Review Article

Regulation of Gonadotropins Synthesis

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The Gonadotropins play important role in the regulation of gonadal development and function. The regulation of synthesis and secretion of these hormones is regulated by variety of factors that originate from different tissues. In this review, we have made an attempt to review the current knowledge on the regulation of gonadotropin synthesis.

Key Words: Gonadotrophins, Glycoprotein harmone genes, Transcriptional regulation

The gonadotropins

The gonadotropins, luteinizing hormone (lutropin, LH) and follicle-stimulating hormone (follitropin, FSH) through their ability to regulate the synthesis of the sex steroids (androgens and estrogens), are indispensable for proper gonadal development and function[1]. The synthesis and secretion of gonadotropins are regulated by the hormones produced by the pituitary itself (such as follistatin and activin), as well as the hormones from the hypothalamus and the gonads[2,3,4,5,6]. This tripartite regulatory network is known as the hypothalamus-pituitary-gonadal (HPG) axis (Fig. 1).

The neurons in the hypothalamus synthesize and secrete the decapeptide hormone, gonadotropin-releasing hormone (GnRH). The secretion of GnRH is positively governed by the neurotransmitters such as the catecholamines, epinephrine and norepinephrine, and negatively by the endogenous opioids such as β-endorphin. The secreted GnRH binds to its receptors present on the gonadotropin secreting cells, the gonadotropes in the pituitary and stimulate the synthesis and secretion of LH and FSH. These gonadotropes comprise of 7-14% of cells in the anterior pituitary gland. Both LH and FSH are secreted by the same gonadotrope cell. FSH and LH belong to the family of glycoprotein hormones, other members of the family being thyroid stimulating hormone (thyrotopin, TSH) that regulates thyroid function and chorionic gonadotropin (CG) that is present only in equines and primates and is critical for successful establishment and maintenance of pregnancy. The members of this family are structurally related glycoprotein hormones and are heterodimeric in nature, comprising of an identical α subunit associated noncovalently with a hormone specific β subunit[7]. An intricate yet balanced, feedback regulation exerted by hormones synthesized and secreted from each endocrine organ is pivotal for the proper reproductive functioning. The actions of LH and FSH on the male and female reproductive system are described below.

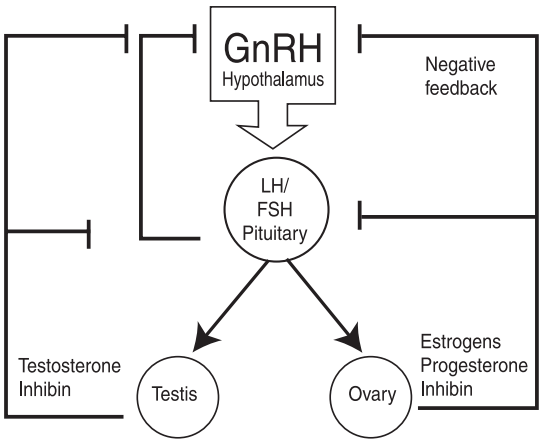

Figure 1: *The hypothalamus-pituitary-gonadal (HPG) axis. The principal hormones involved in the HPG axis are mentioned*

Gonadotropes: The cells that make gonadotropins

As mentioned earlier, LH and FSH are synthesized by gonadotropes present in the anterior pituitary gland. As the secretion pattern of LH and FSH were different, it was suggested earlier that these hormones were made distinctly by different gonadotropes. But with techniques such as immunocytochemistry using LH and FSH antibodies, it was shown that these gonadotropins were present in the same gonadotrope cells[8]. Subsequently, using β subunit specific antibodies, it was confirmed that both the hormones were stored in the same cells. Light microscopic morphometry analysis revealed that the gonadotropes comprised of approximately 14% of the cells of an adult rat pituitary gland. The technique also confirmed that gonadotropes varied considerably in size ranging from 30 to 160 μm^2 in the male rat and 130-170 μm^2 in the female. The difference in sizes has been termed as functional heterogeneity and has been attributed to the gonadotropes being present in several different stages of the secretory cycle, which is under the influence of physiological changes. Subsequent work on purification and characterization of gonadotropes has

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been extensively carried out by Childs and co-workers, where they first noted an increase in the percentage of gonadotropes bearing LHβ and/or FSHβ mRNAs[9]. Although this observation was partly explained by the appearance of new gonadotropes, 60% of the cells also stained positive for growth hormone (GH)[10,11]. Further studies validated this observation and suggested that a subset of the GH secreting cells (somatotropes) get converted to "transitional gonadotropes" just before the proestrous secretory period[10]. Thus, number and size of gonadotropes differ considerably depending on the various physiological states.

Storage and secretion of gonadotropins from the gonadotropes

The synthesis of the gonadotropins occurs under the influence of the GnRH and host of other hormones from the pituitary and the gonads[12]. These are described in detail later. Upon synthesis, the gonadotropins have two modes of release. They can be released immediately (the rate of synthesis is almost equal to that of release). This is called the constitutive pathway. Alternatively, the gonadotropins can be packaged as granules and stored in secretory vesicles. This constitutes the secretory pathway that is generally used for regulated release of stored hormones. Upon appropriate signal(s), these granules are released by exocytosis. FSH is generally secreted via the constitutive pathway. While the basal levels of LH are also released through the constitutive pathway, majority of LH is generally stored in secretory vesicles. LH has been shown to be associated with the secretory protein, secretogranin II as electron dense granules in the secretory vesicles[13,14,15]. During the preovulatory surge, under the influence of GnRH, LH-secretogranin II complex is released as granules from the gonadotropes. Thus, differential packaging of LH and FSH into separate granules is important for their disparate release during the reproductive cycles. In this way, the gonadotropes go through the process of synthesis, storage and release for every cycle[12].

To understand the molecular mechanisms of gonadotropin synthesis, storage and secretion along with regulation of these processes by the HPG axis, a robust gonadotrope model system is required that is lacking at present. Although many studies have been carried out using primary pituitary cell cultures, these are beset with inherent problems. The gonadotropes comprise only 7-14% of the cells and are thus present in a heterogeneous population with other secretory cells such as thyrotropes, somatotropes, mammotropes and other nonendocrine cells such as endothelial, folliculo-stellate and stromal cells. Moreover, the gonadotropes cannot be propagated *in vitro*. To circumvent these problems, approaches to generate immortalized cell lines expressing the gonadotropins along with transgenic mouse models have been established[13,14]. Employing these tools, most of the regulatory aspects of gonadotropin expression have been elucidated. However, limitations of the cell lines themselves, especially the unavailability of a FSH secreting gonadotrope cell line, has greatly hampered the progress to characterize the underlying mechanisms of gonadotropin regulation.

Physiological roles for gonadotropins in the female

FSH is necessary for production of oocytes. It also plays pivotal roles in selection of the dominant Graafian follicle, development of its follicular cavity (the antrum) and for the expression of LH receptors on granulosa cells. Unavailability of the hormone results in follicular death by apoptosis, termed follicular atresia. Folliculogenesis can be restored in such cases by exogenous administration of FSH. FSH, in concert with the midcycle LH surge, is required for ovulation to occur. The subsequent luteinization (formation of corpus luteum) is driven by LH. LH also stimulates steroidogenesis in the follicular thecal cells, which in response to LH synthesize and secrete testosterone. FSH is known to stimulate aromatase activity in the granulosa cells that converts testosterone to estradiol. FSH also stimulates the production of the steroid hormone progesterone by the granulosa cells, while LH is necessary for maintenance of progesterone production by the corpus luteum. Thus, LH and FSH act synergistically to regulate production of steroids by the ovary.

Physiological roles for gonadotropins in the male

The receptors for LH are present on the Leydig cells of the testis. LH acts on the Leydig cells and stimulates production of testosterone, which in turn binds to its receptor present in the Sertoli cells. Testosterone is known to modulate the secretion of Sertoli cell factors such as androgen binding protein (ABP). Thus LH, by controlling testosterone production is indispensable for maintaining spermatogenesis. Testosterone also acts on the pituitary to decrease LH production (Fig. 1).

The role of FSH in the regulation of spermatogenesis has not been unequivocally established. The Sertoli cells express FSH receptors and in response to the hormone synthesize and secrete inhibin and estrogen. These hormones are known to downregulate FSH secretion from the pituitary. FSH has been demonstrated to be essential for immature testis development and has been shown to control Sertoli cell population by regulating its proliferation. But as discussed above, the exact role of FSH in male reproduction has been a subject of debate. Although requirement for follicle-stimulating hormone (FSH) in the initiation of spermatogenesis is well documented, mice lacking FSHβ subunit were found to

be fertile. However, these mice had small testes and displayed reduced sperm number and motility. Similarly, the male FSH receptor knockout (FORKO) mice also showed reduced sperm production and sperm quality and therefore the adult FORKO males have reduced fertility[16]. It was also demonstrated that absence of gonadotropins also resulted in germ cell death by apoptosis. Amongst them, the pachytene spermatocytes were found to be most sensitive to absence of FSH following specific immunoneutralization of FSH[17]. In primates (rhesus, as well as, the bonnet monkeys), passive immunizations against FSH led to a 50% reduction in testicular size and decline in sperm production[18,19] and in some cases even leading to infertility[20,21,22,23,24]. Nevertheless, importance of LH and FSH in gondal function is highlighted by the fact that neutralization of gonadotropins by either active or passive immunization or by administration of GnRH antagonists[24] or active immunization with GnRH[25] results in infertility which is due to the apoptosis of germ cells[17]. It has been suggested that the effect of the lack of FSH/FSH-R signaling on the quality of primate sperm may be much more dramatic than that observed in the rodent sperm[16].

Additional insights into the physiological roles for LH and FSH are demonstrated by some of the clinical cases involving naturally occurring mutations in these hormone subunits and information from the knockout and transgenic mice (reviewed in 1) will be discussed towards the end.

Structural organization, chromosomal location, sequence homology and evolution of glycoprotein hormone genes

The common α subunit and each of the pituitary β subunits are encoded by unique, single-copy genes that are specifically expressed in the pituitary gland[1,3,26]. In humans, the α subunit gene is located on chromosome the 6p21.1-23, the LHβ subunit on chromosome the 19q13.32 and the FSHβ subunit is located on the chromosome 11p13. These genes comprise of four exons and three introns[27]. In the α subunit gene, the first intron is the longest (6.5-14.7 kb across the species) and it separates the 5'untranslated region (UTR) found in exon 1 from that of the leader sequence and the first 9 amino acids of mature polypeptide (exon 2). Exon 3 codes for amino acids 10-71, followed by intron 3 and the terminal amino acids 72-96 and, the 3'UTR harboring exon 4. Although LH has been found in all the mammalian species examined, CG, to date, has been found only in the equine and primate placentas. The LHβ and CGβ are encoded by a cluster of 7 genes with high homology. It appears that LHβ is encoded by one gene while the CGβ is consists of 6 genes or psuedogenes. The LHβ subunit is expressed in the pituitary while atleast 5 of the hCGβ genes are expressed in the placenta and choriocarcinoma cells. Among these, 3, 5 and 8 appear to contribute primarily to the steady state hCGβ mRNA levels. Further differences appear in the transcription initiation sites of the LHβ and CGβ genes. CGβ is transcribed almost 350 base pair upstream of the LHβ start site. This not only makes their 5'UTRs disparate, but also suggests that their promoter might also be distinct with a unique set of transcription regulatory elements.

As in case of the α subunit and the LHβ subunit genes, the FSHβ subunit also is encoded by a single gene, except in the sheep (2 FSHβ genes). Intriguingly, although the glycoprotein hormones have comparable number of amino acids in their polypeptide chains, only FSHβ has a very long mRNA (Fig. 2).

This is contributed by a lengthy 3'UTR. Although 3'UTRs of many mRNAs are involved in posttranscriptional regulation of gene expression, the physiological significance of such a long 3'UTR is unknown. We demonstrated recently that the FSHβ 3' untranslated region (3'UTR) that is highly conserved across the species. Sequence analyses of the mouse, rat, human, bovine and the ovine 3'UTR revealed presence of elements implicated in mRNA instability and translational control such as AU-Rich Element (ARE)

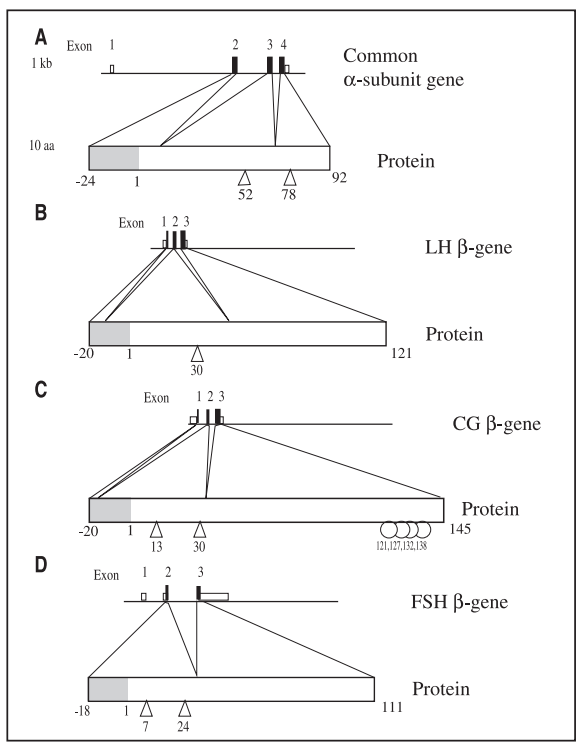

Figure 2: *Structural organization of the human gonadotropin genes Legends: closed bars = coding regions, open bars = UTRs, shaded bar = signal peptide. Numbers indicate start and end of signal peptide and the length of the mature protein product, considering the first amino acid of the mature protein as 1 (1, reproduced with permission of the publisher)*

and LOX-DICE elements. Further, the bovine FSHβ 3'UTR was shown to downregulate reporter expression in cell lines. This was as a result of decreased association of reporter mRNA in presence of the FSHβ 3'UTR, suggesting that the down-regulatory effect may be exerted at the translational level[28].

Interestingly, all of the hormone specific β subunits show amino acid sequence homology with their 12 cysteine residues conserved at identical positions. Among them, human LHβ and hCGβ share the highest relatedness (with 82% homology). The other β subunits range from 25-40% when compared pairwise.

Evolutionary history for the glycoprotein hormone gene family has been predicted by sequence comparison (nucleotide and amino acid) and on the basis of phylogenetic analyses. It has been suggested that all the members of this gene family evolved from a single ancestor through gene duplications. The α subunit and an ancestral β subunit were the result of the first duplication event. Further, duplication of the ancestral gene of the β subunit generated the LHβ subunit gene and the ancestor for the TSHβ and FSHβ groups. Present TSHβ and FSHβ subunit genes evolved from this ancestral gene[29].

Transcriptional regulation of gonadotropin subunit genes

As described earlier, the gonadotropins are expressed in the same gonadotrope cell. Appropriate expression of FSH and LH requires coordinated expression of each of the three subunit genes, namely the common α, LHβ and FSHβ subunits. This is brought about by complex yet intricate transcriptional process that is not completely understood. GnRH from the hypothalamus and gonadal hormones are the factors that modulate individual subunit expression. Studies carried out for these purposes utilize primary pituitary cell cultures, transformed cells lines, αT3-1 and LβT2, along with transgenic and knockout mouse models. Owing to absence of a cell line that expresses FSH constitutively, it is the least characterized of all the three subunit genes.

Ontogeny of expression of gonadotropin subunit genes

During embryonic development, the anterior pituitary gland develops from Rathke's pouch, which is an outgrowth of ectoderm from the buccal cavity. The gonadotrope cell lineage originates from the ventral part of the embryonic pituitary (Fig. 3). At embryonic day 10 (e10.5), the α subunit expression is first detected. This is followed by the expression of LHβ (e16.5) and lastly FSHβ (e17.5) transcripts[30]. A cascade of transcription factors brings about the initiation and later, maintenance of the basal level of gonadotropin subunit expression. Multiple signaling cascade control the spatial and temporal expression of these transcription factors. This sequential expression of these transcription factors determines gonadotrope differentiation. One of the earliest transcription factors expressed that are necessary for the gonadotropin subunit expression are pituitary

The relationship between the organogenesis of the anterior pituitary and the sequential activation of genes encoding transcription factors known to regulate gonadotrophin gene expression in mice. Shaded bars indicate the initiation and duration of gene expression. Rathke's pouch is formed from hypophyseal placode of oral ectoderm that invaginates at approximately embryonic day 9.5 (e9.3). Cell proliferation then occure in the anterior pituitary before pituitary cell-specific genes can be identified. The earliest identifiable marker of pituitary gene expression is the common α-subunit, which is activated at e10.5, a full 6 days before LH β-subunit (e 16.5) and FSH β-subunit (e17.5) gene expression is detected. NE: neuroectoderm.

Figure 3: *Ontogeny of the gonadotrope, gonadotropins and the transcription factors that regulate gonadotropin gene expression (30, © Society for Reproduction and Fertility (1999), reproduced by permission)*

homeobox 1 (Ptx1) and Hesx1-Rpx. These transcription factors are expressed specifically in the Rathke's pouch and are required for cellular expansion of the pituitary before differentiation. The expression of pLIM-Lhx3 homeobox gene determines the specificity of the pituitary lineages. Hesx1-Rpx transactivates LHβ expression *in vitro* whereas pLIM-Lhx3 specifies α subunit expression. Another important transcription factor is steroidogenic factor 1 (SF-1) that has been shown to stimulate α subunit and LHβ subunit expression but not FSHβ expression[31]. The prepubertal expression of gonadotropins seem to require the expression of yet another transcription factor, Otx1, as the knockout mice for Otx1 displayed impaired prepubertal expression of gonadotropins which recovered to normal levels after attaining puberty. A novel role for Otx-related HD protein in gonadotrope maturation during development has also been proposed[32]. Inspite of elucidation of roles of several transcription factors, no specific transcription factor(s) or mechanism has been identified that directs the expression of all the subunits.

Apart from the basal transcriptional activity of the three subunits, the regulated gene expression is brought about by GnRH and other gonadal factors. Although the transcription events that unfold on the promoters of gonadotropin subunit genes remain largely unknown, following information has been obtained regarding some of the critical regions of the promoters and their interacting transcription factors that are involved in the basal and regulated expression of a, LHβ and FSHβ subunit genes.

Transcriptional regulation of α subunit gene

Roberson and co-workers showed that basal gene expression of the α subunit requires a specific region (-337 to –330 bp) in its promoter known as the pituitary glycoprotein hormone basal element (PGBE)[33]. The PGBE is known to interact with a LIM-homeodomain transcription factor to effect basal transcription. Apart from this basal expression, GnRH responsiveness has been localized to –346 to –244 bp in the human and to two sites (-406 to –399 bp and PGBE) in the mouse promoter. The -406 to –399 bp site in the mouse promoter binds to an unknown factor that is regulated by GnRH through the mitogen activated protein kinase (MAPK) pathway. The alpha subunit, apart from the gonadotrope is also expressed in the thyrotrope and the placental trophoblast cells. Several elements and their respective transfactors have been identified and are listed in table 1. Some of these elements are functional exclusively in one of the three cell types while some of them are active in all the three cell types. For example, gonadotrope specific elements such as αBE1 and αBE2 interact with αBP1 and αBP2 respectively, while

Table 1: Elements in the a promoter region and interacting transcription factors (34, reproduced with permission of the publisher)

Element	Position	Cell type	Factor	Family
E box	–21/–16 (h)	G, Th	*αEB1*	Basic helix-loop-helix
E box	–51/–45 (h)	G, Th	USF, *αEB2*	Basic helix-loop-helix
Pitx 1-responsive element	–80/–65 (h) –398/–385 (m)	G G,Th	Pitx1	Homeodomain
CCAAT box	–100/–80 (h)	Tr	*α*CCAAT *binding factor*	CCAAT box
Junctional regulatory element (JRE)	–120/–100 (h)	Tr	Distal-less 3(Dlx3)	Homebox
CRE	–146/–111 (h) –144/–126 (m)	G,Tr Th	CRE-binding proteins (CREB, CREM, cJun, ATF-2)	bZip proteins
Upstream regulatory element (URE)	–182/–141 (h)	Tr	UREB	*Unknown*
*α*ACT	–161/–141 (h)	G, Th, Tr	GATA2, GATA3	GATA (zinc finger)
TSE	–182/–159 (h)	Tr	*TSE-binding protein (TSEB); AP-2*	*Unknown*
GSE	–220/–211 (h) –220/–202 (m) –223/–190 (m)	G G Th	SF-1 *Unknown*	Orphan nuclear receptor
*α*BE2	–296/–285 (h)	G	*αB(E)-binding protein 2 (αBP2)*	*Unknown*
*α*BE1	–316/–302 (h)	G	*αBP1*	*Unknown*
PGBE	–329/–320 (h) –344/–300 (m)	G, Th	LH-2, Lhx3, pLIM	LIM homeodomain
GnRH-RE	–406/–399 (m)	G, Th	*Unknown*	*Unknown*
Msx-1 binding element	–440/–413 (m)	Th	Msx-1	AT-rich homeoprotein
Thyrotrope-specific element	–507/–417 (m)	Th	*Unknown*	*Unknown*
Mouse distal enhancer	–4600/–3700 (m)	G, Th	*Unknown*	*Unknown*

h: human, m: mouse, G: gonadotrope, Th: thyrotrope, Tr: trphoblast, CREM, CRE modulator, USF, upstream stimulatory factor, AP-2, activator protein-2, Italics indicate factor named but not proven or unkonown

Msx-1 binding with Msx-1 element has been seen in thyrotropes and trophoblast-specific element (TSE) interaction with TSEB has been shown to be important for placental expression of the α subunit[34].

Another important element that significantly contributes to α subunit promoter activity is the cAMP response element (CRE). There is one conserved CRE present in the α subunit promoter in all the mammalian species examined thus far. Many transcription factors have been shown to interact with the CRE of α subunit. These include CRE binding protein (CREB), CRE modulator, c-Jun, activating transcription factor (ATF)1, and ATF2. However, CRE interacts with the heterodimer of c-Jun/ATF2 with much higher affinity than CREB and therefore it has been suggested that c-Jun/ATF2 heterodimers are the preferred binding partners for CRE sequences in the gonadotrope. The sex steroids, testosterone and estradiol are known to downregulate LH and FSH levels. To test whether the steroids function by suppressing α subunit expression, cotransfections assays using a human 1,500 bp 5' upstream region (promoter) of α subunit and the steroid receptors were carried out in αT3-1 cells. The α subunit promoter activity was suppressed in presence of androgen receptor but estradiol receptors had no effect. It was therefore suggested that the androgens probably acted directly at the promoter of α subunit while estradiol might downregulate gonadotropin secretion by acting through hypothalamus. Interestingly, the repression mediated by androgen receptor is independent of its DNA binding. Furthermore, this downregulatory effect was localized to the CRE within the α subunit promoter. Therefore investigations were carried out to identify the transcription factors that interacted with AR. These studies revealed that AR interacted with c-Jun and ATF2, individually as well as a heterodimer. However the CREB did not interact with AR, suggesting that specific interaction between the androgen receptor and selective CRE binding proteins is responsible for the suppressing α promoter activity[34,35].

Thus, presence of multiple elements that interact with different transcription factors suggest their synergistic effect on the α subunit promoter activity.

Transcriptional regulation of LHβ subunit gene

As in the case of α subunit expression, LHβ is expressed at basal levels, which is also upregulated by GnRH and modulated by the steroids and peptide hormones. Analysis of the 5' upstream region of the LHβ gene revealed that a proximal 140 bp region in the promoter was important for LHβ expression. Using transgenic approaches, it was shown that the elements in bovine LHβ promoter that bind the transcription factors Pitx1 (*bicoid*-related homeodomain protein), SF-1 (an orphan nuclear receptor) and NF-Y (nuclear transcription factor Y) were required for LHβ expression. Silencing the expression of Pitx1 using antisense RNA resulted in loss of α subunit and Lim3/Lhx3 expression. However, the Pitx1 knockout mice do express α subunit and the LHβ subunit. These observations suggest that the Pitx1 function may be compensated by related proteins that are expressed in the pituitary. The early response growth factor (Erg-1) has been shown to physically interact with SF-1 and synergistically activate LHβ transcription. The Pitx1 also interacts cooperatively with SF-1 and Erg-1 to form a tripartite protein complex that can directly stimulate LHβ promoter activity[34,35]. Apart from these elements, many elements and their respective transcription factors that modulate LHβ expression have been reported which is summarized in table 2.

The basal expression of LHβ is further upregulated by GnRH. Two regions (-490 to –353 bp and -207 to –82 bp) in the promoter of rat LHβ have been demonstrated to confer GnRH responsiveness. The distal region is unique to rat LHβ promoter and harbors two SF-1 elements and an overlapping CArG [$CC(A/T)_6GG$] element that are responsible for GnRH induction of LHβ expression. However, the Pitx1, SF-1 and the Erg-1 elements within the 140 bp region are highly conserved between bovine, equine, rodent and human species and are important for GnRH responsiveness. From these observations, it is implied that cooperation exists between the proximal and distal regions of the LHβ promoter and this coordinated communication is required for the robust GnRH stimulation of the LHβ expression[34].

The LHβ promoter is also regulated by testosterone and estradiol. The rat LHβ promoter contains an estrogen responsive element (ERE) at –1173 to –1159 that has been shown to stimulate the promoter activity. In contrast, the bovine LHβ promoter does not contain ERE or an androgen responsive element but the promoter activity is suppressed by testosterone and estradiol. This indicated that the ligand bound steroid receptors might be interacting with other transcription factors that directly influence LHβ transcription. Co-transfection studies of AR, Pitx1, SF-1 and Erg-1 in the gonadotrope derived cell lines suggested a functional synergistic interaction between these transcription factors in repressing the LHβ promoter. The rat LHβ promoter activity is also suppressed by androgens and this effect requires the presence of the distal Sp1 elements[36]. Although this promoter lacked an androgen responsive element, it was shown that AR physically interacted with Sp1 and this interaction would therefore lead to the androgen mediated effects on LHβ promoter. Thus, overall these studies highlighted the importance of interactions between various transcription factors and show how the formation of such orchestrated complexes can mediate hormonal regulation of the LHβ promoter.

Transcriptional regulation of FSHβ subunit gene

The FSHβ subunit transcription is principally regulated by GnRH, gonadal steroids and the polypeptide hormones activin, inhibin, follistain and BMPs[6,37,38,39,40]. Activins, inhibins and BMPs belong to the family of transforming growth factor (TGF) β and they regulate FSHβ expression while follistatin has been shown to bind to these proteins and neutralize their actions[41].

As there is no cell line available that resembles a FSH secreting gonadotrope, the regulation of FSHβ promoter has been mainly carried out using the gonadotrope cell lines, αT3-1 and LβT2 and transgenic mouse models. The recent observation that LβT2 cells synthesize and secrete FSH if treated with activins and BMPs has made this cell line the only possible *in vitro* model to study FSH regulation. The induction of FSHβ mRNA expression is much higher by activin as compared to GnRH (approximately 25 fold versus 2 fold, respectively) in primary perfused rat pituitary cells. The GnRH regulation of FSHβ transcription has been localized to two AP-1 enhancers in the ovine FSHβ (-120 and –83 bp) and an AP1 half site besides a NF-Y element in mouse FSHβ (-72 bp) promoters[42]. The –120 bp AP-1 element (GnRH enhancer) has been found to be conserved in ovine, bovine, porcine, rabbit and human but with a one base difference in the rodents. To test the potentially important function for the AP-1 element in ovine FSHβ transcription, transient transfections with wild type or mutated AP1 element harboring FSHβ constructs in HeLa cell line expressing GnRHR were carried out. The results indicated that both the AP1 sites were important for GnRH mediated induction of FSHβ expression over basal levels. Intriguingly, transgenic mice with mutated AP1 site also demonstrated GnRH mediated FSHβ expression equal to that of the wild type AP1 element suggesting that the AP1 sites had no impact on *in vivo* regulation of FSHβ expression. Subsequently, these AP1 sites were shown to be critical in the GnRH induced FSHβ expression in the pituitary cell cultures from the transgenic mouse mentioned above. These results not only bring about the complexity of the *in vivo* regulation of FSHβ expression in the gonadotropes but also highlight the limitation of a cell line model in addressing this issue. Nevertheless, these approaches have been instrumental in appreciating the effect of various hormones on FSHβ transcription.

Activin upregulates FSHβ transcription. As signaling through activin receptors have been shown to culminate in increased activity of AP-1 mediated transcriptional events, and the fact that FSHβ promoter has AP-1 elements, it has been suggested that AP-1 may be involved in activin mediated transcriptional upregulation of FSHβ gene. Inhibin, on the other hand, downregulates FSHβ transcription. The exact mechanism of action of inhibin is not clear. BMP6, BMP7 & and BMP15 have been shown to be directly involved in regulation FSHβ expression. Follistatin binds to activins, inhibins and the BMPs and prevents their action on FSHβ expression[37,38,39]. However, the molecular details about the regulation exerted by these molecules on FSHβ

Table 2: Elements in the LHb promoter region and interacting transcription factors (Jorgensen et al. 2004)(reproduced with permission of the publisher)

Element	Position	Factor	Family
TATA box	–31/–27 (b) –30–/26 (r)	TATA-binding protein (TBP)	Minor groove-binding architectural protein
Egr-1-binding element	–50/–42 (b) –49/–41 (r) –46/–38 (e)	Egr-1	Immediate early gene
	–/111/–104 (b) –112/–104 (r) –107/–99 (e)		
GSE	–59/–52 (b) –58/–51 (r)	SF-1	Orphan nuclear factor
	–128/–121 (b) –127/–119 (r)		
Pitx1-binding element	–100/–95 (b) –99/–96 (r)	Pitx1	Homeodomain
NF-Y-binding element	–337/–328 (b) –400/–391 (b)	NF-Y	CCAAT box-binding factor
Sp1-binding element	–366/–354 (r) –400/–391 (b)	sp1	GC box-binding protein
Sp1-binding element	–366/–354 (r) –450/–434 (r)	Sp1	GC box-binding protein
CArG sequences	–443/–434 (r)	Unknown	Serum factor related proteins

b: Bovine, r: rat, e: equine, Italics indicate factor unknown

expression is not clearly elucidated. Future studies would be focused on identifying the respective responsive elements and the transcription factors for the action of these factors on FSHβ expression.

Testosterone stimulates FSHβ expression at the transcriptional level. Estradiol has been shown to downregulate FSHβ expression. Although several animal experiments, especially using the rat model system has been carried out to demonstrate the effect of these steroids on the transcriptional activity of the FSHβ promoter, further insights to the mechanism of action are not currently available. Presence of progesterone response elements (PREs) like sequences have been described for the rat FSHβ gene. Using electrophoretic mobility shift assay and reporter assays employing PRE elements tethered to a heterologous promoter, progesterone receptor binding to the PRE and progesterone responsiveness was demonstrated in rat anterior pituitary cells[43]. But the manner in which progesterone modulates FSHβ expression is controversial.

In the literature there are suggestions for existence of additional level of regulation (post-transcriptional) of the FSHβ mRNA. In certain cases such as the one involving the rapid decrease in the mRNA levels of FSHβ due to inhibin was not completely explained by reduction in FSHβ transcription rate alone[44,45,46]. The decay of the FSHβ mRNA in presence of transcriptional inhibitors accelerated due to the presence of inhibin or follistatin suggesting the involvement of a factor that would "hasten" the degradation process[47]. Conversely, addition of activin A stabilized FSHβ mRNA, providing yet another clue that the post-transcriptional mechanism might be active[48].

Models to understand gonadotropin regulation

Mouse gonadotrope cell lines

Mellon and co-workers have employed the method of targeted expression of oncogenes in transgenic mice to immortalize specific cell types[49]. The immortalized cell lines that are established can then serve as culture model systems to study gonadotropin expression.

α *subunit secreting gonadotrope*

A fusion gene containing 1.8 kb of 5' flanking sequences of the human glycoprotein hormone α subunit gene linked to the protein-coding sequences of the simian virus-40 (SV-40) T antigen oncogene was used to generate transgenic mice[59]. Mice carrying this fusion gene developed tumors of the anterior pituitary that expressed the α subunit and T antigen. A stable cell line, αT3-1 derived from such a pituitary tumor could be maintained in monolayer culture. This cell line was responsive to GnRH, but not TRH suggesting that they were derived from gonadotrope lineage and were not of thyrotrope (that also express α subunit) origin. However, it was found that the αT3-1 cells did not express either of the β subunit suggesting that these cells were developmentally "frozen" as precursors of gonadotropes. This is supported by the observation that during ontogeny, the α subunit appears prior to the expression of the β subunits. These cells have been used extensively to study the mechanism of GnRH action, as well as investigating cell-specific expression of the α subunit[50].

β *subunit secreting gonadotrope*

Using a similar approach, targeted expression of the SV40 T antigen fused to the rat LHβ subunit gene promoter (1.8 kb) was used to generate transgenic mice[51,52]. One of the isolated and established cell lines from the tumors induced by this transgene, LβT2, expressed the LHβ subunit but not FSHβ subunit. Since LβT2 cells expressed both the α and the LHβ subunits, it was suggested that these cells probably arose later in the ontogeny than the αT3-1 cells and therefore represent a more mature gonadotrope precursor than the αT3-1 cells. These cells also demonstrate several characteristics of a mature gonadotrope cell, including expression of GnRHR, steroidogenic factor 1 (SF-1), estrogen and progesterone receptors. Interestingly, it was shown recently that these cells express FSHβ under influence of activin and some bone morphogenetic proteins (BMP6, BMP7 and BMP15)[37,38,39,53,54]. This induced expression can be downregulated by follistatin suggesting that the FSHβ regulatory systems may be functional in these cells. Thus LβT2 cell line has been proven invaluable for studies involving gonadotropin expression and secretion.

RC-4B/C cell line

This anterior pituitary cell line was established from a pituitary adenoma that developed spontaneously in a 3-year-old male rat[55]. Although these cells have the ultrastructural appearance similar to that of the well-differentiated anterior pituitary cells, it has several disadvantages. RC-4B/C cells are a heterogeneous population of pituitary cells and only a fraction of them express gonadotropins. Moreover, the proportion of various cell types was found to be different as compared to their distribution in the rat pituitaries. Furthermore, these cells have not been demonstrated to regulate gonadotropin synthesis and secretion. Thus, this cell is yet to be established as a good model system to study gonadotropin gene expression.

Mouse models to study FSH function

Gonadotrope function in the anterior pituitary is not only influenced by GnRH and other gonadal factors, but

paracrine effects of other cells have been noted[56]. Thus studying regulation of gonadotropin expression and secretion in an isolated cell culture system will not mimic the environment surrounding the gonadotropes in the pituitary. In view of this, transgenic mouse models provide *in vivo* alternative in carrying out such studies. For example, in absence of a gonadotrope cell line that constitutively secretes basal amount of FSH, transgenic mouse model is the only alternative. Kumar and co-workers have generated transgenic mice harboring the human FSHβ gene and showed that the elements conferring gonadotrope specific expression and hormonal responsiveness are contained within a defined 10 kb region[27].

Naturally occurring mutation in the common α subunit gene

Only one mutation in the α subunit has been reported so far although there have been several studies on restriction fragment length polymorphisms (RFLP). None of the RFLPs seem to affect the amino acid sequence. A single amino acid substitution E56A results in the α subunit that fails to associate with the hCGβ subunit. The α subunit has been shown to be secreted ectopically from variety of transformed cells. As described below, lack of α subunit in mice is not lethal, hence it is quite possible that there could be mutations in the common α subunit present in the population that have not been detected.

α knockout mouse

The α subunit knockout mouse apart from being infertile, suffered from stunted growth due to additional absence of TSH. The development of gonads was severely compromised. This indicated that although gonadotropins might be dispensable for sexual differentiation of the gonads, they are necessary for the maturation of the gonads[1].

Naturally occurring mutation in the LHβ gene

Only a single mutation in the LHβ gene in humans has been reported so far. This patient had a homozygous missense mutation Q54R. This mutation did not affect hormone synthesis, heterodimerization or immunoreactivity but the mutated hormone did not bind to its receptor. The patient exhibited delayed puberty, low testosterone and arrested spermatogenesis. Prolonged hCG treatment led to increase in testicular size and increased sperm count along with increased virility[57].

LHβ knockout mouse

LHβ knockout mouse predictably is infertile. Although embryogenesis is normal, there were postnatal defects in gonadal growth and function resulting in infertility. The LHβ knockout males have decreased testes size, Leydig cell hypoplasia and spermatogenesis blocked at the round spermatid stage resulting in a total absence of the elongated spermatids. Also the expression of genes encoding testosterone biosynthesis pathway enzymes is affected in these mice, resulting in reduced serum and intratesticular testosterone levels. The LHβ knockout females are infertile due to hypogonadism with many degenerating antral follicles and absence of corpora lutea. The serum estradiol and progesterone are also subnormal. Fertility of both male and female LHβ knockout mice can be restored by exogenous supplementation of LH suggesting that LH is entirely essential for reproduction function (http://abstracts.co.allenpress.com/pweb/ssr2004/document/?ID=37976).

Naturally occurring mutations in the FSHβ gene

Few cases of homozygous mutations in FSHβ gene have been discovered till now; some of the mutations that have been characterized are described below. All the patients were found to be associated with azoospermia. In one case, a 2 base deletion in the codon 61 (V61) resulted in premature truncated protein due to introduction of a stop codon at the 87th amino acid in the FSHβ subunit. The resulting truncated molecule was unable to heterodimerize with the α subunit. This woman suffered from primary amenorrhea and infertility. A male patient with C82R mutation had normal testosterone levels and pubertal development but spermatogenesis was arrested with complete absence of sperms. As C82 participates in the cysteine knot formation, it was suggested that this mutation might disrupt heterodimerization[58]. Another male patient with V61X mutation displayed reduced testosterone levels and delayed puberty[59].

FSHβ knockout mouse

The disruption of FSHβ gene in female mice renders them infertile. This is due to a block in the folliculogenesis; the follicles do not develop beyond the antral stage. Interestingly, the FSHβ knockout male mouse was fertile. However, testis size, Sertoli cell number and the seminiferous tubule volume were reduced. Leydig cell number did not change. The stages of spermatogenesis were grossly normal and some tubules also had sperms. There was a 40% reduction in the number of motile sperms. It was hence concluded that in rodents, FSH was not essential for fertility[60].

Thus in general, observations from the naturally occurring mutations in the gonadotropin subunit genes and studies on their targeted disruption suggests that impairment of gonadotropin function leads to severe loss in reproductive development and fertility.

Overall, the molecular mechanisms that govern gonadotropin subunit regulation have recently begun to emerge. Effects of hormones and general regulatory mechanisms at the physiological level have been elucidated using various animal models. The details

about the gene regulatory circuits accompanied by the signal transduction pathways that affect gonadotropin subunit expression can now be addressed in greater detail using the transgenic mouse model and gonadotrope cell lines derived from these mice as well as heterologous cell lines. However, a FSH secreting gonadotrope cell line is the immediate requirement to enhance our understanding about FSH gene regulation.

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