as a single band at 29 kD (Fig. 5, *a-c*). Digestion with O-glycanase had no effect on the apparent mol wt of the proteins (Fig. 5 *d*). To learn how these different components are

Table I. Purification of Scatter Factor

Step	Protein*	Activity‡	Specific activity	Yield	Purification factor
	μg	U	U/μg	%	
Serum-free supernatant (10 l)	2.1 × 10 ⁵	200,000	0.95	100	1
Lentil lectin-Sepharose§	4.2 × 10 ³	83,200	19.8	42	20.8
Heparin-Sepharose (0.9–1.2 M NaCl)	364	176,500	484	88	510
Bio-Rex 70 cation exchange (0.7–0.8 M NaCl)	73	141,200	1,930	71	2,032

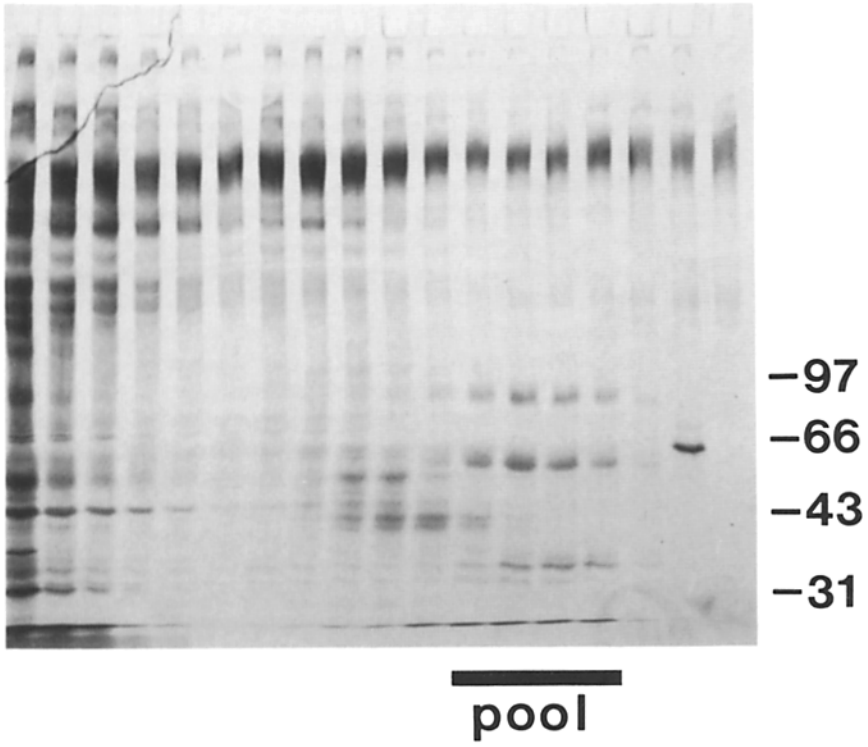
* Protein was determined with the Bio-Rad assay as described by the manufacturer.

‡ One unit represents the minimal activity per ml which shows clear scattering of MDCK cells.

§ This step was not regularly included in the purification, i.e., serum-free supernatant was directly fractionated on heparin-Sepharose.

|| Calculated value (only 10% of the heparin-Sepharose pool was chromatographed in this particular experiment).

B



C

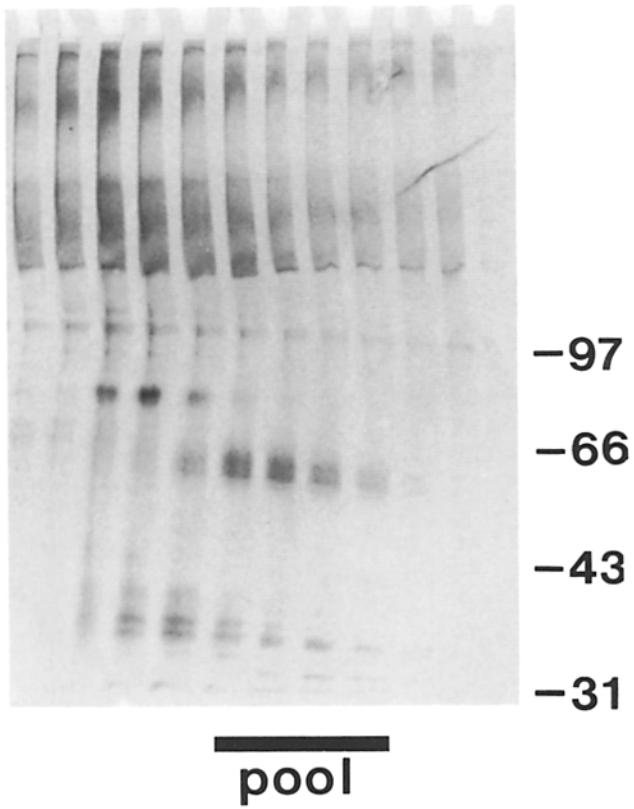


Figure 2.

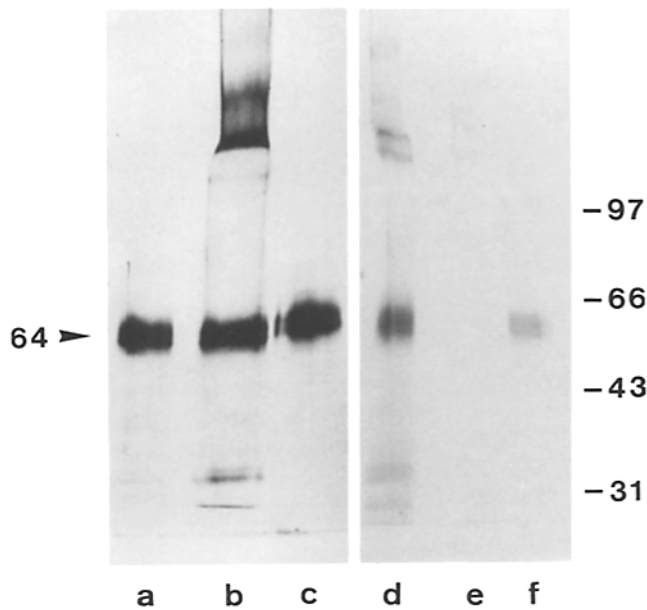


Figure 3. Purified scatter factor electrophoresed on SDS-polyacrylamide gels under nonreducing conditions is a 64-kD protein. Pooled scatter factor from the heparin-Sepharose column (Fig. 2) was further chromatographed by three different methods, i.e., cation exchange chromatography, electroelution after preparative SDS gel electrophoresis, and gel filtration, and the active fractions were analyzed by nonreducing SDS-PAGE (5–15% linear gradient) followed by silver staining. (b and d) Material from the heparin-Sepharose column; (a) active pool from the Bio-Rex 70 cation exchange column; (c) electroeluted 64-kD band exhibiting scatter factor activity; (e) void volume peak from the Sephacryl S-400 gel filtration (no activity could be measured); and (f) active fraction of the gel filtration. Molecular mass markers are as in Fig. 2.

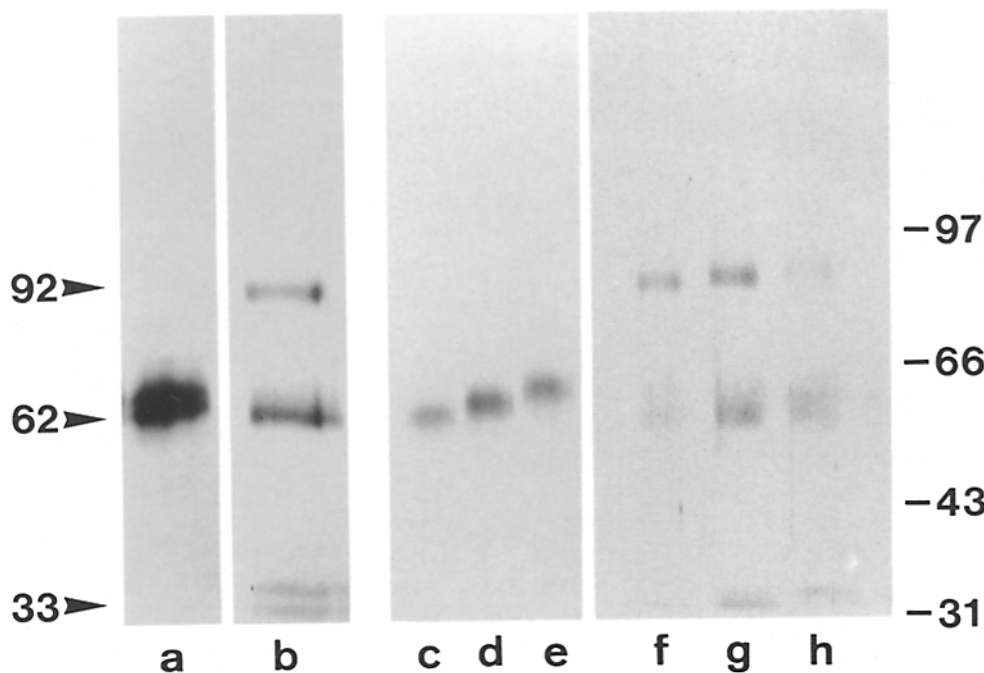


Figure 4. Analysis of the 64-kD scatter factor by electrophoresis on reducing SDS polyacrylamide gels. Purified scatter factor was analyzed on nonreducing (a) and reducing SDS polyacrylamide gels (b). The nonreduced 64-kD band splits into components with apparent molecular masses of 92-, 62-, and a doublet of 34/32-kD. The broad nonreduced 64-kD band (a) was cut into three pieces with increasing molecular mass and the electroeluted material was analyzed by electrophoresis on nonreducing (c–e) and reducing (f–h) SDS gels (5–15% linear gradients). It can be seen that the material with the lowest molecular mass (c) contains mostly the 92-kD component (f), whereas the fraction with the highest molecular mass (e) is mainly composed of the 62- and 34-kD components (h). Molecular mass markers are as in Fig. 2. Analysis of scatter factor activity revealed that the three eluates (c–e) exhibited the same specific activity, i.e., scanning of the protein bands resulted in ratios of 1:1.9:1.4, whereas the ratios of activity were 1:2.0:1.5.

contained within the broad 64-kD band, three regions with increasing mol wt of the nonreduced protein were electroeluted, and the eluates examined for scatter factor activity. All three eluted fractions retained their different mobilities (Fig. 4, c–e) and exhibited the same specific activity (see figure legend) suggesting that all regions of the broad 64-kD band represent scatter factor. However, SDS gel electrophoresis under reducing conditions showed that the fraction with the lowest mol wt (Fig. 4 c) was mainly composed of the 92-kD component (Fig. 4 f), whereas the highest mol wt region (Fig. 4 e) largely dissociated into the 62- and 34-kD bands (Fig. 4 h).

To examine whether the 62- and 34-kD components might be derived from the 92-kD molecule by limited proteolysis, we analyzed tryptic peptides of the three proteins by reversed phase HPLC and by sequence analysis. HPLC showed that the 92- and 62-kD components are clearly related, since seven peptides eluted at identical positions (Fig. 6, arrowheads). In the case of the 92- and the 34-kD proteins, four peptides eluted at the same positions (Fig. 6, arrows). Furthermore, the amino acid sequence of the corresponding peptide pairs a, f, and g, respectively (Fig. 6, asterisks), were found to be identical (see also below). Apparently, no identity exists between the 62- and 34-kD bands. On the basis of these biochemical data we concluded (a) that the 92-kD component is an unprocessed form of scatter factor (which migrates on nonreducing SDS gels at 64 kD due to the presence of intrachain disulfides); (b) that the 62- and 34/32-kD components result from limited proteolytic cleavage of the 92-kD protein and are held together by disulfides under nonreducing conditions; and (c) that different contents of carbohydrates are responsible for the minor shifts in the mol wt of all bands (e.g., for the generation of the 34/32-kD

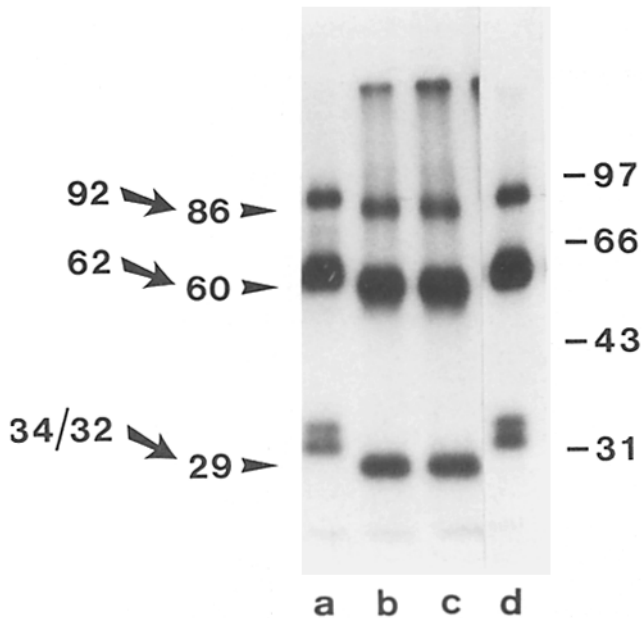


Figure 5. Effect of N- and O-glycanase digestion on the mobility of the components of scatter factor in SDS gels. Iodinated scatter factor was reduced with 2-mercaptoethanol, treated with the two enzymes (see Material and Methods), and analyzed on 12% SDS polyacrylamide electrophoresis followed by autoradiography. (a) Control, no enzymes added; (b) 7 U/ml N-glycanase; (c) 35 U/ml N-glycanase; (d) 1 U/ml neuraminidase followed by 0.1 U/ml O-glycanase. Conditions were as suggested by the manufacturer of the enzymes. Molecular mass markers are as in Fig. 2.

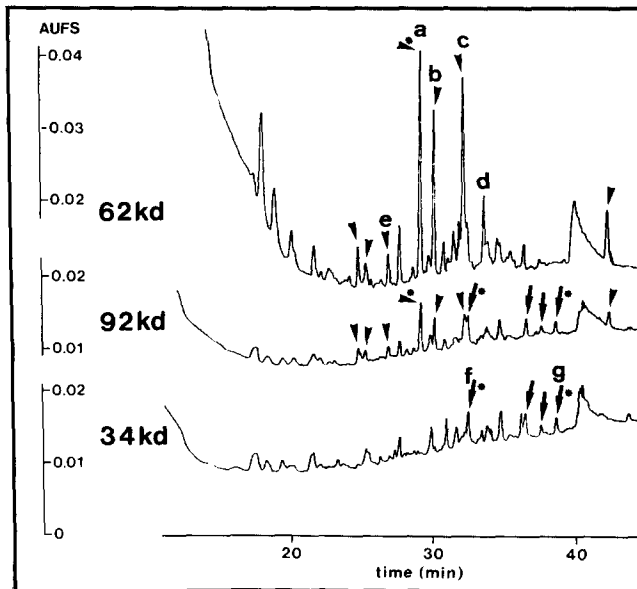


Figure 6. HPLC analysis of tryptic peptides of the 92-, 62-, and 34-kD components of scatter factor. The proteins, blotted onto PVDF membranes, were digested with trypsin in situ, and the released peptides analyzed by reversed phase HPLC (see Materials and Methods). Absorbance of peptides at 214 nm was recorded (AUFS; absorbance units, fraction of full scale). Arrowheads and arrows mark peptide pairs with identical elution properties of the 92- and 62-, and the 92- and 34-kD components, respectively. Asterisks mark the peptide pairs of the 92- and 62-, and of the 92- and 34-kD proteins, respectively, which exhibit an identical amino acid sequence. The sequences of peptides a-g were used for the homology search.

Table II. Amino Acid Sequence of Tryptic Peptides of Scatter Factor

Peptide	Amino acid sequence
a	W D S Q Y P H E (H) D (D) T P E N F (D) (M)
b	H I F W E P D A S K
c	N P D G S E S P X X F T T D P
d	N P D D D (D) X G P (A)
e	A F V F D K
f	D Y E A X L G I H D V
g	Q V L X V S Q L V Y G P

All peptides are from the isolation as shown in Fig. 6. Residues in brackets were equally found in the corresponding sequencing cycle. X indicates the absence of any identifiable residue. Note that W, C, and H are difficult to identify when present in small amounts.

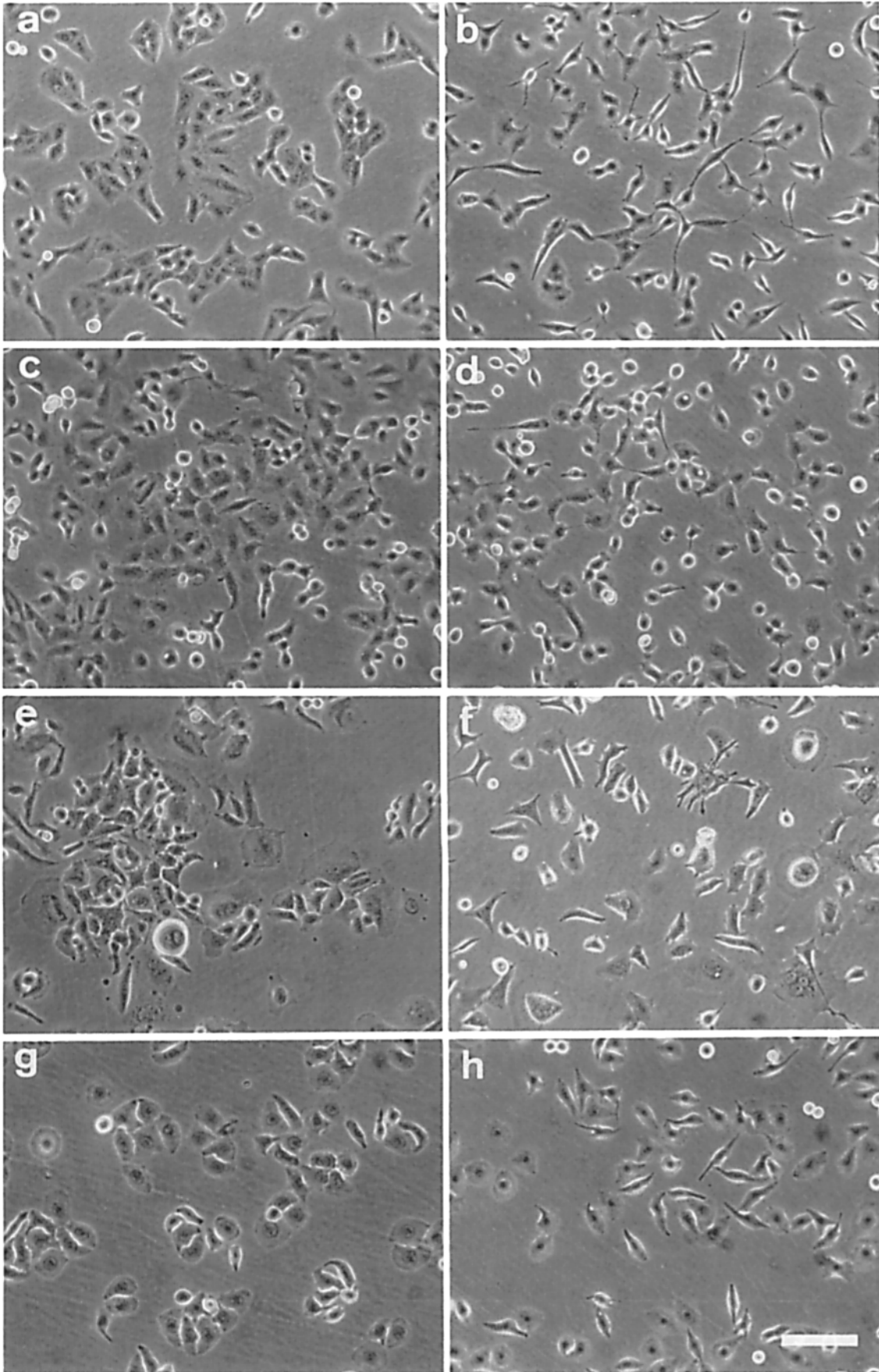
doublet). Possibly due to partial unfolding, the proteolytically processed form of scatter factor migrates as the higher part of the 64-kD band, whereas the nonprocessed form migrates slightly faster.

A homology search with the sequences of seven peptides from the 62- and 34-kD component (peptides a-g; see Fig. 6 and Table II) revealed that scatter factor exhibits sequence similarities to human hepatocyte growth factor and plasminogen, e.g., peptide a extends from amino acids 329 to 344, peptide b from 425 to 434, peptide c from 356 to 370, peptide d from 442 to 450, peptide e from 86 to 91, peptide f from 543 to 553, and peptide g from 563 to 574 in the hepatocyte growth factor (Nakamura et al., 1989; Miyazawa et al., 1989). Within the sequenced area (79 amino acids) the sequence similarity between scatter factor and hepatocyte growth factor is at least 90%. Both hepatocyte growth factor and plasminogen are 90-kD proteins which are proteolytically cleaved into 60-kD (A-chain) and 30-kD (B-chain) subunits held together by interchain disulfides.

Scatter Factor Induces Invasion into Collagen Gels of MDCK Cells and of Various Human Carcinoma Cells

It had previously been reported that scatter factor does not dissociate transformed epithelial cells (Stoker et al., 1987). We found that this is true only for a fraction of human carcinoma cell lines. For instance, the lung carcinoma cell lines A 549 and LXF 289 are clearly dissociated by scatter factor (see Fig. 7, a-d), whereas the lung carcinoma lines LX 1 or A 427 do not respond (not shown). The pancreas carcinoma cell line Hs 766T and the bladder carcinoma line RT 112 are well dissociated by the factor (see Fig. 7, e-h), whereas the pancreas carcinoma line Capan 2 and the bladder carcinoma line RT 4 are not affected (not shown).

We have also examined the effect of scatter factor on the invasiveness of various cell lines of epithelial origin into collagen matrices. We found that scatter factor in fact induces the invasion of MDCK epithelial cells into collagen in a dose-dependent manner (Fig. 8 A and Table III); half-maximum invasiveness was measured at 3-10 U/ml. The degree of invasiveness induced by scatter factor also depends on the density of plated cells; when MDCK and Capan 1 cells were plated at 20-fold lower density than usually (i.e.,



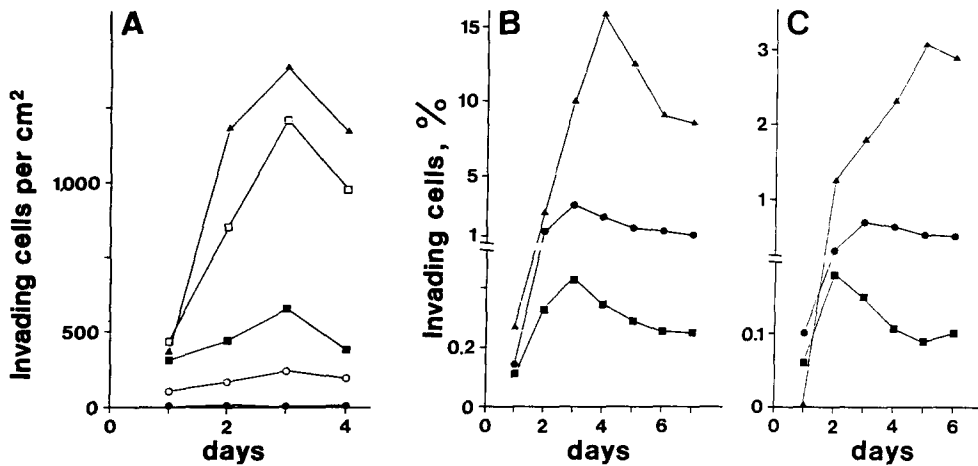


Figure 8. Scatter factor induces the invasiveness of epithelial cells into collagen matrices: effect of factor concentration and cell density. (A) MDCK cells were plated on collagen gels at the usual density (200,000 cells/well) and exposed to scatter factor at concentrations of 20 U/ml (\blacktriangle), 10 U/ml (\square), 3 U/ml (\blacksquare), and 1 U/ml (\circ). Without scatter factor, no invasion was seen (\bullet). Invasion was scored as described in Materials and Methods. (B and C) MDCK and Capan 1 cells, respectively, were plated on collagen gels at densities of 10,000 (\blacktriangle), 50,000 (\bullet), and 200,000 (\blacksquare) cells/well and exposed to scatter factor (10 U/ml). Invasion of cells is expressed in percent of plated cells.

when most cells had no cell-cell contacts), a 15–30-fold higher relative penetration was seen (Fig. 8, B and C). With respect to the invasiveness of the human carcinoma cells, we found again that some lines are sensitive to scatter factor whereas others did not respond (Table III). All cell lines tested which did not exhibit a clearcut epithelial morphology and did not express or expressed low amounts of the cell adhesion molecule Arc-1/uvomorulin (the lung carcinoma cell lines LXF 289 and A 549, the pancreas carcinoma line Hs 766T, and the bladder carcinoma line EJ 28) were strongly induced to invade the collagen matrix, whereas most of the cell lines with pronounced epithelial characteristics (the lung carcinoma line LX 1, the pancreas carcinoma line Capan 2, and the bladder carcinoma line RT 4) were not or only weakly responding.

Since both the action of scatter factor and the interference with cell-cell adhesion by specific antibodies induce similar effects upon epithelial cells, we examined whether scatter factor might exert its effect by modifying Arc-1/uvomorulin. MDCK epithelial cells were incubated with and without scatter factor, and possible changes of the total amount of Arc-1/uvomorulin were measured by Western blotting, de novo synthesis was examined by short pulse metabolic labeling followed by immunoprecipitation, and the effect on phosphorylation of Arc-1/uvomorulin was analyzed in pulse-chase experiments. We found that the presence of scatter factor for 15 h (shorter and longer incubation times were also tested) had no effect on the amount of detergent-soluble and -insoluble Arc-1/uvomorulin (Fig. 9 A), nor did it influence the rate of Arc-1/uvomorulin synthesis and complex formation with the associated proteins at mol wts of 102,000 and

98,000 (Fig. 9 B). Furthermore, we detected no changes in the rate of phosphorylation or dephosphorylation of Arc-1/uvomorulin upon the action of scatter factor (Fig. 9 C).

Discussion

In the present study the secretory protein of human fibroblasts, scatter factor, was purified to homogeneity and found to be a heparin-binding glycoprotein with an apparent mol wt of 92,000 on SDS-PAGE, which can be converted into 62,000 and 34/32,000 mol wt subunits. Under nonreducing conditions, scatter factor exhibits an apparent mol wt of 64,000. The purified factor induces invasiveness of MDCK epithelial cells and of various human carcinoma cell lines into collagen matrices.

Previously, scatter factor was partially purified by sequential molecular sieve and reversed phase FPLC, and bands with mol wts between 40–67,000 were seen on SDS gel electrophoresis (Stoker et al., 1987). In the present study, a major step of purification was accomplished by affinity chromatography taking advantage of the heparin and lectin binding properties of scatter factor. These properties and the mol wt distinguish scatter factor from other well known factors; for instance, the heparin-binding acidic and basic FGFs are non-glycosylated (Maciag et al., 1984; Gospodarowicz et al., 1984); granulocyte macrophage-stimulating factor and interleukin 3 are glycoproteins that bind to heparan sulfate but have different mol wts (23–30,000; Metcalf, 1986; Roberts et al., 1988); keratinocyte growth factor binds to heparin, contains a possible glycosylation site, but again has a different mol wt (28,000; Rubin et al., 1989; Finch et al., 1989);

Figure 7. Effect of scatter factor on the morphology of various human carcinoma cell lines. Cells were incubated with purified scatter factor (b, d, f, and h) and without factor (a, c, e, and g) overnight, and the shape of the cells was analyzed by light microscopy. (a and b) Lung carcinoma cell line A 549; (c and d) lung carcinoma cell line LXF 289; (e and f) pancreas carcinoma cell line Hs 766T; and (g and h) bladder carcinoma cell line RT 112. It can be seen that all four cell lines are clearly dispersed by scatter factor. Bar, 100 μ m.

Table III. Invasion of Cells into Collagen Gels

Cell lines*	Shape‡	Invasion (cells/cm ²)§		Uvomorulin expression
		- Scatter Factor	+ Scatter Factor	
MDCK	E	0	1,250	+
Lung Carcinomas				
LXF 289	E/F	385	3,265	-
A549	E/F	175	3,125	-
LX 1	E	0	0	+
Pancreas Carcinomas				
HS 766 T	E/F	42	420	+/-
Capan 1	E	0	424	+
DAN-G	E	21	231	+
Capan 2†	E	0	0	+
Bladder Carcinomas				
EJ 28	F	8,400	17,570	-
RT 112	E	0	42	+
RT 4†	E	0	0	+

* For the origin of cell lines, see Materials and Methods.

‡ Cell lines were grouped into differentiated (epithelial, *E*), less differentiated (fibroblastoid, *F*), and intermediate forms (*E/F*).

§ For the collagen invasion assay, see Behrens et al. (1989) and Materials and Methods. Cells which entered the collagen matrix within 3 days were counted by light microscopy.

|| Uvomorulin expression was analyzed by Northern-blotting (Frixen et al., 1990).

† Scatter factor did also not induce invasiveness at lower cell density (e.g., at 10,000 cells/well).

migration stimulating factor has a mol wt of 70,000 but elutes from heparin-Sepharose at 0.3–0.6 M NaCl (Grey et al., 1989); fibronectin is a heparin-binding glycoprotein with a grossly different mol wt (Yamada, 1983) and can be well separated from scatter factor (this work). A heparin-Sepharose column was recently also used by Rosen et al. (1989) for the partial purification of a scatter factor-like activity from smooth muscle cells. We have now achieved a further purification of scatter factor by several additional methods, i.e., gel filtration, electroelution from SDS gels, or cation exchange chromatography, which largely resulted in the separation of one major contaminant, fibronectin. We found that the inclusion of Triton X-100 is essential; without detergent during these steps scatter factor activity is rapidly lost, possibly due to nonspecific adsorption.

Purified scatter factor migrated as a broad band at an average mol wt of 64,000 on nonreducing SDS gels, and all parts of this band exhibited the same specific activity. However, reduction of this 64,000 mol wt component followed by SDS gel electrophoresis revealed interesting features: bands with apparent mol wt of 92,000, 62,000, and a doublet at 34/32,000 appeared. It is likely that the 92-kD band is generated from the 64-kD component by conformational changes due to cleavage of intrachain disulfides. Furthermore, the 34/32,000 doublet is converted into a single band after treatment with N-glycanase, i.e., likely representing one primary

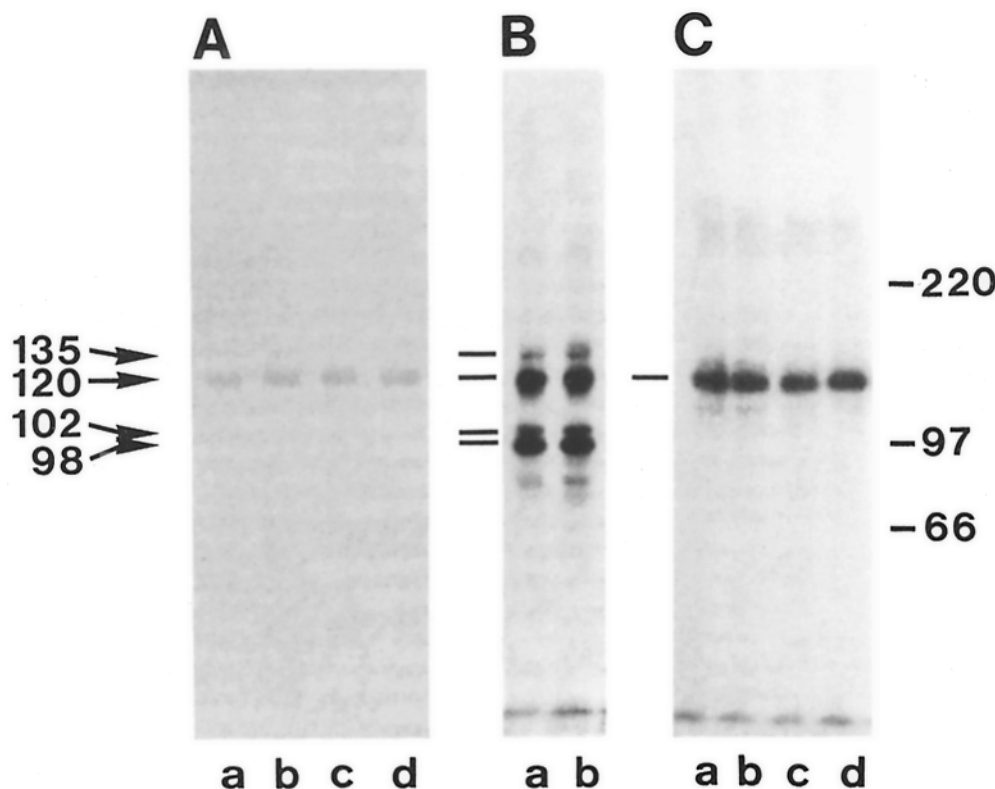


Figure 9. Scatter factor does not influence the metabolism of Arc-1/uvomorulin in MDCK cells. (A) Western blot analysis of detergent-soluble (a and b) and -insoluble (c and d) Arc-1/uvomorulin. Cells were cultured for 15 h with (b and d) or without (a and c) scatter factor, and analyzed for the 120-kD component as described in Materials and Methods. (B) Immunoprecipitation of ³⁵S-methionine metabolically labeled Arc-1/uvomorulin complex. Cells were cultured for 17 h with (b) and without (a) scatter factor followed by a 3-h labeling period. The autoradiogram shows the 135-kD Arc-1/uvomorulin precursor, the 120-kD mature protein, as well as two cytoplasmically associated proteins at 102 and 98 kD (compare Behrens et al., 1989). (C) Immunoprecipitation of ³²Pi-labeled Arc-1/uvomorulin. Cells were metabolically pulse labeled for 4 h with ³²Pi (a) and scatter factor was added to b during the last hour of labeling.

In a further experiment, cells metabolically labeled for 4 h were chased in the absence (c) and presence (d) of scatter factor. A gradual but parallel loss of radioactively labeled Arc-1/uvomorulin was observed within the following 24 h (only the values for the 30-min chase is given in c and d).

translation product. We present here evidence that the 62,000 and 34,000 bands are proteolytic fragments of the 92,000 protein, which are disulfide linked under nonreducing conditions: (a) seven and four tryptic peptides of the 92,000 band were also found in the 62,000 and 34,000 components, respectively, (b) three common peptide pairs of the 92,000 and the 62,000, and the 92,000 and the 34,000 proteins, respectively, were sequenced and found to be identical; and (c) scatter factor exhibits sequence similarities to hepatocyte growth factor and plasminogen which are also 90-kD proteins cleaved into 60- and 30-kD subunits by limited proteolysis.

When this manuscript was being prepared another investigation was published in which scatter factor was purified to homogeneity by different, i.e., nonaffinity purification methods (Gherardi et al., 1989). There, scatter factor is described as a 62,000 mol wt component, which decays into 57- and 30-kD subunits after reduction; a 90-kD component was only seen in some of the preparations. The purified protein turned out to be extremely unstable. Although it is likely that Gherardi et al. (1989) and ourselves have purified the same protein, some significant differences are apparent (a) with our method, scatter factor is stable throughout purification, most likely due to the inclusion of nonionic detergent; (b) large amounts of the factor (70 μ g per 10 liters conditioned medium) could easily be prepared by our procedure, which allowed sequencing of tryptic peptides; and (c) a 92-kD component was always a major constituent of our preparations, and this likely represents the unprocessed form of scatter factor.

Previously, it was shown that scatter factor dissociates target epithelial cell colonies and converts the single cells toward a fibroblastoid and more motile phenotype (Stoker et al., 1987; Stoker, 1989). At a first glance, scatter factor action on epithelial cells thus resembles treatment with anti-Arc-1/uvomorulin antibodies which also leads to the separation of the cells and to the generation of a fibroblast-like morphology (Behrens et al., 1989). However, it is likely that the target of scatter factor on epithelial cells is different from the cell adhesion molecule Arc-1/uvomorulin, since (a) some human carcinoma cell lines are affected by scatter factor although they do not express Arc-1/uvomorulin; and (b) scatter factor action does not seem to influence steady-state level, synthesis, degradation, or phosphorylation of Arc-1/uvomorulin in MDCK cells. Thus, scatter factor does not appear to directly interfere with Arc-1/uvomorulin-mediated cell-cell adhesion, but rather acts as a mobilizer of cells through another mechanism. This is also indicated by the fact that scatter factor-induced invasiveness is increased at lower cell density, i.e., when cell-cell adhesion is negligible.

An important finding of this study is that scatter factor induces or promotes the invasiveness of epithelially derived cells for collagen matrices. We have previously demonstrated that this assay clearly distinguishes between differentiated noninvasive and nondifferentiated invasive epithelial cells (Behrens et al., 1989; Frixen, U. H., J. Behrens, G. Eberle, A. Warda, and W. Birchmeier, manuscript submitted for publication). Interestingly, human carcinoma cells with a weakly expressed epithelial phenotype seem to be more strongly influenced by scatter factor, whereas typically epithelial cell types are less affected. Apparently, scatter factor acts best when carcinoma cells have lost some of their epithe-

lial characteristics, i.e., have progressed to a more malignant phenotype. In vivo scatter factor might be an important host factor produced by mesenchymal tissue which surrounds carcinomas. Alternatively, scatter factor might also be produced by certain cells in the tumors. Our findings suggest that differentiated carcinomas (and possibly also normal epithelia) actually might not respond to scatter factor, and furthermore, heparin-like components present in intact basement membranes could absorb scatter factor before it reaches the epithelial cells.

The analysis of seven tryptic peptides of human scatter factor revealed at least 90% sequence similarity to corresponding regions of human hepatocyte growth factor. We must therefore assume that scatter factor and hepatocyte growth factor represent closely related proteins. Hepatocyte growth factor has previously been identified as a mitogen for mature parenchymal hepatocytes, it is found in the plasma and in the platelets of patients with fulminant hepatic failure, and it is thought to play an important role in liver regeneration (Miyazawa et al., 1989; Nakamura et al., 1989). Hepatocyte growth factor belongs to a family of proteins that are characterized by kringle modules (they form the 60-kD subunit) and by a 30-kD trypsin-like protease domain. It is interesting, however, that two of three important amino acid residues of the active site of the protease domain (i.e., serine and histidine) are replaced by tyrosine and glutamine, respectively, in the hepatocyte growth factor. Apparently, hepatocyte growth factor has lost its proteolytic activity. Scatter factor, on the other hand, has no obvious mitogenic activity on the epithelial cells we have tested (M. Weidner, unpublished results; see also Stoker et al., 1987), and furthermore, its biological activity (i.e., the dissociation of epithelial cells and the induction of motility and invasion) seems to be distinct from the effects of the hepatocyte factor described so far. Future investigations will include the use of scatter factor and hepatocyte growth factor in both assay systems. Furthermore, molecular cloning of scatter factor will resolve the questions concerning the similarities of the two factors.

It is clear from the present and previous reports that scatter factor is an interesting and possibly important molecular modulator of epithelial tissues, e.g., it might be involved in epithelial-mesenchymal interactions during development. In epithelial tumors, the factor seems to promote progression to a more malignant, i.e., invasive phenotype.

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