Transforming growth factor- α -induced DNA synthesis and *c-myc* expression in primary rat hepatocyte cultures is modulated by indomethacin

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Primary hepatocytes stimulated with epidermal growth factor (EGF) secrete prostaglandins into the culture medium as soon as 1 h after the addition of the EGF. Transforming growth factor- α (TGF α), a potent hepatocyte mitogen, shares the same receptor with EGF, and its expression is increased after partial hepatectomy. TGF α is also secreted in culture. We have observed that TGF α induced hepatocyte DNA synthesis (30 h after addition) and at the same time stimulated the production of prostaglandins E_2 and $F_{2\alpha}$ by the cultured hepatocytes. Indomethacin at 20–100 μ M inhibited the TGF α -induced hepatocyte DNA synthesis, and this effect was specifically due to the inhibition of prostaglandin formation. Indomethacin also inhibited a TGF α -induced increase in hepatocyte *c-myc* expression, indicating that prostaglandins mediate this increase, as previously shown for EGF. TGF α increased the expression of the EGF receptor gene, and this was prevented by the presence of an antibody against TGF α in the culture medium. We therefore suggest that TGF α induces hepatocyte proliferation either through coupling with its receptor (i.e. the EGF receptor) or by subsequent phosphorylation of lipocortin I. This leads to activation of phospholipase A_2 , which seems to regulate the metabolism of arachidonic acid and the formation of prostaglandins. Thus hepatocyte proliferation *in vitro* appears to be controlled by a self-regulatory autocrine pathway involving activation of phospholipase A_2 and secretion of prostaglandins and TGF α .

INTRODUCTION

Transforming growth factor- α (TGF α) is a polypeptide which has mitogenic activity in mesenchymal and epithelial cells (Derynck *et al.*, 1984). This factor, which shares structural similarity with epidermal growth factor (EGF), has the ability to compete with EGF for binding to the EGF cell-surface receptor (Massague, 1983). TGF α is a complete hepatocyte mitogen, as recent studies have shown, and moreover it seems to promote a more intense DNA synthetic response than that produced by EGF (Mead & Fausto, 1989). TGF α is secreted by hepatocytes, constituting in this way an autocrine loop regulating hepatocyte proliferation (Mead & Fausto, 1989; Michalopoulos, 1990). EGF and TGF α bind to a common receptor, activating a tyrosine kinase which subsequently leads to receptor autophosphorylation, internalization and down-regulation (Reynolds *et al.*, 1981; Pike *et al.*, 1982). We have found that stimulation of adult hepatocyte DNA synthesis by EGF is mediated in part by arachidonic acid derivatives (Skouteris *et al.*, 1988). Liver regeneration results in the local production of prostaglandins, presumably after stimulation of arachidonic acid metabolism by specific growth factors (Little *et al.*, 1988). In the present study we have examined the involvement of arachidonic acid derivatives in TGF α -induced hepatocyte proliferation. Indomethacin, a known cyclo-oxygenase inhibitor, inhibited TGF α -induced hepatocyte DNA synthesis and at the same time decreased hepatocyte *c-myc* expression. *c-myc*, a gene which is regulated in a cell-cycle-dependent manner, is activated after partial hepatectomy and also after addition of EGF or prostaglandins E_2 and $F_{2\alpha}$ (PGE $_2$ and PGF $_{2\alpha}$) to hepatocyte cultures (Makino *et al.*, 1984; Etienne *et al.*, 1988; Skouteris & Kaser, 1991). The mitogenic activity of

TGF α is mediated through activation of arachidonic acid metabolism and production of prostaglandins, in a manner similar to that observed for EGF. Prostaglandins added to hepatocyte cultures supplemented with indomethacin and TGF α restored the ability of TGF α to stimulate DNA synthesis and to increase *c-myc* expression. We therefore suggest that the stimulatory effect of TGF α on primary hepatocyte proliferation is transduced through phospholipase A_2 (PLA $_2$) activation.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma Chemical Co. Radiochemicals were from Amersham, and prostaglandins were kindly donated by Dr. J. E. Pike, The Upjohn Company, Kalamazoo, MI, U.S.A. EGF was from Collaborative Research, and TGF α and anti-TGF α antisera were from Peninsula Laboratories, Belmont, CA, U.S.A.

Isolation and culture of primary rat hepatocytes

Hepatocytes from adult rats (130–150 g) were prepared using the two-step collagenase perfusion method (Seglen, 1976). The purified hepatocytes were plated on collagen-coated tissue culture dishes (60 mm diameter) at a density of $(3\text{--}3.5) \times 10^5$ cells/2 ml (Nakamura & Ichihara, 1985). The viability of the cells used in all experiments was greater than 85%. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% essential and non-essential amino acids ($10\times$), 0.5 mM-L-ornithine, 1.63 μ M-glutathione, 10 mM-sodium pyruvate and 50 μ g of gentamycin/ml, was used in all experiments. Hepatocytes were allowed to attach for 2 h in a humidified 5% CO $_2$ /95% air atmosphere

Abbreviations used: TGF α , transforming growth factor- α ; EGF, epidermal growth factor; PGE $_2$ /F $_{2\alpha}$, prostaglandin E_2 /F $_{2\alpha}$; PLA $_2$, phospholipase A_2 ; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline (NaCl, 8 g/l; KCl, 0.2 g/l; Na $_2$ HPO $_4$ ·2H $_2$ O, 1.44 g/l; KH $_2$ PO $_4$, 0.2 g/l, pH 7.4).

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in DMEM additionally supplemented with 0.5 μg of insulin/ml. After attachment, the medium was changed and various additions were made. The medium was thereafter changed every 24 h. Where indicated, prostaglandins and indomethacin were dissolved in ethanol and added to the cultures after dilution with phosphate-buffered saline (PBS). The concentration of ethanol to which the cultures were exposed (2 μl /dish) did not affect the cell response.

DNA synthesis in hepatocyte cultures

[methyl- ^3H]Thymidine (5 μCi ; sp. radioactivity > 90 Ci/mmol) was added to the cultures for 2 h. Briefly, DNA was estimated using a fluorimetric procedure (Kapp *et al.*, 1974). An aliquot was counted for radioactivity in an LKB liquid scintillation counter and specific radioactivities were determined as previously described (Nakamura *et al.*, 1983; Skouteris *et al.*, 1988).

RNA isolation and hybridization conditions

Total RNA from cultured cells was isolated using the hot phenol method (Maniatis *et al.*, 1989). Poly(A)⁺ RNA from cultures treated under different conditions was obtained using oligo(dT)-cellulose affinity chromatography. RNA (5 μg /lane) was electrophoresed on a 1.0% agarose/formaldehyde denaturing gel and transferred on to Hybond-N membranes (Amersham). [α - ^{32}P]CTP-labelled probes originating from pHSR1 (a 0.9 kb *Cl*AI-*E*coRI fragment of *c-myc*) (Alitalo *et al.*, 1983) and from a pE7 cDNA probe of the human EGF receptor (2.4 kb fragment) (Xu *et al.*, 1984) were used. The membranes were hybridized for 48 h at 52 °C and subsequently washed with 0.1% SDS/0.1 $\times$ SSC (1 $\times$ SSC = 0.15 M-NaCl/0.015 M-sodium citrate). The membranes were then exposed to film (Kodak XAR-2) at -70 °C using two intensifying screens. Hybridization with actin cDNA was used as an RNA loading control. The autoradiograms were quantified and compared with the stated controls using a Perkin-Elmer densitometer.

Immunoprecipitation of hepatocyte proteins and SDS/PAGE analysis

Cultured hepatocytes were labelled with 100 μCi of [^{35}S]methionine/ml (sp. radioactivity > 800 Ci/mmol) in methionine-free DMEM (Gibco), or with 100 μCi of [^{32}P]P_i in ordinary DMEM, for 2 h. Hepatocytes were scraped off using a rubber policeman and washed three times with cold PBS, pH 7.4. The cell pellet was homogenized in lysis buffer, pH 7.4, containing 20 mM-Hepes, 5 mM-KCl, 5 mM-MgCl₂, 0.5% Triton X-100, 0.1% sodium deoxycholate and 0.1 mM-phenylmethane-sulphonyl fluoride. The lysate was sheared until no viscous material was present and the appropriate antibodies were added. Control experiments confirmed that the immunoprecipitation reactions were performed in antibody excess. The lysates were incubated for 3 h with gentle rocking (60 rev./min). Then, 40 μl of Protein A-Sepharose (1:1 slurry in lysis buffer) or 50% Pansorbin (Calbiochem) was added and the incubation proceeded for an additional 1 h. The beads were washed three times with cold lysis buffer and twice with PBS. The beads were then resuspended in sample buffer containing 10 mM-dithiothreitol, heated at 100 °C for 4 min and loaded on to a discontinuous gel system (10% polyacrylamide separating gel, 4.5% stacking gel) (Laemmli, 1970). Before loading, the samples were adjusted to have approximately equal protein contents (30 μg /slot). The antibodies used in this study were CT14-GT.3, an anti-*c-myc* monoclonal (Evan *et al.*, 1985; ATCC hybridoma collection) and the anti-(lipocortin I) antibody a-646 (Pepinsky & Sinclair, 1986), kindly provided by Dr. B. Pepinsky, Biogen Research Corp., Cambridge, MA, U.S.A.

Prostaglandin assays in the hepatocyte culture supernatant

Collection of hepatocyte culture supernatants and radio-immunoassay of PGE₂ and PGF_{2 α} was performed as previously shown (Bothwell *et al.*, 1982; Rosengurt *et al.*, 1983; Skouteris *et al.*, 1988).

RESULTS

Effects of TGF α and prostaglandins on primary hepatocyte proliferation

Primary hepatocytes were plated at 5×10^5 cells/dish, and after cell attachment the cultures were supplemented with either EGF or TGF α . DNA synthesis was measured throughout the culture period (up to 96 h) (Fig. 1). The DNA synthetic activity started at approx. 30 h after addition of either factor at all concentrations used (Fig. 1). TGF α at 50 ng/dish stimulated maximal hepatocyte DNA synthesis at 48 h. At 20 ng/dish,

Fig. 1. Effects of TGF α on hepatocyte DNA synthesis

Hepatocyte cultures were supplemented with 50 ng of EGF/dish (plot a), 50 ng of TGF α /dish (b), 20 ng of TGF α /dish (c) or nothing (d). All dishes contained insulin (0.5 μg /ml). Points represent means $\pm$ S.E.M. from triplicate dishes from at least three independent experiments.

Fig. 2. Effects of co-addition of prostaglandins and TGF α upon the DNA synthetic activity of cultured hepatocytes

Hepatocyte cultures were supplemented with 20 ng of TGF α /dish plus 6 μg of PGE₂/dish (plot a) or 6 μg of PGF_{2 α} /dish (b). Separate cultures were supplemented with 6 μg of PGE₂/dish (c) or 6 μg of PGF_{2 α} /dish (d) alone. Points represent means $\pm$ S.E.M. of triplicate dishes from at least three independent experiments. Control values for TGF α addition in hepatocyte cultures are shown in Fig. 1 (plot c).

TGF α induced hepatocyte DNA synthesis by approx. 14-fold between 48 and 72 h in culture. It should also be noted that both factors needed to be present at the beginning of the culture to induce such DNA synthetic responses. TGF α -induced hepatocyte DNA synthesis was slightly higher than that induced by EGF at 48 and 72 h in culture (approx. 20%). Thymidine incorporation in hepatocyte cultures was not very different when a TGF α concentration of either 20 or 50 ng/dish was used.

In previous experiments performed in our laboratory, we observed that the hepatocyte DNA synthetic response elicited after stimulation by EGF is mediated in part by prostaglandins. At the same time, PGE $_2$ and PGF $_{2\alpha}$ by themselves stimulate hepatocytes (Skouteris *et al.*, 1988). Addition of PGE $_2$ or PGF $_{2\alpha}$ at 6 μ g/dish, together with 20 ng of TGF α , stimulated hepatocyte DNA synthesis in an additive manner (Fig. 2). Thus addition of PGE $_2$ + TGF α increased hepatocyte thymidine incorporation by 30%, whereas TGF α plus PGF $_{2\alpha}$ increased thymidine incorporation by 35%. Hepatocyte cultures not stimulated with growth factors did not show significant DNA synthesis. Control values for unstimulated hepatocyte cultures are shown in Fig. 1 (plot d). Hepatocyte DNA synthesis in prostaglandin-supplemented cultures co-stimulated with TGF α (20 ng/dish) reached the levels observed using TGF α at 50 ng/dish.

TGF α induces production of PGE $_2$ and PGF $_{2\alpha}$ by cultured hepatocytes

We investigated the relationship between TGF α -induced hepatocyte DNA synthesis and the production of prostaglandins (Table 1). Addition of TGF α at 20 ng/dish resulted in the production of prostaglandins 1 and 6 h later. Unstimulated hepatocyte cultures did not produce significant amounts of prostaglandins. The levels of the prostaglandins after TGF α stimulation were comparable with those produced in cultures stimulated with EGF (Skouteris *et al.*, 1988; Little *et al.*, 1988). Prostaglandin levels measured at 24 h were similar to those at 1 h (results not shown). In repeated experiments, prostaglandins were not detectable at later times in culture, thus showing that TGF α induced a transient increase. Indomethacin, a fatty acid cyclo-oxygenase inhibitor, was tested for its ability to modulate the TGF α -induced production of prostaglandins by cultured hepatocytes (Table 1). Indomethacin at 20 μ M inhibited TGF α -induced PGE $_2$ production at 1 and 6 h in culture by approx. 75–90%. Addition of indomethacin (20 μ M) inhibited the production of PGF $_{2\alpha}$ by 70–84% after TGF α stimulation (Table 1). Indomethacin at concentrations between 10 and 100 μ M was effective in inhibiting both prostaglandin production and DNA synthesis in hepatocyte cultures. However, its maximal inhibitory effect without interfering with cell viability was observed at 100 μ M (Table 1). Addition of aspirin, which acts in a manner similar to indomethacin, also lowered the production of prostaglandins to levels comparable with those in indomethacin-treated cultures (results not shown).

Indomethacin modulates TGF α -induced DNA synthesis and *c-myc* expression in hepatocyte cultures

TGF α was added to hepatocyte cultures at 50 or 20 ng/dish together with indomethacin (20 or 100 μ M) (Table 2). Thymidine incorporation was then measured after 48 h of culture. Indomethacin inhibited by 70–81% thymidine incorporation into hepatocyte cultures treated with 50 ng of TGF α . When TGF α was used at 20 ng/dish, indomethacin inhibited DNA synthesis by more than 80% (Table 2). Together with the addition of indomethacin (20 μ M) and TGF α , we added a mixture of PGs (6 or 9 μ g) to the cultures and then measured thymidine incorporation at 48 h. Prostaglandins used at both concentrations restored the ability of TGF α to induce hepatocyte DNA synthesis

Table 1. Effects of TGF α on prostaglandin production by cultured hepatocytes

All dishes contained insulin (0.5 μ g/ml). Values represent means $\pm$ S.E.M. of duplicate measurements from at least three independent experiments.

Additions	Prostaglandin production (pg/ml)			
	1 h		6 h	
	PGE $_2$	PGF $_{2\alpha}$	PGE $_2$	PGI $_{2\alpha}$
None	13 $\pm$ 0.1	51 $\pm$ 0.9	16 $\pm$ 0.1	48 $\pm$ 0.4
TGF α (20 ng/dish)	182 $\pm$ 10	624 $\pm$ 31	255 $\pm$ 21	653 $\pm$ 44
TGF α + indomethacin (20 μ M)	64 $\pm$ 0.6	160 $\pm$ 9	48 $\pm$ 0.2	79 $\pm$ 0.9
TGF α + indomethacin (100 μ M)	19 $\pm$ 0.1	33 $\pm$ 0.6	8.0 $\pm$ 0.1	21 $\pm$ 0.2

Table 2. Effects of indomethacin on TGF α -induced hepatocyte DNA synthesis

Hepatocyte DNA synthesis was measured 48 h after cell attachment. All dishes contained insulin (0.5 μ g/ml). Values represent means $\pm$ S.E.M. of triplicate determinations from at least three independent experiments.

Additions	DNA synthesis (c.p.m./ μ g)
TGF α (50 ng/dish)	10730 $\pm$ 665
TGF α (20 ng/dish)	7184 $\pm$ 283
TGF α (50 ng/dish) + indomethacin (20 μ M)	3390 $\pm$ 165
TGF α (50 ng/dish) + indomethacin (100 μ M)	2238 $\pm$ 327
TGF α (20 ng/dish) + indomethacin (20 μ M)	1500 $\pm$ 285
TGF α (20 ng/dish) + indomethacin (100 μ M)	1160 $\pm$ 216
TGF α (20 ng/dish) + indomethacin (20 μ M) + PGE $_2$ /PGF $_{2\alpha}$ (6 μ g/dish)	6116 $\pm$ 420
TGF α (20 ng/dish) + indomethacin (20 μ M) + PGE $_2$ /PGF $_{2\alpha}$ (9 μ g/dish)	6870 $\pm$ 389

(Table 2). However, prostaglandins not present at the start of the culture failed to restore the ability of TGF α to induce DNA synthesis (results not shown).

EGF and prostaglandins induced changes in *c-myc* expression in hepatocyte cultures (Skouteris & Kaser, 1991). *c-myc* is a target gene in primary hepatocytes stimulated with growth factors and is regulated in a cell-cycle-dependent manner. However, in cultured hepatocytes this gene seems to be expressed throughout the cell cycle, independently of the growth stage (Etienne *et al.*, 1988). TGF α induced an approx. 10-fold increase in *c-myc* expression (compared with the insulin-supplemented hepatocytes) 2 h after addition (Fig. 3a, lanes b and c). *c-myc* expression was above baseline levels at 24 h in culture (Fig. 3a, lane d) and was increased by approx. 13-fold when the cultures were supplemented with TGF α (20 ng/dish) together with a mixture of prostaglandins (6 μ g) (Fig. 3a, lane e). Indomethacin (20 μ M) decreased *c-myc* levels in TGF α -induced hepatocyte cultures by more than 9-fold (Fig. 3b, lane f). At the same time, indomethacin did not affect significantly the basal expression of *c-myc* if added to unstimulated cultures (G. G. Skouteris & M. R. Kaser, unpublished work). Addition of a prostaglandin mixture (3, 6 or 9 μ g) to cultures supplemented with both TGF α and indomethacin restored (in part) the ability of the growth factor to activate *c-myc* expression (Fig. 3b, lanes g–i). As expected,

Fig. 3. Stimulation of *c-myc* expression in hepatocyte cultures treated with TGF α : modulation of *c-myc* expression by indomethacin and prostaglandins

In both (a) and (b), 5 μ g of poly(A)⁺-containing hepatocyte RNA was electrophoresed in 1% agarose/formaldehyde denaturing gels as described in the Materials and methods section. Membranes were hybridized with a ³²P-labelled *c-myc* probe (*Clal*-*Eco*RI fragment of pHSR1). (a) *c-myc* expression in hepatocyte cultures treated with: lane a, insulin alone (0.5 μ g/ml) 1 h after cell plating; lane b, as for a, but 2 h after cell attachment; lane c, as for b, but in the presence of TGF α (20 ng/dish); lane d, as for c, but at 24 h in culture; lane e, as in c, but in cultures supplemented with PGE₂/PGF_{2 α} (6 μ g). (b) Lane f, as in lane c, (a) in the presence of 20 μ M-indomethacin; lanes g-i, as for f, but in the presence of 3, 6 and 9 μ g respectively of PGE₂ + PGF_{2 α} ; lane j, as in d, but in the presence of indomethacin (20 μ M); lane k, as in c, but at 48 h in culture; lane l, as in k, but in the presence of indomethacin (20 μ M). (c) [³⁵S]Methionine-labelled hepatocyte lysates from 2 h cultures supplemented with insulin (lane a) or insulin plus TGF α (20 ng/dish) (lane b), and treated with monoclonal antibody CT14-GT.3. The arrowhead indicates the main bands of anti-*c-myc*-reacting hepatocyte polypeptides (62–67 kDa).

Fig. 4. Phosphorylation of lipocortin I and modulation of EGF receptor expression by TGF α

(a) Hepatocyte cultures were labelled with [³²P]P_i as described in the Materials and methods section and the lysates were then treated with the a-646 anti-(lipocortin I) antibody. Hepatocyte immunoprecipitates are shown from: lane a, insulin-supplemented cultures (2 h); lane b, cultures as for lane a, but supplemented with TGF α (20 ng/dish); lane c, as for b, but in the presence of an antibody against the EGF receptor in the culture medium of the hepatocytes from the beginning of the culture. Lipocortin I is indicated by the arrow. (b) Expression of the EGF receptor in hepatocyte cultures supplemented with: lane a, a mixture of PGE₂ + PGF_{2 α} (6 μ g) (2 h in culture); lane b, insulin alone for 2 h in culture; lane c, as for b, but in the presence of TGF α (20 ng/dish); lane d, as for c, but in the presence of an antibody against TGF α ; lane e, as for b, but in the presence of EGF (20 ng/dish).

myc polypeptides range in molecular mass between 55 and 67 kDa in SDS/PAGE (Skouteris & Michalopoulos, 1991). It is believed that this heterogeneity in molecular size is due to proteolytic cleavage of the main gene product (Muakkassah-Kelly *et al.*, 1988). The synthesis of *c-myc* polypeptides in hepatocyte cultures stimulated with TGF α (20 ng/dish) was increased compared with that in control insulin-supplemented cultures (Fig. 3c).

TGF α modulates phosphorylation of lipocortin I and expression of the EGF receptor

One of the inhibitors of PLA₂, lipocortin I, was shown to be a substrate for the EGF receptor tyrosine kinase (Pepinsky & Sinclair, 1986). PLA₂ is activated when lipocortin I becomes phosphorylated. Because EGF and TGF α share a common receptor, we carried out some experiments to explore the involvement of lipocortin I in the transduction of TGF α -generated signals. Lipocortin I was actively phosphorylated in lysates derived from cultures treated with TGF α (Fig. 4a). In contrast, no phosphorylated lipocortin I was detected in control cultures supplemented with insulin. Addition of a monoclonal antibody against the EGF receptor to the hepatocyte culture medium prevented phosphorylation of lipocortin I (Fig. 4a, lane c). Phosphorylation of lipocortin I became detectable 70 min after the addition of TGF α , independent of the concentration of TGF α used (results not shown). The EGF receptor gene was expressed in hepatocyte cultures stimulated with either TGF α or EGF (Fig. 4b). Expression of the EGF receptor (10 kb) was increased by approx. 15- and 9-fold in cultures supplemented with TGF α and EGF (20 ng/dish) respectively. Presence of an antibody against TGF α in cultures stimulated with TGF α lowered the expression of the EGF receptor to control levels (Fig. 4b, lane d).

DISCUSSION

In this study we have investigated the effects of TGF α on hepatocyte proliferation and its potential involvement in the activation of arachidonic acid metabolism. Previous studies have shown that the stimulation of hepatocyte proliferation by EGF is mediated at least in part through activation of arachidonic acid hydrolysis (Skouteris *et al.*, 1988). TGF α is a complete mitogen for primary hepatocytes (Mead & Fausto, 1989), and shares the

indomethacin inhibited TGF α -induced *c-myc* expression observed at 24 or 48 h in culture (Fig. 3b, lanes j-l). Hepatocyte lysates labelled with [³⁵S]methionine were treated with the CT14-GT.3 monoclonal anti-*c-myc* antibody and the immunoprecipitates were analysed on SDS/PAGE. *c-myc* polypeptides resolved in SDS/PAGE were found to migrate between 62 and 67 kDa (Fig. 3c). Previous studies have shown that hepatocyte *c-*

same receptor as EGF (Derynck, 1988). TGF α at 20–50 ng/dish was effective in stimulating hepatocyte DNA synthesis approx. 30 h after addition. At the same time, TGF α was found to be capable of increasing prostaglandin levels in the hepatocyte culture supernatant as soon as 1 h after addition. Both prostaglandin production and DNA synthesis were stimulated to levels comparable with those observed using EGF (Skouteris *et al.*, 1988). Mitogenic signalling by EGF was shown to require arachidonic acid metabolism in BALB/c 3T3 fibroblasts (Handler *et al.*, 1990). Addition of prostaglandins (6 μ g) together with TGF α (20 ng/dish) resulted in a co-operative effect in stimulating thymidine incorporation. At the same time, indomethacin (20–100 μ M) inhibited TGF α -induced hepatocyte DNA synthesis. Indomethacin used at these concentrations does not inhibit cyclic AMP-dependent protein kinase (protein kinase A) (Goueli & Ahmed, 1980), suggesting that the observed effect on TGF α -induced DNA synthesis was due to selective inhibition of prostaglandin formation. Furthermore, addition of a prostaglandin mixture restored the ability of TGF α to stimulate hepatocyte DNA synthesis.

Hepatocyte cell-cycle traversal after stimulation with a growth factor is associated with modulation of *c-myc* levels. TGF α increased the levels of *c-myc* transcripts early in culture (2 h), and this effect was overcome by the presence of an antibody against TGF α in the hepatocyte culture medium. A clear link emerged between *c-myc* activation and coupling of TGF α with its receptor. Furthermore, addition of prostaglandins into cultures supplemented with TGF α + indomethacin restored the ability of TGF α to enhance thymidine incorporation. These data suggest that prostaglandins mediate the transduction of TGF α -generated signals. Addition of prostaglandins alone to hepatocyte cultures stimulates DNA synthesis and increases *c-myc* expression (Skouteris *et al.*, 1988, Skouteris & Kaser, 1992).

Phosphorylation of lipocortin I, as reported for the EGF receptor tyrosine kinase (Fava & Cohen, 1984), was observed in our studies 70 min after the addition of TGF α . PLA₂ was shown to be activated very quickly after stimulation, within times not consistent with the phosphorylation of lipocortin I (Pepinsky & Sinclair, 1986). TGF α increased the expression of the EGF receptor gene, and this was inhibited by the presence of an antibody against TGF α in the hepatocyte medium. It must also be noted that stimulation of hepatocytes with growth factors results in the production of TGF α and its secretion into the medium (Mead & Fausto, 1989). Thus stimulation of primary hepatocytes with EGF and subsequent activation of arachidonic acid metabolism through PLA₂ and not through phospholipase C (Taylor *et al.*, 1985) results in the generation of an autocrine circuit comprising prostaglandins and TGF α . In this way hepatocytes enter the growth cycle for limited periods only, probably because of a contemporary activation of growth-inhibitory pathways such as that of TGF β .

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