Transglutaminase induced by epidermal growth factor negatively regulates the growth signal in primary cultured hepatocytes

Shinsuke KATO^{H,*}, Noriko NAKAGAWA[†], Yoshiko YANO[†], Kumiko SATO^H, Hiroyuki KOHNO[†] and Yasuhito OHKUBO[†]

^HRadioisotope Research Institute, Faculty of Medicine, University of Tokyo, Hongo, 7-3-1, Bunkyo-ku, Tokyo 113, Japan, and [†]Department of Radiopharmacy, Tohoku College of Pharmacy 4-4-1, Komatsushima, Aoba-ku, Sendai 981, Japan

Transglutaminase (TGase) activity increased 2.5-fold at 6 h after treatment of rat hepatocytes with 17 nM epidermal growth factor (EGF). In the same manner, putrescine incorporation into the proteins of cells occurred in EGF-treated cells, but not in those pretreated with monodansylcadaverine (MDC), a TGase inhibitor, even in the presence of EGF. These results suggest that EGF-induced TGase was active and catalysed some cross-linkage reaction. Cycloheximide completely blocked the increase in TGase activity induced by EGF, suggesting that EGF stimulated *de novo* synthesis of TGase within 6 h. Furthermore, Northern

blotting analysis indicated that EGF increased the expression of TGase mRNA. Pretreatment of cells with MDC additionally increased EGF-induced DNA-synthesis and the ratio of cells in S-phase. TGase antisense oligonucleotide inhibited *de novo* synthesis of TGase, resulting in increases in the ratios of S- and G₂/M-phase cells in the presence of EGF. This effect was the result of inhibition of EGF-induced down-regulation of high-affinity receptor expression. These results suggest that the EGF-induced increase in TGase activity is a negative regulator of a growth signal in rat hepatocytes.

INTRODUCTION

Transglutaminase (TGase) is a Ca²⁺-dependent enzyme which catalyses the post-translational modification of proteins through the formation of γ -glutamyl- ϵ -lysine isopeptides [1,2]. This enzyme is widely distributed in various mammalian tissues and catalyses the modification of many cellular proteins [1,2]. Actin, which is a prominent component of the cytoskeleton in mammalian cells, is a good substrate for TGase *in vitro* [3,4]. However, the physiological effects of TGase on the functions of actin remain unknown. It has been reported that actin is associated with high-affinity binding sites of the epidermal growth factor (EGF) receptor [5–8], and a subclass of high-affinity EGF receptors contributes to activation of the EGF-receptor signal-transduction cascade [9,10]. These findings raise the possibility that modification of actin affects the binding affinity of high-affinity EGF receptor, resulting in alteration of the EGF-receptor signal-transduction cascade. As actin is a good substrate for TGase [3,4], this enzyme may regulate the EGF-receptor signal-transduction cascade through modification of actin.

Recently, we reported the disappearance of high-affinity EGF receptor in the Ca²⁺-pretreated liver membrane [11] and demonstrated reduction in affinity of EGF receptor by modification of actin with TGase *in vitro* [12]. However, it remains unclear whether these results obtained *in vitro* are relevant physiologically. Therefore, further detailed studies using whole-cell systems are necessary to explain the physiological role of TGase on the growth signal of EGF.

In the present study, we investigated the effect of EGF on TGase activity in primary cultured rat hepatocytes, and found that the EGF-induced increase in *de novo* synthesis of TGase and the inhibition of TGase synthesis increased the ratios of S- and G₂/M-phase cells. These results suggest that the EGF-induced

increase in TGase activity is a negative regulator of a growth signal in hepatocytes.

MATERIALS AND METHODS

Materials

Mouse EGF was obtained from Biomedical Technologies, Inc. (Stoughton, MA, U.S.A.), [1,4-¹⁴C]putrescine dihydrochloride ([¹⁴C]putrescine, 3.3 GBq/mmol) and 5-[¹²⁵I]iododeoxyuridine ([¹²⁵I]dU, 81.4 TBq/mmol) were from New England Nuclear (Boston, MA, U.S.A.), adenosine 5'-[γ -³²P]triphosphate ([γ -³²P]ATP, 110 TBq/mmol) was from Amersham International plc (Kent, U.K.), and monodansylcadaverine (MDC) was from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Treatment of hepatocytes with EGF

Rat hepatocytes were prepared as described previously [13]. Isolated hepatocytes (3×10^5 cells/ml) were plated in 35-mm or 60-mm collagen-coated culture dishes (Corning, NY, U.S.A.). After 3 h of incubation in Williams' medium E (Flow Laboratories, North Ryde, Australia) containing 10% (v/v) fetal calf serum, the culture medium was replaced with serum-free medium containing 10⁻⁹ M insulin, 10⁻⁹ M dexamethasone and 0.1 μ g/ml aprotinin. After 16 h of incubation, hepatocytes were incubated in culture medium containing EGF for the indicated time periods.

Measurement of TGase activity

After the indicated time periods of incubation, the culture medium was removed and the cells were scraped with a rubber policeman. Scraped cells were homogenized with Ca²⁺-, Mg²⁺-free Hanks balanced salt solution containing 10 mM Hepes

Abbreviations used: TGase, transglutaminase; EGF, epidermal growth factor; MDC, monodansylcadaverine; dU, iododeoxyuridine; SSC, 150 mM NaCl/15 mM sodium citrate.

‡ To whom correspondence should be addressed.

(pH 7.4), 1 mM EGTA, 10 μ g/ml aprotinin, 10 μ g/ml chymostatin, 10 μ g/ml bestatin and 10 μ g/ml pepstatin (HBSS buffer). The homogenates were centrifuged for 10 min at 20000 g. Aliquots of the resultant supernatants were used for the TGase assay. TGase activity was assayed by the incorporation of [14 C]putrescine into *N,N*-dimethylcasein using the filter paper technique as described previously [11] and expressed as nmol of [14 C]putrescine incorporated into *N,N*-dimethylcasein/min per mg of protein.

Putrescine incorporation assay

Hepatocytes were incubated with 17 nM EGF for the time periods indicated. [14 C]Putrescine (10 μ M) was added to the culture medium 1 h prior to termination of incubation with EGF. After the incubation, the cells were washed with 20 mM PBS (pH 7.4), and fixed with 10% trichloroacetic acid and solubilized in 1 M NaOH. The radioactivity of [14 C]putrescine incorporated into proteins was measured using a liquid scintillation counter.

Northern blot analysis

RNA was isolated and purified by the guanidinium thiocyanate-phenol-chloroform method [14] using oligo(dT)-latex. RNA samples (5 μ g) were separated by electrophoresis on 1% agarose gels containing 0.66 M formaldehyde and transferred on to positively charged nylon membranes (Amersham) for 16 h. λ -Eco T14 digest (Takara Shuzo Co., Otsu, Japan) was used as a size marker. Equivalent loading of samples was verified by hybridization with a probe for rat β -actin (Clontech Laboratories, Palo Alto, CA, U.S.A.). An 87-mer oligonucleotide probe was synthesized from a guinea-pig liver TGase cDNA (bp 860–946) [15]. Probes were labelled with [γ - 32 P]ATP using a 5' end-labelling kit (Megalabel, Takara), and hybridization with the 32 P-labelled probes was performed in a rapid hybridization system (Amersham). The membranes were washed twice with 2 $\times$ SSC (1 $\times$ SSC: 150 mM NaCl and 15 mM sodium citrate) for 10 min, and twice with 0.2 $\times$ SSC containing 0.1% SDS for 30 min at room temperature. The membranes were exposed to X-ray film with intensifying screens at -80°C .

Pretreatment of hepatocytes with MDC, sense or antisense oligodeoxynucleotide

Hepatocytes were pretreated with the indicated concentrations of MDC for 30 min. After the cells were washed three times with serum-free medium, EGF was then added. The cells were subjected to Scatchard analysis, assay of DNA synthesis or cell cycle analysis after 6 h, 24 h or 30 h respectively.

Antisense or sense phosphothioate oligodeoxynucleotides (17-mers) were designed to symmetrically cover the catalytically active region of guinea-pig liver TGase [15] (antisense: 5'-ACCCAGCACTGGCCGTA-3' and sense: 5'-TACGGCCAG-TGCTGGGT-3'). After incubation of hepatocytes for 3 h, the culture medium was replaced with serum-free medium containing antisense or sense oligonucleotide (10 or 30 μ M). After incubation for 16 h, EGF (17 nM) was added to the culture medium. The cells were subjected to TGase assay, Scatchard analysis or cell cycle analysis after 6 h, 6 h or 30 h respectively.

Assay of DNA synthesis

DNA synthesis in primary cultured hepatocytes was determined by the method of Mizuno et al. [16]. After the addition of 17 nM EGF (24 h), the cells were pulse-labelled with 74 kBq/ml of

[125 I]dU for 6 h. The cells were washed with 20 mM PBS (pH 7.4) and solubilized in 1 M NaOH. The radioactivity incorporated into DNA was measured using a well-type γ -counter. As a control, pulse-labelling was performed in the presence of 10 μ g/ml aphidicolin. The control value was subtracted from each value and net [125 I]dU incorporation into DNA was obtained.

Cell cycle analysis

After incubation of MDC-, sense- or antisense-pretreated hepatocytes for 30 h with 17 nM EGF, the cell cycle was analysed using Cycle TEST (Becton Dickinson, Lincoln Park, NJ, U.S.A.). Briefly, the cell membranes were digested with trypsin for 10 min at room temperature. Digestion was stopped by adding a trypsin inhibitor, and RNA was digested by ribonuclease A for 10 min at room temperature. DNA in the cells was then stained by propidium iodide and analysed by FACScan (Becton Dickinson).

Scatchard plot analysis

After incubation of MDC-, sense- or antisense-pretreated hepatocytes for 6 h with 17 nM EGF, the EGF receptors of these cells were examined by Scatchard plot analysis as described previously [17].

RESULTS

EGF-induced TGase activity

In the cytosolic fraction from hepatocytes, TGase activity increased gradually and reached a maximum 6 h after the addition of 17 nM EGF (Figure 1a). The increased TGase activity of EGF-treated cells returned to the control level at 12 h (Figure 1a). Similarly, putrescine incorporation into cellular proteins was increased at 6 h after the addition of 17 nM EGF (Figure 1b). The increase in putrescine incorporation disappeared when the cells were pretreated with MDC, a specific TGase inhibitor [18] (Figure 1b), indicating that putrescine incorporation was catalysed by TGase. In the untreated cells, putrescine incorporation did not change (Figure 1b).

Effect of EGF on TGase synthesis

EGF enhanced cytosolic TGase activity in a dose-dependent manner (Figure 2a). This increase in TGase activity was completely blocked by 30 μ M cycloheximide (Figure 2a), suggesting that EGF enhances *de novo* synthesis of TGase. Northern blotting analysis supported this suggestion. EGF increased TGase mRNA expression in a dose-dependent manner at 6 h after its addition (Figure 2b). In contrast, the expression of β -actin mRNA was not affected by EGF (Figure 2b). These results indicated that the EGF-induced increase in TGase activity was the result of an increase in *de novo* synthesis of TGase.

Effect of MDC on cell growth

It was of interest to determine whether TGase functions in transducing the growth-promoting signal of EGF. We studied the effects of pretreatment with MDC on EGF-induced DNA synthesis. EGF increased DNA synthesis to 15-fold of control cells (Table 1). Interestingly, pretreatment with MDC additionally increased EGF-induced DNA synthesis in a dose-dependent manner, while the pretreatment did not affect DNA synthesis in control cells (Table 1). Furthermore, we analysed the cell cycle of EGF-stimulated hepatocytes pretreated with MDC. EGF increased the ratio of hepatocytes in S-phase to 2-fold that of

Figure 1 Effect of EGF on TGase activity in primary cultured hepatocytes

(a) Time courses of TGase activity of EGF-stimulated hepatocytes; hepatocytes were treated with (●) or without (○) 17 nM EGF. After the indicated time periods, cytosolic TGase activity was measured as described in the Materials and methods section. (b) Time courses of putrescine (PUT) incorporation into proteins of cells; hepatocytes were pretreated with (○) or without (●, ■) 0.5 mM MDC for 30 min and were incubated with (●, ○) or without (■, □) 17 nM EGF. At 1 h prior to termination of incubation with EGF, 10 μ M [14 C]putrescine was added to the culture medium. After the incubation, putrescine incorporated into proteins of cells was determined as described in the Materials and methods section. Each point represents the mean $\pm$ S.E.M. of three samples.

untreated cells (Table 2). Pretreatment with MDC did not affect the cell cycle of untreated hepatocytes (Table 2). In EGF-stimulated cells, the pretreatment with MDC obviously increased the ratio of S-phase cells (Table 2).

Effects of sense and antisense oligonucleotides on cell growth

The cells were exposed prior to growth stimulation by EGF to sense or antisense phosphothioate oligodeoxynucleotides (bp 888–905) to inhibit the synthesis of TGase. When added to the culture medium of quiescent cells 16 h before growth stimulation by EGF, the antisense oligomer inhibited the EGF-induced increase in TGase activity in a dose-dependent manner (Table 3). In contrast, the sense oligomer had no effect on TGase activity of EGF-stimulated cells (Table 3). Neither the antisense nor the sense oligonucleotide affected the cell cycle of quiescent cells (Table 2). In the EGF-treated cells, the ratio of S-phase cells increased in the presence of the antisense oligonucleotide, similarly to pretreatment with MDC (Table 2). Furthermore, the antisense oligonucleotide also increased the ratio of G₂/M-phase

Figure 2 EGF induces *de novo* synthesis of TGase in primary cultured hepatocytes

(a) Effects of cycloheximide on EGF-induced TGase activity; hepatocytes were treated with EGF at the indicated concentrations for 6 h in the absence or presence of 30 μ M cycloheximide. Each point represents the mean $\pm$ S.E.M. of three samples. (b) Northern blotting analysis of TGase expression in EGF-treated cells; after hepatocytes were treated with or without EGF for 6 h, Northern blotting was performed as described in the Materials and methods section. RNA samples (5 μ g) were separated by electrophoresis on 1% agarose gels containing 0.66 M formaldehyde and transferred on positively charged nylon membranes for 16 h. λ -Eco T14 digest was used as a size marker. Equivalent loading of samples was verified by hybridization with a probe for rat β -actin. An 87-mer oligonucleotide probe was synthesized from guinea-pig liver transglutaminase cDNA [15]. Abbreviation: PUT, putrescine.

cells in the EGF-treated hepatocytes (Table 2). In contrast, the sense oligonucleotide had no effect on the cell cycle (Table 2).

Effects of sense and antisense oligonucleotides on the high-affinity EGF receptor

A Scatchard plot of the binding data for EGF in hepatocytes was curvilinear and yielded two apparent dissociation constants of high- and low-affinity binding sites (Table 4). The high-affinity EGF receptor expression was down-regulated and completely disappeared 6 h after the addition of EGF (Table 4). In this case, a Scatchard plot indicated a straight line and B_{max} or K_D of the high-affinity receptor were not detected (indicated as 'ND' in Table 4). However, the addition of EGF did not affect B_{max} or

Table 1 Effect of pretreatment with MDC on EGF-induced DNA synthesis in primary cultured hepatocytes

Hepatocytes were pretreated with MDC at the indicated concentrations for 30 min and then incubated with 17 nM EGF. After 24 h, the cells were further incubated in the presence of [125 I]dU for 6 h. [125 I]dU incorporated into DNA was determined as described in the Materials and methods section. Each point represents the mean $\pm$ S.E.M. of three samples.

MDC pretreated (mM)	[125 I]dU uptake (c.p.m./mg of protein per 10^6 cells)	
	EGF (—)	EGF (+)
0	0.74 $\pm$ 0.09	11.3 $\pm$ 0.79
0.05	0.48 $\pm$ 0.07	13.6 $\pm$ 0.7
0.15	0.65 $\pm$ 0.07	16.6 $\pm$ 0.6
0.5	0.75 $\pm$ 0.07	18.9 $\pm$ 0.4

K_D of the low-affinity receptor (Table 4). When added to the culture medium of quiescent cells 16 h before growth stimulation by EGF, the antisense oligomer inhibited the EGF-induced down-regulation of high-affinity-receptor expression (Table 4). A similarly effect was obtained by pretreatment with MDC for 30 min (Table 4). In contrast, the sense oligonucleotide did not affect B_{Max} or K_D of the receptor (Table 4).

DISCUSSION

In the present study, EGF increased the cytosolic TGase activity within 6 h in primary cultured rat hepatocytes. Moreover, this EGF-induced TGase could catalyse the intracellular cross-linkage and increase the incorporation of putrescine (a TGase substrate) into cellular proteins. This indicates that EGF-induced TGase is biologically active. Cycloheximide completely blocked the increase in TGase activity, suggesting that EGF stimulated *de novo* synthesis of TGase. Northern blotting analysis also indicated that EGF increased the expression of TGase mRNA. The present findings provide the first evidence that EGF induces *de novo* synthesis of TGase in adult rat hepatocytes.

Putrescine incorporation into proteins of EGF-stimulated cells was not observed in those cells pretreated with MDC, a specific TGase inhibitor [18], suggesting that MDC inhibited the TGase-

Table 3 Effect of the antisense oligonucleotide on the EGF-induced increase in TGase activity in primary cultured hepatocytes

After incubation of hepatocytes for 3 h, the medium was replaced with serum-free medium containing antisense or sense oligomers (10 or 30 μ M). After incubation for 16 h, EGF (17 nM) was added to the culture medium. The cells were applied to TGase assay after 6 h. TGase activity was expressed as nmol of [14 C]putrescine incorporated into *N,N'*-dimethylcasein/min per mg of protein. Each point represents the mean $\pm$ S.E.M. of three samples.

	TGase activity (pmol of putrescine/min per mg of protein)
Control	136 $\pm$ 6.5
EGF	320 $\pm$ 1.6
EGF + 10 μ M antisense	263 $\pm$ 2.2
EGF + 30 μ M antisense	171 $\pm$ 1.5
EGF + 30 μ M sense	289 $\pm$ 2.4

related response to EGF. In the EGF-stimulated cells, pretreatment with MDC additionally increased EGF-induced DNA synthesis and the ratio of S-phase cells, but did not affect those in EGF-untreated cells. Furthermore, an antisense oligonucleotide directed against TGase mRNA additionally increased the ratios of S- and G₂/M-phase cells, whereas the sense oligomer did not. These results suggest that newly synthesized TGase negatively regulates the growth signal of EGF in hepatocytes.

It is known that a subclass of high-affinity EGF receptors contributes to the activation of the EGF-receptor signal-transduction cascade [9,10] and that only the high-affinity EGF binding site is associated with the cytoskeleton [5–8]. Previously, we showed that TGase decreased the binding of EGF to the high-affinity receptor in isolated liver membranes through modification of actin [11,12]. These observations raise the possibility that newly synthesized TGase induces the down-regulation of high-affinity EGF-receptor expression and decreases the growth signal of EGF. Indeed, the present results showed that inhibition of TGase synthesis by the antisense oligonucleotide resulted in the inhibition of EGF-induced down-regulation of high-affinity-receptor expression in the cultured hepatocytes, supporting the mechanism suggested above.

In previous immunofluorescence studies, EGF was observed to stimulate the polymerization of cellular actin, and polymerized

Table 2 Effect of MDC or the antisense oligonucleotide on the cell cycle of primary cultured hepatocytes

Treatment	Concentration	Phase of cell cycle (%)					
		EGF (—)			EGF (+)		
		G1	S	G2/M	G ₁	S	G2/M
Control	—	37.3 $\pm$ 2.0	19.6 $\pm$ 1.2	43.2 $\pm$ 3.4	19.9 $\pm$ 0.8	35.9 $\pm$ 2.2 ^a	44.3 $\pm$ 1.3
MDC*	0.5 mM	37.0 $\pm$ 1.1	19.2 $\pm$ 0.9	43.8 $\pm$ 1.6	11.8 $\pm$ 1.8	43.0 $\pm$ 2.0 ^b	45.3 $\pm$ 1.6
Control	—	37.6 $\pm$ 1.5	19.6 $\pm$ 1.1	42.9 $\pm$ 1.7	22.4 $\pm$ 1.5	34.9 $\pm$ 3.1 ^a	42.8 $\pm$ 2.4
Antisense**	10 μ M	37.7 $\pm$ 1.9	19.6 $\pm$ 1.1	42.8 $\pm$ 2.1	16.6 $\pm$ 1.2	38.5 $\pm$ 1.9	44.9 $\pm$ 2.9
	30 μ M	37.8 $\pm$ 2.3	19.8 $\pm$ 1.0	42.4 $\pm$ 2.1	10.2 $\pm$ 1.3	43.6 $\pm$ 2.4 ^b	46.2 $\pm$ 2.1 ^b
Sense**	30 μ M	37.7 $\pm$ 1.6	19.8 $\pm$ 1.4	42.5 $\pm$ 1.9	21.1 $\pm$ 2.3	36.9 $\pm$ 1.5	42.1 $\pm$ 3.1

* Hepatocytes were pretreated with 0.5 mM MDC for 30 min. Thirty hours after EGF (17 nM) was added to the culture medium, the cells were subjected to cell cycle analysis. **After incubation of hepatocytes for 3 h, the medium was replaced with serum-free medium containing antisense or sense oligomers (10 or 30 μ M). After incubation for 16 h, EGF (17 nM) was added to the culture medium. The cells were subjected to cell cycle analysis after 30 h. Each value is the mean $\pm$ S.E.M. of four samples.

^a Significantly different from 'EGF (—)' ($P < 0.01$).

^b Significantly different from 'Control' ($P < 0.05$).

Table 4 Effect of the antisense oligonucleotide or MDC on high-affinity EGF receptor in primary cultured hepatocytes

Incubation with the antisense oligonucleotide or MDC pretreatment was performed as described in the footnote for Table 2. Six hours after EGF (17 nM) was added to the culture medium, Scatchard analyses were performed as described previously [17]. Each value is the mean $\pm$ S.E.M. of three samples. ND, not detected.

Treatment	EGF	Low		High	
		K_D (nM)	B_{Max} (pmol/ 10^7 cells)	K_D (nM)	B_{Max} (pmol/ 10^7 cells)
—	(—)	6.90 $\pm$ 0.52	1.49 $\pm$ 0.18	1.20 $\pm$ 0.14	0.48 $\pm$ 0.04
	(+)	6.80 $\pm$ 0.32	1.28 $\pm$ 0.06	ND	ND
Antisense	(—)	7.10 $\pm$ 0.65	1.48 $\pm$ 0.11	1.40 $\pm$ 0.17	0.41 $\pm$ 0.04
	(+)	7.80 $\pm$ 0.75	1.22 $\pm$ 0.11	1.50 $\pm$ 0.27	0.42 $\pm$ 0.06
Sense	(—)	6.70 $\pm$ 0.55	1.50 $\pm$ 0.16	1.30 $\pm$ 0.18	0.55 $\pm$ 0.04
	(+)	7.00 $\pm$ 0.68	0.74 $\pm$ 0.66	ND	ND
MDC	(—)	7.30 $\pm$ 0.17	1.56 $\pm$ 0.20	1.30 $\pm$ 0.12	0.46 $\pm$ 0.02
	(+)	7.20 $\pm$ 0.69	1.09 $\pm$ 0.09	1.30 $\pm$ 0.20	0.43 $\pm$ 0.03

actin is associated with EGF receptors [19,20]. Furthermore, Takashi [4] reported that the polymerization rate of TGase-treated actin was greater and the critical concentration for polymerization was less than for intact actin *in vitro*. Combining the results of these reports with our previous observations that TGase-modified actin was associated with EGF receptors and decreased the binding of EGF, it is suggested that EGF-induced newly synthesized TGase negatively regulates the growth signal of EGF by down-regulation of high-affinity receptor through stimulation of actin polymerization.

It has been reported that intracellular TGase may be involved in the suppression of cell growth. Birckbichler et al. [21] proposed a model in which an increase in formation of TGase-catalysed isopeptide bonds in cells causes a shift from the proliferative to non-proliferative state. Piacentini et al. [22] reported that the induction of TGase synthesis and the formation of TGase-catalysed isopeptide bonds were directly correlated with the formation of apoptotic bodies. Furthermore, Melino et al. [23] demonstrated that the overexpression of TGase resulted in a drastic reduction in the proliferative capacity of cells. However, the TGase-related mechanism by which growth suppression is induced remains unknown. Furthermore, the increases in TGase activity induced by EGF were demonstrated in other cell types, but these previous reports did not explain the role of TGase on EGF-mediated growth signal [22,24]. Therefore, the present results are important for elucidation of the mechanism of growth suppression.

In the present study, we demonstrated that EGF increased TGase activity in primary cultured hepatocytes, and the newly synthesized TGase negatively regulated the growth signal of EGF through down-regulation of high-affinity receptor, possibly the result of modification of EGF-receptor-associated actin. The present results suggest that the response of TGase to EGF may provide a general system for down-regulation of cell growth.

We would like to acknowledge the helpful suggestions of Professor Y. Sugiyama. We are grateful to Dr. T. Suzuki for comments and critical reading of the manuscript.

REFERENCES

- Folk, J. E. (1980) *Annu. Rev. Biochem.* **49**, 517–531
- Greenberg, C. S., Birckbichler, P. J. and Rice, R. S. (1991) *FASEB J.* **5**, 3071–3077
- Derrick, N. and Laki, K. (1966) *Biochem. Biophys. Res. Commun.* **22**, 82–88
- Takashi, R. (1988) *Biochemistry* **27**, 938–943
- Van Bergen en Henegouwen, P. M. P., Defize, L. H. K., De Kroon, J., Van Damme, J., Verkleij, A. J. and Boonstra, J. (1989) *J. Cell. Biochem.* **39**, 455–465
- Van Belzen, N., Spaargaren, M., Verkleij, A. J. and Boonstra, J. (1990) *J. Cell. Physiol.* **145**, 365–375
- Roy, L. M., Gitlinger, C. K. and Landreth, G. E. (1991) *J. Cell. Physiol.* **146**, 63–72
- Van Bergen en Henegouwen, P. M. P., Den Hartigh, J. C., Romeyn, P., Verkleij, A. J. and Boonstra, J. (1992) *Exp. Cell Res.* **199**, 90–97
- Defize, L. H. K., Boonstra, J., Meisenhelder, J., Kruijer, W., Tertoolen, L. G. J., Tilly, B. C., Hunter, T., Van Bergen en Henegouwen, P. M. P., Moolenaar, W. H. and De Laat, S. W. (1989) *J. Cell Biol.* **109**, 2495–2507
- Bellot, F., Moolenaar, W., Kris, K., Mirakhor, B., Verlaan, I., Ullrich, A., Schlessinger, J. and Felder, S. (1990) *J. Cell Biol.* **110**, 491–502
- Katoh, S., Hashimoto, M., Kohno, H. and Ohkubo, Y. (1993) *Arch. Biochem. Biophys.* **303**, 421–428
- Katoh, S., Inoue, T., Kohno, H. and Ohkubo, Y. (1994) *Biomed. Res.* **15**, 1–8
- Katoh, S., Midorikami, J., Kohno, H. and Ohkubo, Y. (1991) *Chem. Pharmaceut. Bull.* **39**, 3299–3302
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- Ikura, K., Nasu, T., Yokota, H., Tsuchiya, Y., Sasaki, R. and Chiba, H. (1988) *Biochemistry* **27**, 2898–2905
- Mizuno, K., Higuchi, O., Tajima, H., Yonemasu, T. and Nakamura, T. (1993) *J. Biochem. (Tokyo)* **114**, 96–102
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Davies, P. J. A., Davies, D. R., Levitzki, A., Maxfield, F. R., Milhaud, P., Willingham, M. C. and Pastan, I. H. (1980) *Nature (London)* **283**, 162–167
- Van Bergen en Henegouwen, P. M. P., Defize, L. H. K., De Kroon, J., Van Damme, H., Verkleij, A. J. and Boonstra, J. (1989) *J. Cell. Biochem.* **39**, 455–465
- Rijken, P. J., Hage, W. J., Van Bergen en Henegouwen, P. M. P., Verkleij, A. J. and Boonstra, J. (1991) *J. Cell Sci.* **100**, 491–499
- Birckbichler, P. J., Orr, G. R., Patterson, M. K., Jr., Conway, E. and Carter, H. A. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5005–5008
- Piacentini, M., Autuori, F., Dini, L., Farrace, M. G., Ghibelli, L., Piredda, L. and Fesus, L. (1991) *Cell Tissue Res.* **263**, 227–235
- Melino, G., Annichiarico-Petruzzelli, M., Piredda, L., Candi, E., Gentile, V., Davies, P. J. A. and Piacentini, M. (1994) *Mol. Cell. Biol.* **14**, 6584–6596
- Dadabay, C. Y. and Pike, L. J. (1987) *Biochemistry* **26**, 6587–6591