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Type β transforming growth factor/growth inhibitor stimulates entry of monolayer cultures of AKR-2B cells into S phase after a prolonged prereplicative interval

(epidermal growth factor/fibroblast growth factor/insulin/platelet-derived growth factor)

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ABSTRACT **Type β transforming growth factor/growth inhibitor (TGF-β/GI) is demonstrated to be a potent stimulator of DNA synthesis in AKR-2B mouse embryo cells with a prolonged (>24 hr) prereplicative phase when compared with other growth factors (epidermal growth factor, platelet-derived growth factor, or fibroblast growth factor) that induce DNA synthesis 12–14 hr after stimulation. In addition, TGF-β/GI inhibits the early peak of DNA synthesis produced by EGF and insulin before the later stimulatory effects of TGF-β/GI become manifest. TGF-β/GI induces a marked morphologic transformation in these cells prior to their entry into S phase. Like the other growth factors, TGF-β/GI stimulates an early increase in the rate of protein synthesis in AKR-2B cells and its stimulatory effect on DNA synthesis is enhanced by insulin. The data show that this molecule is a growth factor for certain mesenchymal cells in monolayer culture but only after a prereplicative phase that is significantly longer than that of other growth factors.**

Transforming growth factor, type β (TGF-β), is a potent stimulator of growth in soft agar of mouse AKR-2B (1, 2) and normal rat kidney (NRK) cells (3). This growth factor has been isolated from human platelets (3), human placenta (4), and bovine kidney (5). TGF-β from all of these sources has a molecular weight of approximately 25,000 and is composed of two apparently identical subunits held together by interchain disulfide bonds. The ability of TGF-β to support the formation of colonies in soft agar by NRK fibroblasts requires the presence of epidermal growth factor (EGF) (6). However, AKR-2B cells, human diploid fibroblasts, and EGF-receptorless NR-6 cells are stimulated to form colonies by TGF-β in the absence of EGF (1, 7–9).

Recent studies (2, 7) have shown that TGF-β can be inhibitory for cell growth in monolayer cultures under certain circumstances and is very similar, if not identical, to the growth inhibitor (GI) isolated from medium conditioned by BSC-1 cells (10). The inhibitory factor from BSC-1 cells is a protein with a molecular weight of approximately 24,000 composed of two apparently identical polypeptide chains (11, 12). GI causes the reversible G_1-phase arrest of low-density cultures of BSC-1 cells (10) and certain lung and mammary cell lines (13) but does not inhibit the growth of mouse 3T3 cells or human skin fibroblasts (11). GI has been shown to stimulate the growth of AKR-2B cells in soft agar and to compete as effectively for ^{125}I-labeled TGF-β binding to AKR-2B cells as purified TGF-β from human platelets (2). For these reasons, the term TGF-β/GI is used here to denote this molecule(s).

A variety of cell types of both mesenchymal and epithelial origin have recently been shown to have high affinity, specific cell membrane receptors for TGF-β/GI from human platelets (9, 14). EGF, PDGF (platelet-derived growth factor), FGF (fibroblast growth factor) and insulin did not compete with ^{125}I-labeled TGF-β/GI for binding to its receptor on AKR-2B cells. Partially purified preparations of transforming growth factor from mouse embryos (15) and the conditioned medium from a chemically transformed derivative of AKR-2B cells (16) did compete with ^{125}I-labeled TGF-β/GI binding indicating that the active factor in these preparations is TGF-β/GI (9).

Confluent monolayer cultures of AKR-2B cells maintained in serum-free medium for 2 days were stimulated to enter DNA synthesis after a prereplicative phase of 12–14 hr by the addition of EGF (17). Insulin alone had little mitogenic effect on these cells but was highly synergistic with EGF under the above conditions. Partially purified TGF-β/GI preparations from serum or mouse embryos were not mitogenic for AKR-2B cells when tested under the same conditions during the first 24 hr following stimulation, although these preparations did stimulate an increase in the rate of protein synthesis and a striking morphologic change (17, 18). TGF-β/GI from human platelets is not mitogenic for NRK cells in confluent monolayer cultures (3, 5) but a mitogenic effect of TGF-β/GI in subconfluent cultures of these cells in serum-containing medium has been reported (19).

In the present study we demonstrate that pure TGF-β/GI from human platelets is a potent mitogen for mesenchymal AKR-2B cells in defined, serum-free monolayer culture in the absence of other macromolecules. The mitogenic effect of TGF-β/GI takes place after a prolonged lag phase relative to the stimulation obtained with EGF, PDGF, or FGF.

MATERIALS AND METHODS

Cell Culture. AKR-2B is a continuous clonal cell line derived from AKR mouse embryos. This cell line is aneuploid, has a high serum requirement for monolayer growth in conventional culture media, does not form tumors in nude mice, and does not form colonies in soft agar in the absence of TGF-β/GI (16, 20, 21). The cells were maintained in McCoy's 5A medium supplemented with 5% (vol/vol) fetal bovine serum.

DNA Synthesis Assays. [^{3}H]Thymidine incorporation assay and autoradiography were performed as described (17) using AKR-2B cells. Briefly, the cells were plated in 24-well multiwell dishes and grown to confluency in serum-containing medium which was replaced after 5 days with unsupplemented medium MCDB 402 (22). [MCDB 402 is an optimized culture medium developed for the improved survival of

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Abbreviations: EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF-β/GI, type β transforming growth factor/growth inhibitor.
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mouse embryo-derived cells in serum-free medium. It supports the clonal growth of these cells in low serum concentrations (22).] Cells were incubated 2 days in serum-free medium, then the medium was changed again to unsupplemented MCDB 402 and the growth factors to be tested were added. [^{3}H]Thymidine (1.0 μCi/ml, 70 Ci/mmol; 1 Ci = 37 GBq) was added to the cultures as described in the figure legends and the relative amount of incorporated [^{3}H]thymidine was determined by liquid scintillation. Autoradiography was performed as described (17) except that the percentage of labeled nuclei in the cultures was determined using a Quantimet 800 digital image analyzer after the nuclei were counterstained with hematoxylin. At least 1000 cells were examined per dish. The data represent the mean of triplicate determinations +/−1 standard deviation.

Protein Synthesis Assay. The cells were plated and grown in 24-well plates as described above. Cells were incubated 2 days in serum-free medium after which the medium was changed to unsupplemented MCDB 402 containing 10.0 μM methionine for 2 hr, then the factors to be tested and [^{35}S]methionine at 10.0 μCi/ml (New England Nuclear) were added. At the end of 1 hr the reaction was stopped by addition of 10% trichloroacetic acid. After several washes in 10% trichloroacetic acid, the cells were solubilized in 0.2 M NaOH containing 1.0% sodium dodecyl sulfate and bovine serum albumin at 1.0 mg/ml and the relative amount of incorporated [^{35}S]methionine was determined by liquid scintillation.

Growth Factors. Human TGF-β/GI was purified to homogeneity from outdated platelets as described (3) with the addition of a final purification step using a reversed-phase C-18 column (9). TGF-β/GI preparations were shown to be homogeneous on 12.5% silver-stained polyacrylamide/$NaDodSO_4$ gels under both reducing and nonreducing conditions (7). EGF was purified from mouse submaxillary glands as described (23). Purified PDGF was the gift of Russell Ross. Bovine insulin was purchased from Sigma. FGF was purified by ion exchange chromatography and high pressure liquid chromatography from bovine pituitary glands (unpublished results). Fetal bovine serum was purchased from K.C. Biological (Lenexa, KS). Platelet-poor plasma-derived serum was prepared from fresh human blood as described (24).

RESULTS

Effect of Growth Factors on DNA Synthesis in AKR-2B Cells During the First 24 Hr After Stimulation. When nontransformed AKR-2B cells are grown to confluence in medium that contains 5% serum and then placed in serum-free MCDB 402, the cells form a continuous monolayer of nonoverlapping cells with indistinct borders as illustrated previously (17). After being shifted to the serum-free medium, the cells flatten and cover a large surface area with resulting loss of some cells. By 72 hr the number of cells in the monolayers remains essentially stable and the cells are capable of reentering the cell cycle for at least 1 week on the addition of growth factors to the unsupplemented medium. These quiescent cells are highly sensitive to the mitogenic stimulation of EGF and insulin. Greater than 80% of the cells enter DNA synthesis in the first 24 hr after stimulation (Table 1) and cell number nearly doubles by 48 hr after stimulation (data not shown). We have shown previously that the addition of EGF and insulin causes a peak of DNA synthesis that occurs 22–23 hr after stimulation (17).

In this study, the stimulatory effect of EGF and insulin was compared to that of PDGF, FGF, or TGF-β/GI added alone or in the presence of insulin. Unlike EGF, either PDGF alone or FGF alone stimulated maximal levels of DNA synthesis in AKR-2B cells (Fig. 1). The mitogenic effect of PDGF and FGF was synergistic with insulin at lower concentrations. The addition of FGF or PDGF to optimal levels of EGF and insulin caused a small (approximately 20%) but reproducible increase in the level of DNA synthesis (data not shown). Because these assays were carried out by measuring the level of [^{3}H]thymidine incorporation between 22 and 23 hr after stimulation, we conclude that both PDGF and FGF cause AKR-2B cells to enter S phase with approximately the same kinetics as the combination of EGF and insulin. In contrast to other growth factors (EGF, PDGF, FGF), purified TGF-β/GI stimulated minimal DNA synthesis in these cells when added alone or with insulin at 500 ng/ml under similar conditions (Fig. 1).

Table 1. Stimulation of DNA synthesis in AKR-2B cells

Addition(s)	% labeled nuclei	
	24 hr	48 hr
Medium alone	0.7 ± 0.5	ND*
TGF-β/GI	3.4 ± 1.3	39.2 ± 1.9
EGF	7.4 ± 3.0	ND*
Insulin	3.6 ± 1.5	ND*
EGF + insulin	87.9 ± 5.4	ND*
TGF-β/GI + EGF	10.2 ± 5.4	71.1 ± 5.1
TGF-β/GI + insulin	2.6 ± 0.8	65.0 ± 5.1
TGF-β/GI + EGF + insulin	16.6 ± 5.9	83.4 ± 8.5

Confluent cells were cultured in serum-free MCDB 402 medium for 48 hr, the medium was changed at zero time to fresh unsupplemented MCDB 402 containing the factors listed, and the cells were incubated for 24 or 48 hr. The concentrations used were as follows: TGF-β/GI, 10 ng/ml; EGF, 10 ng/ml; insulin, 500 ng/ml. Cells were exposed to [^{3}H]thymidine at 3.0 μCi/ml for 24 or 48 hr from the time of the final additions and then processed for autoradiography as described.
*Not done in this experiment. In a separate experiment, less than 1.0% labeled nuclei was obtained with medium alone, 5.1 ± 2.9% was obtained with insulin, and 29.1 ± 8.5 was obtained with EGF.

We have shown previously that when TGF-β/GI is added to cultures together with EGF and insulin, it inhibits the response of AKR-2B cells to these mitogens in a dose-dependent fashion as measured by [^{3}H]thymidine uptake 22–23 hr after stimulation (2). These results are confirmed by autoradiographic analysis (Table 1). The number of labeled nuclei present in cultures 24 hr after stimulation with EGF and insulin was reduced by a factor of 5 by the addition of TGF-

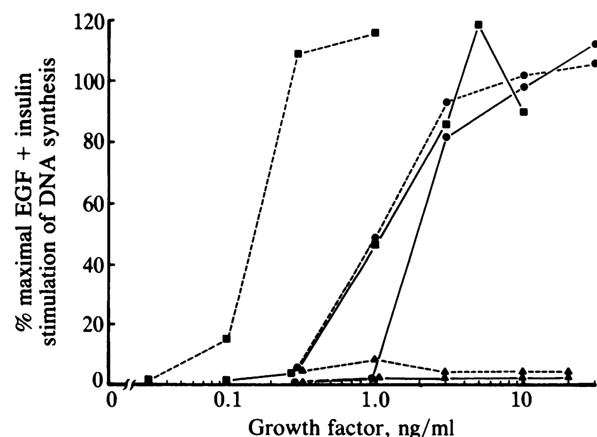

FIG. 1. Effect of TGF-β/GI, FGF, and PDGF on DNA synthesis in AKR-2B cells 22–23 hr after stimulation. DNA synthesis is graphed as a percentage of the response to the addition of EGF (10 ng/ml) and insulin (500 ng/ml). Confluent, quiescent AKR-2B cells maintained in 24-well dishes in serum-free medium for 2 days were stimulated with various concentrations of growth factors either in the presence (dashed lines) or the absence (solid lines) of insulin (500 ng/ml). DNA synthesis was measured by the incorporation of [^{3}H]thymidine 22–23 hr after addition of the growth factors. ▲, TGF-β/GI; ■, FGF; ●, PDGF.

β/GI at 10 ng/ml. This initial inhibitory effect of TGF-β/GI also occurred when the cells were stimulated to enter DNA synthesis by FGF or PDGF (data not shown).

Because serum is included in the soft agar assays used for the detection of TGF-β/GI (6, 18) but not in the monolayer assays reported here, we examined the effect of TGF-β/GI in the presence of serum or plasma proteins. Fetal bovine serum or human platelet-poor plasma-derived serum did not complement TGF-β/GI in the stimulation of DNA synthesis after 24 hr of stimulation. At the same concentrations, however, serum or plasma complemented EGF activity (data not shown).

Delayed Mitogenic Effect of TGF-β/GI on AKR-2B Cells. Although all of the known monolayer mitogens and serum induce fibroblast-like cells (including AKR-2B cells) to enter S phase after a lag period of 9–15 hr, it seemed possible that the TGF-β/GI might stimulate the entry of cells into DNA synthesis after a longer delay. After 48 hr of exposure to TGF-β/GI at 10 ng/ml, approximately 40% of the cells had entered S phase (Table 1). The effect of TGF-β/GI on DNA synthesis was significantly enhanced by the addition of EGF or insulin when measured 48 hr after the addition of the growth factors. While the number of labeled nuclei in EGF/insulin-treated cultures was reduced by a factor of 5 during the first 24 hr when the cells were stimulated in the presence of TGF-β/GI, more than 80% of the cells entered S phase when this treatment was continued for 48 hr (Table 1). When cells were incubated in medium containing 0.1% serum instead of serum-free medium for 2 days before the addition of growth factors, results similar to those shown in Table 1 were obtained.

If the cells were treated with TGF-β/GI for 24 hr, then washed, and incubated with fresh serum-free medium for a second 24-hr period, the number of cells entering S phase during the second 24-hr period was enhanced to approximately 64% (Table 2). If the cells were washed and the medium was then replaced with medium containing TGF-β/GI, the percentage of labeled nuclei was increased even further (approximately 77%, Table 2). The data presented in Tables 1 and 2 show that TGF-β/GI alone stimulates AKR-2B cells to enter S phase in the absence of serum or other macromolecules. The reason for the increase in the percentage of labeled nuclei when fresh medium was added is not clear but it could be due to replenishment of nutrients. Alternatively, the lower values listed in Table 1 could be the result of exposure of these cells to [^{3}H]thymidine for 48 hr. The addition of EGF or insulin to cells pretreated for 24 hr with TGF-β/GI also enhanced the percentage of labeled nuclei (83–87%, Table 2). Further, treatment of the cells with EGF/insulin during the second 24 hr after TGF-β/GI treatment resulted in >90% of the cells entering S phase, indicating that the initial inhibition of EGF/insulin-stimulated DNA synthesis by TGF-β/GI was transient. This result was obtained with or without the addition of TGF-β/GI during the second 24-hr period (Table 2). Control cultures that received fresh medium or medium and insulin after 24 hr had approximately 4% labeled nuclei.

Table 2. Stimulation of DNA synthesis in AKR-2B cells after 24-hr TGF-β/GI pretreatment

Addition(s) at 24 hrs	% labeled nuclei
Control	4.0 ± 1.0
Medium alone	63.6 ± 1.4
TGF-β/GI	76.8 ± 1.0
EGF	82.6 ± 3.7
Insulin	87.3 ± 4.5
EGF + insulin	94.6 ± 2.9
TGF-β/GI + EGF + insulin	96.7 ± 1.7

Confluent cells quiescent in serum-free medium for 2 days were changed at zero time to unsupplemented MCDB 402 containing TGF-β/GI at 10 ng/ml, incubated for 24 hr, rinsed with unsupplemented medium, and exposed for 24 hr to serum-free medium containing the factors listed. Concentrations were as listed for Table 1. Control cells were treated at zero time with serum-free MCDB 402 medium without TGF-β/GI for 24 hr, rinsed with serum-free medium, and exposed to serum-free medium alone for an additional 24 hr. Cells were exposed to [^{3}H]thymidine at 3.0 μCi/ml from 24–48 hr and processed for autoradiography as described.

Kinetics of TGF-β Stimulation of DNA Synthesis. When quiescent cells were stimulated by the addition of EGF and insulin, they began to synthesize DNA after a lag period of 12–13 hr (Fig. 2). However, adding TGF-β/GI with EGF and insulin delayed the onset and peak of DNA synthesis and caused the peak of incorporation to be broader (Fig. 2). These results confirm and further explain our previous observations that TGF-β/GI inhibits the EGF/insulin-stimulation of DNA synthesis (2).

The results of autoradiographic analysis (Tables 1 and 2) suggested that AKR-2B cells exposed to TGF-β/GI or TGF-β/GI and insulin enter DNA synthesis in large numbers between 24 and 48 hr after stimulation. The kinetics of these responses are shown in Fig. 3. During the first 24 hr after stimulation little DNA synthesis was detected but shortly after 24 hr there was an increase in the rate of [^{3}H]thymidine incorporation that reached a peak at approximately 36 hr. The response of these cells to TGF-β/GI during this time period was enhanced by the addition of insulin (see also Table 1).

TGF-β/GI Stimulation of Early Protein Synthesis. One of the early events that occurs after growth-factor binding to quiescent cells [including AKR-2B cells (17)] is an increase in the rate of protein synthesis. This increase is a prerequisite for entry into DNA synthesis (unpublished results). We measured the rate of protein synthesis in cultures of AKR-2B cells at various times after stimulation by TGF-β/GI alone, EGF/insulin, or EGF, insulin, and TGF-β/GI (Fig. 4). TGF-β/GI stimulated the rate of incorporation of [^{35}S]methionine into these cells within 2–3 hr after the addition of the growth factor. By comparison, the combination of EGF and insulin stimulated a slightly higher rate of protein synthesis and this higher rate was not inhibited by the addition of TGF-β/GI at 10 ng/ml.

Purified TGF-β/GI Causes a Rapid Change in the Morphology of AKR-2B Cells. In addition to its effect on protein

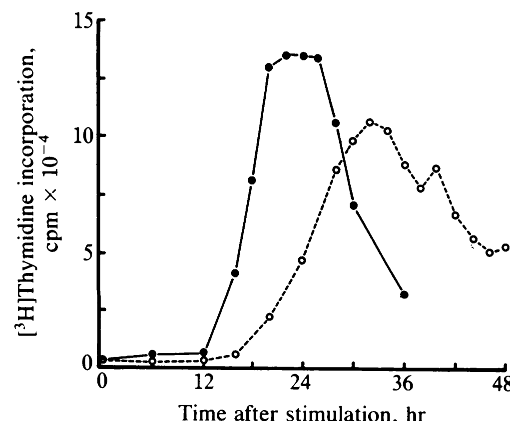

FIG. 2. Kinetics of stimulation of DNA synthesis by EGF and insulin or by EGF, insulin, and TGF-β/GI. Confluent, quiescent AKR-2B cells maintained in 35-mm dishes in serum-free medium for 2 days were stimulated by growth factors at time zero. [^{3}H]Thymidine (1.0 μCi/ml) was added 2 hr preceding the indicated time. ●, EGF (10 ng/ml) and insulin (500 ng/ml); ○, EGF (10 ng/ml), insulin (500 ng/ml), and TGF-β/GI (10 ng/ml).

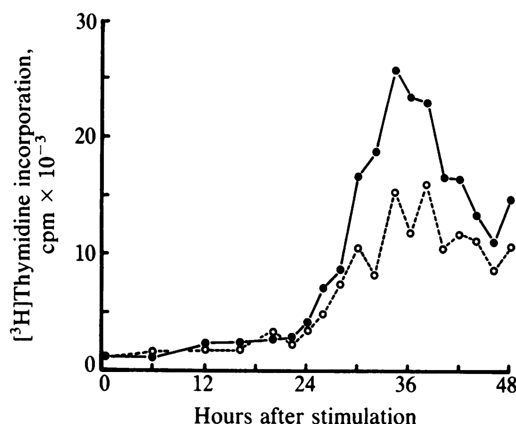

FIG. 3. Kinetics of stimulation of DNA synthesis by TGF-β/GI alone or by TGF-β/GI and insulin. Confluent, quiescent AKR-2B cells maintained in 24-well dishes in serum-free medium for 2 days were stimulated at time zero with TGF-β/GI (10 ng/ml) alone (○) or with TGF-β/GI (10 ng/ml) and insulin (500 ng/ml) together (●). [3H]Thymidine was added 2 hr preceding indicated times.

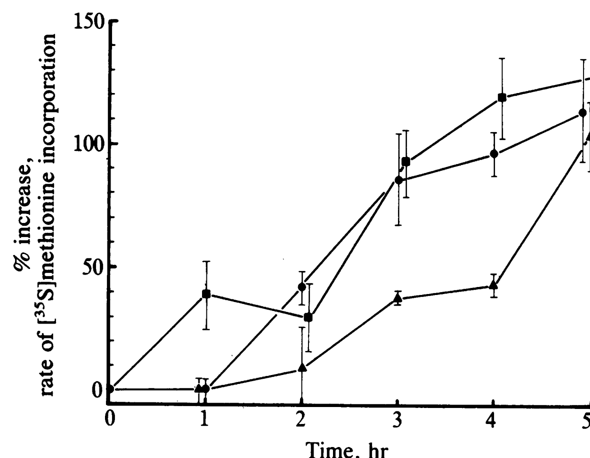

FIG. 4. Effect of growth factors on rate of protein synthesis in AKR-2B cells. Confluent, quiescent cells maintained in 24-well plates in serum-free medium for 2 days were changed to low-methionine medium. The percent increase in rate of protein synthesis relative to cultures receiving no growth factor was determined for the hour preceding the indicated times. Vertical bars represent ±1 SD for triplicate cultures. At time zero the cultures were treated as follows: ▲, TGF-β/GI (10 ng/ml); ●, EGF (10 ng/ml) and insulin (500 ng/ml); ■, TGF-β/GI (10 ng/ml), EGF (10 ng/ml), and insulin (500 ng/ml).

synthesis, purified TGF-β/GI produced a marked change in the morphological appearance of monolayers of AKR-2B cells. This morphological change was apparent by 16 hr after stimulation and occurred, therefore, before the onset of DNA synthesis. The appearance of the cells was similar to that induced by impure preparations of TGF-β/GI as illustrated previously (17) and was characterized by overlapping bipolar, spindle-shaped cells. By comparison, EGF and insulin, alone or in combination, had much less effect on the morphology of the cultures (17).

DISCUSSION

In addition to its ability to stimulate the growth of cells in soft agar in the presence of serum, TGF-β/GI is now shown to be an effective mitogen for mouse embryo-derived cells in serum-free monolayer cultures. Furthermore, TGF-β/GI is demonstrated to be a unique monolayer growth factor with the following properties: (*i*) the prereplicative interval between stimulation and the onset of DNA synthesis in AKR-2B cells is approximately 24 hr as compared to 12–14 hr with EGF, PDGF, and FGF and (*ii*) the "early" peak of DNA synthesis stimulated by the other growth factors in these cells is inhibited by TGF-β/GI before the later stimulatory effect of TGF-β/GI is manifest.

TGF-β/GI isolated from platelets or serum was previously reported to have no stimulatory effect on DNA synthesis in confluent monolayer cultures of AKR-2B cells (17) or NRK cells (3, 5) even though this factor is a potent mitogen for these cells in soft agar. Massague (19) has reported that TGF-β/GI isolated from the conditioned medium of rat cells transformed with feline sarcoma virus stimulated an increase in [3H]thymidine incorporation in NRK cells maintained at subconfluent densities in serum-containing medium. In these experiments, a 7-fold increase in DNA synthesis was measured during 16–24 hr after the addition of TGF-β/GI. The data were not supported by autoradiography, thus the percentage of the cells in the population that were responding cannot be determined. In the absence of kinetic data, it is difficult to determine whether there is a significant delayed mitogenic effect of TGF-β/GI in these cells. The results presented here indicate that in the case of AKR-2B cells, the earlier studies (2, 17) were misleading because of the extended lag phase in TGF-β/GI-stimulated cells. [3H]Thymidine incorporation was assayed in earlier studies within 24 hr after the addition of growth factor but now the peak of stimulation of DNA synthesis by TGF-β/GI is shown to occur at approximately 36 hr after stimulation. When confluent monolayer cultures of AKR-2B cells were maintained in 0.1% serum for 2 days, the growth factors added and the number of cells per dish determined at subsequent times, the cell number nearly doubled within 48 hr after addition of EGF/plus insulin and 72 hr after addition of TGF-β/GI plus insulin (data not shown).

The reason for the extended lag phase before the onset of DNA synthesis after TGF-β/GI stimulation is not clear at this time. One hypothesis is that the cytoskeletal rearrangements that are caused by TGF-β/GI treatment in these cells override the mitogenic signal until the cells have assumed a new morphology. Another possibility is that TGF-β/GI stimulates the synthesis of another endogenous growth factor that is directly responsible for the stimulation of DNA synthesis. The initial inhibition by TGF-β/GI of the DNA synthesis stimulated by other growth factors is of interest although the mechanism of this inhibition is unknown at this time.

Our results on the effect of TGF-β/GI on protein synthesis in AKR-2B cells are in contrast to those reported for NRK fibroblasts by Assoian *et al.* (25), who found that TGF-β/GI did not induce a higher rate of protein synthesis. This may reflect a response specific to the cell type. Other differences in the responsiveness of AKR-2B cells and NRK cells have been noted previously, including the ability of TGF-β/GI to stimulate the growth of AKR-2B cells but not NRK cells in soft agar in the absence of EGF (or TGF-α) (7, 18). Another explanation for this apparent discrepancy is that the AKR-2B cells in this study were cultured in the absence of serum for 2 days before the start of the assay and had a low basal rate of protein synthesis, whereas the NRK cells in the previous study (25) were exposed to medium containing 10% serum until the beginning of the assay period. Thus, the NRK cells may have been synthesizing proteins at a maximal rate due to the influence of the growth factors present in the serum-containing medium and been unable to respond differentially to added growth factors during the assay period. The inability of TGF-β/GI to inhibit the early stimulation of protein synthesis by EGF and insulin in the current study indicates that this is not the mechanism of TGF-β/GI inhibition of early DNA synthesis in AKR-2B cells. It has been

demonstrated that TGF-β/GI causes a transient decrease in the level of ^{125}I-labeled EGF binding to NRK cells (25). We have examined the ability of AKR-2B cells to bind and degrade ^{125}I-labeled EGF in the presence of TGF-β/GI and found that TGF-β/GI had no detectable effect on these processes during the first 8 hr after treatment.

The results presented here show that TGF-β/GI is a growth factor for certain types of cells when a standard assay for the detection of growth factors is used (i.e., the stimulation of DNA synthesis in monolayer cultures of mesenchymal cells). The prolonged prereplicative period following TGF-β/GI stimulation may indicate that the mechanism of stimulation by this growth factor is unique when compared to other growth factors (EGF, PDGF, FGF). Previous studies have shown that TGF-β/GI stimulates the growth of mouse, rat, and human mesenchymal cells in soft agar (2, 3, 7) and inhibits the growth of certain human carcinoma cell lines under similar conditions (7). TGF-β/GI is an inhibitor of replication in epithelial cells from several species in monolayer culture (7, 10–12). These investigations indicate a need for reevaluating the role of TGF-β/GI in the control of proliferation of normal cells as well as the role of this factor in neoplastic transformation. Current data indicate that autocrine activity of TGF-β/GI could account for many of the characteristics of the transformed phenotype in mesenchymal cells and that paracrine secretion of TGF-β/GI by epithelial cell tumors could account for the proliferation of surrounding stromal elements frequently associated with these types of tumors. In addition, the inability of some epithelial cell tumors to respond to the inhibitory effects of TGF-β/GI could be the reason for altered growth characteristics in carcinoma cells.

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