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Growth factors from murine sarcoma virus-transformed cells

(epidermal growth factor/polypeptide hormones/cell transformation/radioreceptor assays)

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ABSTRACT **Murine sarcoma virus-transformed mouse fibroblasts produce polypeptide growth factors and release them into serum-free medium. These factors stimulate cells to divide in monolayer cultures and also to form colonies that grow progressively in soft agar. Three major peaks of activity are seen, with apparent molecular weights of 25,000, 12,000, and 7000. The sarcoma growth factors are heat-stable, trypsin-sensitive, and active in nanogram quantities when tested for growth stimulation of untransformed rat and mouse fibroblasts. All three molecular species are also capable of competing for membrane epidermal growth factor (EGF) receptors when tested with ^{125}I-labeled EGF. They differ from mouse EGF, however, in their molecular weights, in their inability to react with anti-EGF antibodies, and in their ability to convert cells to anchorage independent (agar) growth. For the above reasons, we conclude that the sarcoma growth factors are a new class of polypeptide tropic factors that confer on fibroblasts *in vitro* properties associated with the transformed phenotype.**

Previous studies (1, 2) with murine sarcoma virus (MuSV)-transformed cells have suggested that these cells produce growth factors that stimulate cell division in normal fibroblasts and interact with the membrane receptors for epidermal growth factor (EGF). The basis for this suggestion was the finding that MuSV-transformed cells no longer display EGF receptors on their cell surfaces; in contrast, DNA virus-transformed cells and most chemical carcinogen-transformed cells continue to express their EGF receptors. Therefore, the substances produced by the MuSV-transformed cells may specifically interact with the EGF receptor system in fibroblast membranes.

In this report we describe the partial purification of a family of growth-stimulating polypeptides, sarcoma growth factors (SGFs), from the supernatant fluids of Moloney MuSV-transformed mouse 3T3 cells. These factors have EGF-competing activity and growth-stimulating activity that coelute in gel filtration. SGFs not only stimulate fibroblasts to divide and overgrow in monolayer cultures but also produce a profound morphologic alteration in the cells and allow them to form progressively growing colonies in soft agar, a property closely associated with the transformed phenotype (3, 4). The morphological alterations produced in monolayer cultures and the ability to induce anchorage-independent growth are observable within a few days after treatment with nanogram quantities of partially purified SGFs and continue as long as the peptides are supplied. There is as yet no evidence that SGFs produce permanent genetic changes in the responding fibroblasts. Upon removal of SGFs from the medium, the cells resume their normal growth properties. These cells can be cycled back and forth between the untransformed and the transformed phenotype, without an apparent genetic change, by growing them in the absence or presence of the SGFs. It appears, therefore, that these polypeptides from the medium of MuSV-transformed cells are able to act as effectors of fibroblastic cell transformation. The 12,000 molecular weight protein is the most active in stimulating cells to divide in soft agar.

MATERIALS AND METHODS

Sarcoma-Conditioned Medium. The Moloney MuSV-transformed 3T3 cell line, 3B11-IC (5), was grown in roller bottles containing Dulbecco's modification of Eagle's medium with 10% calf serum (Colorado Serum Co.). The cells were washed for 1 hr with 50 ml of serum-free Waymouth's medium (GIBCO, MD 705/1) (6). This was discarded, as was a second wash 12 hr later. Two subsequent 48-hr collections were taken; this medium will be referred to as "sarcoma-conditioned medium." The viability of the cells maintained either in medium containing 10% serum or in serum-free medium for 5 days with four changes of medium was greater than 80% as determined by trypan blue exclusion. The supernatant, after centrifugation at 100,000 × *g* for 45 min, was saved and stored at −20° until used. Conditioned media from the uninfected normal rat kidney fibroblastic clone 49F (7), a MuSV-transformed derivative of this clone, designated 49F-MuSV, a simian virus 40-transformed clone of 3T3, and an NIH/3T3 line producing Moloney leukemia "helper" virus were processed as above.

Assay for EGF-Competing Activity. The ^{125}I-labeled EGF binding assays were performed on subconfluent cell cultures of Mv1Lu (or CCl 64) (8) or human carcinoma cells (A431) (9) as described (1). Competitions were initiated by the addition of 0.2 ml of binding buffer with or without the potential inhibitor. The mixtures were incubated with the monolayer at 22° for 1 hr, the fluid was removed, and the cells were washed four times with 1-ml portions of binding buffer. Binding of EGF was initiated immediately by adding 0.4 ng of ^{125}I-labeled EGF in 0.2 ml of binding buffer (18,600 dpm). After incubation for 1 hr at 22°, the quantity of ^{125}I-labeled EGF specifically bound was determined (1).

Membrane receptor assays for nerve growth factor (9), multiplication-stimulating activity (MSA) (2), and Rauscher leukemia viral envelope glycoprotein (gp71) (1) were performed as described.

Assay for Growth-Promoting Activity. Serum-deprived, subconfluent, normal rat kidney cells (10) were prepared by trypsinizing the fibroblastic clone 49F (7). They were seeded at 2.5 × 10^4 cells per 16-mm Linbro well (FB-16-24TC) in Dulbecco's modified Eagle's medium containing 10% calf serum. After 24 hr the medium was removed, and the cells were washed with fresh medium and then incubated with 1 ml of Waymouth's medium containing 0.1% fetal calf serum per well. Three days later, 0.1 ml of binding buffer containing the sample to be tested was added. Sixteen hours after the addition, the cells

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Abbreviations: MuSV, murine sarcoma virus; EGF, epidermal growth factor; SGFs, sarcoma growth factors; MSA, multiplication-stimulating activity.

were exposed for 8 hr to 2.5 μCi of [^{3}H]thymidine (specific activity, 6.7 mCi/mmol). The medium, containing the radiolabeled thymidine, was then removed, and the cultures were washed twice with 1 ml of Dulbecco's modified Eagle's medium containing 100 μg of unlabeled thymidine and incubated for 30 min. After the incubation, the monolayers were washed three times, the cells were lysed with 0.5% sodium dodecyl sulfate/1 mM EDTA, and the DNA was precipitated by adding the lysate to 3 vol of cold 10% trichloroacetic acid. The precipitated DNA was removed by filtration (Millipore, HA, 0.45 μM); the filters were dried and added to counting vials with 5 ml of toluene/Liquifluor (New England Nuclear, NEF 903), and the radioactivity was measured in a Beckman LS-250 liquid scintillation counter.

Assay for Growth in Soft Agar. Agar plates were prepared in 60-mm petri dishes (Costar no. 3060) by first applying a 2-ml base layer of 0.5% agar (Difco, Agar Noble) in Dulbecco's modified Eagle's medium containing 10% calf serum. Over this basal layer, an additional 2-ml layer of 0.3% agar in the same medium/calf serum mixture, the appropriate concentration of protein isolated from the sarcoma-conditioned medium, and 2×10^3 cells were added. The cells were incubated at 37° in a humidified atmosphere of 5% CO_2 in air and refed after 7 days by addition of 2 ml of 0.3% agar containing the appropriate supplements. Colonies were measured unfixed and unstained by using a microscope with a calibrated grid. Preliminary experiments showed that the agar growth assay could be scored at 1 week. Normal rat fibroblasts from clone 49F showed predominantly single cells with rare (<10%) two- to four-cell colonies. Clones with greater than 20 cells were scored as positive; by 2 weeks, many of these contained several hundred cells.

Partial Purification of the Growth-Promoting Activity and EGF-Competing Activity. The clarified serum-free sarcoma-conditioned medium was dialyzed against 1% acetic acid (five changes of 5 vol) in Spectrapor 3 dialysis tubing (molecular weight cutoff, approximately 3500; Spectrum Medical Industries, no. 132720). The dialysate was concentrated by lyophilization and extracted with 15 ml of 1 M acetic acid per liter of medium. The extract was clarified by centrifuging at 100,000 × *g* for 30 min. The supernatant fluid contained virtually all the activity when tested for the ability to stimulate [^{3}H]thymidine incorporation and to compete with ^{125}I-labeled EGF for cell surface receptors. A 30-ml aliquot of the 100,000 × *g* supernatant, from 2 liters of conditioned medium, was lyophilized, dissolved in 15 ml of 1 M acetic acid, clarified, and separated by gel filtration chromatography. The sample was applied to a 5 × 90 cm column of Bio-Gel P-60 equilibrated with 1 M acetic acid and eluted at a flow rate of 36 ml/hr at room temperature. Three-hundred-drop fractions were collected. Aliquots were lyophilized for protein determination (11), EGF competition, stimulation of thymidine incorporation, and colony-forming activity in soft agar.

Chemical Treatment of SGF. One-milligram portions of the dialyzed, lyophilized material were dissolved in phosphate-buffered saline (pH 7.2) and tested for heat stability by treating one aliquot for 30 min at 56° and another in a boiling water bath for 3 min. Trypsin sensitivity was tested by incubating an aliquot of SGF with 50 μg of L-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington Biochemical Corp.) at 37° for 2 hr. The trypsin was inactivated by adding 100 μg of soybean trypsin inhibitor (Worthington). As a control, 50 μg of trypsin was incubated with 100 μg of soybean trypsin inhibitor for 30 min and then this mixture was incubated at 37° with an aliquot of SGF for 2 hr. The effect of reducing agents was tested by using 0.065 M dithiothreitol (Calbiochem) in 0.1 M NH_4HCO_3 and incubating at room temperature for 1 hr. At the end of the treatments, all samples, including the control, were extensively dialyzed against 1% acetic acid (vol/vol), lyophilized, and dissolved in binding buffer for testing.

RESULTS

Serum-free conditioned media from normal and MuSV-transformed cells were tested for their ability to compete in an EGF receptor assay. As shown in Table 1, the conditioned medium from 49F had little EGF-competing activity whereas the supernatant medium from the transformant 49F-MuSV competed with 88% of the ^{125}I-labeled EGF for membrane receptor sites. The conditioned medium from 49F contained no detectable growth-stimulating activity but that from the 49F-MuSV transformant increased the thymidine incorporation to 750% above that of the control. NIH/3T3-producing Moloney leukemia virus was compared with 3B11-IC, which produces both MuSV and the leukemia helper. Supernatants from the cells producing only the leukemia virus contained minimal levels of either activity; the supernatant from the MuSV-transformed cell had high levels of both, inhibiting 97% of the EGF binding and stimulating [^{3}H]thymidine incorporation to over 100% above the control level. The conditioned medium from the MuSV-transformed 3B11-IC line stimulated [^{3}H]-thymidine incorporation into cell lines derived from several species, including those from rat, mouse, and man. These results show that substances capable of stimulating cells to divide and of competing with EGF for membrane receptors are produced by MuSV-transformed cells.

Ability to grow in soft agar has been shown to correlate well with other parameters of cell transformation and with tumorigenicity in animals (3, 4). Crude SGF, from the conditioned medium of 3B11-IC cells, was tested for its effect on agar colony formation. Two thousand clone 49F cells were seeded in petri dishes containing soft agar and the appropriate concentration of crude SGF (Table 2). Three days after seeding, small colonies could be seen in the SGF-treated plates. Colony growth was dose-dependent. Many of the colonies were found to contain well over 500 cells.

Several other growth factors were tested for their ability to stimulate colony formation in soft agar (Table 3). All of these were able to stimulate thymidine incorporation; none stimulated the rat fibroblast cells to form colonies in the soft agar. These studies led to the conclusion that SGFs differ from the previously described growth factors in ability to convert cells to anchorage-independent growth.

To test the possibility that the soft-agar growth-promoting activity present in the SGFs was due to a nonspecific protease,

Table 1. EGF-competing activity and growth-stimulating activity in supernatants from cells grown in serum-free medium

Cell line *	% EGF competition/ 18 μg	% stimulation thymidine incorp.
49F (NRK)	<10	<20
49F-MuSV (MuSV-transformed NRK)	88	750
MJD-54 (mouse cells producing MuLV)	<20	<50
3B11-IC (MuSV-transformed mouse cells producing MuSV and MuLV)	97	1050

Forty-eight-hour serum-free conditioned media were collected from the cell lines, filtered, serially diluted, and tested. The EGF competition was determined by using mink lung CCL 64 cells in a radioreceptor assay; stimulation of thymidine incorporation was determined by using rat 49F cells. The thymidine incorporation data are given as the percentage incorporation above the control (23,200 cpm).
*NRK, normal rat kidney; MuLV, murine leukemia virus.

Table 2. Effect of SGF from MuSV-transformed 3T3 cells on growth of rat fibroblasts in soft agar

Substance and concentration, μg/ml	Distribution by colony size, no. <0.032 mm	0.032–0.064 mm	0.065–0.130 mm	>0.130 mm
SGF:				
56	1	9	60	30
14	27	42	23	3
3.5	32	61	7	0
EGF				
100 ng/ml	100	0	0	0
None	100	0	0	0

The soft agar was made up to contain the final concentration of protein listed. The SGFs used were the acid-soluble proteins obtained from serum-free medium conditioned by MuSV-transformed NIH/3T3 cells. This medium was clarified, dialyzed against 1% acetic acid, lyophilized, and dissolved in 1 M acetic acid. Two liters of this medium gave 74 mg of acetic acid-soluble protein. Aliquots were lyophilized and tested directly for soft agar growth. One week later, each dish was fed with 2 ml of 0.3% agar containing the appropriate concentration of factor. At the end of the second week, the sizes of 100 unstained, unfixed colonies were measured; the number of colonies in each size-category are recorded. The size of a single cell ranged between 0.010 and 0.022 mm.

Table 3. Effect of various growth factors and SGF on growth of rat fibroblasts in soft agar

Test cells	Colonies, no./plate	% stimulation thymidine incorp.*
NRK clone 49F		
No treatment	0	0
Calf serum (6 mg/ml)	0	640
EGF (100 ng/ml)	0	NT
(10 ng/ml)	0	530
(1 ng/ml)	0	620
MSA (100 ng/ml)	0	180
FGF (100 ng/ml)	0	480
Human sarcoma factor (25 μg/ml)	0	630
SGF untreated (50 μg/ml)	1350	1050
SGF heat-treated (100° for 3 min; 50 μg/ml)	1200	990
SGF trypsin treated (equivalent of 50 μg/ml)	0	10
SGF dithiothreitol-treated (50 μg/ml)	0	0
NRK clone 49F transformed by MuSV	1600	NT

Soft agar assays were set up by using rat fibroblast 49F cells at 2 × 10^3 cells per plate in the presence of the listed concentrations of proteins. Seven days after seeding, colonies containing >20 cells were scored as positive. The growth factors used were tested at or near their optimal concentration for stimulation of thymidine incorporation. The SGF preparation was that described in the legend to Table 2. Concentrations of EGF >100 ng/ml began to show toxic effects. The human sarcoma factor is the partially purified 22,000 molecular weight peak of MSA-competing activity isolated from conditioned medium produced by a human fibrosarcoma line (12). The stimulation of thymidine incorporation listed is the percentage of [^{3}H]thymidine incorporated above the control (19,700 cpm).
*NT, not tested.

the dialyzed, lyophilized SGFs were tested for their ability to hydrolyze hide powder azure, and the fibroblastic cells were tested for their ability to grow in soft agar in the presence of varying concentrations of proteases. Protein (250 μg) from the conditioned medium showed no detectable hydrolysis of hide powder azure under conditions such that 0.1 μg of trypsin scored as strongly positive. With trypsin, chymotrypsin, and Pronase incorporated into a soft agar assay at final concentrations of 0.01–10 μg/ml, there were no colonies formed. Supernatants prepared from various other kinds of transformed cells, including one simian virus 40-transformed line (SV-3T3-6), one polyoma-transformed line (PY-3T3-4a), and several chemically transformed cells, failed to compete in the radioreceptor assay for EGF binding.

The activity responsible for soft agar colony formation was stable to boiling at pH 7.2 for 3 min, but treatment with either trypsin or the disulfide reducing agent dithiothreitol destroyed the activity (Table 3). Thus, the soft agar colony-stimulating activity is a heat stable protein containing disulfide bonds.

When the concentrated sarcoma-conditioned medium was fractionated on a column of Bio-Gel P-60, most of the eluted protein had an apparent molecular weight greater than 30,000 (Fig. 1). Three major size classes of EGF-competing activity were observed. The largest and most heterogeneous had an apparent molecular weight of 20,000–27,000. The other two considerably sharper activities eluted with apparent molecular weights of 12,000 and 7000.

The activity that stimulated cells to form colonies in soft agar coeluted in each case with a peak of EGF-competing activity. The peak at 12,000 had more soft-agar colony-forming activity per unit of EGF-competing activity than did the other peaks; it contained 5 × 10^5 agar colony growth units per mg of protein. The specific colony-forming activity of the protein in this peak

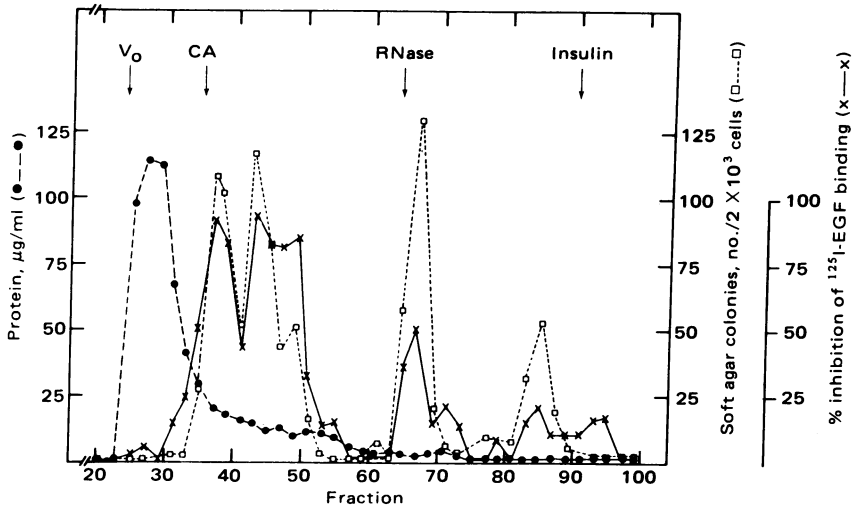

FIG. 1. Bio-Gel P-60 chromatography of the acetic acid-soluble concentrate from MuSV-transformed cell line 3B11-IC (5). The soluble concentrate from 2 liters of serum-free conditioned tissue culture medium was dissolved in 15 ml of 1 M acetic acid and chromatographed on a column of Bio-Gel P-60 (5 × 90 cm). The column was eluted with 1 M acetic acid at 36 ml/hr, and 300-drop fractions were collected. The markers were: Vo, blue dextran for void volume; CA, carbonic anhydrase, 29,000; RNase, 13,800; and insulin, 6,000.

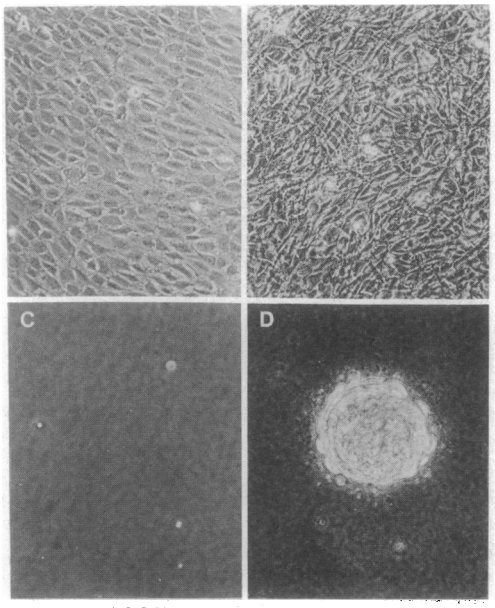

FIG. 2. Effect of SGFs on morphology and growth in soft agar. (*A*) Rat clone 49F seeded 6 days previously at 1×10^5 cells per 30-cm^2 tissue culture flask in Dulbecco's modified Eagle's medium containing 10% calf serum (the medium was changed once, 3 days after seeding). (*B*) Parallel culture treated with SGF (2.5 μg of protein per ml, fraction 43 from Fig. 1); the medium was changed 3 days after seeding and new SGF (fraction 43) was added. (*C*) Clone 49F cells at 2 weeks after seeding in soft agar without the addition of SGF. The cells were seeded at 1×10^4 cells per 20-cm^2 petri dish and were fed with a 2-ml overlay of 0.3% agar after 7 days. More than 10^7 rat fibroblast cells (clone 49F) have been plated in soft agar and, in the absence of SGFs, no spontaneous colony formation has been seen with this clone. (*D*) Cells in *C* except that 0.4 μg of protein from fraction 67 of Fig. 1 was incorporated per ml of the soft agar. After 1 week these cells were refed with 2 ml of 0.3% agar containing 0.4 μg of protein from the same fraction per ml. (*A*, ×45; *B*, ×45; *C*, ×90; *D*, ×90.)

was approximately 200 times greater than that in the conditioned medium. The low molecular weight peak contained 2.5 $\times 10^5$ agar colony growth units and the larger peak had 10^4–10^5. The column eluate was also tested for its ability to stimulate thymidine incorporation. Peaks of activity coeluted with EGF-1 competing activity almost exactly and were highest in fractions 37, 67, and 85, corresponding to molecular weights of 27,000, 12,000, and 7000, respectively.

The phenotypic changes in cellular morphology seen in the presence of SGFs are illustrated in Fig. 2. The untreated fibroblastic cells were present as a flat monolayer containing a regular growth pattern, whereas the treated cells were refractile, displayed no discernible overall growth pattern, crisscrossed in an apparently random fashion, and grew in multiple layers. In soft agar, the untreated cells (Fig. 2*C*) were present as single cells. During the course of these experiments the indicator cells had a frequency of spontaneous transformation, as determined by colony formation in soft agar, of less than $1/10^6$. Fig. 2*D* illustrates a typical soft agar colony 2 weeks after seeding the cells in the presence of protein from column fraction 67 (Fig. 1) at 0.4 μg/ml. The colonies continued to grow as long as they are fed with fresh medium and factor weekly. Colonies picked from soft agar and grown as monolayers showed the phenotype of the untreated parent and would not grow in soft agar unless SGFs were added to the medium. The phenotypically transformed cells from the monolayers could be cycled back and forth between the transformed and untransformed states by seeding in the presence or absence of SGFs, respectively.

When the biological properties of EGF and the SGFs obtained from the 12,000 molecular weight material (Fig. 1) were compared, the radioreceptor assay (Fig. 3*A*) showed that both EGF and SGFs competed with ^{125}I-labeled EGF for the receptor sites. The slopes of the competition curves were similar although the EGF was approximately 3 orders of magnitude more potent on a weight basis. In a radioimmunoassay using anti-EGF antibody and ^{125}I-labeled EGF, approximately 0.1 ng of competing unlabeled EGF gave 50% competition whereas the SGF fraction gave no competition when up to 30 μg of protein was used (Fig. 3*B*). Both EGF and the 12,000 M_r SGF were specific in the radioreceptor assays; neither showed competition when tested in specific radioreceptor assays using ^{125}I-labeled nerve growth factor, MSA, or mouse virus gp71 (1, 2, 9).

Both the EGF and SGF were potent stimulators of [^{3}H]thymidine incorporation (Fig. 3*C*). At approximately 1 ng, EGF gave a value of 600% of the control; SGF required approximately 750 ng for the same degree of stimulation. The EGF, however, reached a maximum stimulation of approximately 750% of the control and at higher concentrations showed less

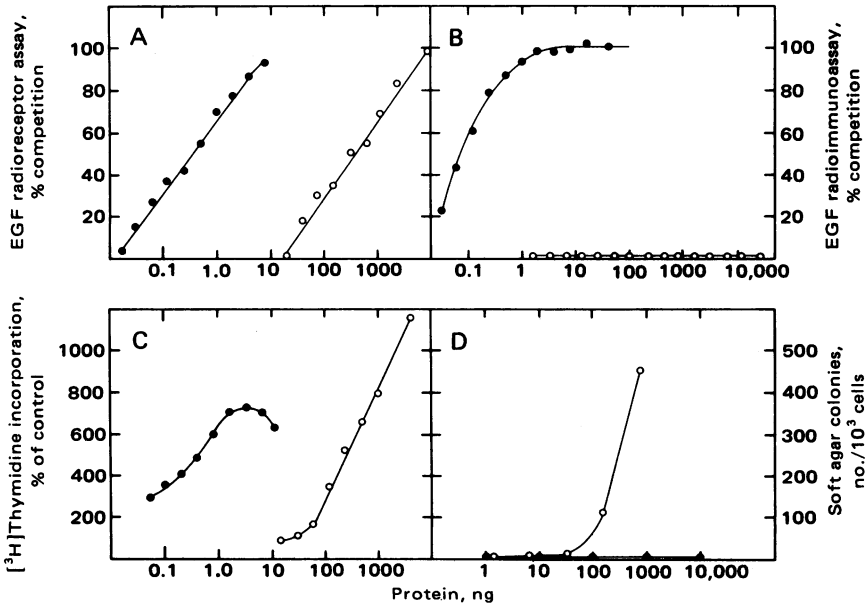

FIG. 3. Properties of EGF (●) and the 12,000 molecular weight peak of SGF (○) from Fig. 1. (*A*) EGF radioreceptor competition assays were performed with 2.5×10^4 mink lung cells and a sequential binding assay in which the competitor is allowed to preincubate with the cells at 22° for 1 hr. It is then removed, the cells are washed four times with binding buffer, and the ^{125}I-labeled EGF is incubated with the cells for 1 hr at 22°. (*B*) Radioimmunoassay using anti-EGF antibody and ^{125}I-labeled EGF. The antibody to mouse salivary gland EGF was produced in rabbits and the precipitating antibody was goat anti-rabbit IgG. (*C*) Thymidine incorporation studies using serum-depleted fibroblastic clone 49F. (*D*) Soft-agar growth-promoting activity. Rat clone 49F was seeded in the presence of varying concentrations of the peptides. The cells were fed 1 week after seeding and the colonies were counted at the end of 2 weeks.

growth stimulation, whereas the effect of SGF increased with dose to greater than 1100% of the control. EGF did not stimulate growth of these cells in soft agar; the SGF began to stimulate soft agar growth at concentrations similar to those required for [^{3}H]thymidine incorporation (Fig. 3*D*). Mouse submaxillary gland EGF behaves anomalously on acidic Bio-Gel columns in that it is adsorbed to the resin and is eluted after the salt peak (13). On our column the peak of the EGF elution profile was in fraction 135. Fractions 105–141 were tested for their EGF competing ability, their ability to stimulate [^{3}H]thymidine incorporation, and soft agar colony formation; none of these fractions contained activity. We therefore conclude that none of the EGF-competing activities or growth-stimulating activities produced by these cells are the same as those of EGF purified from mouse submaxillary gland.

DISCUSSION

The factor(s) we have characterized differ from mouse EGF purified from male mouse salivary glands. First, each of the three fractions, even the smallest, appears to be significantly larger than EGF. Second, antiserum prepared against mouse EGF had no activity against the SGFs as measured in radioimmunoassays, in direct immunoprecipitation, or as an inhibitor of the growth-stimulating activity. Thus, despite the fact that they each can compete with the EGF receptor systems, SGFs and EGF are not closely related immunologically. Antisera prepared against SGFs will allow testing of the reciprocal antigenic response. Third, and perhaps most importantly, SGFs will permit fibroblasts to grow progressively in agar but EGF, even at much higher concentrations as measured by EGF receptor competing activity, has no such effect. EGF, and other normal growth factors such as fibroblast growth factor and MSA, will allow the rat fibroblast cells used as test cells in this study to pass through one or two extra cell divisions in agar but will not produce progressively growing agar colonies. We have, as yet, no evidence that the three size classes described are different molecular forms of related peptides. Nevertheless, the fact that the EGF-competing activity and the agar growth activity coelute suggests that EGF receptor binding can be used to follow the purification of the agar growth activity. Although they are able to compete with EGF binding, SGF may act through a separate receptor system that does not bind EGF. This would be analogous to insulin and nonsuppressible insulin-like activity: the two are immunologically distinct, each has its own receptor system, and yet they can compete for one another's receptors in chicken and rat cells (14).

Even though known proteases incorporated into the soft agar assays were unable to stimulate growth of the fibroblastic cells in soft agar, the possibility remains that the SGFs are specific proteases having properties that are distinct from those tested. To establish the SGFs as specific proteases one would have to know their substrate specificity. A possible substrate for this type of activity would be a membrane component, such as a growth factor receptor, which, once "cleaved," would activate a cellular mechanism that stimulates DNA synthesis or a morphological change. Even if the activities present in these preparations are due to proteases they are specific proteases, not general factors released by all cells or even all transformed cells, because neither the untransformed parental cell nor the DNA virus-transformed cells released similar products.

The hypothesis put forward previously that MuSV transformation involved the production of polypeptide growth hormones (1, 2) receives strong support from the experiments described here. Polypeptide hormones are commonly produced on larger precursors that are subsequently cleaved. Corticotropin (ACTH), for example, is produced by normal pituitary and by a pituitary tumor line as a 31,000 molecular weight precursor that is cleaved into active molecules with molecular weights of 23,000, 12,000, and 4500 (15, 16). Insulin and growth hormone represent other examples of peptide growth factors made as larger precursors. In our experiments, the 25,000, 12,000, and 7000 molecular weight forms of SGF all are biologically active. Because they are produced by transformed fibroblasts and act on untransformed fibroblasts, it is reasonable to assume that the producing cells are able to respond to their own growth factors.

The proteins we have partially characterized appear to be direct effectors of cell transformation, if cell morphology and anchorage-independent growth in agar are taken as indices of the transformed phenotype. The SGFs produce a morphological change seen within hours after treatment in monolayer cultures; within a few days after seeding in agar, SGF-responsive cells are recognized. A large number of factors have been described that stimulate cell division in arrested fibroblast populations (13, 17–19), including ones that cause the cells to overgrow the monolayer (20). This report, however, shows that a peptide produced by sarcoma virus-transformed mammalian cells causes normal cells to assume the *in vitro* properties of transformed cells. When the peptides become available in a more purified form, it should be possible to determine if the protein is a direct sarcoma virus gene product or a growth factor produced from the cellular genome in response to MuSV transformation.

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