

# The regulation of TGF $\beta$ signal transduction

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Transforming growth factor  $\beta$  (TGF $\beta$ ) pathways are implicated in metazoan development, adult homeostasis and disease. TGF $\beta$  ligands signal via receptor serine/threonine kinases that phosphorylate, and activate, intracellular Smad effectors as well as other signaling proteins. Oligomeric Smad complexes associate with chromatin and regulate transcription, defining the biological response of a cell to TGF $\beta$  family members. Signaling is modulated by negative-feedback regulation via inhibitory Smads. We review here the mechanisms of TGF $\beta$  signal transduction in metazoans and emphasize events crucial for embryonic development.

## Introduction

The human transforming growth factor  $\beta$  (TGF $\beta$ ) family consists of 33 members, most of which encode dimeric, secreted polypeptides that control developmental processes, ranging from gastrulation and body axis asymmetry to organ-specific morphogenesis and adult tissue homeostasis (reviewed by Derynck and Miyazono, 2008). In addition to TGF $\beta$ s, this family includes the bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins and nodal. The TGF $\beta$  family is conserved throughout metazoan evolution. At the cellular level, TGF $\beta$  family members regulate cell growth, differentiation, adhesion, migration and death, in a developmental context-dependent and cell type-specific manner. For example, TGF $\beta$  more often inhibits, but sometimes also stimulates, cell proliferation (reviewed by Yang and Moses, 2008). Furthermore, nodal signaling sometimes inhibits, whereas BMP promotes, cell differentiation, as in stem cells (Watabe and Miyazono, 2009). As TGF $\beta$  ligands act multifunctionally in numerous tissue types, they also play complex roles in various human diseases, ranging from autoimmune to cardiovascular diseases and cancer (reviewed by Gordon and Blobe, 2008; Massagué, 2008).

Here we review the core components of the TGF $\beta$  family and their signaling engines, as part of a Minifocus in this issue on TGF $\beta$  signaling (see Box 1), and discuss emerging concepts concerning the regulatory mechanisms of TGF $\beta$  pathways at the receptor, cytoplasmic and nuclear level. We also highlight recent discoveries that are of particular developmental relevance.

## The TGF $\beta$ family

The development of the axes and the asymmetry of the animal body depends on the localized action of extracellular signals, such as the Wnt, nodal and BMP ligands. Gradients of these ligands, their extracellular regulators and the competence of receptors in responding cells, play important roles during tissue morphogenesis (Affolter and Basler, 2007; Smith and Gurdon, 2004). TGF $\beta$  family members also contribute to tissue patterning and are important regulators of stem cell self-renewal and differentiation (see Box 2) (De Robertis and Kuroda, 2004; Watabe and Miyazono, 2009).

The TGF $\beta$  morphogens include numerous secreted and conserved polypeptides (Table 1), which emerged at the onset of multicellular (metazoan) life (Huminięcki et al., 2009). Structurally, this family is characterized by a specific three-dimensional fold and by a conserved number and spacing of cysteine residues in the C-terminus of the mature polypeptide (Derynck and Miyazono, 2008). The prototypic TGF $\beta$  isoforms (TGF $\beta$ 1,  $\beta$ 2,  $\beta$ 3), and the related inhibin  $\beta$  polypeptides that make up the activin and inhibin members, have nine characteristic cysteines, eight of which form four intramolecular disulfide bridges, while one intermolecular bond links the two monomers. The inhibin  $\alpha$  polypeptides, BMPs and GDFs have seven cysteines, of which six form intramolecular and one intermolecular bridges. The lefty proteins, GDF3, GDF9 and BMP15A have six cysteines in their mature sequence and lack the intermolecular bridge between the two monomers. The lack of covalent dimers provides regulatory flexibility; for example, lefty forms non-covalent complexes with nodal and binds to the glycosylphosphatidylinositol (GPI)-anchored co-receptor of the epidermal growth factor-Cripto/FRL-1/Cryptic (EGF-CFC) family, leading to the inhibition of nodal signaling (Chen and Shen, 2004).

*Xenopus laevis* expresses TGF $\beta$ s, nodal, activins, BMPs and GDFs (De Robertis and Kuroda, 2004), and additional unique family members, such as the mesoderm-inducer Derrière, and the six nodal-related proteins XNR1-6 (Eimon and Harland, 2002; Onuma et al., 2002; Ramis et al., 2007). *Drosophila melanogaster* has only seven TGF $\beta$  family members (Table 1). The BMP-like ligands Decapentaplegic (Dpp) and Screw (Scw) regulate dorsoventral patterning and the differentiation of imaginal discs, such as the wing disc (Affolter and Basler, 2007; Serpe et al., 2005). The BMP-like Glass bottom boat (Gbb) regulates brain and wing disc differentiation (Bangi and Wharton, 2006; Goold and Davis, 2007). The activin-like dActivin (Act $\beta$  – FlyBase) and Dawdle (Daw) ligands have tissue-specific roles (for example, in the larval brain), whereas much remains to be understood about the functions of Maverick, the GDF8 (myostatin)-like ligand, and of Myoglianin, which are expressed in endodermal and mesodermal cells (Lee-Hoeflich et al., 2005; Nguyen et al., 2000; Zhu et al., 2008). In *Caenorhabditis elegans*, the BMP-like DBL-1, and the TGF $\beta$ -like DAF-7, regulate body length and the dauer pathway, a special environmental adaptation of earthworms, respectively (Table 1) (reviewed by Savage-Dunn, 2005). The other three ligands, TIG-2, TIG-3 and UNC-129, are as yet unexplored.

### Box 1. Minifocus on TGF $\beta$ signaling

This article is part of a Minifocus on TGF $\beta$  signaling. For further reading, please see the accompanying articles in this collection: 'The extracellular regulation of bone morphogenetic protein signaling' by David Umulis, Michael O'Connor and Seth Blair (Umulis et al., 2009); 'Informatics approaches to understanding TGF $\beta$  pathway regulation' by Pascal Kahlem and Stuart Newfeld (Kahlem and Newfeld, 2009); and 'TGF $\beta$  family signaling: novel insights in development and disease', a review of a recent FASEB Summer Conference on TGF $\beta$  signaling by Kristi Wharton and Rik Derynck (Wharton and Derynck, 2009).

### Box 2. Role of TGF $\beta$ /BMP signaling in embryonic stem cells

Stem cells exhibit self-renewing capacity and pluripotency in generating the multitude of embryonic and adult cell types of the metazoan body (reviewed by Rossi et al., 2008). Growth factors, such as TGF $\beta$  and FGF, regulate stem cell self-renewal and differentiation. FGF2, the most widely used growth factor that supports mouse and human embryonic stem cell (ESC) self-renewal in culture, induces TGF $\beta$ /activin ligands and receptors while suppressing BMP-like activities (Greber et al., 2007; Ogawa et al., 2007). Furthermore, pharmacological inhibitors of the TGF $\beta$ /nodal type I receptor family suppress human and mouse ESC self-renewal (Ogawa et al., 2007). In general, TGF $\beta$  inhibits differentiation of pluripotent progenitor cells, whereas BMP induces their differentiation (Watabe and Miyazono, 2009) (Fig. 7A,B).

To promote self-renewal of ESCs, TGF $\beta$ /nodal signaling activates SMAD2 and SMAD3, which directly induce *Nanog*, one of the crucial stem cell transcription factors (Xu, R. H. et al., 2008). TGF $\beta$  and FGF signaling synergize by enhancing binding of Smad complexes to the *Nanog* promoter. Interestingly, NANOG provides a molecular link for the antagonism between TGF $\beta$  (the self-renewing factor) and BMP (the differentiation factor) in ESCs. NANOG binds to SMAD1, inhibiting its transcriptional activity and limiting the BMP signaling potential that promotes early mesodermal differentiation or tissue-specific differentiation later in development (Suzuki et al., 2006). This example is likely to be expanded to additional regulators of ESC self-renewal and differentiation as a result of genome-wide screens for the transcription and signaling factors of these pathways (Chen et al., 2008).

Although specification of body asymmetry is a fundamental function of TGF $\beta$ -like proteins during early embryogenesis, the identification of genes that encode a complete TGF $\beta$  pathway in the primitive metazoan *Trichoplax adhaerens*, a two-cell-layered animal that lacks obvious body asymmetry, suggests that these morphogens might have played a fundamental role in the specification of the multicellularity that precedes body asymmetry during animal evolution (Huminiacki et al., 2009).

### TGF $\beta$ secretion and extracellular regulation

All TGF $\beta$  ligands are synthesized as precursor proteins with a longer N-terminal pro-peptide followed by a shorter C-terminal mature polypeptide (reviewed by ten Dijke and Arthur, 2007). Intermolecular disulfide linkages pair dimers of these precursors via conserved cysteine residues in the pro-peptide and mature peptide sequence. While precursor proteins are in the secretory pathway, furin-like proteases cleave the pro-peptide from the mature peptide. The TGF $\beta$  pro-peptide, called the latency-associated peptide (LAP), continues to scaffold the smaller mature peptide within its core, serving as a chaperone during exocytosis of the complex. It also mediates the deposition of TGF $\beta$  in the extracellular matrix (ECM) through its covalent association with large secreted proteins called latent TGF $\beta$ -binding proteins (LTBPs), and with ECM proteins, such as fibronectin and fibrillin 1 (reviewed by Rifkin, 2005). Activation of the mature C-terminal dimeric ligands from their matrix-deposited, multi-protein 'cages' relies on several proteases, including elastase (which cleaves fibrillin 1), BMP1/Tolloid family proteases (which cleave LTBPs), and matrix metalloproteases, such as MMP2 (which cleave TGF $\beta$  LAPs) (reviewed by ten Dijke and Arthur, 2007).

The ability of the TGF $\beta$  LAP to maintain the ligand in an inactive state is conserved among some TGF $\beta$  family ligands, such as GDF8 and GDF11 (Ge et al., 2005; Wolfman et al., 2003). However, the nodal, BMP4 and BMP7 pro-peptides do not act as extracellular

antagonists, but instead regulate mature ligand stability and processing, including ligand degradation in lysosomes, which limits ligand availability (Degnin et al., 2004; Dick et al., 2000; Le Good et al., 2005). Similarly, the nodal pro-peptide associates with its EGF-CFC family co-receptor Cripto in secretory vesicles near the cell surface (Blanchet et al., 2008). Cripto also forms complexes with mature nodal and enhances signaling via the receptor kinase complex (see below) (Bianco et al., 2004). Recent evidence demonstrates that the signaling Cripto-nodal-receptor complex enters a specialized endocytic pathway that is characterized by the protein flotillin, possibly en route to its final degradation. Interestingly, many other TGF $\beta$  ligands are inactivated in the extracellular space by antagonists, such as noggin and chordin, which inhibit BMPs, and follistatin, which inhibits activins (Gazzerro and Canalis, 2006; Harrison et al., 2005). These extracellular antagonists help to establish the morphogen gradients that pattern early embryos, as discussed in an accompanying review (Umulis et al., 2009) (see Box 1).

### The TGF $\beta$ receptor family

All TGF $\beta$  ligands transmit biological information to cells by binding to type I and type II receptors that form heterotetrameric complexes in the presence of the dimeric ligand (reviewed by Wrana et al., 2008). Five type II and seven type I receptors exist in humans and other mammals, and are characterized by a cytoplasmic kinase domain that has strong serine/threonine kinase activity and weaker tyrosine kinase activity, which classifies them as being dual-specificity kinases (Table 1) (reviewed by ten Dijke and Heldin, 2006). The type I receptors are also known as activin receptor-like kinases (ALKs), a nomenclature that is employed to tackle the problem of one ligand signaling via many receptors, or many ligands signaling via the same receptor. TGF $\beta$  ligands also interact with co-receptors that either facilitate or limit receptor kinase signaling. In addition to the EGF-CFC/Cripto co-receptors discussed above, type III receptors, such as endoglin and the proteoglycan betaglycan (TGF $\beta$ R3; T $\beta$ RIII), regulate TGF $\beta$  signaling in mammals, as does the repulsive guidance molecule (RGM, also known as Dragon) family of co-receptors (reviewed by Wrana et al., 2008) (Table 1).

Ligand binding links the constitutively active type II receptor kinases to the dormant type I receptor kinases, allowing the type II receptor to phosphorylate the juxtamembrane part of the cytoplasmic domain of the type I receptor (Fig. 1), turning on receptor kinase activity (reviewed by Wrana et al., 2008). Recent structural analysis of TGF $\beta$  and BMP ligands bound to their respective type I and type II receptor ectodomains shows that TGF $\beta$  ligands contact both receptors tightly, whereas the evolutionarily more ancient BMPs associate more loosely with their receptors (Groppe et al., 2008). Binding of TGF $\beta$  to T $\beta$ RII (TGF $\beta$ R2) creates the interface required for T $\beta$ RI (ALK5; TGF $\beta$ R1) type I receptor recruitment to the complex.

*D. melanogaster* has five TGF $\beta$  family receptors, including the type II receptors Punt (Put) and Wishful thinking (Wit), which bind the BMP-like ligands Dpp, Gbb and Scw during fly development and which form complexes with the type I receptors Thickveins (Tkv) and Saxophone (Sax) (Table 1) (Affolter and Basler, 2007; Goold and Davis, 2007; Serpe et al., 2005). Put and Wit also pair with the type I receptor Baboon (Babo) to mediate activin-like signals from dActivin and Daw (Zhu et al., 2008). The accompanying review by Umulis et al. (Umulis et al., 2009) discusses how, in the developing wing disc, a gradient of BMP-like signaling activity is achieved by the dual contribution of Dpp and Gbb, which differentially bind to distinct receptor complexes.

Table 1. TGF $\beta$  pathways in humans, flies and worms

Pathway	<i>H. sapiens</i>					
	BMP	GDF	Activin	TGF $\beta$	AMH	Inhibitors
Ligand	BMP2, 4 BMP5, 6, 7 BMP8A, 8B BMP9, 10	GDF5, 6, 7 GDF9b GDF10, 11 GDF15 (MIC1) ----- GDF1, 3 GDF8 (MYO) GDF9	Inhibin $\beta$ A Inhibin $\beta$ B Nodal	TGF $\beta$ 1 TGF $\beta$ 2 TGF $\beta$ 3	AMH (MIS)	BMP3 Inhibin $\alpha$ Inhibin $\beta$ C Inhibin $\beta$ E LEFTYA LEFTYB
RII	BMPRII ActRIIA, ActRIIB	BMPRII ActRIIA, ActRIIB	ActRIIA ActRIIB	T $\beta$ RII	AMHRII	N/A
RI	BMPRIA (ALK3) BMPRIB (ALK6) ALK2 ALK1	BMPRIA (ALK3) BMPRIB (ALK6) ALK2 ----- ActRIB (ALK4) ALK7 T $\beta$ RI (ALK5)	ActRIB (ALK4) ALK7	T $\beta$ RI (ALK5) ----- ALK1 ALK2 BMPRIA (ALK3)	BMPRIA (ALK3) BMPRIB (ALK6) ALK2	N/A
RIII	RGMa, b, c (+)	Cripto 3 (+)	Cripto 3 (-) Cripto 1 (+)	T $\beta$ RIII (+) Endoglin (+) Cripto 3 (-)	?	T $\beta$ RIII (-) Cripto 3 (-)
R-Smad	SMAD1, 5, 8	SMAD1, 5, 8 ----- SMAD2, 3	SMAD2, 3	SMAD2, 3 ----- SMAD1, 5, 8	SMAD1, 5, 8	N/A
Co-Smad	SMAD4	SMAD4	SMAD4	SMAD4	SMAD4	N/A
I-Smad	SMAD6, 7	SMAD6, 7	SMAD7	SMAD7	SMAD6, 7	N/A
Pathway	<i>D. melanogaster</i>			<i>C. elegans</i>		
	BMP	Activin	Other	Sma/Mab	Dauer	
Ligand	Dpp Gbb Scw	dActivin Daw	Mav Myo	DBL-1	DAF-7	
RII	Put Wit	Put Wit	?	DAF-4	DAF-4	
RI	Tkv Sax	Babo	?	SMA-6	DAF-1	
RIII	?	?	?	?	?	
R-Smad	Mad	dSmad2	?	SMA-2 SMA-3	DAF-8 DAF-14	
Co-Smad	Medea	Medea	?	SMA-4	DAF-3 (?)	
I-Smad	Dad	?	?	TAG-68 (?)	TAG-68 (?)	

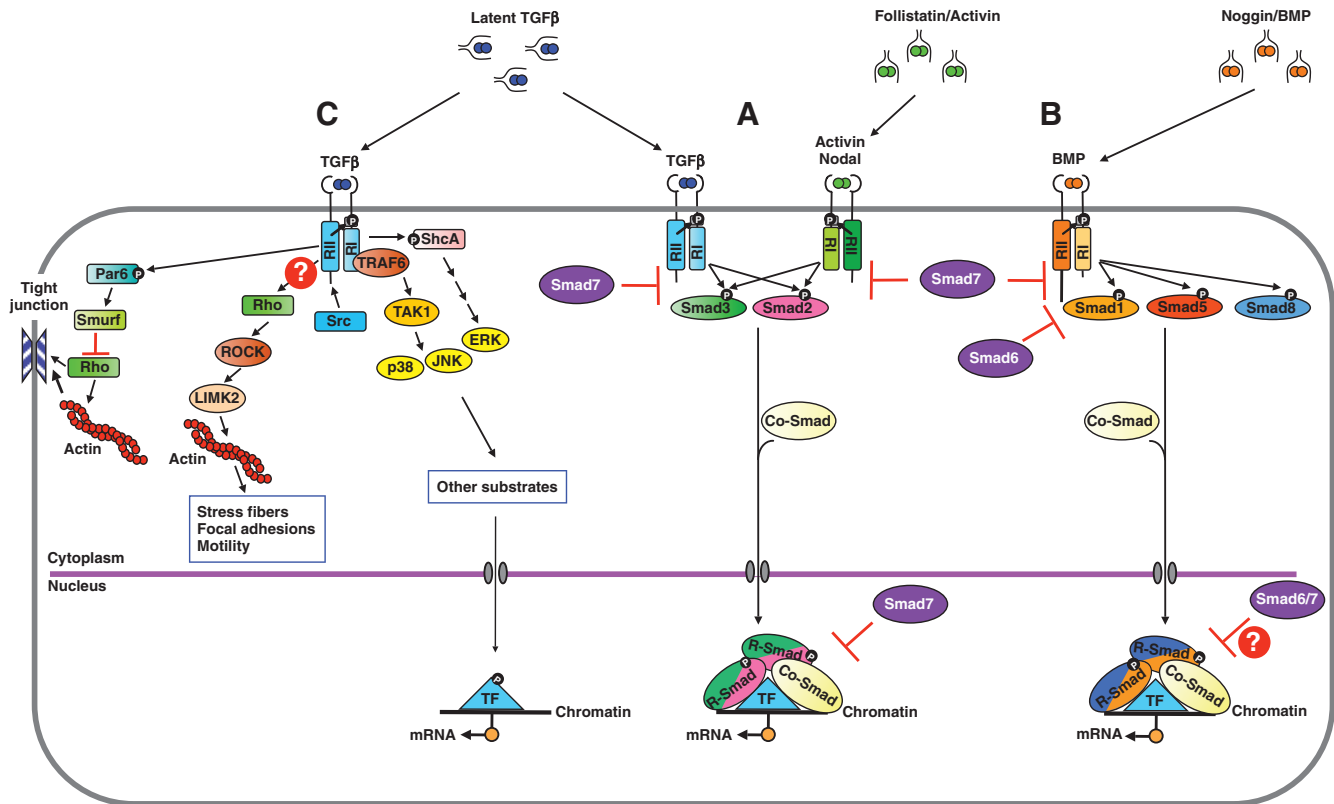
Receptors are listed as type II (RII), type I (RI) and type III (RIII) co-receptors. Dashed lines separate groups of ligands or receptors based on the division into BMP and TGF $\beta$ /activin-like pathways. Ligands, type I receptors and R-Smads are color-coded: blue, BMP-like pathways; red, TGF $\beta$ /activin-like pathways. Question marks indicate unassigned signaling relationships. For two human ligands, GDF8 and GDF15, we provide their alternative names [myostatin (MYO) and macrophage inhibitory cytokine 1 (MIC1)], as the latter are more commonly used in the literature. LEFTYA and B are also known as LEFTY2 and 1, respectively. The co-receptor T $\beta$ RIII is also known as betaglycan. Cripto 1 and Cripto 3 are also known as TDGF1 and TDGF3, respectively. In the RIII group (+) or (-) indicates positive or negative effects, respectively, on signaling by each co-receptor. N/A, not applicable.

*C. elegans* has three TGF $\beta$  family receptors (Table 1) (Patterson and Padgett, 2000). In the dauer pathway, DAF-7 signals via the type II receptor DAF-4 and the type I receptor DAF-1. In the Sma/Mab pathway, which regulates body length, tail development and innate immunity, DBL-1 signals via DAF-4 and the SMA-6 type I receptor. Which of these receptors mediate signals by UNC-129, TIG-2 and TIG-3 remains unknown.

Finally, *T. adhaerens* has one type II and three type I receptors (Huminiacki et al., 2009), which is compatible with a model in which the type II receptor represents the ligand-recognizing core, whereas the type I receptor is the downstream signaling effector that defines biological responses and that has diverged more rapidly to serve the new developmental processes of more complex organisms.

### The Smad family

The activated type I receptor phosphorylates cytoplasmic proteins of the Smad family in their C-terminal regions (Figs 1 and 2). Smads consist of three domains: (1) an N-terminal Mad-homology 1 (MH1) domain that can interact with other proteins and carries nuclear localization signals (NLSs) and a DNA-binding domain; (2) a middle linker domain that interacts with prolyl-isomerases and ubiquitin ligases and that is enriched in prolines and phosphorylatable serines or threonines; and (3) a C-terminal MH2 domain that binds to type I receptors and can interact with other proteins, and that mediates Smad homo- and hetero-oligomerization and mediates the transactivation potential of nuclear Smad complexes (Fig. 2) (reviewed by ten Dijke and Heldin, 2006). The C-terminal phosphorylation of receptor-activated (R) Smads allows



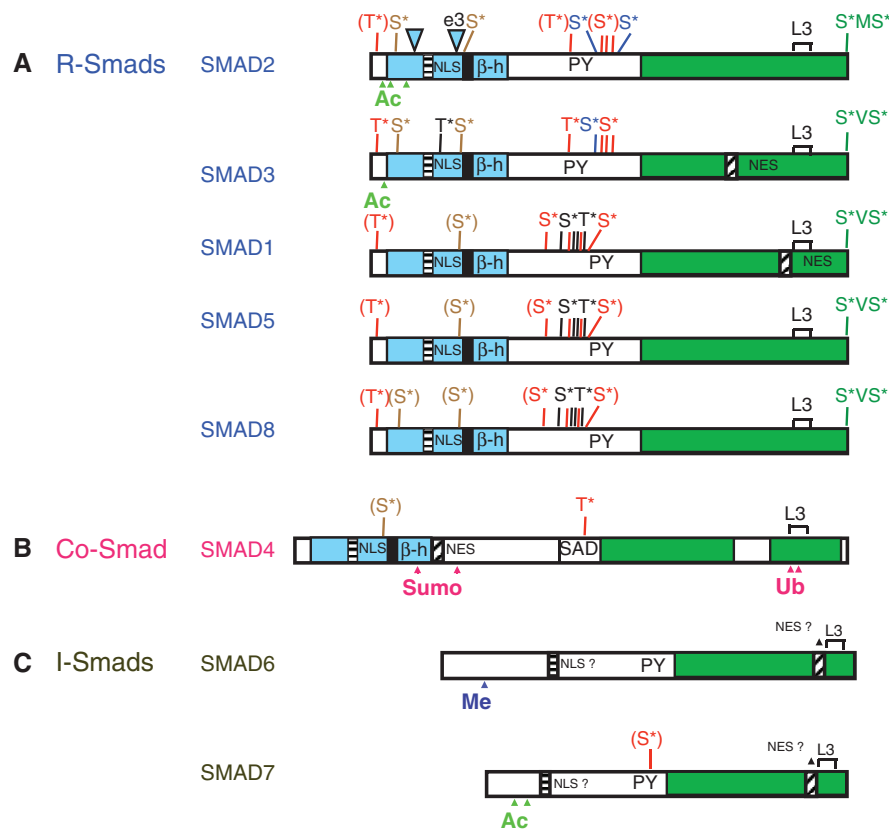
**Fig. 1. TGF $\beta$  and BMP signaling.** (A, B) The (A) TGF $\beta$  and activin/nodal and (B) BMP pathways, with their corresponding Smad proteins and mechanisms of inhibition by I-Smads (Smad6/7). The latent TGF $\beta$  complex and the extracellular antagonists, follistatin (bound to activin) and noggin (bound to BMP) are shown. (C) Non-Smad signaling pathways downstream of the TGF $\beta$  receptors [RI, T $\beta$ RI (ALK5) and RII, T $\beta$ RII (TGF $\beta$ R2)]. The nuclear Smad complexes that lead to gene regulation are shown for each pathway. In these complexes, the Smad trimer most likely contains two R-Smad (identical or different) and one Co-Smad subunit. In addition to the major signaling pathways shown, TGF $\beta$  also activates BMP R-Smads in certain contexts (see text). BMP (bone morphogenetic protein), Erk (extracellular signal-regulated kinase), LIMK2 (LIM domain kinase 2), JNK (Jun N-terminal kinase), p38 (p38 MAPK), PAR6 (partitioning-defective 6 homolog), Rho (Ras homolog), ROCK (Rho-associated, coiled-coil-containing protein kinase), SHCA (SH2 domain-containing sequence A), Smurf (Smad ubiquitylation regulatory factor), Src (Rous sarcoma virus oncoprotein), TGF $\beta$  (transforming growth factor  $\beta$ ), TAK1 (TGF $\beta$ -activated kinase 1), TF (transcription factor), TRAF6 (tumor necrosis factor  $\alpha$  receptor-associated factor 6).

them to associate with the common-mediator (Co) Smad, SMAD4. The resulting Smad oligomer is thought to consist of a trimer of two R-Smads and a single SMAD4 (such as a SMAD2-SMAD2-SMAD4 complex, a SMAD3-SMAD3-SMAD4 complex, or a SMAD2-SMAD3-SMAD4 complex), which is then shuttled into the nucleus. Nuclear Smad complexes bind to chromatin, and, together with other transcription factors, regulate target gene expression (Fig. 1) (reviewed by Massagué et al., 2005; Schmierer and Hill, 2007). TGF $\beta$ - or BMP-specific Smad complexes induce the expression of the inhibitory (I) Smads, SMAD6 and SMAD7 (Figs 1 and 2), which negatively regulate signaling strength and duration, thus forming a negative-feedback loop (reviewed by Itoh and ten Dijke, 2007).

All genomes sequenced to date possess the three fundamental classes of Smad proteins: R-, Co- and I-Smads (Table 1) (Huminięcki et al., 2009). In *D. melanogaster*, BMP-like signaling is mediated by a single R-Smad (Mad) and a single Co-Smad (Medea), despite the existence of two type I receptors (Tkv, Sax) (Affolter and Basler, 2007). In the activin-like pathways, the type I receptor Babo signals via dSmad2 (Smox) (an R-Smad) and Medea. A single I-Smad (Dad) also operates during wing imaginal disc development (Tsuneizumi et al., 1997). Dad binds and inhibits signaling from the Dpp/Scw/Gbb type I receptors Tkv and Sax, but not from the dActivin type I receptor Babo (Kamiya et al., 2008).

In *C. elegans*, the Sma/Mab pathway engages two R-Smads, SMA-2 and SMA-3, that signal with a single Co-Smad, SMA-4 (Patterson and Padgett, 2000). The dauer pathway has two R-Smads, DAF-8 and DAF-14, which have more divergent MH1 domains but conserved MH2 domains that suggest activation by the corresponding type I receptors. The dauer pathway Co-Smad is possibly DAF-3, which presents peculiar developmental characteristics. Unlike the Co-Smads in other organisms and SMA-4 in *C. elegans*, the DAF-3 loss-of-function phenotype is distinct from those of loss-of-function mutations in the ligand, receptors or R-Smads of this pathway (Patterson et al., 1997). Furthermore, the receptors and R-Smad seem to negatively regulate the function of DAF-3. Thus, DAF-3 is classified as a Co-Smad only on the basis of sequence similarity to other Co-Smads (Huminięcki et al., 2009). Finally, TAG-68 is classified as an I-Smad based on phylogenetic arguments, although functional evidence for such a role is currently absent (Savage-Dunn et al., 2003). *T. adhaerens* also has three distinct Smad classes, which suggests that the three different functional features of Smad proteins evolved early during metazoan evolution (Huminięcki et al., 2009).

A fundamental feature of all TGF $\beta$  signaling pathways is their division into TGF $\beta$ -like and BMP-like cascades, a classification based on the specificity of interaction between the so-called L45 loop of the



**Fig. 2. The Smad family.** Simplified structures of the eight human Smad proteins divided into **(A)** Receptor-activated (R) Smads; **(B)** common-mediator (Co) Smad; and **(C)** inhibitory (I) Smads. The conserved N-terminal Mad-homology 1 (MH1) (blue) and C-terminal MH2 (green) domains are shown. Highlighted are the nuclear localization signal (NLS, striped box); the two unique inserts of SMAD2 (triangles), the second of which corresponds to exon 3 (e3); the  $\beta$ -hairpin domain that binds to DNA ( $\beta$ -h, black box); the proline-tyrosine (PY) motif in the linker domain that is recognized by the WW domain of Smurf family proteins; the Smad activation domain (SAD) at the linker-MH2 border; the nuclear export signal (NES, hatched box); and the L3 loop of the MH2 domain. Phosphorylatable serine and threonine residues are shown; S/T\* indicates experimentally proven phosphorylation sites; (S/T\*) indicates a conserved residue with a predicted phosphorylation motif that awaits experimental validation. The C-terminal serines that are phosphorylated by the type I receptor kinases are shown in green (S\*VS\*, S\*MS\*); red S/T residues are phosphorylated by the MAPKs ERK1/2; brown S/T residues are phosphorylated by protein kinase C and by calmodulin-dependent kinase II; blue S/T residues are phosphorylated by cyclin-dependent kinases; and black S/T residues are phosphorylated by glycogen synthase kinase 3 $\beta$ . Sumoylation (Sumo), ubiquitylation (Ub), methylation (Me) and acetylation (Ac) sites are indicated with colored arrowheads.

type I receptors and the L3 loop of the MH2 domains of R-Smads (Fig. 2) (reviewed by ten Dijke and Heldin, 2006). TGF $\beta$ /activin pathways signal via SMAD2, SMAD3, and BMP/GDF pathways via SMAD1, SMAD5 and SMAD8. However, in a diversity of cell types in culture, such as endothelial, immortalized epithelial, adenoma and carcinoma cell lines and even NIH-3T3 fibroblasts and chondrocytes (Daly et al., 2008; Finnson et al., 2008; Goumans et al., 2003; Liu et al., 2008), TGF $\beta$  signaling can also activate SMAD1 and SMAD5. Originally, TGF $\beta$  was shown to bind to two type I receptors, thus activating SMAD2 and SMAD3 via T $\beta$ RI, and SMAD1, SMAD5 and SMAD8 via the BMP type 1 receptor ALK1 (ACVRL1). ALK1 is expressed mainly in endothelial cells, where SMAD2, SMAD3 signaling inhibits and SMAD1, SMAD5, SMAD8 signaling promotes proliferation and migration (Goumans et al., 2003). However, TGF $\beta$  can also activate SMAD1 and SMAD5 via two additional mechanisms. First, in immortalized EpH4 mouse mammary epithelial cells and in MDA-MB-231 human mammary carcinoma cells, TGF $\beta$  induces SMAD1 and SMAD5 C-terminal phosphorylation via heteromeric receptor complexes that form between T $\beta$ RII and T $\beta$ RI, as well as between T $\beta$ RII and the BMP type I receptors ALK2 (ACVR1) and BMPRIA (ALK3) (Daly et al., 2008). Second, in 4T1

mouse mammary carcinoma cells, TGF $\beta$  leads to SMAD1 and SMAD5 phosphorylation without the requirement of a BMP-like type I receptor because the T $\beta$ RI receptor kinase can directly phosphorylate SMAD1/5 (Liu et al., 2008). These findings suggest that a re-evaluation of TGF $\beta$  family signaling is needed through the elucidation of the type I receptors and Smad pathways that function in specific physiological and developmental contexts.

### TGF $\beta$ family signaling via non-Smad signaling proteins

Other proteins mediate TGF $\beta$  signaling in addition to Smads (see also Moustakas and Heldin, 2005), and here we describe the mechanistically best-established examples (Fig. 1C). T $\beta$ RII phosphorylates the polarity protein PAR6, which regulates the local degradation of the RHOA small GTPase that controls the assembly of intercellular tight junctions in mammalian cells (Ozdamar et al., 2005). As tight junctions disassemble, epithelial architecture disintegrates, followed by de-differentiation known as the epithelial-to-mesenchymal transition (EMT), an important developmental and disease-associated process that is regulated by TGF $\beta$  signaling (reviewed by Moustakas and Heldin, 2007). During EMT, in

addition to tight junctions, adherens junctions and desmosomes of polarized epithelial cells are destroyed and remodeled to give rise to mesenchymal-like cells that are motile and invasive. It should be noted that, in addition to the above direct mechanism of tight junction disassembly, TGF $\beta$  elicits EMT via Smad signaling, leading to the transcriptional induction of major inducers of this differentiation process (Thuault et al., 2008).

Whereas the TGF $\beta$ -PAR6 pathway locally degrades RHOA in a breast epithelial cell culture model, other studies have demonstrated the positive activation of Rho GTPase signaling by TGF $\beta$  and BMP receptors in diverse cell types (reviewed by Kardassis et al., 2009). However, the mechanism of Rho activation by TGF $\beta$  receptors remains unclear (Fig. 1C).

In a distinct mechanism, the TGF $\beta$  type I receptor phosphorylates both serine and tyrosine residues in the SHCA (SHC1) adaptor, which then recruits the adaptor protein GRB2 and the Ras guanine exchange factor (GEF) son of sevenless (SOS) in mammalian cells (Fig. 1C) (Lee, M. K. et al., 2007). This leads to activation of the Ras-Raf-MEK-Erk mitogen-activated protein kinase (MAPK) signaling cascade, which can regulate cell proliferation or migration. Future work might decipher to what extent a specific biological response to TGF $\beta$  receptor signaling depends on its strong serine/threonine, or on its weaker tyrosine kinase, activity.

The tyrosine kinase Src can phosphorylate Tyr284 in the cytoplasmic domain of the T $\beta$ RII receptor, leading to GRB2 and SHC recruitment and to the activation of the p38 MAPK pathway (Galliher and Schiemann, 2007). Src-dependent T $\beta$ RII phosphorylation regulates breast cancer cell proliferation and invasiveness, possibly without affecting the Smad signaling output (Galliher-Beckley and Schiemann, 2008).

As a final example, TGF $\beta$ -induced receptor heterotetramers recruit the ubiquitin ligase tumor necrosis factor  $\alpha$  receptor-associated factor 6 (TRAF6) to the T $\beta$ RI cytoplasmic domain in mammalian cells (Fig. 1C). TRAF6 ubiquitylates and activates the catalytic activity of the TGF $\beta$ -activated kinase 1 (TAK1; MAP3K7), leading to activation of the p38 and c-Jun N-terminal kinase (JNK) cascades, which regulate apoptosis or cell migration (Sorrentino et al., 2008; Yamashita et al., 2008). The T $\beta$ RI kinase activity is dispensable for this pathway (Sorrentino et al., 2008).

The developmental significance of these non-Smad pathways remains to be elucidated. However, it has recently been shown that both p38 MAPK and Smad signaling play important roles downstream of TGF $\beta$  during mouse palate and tooth development (Xu, X. et al., 2008). In *Xenopus*, the adaptor protein TRAF4 has positive signaling roles in mediating both the BMP and nodal signals that regulate neural crest differentiation and migration (Kalkan et al., 2009). TRAF4 is a substrate of the ubiquitin ligase Smad ubiquitylation regulatory factor 1 (SMURF1), which polyubiquitylates and promotes TRAF4 degradation, thus limiting the activity of the BMP and nodal pathways in the *Xenopus* neural plate. Signaling via multiple effectors enables the TGF $\beta$  pathways to be controlled by other pathways, as we discuss below, through the mechanisms that control Smad function in different cell compartments.

### TGF $\beta$ receptor regulation and endocytosis

Receptor phosphorylation is important for TGF $\beta$  family signal transduction, and thus receptor dephosphorylation might also be important. New evidence shows that TGF $\beta$ /nodal receptors are reciprocally regulated by B $\alpha$  and B $\delta$ , two isoforms of regulatory subunit B of the protein phosphatase 2A (PP2A). PP2A that contains the B $\alpha$  subunit positively, whereas PP2A that contains the B $\delta$  subunit negatively, regulates receptor signaling in *Xenopus* embryos

and in mammalian cells (Batut et al., 2008). The direct molecular targets of PP2A and the serine or threonine residues that they dephosphorylate await further analysis.

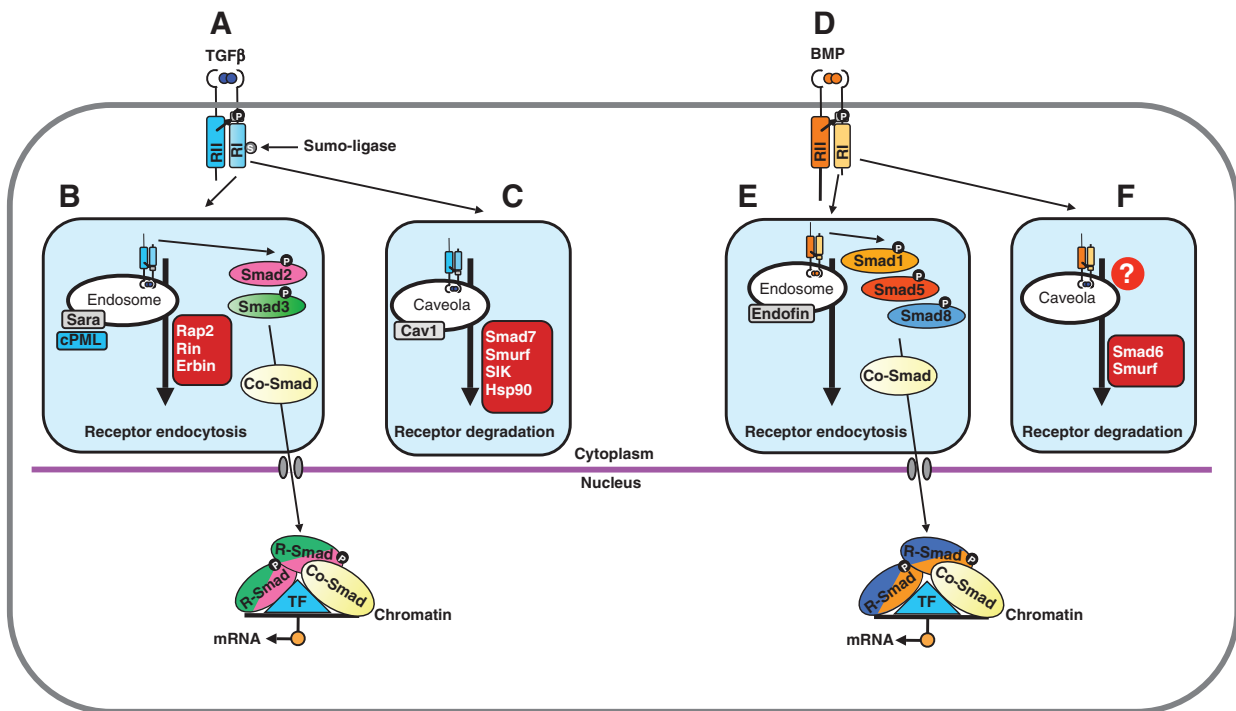
In addition, T $\beta$ RI can be sumoylated by an as yet unknown sumo-ligase in mammalian cells (Fig. 3A), which enhances TGF $\beta$  signaling by facilitating SMAD3 recruitment to the receptor for phosphorylation (Kang et al., 2008). T $\beta$ RI sumoylation might provide a docking site for an adaptor that mediates SMAD3 binding to the receptor, might induce a conformational change in the receptor that is required for SMAD3 binding, or might be coupled to the internalization mechanism.

The activated TGF $\beta$  receptors are internalized via clathrin-coated pits into early endosomes, where the receptors encounter Smad anchor for receptor activation (SARA; ZFYVE9), which facilitates the recruitment of SMAD2 and SMAD3 to T $\beta$ RI and their subsequent phosphorylation (Fig. 3A,B) (Tsukazaki et al., 1998). SARA binds SMAD2 and SMAD3, but not BMP R-Smads, and also contacts the T $\beta$ RI receptor. A homolog of SARA, endofin (ZFYVE16), plays a similar role in BMP pathways (Fig. 3D,E) (Shi et al., 2007). However, endofin can also operate in TGF $\beta$  pathways by scaffolding SMAD4 and by mediating the formation of complexes of SMAD2, SMAD3 and SMAD4 in association with the type I receptor (Chen et al., 2007).

In mammals, SARA cooperates with the cytoplasmic promyelocytic leukemia (cPML) tumor suppressor protein, which stabilizes the SARA-Smad complex (Fig. 3B) (Lin et al., 2004). This process also involves another adaptor, the PML competitor for TGIF association (PCTA), which binds to the nuclear homeodomain repressor protein TGIF (5'TG3'-interacting factor) and retains cPML in the nucleus (Faresse et al., 2008). In response to TGF $\beta$ , PCTA releases cPML to translocate to the cytoplasm, reach SARA and promote TGF $\beta$  signaling.

The *Drosophila* ortholog, dSARA, plays a similar role to mammalian SARA during Dpp/Mad signaling (Bökel et al., 2006). Since dSARA has no other homolog in *Drosophila*, it might mediate both Mad and dSmad2 signaling, although this remains to be established. In the epithelial cells of the *Drosophila* wing, which undergo apical and basolateral differentiation, Dpp receptor-dSARA complexes reside in apically located endosomes and are precisely segregated during cell division (Bökel et al., 2006). In this way, the daughter cells receive equal numbers of signaling complexes, which ensures the conservation of signaling strength from mother to daughter cells.

Many additional cytoplasmic regulators of early TGF $\beta$  receptor signaling have been described recently (Fig. 3B). Most notably, the small GTPase RAP2 inhibits activin/nodal receptor recycling, thus controlling receptor levels on the surface of *Xenopus* embryonic cells (Choi et al., 2008). During signaling, RAP2 antagonizes the negative effects of SMAD7, thus positively contributing to nodal signal propagation and the onset of gastrulation. In addition, RIN1, a RAB5 GEF, promotes TGF $\beta$  receptor endocytosis and overall signaling, which contributes to the pro-tumorigenic action of TGF $\beta$  in breast epithelial cells (Hu et al., 2008). The PDZ-containing protein erbin (ERBB2IP) binds to phosphorylated SMAD2, SMAD3, prevents their association with SMAD4 in mammalian cells, and produces opposite effects on signaling to SARA or endofin (Dai, F. et al., 2007). The protein Dapper 2 (DACT2) contributes to TGF $\beta$  receptor downregulation, which modulates nodal signaling in *Xenopus*, zebrafish, and mice (Su et al., 2007), although its partners and mechanism of action require further exploration. It would be interesting to elucidate the mechanism that regulates the



**Fig. 3. TGF $\beta$  receptor endocytosis and downregulation.** (A,D) The (A) TGF $\beta$  and (D) BMP receptor complexes are shown at the plasma membrane. Type II receptors are labeled as RII, type I receptors as RI. Sumoylation (S) of the T $\beta$ RI that positively influences Smad signaling is highlighted. (B) TGF $\beta$  receptors are internalized in endosomes characterized by the presence of the endocytic protein SARA and the regulatory adaptor cPML. The receptors can signal by SMAD2 and SMAD3 phosphorylation and activation of the Co-Smad and by accumulation of nuclear Smad complexes on chromatin. Key regulatory proteins involved in the process of receptor endocytosis are listed on the side of the arrow that indicates the flow of endosomal trafficking. (C) The TGF $\beta$  receptor degradation pathway via caveolae, which are characterized by the presence of caveolin 1 (CAV1), and the key regulatory proteins involved as shown within the box. (E) The corresponding endocytic pathway for BMP receptors with the endocytic protein endofin is less well understood. (F) The corresponding caveolae-based degradation pathway for the BMP receptors remains unexplored (?). Key established regulatory proteins are shown in the box. BMP (bone morphogenetic protein), cPML (cytoplasmic promyelocytic leukemia protein), HSP90 (heat-shock protein of 90 kDa), RAP2 (Ras-related protein 2), RIN (Ras-like protein expressed in neurons), SARA (Smad anchor for receptor activation), SIK (salt-inducible kinase), Smurf (Smad ubiquitylation regulatory factor), TGF $\beta$  (transforming growth factor  $\beta$ ).

recruitment of positive regulators, such as SARA, endofin and RIN1, and of negative regulators, such as RAP2, erbin and Dapper 2, to the TGF $\beta$  receptor complex to ensure appropriate R-Smad phosphorylation and SMAD4 association.

Receptor endocytosis both controls the flow of signaling and regulates the availability of TGF $\beta$  ligand on the cell surface. An *in vitro* kinetic analysis of TGF $\beta$  ligand bioavailability has shown that constitutive endocytosis of T $\beta$ RII depletes excess ligand (Clarke et al., 2009). This mechanism enables a cell to fine-tune the level of signaling growth factor on the cell surface.

The regulatory proteins described above highlight the link between TGF $\beta$  signaling and the regulation of receptor internalization. However, the developmental relevance of many of these factors awaits further analysis.

### TGF $\beta$ receptor downregulation and the role of I-Smads

TGF $\beta$  ligand-receptor complexes can be additionally internalized via lipid rafts into caveolae, and then into lysosomes, where the ligand-receptor complex is degraded (Di Guglielmo et al., 2003) (Fig. 3C). TGF $\beta$  receptor trafficking via caveolae is marked by their association with I-Smads and SMURF1 or SMURF2 ubiquitin ligases, which negatively regulate the signaling cascade.

The inhibitory Smads, SMAD6 and SMAD7, bind to type I receptors, thereby competitively inhibiting R-Smad phosphorylation and recruiting phosphatases and Smurf ubiquitin ligases to downregulate receptor levels and function (reviewed by Itoh and ten Dijke, 2007). Whereas SMAD7 inhibits both TGF $\beta$  and BMP pathways, SMAD6 more selectively inhibits BMP pathways (Fig. 1) and shows greater selectivity for the BMP type I receptors ALK1, ALK2, ALK3 and ALK6, as demonstrated recently in mammalian cells. Furthermore, SMAD6 binds with even higher affinity to specific amino acid residues in the BMPRIA (ALK3) and BMPRII (ALK6) kinase domains, than to ALK1 and ALK2 domains (Goto et al., 2007). By contrast, SMAD7 shows broader specificity as it binds to all type I receptors via specific lysine residues in its MH2 domain (Mochizuki et al., 2004).

Two regulatory mechanisms that mediate the SMAD7-dependent ubiquitylation and downregulation of the TGF $\beta$  receptor have recently been uncovered in mammalian cells (Fig. 3C). The chaperone protein HSP90 binds to T $\beta$ RII and to T $\beta$ RI and protects them from ubiquitylation by SMURF2, positively contributing to TGF $\beta$  signaling (Wrighton et al., 2008). Conversely, the AMP-regulated kinase member salt-inducible kinase (SIK) is induced at the mRNA and protein levels by TGF $\beta$

signaling, concomitantly with the induction of SMAD7 and SMURF2 (Kowanetz et al., 2008). SIK binds to SMAD7 and to T $\beta$ RI to promote receptor downregulation. The *C. elegans* SIK ortholog, KIN-29, exhibits a conserved function by regulating body size in the Sma/Mab pathway; however, the molecular mechanism of KIN-29 action in worms remains unexplored (Maduzia et al., 2005).

Although SMAD7 primarily acts at the type I receptor level, it also resides in the nucleus, and new evidence suggests that it can bind to DNA and to nuclear complexes of SMAD2, SMAD3 and SMAD4, disrupting their complexes and inhibiting their transcriptional activity (Fig. 4) (Zhang et al., 2007).

Based on the importance of the mechanisms of TGF $\beta$  receptor endocytosis and downregulation, and the links of such mechanisms to the nucleocytoplasmic shuttling of Smads (see below), future studies into the biology of I-Smads promise to reveal interesting and novel findings.

### Regulation of Smad trafficking by motor proteins

In parallel to TGF $\beta$  receptor endocytosis, R-Smads become phosphorylated by the type I receptors and accumulate in the nucleus. However, Smads, like the receptors, show dynamic mobility and shuttle in and out of the nucleus even when they are not activated by receptors (reviewed by Moustakas and Heldin, 2008). The cytoplasmic trafficking of both TGF $\beta$  receptors and Smads is often mediated by motor proteins that are associated with microtubules. Motor proteins are important both before and after R-Smad C-terminal phosphorylation.

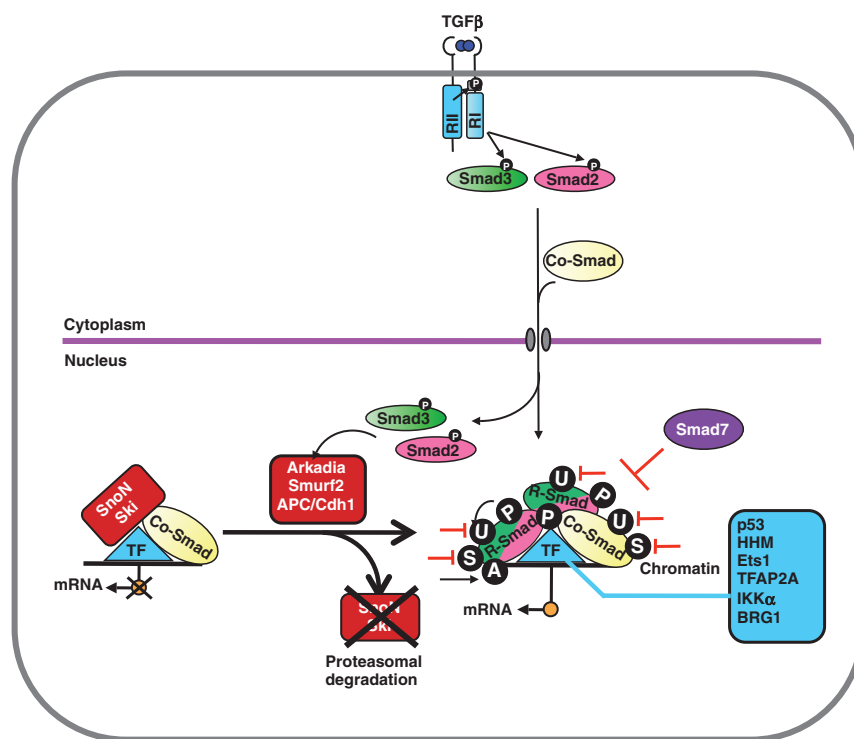
Accordingly, Smads interact with kinesin 1, which mediates the recruitment of SMAD2 to the receptor complex in *Xenopus* and mammalian cells (Batut et al., 2007). Smads are sequestered away from the receptors when bound to microtubules, from where they can be released, for example, by connexin 43 (GJ $\alpha$ 1), which competes with microtubules to bind Smads (Dai, P. et al., 2007). Additional motor proteins, such as the dynein light chain km23-1

(DYNLRB1), also promote Smad traffic towards the nucleus of mammalian cells (Jin et al., 2007). The developmental roles of the connexin 43 and km23-1 mechanisms have not yet been specifically addressed.

Microtubules also transport specialized pools of Smad proteins. For example, the pool of SMAD1 that has been subjected to inhibitory phosphorylation in its linker domain by Erk MAPKs (see below) moves towards the centrosome via microtubules, where it is degraded by proteasomes (Fuentelba et al., 2008). Interestingly, when mammalian embryonic and adult cells, or *Drosophila* blastoderm cells, complete mitosis, phosphorylated SMAD1 and the centrosomal degrading apparatus segregate to only one of the two daughter cells (Fuentelba et al., 2008). Thus, Smad trafficking and segregation to daughter cells is regulated developmentally in a stage-specific manner. Additional studies in *Xenopus* embryos show that SMAD2-SMAD4 complexes are recruited to chromatin during every cell division, when mitosis dissolves the nuclear envelope (Saka et al., 2007). Again, this event is regulated developmentally as it does not occur prior to the mid-blastula transition. Moreover, SMAD3 regulates the activity of the anaphase-promoting complex, a primary initiator of mitosis during the mammalian cell cycle (Fujita et al., 2008). Whether these three mechanisms of Smad regulation during embryonic cell mitosis constitute one and the same process remains to be elucidated.

### Mechanisms of Smad shuttling through the nuclear envelope

Smad nuclear import via nuclear pores is mediated by nucleoporins, which are integral constituents of the pores, and by importins, carrier proteins that bind to both cargo proteins and nucleoporins and that catalyze their nuclear translocation in an energy-dependent manner (reviewed by Moustakas and Heldin, 2008). All Smads have a conserved NLS in their MH1 domain (Fig. 2), which binds to specific importins, such as importin  $\beta$  (in



### Fig. 4. Regulation of the nuclear Smad complex.

Following TGF $\beta$  receptor activation, the activated Smad complex translocates to the nucleus and associates with various Smad partners [represented by the blue, triangular transcription factor (TF) and by examples discussed in the text] and undergoes post-translational modifications (A, acetylation; U, ubiquitylation; S, sumoylation; P, phosphorylation). Black arrows indicate a positive effect of a post-translational modification on Smad transcriptional function, red T-bars indicate a negative effect. (Left) Derepression of Smad target genes when the SNON/SKI co-repressors are proteasomally degraded after being ubiquitylated by the ubiquitin ligases arkadia, SMURF2 or APC/CDH1. APC/CDH1 (anaphase-promoting complex and its ubiquitin ligase subunit CDH1), BRG1 (Brahma-related gene 1), ETS1 (v-ets erythroblastosis virus E26 oncogene homolog 1), HHM (human homolog of Maf), IKK $\alpha$  (I $\kappa$ B kinase  $\alpha$ ), SMURF2 (Smad ubiquitylation regulatory factor 2), TFAP2A (transcription factor activating enhancer-binding protein 2 $\alpha$ ), TGF $\beta$  (transforming growth factor  $\beta$ ).



the case of SMAD1 and SMAD3) and importin  $\alpha$  (in the case of SMAD4). A recent genome-wide study in *Drosophila* S2 cells reported the roles of additional importins, such as Msk, which imports Mad, and of its mammalian orthologs, importin 7 and importin 8, which import SMAD2, SMAD3 and SMAD4 (Xu et al., 2007; Yao et al., 2008).

Smad proteins have characterized nuclear export signals (NESs) in their MH2 (SMAD1, SMAD3) or linker (SMAD4) domains (Fig. 2). The NESs bind specific exportins – exportin 4 for SMAD3 and exportin 1 for SMAD1 and SMAD4 – and export is catalyzed by the small GTPase RAN. Recently, a new exportin was described for SMAD2 and SMAD3, the RAN-binding protein 3 (RANBP3), which is a known exportin family member (Dai et al., 2009). RANBP3 was shown to recognize dephosphorylated nuclear SMAD2 and SMAD3 proteins and export them to the cytoplasm. Importins and exportins for SMAD5 and SMAD8 or for the I-Smads remain to be characterized. However, as we discuss below, our understanding of how Smads are transported into the nucleus has been significantly advanced by recent findings.

### Regulation of Smad nuclear shuttling

The dynamic movement of Smads in and out of the nucleus is highly regulated. A recent mathematical model of SMAD2 and SMAD4 trafficking reported that their nuclear accumulation in response to TGF $\beta$  reflects a shift in the equilibrium between the cytoplasmic and nuclear pools of Smads that is brought about by a decrease in nuclear export (Schmierer et al., 2008). During signaling, however, low-level R-Smad dephosphorylation by nuclear phosphatases, among other factors, continues to ensure their subsequent nuclear export (Schmierer et al., 2008).

R-Smad shuttling is regulated not only by cycles of receptor-mediated C-terminal phosphorylation and dephosphorylation by nuclear phosphatases (reviewed by Wrighton et al., 2009), but also by sumoylation and ubiquitylation. Sumoylation of SMAD3 by the protein inhibitor of activated Stat  $\gamma$  (PIAS $\gamma$ ; PIAS4) sumo-ligase promotes its nuclear export in mammalian cells (Imoto et al., 2008). Sumoylation of Medea, by an as yet unidentified sumo-ligase, also promotes its nuclear export, providing negative regulation that restricts the competence of early *Drosophila* embryonic cells to respond to Dpp (Miles et al., 2008). This mechanism resembles the previously established role of mammalian SMAD4 sumoylation by PIAS ligases (reviewed by Lönn et al., 2009). It is possible that in vivo Smad sumoylation might not negatively regulate TGF $\beta$  signaling; rather, it might promote continuous Smad shuttling. However, under overexpression conditions, sumo-ligases may shift the shuttling equilibrium by pushing Smads to the cytoplasm, thus reducing their time in the nucleus.

SMAD4 can also be monoubiquitylated (Morén et al., 2003) by the nuclear ubiquitin ligase TIF1 $\gamma$  (ectodermin; TRIM33), which promotes its nuclear export and inhibits the formation of nuclear complexes of SMAD2, SMAD3 and SMAD4 (Dupont et al., 2009). Once monoubiquitylated, SMAD4 is exported from the nucleus (Wang et al., 2008), whereupon fat facets in mouse (FAM; USP9X) deubiquitylates it, recharging it for subsequent cycles of shuttling, as demonstrated in *Drosophila*, *Xenopus* and human cells (Dupont et al., 2009).

Other mechanisms also regulate the nuclear residence and function of Smad complexes. Heteromeric complexes of SMAD2, SMAD3 and SMAD4 bind to the shuttling protein transcriptional co-activator with PDZ-binding motif (TAZ) in the nucleus of

human cells, and are then recruited to chromatin via factors such as the activator-recruited co-factor (ARC) protein ARC105 (MED15), a member of the Mediator complex that ensures the progression of gene transcription (Varelas et al., 2008). TAZ is regulated by phosphorylation and by interaction with 14-3-3 family adaptors that control its timely residence in the nucleus. Furthermore, the *Drosophila* nuclear lamin Otefin interacts with Medea and tethers Smad complexes to the nuclear envelope (Jiang et al., 2008). The Otefin-Medea complexes bind to specific gene-regulatory elements that control germline stem cell development (Jiang et al., 2008). Future research into Smad shuttling and the regulation of Smad compartmentalization will bring to light additional regulatory mechanisms of TGF $\beta$  signaling.

### Negative regulation of Smad signaling by phosphorylation and ubiquitylation

In addition to regulating Smad nucleocytoplasmic shuttling, C-terminal tail dephosphorylation, linker domain phosphorylation and ubiquitylation are implicated in the negative regulation of Smad signaling (Figs 2 and 4).

The developmental importance of such post-translational modifications has been recognized during BMP-dependent neurogenesis in *Xenopus* and mouse C<sub>2</sub>C<sub>12</sub> osteoblast differentiation. Fibroblast growth factor (FGF) signaling via Ras-Erk MAPK negatively regulates BMP signaling, as Erk (and GSK3 $\beta$  kinase) directly phosphorylates the SMAD1 linker, leading to recruitment of SMURF1 and to the proteasomal degradation of SMAD1 in perinuclear centrosomes (Fuentealba et al., 2007; Sapkota et al., 2007). During *Xenopus* neurogenesis, SMAD1 degradation is triggered in three ways: by chordin antagonising BMP activity extracellularly; by the Wnt antagonist Dickkopf 1 blocking Wnt activity extracellularly; and by Erk MAPK pathway activation via FGF and insulin-like growth factor (IGF) signaling. Thus, FGF/IGF signaling provides negative feedback to BMP signaling in the developing nervous system. Conversely, Wnt signaling induces GSK3 $\beta$  degradation, leading to decreased SMAD1 linker phosphorylation and to its prolonged signaling, an event that is required for *Xenopus* epidermal differentiation (Fuentealba et al., 2007). This is a good example of positive cross-talk between Wnt and BMP signaling during skin differentiation.

GSK3 $\beta$  also negatively controls TGF $\beta$  signaling, as it directly phosphorylates SMAD3 in its MH1 domain in mammalian cells (Guo et al., 2008a). This phosphorylation is followed by the ubiquitylation and proteasomal degradation of SMAD3, which regulate its steady-state levels. By contrast, upon TGF $\beta$  receptor phosphorylation, SMAD3 can be further phosphorylated in its MH2 domain by casein kinase 1  $\gamma$ 2, which leads to the specific ubiquitylation and degradation of the activated form of SMAD3 (Guo et al., 2008b).

A recent report has shed more light on the complexity of Smad regulation through the phosphorylation of its linker domain (Wang et al., 2009). After SMAD3 C-terminal phosphorylation by T $\beta$ RI in mammalian cells, nuclear GSK3 $\beta$  and cyclin-dependent kinases phosphorylate three distinct SMAD3 linker residues, downregulating its transcriptional activity. Thus, TGF $\beta$  signaling tightly controls the activity of one of its main transducers through highly regulated phosphorylation events.

Recent evidence also shows that during mitosis of mammalian cells in culture, the kinase MPS1 (TTK) can directly C-terminally phosphorylate SMAD2 and SMAD3, thus activating their nuclear activities in the absence of TGF $\beta$  receptor activation (Zhu et al.,

2007). This is one of the first clear TGF $\beta$ -independent mechanisms that engages Smads and mimics the action of TGF $\beta$ . Thus, whereas R-Smad C-terminal phosphorylation by type I receptor kinases is a positive regulator of TGF $\beta$  family signaling, Smad phosphorylation by other kinases can negatively affect TGF $\beta$  family signaling in a cell cycle-, developmental- or tissue-specific manner.

### Transcriptional regulation by Smads

The list of transcription factors to which Smads bind to regulate gene expression continues to grow (see Table S1 in the supplementary material) (reviewed by Feng and Derynck, 2005). Nuclear Smad complexes bind with weak affinity to Smad-binding elements (SBEs) on DNA (reviewed by Schmierer and Hill, 2007). Notably, the most common isoform of SMAD2 fails to bind to SBEs owing to an insertion within its DNA-binding domain, which resides in the MH1 domain of all Smads (see Fig. 2). SMAD3 recognizes 5'-GTCTG-3' as its SBE. By contrast, the BMP Smads and SMAD4 recognize GC-rich sequences that have less conserved motifs, which are sometimes in close proximity to an SBE. In general, recruitment of Smad complexes to chromatin is dependent on their direct interaction with transcription factors that bind to DNA with higher affinity (Fig. 4).

Upon binding to DNA and to their transcriptional partners, Smads recruit co-activators and histone acetyltransferases, such as p300, C/EBP-binding protein (CBP) and p300/CBP-associated factor (P/CAF), facilitating the initiation of transcription (reviewed by Schmierer and Hill, 2007). Recent evidence has shown that p300/CBP also acetylates SMAD2/3, enhancing their DNA-binding activity in mammalian cells (Simonsson et al., 2006; Tu and Luo, 2007). Conversely, histone deacetylases inhibit SMAD1 transcriptional activity during neuronal differentiation in the mouse embryonic brain (Shak ed et al., 2008). However, direct acetylation or deacetylation of BMP-specific Smads has yet to be demonstrated.

The negative regulation of Smad signaling by Smad ubiquitylation was summarized above. Positive regulation of nuclear Smad signaling by ubiquitylation has more recently come to light from studies in mouse embryos and in mammalian cells (Mavrikakis et al., 2007). Nuclear Smad complexes associate with the ubiquitin ligase arkadia (RNF111) in a ligand-dependent manner and promote the ubiquitylation and degradation of their interacting co-repressors SKI and SNON (SKIL) (Le Scolan et al., 2008; Levy et al., 2007; Nagano et al., 2007). This mechanism brings about the derepression and transcriptional induction of target genes by nuclear Smads (Fig. 4). Interestingly, the proteasomal degradation of SNON depends on its phosphorylation by TAK1, the non-Smad effector of TGF $\beta$  signaling (see Fig. 1C) (Kajino et al., 2007). However, it is unclear whether SNON ubiquitylation by arkadia requires its prior phosphorylation by TAK1. Arkadia also ubiquitylates the inhibitory SMAD7 (Koinuma et al., 2003), but whether this process takes place in the nucleus or in the cytoplasm awaits clarification.

Genome-wide screens have revealed an association between Smads and the SWI/SNF family chromatin remodeling protein Brahma-related gene 1 (BRG1; SMARCA4) and the DNA-binding proteins ETS1 and transcription factor activating enhancer-binding protein 2 $\alpha$  (TFAP2 $\alpha$ ) (Koinuma et al., 2009; Xi et al., 2008). A current model suggests that chromatin-bound Smads cannot perform transcriptional work in the absence of essential chromatin remodeling factors, such as BRG1 and the mediator component ARC105 (reviewed by Schmierer and Hill, 2007). Interestingly, ARC105 localization in distinct chromatin domains is regulated by TAZ, the nuclear Smad-tethering factor (Varelas et al., 2008). These early reports open the door to future studies that might demonstrate

how TGF $\beta$  alters the dynamic architecture of chromatin, leading to gene-specific transcriptional induction or repression. Such research might, for the first time, establish links between the epigenetic regulation of chromatin and the function of the TGF $\beta$  pathways.

### Regulatory mechanisms of Smad transcriptional co-factors

From the numerous Smad-transcription factor complexes and their resulting mechanisms of target gene regulation (see Table S1 in the supplementary material) (Feng and Derynck, 2005), we highlight here a few selected examples that are of demonstrated or potential developmental relevance.

*Xenopus* mesoderm specification is driven by the concerted action of TGF $\beta$ /activin and FGF-Ras-Erk MAPK signaling (Cordenonsi et al., 2007). The FGF-Ras-Erk MAPK pathway acts in distinct regions of the developing *Xenopus* embryo, such as in the marginal zone, and induces, via phosphorylation, the activity of casein kinases, which then phosphorylate serines 6 and 9 of the tumor suppressor p53, contributing to mesoderm development. This phosphorylation activates the transcriptional activity of p53, making it competent to pair with Smads. This interaction leads to the transcriptional induction of mesoderm-defining genes, such as the transcription factors *Snail*, *Xbra* (*brachyury*) and *Mix.2*. Conversely, during *Xenopus* ectoderm specification, p53 is inhibited by the zinc-finger protein XFDL156 (Sasai et al., 2008). This mechanism is essential for preventing the aberrant activation of nodal signaling in the ectoderm, the developmental fate of which depends primarily on the activity of BMP pathways. Although the above example emphasizes positive cross-talk between FGF and activin signaling during *Xenopus* mesoderm specification, this should not be interpreted as the only developmental FGF signaling mechanism during frog mesoderm induction. The FGF response is multifactorial and multigenic, as revealed by recent genome-wide transcriptomic screens (Branney et al., 2009). Interestingly, although Smads cooperate with wild-type p53 to promote developmental processes, they also cooperate with mutant p53, which often accumulates in human cancers (Adorno et al., 2009; Kalo et al., 2007). The Smad-mutant p53 complex represses *TGFBR2* transcription, leading to the induction of pro-metastatic genes.

An unexpected transcriptional partner of SMAD2 and SMAD3 is the well-characterized I $\kappa$ B kinase  $\alpha$  (IKK $\alpha$ ; CHUK), which participates in the nuclear factor  $\kappa$  B (NF $\kappa$ B) pathway (Descargues et al., 2008). In the epidermis of *Smad4*-null mice, a complex of SMAD2, SMAD3 and IKK $\alpha$  forms in the absence of SMAD4 and regulates mammalian keratinocyte differentiation by binding to the regulatory sequences of the transcription factor genes *Mad1* (*Mxd1* – Mouse Genome Informatics) and *Ovoll*, which induce epidermal differentiation. Transcriptional induction mediated by the complex of SMAD2, SMAD3 and IKK $\alpha$  is independent of the kinase activity of IKK $\alpha$ . Interestingly, invasive squamous cell carcinomas that are resistant to the tumor suppressor action of TGF $\beta$  have defective IKK $\alpha$  that cannot enter the nucleus and act as a Smad co-factor (Marinari et al., 2008).

Genes that are either transcriptionally co-regulated at the same developmental time, or in the same tissue, are referred to as synexpression groups (Niehrs and Pollet, 1999). One such group is the inhibitor of differentiation (Id) family of genes, which like other TGF $\beta$ -responsive synexpression groups, respond to TGF $\beta$  family members via specific regulatory sequences present in their genes (see Table S1 in the supplementary material) (Karaulanov et al., 2004). Synexpression groups also require specific Smad-interacting transcriptional co-factors for their expression. For example, the

mammalian FoxO transcription factors bind to Smads and coordinate the regulation of 11 genes that define the cyostatic, apoptotic and adaptive signaling responses of keratinocytes to TGF $\beta$  (see Table S1 in the supplementary material) (Gomis et al., 2006a). The helix-loop-helix HHM (human homolog of Maid; CCNDBP1) protein regulates a specific synexpression group of cell cycle and cell migration regulators (see Table S1 in the supplementary material), and, accordingly, regulates growth inhibition and migration in mammalian epithelial cells in response to TGF $\beta$ , while mediating other responses in different cells (Ikushima et al., 2008).

In the *Drosophila* wing imaginal disc, the BMP ligand Dpp activates Mad (an R-Smad) and Medea (its Co-Smad) (see Table 1), which then directly repress certain transcription factor genes, including the transcriptional repressor *brinker* (*brk*). This repression of *brk* by Mad-Medea leads to the derepression of *optomotor blind* (*omb*; *bifid* – FlyBase), *zerknüllt* (*zen*) and *spalt* (*sal*), which encode transcription factors that regulate the expression of other transcription factors, to provide patterning and morphogenetic information to the developing wing (de Celis and Barrio, 2000; Shen et al., 2008). *sal*, however, additionally requires direct binding and transactivation by the Mad-Medea complex.

In *C. elegans*, the *sma-9* gene is involved in neuronal specification within a restricted group of rays in the tail of the developing worm and is expressed during early larval stages (Liang et al., 2003). SMA-9 is the ortholog of the *Drosophila* zinc-finger transcription factor Schnurri, a co-factor of the Mad-Medea complex that represses *brk* expression (Marty et al., 2000). By analogy with *Drosophila*, SMA-9 might mediate BMP-like DBL-1 signaling by complexing with SMA-2, SMA-3 or SMA-4. Indeed, SMA-9 acts as both a transcriptional repressor and an activator downstream of DBL-1. Newly identified targets of this pathway are orthologs of transcription factors that are already implicated in mammalian BMP signaling, such as Runx and Fos, or orthologs of Hedgehog signaling proteins (Liang et al., 2007). Interestingly, mouse Schnurri-2 (HIVEP2 – Mouse Genome Informatics) also regulates TGF $\beta$ /BMP-dependent gene expression. It binds to SMAD1-SMAD4 and to the transcriptional co-factor C/EBP $\alpha$  (CCAAT/enhancer-binding protein  $\alpha$ ) to induce the *PPAR $\gamma$ 2* (peroxisome proliferator-activated receptor  $\gamma$ 2; *Pparg*) gene that regulates adipocyte differentiation (Jin et al., 2006). Thus, mice that lack the *Schnurri-2* gene have reduced fat.

Finally, as we discuss in Box 3, TGF $\beta$  family transcriptional regulation in development also occurs via the regulation of microRNA (miRNA) genes, and via the reciprocal regulation of TGF $\beta$  signaling by miRNAs.

## Conclusions

As TGF $\beta$  research continues with ever increasing speed, we foresee important novel findings regarding the mechanisms of TGF $\beta$  receptor regulation and specificity of signaling, cytoplasmic trafficking of receptors and Smads, nuclear dynamics of Smad-chromatin associations and their relationship to developmental processes. The functional implications of ‘promiscuous’ signaling by TGF $\beta$  family receptor kinases that simultaneously activate TGF $\beta$ - and BMP-like Smad pathways, as well as MAPK and other pathways, needs to be analyzed carefully and with quantitative methods. The area of post-translational modifications of TGF $\beta$  receptors and Smads will continue its prolific expansion. More sensitive proteomic approaches will be useful in dissecting all the components of signaling complexes and their dynamic nature, especially if coupled to multi-protein imaging in real time. Progress in the modeling of signaling dynamics and of the protein networks

### Box 3. TGF $\beta$ /BMP signaling and microRNAs

TGF $\beta$  regulates micro-RNA (miRNA) gene expression in mammalian cells (Zavadil et al., 2007). TGF $\beta$  promotes the epithelial-to-mesenchymal transition (EMT) by repressing transcription of the *miR-200* family (Burk et al., 2008; Gregory et al., 2008; Korpala et al., 2008), which downregulates the pro-EMT transcription factors zinc-finger E-box-binding homeobox 1 (ZEB1) and ZEB2. Transcriptional repression of *miR-24*, a positive regulator of myogenesis, in part explains the anti-myogenic effects of TGF $\beta$  (Sun et al., 2008). BMP induces osteoblast differentiation from mesenchymal progenitors and concomitantly regulates expression of 22 miRNAs (Li et al., 2008). Among these, *miR-133* targets the transcription factor RUNX2, a known target of BMP/Smad signaling that promotes osteoblast differentiation, whereas *miR-135* targets SMAD5. These miRNAs progressively inhibit BMP signaling to fine-tune bone development.

In addition, SMAD1, SMAD3 and SMAD5, but not SMAD4, directly bind to and regulate components of the DROSHA microprocessor complex, which regulates miRNA biogenesis (Davis et al., 2008). This post-transcriptional mechanism selectively regulates only certain miRNAs during vascular smooth muscle differentiation. Understanding the mechanism of this selectivity and its developmental relevance is important.

miRNAs also target TGF $\beta$  signaling components. In *Xenopus*, *miR-15* and *miR-16* downregulate *ActRIIA*, which restricts receptor expression to specific cells, thus defining the embryonic territory that responds to activin/nodal (Martello et al., 2007). In zebrafish, *miR-430* downregulates the nodal-like ligand *squint* (*ndr1* – Zebrafish Information Network) and its extracellular antagonist *lefty*, which is required for proper patterning of the early fish embryo (Choi et al., 2007).

that participate in the TGF $\beta$  family cascades should provide fresh ideas about new regulatory nodes in the network, and should also define more quantitatively critical parameters that govern the behavior of the network. A major challenge is to decipher the roles of the TGF $\beta$  pathways during late stages of embryogenesis and during neonatal life by conditional activation and inactivation of TGF $\beta$  signaling components in model organisms. The importance of cross-talk during different developmental stages between TGF $\beta$  and Wnt, Hedgehog, FGF or other pathways should be another focus for future research. Finally, in the context of development, more complete circuits of target genes, and their corresponding protein or RNA regulators, will need to be delineated in the global effort to provide a systems-level description of TGF $\beta$  pathways in every tissue and organ.

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### Supplementary material

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/136/22/3699/DC1>

### References

- Adorno, M., Cordenonsi, M., Montagner, M., Dupont, S., Wong, C., Hann, B., Solari, A., Bobisse, S., Rondina, M. B., Guzzardo, V. et al. (2009). A Mutant-p53/Smad complex opposes p63 to empower TGF $\beta$ -induced metastasis. *Cell* **137**, 87–98.
- Affolter, M. and Basler, K. (2007). The Decapentaplegic morphogen gradient: from pattern formation to growth regulation. *Nat. Rev. Genet.* **8**, 663–674.

- Anttonen, M., Parviainen, H., Kyronlahti, A., Bielinska, M., Wilson, D. B., Ritvos, O. and Heikinheimo, M. (2006). GATA-4 is a granulosa cell factor employed in inhibin-alpha activation by the TGF- $\beta$  pathway. *J. Mol. Endocrinol.* **36**, 557-568.
- Bangi, E. and Wharton, K. (2006). Dpp and Gbb exhibit different effective ranges in the establishment of the BMP activity gradient critical for *Drosophila* wing patterning. *Dev. Biol.* **295**, 178-193.
- Barrio, R. and de Celis, J. F. (2004). Regulation of spalt expression in the *Drosophila* wing blade in response to the Decapentaplegic signaling pathway. *Proc. Natl. Acad. Sci. USA* **101**, 6021-6026.
- Batut, J., Howell, M. and Hill, C. S. (2007). Kinesin-mediated transport of Smad2 is required for signaling in response to TGF- $\beta$  ligands. *Dev. Cell* **12**, 261-274.
- Batut, J., Schmierer, B., Cao, J., Rafferty, L. A., Hill, C. S. and Howell, M. (2008). Two highly related regulatory subunits of PP2A exert opposite effects on TGF- $\beta$ /Activin/Nodal signalling. *Development* **135**, 2927-2937.
- Benchabane, H. and Wrana, J. L. (2003). GATA- and Smad1-dependent enhancers in the Smad7 gene differentially interpret bone morphogenetic protein concentrations. *Mol. Cell. Biol.* **23**, 6646-6661.
- Bianco, C., Normanno, N., Salomon, D. S. and Ciardiello, F. (2004). Role of the cripto (EGF-CFC) family in embryogenesis and cancer. *Growth Factors* **22**, 133-139.
- Blanchet, M. H., Le Good, J. A., Mesnard, D., Oorschot, V., Baflost, S., Minchiotti, G., Klumperman, J. and Constam, D. B. (2008). Cripto recruits Furin and PACE4 and controls Nodal trafficking during proteolytic maturation. *EMBO J.* **27**, 2580-2591.
- Blokzijl, A., ten Dijke, P. and Ibanez, C. F. (2002). Physical and functional interaction between GATA-3 and Smad3 allows TGF-beta regulation of GATA target genes. *Curr. Biol.* **12**, 35-45.
- Blokzijl, A., Dahlqvist, C., Reissmann, E., Falk, A., Moliner, A., Lendahl, U. and Ibanez, C. F. (2003). Cross-talk between the Notch and TGF- $\beta$  signaling pathways mediated by interaction of the Notch intracellular domain with Smad3. *J. Cell Biol.* **163**, 723-728.
- Bökel, C., Schwabedissen, A., Entchev, E., Renaud, O. and González-Gaitán, M. (2006). Sara endosomes and the maintenance of Dpp signaling levels across mitosis. *Science* **314**, 1135-1139.
- Branney, P. A., Faas, L., Steane, S. E., Pownall, M. E. and Isaacs, H. V. (2009). Characterisation of the fibroblast growth factor dependent transcriptome in early development. *PLoS ONE* **4**, e4951.
- Brodin, G., Ahgren, A., ten Dijke, P., Heldin, C.-H. and Heuchel, R. (2000). Efficient TGF- $\beta$  induction of the Smad7 gene requires cooperation between AP-1, Sp1, and Smad proteins on the mouse Smad7 promoter. *J. Biol. Chem.* **275**, 29023-29030.
- Brown, C. O., 3rd, Chi, X., Garcia-Gras, E., Shirai, M., Feng, X. H. and Schwartz, R. J. (2004). The cardiac determination factor, Nkx2-5, is activated by mutual cofactors GATA-4 and Smad1/4 via a novel upstream enhancer. *J. Biol. Chem.* **279**, 10659-10669.
- Bruna, A., Darken, R. S., Rojo, F., Ocana, A., Penuelas, S., Arias, A., Paris, R., Tortosa, A., Mora, J., Baselga, J. et al. (2007). High TGF $\beta$ -Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. *Cancer Cell* **11**, 147-160.
- Burk, U., Schubert, J., Wellner, U., Schmalhofer, O., Vincan, E., Spaderna, S. and Brabletz, T. (2008). A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. *EMBO Rep.* **9**, 582-589.
- Chen, C. and Shen, M. M. (2004). Two modes by which Lefty proteins inhibit nodal signaling. *Curr. Biol.* **14**, 618-624.
- Chen, C. R., Kang, Y., Siegel, P. M. and Massague, J. (2002). E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. *Cell* **110**, 19-32.
- Chen, D. and McKearin, D. M. (2003). A discrete transcriptional silencer in the bam gene determines asymmetric division of the *Drosophila* germline stem cell. *Development* **130**, 1159-1170.
- Chen, X., Rubock, M. J. and Whitman, M. (1996). A transcriptional partner for MAD proteins in TGF- $\beta$  signalling. *Nature* **383**, 691-696.
- Chen, Y., Blom, I. E., Sa, S., Goldschmeding, R., Abraham, D. J. and Leask, A. (2002). CTGF expression in mesangial cells: involvement of SMADs, MAP kinase, and PKC. *Kidney Int.* **62**, 1149-1159.
- Chen, Y. G., Wang, Z., Ma, J., Zhang, L. and Lu, Z. (2007). Endofin, a FYVE domain protein, interacts with Smad4 and facilitates transforming growth factor- $\beta$  signaling. *J. Biol. Chem.* **282**, 9688-9695.
- Chen, X., Xu, H., Yuan, P., Fang, F., Huss, M., Vega, V. B., Wong, E., Orlov, Y. L., Zhang, W., Jiang, J. et al. (2008). Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell* **133**, 1106-1117.
- Chi, X. Z., Yang, J. O., Lee, K. Y., Ito, K., Sakakura, C., Li, Q. L., Kim, H. R., Cha, E. J., Lee, Y. H., Kaneda, A. et al. (2005). RUNX3 suppresses gastric epithelial cell growth by inducing p21<sup>WAF1/Cip1</sup> expression in cooperation with transforming growth factor  $\beta$ -activated SMAD. *Mol. Cell. Biol.* **25**, 8097-8107.
- Cho, G., Lim, Y., Zand, D. and Golden, J. A. (2008). Szn1 is a novel protein that functions as a transcriptional coactivator of bone morphogenetic protein signaling. *Mol. Cell. Biol.* **28**, 1565-1572.
- Choi, S. C., Kim, G. H., Lee, S. J., Park, E., Yeo, C. Y. and Han, J. K. (2008). Regulation of activin/nodal signaling by Rap2-directed receptor trafficking. *Dev. Cell* **15**, 49-61.
- Choi, W. Y., Giraldez, A. J. and Schier, A. F. (2007). Target protectors reveal dampening and balancing of Nodal agonist and antagonist by miR-430. *Science* **318**, 271-274.
- Chou, W. C., Prokova, V., Shiraishi, K., Valcourt, U., Moustakas, A., Hadzopoulou-Cladaras, M., Zannis, V. I. and Kardassis, D. (2003). Mechanism of a transcriptional cross talk between transforming growth factor- $\beta$ -regulated Smad3 and Smad4 proteins and orphan nuclear receptor hepatocyte nuclear factor-4. *Mol. Biol. Cell* **14**, 1279-1294.
- Clarke, D. C., Brown, M. L., Erickson, R. A., Shi, Y. and Liu, X. (2009). Transforming growth factor  $\beta$  depletion is the primary determinant of Smad signaling kinetics. *Mol. Cell. Biol.* **29**, 2443-2455.
- Cohen, T. V., Kosti, O. and Stewart, C. L. (2007). The nuclear envelope protein MAN1 regulates TGF $\beta$  signaling and vasculogenesis in the embryonic yolk sac. *Development* **134**, 1385-1395.
- Cordenonsi, M., Dupont, S., Maretto, S., Insinga, A., Imbriano, C. and Piccolo, S. (2003). Links between tumor suppressors: p53 is required for TGF- $\beta$  gene responses by cooperating with Smads. *Cell* **113**, 301-314.
- Cordenonsi, M., Montagner, M., Adorno, M., Zacchigna, L., Martello, G., Mamidi, A., Soligo, S., Dupont, S. and Piccolo, S. (2007). Integration of TGF- $\beta$  and Ras/MAPK signaling through p53 phosphorylation. *Science* **315**, 840-843.
- Costamagna, E., Garcia, B. and Santisteban, P. (2004). The functional interaction between the paired domain transcription factor Pax8 and Smad3 is involved in transforming growth factor- $\beta$  repression of the sodium/iodide symporter gene. *J. Biol. Chem.* **279**, 3439-3446.
- Dahlqvist, C., Blokzijl, A., Chapman, G., Falk, A., Dannaes, K., Ibanez, C. F. and Lendahl, U. (2003). Functional Notch signaling is required for BMP4-induced inhibition of myogenic differentiation. *Development* **130**, 6089-6099.
- Dai, F., Chang, C., Lin, X., Dai, P., Mei, L. and Feng, X. H. (2007). Erbin inhibits transforming growth factor  $\beta$  signaling through a novel Smad-interacting domain. *Mol. Cell. Biol.* **27**, 6183-6194.
- Dai, F., Lin, X., Chang, C. and Feng, X. H. (2009). Nuclear export of Smad2 and Smad3 by RanBP3 facilitates termination of TGF- $\beta$  signaling. *Dev. Cell* **16**, 345-357.
- Dai, P., Nakagami, T., Tanaka, H., Hitomi, T. and Takamatsu, T. (2007). Cx43 mediates TGF- $\beta$  signaling through competitive Smads binding to microtubules. *Mol. Biol. Cell* **18**, 2264-2273.
- Daly, A. C., Randall, R. A. and Hill, C. S. (2008). Transforming growth factor  $\beta$ -induced Smad1/5 phosphorylation in epithelial cells is mediated by novel receptor complexes and is essential for anchorage-independent growth. *Mol. Cell. Biol.* **28**, 6889-6902.
- Datta, P. K., Blake, M. C. and Moses, H. L. (2000). Regulation of plasminogen activator inhibitor-1 expression by transforming growth factor- $\beta$ -induced physical and functional interactions between smads and Sp1. *J. Biol. Chem.* **275**, 40014-40019.
- Davis, B. N., Hilyard, A. C., Lagna, G. and Hata, A. (2008). SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* **454**, 56-61.
- de Celis, J. F. and Barrio, R. (2000). Function of the spalt/spalt-related gene complex in positioning the veins in the *Drosophila* wing. *Mech. Dev.* **91**, 31-41.
- De Robertis, E. M. and Kuroda, H. (2004). Dorsal-ventral patterning and neural induction in *Xenopus* embryos. *Annu. Rev. Cell Dev. Biol.* **20**, 285-308.
- Degnin, C., Jean, F., Thomas, G. and Christian, J. L. (2004). Cleavages within the prodomain direct intracellular trafficking and degradation of mature bone morphogenetic protein-4. *Mol. Biol. Cell* **15**, 5012-5020.
- Derynck, R. and Miyazono, K. (2008). TGF- $\beta$  and the TGF- $\beta$  family. In *The TGF- $\beta$  Family* (ed. R. Derynck and K. Miyazono), pp. 29-43. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Descargues, P., Sil, A. K., Sano, Y., Korchynski, O., Han, G., Owens, P., Wang, X. J. and Karin, M. (2008). IKK $\alpha$  is a critical coregulator of a Smad4-independent TGF $\beta$ -Smad2/3 signaling pathway that controls keratinocyte differentiation. *Proc. Natl. Acad. Sci. USA* **105**, 2487-2492.
- Di Guglielmo, G. M., Le Roy, C., Goodfellow, A. F. and Wrana, J. L. (2003). Distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover. *Nat. Cell Biol.* **5**, 410-421.
- Dick, A., Hild, M., Bauer, H., Imai, Y., Maifeld, H., Schier, A. F., Talbot, W. S., Bouwmeester, T. and Hammerschmidt, M. (2000). Essential role of Bmp7 (snailhouse) and its prodomain in dorsoventral patterning of the zebrafish embryo. *Development* **127**, 343-354.
- Dupont, S., Mamidi, A., Cordenonsi, M., Montagner, M., Zacchigna, L., Adorno, M., Martello, G., Stinchfield, M. J., Soligo, S., Morsut, L. et al. (2009). FAM/USP9x, a deubiquitinating enzyme essential for TGF $\beta$  signaling, controls Smad4 monoubiquitination. *Cell* **136**, 123-135.
- Eimon, P. M. and Harland, R. M. (2002). Effects of heterodimerization and proteolytic processing on Derrière and Nodal activity: implications for mesoderm induction in *Xenopus*. *Development* **129**, 3089-3103.
- Faresse, N., Colland, F., Ferrand, N., Prunier, C., Bourgeade, M. F. and Atfi, A.

- (2008). Identification of PCTA, a TGF $\beta$  antagonist that promotes PML function in TGF $\beta$  signalling. *EMBO J.* **27**, 1804-1815.
- Feng, X. H. and Derynck, R.** (2005). Specificity and versatility in TGF $\beta$  signaling through Smads. *Annu. Rev. Cell Dev. Biol.* **21**, 659-693.
- Feng, X. H., Lin, X. and Derynck, R.** (2000). Smad2, Smad3 and Smad4 cooperate with Sp1 to induce p15<sup>Ink4b</sup> transcription in response to TGF $\beta$ . *EMBO J.* **19**, 5178-5193.
- Finsson, K. W., Parker, W. L., ten Dijke, P., Thorikay, M. and Philip, A.** (2008). ALK1 opposes ALK5/Smad3 signaling and expression of extracellular matrix components in human chondrocytes. *J. Bone Miner. Res.* **23**, 896-906.
- Fuentealba, L. C., Eivers, E., Ikeda, A., Hurtado, C., Kuroda, H., Pera, E. M. and De Robertis, E. M.** (2007). Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal. *Cell* **131**, 980-993.
- Fuentealba, L. C., Eivers, E., Geissert, D., Taelman, V. and De Robertis, E. M.** (2008). Asymmetric mitosis: unequal segregation of proteins destined for degradation. *Proc. Natl. Acad. Sci. USA* **105**, 7732-7737.
- Fujita, T., Epperly, M. W., Zou, H., Greenberger, J. S. and Wan, Y.** (2008). Regulation of the anaphase-promoting complex-separase cascade by transforming growth factor- $\beta$  modulates mitotic progression in bone marrow stromal cells. *Mol. Biol. Cell* **19**, 5446-5455.
- Furumatsu, T., Tsuda, M., Taniguchi, N., Tajima, Y. and Asahara, H.** (2005). Smad3 induces chondrogenesis through the activation of SOX9 via CREB-binding protein/p300 recruitment. *J. Biol. Chem.* **280**, 8343-8350.
- Gallagher, A. J. and Schiemann, W. P.** (2007). Src phosphorylates Tyr284 in TGF $\beta$  type II receptor and regulates TGF $\beta$  stimulation of p38 MAPK during breast cancer cell proliferation and invasion. *Cancer Res.* **67**, 3752-3758.
- Gallagher-Beckley, A. J. and Schiemann, W. P.** (2008). Grb2 binding to Tyr284 in T $\beta$ R-II is essential for mammary tumor growth and metastasis stimulated by TGF $\beta$ . *Carcinogenesis* **29**, 244-251.
- Gazzerro, E. and Canalis, E.** (2006). Bone morphogenetic proteins and their antagonists. *Rev. Endocr. Metab. Disord.* **7**, 51-65.
- Ge, G., Hopkins, D. R., Ho, W. B. and Greenspan, D. S.** (2005). GDF11 forms a bone morphogenetic protein 1-activated latent complex that can modulate nerve growth factor-induced differentiation of PC12 cells. *Mol. Cell. Biol.* **25**, 5846-5858.
- Germain, S., Howell, M., Esslemont, G. M. and Hill, C. S.** (2000). Homeodomain and winged-helix transcription factors recruit activated Smads to distinct promoter elements via a common Smad interaction motif. *Genes Dev.* **14**, 435-451.
- Gomis, R. R., Alarcon, C., He, W., Wang, Q., Seoane, J., Lash, A. and Massagué, J.** (2006a). A FoxO-Smad synexpression group in human keratinocytes. *Proc. Natl. Acad. Sci. USA* **103**, 12747-12752.
- Gomis, R. R., Alarcon, C., Nadal, C., Van Poznak, C. and Massagué, J.** (2006b). C/EBP $\beta$  at the core of the TGF $\beta$  cytoskeletal response and its evasion in metastatic breast cancer cells. *Cancer Cell* **10**, 203-214.
- Goold, C. P. and Davis, G. W.** (2007). The BMP ligand Gbb gates the expression of synaptic homeostasis independent of synaptic growth control. *Neuron* **56**, 109-123.
- Gordon, K. J. and Blobel, G. C.** (2008). Role of transforming growth factor- $\beta$  superfamily signaling pathways in human disease. *Biochim. Biophys. Acta* **1782**, 197-228.
- Goto, K., Kamiya, Y., Imamura, T., Miyazono, K. and Miyazawa, K.** (2007). Selective inhibitory effects of Smad6 on bone morphogenetic protein type I receptors. *J. Biol. Chem.* **282**, 20603-20611.
- Goumans, M. J., Valdimarsdottir, G., Itoh, S., Lebrin, F., Larsson, J., Mummery, C., Karlsson, S. and ten Dijke, P.** (2003). Activin receptor-like kinase (ALK1) is an antagonistic mediator of lateral TGF $\beta$ /ALK5 signaling. *Mol. Cell* **12**, 817-828.
- Greber, B., Lehrach, H. and Adjaye, J.** (2007). Fibroblast growth factor 2 modulates transforming growth factor  $\beta$  signaling in mouse embryonic fibroblasts and human ESCs (hESCs) to support hESC self-renewal. *Stem Cells* **25**, 455-464.
- Gregory, P. A., Bert, A. G., Paterson, E. L., Barry, S. C., Tszyk, A., Farshid, G., Vadas, M. A., Khew-Goodall, Y. and Goodall, G. J.** (2008). The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.* **10**, 593-601.
- Grieder, N. C., Marty, T., Ryoo, H. D., Mann, R. S. and Affolter, M.** (1997). Synergistic activation of a *Drosophila* enhancer by HOM/EXD and DPP signaling. *EMBO J.* **16**, 7402-7410.
- Grienerberger, A., Merabet, S., Manak, J., Iltis, I., Fabre, A., Berenger, H., Scott, M. P., Pradel, J. and Graba, Y.** (2003). Tgf $\beta$  signaling acts on a Hox response element to confer specificity and diversity to Hox protein function. *Development* **130**, 5445-5455.
- Grinberg, A. V. and Kerppola, T.** (2003). Both Max and TFE3 cooperate with Smad proteins to bind the plasminogen activator inhibitor-1 promoter, but they have opposite effects on transcriptional activity. *J. Biol. Chem.* **278**, 11227-11236.
- Groppe, J., Hinck, C. S., Samavarchi-Tehrani, P., Zubieta, C., Schuermann, J. P., Taylor, A. B., Schwarz, P. M., Wrana, J. L. and Hinck, A. P.** (2008). Cooperative assembly of TGF $\beta$  superfamily signaling complexes is mediated by two disparate mechanisms and distinct modes of receptor binding. *Mol. Cell* **29**, 157-168.
- Guo, X., Ramirez, A., Waddell, D. S., Li, Z., Liu, X. and Wang, X. F.** (2008a). Axin and GSK3 $\beta$  control Smad3 protein stability and modulate TGF $\beta$  signaling. *Genes Dev.* **22**, 106-120.
- Guo, X., Waddell, D. S., Wang, W., Wang, Z., Liberati, N. T., Yong, S., Liu, X. and Wang, X. F.** (2008b). Ligand-dependent ubiquitination of Smad3 is regulated by casein kinase 1 $\gamma$ 2, an inhibitor of TGF $\beta$  signaling. *Oncogene* **27**, 7235-7247.
- Hanai, J., Chen, L. F., Kanno, T., Ohtani-Fujita, N., Kim, W. Y., Guo, W. H., Imamura, T., Ishidou, Y., Fukuchi, M., Shi, M. J. et al.** (1999). Interaction and functional cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline  $\alpha$  promoter. *J. Biol. Chem.* **274**, 31577-31582.
- Harrison, C. A., Gray, P. C., Vale, W. W. and Robertson, D. M.** (2005). Antagonists of activin signaling: mechanisms and potential biological applications. *Trends Endocrinol. Metab.* **16**, 73-78.
- Hata, A., Seoane, J., Lagna, G., Montalvo, E., Hemmati-Brivanlou, A. and Massagué, J.** (2000). OAZ uses distinct DNA- and protein-binding zinc fingers in separate BMP-Smad and Olf signaling pathways. *Cell* **100**, 229-240.
- Hu, H., Milstein, M., Bliss, J. M., Thai, M., Malhotra, G., Huynh, L. C. and Colicelli, J.** (2008). Integration of transforming growth factor  $\beta$  and RAS signaling silences a RAB5 guanine nucleotide exchange factor and enhances growth factor-directed cell migration. *Mol. Cell. Biol.* **28**, 1573-1583.
- Hu, M. C. and Rosenblum, N. D.** (2005). Smad1,  $\beta$ -catenin and Tcf4 associate in a molecular complex with the Myc promoter in dysplastic renal tissue and cooperate to control Myc transcription. *Development* **132**, 215-225.
- Hua, X., Miller, Z. A., Wu, G., Shi, Y. and Lodish, H. F.** (1999). Specificity in transforming growth factor  $\beta$ -induced transcription of the plasminogen activator inhibitor-1 gene: interactions of promoter DNA, transcription factor  $\mu$ E3, and Smad proteins. *Proc. Natl. Acad. Sci. USA* **96**, 13130-13135.
- Hua, X., Miller, Z. A., Benchabane, H., Wrana, J. L. and Lodish, H. F.** (2000). Synergism between transcription factors TFE3 and Smad3 in transforming growth factor-beta-induced transcription of the Smad7 gene. *J. Biol. Chem.* **275**, 33205-33208.
- Huminiacki, L., Goldovsky, L., Freilich, S., Moustakas, A., Ouzounis, C. and Heldin, C. H.** (2009). Emergence, development and diversification of the TGF $\beta$  signalling pathway within the animal kingdom. *BMC Evol. Biol.* **9**, 28.
- Hussein, S. M., Duff, E. K. and Sirard, C.** (2003). Smad4 and  $\beta$ -catenin co-activators functionally interact with lymphoid-enhancing factor to regulate graded expression of Msx2. *J. Biol. Chem.* **278**, 48805-48814.
- Ikushima, H., Komuro, A., Isogaya, K., Shinozaki, M., Hellman, U., Miyazawa, K. and Miyazono, K.** (2008). An Id-like molecule, HHM, is a synexpression group-restricted regulator of TGF $\beta$  signalling. *EMBO J.* **27**, 2955-2965.
- Imoto, S., Ohbayashi, N., Ikeda, O., Kamitani, S., Muromoto, R., Sekine, Y. and Matsuda, T.** (2008). Sumoylation of Smad3 stimulates its nuclear export during PI3K-mediated suppression of TGF $\beta$  signaling. *Biochem. Biophys. Res. Commun.* **370**, 359-365.
- Ishimura, A., Ng, J. K., Taira, M., Young, S. G. and Osada, S.** (2006). Man1, an inner nuclear membrane protein, regulates vascular remodeling by modulating transforming growth factor  $\beta$  signaling. *Development* **133**, 3919-3928.
- Ishimura, A., Chida, S. and Osada, S.** (2008). Man1, an inner nuclear membrane protein, regulates left-right axis formation by controlling nodal signaling in a node-independent manner. *Dev. Dyn.* **237**, 3565-3576.
- Itoh, F., Itoh, S., Goumans, M. J., Valdimarsdottir, G., Iso, T., Dotto, G. P., Hamamori, Y., Kedes, L., Kato, M. and ten Dijke, P.** (2004). Synergy and antagonism between Notch and BMP receptor signaling pathways in endothelial cells. *EMBO J.* **23**, 541-551.
- Itoh, S. and ten Dijke, P.** (2007). Negative regulation of TGF $\beta$  receptor/Smad signal transduction. *Curr. Opin. Cell Biol.* **19**, 176-184.
- Jiang, X., Xia, L., Chen, D., Yang, Y., Huang, H., Yang, L., Zhao, Q., Shen, L. and Wang, J.** (2008). Otefin, a nuclear membrane protein, determines the fate of germline stem cells in *Drosophila* via interaction with Smad complexes. *Dev. Cell* **14**, 494-506.
- Jin, Q., Ding, W. and Mulder, K. M.** (2007). Requirement for the dynein light chain km23-1 in a Smad2-dependent transforming growth factor- $\beta$  signaling pathway. *J. Biol. Chem.* **282**, 19122-19132.
- Jin, W., Takagi, T., Kanesashi, S. N., Kurahashi, T., Nomura, T., Harada, J. and Ishii, S.** (2006). Schnurri-2 controls BMP-dependent adipogenesis via interaction with Smad proteins. *Dev. Cell* **10**, 461-471.
- Kahlem, P. and Newfeld, S. J.** (2009). Informatics approaches to understanding TGF $\beta$  pathway regulation. *Development* **136**, 3729-3740.
- Kajino, T., Omori, E., Ishii, S., Matsumoto, K. and Ninomiya-Tsuji, J.** (2007). TAK1 MAPK kinase mediates transforming growth factor- $\beta$  signaling by targeting SnO oncoprotein for degradation. *J. Biol. Chem.* **282**, 9475-9481.
- Kalkan, T., Iwasaki, Y., Park, C. Y. and Thomsen, G. H.** (2009). TRAF4 is a positive regulator of TGF $\beta$  signaling that affects neural crest formation. *Mol. Biol. Cell* **20**, 3436-3450.
- Kalo, E., Buganim, Y., Shapira, K. E., Besserglick, H., Goldfinger, N., Weisz, L., Stambolsky, P., Henis, Y. I. and Rotter, V.** (2007). Mutant p53 attenuates

- the SMAD-dependent transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) signaling pathway by repressing the expression of TGF- $\beta$  receptor type II. *Mol. Cell. Biol.* **27**, 8228-8242.
- Kamiya, Y., Miyazono, K. and Miyazawa, K. (2008). Specificity of the inhibitory effects of Dad on TGF- $\beta$  family type I receptors, Thickveins, Saxophone, and Baboon in *Drosophila*. *FEBS Lett.* **582**, 2496-2500.
- Kang, J. S., Saunier, E. F., Akhurst, R. J. and Derynck, R. (2008). The type I TGF- $\beta$  receptor is covalently modified and regulated by sumoylation. *Nat. Cell Biol.* **10**, 654-664.
- Kang, Y., Chen, C. R. and Massagué, J. (2003). A self-enabling TGF $\beta$  response coupled to stress signaling: Smad engages stress response factor ATF3 for Id1 repression in epithelial cells. *Mol. Cell* **11**, 915-926.
- Karaulanov, E., Knochel, W. and Niehrs, C. (2004). Transcriptional regulation of BMP4 synexpression in transgenic *Xenopus*. *EMBO J.* **23**, 844-856.
- Kardassis, D., Murphy, C., Fotsis, T., Moustakas, A. and Stournaras, C. (2009). Control of transforming growth factor  $\beta$  signal transduction by small GTPases. *FEBS J.* **276**, 2947-2965.
- Katagiri, T., Imada, M., Yanai, T., Suda, T., Takahashi, N. and Kamijo, R. (2002). Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis. *Genes Cells* **7**, 949-960.
- Kato, Y., Habas, R., Katsuyama, Y., Näär, A. M. and He, X. (2002). A component of the ARC/Mediator complex required for TGF $\beta$ /Nodal signalling. *Nature* **418**, 641-646.
- Koinuma, D., Shinozaki, M., Komuro, A., Goto, K., Saitoh, M., Hanyu, A., Ebina, M., Nukiwa, T., Miyazawa, K., Imamura, T. et al. (2003). Arkadia amplifies TGF- $\beta$  superfamily signalling through degradation of Smad7. *EMBO J.* **22**, 6458-6470.
- Koinuma, D., Tsutsumi, S., Kamimura, N., Taniguchi, H., Miyazawa, K., Sunamura, M., Imamura, T., Miyazono, K. and Aburatani, H. (2009). Chromatin immunoprecipitation on microarray analysis of Smad2/3 binding sites reveals roles of ETS1 and TFAP2A in transforming growth factor  $\beta$  signaling. *Mol. Cell. Biol.* **29**, 172-186.
- Korchynskyi, O. and ten Dijke, P. (2002). Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. *J. Biol. Chem.* **277**, 4883-4891.
- Korpala, M., Lee, E. S., Hu, G. and Kang, Y. (2008). The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J. Biol. Chem.* **283**, 14910-14914.
- Kowanetz, M., Valcourt, U., Bergström, R., Heldin, C. H. and Moustakas, A. (2004). Id2 and Id3 define the potency of cell proliferation and differentiation responses to transforming growth factor  $\beta$  and bone morphogenetic protein. *Mol. Cell. Biol.* **24**, 4241-4254.
- Kowanetz, M., Lönn, P., Vanlandewijck, M., Kowanetz, K., Heldin, C. H. and Moustakas, A. (2008). TGF $\beta$  induces SIK to negatively regulate type I receptor kinase signaling. *J. Cell Biol.* **182**, 655-662.
- Ku, M., Howard, S., Ni, W., Lagna, G. and Hata, A. (2006). OAZ regulates bone morphogenetic protein signaling through Smad6 activation. *J. Biol. Chem.* **281**, 5277-5287.
- Labbé, 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. USA* **97**, 8358-8363.
- Le Good, J. A., Joubin, C., Giraldez, A. J., Ben-Haim, N., Beck, S., Chen, Y., Schier, A. F. and Constam, D. B. (2005). Nodal stability determines signaling range. *Curr. Biol.* **15**, 31-36.
- Le Scolan, E., Zhu, Q., Wang, L., Bandyopadhyay, A., Javelaud, D., Mauviel, A., Sun, L. and Luo, K. (2008). Transforming growth factor- $\beta$  suppresses the ability of Ski to inhibit tumor metastasis by inducing its degradation. *Cancer Res.* **68**, 3277-3285.
- Lee, H. H. and Frasch, M. (2005). Nuclear integration of positive Dpp signals, antagonistic Wg inputs and mesodermal competence factors during *Drosophila* visceral mesoderm induction. *Development* **132**, 1429-1442.
- Lee, H. J., Yun, C. H., Lim, S. H., Kim, B. C., Baik, K. G., Kim, J. M., Kim, W. H. and Kim, S. J. (2007). SRF is a nuclear repressor of Smad3-mediated TGF- $\beta$  signaling. *Oncogene* **26**, 173-185.
- Lee, M. K., Pardoux, C., Hall, M. C., Lee, P. S., Warburton, D., Qing, J., Smith, S. M. and Derynck, R. (2007). TGF- $\beta$  activates Erk MAP kinase signalling through direct phosphorylation of ShcA. *EMBO J.* **26**, 3957-3967.
- Lee-Hoeflich, S. T., Zhao, X., Mehra, A. and Attisano, L. (2005). The *Drosophila* type II receptor, Wishful thinking, binds BMP and myoglianin to activate multiple TGF $\beta$  family signaling pathways. *FEBS Lett.* **579**, 4615-4621.
- Lei, S., Dubeykovskiy, A., Chakladar, A., Wojtukiewicz, L. and Wang, T. C. (2004). The murine gastrin promoter is synergistically activated by transforming growth factor- $\beta$ /Smad and Wnt signaling pathways. *J. Biol. Chem.* **279**, 42492-42502.
- Levy, L., Howell, M., Das, D., Harkin, S., Episkopou, V. and Hill, C. S. (2007). Arkadia activates Smad3/Smad4-dependent transcription by triggering signal-induced SnoN degradation. *Mol. Cell. Biol.* **27**, 6068-6083.
- Li, C., Zhu, N. L., Tan, R. C., Ballard, P. L., Derynck, R. and Minoo, P. (2002). Transforming growth factor- $\beta$  inhibits pulmonary surfactant protein B gene transcription through SMAD3 interactions with NKX2.1 and HNF-3 transcription factors. *J. Biol. Chem.* **277**, 38399-38408.
- Li, Z., Hassan, M. Q., Volinia, S., van Wijnen, A. J., Stein, J. L., Croce, C. M., Lian, J. B. and Stein, G. S. (2008). A microRNA signature for a BMP2-induced osteoblast lineage commitment program. *Proc. Natl. Acad. Sci. USA* **105**, 13906-13911.
- Liang, J., Lints, R., Foehr, M. L., Tokarz, R., Yu, L., Emmons, S. W., Liu, J. and Savage-Dunn, C. (2003). The *Caenorhabditis elegans schnurri* homolog *sma-9* mediates stage- and cell type-specific responses to DBL-1 BMP-related signaling. *Development* **130**, 6453-6464.
- Liang, J., Yu, L., Yin, J. and Savage-Dunn, C. (2007). Transcriptional repressor and activator activities of SMA-9 contribute differentially to BMP-related signaling outputs. *Dev. Biol.* **305**, 714-725.
- Lin, F., Morrison, J. M., Wu, W. and Worman, H. J. (2005). MAN1, an integral protein of the inner nuclear membrane, binds Smad2 and Smad3 and antagonizes transforming growth factor- $\beta$  signaling. *Hum. Mol. Genet.* **14**, 437-445.
- Lin, H. K., Bergmann, S. and Pandolfi, P. P. (2004). Cytoplasmic PML function in TGF- $\beta$  signalling. *Nature* **431**, 205-211.
- Liu, D., Kang, J. S. and Derynck, R. (2004). TGF- $\beta$ -activated Smad3 represses MEF2-dependent transcription in myogenic differentiation. *EMBO J.* **23**, 1557-1566.
- Liu, I. M., Schilling, S. H., Knouse, K. A., Choy, L., Derynck, R. and Wang, X. F. (2008). TGF $\beta$ -stimulated Smad1/5 phosphorylation requires the ALK5 L45 loop and mediates the pro-migratory TGF $\beta$  switch. *EMBO J.* **28**, 88-98.
- Lönn, P., Morén, A., Raja, E., Dahl, M. and Moustakas, A. (2009). Regulating the stability of TGF $\beta$  receptors and Smads. *Cell Res.* **19**, 21-35.
- Lopez-Rovira, T., Chaloux, E., Rosa, J. L., Bartrons, R. and Ventura, F. (2000). Interaction and functional cooperation of NF- $\kappa$ B with Smads. Transcriptional regulation of the junB promoter. *J. Biol. Chem.* **275**, 28937-28946.
- Lopez-Rovira, T., Chaloux, E., Massagué, J., Rosa, J. L. and Ventura, F. (2002). Direct binding of Smad1 and Smad4 to two distinct motifs mediates bone morphogenetic protein-specific transcriptional activation of Id1 gene. *J. Biol. Chem.* **277**, 3176-3185.
- Maduzia, L. L., Roberts, A. F., Wang, H., Lin, X., Chin, L. J., Zimmerman, C. M., Cohen, S., Feng, X. H. and Padgett, R. W. (2005). *C. elegans* serine-threonine kinase KIN-29 modulates TGF $\beta$  signaling and regulates body size formation. *BMC Dev. Biol.* **5**, 8.
- Major, M. B. and Jones, D. A. (2004). Identification of a gadd45 $\beta$  3' enhancer that mediates SMAD3- and SMAD4-dependent transcriptional induction by transforming growth factor  $\beta$ . *J. Biol. Chem.* **279**, 5278-5287.
- Marinari, B., Moretti, F., Botti, E., Giustizieri, M. L., Descargues, P., Giunta, A., Stolfi, C., Ballaro, C., Papoutsaki, M., Alema, S. et al. (2008). The tumor suppressor activity of IKK $\alpha$  in stratified epithelia is exerted in part via the TGF- $\beta$  antiproliferative pathway. *Proc. Natl. Acad. Sci. USA* **105**, 17091-17096.
- Martello, G., Zacchigna, L., Inui, M., Montagner, M., Adorno, M., Mamidi, A., Morsut, L., Soligo, S., Tran, U., Dupont, S. et al. (2007). MicroRNA control of Nodal signalling. *Nature* **449**, 183-188.
- Marty, T., Muller, B., Basler, K. and Affolter, M. (2000). Schnurri mediates Dpp-dependent repression of brinker transcription. *Nat. Cell Biol.* **2**, 745-749.
- Massagué, J. (2008). TGF $\beta$  in cancer. *Cell* **134**, 215-230.
- Massagué, J., Seoane, J. and Wotton, D. (2005). Smad transcription factors. *Genes Dev.* **19**, 2783-2810.
- Mavrikakis, K. J., Andrew, R. L., Lee, K. L., Petropoulou, C., Dixon, J. E., Navaratnam, N., Norris, D. P. and Episkopou, V. (2007). Arkadia enhances Nodal/TGF- $\beta$  signaling by coupling phospho-Smad2/3 activity and turnover. *PLoS Biol.* **5**, e67.
- Messenger, N. J., Kabitschke, C., Andrews, R., Grimmer, D., Nunez Miguel, R., Blundell, T. L., Smith, J. C. and Wardle, F. C. (2005). Functional specificity of the *Xenopus* T-domain protein Brachyury is conferred by its ability to interact with Smad1. *Dev. Cell* **8**, 599-610.
- Miles, W. O., Jaffray, E., Campbell, S. G., Takeda, S., Bayston, L. J., Basu, S. P., Li, M., Raftery, L. A., Ashe, M. P., Hay, R. T. et al. (2008). Meada SUMOylation restricts the signaling range of the Dpp morphogen in the *Drosophila* embryo. *Genes Dev.* **22**, 2578-2590.
- Mochizuki, T., Miyazaki, H., Hara, T., Furuya, T., Imamura, T., Watabe, T. and Miyazono, K. (2004). Roles for the MH2 domain of Smad7 in the specific inhibition of transforming growth factor- $\beta$  superfamily signaling. *J. Biol. Chem.* **279**, 31568-31574.
- Morén, A., Hellman, U., Inada, Y., Imamura, T., Heldin, C. H. and Moustakas, A. (2003). Differential ubiquitination defines the functional status of the tumor suppressor Smad4. *J. Biol. Chem.* **278**, 33571-33582.
- Moustakas, A. and Heldin, C. H. (2005). Non-Smad TGF- $\beta$  signals. *J. Cell Sci.* **118**, 3573-3584.
- Moustakas, A. and Heldin, C. H. (2007). Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. *Cancer Sci.* **98**, 1512-1520.
- Moustakas, A. and Heldin, C. H. (2008). Dynamic control of TGF- $\beta$  signaling and its links to the cytoskeleton. *FEBS Lett.* **582**, 2051-2065.

- Nagano, Y., Mavrikis, K. J., Lee, K. L., Fujii, T., Koinuma, D., Sase, H., Yuki, K., Isogaya, K., Saitoh, M., Imamura, T. et al. (2007). Arkadia induces degradation of SnoN and c-Ski to enhance transforming growth factor- $\beta$  signaling. *J. Biol. Chem.* **282**, 20492-20501.
- Nakashima, A., Katagiri, T. and Tamura, M. (2005). Cross-talk between Wnt and bone morphogenetic protein 2 (BMP-2) signaling in differentiation pathway of C2C12 myoblasts. *J. Biol. Chem.* **280**, 37660-37668.
- Nguyen, M., Parker, L. and Arora, K. (2000). Identification of maverick, a novel member of the TGF- $\beta$  superfamily in *Drosophila*. *Mech. Dev.* **95**, 201-206.
- Niehrs, C. and Pollet, N. (1999). Synexpression groups in eukaryotes. *Nature* **402**, 483-487.
- Niimi, H., Pardali, K., Vanlandewijck, M., Heldin, C. H. and Moustakas, A. (2007). Notch signaling is necessary for epithelial growth arrest by TGF- $\beta$ . *J. Cell Biol.* **176**, 695-707.
- 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**, 781-785.
- Ogawa, K., Saito, A., Matsui, H., Suzuki, H., Ohtsuka, S., Shimosato, D., Morishita, Y., Watabe, T., Niwa, H. and Miyazono, K. (2007). Activin-Nodal signaling is involved in propagation of mouse embryonic stem cells. *J. Cell Sci.* **120**, 55-65.
- Onuma, Y., Takahashi, S., Yokota, C. and Asashima, M. (2002). Multiple nodal-related genes act coordinately in *Xenopus* embryogenesis. *Dev. Biol.* **241**, 94-105.
- Osada, S., Ohmori, S. Y. and Taira, M. (2003). XMAN1, an inner nuclear membrane protein, antagonizes BMP signaling by interacting with Smad1 in *Xenopus* embryos. *Development* **130**, 1783-1794.
- Osada, S. I., Saijoh, Y., Frisch, A., Yeo, C. Y., Adachi, H., Watanabe, M., Whitman, M., Hamada, H. and Wright, C. V. (2000). Activin/nodal responsiveness and asymmetric expression of a *Xenopus* nodal-related gene converge on a FAST-regulated module in intron 1. *Development* **127**, 2503-2514.
- Ozdamar, B., Bose, R., Barrios-Rodiles, M., Wang, H. R., Zhang, Y. and Wrana, J. L. (2005). Regulation of the polarity protein Par6 by TGF $\beta$  receptors controls epithelial cell plasticity. *Science* **307**, 1603-1609.
- Pan, D., Estevez-Salmeron, L. D., Stroschein, S. L., Zhu, X., He, J., Zhou, S. and Luo, K. (2005). The integral inner nuclear membrane protein MAN1 physically interacts with the R-Smad proteins to repress signaling by the transforming growth factor- $\beta$  superfamily of cytokines. *J. Biol. Chem.* **280**, 15992-16001.
- Pardali, E., Xie, X. Q., Tzapogas, P., Itoh, S., Arvanitidis, K., Heldin, C. H., ten Dijke, P., Grundstrom, T. and Sideras, P. (2000). Smad and AML proteins synergistically confer transforming growth factor  $\beta$ 1 responsiveness to human germ-line IgA genes. *J. Biol. Chem.* **275**, 3552-3560.
- Pardali, K., Kurisaki, A., Morén, A., ten Dijke, P., Kardassis, D. and Moustakas, A. (2000). Role of Smad proteins and transcription factor Sp1 in p21<sup>Waf1/Cip1</sup> regulation by transforming growth factor- $\beta$ . *J. Biol. Chem.* **275**, 29244-29256.
- Patterson, G. I. and Padgett, R. W. (2000). TGF  $\beta$ -related pathways. Roles in *Caenorhabditis elegans* development. *Trends Genet.* **16**, 27-33.
- Patterson, G. I., Koweeck, A., Wong, A., Liu, Y. and Ruvkun, G. (1997). The DAF-3 Smad protein antagonizes TGF- $\beta$ -related receptor signaling in the *Caenorhabditis elegans* dauer pathway. *Genes Dev.* **11**, 2679-2690.
- Peñuelas, S., Anido, J., Prieto-Sánchez, R. M., Folch, G., Barba, I., Cuartas, I., García-Dorado, D., Poca, M. A., Sahuquillo, J., Baselga, J. et al. (2009). TGF- $\beta$  increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell* **15**, 315-327.
- Pyrowolakis, G., Hartmann, B., Muller, B., Basler, K. and Affolter, M. (2004). A simple molecular complex mediates widespread BMP-induced repression during *Drosophila* development. *Dev. Cell* **7**, 229-240.
- Qiu, P., Feng, X. H. and Li, L. (2003). Interaction of Smad3 and SRF-associated complex mediates TGF- $\beta$ 1 signals to regulate SM22 transcription during myofibroblast differentiation. *J. Mol. Cell. Cardiol.* **35**, 1407-1420.
- Raju, G. P., Dimova, N., Klein, P. S. and Huang, H. C. (2003). SANE, a novel LEM domain protein, regulates bone morphogenetic protein signaling through interaction with Smad1. *J. Biol. Chem.* **278**, 428-437.
- Ramis, J. M., Collart, C. and Smith, J. C. (2007). Xnrs and activin regulate distinct genes during *Xenopus* development: activin regulates cell division. *PLoS ONE* **2**, e213.
- Rifkin, D. B. (2005). Latent transforming growth factor- $\beta$  (TGF- $\beta$ ) binding proteins: orchestrators of TGF- $\beta$  availability. *J. Biol. Chem.* **280**, 7409-7412.
- Rossi, D. J., Jamieson, C. H. and Weissman, I. L. (2008). Stems cells and the pathways to aging and cancer. *Cell* **132**, 681-696.
- Saijoh, Y., Adachi, H., Mochida, K., Ohishi, S., Hirao, A. and Hamada, H. (1999). Distinct transcriptional regulatory mechanisms underlie left-right asymmetric expression of lefty-1 and lefty-2. *Genes Dev.* **13**, 259-269.
- Saka, Y., Hagemann, A. I., Piepenburg, O. and Smith, J. C. (2007). Nuclear accumulation of Smad complexes occurs only after the midblastula transition in *Xenopus*. *Development* **134**, 4209-4218.
- Sanchez-Elsner, T., Botella, L. M., Velasco, B., Corbi, A., Attisano, L. and Bernabeu, C. (2001). Synergistic cooperation between hypoxia and transforming growth factor- $\beta$  pathways on human vascular endothelial growth factor gene expression. *J. Biol. Chem.* **276**, 38527-38535.
- Sapkota, G., Alarcon, C., Spagnoli, F. M., Brivanlou, A. H. and Massagué, J. (2007). Balancing BMP signaling through integrated inputs into the Smad1 linker. *Mol. Cell* **25**, 441-454.
- Sasai, N., Yakura, R., Kamiya, D., Nakazawa, Y. and Sasai, Y. (2008). Ectodermal factor restricts mesoderm differentiation by inhibiting p53. *Cell* **133**, 878-890.
- Savage-Dunn, C. (2005). TGF- $\beta$  signaling. *WormBook*, 1-12. <http://www.wormbook.org/>.
- Savage-Dunn, C., Maduzia, L. L., Zimmerman, C. M., Roberts, A. F., Cohen, S., Tokarz, R. and Padgett, R. W. (2003). Genetic screen for small body size mutants in *C. elegans* reveals many TGF $\beta$  pathway components. *Genesis* **35**, 239-247.
- Schmierer, B. and Hill, C. S. (2007). TGF $\beta$ -SMAD signal transduction: molecular specificity and functional flexibility. *Nat. Rev. Mol. Cell Biol.* **8**, 970-982.
- Schmierer, B., Tournier, A. L., Bates, P. A. and Hill, C. S. (2008). Mathematical modeling identifies Smad nucleocytoplasmic shuttling as a dynamic signal-interpreting system. *Proc. Natl. Acad. Sci. USA* **105**, 6608-6613.
- Seoane, J., Pouponnot, C., Staller, P., Schader, M., Eilers, M. and Massagué, J. (2001). TGF $\beta$  influences Myc, Miz-1 and Smad to control the CDK inhibitor p15<sup>INK4b</sup>. *Nat. Cell Biol.* **3**, 400-408.
- Seoane, J., Le, H. V., Shen, L., Anderson, S. A. and Massagué, J. (2004). Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. *Cell* **117**, 211-223.
- Serpe, M., Ralston, A., Blair, S. S. and O'Connor, M. B. (2005). Matching catalytic activity to developmental function: tolloid-related processes Sog in order to help specify the posterior crossvein in the *Drosophila* wing. *Development* **132**, 2645-2656.
- Shakéd, M., Weissmüller, K., Svoboda, H., Hortschansky, P., Nishino, N., Wolff, S. and Tucker, K. L. (2008). Histone deacetylases control neurogenesis in embryonic brain by inhibition of BMP2/4 signaling. *PLoS ONE* **3**, e2668.
- Shen, J., Dorner, C., Bahlo, A. and Pflügfelder, G. O. (2008). optomotor-blind suppresses instability at the A/P compartment boundary of the *Drosophila* wing. *Mech. Dev.* **125**, 233-246.
- Shi, W., Chang, C., Nie, S., Xie, S., Wan, M. and Cao, X. (2007). Endofin acts as a Smad anchor for receptor activation in BMP signaling. *J. Cell Sci.* **120**, 1216-1224.
- Shimizu, K., Bourillot, P. Y., Nielsen, S. J., Zorn, A. M. and Gurdon, J. B. (2001). Swift is a novel BRCT domain coactivator of Smad2 in transforming growth factor  $\beta$  signaling. *Mol. Cell Biol.* **21**, 3901-3912.
- Shiratori, H., Sakuma, R., Watanabe, M., Hashiguchi, H., Mochida, K., Sakai, Y., Nishino, J., Saijoh, Y., Whitman, M. and Hamada, H. (2001). Two-step regulation of left-right asymmetric expression of Pitx2: initiation by nodal signaling and maintenance by Nkx2. *Mol. Cell* **7**, 137-149.
- Simonsson, M., Kanduri, M., Grönroos, E., Heldin, C.-H. and Ericsson, J. (2006). The DNA binding activities of Smad2 and Smad3 are regulated by coactivator-mediated acetylation. *J. Biol. Chem.* **281**, 39870-39880.
- Sivasankaran, R., Vignano, M. A., Muller, B., Affolter, M. and Basler, K. (2000). Direct transcriptional control of the Dpp target omb by the DNA binding protein Brinker. *EMBO J.* **19**, 6162-6172.
- Smith, J. C. and Gurdon, J. B. (2004). Many ways to make a gradient. *BioEssays* **26**, 705-706.
- Sorrentino, A., Thakur, N., Grimsby, S., Marcusson, A., von Bulow, V., Schuster, N., Zhang, S., Heldin, C. H. and Landström, M. (2008). The type I TGF- $\beta$  receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nat. Cell Biol.* **10**, 1199-1207.
- Sowa, H., Kaji, H., Hendy, G. N., Canaff, L., Komori, T., Sugimoto, T. and Chihara, K. (2004). Menin is required for bone morphogenetic protein 2- and transforming growth factor  $\beta$ -regulated osteoblastic differentiation through interaction with Smads and Runx2. *J. Biol. Chem.* **279**, 40267-40275.
- Su, Y., Zhang, L., Gao, X., Meng, F., Wen, J., Zhou, H., Meng, A. and Chen, Y. G. (2007). The evolutionarily conserved activity of Dapper2 in antagonizing TGF- $\beta$  signaling. *FASEB J.* **21**, 682-690.
- Sun, Q., Zhang, Y., Yang, G., Chen, X., Cao, G., Wang, J., Sun, Y., Zhang, P., Fan, M., Shao, N. et al. (2008). Transforming growth factor- $\beta$ -regulated miR-24 promotes skeletal muscle differentiation. *Nucleic Acids Res.* **36**, 2690-2699.
- Suzuki, A., Raya, A., Kawakami, Y., Morita, M., Matsui, T., Nakashima, K., Gage, F. H., Rodriguez-Esteban, C. and Izpisua Belmonte, J. C. (2006). Nanog binds to Smad1 and blocks bone morphogenetic protein-induced differentiation of embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **103**, 10294-10299.
- Takebayashi-Suzuki, K., Funami, J., Tokumori, D., Saito, A., Watabe, T., Miyazono, K., Kanda, A. and Suzuki, A. (2003). Interplay between the tumor suppressor p53 and TGF  $\beta$  signaling shapes embryonic body axes in *Xenopus*. *Development* **130**, 3929-3939.
- ten Dijke, P. and Heldin, C. H. (2006). The Smad family. In *Smad Signal Transduction*, vol. 5 (ed. P. ten Dijke and C. H. Heldin), pp. 1-13. Dordrecht, The Netherlands: Springer.

- ten Dijke, P. and Arthur, H. M. (2007). Extracellular control of TGF $\beta$  signalling in vascular development and disease. *Nat. Rev. Mol. Cell Biol.* **8**, 857-869.
- Thuault, S., Tan, E. J., Peinado, H., Cano, A., Heldin, C. H. and Moustakas, A. (2008). HMG2 and Smads co-regulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. *J. Biol. Chem.* **283**, 33437-33446.
- 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 $\beta$  receptor. *Cell* **95**, 779-791.
- Tsuneizumi, K., 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.
- Tu, A. W. and Luo, K. (2007). Acetylation of Smad2 by the co-activator p300 regulates activin and transforming growth factor  $\beta$  response. *J. Biol. Chem.* **282**, 21187-21196.
- Umulis, D., O'Connor, M. B. and Blair, S. S. (2009). The extracellular regulation of bone morphogenetic protein signaling. *Development* **136**, 3715-3728.
- Valcourt, U., Thuault, S., Pardali, K., Heldin, C. H. and Moustakas, A. (2007). Functional role of Meox2 during the epithelial cytoskeletal response to TGF- $\beta$ . *Mol. Oncol.* **1**, 55-71.
- Van Beek, J. P., Kennedy, L., Rockel, J. S., Bernier, S. M. and Leask, A. (2006). The induction of CCN2 by TGF $\beta$ 1 involves Ets-1. *Arthritis Res. Ther.* **8**, R36.
- Varelas, X., Sakuma, R., Samavarchi-Tehrani, P., Peerani, R., Rao, B. M., Dembowy, J., Yaffe, M. B., Zandstra, P. W. and Wrana, J. L. (2008). TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. *Nat. Cell Biol.* **10**, 837-848.
- Wang, B., Suzuki, H. and Kato, M. (2008). Roles of mono-ubiquitinated Smad4 in the formation of Smad transcriptional complexes. *Biochem. Biophys. Res. Commun.* **376**, 288-292.
- Wang, G., Matsuura, I., He, D. and Liu, F. (2009). Transforming growth factor- $\beta$ -inducible phosphorylation of Smad3. *J. Biol. Chem.* **284**, 9663-9673.
- Wang, Q., Wei, X., Zhu, T., Zhang, M., Shen, R., Xing, L., O'Keefe, R. J. and Chen, D. (2007). Bone morphogenetic protein 2 activates Smad6 gene transcription through bone-specific transcription factor Runx2. *J. Biol. Chem.* **282**, 10742-10748.
- Watabe, T. and Miyazono, K. (2009). Roles of TGF- $\beta$  family signaling in stem cell renewal and differentiation. *Cell Res.* **19**, 103-115.
- Watanabe, M., Rebbert, M. L., Andreazzoli, M., Takahashi, N., Toyama, R., Zimmerman, S., Whitman, M. and Dawid, I. B. (2002). Regulation of the *Lim-1* gene is mediated through conserved FAST-1/FoxH1 sites in the first intron. *Dev. Dyn.* **225**, 448-456.
- Wharton, K. and Derynck, R. (2009). TGF $\beta$  family signaling: novel insights in development and disease. *Development* **136**, 3691-3697.
- Wilkinson, D. S., Tsai, W. W., Schumacher, M. A. and Barton, M. C. (2008). Chromatin-bound p53 anchors activated Smads and the mSin3A corepressor to confer transforming-growth-factor- $\beta$ -mediated transcription repression. *Mol. Cell Biol.* **28**, 1988-1998.
- Wolfman, N. M., McPherron, A. C., Pappano, W. N., Davies, M. V., Song, K., Tomkinson, K. N., Wright, J. F., Zhao, L., Sebald, S. M., Greenspan, D. S. et al. (2003). Activation of latent myostatin by the BMP-1/tolloid family of metalloproteinases. *Proc. Natl. Acad. Sci. USA* **100**, 15842-15846.
- Wrana, J. L., Ozdamar, B., Le Roy, C. and Benchabane, H. (2008). Signaling receptors of the TGF- $\beta$  family. In *The TGF- $\beta$  Family* (ed. R. Derynck and K. Miyazono), pp. 179-202. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Wrighton, K. H., Lin, X. and Feng, X. H. (2008). Critical regulation of TGF $\beta$  signaling by Hsp90. *Proc. Natl. Acad. Sci. USA* **105**, 9244-9249.
- Wrighton, K. H., Lin, X. and Feng, X. H. (2009). Phospho-control of TGF- $\beta$  superfamily signaling. *Cell Res.* **19**, 8-20.
- Xi, Q., He, W., Zhang, X. H., Le H. V. and Massagué, J. (2008). Genome-wide impact of the BRG1 SWI/SNF chromatin remodeler on the transforming growth factor  $\beta$  transcriptional program. *J. Biol. Chem.* **283**, 1146-1155.
- Xu, L., Yao, X., Chen, X., Lu, P., Zhang, B. and Ip, Y. T. (2007). Msk is required for nuclear import of TGF- $\beta$ /BMP-activated Smads. *J. Cell Biol.* **178**, 981-994.
- Xu, M., Kirov, N. and Rushlow, C. (2005). Peak levels of BMP in the *Drosophila* embryo control target genes by a feed-forward mechanism. *Development* **132**, 1637-1647.
- Xu, R. H., Sampsel-Barron, T. L., Gu, F., Root, S., Peck, R. M., Pan, G., Yu, J., Antosiewicz-Bourget, J., Tian, S., Stewart, R. et al. (2008). NANOG is a direct target of TGF $\beta$ /activin-mediated SMAD signaling in human ESCs. *Cell Stem Cell* **3**, 196-206.
- Xu, X., Yin, Z., Hudson, J. B., Ferguson, E. L. and Frasch, M. (1998). Smad proteins act in combination with synergistic and antagonistic regulators to target Dpp responses to the *Drosophila* mesoderm. *Genes Dev.* **12**, 2354-2370.
- Xu, X., Han, J., Ito, Y., Bringas, P., Jr, Deng, C. and Chai, Y. (2008). Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF- $\beta$ /BMP signaling during tooth and palate development. *Dev. Cell* **15**, 322-329.
- Yamashita, M., Fatyol, K., Jin, C., Wang, X., Liu, Z. and Zhang, Y. E. (2008). TRAF6 mediates Smad-independent activation of JNK and p38 by TGF- $\beta$ . *Mol. Cell* **31**, 918-924.
- Yang, G., Li, Y., Nishimura, E. K., Xin, H., Zhou, A., Guo, Y., Dong, L., Denning, M. F., Nickoloff, B. J. and Cui, R. (2008). Inhibition of PAX3 by TGF- $\beta$  modulates melanocyte viability. *Mol. Cell* **32**, 554-563.
- Yang, L. and Moses, H. L. (2008). Transforming growth factor  $\beta$ : tumor suppressor or promoter? Are host immune cells the answer? *Cancer Res.* **68**, 9107-9111.
- Yano, T., Ito, K., Fukamachi, H., Chi, X. Z., Wee, H. J., Inoue, K., Ida, H., Bouillet, P., Strasser, A., Bae, S. C. et al. (2006). The RUNX3 tumor suppressor upregulates Bim in gastric epithelial cells undergoing transforming growth factor  $\beta$ -induced apoptosis. *Mol. Cell Biol.* **26**, 4474-4488.
- Yao, X., Chen, X., Cottonham, C. and Xu, L. (2008). Preferential utilization of Imp7/8 in nuclear import of Smads. *J. Biol. Chem.* **283**, 22867-22874.
- Yoo, J., Ghiassi, M., Jirmanova, L., Balliet, A. G., Hoffman, B., Fornace, A. J., Jr, Liebermann, D. A., Böttinger, E. P. and Roberts, A. B. (2003). Transforming growth factor- $\beta$ -induced apoptosis is mediated by Smad-dependent expression of GADD45b through p38 activation. *J. Biol. Chem.* **278**, 43001-43007.
- Zavadil, J., Cermak, L., Soto-Nieves, N. and Böttinger, E. P. (2004). Integration of TGF- $\beta$ /Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. *EMBO J.* **23**, 1155-1165.
- Zavadil, J., Narasimhan, M., Blumenberg, M. and Schneider, R. J. (2007). Transforming growth factor- $\beta$  and microRNA:mRNA regulatory networks in epithelial plasticity. *Cells Tissues Organs* **185**, 157-161.
- Zhang, S., Fei, T., Zhang, L., Zhang, R., Chen, F., Ning, Y., Han, Y., Feng, X. H., Meng, A. and Chen, Y. G. (2007). Smad7 antagonizes transforming growth factor  $\beta$  signaling in the nucleus by interfering with functional Smad-DNA complex formation. *Mol. Cell Biol.* **27**, 4488-4499.
- Zhang, Y. and Derynck, R. (2000). Transcriptional regulation of the transforming growth factor- $\beta$ -inducible mouse germ line Ig  $\alpha$  constant region gene by functional cooperation of Smad, CREB, and AML family members. *J. Biol. Chem.* **275**, 16979-16985.
- Zhang, Y. W., Yasui, N., Ito, K., Huang, G., Fujii, M., Hanai, J., Nogami, H., Ochi, T., Miyazono, K. and Ito, Y. (2000). A RUNX2/PEBP2 $\alpha$ A/CBFA1 mutation displaying impaired transactivation and Smad interaction in cleidocranial dysplasia. *Proc. Natl. Acad. Sci. USA* **97**, 10549-10554.
- Zheng, X., Wang, J., Haerry, T. E., Wu, A. Y., Martin, J., O'Connor, M. B., Lee, C. H. and Lee, T. (2003). TGF- $\beta$  signaling activates steroid hormone receptor expression during neuronal remodeling in the *Drosophila* brain. *Cell* **112**, 303-315.
- Zhu, C. C., Boone, J. Q., Jensen, P. A., Hanna, S., Podemski, L., Locke, J., Doe, C. Q. and O'Connor, M. B. (2008). *Drosophila* Activin- and the Activin-like product Dawdle function redundantly to regulate proliferation in the larval brain. *Development* **135**, 513-521.
- Zhu, S., Wang, W., Clarke, D. C. and Liu, X. (2007). Activation of Mps1 promotes transforming growth factor- $\beta$ -independent Smad signaling. *J. Biol. Chem.* **282**, 18327-18338.