Stimulation of hyaluronan biosynthesis by platelet-derived growth factor-BB and transforming growth factor- β 1 involves activation of protein kinase C

Masanobu SUZUKI,* Tomas ASPLUND,* Hidetoshi YAMASHITA,*† Carl-Henrik HELDIN† and Paraskevi HELDIN*‡

* Department of Medical and Physiological Chemistry, Biomedical Center, Uppsala University, Box 575, S-751 23 Uppsala, Sweden,

and † Ludwig Institute for Cancer Research, Biomedical Centre, Box 595, S-751 24 Uppsala, Sweden

The intracellular signal transduction pathways that mediate the stimulatory effects of platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)- β on hyaluronan biosynthesis in human fibroblasts were investigated. The stimulatory effects of both PDGF-BB and TGF- β 1 were dependent on protein kinase C (PKC), since the PKC inhibitor calphostin C inhibited the stimulation by the growth factors. Direct activation of PKC by phorbol 12-myristate 13-acetate (PMA) also stimulated hyaluronan production, and the combination of either PDGF-BB or TGF- β 1 and PMA gave an increased effect. One possible mechanism for activation of PKC is via induction of phospholipase C (PLC) activity; U-17322, an inhibitor of PLC- γ , was found to inhibit partially PDGF-BB-stimulated hyaluro-

nan synthesis. PDGF-BB is known to activate PLC- γ through tyrosine phosphorylation; however, a PDGF β -receptor mutant unable to interact with and activate PLC- γ was still able to mediate induction of hyaluronan biosynthesis, indicating that PDGF-mediated stimulation is not entirely dependent on PLC- γ . The stimulations by PDGF-BB and TGF- β 1 were partly dependent on protein synthesis, since parts of the effects were inhibited by cycloheximide; in contrast, the effects mediated by PMA were not. Our results indicate that PKC is involved in the transduction of the effects of growth factors on hyaluronan biosynthesis, and that the effects involve direct or indirect activation of existing hyaluronan synthetase molecules, as well as induction of new enzyme molecules.

INTRODUCTION

Hyaluronan is a linear polysaccharide of alternating glucuronic acid and *N*-acetylglucosamine residues, which provides stability and elasticity to the extracellular matrix by interaction with chondroitin sulphate proteoglycans and other matrix components [1]. Moreover, by virtue of its viscosity, hyaluronan acts as a lubricant in joints. Furthermore, hyaluronan has been directly or indirectly implicated in cell growth [2], cell motility [3], angiogenesis [4], cell differentiation [5] and cell aggregation [6,7]. Underlying the biological effects of hyaluronan is its interaction with cell surface receptors [8,9]. Several invasive tumour cells exhibit elevated amounts of cell surface hyaluronan receptors [10,11] and surround themselves with a hyaluronan-enriched extracellular matrix [12]. However, the production of tumour-associated hyaluronan is often not due to synthesis of hyaluronan by the tumour cells on their own, but because they secrete factors that stimulate hyaluronan synthesis by adjacent normal connective tissue cells [12,13].

Hyaluronan is synthesized by a membrane-bound enzyme; monosaccharide units are added to the reducing end of the polysaccharide as it protrudes through the plasma membrane [14,15]. The synthesis of hyaluronan is stimulated by growth regulatory factors such as platelet-derived growth factor (PDGF), epidermal growth factor, fibroblast growth factor, transforming growth factor- β (TGF- β) and insulin-like growth factor I [16,17]. An increased activity of hyaluronan synthetase was demonstrated in membrane fractions of human mesothelial cells about 6 h after stimulation with PDGF and about 24 h after stimulation with TGF- β 1 [18].

PDGF is a major mitogen for connective tissue cells (for

reviews see [19,20]). Structurally, PDGF is a family of homo- or hetero-dimers of disulphide-bonded A- or B-polypeptide chains. The PDGF isoforms exert their effects on cells by binding to two structurally related receptors, denoted the α - and β -receptors. The receptors are protein tyrosine kinases that become dimerized and activated after ligand binding. This results in receptor phosphorylation and the phosphorylation of downstream components in the signal transduction pathway.

TGF- β is a family of related dimeric molecules, called TGF- β 1, - β 2 and - β 3 (for a review see [21]). TGF- β s exert their cellular effects by binding to a number of different receptor types; a family of protein serine/threonine kinase receptors is likely to be of particular importance in signal transduction (reviewed in [22]).

In the present study, we have attempted to elucidate the intracellular signal transduction pathways that mediate the stimulatory effects of growth regulatory factors on hyaluronan biosynthesis. We have focused on the role of protein kinases as signal transducers, and have compared one growth stimulator, PDGF-BB, and one growth inhibitor, TGF- β 1. We show that protein kinase C (PKC) is involved in the regulation of hyaluronan synthesis in human fibroblasts.

MATERIALS AND METHODS

Materials

PDGF-BB was recombinant material produced by transfected yeast cells [23]. TGF- β 1 was purified from the conditioned medium of CHO cells stably transfected with a TGF- β 1 DNA construct (kindly provided by Dr. K. Miyazono, Ludwig Institute for Cancer Research, Uppsala, Sweden). Antibodies against PDGF homodimers were made in rabbits (these antibodies

Abbreviations used: PDGF, platelet-derived growth factor; TGF, transforming growth factor; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PLC, phospholipase C; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum.

‡ To whom correspondence should be addressed.

neutralize homo- as well as hetero-dimers of PDGF [24]. 4-Phorbol 12-myristate 13-acetate (PMA), cycloheximide and forskolin were purchased from Sigma. The PKC inhibitor calphostin C and the phospholipase C (PLC) inhibitor U-73122 were purchased from Calbiochem and Upjohn respectively. The phosphatidylinositol 3'-kinase blocker wortmannin was obtained from Kamiya Biomedical Company. *Streptomyces* hyaluronidase was purchased from Seikagaku Kogyo, Tokyo, Japan. Dulbecco's modified Eagle's medium (DMEM) and other cell culture reagents were obtained from SVA (Statens Veterinärmedicinska Anstalt, Uppsala, Sweden).

Determination of hyaluronan synthesis

Human foreskin fibroblasts (AG1518; obtained from the Human Mutant Cell Repository, Camden, NJ, U.S.A.) were cultured in DMEM containing 10% fetal calf serum (FCS), 4 mmol/l L-glutamine, 100 i.u./ml penicillin and 100 µg/ml streptomycin. At 24 h after plating in 12-well Falcon plates (40 000 cells/well), the cell cultures were rinsed once with DMEM containing 0.1% FCS (starvation medium) and then incubated for 2 days in 2 ml of this medium. Then the medium was changed and fresh starvation medium was added (0.4 ml/well) in the absence or presence of different inhibitors (cycloheximide, calphostin C, U-73122, wortmannin, and antibodies against PDGF) and stimulators (PDGF-BB, TGF-β1, PMA and forskolin) added 1 h later. The stimulators and inhibitors alone or in combination at the concentrations used were not toxic for the cells, as investigated using a commercial cytotoxicity assay which measures lactate dehydrogenase, a cytosolic enzyme that is released upon cell lysis (CytoTox 96; Promega). At 24 h after addition of stimulators or inhibitors, the hyaluronan content in the culture medium was measured using the hyaluronan Test 50 (Kabi Pharmacia Diagnostics, Uppsala, Sweden). The hyaluronan associated with the cell layer was also estimated. After washing once with starvation medium, the cell layers were dissolved in 0.3 ml of 0.1 M NaOH for 15 min followed by neutralization with 0.3 ml of 0.1 M HCl. The hyaluronan content was then measured with the hyaluronan Test 50. Under the conditions used, a major part of the synthesized hyaluronan (about 80%) was found in the conditioned medium, consistent with previous findings [25]. The results are given as the means of duplicates and the ranges are indicated. Each experiment was repeated at least three times with similar results.

Analysis of PDGF receptor mutants

The assay described above was also used to estimate the hyaluronan synthesized by dog kidney epithelial cells transfected with wild-type PDGF β-receptor or receptor mutants in which certain autophosphorylated tyrosine residues were mutated to phenylalanine residues (kindly provided by J. A. Cooper, Fred Hutchinson Cancer Research Center, Seattle, WA, U.S.A. [26]. The abilities of the receptor mutants to phosphorylate PLC-γ were determined as described [27].

RESULTS

Effect of activation of PKC and PKA on growth factor-stimulated hyaluronan synthesis

Previous studies have shown that PDGF-BB and TGF-β stimulate hyaluronan synthesis in human fibroblasts [16]. PMA, a stimulator of PKC, also induces increased synthesis of hyaluronan [18]. We therefore investigated further the possible role of PKC in growth factor-mediated hyaluronan

Figure 1 Effects of growth factors, PMA and forskolin on hyaluronan synthesis in fibroblast cultures

The amounts of hyaluronan released into the culture media after 24 h of stimulation by PDGF (50 ng/ml), TGF-β1 (5 ng/ml), PMA (10 nM) or forskolin (10 nM), or combinations thereof, were measured as described in the Materials and methods section.

biosynthesis. Figure 1 shows the effects of PDGF-BB, TGF-β1, PMA and forskolin, and combinations thereof, on the synthesis of hyaluronan by human fibroblasts over a 24 h period, assayed at concentrations found to give maximum effects (results not shown). PMA stimulated hyaluronan synthesis even more efficiently than 10% FCS. PDGF-BB also gave a clear stimulation, and the combination of PDGF-BB and PMA gave a synergistic effect. TGF-β1 showed a somewhat lower stimulatory effect on hyaluronan synthesis compared with that of PDGF-BB, and the combination of TGF-β1 and PMA did not give any synergistic stimulation. However, TGF-β1 showed an additive stimulation of hyaluronan synthesis in combination with PDGF-BB (Figure 1). The observed effects of PDGF-BB, TGF-β1 and PMA were not due to an increased release of stored hyaluronan, since the fibroblast cell layer contained only a low amount of hyaluronan, before and after stimulation (results not shown).

We then investigated the possible involvement of PKA in stimulation of hyaluronan biosynthesis. Forskolin stimulates the production of cyclic AMP in human fibroblasts [28] and would thus be expected to stimulate PKA. Forskolin treatment of the cells induced only a moderate stimulation of hyaluronan production (Figure 1). Moreover, there was a difference in the effect of activation of PKA in the signal transduction pathway of PDGF-BB compared with TGF-β1; the effect of TGF-β1 was slightly potentiated by forskolin, but there was an inhibition by forskolin of the PDGF-BB- and PMA-induced hyaluronan biosynthesis. These data support the notion that PKC, but not PKA, has a major role in the activation of hyaluronan synthetase.

Effect of inhibition of protein synthesis on hyaluronan synthesis stimulated by growth factors and kinase activators

The inhibitor of protein synthesis, cycloheximide, was used to determine whether the effects on hyaluronan synthesis of the

Figure 2 Stimulation of hyaluronan biosynthesis by growth factors and PMA: dependence on protein synthesis

Quiescent human fibroblasts were incubated in the absence (medium containing 0.1% FCS; control) or presence of 10% FCS, PDGF-BB (50 ng/ml), PMA (10 nM), TGF-β1 (5 ng/ml) or cycloheximide (CH; 1 µg/ml), or combinations thereof. Cycloheximide was added in 0.01% DMSO. The hyaluronan concentration in the cell culture media was determined after 24 h of incubation.

factors required new synthesis of protein or not. The stimulation of hyaluronan biosynthesis by PMA was virtually unaffected by cycloheximide, whereas the majority of the stimulation by PDGF-BB and TGF-β1 was inhibited (Figure 2). This suggests that the effect of PKC is not dependent on synthesis of new protein, and thus may involve a direct, or indirect, activation of already existing hyaluronan synthetase molecules, whereas the stimulation by PDGF-BB and TGF-β1 in part involves the synthesis of new hyaluronan synthetase enzyme.

Involvement of PKC in stimulation of hyaluronan synthesis by PDGF-BB and TGF-β1

In order to investigate whether the effects of PDGF-BB and TGF-β1 on hyaluronan synthesis were mediated through activation of PKC, the inhibitor calphostin C was used. Calphostin C at a concentration of 1 µM inhibited most of the hyaluronan synthesis induced by PDGF-BB or TGF-β (Figure 3); at this concentration, calphostin C had no effect on protein synthesis, as revealed by control experiments using [³⁵S]methionine/cysteine-labelled cells (results not shown). As expected, calphostin C also inhibited the effect of PMA; however, at this high concentration of PMA (10 nM), the inhibition was only partial (Figure 3). These results are consistent with the notion that PKC has an important role in the growth factor-induced stimulation of hyaluronan synthesis. Calphostin C also lowered the background hyaluronan synthesis seen in the absence of growth factors (Figure 3), suggesting that PKC activity is important also for the background hyaluronan synthesis.

Effects of inhibitors of PLC-γ and PtdIns 3-kinase on growth factor-induced hyaluronan synthesis

PDGF exerts its cellular effects by activation of several intracellular signal transduction pathways. Two of the more well characterized pathways start with the activation of PLC-γ and PtdIns 3-kinase respectively. In order to investigate whether stimulation of hyaluronan biosynthesis is mediated by any of

Figure 3 Stimulation of hyaluronan synthesis by growth factors: effect of inhibition of PKC

Quiescent fibroblast cultures were incubated in PMA (10 nM), PDGF-BB (50 ng/ml) or TGF-β1 (5 ng/ml), in the absence or presence of calphostin C (CphC; 1 µM). The concn. of DMSO used was 0.02%. The amount of hyaluronan accumulated in the medium after 24 h was determined.

Figure 4 Stimulation of hyaluronan biosynthesis by PDGF and TGF-β: effect of inhibition of PtdIns 3-kinase and PLC-γ

Quiescent fibroblasts were incubated in the absence or presence of 10% FCS, PDGF-BB (50 ng/ml), TGF-β1 (5 ng/ml), wortmannin (wort.; 100 nM) or U-73122 (10 µM), or combinations thereof. The concn. of DMSO used was 0.2%. The amount of hyaluronan released into the culture media was determined after 24 h of incubation.

these pathways, the effects of U-17322 and wortmannin, inhibitors of PLC-γ and PtdIns 3-kinase respectively, were investigated. Whereas wortmannin, at a concentration which efficiently inhibits PtdIns 3-kinase (100 nM), had no effect on PDGF-BB-stimulated synthesis, U-73122 gave a slight inhibition (Figure 4).

Figure 5 PDGF-BB-stimulated hyaluronan synthesis in dog epithelial cells transfected with PDGF β -receptor mutants unable to activate PLC- γ

(a) Quiescent cultures of cells expressing wild-type PDGF β -receptor (WT) or mutant receptors with specific tyrosine residues replaced by phenylalanine residues (Y1009F, Y1021F or Y1009/1021F), or untransfected cells (UT), were grown for 24 h in the absence (□) or presence of PDGF-BB (50 ng/ml) (■) or 10% FCS (▨), after which the concentration of hyaluronan in the media was determined. (b) Tyrosine phosphorylation of PLC- γ in the different cell lines was investigated by immunoprecipitation of PLC- γ before or after stimulation with PDGF-BB; samples were then subjected to immunoblotting using an anti-phosphotyrosine antibody. Results are expressed as the fold increase in tyrosine phosphorylation of PLC- γ after PDGF-BB stimulation.

No significant inhibition of the effect of TGF- β 1 was obtained with either wortmannin or U-73122 (Figure 4).

Since the results of Figure 4 suggested that activation of PLC- γ by PDGF could mediate part of the PDGF-BB-stimulated increase in hyaluronan synthesis, we explored further the possible involvement of PLC- γ by use of PDGF β -receptor mutants specifically defective in their ability to associate with and activate PLC- γ [26,27]. Since PLC- γ binds to autophosphorylated tyrosine residue 1021, and with lower affinity to Tyr-1009, receptor mutants with these residues mutated to phenylalanine residues are unable to activate PLC- γ [26,27]. Whereas untransfected dog epithelial kidney cells showed no increase in hyaluronan production after PDGF-BB-stimulation, these cells transfected with wild-type PDGF β -receptor responded (Figure 5a). Likewise, receptor mutants in which Tyr-1009 or Tyr-1021, or both of these residues, were mutated to phenylalanine residues also mediate stimulation of hyaluronan synthesis (Figure 5a). All cell lines responded to stimulation by 10% FCS. Control experiments verified that PLC- γ was phosphorylated on tyrosine in response to PDGF-BB in cells transfected with wild-type PDGF β -receptors and with Tyr-1009 $\rightarrow$ Phe mutant receptors, whereas no phosphorylation occurred in cells transfected with Tyr-1021 $\rightarrow$ Phe or Tyr-1009-1021 $\rightarrow$ Phe mutants (Figure 5b). Taken

Figure 6 Lack of effect of anti-PDGF antibodies on TGF- β 1 induced hyaluronan biosynthesis

Quiescent fibroblasts were incubated in the absence or presence of 10% FCS, TGF- β 1 (5 ng/ml), PDGF-BB (50 ng/ml) and antibodies against PDGF [a mixture of immunoglobulin fractions of rabbit antisera made against PDGF-AA (40 μ g/ml) and against PDGF-BB (40 μ g/ml)]. The hyaluronan concentration in the cell culture media was determined after 24 h of culture.

together, these data indicate that PLC- γ partially mediates, but is not essential for, the stimulation of hyaluronan synthesis by PDGF-BB.

TGF- β does not stimulate hyaluronan synthesis through induction of PDGF synthesis

TGF- β has been shown to induce the synthesis of PDGF in certain cell types, including fibroblasts [29,30]. In addition, it has been observed that TGF- β 1 stimulates hyaluronan synthetase activity with a peak of activity later than that obtained after PDGF stimulation [18]. Therefore it was of importance to investigate whether the stimulatory effect of TGF- β 1 on human fibroblasts involved the production of PDGF. However, a mixture of antibodies which would neutralize all isoforms of PDGF did not inhibit the stimulation of hyaluronan production by TGF- β (Figure 6), thus excluding PDGF as a mediator of the TGF- β effect.

DISCUSSION

We show in the present paper that PDGF-BB and TGF- β 1 stimulate the synthesis of hyaluronan in processes which are partly dependent on protein synthesis, and thus involve the induction of hyaluronan synthetase mRNA and protein. Based on the results with specific inhibitors, the signal transduction pathways involve activation of PKC. Moreover, direct activation of PKC stimulated an increase in hyaluronan production which was not dependent on protein synthesis. It is thus possible that PKC can directly phosphorylate and activate hyaluronan synthetase, or phosphorylates a component which in turn activates the enzyme.

PDGF-BB stimulates the growth of human fibroblasts, whereas TGF- β 1 inhibits the growth of these cells [30], yet both factors stimulate hyaluronan production [16]. PKC seems to be of major importance in the signal transduction pathways of both factors; most of the stimulation of hyaluronan production induced by PDGF-BB or TGF- β 1 was inhibited by the PKC inhibitor

calphostin C (Figure 3). A possible mechanism for activation of PKC after PDGF-stimulation is via activation of PLC- γ , which is known to interact with and be phosphorylated by the PDGF receptors. PLC- γ degrades phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate, which mobilizes Ca^{2+} from internal stores (reviewed in [31]); together Ca^{2+} and diacylglycerol activate PKC (reviewed in [32]). However, our data indicate that PLC- γ is not uniquely important for PDGF-stimulation of hyaluronan synthesis, since PDGF-stimulated hyaluronan production also occurs in cells with PDGF receptor mutants specifically altered to prevent activation of PLC- γ (Figure 5). It is possible that PKC is activated after PDGF-stimulation via alternative routes, e.g. involving other phospholipases. Interestingly, PtdIns 3-kinase, which is a signal transduction molecule that is activated by PDGF and which has been shown to be important for mediation of many effects of PDGF, including chemotaxis and actin re-organization [33] and to some extent mitogenicity [34,35], was found not to be important for stimulation of hyaluronan synthesis, as judged from the lack of inhibition by the specific inhibitor wortmannin (Figure 4). The fact that forskolin gave a small stimulation of hyaluronan synthesis (Figure 1) is consistent with the possibility that PKA can phosphorylate and activate hyaluronan synthetase, or activate the enzyme indirectly. The observation that forskolin inhibited PDGF-BB-induced hyaluronan synthesis is consistent with the finding that PKA negatively regulates PDGF signal transduction pathways, such as activation of the mitogen-activated protein (MAP) kinase cascade [36], which thus could inhibit the PDGF-stimulated induction of hyaluronan synthetase mRNA and protein.

Very little is known about the signal transduction pathways that originate at the activated TGF- β 1 receptor complex. Calphostin C, but not wortmannin or U-73122, inhibited the stimulatory effect of TGF- β 1 on hyaluronan synthesis, suggesting an important role for PKC or a related enzyme, but not for PtdIns 3-kinase or PLC- γ (Figure 4). The fact that the peak of stimulatory activity occurs later for TGF- β 1 compared with PDGF-BB [18], and that PKC appears to be important for the stimulations by both TGF- β 1 and PDGF-BB (Figures 1 and 3), led to the question of whether the effect of TGF- β 1 on hyaluronan synthesis is indirect via stimulation of PDGF-BB production. Such an induction of PDGF B-chain by TGF- β 1 has been reported to occur in AKR2B fibroblasts [29]. However, it is not likely that induction of PDGF accounts for the stimulatory effect of TGF- β 1 on hyaluronan synthesis in human fibroblasts, since anti-PDGF antibodies were without effect (Figure 6). The possibility has not been excluded, however, that TGF- β 1 induces other factors which in turn stimulate hyaluronan production.

The further characterization of the signal transduction pathways involved in stimulation of hyaluronan synthesis will be facilitated by cloning of cDNA for the enzyme, which will make studies on mRNA expression possible. Moreover, it would allow the production of recombinant hyaluronan synthetase, and make it possible to directly investigate the effects on enzyme activity of phosphorylation by different kinases. Information about the mechanism behind the stimulation of hyaluronan synthesis would make it possible to modulate hyaluronan synthesis in clinical conditions characterized by over- or under-production of hyaluronan.

We thank Professor Torvard Laurent for support and constructive criticism of this work, Dr. Lena Claesson-Welsh for advice on the PLC- γ assay, and Ingegärd Schiller for secretarial assistance. This work was supported in part by grants from the Swedish Cancer Society, Gustaf V:s 80-årsfond and the Swedish Medical Research Council (03X-4).

REFERENCES

- Laurent, T. C. and Fraser, J. R. E. (1992) *FASEB J.* **6**, 2397–2404
- Brecht, M., Mayer, U., Schlosser, E. and Prehm, P. (1986) *Biochem. J.* **239**, 445–450
- Turley, E. A. (1989) *Ciba Found. Symp.* **143**, 121–137
- West, D. C. and Kumar, S. (1989) *Ciba Found. Symp.* **143**, 187–207
- Kujawa, M. J., Pechak, D. G., Fizman, M. Y. and Caplan, A. I. (1986) *Dev. Biol.* **113**, 10–16
- Underhill, C. B. (1982) *J. Cell Sci.* **56**, 177–189
- Wasteson, Å., Westermark, B., Lindahl, U. and Pontén, J. (1973) *Int. J. Cancer* **12**, 169–178
- Toole, B. P. (1990) *Curr. Opin. Cell Biol.* **2**, 839–844
- Knudson, C. B. and Knudson, W. (1993) *FASEB J.* **7**, 1233–1241
- Nemec, R. E., Toole, B. P. and Knudson, W. (1987) *Biochem. Biophys. Res. Commun.* **149**, 249–257
- Asplund, T. and Heldin, P. (1994) *Cancer Res.* **54**, 4516–4523
- Knudson, W., Biswas, C., Li, X.-Q., Nemec, R. E. and Toole, B. P. (1989) *Ciba Found. Symp.* **143**, 150–169
- Asplund, T., Versnel, M., Laurent, T. C. L. and Heldin, P. (1993) *Cancer Res.* **53**, 388–392
- Prehm, P. (1983) *Biochem. J.* **211**, 181–189
- Prehm, P. (1983) *Biochem. J.* **211**, 191–198
- Heldin, P., Laurent, T. C. and Heldin, C.-H. (1989) *Biochem. J.* **258**, 919–922
- Honda, A., Noguchi, N., Takehara, H., Ohashi, Y., Asuwa, N. and Mori, Y. (1991) *J. Cell Sci.* **98**, 91–98
- Heldin, P., Asplund, T., Ytterberg, D., Thelin, S. and Laurent, T. C. (1992) *Biochem. J.* **283**, 165–170
- Heldin, C.-H. and Westermark, B. (1990) *Cell Regul.* **1**, 555–566
- Raines, E. W., Bowen-Pope, D. F. and Ross, R. (1990) *Handb. Exp. Pharmacol.* **95**, 173–262
- Roberts, A. B. and Sporn, M. B. (1990) *Handb. Exp. Pharmacol.* **95**, 419–472
- Miyazono, K., ten Dijke, P., Ichijo, H. and Heldin, C.-H. (1994) *Adv. Immunol.* **55**, 181–220
- Östman, A., Bäckström, G., Fong, N., Betsholtz, C., Wernstedt, C., Hellman, U., Westermark, B., Valenzuela, P. and Heldin, C.-H. (1989) *Growth Factors* **1**, 271–281
- Thyberg, J., Östman, A., Bäckström, G., Westermark, B. and Heldin, C.-H. (1990) *J. Cell Sci.* **97**, 219–229
- Engström-Laurent, A., Feltelius, N., Hällgren, R. and Wasteson, Å. (1985) *Ann. Rheum. Dis.* **44**, 614–620
- Kashishian, A. and Cooper, J. A. (1993) *Mol. Biol. Cell* **4**, 49–57
- Rönstrand, L., Mori, S., Arvidsson, A.-K., Eriksson, A., Wernstedt, C., Hellman, U., Claesson-Welsh, L. and Heldin, C.-H. (1992) *EMBO J.* **11**, 3911–3919
- Heldin, N.-E., Paulsson, Y., Forsberg, K., Heldin, C.-H. and Westermark, B. (1989) *J. Cell. Physiol.* **138**, 17–23
- Leof, E. B., Proper, J. A., Goustin, A. S., Shipley, G. D., Dicorleto, P. E. and Moses, H. L. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2453–2457
- Paulsson, Y., Hammacher, A., Heldin, C.-H. and Westermark, B. (1987) *Nature (London)* **328**, 715–717
- Berridge, M. J. and Irvine, R. F. (1984) *Nature (London)* **312**, 315–321
- Nishizuka, Y. (1988) *Nature (London)* **334**, 661–668
- Wennström, S., Siegbahn, A., Yokote, K., Arvidsson, A.-K., Heldin, C.-H., Mori, S. and Claesson-Welsh, L. (1994) *Oncogene* **9**, 651–660
- Fanti, W. J., Escobedo, J. A., Martin, G. A., Turck, C. W., del Rosario, M., McCormick, F. and Williams, L. T. (1992) *Cell* **69**, 413–423
- Kazlauskas, A., Kashishian, A., Cooper, J. A. and Valius, M. (1992) *Mol. Cell. Biol.* **12**, 2534–2544
- Graves, L. M., Bornfeldt, K. E., Raines, E. W., Potts, B. C., Macdonald, S. G., Ross, R. and Krebs, E. G. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10300–10304