Minireview

New intracellular components of bone morphogenetic protein/Smad signaling cascades

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Abstract Bone morphogenetic proteins (BMPs) regulate many processes in the embryo, including cell type specification, patterning, apoptosis, and epithelial–mesenchymal interaction. They also act in soft and hard tissues in adult life. Their signals are transduced from the plasma membrane to the nucleus through a limited number of Smad proteins. The list of Smad-interacting proteins is however growing and it is clear that these partners determine the outcome of the signal. We summarize the present status in BMP/Smad signaling, with emphasis on recently identified Smad partners and how these proteins may cooperate in the regulation of the expression of BMP target genes.

Key words: Bone morphogenetic protein; Signal transduction; Smad; Transforming growth factor-β

1. Introduction

Bone morphogenetic proteins (BMPs) form a large subgroup within the transforming growth factor-β (TGF-β) family of growth and differentiation regulatory polypeptides. BMPs were originally identified as proteins capable of inducing ectopic cartilage and bone upon non-systemic injection in mammals [1]. Later, they were shown to be crucial regulators of early embryogenesis and subsequent organogenesis, and tissue homeostasis in the adult [2]. Like other members of the TGF-β family, BMPs elicit their effects through activation of combinations of two type I and two type II serine/threonine kinase receptors (Fig. 1). Type I receptors within such complexes act downstream of type II receptors and determine the specificity of the signal [3]. Three distinct type II receptors, BMP type II (BMPRII) and activin type IIA and IIB receptors (ActRIIA and ActRIIB), and three type I receptors (activin receptor-like kinases (or ALKs) ALK2, ALK3/BmprIA and ALK6/BmprIB) have been identified for ligands of the BMP subgroup. ALK3 and ALK6 are activated by BMP2, BMP4 and BMP7, whereas ALK2 binds BMP6 and BMP7 but neither BMP2 nor BMP4.

The type I receptors within the tetrameric receptor complex initiate signal propagation by phosphorylation of the receptor-activated Smads (R-Smads). In addition to the R-Smads, two other types of Smad have been identified, the common mediator Smads (co-Smads, Smad4, or Smad4α and 4β in *Xenopus laevis*) and the inhibitory Smads (I-Smads, Smad6 and Smad7). BMP receptors activate Smad1, Smad5 and Smad8 (named here BR-Smads), whereas Smad2 and Smad3 are phosphorylated by the activin or TGF-β receptors (AR-Smads). Remarkably, ALK3 and ALK6 can activate all three BR-Smads, but ALK2 only phosphorylates Smad1 and Smad5 [4]. The BR-Smads can however also transduce TGF-β signals from the ALK1 type I receptor (for TGF-β) in endothelium and hematopoietic cells [5]. Similarly, Müllerian inhibiting substance (MIS; or anti-Müllerian hormone, AMH) acts through MISRII (AMHRII) with ALK1 or ALK6 [6,7], and some BMP-like growth/differentiation factors (GDF5, 6 and 7) act through activated ALK6 [8]. For many ligands (e.g. BMP10 and 11, GDF1), it is still unclear through which receptors and R-Smads they signal, particularly in vivo.

Activated R-Smads assemble into heteromeric complexes with Smad4 in the cytoplasm and these accumulate in the nucleus (for recent insights into nucleocytoplasmic shuttling of Smads, see [9]) where they participate directly in the modulation of gene expression (Fig. 1). They do this by binding to DNA, interacting with DNA binding transcription factors, recruiting co-repressors or co-activators to specific promoters or mobilizing components of the chromatin-modifying machinery, or a combination of these modes [10,11](Table 1). BMPs (and TGF-βs) also initiate other non-Smad intracellular pathways, e.g. they can activate Jun N-terminal kinase (JNK), p38 kinase/Akt and PI3 kinase pathways, as well as caspases [12–14]. These pathways will not be discussed here.

2. Present status

Only five R-Smads mediate the majority of the complex responses elicited in different tissues by ligands of the TGF-β family. Depending on the cellular context, i.e. the spatial and temporal-specific cytoplasmic partners of Smads and,

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Abbreviations: ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; BRE, BMP response element; CIZ, Cas-interacting zinc finger protein; co-Smad, common mediator Smad; GDF, growth/differentiation factor; Id, inhibitor of differentiation; I-Smad, inhibitory Smad; R-Smad, receptor-activated Smad; SARA, Smad anchor for receptor activation; SANE, Smad1 antagonistic effector; SBE, Smad binding element; SNIP, Smad nuclear interacting protein; TGF-β, transforming growth factor-β; OAZ, Olf-1/EBF-associated zinc finger

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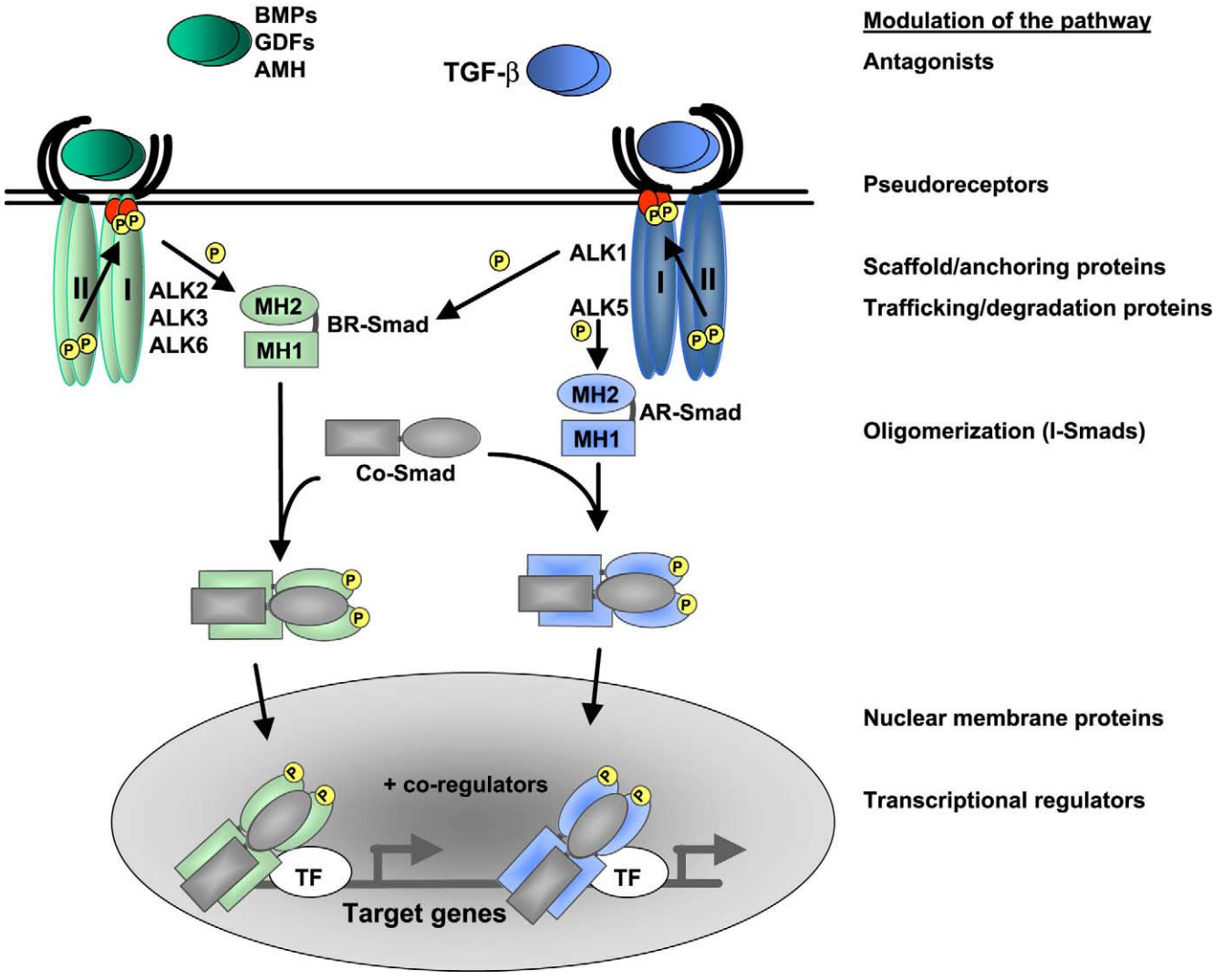

Fig. 1. BMP and TGF-β signaling pathways mediated by BR-Smads. Ligand-induced heteromeric receptor complex formation and phosphorylation of the type I receptor lead to phosphorylation of R-Smads. Activated R-Smads form complexes with co-Smad and accumulate in the nucleus, where they participate, together with transcription factors and co-regulators, in the transcriptional regulation of target genes. It is peculiar that TGF-β binds in endothelium in the presence of TβRII to ALK5 as well as to ALK1. The former activates the classical TGF-β/AR-Smad pathway, whereas ALK1 activates BR-Smads and transmits therefore BMP-like signals. Different levels of modulation of the BMP/BR-Smad signaling cascade are indicated schematically, see for details Table 1 and text.

likewise, nuclear transcription factors and cofactors, the transcriptional outcome of Smad activation can be specified and/or fine-tuned. Differences in stability of signaling components and their subcellular localization may as well affect the cellular response. In addition, the modulation of BMP bioavailability by BMP antagonists, or a pseudoreceptor (BAMBI; [15]) add to the attenuation or complete silencing of BMP elicited responses [16].

Smad proteins have three domains, the N-terminal Mad homology domain (MH1), a divergent proline-rich linker domain, and the C-terminal MH2 domain [13]. The MH2 domain, which is highly conserved among all Smads, is in R-Smads involved in type I receptor recognition and becomes directly phosphorylated in its C-terminal SSXS motif by type I receptors. This domain is also required for Smad oligomerization and interaction with Smad4, and is shown to interact with cytoplasmic adapters and nuclear transcription factors (Table 1). The MH1 domain is conserved among R- and co-Smads, binds to several cytoplasmic partners, and is required for nuclear import of Smads through its N-terminally located nuclear localization signal. The MH1 domain of some R-Smads has been shown to bind to DNA and to interact with several nuclear proteins (Table 1).

At present, about 65 AR-Smad-interacting proteins have been described [17,18], whereas few partners for BR-Smads are known (Table 1). The strikingly less charged surface of the BR-Smad trimeric complexes, as compared to AR-Smad trimers, has been proposed to render BR-Smads less efficient for interaction and therefore restrict the number of BR-Smad partners [17]. In addition, the most extensively BR-Smad that is being studied is Smad1, and not many of its partners have been tested for their interaction with Smad5 or Smad8 and for the biological consequences of this interaction.

3. Interaction of BR-Smads with proteins in the cytoplasm

Several accessory/scaffolding proteins, like SARA (Smad anchor for receptor activation), axin and disabled2 (Dab2),

Table 1
BR-Smad-interacting proteins

Function	MH1 domain	Smad 1	Smad 5	Smad 8	Linker domain	Smad 1	Smad 5	Smad 8	MH2 domain	Smad 1	Smad 5	Smad 8
Receptors									ALK1	■	■	■
									ALK2	■	■	□
									ALK3	■	■	■
									ALK6	■	■	■
Oligomerization									R-Smads, Co-Smad, I-Smads			
Cytoplasmic adapters, effectors	calmodulin	■			filamin	■	■		SANE	■	■	
	filamin	■	■		Hgs/Hrs	♦			HsN3	■		
	HEF1	♦			Smurf1	■	■		Az	■		
	Erbin	■			Smurf2	■						
	Dvl1	■										
	Par3	■										
Nuclear membrane proteins									MAN1	■	■	■
Transcriptional regulators	Hoxc8	■			*Gli3Δ-ter*	■			BF-1	■	♦	
	Vent2	■							CIZ	■	■	■
	Znf8	■	■		Hoxc8	■			E1A	■	■	
					JunB	♦	♦		Lef1/Tcf	♦	■	
					menin	■	■		OAZ	■		
					Ngn	■			p300/CBP	♦		
					Swift	♦			Runx1/PebpαB/CBFA2/AML1	■	■	
									Runx2/PebpαB/CBFA1/AML3	■	■	
									Runx3/PebpαC/CBFA3/AML2	■	■	
									c-Ski	♦	♦	
									SIP1	■	■	
									SNIP1	♦	□	
									TGIF	♦		
									Tob	■	■	■
									Znf8	■	■	

Table of the BR-Smad protein–protein interactions known to occur in each domain. Entries in more than one domain indicate interactions with the same factor at multiple domains. If the specific Smad domain that interacts with the protein is not yet determined, the protein is indicated in italic. The specificity of the interaction to Smad1, Smad5 or Smad8 is indicated by symbols: ■: positive interaction, ♦: weak interaction, □: no interaction, blank: not determined. Names and references of proteins not discussed in the text are: BF-1: brain factor 1 [64]; calmodulin [65]; E1A: early region [66]; GliΔ-ter [67]; HEF1: human enhancer of filamentation [68]; JunB [69]; Lef1/Tcf: lymphoid enhancer binding factor 1/T-cell factor [70]; Ngn1: neurogenin [71]; Swift [72].

have been described to interact with non-activated AR-Smads, bridge them with the receptor complex, and assist in AR-Smad activation [19]. SARA can anchor AR-Smads through cooperation with Hgs/Hrs (hepatic growth factor-regulated tyrosine kinase substrate) and their FYVE domain to the inner leaflet of the plasma membrane or to early endosomes. SARA-like adapter proteins have not yet been reported in the BMP cascade, but Hgs/Hrs likely interacts with Smad1 [20], indicating that there may be shared mechanisms that organize R-Smad signaling centers at the plasma membrane but also link their activity to membrane trafficking (endocytosis) and to degradation. In addition, the interaction of filamin (an actin cross-linking factor and scaffolding protein) with Smad1 and Smad5 enhances their signaling presumably by serving as a track for intracellular Smad movement [21].

The observation that Smad1 can directly interact, although weaker than Smad3, with proteins involved in epithelial cell polarity (like Erbin, Par3, Dishevelled-1 (Dvl1); [22]), suggests a mean by which BMPs could alter cell polarity. Dvl1 is an intracellular protein involved in Wnt signaling, and its interaction with Smads may provide another level of cross-talk between Wnt and TGF-β signal transduction. Par3, originally identified in *Caenorhabditis elegans*, is involved in partitioning (cell polarity). Erbin is a protein that binds to the receptor ErbB2 and to several proteins that are involved in cytoskeleton and desmosome formation. Erbin also suppresses the mitogen-activated protein kinase (MAPK) pathway [23]. All three proteins contain at least one PDZ domain, which appears not to be involved in Smad binding, but they can provide a platform for assembly of multi-protein complexes that are targeted to specific subcellular locations. Interestingly, also Smad4 and Smad7 interact with Erbin, Dvl1 and Par3. It is not clear yet whether these proteins interact at endogenous levels in a ligand-dependent manner and how these interactions modulate signaling [22].

Additional cytoplasmic or shuttling BR-Smad-interacting partners, mainly involved in the negative regulation of Smads, are discussed further below.

4. BR-Smad-interacting activators in the nucleus

The direct role of Smads in gene transcription is dependent on their physical interaction with other DNA binding transcription factors [24] and/or interaction with proteins like the general transcriptional co-activator CBP/p300 [25]. Smad4 is accepted as an essential functional partner in Smad-regulated transcription, and has been shown to interact with CBP/p300. Since CBP/p300 has intrinsic histone acetyl transferase (HAT) activity and participates in chromatin remodeling, the recruitment of CBP/p300 into the DNA binding complexes of Smad1 and Smad4 has been claimed as a critical step in Smad-regulated gene activation. However, our current knowledge of the molecular details of the recruitment of CBP/p300 and other transcriptional modulators by Smads is still very limited.

The multifunctional multi-zinc finger protein OAZ (Olf-1/EBF-associated zinc finger) functions in BMP signaling as a regulator of Smad1-dependent *Xvent* target gene transcription

and acts, through other zinc finger-rich domains, in a Smad-independent fashion as an inhibitory partner of the Olf-1/EBF bHLH activator in pre-B-lymphocytes and olfactory epithelium. OAZ may in fact provide three levels of specificity in BMP signaling. It is able to cooperate with Smad1 and not with Smad2. OAZ seems to provide target gene specificity in BMP-stimulated cells, because it recognizes target genes that contain appropriate BMP response elements (BRE) (the 3′-flanking box in the *Xvent2* gene) but not other genes such as *Tlx2*. Finally, by being expressed in some cell types only, OAZ provides cell-type specificity to the response of OAZ-dependent BMP target genes [26].

Genes of the Xvent family of homeodomain-containing transcription factors, like Xvent2, are not only immediate response genes to BMP4 signaling in the ventral mesoderm of the *Xenopus* embryo. A recent study identified Xvent2 as a Smad1 binding protein, and provided evidence that Xvent2 maintains and/or amplifies its own expression by binding to its own promoter, and autoregulates its own transcription and in a Smad-dependent fashion [27].

BR-Smads have been documented to interact, in contrast to AR-Smads, only with a limited number of transcription factors, including the family with a Runt domain (Runx1 to 3) that are integral components of AR- and BR-Smad signaling cascades in hematopoiesis and in osteogenesis [28]. The interaction of Runx2 with Smad1 or Smad5 enhances the osteogenic actions of Runx2 significantly, and its expression is dependent on Smad1/Smad5 activation [29] (Table 2).

The tumor suppressor menin interacts with Smad3 and many transcription factors, shuttles between cytoplasm and nucleus, and is likely involved in cell cycle regulation, and DNA repair or synthesis [30]. Recently, the functional interaction of menin with Smad1 and Smad5 was documented [31]. Knockdown studies in C3H10T1/2 cells revealed interestingly that menin did not affect BMP2-induced chondrogenesis and adipogenesis but is required for ligand-induced commitment into the osteoblast lineage, and early differentiation of osteoblasts.

5. BR-Smad-interacting repressors in the nucleus

Several nuclear proteins have been implicated in transcriptional repression of TGF-β/BMP target genes. The homeodomain protein TGIF and the c-Ski protein were identified as transcriptional co-repressors of Smad2 and Smad3 in a TGF-β-dependent manner [32], but they interact only weakly with BR-Smads. The protein Tob, on the other hand, interacts specifically with Smad1, Smad5 and Smad8, as well as with Smad4, recruits them to nuclear bodies, and represses BMP-induced and Smad-dependent transcription in osteoblasts [33].

Smad1 interacts also with Hoxc8, a homeodomain transcriptional repressor, and dislodges Hoxc8 from its DNA binding element, resulting in the induction of specific BMP target genes [34,35]. Smad-interacting protein-1 (SIP1), a member of the Zfhx family, is a multi-zinc finger DNA binding protein that acts as a repressor of a number of promoters of embryonically relevant genes [36]. A novel Krüppel-type zinc finger protein, mZnf8, endogenously interacts with R- and co-Smads, and shows highest affinity for Smad1/5 [37]. Like many other Krüppel-type zinc finger proteins, Znf8 appears as a transcriptional repressor of BMP and to a lesser extent TGF-β reporter genes. In addition, knockdown of endogenous Znf8 revealed its function in selective repression of certain BMP reporter genes (e.g. 12xGCGC based) and not of other reporter genes (e.g. Tlx2 based). At present, different modes of action of Znf8 may be envisaged. First, like Hoxc8 and as proposed for Brinker in *Drosophila*, Znf8 may function as a transcriptional repressor of specific BMP target genes. Binding of activated Smad to Znf8 could dislodge it from its binding site, allowing the transcription of target genes. This is however unlikely because knockdown of Znf8 should then result in BMP-independent induction of target genes, which was not seen. Alternatively, Znf8 may rather serve as a general inhibitor that blocks the activity of Smad proteins. A similar mechanism was proposed for Smad nuclear interacting protein-1 (SNIP1, [38]). Znf8 may also take part in a transcriptional co-repressor complex with Smads to repress target genes, similar to Ski and TGIF for AR-Smads. It is particularly interesting that Znf8 can interact, unlike the other Smad-interacting co-repressors, with both the MH1 and MH2 domain of Smad1. Consequently, the inhibition of Smad1 activity by Znf8 may have two effects by blocking the Smad1 DNA binding activity through interaction with the MH1 domain and by blocking Smad1 *trans*-activation

Table 2
Selection of BMP/BR-Smad target genes

Target gene	Function	Reference
BIG-3	seven WD-40 repeat protein	[73]
BIKe	Ser/Thr protein kinase	[74]
Runx2/cbfa1/OSF2	DNA binding subunit of core binding transcription factor CBP	[29]
Dlx-2, Dlx-3, Dlx-5	homeobox proteins	[75–77]
GATA2, GATA4	zinc finger transcription factors	[78–81]
Hex	homeobox protein	[82]
Id1, Id2, Id3	inhibitors of basic helix-loop-helix proteins	[83]
JunB	proto-oncogene	[84]
Msx-1	homeobox protein	[83]
Msx-2	homeobox protein	[83]
Nkx2.5	homeobox protein	[50,51]
Noggin	BMP antagonist	[85]
Osteopontin (OPN)	bone matrix protein	[86]
Osteoprotegrin (OPG)	soluble decoy TNF receptor	[87]
Smad6	inhibitory Smad	[47]
Smad7	inhibitory Smad	[45]
Tbx2	T-box transcription factor	[88]
Tlx-2	homeobox protein	[89,90]
Vent2	homeodomain transcription factor	[46]

activity through binding to the MH2 domain. Znf8 could set thresholds for BMP signaling due to the different affinity of Znf8 for different Smads, providing a new mechanism for cells to have different sensitivities to different ligands [37].

The Cas-interacting zinc finger protein (CIZ) has recently been described as a negative regulator in BMP/Smad signaling [39], known as nucleocytoplasmic shuttling protein, which localized in focal adhesions and nuclei, and was suggested to function in cell attachment and concomitant gene expression. A novel finding is that differentiation of osteoblasts turns out to be suppressed by CIZ through interaction with BR-Smads.

The inner nuclear membrane protein MAN1 is a new and recent player in the field. MAN1 specifically interacts with the MH2 domain of BR-Smads and antagonizes BMP effects in *Xenopus* embryos by suppression of known target genes for BMPs, thereby acting as a neuralizing factor [40]. Inner nuclear membrane proteins have been implicated mainly in disassembly and subsequent reassembly of the nuclear envelope in mitosis, based on their proximity to the nuclear lamina and chromatin. The molecular action mechanism of MAN1 in BR-Smad signaling is unknown, but MAN1 may titrate out BR-Smads and prevent their association with other transcription regulators, or alternatively it may recruit a co-repressor on the MH2 domain as has been suggested for the c-Ski oncoprotein [41].

6. Smad4-interacting factors involved in BMP signaling

Although emphasis here is not on Smad4-interacting proteins involved in BMP signaling, they represent yet another mechanism to modulate BMP and TGF-β signaling. For example, SMIF is a Smad4-interacting protein that does not display sequence similarities with known functional domains, including those that bind DNA [42]. The SMIF/Smad4 interaction is BMP and TGF-β dependent, and the complex translocates to the nucleus upon ligand stimulation. SMIF appears as a transcriptional co-activator in Smad4 containing transcriptional complexes, essential for a subset of BMP-induced cellular responses [43].

Msx1 is a target gene and mediator of BMP signaling. Recent data indicate that Msx1 can bind to Smad1 or 2 as well as Smad4α or β in *Xenopus*. Dependent on the presence of Smad4, Msx1 can exclude FAST proteins from Smad2/4 complexes and has been proposed to antagonize nodal signaling [44].

7. Target genes

Although many BMP-induced genes are documented, including *Msx*, *Id* genes (inhibitor of differentiation) *Id1* to *3*, *Smad6*, *Vent2* and *Tlx2* (Table 2), relatively few genes have been identified as BR-Smad target genes (Table 3). Some BR-Smad target genes encode BR-Smad-interacting proteins and are involved in the induction of autoregulatory (Vent2) or negative feedback (Smad6, Tob) loops in BMP signaling, as well as the induction of indirect BMP target genes (e.g. *Osterix*, *EKLF*, *Collagen 2a1*).

The responsive regions of TGF-β/activin target genes contain Smad binding elements (SBEs), consisting of a consensus sequence of CAGAC, or minimally AGAC [32]. But Smad binding to these SBEs is fairly weak and lacks selectivity, therefore requiring additional DNA binding factors for high affinity, specific recruitment of the Smad complex to a distinct target promoter, a mechanism likely to be conserved in both BMP and TGF-β signaling cascades. BR-Smads (e.g. Smad7 promoter), and Smad3 and Smad4 (e.g. PAI-1 promoter) can bind SBE CAGAC sequences, likely depending on the flanking sequences. Remarkably, though Smad5 falls in the same family of BR-Smads as Smad1 and Smad8, Smad5 has DNA SBE binding properties similar to Smad3 in the Smad7 promoter, a feature not shared by Smad1 and Smad8 [45].

BR-Smads have been shown to weakly bind GCAT or GCCG motifs, so-called BREs found in BMP target genes like *Xvent2* and *Smad6* [46,47]. However, BMP inducibility of multimerized GC-rich reporter constructs is very low and requires R-Smad overexpression.

Id proteins play important roles in the multiple biological functions of BMPs as they act as negative regulators of cell differentiation and positive mediators of cell proliferation [48]. Id proteins sequester ubiquitous bHLH transcription factors and inhibit their transcriptional activities. The Id1 promoter contains several BMP-responsive regions that contain SBEs and CAGC and CGCC motifs [49]. Nearly identical CGCC BREs are retrieved in the BMP-responsive Hex and Nkx2.5 promoters [50,51].

Recently, different gene expression profiling analyses have yielded novel BMP target genes involved in osteoblast differentiation of C2C12 mesenchymal progenitor cells [52–54] and in ALK1/Smad5-mediated TGF-β signaling in endothelium [55]. These approaches will continue to give further insight in BR-Smad transcriptional cascades and lead to the identification of their direct target genes.

8. Further modulation of Smad-mediated BMP signaling by BR-Smad-interacting proteins

Smad6 blocks predominantly the BMP pathway by competing with Smad4 for binding to activated Smads [56], and

Table 3
BR-SBE

Gene	BRE	Mechanism	Reference
Dlx-3	GCAT motif	Smad1/Smad4 binding	[76]
Hex	2x GCCGnCGC 2x CAGAC (SBE)	Smad1/Smad4 binding Smad4 binding	[82]
Id1	2× SBE, GGCGCC, CAGC and CGCC	Smad5/Smad4 binding	[49]
Nkx2.5	multiple GTCT/AGAC, GCCGCGC	Smda4 binding, Smad1/5/Smad4 binding	[50,51]
Osteopontin	AGACTGTCTGGAC	Smad1/Smad4 dislodges Hoxc8 from its HRE	[86]
Smad6	GCCGCGCC motif	Smad1/4	[47]
Smad7	SBE GTCTAGAC	Smad2/3/5-specific, no Smad1/8 binding	[45]
Vent2B	GCAT motif (Smad1) AGNC motif (Smad4)	Smad1/5 and OAZ	[26,46]

Smad7 inhibits phosphorylation of Smad1 by interacting with activated BMP type I receptors [57]. Smad6, but not Smad7, interacts with both Hoxc8 and Hoxa9 proteins as a heterodimer when binding to DNA. The Smad6–Hoxc8 complex inhibits interaction of Smad1 with Hoxc8 and, consequently, Smad1-induced transcription. These data suggest that Smad6 interacts with Hox proteins as part of a negative feedback circuit for modulating the magnitude and duration of BMP signaling [43].

Recently, SANE (Smad1 antagonistic effector) was identified [58] and shown to interact specifically with the MH2 domain of BR-Smads and BMP type I receptors (i.e. ALK3 and 6, but not ALK2). The interaction with both a receptor and R-Smad makes it resemble SARA. However, SANE appears to antagonize BMP signaling in *Xenopus* embryos and mammalian cells by inhibiting BR-Smad phosphorylation and blocking nuclear translocation. This protein may represent a new mode of regulation for intracellular BMP signaling. It shows no sequence similarity to other proteins that interact with BR-Smads and/or BMP receptors, with the notable exception of MAN1. The highest sequence similarity is found in the carboxy-terminal region, which includes the Smad binding domain of SANE.

Smad-dependent proteasome-mediated degradation of Smads themselves but also of cytoplasmic and nuclear Smad partners is an important aspect of TGF-β/BMP signaling as well. Smurf1 and Smurf2, which are Hect E3 ubiquitin ligases, selectively interact with BR-Smads (although Smurf2 has also affinity for Smad2) to trigger their ubiquitination and subsequent degradation, and hence their inactivation [59,60]. Smurf1 interacts also with Smad7, which is exported from the nucleus in ligand-treated cells, and induces Smad7 ubiquitination. In addition, Smurf1 associates with ALK5 (TβRI) via Smad7, with subsequent enhancement of TβRI and Smad7 turnover [61]. Ubiquitination, however, is not an obligate step for substrate targeting to the proteasome. Recently, Smad1 was shown to interact with two proteins that function along the degradation pathway of the 26S proteasome, i.e. antizyme (Az) and HsN3 [62,63]. Activation of BMP type I receptor triggers binding of Smad1 to Az and HsN3, and brings them – likely with Smad4 – into the nucleus, where the CBP/p300 repressor SNIP1 is recruited to the complex and co-targeted to the proteasome. Smad4 seems a positive regulator of targeting SNIP1 to the proteasome. SNIP1 is a potent inhibitor of Smad-responsive genes in BMP pathways via inhibiting CBP/p300. The degradation of SNIP1 may be an essential step for Smad1/Smad4-mediated transcriptional activation, since an increase of SNIP1 expression inhibits BMP-induced gene responses [63].

9. Conclusions

The true web of Smad interactions that is triggered when cells perceive a BMP stimulus, and the adequate integration into an appropriate transcriptional modulation of gene expression, is still of a higher order than we can currently review, as aspects of concentration and subcellular localization of Smad partners become critical parameters. Much from the future identification of more components of the BMP pathway and from our insight in the endogenous/physiological interplay between signaling components is likely to come from studies involving knockout and knockdown approaches in entire embryos (or tissues thereof) and in cell cultures specifically designed for this purpose. In addition, TGF-β ligands function in complex growth factor/cytokine environments, and it is anticipated that many studies will have to address the complexity of cross-talk of their signal transduction with the Smad pathway.

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References

[1] Reddi, A.H. (1992) Curr. Opin. Cell Biol. 4, 850–855.
[2] Wozney, J.M. (2002) Spine 27, S2–8.
[3] Massagué, J. (1998) Annu. Rev. Biochem. 67, 753–791.
[4] Aoki, H., Fujii, M., Imamura, T., Yagi, K., Takehara, K., Kato, M. and Miyazono, K. (2001) J. Cell Sci. 114, 1483–1489.
[5] Goumans, M.J., Valdimarsdottir, G., Itoh, S., Rosendahl, A., Sideras, P. and ten Dijke, P. (2002) EMBO J. 21, 1743–1753.
[6] Visser, J.A., Olaso, R., Verhoef-Post, M., Kramer, P., Themmen, A.P. and Ingraham, H.A. (2001) Mol. Endocrinol. 15, 936–945.
[7] Clarke, T.R., Hoshiya, Y., Yi, S.E., Liu, X., Lyons, K.M. and Donahoe, P.K. (2001) Mol. Endocrinol. 15, 946–959.
[8] Baur, S.T., Mai, J.J. and Dymecki, S.M. (2000) Development 127, 605–619.
[9] Inman, G.J., Nicolas, F.J. and Hill, C.S. (2002) Mol. Cell 10, 283–294.
[10] Derynck, R. (1998) Nature 393, 737–739.
[11] van Grunsven, L.A., Huylebroeck, D. and Verschueren, K. (2002) Crit. Rev. Eukaryot. Gene Expr. 12, 101–118.
[12] Hay, E., Lemonnier, J., Fromigue, O. and Marie, P.J. (2001) J. Biol. Chem. 276, 29028–29036.
[13] Heldin, C.H., Miyazono, K. and ten Dijke, P. (1997) Nature 390, 465–471.
[14] Ghosh-Choudhury, N., Abboud, S.L., Nishimura, R., Celeste, A., Mahimainathan, L. and Choudhury, G.G. (2002) J. Biol. Chem. 277, 33361–33368.
[15] Onichtchouk, D., Chen, Y.G., Dosch, R., Gawantka, V., Delius, H., Massagué, J. and Niehrs, C. (1999) Nature 401, 480–485.
[16] Balemans, W. and Van Hul, W. (2002) Dev. Biol. 250, 231–250.
[17] Moustakas, A., Souchelnytskyi, S. and Heldin, C.H. (2001) J. Cell Sci. 114, 4359–4369.
[18] Miyazawa, K., Shinozaki, M., Hara, T., Furuya, T. and Miyazono, K. (2002) Genes Cells 7, 1191–1204.
[19] Tsukazaki, T., Chiang, T.A., Davison, A.F., Attisano, L. and Wrana, J.L. (1998) Cell 95, 779–791.
[20] Miura, S. et al. (2000) Mol. Cell. Biol. 20, 9346–9355.
[21] Sasaki, A., Masuda, Y., Ohta, Y., Ikeda, K. and Watanabe, K. (2001) J. Biol. Chem. 276, 17871–17877.
[22] Warner, D.R., Pisano, M.M., Roberts, E.A. and Greene, R.M. (2003) FEBS Lett. 539, 167–173.
[23] Huang, Y.Z., Zang, M., Xiong, W.C., Luo, Z. and Mei, L. (2003) J. Biol. Chem. 278, 1108–1114.
[24] Attisano, L. and Wrana, J.L. (2000) Curr. Opin. Cell Biol. 12, 235–243.
[25] Pearson, K.L., Hunter, T. and Janknecht, R. (1999) Biochim. Biophys. Acta 1489, 354–364.
[26] Hata, A., Seoane, J., Lagna, G., Montalvo, E., Hemmati-Brivanlou, A. and Massagué, J. (2000) Cell 100, 229–240.
[27] Henningfeld, K.A., Friedle, H., Rastegar, S. and Knochel, W. (2002) J. Biol. Chem. 277, 2097–2103.
[28] Ito, Y. and Miyazono, K. (2003) Curr. Opin. Genet. Dev. 13, 43–47.
[29] Nishimura, R., Hata, K., Harris, S.E., Ikeda, F. and Yoneda, T. (2002) Bone 31, 303–312.
[30] Guo, S.S. and Sawicki, M.P. (2001) Mol. Endocrinol. 15, 1653–1664.

[31] Sowa, H. et al. (2003) J. Biol. Chem. 20, 20.
[32] Massagué, J. and Wotton, D. (2000) EMBO J. 19, 1745–1754.
[33] Yoshida, Y. et al. (2000) Cell 103, 1085–1097.
[34] Shi, X., Yang, X., Chen, D., Chang, Z. and Cao, X. (1999) J. Biol. Chem. 274, 13711–13717.
[35] Yang, X., Ji, X., Shi, X. and Cao, X. (2000) J. Biol. Chem. 275, 1065–1072.
[36] Verschueren, K. et al. (1999) J. Biol. Chem. 274, 20489–20498.
[37] Jiao, K., Zhou, Y. and Hogan, B.L.M. (2002) Mol. Cell. Biol. 22, 7633–7644.
[38] Kim, R.H., Flanders, K.C., Birkey Reffey, S., Anderson, L.A., Duckett, C.S., Perkins, N.D. and Roberts, A.B. (2001) J. Biol. Chem. 276, 46297–46304.
[39] Shen, Z.J., Nakamoto, T., Tsuji, K., Nifuji, A., Miyazono, K., Komori, T., Hirai, H. and Noda, M. (2002) J. Biol. Chem. 277, 29840–29846.
[40] Osada, S., Ohmori, S.Y. and Taira, M. (2003) Development 130, 1783–1794.
[41] Wang, W., Mariani, F.V., Harland, R.M. and Luo, K. (2000) Proc. Natl. Acad. Sci. USA 97, 14394–14399.
[42] Bai, R.Y., Koester, C., Ouyang, T., Hahn, S.A., Hammerschmidt, M., Peschel, C. and Duyster, J. (2002) Nat. Cell Biol. 4, 181–190.
[43] Bai, S., Shi, X., Yang, X. and Cao, X. (2000) J. Biol. Chem. 275, 8267–8270.
[44] Yamamoto, M., Meno, C., Sakai, Y., Shiratori, H., Mochida, K., Ikawa, Y., Saijoh, Y. and Hamada, H. (2001) Genes Dev. 15, 1242–1256.
[45] Li, W., Chen, F., Nagarajan, R.P., Liu, X. and Chen, Y. (2001) Biochem. Biophys. Res. Commun. 286, 1163–1169.
[46] Henningfeld, K.A., Rastegar, S., Adler, G. and Knochel, W. (2000) J. Biol. Chem. 275, 21827–21835.
[47] Ishida, W. et al. (2000) J. Biol. Chem. 275, 6075–6079.
[48] Miyazono, K. and Miyazawa, K. (2002) Sci. STKE 2002, PE40.
[49] Korchynskyi, O. and ten Dijke, P. (2002) J. Biol. Chem. 277, 4883–4891.
[50] Liberatore, C.M., Searcy-Schrick, R.D., Vincent, E.B. and Yutzey, K.E. (2002) Dev. Biol. 244, 243–256.
[51] Lien, C.L., McAnally, J., Richardson, J.A. and Olson, E.N. (2002) Dev. Biol. 244, 257–266.
[52] de Jong, D.S., van Zoelen, E.J., Bauerschmidt, S., Olijve, W. and Steegenga, W.T. (2002) J. Bone Miner. Res. 17, 2119–2129.
[53] Vaes, B.L. et al. (2002) J. Bone Miner. Res. 17, 2106–2118.
[54] Balint, E. et al. (2003) J. Cell Biochem. 89, 401–426.
[55] Ota, T., Fujii, M., Sugizaki, T., Ishii, M., Miyazawa, K., Aburatani, H. and Miyazono, K. (2002) J. Cell Physiol. 193, 299–318.
[56] Hata, A., Lagna, G., Massagué, J. and Hemmati-Brivanlou, A. (1998) Genes Dev. 12, 186–197.
[57] Souchelnytskyi, S., Nakayama, T., Nakao, A., Moren, A., Heldin, C.H., Christian, J.L. and ten Dijke, P. (1998) J. Biol. Chem. 273, 25364–25370.
[58] Raju, G.P., Dimova, N., Klein, P.S. and Huang, H.C. (2003) J. Biol. Chem. 278, 428–437.
[59] Zhu, H., Kavsak, P., Abdollah, S., Wrana, J.L. and Thomsen, G.H. (1999) Nature 400, 687–693.
[60] Lin, X., Liang, M. and Feng, X.H. (2000) J. Biol. Chem. 275, 36818–36822.
[61] Ebisawa, T., Fukuchi, M., Murakami, G., Chiba, T., Tanaka, K., Imamura, T. and Miyazono, K. (2001) J. Biol. Chem. 276, 12477–12480.
[62] Gruendler, C., Lin, Y., Farley, J. and Wang, T. (2001) J. Biol. Chem. 276, 46533–46543.
[63] Lin, Y. et al. (2002) BMC Cell Biol. 3, 15.
[64] Rodriguez, C., Huang, L.J., Son, J.K., McKee, A., Xiao, Z. and Lodish, H.F. (2001) J. Biol. Chem. 276, 30224–30230.
[65] Scherer, A. and Graff, J.M. (2000) J. Biol. Chem. 275, 41430–41438.
[66] Nishihara, A., Hanai, J., Imamura, T., Miyazono, K. and Kawabata, M. (1999) J. Biol. Chem. 274, 28716–28723.
[67] Liu, F., Massagué, J. and Ruiz i Altaba, A. (1998) Nat. Genet. 20, 325–326.
[68] Liu, X., Elia, A.E., Law, S.F., Golemis, E.A., Farley, J. and Wang, T. (2000) EMBO J. 19, 6759–6769.
[69] Liberati, N.T., Datto, M.B., Frederick, J.P., Shen, X., Wong, C., Rougier-Chapman, E.M. and Wang, X.F. (1999) Proc. Natl. Acad. Sci. USA 96, 4844–4849.
[70] Nishita, M., Hashimoto, M.K., Ogata, S., Laurent, M.N., Ueno, N., Shibuya, H. and Cho, K.W. (2000) Nature 403, 781–785.
[71] Sun, Y., Nadal-Vicens, M., Misono, S., Lin, M.Z., Zubiaga, A., Hua, X., Fan, G. and Greenberg, M.E. (2001) Cell 104, 365–376.
[72] Shimizu, K., Bourillot, P.Y., Nielsen, S.J., Zorn, A.M. and Gurdon, J.B. (2001) Mol. Cell. Biol. 21, 3901–3912.
[73] Gori, F., Divieti, P. and Demay, M.B. (2001) J. Biol. Chem. 276, 46515–46522.
[74] Kearns, A.E., Donohue, M.M., Sanyal, B. and Demay, M.B. (2001) J. Biol. Chem. 276, 42213–42218.
[75] Xu, S.C., Harris, M.A., Rubenstein, J.L., Mundy, G.R. and Harris, S.E. (2001) DNA Cell Biol. 20, 359–365.
[76] Park, G.T. and Morasso, M.I. (2002) Nucleic Acids Res. 30, 515–522.
[77] Miyama, K., Yamada, G., Yamamoto, T.S., Takagi, C., Miyado, K., Sakai, M., Ueno, N. and Shibuya, H. (1999) Dev. Biol. 208, 123–133.
[78] Friedle, H. and Knochel, W. (2002) J. Biol. Chem. 277, 23872–23881.
[79] Maeno, M., Mead, P.E., Kelley, C., Xu, R.H., Kung, H.F., Suzuki, A., Ueno, N. and Zon, L.I. (1996) Blood 88, 1965–1972.
[80] Liu, B., Sun, Y., Jiang, F., Zhang, S., Wu, Y., Lan, Y., Yang, X. and Mao, N. (2003) Blood 101, 124–133.
[81] Schultheiss, T.M., Burch, J.B. and Lassar, A.B. (1997) Genes Dev. 11, 451–462.
[82] Zhang, W., Yatskievych, T.A., Cao, X. and Antin, P.B. (2002) J. Biol. Chem. 277, 45435–45441.
[83] Hollnagel, A., Oehlmann, V., Heymer, J., Ruther, U. and Nordheim, A. (1999) J. Biol. Chem. 274, 19838–19845.
[84] Jonk, L.J., Itoh, S., Heldin, C.H., ten Dijke, P. and Kruijer, W. (1998) J. Biol. Chem. 273, 21145–21152.
[85] Narita, T., Saitoh, K., Kameda, T., Kuroiwa, A., Mizutani, M., Koike, C., Iba, H. and Yasugi, S. (2000) Development 127, 981–988.
[86] Hullinger, T.G., Pan, Q., Viswanathan, H.L. and Somerman, M.J. (2001) Exp. Cell Res. 262, 69–74.
[87] Wan, M., Shi, X., Feng, X. and Cao, X. (2001) J. Biol. Chem. 276, 10119–10125.
[88] Yamada, M., Revelli, J.P., Eichele, G., Barron, M. and Schwartz, R.J. (2000) Dev. Biol. 228, 95–105.
[89] Tang, S.J., Hoodless, P.A., Lu, Z., Breitman, M.L., McInnes, R.R., Wrana, J.L. and Buchwald, M. (1998) Development 125, 1877–1887.
[90] Macias-Silva, M., Hoodless, P.A., Tang, S.J., Buchwald, M. and Wrana, J.L. (1998) J. Biol. Chem. 273, 25628–25636.