Activin Receptors

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SUMMARY

Activin receptors are structurally related membrane proteins that belong to the transforming growth factor (TGF)β receptor superfamily. Two main types, I and II activin receptors have been identified which consist of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain containing a serine/threonine kinase region. A dimer of each of these receptors participates in forming a receptor complex on the cell surface with the dimeric ligand. This activated complex signals intracellularly through the kinase domain of type I receptors to recruit and activate the intracellular transducers of activin: receptor-regulated (R)-Smad proteins. The latter form a hetero-oligomeric complex with a common (Co)-Smad protein shared by all TGFβ-induced signaling pathways. This complex then translocates to the nucleus and forms a transcription complex that binds to promoters and regulates the expression of several genes. One of these genes encodes an inhibitory (I)-Smad protein which negatively regulates further signaling. Activin receptors are widely expressed in various organs and cell types. Activin binding to its receptors leads to a plethora of biological functions which are antagonized by inhibin that competes with activin for binding to the receptor. In addition, activin receptors bind additional ligands and thus mediate functions which are not necessarily related to activin. Studies of mutant activin receptors in different species, including mammals, revealed dramatic phenotypes that demonstrate the crucial role of these receptors in early mesoderm induction. Accordingly, activin receptors control the expression of several mesoderm differentiation genes. Furthermore, activin receptors control embryonic axis symmetry determination and organ development and are also involved in the control of specific adult organ function.

BACKGROUND

Discovery

Activin binding sites were initially observed and identified on various cells in the late 1980s (Mathews, 1994). The first successful approach to identify and clone a mammalian activin receptor used the binding of radioactive activin A to an expression cDNA library. Murine activin receptor type IIA (ActRIIA) was cloned and was the first receptor of the TGFβ receptor superfamily to be described (Mathews and Vale, 1991). This later led to the cloning of human ActRIIA (Donaldson *et al.*, 1992). Sequence analysis of the kinase domain identified in this membrane protein showed that two inserts are found in the sequence, similar to those found in the first described transmembrane serine/threonine kinase, the *daf-1* gene product from *Caenorhabditis elegans* (Georgi *et al.*, 1990). These findings indicated the presence of a new family of receptors, later to be called the TGFβ receptor superfamily. The identification of activin receptor type IIB soon followed and the mRNA was cloned from mouse (Attisano *et al.*, 1992) and *Xenopus* (Mathews *et al.*, 1992).

Activin type I receptors were identified in parallel by several groups and were given different names: human SKRI (Matsuzaki *et al.*, 1993), human SKR2 (Xu *et al.*, 1994), rat R1-R4 (He *et al.*, 1993), human ALK-1–ALK-4 (ten Dijke *et al.*, 1993), mouse Tsk-7L (Ebner *et al.*, 1993), human ActR-I (Attisano *et al.*, 1993) and rat ActX1R (Tsuchida *et al.*, 1993).

Alternative names

Activin receptor type IIA/type II/ACVR2.
Activin receptor type IIB/ACVR2B.

Activin receptor type I or IA/ALK-2/ActR-I/SKR1/Tsk-7L/ActX1R/R1/ACVR1.
Activin receptor type IB/ALK-4/SKR2/R2/ACVR1B.

Structure

Activin receptors type I and II are structurally related protein kinases belonging to the TGFβ receptor superfamily. They all contain a hydrophobic signal peptide, a small extracellular ligand binding domain, a single transmembrane domain and a cytoplasmic serine/threonine kinase domain. These receptors are glycosylated and are serine and threonine phosphoproteins (Mathews and Vale, 1993).

The full-length gene for human and murine ActRIIA produces a protein sequence of 513 amino acids (aa): 19 aa signal peptide; 116 aa extracellular domain; 26 aa transmembrane domain; 352 aa intracellular domain; thus the gene encodes a mature protein 494 amino acids long (Mathews and Vale, 1991; Donaldson *et al.*, 1992). Similarly, the ActRIIB open reading frame encodes 536 amino acids: 18 aa signal peptide; 116 aa extracellular domain; 26 aa transmembrane domain; 376 aa intracellular domain; producing a 518 aa polypeptide (Attisano *et al.*, 1992).

The ActRIA/ALK-2 gene is described in several papers as encoding 509 amino acids: 20 aa signal peptide; 103 aa extracellular domain; 23 aa transmembrane domain; 363 aa intracellular domain; giving rise to a 489 aa membrane protein (Tsuchida *et al.*, 1993; Matsuzaki *et al.*, 1993; Ebner *et al.*, 1993). The ActRIB/ALK-4 gene encodes for 505 amino acids: 23 aa signal peptide; 103 aa extracellular domain; 24 aa transmembrane domain; 355 aa intracellular domain; thus producing a 482 aa long mature protein (He *et al.*, 1993).

Main activities and pathophysiological roles

Activins mediate their biological functions by binding to ActRs that initiate intracellular signaling cascades. These cellular functions are reviewed in the chapter on Activin. However, the study of activin A and B and ActRIIA knockout mice (Matzuk *et al.*, 1995a,b) indicated that neither are required for mesoderm formation or for establishment of right–left symmetry during embryogenesis, implicating other ligand/receptor combinations. Indeed, ActRs interact with ligands other than activins, and these may be responsible for several functions mediated by ActRs. Moreover, differences exist in the capacity of different types of activin receptors to elicit biological functions. These issues are discussed below. See Affinity for ligand(s) and Biological consequences of activating or inhibiting receptor and pathophysiology.

GENE

Accession numbers

The accession numbers for cDNAs are:
Activin receptor type IIA:
NCBI reference sequences (human): NM_001616
Human: M93415 (Donaldson *et al.*, 1992)
Mouse: M65287 (Matthews and Vale, 1991)
Xenopus (S70930, S49438), rat (S48190), cow (L21717, U43208), chicken (D31899, U31222), sheep (L19442)
Activin receptor type IIB:
NCBI reference sequences (human): NM_001106
Human: X77533 (Hilden *et al.*, 1994)
Mouse: M84120 (Attisano *et al.*, 1992)
Xenopus (M88594), zebrafish (AF069500), cow (U57707), chicken (U31223)
Drosophila type II receptor Atr-II (L22176) differs from both type IIA and B receptors and binds activin in concert with Atr-I (U04692).
Activin receptor type IA/ALK-2:
NCBI reference sequences (human): NM_001105
Human: Z22534 (ten Dijke *et al.*, 1993), L02911 (Matsuzaki *et al.*, 1993)
Xenopus (U49914), rat (L19341)
Activin receptor type IB/ALK-4:
NCBI reference sequences (human): NM_020327
Human: Z22536 (ten Dijke *et al.*, 1993), L10125 and L10126 (Xu *et al.*, 1994), Xenopus (U60643)
The accession numbers for receptor gene sequences known:
Mouse activin receptor type IIA: S37518 and S37521 (Matzuk and Bradley, 1992)
Human activin receptor type IIB: AB008681 (Ishikawa *et al.*, 1998)
Human activin receptor type IB/ALK-4: L31848 (Xu *et al.*, 1994)

Chromosome location and linkages

Human chromosomes:
Activin receptor type IIA: 2q22.2-q23.3
Activin receptor type IIB: 3p22-p21.3
Activin receptor type IA/ALK-2: 2q23-q24
Activin receptor type IB/ALK-4: 12q13
Mouse:
Activin receptor type I: chromosome 2

PROTEIN

Sequence

Protein sequence information can be retrieved with the accession numbers cited in the previous section.

Description of protein

Activin type II receptors are molecules of approximately 70 kDa consisting of ~60 kDa core protein modified, probably in two sites, by *N*-linked glycosylation. These receptors are constitutively phosphorylated, in part by autocatalytic events. Phosphorylation is found mainly on serine residues and to a lesser extent on threonine residues and remains unaltered by addition of ligand, in cells lacking type I receptors (Attisano *et al.*, 1992; Mathews and Vale, 1993). Experiments with a deglycosylated extracellular-domain recombinant form of ActRII (ActRII-ECD) did not show lower affinity for activin, thus indicating that glycosylation is not essential for the high-affinity recognition and binding of activin with its receptor (Greenwald *et al.*, 1998). Common to all members of this family is an extracellular cysteine-rich motif in proximity to the transmembrane domain containing 10 conserved cysteines with shared spacing. These cysteines are necessary for determining the correct three-dimensional structure required for ligand binding. Crystal structure (15 Å resolution) of the ActRII-ECD ligand-binding domain revealed a fold known as a three-finger toxin fold found in snake toxins, containing eight cysteines that form four disulfide bonds (Greenwald *et al.*, 1999). This has led to the identification of the binding site for activin and inhibin on type II receptors which consists of a cluster of three hydrophobic residues (Gray *et al.*, 2000).

Type II receptors contain proline-rich segments juxtaposed on both sides of the transmembrane domain. The intracellular one resembles SH3 domains known for binding of cytoplasmic signaling proteins. In the case of ActRIIB, which has four alternatively spliced forms: two isoforms include an 8 aa segment containing the extracellular prolines, and two isoforms include an intracellular 24 aa segment inserted between the transmembrane domain and the proline motif preceding the kinase domain (Attisano *et al.*, 1992). The kinase region contains two conserved inserts between subdomains VIA and VIB and subdomains X and XI (Mathews and Vale, 1991). Beyond the kinase region is a ~30 aa C-terminal tail which has high content of serine and threonine residues.

Activin type I receptors have a molecular mass of ~50 kDa. Similar to type II receptors, the extracellular domain includes 10 conserved cysteines and one potential *N*-linked glycosylation site. These receptors also contain a canonical protein kinase domain in the intracellular portion including the two inserts characteristic of the kinase domain of type II receptors. The C-terminal end of the receptor has a 5–15 aa extension, typically shorter than that observed in type II receptors (Ebner *et al.*, 1993; He *et al.*, 1993; Matsuzaki *et al.*, 1993; ten Dijke *et al.*, 1993; Tsuchida *et al.*, 1993). The loop between kinase subdomains IV and V in ALK-4/ActRIB is important for mediating the downstream signaling of this receptor (Armes *et al.*, 1999).

While type II receptors are constitutively phosphorylated, type I receptor phosphorylation is regulated by the presence of type II receptors (Willis *et al.*, 1996). The juxtamembrane intracellular region is termed the GS domain in type I receptors. It is larger than that found in type II receptors (30 aa), is serine and glycine-rich and contains a unique SGSGSG motif. Serine and threonine residues within the region are phosphorylated upon complex formation with type II receptors and ligand. A Leu-Pro motif, common to all type I receptors, is present immediately after the GS sequence and serves as a binding domain for the immunophilin FKBP-12 (FK506-binding protein 12), which has been shown *in vitro* to block downstream signal transduction (Charng *et al.*, 1996; Wang *et al.*, 1996).

Relevant homologies and species differences

Activin type IIA and IIB receptors are closely related. There is 50–60% identity between the extracellular ligand-binding domains and 60–70% identity in the intracellular kinase domains of type IIA and IIB receptors (Mathews *et al.*, 1992). ActRIIA is closely related to TGFβ receptor (TβRII) (Wang *et al.*, 1991) and to the product of the *daf-1* gene of *Caenorhabditis elegans* (Georgi *et al.*, 1990). An alternatively spliced form of activin receptor type IIA was found in differentiation-induced neural cells (ActRIIA-N) and contains a 24 bp insertion in the juxtamembrane domain (Shoji *et al.*, 1998). ActRIIB was cloned from *Xenopus* (Mathews *et al.*, 1992) and two alleles were found, one of which is truncated in the C-terminal kinase domain (Nishimatsu *et al.*, 1992b); yet this truncated protein is capable of transmitting activin signal (Nishimatsu *et al.*, 1992a). Mouse ActRIIB has four splice variants which differ either in the extracelluar or in the intracellular juxtamembrane

regions of the protein and exhibit different affinities to activin (Attisano *et al.*, 1992). The *Drosophila* homolog of the type II receptors, Atr-II, is capable of binding activin with high affinity (Childs *et al.*, 1993).

Type I activin receptors have been cloned from several species. Activin XI receptor from rat brain exhibited 38–40% identity in the kinase domain to ActRIIA and IIB (Tsuchida *et al.*, 1993). Also identified were Tsk-7L from mouse (Ebner *et al.*, 1993) and SKR1 from human (Matsuzaki *et al.*, 1993). This specific receptor (ActRIA) was later termed ALK-2 according to the nomeclature of ten Dijke *et al.* (1993) and is one of eight identified ALKs. ALK-2/ActRIA can complex with ActRII and activin and produce activin signals (Attisano *et al.*, 1993). However, ALK-2/ActRIA can also bind bone morphogenic proteins (BMPs) and induce a transcriptional response (ten Dijke *et al.*, 1994b; Liu *et al.*, 1995). It is therefore debatable whether ALK-2/ActRIA is a physiological receptor for activin and it is possible that this receptor may serve two ligands depending on the type of expressing cell. ALK-4/ActRIB is considered the physiological activin type I receptor (Ile *et al.*, 1993; ten Dijke *et al.*, 1994a; Xu *et al.*, 1994). It forms complexes with ActRII and activin and produces downstream signals (Carcamo *et al.*, 1994). The remaining ALKs are not considered physiological receptors for activin.

ALK-4ActRIB has four predicted alternatively spliced mRNA species, three of which are truncated in their kinase domains (Xu *et al.*, 1994). The naturally occurring truncated forms are expressed exclusively in human pituitary tumors (Alexander *et al.*, 1996) and are found to inhibit activin signaling (Zhou *et al.*, 2000). ALK-1 is thought to signal through binding to the TGF1 or TGF3 ligands together with TRII (Lux *et al.*, 1999). ALK-3 and ALK-6 are receptors for bone morphogenic proteins (BMPs); ALK-3 is the BMP receptor type IA (BMPR-IA) and ALK-6 is the receptor for BMPR-IB (ten Dijke *et al.*, 1994b). ALK-5 is a type I TGF receptor (TRI) which complexes with TRII (Franzen *et al.*, 1993). ALK-7 is predominantly expressed in the central nervous system and can complex with type II receptors (Ryden *et al.*, 1996; Tsuchida *et al.*, 1996). Presently, the ligand for ALK-7 remains unknown. The recently cloned zebrafish ALK-8 is a BMP receptor, although it shares higher similarity with ALK-2 than with ALK-3 or ALK-6 (Yelick *et al.*, 1998). All ALKs are 6085 identical to each other in their kinase domains while the extracellular domains present lower similarity (1530). Atr-I, the *Drosophila* homolog of type I receptors, can complex with mammalian activin type II receptors or with *Drosophila* Atr-II (Wrana *et al.*, 1994) (**Figure 1**).

Affinity for ligand(s)

Activin binds activin type II receptors with high affinity of K_d = 100–500 pM while the affinity of inhibin is 10-fold lower (Mathews and Vale, 1991; Attisano *et al.*, 1992). Three-fold differences in activin affinity are observed between the four isoforms of the ActRIIB receptor and higher affinity is dependent on the presence of an extracellular juxtamembrane proline-rich region (Attisano *et al.*, 1992). Type I receptors cannot bind activin when expressed alone and affinity to activin remains unchanged when type I and type II receptors are coexpressed by cells (Tsuchida *et al.*, 1993). Monomeric activin binds the receptor with an affinity 20% of that of dimeric activin, yet signal transduction is reduced to 1% (Husken-Hindi *et al.*, 1994). Another ligand for activin receptors, OP-1/BMP-7, can bind activin type II receptors with 2–3-fold lower affinity than activin (Yamashita *et al.*, 1995). Inhibin affinity for ActRII is increased 30-fold in the presence of beta-glycan, a surface proteoglycan known to bind TGFβ (Lewis *et al.*, 2000). A membranal protein that associates strongly with inhibin is InhBP (p120). It interacts with ALK-4/ActRIB and thus disrupts the formation of functional activin receptor complexes (Chapman and Woodruff, 2001).

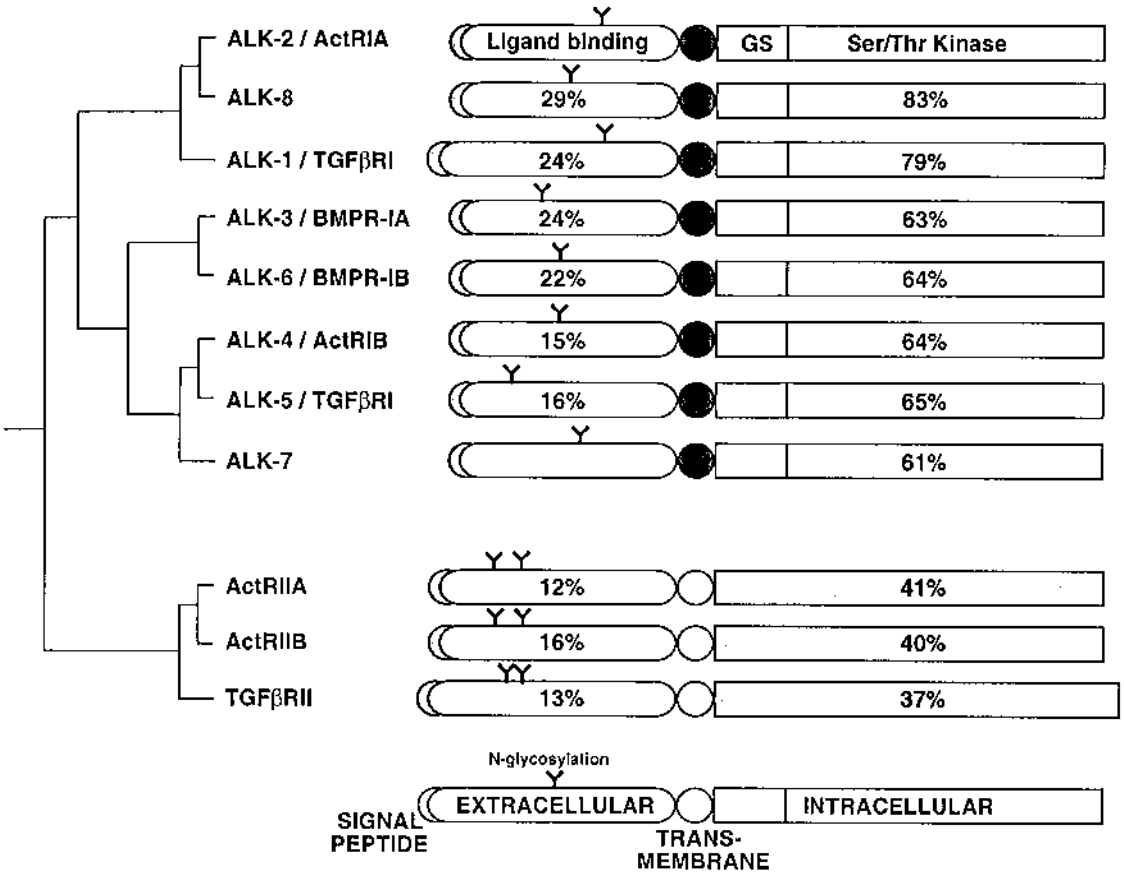
Cell types and tissues expressing the receptor

Activin receptors are expressed at different levels by a wide variety of tissues, cells and tumors of different species. **Table 1** is a nonexhaustive list of expression of these receptors in several species.

Release of soluble receptors

Naturally occurring soluble forms of activin receptors are unknown. Two recombinant forms have been produced and are discussed in the sections on Description of protein and Unique biological effects of activating the receptors. One is the extracellular domain of activin receptor II (ActRII-ECD), which was shown to bind activin and inhibin (Donaldson *et al.*, 1999) and was used in defining structural features of the ligand-binding domain. The other form has been used as a dominant negative molecule to gain an understanding of the role of ActRs during development (Dyson and Gurdon, 1997).

Figure 1 Identities of the various activin receptors in their extracellular and intracellular domains. Percentage identities are relative to ALK-2/ActRIA. The data in this figure is adapted and updated from figure 4 in Gaddy-Kurten *et al.*, 1995.



SIGNAL TRANSDUCTION

Associated or intrinsic kinases

Type II activin receptor is a constitutively active kinase which, upon ligand binding, recruits a type I receptor to form a heteromeric complex. The type I receptor is then phosphorylated in the GS domain by the type II receptor, and thus becomes an activated kinase which propagates the signal by phosphorylation of downstream proteins (**Figure 2**). Although mutations in the GS domain yield molecules that cannot produce downstream signals, indicating the importance of this domain in phosphorylation of type I receptors (Willis *et al.*, 1996), it seems that in order to achieve complete activation of the type I receptor, additional residues outside this region must be phosphorylated (Willis and Mathews, 1997).

The binding of type I receptors to ActRII can be competed for by the pseudoreceptor BAMBI which lacks the kinase domain and cannot be phosphorylated. This complex binds activin but does not signal and thus negatively regulates activin-mediated signaling (Onichtchouk *et al.*, 1999). Another protein shown to complex with ActRIIA and ActRIIB is the membrane glycoprotein endoglin. This interaction occurs with or without ligand. The function of this protein in activin receptor signaling is yet to be determined (Barbara *et al.*, 1999).

Cytoplasmic signaling cascades

Smad proteins have been demonstrated to be the intracellular mediators of the signaling pathway from the receptor serine/threonine kinases to the nucleus.

Table 1 Types of activin receptors expressed by various tissues and cells of different species (detection on mRNA and protein levels are indicated)

Species	Tissues and organs	Receptor type (detection of mRNA or protein)	References
Human	**Tissues and primary cells**		
	Heart, placenta, skeletal muscle, kidney, brain	RIA (mRNA)	ten Dijke *et al.*, 1993
	Pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, heart	RIA (mRNA)	Attisano *et al.*, 1993
	Heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas	RIB (mRNA)	ten Dijke *et al.*, 1993
	Brain, ovary	RII (mRNA)	Peng *et al.*, 1993
	Placenta	RIIA, RIIB (mRNA)	Shinozaki *et al.*, 1995
	Osteoblasts	RI (mRNA) RII (mRNA, protein)	Shuto *et al.*, 1997
	Pancreas	RI, RII (mRNA)	Kleeff *et al.*, 1998
	Oocytes, granulosa cells	RIA, RIB, RIIA, RIIB (mRNA)	Sidis *et al.*, 1998
	Granulosa cells, trophoblast cells from placenta	RIA, RIB, RIIA, RIIB (mRNA)	Peng *et al.*, 1999
	Thyroid	RI, RII (mRNA)	Schulte *et al.*, 2000
	Tumors		
	Brain tumors	RII (mRNA)	Demura *et al.*, 1995
	Pituitary adenomas	RIIA, RIIB, RIA, RIB (+truncated forms) (mRNA)	Alexander *et al.*, 1996
	Testicular germ cell tumors, spermatocytic seminomas	RIA, RIB, RIIA, RIIB (mRNA)	van Schaik *et al.*, 1997
	Pancreatic cancer	RIA, RIB, RII (mRNA)	Kleeff *et al.*, 1998
	Prostate tumor	RIB (mRNA)	van Schaik *et al.*, 2000
	Ovarian epithelial tumors	RIIA, RIIB, RIA (mRNA)	Minegishi *et al.*, 2000
	Thyroid cancer	RI, RII (mRNA)	Schulte *et al.*, 2001
	Cultured cells		
	Hepatoma (HepG2, Hep3B), epidermoid carcinoma (A431), umbilical vein endothelium (HUV-EC), foreskin fibroblasts	RIA, RIB (mRNA)	Matsuzaki *et al.*, 1993; Xu *et al.*, 1994
	Teratocarcinoma cell line	RIIA, RIIB (mRNA)	de Jong *et al.*, 1993
	Trophoblast cells	RII (mRNA)	Peng *et al.*, 1993
	Retinoblastoma	RII (mRNA)	Zhang and Ying, 1995
	Epithelial ovarian cancer cell lines	RII (mRNA)	Di Simone *et al.*, 1996
	Breast cancer cell line	RII (mRNA)	Ying and Zhang, 1996
	Epidermal keratinocyte cell line	RIIA, RIB (protein)	Shimizu *et al.*, 1998
	Erythroleukemia cells	RIA, RIB, RIIA, RIIB (mRNA)	Hilden *et al.*, 1994, 1999

Table 1 *(Continued)*

Species	Tissues and organs	Receptor type (detection of mRNA or protein)	References
	Granulosa cells, surface epithelial cells, ovarian cancer cell lines	RI (mRNA) RII (mRNA, protein)	Ito *et al.*, 2000
	Choriocarcinoma cell line	RIA, RIIA, RIB (mRNA)	Ni *et al.*, 2000
	Pheochromocytomas	RI, RII (mRNA)	Liu *et al.*, 2000
	Fetal lung fibroblasts	RIA, RIB, RII (mRNA, protein)	Ohga *et al.*, 2000
	Prostate epithelial cells	RIA, RIB, RIIA, RIIB (mRNA)	van Schaik *et al.*, 2000
Mouse	**Tissues and primary cells**		
	Brain, liver, kidney, spleen, intestine, pancreas, testis	RIIA (mRNA)	Mathews and Vale, 1991
	Brain, heart, lung, spleen, uterus, skeletal muscle	RIA (mRNA)	Ebner *et al.*, 1993
	Reproductive organs, oocytes	RIIA, RIIB (mRNA)	Wu *et al.*, 1994
	Brain, spinal cord	RIIA-N	Shoji *et al.*, 1998
	Peritoneal macrophages	RIA, RIB, RIIA, RIIB (mRNA)	Ogawa *et al.*, 2000
	Tumors		
	Testicular tumor cells (Leydig)	RII (mRNA)	Chen *et al.*, 1993
	Cell lines		
	Corticotropic cells (AtT20)	RIIA, RIA (mRNA)	Mathews and Vale, 1991; Tsuchida *et al.*, 1993
	3T3 fibroblasts	RIA (mRNA)	Matsuzaki *et al.*, 1993
	Embryonal carcinoma cells (P19)	RII, RIA RIIA-N (mRNA)	Tsuchida *et al.*, 1993; Shoji *et al.*, 1998;
	Leydig cell line	RIIA, RIIB (mRNA)	Ying *et al.*, 1995
	Neural stem cell line (MEB-5)	RI, RII (mRNA)	Satoh *et al.*, 2000
	Plasmacytoma (MPC-11)	RIIA (mRNA, protein)	Shoham *et al.*, 2001
Rat	**Tissues**		
	Brain, lung, heart, liver, intestine, kidney, prostate	RIA (mRNA)	Matsuzaki *et al.*, 1993
	Brain, lung, heart, liver, intestine, kidney	RII (mRNA)	Matsuzaki *et al.*, 1993
	Brain, pituitary, kidney, lung	RIA (mRNA)	Tsuchida *et al.*, 1993
	Fetal tissues: brain, lung, heart, stomach, placenta, testis, ovary	RIA, RIB (mRNA)	He *et al.*, 1993
	Reproductive male and female organs	RIIA, RIIB (mRNA)	Feng *et al.*, 1993
	Liver, heart, lung, spleen, muscle, bone, thymus, intestine, prostate, brain, kidney, prostate tumor cells	RIB (mRNA)	Xu *et al.*, 1994
	Brain, pituitary, ovary, testis	RII, RIIB (mRNA)	Cameron *et al.*, 1994
	Epiblast of pregastrula and gastrula of embryos	RIIA, RIIB (mRNA)	Manova *et al.*, 1995
	Brain	RIB, RIIA (mRNA)	Morita *et al.*, 1996

Table 1 *(Continued)*

Species	Tissues and organs	Receptor type (detection of mRNA or protein)	References
	Prostate	RII (mRNA)	Ying *et al.*, 1997
	Pituitary (prepubertal female rat)	RII, RIIB (mRNA)	Wilson and Handa, 1998
	Intestine	RIIA, RIB (mRNA)	Sonoyama *et al.*, 2000
	Hypothalamus	RIB (mRNA)	Prevot *et al.*, 2000
	Cell lines		
	Dunning tumor (malignant and non-malignant epithel)	RIA (mRNA)	Matsuzaki *et al.*, 1993
	Pituitary adenocarcinoma cell line	RIIA, RIIB, RI (mRNA)	Ying *et al.*, 1996
	Proliferating chondrocytes, osteoblasts (neonatal rats)	RI, RII (mRNA)	Funaba *et al.*, 2001
Goldfish	Ovary	RIIB (mRNA)	Ge *et al.*, 1997
Chicken	Embryonal brain	RIIB (mRNA)	Ohuchi *et al.*, 1992
	Ciliary ganglion	RIIA (mRNA)	Kos and Coulombe, 1997
	Embryonal retina	RIIA, RIIB (protein)	Belecky-Adams *et al.*, 1999
	Dorsal root ganglia	RIIA, RIIB (mRNA, protein)	Kos *et al.*, 2001
Hamster	Ovary cell line (CHO)	RII, RIA (mRNA)	Tsuchida *et al.*, 1993
Porcine	Thyroid follicles	RIB,RII (mRNA)	Franzen *et al.*, 1999
Xenopus	Embryo	Receptor (mRNA)	Kondo *et al.*, 1991

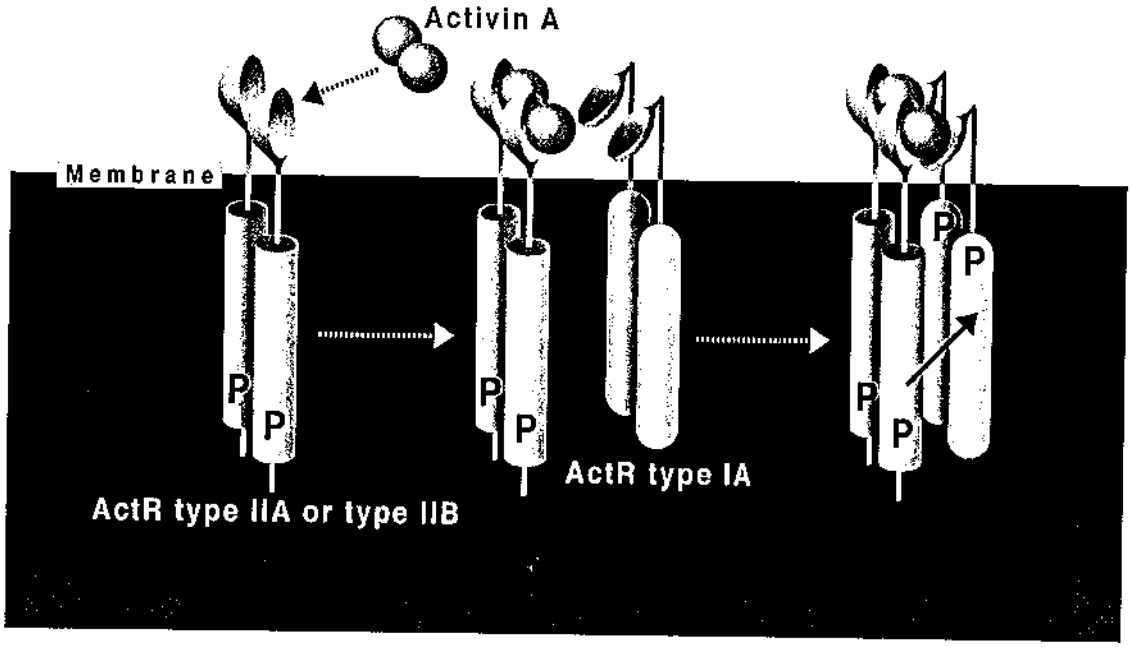
mRNA refers to detection of transcripts in Northern blotting, RNase protection assays and RT-PCR. Protein refers to detection of receptors by antibodies.

Eight Smad proteins have been isolated thus far in mammals and can be classified into three subtypes according to structure and function: receptor-regulated or pathway-restricted Smads (R-Smads), common-mediator Smads (Co-Smads) and inhibitory Smads (I-Smads) (**Figures 3** and **4**). (For an updated scheme of the family and structure of Smad proteins see Itoh *et al.*, 2000.) R-Smads are further divided into those activated by BMP receptors (Smad1, 5 and 8) and those activated by TGFβ and activin receptors (Smad2 and 3). Smad4 is the only Co-Smad identified in mammals, whereas two isoforms of this mediator are found in *Xenopus* (Masuyama *et al.*, 1999; Howell *et al.*, 1999). Smad4 is a tumor suppressor and the mutation or deletion of its gene (*Smad4/DPC4*) is associated with several types of cancer (Hata, 2001). Smad6 and Smad7 are I-Smads (Miyazono, 1999).

The N- and C-terminal regions of Smads contain conserved regions termed Mad homology 1 (MH1) and 2 (MH2) domains. An unconserved linker region connects the MH1 and MH2 regions. Both MH1 and MH2 domains are observed in R- and Co-Smads, but a MH1-like structure is not found in I-Smads.

Interactions between type I receptors and unphosphorylated R-Smads (Smad2 and Smad3) are facilitated by the membrane-associated protein Smad anchor for receptor activation (SARA) (Wu *et al.*, 2000). Specific interactions between Smads and the type I receptor are mediated by a sequence of eight amino acids called the L45 loop found in the kinase domain of the receptor (Feng and Derynck, 1997). Activation of R-Smads occurs by the phosphorylation of two serine residues within a C-terminal SSXS motif by type I receptors. After phosphorylation, R-Smads interact with Co-Smad (Smad4) to form hetero-oligomeric complexes (**Figure 3**), which can then translocate into the nucleus and induce the transcription of various target genes (Miyazono, 1999) (**Figure 5**). Smad2 and 3 may also mediate suppression of transcription

Figure 2 The activin receptor complex. Type II activin receptors interact with activin, complex with type I receptors, and activate them via transphosphorylation.



(Zauberman *et al.*, 2001). Co-Smad is believed to stabilize the structures of the Smad oligomers and is thus required for efficient transcriptional activity of the Smad complexes. I-Smads interact efficiently with the activated type I receptor, thereby preventing access of R-Smads to the type I receptor (Souchelnytskyi *et al.*, 1998; Lebrun *et al.*, 1999). Smad6 inhibits BMP signaling, while Smad7 is an inhibitor of TGFβ/activin signaling (**Figure 4**).

A number of proteins are known to bind Smads. The p300 and CBP co-activators can interact in a ligand-dependent manner with R-Smads (Smad1, 2 and 3) through their MH2 domains and thus promote transcription (Pouponnot *et al.*, 1998; Pearson *et al.*, 1999) (**Figure 5**). Co-repressors of Smads are TGIF and Ski/SnoN which mediate the negative regulation of transcription. Ski inhibits transcription by competing with p300/CBP for Smad interaction (Akiyoshi *et al.*, 1999).

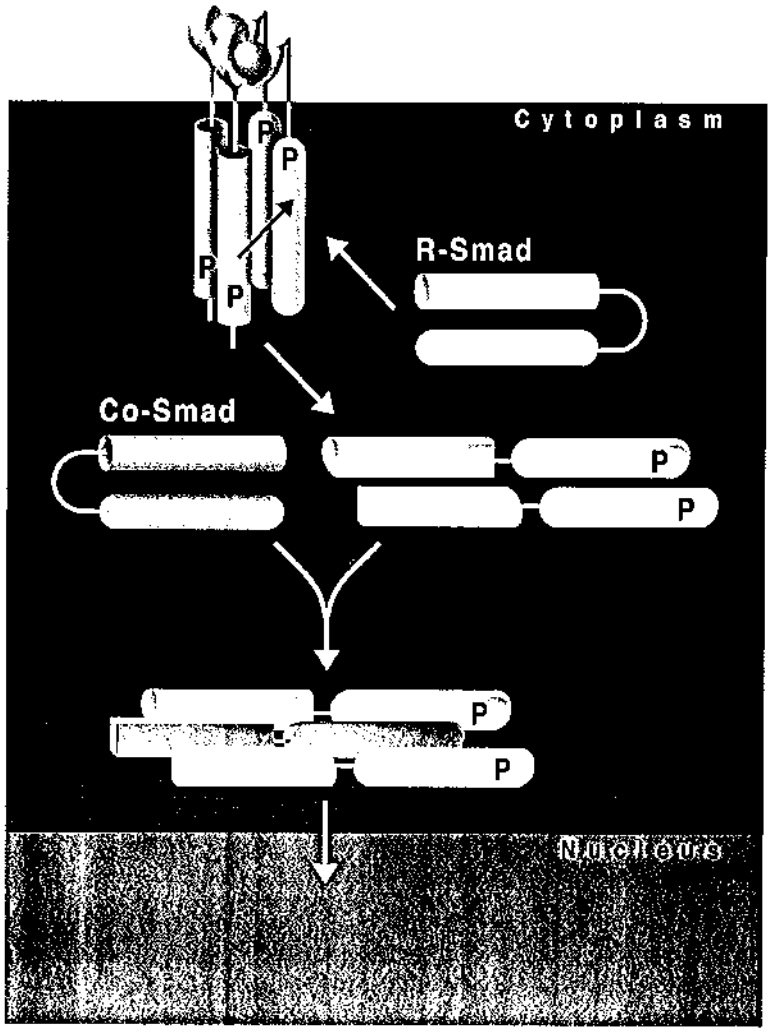
Although the signaling proteins of the activin pathway interact with type I receptors, there are also interactions with type II receptors. ARIP1 interacts with the C-terminal region of ActRIIA and ActRIIA-N via its PDZ domain, and with Smad3 through its WW domains, perhaps contributing into the regulation of signal transduction (Shoji *et al.*, 2000).

DOWNSTREAM GENE ACTIVATION

Transcription factors activated

The Smad2 and Smad3 proteins serve both as the cytoplasmic signaling molecules for TGFβ and activin receptors, and as nuclear transcriptional regulators for this pathway. They become phosphorylated and translocate to the nucleus subsequent to activation, homodimerization and then heterodimerization with Smad4 (Pangas and Woodruff, 2000). Smads apparently can propagate or repress transcription either by direct binding to DNA or by the formation of complexes with other transcription factors. In general, Smads can cooperate with various transcription factors such as c-Fos and c-Jun in binding to AP-1 sites. The association with transcriptional co-activators such as p300 and CBP can lead to

Figure 3 Activation of cytoplasmic Smad proteins. R-Smads interact with the type I receptors, are phosphorylated, and then associate with Co-Smads.



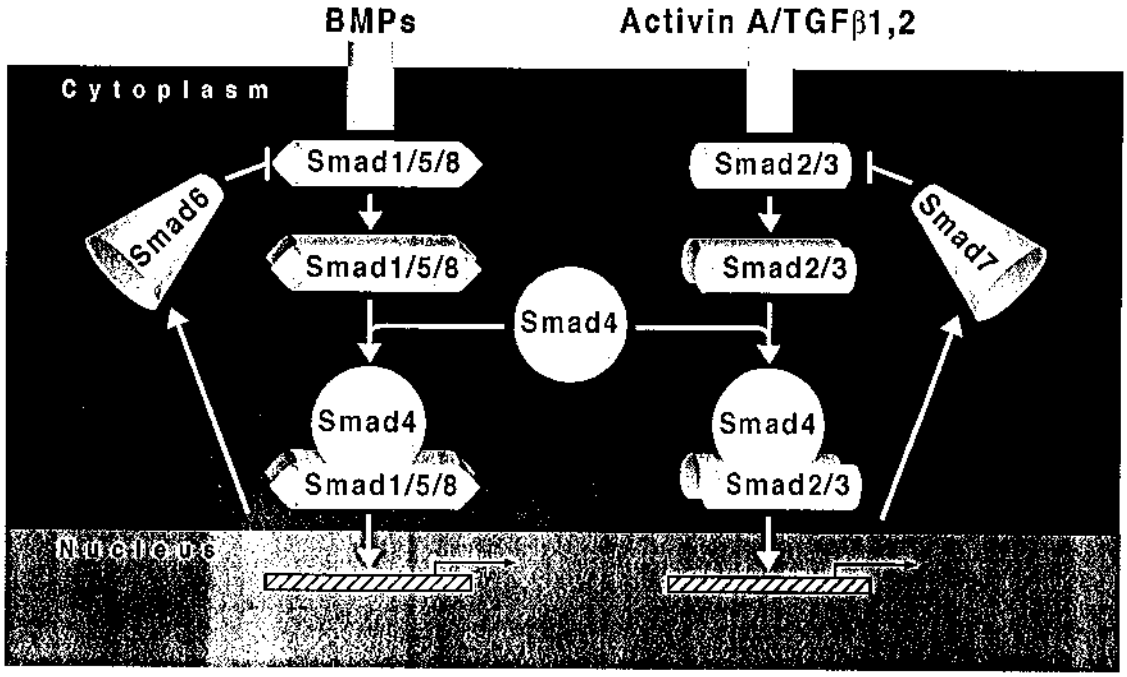
interactions with a variety of transcription factors and thereby link these factors to the basal transcriptional machinery (Piek *et al.*, 1999).

As for specific activin-induced signals, initially it was shown that Smad2 and Smad4 can interact with Forkhead activin signal transducer-1 (FAST-1). This protein is a winged-helix transcription factor that is activated by activin and interacts with the activin response element (ARE) found in the *Xenopus Mix.2* promoter (Chen *et al.*, 1996). It has also been shown that the induction of the p38 MAP kinase pathway by activin leads to the phosphorylation of the transcription factor ATF2 (Cocolakis *et al.*, 2001).

Genes induced

ActR signaling induces the gene expression of Smad7 that in turn inhibits further signaling (Nakao *et al.*, 1997). In addition, activin receptors are involved in the induction of mesoderm during embryogenesis and thus induce the expression of mesodermal genes. In

Figure 4 Signaling pathways of the TGFβ receptor superfamily. Activin and TGFβ receptors signal through the Smad2 and Smad3 proteins. BMP receptors signal through Smad1, 5 or 8. The Co-Smad Smad4 is involved in both pathways.



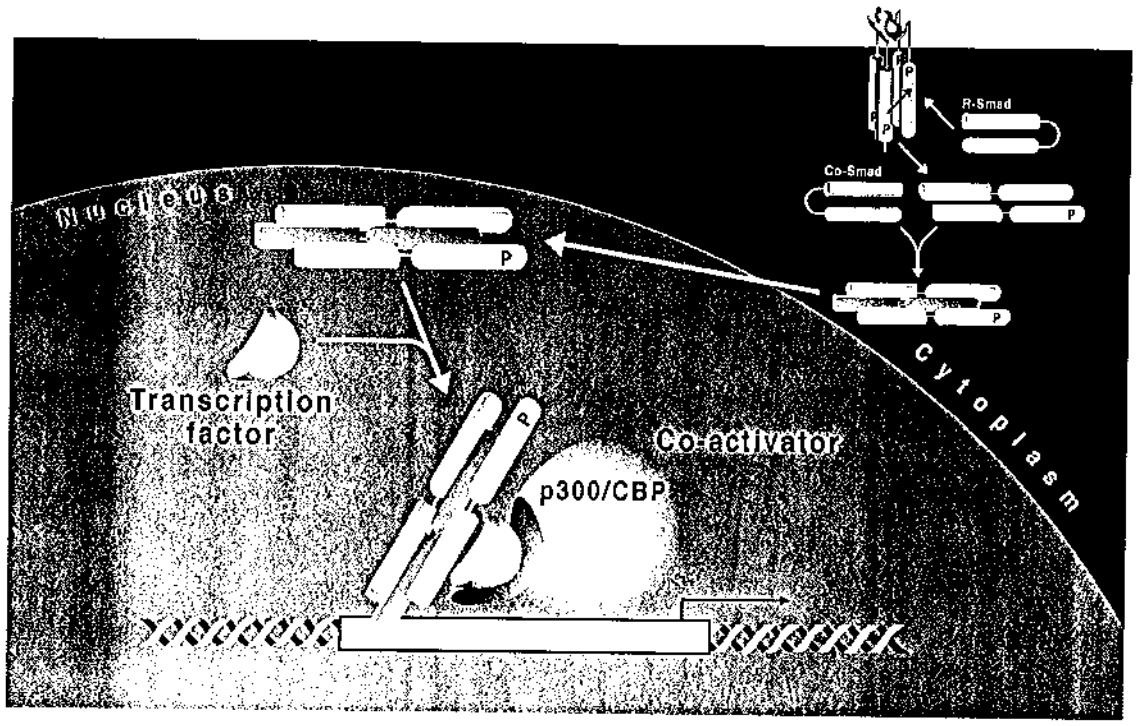
Xenopus the use of truncated dominant negative ActRIIB showed that the expression of the immediate early genes: *brachyury*, *Hox-4* and *comesodermin*, the midgastrula mesoderm marker *gooscoid*, the *wnt-8* ventral mesoderm marker, and muscle *actin*, which marks the end of gastrulation, as well as the Spemann's organizer, BMP antagonists, *chordin* and *noggin*, are all dependent on functional activin receptors (Hemmati-Brivanlou and Melton, 1992; Schultc-Merker *et al.*, 1994; Dyson and Gurdon, 1997; New *et al.*, 1997). A constitutively active form of ActRI induced the expression of mesodermal differentiation genes in *Xenopus* (Armes and Smith, 1997; Chang *et al.*, 1997).

Promoter regions involved

Smad2, Smad4 and FAST-1 are components of the activin-response factor (ARF) found to interact with an activin-response element (ARE) of the *Mix.2* promoter (Chen *et al.*, 1996). Interaction occurs with a 6-base-pair repeat in the promoter (5′-AAATGT-3′). Other AREs have been identified in several genes and remarkably share very little sequence similarity: *goosecoid* (Watabe *et al.*, 1995), *XFKHI/XFD-1* (Kaufmann *et al.*, 1996), *HNFα1* (Weber *et al.*, 1996), *Xlim-1* (Rebbert and Dawid, 1997) and *Xbra2* (Latinkic *et al.*, 1997). The first ARE to be found in a gonadotrope-expressed gene is called GRAS and is located in the *GnRHR* gene promoter 5′ to GRAS and is an inverted repeat that resembles a binding site for Smad3 and Smad4 (Duval *et al.*, 1999).

Activin was shown to activate a TGFβ inducible DNA element termed the CAGA box, which is found in the human PAI-1 promoter. This transcriptional activation was mediated through ALK-4 and Smad proteins (Dennler *et al.*, 1998). Interestingly, there are sequences very similar to CAGA boxes flanking the 6 bp repeats of ARE in the *Mix.2* promoter that binds the FAST-1 protein. These elements might play a role in the binding of the ARF to DNA. A similar sequence was identified in the promoter of the early immediate gene *JunB* that binds Smads and is regulated by activin. This sequence is called the Smad-binding element (SBE) (Jonk *et al.*, 1998). The

Figure 5 Nuclear translocation of Smad complex and formation of transcription activation complex. Interactions of Smad complex and transcription factors with promoter regions are facilitated by the p300/CBP coactivators.



MH1 domain of Smad3 and Smad4 binds to SBE. These elements are commonly found in proximity to DNA-binding elements of other transcription factors, thus allowing the accessibility of other DNA-binding proteins and the efficient interaction of the Smad complex with gene promoters.

BIOLOGICAL CONSEQUENCES OF ACTIVATING OR INHIBITING RECEPTOR AND PATHOPYSIOLOGY

Unique biological effects of activating the receptors

Activin A has been shown to operate as a morphogen in amphibian development. It induces mesoderm and imposes pattern formation in a dose-dependent manner (Green and Smith, 1990); high concentrations induce dorsal and anterior structures (muscle, notochord) while low concentrations induce ventral and posterior tissues (mesenchyme and mesoderm). Expression of kinase domain-deleted, and thus dominant-negative ActRIIB, blocked mesoderm formation (Hemmati-Brivanlou and Melton, 1992). An additional TGFβ superfamily member that induces mesoderm in *Xenopus* is Vg1. Truncated ActRIIB that lacks the kinase domain suppressed signaling of both activin A and Vg1, indicating that these molecules may share this receptor (Schulte-Merker *et al.*, 1994). A dominant-negative soluble ActRIIB lacking the intracellular and transmembrane domains was found to specifically block activin signaling and interfere with mesoderm induction, without affecting Vg1 (Dyson and Gurdon, 1997). In addition, the soluble truncated receptor partially inhibited BMP-4 signaling, suggesting that BMP-4 is a further candidate ligand for ActRIIB. The similarities between the phenotypes of ActRIIB and growth/differentiation factor 11 (Gdf11) deficiencies (Mcpherron *et al.*, 1999), imply that this TGFβ family member which is a secreted protein that controls anterior/posterior axial patterning, may be yet an additional ligand for activin receptors.

Analysis of the contribution of different ActRs to early development showed that both ActRIIA and IIB induce mesoderm upon overexpression. However, the use of dominant-negative truncated receptors revealed that ActRIIA contributes to ventral mesoderm induction in *Xenopus*, whereas ActRIIB mainly contributes to secondary axes formation (New *et al.*, 1997). ActRII is the earliest signal in left–right axis formation in chick development (Levin *et al.*, 1995). Mouse ActRIIB$^{-/-}$ mutants exhibit cardiac, lung, and splenic defects suggesting involvement of ActRIIB in left–right and anterior–posterior axes determination (Oh and Li, 1997). Mice carrying mutations in both ActRIIB and IIA were developmentally arrested at the egg cylinder stage and had severe defects in the gastrulation process. Rare embryos among mutants with a genotype of IIA$^{-/-}$IIB$^{+/-}$ or IIA$^{-/-}$*nodal*$^{+/-}$, were not arrested in early development, underwent relatively normal gastrulation, but had a truncated forebrain. One possible interpretation of these results is that *nodal*, a cytokine of the TGFβ superfamily, is the ligand that signals through ActRIIB to induce forebrain formation (Song *et al.*, 1999).

The study of ActRIIB$^{-/-}$ or ActRIIA$^{+/-}$IIB$^{-/-}$ animals, which do not exhibit an early developmental arrest, revealed the function of ActRII in organogenesis and establishment of organ boundaries. The above mutants had a malformed stomach and the spleen and pancreas were either hypoplastic or completely absent. Some of the knockout mice, such as those harboring ActRIIB$^{-/-}$ or ActRIIA$^{+/-}$IIB$^{+/-}$ mutations were viable and fertile. Nevertheless, they had reduced pancreatic islet size and ActRIIA$^{+/-}$IIB$^{+/-}$ mice exhibited impaired glucose tolerance (Kim *et al.*, 2000). It therefore appears that apart from the role in axial determination, ActRIIs are involved in the development and function of the pancreas and may be involved in gastric epithelium differentiation (Li *et al.*, 1998).

A role in development was also ascribed to type I ActRs (Chang *et al.*, 1997; Armes and Smith, 1997). Overexpression of ActRIB induced dorsal mesoderm in *Xenopus*. Use of constitutively active forms of ActRIA/ALK-2 and ActRIB/ALK-4 in animal cap assays showed that both induce mesodermal markers although to different degrees and in a different pattern; only ActRIB is capable of inducing secondary axes while ActRIA appeared to have an antagonistic role (Armes *et al.*, 1997). The ActRI receptor was also found to be essential for mammalian development. Thus, in ActRIB$^{-/-}$ mice development was blocked at the egg cylinder stage, before gastrulation. Studies of chimeric mice indicated that ActRIB is not essential for mesoderm induction (Gu *et al.*, 1998) while ActRIA was found to be required for gastrulation (Gu *et al.*, 1999).

Human abnormalities

Somatic mutations are found in the *ACVR1B* gene for the ALK-4/ActRIB receptor in cases of pancreatic cancer. A homozygous deletion of 657 bp including entire exon 8 was deleted. In another mutation there was a 5 bp deletion causing a frameshift and early termination of protein translation. Both cases resulted in elimination of part of the kinase domain of the receptor (Su *et al.*, 2001).

Since ActRIIB$^{-/-}$ knockout mice have left–right axis malformations, the human *ACVR2B* gene was screened for mutations in individuals with such malformations. Although several mutations were identified there was no genotype–phenotype correlation. The conclusion of this study was that mutations in *ACVR2B* are uncommon among individuals with left–right malformations (Kosaki *et al.*, 1999). Similarly, no evidence was found for a linkage between mutations in the *ACVR2B* gene and type II diabetes (Dupont *et al.*, 2001) although ActRIIB$^{-/-}$ knockout mice display pancreas-related abnormalities.

References

Akiyoshi, S., Inoue, H., Hanai, J., Kusanagi, K., Nemoto, N., Miyazono, K., and Kawabata, M. (1999). c-Ski acts as a transcriptional co-repressor in transforming growth factor-β signaling through interaction with smads. *J. Biol. Chem.* **274**, 35269 35277.

Alexander, J. M., Bikkal, H. A., Zervas, N. T., Laws, E. R. Jr., and Klibanski, A. (1996). Tumor-specific expression and alternate splicing of messenger ribonucleic acid encoding activin/transforming growth factor-β receptors in human pituitary adenomas. *J. Clin. Endocrinol. Metab.* **81**, 783–790.

Armes, N. A., and Smith, J. C. (1997). The ALK-2 and ALK-4 activin receptors transduce distinct mesoderm-inducing signals during early Xenopus development but do not co-operate to establish thresholds. *Development* **124**, 3797–3804.

Armes, N. A., Neal, K. A., and Smith, J. C. (1999). A short loop on the ALK-2 and ALK-4 activin receptors regulates signaling specificity but cannot account for all their effects on early Xenopus development. *J. Biol. Chem.* **274**, 7929–7935.

Attisano, L., Carcamo, J., Ventura, F., Weis, F. M., Massague, J., and Wrana, J. L. (1993). Identification of human activin and TGFβ type I receptors that form heteromeric kinase complexes with type II receptors. *Cell* **75**, 671–680.

Attisano, L., Wrana, J. L., Cheifetz, S., and Massague, J. (1992). Novel activin receptors: distinct genes and alternative mRNA splicing generate a repertoire of serine/threonine kinase receptors. *Cell* **68**, 97–108.

Barbara, N. P., Wrana, J. L., and Letarte, M. (1999). Endoglin is an accessory protein that interacts with the signaling receptor

complex of multiple members of the transforming growth factor-β superfamily. *J. Biol. Chem.* **274**, 584–594.

Belecky-Adams, T. L., Scheurer, D., and Adler, R. (1999). Activin family members in the developing chick retina: expression patterns, protein distribution, and in vitro effects. *Dev. Biol.* **210**, 107–123.

Cameron, V. A., Nishimura, E., Mathews, L. S., Lewis, K. A., Sawchenko, P. E., and Vale, W. W. (1994). Hybridization histochemical localization of activin receptor subtypes in rat brain, pituitary, ovary, and testis. *Endocrinology* **134**, 799–808.

Carcamo, J., Weis, F. M., Ventura, F., Wieser, R., Wrana, J.L., Attisano, L., and Massague, J. (1994). Type I receptors specify growth-inhibitory and transcriptional responses to transforming growth factor β and activin. *Mol. Cell. Biol.* **14**, 3810–3821.

Chang, C., Wilson, P. A., Mathews, L. S., and Hemmati-Brivanlou, A. (1997). A Xenopus type I activin receptor mediates mesodermal but not neural specification during embryogenesis. *Development* **124**, 827–837.

Chapman, S. C., and Woodruff, T. K. (2001). Modulation of activin signal transduction by inhibin B and inhibin-binding protein (Inhbp). *Mol. Endocrinol.* **15**, 668–679.

Charng, M. J., Kinnunen, P., Hawker, J., Brand, T., and Schneider, M.D. (1996). FKBP-12 recognition is dispensable for signal generation by type I transforming growth factor-β receptors. *J. Biol. Chem.* **271**, 22941–22944.

Chen, C. L., Pignataro, O. P., and Feng, Z. M. (1993). Inhibin/activin subunits and activin receptor are co-expressed in Leydig tumor cells. *Mol. Cell. Endocrinol.* **94**, 137–143.

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

Childs, S. R., Wrana, J. L., Arora, K., Attisano, L., O'Connor, M. B., and Massague, J. (1993). Identification of a Drosophila activin receptor. *Proc. Natl Acad. Sci. USA* **90**, 9475–9479.

Cocolakis, E., Lemay, S., Ali, S., and Lebrun, J.J. (2001). The p38 MAP Kinase pathway is required for cell growth inhibition of human breast cancer cells in response to activin. *J. Biol. Chem.* **276**, 18430–18436.

de Jong, F. H., de Winter, J. P., Wesseling, J. G., Timmerman, M. A., van Genesen, S., van den Eijnden-van Raaij, A. J., and van Zoelen, E. J. (1993). Inhibin subunits, follistatin and activin receptors in the human teratocarcinoma cell line Tera-2. *Biochem. Biophys. Res. Commun.* **192**, 1334–1339.

Demura, R., Tajima, S., Suzuki, T., Yajima, R., Odagiri, E., Demura, H., Kato, H., Uchiyama, T., Kubo, O., and Takakura, K. (1995). Inhibin α,β A subunit and activin type II receptor mRNAs are expressed in human brain tumors. *Endocr. J.* **42**, 307–313.

Dennler, S., Itoh, S., Vivien, D., ten Dijke, P., Huet, S., and Gauthier, J.M. (1998). Direct binding of Smad3 and Smad4 to critical TGFβ-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J.* **17**, 3091–3100.

Di Simone, N., Crowley, W. F. Jr., Wang, Q. F., Sluss, P. M., and Schneyer, A. L. (1996). Characterization of inhibin/activin subunit, follistatin, and activin type II receptors in human ovarian cancer cell lines: a potential role in autocrine growth regulation. *Endocrinology* **137**, 486–494.

Donaldson, C. J., Mathews, L. S., and Vale, W. W. (1992). Molecular cloning and binding properties of the human type II activin receptor. *Biochem. Biophys. Res. Commun.* **184**, 310–316.

Donaldson, C. J., Vaughan, J. M., Corrigan, A. Z., Fischer, W. H., and Vale, W. W. (1999). Activin and inhibin binding to the soluble extracellular domain of activin receptor II. *Endocrinology* **140**, 1760–1766.

Dupont, S., Hani, E. H., Cras-Meneur, C., De Matos, F., Lobbens, S., Lecoeur, C., Vaxillaire, M., Scharfmann, R., and Froguel, P. (2001). No evidence for linkage or for diabetes-associated mutations in the activin type 2B receptor gene (ACVR2B) in French patients with mature-onset diabetes of the young or type 2 diabetes. *Diabetes* **50**, 1219–1221.

Duval, D. L., Ellsworth, B. S., and Clay, C. M. (1999). Is gonadotrope expression of the gonadotropin releasing hormone receptor gene mediated by autocrine/paracrine stimulation of an activin response element? *Endocrinology* **140**, 1949–1952.

Dyson, S., and Gurdon, J. B. (1997). Activin signalling has a necessary function in Xenopus early development. *Curr. Biol.* **7**, 81–84.

Ebner, R., Chen, R. H., Shum, L., Lawler, S., Zioncheck, T. F., Lee, A., Lopez, A. R., and Derynck, R. (1993). Cloning of a type I TGF-β receptor and its effect on TGF-β binding to the type II receptor. *Science* **260**, 1344–1348.

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

Feng, Z. M., Madigan, M. B., and Chen, C. L. (1993). Expression of type II activin receptor genes in the male and female reproductive tissues of the rat. *Endocrinology* **132**, 2593–2600.

Franzen, A., Pick, E., Westermark, B., ten Dijke, P., and Heldin, N. E. (1999). Expression of transforming growth factor-β1, activin A, and their receptors in thyroid follicle cells: negative regulation of thyrocyte growth and function. *Endocrinology* **140**, 4300–4310.

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

Funaba, M., Ogawa, K., and Abe, M. (2001). Expression and localization of activin receptors during endochondral bone development. *Eur.J. Endocrinol.* **144**, 63–71.

Gaddy-Kurten, D., Tsuchida, K., and Vale, W. (1995). Activins and the receptor serine kinase superfamily. *Recent Prog. Horm. Res.* **50**, 109–129.

Ge, W., Tanaka, M., Yoshikuni, M., Eto, Y., and Nagahama, Y. (1997). Cloning and characterization of goldfish activin type IIB receptor. *J. Mol. Endocrinol.* **19**, 47–57.

Georgi, L. L., Albert, P. S., and Riddle, D. L. (1990). daf-1, a C. elegans gene controlling dauer larva development, encodes a novel receptor protein kinase. *Cell* **61**, 635–645.

Gray, P. C., Greenwald, J., Blount, A. L., Kumitake, K. S., Donaldson, C. J., Choe, S., and Vale, W. (2000). Identification of a binding site on the type II activin receptor for activin and inhibin. *J. Biol. Chem.* **275**, 3206–3212.

Green, J. B., and Smith, J. C. (1990). Graded changes in dose of a Xenopus activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* **347**, 391–394.

Greenwald, J., Fischer, W. H., Vale, W. W., and Choe, S. (1999). Three-finger toxin fold for the extracellular ligand-binding domain of the type II activin receptor serine kinase. *Nature Struct. Biol.* **6**, 18–22.

Greenwald, J., Le, V., Corrigan, A., Fischer, W., Komives, E., Vale, W., and Choe, S. (1998). Characterization of the extracellular ligand-binding domain of the type II activin receptor. *Biochemistry* **37**, 16711–16718.

Gu, Z., Nomura, M., Simpson, B. B., Lei, H., Feijen, A., van den Eijnden-van Raaij, J., Donahoe, P. K., and Li, E. (1998). The

type I activin receptor ActRIB is required for egg cylinder organization and gastrulation in the mouse. *Genes Dev.* **12**, 844–857.

Gu, Z., Reynolds, E. M., Song, J., Lei, H., Feijen, A., Yu, L., He, W., MacLaughlin, D. T., van den Eijnden-van Raaij, J., Donahoe, P. K., and Li, E. (1999). The type I serine/threonine kinase receptor ActRIA (ALK2) is required for gastrulation of the mouse embryo. *Development* **126**, 2551–2561.

Hata, A. (2001). TGFβ signaling and cancer. *Exp. Cell. Res.* **264**, 111–116.

He, W. W., Gustafson, M. L., Hirobe, S., and Donahoe, P. K. (1993). Developmental expression of four novel serine/threonine kinase receptors homologous to the activin/transforming growth factor-β type II receptor family. *Dev. Dyn.* **196**, 133–142.

Hemmati-Brivanlou, A., and Melton, D. A. (1992). A truncated activin receptor inhibits mesoderm induction and formation of axial structures in Xenopus embryos. *Nature* **359**, 609–614.

Hilden, K., Tuuri, T., Eramaa, M., and Ritvos, O. (1994). Expression of type II activin receptor genes during differentiation of human K562 cells and cDNA cloning of the human type IIB activin receptor. *Blood* **83**, 2163–2170.

Hilden, K., Tuuri, T., Eramaa, M., and Ritvos, O. (1999). Coordinate expression of activin A and its type I receptor mRNAs during phorbol ester-induced differentiation of human K562 erythroleukemia cells. *Mol. Cell. Endocrinol.* **153**, 137–145.

Howell, M., Itoh, F., Pierreux, C. E., Valgeirsdottir, S., Itoh, S., ten Dijke, P., and Hill, C. S. (1999). Xenopus Smad4β is the co-Smad component of developmentally regulated transcription factor complexes responsible for induction of early mesodermal genes. *Dev. Biol.* **214**, 354–369.

Husken-Hindi, P., Tsuchida, K., Park, M., Corrigan, A. Z., Vaughan, J. M., Vale, W. W., and Fischer, W. H. (1994). Monomeric activin A retains high receptor binding affinity but exhibits low biological activity. *J. Biol. Chem.* **269**, 19380–19384.

Ishikawa, S., Kai, M., Murata, Y., Tamari, M., Daigo, Y., Murano, T., Ogawa, M., and Nakamura, Y. (1998). Genomic organization and mapping of the human activin receptor type IIB (hActR-IIB) gene. *J. Hum. Genet.* **43**, 132–134.

Ito, I., Minegishi, T., Fukuda, J., Shinozaki, H., Auersperg, N., and Leung, P. C. (2000). Presence of activin signal transduction in normal ovarian cells and epithelial ovarian carcinoma. *Br. J. Cancer* **82**, 1415–1420.

Itoh, S., Itoh, F., Goumans, M.J., and ten Dijke, P. (2000). Signaling of transforming growth factor-β family members through Smad proteins. *Eur.J. Biochem.* **267**, 6954–6967.

Jonk, L. J., Itoh, S., Heldin, C. H., ten Dijke, P., and Kruijer, W. (1998). Identification and functional characterization of a Smad binding element (SBE) in the JunB promoter that acts as a transforming growth factor-β, activin, and bone morphogenetic protein-inducible enhancer. *J. Biol. Chem.* **273**, 21145–21152.

Kaufmann, E., Paul, H., Friedle, H., Metz, A., Scheucher, M., Clement, J. H., and Knochel, W. (1996). Antagonistic actions of activin A and BMP-2/4 control dorsal lip-specific activation of the early response gene XFD-1' in Xenopus laevis embryos. *EMBO J.* **15**, 6739–6749.

Kim, S. K., Hebrok, M., Li, E., Oh, S. P., Schrewe, H., Harmon, E. B., Lee, J. S., and Melton, D. A. (2000). Activin receptor patterning of foregut organogenesis. *Genes Dev.* **14**, 1866–1871.

Kleeff, J., Ishiwata, T., Friess, H., Buchler, M. W., and Korc, M. (1998). Concomitant over-expression of activin/inhibin β subunits and their receptors in human pancreatic cancer. *Int. J. Cancer* **77**, 860–868.

Kondo, M., Tashiro, K., Fujii, G., Asano, M., Miyoshi, R., Yamada, R., Muramatsu, M., and Shiokawa, K. (1991). Activin receptor mRNA is expressed early in Xenopus embryogenesis and the level of the expression affects the body axis formation. *Biochem. Biophys. Res. Commun.* **181**, 684–690.

Kos, K., and Coulombe, J. N. (1997). Activin receptor mRNA expression by neurons of the avian ciliary ganglion. *J. Neurobiol.* **32**, 33–44.

Kos, K., Fine, L., and Coulombe, J. N. (2001). Activin type II receptors in embryonic dorsal root ganglion neurons of the chicken. *J. Neurobiol.* **47**, 93–108.

Kosaki, R., Gebbia, M., Kosaki, K., Lewin, M., Bowers, P., Towbin, J. A., and Casey, B. (1999). Left-right axis malformations associated with mutations in ACVR2B, the gene for human activin receptor type IIB. *Am. J. Med. Genet.* **82**, 70–76.

Latinkic, B. V., Umbhauer, M., Neal, K. A., Lerchner, W., Smith, J. C., and Cunliffe, V. (1997). The Xenopus Brachyury promoter is activated by FGF and low concentrations of activin and suppressed by high concentrations of activin and by paired-type homeodomain proteins. *Genes Dev.* **11**, 3265–3276.

Lebrun, J. J., Takabe, K., Chen, Y., and Vale, W. (1999). Roles of pathway-specific and inhibitory Smads in activin receptor signaling. *Mol. Endocrinol.* **13**, 15–23.

Levin, M., Johnson, R. L., Stern, C. D., Kuehn, M., and Tabin, C. (1995). A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* **82**, 803–814.

Lewis, K. A., Gray, P. C., Blount, A. L., MacConell, L. A., Wiater, E., Bilezikjian, L. M., and Vale, W. (2000). Betaglycan binds inhibin and can mediate functional antagonism of activin signalling. *Nature* **404**, 411–414.

Li, Q., Karam, S. M., Coerver, K. A., Matzuk, M. M., and Gordon, J. I. (1998). Stimulation of activin receptor II signaling pathways inhibits differentiation of multiple gastric epithelial lineages. *Mol. Endocrinol.* **12**, 181–192.

Liu, F., Ventura, F., Doody, J., and Massague, J. (1995). Human type II receptor for bone morphogenic proteins (BMPs): extension of the two-kinase receptor model to the BMPs. *Mol. Cell. Biol.* **15**, 3479–3486.

Liu, J., Heikkila, P., Kahri, A. I., and Voutilainen, R. (2000). Expression of activin A and its receptors in human pheochromocytomas. *J. Endocrinol.* **165**, 503–508.

Lux, A., Attisano, L., and Marchuk, D. A. (1999). Assignment of transforming growth factor β1 and β3 and a third new ligand to the type I receptor ALK-1. *J. Biol. Chem.* **274**, 9984–9992.

Manova, K., De Leon, V., Angeles, M., Kalantry, S., Giarre, M., Attisano, L., Wrana, J., and Bachvarova, R. F. (1995). mRNAs for activin receptors II and IIB are expressed in mouse oocytes and in the epiblast of pregastrula and gastrula stage mouse embryos. *Mech. Dev.* **49**, 3–11.

Masuyama, N., Hanafusa, H., Kusakabe, M., and Shibuya, H., Nishida E. (1999). Identification of two Smad4 proteins in Xenopus. Their common and distinct properties. *J. Biol. Chem.* **274**, 12163–12170.

Mathews, L. S. (1994). Activin receptors and cellular signaling by the receptor serine kinase family. *Endocr. Rev.* **15**, 310–325.

Mathews, L. S., and Vale, W. W. (1991). Expression cloning of an activin receptor, a predicted transmembrane serine kinase. *Cell* **65**, 973–982.

Mathews, L. S., and Vale, W. W. (1993). Characterization of type II activin receptors. Binding, processing, and phosphorylation. *J. Biol. Chem.* **268**, 19013–19018.

Mathews, L. S., Vale, W. W., and Kintner, C. R. (1992). Cloning of a second type of activin receptor and functional characterization in Xenopus embryos. *Science* **255**, 1702–1705.

Shoji, H., Nakamura, T., van den Eijnden-van Raaij, A. J., and Sugino, H. (1998). Identification of a novel type II activin receptor, type IIA-N, induced during the neural differentiation of murine P19 embryonal carcinoma cells. *Biochem. Biophys. Res. Commun.* **246**, 320–324.

Shoji, H., Tsuchida, K., Kishi, H., Yamakawa, N., Matsuzaki, T., Liu, Z., Nakamura, T., and Sugino, H. (2000). Identification and characterization of a PDZ protein that interacts with activin type II receptors. *J. Biol. Chem.* **275**, 5485–5492.

Shuto, T., Sarkar, G., Bronk, J. T., Matsui, N., and Bolander, M. E. (1997). Osteoblasts express types I and II activin receptors during early intramembranous and endochondral bone formation. *J. Bone Miner. Res.* **12**, 403–411.

Sidis, Y., Fujiwara, T., Leykin, L., Isaacson, K., Toth, T., and Schneyer, A. L. (1998). Characterization of inhibin/activin subunit, activin receptor, and follistatin messenger ribonucleic acid in human and mouse oocytes: evidence for activin's paracrine signaling from granulosa cells to oocytes. *Biol. Reprod.* **59**, 807–812.

Song, J., Oh, S. P., Schrewe, H., Nomura, M., Lei, H., Okano, M., Gridley, T., and Li, E. (1999). The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. *Dev. Biol.* **213**, 157–169.

Sonoyama, K., Rutatip, S., and Kasai, T. (2000). Gene expression of activin, activin receptors, and follistatin in intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver. Physiol.* **278**, G89–97.

Souchelnytskyi, S., Nakayama, T., Nakao, A., Moren, A., Heldin, C. H., Christian, J. L., and ten Dijke, P. (1998). Physical and functional interaction of murine and Xenopus Smad 7 with bone morphogenetic protein receptors and transforming growth factor-β receptors. *J. Biol. Chem.* **273**, 25364–25370.

Su, G. H., Bansal, R., Murphy, K. M., Montgomery, E., Yeo, C. J., Hruban, R. H., and Kern, S. E. (2001). ACVR1B (ALK4, activin receptor type 1B) gene mutations in pancreatic carcinoma. *Proc. Natl Acad Sci. USA* **98**, 3254–3257.

ten Dijke, P., Ichijo, H., Franzen, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C. H., and Miyazono, K. (1993). Activin receptor-like kinases: a novel subclass of cell-surface receptors with predicted serine/threonine kinase activity. *Oncogene* **8**, 2879–2887.

ten Dijke, P., Yamashita, H., Ichijo, H., Franzen, P., Laiho, M., Miyazono, K., and Heldin, C. H. (1994a). Characterization of type I receptors for transforming growth factor-β and activin. *Science* **264**, 101–104.

ten Dijke, P., Yamashita, H., Sampath, T. K., Reddi, A. H., Estevez, M., Riddle, D. L., Ichijo, H., Heldin, C. H., and Miyazono, K. (1994b). Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. *J. Biol. Chem.* **269**, 16985–16988.

Tsuchida, K., Mathews, L. S., and Vale, W. W. (1993). Cloning and characterization of a transmembrane serine kinase that acts as an activin type I receptor. *Proc. Natl Acad. Sci. USA* **90**, 11242–11246.

Tsuchida, K., Sawchenko, P. E., Nishikawa, S., and Vale, W. W. (1996). Molecular cloning of a novel type I receptor serine/threonine kinase for the TGF β superfamily from rat brain. *Mol. Cell. Neurosci.* **7**, 467–478.

van Schaik, R. H., Wierikx, C. D., Looijenga, L. H., Oosterhuis, J. W., and de Jong, F. H. (1997). Human testicular germ cell tumours express inhibin subunits, activin receptors and follistatin mRNAs. *Br. J. Cancer* **76**, 1191–1198.

van Schaik, R. H., Wierikx, C. D., Timmerman, M. A., Oomen, M. H., van Weerden, W. M., van der Kwast, T. H., van Steenbrugge, G. J., and de Jong, F. H. (2000). Variations in activin receptor, inhibin/activin subunit and follistatin mRNAs in human prostate tumour tissues. *Br. J. Cancer* **82**, 112–117.

Wang, T., Li, B. Y., Danielson, P. D., Shah, P. C., Rockwell, S., Lechleider, R. J., Martin, J., Manganaro, T., and Donahoe, P. K. (1996). The immunophilin FKBP12 functions as a common inhibitor of the TGF β family type I receptors. *Cell* **86**, 435–444.

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

Watabe, T., Kim, S., Candia, A., Rothbacher, U., Hashimoto, C., Inoue, K., and Cho, K. W. (1995). Molecular mechanisms of Spemann's organizer formation: conserved growth factor synergy between Xenopus and mouse. *Genes Dev.* **9**, 3038–3050.

Weber, H., Holewa, B., Jones, E. A., and Ryffel, G. U. (1996). Mesoderm and endoderm differentiation in animal cap explants: identification of the HNF4-binding site as an activin A responsive element in the Xenopus HNF1alpha promoter. *Development* **122**, 1975–1984.

Willis, S. A., and Mathews, L. S. (1997). Regulation of activin type I receptor function by phosphorylation of residues outside the GS domain. *FEBS Lett.* **420**, 117–120.

Willis, S.A., Zimmerman, C.M., Li, L.I., and Mathews, L.S. (1996). Formation and activation by phosphorylation of activin receptor complexes. *Mol. Endocrinol.* **10**, 367–379.

Wilson, M. E., and Handa, R. J. (1998). Activin subunit, follistatin, and activin receptor gene expression in the prepubertal female rat pituitary. *Biol. Reprod.* **59**, 278–283.

Wrana, J. L., Tran, H., Attisano, L., Arora, K., Childs, S. R., Massague, J., and O'Connor, M. B. (1994). Two distinct transmembrane serine/threonine kinases from Drosophila melanogaster form an activin receptor complex. *Mol. Cell. Biol.* **14**, 944–950.

Wu, G., Chen, Y. G., Ozdamar, B., Gyuricza, C. A., Chong, P. A., Wrana, J. L., Massague, J., and Shi, Y. (2000). Structural basis of Smad2 recognition by the Smad anchor for receptor activation. *Science* **287**, 92–97.

Wu, T. C., Jih, M. H., Wang, L., and Wan, Y. J. (1994). Expression of activin receptor II and IIB mRNA isoforms in mouse reproductive organs and oocytes. *Mol. Reprod. Dev.* **38**, 9–15.

Xu, J., Matsuzaki, K., McKeehan, K., Wang, F., Kan, M., and McKeehan, W. L. (1994). Genomic structure and cloned cDNAs predict that four variants in the kinase domain of serine/threonine kinase receptors arise by alternative splicing and poly(A) addition. *Proc. Natl Acad. Sci. USA* **91**, 7957–7961.

Yamashita, H., ten Dijke, P., Huylebroeck, D., Sampath, T. K., Andries, M., Smith, J.C., Heldin, C.H., and Miyazono, K. (1995). Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J. Cell Biol.* **130**, 217–226.

Yelick, P. C., Abduljabbar, T. S., and Stashenko, P. (1998). zALK-8, a novel type I serine/threonine kinase receptor, is expressed throughout early zebrafish development. *Dev. Dyn.* **211**, 352–361.

Ying, C., Zhang, Z., and Ying, S. Y. (1995). Expression and localization of activin β A-subunit and activin receptors in TM3, a mouse Leydig cell line. *Endocr. Res.* **21**, 815–24.

Ying, C., Zhang, Z., Huang, G., Li, S. Q., and Ying, S. Y. (1996). Expression and localization of inhibin/activin and activin receptors in GH3 cells, a rat pituitary adenocarcinoma cell line. *J. Endocrinol. Invest.* **19**, 6–11.

Ying, S. Y., and Zhang, Z. (1996). Expression and localization of inhibin/activin subunits and activin receptors in MCF-7 cells, a

human breast cancer cell line. *Breast Cancer Res. Treat.* 37, 151–160.

Ying, S. Y., Zhang, Z., and Huang, G. (1997). Expression and localization of inhibin/activin subunits and activin receptors in the normal rat prostate. *Life Sci.* 60, 397–401.

Zauberman, A., Lapter, S., and Zipori, D. (2001). Smad proteins suppress C/EBPβ and STAT3 mediated transcriptional activation of the haptoglobin promoter. *J. Biol. Chem.*In press.

Zhang, Z., and Ying, S. Y. (1995). Expression of activins and activin receptors in human retinoblastoma cell line Y-79. *Cancer Lett.* 89, 207–214.

Zhou, Y., Sun, H., Danila, D. C., Johnson, S. R., Sigai, D. P., Zhang, X., and Klibanski, A. (2000). Truncated activin type I receptor Alk4 isoforms are dominant negative receptors inhibiting activin signaling. *Mol. Endocrinol.* 14, 2066–2075.

LICENSED PRODUCTS

Patents filed for activin receptors are listed in **Table 2**.

Table 2 Patents filed for activin receptors

Patent no.	Title	Assignee
6162896	Activin receptor useful for diagnosis and treatment of cancer, wound healing, immune, reproductive or CNS disorders	Salk Institute for Biological Studies
5216126	Activin receptor that does not bind TGFβ can be used to purify ligands and in assays	Genentech Inc.
9946386	ALK-1 can be used to develop products for treating fibrosis, cancer, rheumatoid arthritis and golemrulonephritis	Ludwig Institute for Cancer Research
5885794	Activin receptor nucleotide sequences useful for investigating receptor function	Salk Institute for Biological Studies
6132988	Neuronal activin receptor for use in drug screening assays and diagnosis of neurodegenerative diseases	Takeda Chemical Industries Ltd.
9611259	Activin receptors useful for detection of cancer and prevention of liver fibrosis and treatment of male infertility	Human Genome
6207814	ALK's can be used in diagnosis or therapy of rheumaoid arthiritis, glomerular nephritis and fibrosis	Ludwig Institue for Cancer Research
6207814	Sequences for ALK-3 and ALK-6	Ludwig Institute
5976815	ALK-7 use in bioassay for assessing candidate drugs and ligands	Assigned to individual
5891638	Method for detection of ALK-7	Assigned to individual