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Mechanisms for TGF-ß-Mediated Regulation of the Actin Filament System and Apoptosis

BY

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ABSTRACT

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Transforming growth factor-β (TGF-β) is a member of a large superfamily of cytokines which participate in many different types of cellular processes, such as growth inhibition, cell migration, differentiation, cell adhesion, wound healing and immunosuppression. Alterations of TGF-β superfamily signalling results in several different disorders, including bone disease, vascular disease and cancer. The TGF-β signalling pathways involve several different proteins, such as the Smad proteins, which upon receptor activation are translocated to the nucleus, where they affect transcriptional responses.

The actin cytoskeleton is an organised network of filaments with a highly dynamic structure, which is under a continuous reconstruction to control the morphology, survival, growth and motility of eukaryotic cells. The members of the family of small GTP-binding proteins have been shown to be important regulators of the actin cytoskeleton.

TGF-β was found to induce short term as well as long term actin reorganisation in prostate cancer cells. The short term response included membrane ruffling, and required signalling by the small GTPases Cdc42 and Rho as well as, the involvement of the mitogen-activated protein kinases p38 (p38 MAPK). The long term response included formation of stress fibers and required a cooperation between Smad and Rho GTPase signalling pathways involving the Rho-associated coiled-coil-containing protein kinase 1 (ROCK1).

The TGF-β-induced activation of Cdc42 was, furthermore, shown to require the inhibitory Smad7 and p38 MAP kinase, via a PI3K-dependent pathway. Mixed lineage kinase 3 (MLK3), a mediator downstream of Cdc42, was necessary for the Cdc42-dependent actin filament reorganisation.

Apoptosis is an important and carefully regulated process in human development and disease, which allows the multicellular organisms to remove cells that are in excess or potentially dangerous. TGF-β family members can induce apoptosis in many different cell types, in the presence or absence of other growth factors. Smad7 had previously been shown to be necessary for TGF-β-induced apoptosis of epithelial cells. We could show that Smad7 is required for TGF-β-induced activation of the TGF-β activated kinase 1 (TAK1)-mitogen-activated protein kinase kinase 3 (MKK3)-p38 MAPK pathway, which subsequently leads to apoptosis in prostate cancer cells.

Members of the lymphoid enhancer factor-1/T-cell factor (LEF1/TCF) family of transcription factors have, together with β-catenin, been shown to be nuclear effectors in the Wnt-signalling pathway. We investigated a possible cross-talk between the TGF-β and Wnt signalling pathways. We found that TGF-β, in a Smad7-dependent manner induced a nuclear accumulation of β-catenin and enhanced the transcriptional activity of β-catenin and the induction of the downstream target gene *c-myc*. Since β-catenin and c-Myc has been shown to promote apoptosis, our results suggests the possibility that β-catenin contributes to TGF-β-induced apoptosis

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I. **Edlund, S.,** Landström, M., Heldin, C.-H., and Aspenström, P. (2002) Transforming growth factor-β-induced mobilization of actin cytoskeleton required signaling by small GTPases Cdc42 and RhoA. *Mol Biol Cell* **13**, 902-914.

II. **Edlund, S.,** Landström, M., Heldin, C.-H., and Aspenström, P. Smad7 is required for TGF-β-induced activation of the small GTPase Cdc42. Manuscript.

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IV. **Edlund, S*.,** Tagami, S*., Kozakai, T., Aspenström, P., Heldin, C.-H., and Landström, M. TGF-β promotes nuclear accumulation and activation of β-catenin in a Smad7 dependent manner.
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ABBREVIATIONS

ACK	activating Cdc42-associated tyrosine kinase
ALK	activin receptor-like kinase
AMH	anti-Müllerian hormone
APC	adenomatous polyposis coli
ASK1	apoptosis signal-regulating kinase 1
ATF2	activating transcription factor 2
BMPs	bone morphogenetic proteins
CRIB	Cdc42/Rac interactive binding
Dab-2	disabled-2
Dia	diaphanous
EGF	epidermal growth factor
EMT	epithelial to mesenchymal transdifferentiation
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FGF	fibroblast growth factor
GAPs	GTPase-activating proteins
GDIs	guanine dissociation inhibitors
GEFs	GDP/GTP exchange factors
GSK-3	glycogenesynthetase kinase-3
HGF	hepatocyte growth factor
HPK1	hematopoietic progenitor kinase-1
HSP27	heat shock protein 27
LPA	lysophosphatidic acid
LEF1/TCF	lymphoid enhancer-binding factor 1/T-cell specific factor
MAPK	mitogen-activated protein kinase
MKK	mitogen-activated protein kinase kinase
MLK3	mixed lineage kinase 3
PAK	p21-activated kinase
PAR-6	partitioning defective-6
PDGF	platelet-derived growth factor
PI3K	phosphatidylinositol-3-OH kinase
Pix	PAK-interacting exchange factor
ROCK	Rho-associated coiled-coil-containing protein kinase
SAPK/JNK	stress-activated protein kinase/Jun N-terminal kinase
SARA	smad anchor for receptor activation
TAB1	TAK1 binding protein
TAK1	TGF-β-activated kinase 1
TβR	TGF-β receptor
TCF-4	T-cell factor-4
TGF-β	transforming growth factor-β
TNF	tumor necrosis factor
TRAP-1	TGF-β receptor-associated protein-1
WASP	Wiskott-Aldrich syndrome protein
XIAP	X-chromosome-linked inhibitor of apoptosis protein

INTRODUCTION

The communication between different cells in the human body, through highly defined signalling networks preserved throughout the evolution, is crucial for the development from a single cell in the early embryo to the fully developed human being. Perturbations of these signalling networks cause pathological conditions, resulting in defective development of an organism, often leading to premature death or physiological disability and disease. The cell-cell communication is achieved through direct cell-cell interaction, cell contact with extracellular matrix components or by the binding of secreted soluble signalling factors to specific receptors at the plasma membrane. The soluble signalling factors involve neurotransmitters, cytokines, hormones and growth factors, such as the transforming growth factor-β (TGF-β). Signalling is then achieved through the receptors to the cell interior, where different signalling cascades convey signals to the nucleus or to the cytoskeleton resulting in specific cellular responses.

Members of the TGF-β family are involved in many different types of cellular processes, such as, growth inhibition, migration, differentiation, adhesion, wound healing, apoptosis and immunosuppression. TGF-β has a dual role during tumorigenesis, in the early phase acting as a tumor suppressor, but in the later phase stimulating cancer progression. Cancer cells are often refractile to growth inhibition either because of genetic loss of TGF-β-signalling components or, more commonly, because of downstream perturbation of signalling pathways, often involving activation of the Ras family of proto-oncogens. Mutations in TGF-β receptors as well as in Smad proteins have been found in tumors. In addition, defects in the TGF-β signalling pathway have been associated with different developmental disorders. Therapeutic approaches should aim at inhibiting the late TGF-β-induced invasive phenotype, but also to retain the growth-inhibitory and pro-apoptotic effects.

The actin cytoskeleton is an organised network of actin filaments with highly dynamic organisation, which is under a continuous reconstruction and, together with myosin and a huge number of actin-binding proteins it controls the morphology, motility, growth, apoptosis and survival of eukaryotic cells. Cell locomotion plays a key role in normal physiology, for organ development and remodeling, wound healing, as well as during disease. Protein tyrosine kinase receptors, such as the receptors for epidermal growth factor (EGF) or platelet-derived growth factor (PDGF), have been known for two decades to be potent regulators of the actin cytoskeleton. The correlation between TGF-β signalling and the dynamic organisation of the actin filament system have been less characterised.

Apoptosis, also called programmed cell death, is an important and carefully regulated process in human development and disease. The death program allows the multicellular organisms to remove cells that are in excess or potentially dangerous. The coordination and balance between cell survival and apoptosis is crucial for normal development and homeostasis of multicellular organisms. Defects in the control of this balance may contribute to a variety of diseases, including cancer, autoimmune diseases and neurodegenerative conditions. TGF-β is an important component in the cell death

program of a large number of different cell types. However, the molecular mechanisms underlying TGF-β-dependent apoptosis are still not clear.

The aim of this thesis was to examine the effects of TGF-β on the organisation of the actin cytoskeleton and to determine the molecular mechanism whereby these effects are exerted. A second aim was to determine the molecular mechanisms whereby TGF-β induces apoptosis.

1. Transforming growth factor-β (TGF-β)

TGF-β is a member of a large superfamily of cytokines, including activins, inhibins, nodals, leftys, bone morphogenetic proteins (BMPs), anti-Müllerian hormone (AMH) also known as Müllerian inhibiting substance (MIS) and growth and differentiation factors (GDFs) (Heldin *et al*., 1997; Massagué, 2000; Chang *et al*., 2002). All of these growth factors participate in many different types of cellular processes, such as, growth inhibition, cell migration, differentiation, cell adhesion, wound healing, apoptosis and immunosuppression (Massagué and Wotton, 2000).

TGF-β was originally identified by de Larco and Todaro in the late seventies. They discovered that a pool of polypeptide growth factors released from mouse fibroblasts transformed with murine arcoma virus, were able to induce foci formation of cells in a soft agar assay (De Larco and Todaro, 1978). Later it was found that theses active factors consisted of two distinct polypeptide growth factors, transforming growth factor (TGF)-α and -β (Roberts *et al*., 1981; Anzano *et al*., 1983). TGF-α belongs to the EGF family of ligands and was shown to have a mitogenic activity, whereas TGF-β served as a potent regulator of cell proliferation and differentiation in most cell types (Roberts and Sporn, 1990, 1993).

a) TGF-β superfamily of ligands

TGF-β

There exist three different isoforms of TGF-β, TGF-β1 (Derynck *et al*., 1985), TGF-β2 (de Martin *et al*., 1987; Madisen *et al*. 1988), and TGF-β3 (Dernyck *et al*., 1988). They are all 25 kDa homodimers, but heterodimers between TGF-β1 and TGF-β2, and between TGF-β2 and TGF-β3, have also been reported (Cheifetz *et al*., 1987; Ogawa *et al*., 1992). TGF-β is a potent growth inhibitor for most cells types, including fibroblasts, epithelial cells, lymphoid cells, neuronal cells, osteoblast and hematopoietic cells. However, TGF-β has also been shown to regulate cell proliferation and differentiation of mesenchymal cells (Roberts and Sporn, 1990, 1993). TGF-βs tightly regulates the production of the extracellular matrix (ECM) and are involved in wound healing and immunosuppression (Roberts and Sporn, 1990, 1993; Hartsough and Mulder, 1997, Roberts, 1998). Deregulated TGF-β signalling has also been implicated in different human diseases including vascular diseases, fibrosis, autoimmune diseases, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and cancer (reviewed in Blobe *et al*, 2000). Although the three TGF-β isoforms share the same receptors and have similar cellular effects *in vitro*, they are all differentially expressed during embryogenesis (Roberts and Sporn, 1992). Each of these TGF-β isoforms is controlled by unique

promoters which determine their different expression patterns and responses to various stimuli (Roberts, 1998).

Activins/Inhibins
Activin is a dimeric protein comprised of hetero- or homodimers between different inhibin β chains (Gaddy-Kurten *et al*., 1995; Ball and Risbridger, 2001). Activin members were originally identified as endocrine regulators for pituitary function, inducing follicle-stimulating hormone (FSH) production, however mesoderm induction was the first role identified for activin during development in *Xenopus* embryos (reviewed in Ball and Risbridger, 2001). Activin modulate branching morphogenesis in the kidney, prostate and other branched organs (reviewed in Ball and Risbridger, 2001).

Inhibin, a heterodimeric glycoprotein, is comprised of an α chain which dimerizes with one of the activin β subunits. Inhibin antagonizes the action of activin and inhibits follicle-stimulating hormone production (Gaddy-Kurten *et al*., 1995).

BMPs
Bone morphogenetic proteins (BMPs) are disulfide-linked dimeric proteins, which consists of over 20 family members. They are grouped into subfamilies according to their amino acid sequence similarity. BMPs were originally identified as osteogenic proteins involved in the formation of new bone (reviewed in Kawabata *et al*., 1998). BMPs play an important role in diverse biological processes, including cell differentiation, cell-fate determination, cell growth, neurogenesis, morphogenesis, apoptosis and early embryonic development (Hogan, 1996). The best characterised family members are the BMP2/4 proteins. The others are BMP5, BMP7/OP1 (osteogenic protein-1), BMP8/OP2 and BMP6, with its *Xenopus* homologue, Vgr1. BMP-like proteins have been identified in various species. The most studied is *decapentaplegic* (*dpp*), which is the *Drosophila melanogaster* homologue of the mammalian BMP2/4. All BMP family members play a pivotal role in embryonic development in several organs (Hogan, 1996; Kawabata *et al*., 1998).

Other members
Growth and differentiation factors (GDFs), with GDF5/CDMP1 (cartilage-derived morphogenetic protein-1) and its structurally related members GDF6/CDMP2 and GDF7 are all involved in chondrogenesis, the morphogenesis of limb skeleton (Kingsley, 1994).

GDF9 and GDF8 are structurally distantly related BMPs involved in the regulation of skeletal muscle cells and ovarian folliculogenesis, respectively (Kawabata *et al*., 1998).

The BMP3 subfamily members influence osteogenic differentiation, endochondral bone formation and monocyte chemotaxis (Cunningham *et al*., 1992).

Anti-Müllerian hormone (AMH) also known as Müllerian inhibiting substance (MIS) are distantly related TGF-β family members, which regulates regression of the Müllerian duct and blocks formation of the uterus and oviducts (Cate *et al*., 1986; Donahoe, 1992).

Nodal plays an important role for mesoderm formation and the determination of the left-right axis during embryonic development in vertebrates, a process by which vertebrates lateralize unpaired organs (Levin *et al*., 1995; Hogan, 1996).

Lefty, which is yet another distantly related TGF-β superfamily member plays an important role in vertebrate embryogenesis, regulating the dorsal mesoderm patterning and axial morphogenesis. Lefty has also been suggested to function as a negative inhibitor of Nodal signalling during vertebrate gastrulation (Hogan, 1996).

Glial cell-divergent neurotrophic factor (GDNF) is the most divergent TGF-β superfamily member; it promotes dopaminergic neuron survival, differentiation and kidney development (Massagué, 1998).

TGF-β production, activation and associated proteins
The TGF-β production and activation is triggered by the cleavage of the inactive dimeric TGF-β precursor into an amino-terminal propeptide, called latency-associated protein (LAP) and a carboxy-terminal fragment that constitutes the mature growth factor. For stabilisation and correct folding of TGF-β, LAP is bound by disulphide bonds to the latent TGF-β-binding protein (LTBP), resulting in large latent complex (LLC), which is targeted either to the cell surface for activation, or to the extracellular matrix for storage (Roberts and Sporn, 1990; Munger *et al*., 1997; Taipale and Keski-Oja, 1997; Crawford *et al*., 1998; Cui *et al*., 1998;: Massagué and Chen, 2000). A conformational change in the LLC complex by thrombospondin-1 (TSP-1), or cleavage by proteases, leads to the activation of TGF-β (Taipale and Keski-Oja, 1997; Crawford *et al*., 1998). The latency-associated protein (LAP) has been shown to communicate with other signalling molecules, such as the integrins. It has been suggested that the interaction between latent TGF-β and integrin $\alpha_v\beta_1$ at the plasma membrane, may initiate integrin-dependent signalling pathways (Munger *et al*., 1998).

TGF-β-associated proteins have been shown to have important roles in regulating the bioactivity of TGF-β (Piek *et al*., 1999a). Three extracellular matrix proteins have all been shown to inhibit TGF-β activity; biglycan, decorin and a yet uncharacterised 60-kDa protein (Yamaguchi *et al*., 1990; Piek *et al*., 1997). Noggin, chordin, DAN, follistatin, or gremlin has been shown to bind to BMP ligands and prevent them from interacting with their receptors (Zimmerman *et al*., 1996; Piccolo *et al*., 1996; Hsu *et al*., 1998a). In addition, follistatin can bind directly with activin, and prevent its receptor interaction (Nakamura *et al*., 1990).

b) TGF-β superfamily receptors, activation and Smad signalling pathway

The TGF-β superfamily receptors and activation
The TGF-β family receptors are divided into three groups, known as the type I, type II and type III receptors. The basis for this categorization is the structural and functional characteristics of the receptors. The type I and type II receptors, are signalling receptors, whereas the type III receptor regulate the accessibility of TGF-β to the signalling receptors (reviewed in Derynck and Feng, 1997; Heldin *et al*., 1997). All type I and type

II receptors are transmembrane serine/threonine kinases, with structural regions important for their activation. The GS-rich region with the amino acid sequence motif, TTSGSGSG, which immediately precedes the kinase domain in the type I receptor (Wrana *et al*., 1994), is required for the phosphorylation and thereby activation of the type I receptor by the constitutive activated type II receptor (Wrana *et al*., 1994; Souchelnytskyi *et al*., 1996).

The type I receptors are also referred to as activin receptor like kinase (ALK) 1-7. The TGF-β type I receptor, TβR-I/ALK5 (Franzén *et al*., 1993), can only bind the TGF-β ligand. ALK1 can bind to both TGF-β and activins, but its physiological functions is unknown (Attisano *et al*., 1993; ten Dijke *et al*., 1994). There are two activin type I receptors, ActR-IA and ActR-IB (ten Dijke *et al*., 1993). ActR-IB/ALK4 can only bind activins, whereas ActR-IA/ALK2 (Attisano *et al*., 1993) can bind various ligands, including TGF-β, activins and BMP (reviewed in Derynck and Feng, 1997). The BMP type I receptors, BMPR-IA/ALK3 and BMPR-IB/ALK6 bind different BMP ligands, such as BMP2/4, BMP7/OP1 and GDF-5 (Koenig *et al*., 1994; reviewed in Derynck and Feng, 1997).

The type II receptors bind to specific sets of ligands (reviewed in Derynck and Feng, 1997). The type II receptor, TβR-II binds specifically to TGF-β isoforms, with a higher affinity for TGF-β1 and TGF-β3 and a lower affinity for TGF-β2. ActR-IIA and ActR-IIB are type II activin receptors. The ligands for ActR-IIA include activins, BMP7/OP1 and GDF-5, whereas the ligands for ActR-IIB also include BMP2. The type II BMP receptor, BMPR-II binds exclusively BMP2/4 and BMP7/OP1 (reviewed in Derynck and Feng, 1997).

The type III receptors are accessory receptors, which have short intracellular domains and have a more indirect role in TGF-β signalling. Betaglycan, endoglin and crypto are three examples of type III receptors. These receptors regulate the TGF-β access to the signalling receptors and facilitate binding to the TβR-I/TβR-II complex (Gougos and Letarte, 1990; Cheifetz and Massagué, 1991; López-Casillas *et al*., 1993)

All TGF-β type I and type II receptors exist as homodimers in the absence of ligand. The extracellular domains of both receptors are required for ligand binding, whereas the cytoplasmic domains are important for the dimerization of the receptors (reviewed in Derynck and Feng, 1997). Upon TGF-β ligand binding to the constitutive active TβR-II, the TβR-I is recruited and form a heterotetrameric complex (Figure 1) (Wrana *et al*., 1994). The formation of the heterotetrameric complex results in the activation of the TβR-I through phosphorylation at the serine and threonine residues located in the GS-domain (Franzén *et al*., 1993; Wrana *et al*., 1994; Souchelnytskyi *et al*., 1996). The activated TβR-I kinase subsequently phosphorylates members of the Smad signalling pathway (Figure 1), through interactions mediated by its L45 loop/Smad binding domain (Feng and Dernyck, 1997; Heldin *et al*., 1997; Kretzschmar *et al*., 1997b; Souchelnytski *et al*., 1997). Activin binds to their receptors in a similar manner as TGF-β, whereas BMPs need to simultaneously bind to both type I and type II receptors in order to form an active heterotetrameric complex (Figure 1) (reviewed in Derynck and Feng, 1997).

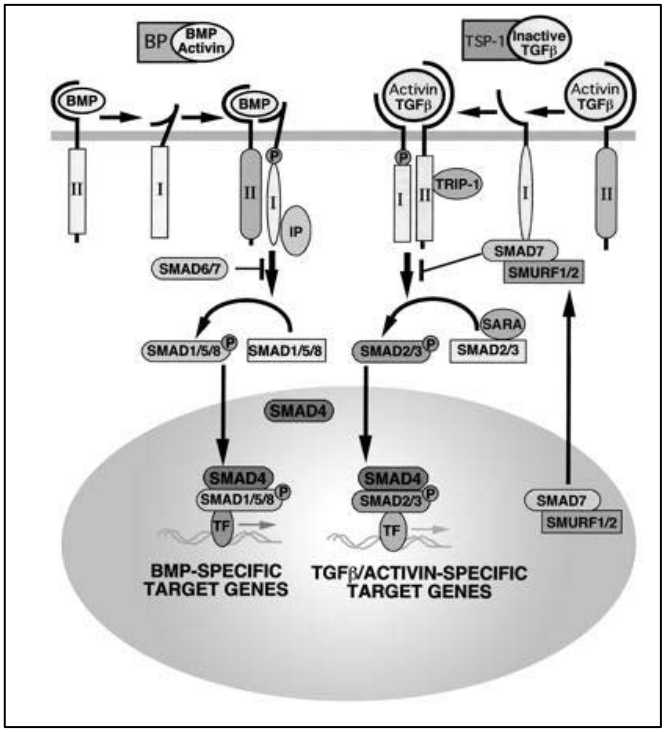

Figure 1. The regulation of TGF-β superfamily signal transduction (Chang *et al*., 2002)

The Smad family

The Smad proteins are the most characterised signalling molecules downstream of the TGF-β superfamily receptors. The first member of the Smad family, Mad [mothers against dpp (decapentaplegic)], was isolated through a genetic screening for genes that enhanced the effect of weak dpp alleles in *Drosophila melanogaster* (Raftery *et al*., 1995; Sekelsky *et al*., 1995). Three *Caenorhabditis elegans* homologues of Mad, *sma-2, sma-3* and *sma-4*, were later identified. Mutations in these genes showed similar phenotypes as mutations in *Daf-4*, a gene encoding a TGF-β type II receptor in *C. elegans* (Savage *et al*., 1996). The vertebrate homologues were thereafter identified and received the name Smads, which is a combination of sma and Mad (Derynck *et al*., 1996).

The Smad family consists of proteins with molecular masses of 42 kDa - 65 kDa. The Smads are highly evolutionary conserved and can be divided into three subgroups according to structural and functional criteria. Smads that directly bind to and become phosphorylated by the receptor are called receptor-activated Smads (R-Smads), Smads that are involved in the signalling by associating to the receptor-activated Smads are called common partner Smads (Co-Smads) and Smads that obstruct the signalling

function of the first two subfamilies are called inhibitory Smads (I-Smads) (Massagué, 1998). The R- and Co-Smads share two regions of homology at the amino- and carboxy-terminal ends, called Mad-homology domain-1 (MH1) and -2 (MH2), respectively. The MH1 and MH2 domains are separated by a proline-rich linker region (reviewed in Heldin *et al*., 1997). The MH1 and MH2 domains mediate a large number of specific protein-protein interactions. Smad proteins reside in the cytoplasm in a resting autoinhibited conformation, caused by the MH1 domain binding to the MH2 domain, suppressing its function. In the active conformation, however, the MH1 domain functions as DNA-binding domain (Hata *et al*., 1997). The I-Smads have a conserved MH2 domain, but their amino-terminal region (N-domain) is highly divergent from the MH1 domain of other Smads. The R-Smads contains a domain which is not present in the other Smad proteins, an -SSXS motif in the carboxy-terminal end. This domain forms the binding motif and activation site for the R-Smads to the receptor (reviewed in Heldin *et al*., 1997).

The Smad signalling pathway

In order for the Smad proteins to be activated by the TGF-β receptor, they need to come in proximity to the activated receptor. There are several proteins which regulate and facilitate the recruitment of Smad proteins to the receptor complex. One example is the FYVE-domain containing protein, SARA (Smad anchor for receptor activation), which helps to present Smad2 and Smad3, to the TGF-β receptor (Figure 1) (Tsukazaki *et al*., 1998). The Smad-binding domain (SBD) of SARA binds to the MH2 domain of Smad2 (Wu *et al*., 2000). SARA can only interact with Smad2 and Smad3, but not with the other R-Smads (Smads 1, 5 and 8). This is due to the unique presence of five amino-acid residues in the β-sheet within the MH2 domain of Smad2 and Smad3, which determine binding specificity (Wu *et al*., 2000). The differences in SARA binding can be one reason for the differences in the receptor-binding specificity between the Smad proteins. Other proteins which aid in the activation of Smads are the adaptor molecule disabled-2 (Dab-2) (Hocevar *et al*., 2001), TGF-β receptor-associated protein-1 (TRAP-1) (Wurthner *et al*., 2001), filamin-1 (Sasaki, *et al*., 2001) and axin (Furuhashi *et al*., 2001). Dab-2 directly interacts with Smad2/Smad3 and the TGF-β receptors, similarly to SARA, serving to bridge the TGF-β receptor complex to the Smad pathway. In this case the interaction with the receptor complex is constitutive and the interaction between Dab-2 and Smads is ligand-dependent (Hocevar *et al*., 2001). Both SARA (Itoh *et al*., 2002) and Dab-2 (Oleinikov *et al*., 2000), have been shown to associate with the endocytic machinery together with the TGF-β receptors (Doré *et al*., 1998), and thereby regulate the TGF-β-Smad signalling efficiency. TRAP-1 is associated with the inactive TGF-β receptor and upon receptor activation, TRAP-1 dissociates from the receptor and interacts with Smad4, and thereby facilitates the Smad4 binding to the Smad2/Smad3 complex (Wurthner *et al*., 2001). Filamin-1 is an actin-binding protein which acts as a scaffold protein for signal molecules, such as Smads, and thereby forms a link between trans-membrane receptors and the actin cytoskeleton (Sasaki, *et al*., 2001). In addition, a negative regulator of Wnt-signalling, axin, was shown to act as an adaptor of Smad3 to facilitate TGF-β-induced Smad3 activation (Furuhashi *et al*., 2001). There also exist other proteins, which have roles in regulating the Smad subcellular localisation, one example is the microtubules. Recently, it was reported that Smad proteins require an

intact microtubuli system, in order to be translocated to the nucleus and affect transcription (Dong *et al*., 2000).

After the recruitment of Smad proteins to the receptor complex, the L3 loop within the MH2 domain of R-Smads binds to a specific motif in TβR-I, known as the L45 loop (Feng and Dernyck, 1997; Souchelnytski *et al*., 1997). These two motifs, the L3 and L45 loops play an important role in giving the specificity of the R-Smads for recognition to TβR-I. The L45 loop differs significantly between BMP, TGF-β and activin type I receptors, and the L3 loop differs between the Smad1, -5, -8 and Smad2, -3, allowing discrimination between the receptors (Chen *et al*., 1998b; Lo *et al*., 1998). The binding of R-Smads to the receptor then leads to the phosphorylation of the C-terminal -SSXS motif (Feng and Dernyck, 1997; Heldin *et al*., 1997; Souchelnytski *et al*., 1997; Kretzschmar *et al*., 1997b). This phosphorylation, in turn, leads to a decreased affinity of R-Smads for SARA and an increased affinity for Smad4. A heteromeric complex of oligomerized R-Smads and Smad4 then translocates to the nucleus (Figure 1) (Heldin *et al*., 1997; Massagué and Wotton, 2000; Gorelik and Flavell, 2001; Chang *et al*., 2002), where it affects transcription by binding to specific gene promoters and recruit transcription factors, such as AP-1 (Liberati *et al*., 1999), DNA-binding adaptors, such as FAST-1 (Chen *et al*., 1996b) and co-activators, such as CBP/p300 (Feng *et al*., 1998; Dernyck *et al*., 1998).

Other members of the TGF-β superfamily, BMPs and activin were shown to activate Smad1, -5, -8 through BMP type I and type II receptors (Thomsen, 1996; Macías-Silva *et al*., 1998) and Smad2, -3 through activin type I and type II receptors (Eppert *et al*., 1996; Zhang *et al*., 1996), respectively (Figure 1). After oligomerization of Smad1, -5, -8 with Smad4, the complexes were translocated to the nucleus in the same manner as the Smad2, -3 and Smad4 complex in TGF-β activation (Figure 1) (Chang *et al*., 2002).

The third group of Smads, Smad6 and Smad7 (Imamura *et al*., 1997; Nakao *et al*., 1997; Topper *et al*., 1997) are known as inhibitory Smads (I-Smads) since they mediate negative feedback mechanisms within the TGF-β/BMP signalling pathways (Heldin *et al*., 1997; Gorelik and Flavell, 2001). The expression of I-Smads is upregulated by TGF-β, activin, as well as by BMP signalling (Tsuneizumi *et al*., 1997; Afrakhte *et al*., 1998; Ishisaki *et al*., 1998; Takase *et al*., 1998; Miyazono, 2000). The I-Smads exert their negative effects on R-Smads by interacting with the activated TβR-I, thereby preventing phosphorylation of the R-Smads (Figure 1) (Imamura *et al*., 1997; Nakao *et al*., 1997; Hata *et al*., 1998; Souchelnytskyi *et al*., 1998). The carboxyl-terminal MH2 domains of Smad6 and Smad7 are essential for the inhibition of TGF-β and BMP signalling. In addition, the N domain of Smad7 has been shown to physically interact with the MH2 domain, resulting in enhancement of the inhibitory activity of Smad7 through facilitation of the interaction with TGF-β receptor (Hanyu *et al*., 2001). Smad7 is a general inhibitor of the TGF-β superfamily pathway, whereas Smad6 predominantly inhibits the BMP pathway (Itoh *et al*., 1998; Hanyu *et al*., 2001). The I-Smads, as well as the other Smads, shuttle between the nucleus and the cytoplasm (Itoh *et al*., 1998; Hanyu *et al*., 2001; Kavasak *et al*., 2000). In resting cells, Smad7 is localised in the nucleus, but upon TGF-β stimulation Smad7 is exported to the cytoplasm (Itoh *et al*., 1998). Recently, a

mechanism was proposed where Smurf1 helped to translocate Smad7 out of the nucleus and to the plasma membrane. Smurf1 targeted with nuclear Smad7 and formed a complex which then was translocated out of the nucleus and to the plasma membrane, where it was targeted for ubiquitin-dependent degradation (Suzuki *et al*, 2002).

Smads in the cytoplasm and the nucleus are subjected to ubiquitination and proteasomal degradation (Lo and Massagué, 1999; Zhu *et al*., 1999; Kavsak *et al*., 2000; Lin *et al*., 2000b; Ebisawa *et al*., 2001). The ubiquitin-ligase proteins Smurf1 and Smurf2, which targets the cytoplasmic Smad1 and Smad2, respectively (Lin *et al*., 2000b; Zhu *et al*., 1999) and the ubiquitin-conjugating protein, HbcH5, which targets the nuclear Smad2 (Lo and Massagué, 1999), are proteins involved in the proteasomal degradation of Smads. In addition, Smad7 recruits Smurf1 and Smurf2 to TβR-I at the plasma membrane, leading to ubiquitin-dependent degradation of the TGF-β receptor complexes (Kavsak *et al*., 2000; Ebisawa *et al*., 2001). However, ubiquitin-dependent degradation of Smad7 can be prevented by the acetylation of Smad7 performed by the transcriptional co-activator p300, leading to stabilisation of Smad7 (Grönroos *et al*., 2002).

c) Non-Smad signalling pathways and cross-talk with other signalling pathways
In addition to the Smad pathways, TGF-β also activates other signalling pathways, e.g. the ERK, SAPK/JNK and p38 MAPK pathways.

TGF-β or BMP stimulation result in activation of TAK1 (Yamaguchi *et al*., 1995; Kimura *et al*., 2000), which in turn either activate JNK/SAPK, via MKK4 (Shirakabe *et al*., 1997; Hocevar *et al*., 1999), or p38 MAPK, via MKK3 (Morigouchi *et al*., 1996; Hanafusa *et al*., 1999) (Figure 2). This activation leads to enhanced activity of transcription factors, such as c-Jun and ATF2 (Sano *et al*., 1999) (Figure 2). There are three known activators of TAK1, X-chromosome-linked inhibitor of apoptosis protein (XIAP) (Yamaguchi *et al*., 1999) (Figure 2), the hematopoietic progenitor kinase-1 (HPK1) (Zhou *et al*., 1999) (Figure 2) and the TAK1 binding protein (TAB1) (Shibuya *et al*., 1996; Shibuya *et al*., 1998). TAB1 is activated in both TGF-β and BMP signalling (Shibuya *et al*., 1996; Shibuya *et al*., 1998), whereas XIAP is activated only by BMP, and has been proposed to form the link between the activated BMP receptor and the TAB1-TAK1 complex (Yamaguchi *et al*., 1999). Furthermore, it has been shown that HPK1 is important in TGF-β signalling (Zhou *et al*., 1999).

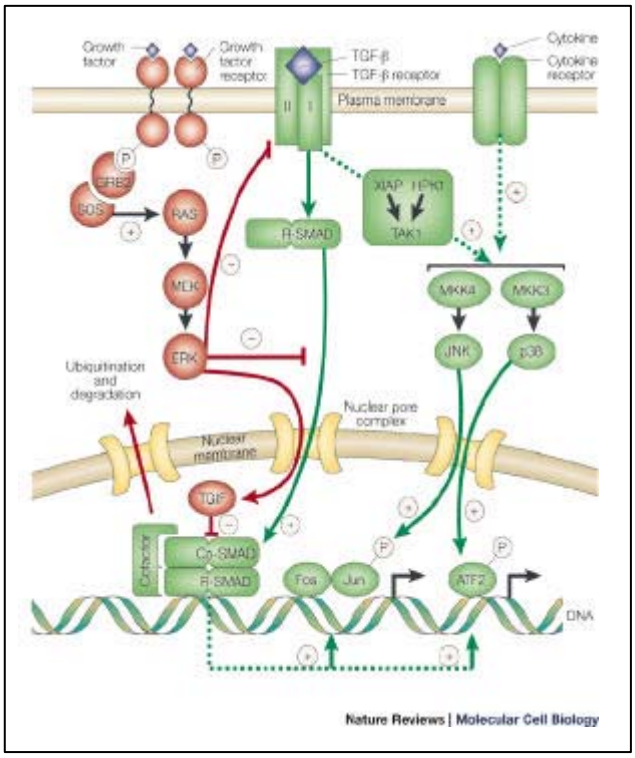

Figure 2. Crosstalk between the SMAD and mitogen-activated protein kinase pathways (Massagué, 2000).

In addition to the SAPK/JNK and p38 MAPK pathway, TGF-β also activates the Ras-MEK-ERK MAPK pathway (reviewed in Mulder, 2000). There exist cross-talks between Smad and the p38 MAPK pathway (Engel *et al*., 1999; Hanafusa *et al*., 1999; Sano *et al*., 1999; Watanabe *et al*., 2001), as well as between Smad and Ras-MEK-ERK signalling pathways (Figure 2) (Oft *et al*., 1996; Kretzschmar *et al*., 1999; Mulder, 2000; Lo *et al*., 2001). The small GTP binding protein, Ras and Smad pathways can communicate at different levels of the signalling cascade, and depending on the timing result in different signalling effects (Figure 2). H-Ras transformation of rat intestinal epithelial cells have been shown to result in downregulation of TGF-β receptors (Zhao and Buick, 1995), as well as, inhibition of BMP (Kretzschmar *et al*., 1997a) and TGF-β (Kretzschmar *et al*., 1999) signalling by inducing a phosphorylation of Smad1 or Smad2, Smad3, respectively. This phosphorylation occurs on specific phosphorylation sites within the linker region to prevent the accumulation of Smad1 and Smad2, Smad3 in the nucleus and thereby interfere with transcriptional activities. Phosphorylation of these sites can also be induced by other growth factors. Both EGF and hepatocyte growth factor (HGF) have been shown to inhibit BMP induced Smad1 nuclear accumulation (Kretzschmar *et al*., 1997a). In contrast, it has been shown that the Ras-MEK-ERK pathway is required for the ability of TGF-β to positively activate Smad1 (Yue *et al*., 1999a; 1999b) and that

HGF induces both phosphorylation and nuclear translocation of Smad2, thereby leading to increased Smad2 transcriptional activity (de Caestecker *et al*., 1998; Brown *et al*., 1999).

The Ras-related family of Rho GTPases has also been shown to have roles in TGF-β signalling (Hartsough *et al*., 1996; Mucsi *et al*., 1996; Atfi *et al*., 1997b). One study showed that Rac1 contributes to TGF-β-mediated gene transcription (Mucsi *et al*., 1996). Furthermore, in *Drosophila*, the TGF-β ortholog Dpp has been implicated as an activator of Dcdc42 (Ricos *et al*., 1999). It has also been suggested that RhoA, but not Cdc42, has a role in epithelial to mesenchymal transdifferentiation (EMT) of NMuMG cells (Bhowmick *et al*., 2001a). One of the aims of this thesis was to explore the role of small GTPases in the mobilisation of the actin cytoskeleton (see paper I)

Another pathway which has been shown to cooperate with the Smads is the Wnt/β-catenin pathway. Components within this pathway physically interact with Smad proteins to activate different transcription factors, such as lymphoid enhancer-binding factor 1/T-cell specific factor (LEF1/TCF) (Labbe *et al*., 2000; Nishita *et al*., 2000). In addition, there exist cross-talk between the TGF-β and the IL-6 signalling cascades, which occurs by physical and functional interactions between STAT3 and Smad3, bridged by p300 in a hepatoma cell line (Yamamoto *et al*., 2001).

In conclusion, the ultimate biological response to TGF-β depends on a balance between multiple signalling pathways, each activated in a cell type- and context-dependent manner, involving both Smad-dependent and Smad-independent pathways.

2. The cytoskeleton

a) The cytoskeleton

The cytoskeleton of vertebrate cells is formed by three different types of filament systems; microfilaments, microtubules and intermediate filaments. Microfilaments are formed by actin subunits, microtubules are formed by tubulin subunits, whereas intermediate filaments are formed from different proteins in a cell type-specific fashion. Microfilaments are, together with actin-binding proteins, essential for cell movement and cell shape reorganisation, microtubules are essential for intracellular trafficking as well as cytokinesis, and finally, the intermediate filaments provide cells with mechanical strength (Amos, 1991, Gelfand and Bershadsky, 1991; Albers and Funchs, 1992). The microfilaments and microtubules have also been shown to work together to form an active transport mechanism for organelle transport (Goode *et al*., 2000; Rogers and Gelfand, 2000). Recently cell migration, as well as the establishment of cell polarity has been found to be dependent on both the microfilaments and microtubules in a process involving the Rho GTPases (Magdalena *et al*., 2002; Wittmann and Waterman-Storer, 2001; reviewed in Kaverina *et al*., 2002).

The microfilament system and actin polymerisation
The microfilament system, also known as the actin cytoskeleton, is a highly dynamic structure, which is under a continuous reconstruction to control the morphology, survival, growth and motility of eukaryotic cells (Pollard *et al*., 2000). Migration of vertebrate cells depends on the formation of specific cellular protrusions which aid in the locomotion process.

Lamellipodia, also known as membrane ruffles, is formed at the leading edge of a crawling cell, with thin, flat, sheet-like structures of actin filaments, first described by Abercrombie and Ambrose in the late fifties (Abercrombie *et al*., 1970; reviewed in Small *et al*., 2002). Filopodia, also called microspikes, are thin protrusions, of tightly packed parallel bundles of actin filaments, extending out from the leading edge (Small, 1989; Small *et al*., 1999), first described at the beginning of the sixties in studies on the embryonic development of sea urchins (reviewed in Wood and Martin, 2002). Filopodia was later also shown to be involved in cell-cell communication during development (Karp and Solurch 1985; Malinda *et al*., 1995) and sensing of external gradients of chemoattractant molecules (Allen *et al*., 1998; reviewed in Wood and Martin, 2002). Another type of actin filament-containing structure, the stress fibres, consists of bundles of actin filaments and myosin-II filaments. The ends of the stress fibres are attached to the plasma membrane at special sites called focal contacts or focal adhesions, which are in association with the extracellular matrix (Langanger *et al*., 1986; Burridge *et al*., 1988; Bretscher, 1991; Burridgde *et al*., 1992; Small *et al*., 1999). The main transmembrane linker proteins of focal contacts are members of the integrin family (Burridge *et al*., 1988; Burridgde *et al*., 1992; Juliano, 2002).

Actin, the major component of the microfilaments, is an ATP-binding protein that exists in two forms in the cell, as monomers [globular actin (G-actin)], or as filaments [filamentous actin (F-actin)].
Actin monomers polymerise by a strictly regulated process into helical polar filaments (Schmidt and Hall, 1998; Pollard *et al*., 2000). The two ends of the actin filament have distinct features; the fast growing end, also called the barbed end and the slow growing end, also called the pointed end (Bonder *et al*., 1983; Small, 1989; Janemey, 1991). In lamellipodia the actin filaments are organised with the barbed ends facing toward the plasma membrane. The continuous reconstruction of actin filaments is responsible for the ruffling activity of the lamellipod. The dynamic reconstruction of actin filaments is in turn regulated by actin-binding proteins, such as myosin, tropomyosin, filamin, fimbrin, α-actinin, gelsolin, villin and profilin (Bretscher, 1991; Hartwig and Kwiatkowski, 1991; Schmidt and Hall, 1998; Pollard *et al*., 2000).

The dynamic reorganisation of the microfilaments has been shown to be important for different cellular processes, such as internalisation by phagocytosis and endocytocis, as well as the formation of acto-myosin contractile fibers in nonmuscle cells (reviewed in Welch and Mullins, 2002). In living cells, actin polymerisation occurs predominantly at the leading edge (Wang, 1985; Watanabe and Mitchison, 2002). In resting cells, the actin filaments are capped at their barbed ends with capping proteins, to prevent spontaneous actin polymerisation. Free barbed ends are created by filament severing by cofilin, also

called actin depolymerising factor (ADF) (Ichetovkin *et al*., 2002). The creation of free barbed ends, which occurs in the initiatial phase of actin polymerisation, involves three general mechanisms; uncapping of pre-existing filaments, severing of filaments, and de novo nucleation (Welch and Mullins, 2002). The specific contribution of each mechanism may be cell type specific, but most often all three mechanisms are involved. De novo nucleation is the best characterised of the mechanisms for the initiation of the polymerisation (Welch and Mullins, 2002). The most important cellular factor known to nucleate new actin filaments with free barbed ends is the seven-subunit Arp2/3 complex, in collaboration with its activators, the Wiskott-Aldrich syndrome protein (WASP), Scar (also called WAVE), and ATP actin filaments (Machesky *et al*., 1999; Rohatgi *et al*., 1999; Winter *et al*., 1999; Yarar *et al*., 1999; Pollard *et al*., 2000).

The actin polymerisation is tightly regulated by transmembrane signalling and one possible link between the cell surface receptors and actin assembly is the WASP and Scar proteins (Machesky *et al*., 1999; Rohatgi *et al*., 1999; Winter *et al*., 1999; Yarar *et al*., 1999; Pollard *et al*., 2000). WASP, one of the members, was first discovered as a protein defective in a human genetic disease with deficiencies in the actin cytoskeleton of platelets and leukocytes (Derry *et al*., 1994a, 1994b; Ochs, 1998). The WASP and Scar family of proteins regulates the actin nucleation activity (Mullins *et al*., 1997; Welch *et al*., 1997; Winter *et al*., 1997; Mullins *et al*., 1998; Ma *et al*., 1998; Machesky *et al*., 1999; Pollard *et al*., 2000). All WASP and Scar/WAVE family members have a homologous carboxyl terminus, the verprolin-homology and cofilin-like acidic (WCA) region, which can bind to, and activate the Arp2/3 complex (Machesky *et al*., 1999; Rohatgi *et al*., 1999; Higgs and Pollard, 2001). The Rho GTPase Cdc42 interacts with WASP and N-WASP and their binding partner the Arp2/3 complex and thus recruits the whole actin nucleation/elongation machinery to a site on the membrane where the polymerisation then is started by the activation of the Arp2/3 nucleation (Aspenström *et al*., 1996; Rohatgi *et al*., 1999; Higgs and Pollard, 2001).

Another protein found to promote actin assembly is the insulin receptor substrate protein 53 (IRSp53) (Krugmann *et al*., 2001), which interacts with WAVE2/Scar2 (Miki *et al*., 2000) and Mena, a member of the Ena/VASP family proteins (Krugmann *et al*., 2001), to promote Arp2/3 activation. IRSp53 can also associate with the activated Rho GTPase Rac and thereby connect the Rho GTPases to Arp2/3 complex-mediated actin polymerisation (Miki *et al*., 2000).

Recent evidence has also indicated that proteins other than the WASP family can interact with the Arp2/3 complex. One example is cortactin, which can bind Arp2/3 and stimulate nucleation/polymerisation (Uruno, *et al*., 2001), and stabilisation of newly generated actin filaments (Bowden *et al*., 1999; Weaver *et al*., 2001). Cortactin has also been shown to bind directly to the endocytic protein dynamin2 (McNiven *et al*., 2000). Dynamin has in turn been shown to bind to the proteins syndapin and N-WASP (Qualmann *et al*., 1999). Dynamin might therefore stimulate the Arp2/3 complex and actin polymerisation either via binding cortactin or via binding syndapin and N-WASP.

The nucleation and thereby also the actin polymerisation can occur at special nucleation sites at the plasma membrane, which interconnects the extracellular matrix, the plasma membrane, and the microfilaments (reviewed in Schmidt and Hall, 1998). These sites are found essentially in two types of membrane-associated complexes: focal adhesions and adherens junctions (Yamada and Geiger, 1997). The focal adhesions consists of integrin-type receptors, which connect the extracellular matrix with intracellular proteins, such as vinculin, talin, α-actinin, paxillin, zyxin, tensin, and focal adhesion kinase (FAK) (Burridge *et al*., 1990). The adherens junctions consists of clusters of cadherins, intracellularly interacting with α-actinin, catenin, filamin and ezrin, radixin, moesin, also called the ERM proteins (Geiger *et al*., 1990). The Rho GTPases, as well as WASP and phosphatidylinositol-3-OH kinase (PI3K), have also been shown to be activated by E-cadherin-mediated intracellular adhesion (reviewed in Jamora and Fuchs, 2002).

Cell motility
Protein tyrosine kinase receptors, such as the receptors for EGF or PDGF have been known for two decades to be potent regulators of the actin cytoskeleton (Chinkers *et al*., 1979; Mellström *et al*., 1983; Rönnstrand and Heldin, 2001). The correlation between TGF-β signalling and the dynamic organisation of the microfilament system has been less characterised and has only recently been studied in some detail in this thesis (paper I and II).

Cell locomotion plays a key role in normal physiology, for organ development and remodeling, wound healing, as well as during disease, with cancer as one example. Cancer cells proliferate and invade tissues in defiance of normal control (Mitchison and Cramer, 1996). Cell migration involves changes in the cytoskeleton, cell-substrate adhesion and extracellular matrix. In migrating cells the actin filaments are organised with the barbed ends facing toward the plasma membrane, in the direction of migration (Bonder *et al*., 1983; Janemey, 1991; Pollard *et al*., 2000). Cell migration is believed to be divided into four different actin-dependent processes: formation of membrane protrusive structures, adhesion to the substratum, cell body contraction, and finally, deadhesion/tail detachment (Mitchison and Cramer, 1996; Pollard *et al*., 2000; Ridley, 2001b, 2001c). Many different molecules have been implicated in cell migration, including the family of Rho GTPases, MAPK pathways, protein kinase C (PKC), phosphatidylinositide kinases and tyrosine kinases (reviewed in Ridley, 2001c). Rac has been shown to be required for protrusive lamellipodial activity and Cdc42 for maintaining cell migration polarity, which includes the localisation of lamellipodial activity to the leading edge and the reorientation of the Golgi apparatus in the direction of movement. Rho is required to maintain cell adhesion during movement, and together with Ras they regulate focal adhesion and stress fibre turnover (Hall and Nobes, 2000). Although the proteins involved in the actin dynamics are well identified, it is not yet clear how these proteins collaborate to orchestrate signals that initiate cell motility or maintenance of chemotaxis.

b) Rho GTPases, growth factors and cytoskeletal control
The Rho GTPases are important links between extracellular growth signalling pathways and the cytoskeleton, controlling both the polymerisation and branching of actin

filaments and thereby cell locomotion, tumor growth, cell cycle progression, gene transcription and cell survival (Ridley and Hall, 1992; Hall, 1998; Aspenström, 1999a; Bishop and Hall, 2000; Pollard *et al*., 2000; Frame and Brunton, 2002). The best-characterised proteins in this family are RhoA, Rac1 and Cdc42, which have been conserved through evolution from yeast to mammals (reviewed in Wherlock and Mellor, 2002). Their activity is regulated by signals originated from different classes of surface receptors including G-protein-coupled receptors, tyrosine kinase receptors, cytokine receptors and adhesion receptors.

Important tools in the analyses of Rho protein functions are point mutated molecules making Rho constitutive active or dominant negative, and a number of bacterial toxins which covalently modifies the activity of the different Rho GTPases, either by activation or inactivation. The constitutively active mutant Rho GTPases are constitutively GTP-bound because the GTPase activity is inhibited, preventing intrinsic and GAP-induced GTP hydrolysis. The dominant negative mutant Rho GTPases inhibit the action of the Rho GTPase by competing with endogenous GTPases for binding to cellular GEFs and thereby keeping the GTPase in the inactive GDP-bound state (reviewed in Bishop and Hall, 2000). The *Clostridium botulinum* and *Clostridium difficile* on the other hand, either activate, through deamidation or inactivate, through glucosylation or ribosylation the different Rho GTPases. The exoenzyme C3 ADP-ribosyltransferase from *Clostridium botulinum* inactivates RhoA, RhoB, and RhoC by ribosylation (Aktories, 1997), whereas the *Clostridium difficile* toxin B-10463 (TcdB-10463) inhibit RhoA, Rac1 and Cdc42 (Aktories and Just, 1995; Just *et al*., 1995), and toxin B-1470 (TcdB-1470) inhibit Rac1, Rab, Ral and R-Ras (Eichel-Streiber *et al*., 1995; Depitre *et al*., 1993), by glucosylation.

The Rho GTPase family

The Ras-homologous (Rho) GTPases are monomeric 20-30 kDa proteins closely related to the Ras proteins. Ras genes (*H-Ras, Ki-Ras* and *N-Ras*) were discovered in the early 1980s as oncogenes mutated in human tumors (reviewed in Ridley, 2001a). The *Rho* gene was first identified in the sea-slug *Aplysia,* and subsequently in human, which has three homologues, *RhoA, RhoB* and *RhoC* (reviewed in Ridley, 2001a). This initiated a fast growing list of Rho GTPase members by the identifications of Ras-related C3 botulinum toxin substrate (Rac) isoforms, Rac1 and Rac2, and *Saccharomyces cervisiae* as well as human cell division cycle 42 (Cdc42) (reviewed in Ridley, 2001a). Up to this date, 8 distinct subfamily groups of Rho GTPases have been found in mammalian cells: Rac (Rac1, Rac2, Rac3, RhoG), Cdc42 (Cdc42Hs/G25K, TC10, TCL, Chp1, Chp2/Wrch), RhoBTB (RhoBTB1, RhoBTB2), Rho (RhoA, RhoC, RhoB), Rnd (Rnd2/Rho7, RhoE/Rnd3, Rnd1/Rho6), RhoD (RhoD/HP1, Rif), RhoH (TTF/RhoH) and Miro (Miro-1, Miro-2) (Aspenström, 1999a; Ridley, 2001b; Wherlock and Mellor, 2002; Fransson *et al*., 2003).

The Rho GTPase switch

The Rho GTPases cycle between an inactive GDP-bound and an active GTP-bound state (Figure 3). This cycling is regulated by GDP/GTP exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine dissociation inhibitors (GDIs) (Schmidt and

Hall, 1998; Frame and Brunton, 2002; Schmidt and Hall, 2002). GEFs bind and stabilise the nucleotide-free form of the protein and thereby catalyse the exchange of GDP for GTP and activate Rho (Hart *et al*., 1991). These proteins have two important types of domains, the Dbl (diffuse B-cell lymphoma) homology (DH) domain, which facilitates the exchange of the GTPase (Hart *et al*., 1991), and the pleckstrin homology (PH) domain, which confers membrane targeting (Zheng *et al*., 1996; Schmidt and Hall, 1998). All GAPs have a conserved catalytic domain, which bind to the GTP-loaded form of the Rho GTPase and aid in the hydrolysis, converting the Rho GTPase to their inactive, GDP-bound form (Garrett, *et al*., 1991; Lamarche and Hall, 1994; Schmidt and Hall, 1998). GDIs are inhibitors for both GEFs and GAPs (Fukumoto *et al*., 1990; Schmidt and Hall, 1998) and suppress the release of GDP, thereby keeping the Rho GTPase inactive, thereby preventing their activation (Schmidt and Hall, 2002). In resting cells, the Rho GTPases are thought to reside in the cytoplasm in an inactive state complexed by RhoGDI. In response to extracellular signals the GTPases are released form the GDIs and translocate to the membrane (Takai *et al*., 1995). In order for the Rho GTPases to be targeted to the membrane, they need to be post-translationally prenylated (Schmidt and Hall, 2002).

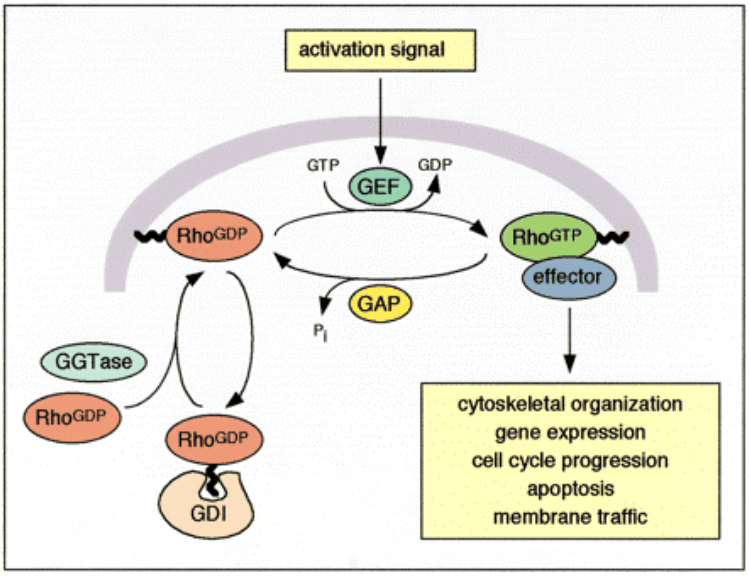

Figure 3. The Rho GTPase switch (Schmidt and Hall, 2002)

Rho GTPase and growth factor signalling, in cytoskeletal control
A substantial part of the initial work, which identified the relation between transmembrane receptors, Rho GTPases and the cytoskeletal reorganisation was performed in Swiss 3T3 fibroblasts and subsequentially in other cell types (Figure 4) (reviewed in Machesky and Hall, 1997; Hall, 1998; Schmidt and Hall, 1998; Ridley, 2001a). Microinjections of constitutively active Rac1, as well as activation of Rac1 elicited by tyrosine kinase receptors, such as the receptors for EGF, PDGF and insulin,

lead to the formation of lamellipodia (Ridle y *et al*., 1992; Nobes and Hall, 1995). Cdc42 has been shown to be activated via the bradykinin G-protein-coupled receptor, leading to formation of filopodia (Kozma *et al*., 1995; Nobes and Hall, 1995). The cytokines TNF-α and IL-1 have also been shown to activate Cdc42 (reviewed in Kjøller and Hall, 1999). Finally, Rho was found to be activated upon stimulation of the lysophosphatidic acid (LPA) and bombesin G-protein-coupled receptors, leading to the formation of focal adhesions and stress fibers (Ridley and Hall, 1992), a response seen also by microinjecting constitutively active RhoA (Paterson *et al*., 1990).

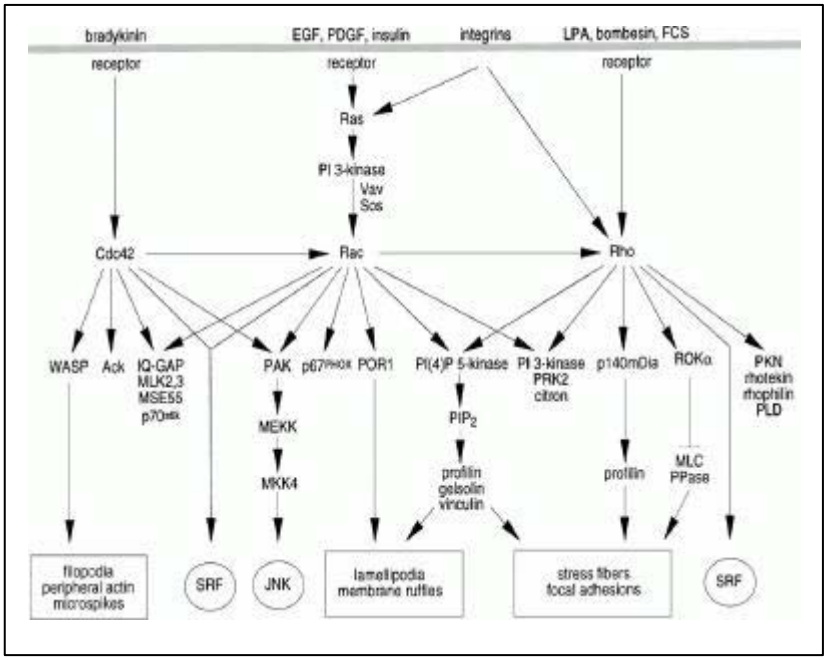

Figure 4. Rho, Rac and Cdc42 signalling pathways in mammalian cells, primarily swiss 3T3 fibroblasts (Schmidt and Hall, 1998).

Although several studies have shown that Rac is critical for the formation of lamellipodia, recent reports have shown that there exist Rac-independent pathways for the formation of lamellipodia. Expression of active Rab5 (which regulates endocytosis) can induce ruffle-like structures independently of Rac (Spaargaren and Bos, 1999). Rac is neither needed for lamellipodia formation in immature dendric cells (West *et al*., 2000), or in colon carcinoma cells plated on laminin, which instead need Rho for lamellipodia extension (O´Connor *et al*., 2000). Chp, a homologue of the GTPase Cdc42Hs has also been seen to induce lamellipodia formation, prior to filopodia formation (Aronheim, *et al*., 1998). As part of this thesis work, we show that TGF-β-induced activation of Cdc42 and Rho regulates lamellipodia formation without the involvement of Rac (paper I).

There is a large group of downstream targets, identified for the Rho GTPases reviewed in figure 4. The activated Cdc42 tyrosine kinase (ACK) and the serine/threonine kinase, p21-activated kinase (PAK) were the first downstream targets found for the Rho GTPases, as targets for Cdc42 and Rac, respectively (Manser *et al*., 1993; 1994), followed by a still increasing number of proteins binding to Rho GTPases. ACK and PAK are apart of a group of Cdc42 and Rac targets, which all share Cdc42 and Rac interactive binding (CRIB) domains. The other members are WASP, PAR-6 for partitioning defective, and mixed lineage kinase 3 (MLK3) (Burbelo *et al*., 1995; Schmidt and Hall, 1998; Daniels and Bokoch, 1999; Aspenström *et al*., 1996; Mott *et al*., 1999; Johansson *et al*., 2000; Gallo and Johnson, 2002).

PAK1 is one of the best characterised Rac and Cdc42 binding protein (Figure 4), and has been shown to induce filopodia-like structures, as well as lamellipodia, in fibroblasts (Van Aelst and D`Souza-Schorey, 1997; Hall, 1998). In addition, HGF and PDGF have been shown to activate PAK1 and stimulate migration (Bottaro *et al*., 1991; Royal *et al*., 2000; Dechert *et al*., 2001).

A ubiquitously expressed isoform of WASP, N-WASP was shown to bind to both actin and profilin, and to induce filopodia formation in fibroblasts (Miki *et al*., 1998), whereas WASP was shown to link Cdc42 to the formation of filopodia in haematopoietic cells (Figure 4) (Brickell *et al*., 1998; Aspenström, 1999b).

PAR-6/PAR-3 complexes together with atypical PKC were shown to be involved in the establishment of cell polarity in epithelial cells (Joberty *et al*., 2000; Johansson *et al*., 2000; Lin *et al*., 2000).

MLK3 is a member of the serine/threonine kinase family involving MLK1, MLK2/MST, MLK3/SPRK/PTK1 and DLK, which has been shown to bind to Cdc42 and to a lesser extent to Rac (Figure 4) (Burbelo *et al*., 1995; Tibbles *et al*., 1996; Nagata *et al*., 1998). It has been suggested that activated Cdc42 or Rac might in turn activate and target MLK3 to membrane compartments in the cell to induce a localised activation of MAPK pathways (reviewed in Gallo and Johnson, 2002). Recently, both MLK2 and MLK3 were shown to interact with Cdc42 and thus activate SAPK/JNK, ERK and p38 MAPK pathways (Nagata *et al*., 1998). MLK3 has also been shown to activate the p38 MAPK pathway via the mitogen-activated protein kinase kinase 3/6 (MKK3/6) (Tibbles *et al*., 1996). In addition, the MLK2 has been shown to be associated with the microtubules, where it co-localises with activated SAPK/JNK and KIF3, a member of the kinesin superfamily motor proteins (Nagata *et al*., 1998).

There also exists downstream effectors for Cdc42 and Rac which do not have CRIB-domains, e.g. IQGAP and POR1 (Figure 4). IQGAP interacts with both Cdc42 and Rac and is involved in the formation of lamellipodia (Brill *et al*., 1996; Hart *et al*., 1996; Schmidt and Hall, 1998), as well as the regulation of cadherin-dependent cell-cell adhesion (Erickson *et al*., 1997; Kuroda *et al*., 1998; Fukata *et al*., 1999), whereas POR1 is involved in the formation of lamellipodia (Van Aelst and D`Souza-Schorey, 1997; Schmidt and Hall, 1998). Cdc42-interacting protein 4 (CIP4) also binds Cdc42 via a

domain motif unrelated to the CRIB domain (Aspenström, 1997). CIP4 has recently in turn been shown to interact with the SH3 domain of the protein RhoGAP interacting with CIP4 homologues (RICH-1) (Richnau and Aspenström, 2001).

Examples of targets for Rho are the PKC-related Ser/Thr kinases PKN and PRK2, rhotekin, rhophilin, Rho-associated coiled-coil-containing protein kinase (ROCK), also called ROK, and the mammalian diaphanous protein, p140mDia (Figure 4) (Reid *et al*., 1996; Watanabe *et al*., 1996; Bishop and Hall, 2000). ROCK is important for stress fibre formation, while p140mDia is important for actin polymerisation (reviewed in Schmidt and Hall, 1998), as well as stress fibre formation in cooperation with ROCK and the actin-binding protein, profilin (Watanabe *et al*., 1999; Tominaga *et al*., 2000; Ishizaki *et al*., 2001). ROCK and Dia have also been shown to have effects on adherent junctions, with Dia as a stabilisor and ROCK as an inhibitor of cell-cell contacts (Sahai and Marshall, 2002). Downstream targets for ROCK include myosin light chain (MLC) (Amano, *et al*., 1996), involved in actin-myosin filament assembly and LIM kinase (LIMK) (Maekawa *et al*., 1999). Recently, it was shown that the human brahma-related gene 1 (BRG1) protein, a component of the SWI/SNF family of the ATP-dependent chromatin remodeling complex, affected the RhoA pathway by increasing the protein level of ROCK1 and thereby induced the formation of stress fibers (Asp *et al*., 2002). The SWI/SNF family of proteins has also been seen to bind to histone acetyltransferase complexes (HATs), involved in transcriptional regulation and protein stability (Hassan *et al*., 2001).

Other proteins important in the regulation of the actin cytoskeleton are phosphoinositide kinases (PI-kinases) including PI3K (Figure 4) (Schmidt and Hall, 1998). PI3K is an enzyme that catalyzes the conversion of phosphatidyl-inositol-4,5-bisphosphate (PIP_2) into phosphatidyl-inositol-3,4,5-triphosphate (PIP_3). The activation of the Rho GTPases by PI3K is probably achieved through binding of PIP_3, the product of PI3K, to the PH domain of a Rho-GEF, which thereby is activated (reviewed in Scita *et al*., 2000). Constitutive activated PI3K induces Rac-dependent lamellipodia and Rho-dependent stress fibers (reviewed in Scita *et al*., 2000). PI3K can act both upstream and downstream of Rac in the Rho GTPase signalling pathway, whereas the other Rho GTPases, Cdc42 and Rho have yet only been seen to be downstream of PI3K (Figure 4). These differences are likely to be dependent on cell type, external stimuli and involved proteins (reviewed in Schmidt and Hall, 1998). Recently, however it has been suggested that activation of type I PI3Ks by Rho-family GTPases is a manifestation of a positive-feedback loop, in which PI3K acts upstream of the Rho GTPases, involving cell polarity (Rickert *et al*., 2000; Stephens *et al*., 2002). c-Akt, a serine/threonine protein kinase whose activity has been shown to depend on PI3K, has been shown to co-localise with Rac and Cdc42 at the leading edge of mammalian fibroblasts and to be essential for Rac and Cdc42-regulated cell motility (Higuchi, *et al*., 2001).

Several activators for the Rho GTPases have been found which include more then 30 mammalian GEFs (also known as Dbl family proteins) (reviewed in Schmidt and Hall, 2002). The first mammalian GEF found was Dbl (Eva and Aaronson, 1985), catalyzing the exchange of human Cdc42 (Hart *et al*., 1991). There are several other potential

Cdc42-specific GEFs in mammalian cells. One GEF candidate is the cloned-out of library/PAK-interacting exchange factor (Cool/βPix), which is a PAK-interacting guanine nucleotide exchange factor for both Cdc42 and Rac (Manser *et al*., 1998). The Pix family contains of two members, αPix and βPix (Koh *et al*., 2001) and they all share Src homology 3 (SH3), Dbl homology (DH), pleckstrin homology (PH), GIT1-binding (GB) domains, and proline-rich regions (Whitehead *et al*., 1997). In addition to all those domains, βPix also contains a putative leucine zipper domain at the C-terminal end, which has been shown to be critical for βPix homodimerization and lamellipodia formation (Kim *et al*., 2001). These domains presumably function to mediate protein/protein or protein/lipid interactions and serve to link GEFs to upstream regulators and downstream effectors (Whitehead *et al*., 1997). βPix has recently also been shown to enhance the p38 MAPK activation by a Cdc42, Rac, PAK, and MKK3/6-mediated pathway, implicated in the regulation of lamellipodia (Lee *et al*., 2001) and in basic fibroblast growth factor (FGF)-induced neurite outgrowth via the Ras, ERK, and PAK2 pathway (Shin *et al*., 2002).

c) p38 MAPK and cytoskeletal control

Four distinct groups within the MAPK family of intracellular serine/threonine kinases have been described: ERK, SAPK/JNK, ERK5/big MAP kinase 1 (BMK1) and p38 MAPK (reviewed in Ono and Han, 2000).

p38 MAPK

The MAPK family member of intracellular serine/threonine kinases, p38 MAPK, was first identified as a 38-kDa protein, activated by tyrosine phosphorylation upon extracellular endotoxic LPS stimulation (reviewed in Ono and Han, 2000). Similar to the other MAPK, the p38 MAPK members are activated by a MAP kinase kinase (MKK) at conserved Thr-Xaa-Tyr (TXY) dual phosphorylation sites (reviewed in Ono and Han, 2000; Davis, 2000). The p38 MAPK pathway is activated by cellular stress, pro-inflammatory cytokines and growth factors (reviewed in Ono and Han, 2000).

The upstream kinases for p38 MAPK are MKKKs, such as the apoptosis signal-regulating kinase 1 (ASK1) (Ichijo *et al*., 1997) and TAK1 (Moriguchi *et al*., 1996), which in turn activate MKK proteins, such as MKK3 and MKK6, which then activate p38 MAPK (Ono and Han, 2000). The Rho GTPases, Rac and Cdc42 are important regulators of the p38 MAPK pathway. Dominant negative variants of Rac and Cdc42 inhibited IL-1 dependent p38 MAPK activation (Bagrodia *et al*., 1995; Zhang *et al*., 1995). Rho, Rac and Cdc42 were also shown to bind and activate both MKK3 and MKK6, inducing p38 MAPK activation (Yamauchi *et al*., 2001). In addition, PAK was shown to be involved in the MAPK pathway, since dominant negative, catalytically inactive, PAK inhibited the p38 MAPK activation by IL-1, Rac and Cdc42 (Zhang *et al*., 1995). Recently, it was also found that a scaffolding protein for p38 MAPK, IB2/JIP2, binds both the Rac exchange factor Tiam1, Ras-GRF1, MLK3, MKK3 and p38 MAPK, leading to activation of the p38 MAPK signalling cascade (Buchsbaum *et al*., 2002).

p38 MAPK and cytoskeletal control
Platelet-derived growth factor-BB (PDGF-BB) has been shown to regulate the cytoskeleton via activation of the p38 MAPK pathway. PDGF-BB stimulated the migration as well as lamellipodia formation of hepatic myofibroblasts (HMFs), by activation of the p38 MAPK pathway, involving the heat shock protein 27 (HSP27), ERK1, -2 and FAK (Tangkijvanich *et al*., 2002). p38 MAPK has also been found to be involved in cytoskeletal control and cell migration via phosphorylation of paxillin and HSP27 (Hedges *et al*., 1999). Recently, p38 MAPK and HSP27 were shown to be activated by TGF-β in osteoblast-like MC3T3-E1 cells (Hatakeyama *et al*., 2002). p38 MAPK has previously been shown to be involved in a TGF-β-induced pathway in *Drosophila*, where p38 MAPK and MKK3 were needed during wing morphogenesis (Adachi-Yamada *et al*., 1999).

d) TGF-β signalling and cytoskeletal control
TGF-β-induced effects on the microfilaments as well as the microtubules have been reported. The microtubule system was shown to be important for the regulation of TGF-β-induced gene transcription and Smad translocation. In one report, Smad2, Smad3 and Smad4 were seen to bind microtubules in endothelial and epithelial cells in the absence of TGF-β. TGF-β treatment then resulted in phosphorylation and dissociation of the Smads from microtubules to be translocated into the nucleus where they affected gene transcription (Dong *et al*., 2000).

Effects of TGF-β on the formation of focal adhesions and stress fibers have also been reported (Like and Massagué, 1986; Koyasu *et al*., 1988; Lee *et al*., 1999). TGF-β-mediated stress fiber formation was recently shown to involve the Rho-specific guanine exchange factor NET1 in Swiss 3T3 cells (Shen *et al*., 2001). In addition, TGF-β has been shown to induce migration of different cell types such as epithelial cells (Boland *et al*., 1996; Zicha *et al*., 1999), and mast cells (Gruber *et al*., 1994; Olsson *et al*., 2000), and could thus contribute to tumor invasiveness and metastasis. The TGF-β superfamily member activin A was shown to induce migration as well as formation of focal adhesions and stress fibers in vascular smooth muscle cells, through a mechanism that involved phosphorylation of the focal adhesion proteins paxillin and $p130^{CAS}$ (Riedy *et al*., 1999). TGF-β induced similar cytoskeletal reorganisations, but in contrast to Activin, TGF-β did not induce migration (Riedy *et al*., 1999).

Treatment of cells in tissue culture with TGF-β has been reported to affect the morphology of a number of different cell types such as mink lung epithelial Mv1Lu cells (Like and Massagué, 1986), Swiss 3T3 cells (Lee *et al*., 1999) and human epidermoid carcinoma KB cells (Koyasu *et al*., 1988). This effect was at least partially dependent on changes in the cytoskeleton caused by altered expression of cytoskeletal proteins, such as α-smooth muscle actin (Nakajima *et al*., 1999), vimentin or tubulin (Lomri and Marie, 1990). This response occurred after several hours of TGF-β stimulation and required new RNA and protein synthesis (Koyasu *et al*., 1988; Baghdassarian *et al*., 1993). However, in the course of this thesis work short time responses of TGF-β were noticed as lamellipodia formation in prostate cancer cells, through the activation of Cdc42, Rho and p38 MAPK (paper I).

TGF-β-mediated effects on cell morphology also occur in the process of epithelial to mesenchymal transdifferentiation (EMT). This phenomenon was first reported in mouse breast epithelial NMuMG cells, where TGF-β stimulation resulted in a dramatic morphological alteration: the rhomboid epithelial cells lost their cell-cell contacts and cell polarity, flattened out and obtained a fibroblast-like morphology (Miettinen *et al*., 1994). This process occurred after 48 to 72 hours and was accompanied by a down-regulation of E-cadherin and formation of stress fibres (Piek *et al*., 1999b). This response was shown to be dependent on gene transcription via the Smad signalling pathway (Piek *et al*., 1999b). However, contradictory to these findings, other studies have suggested that the Smad proteins are not involved in EMT. Overexpression of the inhibitory Smad7 as well as a dominant negative Smad3 did not interfere with EMT (Bhowmick *et al*., 2001a). Therefore it is still an important task to study the molecular mechanism underlying the involvement of Smad signalling in regulation of actin reorganisation or migration. The Rho GTPases have been shown to be involved in TGF-β-induced EMT. TGF-β-mediated effects in EMT were recently shown to involve the induction of stress fibers in H-Ras-transformed fibroblasts in a Rho-dependent manner (Moustakas and Stournaras, 1999). Furthermore, expression of a dominant negative mutant of RhoA or its effector ROCK, were shown to inhibit TGF-β-mediated EMT (Bhowmick *et al*., 2001a). Recently, TGF-β3-induced activation of the RhoA/Rho-kinase pathway was also shown to be involved in EMT (Kaartinen *et al*., 2002). In addition, TGF-β-mediated EMT and cell migration was shown to require PI3K (Bakin *et al*., 2000; Kang and Svoboda, 2002), as well as Rac1-mediated p38 MAPK activation (Bakin *et al*., 2002). However, the PI3K pathway is possibly not always necessary for TGF-β-mediated Rho GTPase activation. Activation of Rho GTPases was shown to be essential for PDGF as well as TGF-β-induced contraction of human dermal fibroblast, but no PI3K activation was detected (Han *et al*., 2002).

Most certainly, cross-talk between TGF-β and integrin signal transduction pathways exists. Integrin β1 has been shown to be necessary for TGF-β induced activation of p38 MAPK and epithelial plasticity (Bhowmick *et al*., 2001b). TGF-β was also shown to significally up-regulate the expression levels of different integrin α subunits in peritoneal fibroblasts, leading to increased levels of the focal adhesion molecule vinculin, in focal contacts. This response was also associated with a reorganisation of the microfilament system and an increased migration (Rout *et al*., 2002).

3. TGF-β-induced apoptosis

a) Apoptosis

Cell death can occur by two distinct mechanisms, necrosis and apoptosis. Apoptosis, also called programmed cell death, is an important and very carefully regulated process in human development and disease. This program allows the multicellular organisms to remove cells that are in excess or potentially dangerous. The coordination and balance between cell survival and apoptosis is crucial for normal development and homeostasis of multicellular organisms. Defects in control of this balance may contribute to a variety of diseases, including cancer, autoimmune disease and neurodegenerative conditions

(reviewed in Kroemer *et al*., 1998). The apoptotic process of cells is characterised by cell shrinkage, chromatin condensation, fragmentation of DNA and the formation of cytoplasmic blebs (reviewed in Hengartner, 2000). These characteristic morphological alterations occurring in cells undergoing apoptosis, originally described by Kerr *et al*., (1972), were further on shown to be mainly caused by a group of cysteine proteases, called caspases, that are activated specifically in apoptotic cells (reviewed in Green, 1998; Hengartner, 2000). Blockage of the caspase activity can prevent, or even rescue, cells from apoptosis. Procaspases are synthesised as inactive enzymes that are activated by cleavage, leading to a cascade of caspase activations. Caspase-8 is the initiator in the caspase cascade, with caspase-3, -6 and -7 as downstream caspase substrates. Caspases can then activate or inactivate a set of target proteins through proteolytic cleavage (reviewed in Hengartner, 2000). In contrast, necrosis, also called accidental cell death, does not involve any regular DNA and protein degradation patterns. The necrotic process is characterised by the damaging of the plasma membrane through the swelling and rupturing of the entire cell, often leading to tissue damage and inflammation. Because of the distinct mechanisms for necrotic and apoptotic cell death, it has been possible to develop methods for studying necrosis and apoptosis as separate phenomena.

There exist two major apoptotic pathways in mammalian cells, the death-receptor pathway and the mitochondrial pathway (Figure 5) (reviewed in Hengartner, 2000). The death-receptor pathway starts with CD95 ligand binding, leading to the clustering of either CD95 receptors or tumor necrosis factor receptor I receptor complexes. These complexes then recruit several procaspase-8 molecules via the adaptor protein, Fas-associated death domain protein (FADD), and thus initiating the downstream caspase cascade involving activation of caspase-8 and -3 (Figure 5). The caspase-8 activation can be inhibited by the degenered caspase homologue c-FLIP (Figure 5). The death-receptor pathway can interfere with the mitochondrial pathway, by the caspase-8-mediated cleavage of a member of the Bcl-2 superfamily, Bid, which then binds to, and accelerates the release of the pro-apoptotic agent cytochrome c from the mitochondria, which promotes cell death (Figure 5) (reviewed in Green, 1998; Hengartner, 2000). The released Cytochrome c then functions to activate Apaf1 and procaspase-9, to form the apoptosome complex, which in turn can activate procaspase-3 leading to caspase-3 activation and eventually to apoptosis (figure 5).

The mitochondrial pathway is activated by extracellular as well as intracellular signals, such as DNA damage, which is known to activate p53, and in turn leading to induction of Bax (Figure 5) (Miyashita and Reed, 1995). The Bcl-2 family members, which consists of three functional subgroups, are important intermediates in this pathway. The first group with Bcl-2 and Bcl-x_L show anti-apoptotic activity, inhibiting cell death, whereas the second group with Bax, and the third with Bid and Bik, show pro-apoptotic activity, activating cell death. Other molecules which can be released from the mitochondria after an apoptotic stimuli include Smac/DIABLO and apoptosis inducing factor (IAF), with pro-apototic activity, and inhibitors-of-apoptosis (IAPs), with anti-apoptotic activity (Figure 5) (reviewed in Green, 1998; Hengartner, 2000).

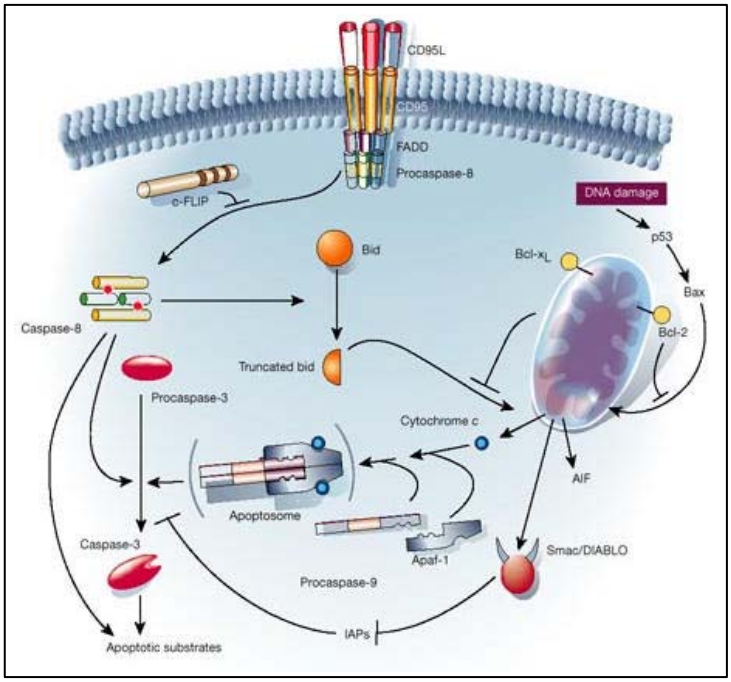

Figure 5 The two major apoptotic pathways in mammalian cells (Hengartner, 2000).

The apoptotic cascade can be divided into three phases, initiation, integration and execution. The initiation phase involves the triggering of stress and growth factors. The intergration phase involves the coordination and balance between cell survival and apoptosis involving different protein-protein interactions of signalling molecules, and finally, the execution phase involves the activation and degradation of the caspase family (Schuster and Krieglstein, 2002).

b) TGF-β-mediated apoptosis and the involvement of other growth factors
TGF-β family members can induce apoptosis in many different cell types, in the presence or absence of other growth factors. TGF-β was shown to induce apoptosis and proliferation of T- and B-cells in the immune system, through the up- or downregulation of the Bcl-2 family members and activation of caspases (reviewed in Schuster and Krieglstein, 2002). Furthermore, the selective expression of several proteins was found to be up-regulated in a microarray study in haematopoietic cells after TGF-β and activin induced apoptosis. These proteins included inositol phosphatase SHIP [Src homology 2 (SH2) domain-containing 5´inositol phosphatase], and Smad2,

Smad3 and Smad4 (Valderrama-Carvajal *et al*., 2002). TGF-β has also been shown to have a functional role in the development of the liver. Activation of TGF-β caused both growth arrest and apoptosis in rat liver epithelial cells (Teramoto *et al*., 1998). In addition, TGF-β induced apoptosis in FaO hepatoma cells, mediated by cytochrome c release, caspase activation and the up-regulation of certain genes, such as *jun* and *ATF3* and genes encoding cytoskeletal proteins and extracellular matrix proteins (Coyle *et al*., 2003).
Furthermore, TGF-β has been shown to induce apoptosis in normal prostate cells as well as prostatic carcinoma cells *in vitro* and *in vivo* (Landström *et al*., 1996; Rajah *et al*., 1997). Moreover, it exist both positive and negative cross-talk between different growth factors and TGF-β in the apoptotic pathway. Insulin-mediated survival signals via activation of PI3K and c-Akt prevented the TGF-β-induced apoptotic effect in Hep3B cells (Chen and Chang, 1997; Chen *et al*., 1998a). However, both TGF-β and activin A induced apoptosis and growth inhibition via a Smad-dependent pathway in the same cell line (Kanamaru *et* al., 2002). In addition, TNF-α and EGF suppressed the pro-apoptotic effect of TGF-β, in primary hepatocytes, by a mechanism involving both PI3K and MAPK pathways (Roberts *et al*., 2000).

TGF-β is an important component in tissue formation, through the regulation of apoptotic cell death during the animal life, in processes such as postlactational involution (Rosfjord and Dickson, 1999), embryogenetic tissue modeling (Choi and Ballerman, 1995) and wound healing (Crowe *et al*., 2000). Furthermore, TGF-β was shown to induce cell death in c-myc overexpressing mouse mammary epithelial cells (Amundadottir *et al*., 1996; Nass *et al*., 1996), as well as in MCF-7 breast cancer cells (Chen *et al*., 1996). TGF-β has, moreover, been shown to be an important component in regulating the balance of neuron survival and death, supporting those neurons that have successfully reached their target area and promote cell death of the others (de Luca *et al*., 1996).

Finally, apoptosis can also occur by the treatment of other stimuli, either promoted by serum withdrawal and Fas/APO-1, or inhibited by insulin and PDGF (reviewed in Schuster and Krieglstein, 2002).

All these observations demonstrate that TGF-β is an important component in apoptosis of a large number of cell types, involving the regulation of many different types of proteins.

c) TGF-β-mediated apoptosis involving TAK1 and TAB
TAK1 was originally identified as a MAPKKK, activated downstream of the TGF-β and BMP receptors, positively regulating the SAPK/JNK and p38 MAPK pathways (Figure 6) (Yamaguchi *et al.,* 1995). TAK1 has been shown to significantly contribute to an apoptotic signal in several different organisms: in the retina of *Drosophila melanogaster* through activation of JNK (Takatsu *et al.,* 2000), in early embryos of *Xenopus* with ectopic expression of TAK1 (Shibuya *et al.,* 1998), and in the heart of transgenic mice expressing an activating mutation of TAK1, through the activation of p38 MAPK (Zhang and Derynck, 2000). The upstream activator of TAK1, TAB1 (Shibuya *et al*.,

1996) is linked to TGF-β, as well as BMP receptor activation via its upstream activators, HPK1 (Wang *et al*., 1997) or XIAP (Yamaguchi *et al*., 1999), leading to downstream SAPK/JNK-p38 MAPK activation and apoptosis (Figure 6) (ten Dijke *et al*., 2002). Recently, TAB1 was also shown to be up-regulated after TGF-β-induced apoptosis in FaO hepatoma cells (Coyle *et al*., 2003). Smad proteins have also been suggested to be involved in TGF-β-mediated apoptosis, both involving pro-apoptotic as well as anti-apoptotic effects (Figure 6). Smad6 and Smad7 were also found to be able to interact with TAB1 (Yanagisawa *et al.,* 2001). In the course of the present thesis work, we found that TAK1 interacts with Smad7 (paper III).

A newly identified apoptosis-related protein in TGF-β signalling (ARTS), which translocates from the mitochondria to the nucleus upon TGF-β-stimulation, has been found to be an important downstream effector for TGF-β-mediated apoptosis (Figure 6) (Larisch *et al*., 2000). Another essential component of TGF-β-induced apoptosis is the Fas-receptor-associated adaptor protein, Daxx. It binds directly to the cytoplasmic domain of TGFβR-II and activates the SAPK/JNK pathway and Fas-induced apoptosis (Figure 6) (Perlman *et al*., 2001).

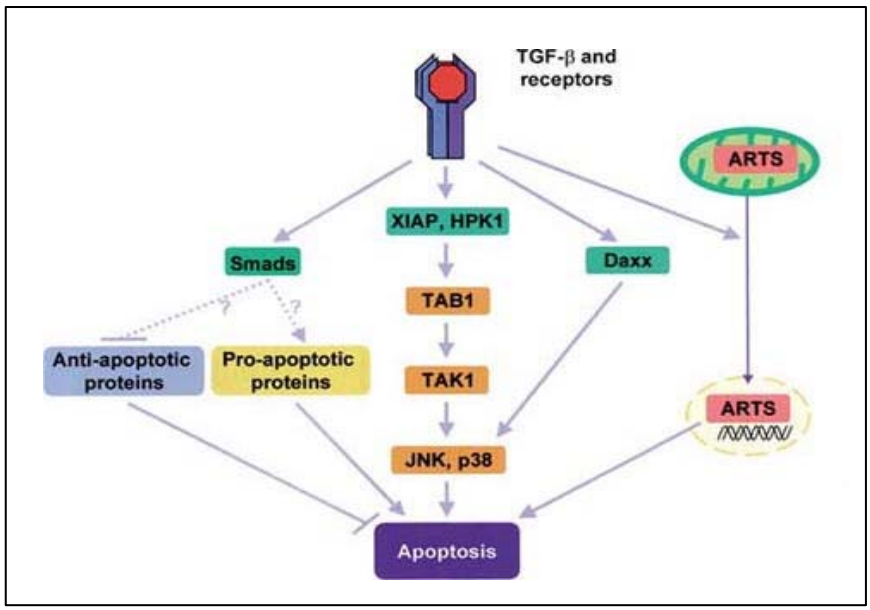

Figure 6 Gene regulations in TGF-β-induced apoptosis (ten Dijke *et al*., 2002).

d) Smad7 and apoptosis

Increased apoptosis was detected in Madine-Darby canine kidney (MDCK) cells, since coexpression of Smad3 and Smad4 (also called DPC4), in these cells, resulted in apoptosis through the involvement of the SAPK/JNK signalling pathway (Atfi *et al*., 1997a). Moreover, overexpression of Smad7 has been detected to enhance apoptosis of

MDCK cells caused by treatment with TGF-β or tumor necrosis factor α (TNF-α), growth factor withdrawal, or loss of cell adhesion (Lallemand *et al*., 2001). This effect was found to be mediated by a decreased expression of NF-κB, a molecule known to promote survival of cells. Furthermore, in normal and malignant prostate epithelial cells, enhanced Smad expression, seen as activated Smad2 and nuclear Smad6 and Smad7, were found in areas with large numbers of apoptotic cells after androgen withdrawal (Brodin *et al*., 1999). However, overexpression of dominant negative Smad3 and wild-type Smad7 were also shown to inhibit TGF-β-induced apoptosis (Yamamura *et al*., 2000). In contrast, Smad7 was shown to be necessary for TGF-β-induced apoptosis of epithelial cells (Landström *et al*., 2000). Podocytes, which is a highly specialized cell type in the kidney, has also been shown to undergo apoptosis after TGF-β1 stimulation or overexpression of Smad7 through adenoviral infection (Schiffer *et al*., 2001). Recently, TGF-β-induced expression of Smad7 in rat mesanglian cells, induced apoptosis (Okado *et al*., 2002), whereas the opposite effect was seen in BMP and activin signalling; both Smad6 and Smad7 were shown to inhibit BMP-induced apoptosis, whereas Smad7 inhibited activin A-induced apoptosis in a mouse cell hybridoma HS-72 cell line (Ishisaki *et al*., 1999). All these findings suggest different functions of the I-Smads in the TGF-β superfamily receptor activated apoptotic pathway.

The two I-Smads seem to have different roles in the regulation of the MAPK pathways (Mazars *et al*., 2001). Smad6 has been reported to inhibit BMP-TAK1-induced phosphorylation of p38 MAPK and apoptosis in a mouse hybridoma cell line, MH60 cells (Kimura *et al.,* 2000). In contrast, overexpression of Smad7 has been demonstrated to cause activation of the JNK/SAPK pathway and apoptosis in MvLu1, MDCK and COS-7 cells (Mazars *et al.,* 2001). One of the aims of the present thesis work was to further characterise the involvement of the Smad proteins and the p38 MAPK pathway in TGF-β-induced apoptosis (paper III).

e) The MAPK p38, the nuclear substrate for p38, ATF-2 and apoptosis

p38 MAPK and apoptosis

The involvement and activation of the MAPKs pathways in apoptosis is well studied. But how these MAPKs modify the rate of apoptosis is unclear and the roles of the different MAPKs are highly cell type- and stimulus-dependent. The activation of SAPK/JNK1, -2, -3, and p38 MAPK, are shown to promote apoptosis, while extracellular signal-related kinase ERK1, -2 inhibits apoptosis (Xia *et al*., 1995; Cross *et al*., 2000; Chang and Karin, 2001). A recent report confirms this notion by showing that disruption of one of the MAPK pathways affects the TGF-β-induced activation of other MAPK pathways in FaO rat hepatoma cells; inhibition of ERK enhanced both TGF-β-induced p38 MAPK and JNK activation (Park *et al*., 2002). The dynamic balance between growth factor-activated ERK and stress-activated SAPK/JNK-p38 MAPK pathways might determine whether a cell survives or undergoes apoptosis. Furthermore, p38 MAPK was shown to induce c-Jun phosphorylation and apoptosis in neurons (Yamagishi *et al*., 2001). In addition, the withdrawal of growth factors has been shown to activate the p38 MAPK pathway in adipocytes and in neurons (Xia *et al*., 1995; Ichijo *et al.,* 1997; Kummer *et al.,* 1997; Yamagishi *et al.,* 2001), whereas stimulation by TNF-α activates both the SAPK/JNK and p38 MAPK pathways, leading to apoptosis

(Kyriakis *et al*., 1994; Raingeaud *et al*., 1995), which could in some cases be inhibited by insulin (Kummer *et al*., 1997). These data further support the role of p38 MAPK in cellular apoptosis and support the hypothesis that insulin promotes cell survival. However, the opposite situation in which p38 MAPK was involved in cell survival has also been demonstrated; p38 MAPK was shown to protect human melanoma cells from UV-induced apoptosis through the down-regulation of NF-κB activity and Fas expression (Ivanov and Ronai, 2000). Moreover, another study has indicated that the activation of SAPK/JNK or p38 MAPK may be involved in the protection of TNF-α-induced apoptosis, through the requirement of p38β, but not p38α (Guo *et al*., 2001). Recently, yet another study, in human neutrophils, suggested that p38 MAPK was involved in a survival pathway. Early and transient inhibition of the p38 MAPK activation during spontaneous and Fas-induced apoptosis, lead to induced caspase activation and thereby apoptosis. This study showed that the PI3K had the opposite effect to p38 MAPK, involved in a pro-apoptotic signal through the activation of Fas leading to spontaneous apoptosis (Alvarado-Kristensson *et al*., 2001). PI3K has otherwise mostly been linked to survival pathways, via its downstream target protein c-Akt. One recent study demonstrated the involvement of PI3K-Akt in protection of endothelial cells from apoptosis through the inhibition of p38 MAPK-dependent apoptosis (Gratton *et al*., 2001). MAPKs are also known to be able to activate p53, a protein which can activate almost all apoptotic pathways in the cell (Vousden, 2000).

These different involvements of p38 MAPK in both cell survival and apoptosis could probably reflect the multiple and complex activities of this signalling pathway, which acts simultaneously on different targets and thus can yield distinct end effects depending on the cellular context. This is not something unique to p38 MAPK, many growth-promoting pathways can be either pro- or anti-apoptotic, depending on the cellular context. Several studies have found an increased phosphorylation of the p38 MAPK after TGF-β treatment, as well as increased apoptosis in different cell lines, which could be inhibited by the use of specific MAPK inhibitors (Liao *et al*., 2001; Schrantz *et al*., 2001; Hyman *et al*., 2002; Pelaia *et al*., 2003). Recently, it was shown that TGF-β-induced activation of p38 MAPK was required for TGF-β-induced apoptosis as well as EMT, but not growth arrest (Yu *et al*., 2002). They further demonstrated the p38 MAPK activation by TGF-β treatment was independent of Smads. Furthermore, we report in this thesis that TAK1 is activated downstream of the TGF-β receptors, positively regulating the p38 MAPK pathway and Smad7-dependent apoptosis (paper III).

ATF-2 and apoptosis

The nuclear substrate for p38 activating transcription factor 2 (ATF2), is a basic region-leucine zipper transcription factor which can mediate a diverse range of transcriptional responses including those generated by various forms of cellular stress (Davis, 2000). Recently, it was reported that ATF2 is phosphorylated and activated after TGF-β1 stimulation, in a TAK1- and p38 MAPK-dependent manner (Sano *et al.,* 1999). Moreover, Smad3 and Smad4 were reported to interact with ATF2, and act in synergy with TAK1, MKK6 and ATF2 in a transcriptional assay (Hanafusa *et al.,* 1999; Sano, *et al.,* 1999). In addition, phosphorylation of both c-Jun and ATF2 has been shown to correlate with apoptosis, induced by stress-inducing factors, such as ischemia or

treatment with cyclosporine-A (Walton *et al*., 1998; Pyrzynska *et al*., 2000). Furthermore, upregulation of ATF3 after TGF-β-induced apoptosis were recently shown in several different cell lines (Zimmerman *et al*., 2000; Coyle *et al*., 2003).

f) MKK3/6 and apoptosis

ASK1, mediates apoptosis through activation of SEK1/MKK4 or MKK3/6, which, in turn, activates SAPK/JNK and p38 MAPK (Ichijo *et al*., 1997; Tobiume *et al*., 2001). The ASK1-JNK-p38 pathway mainly mediates apoptosis by the activation of the proinflammatory cytokine TNF-α, the death receptor family member Fas, or oxidative stress-activated kinases (reviewed in Matsuzawa and Ichijo, 2001). However, ASK1-MKK6-p38 MAPK has also been shown to alter the degree and nature of the UV-induced apoptosis of melanoma cells, which promoted cell survival (Ivanov and Ronai, 2000). Furthermore, MKK6b was shown to be necessary for Fas-induced apoptosis in jurkat cells, whereas p38 MAPK did not participate in this pathway (Huang *et al*., 1997). The serine/threonine kinase MLK3, has also been shown to be involved in apoptosis through the activation of SAPK/JNK or p38 MAPK in neuronal cells (Mota *et al*., 2001; Xu *et al*., 2001).

g) Rho GTPases and apoptosis

Cdc42 has been shown to induce apoptosis in Jurkat cells through the activation of SAPK/JNK (Chuang *et al*., 1997). In addition, Rac has been shown to be involved in TNF-α-induced apoptosis in U937 cells (Esteve *et al*., 1998). However, Rac has also been shown to be involved in cells survival, in Rat 1 fibroblasts, possibly via activation of PI3K (Ruggieri *et al*., 2001) and in NIH3T3 cells, where Rac1 prevented cisplatin-induced activation of p38 MAPK and apoptosis (Jeong *et al*., 2002). Cdc42 has also been demonstrated to be involved in apoptosis through the activation of PAK1 and SAPK/JNK, promoted by p53 in baby rat kidney cells (Thomas *et al*., 2000). p53 is a much investigated component in tumor growth, through its ability to inhibit cell cycle progression and promote apoptosis. Recently, new members of the family of Rho GTPases, called Miro-1 and Miro-2 (for mitochondrial Rho) were found to be present in mitochondria and to have roles in mitochondrial homeostasis and apoptosis (Fransson *et al*., 2003). All these results demonstrate that the Rho GTPases can either have anti-apoptotic or pro-apoptotic roles, depending on the cellular context.

h) Membrane blebbing

One of the characteristically features of apoptosis is blebbing, which can occur in migrating cells and in cells undergoing mitosis. The formation of blebs is controlled by different processes, such as microtubule disassembly, local actin depolymerisation and increased cellular pressure (Keller *et al*., 2002). Two different form of blebs has been described, membrane dissociation blebs and cortical actin disassembly blebs (Keller *et al*., 2002). This phenomenon has been shown to be dependent on p38 MAPK-sensitive changes in microfilament dynamics, mediated by the phosphorylation of HSP27, as well as caspase-dependent and -independent nuclear condensation and fragmentation (Deschesnes *et al*., 2001). Furthermore, the p21-activated kinase PAK2 is activated by caspase cleavage leading to activation of membrane blebbing and increased apoptosis (Rudel and Bokoch, 1997). In addition, ecotopic expression of ROCK I (Coleman *et al*.,

2001) and ROCK II (Song *et al*., 2002), were also shown to induce membrane blebbing and chromatin condensation and increased apoptosis.

4. TGF-β- and Wnt-signalling pathways

The Wnt family are cysteine-rich secreted glycoproteins involved in control of several different cellular processes, such as gene expression, cell adhesion, cell polarity, cell proliferation and apoptosis (reviewed in Wodarz and Nusse, 1998; Bienz and Clevers, 2000; Moon *et al*., 2002). Members of the lymphoid enhancer factor-1/T-cell factor (LEF/TCF) family of transcription factors have together with β-catenin been shown to be nuclear effectors in the Wnt-signalling pathway (Barker *et al*., 2000).

a) The Wnt-signalling pathway

The family of Wnt proteins is highly conserved throughout the evolution. Elucidating studies have been made in *Drosophila, Caenorhabditis elegans*, *Danio rerio*, *Xenopus*, as well as in mammals (reviewed in Wodarz and Nusse, 1998; Bienz and Clevers, 2000; Moon *et al*., 2002). The first gene discovered in the Wnt family, was the mouse *Wnt-1*, acting as a proto-oncogene activated by integration of mouse mammary tumor virus in mammary tumors (Nusse and Varmus, 1982). The next gene to be discovered was the *wingless* (*wg*) gene identified in *Drosophila*, as an orthologue of Wnt-1, involved in polarity (Cabera *et al*., 1987). In *Drosophila* and *Xenopus*, Wnt/Wg signalling activates several genes that control axin formation and segmental identity, such as, *Xnr-3*, *Twin* and *Ubi*, which are all regulated by LEF-1/TCF proteins in association with β-catenin (McKendry *et al*., 1997; Hsu *et al*., 1998b). In mammalian cells, the activation of the Wnt-signalling pathway has been shown to be particularly important in colorectal cancer cells, where mutations of β-catenin or adenomatous polyposis coli (APC) results in accumulation of β-catenin in the cytoplasm and in the nucleus (Bienz and Clevers, 2000; Wong and Pignatelli, 2002). The seven-pass transmembrane proteins of the Frizzled (Frz) family, have been found to be receptor for Wnts and to be activated upon binding by the secreted Wnts and to activates downstream signalling components (reviewed in Wodarz and Nusse, 1998; Moon *et al*., 2002). The cellular outcome of the activation of the Wnt/Frz pathway is complex, and is suggested to have an oncogenic effect through reduced degradation of cytosolic β-catenin resulting in an increased nuclear translocation of β-catenin (reviewed in Wong and Pignatelli, 2002).

The canonical Wnt/β-catenin pathway

In the absence of Wnt signalling, the glycogene synthetase kinase-3 (GSK-3) phosphorylates β-catenin, thereby inducing its degradation by the ubiquitin-proteasome pathway (Figure 7) (Fagotto *et al*., 1999; Kitagawa *et al*., 1999). Upon Wnt signalling activation, the cytoplasmic protein Dishevelled is activated, thus destabilising the axin-APC-GSK-3 complex which thereby inhibits the GSK-3 phosphorylation of β-catenin (Figure 7). This promotes β-catenin stabilisation and translocation to the nucleus, where it binds to the family of LEF/TCF transcription factors (Figure 7) (reviewed in Bienz and Clevers, 2000; Sharpe *et al*., 2001; Moon *et al*., 2002). The LEF1/TCF factors are a family of DNA-binding proteins that associate with β-catenin and thereby modulate the expression of defined target genes (Molenaar *et al*., 1996).

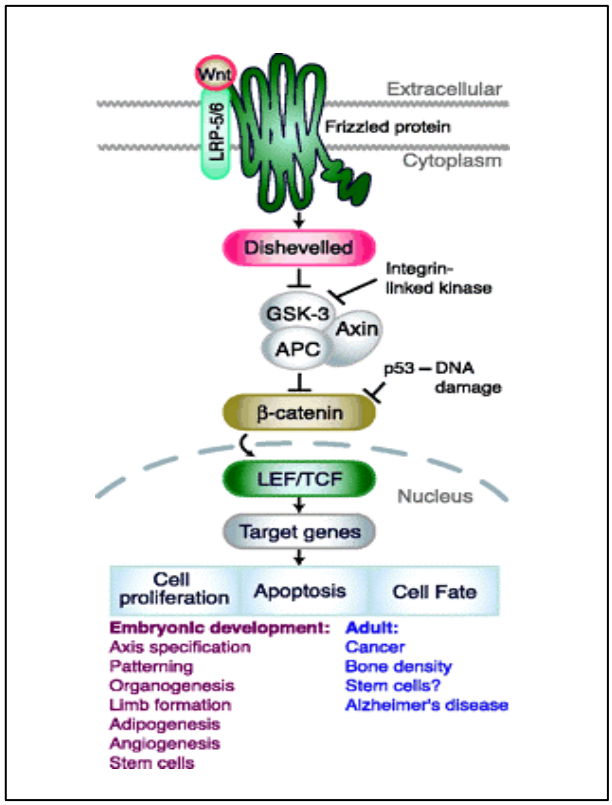

Figure 7 The canonical Wnt/β-catenin pathway (Moon *et al*., 2002)

β-catenin

β-catenin is one of the best characterised components in the Wnt signalling pathway. It was first described in humans as a member of the adherens junction complex (Kemler and Ozawa, 1989). In mammalian cells, the subcellular localisation and protein level of β-catenin are under strict control (Polakis, 2000). Normally, β-catenin is either bound to the intracellular domain of cadherin (calcium-dependent adhesion molecules) and the microfilaments at adherens junctions, or targeted for degradation in the cytoplasm, by binding to APC, axin and GSK-3 (Sharpe *et al*., 2001). A serine/threonine phosphorylation of β-catenin and APC by GSK-3 causes dissociation from the adherence complex, leading to degradation of β-catenin by the ubiquitin-proteasome pathway. The β-catenin degradation is inhibited upon ligand stimulation, thus promoting β-catenin stabilisation and translocation to the nucleus.

Several growth factors, including the HGF, have been shown to promote tyrosine phosphorylation of β-catenin, thereby disrupting its membrane-bound pool and increase the cytosolic and nuclear pool (Hiscox and Jiang, 1999). In recent years, several reports

have indicated that dysregulation of β-catenin, causing increased stability of the protein and thereby increased nuclear accumulation, can lead to apoptosis (Kim *et al*., 2000; Freeman and Bienz, 2001). Studies in several different cell lines have shown an increased cell death induced by components of the frizzled-dishevelled cascade, mediated by β-catenin (van Gijn *et al*., 2001). Disruption of E-cadherin-dependent adhesion and increased accumulation of E-cadherin and β-catenin in the cytoplasm was shown to initiate apoptosis in prostate cancer cells (Vallorosi *et al*., 2000). In addition, overexpression of dishevelled together with APC also induced apoptosis in murine breast tumors (Strovel and Sussman, 1999). Overexpression of nuclear β-catenin, caused by inhibition of its degradation, lead to an increased accumulation of transcripionally active p53 (Damalas *et al*., 1999). β-catenin has also been suggested to have an anti-apoptotic role in colorectal carcinogenesis, however, no plausible mechanism has yet been shown (Satoh and Kuroda, 2000). E-cadherin has been shown to have a negative effect on nuclear β-catenin, since E-cadherin relocates β-catenin to the membrane. Mutation or loss of E-cadherin in cells, leads to loss of cell-cell adhesion but also to loss of suppression of nuclear β-catenin (Ilyas *et al*., 1997). In addition to the effect of APC together with Axin and GSK-3, to guide β-catenin for degradation, APC has also been shown to have an effect on β-catenin shuttling into and out of the nucleus (Henderson, 2000). β-catenin and APC have been found to interact both in the cytoplasm and in the nucleus (McCartney *et al*., 1999; Yu *et al*., 1999). APC have also been suggested to translocate β-catenin out of the nucleus to form a complex with Axin for degradation or to E-cadherin for incorporation in adherens junctions (McCartney *et al*., 1999; Yu *et al*., 1999; Bienz and Clevers, 2000; Henderson, 2000).

c-myc

c-*myc* was the first identified target gene for the activated β-catenin-TCF-4 complex in humans (He *et al*., 1998; Barker *et al*., 2000). The c-*myc* expression was shown to be increased by a non-degradable β-catenin mutant (He *et al*., 1998). β-catenin and c-*myc* was also seen to co-immunostain in the nucleus of colorectal adenocarcinomas (Brabletz *et al*., 2000). In addition, c-*myc* has been implicated in apoptosis, in particular under conditions of stress, genotoxic damage or depletion of survival factors (Evan *et al*., 1992; Evan and Vousden, 2001). These results suggest c-*myc* to be an important candidate in growth factor induced apoptosis involving the Wnt-signalling pathway. Recently, c-*myc* was shown to directly interact with the Smad proteins, Smad2 and Smad3, which could implicate a role for c-*myc* in TGF-β signalling (Feng *et al*., 2002). TGF-β has previously been shown to down-regulate c-*myc* expression in several different cell lines. The loss of TGF-β-induced suppression of c-*myc* has been shown to correlate with resistance of TGF-β-dependent growth inhibition in different cancers. Recently, a possible mechanism for the lost responsiveness to TGF-β-induced growth inhibition of cancer cells was identified; enhanced expression of the lymphoid enhancer binding factor-1 (LEF-1) made the cancer cells resistant to TGF-β-induced repression of c-*myc* (Sasaki *et al*., 2003).

b) TGF-β and Wnt signalling and cross-talk

Members of the TGF-β and Wnt/wingless superfamilies regulate cell fate during development and tissue maintenance (Barker *et al*., 2000; Massagué, 2000). Cooperative

signalling between TGF-β, BMP and Wnt was first detected in *Drosophila*, where it was shown that associations between the Smad proteins and the LEF/TCF factors were essential for full responsiveness of Wnt-inducible genes (Labbe *et al*., 2000; Nishita *et al*., 2000). In addition, a target gene for both Wnt and TGF-β, *Xtwn* was also shown to be activated through its binding to both Smad3 and LEF/TCFs (Letamendia *et al*., 2001). Recently, TGF-β treatment was found to lead to decreased cell-cell contacts, disassembly of adherens and tight junction and increased translocation of β-catenin into the nucleus in epithelial cells; i.e. it resembled the responses seen in Wnt signalling (Tian and Phillips, 2002). An interaction between Smad3, Smad4 and β-catenin in the cytoplasm has been shown to occur upon TGF-β stimulation (Tian and Phillips, 2002). The activation of the Wnt signalling pathway in these studies lead to an increased Smad4/β-catenin interaction and promoted nuclear accumulation of this complex (Tian and Phillips, 2002). These studies demonstrate the importance of Smad proteins in the cooperation between TGF-β and Wnt. The NEMO-like kinase (NLK) is a downstream effector of TAK1, involved in the MAPK pathway (Ishitani *et al*., 1999). NLK was shown to phosphorylate one of the isoforms of TCF, TCF-4 and thereby inhibit the interaction of the β-catenin-TCF-complex with DNA upon TAK1 activation, leading to downregulation of the transcriptional activation mediated by β-catenin and TCF (Ishitani *et al*., 1999). This result suggests that the MAPK pathway negatively regulates the Wnt signalling, but further studies are required to understand the underlying mechanisms.

5. TGF-β and Rho GTPases in physiological and pathological conditions

a) The physiological role for TGF-β

The TGF-β superfamily has been shown to be involved in many different processes in the cells, such as growth and development during embryogenesis, formation of blood vessels, and differentiation and proliferation of immune cells.

The TGF-β superfamily is essential for regulation of several critical steps during the early mouse embryonic development; the formation of the primitive streak, the development of the extraembryonic membranes and the differentiation of the mesoderm (reviewed in Chang *et al*., 2002). Smad2 and Smad4 were both shown to be essential components in the extraembryonic cell development (reviewed in Chang *et al*., 2002). TGF-β has also been shown to be important during mouse embryogenesis in the formation and regulation of the heart development (reviewed in Chang *et al*., 2002). Members of the TGF-β superfamily control the formation of EMT to form the cardiac cushion (reviewed in Chang *et al*., 2002). Defects in heart development have also been seen both in *Smad5* and in *Smad6* knockout mice, which also displayed elevated blood pressure (Chang *et al*., 2000; Galvin *et al*., 2000).

The formation of new blood vessels occurs mainly through two processes, vasculogenesis and angiogenesis (Risau and Flamme, 1995; Beck and D´Amore, 1997). Differentiation of endothelial precursors from the mesoderm to the formation of the primary capillary plexus reflects the vasculogenesis (Risau and Flamme, 1995). In addition, active angiogenesis leads to the formation of new blood vessels from the preexisting vessel plexus through splitting and sprouting (Beck and D´Amore, 1997).

Angiogenesis has been shown to be essential for wound healing and tissue remodeling (Beck and D´Amore, 1997). The members of the TGF-β pathway have been shown to be important regulators of angiogenesis and the development of the vascular system (Pepper, 1997). TGF-β superfamily members were also involved in the craniofacial development, cleft plate formation, in tooth development, in eye structure development as well as in skeletal morphogenesis (reviewed in Chang *et al*., 2002).

The role for TGF-β in the immune system is to prevent inappropriate autoimmune responses in proliferating and differentiating cells and balance the immune cell levels during disease. Dysregulation of TGF-β signalling has been implicated in autoimmunity, opportunistic infections and fibrotic complications (Letterio and Roberts, 1998). Systemic inflammation and early death was observed in mice where TGF-β was deleted (Shull *et al*., 1992).

b) TGF-β in diseases

Alterations of TGF-β superfamily signalling resulted in several different disorders, including bone disease, vascular disease and cancers. Low expression levels of TGF-β have been associated with impaired wound healing and increased diabetes (Sporn and Roberts, 1993). In contrast, overexpression of TGF-β appears also to contribute to many human fibrotic diseases in the kidney, liver, lung, skin and heart (Border and Noble, 1994).

TGF-β has been shown to have a dual role in tumorigenesis. It acts as a tumor suppressor at early stages of tumorigenesis, whereas it acts as a tumor promoter at later stages, when the tumor secretes high levels of TGF-β and the cells tend to increase their resistance to the growth inhibitory response (Gold, 1991; Gold, 1999; de Caestecker *et al*., 2000). Mutations in the TGF-β receptors were found in the late stage of adenoma, apparently correlating with the transition from benign adenoma to malignant carcinoma (Grady *et al*., 1998). Inactivation of both TGF-β receptors and Smads in tumor cells were shown to lead to resistance to TGF-β-induced growth inhibition as well as apoptosis (reviewed in ten Dijke *et al*., 2002). In addition, mutations in the TGF-β type II receptor lead to gastrointestinal and colon cancers through microsatellite instability (reviewed in Massagué, 1998). Inactivation of the Smads caused by mutations or depletions in the Smad genes have been found in several different cancers (reviewed in ten Dijke *et al*., 2002).

Interestingly, recent results suggested that a new soluble TGF-β receptor antagonist (Fc:TβRII), effectively reduced tumor growth metastasis without any major side effect (Muraoka *et al*., 2002; Yang *et al*., 2002). However, it is still too early to introduce any TGF-β antagonist in clinical trials, due to the fact that TGF-β has both inhibiting and promoting effects in tumorigenesis. It will be critical to find a drug, which inhibits TGF-βs effect on metastasis, but retain the growth inhibitory as well as apoptotic effects.

c) Rho GTPases in diseases

Rho GTPases are essential components in cancer-related processes such as cell-proliferation, migration, invasion and metastasis. The dysregulation of the Rho GTPases

may involve either an under- or overactivated pathway (reviewed in Boettner and Van Aelst, 2002). The oncogenic Ras proteins are frequently mutated in human cancers, whereas only one specific Rho mutation has been found, the RhoH/TTF GTPase, detected in patients diagnosed with non-Hodgkin's lymphoma (Proudhomme *et al*., 2000). Mutations have also been seen in genes that are associated with Rho GTPase signalling, such as the different regulators and effectors for the different Rho GTPases (reviewed in Boettner and Van Aelst, 2002). Several of the members of the Dbl-family of Rho GEFs have been classified as proto-onco-proteins, involved in transformation, invasion and metastasis (reviewed in Boettner and Van Aelst, 2002). The Rac-specific exchange factor, the T-cell invasion and metastatic gene (TIAM) promoted high levels of metastasis and invasion of T-lymphoma cells (reviewed in Boettner and Van Aelst, 2002). The downstream effectors for the Rho GTPases in carcinogenesis involve several different cell cycle regulatory factors, often dysregulated in cancer (reviewed in Boettner and Van Aelst, 2002). In addition, several different cancers in colon, breast, pancreas and lung were shown to have transcriptionally upregulated levels of different Rho GTPases (reviewed in Boettner and Van Aelst, 2002). Rac1B, a spliced isoform of Rac1 was shown to be overexpressed in colorectal tumors, whereas RhoC expression was shown to be upregulated in pancreas as well as in inflammatory breast cancer cells, leading to metastatic behavior (reviewed in Boettner and Van Aelst, 2002). The Rho GTPases has also been shown to be over-expressed in several different human tumors (Fritz *et al*., 1999). The level of the RhoA protein was highly increased in colon, breast and lung, whereas Rac and Cdc42 were increased only in breast tumors (Fritz *et al*., 1999). The pathological Rho GTPase pathway may also cooperate with other signalling pathways, like the Wnt and integrin pathways. Recently, the Rho GTPase, Wrch1/Chp2, which is shown to be a Wnt-1 transcriptional target (Tao *et al*., 2001), was suggested to cooperate with the often mutated Wnt-genes, APC or β-catenin in tumorigenesis (reviewed in Boettner and Van Aelst, 2002). Transformed cells show increased integrin expression as well as increased invasiveness (reviewed in Price and Collard, 2001). The α6β4 integrin has been suggested to co-operate with Rac1 via PI3K, for increased invasiveness (Shaw *et al*., 1998), whereas Cdc42 and Rac1 has been shown to induce integrin-mediated cell motility and invasiveness through PI3K (Keely, *et al*., 1997). All these data indicate an important role of the Rho GTPases in tumor development and progression. The coming years will probably witness an increasing number of clinical trials to evaluate new strategies aiming at the inhibition of Rho GTPase pathways, using drugs such as Rho-kinase inhibitors and gene-therapeutic approaches.

The Rho GTPases are involved in other disease conditions; i.e. neurodegenerative disorders, with the nonsynaptic, X-chromosome linked forms of mental retardation and Down syndrome, as two examples (reviewed in Luo, 2000; Boettner and Van Aelst, 2002). The dysregulated neuronal diseases involved either only the Rho GTPases pathway or a cooperation signalling, exclusively through the integrin-specific pathways (reviewed in Luo, 2000; Boettner and Van Aelst, 2002).

Other diseases implicated to be caused by dysregulation of Rho GTPase signalling include; faciogenital dysplasia (FGD1), also known as Aarskog-Scott syndrome (Pasteris *et al*., 1994), Wiskott-Aldrich syndrome (WASP) (Derry *et al*., 1994a, b) and non-

syndromic deafness (Diaphanous) (Lynch *et al*., 1997; Watanabe *et al*., 1997). Faciogenital dysplasia is an X-linked developmental disorder leading to skeletal and urogenital abnormalities (Pasteris *et al*., 1994). The Wiskott-Aldrich syndrome (WASP) is also an X-linked disorder, leading to dysregulated T- and B-cell function and thrombocytopenia (Ochs, 1998). Finally, diaphanous, involved in nonsyndromic deafness, has been suggested to associate with dysregulated actin filaments containing structures of the inner ear (Lynch *et al*., 1997; Boettner and Van Aelst, 2002).

PRESENT INVESTIGATIONS

Transforming Growth Factor-β-induced mobilization of actin cytoskeleton requires signaling by Small GTPases Cdc42 and RhoA (paper I)

In paper I, we investigated the rapid as well as delayed reorganisation of the actin cytoskeleton after TGF-β administration. We found that TGF-β1 treatment of both a rat basophilic leukemia cell line, RBL-2H3, and a human prostate carcinoma cell line, PC-3U, resulted in flattening of the cells and a rapid formation of lamellipodia, accumulating at the cell edges. These ruffle-like structures were visible already after 5-10 minutes of stimulation. In order to exclude that contaminating factors in the TGF-β1 preparation caused the actin reorganisation, RBL-2H3 cells were preincubated with a TGF-β-specific antibody prior to stimulation with the ligand. This neutralising antibody entirely abrogated the TGF-β1-induced actin reorganisation, strongly suggesting that the membrane ruffling was dependent on TGF-β1.

Interestingly, the short-term effect of TGF-β on the actin reorganisation was shown to be independent of the Smad signalling pathway, instead it required the activity of the Rho GTPases Cdc42 and RhoA. Ectopic expression of dominant negative mutants of Cdc42 and RhoA abrogated the membrane ruffling and a GST pull-down activity assay accumulated a changed activity only for the GTPases Cdc42 and Rho. Ectopic expression of dominant negative Smad4 did not abrogate the response. Prolonged treatment of PC-3U cells with TGF-β1 resulted in the formation of stress fibres, as well as cortical actin filaments, seen after 12 to 48 hours of stimulation. This induction of stress fibres seems to be specific for TGF-β, since treatment of the cells with hepatocyte growth factor/scatter factor (HGF/SCF) did not induce a similar response. The response also required new synthesis of proteins, since pre-treatment of the PC-3U cells with the protein synthesis inhibitor cycloheximide, prevented the TGF-β-induced effect. The long-term response required both signalling via Cdc42 and RhoA, and Smad proteins, shown by ectopic expression of dominant negative mutants. A known downstream effector of Cdc42 is p38 MAP kinase; and treatment of the cells with the p38 inhibitor SB203580, as well as ectopic expression of a dominant negative p38, abrogated the TGF-β-induced actin reorganisation of both lamellipodia and stress fibres in PC-3U cells. These findings indicated that p38 was involved both in the long-term and short-term effects on the reorganisation of the actin filament system. Moreover, treatment of cells with the inhibitors of the RhoA target-protein Rho coiled-coil kinase (ROCK) Y-27632 and HA-1077, as well as ectopic expression of kinase-inactive ROCK-1, abrogated the TGF-β1-induced formation of stress fibres in PC-3U cells, whereas the initial flattening out and formation of membrane ruffles was not affected. Collectively, these data indicate that TGF-β-induced membrane ruffling occur via Rho GTPase-dependent pathways, whereas long-term effects require a co-operation between Smad and Rho GTPase signalling pathways.

Smad7 is required for TGF-β-induced activation of the small GTPase Cdc42 (paper II)

In paper II, we investigated the involvement of the inhibitory Smad7 in TGF-β-induced actin reorganisation and Rho GTPase activation. The inhibitory Smads, have been shown

to function in a negative feedback loop, turning down the general Smad signalling cascade. For this reason we studied the potential interference by Smad7 of the activation of Rho GTPases, as well as reorganisation of the actin filament system. We used a cell system with PC-3U stably transfected with Flag-Smad7 under control of the Cd^{2+}-inducible metallothionein promoter (PC-3U/pMEP4-S7), as well as a PC-3U cells stably transfected with an antisense Smad7 construct (PC-3U/AS-S7 cells), which expressed much reduced levels of Smad7 (Landström *et al*., 2000). To our surprise, we found the Smad7 instead had a positive effect on the activation of Cdc42 and RhoA. Cells over-expressing Smad7 induced activation of Cdc42, and to a less extent RhoA, in a Smad7-dependent manner. This response was also seen by the appearance of lamellipodia in cells expressing Smad7. Strikingly enough, no TGF-β-induced activation of Cdc42 could be noticed in the antisense Smad7 cells, whereas RhoA still was activated by TGF-β treatment. Moreover, the short-term TGF-β-induced formation of lamellipodia was abrogated. TGF-β stimulation did not lead to an increase in the activation of Cdc42 above the Smad7-dependent activation, neither any increase in the membrane ruffles, whereas an increased RhoA activation was seen after 6-24 hours of stimulation, which follows the appearance of stress fibers. Collectively, these observations implicate that Smad7 is a component actively inducing actin reorganization by influencing the activation of Cdc42 and, to lesser extent, RhoA. TGF-β induced membrane ruffles involves Cdc42, whereas TGF-β- induced stress fibers involves RhoA.

The PI3K has previously been shown to mediate PDGF-induced activation of Rho GTPases (Tolias *et al*., 1995; Reif *et al*., 1996). Therefore, we decided to look into the possible involvement of PI3K in TGF-β-mediated signalling and actin reorganisation. We used a PI3K inhibitor (LY294002) prior to TGF-β stimulation and observed the actin filaments by phalloidin staining. In our study, an activated PI3K pathway was needed for the formation of the TGF-β-induced membrane ruffles as well as stress fiber formation. We continued the studies by looking at the activated status of the PI3K effector protein Akt after TGF-β stimulation. The TGF-β stimulation was shown to phosphorylate and activate the Akt in a biphasic manner.

We next analysed if Rho GTPases were required for activation of Akt. We used two different clostridial cytotoxins, which previously have been shown to inhibit the different Rho GTPases (Aktories *et al*., 1995; Just *et al*., 1995; Eichel-Streiber *et al*., 1995; Depitre *et al*., 1993). The inhibition of the Rho GTPases Rho, Rac and Cdc42 did not inhibit the TGF-β-induced phosphorylation of Akt activation. In summary, these results suggested that TGF-β-induced lamellipodia and stress fibres were dependent on the PI3K activation and that the PI3K activation is likely to occur upstream of the Rho GTPases.

We have previously shown that p38 MAPK is required for TGF-β-induced rearrangements of the actin filament system (Edlund *et al*., 2002), and a binding partner to Smad7 in TGF-β induced apoptosis (Edlund *et al*., 2003). We observed in this study that p38 MAPK was required for the formation of Smad7-induced membrane ruffles, by the use of the p38 inhibitor (SB203580) which abrogated the effect, whereas an increased stress fiber formation was observed. In conclusion, these data shows that an

activated p38 MAPK pathway is needed for Smad7-induced activation of Cdc42 to form membrane ruffles. p38 MAPK was also shown to be required for the Smad7-induced activation of Cdc42, whereas the PI3K pathway was not involved, as shown by the use of a PI3K inhibitor (LY294002) which did not abrogate the effect. This indicated that PI3K is functioning upstream of Smad7 in the activation of Cdc42.

We wanted to test the potential involvement of the MAPKKK, MLK3 in the Cdc42-induced reorganization of the actin filament system. MLK3 has been shown to be important for both Cdc42 and the p38 MAPK pathway (Burbelo *et al*., 1995; Tibbles *et al*., 1996; Nagata *et al*., 1998). MLK3 was shown to be important both for the formation of short-term membrane ruffles, as well as long-term stress fiber formations after TGF-β stimulation, thus making it a likely candidate to link the activation of Cdc42 to the rearrangements of the actin filament system.
The PI3K cascade has been shown to activate Rho GTPases via activation of GEFs (reviewed in Scita *et al*., 2000). Therefore, we wanted to further investigate the involvement of GEFs upon TGF-β activation. A candidate GEF to mediate TGF-β-dependent activation of Cdc42 is the PAK interacting exchange factor (Pix) (Manser *et al*., 1998). We found that βPix, in contrast to αPix, increased the TGF-β-induced membrane ruffles, as a response not seen in either cells expressing a catalytically inactive βPix or a wild-type αPix.

Collectively, these data indicate that TGF-β-induced activation of Cdc42 requires Smad7 and p38 MAPK via a PI3K dependent pathway. Moreover, MLK3 is a potential mediator downstream of Cdc42 effecting the Cdc42-dependent actin filament reorganisation.

Transforming Growth Factor-β1 (TGF-β1)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-β-activated kinase 1 and mitogen-activated protein kinase kinase 3 (paper III)
In paper III, we investigated the molecular mechanism of TGF-β1- and Smad7-induced apoptosis and the involvement of TAK1, MKK3 and p38 MAPK in this pathway. We demonstrate that the specific activation of the p38 MAP kinase pathway is essential for TGF-β1- and Smad7-induced apoptosis of the human prostate cancer PC-3U cells. TGF-β1 treatment of PC-3U cells specifically activated the MKK3/6-p38 MAPK pathway, while no effect on the ERK or SAPK/JNK pathways were observed. TGF-β stimulation also led to increased levels of endogenous Smad7 protein, as well as to the export of endogenous Smad7 from the nucleus to the cytoplasm, which upon prolonged incubation again accumulated in the nucleus.

Expression of dominant negative p38 MAPK, dominant negative MKK3, or incubation with the p38 MAPK selective inhibitor SB203580, prevented TGF-β1-induced apoptosis. Furthermore, ectopically overexpression of MKK3 enhanced TGF-β-induced phosphorylation of p38 MAPK and apoptosis in PC-3U cells.
Smad7 mediates TGF-β1-induced apoptosis in several cell types. It has previously been demonstrated that Smad7 is necessary for TGF-β1-induced apoptosis in PC-3U and HaCaT cells (Landström *et al.,* 2000). The expression of Smad7 was required for TGF-β-induced activation of MKK3 and p38 MAPKs. Two cell lines were used, PC-3U cells

stably transfected with an antisense Smad7 construct (AS-S7), also called PC-3U/AS-S7 or PC-3U cells stably transfected with pMEP4-Flag-Smad7 (Clone I), also called PC-3U/pMEP4-S7 (Landström *et al*., 2000). In AS-S7 cells, TGF-β treatment did not cause p38 MAPK activation or phosphorylation of MKK3 at any time point investigated, whereas overexpression of Smad7 in Clone I cells caused an activation of both p38 MAPK and MKK3/6. Phosphorylated p38 MAPK and Flag-Smad7 colocalised in the nucleus of Clone I cells upon Smad7 overexpression. Endogenous Smad7 was also found to interact with phosphorylated p38 MAPK in a ligand-dependent way.
The apoptotic rate was increased in Clone I cells upon Smad7 overexpression. Interestingly, simultaneous treatment of cells with SB203580 inhibited Smad7-induced apoptosis. This indicate that Smad7 is required for TGF-β-induced phosphorylation of MKK3/6, as well as p38 MAPK, and that the p38 MAPK pathway is essential for TGF-β1- and Smad7-induced apoptosis in human prostate cancer cells.

TGF-β treatment of PC-3U cells also enhanced phosphorylation of the nuclear substrate for p38 MAPK, ATF2, whereas this was not the case in AS-S7 cells. It has previously been reported that ATF2 is phosphorylated after TGF-β1 stimulation, in a TAK1- and p38 MAPK-dependent way (Sano *et al.,* 1999) and that Smad3 and Smad4 interact with ATF2 (Hanafusa *et al.,* 1999; Sano, *et al.,* 1999). We found that also Smad7 colocalised with phosphorylated p38 MAPK and ATF2 in the nucleus of Smad7 overexpressing cells upon $CdCl_2$ stimulation. These data suggests that phosphorylation of ATF2 by p38 MAPK in TGF-β-treated cells is dependent on the expression of Smad7.

TAK1 is a member of the MAPKKK family previously implicated in TGF-β signalling (Yamaguchi *et al.,* 1995). Ectopic expression of wild-type TAK1 in PC-3U cells promoted TGF-β1-induced phosphorylation of p38 MAPK and apoptosis, while dominant negative TAK1 reduced TGF-β1-induced phosphorylation of p38 MAPK and apoptosis. From these observations, we conclude that ectopic expression of wild-type TAK1 in PC-3U cells, enhances TGF-β-induced phosphorylation of p38 MAPK and apoptosis, while ectopic expression of kinase-inactive TAK1 decreases the TGF-β-activated phosphorylation of p38 MAPK and subsequently apoptosis. Endogenous Smad7 was found to interact with TAK1, and, interestingly, TAK1, MKK3 and p38 MAPK were co-immunoprecipitated with Smad7 in transiently transfected COS1 cells.

We have here reported that Smad7 can interact with TAK1, MKK3 and p38 MAPK. Since Smad7 can also interact with the activated TGF-β receptor complex, it is possible that Smad7 acts as an adaptor protein, bridging between the activated receptor and the TAK1-MKK3-p38 series of kinases, thus facilitating their activation.

TGF-β promotes nuclear accumulation and activation of β-catenin in a Smad7 dependent manner (paper IV)
In paper IV, we investigated a possible cross-talk between the TGF-β and Wnt signalling pathways. β-catenin has been shown to act as a transcriptional regulator together with T-cell factor-4 (TCF-4) downstream in the Wnt-signalling pathway in colorectal carcinoma (Barker *et al*., 2000). We examined whether Smad7 interacted with β-catenin and TCF-4 and found that in transiently transfected COS1-cells, Smad3, Smad4 and Smad7

interacted strongly with endogenous β-catenin. We also observed a weak interaction between endogenous β-catenin and transiently transfected Smad2 and Smad6.

Next, we analysed which domain of Smad7 that interacted with β-catenin. We observed a strong interaction between the wild-type, as well as the N-terminal part of Smad7 to β-catenin, whereas a weak interaction was observed with the C-terminal part of Smad7. Interestingly, the N-terminal domain of Smad7 as well as the wild-type Smad7 was also shown to interact with endogenous TCF-4 in a GST-pull-down assay, using different GST-fusion constructs of full length and deletion mutants of Smad7.

To further investigate whether Smad7 cooperated with TCF-4 and β-catenin in transcriptional regulation, we next examined the effect of Smad7 on a synthetic TCF-4 promoter (TOPFLASH). As expected, TCF-4 and β-catenin together increased the luciferace reporter activity of TOPFLASH in PC-3U cells four-five fold, when compared to mock transfections (pcDNA3). Co-transfection of Smad7 together with TCF-4 and β-catenin resulted in an additional increase of the luciferase reporter activity. In conclusion, we suggest that the synergistic effect by Smad7, TCF-4 and β-catenin on a synthetic TCF-4 promoter (TOPFLASH) probably is a result of Smad7 interacting with both TCF-4 and β-catenin, suggesting that Smad7 can positively regulate transcription in the Wnt-signalling pathway.

We found in paper III that expression of Smad7 is required for TGF-β-induced activation of the TAK1-p38 MAPK pathway and subsequently apoptosis in prostate cancer cells (Edlund *et al*., 2003). Since β-catenin recently has been implicated in regulation of apoptosis (Kim *et al*., 2000; Freeman and Bienz, 2001), we decided to investigate whether TGF-β affected the subcellular localisation of β-catenin in a Smad7-dependent manner and if this correlated to apoptosis. We found that β-catenin accumulated at perinuclear as well as nuclear compartments after 30-120 min of TGF-β stimulation in PC-3U cells, and remained in these compartments up to 24-48 hours. In contrast, an accumulation of β-catenin in the nucleus was not observed in the antisens Smad7 cells (PC-3U/AS-S7) after TGF-β stimulation. Instead, β-catenin accumulated in areas involved in cell-cell contacts. Furthermore, we found that Smad7 overexpression in PC-3U/pMEP4-S7 cells also resulted in a nuclear accumulation of β-catenin, leading to co-localisation of Flag-Smad7 and endogenous β-catenin in the nuclear compartment. Interestingly, morphological signs of apoptosis (i.e. condensation of the nucleus and shrinkage of the cytoplasm) were also observed in PC-3U cells as well as in the Smad7 overexpressing cells, in which high amounts of both Smad7 and β-catenin were detected. Using Western blot analysis, we found increased levels of endogenous β-catenin in Smad7 overexpressing cells. Interestingly, the specific p38 MAPK inhibitor, SB203580, prevented TGF-β-and Smad7-induced accumulation of β-catenin in the nucleus in both PC-3U cells and in PC-3U/pMEP4-S7 cells. In conclusion, these studies show that TGF-β1-treatment of the prostate cancer cells promoted perinuclear and nuclear accumulation of β-catenin in a Smad7 and p38 MAPK dependent manner.

One of the target genes for Wnt-signalling has been shown to be c-*myc* (Barker *et al*., 2000), a proto-oncogene also implicated in apoptosis (Evan *et al*., 1992). We therefore

investigated whether Smad7 could activate the c-*myc* promoter in transiently transfected PC-3U cells. Smad7 significantly enhanced the activity of the Wnt target gene c-*myc* as measured by luciferase activity when compared to mock transfected cells. Furthermore, the level of c-Myc protein was also increased in PC-3U/pMEP4-S7 cells as analysis by Western blotting. Our results suggest that Smad7 positively regulate the expression of c-Myc. Since c-Myc has been shown to potently induce apoptosis, in particular under condition of stress, genotoxic damage or depletion of survival factors (Evan and Vousden, 2001), our finding suggests the possibility that cooperation between Smad7 and components in the Wnt-signalling contributes to the induction of apoptosis in TGF-β stimulated PC-3U cells.

FUTURE PERSPECTIVES

Our studies have shown that TGF-β treatment affect the organisation of the actin filament system. We showed that these effects required the concerted action of the Rho GTPases, p38 MAPK, as well as the Smad signalling pathways. We also observed a correlation between TGF-β-induced actin reorganistation and the GEF, βPix, a possible upstream regulator for Cdc42/Rac, but it is not clear if this is the critical component in the pathway leading from the TGF-β receptor to the Rho GTPases. It is of critical importance to dissect the pathways leading directly from the activated TGF-β receptor to the Rho GTPases. There is an intriguing possibility that the TGF-β receptor could have a direct effect on the organisation of the actin cytoskeleton. The receptor ligation, which leads to a clustering of receptor complexes in close proximity to cytoskeletal components at the plasma membrane, could potentially directly induce actin reorganisation.

It was recently reported that Smad proteins require an intact microtubuli system in order to be translocated to the nucleus and induce gene transcription (Dong *et al*., 2000). Interestingly, it has also been shown that MLK2, which is related to the MAPKKK family, activates JNK and localize along microtubules (Nagata., *et al*., 1998). Furthermore, MLK3 has been shown to be important, for the activation of Cdc42 and p38 MAPK (Gallo and Johnson, 2002), as well as for the actin reorganisation after TGF-β stimulation showed in paper II. It is therefore an intriguing possibility, that the TGF-β/Smad7-induced nuclear accumulation of β-catenin during the onset of apoptosis require the microtubule system. We will therefore continue to examine the involvement of microtubules for the TGF-β/Smad7-induced nuclear accumulation of β-catenin, as well as the involvement of the different Rho GTPases.

The Rho GTPases might be involved in TGF-β-induced apoptosis. They are all of importance in TGF-β signalling through the activation of p38 MAP kinase, as well as mediators of different apoptotic pathways (Aznar and Lacal, 2001). The Rho GTPases Cdc42 and Rac have previously been documented to interact with its target protein IQGAP1, as well as β-catenin, calmodulin, actin and E-cadherin (Kuroda *et al*., 1998), which suggests an interaction of the Rho GTPases through IQGAP with β-catenin and Smad7. Therefore, it would be of great interest to further investigate the involvement of Smad7 and the Rho GTPases in both the p38 MAP kinase pathway and β-catenin/TCF-4 pathway, in conjunction with induction of apoptosis.

Finally, we would like to investigate how the TGF-β-mediated effects on the actin filament system and on apoptosis are connected. It is tempting to speculate that Smad7 can function as a link between the machineries that regulate the actin cytoskeleton and apoptosis, respectively. It is an intriguing possibility, which deserves further exploration, that Rho GTPases, the actin cytoskeleton, Smads and the p38 MAP kinase, together with the microtubule system, have important roles in TGF-β signalling and apoptosis.

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REFERENCES

Abercrombie, M., Heaysman, J. E., and Pegrum, S. M. (1970). The locomotion of fibroblasts in culture. II. "Ruffling", Exp Cell Res *60*, 437-44.

Adachi-Yamada, T., Nakamura, M., Irie, K., Tomoyasu, Y., Sano, Y., Mori, E., Goto, S., Ueno, N., Nishida, Y., and Matsumoto, K. (1999). p38 mitogen-activated protein kinase can be involved in transforming growth factor beta superfamily signal transduction in Drosophila wing morphogenesis, Mol Cell Biol *19*, 2322-9.

Afrakhte, M., Morén, A., Jossan, S., Itoh, S., Sampath, K., Westermark, B., Heldin, C.-H., Heldin, C.-E., and ten Dijke. (1998). Induction of inhibitory Smad6 and Smad7 mRNA by TGF-β family members. Biochem Biophys Res Commun *249*, 505-511.

Aktories, K. (1997). Identification of the catalytic site of clostridial ADP- ribosyltransferases, Adv Exp Med Biol *419*, 53-60.

Aktories, K. and Just, I. (1995). In vitro ADP-ribosylation of Rho by bacterial ADP-ribosyltransferas. Methods Enzymol *256*, 184-195.

Albers, K., and Fuchs, E. (1992). The molecular biology of intermediate filament proteins. Int Rev Cytol *134*, 243-279.

Allen, W. E., Zicha, D., Ridley, A. J., and Jones, G. E. (1998). A role for Cdc42 in macrophage chemotaxis, J Cell Biol *141*, 1147-57.

Alvarado-Kristensson, M., Pörn-Ares, M. I., Grethe, S., Smith, D., Zheng, L., and Andersson, T. (2001). p38 mitogen-activated protein kinase and phosphatidylinositol 3-kinase activities have opposite effects on human neutrophil apoptosis. FASEB J *16*, 129-131.

Amano, M., Ito, M., Kimura, K., Fukata, Y., Chihara, K., Nakano, T., Matsuura, Y., and Kaibuchi, K. (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho- kinase), J Biol Chem *271*, 20246-9.

Amos, L. A. Amos, W. B. (1991). Molecules of the Cytoskeleton. New York, Guilford Press.

Amundadottir, L. T., Nass, S. J., Berchem, G. J., Johnson, M. D., and Dickson, R. B. (1996). Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis, Oncogene *13*, 757-65.

Anzano, M. A., Roberts, A. B., Smith, J. M., Sporn, M. B., and De Larco, J. E. (1983). Sarcoma growth factor from conditioned S A medium of virally transformed cells is composed of both type alpha and type beta transforming growth factors, Proc Natl Acad Sci U *80*, 6264-8.

Aronheim, A., Broder, Y. C., Cohen, A., Fritsch, A., Belisle, B., and Abo, A. (1998). Chp, a homologue of the GTPase Cdc42Hs, activates the JNK pathway and is implicated in reorganizing the actin cytoskeleton, Curr Biol *8*, 1125-8.

Asp, P., Wihlborg, M., Karlen, M., and Farrants, A. K. (2002). Expression of BRG1, a human SWI/SNF component, affects the organisation of actin filaments through the RhoA signalling pathway, J Cell Sci *115*, 2735-46.

Aspenström, P., Lindberg, U., and Hall, A. (1996). Two GTPases, Cdcc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott-Aldrich Syndrome. Curr Biol *6,* 70-75.

Aspenström, P. (1997). A Cdc42 target protein with homology to the non-kinase domain of FER has a potential role in regulating the actin cytoskeleton. Curr Biol *7*, 479-487.

Aspenström, P. (1999a). The Rho GTPases have multiple effects on the actin cytoskeleton. Exp Cell Res *246*, 20-25.

Aspenström, P. (1999b). Effectors for the Rho GTPases. Curr Opin Cell Biol *11*, 95-102.

Atfi, A., Buisine, M., Mazars, A., and Gespach, C. (1997a). Induction of apoptosis by DPC4, a transcriptional factor regulated by transforming growth factor-beta through stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling pathway, J Biol Chem *272*, 24731-4.

Atfi, A., Djelloul, S., Chastre, E., Davis, R., and Gespach, C. (1997b). Evidence for a role of Rho-like GTPases and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) in transforming growth factor-β-mediated signaling. J Biol Chem *12*, 1429-1432.

Attisano, L., and Wrana, J. L. (2000). Smads as transcriptional co-modulators. Curr Opin Cell Biol *12*, 235-243.

Attisano, L., Carcamo, J., Ventura, F., Weis, F. M., Massague, J., and Wrana, J. L. (1993). Identification of human activin and TGF beta type I receptors that form heteromeric kinase complexes with type II receptors, Cell *75*, 671-80.

Aznar, S., and Lacal, J. C, (2001). Rho signals to cell growth and apoptosis. Cancer Lett *165*, 1-10.

Baghdassarian, D., Toru-Delbauffe,D., Gavaret, J.M., and M. Pierre. (1993). Effects of transforming growth factor-beta 1 on the extracellular matrix and cytoskeleton of cultured astrocytes. Glia *7*, 193-202.

Bagrodia, S., Derijad, B., Davis, R. J., Cerione, R. A. (1995). Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activationJ Biol Chem 270, 27995-27998.

Bakin, A. V., Rinehart, C., Tomlinson, A. K., and Arteaga, C. L. (2002). p38 mitogen-activated protein kinase is required for TGF-beta-mediated fibroblasic transdifferentiation and cell migration. J Cell Sci *115*, 3193-3206.

Bakin, A. V., Tomlinson, A. K., Bhowmick, N. A., Moses, H. L., and Arteaga, C. L. (2000). Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration, J Biol Chem*275*, 36803-10.

Ball, E. M., and Risbridger, G. P. (2001). Activins as regulators of branching morphogenesis, Dev Biol *238*, 1-12.

Barker, N., Morin, P. J., and Clevers, H. (2000). The Yin-Yang of TCF/beta-catenin signaling, Adv Cancer Res *77*, 1-24.

Beck, L., Jr., and D'Amore, P. A. (1997). Vascular development: cellular and molecular regulation, Faseb J *11*, 365-73.

Bhowmick, N. A., Ghiassi, M., Bakin, A., Aakre, M., Lundquist, C. A., Engel, M. E., Arteaga, C. L., and Moses, H. L. (2001a). Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism, Mol Biol Cell *12*, 27-36.

Bhowmick, N. A., Zent, R., Ghiassi, M., McDonnell, M., and Moses, H. L. (2001b). Integrin beta 1 signaling is necessary for transforming growth factor- beta activation of p38MAPK and epithelial plasticity, J Biol Chem*276*, 46707-13.

Bienz, M., and Clevers, H. (2000). Linking colorectal cancer to Wnt signaling, Cell *103*, 311-20.

Bishop, A. L., and Hall, A. (2000). Rho GTPases and their effector proteins, Biochem J *348 Pt 2*, 241-55.

Blobe, G. C., Schiemann, W. P., and Lodish, H. F. (2000). Role of transforming growth factor beta in human disease, N Engl J Med *342*, 1350-8.

Boettner, B., and Van Aelst, L. (2002). The role of Rho GTPases in disease development, Gene *286*, 155-74.

Boland, S., Boisvieux-Ulrich, E., Houcine, O., Baeza-Squiban, A., Pouchelet, M., Schoëvaërt, D., and F. Marano. (1996). TGFβ1 promotes actin cytoskeleton reorganization and migratory phenotype in epithelial tracheal cells in primary culture. J Cell Sci *109*, 2207-2219.

Bonder, E. M., Fishkind, D. J., Mooseker, M. S. (1983). Direct measurement of critical concentrations and assembly rate constants at the two ends of an actin filament. Cell *34*, 491-501.

Border, W. A., and Noble, N. A. (1994). Transforming growth factor beta in tissue fibrosis, N Engl J Med *331*, 1286-92.

Bottaro, D. P., Rubin, J. S., Faletto, D. L., Chan, A. M., Kmiecik, T. E., Vande Woude, G. F., and Aaronson, S. A. (1991). Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product, Science *251*, 802-4.

Bowden, E. T., Barth, M., Thomas, D., Glazer, R. I., and Mueller, S. C. (1999). An invasion-related complex of cortactin, paxillin and PKCmu associates with invadopodia at sites of extracellular matrix degradation, Oncogene *18*, 4440-9.

Brabletz, T., Herrmann, K., Jung, A., Faller, G., and Kirchner, T. (2000). Expression of nuclear beta-catenin and c-myc is correlated with tumor size but not with proliferative activity of colorectal adenomas, Am J Pathol *156*, 865-70.

Bretscher, A. (1991). Microfilament structure and function in cortical cytoskeleton. Annu Rev Cell Biol 7, 337-374.

Brickell, P. M., Katz, D. R., and Thrasher, A. J. (1998). Wiskott-Aldrich syndrome: current research concepts, Br J Haematol *101*, 603-8.

Brill, S., Li, S., Lyman, C. W., Church, D. M., Wasmuth, J. J. *et al*. (1996). The Ras GTPase-activating-protein-related human protein IQGAP2 harbors a potential actin binding domain and interacts with calmodulin and Rho family GTPases. Mol Cell Biol *16*, 4869-4878.

Brodin, G., ten Dijke, P., Funa, K., Heldin, C. H., and Landstrom, M. (1999). Increased smad expression and activation are associated with apoptosis in normal and malignant prostate after castration, Cancer Res *59*, 2731-8.

Brown, J. D., DiChiara, M. R., Anderson, K. R., Gimbrone, M. A., Jr., and Topper, J. N. (1999). MEKK-1, a component of the stress (stress-activated protein kinase/c- Jun N-terminal kinase) pathway, can selectively activate Smad2-mediated transcriptional activation in endothelial cells, J Biol Chem *274*, 8797-805.

Buchsbaum, R. J., Connolly, B. A., Feig, L. A. (2002). Interaction of Rac exchange factor Tiam1and Ras-GRF1 with a scaffold for p38 mitogen-activated protein kinase cascade. Mol Cell Biol 22, 4073-4085.

Burbelo, P. D., Drechsel, D., and Hall, A. (1995). A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases, J Biol Chem*270*, 29071-4.

Burridge, K., Nuckolls, G., Otey, C., Pavalko, F., Simon, K., and Turner, C. (1990). Actin-membrane interaction in focal adhesions, Cell Differ Dev *32*, 337-42.

Burridge, K., Fath, K., Kelly, T., Nuckolls, G., Turner, C. (1988). Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol *4*, 487-525.

Burridge, K., Turner, C. E., Romer, L. H. (1992). Tyrosine phosphoryation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. J Cell Biol *119*, 893-903.

Cabrera, C. V., Alonso, M. C., Johnston, P., Phillips, R. G., and Lawrence, P. A. (1987). Phenocopies induced with antisense RNA identify the wingless gene. Cell *50*, 659-663.

Cate, R. L., Mattaliano, R. J., Hession, C., Tizard, R., Farber, N. M., Cheung, A., Ninfa, E. G., Frey, A. Z., Gash, D. J., Chow, E. P.*, et al.* (1986). Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells, Cell *45*, 685-698.

Chang, H., Brown, C. W., and Matzuk, M. M. (2002). Genetic analysis of the mammalian transforming growth factor-beta superfamily, Endocr Rev *23*, 787-823.

Chang, L., and Karin, M. (2001). Mammalian MAP kinase signalling cascade. Nature *410*, 37-40.

Chang, H., Zwijsen, A., Vogel, H., Huylebroeck, D., and Matzuk, M. M. (2000). Smad5 is essential for left-right asymmetry in mice, Dev Biol *219*, 71-8.

Cheifetz, S., and Massague, J. (1991). Isoform-specific transforming growth factor-beta binding proteins with membrane attachments sensitive to phosphatidylinositol-specific phospholipase C, J Biol Chem*266*, 20767-72.

Cheifetz, S., Weatherbee, J. A., Tsang, M. L., Anderson, J. K., Mole, J. E., Lucas, R., and Massague, J. (1987). The transforming growth factor-beta system, a complex pattern of cross- reactive ligands and receptors, Cell *48*, 409-15.

Chen, H., Tritton, T. R., Kenny, N., Absher, M., and Chiu, J. F. (1996). Tamoxifen induces TGF-beta 1 activity and apoptosis of human MCF-7 breast cancer cells in vitro, J Cell Biochem *61*, 9-17.

Chen, R. H., and Chang, T. Y. (1997). Involvement of caspase family proteases in transforming growth factor- beta-induced apoptosis, Cell Growth Differ *8*, 821-7.

Chen, R. H., Su, Y. H., Chuang, R. L., and Chang, T. Y. (1998a). Suppression of transforming growth factor-beta-induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway, Oncogene *17*, 1959-68.

Chen, X., Rubock, M. J., and Whitman, M. (1996b). A transcriptional partner for MAD proteins in TGF-β signalling. Nature *383*, 691-696.

Chen, Y. G., Hata, A., Lo, R. S., Wotton, D., Shi, Y., Pavletich, N., and Massague, J. (1998b). Determinants of specificity in TGF-beta signal transduction, Genes Dev *12*, 2144-52.

Chinkers, M., McKanna, J. A., S. Cohen. (1979). Rapid induction of morphological changes in human carcinoma cells A-431 by epidermal growth factors. J Cell Biol *83*, 260-265.

Choi, M. E., and Ballermann, B. J. (1995). Inhibition of capillary morphogenesis and associated apoptosis by dominant negative mutant transforming growth factor-beta receptors, J Biol Chem*270*, 21144-50.

Chuang, T. H., Hahn, K. M., Lee, J. D., Danley, D. E., and Bokoch, G. M. (1997). The small GTPase Cdc42 initiates an apoptotic signaling pathway in Jurkat T lymphocytes, Mol Biol Cell *8*, 1687-98.

Coleman, M. L., Sahai, E. A., Yeo, M., Bosch, M., Dewar, A., and Olson, M. F. (2001). Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I, Nat Cell Biol *3*, 339-45.

Coyle, B., Freathy, C., Gant, T. W., Roberts, R. A., and Cain, K. (2003). Characterization of the Transforming Growth Factor-beta 1-induced Apoptotic Transcriptome in FaO Hepatoma Cells, J Biol Chem *278*, 5920-8.

Crawford, S. E., Stellmach, V., Murphy-Ullrich, J. E., Riberio, S. M. F., Lawler, J., Hynes, R. O., Boivin, G. P., and Bouck, N. (1998). Thrombospondin-1 is a major activator of TGF-beta1 in vivo. Cell *93*, 1159-1170.

Cross, T. G., Scheel-Toellner, D., Henriquez, N. V., Dacon, E., Salmon, M., and Lord, J. M. (2000). Serine/Threonine protein kinase and apoptosis. Exp Cell Res *256*, 34-41.

Crowe, M. J., Doetschman, T., and Greenhalgh, D. G. (2000). Delayed wound healing in immunodeficient TGF-beta 1 knockout mice, J Invest Dermatol *115*, 3-11.

Cunningham, N. S., Paralkar, V., and Reddi, A. H. (1992). Osteogenin and recombinant bone morphogenetic protein 2B are chemotactic for human monocytes and stimulate transforming growth factor b1 mRNA expression, Proc Natl Acad Sci USA *89*, 11740-11744.

Damalas, A., Ben-Ze'ev, A., Simcha, I., Shtutman, M., Leal, J. F., Zhurinsky, J., Geiger, B., and Oren, M. (1999). Excess beta-catenin promotes accumulation of transcriptionally active p53, Embo J *18*, 3054-63.

Daniel, R. H., and Bokoch, G. M. (1999). p21-activated protein kinase: a crucial component of morphological signaling? Trends Biochem Sci *24*, 350-355.

Davis, R.J. (2000). Signal transduction by the JNK group of MAP kinases. Cell *103*, 239-252.

de Caestecker, M. P., Parks, W. T., Frank, C. J., Castagnino, P., Bottaro, D. P., Roberts, A. B., and Lechleider, R. J. (1998). Smad2 transduces common signals from receptor serine-threonine and tyrosine kinases, Genes Dev *12*, 1587-92.

de Caestecker, M. P., Piek, E., and Roberts, A. B. (2000). Role of transforming growth factor-beta signaling in cancer, J Natl Cancer Inst *92*, 1388-402.

de Larco, J. E., and Todaro, G. J. (1978). Growth factors from murine sarcoma virus-transformed cells, Proc Natl Acad Sci U S A *75*, 4001-5.

de Luca, A., Weller, M., and Fontana, A. (1996). TGF-beta-induced apoptosis of cerebellar granule neurons is prevented by depolarization, J Neurosci *16*, 4174-85.

de Martin, R., Haendler, B., Hofer-Warbinek, R., Gaugitsch, H., Wrann, M., Schlüsener, H., Seifert, J, M., Bodmer, S., Fontana, A., and Hofer, E. (1987). Complementary DNA for human glioblastoma-derived T cell suppressor factor, a novel member of the transforming growth factor-β gene family. EMBO J *6*, 3673-3677.

Dechert, M. A., Holder, J. M., and Gerthoffer, W. T. (2001). p21-activated kinase 1 participates in tracheal smooth muscle cell migration by signaling to p38 Mapk, Am J Physiol Cell Physiol *281*, C123-32.

Depitre, C., Delmee, M., Avesani, V., Haridon, R. L., Roels, A., Popoff, M. and Corthier, G. (1993). Serogroup F Clostridium difficile produce toxin B but not toxin A. J Med Microbiol *6*, 434-441.

Derry, J. M., Ochs, H. D., and Francke, U. (1994a). Isolation of a novel gene mutated in Wiskott-Aldrich syndrome, Cell *79*, following 922.

Derry, J. M., Ochs, H. D., and Francke, U. (1994b). Isolation of a novel gene mutated in Wiskott-Aldrich syndrome, Cell *78*, 635-44.

Derynck, R., and Feng, X. H. (1997). TGF-beta receptor signaling, Biochim Biophys Acta *1333*, F105-50.

Derynck, R., Gelbart, W. M., Harland, R. M., Heldin, C. H., Kern, S. E., Massague, J., Melton, D. A., Mlodzik, M., Padgett, R. W., Roberts, A. B., *et al.* (1996). Nomenclature: vertebrate mediators of TGFbeta family signals, Cell *87*, 173.

Derynck, R., Jarrett, J. A., Chen, E. Y., Eaton, D. H., Bell, J.R., Assoian, R. K., Roberts, A. B., Sporn, M. B., Goeddel, D.V. (1985). Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. Nature *316*, 22-28.

Dernyck, R., Lindquist, P. B., Lee, A., Wen, D., Tamm, J., Graycar, J. L., Rhee, L., Mason, A. J., Miller, D. A., Coffey, R. J., *et al*. (1988). A new type of transforming growth factor-β, TGF-β3. EMBO J *12*, 3737-3743.

Derynck, R., Zhang, Y., and Feng, X.-H. (1998). Smads: transcriptional activators of TGF-β responses. Cell *95*, 737-740.

Deschesnes, R. G., Huot, J., Valerie, K., and Landry, J. (2001). Involvement of p38 in apoptosis-associated membrane blebbing and nuclear condensation, Mol Biol Cell *12*, 1569-82.

Donahoe, P. K. (1992). Mullerian inhibiting substance in reproduction and cancer, Mol Reprod Dev *32*, 168-72.

Dong, C., Li, Z., Alvarez, R., Jr., Feng, X. H., and Goldschmidt-Clermont, P. J. (2000). Microtubule binding to Smads may regulate TGF beta activity, Mol Cell *5*, 27-34.

Dore, J. J., Jr., Edens, M., Garamszegi, N., and Leof, E. B. (1998). Heteromeric and homomeric transforming growth factor-beta receptors show distinct signaling and endocytic responses in epithelial cells, J Biol Chem *273*, 31770-7.

Ebisawa, T., Fukuchi, M., Murakami, G., Chiba, T., Tanaka, K., Imamura, T., and Miyazono, K. (2001). Smurf interacts with transforming growth factor-β type I receptor through Smad7 and induces receptor degradation. J Biol Chem *276*,12477-12480.

Edlund, S., Bu, S., Schuster, N., Aspenström, P., Heuchel, R., Heldin, N. E., ten Dijke, P., Heldin, C. H., and Landström, M. (2003). Transforming growth factor-beta1 (TGF-β)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-beta-activated kinase 1 and mitogen-activated protein kinase 3. Mol Biol Cell. *14*, 529-544.

Edlund, S., Landstrom, M., Heldin, C. H., and Aspenstrom, P. (2002). Transforming growth factor-beta-induced mobilization of actin cytoskeleton requires signaling by small GTPases Cdc42 and RhoA, Mol Biol Cell *13*, 902-14.

Eichel-Streber, C. V., Meyer, D., Habermann, E., and Sartigen, S. (1995). Closing in on the toxic domain through analysis of variant Clostridium difficile cytotoxin B. Mol Microbiol *2*, 313-321.

Engel, M. E., McDonnell, M. A., Law, B. K., and Moses, H. L. (1999). Interdependent SMAD and JNK signaling in transforming growth factor-β-mediated transcription. J Biol Chem *274*, 37413-37420.

Eppert, K., Scherer, S. W., Ozcelik, H., Pirone, R., Hoodless, P., Kim, H., Tsui, L. C., Bapat, B., Gallinger, S., Andrulis, I. L., *et al*. (1996). MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma, Cell *86*, 543-52.

Erickson, J. W., Cerione, R. A., Hart, M. J. (1997). Identification of an actin cytoskeletal complex that includes IQGAP and the Cdc42 GTPase. J Biol Chem *272*, 24443-24447.

Esteve, P., Embade, N., Perona, R., Jimenez, B., del Peso, L., Leon, J., Arends, M., Miki, T., and Lacal, J. C. (1998). Rho-regulated signals induce apoptosis in vitro and in vivo by a p53- independent, but Bcl2 dependent pathway, Oncogene *17*, 1855-69.

Eva, A., and Aaronson, S. A. (1985). Isolation of a new human oncogene from a diffuse B-cell lymphoma, Nature *316*, 273-5.

Evan, G. I., and Vousden, K. H. (2001). Proliferation, cell cycle and apoptosis in cancer, Nature *411*, 342-8.

Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C. (1992). Induction of apoptosis in fibroblasts by c-myc protein, Cell *69*, 119-28.

Fagotto, F., Jho, E., Zeng, L., Kurth, T., Joos, T., Kaufmann, C., and Costantini, F. (1999). Domains of axin involved in protein-protein interactions, Wnt pathway inhibition, and intracellular localization, J Cell Biol *145*, 741-56.

Feng, X.-H., and Dernyck, R. (1997). A kinase subdomain of transforming growth factor-β (TGF-β) type I receptor determines the TGF-β intracellular signaling specificity. EMBO J *16*, 3912-3923.

Feng, X. H., Liang, Y. Y., Liang, M., Zhai, W., and Lin, X. (2002). Direct interaction of c-Myc with Smad2 and Smad3 to inhibit TGF-beta- mediated induction of the CDK inhibitor p15(Ink4B), Mol Cell *9*, 133-43.

Feng, X. H., Zhang, Y., Wu, R. Y., and Derynck, R. (1998). The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for smad3 in TGF-beta-induced transcriptional activation, Genes Dev *12*, 2153-63.

Frame, M. C., and Brunton,V. G. (2002). Advances in Rho-dependent actin regulation and oncogenic transformation. Curr Opin in Gene and develop *12*, 36-43.

Fransson, A., Ruusala, A., and Aspenstrom, P. (2003). Atypical rho GTPases have roles in mitochondrial homeostasis and apoptosis, J Biol Chem*278*, 6495-502.

Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C.-H., and Miyazono, K. (1993). Cloning of TGF beta type I receptor that forms a heteromeric complex with the TGF beta type II receptor. Cell *75*, 681-692.

Freeman, M., and Bienz, M. (2001). EGF receptor/Rolled MAP kinase signalling protects cells against activated Armadillo in the Drosophila eye, EMBO Rep *2*, 157-62.

Fritz, G., Just, I., and Kaina, B. (1999). Rho GTPases are over-expressed in human tumors, Int J Cancer *81*, 682-7.

Fukata, M., Kuruda, S., Nakagawa, M., Kawajiri, A., Itoh, N., Shoji, I., Matsuura, Y., Yonehara, S., *et al*. (1999). Cdc42 and Rac1 regulate the interaction of IQGAP with beta-catenin. J Biol Chem *274*, 26044-26050.

Fukumoto, Y., Kaibuchi, K., Hori, Y., Fujioka, H., Araki, S., Ueda, T., Kikuchi, A., and Takai, Y. (1990). Molecular cloning and characterization of a novel type of regulatory protein (GDI) for the rho proteins, ras p21-like small GTP-binding proteins, Oncogene *5*, 1321-8.

Furuhashi, M., Yagi, K., Yamamoto, H., Furukawa, Y., Shimada, S., Nakamura, Y., Kikuchi, A., Miyazono, K., and Kato, M. (2001). Axin facilitates Smad3 activation in the transforming growth factor beta signaling pathway, Mol Cell Biol *21*, 5132-41.

Gaddy-Kurten, D., Tsuchida, K., and Vale, W. (1995). Activins and the receptor serine kinase superfamily, Recent Prog Horm Res *50*, 109-29.

Gallo, K. A., and Johnson, G. L. (2002). Mixed-lineage kinase control of JNK and p38 MAPK pathways, Nat Rev Mol Cell Biol *3*, 663-72.

Galvin, K. M., Donovan, M. J., Lynch, C. A., Meyer, R. I., Paul, R. J., Lorenz, J. N., Fairchild-Huntress, V., Dixon, K. L., Dunmore, J. H., Gimbrone, M. A., Jr*., et al.* (2000). A role for smad6 in development and homeostasis of the cardiovascular system, Nat Genet *24*, 171-4.

Garrett, M. D., Major, G. N., Totty, N., and Hall, A. (1991). Purification and N-terminal sequence of the p21rho GTPase-activating protein, rho GAP, Biochem J *276*, 833-6.

Geiger, B., Ginsberg, D., Salomon, D., and Volberg, T. (1990). The molecular basis for the assembly and modulation of adherens-type junctions, Cell Differ Dev *32*, 343-53.

Gelfand, V. I., and Bershadsky, A. D. (1991). Microtubule dynamics: mechanisms, regulation, and function. Annu Rev Cell Biol *7*, 93-116.

Gold, L. I. (1999). The role for transforming growth factor-beta (TGF-beta) in human cancer, Crit Rev Oncog *10*, 303-60.

Gold, M. R., Gajewski, T. F., and DeFranco, A. L. (1991). Regulation of anti-immunoglobulin-induced B lymphoma growth arrest by transforming growth factor beta 1 and dexamethasone, Int Immunol *3*, 1091-8.

Goode, B. L., Drubin, D. G., and Barnes, G. (2000). Functional cooperation between the microtubule and actin cytoskeletons, Curr Opin Cell Biol *12*, 63-71.

Gorelik, L., and Flavell, R. A. (2001). Nature Rev Immunol *2*, 46-53.

Gougos, A., and Letarte, M. (1990). Primary structure of endoglin, an RGD-containing glycoprotein of human endothelial cells, J Biol Chem *265*, 8361-4.

Grady, W. M., Rajput, A., Myeroff, L., Liu, D. F., Kwon, K., Willis, J., and Markowitz, S. (1998). Mutation of the type II transforming growth factor-beta receptor is coincident with the transformation of human colon adenomas to malignant carcinomas, Cancer Res *58*, 3101-4.

Gratton, J. P., Morales-Ruiz, M., Kureishi, Y., Fulton, D., Walsh, K., and Sessa, W. C. (2001). Akt down-regulation of p38 signaling provides a novel mechanism of vascular endothelial growth factor-mediated cytoprotection in endothelial cells, J Biol Chem *276*, 30359-65.

Green, D. R. (1998). Apoptotic pathways: The roads to ruin. Cell *94*, 695-698.

Gronroos, E., Hellman, U., Heldin, C. H., and Ericsson, J. (2002). Control of Smad7 stability by competition between acetylation and ubiquitination, Mol Cell *10*, 483-93.

Gruber, B. L., Marchese, M. J., and Kew, R. R. (1994). Transforming growth factor-beta 1 mediates mast cell chemotaxis, J Immunol *152*, 5860-7.

Gundersen, G.G., Kim, I. And Chapin, C.J. (1994). Induction of stable microtubules in 3T3 fibroblasts by TGF-beta and serum. J Cell Sci *107*, 645-659

Guo, Y. L., Kang, B., Han, J., and Williamson, J. R. (2001). p38beta MAP kinase protects rat mesangial cells from TNF-alpha-induced apoptosis, J Cell Biochem *82*, 556-65.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. Science *279*, 509-514.

Hall, A, and Nobes, C. D. (2000). Rho GTPases: molecular switches that contol the organization and dynamics of the actin cytoskeleton. Philos Trans R Soc Lond B Sci *355*, 965-970.

Han, Y. P., Nien, Y. D., and Garner, W. L. (2002). Recombinant human platelet-derived growth factor and transforming growth factor-beta mediated contraction of human dermal fibroblast populated lattices is inhibited by Rho/GTPase inhibitor but does not require phosphatidylinositol-3' kinase, Wound Repair Regen *10*, 169-76.

Hanafusa, H., Ninomiya-Tsuji, J., Masuyama, N., Nishita, M., Fujisawa, J., Shibuya, H., Matsumoto, K., and Nishida, E. (1999). Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-β-induced gene expression. J Biol Chem *274*, 27161-27167.

Hanyu, A., Ishidou, Y., Ebisawa, T., Shimanuki, T., Imamura, T., and Miyazono, K. (2001). The N domain of Smad7 is essential for specific inhibition of transforming growth factor-β signaling. J Cell Biol *155*, 1017-1027.

Hart, M. J., Callow, M. G., Souza, B., Polakis, P. (1996). IQGAP1, a calmodulin-binding protein with a rasGAP-related domain, is a potential effector for cdc42Hs. EMBO J *15*, 2997-3005.

Hart, M. J., Shinjo, K., Hall, A., Evans, T., and Cerione, R. A. (1991). Identification of the human platelet GTPase activating protein for the CDC42Hs protein, J Biol Chem*266*, 20840-8.

Hartsough, M. T., Frey, R. S., Zipfel, P. A., Buard, A., Cook, S. J., McCormick, F., and Mulder, K. M. (1996). Altered transforming growth factor signaling in epithelial cells when ras activation is blocked. J Biol Chem *271*, 22368-22375.

Hartsough, M. T., and Mulder, K. M. (1997). Transforming growth factor-beta signaling in epithelial cells, Pharmacol Ther *75*, 21-41.

Hartwig, J. H., and Kwiatkowski, D. J. (1991). Actin-binding proteins. Curr Rev Cell Biol *3*, 87-97.

Hassan, A. H., Neely, K. E., and Workman, J. L. (2001). Histone acetyltransferase complexes stabilize swi/snf binding to promoter nucleosomes, Cell *104*, 817-27.

Hata, A., Lagna, G., Massagué, J., and Hemmati-Brivanlou, A. (1998). Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. Genes Dev *12*, 186-197.

Hata, A., Lo, R. S., Wotton, D., Lagna, G., and Massague, J. (1997). Mutations increasing autoinhibition inactivate tumour suppressors Smad2 and Smad4, Nature *388*, 82-7.

Hatakeyama, D., Kozawa, O., Niwa, M., Matsuno, H., Ito, H, Kato, K., Tatematsu, N., Shibata, T., Uematsu, T. (2002). Upregulation by retinoic acid of transforming growth factor-b-stimulated heat shock protein 27 induction in oseoblasts: involvement of mitogen-activated protein kinases. Bioch Bioph Acta *1589*, 15-30.

He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998). Identification of c-MYC as a target of the APC pathway, Science *281*, 1509-12.

Hedges, J. C., Dechert, M. A., Yamboliev, I. A., Martin, J. L., Hickey, E., Weber, L. A., and Gerthoffer, W. T. (1999). A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration, J Biol Chem *274*, 24211-9.

Heldin, C-H., Miyazono, K., and ten Dijke, P. (1997). TGF-β signalling from cell membrane to nucleus through SMAD proteins. Nature *390*, 465-471.

Henderson, B. R. (2000). Nuclear-cytoplasmic shuttling of APC regulates beta-catenin subcellular localization and turnover, Nat Cell Biol *2*, 653-60.

Hengartner, M. O. (2000). The biochemistry of apoptosis. Nature *407*, 770-776.

Higgs, H. N., and Pollard, T. D. (2001). Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins, Annu Rev Biochem *70*, 649-76.

Higuchi, M., Masuyama, N., Fukui, Y., Suzuki, A., and Gotoh, Y. (2001). Akt mediates Rac/Cdc42-regulated cell motility in growth factor- stimulated cells and in invasive PTEN knockout cells, Curr Biol *11*, 1958-62.

Hiscox, S., and Jiang, W. G. (1999). Hepatocyte growth factor/scatter factor disrupts epithelial tumour cell- cell adhesion: involvement of beta-catenin, Anticancer Res *19*, 509-17.

Hocevar, B. A., Brown, T. L., and Howe, P. H. (1999). TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. EMBO J *18*, 1347-1356.

Hocevar, B. A., Smine, A., Xu, X. X., and Howe, P. H. (2001). The adaptor molecule Disabled-2 links the transforming growth factor beta receptors to the Smad pathway, Embo J *20*, 2789-801.

Hogan, B. L. M. (1996). Bone morphogenetic proteins: multifunctional regulators of vertebrate development, Genes Dev *10*, 1580-1594.

Hsu, D. R., Economides, A. N., Wang, X., Eimon, P. M., and Harland, R. M. (1998a). The Xenopus dorsalizing factor Gremlin identifies a novel family of secreted proteins that antagonize BMP activities, Mol Cell *1*, 673-83.

Hsu, S. C., Galceran, J., and Grosschedl, R. (1998b). Modulation of transcriptional regulation by LEF-1 in response to Wnt-1 signaling and association with beta-catenin, Mol Cell Biol *18*, 4807-18.

Huang, S., Jiang, Y., Li, Z., Nishida, E., Mathias, P., Lin, S., Ulevitch, R. J., Nemerow, G. R., and Han, J. (1997). Apoptosis signaling pathway in T cells is composed of ICE/Ced-3 family proteases and MAP kinase kinase 6b, Immunity *6*, 739-49.

Hyman, K. M., Seghezzi, G., Pintucci, G., Stellari, G., Kim, J. H., Grossi, E. A., Galloway, A. C., and Mignatti, P. (2002). Transforming growth factor-beta1 induces apoptosis in vascular endothelial cells by activation of mitogen-activated protein kinase, Surgery *132*, 173-9.

Ichetovkin, I., Grant, W., and Condeelis, J. (2002). Cofilin produces newly polymerized actin filaments that are preferred for dendritic nucleation by the Arp2/3 complex, Curr Biol *12*, 79-84.

Ichijo, H., Nishida, E., Irie, K., ten Dijke, P., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K., and Gotoh, Y. (1997). Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science *275,* 90-4.

Ilyas, M., Tomlinson, I. P., Rowan, A., Pignatelli, M., and Bodmer, W. F. (1997). Beta-catenin mutations in cell lines established from human colorectal cancers, Proc Natl Acad Sci U S A *94*, 10330-4.

Imamura, T., Takase, M., Nishihara, A., Oeda, E., Hanai, J., Kawabata, M., and Miyazono, K. (1997). Smad6 inhibits signalling by the TGF-β superfamily. Nature *389*, 622-626.

Ishiazaki, T., Morishima, Y., Okamoto, M., Furuyashiki, T., Kato, T., Narumiya, S. (2001). Coordination of microtubules and the actin cytoskeleton by the Rho effector mDia1. Nat Cell Biol *3*, 8-14.

Ishisaki, A., Yamato, K., Hashimoto, S., Nakao, A., Tamaki, K., Nonaka, K., ten Dijke, P., Sugino, H., and Nishihara, T. (1999). Differential inhibition of Smad6 and Smad7 on bone morphogenetic protein- and activin-mediated growth arrest and apoptosis in B cells, J Biol Chem *274*, 13637-42.

Ishisaki, A., Yamato, K., Nakao, A., Nonaka, K., Ohguchi, M., ten Dijke, P., and Nishihara, T. (1998). Smad7 is an activin-inducible inhibitor of activin-induced growth arrest and apoptosis in mouse B cells. J Biol Chem *273*, 24293-24296.

Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. (1999). The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta- catenin and transcription factor TCF, Nature *399*, 798-802.

Itoh, F., Divecha, N., Brocks, L., Oomen, L., Janssen, H., Calafat, J., Itoh, S., and Dijke Pt, P. (2002). The FYVE domain in Smad anchor for receptor activation (SARA) is sufficient for localization of SARA in early endosomes and regulates TGF-beta/Smad signalling, Genes Cells *7*, 321-31.

Itoh, S., Landström, M., Hermansson, A., Itoh, F., Heldin, C.-H., Heldin, C.-E., and ten Dijke, P. (1998). Transforming growth factor β1 induces nuclear export of inhibitory Smad7. J Biol Chem *273*, 29195-29201.

Ivanov, V. N., and Ronai, Z. (2000). p38 protects human melanoma cells from UV-induced apoptosis through down-regulation of NF-kappaB activity and Fas expression, Oncogene *19*, 3003-12.

Jamora, C., and Fuchs, E. (2002). Intercellular adhesion, signalling and the cytoskeleton, Nat Cell Biol *4*, E101-8.Janmey, P. A. (1991). Mechanical properties of cytoskeletal polymers. Curr opin Cell Biol *3*, 4-11.

Jeong, H. G., Cho, H. J., Chang, I. Y., Yoon, S. P., Jeon, Y. J., Chung, M. H., and You, H. J. (2002). Rac1 prevents cisplatin-induced apoptosis through down-regulation of p38 activation in NIH3T3 cells, FEBS Lett *518*, 129-34.

Joberty, G., Petersen, C., Gao, L., Macara, I. G. (2000). The cell-polarity protein Par6 links Par3 and atypicaly protein kinase C to Cdc42. Nat Cell Biol *2*, 531-539.

Johansson, A., Driessens, M., and Aspenström, P. (2000). The mammalian homologue of the *Caenorhabditis elegance* polarity protein PAR-6 is a binding partner for the Rho GTPases Cdc42 and Rac. J Cell Sci *113*, 3267-3275.

Juliano, R. L. (2002). Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. Annu Rev Pharmacol Toxicol *42*, 283-323.

Just, I., Selzer, J., Wilm, M., von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995). Glucosylation of Rho proteins by Clostridium difficile toxin B, Nature *375*, 500-3.

Kaartinen, V., Haataja, L., Nagy, A., Heisterkamp, N., and Groffen, J. (2002). TGFbeta3-induced activation of RhoA/Rho-kinase pathway is necessary but not sufficient for epithelio-mesenchymal transdifferentiation: implications for palatogenesis, Int J Mol Med *9*, 563-70.

Kanamaru, C., Yasuda, H., and Fujita, T. (2002). Involvement of Smad proteins in TGF-beta and activin A-induced apoptosis and growth inhibition of liver cells. Hepatol Res *23*, 211-219.

Kang, P., and Svoboda, K. K. (2002). PI-3 kinase activity is required for epithelial-mesenchymal transformation during palate fusion, Dev Dyn *225*, 316-21.

Karp, G. C., and Solursh, M. (1985). Dynamic activity of the filopodia of sea urchin embryonic cells and their role in directed migration of the primary mesenchyme in vitro, Dev Biol *112*, 276-83.

Kavasak, P., Rasmussen, R. K., Causing, C. G., Bonni, S., Zhu, H., Thomsen, G. H., and Wrana, J. L. (2000). Smad7 binds to Smurf to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation. Mol Cell *6*, 1365-1375.

Kaverina, I., Krylyshkina, O., and Small, J. V. (2002). Regulation of substrate adhesion dynamics during cell motility, Int J Biochem Cell Biol *34*, 746-61.

Kawabata, M., Imamura, T., and Miyazono, K. (1998). Signal transduction by bone morphogenetic proteins, Cytokine Growth Factor Rev *9*, 49-61.

Keely, P. J., Westwick, J. K., Whitehead, I. P., Der, C. J., and Parise, L. V. (1997). Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K, Nature *390*, 632-6.

Keller, H., Rentsch, P., and Hagmann, J. (2002). Differences in cortical actin structure and dynamics document that different types of blebs are formed by distinct mechanisms, Exp Cell Res *277*, 161-72.

Kemler, R., and Ozawa, M. (1989). Uvomorulin-catenin complex: cytoplasmic anchorage of a Ca2+-dependent cell adhesion molecule, Bioessays *11*, 88-91.

Kerr, J. F., Wyllie, A. H., and Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer *26,* 239-257.

Kim, K., Pang, K. M., Evans, M., and Hay, E. D. (2000). Overexpression of beta-catenin induces apoptosis independent of its transactivation function with LEF-1 or the involvement of major G1 cell cycle regulators, Mol Biol Cell *11*, 3509-23.

Kim, S., Lee, S. H., and Park, D. (2001). Leucine zipper-mediated homodimerization of the p21-activated kinase- interacting factor, beta Pix. Implication for a role in cytoskeletal reorganization, J Biol Chem *276*, 10581-4.

Kimura, N., Matsumo, R., Shibuya, H., Nakashima, K., and Taga, T. (2000). BMP2- induced apoptosis is mediated by activation of the TAK1-p38 kinase pathway that is negatively regulated by Smad6. J Biol Chem *275*, 17647-17652.

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

Kitagawa, M., Hatakeyama, S., Shirane, M., Matsumoto, M., Ishida, N., Hattori, K., Nakamichi, I., Kikuchi, A., and Nakayama, K. (1999). An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of beta-catenin, Embo J *18*, 2401-10.

Kjoller, L., and Hall, A. (1999). Signaling to Rho GTPases, Exp Cell Res *253*, 166-79.

Koenig, B. B., Cook, J. S., Wolsing, D. H., Ting, J., Tiesman, J. P., Correa, P. E., Olson, C. A., Pecquet, A. L., Ventura, F., Grant, R. A., and et al. (1994). Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells, Mol Cell Biol *14*, 5961-74.

Koh, C. G., Manser, E., Zhao, Z. S., Ng, C. P., and Lim, L. (2001). Beta1PIX, the PAK-interacting exchange factor, requires localization via a coiled-coil region to promote microvillus-like structures and membrane ruffles, J Cell Sci *114*, 4239-51.

Koyasu, S., Kadowaki, T., Nishida, E., Tobe, K., Abe, E., Kasuga, M., Sakai, H., and I. Yahara. (1988). Alteration in growth, cell morphology, and cytoskeletal structures of KB cells induced by epidermal growth factor and transforming growth factor-β. Exp Cell Res *176*:107-116.

Kozma, R., Ahmed, S., Best, A., Lim, L. (1995). The Ras-related protein Cdc42Hs and Bradykinin promote formation of peripheral actin mikrospikes and filopodia in Swiss 3T3 fibroblasts. Mol Cell Biol *l5*, 1942-1952.

Kretzschmar, M., Doody, J., and Massague, J. (1997a). Opposing BMP and EGF signalling pathways converge on the TGF-beta family mediator Smad1, Nature *389*, 618-22.

Kretzschmar, M., Doody, J., and Massagué, J. (1999). A mechanism of repression of TGFβ/Smad signaling by oncogenic Ras. Genes Dev *13*, 804-816.

Kretzschmar, M., Lui, F., Hata, A., Doody, J., and Massagué, J. (1997b). The TGF-β family mediator Smad1 is phosphorylated directly and activated functionally by the BMP receptor kinase. Genes Dev *11*, 984-995.

Kroemer, G., Dallaporta, B., and Resche-Rigon, M. (1998). The mitochondrial death/life regulator in apoptosis and necrosis, Annu Rev Physiol *60*, 619-42.

Krugmann, S., Jordens, I., Gevaert, K., Driessens, M., Vandekerckhove, J., and Hall, A. (2001). Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex, Curr Biol *11*, 1645-55.

Kummer, J.L., Rao, P.K., and Heidenreich, K.A. (1997). Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. J Biol Chem *272*, 20490-20494.

Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, M., Ookubo, T., Izawa, I., Nagase, T., Nomura, N., Tani, H., *et al*. (1998). Role of IQGAP, a target of small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell-cell adhesion. Science *281*, 832-835.

Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A.„Ahmad, M. F., Avrush, M. F., and Woodgett, J. R. (1994). The stress-activated protein kinase subfamily of c-Jun kinases Nature *369*, 156-160.

Labbe, E., Letamendia, A., and Attisano, L. (2000). Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor-beta and wnt pathways, Proc Natl Acad Sci U S A *97*, 8358-63.

Lallemand, F., Mazars, A., Prunier, C., Bertrand, F., Kornprost, M., Gallea, S., Roman, S., Cherqui, G., and Atfi, A. (2001). Smad7 inhibits the survival nuclear factor kappaB and potentiates apoptosis in epithelial cells. Oncogene *20*, 879-884.

Lamarche, N., and Hall, A. (1994). GAPs for Rho-related GTPases. Trends Genet *10*, 436-440.

Landström, M., Eklov, S., Colosetti, P., Nilsson, S., Damber, J. E., Bergh, A., and Funa, K. (1996). Estrogen induces apoptosis in a rat prostatic adenocarcinoma: association with an increased expression of TGF-beta 1 and its type-I and type-II receptors, Int J Cancer *67*, 573-9.

Landström, M., Heldin, N.-E., Bu, S., Hermansson, A., Itoh, S., ten Dijke, P., and Heldin, C.-H. (2000). Smad7 mediates apoptosis induced by transforming growth factor β in prostatic carcinoma cells. Curr Biol *10*, 535-538.

Langanger, G., Moeremans, M., Daneels, G., et al. (1986). The molecular organization of myosine in stress fibers of cultured cells. J Cell Biol *102*, 200-209.

Larisch, S., Yi, Y., Lotan, R., Kerner, H., Eimerl, S., Tony Parks, W., Gottfried, Y.„Birkey, Raffey, S., de Caestecker, M. P., Danielpour, D., Book-Melamed, N., et al. (2000). A novel mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif. Nat Cell Biol *2*, 915-921.

Lee, K.M., Park, J., Kim, J.H, Yie, S.W., Chun, G.-T., Kim, P.-H., and E.Y. Choi. (1999). Reorganisation of myosin and focal adhesion proteins in Swiss 3T3 fibroblasts induced by transforming growth factor beta. Cell Biol Int *7*:507-517.

Lee, S. H., Eom, M., Lee, S. J., Kim, S., Park, H. J., and Park, D. (2001). BetaPix-enhanced p38 activation by Cdc42/Rac/PAK/MKK3/6-mediated pathway. Implication in the regulation of membrane ruffling, J Biol Chem *276*, 25066-72.

Letamendia, A., Labbe, E., and Attisano, L. (2001). Transcriptional regulation by Smads: crosstalk between the TGF-beta and Wnt pathways, J Bone Joint Surg Am*83-A*, S31-9.

Letterio, J. J., and Roberts, A. B. (1998). Regulation of immune responses by TGF-beta, Annu Rev Immunol *16*, 137-61.

Levin, M., Johnson, R. L., Stern, C. D., Kuehn, M., and Tabin, C. (1995). A molecular pathway determining left-right asymmetry in chick embryogenesis, Cell *82*, 803-14.

Liao, J. H., Chen, J. S., Chai, M. Q., Zhao, S., and Song, J. G. (2001). The involvement of p38 MAPK in transforming growth factor beta1-induced apoptosis in murine hepatocytes, Cell Res *11*, 89-94.

Liberati, N. T., Datto, M. B., Fredrick, J. P., Shen, X., Wong, C., Rougier-Chapman, E. M., and Wang, X.-F. (1999). Smads bind directly to the Jun family of AP-1 transcription factors. Proc Natl Acad Sci USA *96*, 4844-4849.

Like, B., and J. Massagué. (1986). The antiproliferative effect of type beta transforming growth factor occurs at a level distal from receptors for growth-activating factors. J Biol Chem 261:13426-13429.

Lin, D., Edwards, A. S., Fawsett, J. P., Mbamalu, G., Scott, J. D., Pawson, T. (2000). A mammalian PAR-3-RAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat Cell Biol *2*, 540-547.

Lin, X., Liang, M., and Feng, X. H. (2000). Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor-β signaling. J Biol Chem *275*, 36818-36822.

Lo, R. S., Chen, Y. G., Shi, Y., Pavletich, N. P., and Massague, J. (1998). The L3 loop: a structural motif determining specific interactions between SMAD proteins and TGF-beta receptors, Embo J *17*, 996-1005.

Lo, R. S., and Massagué, J. (1999). Ubiquitin-dependent degradation of TGF-β-activated Smad2. Nature Cell Biol *I,* 472-478.

Lo, R. S., Wotton, D., and J. Massagué. (2001). Epidermal growth factor signalling via Ras controls the Smad transcriptional co-repressor TGIF. EMBO J *20,* 128-136.

Lomri, A., and P.J. Marie. (1990). Effects of tranforming growth factor type beta on expression ofcytoskeletal proteins in endosteal mouse osteoblastic cells. Bone *11*, 445-451.

Lopez-Casillas, F., Wrana, J. L., and Massague, J. (1993). Betaglycan presents ligand to the TGF beta signaling receptor, Cell *73*, 1435-44.

Luo, L. (2000). Rho GTPases in neuronal morphogenesis, Nat Rev Neurosci *1*, 173-80.

Lynch, E. D., Lee, M. K., Morrow, J. E., Welcsh, P. L., Leon, P. E., and King, M. C. (1997). Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the Drosophila gene diaphanous, Science *278*, 1315-8.

Ma, L., Rhotagi, R., Kirshner, M. W. (1998). The Arp2/3 complex mediates actin polymerizationinduced by the Small GTP-binding protein Cdc42. Proc Natl Acad Sci USA *95*, 15362-15367.

Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J., Pollard, T. D. (1994).Purification of chromatography on profilin agarose. J Cell Biol *127*, 107-115.

Machesky, L. M., and Hall, A. (1997). Role of actin polymerization and adhesion to extracellularmatrix in Rac- and Rho-induced cytoskeletal reorganization. J Cell Biol *138*, 913-926.

Machesky, L. M., Mullins D. M., Higgs, H. N., Kaiser, D. A., Blanchoin, L., *et al*. (1999). Scar, a WASP-related protein, activates nucleation of actin filaments by the Arp2/3 complex. Proc Natl Acad Sci USA *96*, 3739-3744.

Macias-Silva, M., Hoodless, P. A., Tang, S. J., Buchwald, M., and Wrana, J. L. (1998). Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2, J Biol Chem *273*, 25628-36.

Madisen, L., Webb, N. R., Rose, T. M., Marquardt, H., Ikeda, T., Twardzik, D., Seyedin, S., and Puricho, A. F. (1988). Transforming growth factor-beta 2: cDNA cloning and sequence analysis. DNA *7*, 1-8.

Maekawa, M., Ishizaki, T., Boku, S., Watanabe, N., Fujita, A., Iwamatsu, A., Obinata, T., Ohashi, K., Mizuno, K., and Narumiya, S. (1999). Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase, Science *285*, 895-8.

Magdalena, J., and Goldberg, M. B. (2002). Quantification of Shigella IcsA required for bacterial actin polymerization, Cell Motil Cytoskeleton *51*, 187-96.

Malinda, K. M., Fisher, G. W., and Ettensohn, C. A. (1995). Four-dimensional microscopic analysis of the filopodial behavior of primary mesenchyme cells during gastrulation in the sea urchin embryo, Dev Biol *172*, 552-66.

Manser, E., Leung, T., Salihuddin, H., Tan, L., and Lim, L. (1993). A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42, Nature *363*, 364-7.

Manser, E., Leung, T., Salihuddin, H., Zhao, Z. S., and Lim, L. (1994). A brain serine/threonine protein kinase activated by Cdc42 and Rac1, Nature *367*, 40-6.

Manser, E., Loo, T. H., Koh, C. G., Zhao, Z. S., Chen, X. Q., Tan, L., Tan, I., Leung, T., and Lim, L. (1998). PAK kinases are directly coupled to the PIX family of nucleotide exchange factors, Mol Cell *1*, 183-92.

Massagué, J. (1998). TGF-beta signal transduction. Annu Rev Biochem *67*, 753-791.

Massagué, J. (2000). How cells read TGF-β signals. Nature Rev Mol Cell Biol *1*, 169-178.

Massagué, J., and Chen, Y. G. (2000). Controlling TGF-beta signaling. Genes Dev *14*, 627-644.

Massagué, J., and Wotton, D. (2000). Transcriptional control by the TGF-β/Smad signaling system. EMBO J *19*, 1745-1754.

Matsuzawa, A., and Ichijo, H. (2001). Molecular mechanisms of the decision between life and death: regulation of apoptosis by apoptosis signal-regulating kinase 1, J Biochem (Tokyo) *130*, 1-8.

Mazars, A., Lallemand, F., Prunier, C., Marais, J., Ferrand, N., Pessah, M., Cherqui, G., and Atfi ,A. (2001). Evidence for a role of the JNK cascade in Smad7-mediated apoptosis. J Biol Chem *276*, 36797-36803.

McCartney, B. M., Dierick, H. A., Kirkpatrick, C., Moline, M. M., Baas, A., Peifer, M., and Bejsovec, A. (1999). Drosophila APC2 is a cytoskeletally-associated protein that regulates wingless signaling in the embryonic epidermis, J Cell Biol *146*, 1303-18.

McKendry, R., Hsu, S. C., Harland, R. M., and Grosschedl, R. (1997). LEF-1/TCF proteins mediate wnt-inducible transcription from Xenopus nodal-related 3 promotor. Dev Biol *192,* 420-431.

McNiven, M. A., Kim, L., Krueger, E. W., Orth, J. D., Cao, H., and Wong, T. W. (2000). Regulated interactions between dynamin and the actin-binding protein cortactin modulate cell shape, J Cell Biol *151*, 187-98.

Mellström, K., Höglund, A.-S., Nistér, M., Heldin, C.-H., Westermark, B. and U. Lindberg. (1983). The effect of platelet-derived growth factor on morphology and motility of human glial cells. J Muscle Res Cell Motil *4,* 589-609.

Miettininen, P.J., Ebner, R., Lopez, A.R., and R. Derynck. (1994). TGF-beta inducedtransdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. J Cell Biol *127*, 2021-2036.

Miki, H., Sasaki, T., Takai, Y., and Takenawa, T. (1998). Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP, Nature *391*, 93-6.

Miki, H., Yamaguchi, H., Suetsugu, S., and Takenawa, T. (2000). IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling, Nature *408*, 732-5.

Mitchison, T. J. and L. P. Cramer. (1996). Actin-based cell motility and cell locomotion. Cell *84*, 371-379.

Miyashita, T., and Reed, J. C. (1995). Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell *80*, 293-299.

Miyazono, K., ten Dijke, P., and Heldin, C.-H. (2000). TGF-β signaling by Smad proteins. AdvImmunol 75, 115-157.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos, Cell *86*, 391-9.

Moon, R. T., Bowerman, B., Boutros, M., and Perrimon, N. (2002). The promise and perils of Wnt signaling through beta-catenin, Science *296*, 1644-6.

Moriguchi, T., Kuroyanagi, N., Yamaguchi, K., Gotoh, Y., Irie, K., Kano, T., Shirakabe, K., Muro, Y., Shibuya, H., Matsumoto, K., *et al*. (1996). A novel kinasecascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. J Biol Chem *271*, 13675-13679.

Mota, M., Reeder, M., Chernoff, J., and Bazenet, C. E. (2001). Evidence for a role of mixed lineage kinases in neuronal apoptosis, J Neurosci *21*, 4949-57.

Mott, H. R., Owen, D., Nietlispach, D., Lowe, P. N., Manser, E., Lim, L., and Laue, E. D. (1999). Structure of the small G protein Cdc42 bound to the GTPase-binding domain of ACK, Nature *399*, 384-8.

Moustakas, A., and Stournaras, C. (1999). Regulation of actin organization by TGF-β in H-ras-transformed fibroblasts. J Cell Biol *127*, 2021-2036.

Mucsi, I., Skorecki, K. L., and H. J. Goldberg. (1996). Extracellular signal-regulated kinase and the small GTP-binding protein, Rac, contribute to the effects of transforming growth factor-β1 on gene expression. J Biol Chem *271*, 16567-16572.

Mulder K. M. (2000). Role of ras and Mapks TGFβ sinaling. Cytokine Growth Factor Rev *11*, 23-35.

Mullins, R. D., Heuser, J. A., Pollard, T. D. (1998). The interaction of Arp2/3 complex with actin:nucleation, high-affinity pointed end capping, and formation of branching networks of filaments. Proc Natl Acad Sci USA *95*, 6181-6186.

Mullins, R. D., Stafford, W. F., Pollard, T. D. (1997). Strukture, subunit topology, and actin-binding activity of the Arp2/3 complex from Acanthamoeba. J Cell Biol *136*, 331-343.

Munger, J. S., Harpel, J. G., Giancotti, F. G., and Rifkin, D. B. (1998). Interactions between growth factors and integrins: Latent forms of transforming growth factor-beta are ligands for the integrin alphavbeta1, Mol Biol Cell *9*, 2627-2638.

Munger, J. S., Harpel, J. G., Gleizes, P. E., Mazzieri, R., Nunes, I., and Rifkin, D. B. (1997). Latent transforming growth factor-beta: Structural features and mechanisms of activation, Kidney Int *51*, 1376-1382.

Muraoka, R. S., Dumont, N., Ritter, C. A., Dugger, T. C., Brantley, D. M., Chen, J., Easterly, E., Roebuck, L. R., Ryan, S., Gotwals, P. J., *et al.* (2002). Blockade of TGF-beta inhibits mammary tumor cell viability, migration, and metastases, J Clin Invest *109*, 1551-9.

Nagata, K., Puls, A., Futter, C., Aspenstrom, P., Schaefer, E., Nakata, T., Hirokawa, N., and Hall, A. (1998). The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3, Embo J *17*, 149-58.

Nakajima, Y., Yamagishi, T., Yoshimura, K., Nomura, M., and H. Nakamura. (1999). Antisenseoligonucleotide complementary to smooth muscle α-actin inhibits endothelial-mesenchymal transformation during chick cardinogenesis. Developmenal Dynamics. *216*, 489-498.

Nakamura, T., Takio, K., Eto, Y., Shibai, H., Tiani, K., and Sugino, H. (1990). Activin-binding protein from rat ovary is follistatin. Science *247*, 836-838.

Nakao, A., Afrakhte, M., Morén, A., Nakayama, T., Christian, J, L., Heuchel, R., Itoh, S., Kawataba, M., Heldin, C.-E., Heldin, C.-H., and ten Dijke, P. (1997). Identification of Smad7, a TGF-β-inducible antagonist of TGF-β signalling. Nature *389*, 631-635.

Nass, S. J., Li, M., Amundadottir, L. T., Furth, P. A., and Dickson, R. B. (1996). Role for Bcl-xL in the regulation of apoptosis by EGF and TGF beta 1 in c-myc overexpressing mammary epithelial cells, Biochem Biophys Res Commun *227*, 248-56.

Nishita, M., Hashimoto, M. K., Ogata, S., Laurent, M. N., Ueno, N., Shibuya, H., and Cho, K. W. (2000). Interaction between Wnt and TGF-beta signalling pathways during formation of Spemann's organizer, Nature *403*, 781-5.

Nobes, C.D., and Hall, A. (1995). Rho, rac, and cdc42 GTPases regulate the assembly of multi-molecular focal complexes associated with actin stress fibres, lamellipodia, and filopodia. Cell *81*, 53-62.

Ochs, H. D. (1998). The Wiskott-Aldrich syndrome. Semin Hematol 35, 332-345.

O´Connor, K. L., Nguyen, B. K., and Mercurio, A. M. (2000). RhoA function in lamellae formation and migration is regulated by the alpha6beta4 integrin and cAMP metabolism. J Cell Biol *148*, 253-258.

Oft, M., Peli, J., Rudaz, C., Schwartz, H., Beug, H., and Reichmann, E. (1996). TGF- beta1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells. Genes Dev *10*, 2462-2477.

Ogawa, Y., Schmidt, D. K., Dasch, J. R., Chang, R. J., and Glaser, C. B. (1992). Purification and characterization of transforming growth factor-beta 2.3 and -beta 1.2 heterodimers from bovine bone, J Biol Chem*267*, 2325-8.

Okado, T., Terada, Y., Tanaka, H., Inoshita, S., Nakao, A., and Sasaki, S. (2002). Smad7 mediates transforming growth factor-beta-induced apoptosis in mesangial cells, Kidney Int *62*, 1178-86.

Oleinikov, A. V., Zhao, J., and Makker, S. P. (2000). Cytosolic adaptor protein Dab2 is an intracellular ligand of endocytic receptor gp600/megalin, Biochem J *347 Pt 3*, 613-21.

Olsson, N., Piek, E., ten Dijke, P., and G. Nilsson. (2000). Human mast cell migration in response tomembers of the transforming growth factor-beta family. J Leukoc Biol *67*, 350-356

Ono, K., and Han, J. (2000). The p38 signal transduction pathway activation and function. Cell Sign *12*, 1-13.

Park, H. J., Kim, B. C., Kim, S. J., and Choi. (2002). Role of MAP kinases and their cross-talk in TGF-beta1-induced apoptosis in FaO rat hepatoma cell line. Hepatology *35,* 1360-1371.

Pasteris, N. G., Cadle, A., Logie, L. J., Porteous, M. E., Schwartz, C. E., Stevenson, R. E., Glover, T. W., Wilroy, R. S., and Gorski, J. L. (1994). Isolation and characterization of the faciogenital dysplasia (Aarskog- Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor, Cell *79*, 669-78.

Paterson, H. F., Self, A. J., Garrett, M. D., Just, I., Aktories, K., and Hall, A. (1990). Microinjection of recombinant p21rho induces rapid changes in cell morphology, J Cell Biol *111*, 1001-7.

Pelaia, G., Cuda, G., Vatrella, A., Fratto, D., Grembiale, R. D., Tagliaferri, P., Maselli, R., Costanzo, F. S., and Marsico, S. A. (2003). Effects of TGF-{beta} and Budesonide on MAPK activation and apoptosis in airway epithelial cells, Am J Respir Cell Mol Biol *10*, 10.

Pepper, M. S. (1997). Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity, Cytokine Growth Factor Rev *8*, 21-43.

Perlman, R., Schiemann, W. P., Brooks, M. W., Lodish, H. F., Weinberg, R. A. (2001). TGF-β-induced apoptosis is mediated by the adaptor protein Daxx that facilitates JNK activation. Nat Cell Biol *3*, 708-714.

Piccolo, S., Sasai, Y., Lu, B., and De Robertis, E. M. (1996). Dorsoventral patterning in Xenopus: inhibition of ventral signals by direct binding of chordin to BMP-4, Cell *86*, 589-98.

Piek, E., Heldin, C.-H., and ten Dijke, P. (1999a). Specificity, diversity, and regulation in TGF-β superfamily signaling. FASEB J *13*, 2105-2124.

Piek, E., Moustakas, A., Kurisaki, A., Heldin, C.-H., and P. ten Dijke. (1999b). TGF-β type Ireceptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NmuMG breast epithelial cells. J Cell Sci *112*, 4557-4568.

Piek, E., Franzen, P., Heldin, C. H., and ten Dijke, P. (1997). Characterization of a 60-kDa cell surface-associated transforming growth factor-beta binding protein that can interfere with transforming growth factor-beta receptor binding, J Cell Physiol *173*, 447-59.

Polakis, P. (2000). Wnt signaling and cancer. Genes Dev. *14*, 1837-1851.

Pollard, T. D., Blanchoin, L., and R. D. Mullins. (2000). Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. Annu Rev Biophys Biomol Struct *29*, 545-576.

Preudhomme, C., Roumier, C., Hildebrand, M. P., Dallery-Prudhomme, E., Lantoine, D., Lai, J. L., Daudignon, A., Adenis, C., Bauters, F., Fenaux, P., *et al.* (2000). Nonrandom 4p13 rearrangements of the RhoH/TTF gene, encoding a GTP-binding protein, in non-Hodgkin's lymphoma and multiple myeloma, Oncogene *19*, 2023-32.

Price, L. S., and Collard, J. G. (2001). Regulation of the cytoskeleton by Rho-family GTPases: implications for tumour cell invasion, Semin Cancer Biol *11*, 167-73.

Price, W. A. (1999). Peptide growth factors regulate insulin-like growth factor binding protein production by fetal rat lung fibroblasts, Am J Respir Cell Mol Biol *20*, 332-41.

Pyrzynska, B., Mosieniak, G., and Kaminska, B. (2000). Changes of the trans-activating potentialof AP-1 transcription factor during cyclosporin A-induced apoptosis of glioma cells are mediated by phosphorylation and alterations of AP-1 composition. J Neurochem *74*, 42-51.

Qualmann, B., Roos, J., DiGregorio, P. J., and Kelly, R. B. (1999). Syndapin I, a synaptic dynamin-binding protein that associates with the neural Wiskott-Aldrich syndrome protein, Mol Biol Cell *10*, 501-13.

Raftery, L. A., Twombly, V., Wharton, K., and Gelbart, W. M. (1995). Genetic screens to identify elements of the decapentaplegic signaling pathway in Drosophila, Genetics *139*, 241-54.

Raingeaud, J., Gupta, S., Roger, J. S., Dickens, M., Han, J., Ulevitch, R. J., David, R. J. (1995).Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine JBiol Chem 270, 7420-7426.

Rajah, R., Valentinis, B., and Cohen, P. (1997). Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism, J Biol Chem*272*, 12181-8.

Reid, T., Furuyashiki, T., Ishizaki, T., Watanabe, G., Watanabe, N., Fujisawa, K., Morii, N., Madaule, P., and Narumiya, S. (1996). Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, PKN, and rhophilin in the rho-binding domain, J Biol Chem*271*, 13556-60.

Richnau, N., and Aspenström, P. (2001). RICH, a Rho GTPase-activating protein domain-containing protein involved in signaling by Cdc42 and Rac1. J Biol Chem *37*, 35060-35070.

Rickert, P., Weiner, O. D., Wang, F., Bourne, H. R., and Servant, G. (2000). Leukocytes navigate by compass: roles of PI3Kgamma and its lipid products, Trends Cell Biol *10*, 466-73.

Ricos, M. G., Harden, N., Sem, K. P., Lim, L., and W. Cha. 1999. Dcdc42 acts in TGF-β signaling during *Drosophila* morphogenesis: distinct roles for the Drac1/JNK and Dcdc42/TGF-β cascades in cytoskeletal regulation. J Cell Sci *112*, 1225-1235.

Ridley, A.J. (2001a). Rho family proteins: coordinating cell responses. Trends Cell Biol *11*, 471-477.

Ridley, A. J. (2001b). Rho GTPases and cell migration, J Cell Sci *114*, 2713-22.

Ridley, A. J. (2001c). Rho proteins, PI 3-kinases, and monocyte/macrophage motility, FEBS Lett *498*, 168-71.

Ridley, A. J., and Hall, A. (1992). The Small GTP-binding protein rho regulates the assembly of focal adhesion and actin stress fibers in response to growth factors. Cell *70*, 389-399.

Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling, Cell *70*, 401-10.

Riedy, M. C., Brown, M. C., Molloy, C. J., and Turner, C. E. (1999). Activin A and TGF-beta stimulate phosphorylation of focal adhesion proteins and cytoskeletal reorganization in rat aortic smooth muscle cells, Exp Cell Res *251*, 194-202.

Risau, W., and Flamme, I. (1995). Vasculogenesis, Annu Rev Cell Dev Biol *11*, 73-91.

Roberts, A. B. (1998). Molecular and cell biology of TGF-beta, Miner Electrolyte Metab *24*, 111-9.

Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M., and Sporn, M. B. (1981). New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues, Proc Natl Acad Sci U S A *78*, 5339-43.

Roberts, A. B., and Sporn, M. B. (1990) The transforming growth factor-βs. In peptide growth factors and there receptors, Part I, M. B. Sporn, and A. B. Roberts, eds. (Berlin, Springer-Verlag), pp.419-472.

Roberts, A. B., and Sporn, M. B. (1992). Differential expression of the TGF-beta isoforms in embryogenesis suggests specific roles in developing and adult tissues, Mol Reprod Dev *32*, 91-8.

Roberts, R. A., James, N. H., and Cosulich, S. C. (2000). The role of protein kinase B and mitogen-activated protein kinase in epidermal growth factor and tumor necrosis factor alpha-mediated rat hepatocyte survival and apoptosis, Hepatology *31*, 420-7.

Rogers, S. L., and Gelfand, V. I. (2000). Membrane trafficking, organelle transport, and the cytoskeleton, Curr Opin Cell Biol *12*, 57-62.

Rohatgi, R., Ma, L., Miki, H., Lopez, M., Kirchhausen, T., *et al*. (1999). The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell *97*, 221-231.

Rosfjord, E. C., and Dickson, R. B. (1999). Growth factors, apoptosis, and survival of mammary epithelial cells, J Mammary Gland Biol Neoplasia *4*, 229-37.

Rout, U. K., Saed, G. M., and Diamond, M. P. (2002). Transforming growth factor-beta1 modulates expression of adhesion and cytoskeletal proteins in human peritoneal fibroblasts, Fertil Steril *78*, 154-61.

Royal, I., Lamarche-Vane, N., Lamorte, L., Kaibuchi, K., and Park, M. (2000). Activation of cdc42, rac, PAK, and rho-kinase in response to hepatocyte growth factor differentially regulates epithelial cell colony spreading and dissociation, Mol Biol Cell *11*, 1709-25.

Rudel, T., and Bokoch, G. M. (1997). Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2, Science *276*, 1571-4.

Ruggieri, R., Chuang, Y. Y., and Symons, M. (2001). The small GTPase Rac suppresses apoptosis caused by serum deprivation in fibroblasts, Mol Med *7*, 293-300.

Rönnstrand, L., and C.-H. Heldin. 2001. Mechanisms of platelet-derived growth factor-induced chemotaxis. Int J Cancer *91*, 757-762.

Sahai, E., and Marshall, C. J. (2002). ROCK and Dia have opposing effects on adherens junctionsdownstream of Rho. Nature Cell Biol *4*, 408-415.

Sano, Y., Harada, J., Tashiro, S., Gotoh-Mandeville, R., Maekawa, T., and Ishii, S. (1999). ATF-2 is a common nuclear target of Smad and TAK1 pathways in transforming growth factor-β signaling. J Biol Chem *274*, 8949-8957.

Sasaki, A., Masuda, Y., Ohta, Y., Ikeda, K., and Watanabe, K. (2001). Filamin associates with Smads and regulates transforming growth factor- beta signaling, J Biol Chem *276*, 17871-7.

Sasaki, T., Suzuki, H., Yagi, K., Furuhashi, M., Yao, R., Susa, S., Noda, T., Arai, Y., Miyazono, K., and Kato, M. (2003). Lymphoid enhancer factor 1 makes cells resistant to transforming growth factor beta-induced repression of c-myc, Cancer Res *63*, 801-6.

Satoh, J., and Kuroda, Y. (2000). Beta-catenin expression in human neural cell lines following exposure to cytokines and growth factors, Neuropathology *20*, 113-23.

Savage, C., Das, P., Finelli, A. L., Townsend, S. R., Sun, C. Y., Baird, S. E., and Padgett, R. W. (1996). Caenorhabditis elegans genes sma-2, sma-3, and sma-4 define a conserved family of transforming growth factor beta pathway components, Proc Natl Acad Sci U S A *93*, 790-4.

Schrantz, N., Bourgeade, M. F., Mouhamad, S., Leca, G., Sharma, S., and Vazquez, A. (2001). p38-mediated regulation of an Fas-associated death domain protein- independent pathway leading to caspase-8 activation during TGFbeta- induced apoptosis in human Burkitt lymphoma B cells BL41, Mol Biol Cell *12*, 3139-51.

Schiffer, M., Bitzer, M., Roberts, I.S., Kopp, J.B., ten Dijke, P., Mundel, P., and Böttinger, E.P.(2001). Apoptosis in podocytes induced by TGF-β and Smad7. J Clin Invest *108,* 807-816.

Schmidt, A., and Hall, M. N. (1998). Signaling to the actin cytoskeleton. Annu Rev Cell Dev Biol *14*, 305-338.

Schmidt, A., and Hall, A. (2002). Guanine nucleotide exchange factors for Rho GTPases: turningon the switch. Gene Develop *16*, 1587-1609.

Schuster, N., and Krieglstein, K. (2002). Mechanisms of TGF-□-mediated apoptosis. Cell TissueRes *307*, 1-14.

Schäfer, C., and Williams, J.A. (2000). Stress kinases and heat shock proteins in the pancreas:possible roles in normal function and disease. J Gastroenterol *35*, 1-9.

Scita, G., Tenca, P., Frittoli, E., Tocchetti, A., Innocenti, M., Giardina, G., and Di Fiore, P. P. (2000). Signaling from Ras to Rac and beyond: not just a matter of GEFs, Embo J *19*, 2393-8.

Sekelsky, J. J., Newfeld, S. J., Raftery, L. A., Chartoff, E. H., and Gelbart, W. M. (1995). Genetic characterization and cloning of mothers against dpp, a gene required for decapentaplegic function in Drosophila melanogaster, Genetics *139*, 1347-58.

Sharpe, C., Lawrence, N., and Martinez Arias, A. (2001). Wnt signalling: a theme with nuclear variations, Bioessays *23*, 311-8.

Shaw, R. J., Henry, M., Solomon, F., and Jacks, T. (1998). RhoA-dependent phosphorylation and relocalization of ERM proteins into apical membrane/actin protrusions in fibroblasts, Mol Biol Cell *9*, 403-19.

Shen, X., Li, J., Pei-chih, P., Waddel, D., Zhang, J., Wang, X.-F. (2001). The activity of guanineexchange factor NET1 is essential for transforming factor-b-mediated stress fiber formation. J Biol Chem *276*, 15362-15368.

Shibuya, H., Iwata, H., Matsuyama, N., Gotoh, Y., Yamaguchi., Irie, K., Matsumoto, K., Nishida, E., and Ueno, N. (1998). Role of TAK1 and TAB1 in BMP signaling in early *Xenopus* development. EMBO J *17*, 1019-1028.

Shibuya, H., Yamaguchi, K., Shirakabe, K., Tonegawa, A., Gotoh, Y., Ueno, N., Irie, K., Nishida, E., and Matsumoto, K. (1996). TAB1: An activator of the TAK1 MAPKKK in TGF-β signal transduction. Science *272*, 1179-1182.

Shin, E. Y., Shin, K. S., Lee, C. S., Woo, K. N., Quan, S. H., Soung, N. K., Kim, Y. G., Cha, C. I., Kim, S. R., Park, D., *et al.* (2002). Phosphorylation of p85 beta PIX, a Rac/Cdc42-specific guanine nucleotide exchange factor, via the Ras/ERK/PAK2 pathway is required for basic fibroblast growth factor-induced neurite outgrowth, J Biol Chem *277*, 44417-30.

Shirakabe, K., Yamaguchi, K., Shibuya, H., Irie, K., Matsuda, S., Moriguchi, T., Gotoh, Y., Matsumoto, K., and Nishida, E. (1997). TAK1 mediates the ceramide signaling to stress-activated protein kinase/c-Jun N-terminal kinase. J Biol Chem *272*, 8141-8144.

Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., and et al. (1992). Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease, Nature *359*, 693-9.

Small, J. V. (1989). Microfilament-based motility in non-muscle cells. Curr Opin Cell Biol *1*, 75-79.

Small, J. V., Rottner, K., Kaverina, I. (1999). Functional design in the actin cytoskeleton. Curr OpinCell Biol *11*, 54-60.

Small, J. V., Stradal, T., Vignal, E., and Rottner, K. (2002). The lamellipodium: where motility begins, Trends Cell Biol *12*, 112-20.

Song, Y., Hoang, B. Q., and Chang, D. D. (2002). ROCK-II-induced membrane blebbing and chromatin condensation require actin cytoskeleton, Exp Cell Res *278*, 45-52.

Souchelnytskyi, S., Nakayama, T., Nakao, A., Moren, A., Heldin, C. H., Christian, J. L., and ten Dijke, P. (1998). Physical and functional interaction of murine and Xenopus Smad7 with bone morphogenetic protein receptors and transforming growth factor- beta receptors, J Biol Chem *273*, 25364-70.

Souchelnytskyi, S., ten Dijke, P., Miyazono, K., and Heldin, C.-H. (1996). Phosphorylation of Ser165 in TGF-β type I receptor modulates TGF-β1-induced cellular responses. EMBO J *15*, 6231-6240.

Souchelnytskyi, S., Tamaki, K., Engström, U, Wernstedt, C., ten Dijke, P., and Heldin, C.-H. (1997). Phosphorylation of Ser^{465} and Ser^{467} in the C terminus of Smad2 mediates interaction with Smad4 and is required for transforming growth factor–β signaling. J Biol Chem *272*, 28107-28115.

Spaargaren, M., and Bos, J. L. (1999). Rab5 induces Rac-independent lamellipodia formation and cell migration, Mol Biol Cell *10*, 3239-50.

Sporn, M. B., and Roberts, A. B. (1993). A major advance in the use of growth factors to enhance wound healing, J Clin Invest *92*, 2565-6.

Stephens, L., Ellson, C., and Hawkins, P. (2002). Roles of PI3Ks in leukocyte chemotaxis and phagocytosis, Curr Opin Cell Biol *14*, 203-13.

Suzuki, C., Murakami, G., Fukuchi, M., Shimanuki, T., Shikauchi, Y., Imamura, T., and Miyazono, K. (2002). Smurf1 regulates the inhibitory activity of Smad7 by targeting Smad7 to the plasma membrane, J Biol Chem *277*, 39919-25.

Tabata, T. (1997). *Daughters against dpp* modulates *dpp* organizing activity in Drosophila wing development. Nature *389*, 627-631

Taipale, J., and Keski-Oja. (1997). Growth factors in the extracellular matrix. Faseb J *11*, 51-59.

Takai, Y., Sasaki, T., Tanaka, K., Nakanishi, H. (1995). Rho as a regulator of the cytoskeleton.Trends Biochem Sci *20*, 227-231.

Takase, M., Imamura, T., Sampath, T. K., Takeda, K., Ichijo, H., Miyazono. K., and Kawabata, M. (1998). Induction of Smad6 mRNA by bone morphogenetic proteins. Biochem Biophys Res Commun *244*, 26-29.

Takatsu, Y., Nakamura, M., Stapleton, M., Danos, M.C., Matsumoto, K., O'Connor, M.B., Shibuya,H., and Ueno, N. (2000). TAK1 participates in c-Jun N-terminal kinase signaling during Drosophila development. Mol Cell Biol *20,* 3015-3026.

Tamaki, K., Souchelnytskyi, S., Itoh, S., Nakao, A., Sampath, K., Heldin, C. H., and ten Dijke, P. (1998). Intracellular signaling of osteogenic protein-1 through Smad5 activation, J Cell Physiol *177*, 355-63.

Tao, W., Pennica, D., Xu, L., Kalejta, R. F., and Levine, A. J. (2001). Wrch-1, a novel member of the Rho gene family that is regulated by Wnt- 1, Genes Dev *15*, 1796-807.

Tangkijvanich, P., Santiskulvong, C., Melton, A. C., Rozengurd, E., Yee, H. F. Jr. (2002). p38MAP kinase mediates platelet-derived growth factor stimulated migration of hepatic myofibroblasts. J Cell Physiol *191*, 351-361.

ten Dijke, P., Goumans, M.-J., Itoh, F., and Itoh, S. (2002). Regulation of cell proliferation bySmad proteins. J Cell Physiol *191*, 1-16.

ten Dijke, P., Ichijo, H., Franzen, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C. H., and Miyazono, K. (1993). Activin receptor-like kinases: a novel subclass of cell-surface receptors with predicted serine/threonine kinase activity, Oncogene *8*, 2879-87.

ten Dijke, P., Yamashita, H., Ichijo, H., Franzen, P., Laiho, M., Miyazono, K., and Heldin, C. H. (1994). Characterization of type I receptors for transforming growth factor- beta and activin, Science *264*, 101-4.

Teramoto, T., Kiss, A., and Thorgeirsson, S. S. (1998). Induction of p53 and Bax during TGF-beta 1 initiated apoptosis in rat liver epithelial cells, Biochem Biophys Res Commun *251*, 56-60.

Tetsu, O., and McCormick, F. (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells, Nature *398*, 422-6.

Thomas, A., Giesler, T., and White, E. (2000). p53 mediates bcl-2 phosphorylation and apoptosis via activation of the Cdc42/JNK1 pathway, Oncogene *19*, 5259-69.

Thomsen, G. H. (1996). Xenopus mothers against decapentaplegic is an embryonic ventralizing agent that acts downstream of the BMP-2/4 receptor, Development *122*, 2359-66.

Tian, Y. C., and Phillips, A. O. (2002). Interaction between the transforming growth factor-beta type II receptor/Smad pathway and beta-catenin during transforming growth factor-beta1-mediated adherens junction disassembly, Am J Pathol *160*, 1619-28.

Tibbles, L. A., Ing, Y. L., Kiefer, F., Chan, J., Iscove, N., Woodgett, J. R., and Lassam, N. J. (1996). MLK-3 activates the SAPK/JNK and p38/RK pathways via SEK1 and MKK3/6, Embo J *15*, 7026-35.

Tobiume, K., Matsuzawa, A., Takahashi, T., Nishitho, H., Morita, K.-I., Takeda, K., Minowa, O.,Miyazono, K. *et al*. (2001). ASK1 is required for sustained activators of JNK/p38 MAP kinases and apoptosis. EMBO *2*, 222-228.

Tominaga, T., Sahai, E., Chardin, P., McCormick, F., Courtneidge, S. A., Alberts, A. S. (2000).Diaphanous-related formins bridge Rho GTPases and Src tyrosine kinase signaling. Mol Cell 5, 13-25.

Topper, J. N., Cai, J., Qui, Y., Anderson, K, R., Xu, Y.-Y., Deeds, J. D., Freely, R., Gimeno, C. J., Woolf, E. A., Tayber, O., *et al*. (1997). Vascular *MADs*: Two novel MAD-related genes selectively inducible by flow in human vascular endothelium. Proc Natl Acad Sci USA *94*, 9314-9319.

Tsukazaki, T., Chiang, T. A., Davison, A. F., Attisano, L., and Wrana, J. L. (1998). SARA, a FYVE domain protein that recruits Smad2 to the TGF receptor. Cell*95*, 779-791.

Tsuneizumi, T., Nakayama, T., Kamoshida, Y., Kornberg, T. B., Christian, J. L., and Tabata, T. (1997). *Daughters against dpp* modulates *dpp* organizing activity in Drosophila wing development. Nature *389*, 627-631.

Uruno, T., Liu, J., Zhang, P., Fan, Y., Egile, C., Li, R., Mueller, S. C., and Zhan, X. (2001). Activation of Arp2/3 complex-mediated actin polymerization by cortactin, Nat Cell Biol *3*, 259-66.

Van Aelst, L., and D`Souza-Schorey, C. (1997). Rho GTPases and signaling networks. Genes Dev*11*, 2295-2322.

Valderrama-Carvajal, H., Cocolakis, E., Lacerte, A., Lee, E. H., Krystal, G., Ali, S., and Lebrun, J. J. (2002). Activin/TGF-beta induce apoptosis through Smad-dependent expression of the lipid phosphatase SHIP, Nat Cell Biol *4*, 963-9.

Vallorosi, C. J., Day, K. C., Zhao, X., Rashid, M. G., Rubin, M. A., Johnson, K. R., Wheelock, M. J., and Day, M. L. (2000). Truncation of the beta-catenin binding domain of E-cadherin precedes epithelial apoptosis during prostate and mammary involution, J Biol Chem *275*, 3328-34.

van Gijn, M. E., Snel, F., Cleutjens, J. P., Smits, J. F., and Blankesteijn, W. M. (2001). Overexpression of components of the Frizzled-Dishevelled cascade results in apoptotic cell death, mediated by beta-catenin, Exp Cell Res *265*, 46-53.

Vousden, K. H. (2000). p53: death star. Cell *103*, 691-694.

Walton, M., Woodgate, A.M., Sirimanne, E., Gluckman, P., and Dragunow, M. (1998). ATF-2phosphorylation in apoptotic neuronal death. Brain Res Mol Brain Res *63*, 198-204

Wang, X. F., Lin, H. Y., Ng-Eaton, E., Downward, J., Lodish, H. F., and Weinberg, R. A. (1991). Expression cloning and characterization of the TGF-beta type III receptor, Cell *67*, 797-805.

Wang, Y. L. (1985). Exchange of actin subunits at the leading edge of living fibroblasts: possible role of treadmilling, J Cell Biol *101*, 597-602.

Wang, W., Zhou, G., Hu, M.C.-T., Yao, Z., and Tan, T.-H. (1997). Activation of the hematopoieticprogenitor kinase-1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor β (TGF-β)-activated kinase (TAK1), a kinase mediator of TGF β signal transduction. J Biol Chem *272,* 22771-22775.

Watanabe, N., Kato, T., Fujita, A., Ishizaki, T., Narumiya, S. (1999). Cooperation between mDiaand ROCK in Rho-induced actin reorganization. Nat Cell Biol *1*, 136-143

Watanabe, H., de Caestecker, M.P., and Y. Yamada. (2001). Transcriptional cross-talk between Smad, ERK1/2, and p38 Mitogen-activated protein kinase pathway regulates transforming growth factor-β-induced aggrecan gene expression in chondrogenic ATDC5 cells. J Biol Chem *276*, 14466-14473.

Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Fujisawa, K., Morii, N., Mukai, H., Ono, Y., Kakizuka, A., and Narumiya, S. (1996). Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho, Science *271*, 645-8.

Watanabe, N., Madaule, P., Reid, T., Ishizaki, T., Watanabe, G., Kakizuka, A., Saito, Y., Nakao, K., Jockusch, B. M., and Narumiya, S. (1997). p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin, Embo J *16*, 3044-56.

Watanabe, N., and Mitchison, T. J. (2002). Single-molecule speckle analysis of actin filament turnover in lamellipodia, Science *295*, 1083-6.

Weaver, A. M., Karginov, A. V., Kinley, A. W., Weed, S. A., Li, Y., Parsons, J. T., and Cooper, J. A. (2001). Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation, Curr Biol *11*, 370-4.

Welch, M. D., Iwamatsu, A., Mitchison, T. J. (1997). Actin polymerization is induced by Arp2/3complex at he surface of *Listeria monocytogenes*. Nature *385*, 265-269.

Welch, M. D, and Mullins, R. D. (2002) Cellular control of actin nucleation. Annu Rev Cell Dev Biol *18*, 247-288.

West, M. A., Prescott, A. R., Eskelinen, E. L., Ridley, A. J., and Watts, C. (2000). Rac is required for constitutive macropinocytosis by dendritic cells but does not control its downregulation, Curr Biol *10*, 839-48.

Wherlock, M., and Mellor, H. (2002). The Rho GTPase family: Racs to Wrchs story. J Cell Sci *115*,239-240.

Whitehead, I. P., Campbell, S., Rossman, K. L., and Der, C. J. (1997). Dbl family proteins, Biochim Biophys Acta *1332*, F1-23.

Winter, D., Lechler, T., and Li, R. (1999). Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein. Curr Biol *9*, 501-504.

Winter, D., Podtelejnikov A. V., Mann, M., Li, R. (1997). The complex containing actin-relatedproteins Arp2 and Arp3 is required for the motility and integrity of yeast actin patches. Curr Biol *7*, 519-529.

Wittmann, T., and Waterman-Storer, C. M. (2001). Cell motility: can Rho GTPases and microtubules point the way?, J Cell Sci *114*, 3795-803.

Wodarz, A., and Nusse, R. (1998). Mechanisms of Wnt signaling in development, Annu Rev Cell Dev Biol *14*, 59-88.

Wong, C., Rougier-Chapman, E. M., Frederick, J. P., Datto, M. B., Liberati, N. T., Li, J. M., and Wang, X. F. (1999). Smad3-Smad4 and AP-1 complexes synergize in transcriptional activation of the c-Jun promoter by transforming growth factor beta, Mol Cell Biol *19*, 1821-30.

Wong, N. A., and Pignatelli, M. (2002). Beta-catenin--a linchpin in colorectal carcinogenesis?, Am J Pathol *160*, 389-401.

Wood, W., and Martin, P. (2002). Structures in focus--filopodia, Int J Biochem Cell Biol *34*, 726-30.

Wrana, J. L., Attisano, L., Weiser, R., Ventura, F., Massagué, J. (1994). Mechanism of activation of the TGF-β receptor. Nature *370*, 341-347.

Wu, G., Chen, Y. G., Ozdamar, B., Gyuricza, C. A., Chong, P. A., Wrana, J. L., Massague, J., and Shi, Y. (2000). Structural basis of Smad2 recognition by the Smad anchor for receptor activation, Science *287*, 92-7.

Wurthner, J. U., Frank, D. B., Felici, A., Green, H. M., Cao, Z., Schneider, M. D., McNally, J. G., Lechleider, R. J., and Roberts, A. B. (2001). Transforming growth factor-beta receptor-associated protein 1 is a Smad4 chaperone, J Biol Chem *276*, 19495-502.

Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995). Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis, Science *270*, 1326-31.

Xu, Z., Maroney, A. C., Dobrzanski, P., Kukekov, N. V., and Greene, L. A. (2001). The MLK family mediates c-Jun N-terminal kinase activation in neuronal apoptosis, Mol Cell Biol *21*, 4713-24.

Yamada, K. M., and Geiger, B. (1997). Molecular interactions in cell adhesion complexes, Curr Opin Cell Biol *9*, 76-85.

Yamagishi, S., Yamada, M., Ishikawa, Y., Matsumoto, T., Ikeuchi, T., and Hatanaka, H. (2001). p38 mitogen-activated protein kinase regulates low potassium-induced c- Jun phosphorylation and apoptosis in cultured cerebellar granule neurons, J Biol Chem*276*, 5129-33.

Yamaguchi, K., Nagai, S.-i., Ninomiya-Tsuji, J., Nishita, M., Tamai, K., Irie, K., Ueno, N., Nishida, E., Shibuya, H., and Matsumoto, K. (1999). XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. EMBO J *18*, 179-187.

Yamaguchi, K., Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E., and Matsumoto, K. (1995). Identification of a member of the MAPKKK family as a potential mediator of TGF-β signal transduction. Science *270*, 2008-2011.

Yamaguchi, Y., Mann, D. M., and Ruoslahti, E. (1990). Negative regulation of transforming growth factor-b by the proteoglycan decorin, Nature *346*, 281-284.

Yamamura, Y., Hua, X., Bergelson, S., and Lodish, H. F. (2000). Critical role of Smads and Ap-1 complex in transforming growth factor-beta-dependent apoptosis. J Biol Chem *275*, 36295-36302.

Yamamoto, T., Matsuda, T., Muraguchi, A., Miyazono, K., and Kawabata, M. (2001). Cross-talk between IL-6 and TGF-beta signaling in hepatoma cells, FEBS Lett *492*, 247-53.

Yamashita, H., ten Dijke, P., Franzen, P., Miyazono, K., and Heldin, C. H. (1994). Formation of hetero-oligomeric complexes of type I and type II receptors for transforming growth factor-beta, J Biol Chem*269*, 20172-8.

Yamauchi, J., Tsujimoto, G., Kaziro, Y., and Itoh H. (2001). Paralell regulation of mitogen-activated protein kinase kinase 3 (MKK3) and MKK6 in Gq-signaling cascade. J Biol Chem *276*, 23362-23372.

Yanagisawa, M., Nakashima, K., Takeda, K., Ochiai, W., Takizawa, T., Ueno, M., Takizawa, M.,Shibuya, H., and Taga, T. (2001). Inhibition of BMP2-induced, TAK1 kinase-mediated neurite outgrowth by Smad6 and Smad7. Genes Cells *6,* 1091-1099.

Yang, Y. A., Dukhanina, O., Tang, B., Mamura, M., Letterio, J. J., MacGregor, J., Patel, S. C., Khozin, S., Liu, Z. Y., Green, J.*, et al.* (2002). Lifetime exposure to a soluble TGF-beta antagonist protects mice against metastasis without adverse side effects, J Clin Invest *109*, 1607-15.

Yarar, D., To, W., Abo, A., and Welch, M. D. (1999). The Wiskott-Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. Curr Biol *9*, 555-558.

Yu, L., Herbert, M. C., and Zhang, Y. E. (2002). TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. EMBO J. *21*, 3749-3756.

Yu, X., Waltzer, L., and Bienz, M. (1999). A new Drosophila APC homologue associated with adhesive zones of epithelial cells, Nat Cell Biol *1*, 144-51.

Yue, J., Frey, R. S., and Mulder, K. M. (1999a). Cross-talk between the Smad1 and Ras/MEK signaling pathways for TGFbeta, Oncogene *18*, 2033-7.

Yue, J., Hartsough, M. T., Frey, R. S., Frielle, T., and Mulder, K. M. (1999b). Cloning and expression of a rat Smad1: regulation by TGFbeta and modulation by the Ras/MEK pathway, J Cell Physiol *178*, 387-96.

Zicha, D., Genot, E., Dunn, G.A., and I.M. Kramer. (1999). TGFβ1 induces a cell-cycle-dependentincrease in motility of epithelial cells. J Cell Sci *11*,447-454.

Zhang, S., Han, J., Sells, M. A., Chernoff, J., Knause, U. G., Ulevitch, R. J., Bokoch, G. M. (1995).Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. J Biol Chem *270*, 23034-23936.

Zhang, Y., and Dernyck, R. (1999). Regulation of Smad signaling by protein associations and signaling crosstalk. Trends cell Biol *9*, 274-279.

Zhang, Y., and Derynck, R. (2000). Transcriptional regulation of the transforming growth factor-β-inducible mouse germ line Ig α constant region gene by functional cooperation of Smad, CREB and AML family members. J Biol Chem *275,* 16979-16985.

Zhang, Y., Feng, X., We, R., and Derynck, R. (1996). Receptor-associated Mad homologues synergize as effectors of the TGF-beta response, Nature *383*, 168-72.

Zhao, J., and Buick, R. N. (1995). Regulation of transforming growth factor beta receptors in H-ras oncogene-transformed rat intestinal epithelial cells, Cancer Res *55*, 6181-8.

Zheng, Y., Zangrilli, D., Cerione, R. A., and Eva, A. (1996). The pleckstrin homology domain mediates transformation by oncogenic dbl through specific intracellular targeting, J Biol Chem*271*, 19017-20.

Zhou, G., Lee, S. C., Yao, Z., and Tan, T.H. (1999). Hematopoietic progenitor kinase 1 is acomponent of transforming growth factor beta-induced c-Jun N-terminal kinase signaling cascade. J Biol Chem *274*, 13133-13138.

Zhu, H., Kavasak, P., Abdollah, S., Wrana, J. L., and Thomsen, G. H. (1999). A Smad ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. Nature *400*, 687-693.

Zimmerman, C. M., and Padgett, R. W. (2000). Transforming growth factor beta signaling mediators and modulators, Gene *249*, 17-30.

Zimmerman, L. B., De Jesus-Escobar, J. M., and Harland, R. M. (1996). The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4, Cell *86*, 599-606.