Development 136, 3691-3697 (2009) doi:10.1242/dev.040584

TGFβ family signaling: novel insights in development and disease

Kristi Wharton[1] and Rik Derynck[2]

Advances in our understanding of the many levels of regulation of TGFβ and BMP signaling were reported at the recent FASEB Summer Conference entitled 'The TGFβ Superfamily: Development and Disease', which was held in Carefree, Arizona, USA, on the northern edge of the Sonoran Desert. This conference was the fifth meeting in a biannual FASEB conference series and, as with the previous meetings, brought together biochemists, geneticists, developmental and tissue biologists interested in the inter-workings of TGFβ/BMP signaling pathways and in the consequences of these pathways going awry.

Introduction

In vertebrates, 33 genes encode transforming growth factor (TGF) β-related polypeptides, which are processed and secreted as homodimers or heterodimers (Derynck and Miyazono, 2008). The functions of the TGFβ family proteins in development and disease are the subject of an overwhelming array of studies in numerous labs that continue to yield interesting insights into the diverse roles of these secreted factors and the mechanisms of their actions [as reviewed by Wu and Hill (Wu and Hill, 2009) and Massagué (Massagué, 2008), and as discussed in the accompanying reviews of this Minifocus on TGFβ signaling (see Box 1)]. Cell biological studies, supported by epistasis analyses in *Drosophila*, have established the central mechanisms of how signals from TGFβ family proteins are transduced to regulate gene expression (Fig. 1). A secreted dimeric ligand binds to a heterotetrameric cell surface complex of two type II and two type I kinase receptors. In these complexes, ligand occupation induces the type II receptors to phosphorylate, and thereby activate, the type I receptor kinases, which in turn activate, through direct phosphorylation at C-terminal serines, Smads that have been recruited to the receptor complex. Two receptor-activated (R) Smads form a trimeric complex with the common-mediator (co) Smad (SMAD4 in vertebrates), and these complexes translocate into the nucleus and participate in nucleoprotein complexes with sequence-specific transcription factors, co-activators and co-repressors at gene regulatory sequences, thereby executing ligand-induced transcriptional activation or repression of responsive target genes. The TGFβ family can be divided into two groups: (1) the bone morphogenetic proteins (BMPs) and certain 'growth and differentiation factors' (GDFs), which act through SMAD1, 5 and 8; and (2) the TGFβs, activins, nodal and myostatin, which act through SMAD2 and SMAD3 (Derynck and Miyazono, 2008). Which Smads are activated in response to a ligand depends on the identity of the type I receptor and the composition of the receptor complex. Despite the substantial effort focused on TGFβ family signaling, the pathways activated by a number of TGFβ family proteins remain to be fully characterized, even to the extent that we do not know which Smads mediate their signals.

It is against this well-established 'central' mechanism of signaling that progress during the last few years, and communicated at the conference, has to be viewed. We are gaining a better understanding of how ligand activation is controlled and what the consequences of these controls are in development and disease. We have also come to appreciate the roles of many proteins and post-translational modifications that regulate receptor presentation and function as well as Smad signaling (reviewed by Kahlem and Newfeld, 2009; Kang et al., 2009; Moustakas and Heldin, 2009). It is now clear that signaling specificity is not as straightforward as portrayed above, and our knowledge is rapidly expanding concerning how Smad signaling is regulated through cross-talk with other signaling pathways, as well as how non-Smad signaling pathways can be initiated by TGFβ ligands. This conference, organized by Mike O'Connor (University of Minnesota/HHMI, Minneapolis, MN, USA) and Kunxin Luo (UC Berkeley, CA, USA), gave us a flavor of the rapid progress that researchers have made in defining the TGFβ signaling pathway as a highly versatile and finely tuned system. Responses to TGFβ/BMP signals are dictated by the developmental stage and physiological state of the receiving cells and tissues. We are struck by the fact that seemingly slight dysregulations can lead, or contribute, to changes in development, as well as to a multiplicity of syndromes and diseases.

Box 1. Minifocus on TGFβ signaling

This article is part of a Minifocus on TGFβ 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 TGFβ pathway regulation' by Pascal Kahlem and Stuart Newfeld (Kahlem and Newfeld, 2009); and 'The regulation of TGFβ signal transduction' by Aristidis Moustakas and Carl-Henrik Heldin (Moustakas and Heldin, 2009).

Regulation of ligand presentation

Initiation of TGFβ/BMP signaling starts with the binding of a secreted dimeric ligand to a heterotetrameric receptor complex at the cell surface. However, it has long been known that TGFβ ligands are synthesized as pre-pro-polypeptides that require proteolytic cleavage, and that they are often secreted as inactive complexes made up of pro-domains that are non-covalently associated with the fully processed ligand dimer, thus preventing ligand binding to the receptor complex (Rifkin, 2005). Thus far, not much attention has been given to the regulation of the cleavage of the mature ligand from its precursor by furin proprotein convertases. Jan Christian (Oregon Health and Science University, Portland, OR, USA) has previously reported that differential processing of the BMP4 precursor by furin proprotein convertases leads to forms of the ligand that exhibit differences in stability and in their ability to act over long distances in *Xenopus* assays (Cui et al., 2001; Degnin et

[1]Department of Molecular Biology, Cell Biology and Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912, USA. [2]Department of Cell and Tissue Biology, Programs in Cell Biology and Developmental Biology, University of California at San Francisco, San Francisco, CA 94143, USA.

Kristi_Wharton@brown.edu; rik.derynck@ucsf.edu

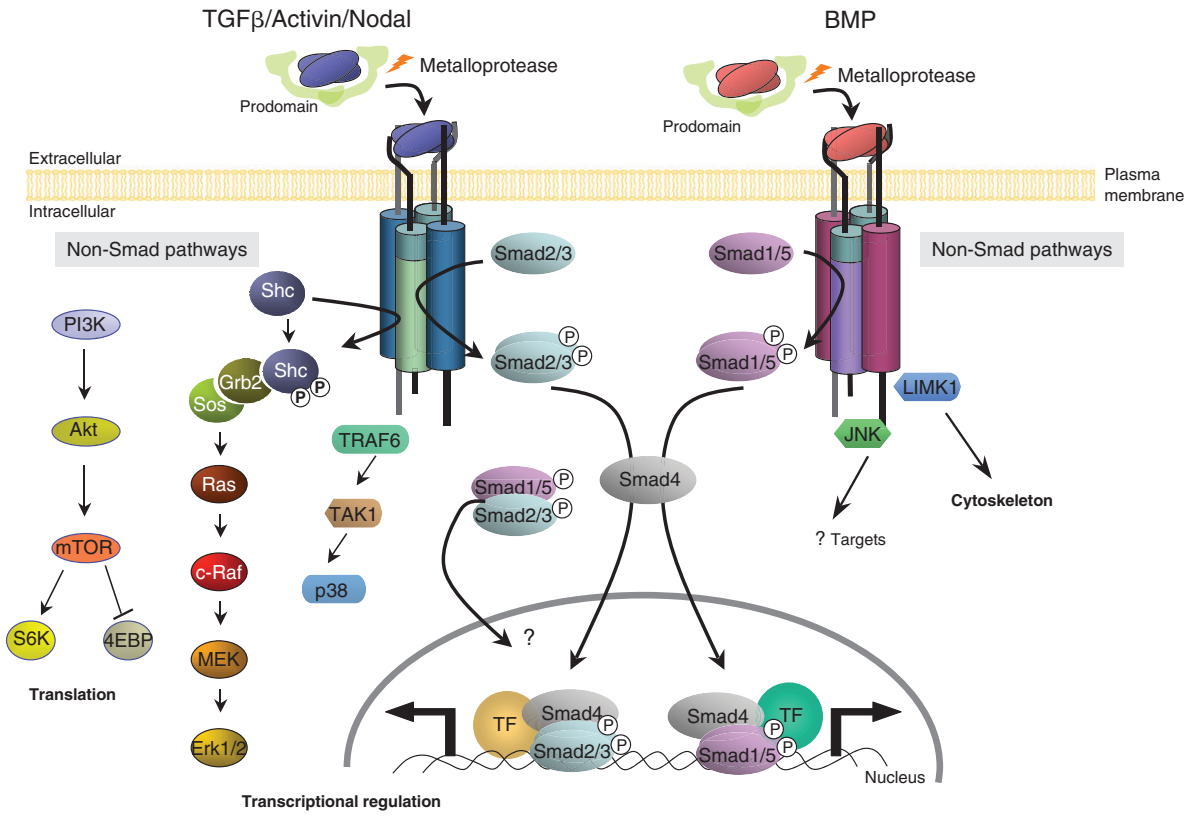

Fig. 1. An overview of TGFβ family signaling. The central Smad-dependent pathways for the TGFβ/Activin/Nodal and BMP ligand subfamilies are shown, as well as some of the non-Smad-dependent pathways relevant to data presented at the conference. The majority of factors and components involved in extracellular regulation have been omitted as they are covered in detail in the accompanying review (Umulis et al., 2009). BMP, bone morphogenetic protein; TF, transcription factor; P, phosphate group.

al., 2004). At this meeting, she and Osamu Shimmi (University of Helsinki, Finland) reported on recent studies that have revealed the importance of differential cleavage for the activity of the BMP2/4 ortholog Decapentaplegic (Dpp) during *Drosophila* development (J. Christian, personal communication) (Künnapuu et al., 2009). Not only is Dpp processed in a tissue-dependent manner, but different cleavage products are also required to provide sufficient function for wing and leg versus gut development.

Similarly, Kristi Wharton (Brown University, Providence, RI, USA) reported on the crucial role of differential processing of Glass bottom boat (Gbb), the *Drosophila* ortholog of BMPs 5, 6, 7 and 8. As with Dpp, Gbb is subject to differential cleavage at multiple furin cleavage sites in a tissue-dependent manner. However, unlike Dpp and BMP4, one of the two resulting Gbb forms has a long N-terminal extension, and Wharton's results show that this secreted large form elicits Mad-dependent signaling (Mad is a SMAD1/5 ortholog). How the receptor-binding properties of the large and small Gbb forms compare remains to be determined. Receptor binding of an incompletely processed ligand is not unprecedented, as nodal has been shown to be secreted as a full-length, uncleaved precursor in the mouse embryo, where it binds the activin receptor to maintain the expression of proprotein convertases (Ben-Haim et al., 2006). The furin-like proteases then act at the cell surface to cleave nodal extracellularly. Clearly, cleavage of the ligand precursor represents an important and regulated event, with much to be learned about the precise mechanisms governing this process in different developmental contexts.

Many TGFβ family ligands are secreted as latent complexes with other proteins that prevent the ligand from binding to its receptor complex. For example, in addition to being non-covalently associated with its pro-domain, TGFβ is complexed with the latent TGFβ-binding protein, whereas activins are bound efficiently by follistatin, both resulting in ligand inactivation (Rifkin, 2005; Chang, 2008). Perhaps the most intricate and complex regulation of ligand activation is apparent in the case of BMPs, in which the BMP-binding proteins Chordin (Sog), Twisted gastrulation, Noggin and Cerberus are involved. The importance of these binding proteins, and of the metalloproteases (Tolloid, Xolloid and BMP1) that cleave them in order to precisely regulate ligand activity, has been demonstrated in several developmental contexts (De Robertis and Kuroda, 2004; Little and Mullins, 2006). Eddy De Robertis (UCLA/HHMI, Los Angeles, CA, USA) and David Umulis (Purdue University, West Lafayette, IN, USA) elaborated on the complexities of regulating ligand activity, and on the roles of such extracellular regulators in defining the BMP morphogen activity gradients in early *Xenopus* and *Drosophila* development, respectively (Umulis et al., 2008) (reviewed by Umulis et al., 2009). Remarkably, De Robertis showed that BMP4 can itself act as a non-competitive inhibitor of Tolloid/BMP1 enzyme activity (see Fig. 2D). Both Umulis and Marcos Gonzalez-Gaitan (University of Geneva, Switzerland) made use of mathematical modeling to test possible mechanisms by which BMPs might generate gradients of morphogenetic information in different tissues, and Gonzalez-Gaitan elaborated on how the growth of tissues appears to be coordinated with BMP activity gradients. Hillary Ashe (University of Manchester,

UK) discussed the roles of other extracellular proteins – the two type IV collagen proteins, Viking (Vkg) and Dcg1 (Cg25C – FlyBase) – in restricting the signaling range of Dpp in the germline stem cell niche in *Drosophila* (Wang et al., 2008). Interestingly, Ashe showed that Vkg also acts in the dorsal midline of the embryo to promote Dpp and Screw (Scw) signaling, presumably by promoting ligand-receptor interactions. By incorporating bio-imaging data from embryo populations, a model developed by Umulis predicts that Dpp-Scw must bind at a faster rate to the Sog antagonist than has been observed from in vitro binding assays. It is possible that in the embryo, collagen IV serves to mediate Dpp-Scw–Sog complex formation and that this function is more important than a role in limiting transport of Sog or Dpp-Scw (see Fig. 2D). Altogether, these new studies highlight the importance of ligand processing and extracellular activation as critical steps used by the cell to control ligand presentation and subsequent signaling.

Receptor presentation and function

Previous studies in *Drosophila* and mouse embryos have provided definitive evidence for the formation of heteromeric ligands (Shimmi et al., 2005; Tanaka et al., 2007), which appear to have more potent activities than homodimeric ligands (Israel et al., 1996), although the nature of their receptors has remained unclear. Studying the role of BMPs in dorsoventral patterning in zebrafish, Mary Mullins (University of Pennsylvania, Philadelphia, PA, USA) reported that Bmp2-Bmp7 heterodimers and not Bmp2 or Bmp7 homodimers, activate BMP signaling in the early zebrafish embryo. Their activity is mediated by BMP receptor complexes that combine two distinct type I receptors, Alk3/6 (Bmpr1a/b) and Alk8, the functional homolog of ALK2 (ACVR1; ActRI) in mammals (Little and Mullins, 2009) (see Fig. 2D).

The combination of different type I receptors in the same receptor complex, as observed by Mullins and colleagues, is consistent with previous observations by Peter ten Dijke (Leiden University Medical Center, The Netherlands), who reported that in endothelial cells TGFβ can act through both ALK5 (TβRI; TGFβR1) and ALK1 (ACVRL1), resulting in activation of SMAD2/3 by ALK5 and of SMAD1 by ALK1 (Goumans et al., 2002). The dual activation of the classical TGFβ-assigned SMAD2/3 pathway as well as of the SMAD1/5 pathway, which was hitherto thought to be restricted to BMP signaling, appears to be no longer exclusive to endothelial cells. Indeed, Caroline Hill (Cancer Research UK London Research Institute, UK) described the activation of SMAD1 and SMAD5, in addition to SMAD2 and

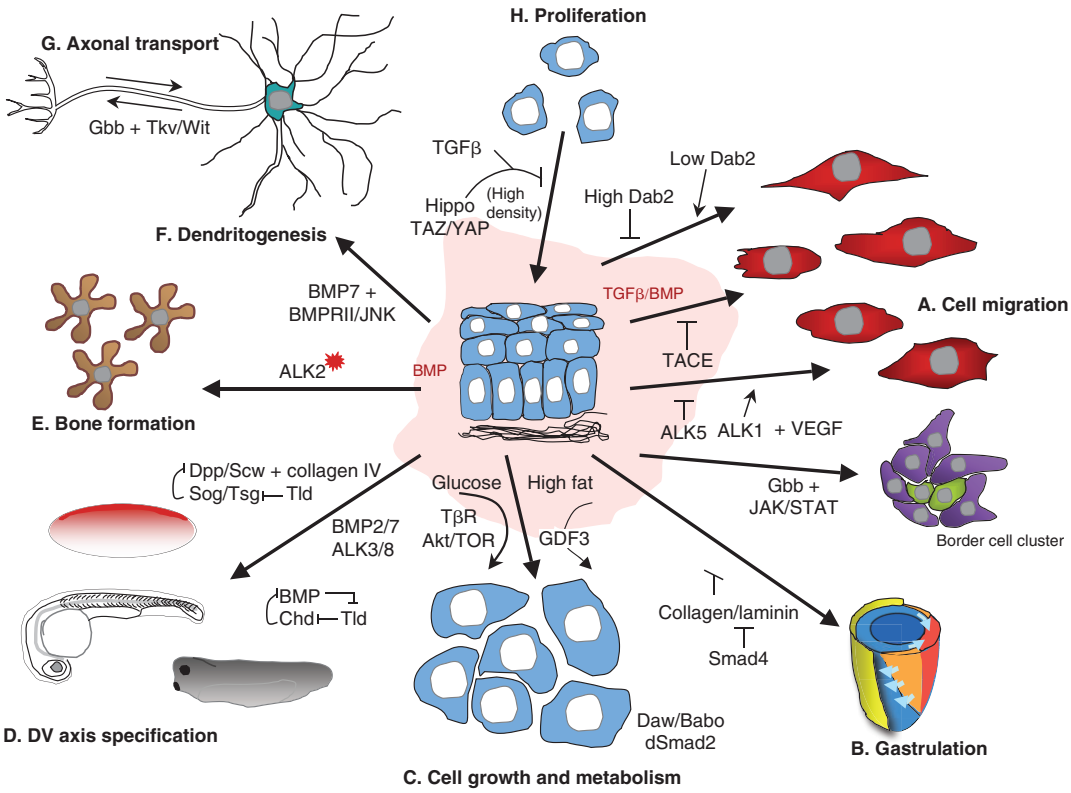

Fig. 2. A summary of biological responses to TGFβ family signaling discussed at this meeting. This illustration is not intended to be inclusive of all work presented at the conference and is organized around a generic tissue (center) exposed to TGFβ and/or BMP signals. (**A**) A number of studies elaborated on the role of TGFβ family signaling in both individual cell and cluster migration. (**B**) The importance of the extracellular environment, including basal lamina and the extracellular matrix, was discussed as both a target and modulator of signaling, e.g. during gastrulation. (**C**) Growing evidence was provided for a role of TGFβ family signaling coupled with diet in the regulation of cell size and metabolic processes. (**D**) Data were presented that refines our understanding of extracellular protein interactions with specific ligand-receptor combinations that are crucial for dorsoventral (DV) axis specification in a number of model organisms. (**E**) A mutation in the ALK2 receptor (red star) leads to hyperactive signaling and heterotopic bone deposition in patients with fibrodysplasia ossificans progressive (FOP). (**F**,**G**) The role of BMP signaling was discussed in both dendritogenesis and axonal transport. (**H**) TGFβ signaling has long been known to impact cell proliferation. New evidence was presented by a number of conference participants on cross-talk between other pathways and the TGFβ family signaling pathway in this cellular response. See text for further details.

SMAD3, in response to TGFβ in both epithelial and cancer cell lines. Hill provided evidence that this might result from the use of two distinct type I receptors, i.e. ALK5 and ALK2 or ALK3, in complex with a TβRII (TGFβR2) homodimer (Daly et al., 2008). However, a separate study proposed that activation of SMAD3 and SMAD1 in response to TGFβ is mediated by the same TβRI receptor (Liu, I. M. et al., 2009). Taken together, these findings raise the possibility that heteromeric combinations of SMAD2 or SMAD3 with SMAD1 or SMAD5 might mediate distinct gene expression responses.

Finally, evidence for 'mixed' Smad signaling was also provided by Mike O'Connor (University of Minnesota/HHMI, Minneapolis, MN, USA), who reported on his studies of the roles of activin ligands in *Drosophila*. Among these, Dawdle (Daw) was shown to activate both dSmad2 (Smox) and Mad through a specific isoform of the type I receptor Baboon, BaboC. Constitutively active BaboC primarily activates dSmad2, but elimination of dSmad2 expression results in significant upregulation of Mad activation. It appears that the output of Daw signaling may depend on a balance between dSmad2 and Mad, and any selective Smad turnover could then impact the ability of a ligand to activate different sets of target genes. Clearly, TGFβ/activin and BMP signaling should no longer be seen as being transduced by two independent Smad pathways.

Crucial for the cellular response to TGFβ family proteins is the presentation of receptors at the cell surface, resulting from both regulated transport and the endosomal routing of internalized receptors (Kang et al., 2009). Several talks provided new information on these subjects. Ed Leof (Mayo Clinic, Minnesota, MN, USA) demonstrated that in polarized cells, both the type II and type I receptors are localized at the basolateral cell surface, and that the basolateral localization of TβRII is mediated by a defined motif in its cytoplasmic domain (Murphy et al., 2007). This motif binds the retromer complex, which has been shown to be involved in retrograde sorting from endosomes to the trans-Golgi network. Guillermo Marques (University of Alabama, Birmingham, AL, USA) demonstrated the transport of vesicles that contain both the Wishful thinking (Wit) type II receptor and the Thickveins (Tkv) type I receptor in *Drosophila* axons (see Fig. 2G). Binding of the Gbb ligand results in the stabilization of these type II-type I receptor complexes, as assessed by fluorescence resonance energy transfer (FRET), and in a substantial change in the relative ratios of vesicles that show retrograde versus anterograde transport. Yoshiki Sasai (RIKEN Center for Developmental Biology, Kobe, Japan) reported the identification in *Xenopus* of a transmembrane protein that localizes to the endoplasmic reticulum in neural plate cells, and retains the BMP type II receptor there by interacting with the receptor's long cytoplasmic domain. The abundance of this protein is an important determinant of the abundance of cell surface BMP receptors and, thus, of BMP responsiveness. Finally, Rik Derynck (UC San Francisco, CA, USA) presented evidence that the cell surface presentation of TβRI and, thus, the responsiveness of cells to TGFβ, is regulated by the transmembrane metalloprotease TACE (also known as ADAM17), which cleaves TβRI to remove its ectodomain, thus downregulating the levels of functional cell surface TGFβ receptors (see Fig. 2A). Carcinoma cells often have increased TACE expression and enhanced Erk MAP kinase signaling, which was shown to activate TACE. Consequently, the downregulation of TGFβ responsiveness attenuates the autocrine growth inhibition by TGFβ, which complements the increased proliferation executed by increased TGFα release and epidermal growth factor (EGF) receptor signaling in response to TACE activation. Inhibition of TACE leads to enhanced TGFβ signaling, resulting not only in increased growth inhibition by TGFβ, but also in increased sensitivity of the cells to epithelial-to-mesenchymal transition (Liu, C. et al., 2009).

Protein interactions and signaling cross-talk define Smad function

It is well established that Smads shuttle between the nucleus and cytoplasm, and that Smad complexes accumulate in the nucleus in response to ligand (Schmierer et al., 2008). Although this paradigm represents a central aspect of the mechanism of Smad action, much remains to be learned about the factors that mediate the nuclear import/export and turnover of these large complexes. Using a genome-wide RNAi screen in *Drosophila* S2R$^+$ cells, Lan Xu (University of Massachusetts Medical School, Worcester, MA, USA) has been identifying components that are essential for, and/or that regulate, the nuclear import of Mad and Medea (the SMAD4 ortholog) in response to BMP signaling. This analysis identified Moleskin (Msk), the *Drosophila* ortholog of importin 7/8 (Xu et al., 2007), and the nucleoporin Sec13 as essential proteins with distinct roles in nuclear import of the Smads. Overall, the results from this screen indicate that a number of nucleopore and associated proteins are required specifically for Smad nuclear accumulation and that each appears to serve a different function.

Stefano Piccolo (University of Padua, Italy) has shown that the function of SMAD4 is regulated by E3 ubiquitin ligase ectodermin (TIF1γ; TRIM33)-mediated monoubiquitylation and USP9x (FAM)-mediated deubiquitylation (Dupont et al., 2009). He described the developmental consequences of inactivating ectodermin in the mouse embryo, which are consistent with a role of ectodermin as an antagonist of SMAD4-dependent signaling in both epiblast and extra-embryonic ectoderm development. Using a conditional mouse strain, Laurent Bartholin (INSERM, Lyon, France) showed that loss of functional TIF1γ cooperates with activated KRAS to induce cystic tumors of the pancreas that resemble human intraductal papillary mucinous neoplasms. Coupled with their demonstration that TIF1γ expression is downregulated in human pancreatic tumors, these data suggest that TIF1γ plays a crucial role in preventing tumor progression in the pancreas (Vincent et al., 2009). This work represents a promising model with which to explore further the role of TIF1γ in TGFβ signaling, especially during pancreatic tumorigenesis, and to understand the complex relationship between TIF1γ and SMAD4, the loss-of-function of which is also associated with pancreatic cancers. These two reports, together with a previous report that TIF1γ can compete with SMAD4 for complex formation with SMAD2 and SMAD3 in response to TGFβ and can thus activate distinct responses (He et al., 2006), suggest that the functions of TIF1γ and its intersection with SMAD4 might be context-dependent and result in different molecular read-outs.

Smad complexes regulate gene expression through interactions with a variety of high-affinity, DNA sequence-specific transcription factors, co-activators and co-repressors (Feng and Derynck, 2005; Ross and Hill, 2008). However, little is known about whether or how Smads affect higher order chromatin organization and presentation. Aristidis Moustakas (Ludwig Institute for Cancer Research, Uppsala, Sweden) presented evidence that SMAD3 specifically interacts with the chromatin insulator CTCF, a zinc-finger protein that has been implicated in various regulatory functions, including transcriptional activation and repression, chromatin insulation and imprinting, and the overall organization of chromatin at diverse genomic loci (Phillips and Corces, 2009). He showed that, in

response to TGFβ, the Smad-CTCF complex occupies the *H19* imprinting control region (ICR) insulator that is associated with the imprinted *Igf2*/*H19* locus. This allele-specific recruitment of the Smad-CTCF complex results in the allele-specific regulation of expression of the gene that encodes insulin-like growth factor II (IGF2).

While R-Smads are activated by type I receptor-mediated phosphorylation at their C-termini, their activity and stability are further regulated by downstream kinases of other signaling pathways via phosphorylation at various sites in the linker between the MH1 and MH2 domains, and by protein-protein interactions (reviewed by Moustakas and Heldin, 2009). Such pathway cross-talk was discussed by Jeff Wrana (Lunenfeld Research Institute, Toronto, Canada), as he reported findings on the cross-talk between the Smad pathway and the Hippo pathway that controls organ size. TAZ (transcriptional coactivator with PDZ-binding motif; also known as WWTR1), a relative of YAP, is a transcriptional co-activator that functions as an effector in the Hippo pathway, regulates SMAD2/3 signaling and is required for ligand-induced retention of the SMAD2-SMAD4 complex in the nucleus (Varelas et al., 2008). Wrana presented evidence that silencing TAZ expression results in enhanced β-catenin levels, illustrating cross-talk with the Wnt signaling pathway. Furthermore, whereas unphosphorylated TAZ promotes retention of the Smad complex in the nucleus, enhanced Hippo pathway signaling in response to high cell density results in TAZ phosphorylation and in the inhibition of Smad nuclear translocation. These results illustrate an important role for TAZ in coupling TGFβ responsiveness to cell density (see Fig. 2H). Perhaps a similar coupling of the Hippo and TGFβ family pathways is important in coordinating tissue growth with patterning during development.

Kunxin Luo (UC Berkeley, CA, USA) discussed the role of SnoN (SKIL – Mouse Genome Informatics) in the regulation of embryonic and postnatal development, as revealed using a knock-in mouse line that expresses a mutant SnoN protein defective in binding and repressing Smad proteins, and a transgenic line that overexpresses SnoN. Her findings indicate that SnoN regulates yolk sac angiogenesis during embryogenesis, as well as mammary gland development postnatally. These results provide the first description of specific functions of SnoN in modulating Smad signaling during mammalian developmental events.

Finally, research in the laboratory of Akiko Hata (Tufts University School of Medicine, Boston, MA, USA) has revealed that, in addition to their functions as transcription factors, TGFβ- and BMP-activated Smads also act as RNA-binding proteins. In association with the RNA helicase p68 (DDX5) in the DROSHA complex, Smads enhance the processing of the primary transcript for the microRNA *miR-21* (Davis et al., 2008). This regulation requires that the Smad complex binds to its specific recognition sequence, which is found in the primary transcripts of at least 20 other microRNAs. Her results suggest that Smads might function as regulators of microRNA processing in response to TGFβ family signals.

Novel insights into the actions of TGFβ proteins

TGFβ family proteins impact multiple cell processes and behaviors in development and homeostasis, including the control of cell proliferation, cell survival or apoptosis, extracellular matrix deposition, the acquisition of specific cell fates and progression of cell differentiation. During this meeting, novel insights into the different actions of TGFβ family proteins were discussed, some of which have already been summarized above. Liliana Attisano (University of Toronto, Canada) previously reported on the signaling mechanisms that allow BMP7 to induce dendrite formation. Specifically, LIM kinase 1 (LIMK1), a downstream effector of Rho GTPases, interacts with the long cytoplasmic sequence of the type II BMP receptor BMPRII (BMPR2) downstream from the kinase domain, thus linking BMP signaling to actin reorganization (Lee-Hoeflich et al., 2004) (see Fig. 1). She now reported that BMP7 induces JNK activation at the tips of dendrites where BMPRII is localized, and that JNK associates with a specific sequence in the cytoplasmic domain of BMPRII. Inhibiting JNK activation or preventing JNK from binding to BMPRII blocks BMP7-induced dendritogenesis (see Fig. 2F). These findings illustrate important roles for non-Smad pathways in BMP-induced differentiation, which in this context are linked to the roles of the cytoplasmic domain of BMPRII. Ed Leof showed that an important aspect of TGFβ activity in mesenchymal cells may result from its ability to induce the expression of EGF receptor ligands, leading to activation of Erk MAP kinase signaling as a consequence of EGF receptor activation.

In addition to Piccolo's discussion of SMAD4 control, Liz Robertson (University of Oxford, UK) elaborated on the role of SMAD4 in cell fate specification and epiblast growth in the early mouse embryo. She showed that loss of SMAD4 from the epiblast results in an excess of basement membrane proteins, such as collagen and laminin, which physically constrains cell migration during anterior primitive streak formation and thus affects primitive endoderm specification. Robertson suggested that continuous remodeling of the basement membrane is regulated by TGFβ family signaling and is crucial for early cell movements (see Fig. 2B). In the same vein, Kristi Wharton provided evidence for the control of border cell specification and cluster migration by Gbb signaling in the *Drosophila* egg chamber. In this case, the distribution of E-cadherin is regulated by Gbb signaling, as is the activity of JAK/STAT signaling (see Fig. 2A). These studies underscore the possible coordination of cell fate specification and cell movement through the control of both processes by TGFβ family signaling.

During tissue growth and regeneration, multi-fate cell lineages are established, as was discussed by Arthur Lander (UC Irvine, CA, USA) in the context of self-renewing adult mouse olfactory epithelium (Lander et al., 2009). Using experimental data and mathematical modeling, Lander demonstrated that the progression from stem cell to olfactory receptor neuron, which requires the action of the activin-related ligands GDF11 and activin, involves elegant negative-feedback controls, reminiscent of a finely tuned machine.

Roles for TGFβ family proteins in metabolism were discussed by several speakers. Mike O'Connor demonstrated that, as one of its functions, a *Drosophila* activin, Daw, appears to act in metabolic homeostasis. Consistent with this role, Daw is expressed in the circulating hemolymph and signals through the BaboC type I receptor. Interestingly, lethality due to *daw* inactivation can be rescued by varying the diet and by the function of *dilp6* (*Ilp6* – FlyBase), a gene that encodes an insulin-like growth factor. Chester Brown (Baylor College of Medicine, Houston, TX, USA) showed that in mice a high-fat diet increases GDF3 expression and that *Gdf3*$^{-/-}$ mice are resistant to obesity induced by diet (Shen et al., 2009) (see Fig. 2C). Finally, Rik Derynck reported that glucose induces a rapid increase in cell surface TGFβ receptor presentation and a rapid activation of TGFβ ligand by matrix metalloproteinases (MMPs) 2 and/or 9 (see Fig. 2C). Consequently, glucose induces autocrine TGFβ signaling through Smads and the Akt-TOR pathway, leading to increased cell size. Thus, activation of TGFβ

signaling plays an essential role in the control of cell size and in cell hypertrophy induced by high glucose, as is observed in diabetes (Wu and Derynck, 2009).

TGFβ signaling has been shown to regulate cell survival as a key regulator of cell death through apoptosis. Using a cell culture model system, Kohei Miyazono (University of Tokyo, Japan) demonstrated that TGFβ can also induce autophagy. In addition to a conversion of microtubule-associated protein light chain 3 (LC3; MAP1LC3) I to II, an increase in the formation of autophagic vacuoles and in the degradation of long-lived proteins were observed in response to TGFβ and appeared to require SMAD4. Miyazono speculated that the role of TGFβ as both a tumor promoter and tumor suppressor might relate to its effects on autophagy. In tumors insensitive to TGFβ, the reduction in autophagy that leads to cell death could allow tumor survival and promote growth, whereas the growth inhibition by TGFβ could be partially explained by an increase in autophagic cell death.

Dysregulation of TGFβ family signaling in disease and cancer

Consistent with the many developmental defects that result from experimentally dysregulated TGFβ family signaling, moderate alterations in TGFβ family protein function have been linked to many diseases and developmental syndromes in humans. Illustrating the key roles of TGFβ family signaling in skeletal disease, Eileen Shore (University of Pennsylvania School of Medicine, Philadelphia, PA, USA) discussed the linkage of fibrodysplasia ossificans progressiva (FOP), which results in the progressive formation of extra-skeletal bone, to a single amino acid replacement in the GS activation domain of the BMP receptor ALK2 that confers enhanced basal signaling (Shore et al., 2006) (see Fig. 2E). In zebrafish, the orthologous mutation results in enhanced chondrogenesis, and, in mice, introducing the same Arg206His mutation into the *Alk2* gene results in toe abnormalities, gradual extra-skeletal bone formation and other phenotypic changes that mimic the human FOP syndrome.

Peter ten Dijke reported his studies on the roles of TGFβ signaling in angiogenesis. Inhibiting TGFβ signaling through ALK5 results in increased endothelial cell migration and proliferation, which are further enhanced in the presence of vascular endothelial growth factor (VEGF) (Liu, Z. et al., 2009) (see Fig. 2A). His results also emphasize a key role for the ALK1 receptor in angiogenesis. For example, inhibition of ALK1 function impairs VEGF-induced angiogenesis and cancer progression in mouse models. Rosemary Akhurst (UC San Francisco, CA, USA) described progress in the characterization of polymorphic genetic loci that determine the penetrance of embryonic lethality in $Tgfb1^{-/-}$ mice (Mao et al., 2006). Two of these genetic loci have been characterized in detail and are composed of gene clusters that encode proteins with related functions in cell proliferation, migration and plasticity, including some that are known to interact with TGFβ signaling components. Each locus contains more than one polymorphic genetic element that influences TGFβ-dependent processes. Both loci influence basal phopho-SMAD2 levels in mouse tissue, and both modify tumor susceptibility in a chemically induced mouse skin carcinogenesis model. One of these possesses both positive and negative genetic elements that regulate developmental angiogenesis, tumor susceptibility and tumor metastasis in mouse models.

As illustrated above, dysregulated TGFβ signaling is thought to be of importance for malignant cell transformation, cancer initiation and progression. That the presentation and function of TGFβ receptors is a determinant in cancer progression was illustrated by the findings of Gareth Inman (The Beatson Institute for Cancer Research, Glasgow, UK) on disabled 2 (DAB2), a PTB domain protein that interacts with the TGFβ receptor complex (Hocevar et al., 2001). He showed that high levels of DAB2 are required for TGFβ to inhibit cell proliferation and migration, whereas low levels of DAB2 enable TGFβ to promote migration and anchorage-independent growth (see Fig. 2A). Furthermore, *DAB2* expression, which is often decreased in squamous carcinomas, may be indicative of poor prognosis, as supported by the observation that its levels inversely correlate with breast cancer relapse to the lung and brain.

Hal Moses (Vanderbilt-Ingram Cancer Center, Nashville, TN, USA) showed that the conditional knockout of the type II TGFβ receptor in six different mammalian epithelial cell systems gave a minimal phenotype overall without the development of carcinomas. However, when the knockout mouse model was challenged with oncogene expression or when a tumor suppressor gene was mutated, the development of invasive and metastatic carcinomas was greatly enhanced. Studies with mammary carcinomas demonstrate that a major mechanism underlying this carcinoma progression is the enhanced expression of chemokines by carcinoma cells lacking a functional *Tgfbr2* gene, resulting in the recruitment of immune suppressor cells that express abundant TGFβ and matrix metalloproteases in the tumor microenvironment, promoting invasion and metastasis (Yang and Moses, 2008; Bierie et al., 2009). These findings were complemented by the presentation by Makoto Mark Taketo (Kyoto University, Japan), who discussed cancer progression in his *cis-*$Apc^{+/\Delta 716}$ $Smad4^{+/-}$ mouse model, in which defective SMAD4-dependent TGFβ signaling turns intestinal adenomas into invasive adenocarcinomas. He demonstrated that $CD34^{+}$ immature myeloid cells are recruited from the bone marrow to the tumor invasion front, apparently driven by the interaction of the chemokine receptor CCR1, which is expressed in myeloid cells, with its ligand CCL9, which is highly expressed in the tumor epithelium (Kitamura et al., 2007). Using a mouse model, in which cancer cells have been injected into the spleen and allowed to disseminate to the liver, he further showed that colon cancer cells recruit these immature myeloid cells and help intrahepatic colonization of the disseminated cells. This metastatic expansion is dependent on both the CCL9 ligand and its receptor CCR1.

Conclusions

Advances in the TGFβ signaling field continue to expand the number of processes in which it is recognized as required, and clarify the mechanisms that underlie the exquisitely finely tuned transduction of its signals. As we consolidate our understanding of the flexibility within the pathway and of the interplay between TGFβ family signaling and other pathways, it has become clear that there are astounding similarities between its roles in different processes and organisms. Despite progress in the field, further insights into the context-dependent nature of the pathway will depend on continued in vivo studies in developmental and disease systems. Definitive measurements of the molecular properties that govern the expression, presentation and turnover of signaling components are needed to make full use of modeling and systems approaches that are aimed towards the successful development of therapeutics for the treatment of syndromes and diseases arising from the dysregulation of TGFβ family signaling. Much work remains to be done. The convergence of cell biologists, developmental biologists, biochemists and geneticists at future TGFβ FASEB meetings will continue to ensure success in these endeavors.

Acknowledgements
We thank Mike O'Connor and Kunxin Luo for their efforts in organizing a highly interactive and exciting meeting, and acknowledge the many talks and posters that could not be covered in this review owing to space constraints, as they also stimulated many discussions by meeting attendees throughout the week. K.W. is supported by a grant from NIGMS and R.D. is supported by grants from NCI, NHLBI and NIAMS.

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