Molecular and Cellular Endocrinology 314 (2010) 204–212

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

Hormonal signaling to follicle stimulating hormone β-subunit gene expression

Philippa Melamed [a,b,*]

[a] Faculty of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel
[b] Dept Biological Sciences, National University of Singapore, Science Drive 4, 117543, Singapore

ARTICLE INFO

Article history:
Received 8 March 2009
Received in revised form 20 May 2009
Accepted 21 May 2009

Keywords:
Pituitary
FSH
GnRH
Activin
Steroids
Transcription
Gonadotrope

ABSTRACT

Expression of the hormone-specific β-subunit of follicle stimulating hormone (FSHβ) is regulated primarily by gonadotropin releasing hormone (GnRH) and activin, with additional feedback by various steroids. While the nature of this hormonal regulation appears conserved, the molecular mechanisms mediating these effects appear less so. This is apparent from the diverse *cis*-elements required for hormonal stimulation in different species, distinct transcription factors that seem to mediate the effects, as well as the lack of conservation of several reportedly functional *cis*-elements across species. Recent additional information on the molecular mechanisms through which these regulatory hormones exert their effects, supports the possibility of species-specific mechanisms of regulation, while some redundancy may exist in signaling by the activated transcription factors which allows preservation of the hormonal regulation in these different promoter contexts.

Contents

Abbreviations: AF-1, activation function-1; AP-1, activator protein-1; BMK, big MAPK; BMP, bone morphogenic protein; CaMK, calmodulin kinase; cAMP, 3′–5′-cyclic adenosine monophosphate; CBP, CREB binding protein; ChIP, chromatin immunoprecipitation; CoIP, co-immunoprecipitation; CREB, cyclic AMP response element binding protein; CRH, corticotrophin releasing hormone; DBD, DNA binding domain; Eif4A3, eukaryotic translation initiation factor 4A, isoform 3; ERK, extracellular signal-regulated kinase; ERα, estrogen receptor α; FSHβ, follicle stimulating hormone β-subunit; GnRH, gonadotropin releasing hormone; GnRH-R, GnRH receptor; GSK3β, glycogen synthase kinase-β; HDAC, histone deacetylase; ICER, inducible cAMP early repressor; JNK, c-jun N-terminal kinase; KO, knockout; LHβ, luteinizing hormone β-subunit; MAPK, mitogen-activated protein kinase; MEF, myocyte enhancer factor; NFAT, nuclear factor of activated T-cells; NFY, nuclear transcription factor Y; Pitx, paired-like homeodomain transcription factor; PKA, protein kinase A; PKC, protein kinase C; SBE, SMAD binding element; Sf-1, steroidogenic factor-1; siRNA, small inhibitory RNA; SMAD, mothers against decapentaplegic homolog; TGFβ, transforming growth factor β; TORC, transducer of regulated CREB; tss, transcriptional start site; αGSU, glycoprotein α-subunit.

* Correspondence address: Faculty of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel. Tel.: +972 4 8293760; fax: +972 4 8225153.
E-mail address: philippa@tx.technion.ac.il.

0303-7207/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2009.05.012

1. Introduction

Mammalian reproduction is regulated by the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which travel via the circulation to the gonads, where they regulate gonadal growth and function. Although the synthesis of both hormones is stimulated by the hypothalamic gonadotropin releasing hormone (GnRH) and they are produced in the same cells, the expression and circulating levels of each hormone are distinct, in accordance with their different roles in reproductive function. In the female ovulatory cycle, FSH is responsible for inducing follicular growth, while also increasing estrogen production, while LH, apart from enhancing cholesterol availability for steroidogenesis, also signals ovulation following its peak mid-cycle.

The differential regulation of gonadotropin hormone gene expression has been the subject of much speculation, and likely relates to changing pulse frequency of GnRH administration: increasing pulse frequency stimulates LH β-subunit (LHβ) gene expression, and lowering it results in a decline in LHβ but a rise in FSH β-subunit (FSHβ) gene expression, while expression of the common α-subunit (αGSU) appears less stringently regulated (Ferris and Shupnik, 2006; Lim et al., submitted for publication and references therein). In addition, FSHβ gene expression is selectively regulated by the gonadal proteins, activin and inhibin, which are also produced locally in the pituitary. Activin stimulates FSHβ production via a membrane-bound receptor on the gonadotropes which is antagonized by inhibin binding. Circulating levels of these proteins also vary throughout the ovulatory cycle and clearly have a major impact in determining FSH levels (Bilezikjian et al., 2004; Hall, 2004). Expression of both hormones is also regulated by gonadal steroids which exert their effects at the level of the hypothalamus, by altering GnRH secretion, and also directly at the level of the gonadotropes, where they exert distinct effects on the various subunit genes (Hall, 2004; Melamed et al., 2006a). Clearly the convergence of these various endocrine signals at the gonadotrope cells results also in cross-talk between the various signaling pathways.

The molecular mechanisms through which these hormones signal transcription of the gonadotropin genes have been studied extensively. However, although the mechanisms regulating LHβ gene transcription have been clarified and appear to be relatively conserved across those mammals studied (reviewed by Jorgensen et al., 2004), an understanding of the mechanisms regulating FSHβ gene transcription has been slower to emerge.

This aim of this review is to collate the recent findings on the molecular regulation of FSHβ gene transcription by its regulatory hormones and to try to reconcile the considerable amount of conflicting data. For this purpose, the review focuses primarily on studies in murine gonadotrope cell lines, or other models which allow conclusions regarding events occurring specifically in the gonadotropes.

2. Regulation by GnRH

2.1. GnRH targets FSHβ gene expression through various transcription factors

The expression of a number of transcription factors markedly increases in response to GnRH; these include the early response genes which are up-regulated within the first hour of treatment, and some of which target the gonadotropin subunit genes (Wurmbach et al., 2001; Kakar et al., 2003; Jorgensen et al., 2004; Melamed et al., 2006a). However, understanding the roles of these factors in mediating the GnRH response has been slow as many earlier studies were carried out in non-gonadotrope cell lines which were engineered to express the GnRH receptor; the degree to which the signaling cascades activated in response to GnRH in these cells resemble those in the gonadotrope cells, is not known. Moreover, the FSHβ gene promoter shows some divergence across species, particularly in several regions that have been reported to have a specific function (Fig. 1). The lack of conservation of these *cis*-elements inevitably sheds some doubt on their degree of importance and/or indicates the possibility of species-specific mechanisms of regulation, the reasons for which are not clear.

The AP-1 factors, Jun and Fos, are early response genes that are up-regulated rapidly after GnRH exposure and have been indicated in GnRH activation of the FSHβ gene in a number of studies; they also mediate the GnRH response of the LHβ gene in some non-mammalian vertebrates (Melamed et al., 2006b). On mammalian FSHβ genes, there are several potential binding sites for these factors, some of which have been shown to be functional, although differences were seen in promoters from various species and/or under different experimental conditions in *in vivo* or *in vitro* models. Initially two putative AP-1 binding sites on the ovine FSHβ promoter were indicated (at −83 and −120 bp from the transcriptional start site [tss]) which were shown to mediate part of the GnRH response in luciferase assays in non-gonadotrope cells, but this was not recapitulated *in vivo* in transgenic mice (Miller et al., 2002). Interestingly, the promoter of the Chinook salmon also contains a putative AP-1 binding site (AGCAGTCA) in a similar location (at −84 bp) and this promoter too is responsive to over-expression of c-Jun alone or together with c-Fos (Chong et al., 2004; Wang et al., 2009).

Subsequent studies on the murine FSHβ gene promoter revealed that the corresponding sequence (ATTGGTCA, between −76 and −69 bp from the tss) comprises a composite half AP-1/NFY site that was shown to be required for the full GnRH response in luciferase assays in LβT2 gonadotropes. It was also required for the induction of promoter activity by c-Fos or c-Jun over-expression, from where the authors concluded that it is the only functional AP-1 site on this promoter. They also showed, using chromatin immunoprecipitation (ChIP) assays, binding of these factors to the promoter and that the effect of GnRH was abolished by over-expressing a dominant negative c-Fos, lending further weight to the concept that the GnRH effect is mediated by AP-1 factors (Coss et al., 2004). An identical AP-1/NFY putative binding site sequence is also present on the Chinook salmon FSHβ gene promoter (at −607 bp from the tss) although its functionality has yet to be tested (Wang et al., 2009).

These findings on the likely importance of AP-1 factors were challenged in a more recent study which showed that over-expression of c-Jun or c-Fos, or a dominant negative c-Fos that sequesters and thus inactivates also c-Jun, had no effect on GnRH-induced rat FSHβ promoter activity, although the expression levels of the proteins in these transfection assays were not shown (Ciccone et al., 2008). The same study suggested that CREB binds this AP-1/NFY binding site to mediate at least part of the GnRH effect: the CREB consensus binding sequence (CRE: TGACGTCA) is almost identical to that of AP-1. Although CREB over-expression had no effect on promoter activity, the effect of GnRH is likely via CREB phosphorylation (Section 2.2), and use of siRNA-mediated knock-down or a dominant negative CREB construct significantly reduced, although it did not abolish, the GnRH effect (Ciccone et al., 2008). The specific binding was demonstrated by gel-shift assays, and plasmid IP confirmed association of CREB with the transfected rat FSHβ gene promoter, while gel-shift assays indicated that NFY does not bind this site on the rat promoter, although some association of AP-1 factors was seen. It was suggested that the CREB might heterodimerize with AP-1 factors at this location (Ciccone et al., 2008).

Nur77 (Nr4a1) is another early response gene, whose expression and activity are rapidly up-regulated by GnRH (Wurmbach et

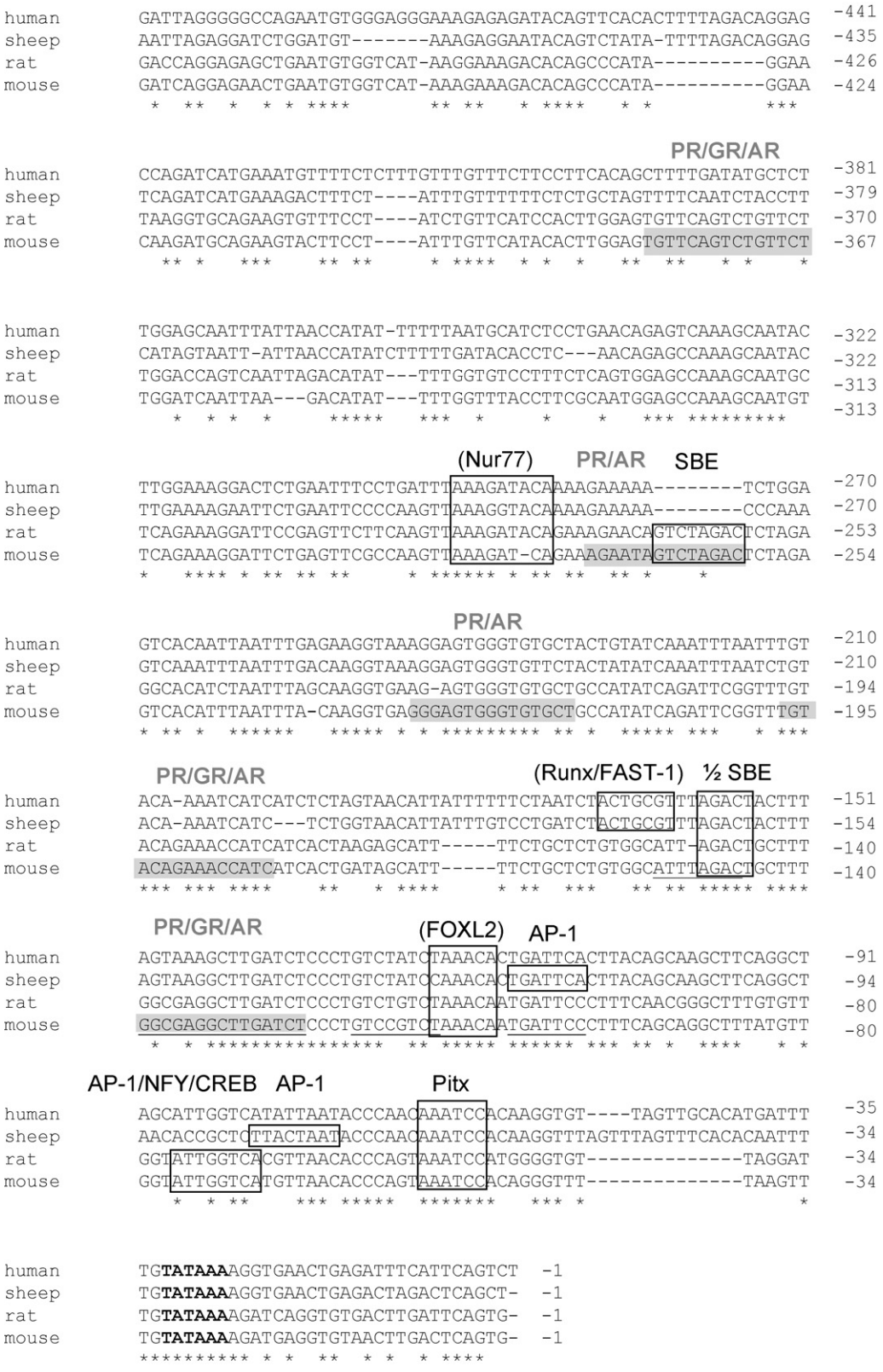

Fig. 1. Alignment of the 5′ flanking regions of mammalian FSHβ genes showing reported functional and putative response elements. The 5′ flanking regions were aligned using ClustalW; asterisks below the sequence mark bases conserved in all four species. *Cis*-elements are boxed and factors are labeled above the sequence; putative factors are bracketed. Underlined sequences represent those shown to impart activin/SMAD responsiveness to the murine promoter. Steroid hormone binding elements (shown functional in mice) are highlighted and labeled in grey. The TATA element is shown in bold typeface.

al., 2001; Kakar et al., 2003; Mazhawidza et al., 2006; Lim et al., 2007). Nur77 plays a vital role in regulating GnRH-induced derepression of the murine FSHβ gene in αT3-1 cells, as seen by the use of a dominant negative expression construct which abolished the GnRH effect, while its over-expression was sufficient to induce FSHβ transcription, and increased also murine FSHβ promoter activity in luciferase assays (Lim et al., 2007). Nur77 is found at the murine FSHβ gene promoter, and its consensus binding site (AAAGA/GTCA) is found on the murine promoter between −284 and −277 bp from the tss, although it appears as AAAGATACA in other mammals. Nur77 has also been shown to bind variants of this sequence, and on the GnRH receptor gene promoter it competes with Sf-1 for the same Sf-1 binding site (CCAAGGACA, in reverse orientation; Sadie et al., 2003). The Chinook salmon FSHβ gene promoter also contains a near consensus site (AAAGGTCC) at −104 to −98 bp from the tss, and this promoter is also highly responsive to Nur77 over-expression (Wang et al., 2009). The functionality of the putative binding site on the rodent FSHβ promoter has yet to be tested, although this region of the promoter (−304 to −230) was indicated to have a role in the GnRH response in LβT2 cells (Coss et al., 2004).

Despite extensive work, a consensus has not been reached on the role of most of these factors in mediating the GnRH response to activate FSHβ gene transcription. However it seems likely that under different physiological conditions, GnRH may signal to the promoter through various pathways, while some of these may be redundant. Further studies involving ChIP and siRNA-mediated knock-down in the gonadotrope cell lines, as well as more work in transgenic animals are required to clarify the role of these factors and their described *cis*-binding elements.

2.2. GnRH signaling to the FSHβ gene involves several kinase pathways

The GnRH-stimulated pathways involved in activation of some of the above-mentioned transcription factors have been demonstrated, while for others, the picture is less clear. The expression of the AP-1 proteins, c-Fos and FosB, is activated by GnRH-stimulated ERK1/2 and this appears to be crucial in GnRH stimulation of the human, murine and ovine FSHβ gene promoters. This was shown using a selective MEK inhibitor, or a dominant negative construct, both of which abolished the expression of the Fos genes and the activity of the FSHβ promoters (Bonfil et al., 2004; Coss et al., 2007; Wang et al., 2008). C-Fos was also shown to be activated by p38 MAPK, although this may be less crucial than its activation by ERK1/2; the differences seen could also relate to the efficacy of the inhibitors that were used in these studies (Roberson et al., 1999; Wang et al., 2008). A role for ERK1/2-independent kinases in the activation of the gonadotropin genes was indicated in transgenic mice in which the ERK1 and 2 were knocked-out in gonadotropes and thyrotropes. Following ovariectomy, which increased expression levels of FSHβ and LHβ in the wild type mice, presumably as a result of GnRH activation, FSHβ expression was lower in the knock-out (KO) mice than in the wild types, but levels were still clearly higher than in the intact (not ovariectomized) KO mice. This suggests that additional ERK1/2-independent GnRH-activated kinases play a role in inducing murine FSHβ gene expression (Bliss et al., in press).

A role for p38 MAPK in the GnRH-activated murine FSHβ promoter activity has been demonstrated in LβT2 cells, although whether c-Fos is the only downstream target, is not yet clear (Bonfil et al., 2004; Coss et al., 2007; Wang et al., 2008). Notably, in similar experiments in cultured rat pituitary cells, the GnRH response of the FSHβ primary transcript was not affected by the p38 MAPK inhibitor (Haisenleder et al., 2008). In the LβT2 cells, p38 MAPK was also shown to play a role in the synergy between GnRH and activin signaling (Section 5) in which its effects on c-Fos were again implicated (Coss et al., 2007).

The expression of c-Jun and JunB, as well as the phosphorylation of c-Jun, appear to be up-regulated by GnRH-activated JNK (Wang et al., 2008). Constructs expressing a dominant negative form of the upstream activator, CDC42, reduced the GnRH effect on activity of the ovine FSHβ promoter, indicating that JNK contributes to GnRH-activated FSHβ gene transcription (Bonfil et al., 2004). Notably, however, doses of a JNK inhibitor (SP600125) that were sufficient to abolish GnRH-induced phosphorylation of c-Jun, did not appear to affect activity of the human FSHβ promoter (Wang et al., 2008), possibly indicating a degree of species-specificity in the activation of the FSHβ gene by Jun proteins (Section 2.1). Given that higher doses of the JNK inhibitor did abolish the GnRH effect on human FSHβ promoter activity (Wang et al., 2008), it seems likely that JNK has additional downstream targets that are required for the GnRH effect on this gene. In perifused rat pituitary cells, the same JNK inhibitor significantly reduced basal levels of the primary transcript, although the GnRH-induced response (to 200 pM pulses every 60 min) was unaltered (Haisenleder et al., 2008). Although the reasons for these conflicting results are not known, paracrine effects may well be involved with possibly inefficient inhibition of the higher levels of activated JNK in these mixed-cell cultures. An additional pathway for GnRH activation of Jun was recently demonstrated involving phosphorylation of GSK3β, leading to accumulation of β-catenin which increases Jun expression. In other contexts, GSK3β can be phosphorylated by p38 MAPK (Salisbury et al., 2009). Although β-catenin knock-down was associated with reduced murine FSHβ expression levels, this could have resulted from a reduction in inhibin levels (Hernandez Gifford et al., 2009), and the direct effect of β-catenin on FSHβ gene expression in the gonadotrope awaits further study.

We have shown that GnRH also activates an additional MAPK, ERK5 (BMK or MAPK7), which increases murine FSHβ promoter activity and mRNA levels (Lim et al., submitted for publication). In T-cells, ERK5 both up-regulates expression and phosphorylates Nur77 allowing it to transactivate various target genes. The increase in Nur77 transcription involves ERK5-mediated phosphorylation of MEF2D which activates the Nur77 promoter (Kasler et al., 2000; Kato et al., 2000). This indicates a likely role for ERK5 in GnRH induction of murine FSHβ gene transcription by Nur77. The site of ERK5-induced phosphorylation on the Nur77 protein has yet to be reported, however, it is phosphorylated on multiple residues; phosphorylation of various residues in the AF-1 domain was seen to stimulate Nur77 activity, while those targeting the DNA binding domain can repress transcription by inhibiting DNA binding (Kasler et al., 2000; Maira et al., 2003; Fujii et al., 2008; Section 2.3). Notably, CRH-induced PKA activation in corticotropes stimulates phosphorylation at the AF-1 domain, which increases transactivation by dimeric but not momomeric Nur77, as a result of the phosphorylation increasing interaction with dimer-specific coactivators (Maira et al., 2003). This indicates promoter-specific effects on the outcome of these signaling pathways. Only when the specific residues targeted by these GnRH-activated kinases are mapped, and the nature of Nur77 binding the murine FSHβ promoter elucidated, will the effects of the various Nur77 phosphorylations and the resulting activation of the FSHβ gene become clearer.

GnRH activation of the calmodulin kinases, CaMKI and CaMKII, occurs as a result of the activation of calmodulin following the GnRH-induced increase in calcium levels (Haisenleder et al., 2003; Lim et al., 2007). Calmodulin was seen to be essential for GnRH-induced ERK activation, although there have been conflicting reports on the role of calcium in mediating GnRH activation of the murine FSHβ gene promoter in LβT2 cells (Roberson et al., 2005; reviewed by Naor, 2009). We have shown that activation of this pathway is crucial in the de-repression of the murine FSHβ gene

in αT3-1 cells (Lim et al., 2007; Melamed, 2008). In its repressed state, this gene is bound by several histone deacetylases (HDACs) which are removed following GnRH-activated CaMKI phosphorylation of HDAC4. The CaMKI-mediated phosphorylation of the class IIa HDACs promotes their association with 14-3-3 proteins, which chaperone them out of the nucleus (Lim et al., 2007; Melamed, 2008). The HDAC4 appears to function as a scaffold for other HDACs in the repressive complex on the murine FSHβ gene promoter and its phosphorylation is followed by its dissociation, along with Sin3A and HDAC3, from the promoter (Lim et al., 2007).

CREB is phosphorylated at Ser133 within 5 min of GnRH treatment in LβT2 cells. Although the kinase responsible for this has not been demonstrated, CREB can be phosphorylated by a number of kinases including various MAPKs, PKA and the CaMKs (Dash et al., 1991; Sheng et al., 1991; Xing et al., 1996). Phosphorylation on this residue enhances CREB interaction with the coactivator CBP and although GnRH did not appear to alter association of total amounts of CREB with the rat FSHβ promoter, the levels of associated pCREB and CBP were seen to increase (Ciccone et al., 2008).

2.3. The GnRH-activated phosphatase, calcineurin, targets both transcription factors and coactivators

GnRH increases expression of the phosphatase calcineurin, and we have shown that calcineurin has a crucial role in GnRH-induced de-repression of the murine FSHβ gene in αT3-1 cells (Lim et al., 2007). This likely relates in part to the ability of GnRH to increase Nur77 expression. In T-cells, calcineurin increases Nur77 expression by dephosphorylating NFAT transcription factors causing their translocation into the nucleus where they interact with MEF2 to activate the Nur77 gene promoter (Blaeser et al., 2000; Youn et al., 2000; McKinsey et al., 2002; Winoto and Littman, 2002). A similar mechanism is indicated for GnRH activation of Nur77 in the gonadotropes, as GnRH causes nuclear translocation of NFAT factors, and the inhibition of calcineurin by cyclosporin A or using specific siRNA was seen to inhibit GnRH-induced expression of Nur77 (Lim et al., 2007 and unpublished). This contrasts with a recent study in which activation of PKA was proposed to be the main pathway leading to activation of Nur77 expression, although the data in that publication supports a strong role of calcium, and the roles of calcineurin were not tested in that study (Hamid et al., 2008).

Calcineurin also targets the Nur77 protein, dephosphorylating serine 354 which is found in the DNA binding domain. Phosphorylation at this residue is inhibitory to its activation of the murine FSHβ gene, and only Nur77 with an unphosphorylated S354 is able to activate transcription (Lim et al., 2007). Previous studies have indicated that this is due to the fact that phosphorylation prevents DNA binding, although we have seen the phospho-S354 bound to the murine FSHβ gene promoter in its repressed state. However, after GnRH treatment, only Nur77 unphosphorylated at this site was detected associated with the promoter (Lim et al., 2007). We consider it likely that the Nur77 is in a larger repressive complex facilitating its association with the DNA through multiple protein–protein and protein–DNA interactions with other repressive factors, including possibly SMRT and various HDACs, some of which interact with the DBD only when it is phosphorylated at this site. The GnRH-induced increase in Nur77 protein levels, coupled with the calcineurin activity ensure that the pS354 Nur77 is replaced at the promoter with the active form, and presumably association with other repressors and co-repressors is lost (Melamed, 2008).

Calcineurin may well have additional targets through which it regulates expression of the FSHβ gene. One example is the CREB coactivator, transducer of regulated CREB (TORC). TORC proteins are expressed in the gonadotropes and over-expression of TORC1 increases murine FSHβ primary transcript levels (unpublished). TORC activity is reportedly regulated by phosphorylation which causes its nuclear export, and its dephosophorylation by calcineurin allows nuclear retention so increasing its activity as a transcriptional coactivator (Bittinger et al., 2004; Takemori et al., 2007). It appears highly likely that GnRH modulates CREB activity through regulating the activity of TORC by calcineurin-mediated dephosphorylation.

3. Regulation by activin

3.1. Activin stimulation of FSHβ gene expression includes signaling via SMAD proteins which interact with various transcription factors, including Pitx proteins

Activin, a member of the TGFβ superfamily, activates rodent FSHβ gene transcription through a pathway of SMAD proteins, involving SMADs 3 and 4, and possibly also SMAD 2 (Bernard, 2004; Suszko et al., 2005). The activated SMADs translocate into the nucleus and bind the DNA, likely recruiting also additional transcription factors to increase binding specificity and rates of transcription (Schmierer and Hill, 2007). A consensus SMAD binding element (SBE; GTCTAGAC) is located between −266 and −259 bp from the tss in the rodent FSHβ gene promoters and appears responsible for their short term response to activin (Suszko et al., 2003; Gregory et al., 2005; Lamba et al., 2006). Two additional half SBEs (at −153 and −120 bp) have also been reported for the murine promoter, as well as other regions (at −139 and −106 bp) that do not contain SBE-like sequences but are required for the full activin effect (McGillivray et al., 2007). Notably, the full SBE sequence is lacking in other mammalian FSHβ gene promoters, and activin-stimulated activation of the ovine FSHβ gene promoter in murine cell lines or in transgenic mice, was shown to be dependent on a different putative SBE element (AGACT), found between −162 and −159 bp from the tss, which is conserved across species (Su et al., 2007). However definitive SMAD binding to this element has not yet been demonstrated and the effect of activin on this promoter was earlier proposed to be SMAD-independent (Safwat et al., 2005).

The human FSHβ gene promoter responds more moderately to activin exposure than that of mice, and requires considerably longer incubation times, while not responding to SMADs 2, 3 and 4 over-expression (Lamba et al., 2006). This may be due to SMAD-independent actions that are induced by activin. A novel role for FOXL2 was recently shown in activating the porcine FSHβ promoter via two binding sites, one of which is conserved across species (Lamba et al., in press, Fig. 1). Although this factor also appears to play a role in activin activation of the murine FSHβ promoter, albeit to a lesser extent, it binds with lower affinity to the human FSHβ promoter. The precise role of FOXL2 in activin-stimulation of these genes is not understood, but FOXL2 has been shown to interact with SMAD 3 and these factors bind adjacent sites in the GnRH-R and follistatin genes (Lamba et al., in press).

A requirement for other distinct SMAD-interacting proteins, that differs across species due to sequence variations in the promoter, might well be responsible for the varying responses to activin. The activin response of the ovine promoter was seen to depend upon a sequence (ACTGCGT; at −171 to −165) found 5′ to the ovine SBE element (Fig. 1). This contains a possible binding site for Runx1 or FAST-1, both of which can interact with SMAD proteins, although the protein binding this sequence has yet to be identified (Su et al., 2007). This sequence is not found in rodents, reinforcing the concept that activin signaling pathways to FSHβ gene promoters of different species are not conserved, both regarding the transactivating factors and the *cis*-binding elements.

The effect of activin on the rodent FSHβ promoter activity was seen to involve interactions with Pitx proteins. Pitx1 was reported

to activate the rat FSHβ gene promoter, through binding a sequence located from −54 to −48 bp from the tss which is highly conserved across species and also binds Pitx2c (Zakaria et al., 2002; Lamba et al., 2008a). The association of Pitx1 with the murine FSHβ promoter was confirmed by ChIP analysis (Lamba et al., 2008a).

The same sequence was also shown to bind Pitx2c in gel-shift assays, and DNA precipitation assays confirmed that at least three Pitx2 isoforms could bind the labeled probe. The role of this proximal Pitx-binding element was seen to be similar, although less obvious, in the human FSHβ gene (Lamba et al., 2008a). Moreover, siRNA-mediated knock-down of Pitx2, or a Pitx2c dominant negative reduced the effect of activin and/or SMAD 3. Conversely, down regulation of SMAD 3 reduced Pitx2c-activated promoter activity, and these proteins were also seen to co-precipitate (Zakaria et al., 2002; Suszko et al., 2003, 2008; Lamba et al., 2008a). As the siRNA targeted all three isoforms of Pitx2 (a, b and c isoforms), and over-expression and Co-IP studies were carried out only for the Pitx2c isoform, functional interactions of SMAD 3 with other Pitx2 isoforms are not known. However all three isoforms can activate the murine FSHβ gene promoter and SMAD 3 was also seen to interact with Pitx2a in two-hybrid assays: the interaction of these proteins is through the highly conserved Pitx homeodomain (Lamba et al., 2008b; Suszko et al., 2003, 2008).

The family of Pitx2 proteins is even more diverse than previously thought, due to alternative promoter usage and various splicing events giving rise to protein isoforms with quite different characteristics; for example Pitx2D, which has a truncated homeodomain preventing its binding to DNA, causes it to act as a transcriptional repressor. A recent report identified two more variants of Pitx2c which result from alternative translation initiation or alternative splicing (Lamba et al., 2008b). Although both encode proteins that can bind the DNA and activate the murine FSHβ gene promoter, they likely form distinct protein–protein interactions and may be subject to different post-translational modifications. The consequences of producing these isoforms form an interesting basis for regulating their activity on the FSHβ and other Pitx2 target genes, and perhaps moderating the effects of activin.

3.2. Activin-activated SMADs interact with steroid receptors

Steroids are powerful determinants of gonadotropin levels and exert direct and indirect effects at both the hypothalamus and the pituitary to alter gonadotropin gene expression. The murine FSHβ gene has been shown to be regulated by androgens, progestins and glucocorticoids whose receptors were found at its promoter in LβT2 cells and, based on gel-shift assays, appear able to bind a number of binding sites (Thackray et al., 2006). These steroid receptors exert synergistic effects together with activin and/or SMAD 3 on the murine and/or ovine promoters (Spady et al., 2004; McGillivray et al., 2007; Thackray and Mellon, 2008). All three steroid receptors interact and/or co-precipitate with SMAD proteins, indicating a direct cross-talk between activin- and steroid-mediated activation at these gene promoters (Melamed et al., 2006a; McGillivray et al., 2007; Thackray and Mellon, 2008). Although a number of sites on the murine and ovine FSHβ promoters have been demonstrated to be able to bind these steroid receptors (Fig. 1), the site at −381 bp on the murine FSHβ promoter appears the most crucial for the steroid receptor-mediated activation and its synergy with activin (Thackray et al., 2006; McGillivray et al., 2007; Thackray and Mellon, 2008). The synergy was shown to be SMAD-dependent and likely involves SMADs binding directly to the DNA, in the same complex as the steroid receptors (Thackray and Mellon, 2008). Interestingly, the binding of the progesterone receptor to this site is induced by GnRH treatment, possibly as a result of its GnRH-stimulated phosphorylation via PKC (An et al., 2009).

We have shown an inhibitory effect of ERα on activin-stimulated murine FSHβ transcription, which appears to occur as a result of ERα competing with SMAD 3 for interaction with SMAD 4 (Melamed et al., 2006a). Although the physiological relevance of this interaction is not known, estradiol treatment was seen to reduce FSHβ primary transcript levels in male castrated rats, although lower doses did not have the same effect in females (Burger et al., 2004, 2007). Unlike the LHβ and αGSU gene promoters, the murine FSHβ gene promoter does not appear to be associated with the ERα, so any effects of estrogen exposure on murine FSHβ transcription are likely indirect (Luo et al., 2005 and unpublished).

Some of the synergistic effects of the steroids have been attributed to their ability to alter levels of transcription or phosphorylation of the SMAD proteins and/or to affect production of the activin antagonist, follistatin (Burger et al., 2004, 2007). Most notably, a 6–10-fold increase was seen in pSMAD 2 levels in cultured male rat primary pituitary cells after testosterone treatment, that correlated with a 2–3-fold increase in FSHβ primary transcript levels (Burger et al., 2007). However, studies have shown that the steroid–activin synergy on activation of the murine FSHβ gene is not due to global effects, as other genes such as the GnRH-R and LHβ were not affected (Thackray and Mellon, 2008). Moreover, although follistatin was shown to reduce the synergy by reducing amount of available activin, it did not completely block gonadal steroid induction, neither did inhibition of SMAD signaling with a chemical inhibitor or through over-expression of SMAD 7. These findings indicate that the effect of the steroids is not dependent on the autocrine effects of activin, although the synergy is clearly SMAD-dependent (Thackray and Mellon, 2008).

It was proposed that the ability of steroids to interact synergistically with activin signaling towards FSHβ promoter activation provides an additional means of differential regulation of gonadotropin hormones production. More specifically, it was suggested that the second peak in FSH levels during the ovulatory cycle, which occurs independently of GnRH and without accompanying increases in LH, relates to increased FSHβ transcription as a result of higher levels of available activin and a rise in steroids, particularly progestins and androgens (Thackray and Mellon, 2008 and references therein).

3.3. Activin also promotes FSHβ splicing

An additional aspect of activin regulation of murine FSHβ gene expression that we have reported, but has not been studied in detail, is its ability to affect processing of the mRNA. Activin treatment of LβT2 cells was seen to increase the ratio of spliced to non-spliced murine FSHβ transcript, which was accompanied by an increase in expression levels of the splice factor, eiF4A3. Given that we also found that over-expression of eif4A3 induces splicing of the murine FSHβ gene and its siRNA-mediated knock-down has the opposite effect, it seems likely that the ability of activin to increase splicing of the murine FSHβ transcript involves eiF4A3 (Feng et al., 2008). Eif4A3 is a DEAD (Asp-Glu-Ala-Asp)-box RNA helicase and a core member of the exon–exon junction complex that associates with the mRNA and directs or moderates downstream RNA processing. Although it was shown to play a role in nonsense mediated decay of mRNAs, it clearly recruits other RNA binding proteins and/or anchors them to the RNA (Palacios et al., 2004; Ferraiuolo et al., 2004).

Mechanisms regulating alternative splicing are poorly understood, but splicing clearly can be regulated through extracellular signaling, and is mediated through phosphorylation by various kinases (Shin and Manley, 2004; Stamm, 2008). Moreover, other TGFβ family members have previously been reported to regulate splicing: during cartilage development, TGFβ-regulated splicing

of the fibronectin gene correlates with the alternative splicing of SRp40, which appears to regulate this splicing event (Han et al., 2007). Interestingly, in *Xenopus*, the TGFβ family member, bone morphophogenic protein (BMP) was shown specifically to up-regulate eif4A3, which induces differentiation to epidermis of cells otherwise destined to a neural fate, mimicking the effects of BMP-4 (Weinstein et al., 1997). This field of hormonal regulation of alternative spicing is in its infancy and clearly warrants further study.

4. GnRH–activin interactions

Microarray analysis of LβT2 cells treated with activin and/or GnRH have demonstrated that the combination of treatments affects expression of a number of genes in either a synergistic or antagonistic manner (Mazhawidza et al., 2006; Zhang et al., 2006). The consequence of such combined treatments on the expression of FSHβ in various species has shown conflicting results. In several studies, chronic GnRH treatment (more than 4–6 h continuous exposure) was seen to reduce activin-stimulated murine and ovine FSH promoter activity (e.g. Melamed et al., 2006a; Shafiee-Kermani et al., 2007). It was proposed that this GnRH inhibitory effect on the activin-stimulated FSHβ transcription reflects the physiological dramatic and prolonged (12–20 h) increase in GnRH levels that is seen just prior to ovulation in sheep, which is accompanied by a drop in FSHβ mRNA levels (Shafiee-Kermani et al., 2007). Given that the GnRH-induced inhibition of the activin effect was mimicked by constitutively active CREB and reversed by ICERII (a CREB inhibitor), it was proposed that CREB is involved, although the exact mechanisms have not been elucidated (Shafiee-Kermani et al., 2007).

It was also suggested that some of the repressive effects of GnRH on stimulation by activin are through GnRH up-regulation of follistatin (Fujii et al., 2002; Burger et al., 2002). However, GnRH-induced changes in follistatin transcript levels were not always detected in conditions in which GnRH inhibited the stimulation by activin, while the quantities of endogenous follistatin are unlikely able to overcome the large amount of administered activin in these experiments (Shafiee-Kermani et al., 2007). Furthermore, this does not explain the lack of antagonism of GnRH on activin stimulation of the LHβ gene (Melamed et al., 2006a).

We have also seen that activin reduces the GnRH effect on murine FSHβ gene expression which is mimicked by SMAD 4 overexpression, while siRNA targeted knock-down of SMAD 4 had the opposite effect (Melamed et al., 2006a). This likely results from the ability of SMAD 4 to interact with a number of transcription factors and compete with them for the same coactivators, as shown in other systems (Verrecchia et al., 2000; Melamed et al., 2006a). These antagonistic effects may be partially due to activin inhibition of ERK activation, which is an immediate response to GnRH and may be down-regulated by 24 h, during which time additional responses would be implemented. Indeed microarray analysis of the effect of activin pre-treatment on the responsiveness of LβT2 cells to acute or chronic GnRH exposure, revealed that the genes affected by the activin pre-treatment after GnRH for 24 h were markedly different from those affected after more acute exposure, and included those encoding proteins involved in secretion and processing of extracellular proteins (Zhang et al., 2006).

Other reports have shown additive or even synergistic interactions of activin and GnRH on human and rodent FSHβ expression, after 4–6 h GnRH treatment (e.g. Gregory et al., 2005; Coss et al., 2007; Wang et al., 2008). This synergy appears to occur via SMAD signaling, as SMAD 7 which acts as a dominant negative SMAD, abolished the stimulatory effect of activin on GnRH-induced promoter activity (Coss et al., 2007). The AP-1 binding sites at −76 bp on the murine FSHβ promoter and at −117 on the human FSHβ promoter were implicated in the synergistic effects (Coss et al., 2007; Wang et al., 2008). Furthermore, activin was seen to increase the AP-1 effect on the human FSHβ promoter activity, although it did not increase GnRH-induced binding of AP-1 factors to the promoter at this site; these effects were mimicked by SMAD 2 (Wang et al., 2008).

One interpretation of these conflicting reports is that chronic and acute GnRH treatments exert opposing effects on the expression of FSHβ, and perhaps on activin signaling pathways (Shafiee-Kermani et al., 2007). This may be further complicated by species-dependent kinetics and pathways in activin signaling: the acute activin effects seen in rodents appear to be dependent on SMAD-dependent signaling, but chronic stimulation, as seen in human FSHβ gene was proposed to be less SMAD-dependent (Lamba et al., 2006). Although the molecular pathways responsible for these different outcomes have not been elucidated, it seems likely that the nature of the GnRH response determines the outcome of the combined treatment, and this may be dependent on the GnRH administration or other factors affecting the behavior of the murine cell lines. The basal levels of FSHβ gene expression do vary in these cells, without any intentional or apparent perturbation. From here we can conclude that the outcome of the cross-talk between GnRH and activin signaling pathways is likely highly dependent on the physiology of the animal which determines the basal activity of the gonadotrope cells and the levels of FSHβ transcription.

5. Conclusions

Although much is known about the GnRH- and activin-stimulated signaling in the gonadotropes, the pathways specifically targeting expression of the FSHβ gene are still somewhat controversial. While some of this may be due to technical reasons, there appears to be a degree of redundancy in the GnRH-induced signaling that activates transcription of this gene, whereby diverse GnRH-activated transcription factors can bind various sequences on the promoter to activate transcription. It seems clear also, based on the divergence of sequence in functional regions of the promoters from different species, that there is some species-specificity in the nature of the response to these regulatory hormones. This is particularly obvious for the full consensus SMAD binding element which is present only in rodents. Given that SMAD proteins usually dimerize, the implication is that the interacting protein partners will also not be conserved across species. While work published in the past few years has clarified some of these ambiguities, more work is required to elucidate the molecules that mediate the hormonal activation of FSHβ genes in different species and under various physiological settings, and not just those than can bind and activate transcription *in vitro*. This will inevitably require greater use of primary cells and transgenic animals.

References

An B.S., Poon, S.L., So, W.K., Hammond, G.L., Leung, P.C., 2009. Rapid effect of GnRH1 on follicle-stimulating hormone β gene expression in LβT2 mouse pituitary cells requires the progesterone receptor. Biol. Reprod., in press.

Bernard, D.J., 2004. Both SMAD2 and SMAD3 mediate activin-stimulated expression of the follicle-stimulating hormone β subunit in murine gonadotrope cells. Mol. Endocrinol. 18, 606–623.

Bonfil, D., Chuderland, D., Kraus, S., Shahbazian, D., Friedberg, I., Seger, R., Naor, Z., 2004. Extracellular signal-regulated kinase, Jun N-terminal kinase, p38, and c-Src are involved in gonadotropin-releasing hormone-stimulated activity of the glycoprotein hormone follicle-stimulating hormone β-subunit promoter. Endocrinology 145, 2228–2244.

Bilezikjian, L.M., Blount, A.L., Leal, A.M.O., Donaldson, C.J., Fischer, W.H., Vale, W.W., 2004. Autocrine/paracrine regulation of pituitary function by activin, inhibin and follistatin. Mol. Cell. Endocrinol. 225, 29–36.

Bittinger, M.A., McWhinnie, E., Meltzer, J., Iourgenko, V., Latario, B., Liu, X.L., Chen, C.H., Song, C.Z., Garza, D., Labow, M., 2004. Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. Curr. Biol. 14, 2156–2161.

Blaeser, F., Ho, N., Prywes, R., Chatila, T.A., 2000. Ca2+-dependent gene expression mediated by MEF2 transcription factors. J. Biol. Chem. 275, 197–209.

Bliss, S.P., Miller, A., Navratil, A.M., Xie, J.J., McDonough, S.P., Fisher, P.J., Landreth, G.E., Roberson, M.S., in press. ERK signaling in the pituitary is required for female but not male fertility. Mol. Endocrinol.

Burger, L.L., Haisenleder, D.J., Wotton, G.M., Aylor, K.W., Dalkin, A.C., Marshall, J.C., 2007. The regulation of FSH beta transcription by gonadal steroids: testosterone and estradiol modulation of the activin intracellular signaling pathway. Am. J. Physiol.: Endocrinol. Metab. 293, E277–E285.

Burger, L.L., Haisenleder, D.J., Aylor, K.W., Dalkin, A.C., Prendergast, K.A., Marshall, J.C., 2004. Regulation of luteinizing hormone-β and follicle-stimulating hormone (FSH)-β gene transcription by androgens: testosterone directly stimulates FSH-β transcription independent from its role on follistatin gene expression. Endocrinology 145, 71–78.

Burger, L.L., Dalkin, A.C., Aylor, K.W., Haisenleder, D.J., Marshall, J.C., 2002. GnRH pulse frequency modulation of gonadotropin subunit gene transcription in normal gonadotropes—assessment by primary transcript assay provides evidence for roles of GnRH and follistatin. Endocrinology 143, 3243–3249.

Chong, K.L., Wang, S., Melamed, P., 2004. Isolation and characterization of the follicle-stimulating hormone β subunit gene and 5′ flanking region of the Chinook salmon. Neuroendocrinology 80, 158–170.

Ciccone, N.A., Lacza, C.T., Hou, M.Y., Gregory, S.J., Kam, K.Y., Xu, S.Y., Kaiser, U.B., 2008. A composite element that binds basic helix loop helix and basic leucine zipper transcription factors is important for gonadotropin-releasing hormone regulation of the follicle-stimulating hormone β gene. Mol. Endocrinol. 22, 1908–1923.

Coss, D., Hand, C.M., Yaphockun, K.K.J., Ely, H.A., Mellon, P.L., 2007. p38 mitogen-activated protein kinase is critical for synergistic induction of the FSH β gene by gonadotropin-releasing hormone and activin through augmentation of c-Fos induction and smad phosphorylation. Mol. Endocrinol. 21, 3071–3086.

Coss, D., Jacobs, S.B., Bender, C.E., Mellon, P.L., 2004. A novel AP-1 site is critical for maximal induction of the follicle-stimulating hormone β gene by gonadotropin-releasing hormone. J. Biol. Chem. 279, 152–162.

Dash, P.K., Karl, K.A., Colicos, M.A., Prywes, R., Kandel, E.R., 1991. cAMP response element-binding protein is activated by Ca^{2+}/calmodulin as well as cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. U.S.A. 88, 5061–5065.

Feng, J., Lawson, M.A., Melamed, P., 2008. A proteomic comparison of immature and mature gonadotropes reveals differentially expressed proteins involved in regulating gonadotropin gene expression, RNA processing and cell survival. Biol. Reprod. 79, 546–561.

Ferraiuolo, M.A., Lee, C.S., Ler, L.W., Hsu, J.L., Costa-Mattioli, M., Luo, M.J., Reed, R., Sonenberg, N., 2004. A nuclear translation-like factor eIF4AIII is recruited to the mRNA during splicing and functions in nonsense-mediated decay. Proc. Natl. Acad. Sci. U.S.A. 101, 4118–4123.

Ferris, H.A., Shupnik, M.A., 2006. Mechanisms for pulsatile regulation of the gonadotropin subunit genes by GnRH1. Biol. Reprod. 74, 993–998.

Fujii, Y., Okada, Y., Moore Jr., J.P., Dalkin, A.C., Winters, S.J., 2002. Evidence that PACAP and GnRH down-regulate follicle-stimulating hormone-β mRNA levels by stimulating follistatin gene expression: effects on folliculostellate cells, gonadotrophs and LβT2 gonadotroph cells. Mol. Cell. Endocrinol. 192, 55–64.

Fujii, Y., Matsuda, S., Takayama, G., Koyasu, S., 2008. ERK5 is involved in TCR-induced apoptosis through the modification of Nur77. Genes Cells 13, 411–419.

Gregory, S.J., Lacza, C.T., Detz, A.A., Xu, S., Petrillo, L.A., Kaiser, U.B., 2005. Synergy between activin A and gonadotropin-releasing hormone in transcriptional activation of the rat follicle-stimulating hormone-β gene. Mol. Endocrinol. 19, 237–254.

Haisenleder, D.J., Burger, L.L., Walsh, H.E., Stevens, J., Aylor, K.W., Shupnik, M.A., Marshall, J.C., 2008. Pulsatile gonadotropin-releasing hormone stimulation of gonadotropin subunit transcription in rat pituitaries: evidence for the involvement of Jun N-terminal kinase but not p38. Endocrinology 149, 139–145.

Haisenleder, D.J., Ferris, H.A., Shupnik, M.A., 2003. The calcium component of gonadotropin-releasing hormone-stimulated luteinizing hormone subunit gene transcription is mediated by calcium/calmodulin-dependent protein kinase type II. Endocrinology 144, 2409–2416.

Hall, J.H., 2004. Neuroendocrine controlof the menstrual cycle. In: Strauss, J.F., Barbieri, R.L. (Eds.), Yen and Jaffe's Reproductive Endocrinology, 5th Ed. Elsevier Saunders, Philadelphia, PA, pp. 195–211.

Hamid, T., Malik, M.T., Millar, R.P., Kakar, S.S., 2008. Protein kinase A serves as a primary pathway in activation of Nur77 expression by gonadotropin-releasing hormone in the LβT2 murine pituitary gonadotroph tumor cell line. Int. J. Oncol. 33, 1055–1064.

Han, F., Gilbert, J.R., Harrison, G., Adams, C.S., Freeman, T., Tao, Z., Zaka, R., Liang, H., Williams, C., Tuan, R.S., Norton, P.A., Hickok, N.J., 2007. Transforming growth factor-beta1 regulates fibronectin isoform expression and splicing factor SRp40 expression during ATDC5 chondrogenic maturation. Exp. Cell Res. 313, 1518–1532.

Hernandez Gifford, J.A., Hunzicker-Dunn, M.E., Nilson, J.H., 2009. Conditional deletion of β-catenin mediated by *Amhr2cre* in mice causes female infertility. Biol. Reprod. 80, 1282–1292.

Jorgensen, J.S., Quirk, C.C., Nilson, J.H., 2004. Multiple and overlapping combinatorial codes orchestrate hormonal responsiveness and dictate cell-specific expression of the genes encoding luteinizing hormone. Endocr. Rev. 25, 521–542.

Kakar, S.S., Winters, S.J., Zacharias, W., Miller, D.M., Flynn, S., 2003. Identification of distinct gene expression profiles associated with treatment of LβT2 cells with gonadotropin-releasing hormone agonist using microarray analysis. Gene 308, 67–77.

Kasler, H.G., Victoria, J., Duramad, O., Winoto, A., 2000. ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. Mol. Cell. Biol. 20, 8382–8399.

Kato, Y., Zhao, M., Morikawa, A., Sugiyama, T., Chakravortty, D., Koide, N., Yoshida, T., Tapping, R.I., Yang, Y., Yokochi, T., Lee, J.D., 2000. Big mitogen-activated kinase regulates multiple members of the MEF2 protein family. J. Biol. Chem. 275, 18534–18540.

Lamba, P., Santos, M.M., Philips, D.P., Bernard, D.J., 2006. Acute regulation of murine follicle-stimulating hormone beta subunit transcription by activin A. J. Mol. Endocrinol. 36, 201–220.

Lamba, P., Khivansara, V., D'Alessio, A.C., Santos, M.M., Bernard, D.J., 2008a. Paired-like homeodomain transcription factors 1 and 2 regulate follicle-stimulating hormone β-subunit transcription through a conserved cis-element. Endocrinology 149, 3095–3108.

Lamba, P., Hjalt, T.A., Bernard, D.J., 2008b. Novel forms of paired-like homeodomain transcription factor 2 (PITX2): generation by alternative translation initiation and mRNA splicing. BMC Mol. Biol. 9, No. 31.

Lamba, P., Fortin, J., Tran, S., Wang, Y., Bernard, D.J., in press. A novel role for the forkhead transcription factor FOXL2 in activin A-regulated follicle-stimulating hormone beta subunit transcription. Mol. Endocrinol.

Lim, S., Tan, J.H., Naor Z., Rajagopal, G., Melamed P., submitted for publication. Negative feedback governs gonadotrope frequency-decoding of gonadotropin releasing hormone pulse-frequency.

Lim, S., Luo, M., Koh, M., Yang, M., Abdul Kadir, M.N., Tan, J.H., Ye, Z., Wang, W., Melamed, P., 2007. Distinct mechanisms involving diverse histone deacetylases repress expression of the two gonadotropin β-subunit genes in immature gonadotropes, and their actions are overcome by GnRH. Mol. Cell. Biol. 27, 4105–4120.

Luo, M., Koh, M., Feng, J., Wu, Q., Melamed, P., 2005. Cross talk in hormonally regulated gene transcription through induction of estrogen receptor ubiquitylation. Mol. Cell. Biol. 25, 7386–7398.

Maira, M., Martens, C., Batsche, E., Gauthier, Y., Drouin, J., 2003. Dimer-specific potentiation of NGFI-B (Nur77) transcriptional activity by the protein kinase A pathway and AF-1-dependent coactivator recruitment. Mol. Cell. Biol. 23, 763–776.

Mazhawidza, W., Winters, S.J., Kaiser, U.B., Kakar, S.S., 2006. Identification of gene networks modulated by activin in LβT2 cells using DNA microarray analysis. Histol. Histophathol. 21, 167–178.

McGillivray, S.M., Thackray, V.G., Coss, D., Mellon, P.L., 2007. Activin and glucocorticoids synergistically activate follicle-stimulating hormone β-subunit gene expression in the immortalized LβT2 gonadotrope cell line. Endocrinology 148, 762–773.

McKinsey, T.A., Zhang, C.L., Olson, E.N., 2002. MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem. Sci. 27, 40–47.

Melamed, P., 2008. The role of histone deacetylases in expression of gonadotropin subunit genes. Trends Endocrinol. Metab. 19, 25–31.

Melamed, P., Abdul Kadir, M.N., Wijeweera, A., Seah, S., 2006a. Transcription of gonadotropin β subunit genes involves cross-talk between the transcription factors and co-regulators that mediate actions of the regulatory hormones. Mol. Cell. Endocrinol. 252, 167–183.

Melamed, P., Zhu, Y., Tan, S.H., Xie, M., Koh, M., 2006b. Gonadotropin releasing hormone activation of c-Jun, but not Egr-1, stimulates transcription of a luteinizing hormone β subunit gene. Endocrinology 147, 3598–3605.

Miller, W.L., Shafiee-Kermani, F., Strahl, B.D., Huang, H.J., 2002. The nature of FSH induction by GnRH. Trends Endocrionol. Metab. 13, 257–263.

Naor, Z., 2009. Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. Front. Neuroendocrinol. 30, 10–29.

Palacios, I.M., Gatfield, D., St Johnston, D., Izaurralde, E., 2004. An eIF4AIII-containing complex required for mRNA localization and nonsense-mediated mRNA decay. Nature 427, 753–757.

Roberson, M.S., Zhang, T., Li, H.L., Mulvaney, J.M., 1999. Activation of the p38 mitogen-activated protein kinase pathway by gonadotropin-releasing hormone. Endocrinology 140, 1310–1318.

Roberson, M.S., Bliss, S.P., Xie, J.J., Navratil, A.M., Farmerie, T.A., Wolfe, M.W., Clay, C.M., 2005. Gonadotropin-releasing hormone induction of extracellular-signal regulated kinase is blocked by inhibition of calmodulin. Mol. Endocrinol. 19, 2412–2423.

Sadie, H., Styger, G., Hapgood, J., 2003. Expression of the murine gonadotropin-releasing hormone receptor gene in alpha T3-1 gonadotrope cells is stimulated by cyclic 3′,5′-adenosine monophosphate and protein kinase A, and is modulated by steroidogenic factor-1 and Nur77. Endocrinology 144, 1958–1971.

Safwat, N., Ninomiya-Tsuji, J., Gore, A.J., Miller, W.L., 2005. Transforming growth factor beta-activated kinase 1 is a key mediator of ovine follicle-stimulating hormone beta-subunit expression. Endocrinology 146, 4814–4824.

Salisbury, T.B., Binder, A.K., Grammer, J.C., Nilson, J.H., 2009. GnRH-regulated expression of Jun and JUN target genes in gonadotropes requires a functional interaction between TCF/LEF family members and β-catenin. Mol. Endocrinol. 23, 402–411.

Schmierer, B., Hill, C.S., 2007. TGF β-SMAD signal transduction: molecular specificity and functional flexibility. Nat. Rev. Mol. Cell. Biol. 8, 970–982.

Shafiee-Kermani, F., Han, S.O., Miller, W.L., 2007. Chronic gonadotropin-releasing hormone inhibits activin induction of the ovine follicle-stimulating hormone β-subunit: involvement of 3′,5′-cyclic adenosine monophosphate response element binding protein and nitric oxide synthase type I. Endocrinology 148, 3346–3355.

Sheng, M., Thompson, M.A., Greenberg, M.E., 1991. CREB: a Ca^{2+}-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252, 1427–1430.

Shin, C., Manley, J.L., 2004. Cell signalling and the control of pre-mRNA splicing. Nat. Rev. Mol. Cell. Biol. 5, 727–738.

Spady, T.J., Shayya, R., Thackray, V.G., Ehrensberger, L., Bailey, J.S., Mellon, P.L., 2004. Androgen regulates follicle-stimulating hormone β gene expression in an activin-dependent manner in immortalized gonadotropes. Mol. Endocrinol. 18, 925–940.

Stamm, S., 2008. Regulation of alternative splicing by reversible protein phosphorylation. J. Biol. Chem. 283, 1223–1227.

Su, P., Shafiee-Kermani, F., Gore, A.J., Jia, J.J., Wu, J.C., Miller, W.L., 2007. Expression and regulation of the β-subunit of ovine follicle-stimulating hormone relies heavily on a promoter sequence likely to bind smad-associated proteins. Endocrinology 148, 4500–4508.

Suszko, M.I., Antenos, M., Balkin, D.M., Woodruff, T.K., 2008. SMAD3 and Pitx2 cooperate in stimulation of FSH β gene transcription. Mol. Cell. Endocrinol. 281, 27–36.

Suszko, M.I., Balkin, D.M., Chen, Y., Woodruff, T.K., 2005. SMAD3 mediates activin-induced transcription of follicle-stimulating hormone β-subunit gene. Mol. Endocrinol. 19, 1849–1858.

Suszko, M.I., Lo, D.J., Suh, H., Camper, S.A., Woodruff, T.K., 2003. Regulation of the rat follicle-stimulating hormone β-subunit promoter by activin. Mol. Endocrinol. 17, 318–332.

Takemori, H., Kajimura, J., Okamoto, M., 2007. TORC-SIK cascade regulates CREB activity through the basic leucine zipper domain. FEBS J. 274, 3202–3209.

Thackray, V.G., Mellon, P.L., 2008. Synergistic induction of follicle-stimulating hormone β-subunit gene expression by gonadal steroid hormone receptors and smad proteins. Endocrinology 149, 1091–1102.

Thackray, V.G., McGillivray, S.M., Mellon, P.L., 2006. Androgens, progestins, and glucocorticoids induce follicle-stimulating hormone β-subunit gene expression at the level of the gonadotrope. Mol. Endocrinol. 20, 2062–2079.

Verrecchia, F., Pessah, M., Atfi, A., Mauviel, A., 2000. Tumor necrosis factor-alpha inhibits transforming growth factor-beta/Smad signaling in human dermal fibroblasts via AP-1 activation. J. Biol. Chem. 275, 30226–30231.

Wang, S., Zhu, Y., Melamed, P., 2009. The molecular regulation of Chinook salmon gonadotropin β-subunit gene transcription. Gen. Comp. Endocrinol. 161, 34–41.

Wang, Y., Fortin, J., Lamba, P., Bonomi, M., Persani, L., Roberson, M.S., Bernard, D.J., 2008. Activator protein-1 and smad proteins synergistically regulate human follicle-stimulating hormone β-promoter activity. Endocrinology 149, 5577–5591.

Weinstein, D.C., Honore, E., Hemmati-Brivanlou, A., 1997. Epidermal induction and inhibition of neural fate by translation initiation factor 4AIII. Development 124, 4235–4242.

Winoto, A., Littman, D.R., 2002. Nuclear hormone receptors in T lymphocytes. Cell 109, S57–S66.

Wurmbach, E., Yuen, T., Ebersole, B.J., Sealfon, S.C., 2001. Gonadotropin-releasing hormone receptor-coupled gene network organization. J. Biol. Chem. 276, 47195–47201.

Xing, J., Ginty, D.D., Greenberg, M.E., 1996. Coupling of the RAS–MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 273, 959–963.

Youn, H.D., Grozinger, C.M., Liu, J.O., 2000. Calcium regulates transcriptional repression of myocyte enhancer factor 2 by histone deacetylase 4. J. Biol. Chem. 275, 22563–22567.

Zakaria, M.M., Jeong, K.H., Lacza, C., Kaiser, U.B., 2002. Pituitary homeobox 1 activates the rat FSHβ (rFSHβ) gene through both direct and indirect interactions with the rFSHβ gene promoter. Mol. Endocrinol. 16, 1840–1852.

Zhang, H., Bailey, J.S., Coss, D., Lin, B., Tsutsumi, R., Lawson, M.A., Mellon, P.L., Webster, N.J.G., 2006. Activin modulates the transcriptional response of LβT2 cells to gonadotropin-releasing hormone and alters cellular proliferation. Mol. Endocrinol. 20, 2909–2930.