Histol Histopathol (1999) 14: 295-304

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Invited Review

Activin: A novel player in tissue repair processes

G. Hübner[1], C. Alzheimer[2] and S. Werner[1]

[1]Max-Planck-Institut für Biochemie, Martinsried, Germany and [2]Physiologisches Institut der Universität, München, Germany

Summary. Recent studies have demonstrated a strong expression of activin in repair processes of various tissues and organs, including the skin, the lung, the intestine, the cardiovascular system, and even the brain. Although little is as yet known about the function of activin in tissue repair, first results suggest a role of activin in epithelial differentiation, fibroblast proliferation and expression of matrix molecules by these cells, and also in neuroprotection. Whereas a transient overexpression of activin after tissue injury might be beneficial for the repair process, sustained expression of activin could lead to fibrotic processes. Therefore, the modulation of the availability or biological activity of activin could be of particular importance for the treatment of impaired tissue repair on the one hand and tissue fibrosis on the other hand.

Key words: Activin, Inflammation, Injury, Tissue repair

Introduction

The members of the transforming growth factor ß (TGF-ß) superfamily are known to be involved in the control of cellular proliferation, differentiation and metabolism (for review see Massagué, 1990; Kingsley, 1994). Therefore, they play a key role during embryonic development and in tissue repair processes. This has been demonstrated in detail for the transforming growth factors ß1, ß2, and ß3 (for review see Roberts and Sporn, 1996). In addition, a series of studies has provided evidence for the involvement of other members of the TGF-ß superfamily - bone morphogenetic proteins, activins, and inhibins - in embryonic development. By contrast, little is known about the role of these members in tissue repair processes. The injury response resembles basic patterns of embryonic development, in that certain molecules which are expressed in development of a particular tissue or organ are reexpressed or upregulated after tissue injury. Since activins are known to be widely expressed during embryonic development, and are involved in early mesoderm induction as well as in organ formation (for review see Ying et al., 1997), we speculated on a possible role of activin in wound repair. Like the other members of the TGF-ß superfamily, activins are dimeric proteins consisting of two disulphide-linked $ß_A$ and $ß_B$ subunits. Homomers (activin A: $ß_Aß_A$; activin B: $ß_Bß_B$) as well as heteromers (activin AB: $ß_Aß_B$) composed of these ß-chains have been identified *in vitro* and *in vivo* (for review see Vale et al., 1990). The closely related ß-chains can also form dimers with a more distantly related α-chain generating inhibin A ($αß_A$) and inhibin B ($αß_B$). In some systems activins and inhibins act as functional antagonists (Ling et al., 1986; Vale et al., 1986). Additional isoforms may arise from the recently cloned $ß_C$-, $ß_D$-, and $ß_E$-chains (Hötten et al., 1995; Oda et al., 1995; Fang et al., 1996).

Cellular responses to activin are mediated by a heteromeric receptor complex composed of two different types of transmembrane receptors with intracellular serine/threonine kinase activity (for review see Massagué, 1996). Activin type II receptors (ARII, $ARII_B$) are constitutively active kinases and - after recruitment of the ligand - they phosphorylate a type I receptor (ARI, ARI_B) which then mediates signal transduction (Wrana et al., 1994; Attisano et al., 1996). Follistatin, a soluble activin binding protein unrelated to the activin receptors, presumably inhibits activin function after binding (de Winter et al., 1996; for review see Mather, 1996).

Activins and activin binding proteins have been localized in various organs during embryonic development of mammals (Roberts et al., 1991; Feijen et al., 1994; Roberts and Barth, 1994; Verschueren et al., 1995; Harkness and Baird, 1997). However, expression of the ligands is significantly downregulated in most of these tissues after completion of development. A series of recent studies from our laboratories has demonstrated a significant reexpression of activin after injury of adult tissue (Hübner et al., 1996a, 1997; Tretter et al., 1996). These results strongly indicate a novel and important role of activin in tissue repair processes, and these data will be summarized in our review.

Offprint requests to: Sabine Werner, Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, D-82152 Martinsried, Germany. Fax: 49-89-8578 2814. e-mail: werner@biochem.mpg.de

The expression of activin during wound repair of the skin

Recent studies using knockout mice have suggested a role of activin in skin morphogenesis. Thus, mice missing the $ß_A$-chain lacked whiskers and had abnormal whisker follicles (Matzuk et al., 1995a). Furthermore, mice deficient in follistatin showed disturbed whisker development (Matzuk et al., 1995b). Their skin was hyperkeratotic, as indicated by the thickened granular layer and stratum corneum. It appeared taut and shiny, closely resembling the skin phenotype of mice with directed overexpression of TGF-ß1 (Sellheyer et al., 1993). In contrast, activin $ß_B$-deficient mice showed no obvious skin phenotype (Schrewe et al., 1994; Vassalli et al., 1994).

Since activin is expressed in embryonic skin but is hardly detectable in the skin of newborn and adult rodents (Roberts et al., 1991; Roberts and Barth, 1994; Hübner et al., 1996a), we speculated that activin might be reexpressed during repair processes of the skin. Using RNase protection assays we found a strong induction of activin mRNA expression within the first day after injury in a full-thickness excisional wound model in mice (Hübner et al., 1996a), whereby the induction of $ß_A$ mRNA expression (Fig. 1, upper panel) was much more prominent than the induction of $ß_B$ mRNA expression (Fig. 1, lower panel). Whereas the activin $ß_A$ mRNA expression had declined to basal levels two weeks after injury when the proliferative phase was completed, the $ß_B$ mRNA expression was still high at this time point. These observations implicate a role of activin $ß_A$ and $ß_B$ in the inflammatory and proliferative phases of the wound healing process, and, beyond that, the involvement of activin $ß_B$ in the remodeling phase. In contrast, expression of the α-chain could not be detected.

All known activin receptors were expressed in normal skin. However, no significant induction of activin receptor expression was observed after skin injury (Hübner et al., 1996a). Therefore, the function of activin in wound repair seems to be controlled by the regulation of ligand expression, whereas the receptors are expressed constitutively.

We were able to localize the activin $ß_A$ mRNA in the granulation tissue and in the dermis at the wound edge (Fig. 2). These cells presumably represent fibroblasts and activated macrophages which both express activin *in vitro* (Erämaa et al., 1992; Shao et al, 1992; Hübner and Werner, 1996). In contrast, activin $ß_B$ mRNA was detected in the hyperproliferative epithelium at the wound edge and in migrating keratinocytes (Fig. 3).

Possible inducers of activin expression in the skin

To identify possible mediators of activin induction during skin repair, we analysed the regulation of activin

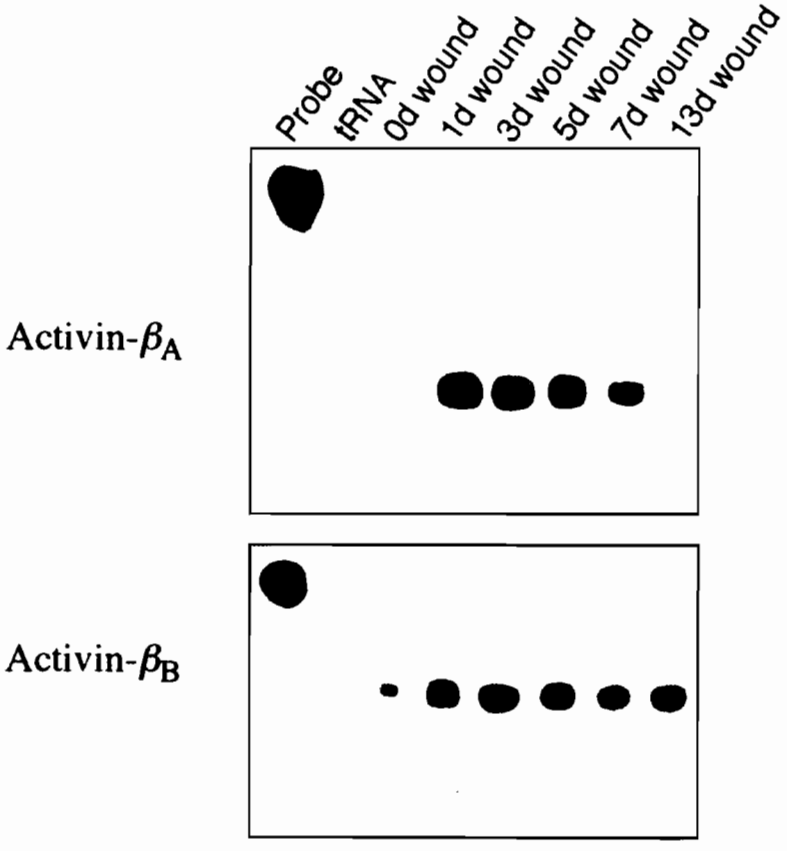

Fig. 1. Expression of activin mRNA in normal and wounded skin. 50 μg total cellular RNA from normal mouse back skin and excisional back skin wounds were analyzed by RNase protection assay for expression of activin $ß_A$ and $ß_B$ chains. The time in days (d; 1, 3, 5, 7, 13) after injury is indicated at the top of each lane. 0d wound refers to nonwounded back skin. For each time point a set of three animals was used. 1000cpm of the hybridization probes were added to the lanes labeled "probe". 50 μg tRNA were used as a negative control. The gels were exposed for 2 days. Reprinted from Hübner et al. (1996a) with permission.

expression in cultured keratinocytes and fibroblasts (Hübner and Werner, 1996). All factors investigated are shown in Table 1. Since activin induction was already detectable 15h after injury, serum growth factors which are released upon hemorrhage could be responsible for the activin induction. Indeed, activin $ß_A$ expression was very low in cultured quiescent fibroblasts and keratinocytes, but was strongly induced upon stimulation with serum or purified growth factors such as TGF-$ß_1$, epidermal growth factor (EGF) or platelet-derived growth factor (PDGF). Furthermore, a strong activin induction by proinflammatory cytokines such as

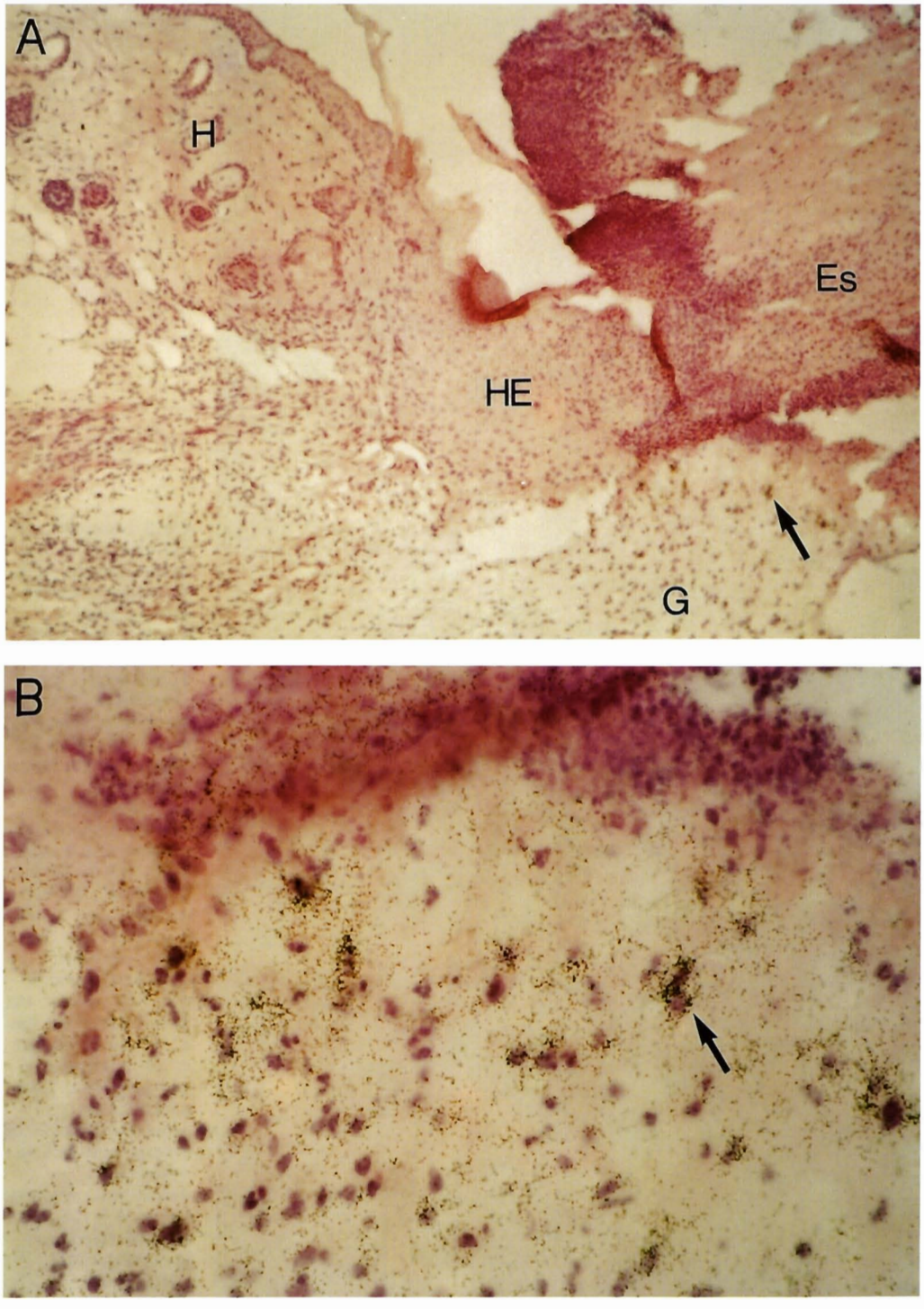

Fig. 2. Localization of activin $ß_A$ mRNA in a 5-day wound by in situ hybridization. Paraffin sections were hybridized with a ^{35}S-labeled mouse activin $ß_A$ antisense riboprobe. A hematoxylin/eosin stain of half of the wound is shown in **A**. The arrow indicates the granulation tissue below the wound where the photograph in **B** was taken. In **A** the letters H, HE, Es, and G indicate hair follicle, hyperproliferative epithelium, eschar, and granulation tissue. The silver grains produced by the radioactive probe appear as black dots. Note the high expression of activin $ß_A$ in a certain population of cells in the granulation tissue below the eschar. A, x 100; B, x 400. Reprinted from Hübner et al. (1996a) with permission.

interleukin (IL) 1ß and tumor necrosis factor (TNF) α was observed.

To determine if these cytokines could be activin inducers *in vivo*, we investigated in detail the time course and localization of their expression during the healing process (Hübner et al., 1996b). These studies revealed a strikingly increased expression of IL-1α, IL-1ß, and TNFα after skin injury. Their temporal expression pattern correlated with the expression of activin $ß_A$ during wound repair. We demonstrated that

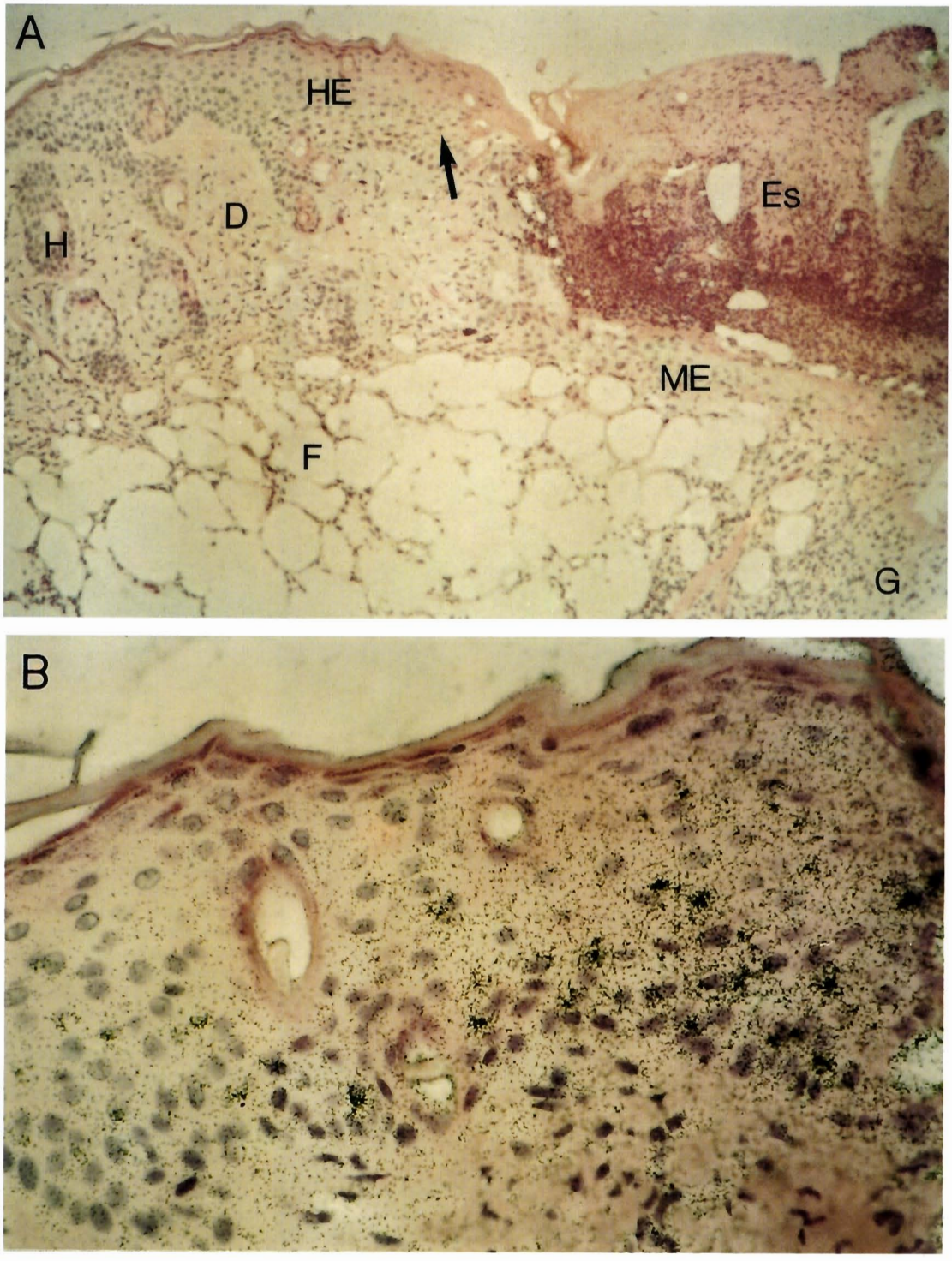

Fig. 3. Localization of activin $ß_B$ mRNA in a 5-day wound by in situ hybridization. Paraffin sections were hybridized with a ^{35}S-labeled mouse activin $ß_B$ antisense riboprobe. A hematoxylin/eosin stain of half of the wound is shown in **A**. The arrow indicates the hyperproliferative epithelium where the photograph in **B** was taken. In **A** the letters D, H, F, HE, ME, Es, and G indicate dermis, hair follicle, fatty tissue, hyperproliferative epithelium, migrating epithelium, eschar, and granulation tissue. The silver grains produced by the radioactive probe appear as black dots. Note the high expression of activin $ß_B$ in keratinocytes of the hyperproliferative epithelium close to the wound. A, x 100; B, x 400. Reprinted from Hübner et al. (1996a) with permission.

these cytokines were predominantly expressed by polymorphonuclear leukocytes (PMLs) during the early inflammatory phase (5-24h after injury) and at later stages by macrophages. The overlapping spatial and temporal expression patterns of activin and pro-inflammatory cytokines strongly suggest that these cytokines might also be inducers of activin expression in keratinocytes and fibroblasts *in vivo*.

In addition to keratinocytes and fibroblasts, macrophages are likely to be another source of activin in the wound tissue. Expression of activin in these cells might be stimulated by bacterial lipopolysaccharide (LPS) and inducers of macrophage maturation which markedly enhance the production of activin $ß_A$ in monocytes *in vitro* (Erämaa et al., 1992; Shao et al., 1992).

A hypothetical model of activin action during wound repair

From these data we constructed a hypothetical model which is shown in Fig. 4, illustrating the regulation of activin expression in a wound. Immediately after injury, growth factors (EGF, PDGF, TGF-ß1) are released from platelets upon hemorrhage. During the early inflammatory phase these blood-derived factors together with proinflammatory cytokines (IL-1ß, IL-6, TNFα) released from PMLs are likely to initiate the large induction of activin expression seen after skin injury in fibroblasts and keratinocytes. During the subsequent proliferative phase of the repair process cytokines and growth factors secreted by activated macrophages and growth factor-stimulated fibroblasts could sustain activin expression in the wound tissue. Finally, macrophages themselves might contribute to the activin production in the wound.

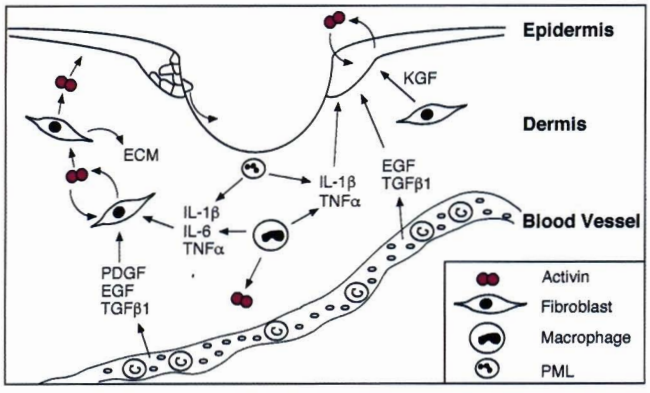

Fig. 4. Hypothetical model illustrating the regulation of activin expression during wound repair. A wound during the proliferative phase (3-5 days after injury) is shown schematically. The activin protein is shown in pink colour as a dimer. Growth factors and cytokines derived from the blood, from polymorphonuclear leukocytes, and from macrophages induce activin expression in fibroblasts and keratinocytes. Activated macrophages themselves contribute to the high activin levels in the wound. Activin might influence the production of extracellular matrix in the dermis and the differentiation of the new epithelium. EGF: epidermal growth factor; ECM: extracellular matrix proteins; IL: interleukin; KGF: keratinocyte growth factor; PDGF: platelet-derived growth factor; PML: polymorphonuclear leukocyte; TGF: transforming growth factor; TNF: tumor necrosis factor.

Possible function of activin during wound repair of the skin

So far we can only speculate about a possible function of activin during wound repair. Preliminary *in vitro* and *in vivo* data of our laboratory suggest a role of activin in connective tissue deposition and keratinocyte differentiation. Follistatin deficient mice show normal epidermal mitotic activity (Matzuk et al., 1995b) suggesting that - in contrast to TGF-ß1 (Sellheyer et al., 1993) - activin might not inhibit keratinocyte proliferation. However, these mice were characterized by hyperkeratosis, indicating that activin normally influences keratinocyte differentiation. Thus activin might also play a role in the redifferentiation process of the keratinocytes in the hyperproliferative epithelium at the wound edge.

Like TGF-ß1 (for review see Roberts and Sporn, 1996), activin might contribute to production of extracellular matrix in the dermis. Thus, Sugiyama et al. (1998) have recently shown that activin A enhances the level of type 1 collagen α1(I) mRNA in cultured kidney fibroblasts. Furthermore, activin stimulates human lung fibroblast proliferation at low concentrations and induces their differentiation into myofibroblasts (Ohga et al., 1996). This cell type is also abundant in the granulation tissue of wounds and is responsible not only for wound contraction but also for the synthesis of extracellular matrix (for review see Desmoulière, 1995). These findings also suggest that increased activin expression could be associated with fibrotic processes. Indeed, Matsuse et al. (1995, 1996) demonstrated the expression of activin A in murine lung injured with bleomycin and

Table 1 . Induction of activin A by growth factors and cytokines

	Balb/c FIBROBLASTS	HUMAN EMBRYONAL FIBROBLASTS	HaCaT KERATINOCYTES
FCS/NCS	+++++	+++	++++
TGFß1	++++	+++	+
KGF	n.d.	n.d.	+
bFGF	+++	n.d.	n.d.
EGF	+++	++	+++
PDGF	+++	++	-
EGF+PDGF	+++++	++++	n.d.
IL-1ß	+++	+++	+
TNF-α	++++	++++	+++
IL-6	++	+++	-

bFGF: basic fibroblast growth factor; EGF: epidermal growth factor; FCS: fetal calf serum; IL-1ß: interleukin 1-ß; IL-6: interleukin 6; KGF: keratinocyte growth factor; NCS: newborn calf serum; PDGF: platelet-derived growth factor; TGF-ß1: transforming growth factor ß1; TNFα: tumor necrosis factor α; n.d.: not done. Reprinted from Hübner et al. (1996b) with permission.

in human pulmonary fibrosis, and studies by Sugiyama et al. (1998) revealed increased levels of activin in cirrhotic livers. These fibrotic tissues are characterized by numerous myofibroblasts which produce excessive amounts of extracellular matrix (for review see Gabbiani, 1996), and activin might act as an important stimulator of this process. Thus, further investigations of activin expression in fibrotic diseases should determine whether activin could serve as a target for anti-fibrotic therapies.

Inflammation is generally accompanied by strong activin expression

The strong activin expression after injury in the skin raised the question of whether this is a tissue-specific phenomenon or rather a general feature of inflammatory processes as suggested by the detection of increased levels of activin in the lung after bleomycin injury (Matsuse et al., 1995). Therefore, we analysed the expression levels of activin in the normal human gut as well as in highly inflamed areas of patients suffering from inflammatory bowel disease (IBD) (Hübner et al., 1997). The latter comprises two major forms, Crohn's disease (CD) and ulcerative colitis (UC). These severe diseases are characterized by their chronic course and by infiltration of the intestinal tissue with activated neutrophils, macrophages and lymphocytes. The etiology of these diseases is still unknown and the factors involved in the pathogenesis of these disorders are poorly characterized (for review see Podolsky, 1991).

No activin expression was detected in normal intestinal tissue. However, expression of activin β_AmRNA was strongly upregulated in affected tissue of patients suffering from UC and CD (Hübner et al., 1997). Most importantly, the levels of activin expression strongly correlated with the degree of inflammation. In situ hybridization studies revealed the highest levels of activin β_A mRNA in the mucosa and submucosa of highly inflamed areas, particularly where the intestinal epithelium was damaged and where a massive accumulation of inflammatory cells was observed (Fig. 5). Furthermore, activin β_A mRNA was localized in a specific fibroblast subpopulation close to crypt abscesses, which are common in UC. Crypt abscesses are characterized by PMLs migrating across the epithelium and collecting in crypts to prevent bacterial immigration into the mucosa (Kumar et al., 1982; Yardley, 1986). These activin-expressing fibroblasts were found to have contact to a fibrin scaffold replacing the damaged epithelial barrier. The fibrin is probably deposited after local hemorrhage, which is often seen in

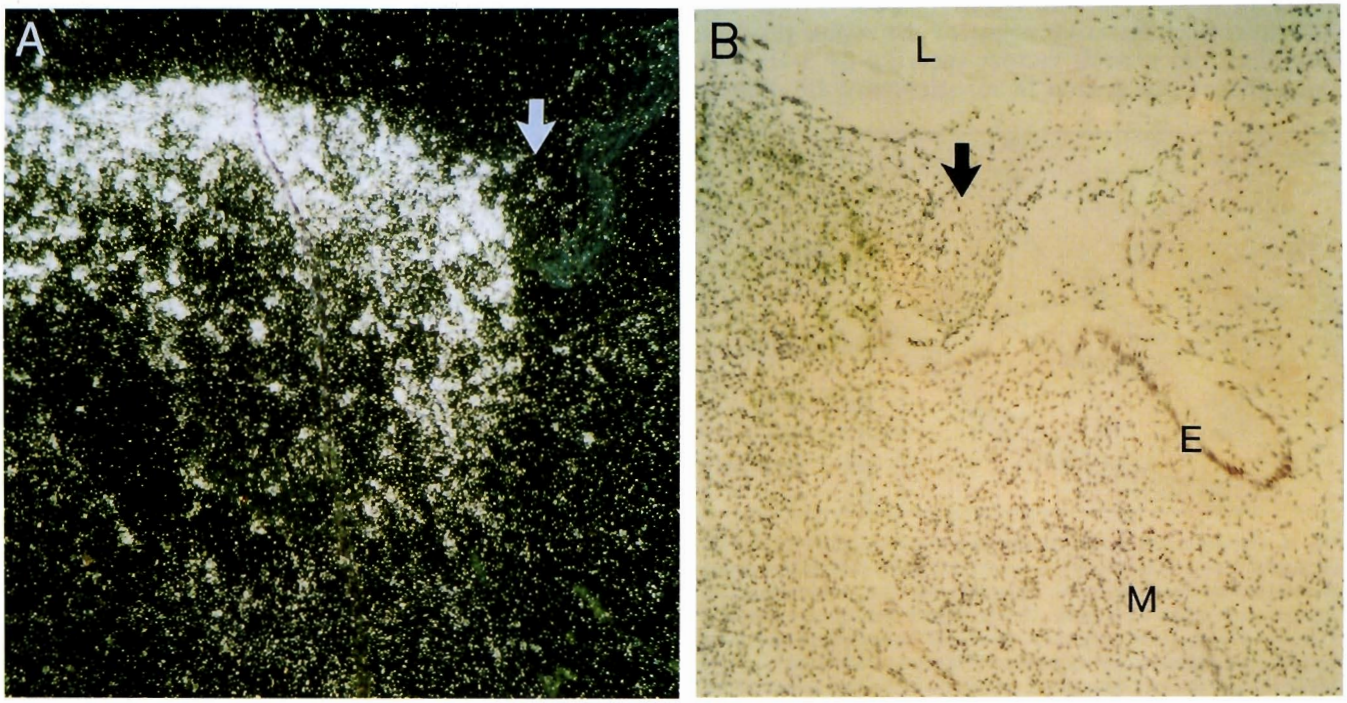

Fig. 5. Detection of activin β_A mRNA in the ileum of a CD patient by in situ hybridization. Surgical specimens were fixed in 4% paraformaldehyde and frozen in OCT. Frozen sections were hybridized with a ^{35}S-labeled human activin β_A antisense riboprobe and counterstained with hematoxylin and eosin. The section was taken from a region of the ileum where highly inflamed tissue (**A and B**, tissue on the left side of the arrow) could be clearly distinguished from relatively normal tissue (**A and B**, tissue on the right side of the arrow). Activin β_A mRNA expressing cells appear white in the dark field photomicrograph (A). E: intestinal epithelium; M: mesenchyme; L: intestinal lumen. x 100. Reprinted from Hübner et al. (1997) with permission.

UC and to a lesser extent in CD (Rubin and Farber, 1994). This situation closely resembles that in wound repair, where blood-derived growth factors and cytokines secreted by inflammatory cells are presumptive inducers of activin expression in fibroblasts. LPS is abundant in the gut lumen and could stimulate activin expression in macrophages in areas where the epithelial barrier is lost.

Activin could be involved in epithelial differentiation, as recently demonstrated for the gastric epithelium (Li et al., 1998). Furthermore, a role of activin in the stimulation of connective tissue deposition seems likely. Particularly in CD, fibrosis is a typical severe long-term complication, and myofibroblasts have been detected in the inflamed gut (Powell, 1994). Therefore, after stimulation by activin, these cells might be involved in fibrotic processes of this tissue. The strong expression of activin in IBD demonstrates that activin expression occurs not only after skin injury, but rather is a general feature of inflammatory processes.

Activin in vascular tissue repair - a possible role in arteriosclerosis

Since activin acts as a mitogen for smooth muscle cells alone and in a synergistic manner with other mitogens (Kojima et al., 1993; Pawlowski et al., 1997) but inhibits proliferation of vascular endothelial cells (McCarthy and Bicknell, 1993), a biological role of activin in injured vascular tissue and pathological processes has been suggested. Recently Pawlowski et al. (1997) demonstrated that angiotensin II and α-thrombin strongly induce activin A expression in quiescent rat aortic smooth muscle cells *in vitro*. Interestingly, they found a striking upregulation of activin mRNA expression within 6 hours after balloon injury of rat carotid arteries, and immunoreactive activin A protein was detected in the expanding neointima 7 and 14 days later. Both neointimal and medial smooth muscle cells were stained with the activin antibody (Pawlowski et al., 1997). Increased levels of activin were also seen in arteriosclerotic lesions of Watanabe heritable hyperlipidemic rabbits (Inoue et al., 1994). Thus activin seems to play a novel role in the response to vascular injury and accompanying neointimal formation. Surprisingly, expression of follistatin was also detected in the media and neointima of experimentally-induced arteriosclerotic lesions in rats and rabbits (Inoue et al., 1993, 1995), suggesting that at least some of the activin might be neutralized by follistatin. The roles of activin and follistatin in the injured artery are presently unknown. The excessive expression of activin at the site of injury might inhibit re-endothelialization and at the same time stimulate smooth muscle and adventitial fibroblast proliferation. Furthermore, activin has been shown to inhibit foam cell formation *in vitro* - a characteristic event in the early stage of arteriosclerosis, whereas follistatin counteracted this effect (Kozaki et al., 1997). Taken together, these data suggest that activin and follistatin could regulate cell proliferation and differentiation of various cell types which are involved in the pathogenesis of arteriosclerosis. However, the precise role of the activin/follistatin system in normal and pathological repair processes of the injured artery

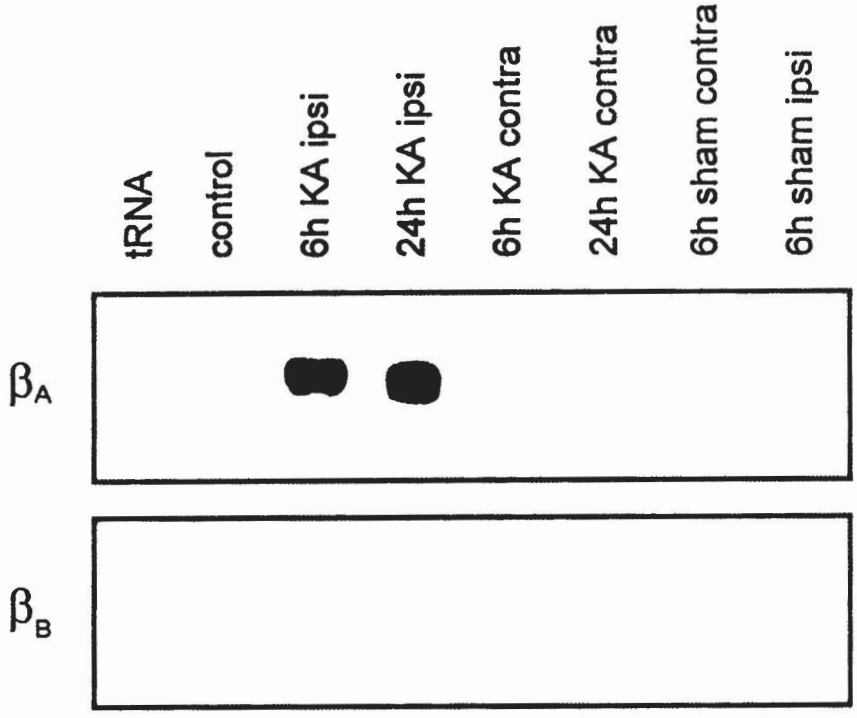

Fig. 6. Expression of activin mRNA in normal and lesioned hippocampi after intracerebroventricular kainic acid injection. Total RNA was isolated from ipsilateral (ipsi) and contralateral (contra) hippocampi at various time points after kainic acid injection as indicated and analyzed by RNase protection assay. For each time point a set of 6 animals was used. Hippocampi from PBS-injected (6h sham ipsi; 6h sham contra) and non-injected animals (control) were used as controls; 50μg tRNA were used as a negative control. For each lane 10μg of total hippocampal RNA were used. The gels were exposed for 2 days (β_A) and 8 days (β_B). Reprinted from Tretter et al. (1996) with permission.

remains to be elucidated.

The role of activin in brain lesion

Over the last decade, a large body of evidence has been accumulated showing that brain lesions upregulate the expression of various growth and differentiation factors, and that their temporal and spatial interplay is crucial for the orchestration of postlesional restructuring (Isackson, 1995). Recent data from our and other laboratories have identified activin as a putative novel player in the early neuronal response to brain injury. In a widely used model of local excitotoxic brain damage, where intracerebroventricular (icv) injection of kainic acid produces selective neuronal death in the hippocampal CA3 region, a striking induction of activin β_A subunit expression in mouse hippocampus was observed (Tretter et al., 1996). RNase protection assays performed at different times post lesion showed that β_A mRNA expression, which is virtually absent in adult hippocampus, was strongly upregulated within 6 h after injury and stayed elevated for approximately 24 h, before it declined to baseline (Fig. 6). Expression of β_B and α-chains was not affected by the lesion, suggesting that the β_A transcripts give rise to activin A, but not to other members of the activin/inhibin family. In situ hybridization and immunohistochemistry from lesioned hippocampal slices demonstrated the presence of β_A mRNA and activin protein, respectively, in neurons adjacent to the site of lesion (Tretter et al., 1996). Since all known activin receptors are present in hippocampus and since the endogenous activin inhibitor, follistatin, is expressed at very low levels in normal and lesioned hippocampus, activin is likely to be functionally active after brain injury (Tretter et al., 1996).

Compared to excitotoxic lesions, a temporally and spatially more complex expression pattern of activin/inhibin subunits was observed after hypoxic-ischemic insult in rat brain (Lai et al., 1996). In addition to the early transient induction of β_A subunit in neuronal cell bodies (resembling the one seen after excitotoxic lesion), two later waves of expression occur, one in the meningeal membrane and one around microvessels of the infarct zone. In both regions, increased β_A expression was closely associated with α-subunit induction, indicating that the late responses (3-7 days post lesion) are mediated by inhibin A, rather than by activin A (Lai et al., 1996).

Although the role of activin A in the early response to brain injury remains to be determined, several findings lend support to the notion that activin A might exert both neuroprotective and neurotrophic actions. Activin A has been shown to influence differentiation of developing neurons where it regulates neurotransmitter phenotype expression (Fann and Patterson, 1994; Darland et al., 1995). Furthermore, activin A promotes survival of midbrain and hippocampus neurons *in vitro* and protects cultured midbrain neurons against neurotoxic damage (Krieglstein et al., 1995; Iwahori et al., 1997). The mechanisms underlying the neurotrophic actions of activin A *in vitro* have not been elucidated in detail, but it appears that a rise in intracellular Ca^{2+}, presumably mediated by an increased Ca^{2+} influx through L-type Ca^{2+} channels, is an essential step in the cascade of events leading to improved neuronal survival in culture (Iwahori et al., 1997). Whether activin employs a similar mechanism in adult brain is not known at present. Future studies should particularly address the issue of whether activin A affects solely neuronal survival and growth, or whether it also promotes glial scar formation, like TGF-ß1 (Logan et al., 1994), thereby impeding neuronal regeneration.

It is noteworthy that β_A subunit induction in adult brain is not restricted to emergency situations such as excitotoxic or hypoxic/ischemic cell death where developmentally relevant programs are reactivated. Transiently increased β_A levels of presumed neuronal origin are also observed after strong synaptic excitation (Andreasson and Worley, 1995; Inokuchi et al., 1996) or after reversible mechanical irritation, giving rise to temporary neuronal hyperexcitability (Lai et al., 1997). An important aspect of these studies is that electrical stimulation paradigms which evoke long-term changes in synaptic efficacy (possibly representing the substrate of memory formation) are capable of inducing appreciable β_A expression (Andreasson and Worley 1995; Inokuchi et al., 1996). This suggests that activin A might not only play a role in brain injury, but might also contribute to neural plasticity during regular brain activity.

Acknowledgements. We would like to thank Thelma Coutts for help with the manuscript. Our work described in this manuscript was supported by the Max-Planck-Gesellschaft, the Deutsche Forschungsgemeinschaft, the German Ministry for Education and Research (BMBF), the Human Frontier Science Program and Boehringer Ingelheim Fellowship to G.H. S.W. is a Hermann-and-Lilly Schilling Professor of Medical Research. C.A. is a Heisenberg Fellow of the Deutsche Forschungsgemeinschaft.

References

Andreasson K. and Worley P.F. (1995). Induction of β_A activin expression by synaptic activity and during neocortical development. Neuroscience 69, 781-796.

Attisano L., Wrana J.L., Montalvo E. and Massagué J. (1996). Activation of signalling by the activin receptor complex. Mol. Cell. Biol. 16, 1066-1073.

Darland D.C., Link B.A. and Nishi R. (1995). Activin A and follistatin expression in developing targets of ciliary ganglion neurons suggests a role in regulating neurotransmitter phenotype. Neuron 15, 857-866.

Desmouliére A. (1995). Factors influencing myofibroblast differentiation during wound healing and fibrosis. Cell. Biol. Int. 19, 471-476.

de Winter J.P., ten Dijke P., de Vries C.J.M., van Achtenberg T.A.E., Sugino H., de Waele P., Huylebroeck D., Verschueren K. and van den Eijnden-van Raaij A.J.M. (1996). Follistatins neutralize activin bioactivity by inhibition of activin binding to its type II receptors. Mol.

Cell. Endocrinol. 116, 105-114.

Erämaa M., Hurme M., Stenman U.-H. and Ritvos O. (1992). Activin A/Erythroid differentiation factor is induced during human monocyte activation. J. Exp. Med. 176, 1449-1452.

Fang J.M., Yin W.S., Smiley E., Wang S.Q. and Bonadio J. (1996). Molecular cloning of the mouse activin beta (e) subunit gene. Biochem. Biophys. Res. Commun. 228, 669-674.

Fann M.-J. and Patterson P.H. (1994). Neuropoietic cytokines and activin A differentially regulate the phenotype of cultured sympathetic neurons. Proc. Natl. Acad. Sci. USA 91, 43-47.

Feijen A., Goumans M.J. and van den Eijnden-van Raaij A.J.M. (1994). Expression of activin subunits, activin receptors and follistatin in postimplantation mouse embryos suggests specific developmental functions for different activins. Development 120, 3621-3637.

Gabbiani G. (1996). The cellular derivation and the life span of the myofibroblast. Pathol. Res. Pract. 192, 708-711.

Harkness L.M. and Baird D.T. (1997). Morphological and molecular characteristics of living human fetuses between Carnegie stages 7 and 23: immunolocalization of inhibin α and β_A subunits. Human Rep. Update 3, 35-57.

Hötten G., Neidhardt H., Schneider C. and Pohl J. (1995). Cloning of a new member of the TGF-ß family: a putative new activin β_C chain. Biochem. Biophys. Res. Commun. 106, 608-613.

Hübner G. and Werner S. (1996). Serum growth factors and pro-inflammatory cytokines are potent inducers of activin expression in cultured fibroblasts and keratinocytes. Exp. Cell. Res. 228, 106-113.

Hübner G., Hu Q., Smola H. and Werner S. (1996a). Strong induction of activin expression after injury suggests an important role of activin in wound repair. Dev. Biol. 173, 490-498.

Hübner G., Brauchle M., Smola H., Madlener M., Fässler R. and Werner S. (1996b). Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. Cytokine 8, 548-556.

Hübner G., Brauchle M., Gregor M. and Werner S. (1997). Activin A: A novel player and inflammatory marker in inflammatory bowel disease? Lab. Invest. 77, 311-318.

Inokuchi K., Kato A., Hiraia K., Hishinuma F., Inoue M. and Ozawa F. (1996). Increase in activin β_A mRNA in rat hippocampus during long-term potentiation. FEBS Lett. 382, 48-52.

Inoue S., Orimo A., Hosoi T., Matsuse T., Hashimoto M., Yamada R., Ouchi Y., Orimo H. and Muramatsu M. (1993). Expression of follistatin, an activin-binding protein, in vascular smooth muscle cells and arteriosclerotic lesions. Arterioscler. Thromb. 13, 1859-1864.

Inoue S., Orimo A., Hosoi T., Igekami A., Kozaki K., Ouchi Y., Nomura S., Muramatsu M. and Orimo H. (1994). Demonstration of activin-A in arteriosclerotic lesions. Biochem. Biophys. Res. Commun. 205, 441-448.

Inoue S., Orimo A., Hosoi T., Matsuse T., Kozaki K., Ouchi Y., Muramatsu M. and Orimo H. (1995). Follistatin as an activin-binding protein expressed in arteriosclerotic lesions. Ann. Acad. Sci. NY 748, 530-533.

Isackson P.J. (1995). Trophic factor response to neuronal stimuli or injury. Curr. Opin. Neurobiol. 5, 350-357.

Iwahori Y., Saito H., Torii K. and Nishiyama N. (1997). Activin exerts a neurotrophic effect on cultured hippocampal neurons. Brain Res. 760, 52-58.

Kingsley D.M. (1994). The TGF-ß superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev. 8, 133-146.

Kojima I., Mogami H., Kawamura N., Yasuda H. and Shibata H. (1993). Modulation of growth of vascular smooth muscle cells by activin A. Exp. Cell Res. 206, 152-156.

Kozaki K., Akishita M., Eto M., Yoshizumi M., Toba K., Inoue S., Ishikawa M., Hashimoto M., Kodama T., Yamada N., Orimo H. and Ouchi Y. (1997). Role of activin-A and follistatin in foam cell formation of THP-1 macrophages. Arterioscler. Thromb. Vasc. Biol. 17, 1289-1394.

Kumar N.B., Nostrant T.T. and Appelman H.D. (1982). The histopathologic spectrum of acute self-limited colitis (acute infectious-type colitis). Am. J. Surg. Pathol. 6, 523-529.

Krieglstein K., Suter-Crazzolara C., Fischer W.H. and Unsicker K. (1995). TGF-ß superfamily members promote survival of midbrain dopaminergic neurons and protect them against MPP^+ toxicity. EMBO J. 14, 736-742.

Lai M., Sirimanne E., Williams C.E. and Gluckman P.D. (1996). Sequential patterns of inhibin subunit gene expression following hypoxic-ischemic injury in the rat brain. Neuroscience 70, 1013-1024.

Lai M., Gluckman P., Dragunow M. and Hughes P.E. (1997). Focal brain injury increases activin β_A mRNA expression in hippocampal neurons. NeuroReport 8, 2691-2694.

Li Q., Karam S.M., Coerver K.A., Matzuk M.M. and Gordon J.I. (1998). Stimulation of activin receptor II signaling pathways inhibits differentiation of multiple gastric epithelial lineages. Mol. Endocrinol. 12, 181-192.

Ling N., Ying S.-Y., Ueno N., Shimasaki S., Esch F., Hotta M. and Guillemin R. (1986). Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. Nature 321, 779-782.

Logan A., Berry M., Gonzales A.M., Frautschy S.A., Sporn M.B. and Baird A. (1994). Effects of transforming growth factor β_1 on scar production in the injured central nervous system of the rat. Eur. J. Neurosci. 6, 355-363.

Massagué J. (1990). The transforming growth factor-ß family. Annu. Rev. Cell. Biochem. 6, 597-641.

Massagué J. (1996). TGFß signaling: Receptors, transducers, and mad proteins. Cell 85, 947-950.

Mather J.P. (1996). Follistatins and α_2-macroglobulin are soluble binding proteins for inhibin and activin. Horm. Res. 45, 207-210.

Matsuse T., Fukuchi Y., Eto Y., Matsui H., Hosoi T., Oka T., Ohga E., Nagase T. and Orimo H. (1995). Expression of immunoreactive and bioactive activin A protein in adult murine lung after bleomycin treatment. Am. J. Respir. Cell Mol. Biol. 33, 17-24.

Matsuse T., Ikegami A., Ohga E., Hosoi T., Oka T., Kida K., Fukayama M., Inoue S., Nagasa T., Ouchi Y. and Fukuchi Y. (1996). Expression of immunoreactive activin A protein in remodeling lesions associated with intestinal pulmonary fibrosis. Am. J. Pathol. 148, 707-713.

Matzuk M.M., Kumar T.R., Vassalli A., Bickenbach J.R., Roop D.R., Jaenisch R. and Bradley A. (1995a). Functional analysis of activins during mammalian development. Nature 274, 354-356.

Matzuk M.M., Lu N., Vogel H., Sellheyer K., Roop D.R. and Bradley A. (1995b). Multiple defects and perinatal death in mice deficient in follistatin. Nature 374, 360-363.

McCarthy S. and Bicknell R. (1993). Inhibition of vascular endothelial cell growth by activin A. J. Biol. Chem. 268, 23066-23071.

Oda S., Nishimatsu S.-I., Murakami K. and Ueno N. (1995). Molecular cloning and functional analysis of a new activin ß subunit: A dorsal

mesoderm-inducing activity in Xenopus. Biochem. Biophys. Res. Commun. 210, 581-588.

Ohga E., Matsuse T., Teramoto S., Katayama H., Nagase T., Fukuchi Y. and Ouchi, Y. (1996). Effects of activin A on proliferation and differentiation of human lung fibroblasts. Biochem. Biophys. Res. Commun. 228, 391-396.

Pawlowski J.E., Taylor D.S., Valentine M., Hail M.E., Ferrer P. and Kowala M.C. (1997). Stimulation of activin A expression in rat aortic smooth muscle cells by thrombin and angiotensin II correlates with neointimal formation in vivo. J. Clin. Invest. 100, 639-648.

Podolsky D.K. (1991). Inflammatory bowel disease. N. Engl. J. Med. 325, 928-937.

Powell D.W. (1994). Neuroimmunophysiology of the gastrointestinal mucosa: implications for inflammatory diseases. Trans. Am. Clin. Climatol. Assoc. 106, 124-138.

Roberts V.J. and Barth S.L. (1994). Expression of messenger ribonucleic acids encoding the inhibin/activin system during mid- and late-gestation rat embryogenesis. Endocrinology 134, 914-923.

Roberts V.J., Sawchenko P.E. and Vale W. (1991). Expression of inhibin/activin subunit mRNAs during rat embryogenesis. Endocrinology 128, 3122-3129.

Roberts A.B. and Sporn M.B. (1996). Transforming growth factor-ß. The molecular and cellular biology of wound repair. 2nd ed. Clark R.A.F. (ed). Plenum Press. New York. pp 275-308.

Rubin E. and Farber J.L. (1994). Inflammatory bowel disease. In Pathology. Rubin E. and Farber J.L. (eds). J.B. Lippincott company. Philadelphia. pp 675-683.

Schrewe H., Gendron-Maguire M., Harbison M.L. and Gridley T. (1994). Mice homozygous for a null mutation of activin $ß_B$ are viable and fertile. Mech. Dev. 47, 43-51.

Sellheyer K., Bickenbach J.R., Rothnagel J.A., Bundman D., Longley M.A., Krieg T., Roche N.S., Roberts A.B. and Roop D.R. (1993). Inhibition of skin development by overexpression of transforming growth factorß1 in the epidermis of transgenic mice. Proc. Natl. Acad. Sci. USA 90, 5237-5241.

Shao L., Frigon N.L., Sehy D.W., Yu A.L., Lofgren J., Schwall R. and Yu J. (1992). Regulation of production of activin A in human marrow stromal cells and monocytes. Exp. Hematol. 20, 1235-1242.

Sugiyama M., Ichida T., Sato T., Ishikawa T., Matsuda Y. and Asakura H. (1998). Expression of activin A is increased in cirrhotic and fibrotic rat livers. Gastroenterology 114, 550-558.

Tretter Y.P., Munz B., Hübner G., ten Bruggencate G., Werner S. and Alzheimer C. (1996). Strong induction of activin expression after hippocampal lesion. NeuroReport 7, 1819-1823.

Vale W., Rivier J., Vaughan J., McClintock R., Corrigan A., Woo W., Karr D. and Spiess J. (1986). Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 321, 776-779.

Vale W., Hsueh A., Rivier C. and Yu J. (1990). The inhibin/activin family of hormones and growth factors. In: Peptide growth factors and their receptors II. Sporn M.B. and Roberts A.B. (eds). Springer-Verlag. Berlin. pp 211-248.

Vassalli A., Matzuk M.M., Gardner H.A.R., Lee K.-F. und Jaenisch R. (1994). Activin/inhibin $ß_B$ subunit gene disruption leads to defects in eyelid development and female reproduction. Genes Dev. 8, 414-427.

Verschueren K., Dewulf N., Goumans M.-J., Lonnoy O., Feijen A., Grimsby S., Spiegle K.V., ten Dijke P., Morén A., Vanscheeuwijck P., Heldin C.-H., Miyazono K., Mummery C., van den Eijnden-van Raaij A.J.M. and Huylebroeck D. (1995). Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. Mech. Dev. 52, 109-123.

Wrana J.L., Attisano L., Wieser R., Ventura F. and Massagué J. (1994). Mechanism of activation of the TGFß receptor. Nature 370, 341-347.

Yardley J.H. (1986). Pathology of idiopathic inflammatory bowel disease and relevance of specific cell findings: an overview. In: Recent developments in the therapy of inflammatory bowel disease, Proceedings of a symposium. Myerhoff Center for Digestive Disease at Johns Hopkins. Baltimore, MD. pp 3-9.

Ying S.-Y., Zhang Z., Furst B., Batres Y., Huang G. and Li G. (1997). Activins and activin receptors in cell growth. Proc. Soc. Exp. Biol. Med. 214, 114-122.

Accepted July 14, 1998