β-catenin signaling in vitro in extracts of *Xenopus* embryos (*48*). With regard to understanding the roles of Wnts in early development, studies in *Xenopus* have established that an asymmetry in β-catenin during the first cell cycles correlates with the dorso-ventral axis (*49*) and is required for axis formation (*50*). The STKE Specific Pathway on the *Xenopus* egg Wnt/β-catenin pathway (*14*) highlights the maternal pathway that is involved in axis specification, and it will be expanded as a consensus is reached regarding the composition and functions of zygotic and noncanonical Wnt and Frizzled pathways.

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VIEWPOINT

Signal Transduction by the TGF-β Superfamily

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Transforming growth factor–β (TGF-β) superfamily members regulate a plethora of developmental processes, and disruption of their activity has been implicated in a variety of human diseases ranging from cancer to chondrodysplasias and pulmonary hypertension. Intense investigations have revealed that SMAD proteins constitute the basic components of the core intracellular signaling cascade and that SMADs function by carrying signals from the cell surface directly to the nucleus. Recent insights have revealed how SMAD proteins themselves are regulated and how appropriate subcellular localization of SMADs and TGF-β transmembrane receptors is controlled. Current research efforts investigating the contribution of SMAD-independent pathways promise to reveal advances to enhance our understanding of the signaling cascade.

The first member of the transforming growth factor–β (TGF-β) superfamily of secreted polypeptide factors, TGF-β1, was discovered approximately 20 years ago. Since then, the family has grown considerably and now comprises over 30 vertebrate members and a dozen or so structurally and functionally related proteins in invertebrates such as worms and flies (*1–6*). TGF-βs control a plethora of cellular functions, and their activity is critical for regulating numerous developmental and homeostatic processes. Mutations in TGF-β family ligands are responsible for a number of human diseases, including hereditary chondrodysplasia and persistent mullerian duct syndrome (*5*). In addition, TGF-β itself plays an important role in cancer progression by functioning both as an antiproliferative factor and as a tumor promoter, and numerous components of the signal transduction pathway are tumor suppressors that are functionally mutated in cancer (*5*, *7*). These diverse activities have prompted intense investigations into understanding how TGF-β family members signal their effects.

Parallel work in vertebrates, worms, and flies has revealed a conserved signaling pathway, which at first glance appears to be surprisingly simple (*1–5*, *7*) [see the TGF-β Pathway (*6*)]. The cell-surface receptor that carries the TGF-β family signal into the cell is a complex of single-pass transmembrane receptors that contain an intracellular kinase domain that phosphorylates serine and threonine residues (Fig. 1). This serine-threonine kinase receptor complex consists of two distinct transmembrane proteins, known as the type I and type II receptors. Ligand binding induces the type I and type II receptors to associate, which leads to a unidirectional phosphorylation event in which the type II receptor phosphorylates the type I receptor, thereby activating its kinase domain. The activated type I receptor then signals to the SMAD family of intracellular mediators. SMAD

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family members were first identified through genetic screens in flies and worms, but the family quickly grew to include eight mammalian counterparts. SMADs can be divided into three distinct classes. The receptor-regulated, or R-Smads (Smads1, -2, -3, -5, and -8), are directly phosphorylated by the type I receptors on two conserved serines at the COOH-terminus. Phosphorylation of R-Smads serves many functions in the pathway. It induces release from the receptor complex as well as from SARA (SMAD anchor for receptor activation), a protein that recruits SMADs to the membrane. Phosphorylation also stimulates R-Smads to accumulate in the nucleus as heteromeric complexes with a second class of SMADs, the Co-Smads, of which Smad4 is the only member. In the nucleus, the SMADs associate with one of many DNA binding partners and various transcriptional coactivators or corepressors, thereby positively or negatively regulating gene expression. In contrast, the third class of SMADs, the inhibitory SMADs (Smad6 and -7), counteract the effects of the R-Smads and thus antagonize TGF-β signaling.

Vertebrates have seven distinct type I receptors, each of which can mix and match with one of five type II receptors to mediate signals for the TGF-β family ligands (*1–7*). Despite this apparent complexity, the biological output appears to be entirely determined by the type I receptor. Even more surprising is that the signal emanating from the type I receptor is funneled at the membrane into one of two intracellular pathways. Three of the receptors phosphorylate the R-Smads Smad2 and Smad3 and thereby transduce TGF-β-like signals, whereas the other four receptors activate the R-Smads Smad1, Smad5, and Smad8 to mediate signals characteristic of those initiated by bone morphogenetic proteins (BMPs). Each of the R-Smads can then interact with a wide array of specific DNA binding proteins to regulate transcriptional responses. Thus, the signaling pathway takes the shape of an hourglass. Because cells are almost always exposed to multiple extracellular signals, an additional level of complexity is achieved through cross talk of the TGF-β pathway with that of other signaling cascades (*2–7*). For instance, activation of mitogen-activated protein kinases (MAPKs) by receptor tyrosine kinases can modify TGF-β signals through the direct phosphorylation of SMADs by MAPKs. In addition, the cooperative interactions of SMADs with transcription factors that function in other signaling pathways provide a molecular explanation of one way TGF-β pathways interact with those of other growth factors.

Because a basic molecular description of how SMADs transmit TGF-β superfamily signals has been achieved, interest has now turned toward investigating how SMAD function is regulated. Structure-based investigations have revealed important determinants that mediate the interaction of SMADs with the receptors, transcriptional partners, and other associating proteins. The identification of various proteins that interact with SMADs and the receptors has suggested that localization of these signaling mediators plays an important role in the pathway. For instance, the membrane-localized FYVE (Fab1p/YOTP/Vac1p/EEA1) domain–containing protein SARA presents Smad2 to the TGF-β receptor complex. TGF-β receptors can associate with caveolin, a protein found in plasma membrane invaginations called caveaolae, and can interact with sorting nexins and TRAP-1, proteins implicated in vesicle transport (*2*, *3*). Abundance of SMAD proteins is also regulated by the ubiquitin-proteasome pathway through association of SMADs with E3 ubiquitin ligases such as Jab1, Roc1, and Smurfs (*2–9*). Smurf proteins are members of the HECT (homologous to E6AP COOH-terminus)–domain containing E3 ubiquitin ligases that interact through their WW domains with a specific proline-tyrosine motif in certain SMADs. However, the Smad-E3 ligase interactions do not function only in regulating SMAD degradation. SMADs can also serve as adapters to bring Smurfs (*2*, *3*, *6*), the anaphase promoting complex (*10–11*), and possibly other E3 ligases to protein targets that include the TGF-β receptor complex, the transcriptional repressor SnoN, and the adapter protein HEF1 (*2*, *3*, *6*). So, in addition to regulating transcription, SMADs can control the turnover of proteins.

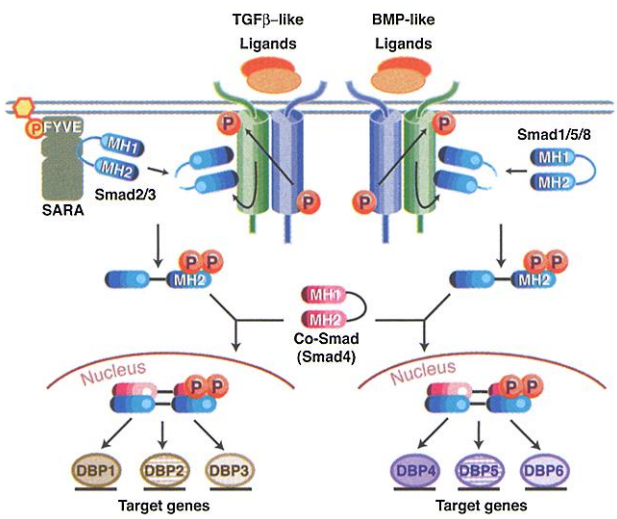

Fig. 1. TGF-β-like and BMP-like ligands signal through distinct receptors and SMADs. TGF-β/activin and BMPs bind to distinct receptor complexes, which then phosphorylate distinct R-Smads. R-Smads then form heteromeric complexes with Smad4, and these complexes translocate to the nucleus. Specific R-Smads recognize different DNA binding proteins (DBPs), regulate distinct target genes, and thereby generate diverse biological responses.

Abundant evidence demonstrates that SMADs are critical for TGF-β family signaling. However, accumulating data suggests that SMAD-independent pathways also exist (*2–7*). For instance, TGF-β rapidly activates Rho family guanosine triphophatases (GTPases); MAPKs, including ERKs, p38, and JNKs through their upstream kinase activators such as TAK1; and protein kinase B (PKB, also called Akt). However, no direct link between these pathways to receptors has yet been made, and this represents an important area for future investigation.

The original premise that elucidation of the TGF-β superfamily signal transduction pathways might provide insights into human disease have been borne out. Various human syndromes and illnesses, both hereditary and spontaneous, have been attributed to mutations in pathway components. Mutations in receptors can cause hereditary hemmorhagic telangiactasia, primary pulmonary hypertension, persistant mullerian duct syndrome, hereditary nonpolyposis colon cancer, and juvenile polyposis syndrome; also, mutations in SMADs have been associated with cancers, particularly those of the colon and gastrointestinal tract (*5*, *7*). Further elaboration of this pathway promises to provide insights into cellular regulation and physiology in health and disease.

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VIEWPOINT

Signaling Pathways for PC12 Cell Differentiation: Making the Right Connections

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A key issue in signal transduction is how signaling pathways common to many systems—so-called canonical signaling cassettes—integrate signals from molecules having a wide spectrum of activities, such as hormones and neurotrophins, to deliver distinct biological outcomes. The neuroendocrine cell line PC12, derived from rat pheochromocytoma, provides an example of how one canonical signaling cassette—the Raf → mitogen-activated protein kinase kinase (MEK) → extracellular signal-regulated kinase (ERK) pathway—can promote distinct outcomes, which in this case include neuritogenesis, gene induction, and proliferation. Two growth hormones, epidermal growth factor (EGF) and nerve growth factor (NGF), use the same pathway to cause PC12 proliferation and differentiation, respectively. In addition, pituitary adenylate cyclase–activating polypeptide (PACAP), a neurotransmitter that also causes differentiation, uses the same canonical cassette as NGF but in a different way. The Connections Map for PC12 Cell Differentiation brings into focus the complex array of specific cellular responses that rely on canonical signal transduction systems.

The PC12 cell line was derived from rat pheochromocytoma, a tumor arising from chromaffin cells of the adrenal medulla. It is a useful model for studying cell signaling for at least two reasons: (i) There are few growth factors, neurotrophins, and hormones to which it does not respond; and (ii) distinct responses of differentiation, proliferation, and survival can all be assessed independently. Differentiation (halted proliferation and neurite outgrowth) of PC12 cells by NGF was described in the first report on the cell line (*1*). NGF signaling through the receptor tyrosine kinase (RTK), TrkA, causes differentiation (*2*). The paradoxical finding that the *src* and *ras* oncogene products enhanced rather than blocked NGF-induced differentiation led to the identification of signaling pathways involving both Ras and Src as part of the total differentiation response to NGF (*3*). A closely related RTK activated by EGF stimulates proliferation, rather than differentiation, of PC12 cells (*4*). The responses to NGF and EGF both require ERK, a mitogen-activated protein kinase (MAPK). Neurite outgrowth stimulated by PACAP, an adrenomedullary neurotransmitter, also occurs through ERK activation, in a process similar to but distinct from NGF signaling (*5*).

These studies put into focus a fundamental question of signal transduction: How are canonical signaling cassettes, such as Raf → MEK (i.e., MAPK kinase) → ERK, accessed by hormones and neurotrophins and differentially integrated into the signaling network (*6*) of PC12 cells to promote distinct outcomes, including neuritogenesis, gene induction, and proliferation (*7*)?

The duration of signaling through ERKs may hold the key to the very different outcomes of EGF and NGF stimulation. EGF induces rapid and transient Ras- and Rap1-dependent ERK phosphorylation, whereas NGF stimulation of ERK is both rapid and sustained, with sustained activation dependent on signaling to ERK through Rap1 (*8, 9*) (Fig. 1). Differential recruitment of phosphatidylinositol 3-kinase (PI3K) and scaffolding components (such as the adaptor FRS2) to activated TrkA, but not to the EGF receptor complex, may be the explanation for sustained Rap1-mediated B-Raf activation by TrkA, but not by the EGF receptor (*8–10*). PI3K also activates the c-Jun NH_2-terminal kinases (JNKs), which, through activation of c-Jun, can promote differentiation or apoptosis, depending on the cell's history of exposure to NGF (*11*). Thus, differentiation, survival, and proliferation may involve a balance among MAPK signaling pathways that depends, in turn, on the combination of neurotrophins and other first messengers present in the cellular milieu.

G protein–coupled receptor (GPCR) activation can also stimulate some aspects of differentiation, especially neurite outgrowth. PACAP signals through the GPCR type 1 PACAP-preferring receptor (PAC1) in PC12 cells (*12, 13*). Both NGF and PACAP cause robust neurite outgrowth, which requires activation of ERK (*14*). NGF requires both Ras- and Rap1-dependent B-Raf activation to stimulate neurite outgrowth, whereas PACAP signaling is Ras-independent (*5*). Does PACAP stimulate a second pathway that substitutes for Ras in neuritogenic signaling? PACAP-stimulated neurite extension is blocked by RpcAMPS, a cyclic adenosine 3′,5′-monophosphate (cAMP) antagonist (*15*). Elevation of cAMP activates ERK through protein kinase A (PKA)–dependent activation of Rap1, which stimulates B-Raf (*16*). However, PACAP-stimulated neuritogenesis is not blocked by the PKA inhibitor H89 (*5, 14*), which suggests that another cAMP sensor besides PKA mediates activation of ERK by PACAP. Finally, cAMP response element (CRE)–mediated transcription is a convergence point for multiple

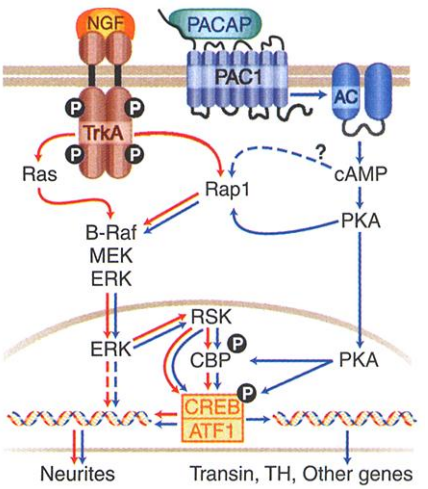

Fig. 1. Signaling pathways for PACAP- and NGF-dependent PC12 cell differentiation. PACAP-dependent signaling is coded in blue, NGF-dependent signaling in red. Arrows are meant to convey major features of information flow through signaling pathways activated differentially by NGF and PACAP. Differences in intensity, duration, and synergy of signaling through a given node or set of nodes, although not indicated, contribute to qualitative differences in PACAP and NGF actions. For example, Ras- and Rap1-dependent signaling are thought to account for immediate and sustained effects, respectively, of NGF mediated through ERK. Rap1-dependent B-Raf activation may also differ in intensity and duration in a stimulus-dependent fashion, perhaps accounting for PKA-dependent and PKA-independent aspects of signaling through ERK. Thus, although the TrkA and PACAP pathways activate several common cellular signaling components, their ultimate effects on gene transcription and cellular phenotype differ substantially. Abbreviations: AC, adenylate cyclase; ATF1, activating transcription factor 1; CBP, CREB binding protein; CREB, cAMP response element–binding protein; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; NGF, nerve growth factor; PAC1, type 1 PACAP-preferring receptor; PKA, protein kinase A; RSK, ribosomal S6 protein kinase; TH, tyrosine hydroxylase.

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