Cold Spring Harbor Perspectives in Biology
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Activins and Inhibins: Roles in Development, Physiology, and Disease

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Since their original discovery as regulators of follicle-stimulating hormone (FSH) secretion and erythropoiesis, the TGF-β family members activin and inhibin have been shown to participate in a variety of biological processes, from the earliest stages of embryonic development to highly specialized functions in terminally differentiated cells and tissues. Herein, we present the history, structures, signaling mechanisms, regulation, and biological processes in which activins and inhibins participate, including several recently discovered biological activities and functional antagonists. The potential therapeutic relevance of these advances is also discussed.

INTRODUCTION, HISTORY AND NOMENCLATURE

The activins and inhibins are among the 33 members of the TGF-β family and were first described as regulators of follicle-stimulating hormone (FSH) secretion and erythropoiesis. However, activins and inhibins have since been implicated in a variety of biological processes, ranging from the early stages of embryonic development, to highly specialized functions in terminally differentiated cells and tissues (Yu et al. 1987; Vassalli et al. 1994; Matzuk et al. 1995c; Aono et al. 1997; Yamaoka et al. 1998; Molloy et al. 1999; Munz et al. 1999a). Herein, we provide an overview of the history, protein structures, signaling mechanisms and their regulation, and the many biological processes in which the activins and inhibins participate, representing some of the most fascinating aspects of TGF-β family biology. We will also incorporate new biological activities that have been recently discovered, the potential clinical relevance of these advances, and therapeutic challenges.

The activins and inhibins comprise integrally linked components of the TGF-β family. The activins were originally recognized for their abilities to augment the gonadotropin-releasing hormone (GnRH)-mediated release of FSH, and were named "activins" because their effects were functionally opposite to those of inhibin in this context (Ling et al. 1986a; Vale et al. 1986). Activins also augment erythropoietin (EPO)-dependent hemoglobin production in K562 erythroleukemia cells and enhance the proliferation of erythrocyte precursors from

Editors: Rik Derynck and Kohei Miyazono
Additional Perspectives on The Biology of the TGF-β Family available at www.cshperspectives.org

Advanced Online Article. Cite this article as *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a021881

human bone marrow cells (Eto et al. 1987; Yu et al. 1987).

Inhibins and activins share common β subunits, with inhibins occurring as αβ heterodimers, whereas activins are ββ homodimers. Because inhibins were discovered and isolated before activins, the β monomers are designated inhibins βA and βB, and the genes are *Inhba* and *Inhbb*, respectively. However, the β monomers are also often referred to as activins βA and βB, the designations that will be used herein (Fig. 1A). Activins βA and βB are closely related peptides, showing 63% identity and 87% similarity within their mature domains. Activins were identified in eluted fractions from porcine follicular fluid and stimulated FSH secretion in vitro from pituitary gonadotropes, which normally produce FSH and luteinizing hormone (LH). The eluted proteins were heterodimers of activin βA and βB monomers (βA βB) (Ling et al. 1986b). Homodimers of the βA or βB monomers (activins A and B, respectively) also stimulated FSH release (Ling et al. 1986a; Vale et al. 1986; Mason et al. 1989), whereas inhibin heterodimers (αβA and αβB, inhibins A and B, respectively) blocked this effect (Setchell and Jacks 1974; De Jong and Sharpe 1976).

Activin C (βC βC) and activin E (βE βE) were later identified with predominant expression in liver but also in several other tissues. Activin βC and βE monomers also have the capacity to heterodimerize among themselves (βC βE) with other activin monomers and inhibin α (Fang et al. 1996, 1997; Schmitt et al. 1996; O'Bryan et al. 2000; Hashimoto et al. 2002; Vejda et al. 2002; Mellor et al. 2003; Gold et al. 2004; Ushiro et al. 2006). Activins C and E are unlikely to signal through activin receptors but rather antagonize activin A signaling by forming βA βC or βA βE heterodimers, as seen in human PC3 prostate tumor cells cotransfected with activins βA and βC (Mellor et al. 2003) and in transgenic mice that overexpress activin βC on an inhibin-α-deficient genetic background, thereby mitigating the cancer-cachexia phenotype of $Inha^{-/-}$ mice (Gold et al. 2013). To our knowledge, the ability of activins C or E to inhibit activin B signaling has not been reported.

PROCESSING, STRUCTURE AND SIGNALING

Processing of Ligands

Activins are formed when larger pro-proteins dimerize, followed by cleavage to produce two mature β monomers, activin A, activin B or activin AB (βA βB) (Ling et al. 1986b; Vale et al. 1986). Dimerization of the βA or βB peptides with α inhibin (encoded by the *Inha* gene) followed by cleavage, results in heterodimeric inhibin A and inhibin B, respectively (Fig. 1A) (Antenos et al. 2008). After the pro-proteins are synthesized, the prodomains aid in folding and assembling of their mature peptides, by holding them in a dimerization-competent conformation (Gray and Mason 1990; Walton et al. 2009, 2012). Three amino acids within the inhibin α prodomain (Leu30, Phe37, Leu41) form a hydrophobic interface with the mature peptide, are required for heterodimer assembly and secretion, and are likely to mediate noncovalent interactions with the mature domain (Walton et al. 2009). A similar relationship exists between the activin βA pro- and mature domains. Mutation of these key amino acids results in greatly reduced production of activin A and inhibin A, because of the failure of dimerization. Similar observations have been made for members of other branches of the TGF-β family and suggest a possible common paradigm for the prodomains in functional assembly of TGF-β family ligands. Also, mutations in the activin βA prodomain greatly reduce its capacity to bind the mature domain as well as its ability to inhibit activin A functions (Walton et al. 2009).

Differences in posttranslational glycosylation of the inhibin α subunit substantially affect the production, secretion, and bioactivity of inhibin A and affect its affinity for betaglycan (Mason et al. 1996; Antenos et al. 2007; Makanji et al. 2007). Failure of normal glycosylation in ovarian granulosa cells results in markedly reduced secretion of inhibin α without impacting activin assembly and release. Therefore, reduction of inhibin α secretion is predicted to favor the production of activins more than inhibins, so glycosylation provides a potential mecha-

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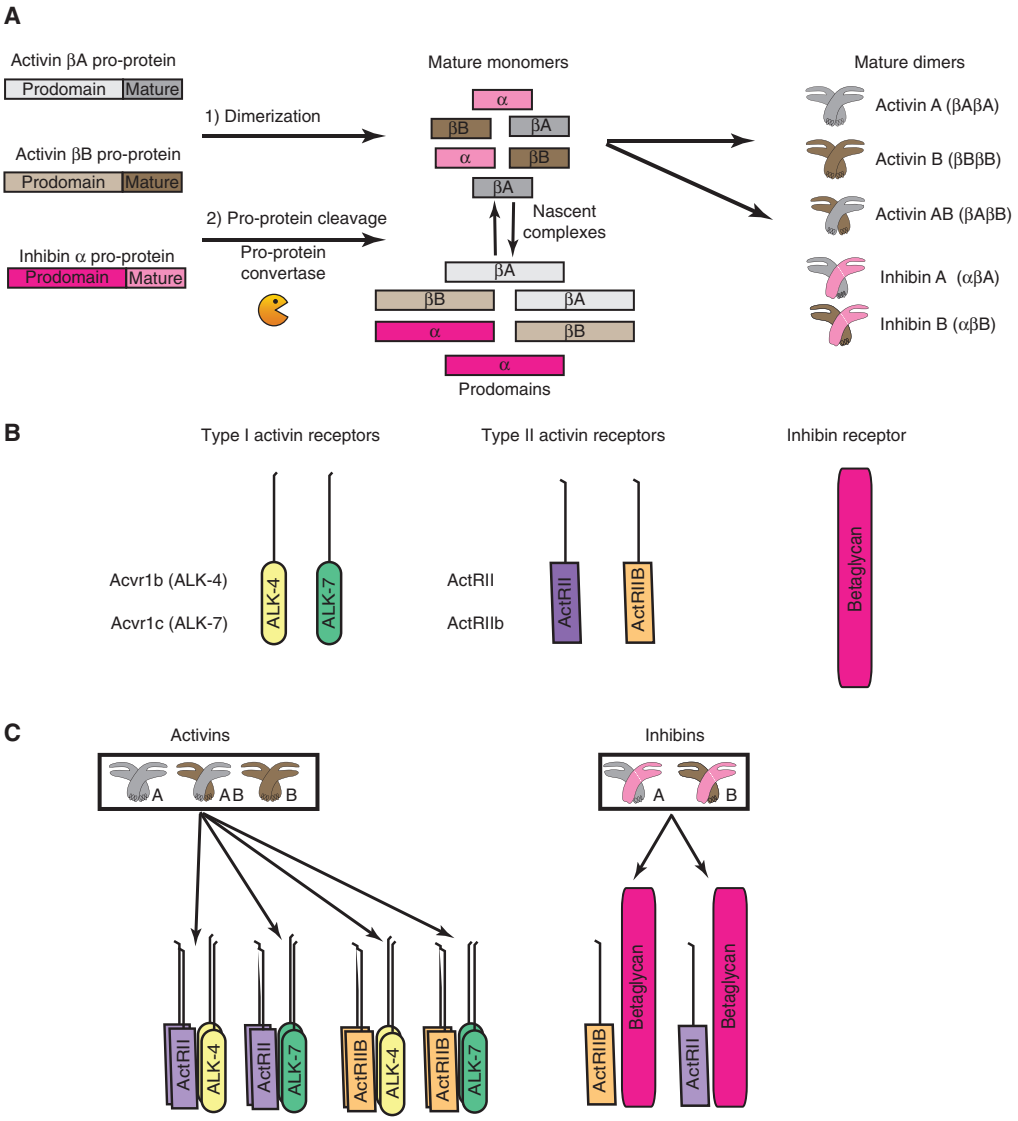

Figure 1. Activin and inhibin ligands and receptors. (*A*) Activin βA, activin βB, and inhibin α are synthesized as pro-proteins that comprise a prodomain and mature domain. The pro-proteins associate to form homo- or heterodimers, which are ultimately processed into activins A, B, AB, and inhibins A and B. The junctions of the pro- and mature domains are cleaved by pro-protein convertases, resulting in dimer complexes that retain the noncovalently linked prodomains. (*B*) The two type I receptors for activins are ActRIB (ALK-4) and ActRIC (ALK-7). The two type II receptors are ActRII and ActRIIB. The inhibins antagonize activin signaling by using one type II receptor and one type III TGF-β receptor, betaglycan. (*C*) The mature activin dimers bind type I and type II receptors to form active signaling complexes. Each activin dimer can bind more than one combination of type I and type II receptors with different affinities, and each type I/type II receptor combination can bind different dimers, including other members of the TGF-β family. The active signaling complex is comprised of one activin dimer, two type I, and two type II receptors. Inhibins competitively antagonize activin signaling by binding one type II receptor and betaglycan, thereby sequestering type II receptors in an inactive complex.

nism to control the relative levels of activins and inhibins in cells in which they are coexpressed.

After the cleavage by convertases, the pro- and mature domains remain associated through noncovalent interactions (Antenos et al. 2008, 2011). Activins and inhibins are secreted from the cell in this fashion, protecting the nascent mature dimers from extracellular degradation (Fig. 2A–C) (Gray and Mason 1990). The prodomains interact extracellularly with specific components of the extracellular matrix, perlecan and agrin, by binding to their heparan sulfate side chains (Fig. 2D) (Li et al. 2010), thereby protecting the activins and inhibins from proteolysis, and perhaps presenting them at higher concentrations to their receptors. Binding of the mature dimers to their receptors displaces the prodomains (Fig. 2E) (Walton et al. 2009).

Structure of Ligands

The structures of activin A alone and in combination with activin receptor type IIB (ActRIIB) are represented in Figure 3 (Thompson et al. 2003; Greenwald et al. 2004). Key elements include the "open hand" or "butterfly-like" configuration of the activin dimer, the "finger-like" and "wrist" domains, comprised of a pair of anti-parallel β-sheets and an α-helix, respectively, and a cystine knot-core (Fig. 3A). The "finger" and "wrist" domains interact with type II and type I receptors, respectively. The amino acids required for these interactions have been determined (Fig. 3B).

The interactions with the type I receptors are inferred from prototypic structural data and site-directed mutagenesis. The activin dimer interacts with the ActRIIB dimer through the convex surfaces of the ligand finger regions and concave surfaces of each of the type II receptors. The interactions are mediated by specific ionic/polar and hydrophobic amino acids (Greenwald et al. 1999; Gray et al. 2000). Activin binding to the type II receptors stabilizes the receptors within the cell membrane. The flexibility of the bound ligand allows it to interact with two type I receptors that bind hydrophobic pockets formed by the activin dimer. The type I receptors are subsequently phosphorylated at their glycine–serine-rich (GS) domains. The phosphorylation activates the type I receptor kinase, resulting in the phosphorylation of downstream signaling components (Harrison et al. 2003; Greenwald et al. 2004; Tsuchida et al. 2004). Differences in specific amino acids of activins βA and βB that affect binding to type II receptors likely explain why activin A binds with higher affinity and has greater biopotency in most contexts (Mathews and Vale 1991; Del Re et al. 2004). The cases in which the bioactivity of activin B is greater (e.g., in pancreatic islet cells) are likely because of the use of the other type I activin receptor, ALK-7 (Tsuchida et al. 2004; Bertolino et al. 2008).

The crystal structure of inhibin has not been determined; however, some aspects can be inferred because the structure of the β subunit and critical regions for the prototypical ligand/receptor interactions are known. Mammalian inhibin α has several additional amino acids at its amino terminus that are not present in other TGF-β family members, and lacks the α-helical configuration in the "wrist" domain. Instead, the "wrist" region contains a proline-rich sequence that not only disrupts the helix, but also facilitates the interaction with betaglycan (Makanji et al. 2008; Zhu et al. 2010).

Signaling in Response to Ligands

Several extra- and intracellular events influence activin signaling (Fig. 4). Strict control is necessary because dysregulated signaling has adverse effects in vivo and at the cellular level (Matzuk et al. 1992; Schwall et al. 1993; Tanimoto et al. 1999; Chen et al. 2000).

Activin Receptors

Like other members of the TGF-β family, signaling of dimeric activins occurs through two type I and two type II serine–threonine kinase receptors (Fig. 1B,C) (Mathews and Vale 1991; Mathews et al. 1992; Cárcamo et al. 1994; Attisano et al. 1996). Each of the seven type I receptors has an additional activin receptor-like kinase (ALK) designation. The type I receptors for the activins are ActRIB and ActRIC (ALK-4

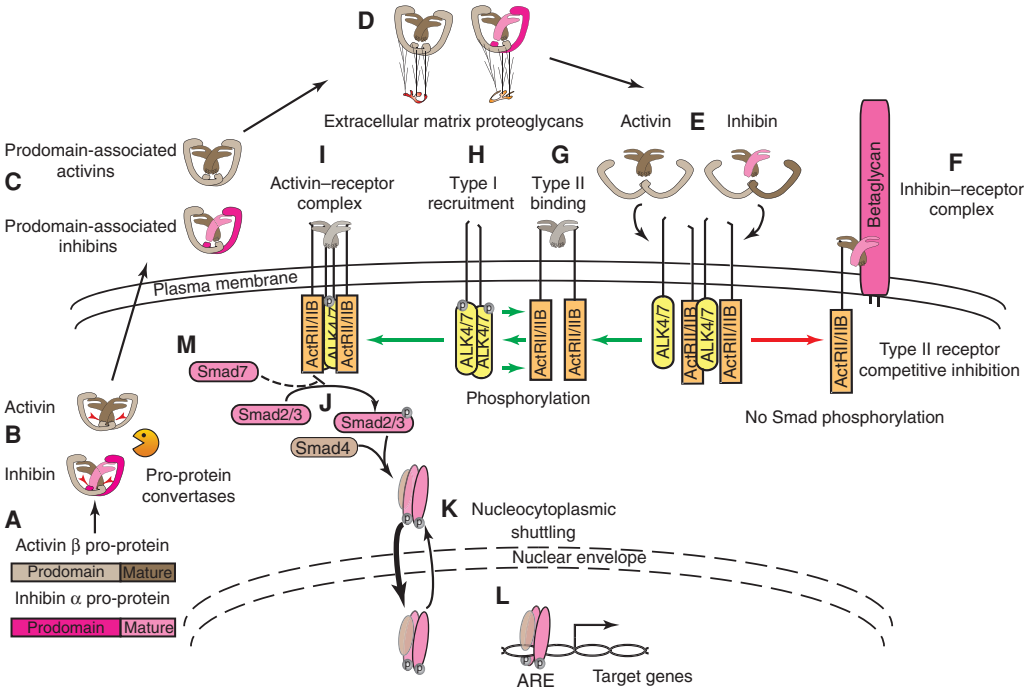

Figure 2. Activin and inhibin processing and signaling. (*A*) Activin and inhibin monomers are synthesized as pro-proteins. (*B*) The pro-proteins associate as homodimers or heterodimers with their intact prodomains. Within the cell, the junctions of the pro- and mature domains (red arrowheads) are cleaved by pro-protein convertases, leaving the noncovalent interactions among the domains intact. (*C*) Prodomain-associated activins and inhibins are released from the cell. (*D*) The intact prodomains enable interactions with glycosaminoglycans on proteins within the extracellular matrix. (*E*) Activins and inhibins compete for type I and II activin receptor binding, and, on receptor binding, release their associated prodomains. (*F*) Inhibin antagonizes activin signaling through association of its single inhibin β subunit with a single type II activin receptor and the association of its single inhibin α subunit with the membrane proteoglycan, betaglycan, thereby forming an inactive inhibin–receptor complex. This complex is incapable of signal transduction and thus inhibits activin signaling by sequestering type II activin receptors (red arrow). (*G*) The activation of activin receptors requires several steps (green arrows). The activin dimer binds two type II activin receptors, and activates type II receptor serine–threonine kinase activity. (*H*) Type II receptor binding results in recruitment and association with two type I activin receptors, ActRIB (ALK-4) or ActRIC (ALK-7), that are subsequently phosphorylated. (*I,J*) The fully assembled, hexameric ligand–receptor complex then initiates Smad-mediated signaling by phosphorylating regulatory Smad2 and/or Smad3 (Smad2 and Smad3) near their carboxyl termini, followed by association of two phosphorylated Smads with a common Smad4. (*K*) Smad complexes are in equilibrium between the cytoplasm and nucleus. Receptor signaling results in a shift in equilibrium toward the nucleus. (*L*) Binding of the Smad complex and transcription coactivators to activin-responsive elements (AREs) results in the transcription of hundreds of genes, a process that is tightly regulated by a variety of proteins that impact nucleocytoplasmic shuttling, Smad phosphorylation status, Smad degradation, and transcriptional activity. (*M*) Inhibitory Smad7 competes with Smad2 and Smad3 for activated type I receptor binding, thereby preventing Smad2 and Smad3 phosphorylation and facilitating proteasomal degradation or dephosphorylation of activin–receptor complexes.

and ALK-7, respectively) (Tsuchida et al. 2004; Bernard et al. 2006). The ability of activin B homodimers and activin AB heterodimers to signal through ALK-7 has been shown in MIN6 pancreatic β cells, whereas activin A uses ALK-4, binding ALK-7 with low affinity (Harrison et al. 2003). ALK-4 is also the type I receptor for growth differentiation factors (GDFs) 1, 3, 8–11 and nodal, and ALK-7 for GDF-1, GDF-3 and nodal (Reissmann et al. 2001; Andersson

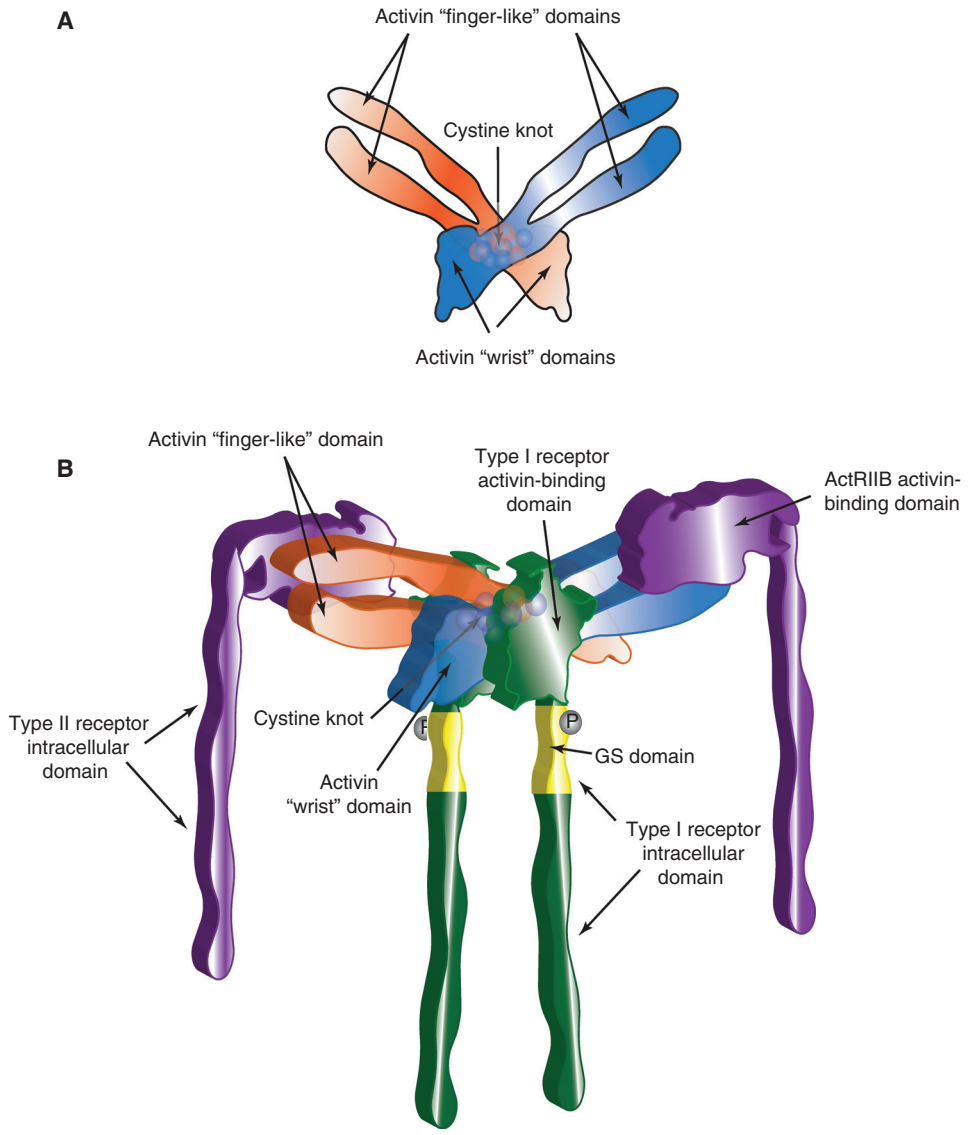

Figure 3. Structures of the activin dimer and activin–receptor complex. (*A*) Two-dimensional representation of an activin dimer. Two activin monomers associate through interactions between the convex surface of the α-helical "wrist" domain of one monomer and the concave surface of the "finger-like" domain, comprised of antiparallel β-sheets, of the second monomer, giving the appearance of an "open hand" or "butterfly." Covalent dimerization occurs through one of the seven conserved cysteines that define TGF-β family ligands. The cysteines are organized into a "cystine knot" (spheres) within the core of the activin dimer. (*B*) Three-dimensional rendition of the activin–receptor complex. The activin dimer and cystine knot are represented as shades of orange and blue. The assembly of the activin dimer with two activin type II receptors occurs through interactions among the convex surfaces of the ligand finger regions with concave surfaces of each of the type II receptors (shown in purple). The binding of the type II receptors by activin stabilizes the receptors within the cell membrane, yet the flexibility of the bound ligand, shown here as a "spreading" of the activin monomers, allows it to further interact with two type I receptors (shown in green). The type I receptors are subsequently phosphorylated (dark gray spheres) at their glycine–serine rich (GS) domains, shown in yellow, thereby activating the type I receptor kinase.

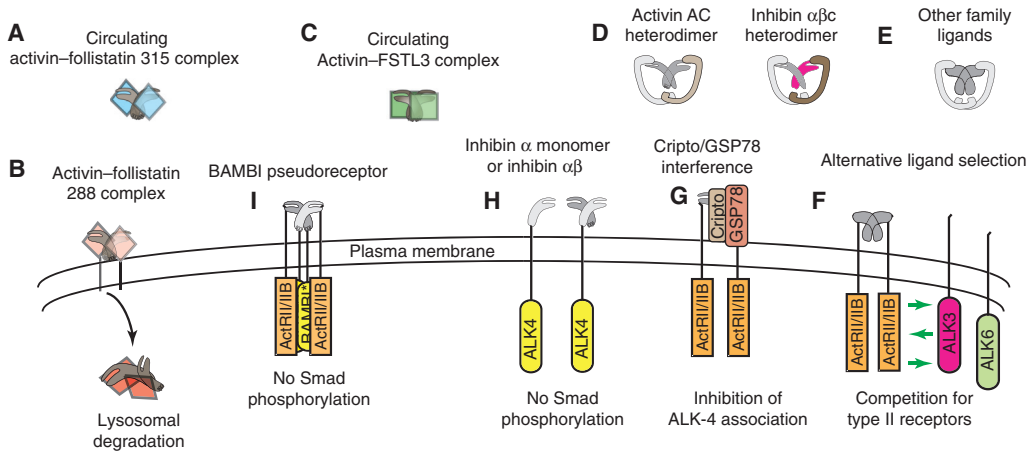

Figure 4. Regulation of activin signaling. (*A*,*B*) Follistatin occurs in circulating (follistatin 315) and membrane-bound (follistatin 288) forms. Activins bind irreversibly to follistatin and the activin–follistatin complex is internalized and degraded in lysosomes (*B*). (*C*) FSTL3 is a structurally similar, follistatin-like protein that also binds circulating activins with high affinity and functions as an activin inhibitor. (*D*) Activin βC can heterodimerize with activins βA and βB as well as inhibin α. Some data suggest that "atypical" activins C and E might antagonize activin A or B signaling by forming nonfunctional heterodimers. (*E*,*F*) Other TGF-β family ligands can bind type II activin receptors, so the potential exists for competition in tissues where ligands and receptors coexpress. (*G*) Although the EGF-CFC coligand/coreceptor Cripto enhances signaling for some TGF-β family ligands, it inhibits functional activin–receptor complexes by binding type II receptor-associated activins, thus preventing the recruitment and phosphorylation of type I receptors. Glucose-regulated protein 78 (GRP78) is also an essential component of this inhibitory complex. (*H*) In addition to associations with betaglycan, inhibin α monomers and αβ dimers also bind directly to ALK-4 and inhibit activin signaling in vitro. (*I*) BMP and activin membrane-bound inhibitor (BAMBI) is a type I pseudoreceptor that cannot phosphorylate Smads. However, it competes with functional type I receptors for inclusion in activin–receptor complexes, thereby inhibiting activin signaling.

et al. 2008; Wakefield and Hill 2013). The *Acvr1b* gene encoding ALK-4 produces three alternatively spliced and polyadenylated transcripts, whereas ALK-7, encoded by *Acvr1c*, is produced from four alternatively spliced transcripts. Two of the proteins lack a transmembrane domain, producing soluble receptors whose functional significance is unknown (ten Dijke et al. 1993; Roberts et al. 2003).

The type II activin receptors are ActRII (also known as ActRIIA) and ActRIIB, encoded by *Acvr2* and *Acvr2b*, respectively. *Acvr2b* produces four alternatively spliced transcripts that affect activin affinity (Attisano et al. 1992). Other TGF-β family ligands, bone morphogenetic proteins (BMPs) -2, -4, -6, and -7 can also bind type II activin receptors. However, the repertoire of type I BMP receptors is different, so the potential exists for a competition for type II activin receptors (Greenwald et al. 2003; Samad et al. 2005; Allendorph et al. 2006, 2007; Koncarevic et al. 2012; Zhang et al. 2013). Binding to the type II receptor results in recruitment and phosphorylation of the type I receptors at several sites within their GS domain, causing activation of type I receptor kinase activity and subsequent phosphorylation of downstream Smad and non-Smad signal transduction proteins. The Smads are the most thoroughly characterized.

Smad-Mediated Signaling

Smads are intracellular mediators of TGF-β family signaling. The first family member, Mothers against decapentaplegic (Mad), was discovered using genetic screens in *Drosophila melanogaster* (Sekelsky et al. 1995). Mutations

in three *Caenorhabditis elegans* orthologs, sma-2, sma-3, and sma-4, all produce developmentally arrested larvae with small body sizes (Savage et al. 1996). Smads were later identified based on their sequence homologies. Vertebrate orthologs of sma and Mad are called Smads (Derynck et al. 1996).

Members of the Smad family play complementary roles in TGF-β family signaling. The receptor-regulated (R-) Smads 1, 2, 3, 5, and 8 are phosphorylated by type I receptors after ligand-induced assembly and activation of type I and type II receptor complexes (Fig. 2J) (Heldin et al. 1997; Massagué 1998; Massagué and Chen 2000). Phosphorylation of R-Smads triggers the association with Smad4, which is common to all R-Smad complexes, followed by a shift in homeostasis toward the nucleus to activate or inhibit the transcription of downstream target genes (Fig. 2J–L) (Hill 2009).

Functionally, TGF-β family ligands are divided into two main branches based on the receptors with which they interact and the R-Smads that mediate their signals. In general, TGF-β, activin, and nodal signaling occur through activation of Smads 2 and 3, whereas the BMPs activate Smads 1, 5, and 8. A noteworthy exception to activin-induced Smad2 and 3 phosphorylation has been reported in the context of liver inflammation: the ability of activin B to induce a BMP-like cascade, including a (combined) pSmad1, 5, 8 western blot signal in human hepatoma cells and primary mouse hepatocytes, and consequential expression of the iron regulatory protein, hepcidin (Besson-Fournier et al. 2012).

Accumulation of the Smad complexes within the nucleus is regulated by nuclear transporters and nuclear phosphatases, followed by dissociation of the heteromeric complexes, allowing the Smads to be recycled to the cytoplasm (Lin et al. 2006; Varelas et al. 2008). Inhibitory Smads (I-Smads, i.e., Smad6 and Smad7) block the association of R-Smads with Smad4 and target ligand/receptor complexes for degradation, thereby antagonizing downstream signaling (Ishisaki et al. 1998, 1999). Smad7 is the predominant inhibitory Smad for activin signaling.

Non-Smad Signaling

In addition to the Smad-mediated signaling pathway, many TGF-β family members, including activins, activate other signaling pathways in a variety of cell types, independent of Smad phosphorylation (Cocolakis et al. 2001; Ogihara et al. 2003; Zhang et al. 2005; de Guise et al. 2006; Huang et al. 2006). Other signaling pathways can also alter the stability of Smads by phosphorylating within the Smad linker, resulting in a variety of outcomes that enhance or reduce activity of the Smad complex (reviewed in Massagué 1998). Activin dramatically inhibits the growth of human breast cancer T47D cells, and this requires an intact p38 mitogen-activated protein kinase (p38 MAPK) pathway, shown by complete reversal of growth inhibition with selective p38 MAPK inhibitors (Cocolakis et al. 2001).

Neurogenin 3 is an essential transcription factor for pancreatic islet cell differentiation. Relaxation of neurogenin 3 transcriptional repression and the consequential differentiation of AR42J-B13 pancreatic progenitor cells is strongly enhanced by activin A and hepatocyte growth factor (HGF) in combination, whereas HGF alone has a modest effect and activin A alone has no effect. The positive effect on differentiation is attenuated by a dominant negative MAPKK3, or by treatment with a p38 MAPK inhibitor. In contrast, overexpression of inhibitory Smad7 has no effect on activin-/HGF-induced differentiation implicating activin-/HGF-induced non-Smad signaling as a contributor to pancreatic islet cell differentiation (Ogihara et al. 2003).

Non-Smad-mediated activin signaling has also been shown in keratinocytes, in which activin-induced MEKK1 activation leads to c-Jun amino-terminal kinase (JNK), c-Jun and p38 MAPK phosphorylation, culminating in stress fiber formation and cell migration. The activin-induced effects are observed in keratinocytes of wild-type mice, but not in those of MEKK1-deficient mice (Zhang et al. 2005). In pituitary prolactin-producing cells an intact p38 MAPK pathway is required for activin inhibition of transcription from the Pit-1 promoter

(de Guise et al. 2006). Finally, erythropoietic gene expression and cytokine-mediated colony formation of K562 erythroleukemia cells are both affected by activin-induced p38 MAPK activation (Huang et al. 2006). Therefore, non-Smad-mediated activin signaling contributes to processes that impact cell migration and differentiation.

Inhibins and Betaglycan

Inhibins antagonize activin functions, and their activities as gonad-derived inhibitors of FSH secretion were well recognized for decades before the peptides and cDNAs were identified (McCullagh 1932; Ling et al. 1985; Vale et al. 1994; Aono et al. 1997). We call attention to a recent, extensive review of the inhibins, which includes their historical, biological, and clinical importance (Makanji et al. 2014).

Bioactive inhibins have been isolated from ovarian follicular fluid and plasma of several species (De Jong and Sharpe 1976; Ling et al. 1985; Miyamoto et al. 1985; Rivier et al. 1985; Robertson et al. 1985). Heterodimers of both full-length and fully processed forms of the α and β subunits are active in pituitary bioassays (Mason et al. 1996). Exogenously administered inhibin is rapidly cleared from circulation with a half-life ranging from 3 to 6 minutes, which is somewhat shorter than activin. Inhibin targets are more widespread than activins, and activins and inhibins are cosynthesized in a variety of contexts (Woodruff et al. 1993a,c; Makanji et al. 2009).

Betaglycan was originally characterized as a membrane-associated proteoglycan and a type III TGF-β receptor that binds TGF-β with high affinity and enhances TGF-β signaling. Betaglycan can also be released as a soluble proteoglycan after cleavage at the membrane attachment site (Lopez-Casillas et al. 1991). Within the context of activin and inhibin biology, however, its most important function is to regulate activin signaling through direct association with inhibin.

Betaglycan is a coreceptor that enhances inhibin's antagonism of activin signaling (Figs. 1C, 2F). The inhibin β subunit binds to one type II activin receptor, competing directly with activin binding. However, the inhibin α subunit cannot bind a second type II receptor. Instead, inhibin α binds betaglycan with high affinity and enhances inhibin's ability to compete with activins for activin type II receptors (Wiater et al. 2009). Betaglycan can also enhance inhibin sensitivity in cells that normally respond poorly to inhibin (Lewis et al. 2000). Silencing betaglycan expression or neutralizing betaglycan antibody in cultured primary anterior pituitary cells blocks the interaction of inhibin A and betaglycan, and reverses inhibin effects without affecting the cellular responses to activin. Repressing betaglycan expression also reduces inhibin antagonism of activin-induced FSH secretion, with a >1000-fold reduction in the response to inhibin A in cells treated with neutralizing antibody (Wiater et al. 2009). In addition to its associations with betaglycan, inhibin α monomers and αβ dimers can associate directly with ALK-4, and an amino-terminal inhibin α peptide is sufficient to inhibit activin signaling and activin-induced FSH production in cultured pituitary gonadotropes (Zhu et al. 2012).

Cripto and GRP78

Cripto is a glycosylphosphatidylinositol-anchored, cell surface coreceptor in the epidermal growth factor-Cripto-1/FRL-1/Cryptic family that participates in embryonic development and cell growth regulation through effects on many TGF-β family ligands, either enhancing (Vg1, nodal, GDF-1, GDF-3) or diminishing (activin A, activin B, TGF-β1) their bioactivities (Cheng et al. 2003; Gray et al. 2003, 2006; Chen et al. 2006; Kelber et al. 2007; Shen 2007). Cripto effects occur through a complex on the cell surface that includes glucose-regulated protein 78 (GRP78) (Shani et al. 2008; Kelber et al. 2009) (Fig. 4G). Cripto forms a complex with activin A or B and either ActRII or ActRIIB, but is incapable of binding activins directly. On association with activin and type II receptors, Cripto blocks the interaction with ALK-4 and inhibits activin signaling in HepG2 and 293T cells (Gray et al. 2003). Disrupting the Cripto-GRP78 interaction by silencing GRP78

expression or using GRP78 neutralizing antibody blocks the modulatory effects of Cripto on activin A and B signaling, confirming an essential role for GRP78 in Cripto-mediated antagonism of activin–receptor complexes (Kelber et al. 2009).

BMP and Activin Membrane-Bound Inhibitor (BAMBI)

BAMBI is structurally related to type I receptors but lacks the kinase domain. Orthologs have been identified in several species (Degen et al. 1996; Tsang et al. 2000; Grotewold et al. 2001; Loveland et al. 2003). BAMBI is coexpressed with BMP-4 during *Xenopus* embryogenesis and its expression requires BMP signaling. The protein stably associates with several TGF-β family receptors, including ALK-4, and inhibits activin, BMP, and TGF-β signaling. BAMBI inhibits ligand signaling through its intracellular domain, which contains a type I receptor homodimerization interface, thereby preventing the assembly of activated receptor complexes (Fig. 4I). To our knowledge, no effects on ligand binding have been reported. Thus, BAMBI is a pseudoreceptor that can inhibit activin signaling (Onichtchouk et al. 1999).

Follistatin and Follistatin-Like 3

Activins are antagonized by follistatin, an activin-binding protein that affects the accessibility of activins to their receptors thereby attenuating activin-mediated FSH release, a function from which its name is derived (Esch et al. 1987; Ueno et al. 1987; Nakamura et al. 1990; Sidis et al. 2006). There are three alternatively spliced products of the single follistatin gene, designated Fst288, Fst303, and Fst315. The carboxyl termini of these isoforms differ. Fst315 is the predominant form in circulation, whereas Fst288 is membrane-bound through its interaction with cell surface heparan sulfate proteoglycans (Fig 4A,B). Fst303 is also membrane-bound but has a lower affinity than Fst288. The membrane association provides the mechanism by which bound activins are internalized and degraded (Fig. 4B), and in part explains the different bioactivities for the follistatin isoforms (Sugino et al. 1993; Hashimoto et al. 1997).

Follistatin was originally isolated from ovarian follicular fluid, the site of production for all isoforms except Fst315, suggesting an alternative source of production for the circulating form, and possibly specialized functions for each isoform (Schneyer et al. 2004). Follistatins have up to three follistatin domains, bind both activin A and activin B, but have a 10-fold higher affinity for activin A (Schneyer et al. 2003). Follistatin also binds GDF-8 (myostatin), GDF-11, BMP-2, BMP-4, BMP-6, and BMP-7 with progressively lower affinities (Glister et al. 2004; Sidis et al. 2006; Schneyer et al. 2008). Structural and mutagenesis data have shown that two follistatin molecules block the sites of interaction between dimeric activin and type I and type II activin receptors (Thompson et al. 2005; Harrington et al. 2006; Harrison et al. 2006). Follistatin-like 3 (FSTL-3) interacts with activin in a similar fashion, but the points of contact differ (Stamler et al. 2008).

FSTL-3, also known as follistatin-related gene (FLRG) (Hayette et al. 1998; Tsuchida et al. 2000) and FST-related protein (FSRP) (Schneyer et al. 2001) (herein, FSTL-3), lacks the third follistatin domain and the heparan-binding motif, thereby restricting it to the circulation (Fig. 4C) (Sidis et al. 2005). Follistatin and FSTL-3 are differentially expressed. Follistatin is generally coexpressed with activins and most highly expressed in the ovary and pituitary (Besecke et al. 1997; Arai et al. 2002; Bilezikjian et al. 2004), but also detected in several other tissues (Ogawa et al. 1993; Inoue et al. 1994; Petraglia et al. 1994; Michel et al. 2000; Sonoyama et al. 2000; Bloise et al. 2009; Lima et al. 2010). FSTL-3 has a different, but overlapping distribution, and is also expressed at high levels in several tissues (Wankell et al. 2001a; Ciarmela et al. 2003; Florio et al. 2004; Xia et al. 2004; Takehara-Kasamatsu et al. 2007; Allen et al. 2008; Bloise et al. 2009). Like follistatin, FSTL-3 has the capacity to bind and regulate other TGF-β family ligands, including myostatin and several BMPs (Tsuchida et al. 2000; Lee and McPherron 2001; Otsuka et al. 2001; Hill et al. 2002; Maguer-Satta et al. 2003).

ROLES OF ACTIVINS AND INHIBINS IN DEVELOPMENT

The expression of activins, inhibins, their receptors and regulatory proteins is dynamic and widespread. Therefore, it is not surprising that activin signaling participates in a variety of biological processes that impact embryonic development, growth, as well as functions of fully differentiated cell types (Table 1).

Spatiotemporal Expression

Although recombinant activin A and activin B have similar biological activities in vitro (Mason et al. 1989; Mathews and Vale 1991), the spatiotemporal expression of activins, their receptors, and regulatory proteins differs greatly during embryonic and fetal development. The mRNAs encoding activin βA and activin βB are present at early stages of embryonic development and in embryonic stem cells (Albano and Smith 1994). Activin signaling contributes to the maintenance of the pluripotent state of human embryonic stem cells (Wu et al. 2008; Xu et al. 2008) and to developmental cell fate decisions (Hay et al. 2008; Pearson et al. 2008; Sumi et al. 2008). Several developing organs and tissues express activins or activin receptors, including the roof of the oral cavity, mesenchyme underlying the tooth primordia and other regions of head mesenchyme, esophagus, developing whiskers, vasculature, vertebral bodies, heart, external genitalia, and the developing limb (Feijen et al. 1994; Roberts and Barth 1994; Merino et al. 1999). In contrast, activin βB mRNA localizes primarily to the gonad, forebrain, hindbrain, spinal cord, stomach, and esophagus in mouse and rat embryos. Type II activin receptors generally colocalize with sites of activin production, and inhibin α localizes to the developing gonad (Feijen et al. 1994; Roberts and Barth 1994). In adult female rats, radiolabled activin A protein binds to the pituitary and ovary, whereas inhibin A localizes to the spleen, adrenal, pituitary, specific stages of ovarian follicles in adult rats and in the bone marrow (Woodruff et al. 1993a,b).

The developing gonad and esophagus are the only tissues, in which activin βA and βB are coexpressed in the mouse embryo, although an overlapping pattern of expression has been observed in the developing chick limb (Merino et al. 1999). In general, activin βA mRNA is more widespread than activin βB in both mouse and rat embryos, and the timing of expression differs.

Mouse Models

Several genetically engineered mouse models have been produced to assess the biological functions of the activins and inhibins. These experiments are summarized in Table 2.

Activins A and B

Genetic studies in mice have shown that activin βA homozygous null mutant mice (*Inhba*$^{-/-}$) are born without whiskers, incisors, and mandibular molars. Approximately 30% also have cleft palate. The pups do not suckle and die within 24 h (Matzuk et al. 1995c). In contrast, *Inhbb*$^{-/-}$ mice are viable and fertile but have eyelid closure defects, prolongation of the gestational period, and are unable to nurse their young (Schrewe et al. 1994; Vassalli et al. 1994). *Inhba*$^{-/-}$; *Inhbb*$^{-/-}$ double mutant mice have phenotypes that are additive of each of the individual null mutants, with no additional abnormalities (Matzuk et al. 1995c).

Mice, in which the mature domain of activin βA is replaced with that of activin βB (*Inhba*^{BK}), display a dosage-sensitive complete or partial rescue of all the phenotypes of *Inhba*$^{-/-}$ mice (Brown et al. 2000), suggesting that activin βB is functionally hypomorphic relative to the wild-type activin βA protein in some developmental contexts.

Follistatin

A homozygous null mutation of the *Fst* gene causes early neonatal lethality as a result of respiratory failure. The mice have taut, shiny skin, hypoplastic respiratory musculature, and craniofacial abnormalities affecting the palate, whiskers, and teeth bearing some similarities to *Inhba*$^{-/-}$ mice (Matzuk et al. 1995d).

Transgenic mice that selectively express either the Fst288 or Fst315 isoform driven by

Table 1. Biological functions of activins and inhibins

Proteins	Tissue/cell types	Biological functions	References
Development			
Activin	Human embryonic stem cells	Maintenance of pluripotency	Wu et al. 2008; Xu et al. 2008
Activin A	Craniofacial (mouse)	Whisker, palate, and tooth development	Matzuk et al. 1995d; Brown et al. 2000
Activin A	Mouse tissues	Supports total body weight gain and the growth of gonads, fat, and liver	Brown et al. 2000; Li et al. 2009
Activin B	Mammary gland	Stroma-supported ductal elongation, alveolar morphogenesis, and luminal expansion	Vassalli et al. 1994; Robinson and Hennighausen 1997
Activin B	Craniofacial (mouse)	Prenatal eyelid fusion	Schrewe et al. 1994; Vassalli et al. 1994
Activin D	All stages, *Xenopus* embryos	Mesoderm induction	Oda et al. 1995
Nervous system			
Activin A	E18 rat retinal progenitors	Induces differentiation into rod photoreceptors	Davis et al. 2000
Activin A	Cultured P19 teratoma cells, rat B50 nerve cells and chick neural retinal cells	Increased survival beyond life span under normal culture conditions	Schubert et al. 1990
Activin A	Rat mesencephalon dopaminergic cells	Protects against *N*-methyl-4-phenylpiridinium ion toxicity	Krieglstein et al. 1995
Activin A	Hippocampus and dorsolateral striatum	Protection against neuronal death induced by hypoxic-ischemic brain injury	Wu et al. 1999
Activin A	Striatal cholinergic interneurons	Protects against chemically induced neurodegeneration	Hughes et al. 1999
Activin B + TGF-β1	Oligodendrocytes	Together enhance proliferation, viability, maturation, and myelination during embryonic development	Dutta et al. 2014
Activin orthologs	*Drosophila* embryos	Promote neuron growth and photoreceptor axon targeting	Zhu et al. 2008
Reproduction			
Activin	Pituitary gonadotropes	Augments GnRH-induced FSH release	Ling et al. 1986a; Vale et al. 1986
Activin A	Cultured ovarian granulosa cells	Increases DNA synthesis, augmented by FSH; increases expression of estrogen receptor; enhances proliferation of cells from human pre-ovulatory follicles	Kipp et al. 2007
Activin A	Cultured ovarian theca cells	Blocks LH-induced androgen production	Hillier et al. 1991b
Activin A	Cultured ovarian luteal cells	Blocks LH-induced progesterone production	Di Simone et al. 1994
Activin B	Developing gonad	Coelomic vessel formation in developing male gonads	Yao et al. 2006
Activin A	Fetal Sertoli cells	Activin A from fetal Leydig cells supports Sertoli cell proliferation and testis cord elongation	Archambeault and Yao 2010

Continued

Table 1. *Continued*

Proteins	Tissue/cell types	Biological functions	References
Activin A	Cultured Sertoli cells	Enhances proliferation alone or in combination with FSH; induces production of inhibin and follistatin; causes reversion to an immature, proliferative state	Buzzard et al. 2003; Nicholls et al. 2012
Activin A	Cultured Leydig cells	Blocks LH-induced androgen production	Mauduit et al. 1991
Activin A	Germ/Sertoli cell coculture	Increases germ cell/Sertoli cell ratio	Meehan et al. 2000
Inhibin	Ovarian granulosa cells	Antagonizes proliferation of granulosa cells at the single-layer follicle stage; ovarian tumor suppression	Matzuk et al. 1992; Wu et al. 2004
Inhibin	Ovarian theca cells	Promotes steroidogenesis	Wu et al. 2004
Inhibin B	Testis	Possible paracrine role in spermatogenesis by supporting germ, Sertoli, and Leydig cell interactions	Clifton et al. 2002; Kumanov et al. 2006
Inhibin	Pituitary gonadotropes	Negative regulator of FSH production and release	Setchell and Jacks 1974; De Jong and Sharpe 1976
Inhibin	Ovarian granulosa cells	Antagonizes proliferation of granulosa cells at the single-layer follicle stage	Wu et al. 2004
Inhibin	Ovarian theca cells	Promotes steroidogenesis	Wu et al. 2004
Inhibin	Placenta	Blocks activin-mediated steroidogenesis and HCG production	Petraglia et al. 1989; Jones et al. 2006
Body composition and energy metabolism			
Activin A	Liver	Induces hepatocyte apoptosis	Schwall et al. 1993
Activin A	Human preadipocytes, mouse 3T3-L1 preadipocytes	Increases proliferation while inhibiting differentiation	Zaragosi et al. 2010
Activin B	3T3-L1 mature adipocytes	Reduces lipolysis and lipase gene expression	Magnusson et al. 2010
Activin A + HGF	AR42J-B13 pancreatic progenitor cells	Relaxation of neurogenin-3 transcriptional repression, enhancing differentiation	Ogihara et al. 2003
ActRIIB signaling	Adipose and muscle	Influences lean/fat body mass ratio, "britening" of white adipose, peripheral glucose uptake and hepatic glucose production	Akpan et al. 2009; Fournier et al. 2012; Koncarevic et al. 2012; Zhang et al. 2012
Activins A and B	Pancreatic islets	Affect islet cell differentiation, growth, calcium signaling, α-/β-cell ratio, and insulin release	Yamaoka et al. 1998; Bertolino et al. 2008; Bonomi et al. 2012
Activin ortholog (dawdle)	*Drosophila* embryos	Regulates triglyceride, glycogen, and glucose metabolism and corresponding Krebs cycle, oxphos, and fatty acid oxidation enzymes	Ghosh and O'Connor 2014
Activin C	Liver	Possible effects on hepatocellular proliferation	Chabicovsky et al. 2003; Takamura et al. 2005; Wada et al. 2005

Continued

Table 1. *Continued*

Proteins	Tissue/cell types	Biological functions	References
Inflammation			
Activin A	Mouse macrophage RAW264.7	Increases IL-1β, IL-6, CD80, macrophage activation, and phagocytosis	Ge et al. 2009
Activin A	Human peripheral blood monocytes	Supports the proinflammatory M2 macrophage phenotype	Sierra-Filardi et al. 2011
Activin B	Human hepatoma and mouse primary hepatocytes	Increases expression of hepcidin during inflammation, mediated by Smad1/5/8 phosphorylation	Besson-Fournier et al. 2012
Erythropoiesis			
Activin A	K562 erythroleukemia cells	Augments EPO-dependent hemoglobin production	Yu et al. 1987
Inhibin	K562 human leukemia and primary human bone marrow cells	Suppresses activin-induced differentiation and EPO-induced CFU formation	Yu et al. 1987
Activin	Human bone marrow–derived erythrocyte precursors	Enhanced proliferation	Yu et al. 1987
Miscellaneous			
Activins A, B, and AB	Myoblasts	Inhibits myotube differentiation	Souza et al. 2008
Activin A	Muscle	Negatively regulates muscle mass	Chen et al. 2015
Activin	Human breast cancer T47D cells	p38 MAPK-mediated growth inhibition	Cocolakis et al. 2001
Activins A and B	Human neuroblastoma and rat pheochromocytoma	Protect against serum withdrawal-induced apoptosis	Kupershmidt et al. 2007
Inhibin	Bone	Positive effects on bone mineral density	Vural et al. 2005; Perrien et al. 2006; Perrien et al. 2007
Activin	Keratinocytes	Stress fiber formation and enhanced cell migration through non-Smad-mediated signaling	Zhang et al. 2005
Inhibin	Adrenal cortex	Tumor suppression	Matzuk et al. 1992; Looyenga and Hammer 2006

endogenous regulatory sequences were generated to determine whether either could rescue the defects of follistatin null mice (Lin et al. 2008). Mice expressing the *Fst315* circulating isoform on a $Fst^{-/-}$ background survive to adulthood, but are small with poor vascular perfusion of the distal tail, and have reproductive abnormalities. However, the survival of mice with the *Fst288* membrane-bound isoform is only marginally improved relative to $Fst^{-/-}$ mice. In contrast, an *Fst* targeted insertion allele that results in the production of Fst288 only, is sufficient for survival. The reason for the difference among these models is unclear, perhaps reflecting the well-described, dosage-sensitive effects of activin-receptor signaling. Nevertheless, both observations support unique roles for follistatin isoforms in development and reproduction.

Activin C and Activin E

Data concerning the functional roles of activin C in the liver are conflicting. Transient reduction of activin C expression occurs after partial

Table 2. Mouse models of activin- and inhibin-related loss- and gain-of-function

Genotypes	Phenotypes	References
	Loss-of-function/hypomorphic	
Single-activin/inhibin mutants		
Inhba$^{-/-}$	Failure to develop whiskers, incisors, mandibular molars; cleft palate (~30%), neonatal lethality	Matzuk et al. 1995d
Inhba$^{-/-}$	Aberrant (absent) whisker development leads to secondary defects in whisker-associated trigeminal sensory function	Jhaveri et al. 1998
Inhba$^{-/-}$	Specific decrease in the number of retinal rod photoreceptors	Davis et al. 2000
Inhbb$^{-/-}$	Eyelid closure defect, prolonged gestation, impaired lactation, normal growth, gonadal differentiation, fertility, and survival; normal whisker and tooth development; no cleft palate	Schrewe et al. 1994; Vassalli et al. 1994
Inhba$^{BK/BK}$	Normal whisker and tooth development; no cleft palate; symmetrical growth deficiency (moderate), enlargement of external genitalia, hypogonadism (moderate), sunken eyes, short hair, delayed hair growth (mild), diminished female fertility, decreased life expectancy (mild/moderate), decreased white adipose (moderate), increased metabolic rate, mitochondrial dysfunction	Brown et al. 2000; Li et al. 2009
Inhba$^{BK/-}$	Short whiskers, normal tooth development; no cleft palate; symmetrical growth deficiency with reduced adiposity (severe), enlargement of external genitalia, hypogonadism (severe), delayed hair growth (moderate); decreased life expectancy (severe)	Brown et al. 2000; Li et al. 2009
Inhbc$^{-/-}$	No effects on development, liver cytoarchitecture, function, or regenerative capacity	Lau et al. 2000
Inhbe$^{-/-}$	No effects on development, liver cytoarchitecture, function, or regenerative capacity	Lau et al. 2000
Inha$^{-/-}$	Gonadal sex cord stromal and adrenal tumors, cachexia, absent parietal cells in the glandular stomach, and hepatocellular necrosis	Matzuk et al. 1992
Activin receptor mutants		
Acvr2$^{-/-}$	25% die perinatally from cleft palate/Pierre Robin sequence; males have delayed fertility; females are infertile	Matzuk et al. 1995b
Acvr2b$^{-/-}$	Perinatal lethal in a subset; left–right asymmetry and anteroposterior axis defects	Oh and Li 1997
Acvr1b$^{-/-}$ (*Alk4*)	Embryonic lethal; disrupted primitive streak	Gu et al. 1998
dnAcvr1b (driven by CaMKII-α)	Dominant negative ActRIB expression in developing forebrain makes hippocampal neurons more susceptible to excitotoxic injury; reduced glutamatergic neurotransmission and impaired long-term potentiation	Muller et al. 2006
Bambi$^{-/-}$	Regulation of mechanisms involved in acute and chronic pain sensitivity; no apparent developmental defects	Chen et al. 2007; Tramullas et al. 2010
Acvr1c$^{-/-}$ (*Alk7*)	Progressive hyperinsulinemia, reduced insulin sensitivity, fatty liver, impaired glucose tolerance, pancreatic islet enlargement, reduced fat accumulation, and partial resistance to diet-induced obesity	Andersson et al. 2008; Bertolino et al. 2008

Continued

Table 2. *Continued*

Genotypes	Phenotypes	References
Fst$^{-/-}$	Neonatal lethality because of respiratory failure, hypoplasia of respiratory musculature, abnormal skin, and craniofacial malformations	Matzuk et al. 1995d
Fst$^{-/-}$	Aberrant (short, curled) whisker development leads to secondary defects in whisker-associated trigeminal sensory function	Jhaveri et al. 1998
Fst$^{-/-}$	Ectopic coelomic vessel formation in developing female gonads	Yao et al. 2004
Fstl3$^{-/-}$	Increased pancreatic islet number and size, with β-cell hyperplasia, diminished white fat, and beneficial effects on glucose metabolism	Mukherjee et al. 2007
Tgfbr3$^{-/-}$	*Tgfbr3* (betaglycan) knockout causes lethal proliferative defects in heart and apoptosis in liver, occurring at E13.5	Stenvers et al. 2003
Double and triple mutants		
Inhba$^{-/-}$; *Inhbb*$^{-/-}$	Combined features of the single mutations with no additional defects	Matzuk et al. 1995d
Inhba$^{BK/BK}$; *Inhbb*$^{-/-}$	Symmetrical growth deficiency (severe), eyelid closure defect, prominence of external genitalia, hypogonadism (severe), delayed hair growth (moderate), decreased life expectancy (severe), decreased adipose (severe)	Brown et al. 2003
Inhbb$^{-/-}$; *Fst*$^{-/-}$	Rescue of *Inhbb*-mediated ectopic coelomic vessel formation in developing female gonads	Yao et al. 2006
Inhbb$^{-/-}$; *Wnt4*$^{-/-}$	Rescue of *Inhbb*-mediated ectopic coelomic vessel formation in developing female gonads	Yao et al. 2006
Inhbc$^{-/-}$; *Inhbe*$^{-/-}$	No effects on development, liver cytoarchitecture, function, or regenerative capacity	Lau et al. 2000
Acvr2$^{+/-}$; *Acvr2b*$^{+/-}$	Abnormal development of stomach, spleen, and endocrine pancreas	Kim et al. 2000
Acvr2$^{-/-}$; *Acvr2b*$^{+/-}$	Embryos fail to form an elongated primitive streak, causing disruption of the mesoderm formation	Song et al. 1999
Acvr2$^{+/-}$; *Acvr2b*$^{-/-}$	Late gestational or early neonatal lethality; modest effect on rostral development	Song et al. 1999
Acvr2$^{-/-}$; *Acvr2b*$^{-/-}$	Arrested at the egg cylinder stage and do not form mesoderm	Song et al. 1999
Inha$^{-/-}$; *Acvr2*$^{-/-}$	Gonadal tumor development but rescue of *Inha*$^{-/-}$ cachexia and hepatocellular necrosis	Coerver et al. 1996
Inha$^{-/-}$; *Fsh*$^{-/-}$	Increased survival and milder tumor/cachexia phenotypes relative to *Inha*$^{-/-}$ mice	Kumar et al. 1999
Inha$^{-/-}$; *Ar*$^{-/-}$	Inhibin/androgen receptor double mutants have increased survival and milder tumor/cachexia phenotypes relative to *Inha*$^{-/-}$ mice	Shou et al. 1997
Inha$^{-/-}$; *Esr1*$^{-/-}$; *Esr2*$^{-/-}$	Protection from early gonadal tumorigenesis in males only. Single estrogen receptor (*Esr*) knockouts are not sufficient to confer a protective effect	Burns et al. 2003a
Inha$^{-/-}$; *Lhb*$^{-/-}$	Increased survival and milder tumor/cachexia phenotypes relative to *Inha*$^{-/-}$ mice	Nagaraja et al. 2008
Inha$^{-/-}$; *Inhbc*$^{TG-CMV}$	Mitigates gonadal tumor progression and prevents cachexia	Gold et al. 2013

Continued

Table 2. *Continued*

Genotypes	Phenotypes	References
Inha$^{-/-}$; *hpg*/*hpg*	Disrupted ovarian folliculogenesis with arrest at the primary antral stage; male and female hypogonadism and sterility; suppression of gonadal/adrenal tumors and cachexia; immature seminiferous tubule-like structures in testes and ovaries	Kumar et al. 1996
Inha$^{-/-}$; *Gdf9*$^{-/-}$	Follicles progress to the multilaminar stage with nonsteroidogenic theca layer	Wu et al. 2004; Myers et al. 2013

Genotypes	Site of inactivation	Phenotypes	References
Conditional knockouts			
Amhr2-Cre; *Inhba*$^{flox/-}$	Ovarian granulosa cells	Disruption results in 35% reduction in female fertility	Pangas et al. 2007
Amhr2-Cre; *Inhba* $^{flox/-}$	Fetal Leydig cells	Failure of fetal testis cord elongation and expansion, decreased Sertoli cell proliferation, smaller adult testes, regional testicular dysgenesis, and reduced sperm production	Archambeault and Yao 2010
Amhr2-Cre; *Inhba* $^{flox/-}$; *Inhbb*$^{-/-}$	Ovarian granulosa cells	Female infertility; increased functional corpora lutea	Pangas et al. 2007
Krt14-Cre; *Acvr1b*flox/flox	Skin epithelium	Variable hairlessness as a result of defects in hair cycling and hair-follicle development	Qiu et al. 2011

Genes	Expression	Promoters	Phenotypes	References
			Gain-of-function	
Activins and inhibins				
Inhba	Testis	Metallothionein	Testicular degeneration	Tanimoto et al. 1999
Inhba	Epidermis	Keratin 14	Small body size, small ears and short tail, severe epidermal thickening, loss of subcutaneous adipose	Munz et al. 1999b
Inhba	Epidermis	Keratin 14	Enhanced skin tumorigenesis and malignancy, differential effects on T-cell proliferation	Antsiferova et al. 2011
Inhba	Forebrain	αCaMKII	Reduced anxiety-related behavior	Ageta et al. 2008
Inhba	Forebrain	Tet-off inducible	Effects on long-term potentiation and maintenance of long-term memory	Ageta et al. 2010
Inhbc	Widespread	CMV promoter	Male infertility, large inflamed liver, prostatic hypertrophy with epithelial hyperplasia	Gold et al. 2009
Inhbc	Liver	Plasmid transfer	Inhibits regenerative DNA synthesis in mouse liver after volume overload injury	Chabicovsky et al. 2003
Inhbc	Liver	Adenovirus transfer	Accelerates liver regeneration after partial hepatectomy in rats	Wada et al. 2005
Inhbe	Widespread	CMV-β actin	Reduced pancreatic weight, adipose replacement of pancreatic acini with islets preserved	Hashimoto et al. 2006
Inhbe	Liver	Plasmid transfer	Inhibits regenerative DNA synthesis in mouse liver after volume overload injury	Chabicovsky et al. 2003

Continued

Table 2. *Continued*

Genes	Expression	Promoters	Phenotypes	References
Inha	Widespread	Metallothionein	Female subfertility because of reduced FSH levels; ovarian cysts, abnormal steroidogenesis, reduced testicular size	Cho et al. 2001; McMullen et al. 2001
Inha	Several tissues including gonads	Metallothionein	FSH reduced, LH increased; females 52% reduction in litter size; reduced ovulation; 50% reduction in sperm count but normal fertility	Cho et al. 2001
Inha	Liver	Inducible	Reduced testis size and block in ovarian folliculogenesis at early antral stage; reduced FSH levels; rescue of the $Inha^{-/-}$ phenotype	Pierson et al. 2000
Activin/inhibin receptors, binding, and regulatory proteins				
Acvr1b	Epidermis	Keratin 14	Delayed wound re-epithelialization	Bamberger et al. 2005
Fst	Liver, testis	Metallothionein	Leydig cell hyperplasia, infertility in males and females, thin uteri, small testes and ovaries, disheveled fur	Guo et al. 1998
Fst288	Normal *Fst* expression pattern	Targeted insertion	*Fst* knockin allele is sufficient for survival but results in female subfertility and premature ovarian follicle depletion	Kimura et al. 2010
Fst288	Normal *Fst* expression pattern	*Fst* regulatory sequences	*Fst288* expression does not rescue the neonatal lethality of $Fst^{-/-}$ mice	Lin et al. 2008
Fst315	Normal *Fst* expression pattern	*Fst* regulatory sequences	Rescue of $Fst^{-/-}$ phenotype; female infertility, cystic ovaries, aberrant corpora lutea, uterine hypoplasia/inflammation, and distal vascular abnormalities	Lin et al. 2008

hepatectomy, suggesting a possible role in the inhibition of hepatocyte proliferation (Esquela et al. 1997; Zhang et al. 1997; Gold et al. 2005; Takamura et al. 2005); however, adenoviral expression of activin C in cultured hepatocytes also enhances proliferation (Wada et al. 2005), whereas transient, low-level expression of either activin C or activin E in mouse liver inhibits regenerative DNA synthesis (Chabicovsky et al. 2003). Moreover, activin C can either increase DNA synthesis or induce apoptosis in vitro depending on the cell type. Neither activin βC nor βE, alone or in combination, however, is required for fertility or development, growth, regeneration, and function of the liver in vivo (Lau 2000). Thus, the cellular context (normal cells versus transformed cells, or tissue of origin), differences in the relative proportions of bioavailable activins, and differences in experimental conditions are all factors that may contribute to the disparate outcomes. Studies in prostate tumor, pituitary cell lines, and transgenic mice suggest that activin C antagonizes activin A signaling (Gold et al. 2009).

Activin Receptors

There are overlapping and unique functions for the two type II activin receptors, ActRII, and ActRIIB. This is perhaps best illustrated during embryonic development, as inactivating either receptor results in a different phenotype. $Acvr2^{-/-}$ mice have small mandibles and cleft palates, reminiscent of the Pierre Robin sequence in humans (Matzuk et al. 1995b). Adult males have reduced fertility and females are in-

fertile. In contrast, *Acvr2b*$^{-/-}$ mice display defects in left–right and anteroposterior axis determination (Oh and Li 1997).

Smad2 and Smad3

Smad2 and Smad3, which transduce signals from several TGF-β family members, including activins, have overlapping and unique functions. *Smad2* null mice display early embryonic lethality as a result of a dosage-sensitive spectrum of defects, which include failure of normal egg cylinder and germ layer formation, with or without mesoderm and severe gastrulation defects. Some heterozygotes have abnormalities in ocular and mandibular development (Nomura and Li 1998; Waldrip et al. 1998). In contrast, *Smad3* null mice are viable and fertile, but have metabolic disturbances and ultimately develop colorectal cancer (Zhu et al. 1998; Yadav et al. 2011). Additional features include accelerated cutaneous wound healing with faster re-epithelialization and reduced local inflammation (Ashcroft et al. 1999) and defects in T-cell mediated and mucosal immunity (Yang et al. 1999). The difference in phenotypic severity between Smad2 and Smad3 null mutants is likely a result, in part, of differences in spatiotemporal expression during development; however, functional differences in tissues in which the two proteins are coexpressed have also been observed. Smad2 and Smad3 play distinct roles in the testis, because activin-induced nuclear Smad accumulation is greatly influenced by the developmental stage of Sertoli cells as well as the activin concentration, reflected by different transcriptional outcomes (Itman et al. 2009). Thus, specific control mechanisms are likely to be in place to direct the use of phosphorylated Smad2 and Smad3 in this context.

Inhibins

Mice with targeted disruption of the inhibin α subunit expression (*Inha*$^{-/-}$), lack of inhibins A and B with a consequential increase in activin signaling. These mice do not have birth defects, but instead develop gonadal and adrenal tumors, a cancer-cachexia phenotype, loss of acid-producing parietal cells in the stomach, and hepatocellular necrosis.

Activins and Development in Other Model Organisms

Morphogen Gradients and Signaling from a Distance in Xenopus laevis and Drosophila melanogaster

Mechanisms that establish morphogen gradients of TGF-β family signaling and their roles in patterning during early embryonic development have been well studied (Green and Smith 1990; Kessler and Melton 1995; Lecuit et al. 1996; Nellen et al. 1996; Gurdon and Bourillot 2001). An important paradigm is that cells respond differently depending on the ligand concentration to which their receptors are exposed, thereby initiating different transcriptional cascades and developmental outcomes. In dissociated *Xenopus* animal cap cells, activin binds to ActRII and ActRIIB, and has downstream effects that are concentration-dependent. The switch in gene expression profile occurs at 2%–6% receptor occupancy (Dyson and Gurdon 1998). This effect depends on the absolute number of receptors that are occupied by ligand per cell, and not by the ratio of occupied to unoccupied receptors (Dyson and Gurdon 1998). These studies provided insight into the mechanisms by which activin morphogen gradients might contribute to differential gene expression and, consequently, developmental patterning.

It is less clear whether activin establishes a gradient by diffusion from its source, thus decreasing in concentration more than several cell diameters, or whether a relay mechanism occurs, whereby cells sequentially send distinct signals to adjacent cells.

In support of the diffusion model, activin can elicit a cellular response at least 10 cell diameters away in experiments using *Xenopus* embryo reconstituted tissue explants (Gurdon et al. 1994). This effect persisted even when protein synthesis was inhibited in the intermediate endothelial cells that were incapable of activin responsiveness, arguing against a cell-to-cell re-

lay mechanism (Gurdon et al. 1994). Also in support of the diffusion model, fluorescently labeled activin travels through the extracellular spaces of reconstituted *Xenopus* animal cap cells. The range of signaling activity from the activin source is inversely proportionate to the number of activin receptors on the cell surface, and activin signal transduction in these cells does not require endocytosis of activin-receptor complexes (Hagemann et al. 2009).

A simple diffusion model is perhaps insufficient, however, to explain how morphogens might overcome deterrents, such as the extracellular matrix and functionally antagonistic proteins to travel several cell diameters. Experiments in intact *Xenopus* blastula cells, as opposed to dissociated and reconstituted cells, support a relay model in which adjacent cells but not more distant ones are capable of ligand-induced signal transduction, a process that includes production of secondary, distinct signals (Reilly and Melton 1996).

In the *Drosophila* wing disc, cells that are incapable of mediating endocytosis and, therefore, unable to transduce ligand-induced signaling are also incapable of establishing a Decapentaplegic (Dpp) gradient (Lecuit et al. 1996). Other experiments examining Dpp functions indicate that simple diffusion and at least one other (possible relay) mechanism may contribute to establishing morphogen gradients (Lecuit et al. 1996). An extension of the relay model posits that morphogen gradients are established with the aid of cytonemes. Cytonemes are specialized signaling filopodia that extend outward from the cytoplasmic membrane of recipient cells to direct cell-to-cell transfer of secreted ligands. Although cytoneme-mediated ligand transfer has been clearly shown for Dpp in the *Drosophila* wing disc (Roy et al. 2014), to our knowledge this phenomenon has not been documented for activins or other TGF-β family ligands in *Drosophila* or in vertebrates.

Activin Signaling in Drosophila Melanogaster

A variety of developmental processes, which include cell proliferation and growth, neuronal remodeling, axon guidance, and dorsal neuron morphogenesis, occur through activin-like signaling pathways in *Drosophila* (Zheng et al. 2003, 2006; Parker et al. 2006; Serpe and O'Connor 2006). Activin-β and Dawdle (Daw) are two activin/TGF-β-like ligands (Parker et al. 2004; Serpe and O'Connor 2006). In contrast to vertebrates, both BMP and activin/TGF-β pathways use common type II receptors, Punt and Wishful Thinking (Wit), whereas the activin pathway specificity is conferred by the type I receptor, Baboon (Babo), which signals through Smad2 (Smox [Smad on X]) (Das et al. 1999; Zheng et al. 2003; Serpe and O'Connor 2006). The diversity of type I/type II receptor combinations in *Drosophila* is considerably less than vertebrates, providing an important tool to dissect the contributions of activin/TGF-β- and BMP-signaling pathways.

PHYSIOLOGICAL FUNCTIONS OF ACTIVINS AND INHIBINS

Reproduction

Activins and inhibins were originally characterized as activators or inhibitors, respectively, of pituitary FSH production and release. The gonads are the major sources of circulating activins and inhibins that provide regulatory feedback to the pituitary, and function as autocrine and paracrine signals that control gonadal function. Conversely, activin and inhibin expression are regulated by endocrine signals that originate in the pituitary. Accordingly, a discussion of the reproductive roles of activins and inhibins requires an understanding of their relationship to the hypothalamic–pituitary–gonadal (HPG) axis (Fig. 5).

The HPG Axis

The anterior pituitary shows cellular heterogeneity, producing a variety of hormones, including FSH and LH. LH and FSH are heterodimeric proteins with the same α subunit but different β subunits, produced by pituitary gonadotropes. The expression and release of LH and FSH are primarily regulated by GnRH that is produced by the hypothalamus, which receives

input from gonadal signals that include inhibins, estrogen, progesterone, and testosterone (Fig. 5A). GnRH is released into a dense capillary plexus, then passes to the adjacent anterior pituitary gland to control the release of LH and FSH, influenced by the pulse frequency of GnRH release (Kaiser et al. 1995; Sealfon et al. 1997; Shacham et al. 2001; Burger et al. 2002). Pituitary activin B expression is also controlled by the GnRH pulse frequency (Burger et al. 2002). GnRH expression in the hypothalamus and GnRH receptors on pituitary gonadotropes is enhanced by activins, and blocked by follistatin (Fig. 5A) (Fernandez-Vazquez et al. 1996; Norwitz et al. 2002).

LH and FSH β subunit expression is regulated by activins and inhibins. Activin B from the anterior pituitary has paracrine effects on gonadotropes, enhancing GnRH-induced FSH expression and release (Fig. 5A) (Corrigan et al. 1991). Similarly, activin A augments GnRH-induced LH production and is antagonized by testosterone (Burger et al. 2003; Yamada et al. 2004; Coss et al. 2005). Inhibin B produced by the gonads plays an important role in the feedback mechanisms that regulate the HPG axis (Fig. 5B,C). Inhibin B and follistatin antagonize many of the aforementioned functions of the activins (Wang et al. 1988; Kaiser et al. 1992; Winters et al. 1996; Burger et al. 2002).

Activins and Inhibins in Female Reproduction

Inhibin B, the major circulating form of inhibin in several animal species, originates from the ovary, as confirmed by markedly reduced plasma levels after gonadectomy (Robertson et al. 1988; Woodruff et al. 1996). It regulates the HPG axis primarily through suppressive effects on activin-mediated FSH expression and release, but also through direct effects in the ovary, impacting ovarian folliculogenesis, steroidogenesis, and the menstrual cycle (Figs. 5 and 6) (Hsueh et al. 1987; McLachlan et al. 1987; Woodruff et al. 1988, 1990; Lenton et al. 1991; Groome et al. 1996).

Activins and inhibins play important autocrine and paracrine roles during several steps of ovarian folliculogenesis (Fig. 6). With each ovarian cycle, follicles are recruited from a primordial pool, and subsequently progress from a single oocyte surrounded by a single layer of epithelial cells to a much larger oocyte surrounded by several layers of granulosa, theca, and stromal cells. This heterogeneous, multicellular structure provides a supportive hormonal environment for folliculogenesis and the early stages of pregnancy.

Activin A contributes to the breakdown of germ cell nests, a process that increases the primordial follicle pool, thereby increasing fertility potential (Fig. 6A) (Bristol-Gould et al. 2006). One or both of the circulating forms of follistatin antagonize this effect (Kimura et al. 2011). Activin and other proteins within and outside the TGF-β family help to drive the early stages of follicular growth, before the contribution of FSH signaling at the early pre-antral stage (Fig. 6B,C) (Trombly et al. 2009). Expression of FSH receptors in granulosa cells of multilayer follicles is essential for normal folliculogenesis. Inhibin antagonizes expression of FSH receptors and has several important functions during the late stages of folliculogenesis. The antral follicle has an abundance of steroidogenic cells that work together to produce and release estrogen. The antral granulosa cells also produce inhibins, with inhibin B the major circulating form. Estrogen production is stimulated by FSH, and pituitary expression of FSH is ultimately down-regulated by circulating inhibin B from ovarian follicles in a negative feedback loop. Inhibin production by granulosa cells is also stimulated by FSH (Hillier et al. 1991a). Inhibin augments FSH-induced estrogen production in granulosa cells and LH-induced androgen production in theca cells (Hillier et al. 1991b; Wrathall and Knight 1995). Androgens are converted to estrogen by granulosa cell aromatase. Inhibin also slows the maturation of oocytes at the antral follicle stage (Fig. 6D) (Silva et al. 1999). Although variable numbers of primordial follicles are recruited for folliculogenesis during each ovarian cycle, only a subset of follicles ultimately proceeds to ovulation. The process of "follicular dominance" is supported in part by activin and antagonized by inhibin (Fig. 6E) (Hillier and Miro 1993). After ovulation, the

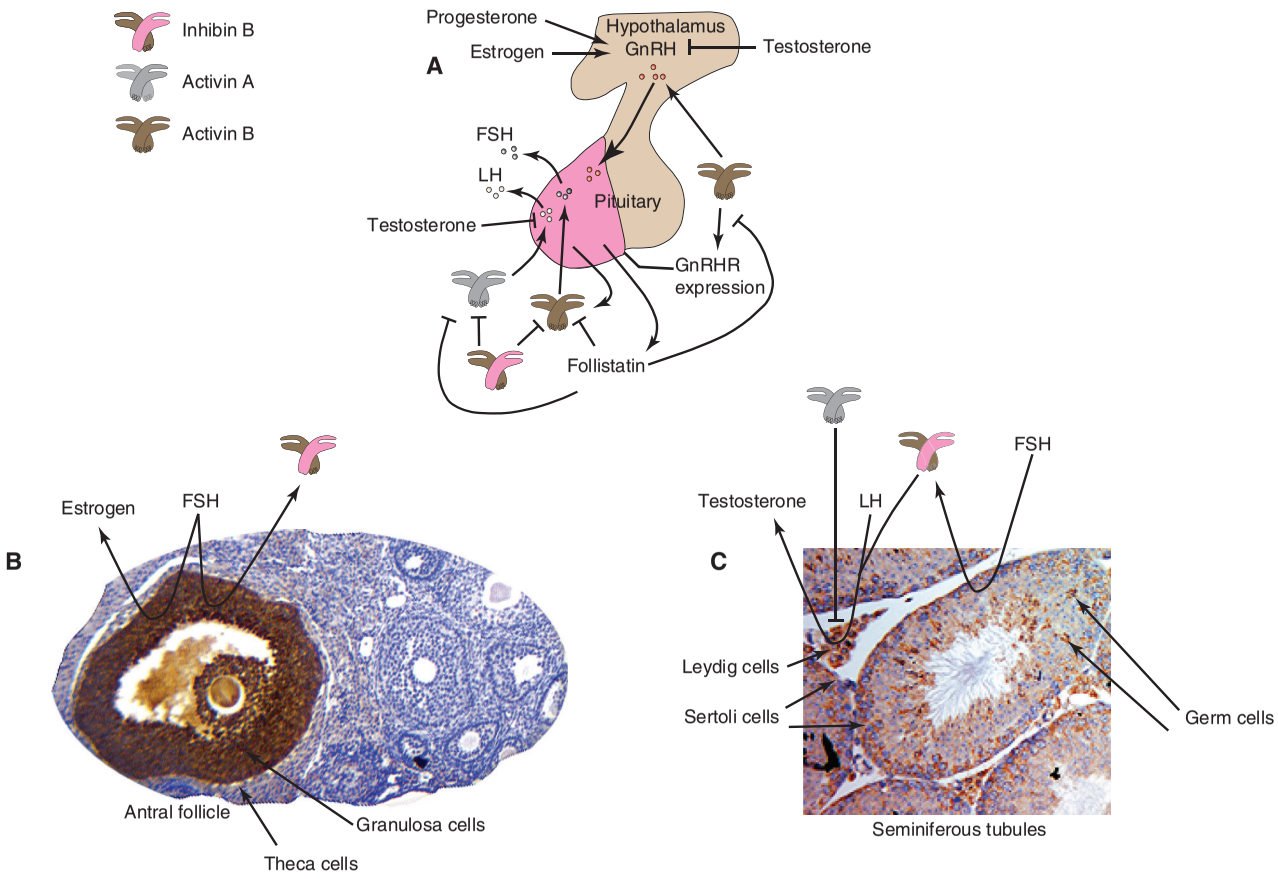

Figure 5. Activins and inhibins in the hypothalamic–pituitary–gonadal (HPG) axis. (*A*) The pituitary gland is divided into anterior (magenta) and posterior (tan) lobes. The anterior pituitary gonadotropes produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The production and release of LH and FSH are primarily regulated by gonadotropin-releasing hormone (GnRH), produced by the hypothalamus, which receives input from gonad-derived signals, inhibins, estrogen, progesterone, and testosterone. The production of GnRH in the hypothalamus and expression of GnRH receptors (GnRHRs) on pituitary gonadotropes are enhanced by activins, and pituitary activin B expression is also controlled by GnRH. (*Legend continued on following page.*)

remaining follicular cells coalesce to form the corpus luteum, whose major function is LH-induced progesterone production to support the early stages of pregnancy. Inhibin is also produced by the corpus luteum at high levels throughout the luteal phase of the ovarian cycle (Fig. 6F) (Roberts et al. 1993).

During the ovarian cycle, granulosa cells differentially express activin and inhibin monomers (Fig. 6G). Activin β subunits are expressed at very low levels in early-stage follicles. Activin βA is most abundant in late antral follicles and in corpora lutea, whereas expression of activin βB is restricted to small antral follicles. The inhibin α subunit is expressed throughout the ovarian cycle, increasing in mature follicles and corpora lutea. All type I and type II activin receptors, betaglycan, and follistatin are expressed in all cell types and at all stages of folliculogenesis (Roberts et al. 1993; Welt and Schneyer 2001; Drummond et al. 2002; Knight and Glister 2006). However, circulating levels of inhibins do not correspond to the levels of expression in granulosa cells. Quickly rising levels of inhibin A can be detected during ovulation and peak levels in the midluteal phase. In contrast, inhibin B shows a biphasic pattern with peak levels at the early follicular and early luteal phases (Fig. 6G) (Groome et al. 1996; Woodruff et al. 1996; Welt 2004).

Important functional roles for activins and inhibins during pregnancy are strongly suspected because circulating activin levels are very low or undetectable under normal physiological conditions except during pregnancy when inhibin, activin, and follistatin levels, likely all of fetal and placental origin, progressively rise and markedly increase during the third trimester (Fig. 6H) (Muttukrishna et al. 1995; O'Connor et al. 1999). Follistatin levels are higher than activin levels throughout pregnancy, whereas basal levels of inhibin are high and decrease slightly before a rapid increase between 25 and 30 weeks gestation. Although the biological roles during pregnancy are unclear, abnormally low maternal inhibin levels are associated with pregnancy loss and other complications (Muttukrishna 2004), whereas abnormally increased activin levels in late pregnancy are associated with pre-eclampsia, preterm labor, and gestational diabetes (Petraglia et al. 1995a,b; Gallinelli et al. 1996).

In cultured granulosa cells from early-stage follicles, FSH stimulates the release of inhibin and estrogen (Fig. 7A) (Hillier et al. 1991a). Inhibin augments FSH-induced estrogen production while inhibiting the expression of FSH receptor (Campbell and Baird 2001; Lu et al. 2009). In contrast, activin A enhances the expression of estrogen receptors and in-

Figure 5. (*Continued*) The LH and FSH β subunits are primary targets for regulation by activins and inhibins. Activin B (brown-colored icon) from the anterior pituitary exerts paracrine effects on gonadotropes, enhancing GnRH-induced FSH production and release. Similarly, activin A (gray-colored icon) augments GnRH-induced LH production, antagonized by testosterone. Activin also enhances the expression of GnRH receptors on gonadotropes, an effect that is blocked by follistatin. Inhibin B (brown-pink icon) produced by the gonads plays an important role in feedback mechanisms that regulate the HPG axis, as inhibin B and follistatin antagonize many functions of activins. (*B*) The antral follicle contains steroidogenic cells that collaborate to produce and release estrogen. The granulosa cells produce estrogen and inhibins, driven by pituitary FSH, with inhibin B the major circulating form. FSH expression is ultimately repressed by circulating inhibin B from ovarian follicles in a negative feedback loop. Activin βA immunostaining (brown) shows abundant expression restricted to the granulosa cells of a single, large antral follicle, but not in follicles at earlier stages (CW Brown, unpubl.). (*C*) The seminiferous tubules are comprised of germ cells, Sertoli cells, Leydig cells, and other cells. All activin and inhibin subunits, activin receptors, betaglycan and follistatin are expressed in the testis, and their expression is controlled by the stage of the seminiferous cycle, age and pubertal stage, and cell type. Activin βA immunostaining (brown) in this adult testis section is apparent in Leydig cells, Sertoli cells and germ cells at more than one stage of development (CW Brown, unpubl.). Inhibin B (brown-pink icon) is the only inhibin produced by the testis and the major circulating form. In Sertoli cells, FSH stimulates inhibin B production, providing negative feedback for pituitary FSH production, whereas LH induces the production of androgens from rodent Leydig cells, a process that is augmented by inhibin and attenuated by activin (gray icon).

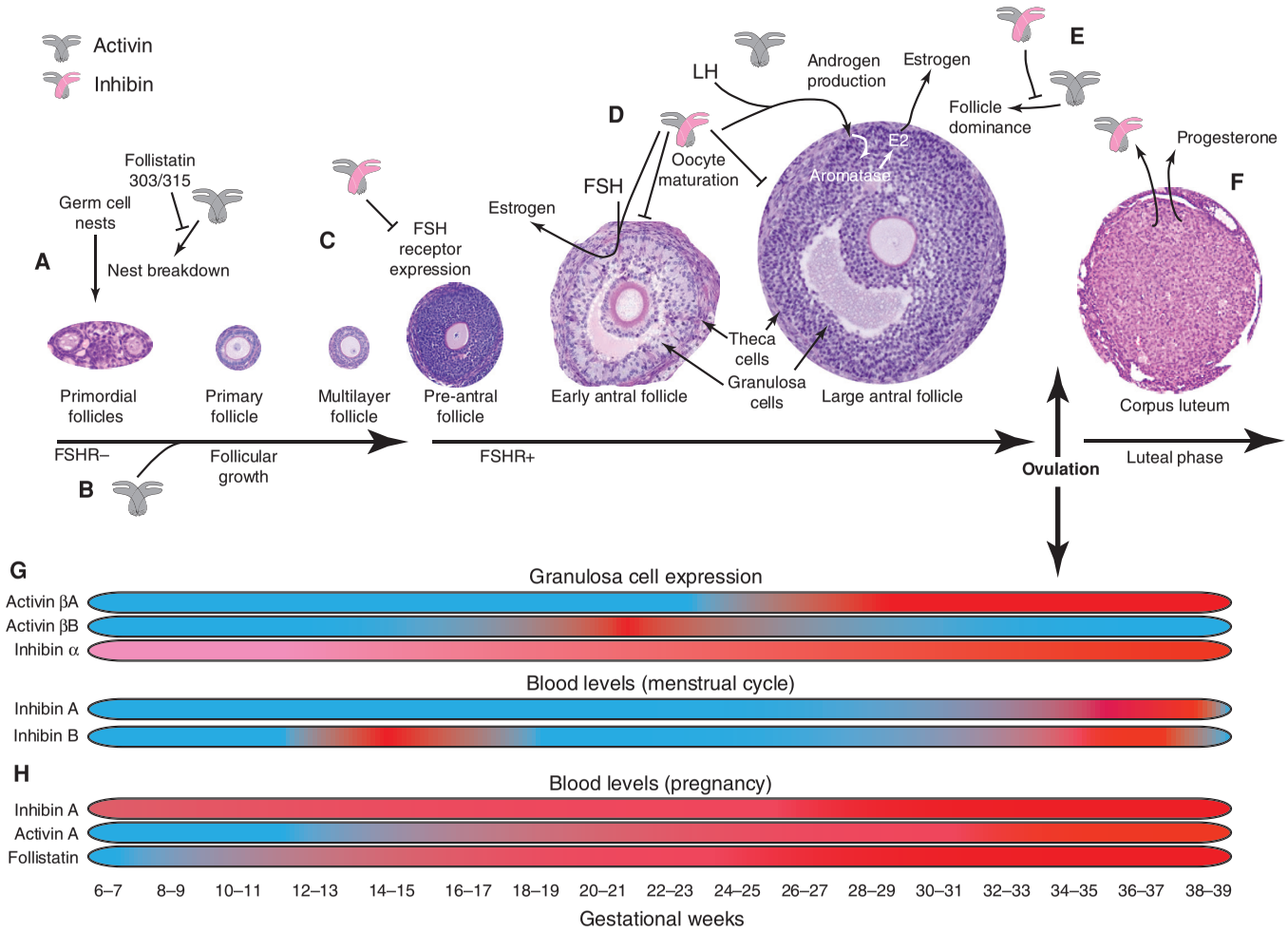

Figure 6. Activins and inhibins in ovarian folliculogenesis and pregnancy. Activins and inhibins play important autocrine/paracrine roles at several steps of ovarian folliculogenesis. (*A*) Activin A (gray icon) contributes to the breakdown of germ cell nests, which increases the primordial follicle pool and fertility potential, antagonized by follistatin. (*Legend continued on following page.*)

creases DNA replication, an effect that is augmented by FSH (Rabinovici et al. 1990; Miro and Hillier 1996; Kipp et al. 2007). In cultured theca cells, activin suppresses LH-induced androgen production, whereas inhibin augments androgen production and antagonizes activin's suppressive effect (Fig. 7B) (Hillier et al. 1991b). In cultured luteal cells, activin suppresses LH-induced progesterone synthesis, whereas inhibin antagonizes activin's effects (Fig. 7C) (Rabinovici et al. 1990; Di Simone et al. 1994).

Genetic approaches to understand the roles of inhibins in reproduction have been confounded by the gonadal tumors that develop in complete inhibin-deficient mice (Matzuk et al. 1992). Nevertheless, some insight has been gained from other inhibin mouse models. Transgenic mice that widely overexpress inhibin α show subfertility with a 52% decrease in litter size, and have reduced LH and FSH levels, ovarian cysts, abnormal steroidogenesis, and a reduced number of ovulated oocytes (Cho et al. 2001; McMullen et al. 2001). Mifepristone-induced expression of inhibin α in the liver of adult mice blocks ovarian folliculogenesis at the early antral stage (Pierson et al. 2000).

GDF-9 is a TGF-β family member whose expression is restricted to oocytes. Ovaries from *Gdf9*$^{-/-}$ mice display an arrest in folliculogenesis at the primary follicle stage, with normal oocyte growth and zona pellucida development, but no progress in the growth of the surrounding follicular cells beyond the single-layer stage (Dong et al. 1996). In contrast, *Gdf9*$^{-/-}$; *Inha*$^{-/-}$ mice have ovarian follicles that progress to the multilaminar stage before the appearance of inhibin-related sex cord stromal tumors (Matzuk et al. 1992), but the theca layer is incapable of steroid production (Wu et al. 2004; Myers et al. 2013). Therefore, inhibins are negative regulators of early follicular development and might contribute to the function of theca cells.

In contrast to the normal fertility of *Inhbb*$^{-/-}$ mice (Vassalli et al. 1994), conditional inactivation of *Inhba* in developing ovarian granulosa cells reduces female fertility by ~38% (Pangas et al. 2007). Conditional loss of one *Inhba* allele in an *Inhbb*$^{-/-}$ genetic background reduces fertility by 90%, and complete loss of activins A and B in granulosa cells causes infertility (Pangas et al. 2007). These findings suggest overlapping, dosage-sensitive functions for activins A and B during ovarian folliculogenesis, with activin A playing a predominant role. Accordingly, female fertility is severely impaired in *Inhba*^{BK} mice, in which *Inhba* is replaced with a functionally hypomorphic activin βB al-

Figure 6. (*Continued*) (*B*) Activin contributes to early stages of follicular growth. (*C*) Expression of the follicle-stimulating hormone (FSH) receptor in granulosa cells of multilayer follicles is required for folliculogenesis. Inhibin (gray-pink icon) antagonizes FSH receptor expression in granulosa cells. (*D*) Inhibin enhances FSH-induced estrogen production in granulosa cells, and luteinizing hormone (LH)-induced androgen production in theca cells, whereas androgen production is antagonized by activin (gray icon). Androgens are converted to estrogen (E2) by aromatase in granulosa cells. Inhibin also slows the maturation of oocytes at the antral follicle stage. (*E*) Although variable numbers of primordial follicles are recruited for folliculogenesis during each ovarian cycle, only a few will proceed to ovulation. The process of "follicular dominance" is supported by activin and antagonized by inhibin. (*F*) After ovulation, the remaining follicular cells coalesce to form the corpus luteum, whose major function is LH-induced progesterone production to support the early stages of pregnancy. Inhibin is also produced by the corpus luteum at high levels throughout the luteal phase of the ovarian cycle. (*G*) Granulosa cell expression and blood levels of activin and inhibin in the context of the ovarian cycle are shown, with low levels in blue and high levels in red. Activin β subunits are expressed at very low levels in early-stage follicles. Activin βA is most abundantly expressed in late antral follicles and in corpora lutea, whereas activin βB is restricted to small antral follicles. The inhibin α subunit is expressed throughout the ovarian cycle, increasing in mature follicles and corpora lutea. However, circulating levels of inhibins do not correlate with the levels of expression in granulosa cells. Inhibin A levels increase rapidly during ovulation and peak in the midluteal phase. In contrast, inhibin B has a biphasic pattern with peak levels at the early follicular and early luteal phases. (*H*) During pregnancy, inhibin, activin, and follistatin levels progressively increase, and markedly increase during the third trimester. Follistatin levels are greater than activin levels throughout pregnancy, whereas basal inhibin levels are higher and decrease slightly before the rapid increase between 25 and 30 weeks gestation.

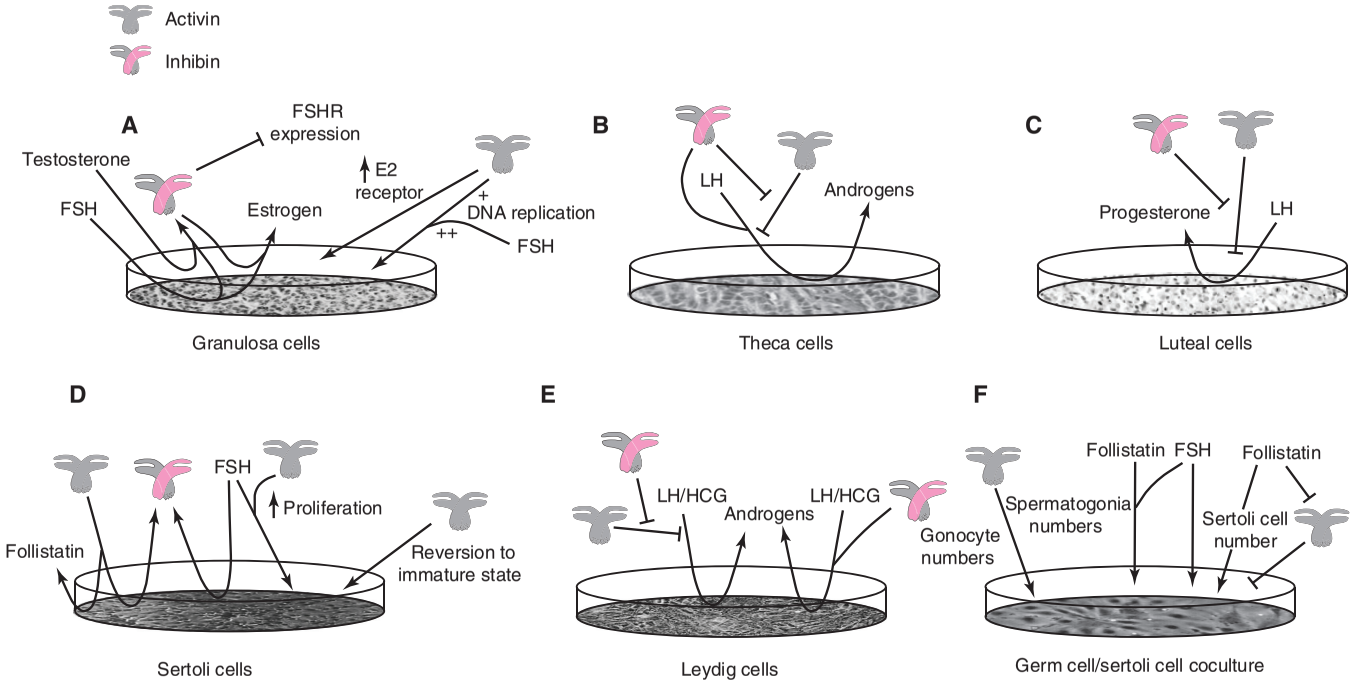

Figure 7. Activin and inhibin effects in cultured gonadal cells. (*A*) In cultured granulosa cells from early-stage follicles, follicle-stimulating hormone (FSH) stimulates the release of inhibin (gray-pink icon) and estrogen (Hillier et al. 1991a). Inhibin augments FSH-induced estrogen production, while inhibiting the expression of FSH receptor (Campbell and Baird 2001; Lu et al. 2009). In contrast, activin A (gray icon) enhances the expression of estrogen receptors and increases DNA replication, an effect that is augmented by FSH (Rabinovici et al. 1990; Miro and Hillier 1996; Kipp et al. 2007). (*B*) In cultured theca cells, activin suppresses luteinizing horm (LH)-induced androgen production, while inhibin augments androgen production and antagonizes activin's suppressive effect (Hillier et al. 1991b). (*C*) In cultured luteal cells, activin suppresses LH-induced progesterone synthesis, whereas inhibin antagonizes activin's effects (Rabinovici et al. 1990; Di Simone et al. 1994). (*D*) Cultured Sertoli cells from young, postnatal rats proliferate in response to FSH and activin A, and FSH increases expression of inhibin. Activin stimulates follistatin and inhibin production and causes adult-derived Sertoli cells to revert to a less differentiated state. (*E*) LH or human chorionic gonadotropin (HCG) induces androgen production in rodent Leydig cells. Inhibin increases and activin antagonizes LH-induced androgen production and inhibin blocks activin's suppressive effect. The activin and inhibin effects on androgen production require LH and HCG. (*F*) When treated with activins, cocultures of germ cells and Sertoli cells from early postnatal rat testis show increased numbers of spermatogonia and gonocyte precursors. The combination of FSH and follistatin also increases the number of spermatogonia. Activin treated co-cultures have fewer Sertoli cells than controls, an effect that is suppressed by follistatin. Follistatin also independently increases the number of Sertoli cells.

lele (Brown et al. 2000). The ovaries of *Inhba*^{BK} mice are smaller and contain fewer large preantral follicles than controls. Serum estrogen and progesterone levels are reduced. Thus, activin B can partially replace activin A as an ovarian growth and maintenance factor, but is insufficient to fully maintain all aspects of ovarian folliculogenesis.

Transgenic mice that overexpress follistatin have thin uteri, small ovaries with defective folliculogenesis, and subfertility, dependent on the level of transgene expression (Guo et al. 1998), and show many similarities to mice with reduced activin signaling. Female transgenic mice that only express *Fst315* are infertile as a result of abnormal corpus luteum formation, cystic ovaries and hypoplastic, inflamed uteri. In contrast to transgenic mice that overexpress the *Fst288* isoform on a Fst$^{-/-}$ background and die as newborns, mice with a *Fst288* insertion allele are viable, but homozygous females are subfertile with excess primordial follicles as a result of a greater number of germ cells and reduced rate of apoptosis. These mice subsequently develop ovarian failure as a result of premature follicle depletion (Kimura et al. 2010, 2011).

Transgenic mice that overexpress human *FSTL3* from an inhibin α promoter show high expression in the gonads. The mice have lower body mass up to 2 months of age. Females have smaller ovaries with fewer large pre-ovulatory follicles, occasional trapped oocytes in corpora lutea, and a 35% reduction in litter size. These features are similar to those of the transgenic mice expressing follistatin and mice that are activin-deficient in granulosa cells, and suggest possible overlapping functions for follistatin and FSTL-3 in reproduction. Taken together, the findings in the *FSTL3* transgenic mice are consistent with important roles for activins in gonadal development and gametogenesis (Xia et al. 2004).

The control of mRNA expression of BAMBI in adult rat gonads suggests potential roles in reproduction with high expression in granulosa and theca cells from adult ovaries. However, *Bambi*$^{-/-}$ mice are viable, fertile and without developmental or reproductive defects. Therefore, BAMBI is not essential for normal female reproduction (Chen et al. 2007).

Activins and Inhibins in Male Reproduction

The seminiferous tubules are comprised of germ cells, Sertoli cells, Leydig cells, and other cell types. Sertoli cells provide a supportive environment for spermatogenesis, and Leydig cells synthesize androgens. Collectively, all activin and inhibin subunits, activin receptors, betaglycan, and follistatin are produced by testis cells, and their expression patterns are influenced by the stage of the seminiferous cycle in adults and the age and pubertal status of the animal. These expression characteristics create a complex, dynamic autocrine and paracrine regulatory network (MacConell et al. 2002; Buzzard et al. 2004; Riccardi et al. 2007). Inhibin B is the only form of inhibin produced by the testis and is also the major circulating inhibin (Illingworth et al. 1996; Marchetti et al. 2003). FSH stimulates inhibin B expression in Sertoli cells, providing negative feedback for pituitary FSH production (Sharpe et al. 1999; Hayes et al. 2001), whereas LH induces the production of androgens from rodent Leydig cells, a process that is augmented by inhibin and attenuated by activin (Hsueh et al. 1987), although these effects may be species-dependent (Lejeune et al. 1997).

Cultured Sertoli cells from postnatal (P6, P9) rats proliferate in response to FSH or activin A, and the effects on proliferation are additive (Fig. 7D) (Buzzard et al. 2003). FSH also induces the expression of inhibin B and free inhibin α subunit precursor, pro-αC. The latter reduces inhibin B bioactivity and likely plays an important regulatory role (Grootenhuis et al. 1990; Hancock et al. 1992). Activin stimulates follistatin and inhibin A and B production and can induce reversion of adult-derived Sertoli cells to a less differentiated state in vitro (Buzzard et al. 2003; Nicholls et al. 2012).

LH or the closely related human chorionic gonadotropin (HCG), which also binds and activates the LH receptor, induces androgen production from cultured rodent Leydig cells (Fig. 7E). Inhibin increases and activin antagonizes

LH-induced androgen production (Hsueh et al. 1987; Mauduit et al. 1991), and inhibin blocks activin's suppressive effect (Lin et al. 1989). It is unclear whether the antagonism of activin signaling by inhibin is the sole mechanism by which inhibin enhances LH-induced androgen secretion. However, no activin or inhibin effects on androgen production occur in the absence of LH/HCG.

When treated with activin A or B, cocultures of germ and Sertoli cells from early postnatal rat testis fragments give rise to increased numbers of spermatogonia and gonocyte precursors, followed by formation of seminiferous tubule-like structures, suggesting that activins provide important signals that direct the positional organization of cells in the developing testis (Fig. 7F) (Mather et al. 1990). The combination of FSH and follistatin also increases the number of spermatogonia. Conversely, activin-treated cocultures have fewer Sertoli cells than untreated control cultures, an effect that is suppressed by follistatin. Follistatin also independently increases Sertoli cell number. In this coculture system, inhibin is unable to block the activin effect (Meehan et al. 2000).

The process of compartmentalization in the prenatal testis causes formation of the testis cord and the surrounding interstitium. Sertoli cells produce signals to direct testis cord formation. Activin A is produced by mouse fetal Leydig cells and acts on Sertoli cells to promote proliferation during late embryogenesis. Silencing activin A expression specifically in fetal Leydig cells causes failure of fetal testis cord elongation and expansion because of decreased Sertoli cell proliferation, a finding reproduced by the conditional silencing of Smad4 expression in fetal Sertoli cells. In mice with absence of activin βA or Smad4 in fetal Leydig or Sertoli cells, respectively, testicular dysgenesis persists to adulthood, with abnormal testis histology and reduced sperm counts. These findings support a model in which activin A produced by Leydig cells during early development acts on Sertoli cells to stimulate proliferation (Archambeault and Yao 2010).

Male *Inhba*^{BK} mice are fertile but their testicular volumes are greatly diminished, and the onset of fertility is later than normal because of developmental effects on Sertoli cell proliferation and delayed germ cell maturation (Brown et al. 2000; Mendis et al. 2010; Mithraprabhu et al. 2010). Adult *Inhba*^{BK} testis histology is normal; however, seminiferous tubule maturation is delayed and the dosage and biopotency of the *Inhba* alleles correlate directly with testicular size. *Inhba*$^{-/-}$ newborn testes are also very small, attributed to the reduced proliferation of Sertoli cells (Mendis et al. 2010). These studies confirm that activin A signaling is required for normal prenatal and postnatal testicular growth, and its function cannot be replaced entirely by activin B.

Transgenic, testis-restricted overexpression of activin βA postnatally results in progressive sterility because of testicular degeneration (Tanimoto et al. 1999). In contrast, transgenic mice that widely overexpress follistatin have reduced testis volume with Leydig cell hyperplasia, arrest of spermatogenesis and seminiferous tubule degeneration leading to infertility (Guo et al. 1998), while adult transgenic male mice that express only *Fst315* are fertile (Kimura et al. 2010, 2011). Mice with transgenic overexpression of inhibin α have a 50% reduction in sperm count but normal fertility (Cho et al. 2001; McMullen et al. 2001). Mice with induced expression of inhibin α in liver also have reduced testis size (Pierson et al. 2000). Thus, transgenic mice overexpressing follistatin or inhibin have findings that are consistent with activin deficiency.

Transgenic male mice that overexpress human *FSTL3* from the inhibin α promoter also have lower testis volumes with ~60% reduction in sperm count, because of irregular seminiferous tubule degeneration with selective loss of germ cells in several tubules, relative preservation of Sertoli cells, Leydig cell hyperplasia and abnormal testosterone production. Collectively, these abnormalities are associated with a 25% reduction in litter size. The findings in *FSTL3* transgenic mice are consistent with important roles for activins in maintaining testicular function and gametogenesis (Xia et al. 2004).

The high level of BAMBI expression in several cell types in adult male rat testes suggests

potential roles for BAMBI in reproduction. BAMBI is expressed in juvenile and adult Sertoli cells, and its expression in germ cells greatly increases as gonocytes mature into spermatogonia shortly after birth. Exogenous activin A decreases the *Bambi* mRNA level in cultures of newborn rat testis fragments. Thus, BAMBI was predicted to contribute to the control of TGF-β family signaling at several stages of gametogenesis (Loveland et al. 2003). However, $Bambi^{-/-}$ males are also viable, fertile, and without identifiable developmental or reproductive defects (Chen et al. 2007).

Erythropoiesis

Activin A was first recognized for its role in erythropoiesis when conditioned media of human leukemic THP-1 cells was seen to induce differentiation of mouse erythroleukemic Friend cells (Eto et al. 1987). Several studies of murine and human bone marrow cells and erythroleukemic cell lines show that activin A robustly induces erythroid differentiation, with increased colonies of erythroid burst forming units (BFU-E), erythroid colony forming units (CFU-E) and an increase in hemoglobin production. These responses to activin require EPO and are sensitive to concentrations of activin and EPO (Yu et al. 1987; Murata et al. 1988; Shiozaki et al. 1992, 1998; Maguer-Satta et al. 2003). In vivo activin A administration also increases BFU-E and CFU-E colonies in a dosage-dependent manner in normal or anemic mice (Shiozaki et al. 1989).

Activin A and activin receptors are expressed in human bone marrow cells (Maguer-Satta et al. 2003; Wu et al. 2012). Activin A induces the expression of EPO receptor, β-globin, $p27^{Kip1}$ and *Bcl-XL* and decreases the expression of *Gata2* (Maguer-Satta et al. 2003). One of the models for the role of activin signaling in erythropoiesis suggests a synergism between activin A and EPO to promote commitment and differentiation of erythroid precursors more than apoptosis. ELM-I-1 mouse leukemic cells show a twofold increase in hemoglobin-positive cells in cultures when treated with activin A and EPO, relative to treatments with either alone (Shiozaki et al. 1998). However, F5-5 mouse leukemia cells with a constitutively active EPO receptor are unaffected by EPO, while treatment with activin A or activin A and EPO increases the number of hemoglobin-positive cells (Shiozaki et al. 1998). Furthermore, ELM-I-1 cells treated with activin A alone were apoptotic when compared with cells treated with EPO and activin A, a phenomenon that was not observed in F5-5 cells (Shiozaki et al. 1998). Together, these results suggest that activin A commits erythroid precursors to the erythroid lineage but requires EPO to suppress apoptosis and possibly to regulate cell-cycle mediators that allow activin-induced differentiation to proceed (Shiozaki et al. 1998).

During erythropoiesis, activin A is regulated by follistatin and inhibin. In vitro and in vivo studies have shown that treatment with either follistatin or inhibin A or cotreatment of follistatin or inhibin A with activin A reduces BFU-E and CFU-E colony formation and hemoglobin accumulation, even in the presence of EPO (Yu et al. 1987; Shiozaki et al. 1992; Maguer-Satta et al. 2003).

The Nervous System

Activins and inhibins are widely expressed in the brain. Activin βA and βB subunits are expressed in neuronal cell bodies in the nucleus of the solitary tract and in the dorsal and ventral medullary reticular nuclei, which control pain sensation and some components of the autonomic nervous system. Activins are also expressed in the nerve fibers and the termini of projection sites for these nuclei. Activin B immunoreactivity is observed in perifornical neurons of the hypothalamus, which include orexin-producing cells, whereas activin A is present in neuronal cell nuclei scattered throughout the central nervous system. Transcripts encoding all inhibin/activin subunits are present in all major brain regions (Roberts et al. 1996). Similarly, type II activin receptors are widely expressed in the rat brain with highest mRNA levels in the hippocampus, amygdala, hypothalamus, and throughout the cortical mantle, including the primary olfactory cortex

(Cameron et al. 1994). Thus, there is potential for wide-ranging activin effects in the brain.

Activin A treatment of embryonic day 18 rat retinal cultures arrests proliferation and induces differentiation of progenitor cells into rod photoreceptors (Davis et al. 2000). The effect is specific to the rod photoreceptor lineage and is dose-dependent. Mice lacking activin A show a decrease in rod photoreceptors (Davis et al. 2000). Blocking activin signaling in *Drosophila* embryos by abrogating activin-β, Daw, Babo, or Smox results in larvae with small brains and aberrant photoreceptor axon targeting (Zhu et al. 2008). The ligands activin β and Daw show functional redundancy in these contexts. Together, these results suggest that activin signaling is required not only for differentiation of rod photoreceptors in the retina, but also for producing the proper number of neurons to enable normal connection of photoreceptor axons to their targets.

Activins are also neuronal survival factors and this effect is brain region-selective. Activin increases the survival of multipotent P19 teratoma cells, the rat B50 nerve cell line and chick neural retinal cells (Schubert et al. 1990). Activin A and B are neuroprotective against serum deprivation and toxin-induced death of human neuroblastoma cells. Transient expression of activin βA or βB protects neuroblastoma and rat pheochromocytoma cells against serum withdrawal-induced apoptosis. Activin A also protects cultured E14 rat mesencephalic dopaminergic cells from *N*-methyl-4-phenylpiridinium ion toxicity (Krieglstein et al. 1995).

Transgenic mice that express a dominant-negative ActRIB (ALK-4) from the CaMKII-α promoter in the forebrain have hippocampal neurons that are more sensitive to intracerebroventricular excitotoxic insult. Glutamatergic neurotransmission is also affected, with reduced *N*-methyl-D-aspartate-induced current response and impaired long-term potentiation (Hughes et al. 1999). In a chemically induced rat model of Huntington disease produced by excitotoxic striatal injection, intrastriatal injections of activin A greatly reduced the degeneration of several populations of striatal neurons of rats. The most potent protective effect was observed in the striatal cholinergic interneuron population, comprised of striatal interneurons and projection neurons (Hughes et al. 1999). Consistent with these observations, intraventricular administration of human activin A reduced neuronal death in the hippocampus and dorsolateral striatum induced by hypoxic-ischemic brain injury (Wu et al. 1999). Activin A also supports the survival of rat hippocampal neurons in vitro (Iwahori et al. 1997).

Activin B plays an important developmental role in spinal cord where TGF-β1 and activin B cooperate to support oligodendrocyte development and myelination (Dutta et al. 2014). Oligodendrocytes produce myelin, the material that surrounds axons and promotes nerve conduction. Cultured oligodendrocyte precursors (OLP) show differential activation of Smad3 and MAPK signaling in response to TGF-β1 and activin B. TGF-β1 increases proliferation while activin B supports maturation. Treatment with both proteins has an additive effect on viability, and enhances proliferation and differentiation, thus increasing the number of mature oligodendrocytes (Dutta et al. 2014). The spinal cords of *Inhbb*$^{-/-}$ embryos display increased apoptosis in the oligodendrocyte lineage and transiently reduced OLP numbers, but cell numbers, maturation, and myelination recover during the first postnatal week. These findings suggest a functional redundancy with other TGF-β family ligands in this process, a contention supported by a more severe and prolonged OLP phenotype in *Smad3*$^{-/-}$ mice (Dutta et al. 2014).

Bambi$^{-/-}$ mice are viable, fertile, and without identifiable developmental defects (Chen et al. 2007), but show increased TGF-β signaling and broadly reduced acute and chronic pain responses. The pain tolerance is reversed by naloxone, an opioid antagonist, through a mechanism involving δ-opioid receptor signaling. Proopiomelanocortin (POMC) and proenkephalin (PENK) are increased in the spinal cords of *Bambi*$^{-/-}$ mice, and treatment of wild-type spinal cord explants with activin A or BMP-7 increases POMC and/or PENK mRNA levels (Tramullas et al. 2010). These findings are con-

sistent with a role for activin signaling in the control of pain sensitivity.

Energy Metabolism

Adipocyte Differentiation

Activins A and B and their receptors are highly expressed in rat and mouse adipose tissues and adipogenic cells (Vejda et al. 2002; Kogame et al. 2006; Allen et al. 2008; Hoggard et al. 2009; Koncarevic et al. 2012). In humans, the high-activin βA mRNA expression in preadipocytes is even higher in adipose tissues from obese individuals. However, activin A mRNA levels decrease when human preadipocytes are induced to differentiate, and exogenous activin A protein inhibits adipogenesis while increasing proliferation of preadipocytes by decreasing the expression of the early adipogenic transcription factor CCAAT/enhancer binding protein-β (C/EBP-β), thereby arresting adipogenesis. Activin A inhibits C/EBP-β through Smad2 signaling (Zaragosi et al. 2010), whereas inhibition by TGF-β1 is transduced through Smad3 (Choy et al. 2000; Tsurutani et al. 2011). Similar outcomes were observed in undifferentiated mouse 3T3-L1 preadipocytes, in which activin A increased cell proliferation and reduced expression of C/EBP-α and peroxisome proliferator-activated receptor γ (PPARγ), the "master regulator" of adipogenesis (Hirai et al. 2005).

Mature Adipocyte Function

Activin βA and βB are expressed in adipose tissues but their abundance differs. Activin βA expression is higher in undifferentiated preadipocytes (Hirai et al. 2005; Zaragosi et al. 2010), whereas activin βB expression is higher in differentiated 3T3-L1 adipocytes and in human adipose tissue. Differentiated 3T3-L1 cells treated with insulin or dexamethasone have increased or decreased activin βB expression, respectively. Activin βB expression is also reduced in leptin-deficient mice after administration of leptin, but no leptin effect is observed in differentiated 3T3-L1 cells (Hoggard et al. 2009).

In mice and humans, activin βB mRNA levels in white adipose correlate positively with obesity (Sjöholm et al. 2006; Hoggard et al. 2009). Diet-induced weight loss reduces *Inhbb* gene expression in obese humans (Sjöholm et al. 2006). Moreover, $Inhbb^{-/-}$ mice have lower body weights than wild-type controls (Bonomi et al. 2012). In differentiated 3T3-L1 cells, treatment with activin B reduces expression of perilipin, hormone-sensitive lipase and adipose triglyceride lipase, important contributors to lipolysis (Magnusson et al. 2010). Thus, activin A and activin B likely play different roles in adipogenesis and mature adipocyte function.

The $Inhba^{BK}$ mice described above have reduced body weight and adiposity with improved insulin sensitivity and increased energy expenditure (Li et al. 2009). These mice are resistant to the obesogenic effects of a high-fat, high-carbohydrate diet. The improved metabolic outcomes are attributed to increased expression of genes involved in energy expenditure, mitochondrial biogenesis, and function in brown adipose tissue, liver, and skeletal muscle. Isolated liver mitochondria show constitutive oxygen consumption as a result of uncoupling of oxidative phosphorylation, suggesting a role for activin signaling in supporting mitochondrial function (Li et al. 2009).

Activin Signaling in Glucose Homeostasis

Activins A and B are expressed in adult rodent pancreatic islet cells, with activin A in α-islet cells and activin B in α- and β-islet cells (Yasuda et al. 1993; Brown et al. 2011). In humans, activin A levels in serum correlate with fasting glucose, fasting insulin, and glycosylated hemoglobin levels (Wu et al. 2013). Loss- and gain-of-function transgenic mouse models for activins, activin receptors, FSTL-3, and Smads have contributed to our understanding of activin signaling in pancreas development and the proliferation, differentiation and function of islet cells (reviewed in Wiater and Vale 2012). Transgenic expression of a dominant negative ActRIIB or a constitutively active ALK-4 receptor in the pancreas results in islet hypoplasia with reduced insulin secretion and reduced glucose tolerance (Yamaoka et al. 1998). In a mouse model of pancreas regeneration, inhibition of

activins by follistatin increases the proliferation of epithelial cells in the pancreatic duct and reduces insulin secretion, most likely by reducing the differentiation of β-islet cells (Zhang et al. 2004). In contrast, *Fstl3*$^{-/-}$ mice show increased pancreatic islet number and size, pancreatic β-cell hyperplasia, lower visceral fat mass, beneficial effects on glucose homeostasis, liver fat accumulation, and mild hypertension. Despite the ability of FSTL-3 to inhibit myostatin, however, *Fstl3*$^{-/-}$ mice show no changes in lean body mass or body weight. Collectively, the findings have been attributed to effects of unsuppressed activin and/or myostatin activity (Mukherjee et al. 2007). The reduction of β-cell mass with streptozotocin or by partial pancreatectomy, results in increased expression of activins in the pancreatic duct, and increased insulin and glucose levels (Zhang et al. 2002). The increased expression of activins in the pancreatic duct after partial pancreatectomy suggests that they may contribute to β-cell neogenesis. Although the factors that regulate activin effects in the duct are not well understood, β-cell regeneration may occur by relief of transcriptional repression of neogenin 3 through non-Smad signaling (Ogihara et al. 2003).

In the *Inhba*^{BK} model, with enhanced insulin sensitivity, our understanding of activin effects on glucose metabolism is confounded by possible systemic effects of activin-B misexpression in activin-A-producing tissues (Brown et al. 2000). *Acvr1c*$^{-/-}$ null mice lacking the ALK-7 receptor are hyperinsulinemic and glucose-intolerant, and have increased islet mass. This is partly because of reduced activin B signaling (Bertolino et al. 2008). However, other ligands, such as activin A, activin-AB, GDF-3, and nodal, also use the ALK-7 receptor so the loss of signaling by one or more of these ligands should also be considered (Tsuchida et al. 2004; Bertolino et al. 2008; Zhao et al. 2012).

Studies on effects of activins on glucose homeostasis have produced disparate results in vivo and in vitro. In *Drosophila*, the activin-like ligand, Daw, maintains glucose homeostasis mediated by Smad signaling. The *daw* mutants have increased triglycerides, glycogen, and glucose levels, and these abnormalities are rescued by overexpressing *daw* selectively in the fat body. Gene expression profiling in *daw* mutants revealed up-regulation of several genes encoding enzymes and other proteins that participate in fatty acid oxidation, Krebs cycle, and oxidative phosphorylation (Ghosh and O'Connor 2014).

Inhbb$^{-/-}$ mice have hyperinsulinemia, glucose intolerance, and insulin resistance, similar to ALK-7-deficient mice. Islets from *Inhbb*$^{-/-}$ mice secrete more insulin and generate more ATP on glucose stimulation, and both processes are reversed after treatment with activin B (Wu et al. 2014). Although activins A and B share common signaling mechanisms, they may have unique functions in glucose homeostasis. Treatment of islets with activin B reduces the influx of calcium ions, insulin secretion, and ATP production after glucose stimulation, whereas activin A has the opposite effect (Bertolino et al. 2008; Wu et al. 2014). The differences in insulin secretion are a result of the selective phosphorylation of Smad2 by activin A and Smad3 by activin B that may have different downstream targets and divergent outcomes on glucose stimulation (Wu et al. 2014). Another study of *Inhbb*$^{-/-}$ mice, however, showed no effect on insulin secretion, insulin sensitivity or glucose tolerance (Bonomi et al. 2012). Additionally, *Inhbb*$^{-/-}$ mice had lower body weights, pancreatic islet mass and a lower α- to β-islet cell ratio (Bonomi et al. 2012); however, a different study found no differences in body weight or pancreatic islet mass at age 2 months (Wu et al. 2014). The difference in genetic background of the mice may explain the conflicting results: a mixed 129/Sv-C57BL/6 background in one study and a backcrossed C57BL/6 background in the other.

Irrespective of these differences, insight has been provided from both studies regarding the pancreatic islet distribution of activins and the different effects of activins A and B on insulin secretion. Ectopic expression of activin B in the *Inhba*^{BK} model results in reduced fasting glucose and insulin levels and enhanced insulin sensitivity and glucose tolerance, suggesting that increased expression of activin B in pancreatic islets may contribute to the enhanced glucose metabolism in this mouse model (Li et al. 2009).

Bone Homeostasis

Activin A mRNA expression by osteoblasts and activin A deposition in the bone matrix are higher than *Inhbb* expression, while inhibin α mRNA is nearly undetectable (Ogawa et al. 2006; Eijken et al. 2007; Wu et al. 2012). Studies of activin A and inhibin A effects on bone development and function have yielded contradictory results. Injection of activin A into newborn rat periosteum increases periosteal and bone matrix layer thickness (Oue et al. 1994). Intramuscular injection of activin A improves bone mass and strength in aged, ovariectomized rats (Sakai et al. 2000). Soluble ActRIIA-mFC inhibits activin signaling but also increases bone formation, mass, and strength in sham-operated and ovariectomized mice, possibly by affecting signaling of other TGF-β family ligands (Pearsall et al. 2008). Treatment of bone marrow–derived stem cells with activin A promotes osteoblastogenesis and osteoclastogenesis, while inhibin A has the opposite effect (Centrella et al. 1991; Fuller et al. 2000; Gaddy-Kurten et al. 2002). Activin A can also inhibit osteoblastogenesis and mineralization in vitro, dependent on the differentiation stage (Ikenoue et al. 1999; Eijken et al. 2007). These results highlight the importance of considering the proper window of responsiveness to activins for osteoblastogenesis, a process that is also regulated by follistatin (Hashimoto et al. 1997; Eijken et al. 2007).

Transgenic mice with mifepristone-induced overexpression of inhibin α in liver have increased bone mineral density, volume, and strength as a consequence of increased osteoblastogenesis and show reversal of gonadectomy-induced bone loss (Perrien et al. 2007). These results seemingly contradict those from in vitro studies, and the disparate outcomes could be explained by compensatory mechanisms in vivo or by differences in the duration of inhibin exposure, since 1-week exposure in vivo inhibits osteoblastogenesis as observed in vitro, whereas 4 weeks overexpression augments osteoblastogenesis and enhances bone quality. Similar to activin A, these results suggest that inhibin A regulates osteoblastogenesis to prime osteoblast precursors for differentiation (Perrien et al. 2007).

In humans, serum inhibin A and inhibin B levels correlate negatively with bone-formation markers in peri- and postmenopausal women and, therefore, can be used as clinical predictors of bone loss before detectable changes in estrogen levels (Perrien et al. 2006).

Inflammation

Activins play early roles in a variety of inflammatory processes (Jones et al. 2004; Werner and Alzheimer 2006; Phillips et al. 2009; Besson-Fournier et al. 2012), and many cells conferring innate and acquired immunity respond to activins (Antsiferova and Werner 2012).

Macrophages

During inflammation, macrophages show different activation states and biological activities (Mantovani et al. 2004). Lipopolysaccharide (LPS) and interferon-γ induce a classical proinflammatory (M1) macrophage profile, whereas interleukin (IL)-4 or IL-13 induce an anti-inflammatory activation state (M2a) with production of immunosuppressive factors, IL-10, IL-1RA (IL-1 receptor antagonist), and arginase (Martinez et al. 2008). Activin A, but not activin B, expression is increased in LPS-stimulated macrophages (Ogawa et al. 2006).

Effects of activin A in the RAW264.7 mouse macrophage cell line include increased IL-1β and IL-6 expression, pinocytosis and phagocytosis, and MHC II expression, but no enhanced expression of MHC I markers and CD80, and no effect on proliferation, suggesting that activin A may affect macrophage-mediated innate and acquired immune responses through macrophage activation (Ge et al. 2009).

M1, but not M2a macrophages, derived from human peripheral blood monocytes, release activin A, and Smad2/3-mediated activin signaling supports the M1 macrophage phenotype, contributing to differential gene expression profiles and functions of the M1 and M2a phenotypes. Activin A suppresses IL-10 production by mature, antiinflammatory M2a macrophages, to inhibit IL-10 production dur-

ing polarization and to inhibit the growth of leukemic cells, a defining feature of M1-polarized macrophages (Sierra-Filardi et al. 2011).

The proinflammatory M1 phenotype of human monocyte–derived macrophages requires activin A induction of *EGLN3*, an oxygen-dependent prolyl hydroxylase whose expression is associated with the M1 macrophage phenotype in a variety of inflammatory conditions, such as Crohn's disease, ulcerative colitis, and solid tumors. *EGLN3* expression is induced by hypoxia and additionally regulated by activin signaling (Escribese et al. 2012). These studies establish important functions for activin A in macrophage biology and are consistent with its contributions to inflammatory processes.

Other Inflammatory Processes and Cell Types

Macrophages are not the only hematopoietic cells that participate in activin-regulated inflammatory processes. Bone marrow–derived neutrophil precursors (BMNPs) express activin A at seven-fold higher levels than mononuclear cells. Tumor necrosis factor α (TNF-α) induces activin A release from BMNPs without de novo synthesis, an effect that is blocked by insulin, suggesting a possible mechanistic link to inflammatory processes in type 2 diabetes (Wu et al. 2013). In contrast, through a different mechanism, LPS has no effect on BMNPs, but stimulates activin A expression and release in total bone marrow cultures (Wu et al. 2013).

In vivo, bone marrow is a major source of circulating activin A after LPS-induced inflammation. Similar to total bone marrow cultures, the increase in activin A is posttranscriptional. This is in contrast to TNF-α and follistatin responses to inflammation, which are transcriptionally regulated. The activin-A-producing cells are bone marrow–derived neutrophils that migrate to the lung and may contribute to LPS-induced lung inflammation and injury (Savov et al. 2002; Wu et al. 2012).

Studies of chronic inflammatory mechanisms that contribute to anemia of chronic disease have revealed an unusual example of promiscuity among activin ligands and receptors. During LPS-induced inflammation, activin B expression is selectively increased in mouse liver (Besson-Fournier et al. 2012), an effect that is not seen for the other activins. Activin B induces BMP type I receptor-mediated combined Smad 1/5/8 phosphorylation (Smads were not assessed individually) in a human hepatoma cell line and primary mouse hepatocytes, resulting in increased *Hamp* (hepcidin) mRNA levels (Besson-Fournier et al. 2012). This activin-B-induced increase occurs synergistically with IL-6. These hepcidin effects are not observed with the other activins, showing that, despite overlapping functions in many contexts, activins A and B also have unique functions that may be transduced by the BMP branch of Smad-mediated TGF-β family signaling.

Adipose Tissue Inflammation and Metabolism

Obesity is characterized by low-grade inflammation. In mice and humans, the expression of inflammatory genes increases with obesity (Hotamisligil et al. 1993, 1995; Engström et al. 2003). Inflammatory cytokines are secreted by immune cells, particularly adipose resident macrophages (Weisberg et al. 2003; Cancello et al. 2005). T cells are also recruited and contribute to the inflammatory milieu (Wu et al. 2007; Feuerer et al. 2009; Winer et al. 2009). Obesity results in recruitment of M1 macrophages to adipose tissues, and also triggers M2 to M1 adipose-resident macrophage conversion (Lumeng et al. 2007, 2008), with higher levels of secreted activin A in M1 than in M2 macrophages (Sierra-Filardi et al. 2011). Proinflammatory cytokines secreted by macrophages inhibit differentiation of preadipocytes (Lacasa et al. 2007; Xie et al. 2010). Activin A inhibits adipocyte differentiation in human and mouse cells (Hirai et al. 2005; Zaragosi et al. 2010), and human preadipocytes cultured in conditioned media from adipose-resident macrophages show increased *Inhba* mRNA expression (Zaragosi et al. 2010). These data are consistent with a model in which activated macrophages in white adipose tissues produce inflammatory cytokines, possibly including activin A, creating an environment that antagonizes adipocyte differentiation.

Chronic Lung Disease

Activin A may participate in airway remodeling, and activin signaling dysregulation may contribute to the normal balance between immunity and tolerance in the airway and asthma in mice and humans (reviewed in Kariyawasam et al. 2011). Activin has shown both beneficial and damaging effects, promoting the growth of airway epithelium and suppressing inflammation in some contexts, while augmenting inflammation, fibrosis, and lung damage in others.

Protective effects for activin were shown in a mouse model of allergic asthma, a process requiring TGF-β and mediated by regulatory T-cell (T-reg) activation that normally suppresses the T helper cell immune response (Semitekolou et al. 2009). Activin A levels and signaling in lung tissues normally acutely increase in response to allergens; however, activin signaling is later reduced, partially by follistatin. In a mouse model of allergy-induced asthma, follistatin blocks mucus production and the allergen-specific immune response in lymph nodes (Hardy et al. 2006). Follistatin also reduces airway inflammation and improves survival in a mouse model of cystic fibrosis (Hardy et al. 2015). Moreover, blocking activin signaling in a bleomycin-induced lung injury model substantially reduces the inflammatory and fibrotic effects of the drug (Aoki et al. 2005). These findings are consistent with a biphasic response to inflammatory stimuli in the lung—an acute phase with increased activin signaling that promotes epithelialization and subsequent suppression that minimizes potentially damaging effects of inflammation. This process is dysregulated in the asthmatic airway, leading to chronic inflammation. Disparate effects among experimental models likely reflect the complexity of the mechanism and the different conditions for administration of irritants, activins, and follistatin.

BIOLOGICAL ROLES IN RESPONSE TO INJURY AND DISEASE

Wound Healing

Activin A expression increases in skin after injury and promotes wound healing (Hübner et al. 1999). Transgenic overexpression of activin A in the skin results in adipose replacement with fibrous connective tissue, a poorly organized, thick epidermis and enhanced proliferation of basal keratinocytes. Injury causes enhanced dermal fibrosis with granulation tissue and robust scar formation. Activins βA and βB normally increase in keratinocytes and stroma after skin injury in mice and humans. All activin receptors are expressed in skin and their levels do not change after injury (Hübner et al. 1996). Recombinant activin B stimulates healing, requiring signaling through the RhoA-JNK pathway (Zhang et al. 2011), and mice expressing a dominant negative ActRIB (ALK-4) receptor in keratinocytes show delayed re-epithelialization after skin injury (Bamberger et al. 2005). Keratinocyte-selective abrogation of follistatin greatly enhances re-epithelialization (Antsiferova et al. 2009), whereas transgenic expression of follistatin in keratinocytes causes thinning of the epidermis and dermis and delayed wound repair, but less scarring (Wankell et al. 2001b). Inhibiting activin signaling in the developing zebrafish fin causes failure of normal fin regeneration after amputation, supporting important roles in proliferation, cell migration, and wound healing (Jazwinska et al. 2007). This finding is also consistent with previously described roles for activins in the developing chick limb (Merino et al. 1999).

Fibrosis

Activins contribute to hepatic, renal, and pulmonary fibrosis. In rats with liver fibrosis, activin A is expressed by collagen-producing, stellate-derived myofibroblasts in connective tissue septae (De Bleser et al. 1997). Follistatin administration during carbon tetrachloride-induced liver fibrosis reduces hepatocyte apoptosis and collagen accumulation (Patella et al. 2006). The profibrotic effects of activin A on hepatic stellate cells in vitro are also mitigated by follistatin (Wada et al. 2004).

In the kidney, activin A participates in the response to ischemic injury, which includes expression in medullary tubules (Maeshima et al. 2002). Follistatin protects against ischemic in-

jury and preserves renal function in vivo, whereas activin A has the opposite effect (Maeshima et al. 2002). Rat kidney fibroblasts, but not renal tubule epithelial cells, express type I collagen and smooth muscle actin when treated with activin A (Yamashita et al. 2004), and cultured rat renal mesangial cells express extracellular matrix proteins when treated with activin A (Gaedeke et al. 2005).

Several medical conditions result in increased activin A expression in the lungs of patients with interstitial pulmonary fibrosis (Matsuse et al. 1996). In vitro studies show that activin A enhances the proliferation of lung fibroblasts, induces their differentiation into myofibroblasts and enhances their ability to contract collagen gels (Ohga et al. 1996, 2000). Moreover, bleomycin-induced lung injury and fibrosis is mitigated by administration of follistatin (Aoki et al. 2005).

In summary, in vitro and in vivo experiments suggest roles for activin signaling in the pathogenesis of fibrosis in a variety of tissues and cell types. Most data corroborate the observation that activin expression increases during acute inflammatory processes, which leads to profibrotic events. Moreover, in vitro experiments show activin-induced up-regulation of a variety of profibrotic factors, including inflammatory cytokines and extracellular matrix components.

Cancer

Activin inhibits the proliferation of breast, liver, prostate, and pancreatic carcinoma cell lines and pituitary adenomas. Many malignant cell types repress the expression of activin receptors, while activins promote the proliferation of cells from testis and ovary (reviewed in Risbridger et al. 2001, 2004). Activins can also indirectly support tumor growth by inducing angiogenesis and by affecting the tumor microenvironment, influencing dendritic cell number and antigen uptake. Elevated circulating activin A is associated with bone metastases in breast and prostate cancer (Leto et al. 2006; Incorvaia et al. 2007), and increased activin A expression in head and neck tumors is associated with poor prognosis for overall and disease-free survival (Chang et al. 2010). Soluble ActRIIB-Fc, an inhibitor of activin signaling, reverses cancer-induced cachexia and improves survival in mice (Zhou et al. 2010).

Activin A overexpression in skin increases susceptibility to chemically induced skin cancer because of effects on stromal cells and loss of antitumor T-cell function. T-reg cells (T cells that normally suppress the function of tumor-suppressing T cells) increase in number in chemically treated activin transgenic mice. The net result of activin A overexpression in this model, therefore, is reduced T-cell antitumor activity and tumor progression (Antsiferova et al. 2011). Taken together, these data suggest a biphasic role for activin, first as an inhibitor of cell proliferation and tumor growth, and later as a promoter of oncogenesis, similar to the actions of TGF-β (reviewed in Akhurst and Hata 2012).

Inha$^{-/-}$ mice with targeted inactivation of inhibin α expression, display early lethality at 12 weeks because of gonadal tumors, hepatocellular injury and a cancer cachexia syndrome. Gonadectomized mice survive longer but succumb to adrenal cortical tumors. Therefore, inhibins are gonadal and adrenal tumor suppressors. Several genes are modifiers of the *Inha*$^{-/-}$ phenotype (Table 2) (Matzuk et al. 1995a; Coerver et al. 1996; Kumar et al. 1996, 1999; Mishina et al. 1996; Shou et al. 1997; Cipriano et al. 2000, 2001; Burns et al. 2003a,b; Li et al. 2007; Nagaraja et al. 2008; Zhou et al. 2010; Gold et al. 2013). With the exception of AMH, AMHRII and p27^{KIP1}, which repress the cancer cachexia syndrome, the double mutants have milder phenotypes than the *Inha*$^{-/-}$ mice, thereby identifying coregulatory processes that influence tumor progression. Collectively, these findings suggest that dysregulated activin signaling contributes to cancer and cachexia, that cachexia and tumor progression are not indelibly linked, and that other signaling pathways and cell-cycle proteins influence inhibin-related cancer progression. The cachexia of *Inha*$^{-/-}$ mice is mediated at least in part by the type II activin receptors, ActRII and ActRIIB, because genetic inactivation (Coerver

et al. 1996) or inhibition (Zhou et al. 2010) of these receptors can block *Inha*$^{-/-}$-induced cachexia.

THERAPEUTIC INITIATIVES TO TARGET ACTIVIN-SIGNALING PATHWAYS: ANTIBODIES, RECEPTOR-BASED LIGAND TRAPS AND MODIFIED PRODOMAINS

Disorders of Energy Metabolism

Obesity

In addition to physical exercise and diet-modification, new drugs aim to treat obesity and its associated morbidities by targeting specific signaling pathways. Targeting the activin-signaling pathways has promising therapeutic potential to influence lean/fat body mass and energy expenditure. Inhibiting ActRIIB using neutralizing antibodies or modified soluble human receptors (ActRIIB-hFc) positively influences body composition and nutrient metabolism (Table 3) (Akpan et al. 2009; Fournier et al. 2012; Koncarevic et al. 2012; Zhang et al. 2012). Administering soluble ActRIIB (RAP-031) improves muscle mass and function, reduces fat mass irrespective of diet composition, enhances peripheral glucose uptake, and suppresses hepatic glucose production. Mice treated with ActRIIB neutralizing antibody have in-

Table 3. Therapeutic strategies to inhibit activin receptor signaling

Disease model	Agent	Benefits	References
Neutralizing antibody			
Obesity/energy metabolism (mouse)	Anti-ActRIIB monoclonal antibody	Increased brown adipose and skeletal mass; no effect on white adipose	Fournier et al. 2012
Ligand traps (soluble receptors)			
Obesity/energy metabolism (mouse)	ActRIIB-hFc (RAP-031); sActRIIB	Increased muscle mass and function; increased glucose uptake; reduced fat-mass and britening of white adipose	Akpan et al. 2009; Koncarevic et al. 2012; Zhang et al. 2012
Cancer cachexia (mouse)	sActRIIB	Increased weight gain and muscle mass; increased survival	Zhou et al. 2010
Duchenne muscular dystrophy (mouse, human)	sActRIIB; ACE-031	Increased muscle mass and function; phase II clinical trial terminated	Pistilli et al. 2011; Wahl 2013
β-Thalassemia (mouse), anemia, and myelodysplastic syndromes (human)	RAP-536 ACE-536; ActRIIA-Fc (ACE-011 [Sotatercept])	Reduced erythroid hyperplasia; improved anemia; phase II clinical trials ongoing	Suragani et al. 2014; clinical trials.gov
Osteoporosis (mouse, cynomologus monkey)	ActRIIA-Fc (ACE-011 [Sotatercept])	Increased osteoblast differentiation, bone mass, and strength; improved noncortical bone volume and increased bone formation; suppressed bone resorption	Pearsall et al. 2008; Chantry et al. 2010; Lotinun et al. 2010
Bone metastasis in breast cancer (mouse)		Reduced bone destruction and bone metastasis	
Multiple myeloma (mouse)		Reduced osteoclastic lesions and prolonged survival	
Modified prodomains			
Muscle wasting (mouse)	Modified activin A and B prodomains	Specific inhibition of activin A and B; improved muscle mass	Chen et al. 2015

creased brown fat and skeletal muscle mass relative to controls, increased energy expenditure, and increased expression of mitochondrial function and energy expenditure genes; however, there are no effects on white adipose tissue mass (Fournier et al. 2012). The antibody treatment also reduces the activation of Smad3 in brown adipose tissue. In contrast, the soluble receptor, ActRIIB-hFC, reverses the effects of diet-induced obesity by increasing energy expenditure and expression of energy expenditure genes in white adipose tissues, including the appearance of brown-like adipocytes (Koncarevic et al. 2012).

Several TGF-β family members, that is, GDF-8, GDF-3, GDF-11, and activins A, B, and AB, bind ActRIIB with high affinity (Souza et al. 2008; Koncarevic et al. 2012) and are coexpressed in adipose tissues, which challenges our ability to separate direct and indirect effects of each ligand on metabolism in vivo and tissue-specific effects in organs that contribute to nutrient metabolism. However, the results above suggest that ActRIIB signaling is a promising target for the treatment of obesity, diabetes, and conditions in which muscle mass is adversely affected.

Cancer-Induced Cachexia

Although the morbidity most commonly associated with cancer results from metastasis and its effects, cancer progression also adversely impacts energy metabolism. In advanced cancer, the release of cytokines and other signaling molecules affect the expression of acute-phase proteins and the ubiquitin-dependent proteasome system, culminating in a hypermetabolic "cancer-cachexia" state, with loss of lean and fat body mass. Cachexia is associated with poor clinical outcomes in a variety of settings (Kotler 2000; Acharyya and Guttridge 2007). The first successful use of a soluble ActRIIB antagonist to prevent and reverse cancer cachexia was in four models of tumor-bearing mice, including $Inha^{-/-}$ mice. Lean body mass and survival improved despite ongoing tumor growth and elevated proinflammatory cytokine levels. The beneficial effects were observed when soluble ActRIIB treatment was initiated before and after the onset of cachexia (Zhou et al. 2010). Although much attention has been given to ActRIIB, ActRII is also an important contributor to the cancer-cachexia syndrome. $Inha^{-/-}$; $Acvr2^{-/-}$ (ActRII) double mutant mice develop tumors but fail to develop $Inha^{-/-}$-induced cachexia (Coerver et al. 1996), suggesting that both type II activin receptors mediate signals that influence energy homeostasis.

Muscular Dystrophies

Because reduction of GDF-8 (myostatin) expression dramatically increases muscle mass in several species, and GDF-8 signals through ActRIIB, an ActRIIB-soluble receptor was reasoned to potentially benefit patients with pathologically reduced muscle mass such as in Duchenne muscular dystrophy (DMD). In the *mdx* mouse model of DMD, 12 weeks of treatment with soluble ActRIIB improved skeletal muscle mass, absolute force production and specific force, with a corresponding decrease in blood creatine kinase (CK) levels, a marker of muscle cell damage. These findings are consistent with an overall improvement in muscle mass, integrity, and function (Pistilli et al. 2011). Accordingly, human clinical trials were initiated in DMD patients, but these were stopped because of off-target effects, which included nosebleeds, gum bleeding, and dilatation of skin vessels (Wahl 2013). These side effects, although mild, underscore the need for agents with high specificity, if therapeutic inhibition strategies are to be successful.

Anemia

One of the earliest identified functions of activins was the ability to augment EPO-dependent hemoglobin production and enhance proliferation of human bone marrow–derived erythrocyte precursors in vitro (Yu et al. 1987). It is therefore counterintuitive that soluble receptor derivatives of the human ActRIIB extracellular domain, ACE-536, or its mouse counterpart, RAP-536, produce rapid and robust increases in erythrocyte numbers under basal conditions, and reduces or prevents anemia in disease mod-

els. Unlike EPO, which acts at early stages of erythrocyte development, soluble receptors promote maturation of late-stage erythrocyte precursors in vivo. Accordingly, cotreatment with ACE-536 and EPO produces a synergistic erythropoietic response. The apparent paradox regarding activin effects on erythropoiesis is resolved by the binding properties of ACE-536 that were modified to affect the ligand-receptor interface: a single amino acid substitution (L79D), an amino-terminal truncation of four amino acids, and a carboxy-terminal truncation of three amino acids (Suragani et al. 2014). ACE-536 binds activin with low affinity but avidly binds GDF-11 and blocks GDF-11-induced Smad2/3 signaling. GDF-11 inhibits erythroid maturation in mice in vivo and ex vivo, and treatment of mice with RAP-536 reduces Smad2/3 activation, improves anemia and reduces erythroid hyperplasia (a marker of ineffective erythropoiesis) in a genetic mouse model of myelodysplastic syndrome (MDS) (Suragani et al. 2014). The beneficial effects of RAP-536 are not a result of enhanced activin signaling as a consequence of GDF-11 sequestration, because a neutralizing activin antibody does not reduce the positive effects of RAP-536 on erythropoiesis. ACE-536 also binds myostatin with high affinity and inhibits myostatin signaling, yet RAP-536 does not impact lean body mass in vivo (Suragani et al. 2014). ACE-536 is now in phase 2 clinical trials for treatment of anemia in β-thalassemia and MDS. Clinical trials for disorders with anemia as a primary or secondary feature are also underway using ACE-011 (Sotatercept), an ActRII-soluble receptor that has not been modified at its ligand-binding interface (clinical trials.gov).

Osteopenia

Diseases that impact bone turnover, such as osteoporosis and cancer metastasis, cause osteopenia and increase fracture risk. Several members of the TGF-β family impact the skeleton structurally and functionally, and are implicated in rare inherited bone disorders (reviewed in Chen et al. 2012). Activin A is present at high levels in bone, yet activin signaling in bone metabolism has not been robustly studied. ActRIIA-Fc administration in mice enhances osteoblast differentiation and increases bone mass and strength in normal and ovariectomized, osteoporotic mice (Pearsall et al. 2008). Moreover, in an osteolytic mouse model of multiple myeloma, ActRIIA-Fc triggers osteoblastogenesis, prevents myeloma-induced suppression of bone formation, prevents osteolytic bone lesions, and increases survival. ActRIIA-Fc also prevents bone destruction and inhibits bone metastases in a mouse model of breast cancer (Chantry et al. 2010). In nonhuman primates, human ActRIIA-Fc (ACE-011) improves medullary (noncortical) bone volume by enhancing bone formation and inhibiting bone resorption (Lotinun et al. 2010). These experiments strongly support the rationale for developing soluble activin receptors for the treatment of conditions with osteopenia as a feature, such as age-related osteoporosis, metastatic bone disease, chronic immobility, and menopause. To our knowledge, there are no ongoing clinical trials to investigate these possibilities.

Inflammation

Activin levels are markedly elevated in humans with life-threatening infections or acute respiratory failure (ARF), and is a poor prognostic indicator in ARF (Michel et al. 2003; de Kretser et al. 2013). In intensive care settings, ARF requires mechanical ventilation for longer than 6 hours, with a 30-day mortality risk of 30% (Esteban et al. 2002; Linko et al. 2009). Abnormally elevated activin A and B levels correlate significantly with mortality within 1 year of hospitalization, suggesting that activins are biomarkers for patients at highest risk for death (de Kretser et al. 2013). In contrast, activins may confer a protective effect in human newborns. Like ARF patients, premature newborns with life-threatening infections have elevated activin A levels. However, activin A suppresses proinflammatory cytokine production in vitro while enhancing the production of anti-inflammatory cytokines in LPS or phytohemagglutinin-treated peripheral leukocytes from human newborns (Petrakou et al. 2013). These observations

have led to the idea that modulating activin signaling during these and other inflammatory processes might be clinically beneficial. Consistent with this hypothesis, mice given lethal doses of LPS show improved clinical outcomes when treated with follistatin, and circulating activin levels directly correlate with proinflammatory cytokine production, and inversely with survival (Jones et al. 2007). Also, administered follistatin protects against several inflammatory processes, such as bleomycin-induced lung injury in rats (Aoki et al. 2005), antigen-induced asthma and airway remodeling in mice (Hardy et al. 2006, 2013), early-stage liver fibrosis (Patella et al. 2006), and inflammatory bowel disease (Dohi et al. 2005). This spectrum of benefits in animal models strongly supports potential clinical use. Important considerations, however, are the biphasic complexity of activin contributions to inflammatory processes and potential off-target effects of systemic follistatin administration. To our knowledge, no ongoing human clinical trials target activin-associated inflammation.

FUTURE DIRECTIONS: INHIBITION OF ACTIVIN SIGNALING—THERAPEUTIC POTENTIAL AND POSSIBLE PITFALLS

Although much attention has been given to GDF-8 as the primary ligand target for beneficial effects of ActRIIB inhibition on body composition, metabolism, and energy expenditure, other ligands and receptors, notably the activins and ActRII, may contribute substantially to these processes (Coerver et al. 1996; Lee and McPherron 2001; Lee et al. 2005; Souza et al. 2008). Moreover, myostatin-deficient mice treated with soluble ActRIIB show a further increase in muscle mass, suggesting that other ligands contribute (Lee et al. 2005), and activins have recently been implicated as negative regulators of muscle mass in vivo (Chen et al. 2015). Therefore, soluble receptors and other "ligand traps" will ultimately impact the functions of all ligand partners, particularly those that bind with high affinity. To be most useful clinically, more selective approaches are needed, such as altering the binding characteristics of the traps through mutagenesis, as in ACE-536, or using ligand-selective modified prodomains. These agents will not only be useful clinically, but also provide tools to dissect the milieu of factors that affect biological outcomes in vivo. Restricting therapeutic inhibition strategies to a single ligand or a selected combination of ligands will help to achieve the most desirable clinical outcomes while minimizing undesirable side effects. It will be interesting to determine whether targeting specific ligands has beneficial effects on body composition, metabolism, and other processes in which activins participate. Efforts to develop such agents are underway, with evidence that selective inhibitors reproduce some characteristics of the soluble receptors in vivo (Schneyer et al. 2008; Datta-Mannan et al. 2013; Suragani et al. 2014; Chen et al. 2015). Based on the early successes of several TGF-β inhibitors, additional strategies for attenuating activin signaling should be explored, which include blocking the expression of specific ligands or the translation of their mRNAs using antisense molecules, producing antibodies that target ligands, receptors, and associated proteins, blocking the conversion from latent to active ligand, identifying and refining ligand-competitive peptide inhibitors, blocking receptor kinases using small molecules, or blocking transcription effects of Smad complexes.

An important challenge facing inhibition of specific ligands, particularly activins that are widely expressed, is that off-target effects because of suppression of activin signaling in other tissues might limit the potential for clinical use. However, this possibility exists for any new therapeutic agent, and the clinical risk to benefit ratio must always be carefully considered. We are encouraged by the progress of several clinical trials that successfully target TGF-β signaling to improve outcomes for a variety of cancers and fibrotic diseases (reviewed in Akhurst and Hata 2012).

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