Published in final edited form as:

Curr Opin Endocrinol Diabetes Obes. 2009 August ; 16(4): 321–327. doi:10.1097/MED.0b013e32832d88fb.

The biology of gonadotroph regulation

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Abstract

Purpose of review—To discuss recent progress in our understanding of pituitary gonadotroph development and gonadotropin gene regulation, with an emphasis on differential luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion and subunit synthesis, and the implications this may have on female reproductive health.

Recent findings—In the mature gonadotroph, there is an emerging concept that differential synthesis of gonadotropin β -subunit genes, essential for cyclic reproductive function, is associated with modification of activation and/or stability of important regulatory proteins and transcription factors. Recent studies suggest that cellular events, which affect histone modification, play an essential role in both gonadotroph development and the ontogeny of gonadotropin subunit gene expression. Such dynamic events are under the orchestration of the hypothalamic neuropeptide gonadotropin-releasing hormone (GnRH), potentially through the ability of GnRH to activate several distinct signaling cascades within the gonadotroph.

Summary—Greater insight into the cellular events that are key to gonadotroph physiology will contribute to our understanding of abnormal gonadotropin secretion in disorders such as hypothalamic amenorrhea and polycystic ovarian syndrome (PCOS), and provide a context for the design of novel therapeutic approaches.

Keywords

follicle-stimulating hormone; gonadotropin-releasing hormone; gonadotroph; luteinizing hormone; reproduction

Introduction

Pulsatile hormone synthesis and secretion are critical for physiological processes, whereas disruption of episodic hormone release is often associated with clinical disorders [1•]. Oscillatory FSH and LH secretion are under the control of pulsatile hypothalamic GnRH [2]. Variations in GnRH pulse pattern are associated with divergent LH and FSH secretion, providing a mechanism by which a single hypothalamic neuropeptide can induce differential changes in two distinct hormones released from the same pituitary cell type, the gonadotroph. How this occurs likely rests on the ability of the gonadotroph to decipher different GnRH input patterns [3–5]. Recent studies in the well characterized α T3-1 and L β T2 gonadotroph cell lines have pointed to the importance of modification of activation and/or stability of regulatory proteins and transcription factors [5] as well as of epigenetic events [6•] in maintaining these dynamic responses of the gonadotroph.

Disruption of normal gonadotroph regulation is associated with several clinical disorders

The tight interrelationship between GnRH release and gonadotropin production is evidenced in patients with Kallmann's syndrome, in which GnRH deficiency results in low gonadotropin levels, absence of pubertal maturation, and infertility [7]. Such control of gonadotropin biosynthesis and secretion by GnRH is critically dependent on the pattern of GnRH delivery to the anterior pituitary. Pulsatile GnRH results in the stimulation of gonadotropin subunit mRNA levels and of LH and FSH secretion, whereas continuous exposure to GnRH downregulates mRNA levels and secretion [2,4,5,8,9]. Increased frequency of pulsatile hypothalamic GnRH release favors LH β gene transcription over FSH β and increases the ratio of secreted LH to FSH [4,5,9–12]. Conversely, decreased GnRH pulse frequency, characteristic of the luteal and early follicular phases of the ovulatory cycle, favors FSH β , allowing for increased pituitary FSH secretion essential for the recruitment and selection of the maturing ovum [4,5,9–12].

The response of the gonadotroph to GnRH in terms of relative FSH and LH production is thus exquisitely sensitive to the pattern of GnRH stimulation. This is exemplified in polycystic ovarian syndrome (PCOS), the most common cause of infertility in women of reproductive age, affecting up to 10% of this population [13–16]. This disorder, which is becoming increasingly prevalent, is often associated with obesity, insulin resistance, and metabolic and cardiovascular abnormalities similar to those of the metabolic syndrome [13,14,17,18]. The pathogenesis of this disorder remains unclear, but one hallmark of PCOS is disrupted reproductive cycles in association with elevated serum LH and depressed FSH levels, leading to an increase in androgen production by ovarian thecal cells [13]. This change in gonadotropin dynamics reflects increased hypothalamic GnRH neuronal activity, which manifests as predominantly high frequency GnRH pulsatility [13,17,19]. Conversely, hypothalamic amenorrhea in women is predominantly associated with low GnRH pulse frequencies and abnormal serum gonadotropin levels [7,20].

Tools for studying gonadotroph function

The anterior pituitary gland is a heterogeneous population of differentiated cell types that each secretes distinct hormones. Despite representing only 5–10% of the total pituitary cell population, the gonadotroph is fundamental to the development and maintenance of fertility through the synthesis and secretion of the gonadotropins (LH and FSH) [21]. Given the poor representation of this cell type within the heterogeneous pituitary, the study of the gonadotroph in primary pituitary cell cultures is a challenging undertaking. To this end, the development of murine gonadotroph-derived cell lines, α T3-1 and L β T2 cells, has provided useful tools for analyzing the molecular and cellular events that underlie the synthesis and secretion of LH and FSH [22–25]. The majority of the data discussed in this review will draw heavily on studies that have used these gonadotroph models. However, it is important to note that immortalized cell lines may have characteristics distinct from those of mature gonadotrophs or may represent and reflect the response of only a subpopulation of this cell type. To overcome such limitations, researchers have recently developed novel strategies for identifying and purifying gonadotrophs from transgenic animals [26•,27]. Although data from these novel approaches are limited, this review will highlight possible avenues of future investigation.

Differential activation of signaling cascades by pulsatile gonadotropin-releasing hormone

The binding of GnRH to its cognate receptor (GnRHR) situated on the cell surface of the gonadotroph has the potential to stimulate a diversity of distinct signaling cascades. GnRH-

stimulated protein kinase C (PKC) isoforms appear to be major mediators of downstream activation of a number of mitogen-activated protein kinase (MAPK) cascades, including extracellular signal-regulated kinase (ERK), jun N-terminal kinase (JNK) and p38 [28,29]. These activated MAPK components phosphorylate both cytoplasmic and nuclear proteins to initiate the transcriptional activation of genes including the gonadotropin subunits. GnRH-induced Ca^{2+} mobilization can initiate rapid gonadotropin secretion and the activation of Ca^{2+} /calmodulin-dependent protein kinases (CaMKs), such as CaMK-I and CaMK-II [30–32], another component of the GnRH signaling network. These diverse arms of the GnRH signaling cascade may enable the gonadotroph to decode GnRH pulse frequencies into differential gonadotropin synthesis and subsequent secretion.

The MAPK cascade has been implicated in mediating the frequency-dependent effects of GnRH. It has been shown that perfused L β T2 cells respond to varying GnRH pulse frequencies by exhibiting differential FSH β and LH β transcription [33]. Activation of phosphorylated (p) ERK1/2 is more rapid and more sustained in L β T2 cells perfused with low, rather than high, GnRH pulse frequencies, suggesting that pERK is important in the preferential stimulation of FSH β transcription [33]. Potential mediators of this differential activity of ERK under pulsatile GnRH are the MAPK phosphatases (MKPs) that inactivate members of the MAPK cascade by dephosphorylation (Fig. 1). In support of this view, GnRH induces MKP-2 expression in gonadotroph cell lines and primary gonadotroph cultures, whereas in-vivo gene profiling has demonstrated that both MKP-1 and MKP-2 are induced by GnRH with distinct temporal profiles that correlate with inactivation of MAPK signaling [34]. Furthermore, addition of the protein translation inhibitor cycloheximide causes more sustained GnRH activation of pERK 1/2 suggesting an important role for inducible MKPs [35•,36]. In this way, MKPs may be integral players in a negative feedback loop to control the level of ERK, JNK and p38 activity in response to different GnRH pulse frequencies (Fig. 1). This is of particular interest as the mammalian GnRHR lacks a C-terminal tail and is not predisposed to conventional G-protein coupled receptor desensitization [37]. In this way, the inactivation or attenuation of GnRH-activated signaling proteins may play an important role in mediating the gonadotroph response to different GnRH pulse frequencies. Nonetheless, knockdown of several distinct MKPs in static L β T2 cell cultures had little effect on pERK levels, suggesting that the role of MKPs in the gonadotroph is complex [35•,36]. The effect of MKP knockdown in L β T2 cells stimulated with pulsatile GnRH remains to be determined. This would be of particular interest as the kinetics of pERK activation in response to pulsatile GnRH may be distinct from static GnRH stimulation, and may represent a more physiologically relevant model.

In addition to the dephosphorylation of activated ERK, another potential rate-limiting step in ERK1/2 signaling is the translocation of these proteins into the nucleus, where they can act on transcription factors to mediate effects on gonadotropin subunit gene expression. It has recently been shown that calcium-dependent proline-rich tyrosine kinase 2 (Pyk2) acts as a mediator of ERK activation and translocation into the nucleus of gonadotrophs [38,39]. This finding highlights the possible crosstalk of MAPK and calcium signaling cascades [29] that may also involve calmodulin [40] and have the potential to be important in mediating differential transcriptional activation of the gonadotropins by pulsatile GnRH.

Transcriptional regulation of gonadotropin subunits by pulsatile gonadotropin-releasing hormone

The gonadotropins are heterodimeric glycoprotein hormones that are composed of a common α -subunit and a unique and biologically specific β -subunit [4,8]. As the α -subunit is produced in excess of the LH and FSH β -subunits in the gonadotroph, this review will focus on the transcriptional events important in the production of the gonadotropin β -subunit mRNAs. The GnRH activation of MAPK proteins such as pERK1/2, JNK and p38 can lead to the activation

of several transcription factors including CREB, c-Fos, c-Jun, and ATFs [41••,42,43] that have the potential to mediate gonadotropin β -subunit gene expression.

FSH is constitutively released from the pituitary gland and the synthesis of FSH β is the rate-limiting step in FSH production [44]. Characterization of the FSH β gene promoter has lagged behind that of the α and LH β subunit genes, largely due to the lack of appropriate cellular models for study. The development of the L β T2 cell line has provided a model for further characterization of the FSH β gene and a subsequent greater understanding of how this gene is regulated. We [41••] and others [45,46] have characterized a major GnRH-responsive element within the proximal FSH β promoter, which contains a partial cAMP response element (CRE)/AP1 site. This GnRH responsive element is fully conserved in humans [46], and so may be of clinical relevance, with protein-DNA interactions at this site a potential focus for therapeutic intervention. This site appears promiscuous with the ability to bind the bZIP transcription family member, CREB, as well as members of the AP1 family, such as c-Fos and c-Jun [41••]. A mechanism by which GnRH stimulates FSH β transcription is by inducing phosphorylation of promoter-bound CREB, leading to the recruitment of the histone acetyltransferase CREB binding protein (CBP) and allowing for the tethering of the basal transcriptional machinery [41••]. However, the role this GnRH-responsive site plays in the differential stimulation of FSH β transcription by GnRH pulse frequencies remains to be determined. A possible mechanism is the increased activation of repressor(s) of CREB/AP1 transcription factors at high GnRH pulse frequencies that causes a reduction in FSH β gene expression (Fig. 2). Potential transcriptional repressors that may fulfill such a role include CCAAT/enhancer-binding protein β [47] and inducible cAMP early repressor (ICER) [48, 49], which have been shown previously to attenuate CREB/AP1 responsive gene expression.

The more rapid and sustained activation of ERK at low GnRH pulse frequencies could be essential in mediating the differential effects on gonadotropin β -subunit transcription. Gonadotroph Egr-1 protein expression is stimulated to a greater extent at high GnRH pulse frequencies in L β T2 cells [33]. Egr-1 may be a key factor in mediating GnRH-dependent transcriptional activation of MKP-2, as Egr-1 *cis*-elements are required for the induction of this gene by GnRH [50]. Therefore, Egr-1 may suppress FSH β transcription by attenuating ERK1/2 phosphorylation and subsequent activation of transcription factors such as CREB or AP1. Furthermore, Egr-1 and Egr-2 have been firmly implicated as being essential in mediating the maximal GnRH stimulation of LH β gene expression (Fig. 2) [51,52] and are both induced by high GnRH pulse frequencies. In contrast, inhibitors of the Egr family, Ngfi-A binding proteins Nab1 and 2, are preferentially induced at low GnRH pulse frequencies and act at the level of the LH β promoter to reduce Egr-1 function and, hence, LH β transcription (Fig. 2) [52]. Thus, it appears that Egr family members may be important transcription factors that play an essential role in mediating the GnRH pulse frequency dependent effects on gonadotropin gene expression through both direct and indirect mechanisms.

Epigenetic regulation of the gonadotroph

Over the past decade significant progress has been made in understanding how the structure of chromatin changes dynamically to induce or repress gene transcription. Posttranslational modification of histone tails by modifying enzymes, including acetylation and methylation, results in changes in gene expression [53]. Recently, histone tail modification has been associated with key events in the pituitary gonadotroph, specifically the ontogeny and GnRH-stimulated transcription of the gonadotropin β -subunits [6••,32,41••,54].

Acetylation and deacetylation of promoter-associated histones is fundamental to transcriptional activation or repression respectively [53]. This dynamic process is dependent on the recruitment and activity of two distinct groups of modifying enzymes, the histone acetyl

transferases (HATs) and the histone deacetylases (HDACs). HATs induce acetylation of the lysine residues of histone tails, which is thought to lead to local chromatin decondensation, resulting in increasing accessibility of gene promoter regions for transcription factors and RNA polymerase complexes to initiate transcription. In contrast, HDACs remove acetyl groups, restoring an overall positive charge to histone tails, thereby resulting in increased compaction of chromatin as a result of tighter interactions with negatively charged DNA [53]. Several recent lines of evidence suggest that GnRH mediates stimulatory transcriptional effects on both *LH β* and *FSH β* genes through modification of the histone acetylation status of the gonadotropin subunit gene promoters [6•,32,41•,53,54].

Due to a lack of LH and FSH β -subunit expression and gonadotropin secretion, the α T3-1 cell line is considered to represent a model of an immature gonadotroph. Distinct HDACs have been reported to occupy the *FSH β* and *LH β* promoters under basal conditions in this cell line to cause transcriptional silencing [6•,32]. GnRH treatment of α T3-1 cells has been shown to cause nuclear export of class IIa HDACs, suggesting that GnRH can potentially alter the acetylation status of gonadotropin promoters and induce gene expression by eliminating repressive markers of transcription [6•,32]. This finding infers that GnRH modulates the activity of histonemodifying enzymes in gonadotrophs. Furthermore, the observation that distinct sets of HDACs are associated with the *LH β* and *FSH β* promoters allows for a potential mechanism by which LH and FSH expression can be differentially regulated within the same cell type [6•,32].

Studies in the more mature L β T2 gonadotroph model, which robustly expresses *LH β* mRNA and to a lesser extent *FSH β* mRNA, have implicated HATs in mediating the GnRH stimulation of both gonadotropin β -subunit genes [41•,54]. The HAT p300 is associated with the *LH β* gene and this observed association is enhanced by GnRH treatment [51,54]. In this way, p300, by acting in synergy with transcription factors SF1, Sp1 and Egr-1, plays an essential role in the augmentation of *LH β* transcription (Fig. 2) [51,54]. In L β T2 cells, the p300 paralogue CREB binding protein (CBP) has been shown to associate with the *FSH β* promoter [41•]. GnRH stimulation can increase this association through increased interaction with promoter-bound phosphorylated CREB to stimulate *FSH β* transcription (Fig. 2) [41•]. The knockdown of p300 causes a reduction in the transcription of both gonadotropin β -subunit genes, further implicating a fundamental role of HATs in gonadotropin gene expression [6•].

Collectively, studies in α T3-1 and L β T2 cells suggest that histone modification through dynamic GnRH control of HDACs and HATs may affect the acetylation status of genes to regulate gonadotroph transcription in general and gonadotropin β -subunit transcription in particular. However, no study to date has shown a correlation between the association of HDACs and HATs with gonadotropin promoters with changes in the histone acetylation status. Future studies focused in this area will further our understanding of the relative importance of epigenetic modifications in terms of gonadotropin regulation, particularly in response to pulsatile GnRH. In addition, a natural expansion of this body of work will be to investigate the relevance of other histone modifications such as methylation and phosphorylation.

Conclusion

The differential modification of signaling cascades by GnRH pulse frequency appears pivotal in differential gonadotropin synthesis and subsequent secretion which involves the modification of both chromatin and transcription factors. This greater perspective of the dynamic biology of the gonadotroph will prove essential in our understanding of the complex physiology of the ovulatory and menstrual cycles, thereby facilitating the development of new therapies for disorders of neuroendocrine reproductive control.

Acknowledgments

This research was supported in part by NIH grants R01 HD33001, HD19938, and by NICHD/NIH through cooperative agreement U54 HD28138 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research (UBK).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 336).

1. Veldhuis JD, Keenan DM, Pincus SM. Motivations and methods for analyzing pulsatile hormone secretion. *Endocr Rev* 2008;29:823–864. [PubMed: 18940916] A comprehensive review of the importance of pulsatile hormone secretion in health and disease.
2. Belchetz PE, Plant TM, Nakai Y, et al. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* 1978;202:631–633. [PubMed: 100883]
3. Ruf F, Park MJ, Hayot F, et al. Mixed analog/digital gonadotrope biosynthetic response to gonadotropin-releasing hormone. *J Biol Chem* 2006;281:30967–30978. [PubMed: 16916798]
4. Burger LL, Haisenleder DJ, Dalkin AC, Marshall JC. Regulation of gonadotropin subunit gene transcription. *J Mol Endocrinol* 2004;33:559–584. [PubMed: 15591020]
5. Ferris HA, Shupnik MA. Mechanisms for pulsatile regulation of the gonadotropin subunit genes by GNRH1. *Biol Reprod* 2006;74:993–998. [PubMed: 16481592]
6. Melamed P. Histone deacetylases and repression of the gonadotropin genes. *Trends Endocrinol Metab* 2008;19:25–31. [PubMed: 18155918] A review of the role that HDACs play in gonadotropin transcription.
7. Marshall JC, Eagleson CA, McCartney CR. Hypothalamic dysfunction. *Mol Cell Endocrinol* 2001;183:29–32. [PubMed: 11604221]
8. Gharib SD, Wierman ME, Shupnik MA, Chin WW. Molecular biology of the pituitary gonadotropins. *Endocr Rev* 1990;11:177–199. [PubMed: 2108012]
9. Haisenleder DJ, Dalkin AC, Ortolano GA, et al. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes: evidence for differential regulation of transcription by pulse frequency in vivo. *Endocrinology* 1991;128:509–517. [PubMed: 1702704]
10. Kaiser UB, Jakubowiak A, Steinberger A, Chin WW. Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels in vitro. *Endocrinology* 1997;138:1224–1231. [PubMed: 9048630]
11. Kaiser UB, Sabbagh E, Katzenellenbogen RA, et al. A mechanism for the differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing hormone. *Proc Natl Acad Sci U S A* 1995;92:12280–12284. [PubMed: 8618885]
12. Reame N, Sauder SE, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion during the human menstrual cycle: evidence for altered frequency of gonadotropin-releasing hormone secretion. *J Clin Endocrinol Metab* 1984;59:328–337. [PubMed: 6429184]
13. Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med* 2005;352:1223–1236. [PubMed: 15788499]
14. Hoffman LK, Ehrmann DA. Cardiometabolic features of polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab* 2008;4:215–222. [PubMed: 18250636]
15. Azziz R, Carmina E, Dewailly D, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* 2009;91:456–488. [PubMed: 18950759]

16. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 1997;18:774–800. [PubMed: 9408743]
17. Blank SK, McCartney CR, Helm KD, Marshall JC. Neuroendocrine effects of androgens in adult polycystic ovary syndrome and female puberty. *Semin Reprod Med* 2007;25:352–359. [PubMed: 17710731]
18. Bremer AA, Miller WL. The serine phosphorylation hypothesis of polycystic ovary syndrome: a unifying mechanism for hyperandrogenemia and insulin resistance. *Fertil Steril* 2008;89:1039–1048. [PubMed: 18433749]
19. Hall JE, Taylor AE, Hayes FJ, Crowley WF Jr. Insights into hypothalamic-pituitary dysfunction in polycystic ovary syndrome. *J Endocrinol Invest* 1998;21:602–611. [PubMed: 9856414]
20. Reame NE, Sauder SE, Case GD, et al. Pulsatile gonadotropin secretion in women with hypothalamic amenorrhea: evidence that reduced frequency of gonadotropin-releasing hormone secretion is the mechanism of persistent anovulation. *J Clin Endocrinol Metab* 1985;61:851–858. [PubMed: 3900122]
21. Lloyd JM, Childs GV. Changes in the number of GnRH-receptive cells during the rat estrous cycle: biphasic effects of estradiol. *Neuroendocrinology* 1988;48:138–146. [PubMed: 2851748]
22. Alarid ET, Windle JJ, Whyte DB, Mellon PL. Immortalization of pituitary cells at discrete stages of development by directed oncogenesis in transgenic mice. *Development* 1996;122:3319–3329. [PubMed: 8898243]
23. Thomas P, Mellon PL, Turgeon J, Waring DW. The L beta T2 clonal gonadotrope: a model for single cell studies of endocrine cell secretion. *Endocrinology* 1996;137:2979–2989. [PubMed: 8770922]
24. Turgeon JL, Kimura Y, Waring DW, Mellon PL. Steroid and pulsatile gonadotropin-releasing hormone (GnRH) regulation of luteinizing hormone and GnRH receptor in a novel gonadotrope cell line. *Mol Endocrinol* 1996;10:439–450. [PubMed: 8721988]
25. Windle JJ, Weiner RI, Mellon PL. Cell lines of the pituitary gonadotrope lineage derived by targeted oncogenesis in transgenic mice. *Mol Endocrinol* 1990;4:597–603. [PubMed: 1704103]
26. Wen S, Schwarz JR, Niculescu D, et al. Functional characterization of genetically labeled gonadotropes. *Endocrinology* 2008;149:2701–2711. [PubMed: 18325995] A transgenic study that labels gonadotrophs for functional analysis.
27. Wu JC, Su P, Safwat NW, et al. Rapid, efficient isolation of murine gonadotropes and their use in revealing control of follicle-stimulating hormone by paracrine pituitary factors. *Endocrinology* 2004;145:5832–5839. [PubMed: 15319358]
28. Liu F, Usui I, Evans LG, et al. Involvement of both G(q/11) and G(s) proteins in gonadotropin-releasing hormone receptor-mediated signaling in L beta T2 cells. *J Biol Chem* 2002;277:32099–32108. [PubMed: 12050161]
29. Mulvaney JM, Roberson MS. Divergent signaling pathways requiring discrete calcium signals mediate concurrent activation of two mitogen-activated protein kinases by gonadotropin-releasing hormone. *J Biol Chem* 2000;275:14182–14189. [PubMed: 10799494]
30. Haisenleder DJ, Burger LL, Aylor KW, et al. Gonadotropin-releasing hormone stimulation of gonadotropin subunit transcription: evidence for the involvement of calcium/calmodulin-dependent kinase II (Ca/CAMK II) activation in rat pituitaries. *Endocrinology* 2003;144:2768–2774. [PubMed: 12810529]
31. Haisenleder DJ, Ferris HA, Shupnik MA. The calcium component of gonadotropin-releasing hormone-stimulated luteinizing hormone subunit gene transcription is mediated by calcium/calmodulin-dependent protein kinase type II. *Endocrinology* 2003;144:2409–2416. [PubMed: 12746302]
32. Lim S, Luo M, Koh M, et al. Distinct mechanisms involving diverse histone deacetylases repress expression of the two gonadotropin beta-subunit genes in immature gonadotropes, and their actions are overcome by gonadotropin-releasing hormone. *Mol Cell Biol* 2007;27:4105–4120. [PubMed: 17371839]
33. Kanasaki H, Bedecarrats GY, Kam KY, et al. Gonadotropin-releasing hormone pulse frequency-dependent activation of extracellular signal-regulated kinase pathways in perfused LbetaT2 cells. *Endocrinology* 2005;146:5503–5513. [PubMed: 16141398]

34. Zhang T, Roberson MS. Role of MAP kinase phosphatases in GnRH-dependent activation of MAP kinases. *J Mol Endocrinol* 2006;36:41–50. [PubMed: 16461925]
35. Armstrong SP, Caunt CJ, McArdle CA. Gonadotropin-releasing hormone and protein kinase C signaling to ERK: spatiotemporal regulation of ERK by docking domains and dual-specificity phosphatases. *Mol Endocrinol* 2009;23:510–519. [PubMed: 19179479] A comprehensive study of the unique activation of MKPs in the gonadotroph by GnRH stimulation.
36. Caunt CJ, Armstrong SP, Rivers CA, et al. Spatiotemporal regulation of ERK2 by dual specificity phosphatases. *J Biol Chem* 2008;283:26612–26623. [PubMed: 18650424]
37. Willars GB, Heding A, Vrecl M, et al. Lack of a C-terminal tail in the mammalian gonadotropin-releasing hormone receptor confers resistance to agonist-dependent phosphorylation and rapid desensitization. *J Biol Chem* 1999;274:30146–30153. [PubMed: 10514504]
38. Dobkin-Bekman M, Naidich M, Pawson AJ, et al. Activation of mitogen-activated protein kinase (MAPK) by GnRH is cell-context dependent. *Mol Cell Endocrinol* 2006;252:184–190. [PubMed: 16682115]
39. Xie J, Allen KH, Marguet A, et al. Analysis of the calcium-dependent regulation of proline-rich tyrosine kinase 2 by gonadotropin-releasing hormone. *Mol Endocrinol* 2008;22:2322–2335. [PubMed: 18635666]
40. Roberson MS, Bliss SP, Xie J, et al. Gonadotropin-releasing hormone induction of extracellular-signal regulated kinase is blocked by inhibition of calmodulin. *Mol Endocrinol* 2005;19:2412–2423. [PubMed: 15890671]
41. Ciccone NA, Lacza CT, Hou MY, et al. 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 beta gene. *Mol Endocrinol* 2008;22:1908–1923. [PubMed: 18550775] Study demonstrating that GnRH can stimulate FSH β transcription by inducing binding of the HAT, CBP.
42. Liu F, Austin DA, Mellon PL, et al. GnRH activates ERK1/2 leading to the induction of c-fos and LHbeta protein expression in LbetaT2 cells. *Mol Endocrinol* 2002;16:419–434. [PubMed: 11875099]
43. Xie J, Bliss SP, Nett TM, et al. Transcript profiling of immediate early genes reveals a unique role for activating transcription factor 3 in mediating activation of the glycoprotein hormone alpha-subunit promoter by gonadotropin-releasing hormone. *Mol Endocrinol* 2005;19:2624–2638. [PubMed: 15961508]
44. Farnworth PG. Gonadotrophin secretion revisited. How many ways can FSH leave a gonadotroph? *J Endocrinol* 1995;145:387–395. [PubMed: 7636423]
45. Coss D, Jacobs SB, Bender CE, Mellon PL. A novel AP-1 site is critical for maximal induction of the follicle-stimulating hormone beta gene by gonadotropin-releasing hormone. *J Biol Chem* 2004;279:152–162. [PubMed: 14570911]
46. Wang Y, Fortin J, Lamba P, et al. Activator protein-1 and smad proteins synergistically regulate human follicle-stimulating hormone beta-promoter activity. *Endocrinology* 2008;149:5577–5591. [PubMed: 18653705]
47. Burkart AD, Mukherjee A, Sterneck E, et al. Repression of the inhibin alphasubunit gene by the transcription factor CCAAT/enhancer-binding proteinbeta. *Endocrinology* 2005;146:1909–1921. [PubMed: 15650079]
48. Foulkes NS, Borjigin J, Snyder SH, Sassone-Corsi P. Transcriptional control of circadian hormone synthesis via the CREM feedback loop. *Proc Natl Acad Sci U S A* 1996;93:14140–14145. [PubMed: 8943074]
49. Foulkes NS, Duval G, Sassone-Corsi P. Adaptive inducibility of CREM as transcriptional memory of circadian rhythms. *Nature* 1996;381:83–85. [PubMed: 8609995]
50. Zhang T, Choy M, Jo M, Roberson MS. Structural organization of the rat mitogen-activated protein kinase phosphatase 2 gene. *Gene* 2001;273:71–79. [PubMed: 11483362]
51. Kaiser UB, Halvorson LM, Chen MT. Sp1, steroidogenic factor 1 (SF-1), and early growth response protein 1 (egr-1) binding sites form a tripartite gonadotropin-releasing hormone response element in the rat luteinizing hormone-beta gene promoter: an integral role for SF-1. *Mol Endocrinol* 2000;14:1235–1245. [PubMed: 10935547]

52. Lawson MA, Tsutsumi R, Zhang H, et al. Pulse sensitivity of the luteinizing hormone beta promoter is determined by a negative feedback loop involving early growth response-1 and Ngfi-A binding protein 1 and 2. *Mol Endocrinol* 2007;21:1175–1191. [PubMed: 17299135]
53. Yang XJ, Seto E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* 2007;26:5310–5318. [PubMed: 17694074]
54. Mouillet JF, Sonnenberg-Hirche C, Yan X, Sadovsky Y. p300 regulates the synergy of steroidogenic factor-1 and early growth response-1 in activating luteinizing hormone-beta subunit gene. *J Biol Chem* 2004;279:7832–7839. [PubMed: 14681221]

Figure 1.

The potential role of MKPs in mediating the differential activation of MAPK components such as ERK1/2 in response to different GnRH pulse frequencies

Figure 2. Low and high GnRH pulse frequencies differentially alter gonadotropin β -subunit transcription through recruitment of histone acetyl transferases (HATs) and induction or modification of transcription factors