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Cell-Type Specific Modulation of Pituitary Cells by Activin, Inhibin and Follistatin

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Abstract

Activins are multifunctional proteins and members of the TGF-β superfamily. Activins are expressed locally in most tissues and, analogous to the actions of other members of this large family of pleiotropic factors, play prominent roles in the regulation of diverse biological processes in both differentiated and embryonic stem cells. They have an essential role in maintaining tissue homeostasis in the adult and are known to contribute to the developmental programs in the embryo. Activins are further implicated in the growth and metastasis of tumor cells. Through distinct modes of action, inhibins and follistatins function as antagonists of activin and several other TGF-β family members, including a subset of BMPs/GDFs, and modulate cellular responses and the signaling cascades downstream of these ligands. In the pituitary, the activin pathway is known to regulate key aspects of gonadotrope functions and also exert effects on other pituitary cell types. As in other tissues, activin is produced locally by pituitary cells and acts locally by exerting cell-type specific actions on gonadotropes. These local actions of activin on gonadotropes are modulated by the autocrine/paracrine actions of locally secreted follistatin and by the feedback actions of gonadal inhibin. Knowledge about the mechanism of activin, inhibin and follistatin actions is providing information about their importance for pituitary function as well as their contribution to the pathophysiology of pituitary adenomas. The aim of this review is to highlight recent findings and summarize the evidence that supports the important functions of activin, inhibin and follistatin in the pituitary.

Keywords

activin; follistatin; inhibin; foxl2; fsh; pituitary; reproduction; gonadotrope

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Conflict Statement

LMB, NJJ, ANB and EW have nothing to report. WWV is a co-founder, consultant, equity holder, member of the Board of Directors and Scientific Advisory Board of Acceleron Pharma, Inc. In accordance with Salk Institute policy, WV derives patent and licensing income in the activin field.

Introduction

The pituitary gland is the source of many hormones and growth factors that directly control or indirectly influence numerous physiologic and homeostatic processes. These include the well-characterized growth-promoting and anabolic actions of growth hormone (GH) secretion from somatotropes, the control of reproduction by gonadotropes via follicle stimulating hormone (FSH) and luteinizing hormone (LH), the effects of corticotrope-derived adrenocorticotropic hormone (ACTH) on the stress axis, as well as the regulation lactation and thyroid hormone production by prolactin (PRL) from lactotropes and thyroid-stimulating hormone (TSH) from thyrotropes, respectively. Not surprisingly, the endocrine functions of the anterior pituitary are tightly controlled and coordinated by cell-type specific actions of hypothalamic factors, feedback signals from peripheral target tissues as well as growth factors and cytokines that originate from populations of non-endocrine (i.e., folliculostellate cells) and endocrine cell types within the pituitary [1]. In addition to short-range control via diffusible factors that are secreted locally, pituitary cell responses are further modulated by various modes of direct cell-to-cell contact within and across populations of distinct cell types [1]. Within the population of folliculostellate cells, propagation of signals via gap junctions has been demonstrated to serve as a mechanism for long-range control within synchronized networks [2]. Preferential paracrine interactions between distinct populations of cell types had previously been suggested based upon functional studies of pituitary cell aggregates [1]. In recent studies that have benefited from advances in imaging technologies, closer examinations of the three-dimensional architecture of the pituitary have provided more detailed information and confirmed the existence of direct cell-to-cell contacts in the adult pituitary as well as during pituitary organogenesis [3]. These studies have led to the hypothesis that direct cell-cell contact during pituitary development is important for the establishment of the overall topography of the pituitary and might dictate the relative sizes of distinct but lineage related populations of pituitary cell types, consistent with the selective populations shifts that occur upon targeted ablation of individual pituitary cell populations [3,4].

Activin and inhibin, members of the TGF-β family of growth factors, as well as follistatin, are now recognized as critical modulators of pituitary gonadotropes and the reproductive axis. In females, the local actions of activin B in the pituitary promote differential FSH synthesis and contribute to the generation of the FSH surge required for normal folliculogenesis [5,6]. In males, pituitary FSH is involved in regulating spermatogenesis [7]. In rodents, it has been demonstrated that activin B, working in conjunction with hypothalamic gonadotropin-releasing hormone (GnRH), feedback gonadal signals including steroids and inhibin and other intrinsic signals such as follistatin and PACAP, regulates the expression of the FSHβ subunit of FSH and thereby promotes the FSH surge that occurs at late proestrus/early estrus [8]. Indeed, assays that monitored the actions of activin and inhibin or follistatin to stimulate or suppress FSH secretion from cultured pituitary cells, respectively, led to the discovery and initial characterization of these factors [9]. As members of the TGF-β family of ligands, activins are now known to exert a broad spectrum of effects and influence the developmental, differentiation and functional programs of most tissues [9]. These diverse actions of activin are antagonized by tissue-selective actions of inhibin and more broadly by follistatin [10–12]. With regards to the pituitary, inhibin functions as an important negative feedback signal from the gonads whereas pituitary-derived follistatin acts locally to regulate activin effects on gonadotropes [5,12]. The current review aims to highlight our current understanding autocrine/paracrine actions of activin on gonadotropes and the mechanisms underlying the modulation of gonadotrope-specific actions of activin by inhibin and follistatin.

Overview of pituitary regulation by activin and other members of the TGF-β family

The ligands of the TGF-β family are important players in the regulation of numerous and diverse biological processes that guide embryonic development and later on establish and maintain homeostasis of mature tissues. Their actions contribute to the maintenance of pluripotency of embryonic stem cells and, in apparent contradiction, also regulate specification of cell fate, differentiation, proliferation, and apoptosis [13–15]. Members of this family are known to exert tumor-suppressive effects in the context of normal cell physiology but to become pro-tumorigenic in cancer cells that have acquired resistance to their anti-cytostatic actions [16]. Similarly, members of the TGF-β superfamily, notably activin, BMP and TGF-β, exert regulatory roles in the embryonic as well as the postnatal pituitary [5]. These factors are further implicated in the pathogenesis of pituitary tumors, as demonstrated by studies of human pituitary adenomas [12,17–23].

During embryonic pituitary development, survival and proliferation of pituitary progenitors and dorsal-ventral patterning of Rathke's pouch are dependant on the establishment of opposing BMP-4 and BMP-2 gradients [24–26]. In the mature pituitary gland, BMPs have been reported to exert cell-type selective actions on lactotropes, gonadotropes and corticotropes. They are implicated in the pathogenesis of prolactinomas or for protection against corticotropinomas [22,27,28]. Variable and species-related effects of BMPs on FSH secretion and FSHβ expression have also been reported [29–31]. Lactotropes might be targets of both activin and TGF-β. Studies of primary rodent pituitary cells indicate that TGF-β1 is involved in the regulation of PRL expression while TGF-β3 from folliculostellate cells mediates the mitogenic actions of estrogen on this cell type, respectively [32,33]. Studies on lactotropic cell lines, on the other hand, suggest that activin modulates PRL expression by influencing PIT-1 function and protects against prolactinomas through a mechanism involving the interaction of activin-mediated Smad3 with the tumor suppressor, menin [34]. Activin has been reported to also modulate somatotrope functions by exerting inhibitory effects on GH production and the proliferation of this cell type [35–37].

Activin effects on gonadotropes are well characterized and documented by numerous studies. Activin is a potent activator of *Fshb* transcription and FSH secretion, as reviewed in detail in the following articles [38,39]. Activin acts in synergy with distinct pulses of hypothalamic GnRH and under the influence of gonadal feedback signals such as steroids and inhibin to regulate FSH production [8]. In female rodents, the actions of activin on *Fshb* are coincident with the differential rise in FSH secretion that occurs late in the estrous cycle and is necessary for follicle maturation [6,8]. These gonadotrope-targeted effects are largely, or perhaps, exclusively, mediated by the autocrine/paracrine actions of activin B secreted by gonadotropes rather than activin A, which seems to be derived from other pituitary cell types [40–46]. Studies of human pituitary adenomas have yielded similar conclusions by demonstrating that higher inhibin βB mRNA levels are found in FSH-producing pituitary adenomas compared to non-functioning adenomas [22].

The activin signaling pathway in gonadotropes not only activates the *Fshb* promoter but also regulates the expression of several other key targets. Activin regulates sensitivity to GnRH by its ability to activate the *Gnrhr* promoter and alter receptor expression [47,48]. Under certain circumstances activin induces LHβ expression through a transcriptional mechanism [49]. Activin-mediated induction of furin has been suggested to serve as a mechanism that promotes signaling by increasing the availability of mature, processed ligand [50]. Smad-dependent induction of Smad7 or truncated forms of Smad3 serve as intracellular mechanisms for negative feedback control of the signaling cascade [51,52]. The induction of follistatin in response to cell-type specific actions of activin on gonadotropes, on the other

hand, serves as an extracellular mechanism for ligand bio-neutralization and attenuation of further signaling [53]. Recent studies that have led to the identification of forkhead box L2 (FoxL2) as a determinant of gonadotrope-selective actions of activin have begun to shed light on the mechanism underlying cell-type specific, Smad-dependent activation of *Fshb*, *Fst* and *Gnrhr* transcription in this cell type, as outlined below.

A variety of genetically altered mouse models have been used to gain information about the role of the activin system within the reproductive axis and its importance for preserving fertility [54]. The inherent complexity, the broad distribution as well as the diverse and overlapping actions of receptors and ligands of the activin signaling system, however, have hampered data interpretation and clear-cut delineation of pituitary level anomalies from those that arise secondary to changes in other tissues, but nevertheless, valuable insight has been gained. Inactivation of *Acvr2a*, for example, was associated with compromised male fertility, female infertility and reductions of FSH levels and GnRH binding through effects at multiple levels of the reproductive axis [55]. By contrast, loss-of-function of *Acvr2b* was associated with postnatal lethality due to anomalies of cardiac and other organs [56]. Female mice with *Inhbb*-deficiency suffered reproductive anomalies that reflected the loss of inhibin B feedback rather than a pituitary-level disruption [57]. Whereas *Smad3* mutant mice did not show overt reproductive defects, studies on isolated pituitary preparations from these mice revealed that Smad3-deficiency compromises FSHβ and LHβ transcriptional responses [49,58,59].

Activin signaling

Activins are synthesized as larger precursor molecules that are assembled into disulfide-linked dimers and secreted as processed, bioactive polypeptides [9]. Activin A and B are dimers of inhibin βA and βB subunits, respectively, whereas inhibin A and B are generated through heterodimeric association of inhibin βA or βB with the inhibin α subunit [9]. Inhibin was first isolated from gonadal fluids and characterized by its inhibitory action on FSH secretion from rodent pituitary cells [9]. Further analysis of purified gonadal fluids led to the identification of activin as an FSH-releasing factor [9]. The discovery of these factors fulfilled the long-held concepts for the existence of a non-steroidal feedback signal that originates from the gonads (i.e., inhibin) as well as for the existence of factors that differentially elevate FSH secretion relative to LH [9]. It is now known that most tissues including the pituitary express inhibin subunits [9]. Whereas activins act primarily as autocrine/paracrine factors at or near sites of expression, inhibins have an additional role as classical endocrine factors [5,9]. In general, inhibins and activins are functional antagonists, but not all activin-responsive cell types display inhibin sensitivity consistent with the requirement for additional components, as outlined below.

Activins utilize the same signaling scheme as that used by other ligands of the TGF-β family [13]. Activin signals are transmitted primarily via two type II receptors, ActRII or ActRIIB, and one type I receptor, ActRIB/ALK4 [13]. ALK4 is the primary type I activin receptor but recent evidence suggests that activin B preferentially signals via ALK7 [60]. An added complexity is that ActRIIA and ActRIIB are shared by a subset of BMP and GDF isoforms, which utilize them to form signaling complexes with BMP/GDF-selective type I receptors, BMPRIA/ALK3 or BMPRIB/ALK6 [61,62]. In the case of TGF-β or activin, type I receptors alone display essentially negligible affinity for ligand and high-affinity ligand binding to the respective type II receptor is a critical event that establishes the interface for type I receptor binding [63,64]. This is in contrast to the mode of BMP/GDF interactions with their receptors in that the type I receptor seems to make the major contribution for high affinity ligand binding [65,66].

The cytoplasmic domains of the single transmembrane spanning type I and type II receptors that transmit signals of the TGF-β family of ligands possess serine/threonine kinase activity [61]. Downstream signaling by receptor-bound ligands is initiated and transmitted by a protein kinase cascade involving the sequential type II-dependent phosphorylation of the cytoplasmic domain of type I receptors and the subsequent phosphorylation-dependent activation of the canonical Smad signaling pathway as well as non-Smad pathways [61,67–69]. Smad2 and/or 3 are signaling mediators of either activin or TGF-β although some data suggest that activin preferentially utilizes Smad3 [70,71]. BMPs, on the other hand, activate Smad1, 5 and/or 8 but Smad1 and Smad5 might also transmit TGF-β signals under certain circumstances [61,62]. Several key structural features define the mode of action of Smads. An N-terminal MH1 (Mothers against decapentaplegic homology domain 1) domain mediates DNA binding but also supports protein interactions [67,68,72]. A C-terminal MH2 domain, separated from the MH1 domain by a linker region, is involved in transactivation and protein:protein interactions [72–75]. Phosphorylation of pathway-restricted Smads at a conserved C-terminal SSXS motif, missing in Smad4, relieves an inhibitory conformation, thereby allowing them to form homo- or hetero-oligomers and associate with Smad4 for subsequent shuttling into the nucleus [72–75]. Smad4, which was first discovered in humans as a pancreatic tumor suppressor gene (*DPC4*), is the only vertebrate common Smad and is shared by all pathway-restricted Smads [76,77]. In the nucleus, further interactions with cofactors facilitates high affinity binding to regulatory DNA elements and brings about gene activation or inhibition depending on whether coactivators or corepressors are recruited, respectively [61]. A major structural difference between Smad2 and Smad3 stems from the presence of a short insert in the MH1 domain of Smad2 that prevents direct DNA interactions in the absence of binding partners such as Smad4 or FoxH1/FAST1/2 [77–80]. Consistent with the latter, targeted inactivation studies confirm that Smad2 and Smad3 have distinct developmental and cellular functions [58,81–84]. Two vertebrate inhibitory Smads, Smad6 and Smad7, are inducible targets of Smads and serve as negative feedback modulators of the pathway [61,85]. Smad functions are subject to modulation through a variety of mechanisms, discussions of which are beyond the scope of this review [86].

Inhibin actions in the pituitary

Following the discovery of inhibin as a member of the TGF-β family and the realization that it functions as an antagonist of activin in a limited number of cell types, including pituitary gonadotropes, led to the search for candidate proteins that function as inhibin receptors [87–90]. The identification of betaglycan (or TβRIII) as a co-receptor for inhibin was somewhat unexpected because this protein had been previously characterized as a TGF-β2 co-receptor and a facilitator of the actions of this isoform of TGF-β [91]. Since its discovery, it has become evident that the picture is even more complicated given the ability of inhibin-bound betaglycan to also antagonize BMP actions [92]. The mechanistic basis of some features of betaglycan-mediated inhibin action has been elucidated through mutagenesis approaches. These studies have shown that inhibin binding to a site on the extracellular domain of betaglycan, separable from that for TGF-β, induces the formation of a ternary complex of betaglycan-inhibin-type II receptors (ActRII, ActRIIB or BMPRII) and prevents signaling downstream of activin or BMP by interfering with type I receptor recruitment and activation [92–94]. A ternary complex of betaglycan-TGF-β2-TβRII, on the other hand, promotes TGF-β2 binding to the type I TGF-β receptor, ALK5, and signaling [95].

Pituitary gonadotropes express betaglycan and exhibit high potency responses to inhibin [96,97]. By contrast, pituitary cell types other than gonadotropes do not display measurable inhibin sensitivity but yet express betaglycan [96]. The importance of betaglycan has been validated by several approaches. In primary cultures of pituitary cells, an immunoneutralizing antibody that recognizes the inhibin-binding domain of betaglycan

interferes with the effects of inhibin on FSH secretion from gonadotropes [98]. It has been suggested that the localization of betaglycan immunoreactivity to gonadotrope membranes is influenced by inhibin feedback signals during the estrous cycle [97]. When betaglycan is introduced into these cells, however, they acquire inhibin sensitivity and display measurable high-affinity cell-surface inhibin binding [91]. Conversely, knockdown of betaglycan levels in gonadotropes using RNAi technology attenuates inhibin binding and compromises the antagonistic action of inhibin on activin-stimulated FSH secretion [98,99]. These observations predict that the level of betaglycan expression is a major determinant of inhibin potency and an important mechanism underlying the high potency of inhibin seen in cell types such as gonadotropes. Several unexpected observations, however, suggest that this model might not fully reflect inhibin action.

Interestingly, although heterologous expression of betaglycan in cell types (i.e., AtT20) that lack detectable expression of this co-receptor induces inhibin sensitivity, the presence of endogenous betaglycan does not seem to be entirely predictive of inhibin sensitivity in cell types that otherwise exhibit activin or TGF-β responsiveness (i.e., lactotropes) [91,96]. Although this discordance between inhibin sensitivity and betaglycan expression is not yet fully understood, the phenomenon has led to the development of several mechanistic models underlying high potency inhibin action. It has been suggested and experimentally demonstrated that high-potency inhibin antagonism of activin signaling is largely determined by the abundance of cell-surface betaglycan levels above a critical threshold [98,99]. Competition between TGF-β and inhibin for binding to betaglycan might be yet another mechanism that affects inhibin potency [100,101]. Alternatively, high potency inhibin antagonism of activin or other ligands such as BMPs might involve the participation of additional membrane components that either interfere with or facilitate inhibin effects [102]. This latter hypothesis has been suggested to also dictate cell-type specific potencies of inhibin isoforms [103,104].

Two major inhibin forms are generated through the association of the common inhibin α subunit with either inhibin βA (inhibin A) or inhibin βB (inhibin B) [9]. Within the reproductive axis, inhibins serve a crucial feedback function as inhibitors of activin-dependent FSH expression and secretion [9]. In females, ovarian inhibin A and B are expressed and secreted in a cyclic manner in response to gonadotropins, with circulating inhibin B predominating during the luteal phase and early follicular phase followed by a rise in circulating inhibin A, which peaks at ovulation [10,105,106]. In males, by contrast, testicular inhibin B is the major circulating form [103,107]. Although early studies showed that *in vivo* administration of purified fractions of inhibin A suppressed FSH secretion, subsequent experiments that utilized recombinant inhibin A raised the possibility that pituitary gonadotropes display preferential sensitivities to inhibin A and B [108]. This possibility has been more recently explored. Comparative studies of the effects of highly purified fractions of inhibins have shown that, despite the lower binding affinity to a betaglycan and ActRII/IIB complex, inhibin B isoforms display higher FSH suppressing bioactivity, when compared to equivalent inhibin A forms [103,104]. Mutagenesis studies have thus far not yielded a structural basis for this apparent discrepancy between affinities and bioactivities of inhibin isoforms on pituitary gonadotropes [103]. Comparative cross-linking experiments, however, have suggested that the high potency of inhibin B actions on LβT2 gonadotropes are determined through high affinity and preferential interactions of inhibin B to an additional 35–40 kDa membrane component rather than betaglycan alone [103,104]. Further validation of this latter model awaits the identification of this novel membrane component, the functional characterization of its role in mediating inhibin actions and determination of its absolute requirement for high potency inhibin B actions.

Significant progress has been made towards a better understanding of the mechanism underlying the ability of inhibin to antagonize activin actions but a number of key questions remain unanswered. For example, it is generally assumed that most of the anomalies of betaglycan mutant mice that lead to embryonic lethality stem from disruptions of TGF-β and possibly BMP signaling [109]. The relative importance of inhibin-specific vs. TGF-β or BMP-related functions of betaglycan during embryonic development, however, has not been fully examined. In recent studies, the characterization of betaglycan expression patterns throughout gonadogenesis has suggested that this protein might have differential roles in the developing male and female gonads [110]. Equivalent studies of betaglycan expression in the developing pituitary are lacking and the extent to which betaglycan anomalies at the pituitary level contribute to reproductive defects is not known. Insight into these questions awaits characterization of the structural features of the inhibin interactions with betaglycan/type II receptors and the development of strategies that can selectively interfere with the inhibin-specific functions of betaglycan in a tissue-selective manner.

Follistatin actions in the pituitary

The cysteine-rich follistatins are extracellular binding proteins that can sequester activin, myostatin and several BMP subtypes and thereby inactivate them [10,111–114]. Structural studies have demonstrated that two molecules of follistatin form a complex with a single activin dimer in such a way that both type I and type II binding sites on activin are masked [115] [115–119]. Mutagenesis studies, however, suggest that the type II binding interface of activin is a more important determinant of high affinity interactions with follistatin [119]. Although first identified in gonadal fluids as FSH-inhibitory factors, follistatins are now known to be widely expressed and exert local regulatory effects in the embryo and in most adult tissues [10,11,120–122]. *Fst* mutant animals do not survive long after birth due to a variety of skeletal and cutaneous abnormalities [123]. Deregulation of follistatin function has been linked to a variety of cancers, including pituitary adenomas, and implicated in tumor metastasis [19,124,125]. Two alternatively spliced isoforms of follistatin that are generated from a single gene seem to have distinct properties and are presumed to act locally (FST-288) or mediate endocrine effects (FST-315) [126,127]. Genetic mouse models engineered to express only one or the other form of follistatin have begun to delineate the roles of each isoform but as yet have not provided clear-cut answers [128,129].

Follistatin is an important local modulator of activin signaling in pituitary gonadotropes [5,130]. Most pituitary cell types express follistatin and the secreted protein is detectable in pituitary cell preparations from a variety of species [44,131–136]. Methods that disrupt or modify follistatin expression or function in pituitary cell preparations have been shown to influence activin responses of gonadotropes [116,136,137]. Moreover, within the pituitary, local variations in the activin/follistatin paracrine loop might contribute to the ability of GnRH to differentially induce FSHβ expression [138–141]. Data from a variety of experimental rodent models suggest that, as a sensor of pulse frequency changes of hypothalamic GnRH release and an integrator of the feedback actions of gonadal inhibin and steroids, the local actions of follistatin contribute to the mechanisms that generate the cyclic variations of FSH production during the estrous cycle [142–147].

The role of FoxL2 in mediating cell-type specific actions of activin in the pituitary

Activin signaling in gonadotropes induces the expression of pituitary cell-restricted markers, such as *Gnrhr* and *Fshb*, but also activates transcription of *Fst*, which is ubiquitously expressed in all pituitary cell types and many other non-pituitary cell types [5]. Whereas activin induces *Fst* expression in primary rodent pituitary cells and gonadotrope-derived

αT3–1 and LβT2 cell lines, it has no effect on the expression of *Fst* in folliculostellate cells, which are otherwise responsive to activin [44,53,148]. Subsequent studies demonstrated that a downstream activin-responsive enhancer located within intron 1 of *Fst* is required for gonadotrope-specific actions of activin on this gene [148]. The enhancer harbors a conserved Smad-binding element (SBE1) that selectively recruits Smad3, not Smad2, as well as an additional site that recruits a cell-type specific factor, subsequently identified to be FoxL2 [148,149]. The function of this intronic enhancer, however, is dispensable in other cell types, such as HepG2 or HEK293, where activin or TGF-β effects on rodent or human *FST* transcription are mediated via upstream Smad2/3 binding sites in the promoter [149–151]. The upstream promoters of *FST* also harbor a number of regulatory elements, which mediate the effects of other signaling pathways such as cAMP/TPA and Wnt/β-catenin in a variety of non-pituitary cell types [152–154] or GnRH, cAMP/TPA, PACAP and PPARα/γ in LβT2 cells [155–159].

An unbiased screen for a factor that works in conjunction with Smad3 to activate *Fst* in gonadotropes ultimately identified Forkhead box L2 (FoxL2) as a candidate protein that fulfilled this function [149]. These efforts revealed that FoxL2 binding to a forkhead-binding element (FKHB) in intron 1 is permissive for Smad3-dependent activation of *Fst* transcription and that the disruption of either site compromises this action of activin on *Fst* in αT3-1 and LβT2 cells [149]. *In vitro* experiments have demonstrated that complex formation between FoxL2 and Smad3 is not regulated by activin and occurs independent of their interactions with DNA elements [149]. This is consistent with the notion that inducible binding of Smad3 to SBE1 brings Smad3 into the vicinity of FoxL2-bound FKHB thereby stabilizing the Smad3:SBE1 complex and activating *Fst* transcription [149].

FoxL2 is an evolutionarily conserved member of a large family of transcription factors characterized by the presence of a highly conserved DNA-binding forkhead domain [160,161]. *FOXL2* mutations are known to cause the Blepherophimosis-Ptosis-Epicanthus Inversus syndrome (BPES) and the associated characteristic eyelid defects and premature ovarian failure (POF) in a subset of females with type I BPES [162]. In the ovary, FoxL2 is known to regulate key enzymes involved in steroidogenesis [163–165]. Loss-of-function of *FoxL2* disrupts folliculogenesis and causes female infertility [166,167]. Conditional inactivation of *Foxl2* in the adult ovary induces ovary to testis reprogramming indicating that the functions of FoxL2 actively maintain the ovary, although this conditional mutant model might have also disrupted pituitary FoxL2 [168]. A variety of FOXL2 mutations have been correlated with ovarian tumors [169]. Moreover, a missense mutation in *FOXL2* (FOXL2C134W) is highly correlated with adult type granulosa cell tumors [170].

FoxL2 (or P-FrK) expression is detectable in the developing pituitary and coincident with that of *Cga* [171–173]. FOXL2 expression has also been immunohistochemically localized to gonadotropin-positive cells of normal human pituitaries as well as the majority of gonadotropinomas [174]. In the pituitaries of adult male or female mice, FoxL2 expression is confined to αGSU-expressing gonadotropes and thyrotropes [149,173,175]. Consistent with this pattern of expression, studies of cell lines derived from these lineages have shown that FoxL2 is indeed involved in the regulation of key targets including *Cga*, *Gnrhr*, *Fshb*, and as already discussed above, *Fst* [48,149,173,176–179]. Of these, *Gnrhr*, *Fshb*, and *Fst* are also targets of the activin/Smad pathway and their expression is facilitated through functional interactions between the Smad and FoxL2 pathways, although FoxL2 effects on *Fshb* might not be strictly or exclusively Smad-dependent [48,149,176–179]. Altogether, these observations have uncovered the possibility that FoxL2 actions in pituitary gonadotropes are involved in the regulation of the reproductive axis and fertility. FOXL2 has well defined functions in the ovary [180–182]. However, genetic *Foxl2* inactivation models have not been able to adequately delineate the contribution of pituitary dysfunction

to infertility of the female, because pituitary FoxL2 function would have also been disrupted in these models [166–168].

Examination of the pituitaries of *Foxl2* mutant mice has provided insight into the importance of this transcription factor in the pituitary [175]. In contrast to the presumed developmental role of FoxL2 in cell type specification in the pituitary due to embryonic expression patterns [171–173], immunohistochemical visualization of pituitary cells using cell-type specific markers confirmed that all pituitary cell types are present and appropriately specified in the pituitaries of adult *Foxl2* mutant animals, both male and female [175]. The importance of FoxL2 to pituitary function was revealed when FSHβ expression in pituitaries of *Foxl2* mutant mice was examined. While *Foxl2* mutant and wild type pituitaries have equivalent numbers of gonadotropes, as defined by LHβ staining, *Foxl2* inactivation is associated with a dramatic impairment of FSHβ expression and persistence of FSHβ immunoreactivity only in a subset of LHβ-positive gonadotropes [175]. In agreement with a reduction in FSHβ immunoreactivity, *Fshb* mRNA levels were significantly lower in pituitaries of *Foxl2* mutant animals compared to wild type [175]. Moreover, primary pituitary cells from mutant animals did not display measurable FSH secretion under either basal conditions or after activin stimulation whereas cells from wild type animals showed the expected induction by activin [175]. Similarly, whereas activin induced *Fst* mRNA levels in pituitary cells from wild type mice, it failed to do so in pituitary cells from *Foxl2* mutant animals (Bilezikjian, unpublished observations).

Altogether, the data from *Foxl2* mutant mice and those from αT3-1 and LβT2 cell lines suggest that FoxL2 has an important function in mature pituitary gonadotropes. Although, it is possible that some of the pituitary changes seen in the *Foxl2* mutant animals are secondary to disruptions of important gonadal feedback signals that normally modulate many aspects of pituitary functions, the data from these mutant animals provide compelling evidence for a role of FoxL2 in the pituitary, in addition to the ovary. As in the ovary, the FOXL2 pathway might also be involved in the regulation of cell growth in the normal pituitary or in pituitary adenomas, as has been suggested by recent studies [174,183]. Whether loss of FSHβ production in the pituitary contributes to the reproductive anomalies seen in either *Foxl2* mutant animals or in BPES patients and whether FoxL2 function is relevant to adenomas that arise from the gonadotrope lineage remain an open question and answers await the development of models for gonadotrope-specific inactivation of *Foxl2* and further studies of human pituitary tumors.

Summary

The importance and roles of activin, inhibin and follistatin for the regulation of pituitary gonadotrope functions are substantiated by numerous studies and observations of a variety of species, including humans. These studies have led to several key conclusions. Activin signaling in gonadotropes is modulated by gonadotrope-selective counter-regulatory mechanisms under the control of inhibin and follistatin. It has been demonstrated that betaglycan, which is expressed by pituitary gonadotropes, is obligatory for the antagonistic actions of inhibin on activin signaling. Is has further been postulated that additional membrane components expressed by gonadotropes facilitates high affinity feedback actions of gonadal inhibin B. The transcription factor, FoxL2, is also expressed in gonadotropes and required for activin-mediated, cell-type specific induction and actions of follistatin. The combined actions of these two distinct mechanisms of antagonism keep activin signaling in check and contribute to the cyclic variations of FSH production that occur across the estrous cycle. A great deal has been learned and many details of the activin/inhibin/follistatin network of the pituitary have been deciphered from numerous *in vivo* and *in vitro* studies of a variety of species, including normal and tumor-derived human pituitary tissues, and

through observations of a variety of human pathologies arising from pituitary anomalies or tumors. However, many issues that remain unresolved await the establishment of more suitable and sensitive models for detailed studies of pituitary cell types. Many discoveries in the field have relied on studies of the rodent pituitary, through both *in vivo* and *in vitro* approaches, or cell lines. Rodent pituitaries have been instructive in many cases but not always predictive of human pituitary functions and pathogenic processes. The field has also been challenged by the inherent heterogeneity and plasticity of the pituitary. Given this heterogeneity, it has often been difficult to pinpoint the exact source or target of endogenous factors. Available cell lines have proven to be tremendously helpful in teasing out cell-type specific modes of regulation but the behavior of these cell lines might not reliably reflect all nuances of pituitary cell functions within the spatial context of the mixed population or the temporal changes that occur throughout developmental stages and subsequent to full differentiation. Lacking are animal models in which temporally controlled and pituitary cell-type specific manipulation of genes of interest provide the opportunity to evaluate their stage-specific and context-relevant functions. This is particularly important given the ubiquitous nature of the expression pattern and actions of the activin/inhibin/follistatin network throughout the reproductive axis as well as in essentially all tissues. The field would also benefit from the development of reporter lines in which promoter elements are used to target cell-type specific expression of a variety of currently available and ever growing list of probes (i.e., fluorescent) that facilitate *in vivo* observations and selective enrichment of individual cell types for further evaluations. The ultimate validation and detailed characterization of the importance of the activin/inhibin/follistatin network within the pituitary, therefore, still awaits the development of such strategies that permit temporal and spatial control of the expression of key ligands, receptors and modulators of this network.

- Activins are important autocrine/paracrine modulators of pituitary gonadotropes.
- The inhibin co-receptor, betaglycan, mediates the feedback actions of gonadal inhibins.
- Follistatins, produced by gonadotropes, are local modulators of activin signaling in gonadotropes.
- FoxL2 is a transcription factor essential for ovarian maintenance and fertility.
- FoxL2 is also expressed in gonadotropes where it mediates cell-type specific actions of activin on *Fst*, *Fshb* and *Gnrhr*.
- FSHβ expression and activin signaling in gonadotropes are severely compromised in *Foxl2* mutant mice.

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elucidation of the key roles of these factors in the regulation of many physiological processes, including pituitary functions and the reproductive axis. Dr. Vale passed away unexpectedly on January 3, 2012, during revision of this manuscript.

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