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# Affinity labeling of a transforming growth factor receptor that does not interact with epidermal growth factor

(membrane receptors/affinity labeling/cell transformation)

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**ABSTRACT** **Membrane components that interact with epidermal growth factor (EGF) and transforming growth factors (TGFs) have been identified by covalent crosslinking to their respective $^{125}$I-labeled ligands. Under appropriate conditions, disuccinimidyl suberate or hydroxysuccinimidyl *p*-azidobenzoate crosslink receptor-bound $^{125}$I-labeled EGF to a 140- to 170-kilodalton (kDal) receptor species in membranes from both A431 human carcinoma cells and normal rat kidney cells. $^{125}$I-Labeled sarcoma growth factor (SGF), a TGF from virally transformed mouse 3T3 cells, also can be affinity-crosslinked to the 140- to 170-kDal EGF receptor species in membranes from A431 and rat kidney cells. The labeling of this receptor is inhibited when either excess unlabeled EGF or SGF is present during incubation of membranes with either $^{125}$I-labeled EGF or $^{125}$I-labeled SGF. In contrast, a second receptor species of 60 kDal is affinity-labeled with $^{125}$I-labeled SGF but not with $^{125}$I-labeled EGF in membranes from both A431 and rat kidney cells. SGF and a TGF from virally transformed rat embryo cells inhibit the labeling of the 60-kDal species when present in excess during incubation of membranes with $^{125}$I-labeled SGF, whereas EGF is completely ineffective in inhibiting the labeling of this receptor. The data suggest that a specific 60-kDal receptor that displays high affinity for TGFs but not for EGF may mediate induction of the transformed phenotype. In addition, SGF and other TGFs interact with the 140- to 170-kDal EGF receptor that appears to mediate normal cell growth effects.**

Transforming growth factors (TGFs) are strongly mitogenic polypeptides that, when added to untransformed cells, induce the cells to grow in soft agar, to overgrow in monolayer cell culture, and to display the full transformed phenotype (1, 2). These *in vitro* properties, especially anchorage-independent growth, correlate well with tumorigenicity *in vivo* (3, 4). DeLarco and Todaro isolated the first TGFs recognized as such—the sarcoma growth factors (SGFs) (2). SGFs are a family of heat- and acid-stable polypeptides, 6–23 kilodaltons (kDal), produced and released into serum-free medium by murine sarcoma virus (MuSV)-transformed mouse 3T3 fibroblasts. Other polypeptides with TGF characteristics have been subsequently isolated from various neoplastic cells and tumor tissues (5–11) and also have been extracted from normal tissues (12).

The initial event in the cellular action of many polypeptide hormones and growth factors is the interaction with specific cell surface receptors. It is generally assumed that, upon binding to the appropriate ligand, receptors generate secondary intracellular signals carrying necessary information to selectively affect cellular functions. A basic hypothesis in the present studies is the existence of cell surface receptors specific for TGFs. Some TGFs, including SGF, have been shown to interact strongly with epidermal growth factor (EGF) receptors (2, 6, 7, 13). Thus, in the process of isolation of TGFs, the ability to compete with $^{125}$I-labeled EGF ($^{125}$I-EGF) for binding to EGF receptors is routinely measured in parallel with anchorage-independent growth-promoting activity to monitor the presence of active TGFs (2, 6, 10). Furthermore, fixed preparations of membranes from the A431 carcinoma cell line rich in EGF receptors are used as a ligand-affinity matrix step for the purification of SGFs (14). However, SGFs are entities chemically different from EGF and do not crossreact with antibodies raised against EGF (2). Although these findings show the ability of TGFs to interact with cell surface receptors, they do not establish through which receptor(s) TGFs elicit the induction of a transformed phenotype. Both the lack of effectiveness of EGF itself to produce cell transformation (2, 5) and the finding of a class of TGFs that does not bind to EGF receptors (9, 12) suggested the possibility that other TGF receptors exist in addition to the EGF receptor.

In the present studies, we have addressed the problem of identifying specific receptor structures for the TGFs in target cell membranes. We have used a technique (15, 16) that was developed to crosslink membrane-bound $^{125}$I-labeled ligands to membrane components and that has been used to affinity-label and characterize receptors specific for insulin (17–19), the insulin-like growth factors (IGFs) I and II (20, 21), and nerve growth factor (NGF) (22). This methodology has been applied to affinity-label membrane components that interact specifically with SGF and with EGF. The data obtained in these experiments indicate that SGF can interact directly with EGF receptors and with a distinct membrane receptor species that does not bind EGF.

## MATERIALS AND METHODS

**Cells.** Cultures of human A431 carcinoma cells and normal rat kidney (NRK) fibroblasts were maintained at 37°C in 100-mm plastic tissue culture dishes with Dulbecco's modified Eagle's medium supplemented with 10% calf serum.

**Membrane Preparation.** Monolayers of A431 cells or NRK cells were mechanically detached and homogenized in the presence of ice-cold 0.25 M sucrose/10 mM Tris/1 mM EDTA/1 mM benzamidine·HCl/0.1 mM phenylmethylsulfonyl fluoride, pH 7.0. This homogenate was centrifuged at 2,000 × *g* for 10 min, and the resulting pellet was rehomogenized and centrifuged at 2,000 × *g* for 10 min. The supernatant was centrifuged at 4,000 × *g* for 15 min to sediment heavy particulate

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Abbreviations: TGF, transforming growth factor; SGF, sarcoma growth factor; EGF, epidermal growth factor; NRK cells, normal rat kidney cells; $^{125}$I-SGF and $^{125}$I-EGF, $^{125}$I-labeled SGF and EGF; kDal, kilodaltons.

material. The resulting supernatant was centrifuged at 30,000 × *g* for 30 min to yield a fraction consisting mainly of membranous material. Protein was determined by the dye-fixation method (Bio-Rad) after resuspension of the membrane preparation.

**Affinity-Labeling Protocols.** Membranes (0.3–1.0 mg of membrane protein per ml) resuspended in binding medium (128 mM NaCl/5 mM KCl/1.3 mM $MgSO_4$/1.3 mM $CaCl_2$/25 mM Hepes/1% bovine serum albumin, pH 7.5) were incubated for 90 min at 10°C in the presence of $^{125}$I-labeled SGF ($^{125}$I-SGF) or $^{125}$I-EGF and unlabeled ligands at the final concentration specified in each experiment. At the end of this incubation, unbound ligands were washed out by a 1:10 dilution with ice-cold binding medium and centrifugation at 30,000 × *g* for 30 min. Membranes were resuspended in ice-cold binding medium (0.3–0.5 mg of membrane protein per ml), and the appropriate crosslinking agent, freshly dissolved in dimethyl sulfoxide, was added at a 1:100 dilution to attain the final concentration indicated in each experiment. Crosslinking reactions were conducted exactly as described (15, 16, 22) and ended by dilution of the reacting mixture with excess ice-cold 10 mM Tris/1 mM EDTA, pH 7.0, and centrifugation at 30,000 × *g* for 30 min. Membrane pellets resulting from the last centrifugation step were resuspended in 70 μl of 10 mM Tris/1 mM EDTA, pH 7.0, and kept for electrophoretic analysis of labeled proteins.

**Electrophoresis and Autoradiography.** Affinity-labeled membranes were solubilized by heating for 2 min at 100°C in the presence of 1% sodium dodecyl sulfate/50 mM dithiothreitol/100 mM Tris, pH 6.8. Samples were electrophoresed in the discontinuous system of Laemmli (23) with 5% (30:0.3 acrylamide/bisacrylamide ratio) to 10% (30:0.8 acrylamide/bisacrylamide ratio) polyacrylamide gradient gels. After electrophoresis, gels were fixed and stained for protein (19) and subjected to autoradiography with Kodak X-Omat R film and Du Pont Lightning Plus intensifying screens. Molecular size protein standards used in the electrophoresis were myosin (200 kDal), β-galactosidase (116 kDal), phosphorylase *b* (94 kDal), bovine serum albumin (68 kDal), and ovalbumin (45 kDal).

**Growth Factors.** EGF isolated from mouse submaxillary glands by the method of Savage and Cohen (24) was labeled with $^{125}$I as described (10). Unless otherwise indicated, the unlabeled SGF preparations used to compete with $^{125}$I-SGF for binding to membrane receptors corresponded to the SGF fractions obtained after the carboxymethylcellulose purification step as described (10). Aliquots of carboxymethylcellulose pools containing SGF were radiolabeled by using $^{125}$I-labeled *N*-succinimidyl 3-(4-hydroxyphenyl)propionate (25). The radiolabeled SGF in these pools was affinity-purified by being bound to and eluted from the EGF receptor on formaldehyde-fixed A431 cells (14) and had a mass of 7.4 kDal (14). In a standard radioreceptor assay, 40–50% of the radioactivity in these purified radiolabeled SGF preparations was bound to fixed A431 cells. When this assay was performed in the presence of excess unlabeled EGF, <4% of the radioactivity in the radiolabeled SGF preparations was bound to the fixed A431 cells.

The unlabeled preparation of TGF used in the experiment in Fig. 3, lanes e and f, was derived from serum-free medium conditioned by Abelson murine leukemia virus-transformed rat embryo cells. This TGF was purified to homogeneity by a method previously used for the purification of human melanoma cell-derived TGF (26). Briefly, a pool of the fraction(s) containing TGF (≈7 kDal) was eluted from a Bio-Gel P-10 column and subjected to various consecutive HPLC steps in trifluoroacetic acid with μ-Bondapak $C_{18}$ columns (Waters Associates), from which it was eluted with a gradient of acetonitrile or propanol. A modification of this method also was applied to further purify SGF from carboxymethylcellulose active fractions. An aliquot of unlabeled SGF prepared by this method was used in the experiment of Fig. 3, lane d. SGF, rat embryo cell-derived TGF, and human melanoma cell-derived TGF have a mass of 7.4 kDal and virtually identical $NH_2$-terminal amino acid sequences (unpublished data).

Due to the scarcity of SGF and TGFs, direct analysis of the amount of peptide was not performed in the preparations of these factors used in the present studies. The amount of TGF in these preparations was estimated by their relative ability, as compared with known amounts of unlabeled EGF, to compete with $^{125}$I-EGF for binding to EGF receptors. "EGF nanogram equivalents" of a given aliquot of the TGF represent the nanograms of EGF that elicit a degree of competition towards $^{125}$I-EGF binding to A431 membranes that is equivalent to the degree of competition that this TGF aliquot exhibits in the same radioreceptor assay.

## RESULTS

Disuccinimidyl suberate and the photoreactive agent hydroxysuccinimidyl *p*-azidobenzoate were used to investigate whether $^{125}$I-SGF and $^{125}$I-EGF that bound to membranes from EGF receptor-rich A431 cells could be crosslinked to specific receptors. Disuccinimidyl suberate and other bishydroxysuccinimide esters have been used before to affinity-label insulin and insulin-like growth factor receptors (15, 17–21), and hydroxysuccinimidyl *p*-azidobenzoate has been used to affinity-label receptors for glucagon and nerve growth factor (16, 22). A431 membranes incubated at 10°C for 90 min with $^{125}$I-EGF or $^{125}$I-SGF were washed at 0°C to remove the excess unbound ligand. They were then crosslinked to bound ligand by incubation with 0.2 mM disuccinimidyl suberate at 0°C for 15 min. Membranes were washed with 10 mM Tris, pH 7.0 (a quenching agent for unreacted suberate), and subjected to gel electrophoresis in the dodecyl sulfate/polyacrylamide gel system of Laemmli (23).

In the autoradiogram from the resulting fixed, dried gel, two major labeled bands (140 and 160 kDal, respectively) appear in the lane corresponding to membranes affinity-labeled with $^{125}$I-EGF (Fig. 1, lane a). The labeling of these species was inhibited when an excess of unlabeled EGF was present during incubation of A431 membranes with $^{125}$I-EGF (Fig. 1, lane b). The apparent mass of these labeled species is similar to those reported for the EGF receptor and a commonly observed proteolytic fragment, respectively, either labeled with $^{125}$I-EGF (27) or purified from A431 cells and human placenta membranes (28–30). Therefore, we identify these species as the high-affinity EGF receptor in A431 cells. These 140- and 160-kDal species were also labeled by $^{125}$I-EGF without the addition of any crosslinking agent (not illustrated), probably because of spontaneous crosslinks between the EGF receptor and the $^{125}$I-EGF preparation (31). The radioactivity associated with the EGF receptor bands in the electrophoretic gels in samples not treated with crosslinking agents and in samples treated with the suberate reagent was 0.3% and 6.8%, respectively, of the receptor-bound $^{125}$I-EGF in these membranes. $^{125}$I-SGF also could be crosslinked to these 140- and 160-kDal species by disuccinimidyl suberate (Fig. 1, lane c). The labeling of these species was inhibited by either unlabeled SGF or unlabeled EGF present in excess during the incubation of membranes with $^{125}$I-SGF (Fig. 1, lanes d and e, respectively). From these data we conclude that SGF binds and can be crosslinked to EGF receptors that are present in large numbers in A431 membranes. The *p*-azidobenzoate reagent was less effective than disuccinimidyl suberate in crosslinking membrane-bound $^{125}$I-EGF (Fig. 1, lane f) or $^{125}$I-SGF (Fig. 1, lane g) to the EGF receptors.

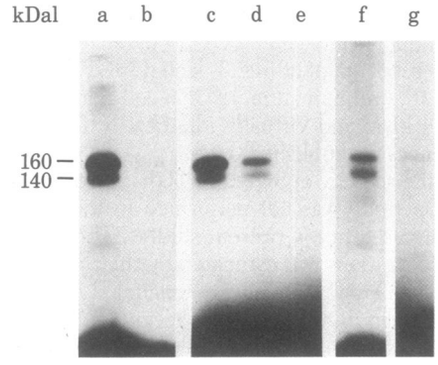


FIG. 1. Affinity labeling of EGF receptors in A431 cell membranes by $^{125}$I-EGF and $^{125}$I-SGF. Membranes from A431 cells were incubated for 90 min at 10°C in the presence of $2 \times 10^6$ cpm of $^{125}$I-EGF (lanes a, b, and f) or $3 \times 10^5$ cpm of $^{125}$I-SGF (lanes c, d, e, and g). Excess unlabeled EGF (1 μg/ml) (lanes b and e) or SGF (30 EGF ng equivalents/ml) (lane d) were also present during this incubation. After the free ligands were washed out, membranes were crosslinked to membrane-bound ligands by incubation in the presence of 0.2 mM disuccinimidyl suberate (lanes a–e) or 75 μM hydroxysuccinimidyl *p*-azidobenzoate (lanes f and g) for 15 min at 0°C. The affinity-labeled samples were subjected to dodecyl sulfate/polyacrylamide gel electrophoresis (150 μg of membrane protein per lane). Shown are autoradiograms corresponding to 4-hr (lanes a, b, and f) or 4-day (lanes c, d, e, and g) exposures of the resulting fixed, dried gels.

The number of EGF binding sites per cell in subconfluent NRK cells is only about 1% of that found in A431 cells (14, 32), and this number may decline when cells attain confluency (32). These cells are highly susceptible to the transforming effects of SGF and other TGFs (2). When we crosslinked membranes from confluent NRK cells to $^{125}$I-SGF and subjected them to electrophoresis and autoradiographic analysis, we could not observe affinity labeling of EGF receptors (Fig. 2). This result was expected because of the low number of EGF receptors in these cells. However, crosslinking of NRK cell membranes to membrane-bound $^{125}$I-SGF by hydroxysuccinimidyl *p*-azidobenzoate effected the intense labeling of a 60-kDal species (59.7 ± 0.6 kDal; $n = 3$)‡ (Fig. 2, lane a). This species was the only membrane component labeled by $^{125}$I-SGF in the 40- to 400-kDal range, and its labeling was inhibited by unlabeled SGF (Fig. 2, lane b). The labeling of the 60-kDal species was not modified by the presence of a large excess of EGF during incubation of membranes with $^{125}$I-SGF (Fig. 2, lane c). Disuccinimidyl suberate was as effective as the *p*-azidobenzoate in crosslinking $^{125}$I-SGF to the 60-kDal component of NRK cell membranes (Fig. 2, lane d).

To further document the specificity of the interaction of this 60-kDal species with TGFs, we tested the ability of TGFs from different origins to inhibit the labeling of this species by $^{125}$I-SGF. The autoradiogram in Fig. 3, lane a, shows such an experiment in which membranes affinity-labeled in the presence of $^{125}$I-SGF alone were electrophoresed. Unlabeled SGF, purified by adsorption to EGF receptor-rich A431 cells, was able to displace most of the radioactivity associated with the 60-kDal labeled species when added at a final concentration of 33 ng equivalents/ml during incubation of membranes with $^{125}$I-SGF (Fig. 3, lane b). Higher concentrations of this preparation displaced essentially all of the radioactivity in the 60-kDal species

‡ By assuming that one single intact molecule of SGF (≈7.4 kDal) is linked per molecule of membrane receptor, the receptor alone would be 52 kDal. However, these values must be taken only as estimates due to the irregularities intrinsic to molecular mass determination in dodecyl sulfate/polyacrylamide gels. For simplicity, we call this membrane component the 60-kDal labeled species.

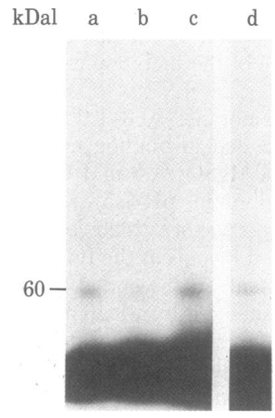


FIG. 2. Affinity labeling of a 60-kDal component of NRK cell membranes by $^{125}$I-SGF. Samples (400 μg of membrane protein) from confluent NRK cells were affinity-labeled by successive incubation with $3 \times 10^5$ cpm of $^{125}$I-SGF and 25 μM hydroxysuccinimidyl *p*-azidobenzoate (lanes a–c) or 0.2 mM disuccinimidyl suberate (lane d). Lanes b and c correspond to conditions in which incubation with $^{125}$I-SGF was done in the presence of 30 ng equivalents of unlabeled SGF or 1 μg of EGF per ml, respectively. The autoradiogram is a 7-day exposure of the fixed, dried electrophoretic gels corresponding to these affinity-labeled samples.

(Fig. 3, lane c). SGF purified by a method that includes HPLC but not cycling over fixed A431 cell preparations was also able to inhibit the labeling of the 60-kDal species, when present at a final concentration of 160 ng equivalents/ml during incubation of membranes with $^{125}$I-SGF (Fig. 3, lane d). A TGF purified from Abelson murine leukemia virus-transformed rat embryo cells was also effective at displacing $^{125}$I-SGF from the 60-kDal species (Fig. 3, lanes e and f), whereas EGF even at 3,300 ng/ml was not effective (Fig. 3, lane g). These observations are consistent with the concept that the 60-kDal species is a TGF receptor that does not interact with EGF.

$^{125}$I-EGF labeled the 150- to 170-kDal EGF receptor present in NRK cell membranes (Fig. 4); this labeling was inhibited by

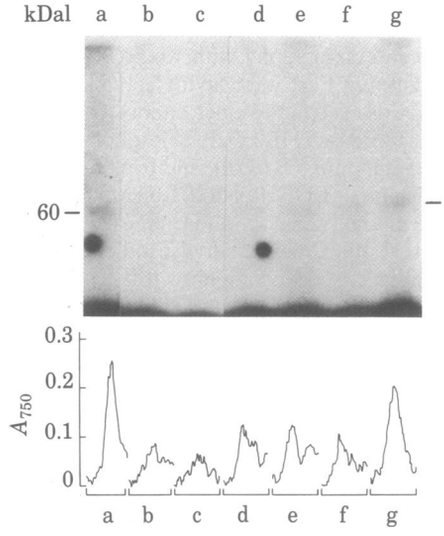


FIG. 3. Interaction of the 60-kDal affinity-labeled species with different growth factors. Samples (250 μg of membrane protein) of NRK cell membranes were affinity-labeled by successive incubation in the presence of $3 \times 10^5$ cpm of $^{125}$I-SGF and 0.2 mM disuccinimidyl suberate. During incubation of membranes with $^{125}$I-SGF, the following unlabeled growth factors were also present: none (lane a); 30 ng equivalents of carboxymethylcellulose-purified SGF per ml (lane b); 130 ng equivalents of this same SGF preparation per ml (lane c); 160 ng equivalents of SGF (purified by a method involving HPLC without adsorption to EGF receptor-rich cells) per ml (lane d); 130 ng equivalents of TGF (from Abelson murine leukemia virus-transformed rat embryo cells) per ml (lane e); 1.1 μg equivalents of this same TGF preparation per ml (lane f); and 1 μg of EGF per ml (lane g). After affinity labeling, membranes were subjected to electrophoresis. The autoradiogram shown was obtained after a 2-wk exposure of the resulting fixed, dried gels. Densitometry of the region containing the 60-kDal band in each lane is shown in the bottom panel.

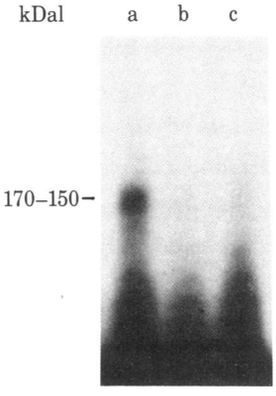


FIG. 4. Affinity labeling of EGF receptors in NRK membranes by $^{125}$I-EGF. NRK membranes (400 $\mu$g of membrane protein) were incubated with $2 \times 10^6$ cpm of $^{125}$I-EGF alone (lane a) or in the presence of 30 ng equivalents of unlabeled SGF (lane b) or 1 $\mu$g of EGF (lane c) per ml. Membrane-bound ligands were crosslinked to membrane components by incubation in the presence of 0.2 mM disuccinimidyl suberate. The autoradiogram shown corresponds to a 7-day exposure of fixed, dried electrophoretic gels corresponding to these affinity-labeled samples.

unlabeled SGF and by unlabeled EGF (Fig. 4). In contrast to membranes from confluent NRK cells, membranes obtained from subconfluent NRK cells showed detectable affinity-labeling of the 150- to 170-kDal EGF receptor species by $^{125}$I-SGF (Fig. 5, lane a); this labeling was inhibited when either unlabeled SGF or unlabeled EGF was present during incubation of membranes with $^{125}$I-SGF (Fig. 5, lanes b and c, respectively). Extensive autoradiographic exposures of gels corresponding to affinity-labeled A431 membranes also show evidence that $^{125}$I-SGF can be crosslinked to a 60-kDal component with specificity for SGF similar to that of the putative SGF receptor component identified in NRK membranes (not illustrated).

Table 1 is a summary of the receptor species for EGF and SGF tentatively identified in the present studies. EGF binds to the abundant 140- to 160-kDal EGF receptor in A431 cells. This receptor is the major A431 membrane species interacting with SGF. However, SGF also binds to a minor 60-kDal receptor species in A431 membranes. Both EGF and SGF bind to the 150- to 170-kDal EGF receptor present in NRK cells. This species is a minor receptor for SGF in NRK cells. The major SGF receptor species in NRK cells is a 60-kDal membrane component that does not bind EGF. From this data we tentatively conclude that, in both a normal cell line (NRK) and a transformed cell line (A431), SGF interacts with 140- to 170-kDal EGF receptors that may mediate normal cell growth effects. More importantly, SGF interacts with a more specific type of receptor (60 kDal) that has little or no affinity for EGF.

## DISCUSSION

The ability of SGF and other TGFs to compete with $^{125}$I-EGF for its receptors had been consistently observed during characterization of these growth factors (2, 5, 6, 9). The binding and elution of SGF from fixed preparations of EGF receptor-rich

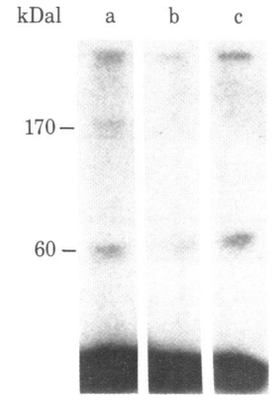


FIG. 5. Affinity labeling of EGF receptors in NRK cell membranes by $^{125}$I-SGF. Membranes (100 $\mu$g of membrane protein per condition) from near confluent NRK cells were incubated in the presence of $2 \times 10^5$ cpm of $^{125}$I-SGF alone (lane a) or in the presence of 25 ng equivalents of SGF (lane b) or 1 $\mu$g of EGF (lane c) per ml. Membranes were then incubated in the presence of 0.2 mM disuccinimidyl suberate. At the end of this treatment, samples were electrophoresed, and the resulting gels were fixed, dried, and subjected to autoradiography. A 3-wk autoradiogram is shown.

Table 1. Receptor species for EGF and SGF affinity-labeled in membranes from A431 and NRK cells by $^{125}$I-EGF and $^{125}$I-SGF

| Cell line | Receptor species, kDal | |
|---|---|---|
| | $^{125}$I-EGF label | $^{125}$I-SGF label |
| A431 | 140–160 | 140–160 (major)<br>60 (minor) |
| NRK | 150–170 | 150–170 (minor)<br>60 (major) |

A431 carcinoma cells (14) and the phosphorylation on tyrosine residues of the EGF receptor in response to SGF (33) also indicated an interaction between SGF and the EGF membrane receptor. The present studies provide direct evidence for this hypothesis by demonstrating that different crosslinking agents are able to link $^{125}$I-SGF to the high-affinity EGF receptor structure that migrates on gels at 140- to 170-kDal (Figs. 1, 4, and 5). Consequently, we consider unlikely the possibility that the displacement of $^{125}$I-EGF from its receptor by TGFs results from interaction of TGFs with a membrane component other than the EGF receptor itself. At present, we cannot rule out the possibility that the EGF receptor contains separate EGF and SGF binding sites that interact with each other to modulate the affinity for their respective ligands.

The experiments reported in this paper show that $^{125}$I-SGF also interacts with and can be crosslinked to a 60-kDal membrane component that shows properties expected for a high-affinity SGF receptor. Unlabeled SGF, purified by binding to and elution from fixed preparations of EGF receptor-rich cells, displaced $^{125}$I-SGF equally well from this 60-kDal species and from the EGF receptor. These observations suggest that the same SGF molecular species can interact with both types of receptors. The affinity labeling of the 60-kDal species is not an artifact of the cycling procedure used in the preparation of SGF and $^{125}$I-SGF, because SGF prepared by an alternative method that does not involve binding to and elution from fixed A431 cells is also able to compete with $^{125}$I-SGF for the labeling of the 60-kDal species (Fig. 3). Furthermore, the ability to interact with the 60-kDal species is not limited to SGF from murine sarcoma virus-transformed mouse 3T3 cells because a TGF purified from Abelson murine leukemia virus-transformed rat embryo cells also competes with $^{125}$I-SGF for the labeling of this species (Fig. 3). We conclude that the 60-kDal affinity-labeled species corresponds to a membrane receptor for TGFs that does not recognize EGF.

It is worth noting that amino acid sequence data of SGF, rat embryo cell-derived TGF, and human melanoma cell-derived TGF show only a distant homology of these factors to EGF. In contrast, these three TGFs share homology in 13 of 15 amino acids in their $NH_2$-terminal end (unpublished data). Control experiments show that the 60-kDal membrane species is labeled by $^{125}$I-SGF only when membranes are present in the incubation medium, indicating that the generation of this labeled species by crosslinking agents is due neither to internal crosslinking of $^{125}$I-SGF aggregates nor to binding and crosslinking of $^{125}$I-SGF to bovine serum albumin (68 kDal) present in the incubation medium (data not shown). Because the scarcity of the most pure TGF preparations used in the present study does not allow an exact protein quantitation, we cannot at present determine precisely the affinity of TGFs for the 60-kDal species. However, by comparison with the ability of these TGF preparations to displace $^{125}$I-EGF from its receptor, we estimate that their $K_d$ for the 60-kDal species is in the nanomolar range.

The observations described above indicate that SGF and other TGFs can interact with at least two types of high-affinity

membrane receptors. One type of receptor interacts only with TGFs, whereas the other type can interact with both TGFs and EGF. The latter type of receptor probably mediates the growth effects of EGF and some TGFs. Cell stimulation through this EGF receptor seems to be essential for TGFs to elicit effective transforming cell growth. This may explain why certain forms of TGF that do not interact with the EGF receptor are dependent on the simultaneous presence of EGF to induce massive cell proliferation (9, 12). Induction of cellular transformation and growth by other forms of TGF, including SGF, is independent of EGF possibly because this class of TGFs can directly interact with EGF receptors (2, 6, 9, 12). In contrast, the inability of EGF to induce cellular transformation (2, 5) supports the interpretation that TGFs induce the transformed phenotype by interaction with receptors that do not bind EGF. The dependence of TGFs on stimulation of EGF receptors to induce cell growth suggests the possibility that the TGF receptors that mediate cell transformation may not mediate cell growth. Therefore, TGFs may capitalize on normal cell growth events mediated through EGF receptors, and perhaps through receptors for other growth factors, to promote transforming cell proliferation.

In conclusion, we have affinity-labeled two types of membrane structures that satisfy expected criteria for receptors for TGFs. One type of TGF receptor corresponds to the 170-kDal EGF receptor and possibly mediates the growth effects of SGF and other TGFs. The other membrane species affinity-labeled by $^{125}$I-SGF is a 60-kDal TGF receptor that may mediate induction of the transformed phenotype. The data presented are consistent with the hypothesis that both receptor types must be activated in order to initiate optimal proliferation of responding cells and the full expression of the transformed phenotype.

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