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Transcriptional regulation of pituitary gonadotrophin subunit genes

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The gonadotrophic hormones, LH and FSH, are synthesized in and secreted from gonadotroph cells in the anterior pituitary and comprise a common α-subunit and a hormone-specific β-subunit. Gonadotrophic gene expression is activated during embryogenesis, independent of GnRH stimulation and increases as GnRH output increases, reaching adult levels at puberty. The transcriptional regulation of pituitary gonadotrophin subunit gene expression is regulated by two types of transcription factor: those that restrict and direct gene expression to gonadotrophs and those that modulate GnRH-regulated gene expression. Synergism between these two types of factor ensures gonadotroph-specific GnRH-regulated gene expression. It is not known whether these two types of transcription factor are mutually exclusive or whether they have overlapping functions. GnRH-regulated gonadotrophin subunit gene expression is modulated by transcription factors controlled by a complex interaction of GnRH, steroids and gonadal peptides, all of which bind to receptors that activate disparate intracellular signalling pathways. It remains to be established how these signalling pathways interact to transduce specific transcriptional activation of common α-subunit and LH and FSH β-subunit gene expression.

The pituitary gonadotrophins, LH and FSH, are heterodimeric molecules comprising a common α-subunit and a hormone-specific β-subunit. LH and FSH are synthesized in and secreted from gonadotroph cells situated in the anterior pituitary gland. Their action is synergistic and pivotal for puberty and subsequent fertility. Gonadotrophin-releasing hormone (GnRH) is released from the hypothalamus and binds to the GnRH receptor (GnRHr) on the gonadotroph cell surface to stimulate the synthesis and release of LH and FSH. In turn, LH and FSH act on the ovary and testes to stimulate spermatogenesis, folliculogenesis and, in females, LH triggers ovulation. The synthesis and secretion of LH and FSH are both positively and negatively regulated by steroids and gonadal peptides (Fig. 1). Investigation of the transcription factors and cognate DNA elements required to activate and direct expression of gonadotrophin subunit genes is currently an active area of research.

Transcriptional regulation of the gonadotrophin subunits involves two key overlaying mechanisms. First, basal gene expression is activated in and targeted to gonadotroph cells and is maintained throughout development. Second, as GnRH secretion increases, basal gonadotrophin gene expression is upregulated, reaching maximum adult values at puberty. Blocking GnRH input returns gonadotrophin gene expression to basal levels (McNeilly *et al.*, 1991). Therefore, it appears that some transcription factors regulate basal tissue-specific gene expression, and some regulate GnRH up-regulation of gene expression. Synergism between the two types of transcription factor ensures gonadotroph-specific GnRH-regulatable gene expression. However, it must be stressed that it has not been established unequivocally that transcription factors that control basal gene expression are not regulated by GnRH, and vice versa, but for the purposes of this review they have been arbitrarily classified into two groups: basal and GnRH-regulated transcription factors. It has also not been established whether there is a basal or GnRH-regulated transcription factor that directs gonadotroph-specific gene expression and specifies the gonadotroph cell type. However, there is evidence that transcriptional regulation of the expression of the LH β-subunit gene is sexually dimorphic, to allow the generation of the preovulatory LH surge in females.

Basal gonadotrophin subunit gene expression

Targeting and activation of gonadotroph-specific gene expression

A controlled cascade of spatially and temporally regulated transcription factors initiates and maintains the basal expression of gonadotrophin genes during embryogenesis (Figs 2 and 3a). This cascade involves multiple signalling pathways that control transcription factor expression and, thus, are responsible for gonadotroph differentiation (Treier *et al.*, 1998). The gonadotroph cell lineage originates in the ventral part of the embryonic pituitary and is characterized initially by the expression of α-subunit transcripts and, after a further 6 days, by the expression of LH and FSH β-subunit transcripts (Japon *et al.*, 1994). Transcription factors that activate gonadotrophin α- and β-subunit gene transcription are expressed during this developmental window (Table 1). It should be noted that most of these transcription factors specify other pituitary cell

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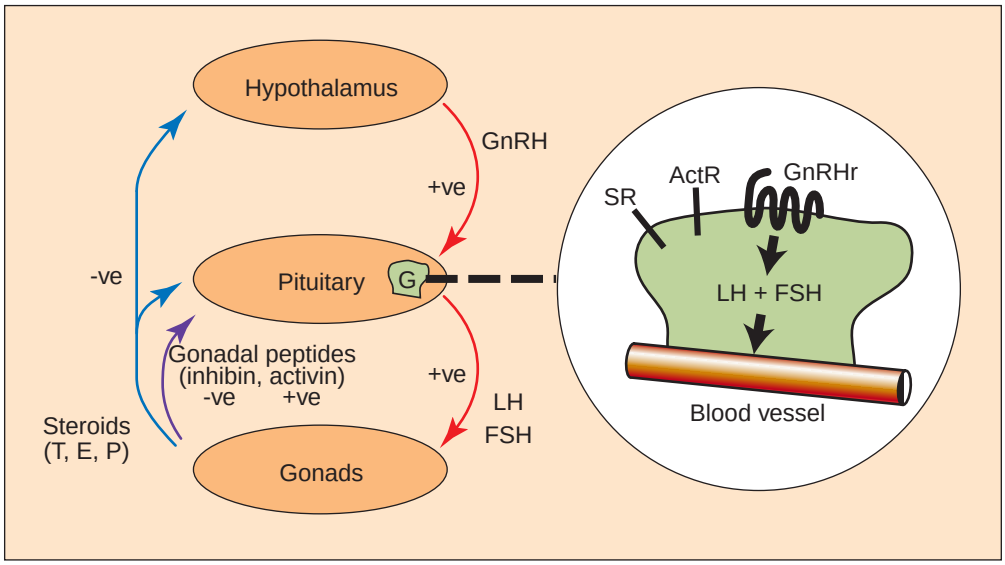

Fig. 1. Schematic representation of the hypothalamic–pituitary–gonadal axis showing positive and negative regulators of gonadotrophin hormone gene expression. Gonadotrophin-releasing hormone (GnRH) synthesized in and released from the hypothalamus binds to GnRH receptor (GnRHr), a seven transmembrane G-protein-coupled receptor located on the surface of the gonadotroph. The binding of GnRH to the GnRHr triggers the synthesis, and ultimately the secretion, of LH and FSH into the vascular system. A stylized steroid receptor (SR) is also indicated on the gonadotroph cell, this represents androgen, oestrogen and progesterone receptor. Testosterone (T), oestrogen (E) and progesterone (P) negatively regulate gonadotrophin synthesis directly at the pituitary and via downregulation of hypothalamic GnRH secretion. The gonadal peptides, inhibin and activin, have opposing roles in regulating gonadotrophin synthesis and seem to regulate production of FSH. Activin transactivates its own receptor (activin receptor; ActR) but it is yet to be determined whether inhibin signals through the same or an unidentified receptor.

lineages in addition to gonadotrophs (for review, see Watkins-Chow and Camper, 1998).

Pituitary homeobox 1 (Ptx1) is expressed in several anterior pituitary cell lineages and transactivates most of the anterior pituitary cell-specific genes, including the α-subunit, LH and FSH β-subunit genes (Tremblay *et al.*, 1998). Hesx1–Rpx expression is restricted to Rathke's pouch during pituitary development (Hermesz *et al.*, 1996), and the 'knock-out' phenotype indicates that it is involved in cellular expansion of the pituitary before differentiation (Dattani *et al.*, 1998). Studies *in vitro* indicate that Hesx1–Rpx transactivates LH β-subunit gene expression (Brown *et al.*, 1999). The pLIM–Lhx3 homeobox gene specifies several pituitary cell lineages (Sheng *et al.*, 1996) and transactivates gonadotrophic α-subunit gene expression (Bach *et al.*, 1995). Steroidogenic factor 1 (SF-1) is specifically found in the gonadotroph cell lineage of anterior pituitary cells, but it is also expressed in other non-pituitary tissues, including the adrenals and gonads (Luo *et al.*, 1994). SF-1 stimulates α-subunit (Horn *et al.*, 1992; Barnhart and Mellon, 1996) and LH β-subunit gene expression (Halvorson *et al.*, 1996; Keri and Nilson, 1996), but does not activate FSH β-subunit gene expression (Tremblay *et al.*, 1998). However, SF-1 does not specify the gonadotroph cell lineage because, although SF-1 'knock-out' mice do not express LH and FSH β-subunit genes, they still have viable gonadotroph cells, and the expression of LH and FSH β-subunit genes are induced when these mice are treated with GnRH (Ikeda *et al.*, 1995). Otx1 transcription factor 'knock-out' mice exhibit the very unusual phenotype of transient dwarfism and hypogonadism (Acampora *et al.*, 1998). Essentially, gonadotroph cells are intact, as in SF-1 'knock-out' mice, but prepubertal expression of gonadotrophins is impaired, only recovering after puberty.

It has not been determined whether there is a pivotal transcription factor that specifies the gonadotroph cell type and activates transcription of all the gonadotrophin subunits. Detailed analysis of the defined DNA elements in the mouse α-subunit promoter reveal that the –337 to –330 bp element, the pituitary glycoprotein hormone basal element (PGBE), binds a LIM-homeodomain transcription factor (LH-2; Roberson *et al.*, 1994) and is required for basal α-subunit gene expression. A transgenic mouse model confirmed that the PGBE was required to direct gonadotroph-specific expression (Brinkmeier *et al.*, 1998). However, the PGBE is also transactivated by p-Lim–Lhx3 (Bach *et al.*, 1995), which specifies multiple anterior pituitary cell lineages (Sheng *et al.*, 1996).

Although SF-1 stimulates α-subunit and LH β-subunit gene expression, SF-1 gene expression is activated at embryonic day 13.5 (e13.5), after the activation of α-subunit gene expression on e10.5, while the earliest detectable LH β-subunit transcripts appear at e16.5 (Fig. 2). It appears that, after activation of α-subunit gene expression, undiscovered transcription factors suppress SF-1 and LH β-subunit gene activation in

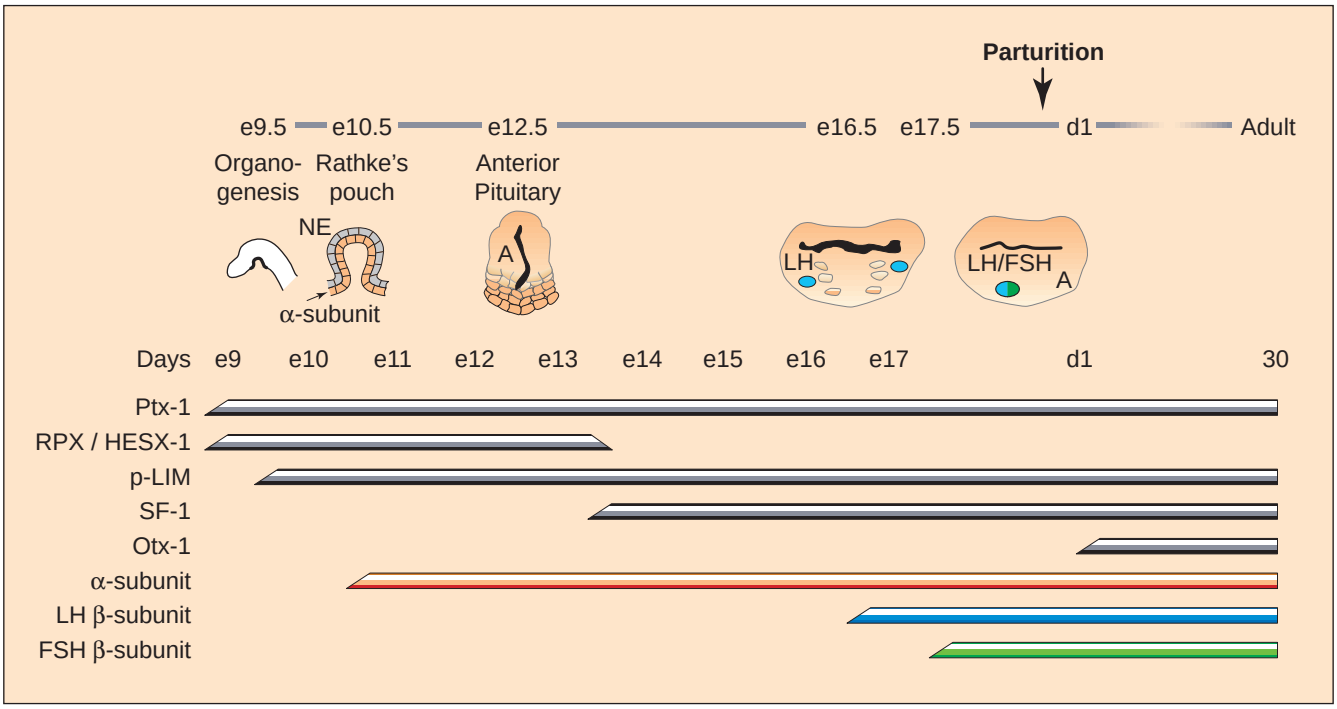

Fig. 2. The relationship between the organogenesis of the anterior pituitary and the sequential activation of genes encoding transcription factors known to regulate gonadotrophin gene expression in mice. Shaded bars indicate the initiation and duration of gene expression. Rathke's pouch is formed from the hypophyseal placode of oral ectoderm that invaginates at approximately embryonic day 9.5 (e9.5). Cell proliferation then occurs 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 (e16.5) and FSH β-subunit (e17.5) gene expression is detected. NE: neuroectoderm.

precursor gonadotrophs. Possible transcription factors that repress gene expression are the dominant negative helix–loop–helix (Id) proteins that inhibit gene activation by basic helix–loop–helix (bHLH) transcription factors. bHLH factors bind to E-boxes, which have a loose consensus sequence of CANNTG. This interaction of Id and bHLH mediated via E-boxes appears to be important for regulating expression of α-subunit in gonadotrophs (Jackson *et al.*, 1995). In addition, GATA factors (so-called because they bind to a central DNA consensus of GATA) may be involved in early pituitary specification of α-subunit gene expression since they transactivate gene expression *in vitro* (Steger *et al.*, 1994).

Upregulation of basal gene expression

GnRH modulation of gonadotrophin gene expression

The acquisition of GnRH responsiveness is dependent on the activation of intracellular signalling transduction pathways stimulated by the binding of GnRH to the GnRHr (Fig. 3b). The GnRHr is a G-protein-coupled seven transmembrane receptor that signals predominantly via the protein kinase C (PKC) pathway (for review, see Anderson 1996). This stimulatory signal is relayed to the nucleus via transcription factors that bind to discrete DNA sequences on the gonadotrophin subunit promoters and, thus, transactivate gene expression. After gonadotrophin subunit gene expression has been activated during embryogenesis, basal expression gradually increases in response to non-pulsatile secretion of GnRH. Activation of the GnRH pulse generator occurs at the onset of puberty and results in increased GnRH release which, in turn, stimulates gonadotrophin subunit gene expression. This dual control of gonadotrophin subunit gene expression is demonstrated in hypogonadal mice, which never progress through puberty because of a mutation in the GnRH gene. These mice have functional gonadotrophs and basal gonadotrophin subunit gene expression, which is upregulated after the administration of GnRH (Cattanach *et al.*, 1977). This observation confirms that GnRH upregulates basal gonadotrophin gene expression.

GnRH regulation of common α-subunit gene expression

GnRH-responsive regions have been mapped in the α-subunit promoter of several species. In humans, cows and mice, the DNA elements that confer GnRH responsiveness all reside in the proximal promoter: human –346 to –244 (Kay and Jameson, 1992), cow –315 (Hamernik *et al.*, 1992) and mouse –406 to –399 and –337 to –330 bp (Schoderbeck *et al.*, 1993). The –337 to –330 bp site in the mouse α-subunit promoter binds a LIM homeodomain transcription factor that directs basal expression and has already been discussed. The second identified site at –406 to –399 binds a factor that was stimulated by GnRH via the mitogen-activated protein kinase pathway (MAPK) (Roberson *et al.*, 1995). Synergism of the transcription factors that bind to

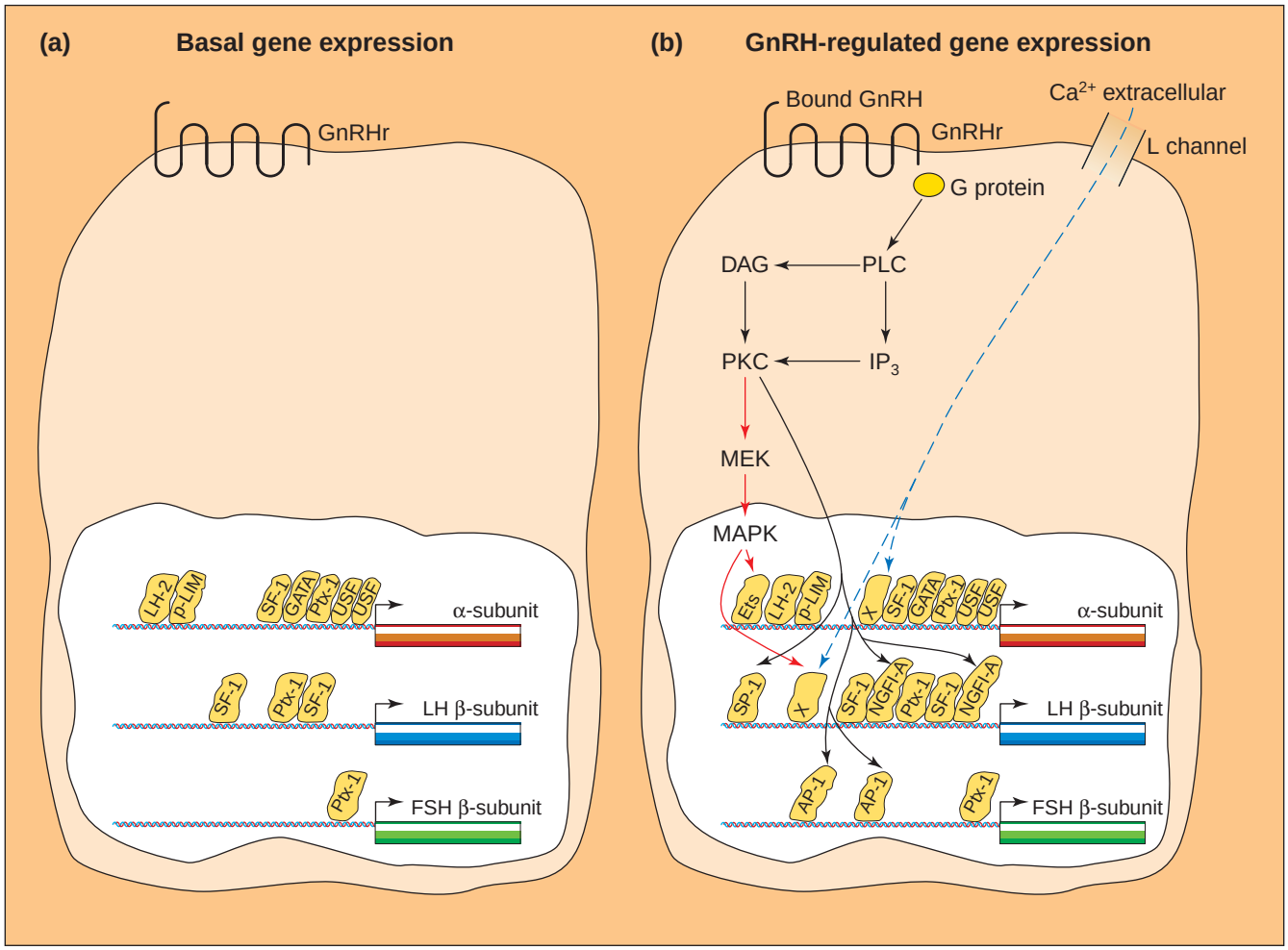

Fig. 3. Proposed schematic model of basal and GnRH-activated gonadotrophin subunit gene expression. (a) Transcription factors involved in basal gonadotrophin subunit gene expression are activated during anterior pituitary development and are shown bound to their cognate DNA elements in the gonadotrophin subunit gene promoters. (b) GnRH-regulated transcription factors, together with their putative second messenger signalling pathways. USF: upstream transcription factor binds to E-box (Jackson *et al.*, 1995); Ptx-1: pituitary homeobox 1 (Tremblay *et al.*, 1998); GATA: binds to GATA DNA consensus (Steger *et al.*, 1994); SF-1: steroidogenic factor 1 (Horn *et al.*, 1992; Barnhart and Mellon, 1996; Halvorson *et al.*, 1996, 1998; Keri and Nilson, 1996); p-LIM: LIM homeobox gene (Bach *et al.*, 1995); LH-2: LIM homeobox gene (Roberson *et al.*, 1994); Ets (Roberson *et al.*, 1995); NGFI-A: nerve growth factor 1A (Lee *et al.*, 1996; Halvorson *et al.*, 1999); SP-1 (Kaiser *et al.*, 1998); AP-1: activating protein 1 (Strahl *et al.*, 1998). Interactions between basal and GnRH-regulated transcription factors are as follows: SF-1 synergises with NGFI-A (Lee *et al.*, 1996; Halvorson *et al.*, 1998); LH-2 synergises with Ets (Roberson *et al.*, 1994); GnRH regulates SP-1 (Kaiser *et al.*, 1998b), NGFI-A (Halvorson *et al.*, 1999), Ets (Roberson *et al.*, 1995) and AP-1 (Strahl *et al.*, 1998). Extracellular Ca^{2+} influx upregulates unknown factor x, which transactivates α-subunit and LH β-subunit promoters (Holdstock *et al.*, 1996; Weck *et al.*, 1998). PLC: phopholipase C; IP_3: inositol 1,4,5 triphosphate; DAG: diacylglycerol; PKC: protein kinase C; MEK: mitogen-activated protein kinase kinase or MAPKK; MAPK: mitogen-activated protein kinase.

these two DNA sites in the α-subunit promoter is required to activate GnRH regulation of gene expression. Cyclic AMP (cAMP) responsive elements (CRE) have been identified in the human α-subunit promoter, but not in that of other species, at –132 to –99 bp (Schroderbeck *et al.*, 1992). Although this element is critical for placental expression of the α-subunit, originally it was not thought to be important for pituitary expression. However, Saunders *et al.* (1998) have shown that cAMP stimulates α-subunit transcription, and that this is additive to the stimulation mediated by the PKC pathway, although it is unclear whether this is mediated via a CRE. GnRHr signalling also increases α-subunit transcription by mobilizing extracellular calcium, and the DNA elements responsible map to between –420 and –244 bp in the human α-subunit promoter (Holdstock *et al.*, 1996). This finding has been confirmed in some studies (Saunders *et al.*, 1998) but not in others (Weck *et al.*, 1998).

Differences in the intracellular signalling of the GnRHr mediated by PKC- and cAMP-activated pathways, and by calcium influx, may be part of the mechanism that ensures adequate amounts of α-subunit are synthesized in different physiological states (Fig 3), especially since α-subunit is always synthesized in excess over the β-subunits.

Table 1. Transcription factors regulating gonadotrophin subunit gene expression during development of the anterior pituitary gland

Transcription factor	Gonadotrophin subunit			'Knock-out' phenotype	Reference
	α	LHβ	FSHβ		
Ptx-1	+	+	+	ND	
Hesx1–Rpx	ND	+	ND	Pituitary dysplasia	Dattani *et al.*, 1998
p-Lim–Lhx3	+	ND	ND	Only corticotrophs remain	Sheng *et al.*, 1996
SF-1	+	+	-	No gonads, adrenals, GnRH, LHβ and FSHβ	Luo *et al.*, 1994; Ikeda *et al.*, 1995
Otx1	+	+	+	Prepubescent hypogonadism	Acampora *et al.*, 1998

ND, not determined; Ptx-1, pituitary homeobox 1; Hesx1–Rpx, Rathke's pouch homeobox; p-LIM–Lhx3, LIM homeobox gene; SF-1, steroidogenic factor-1; Otx1, member of Otx homeobox gene family.

GnRH regulation of LH β-subunit gene expression

LH β-subunit promoter elements required to mediate GnRH-responsiveness have been found in –1786 bp of the sheep promoter (Brown *et al.*, 1993; McNeilly *et al.*, 1996) and –2000 bp of the rat promoter (Fallest *et al.*, 1995) and have been further localized to –776 bp in the cow promoter (Keri *et al.*, 1994). Studies *in vitro* on the regulation of LH β-subunit gene expression by GnRH have been hampered by the lack of suitable cell lines that express the LH β-subunit. The isolation of the LH-expressing gonadotroph cell line, LβT2 (Thomas *et al.*, 1996), will provide a model system to investigate control of LH β-subunit gene expression. LβT2 cells synthesize and secrete LH in response to GnRH stimulation (Turgeon *et al.*, 1996). GnRH responsiveness has been mapped to a region upstream of –650 bp in the sheep LH β-subunit gene promoter using LβT2 cells (P. Brown, unpublished).

LH β-subunit transcriptional stimulation by GnRH is mediated by stimulation of the PKC pathway (Saunders *et al.*, 1998) and, to a lesser extent, by stimulation of the MAPK pathway (Weck *et al.*, 1998) and cAMP pathway (Saunders *et al.*, 1998). Mobilization of extracellular calcium did not increase transcription in one study (Saunders *et al.*, 1998), but significantly increased it in another (Weck *et al.*, 1998). These differences may be due to the cell types used for the studies. Two regions have been identified in the rat LH β-subunit promoter that mediate GnRH stimulation of transcription: at –490 to –352 and –207 to –82 bp (Kaiser *et al.*, 1998a). The –490 to –352 region binds the ubiquitous transcription factor, Sp-1 (Kaiser *et al.*, 1998b).

Transcription factors, which are widely expressed in a number of tissues, have a role in stimulating LH β-subunit transcription (Fig. 3b). A nerve growth factor 1A (NGF1-A) 'knock-out' mouse model had an unusual phenotype, with sterile females and fertile males. The females did not express the LH β-subunit gene and their ovaries had no corpora lutea, indicating the absence of ovulation (Lee *et al.*, 1996). An NGF1-A-binding site in the LH β-subunit promoter is located approximately 10–15 bp upstream of the TATA box. A second NGF1-A site has been identified upstream of this site at –112 bp (Halvorson *et al.*, 1998). The sexually dimorphic difference in the 'knock-out' phenotype prompted speculation that this transcription factor is required to facilitate the LH surge in females (Lee *et al.*, 1996), especially since NGF1-A gene expression is stimulated by GnRH (Halvorson *et al.*, 1999).

SF-1 also plays a role in the GnRH regulation of LH β-subunit gene expression. The SF-1 DNA binding site discovered in LH β-subunit promoters is highly conserved across species and occurs at approximately –130 bp. A second SF-1 site in the rat promoter at –59 bp is also conserved across species and synergises with the NGF1-A site at –112 bp (Halvorson *et al.*, 1998). The stimulatory ligand for SF-1 was thought initially to be GnRH (Haisenleder *et al.*, 1996), but this hypothesis has yet to be confirmed (Brown and McNeilly, 1997; Kaiser *et al.*, 1998a; Halvorson *et al.*, 1999). The high degree of synergism between SF-1 and NGF1-A (Lee *et al.*, 1996; Halvorson *et al.*, 1998) and the stimulation of NGF1-A by GnRH (Halvorson *et al.*, 1999) illustrates the two-tier method of control of β-subunit gene expression. SF-1 directs basal LH β-subunit gene expression, which is synergistically augmented by GnRH-regulated transcription factors (Fig. 3).

GnRH regulation of FSH β-subunit transcription

Experiments *in vitro* have defined the regions responsible for activation of FSH β-subunit gene expression to two activating protein 1 (AP-1) sites, which localized to –215 bp of the promoter at positions –120 and –83 bp (Strahl *et al.*, 1997). The AP-1 sites confer GnRH responsiveness (Strahl *et al.*, 1998) and this is relayed by the PKC pathway (Strahl *et al.*, 1998; Saunders *et al.*, 1998; Fig. 3). Calcium influx appears to have no major role in the regulation of FSH β-subunit gene expression (Saunders *et al.*, 1998).

Modulation of GnRH-regulated gene expression by gonadal steroids and peptides

Gonadotrophin gene expression is negatively regulated by feedback suppression of gonadal steroids that act at the pituitary and the hypothalamus to downregulate the GnRH pulse

generator. Positive feedback of steroids is required to generate the pre-ovulatory LH surge, but this is probably mediated at the level of LH secretion and not synthesis (Currie and McNeilly 1995; Thomas and Clarke, 1997). The gonadal peptides, inhibin and activin, have antagonistic roles in modulating pituitary gonadotrophin gene expression (Fig. 1). The α- and β-subunits show different degrees of response to feedback regulation.

Steroid-mediated repression of gene expression

α-Subunit. Administration of oestrogen *in vivo* profoundly downregulates α-subunit gene expression by a mechanism that is not mediated by receptor binding to the α-subunit promoter (Keri *et al.,* 1991). Similarly, the androgen receptor downregulates human α-subunit gene transcription by interfering with basal expression and with the CRE rather than through binding to the androgen receptor binding site (Heckert *et al.,* 1997).

LH β-subunit. Evidence for a direct inhibitory effect of steroids on LH β-subunit gene expression is weak or contradictory. It appears that there is no direct interaction of oestradiol receptor with the LH β-subunit promoter (Keri *et al.,* 1994), indicating that any inhibitory effects of steroids on LH β-subunit gene expression are not mediated via a classical oestrogen response element (ERE) on the LH β-subunit gene promoter. Studies *in vitro*, in which LβT2 cells were cultured with and without oestradiol, showed that oestradiol did not affect LH β-subunit mRNA concentrations, but did increase GnRHr mRNA concentrations (Turgeon *et al.,* 1996). This observation implies that steroids affect LH β-subunit synthesis by increasing GnRHr gene expression. In contrast, studies *in vivo* suggest that oestradiol negatively regulates LH β-subunit transcription by downregulating expression of SF-1 (Brown and McNeilly, 1997).

FSH β-subunit. FSH β-subunit gene transcription is highly sensitive to the negative feedback effects of steroids. Experiments using nuclear run-on assays demonstrate that oestradiol and progesterone downregulate FSH β-subunit gene transcription (Phillips *et al.,* 1988). Studies *in vitro* of the FSH β-subunit promoter localize the effect of oestrogen not to an ERE but to AP-1 sites, which also confer GnRH responsiveness (Miller and Miller, 1996; Strahl *et al.,* 1998).

Regulation of gonadotrophin subunit gene expression by inhibin and activin. Inhibin and activin are members of the transforming growth factor β (TGF-β) family of cytokines. The signal transduction pathway from the cell membrane to the nucleus used by TGF-β (Heldin *et al.,* 1997) involves the activation of transcription from AP-1 binding sites (Zhang *et al.,* 1998). Activin upregulates FSH β-subunit gene expression (Weiss *et al.,* 1995) and, since there are AP-1 binding sites on the FSH β-subunit promoter (Strahl *et al.,* 1997), these may be used to control FSH β-subunit transcription. Inhibin antagonizes the stimulatory effect of activin on FSH β-subunit gene expression, and this may be mediated by the same signalling pathways used by activin. Inhibin action downregulates transcription of the FSH β-subunit, but has no effect on the transcripton of the LH β-subunit (Clarke *et al.,* 1993). This signalling pathway may also converge on the AP-1 binding sites found in the proximal FSH β-subunit promoter.

Conclusions and future prospects

Transcription factors that control gonadotrophin gene expression can be classified into those that direct basal expression and those that are regulated by GnRH. This biphasic model of control of gonadotrophin gene expression assumes that basal gonadotroph-specific gene expression is further modulated by GnRH. The transcription factors known to direct basal gene expression do not specify the differentiation of the gonadotroph cell type. It is possible that there is no single transcription factor that, if 'knocked-out', would only remove gonadotroph cells or, alternatively, that the gonadotroph cell type may be specified by a multifactor complex. Transcription factors regulated by steroids or gonadal peptides also modulate gonadotrophin subunit gene expression by antagonizing or possibly augmenting the effects of GnRH-induced transcription factors. These effects of transcription factors all rely on either differential activation or crosstalk of intracellular signalling pathways. Since LH and FSH β-subunit genes are both expressed in the same cell type and are both regulated by GnRH, the number of GnRH receptors (Kaiser *et al.,* 1995) and subsequent coupling to G-proteins (Stanislaus *et al.,* 1998) could both contribute to differential coupling to intracellular signalling pathways. In addition, differential modulation by steroids and gonadal peptides also ensures that the two gonadotrophins are differentially synthesized. A future research challenge is to understand how differential activation of transcription factors is controlled to explain the *in vivo* regulation of gonadotrophin gene expression. An example is the differential regulation of LH and FSH gene expression across the female oestrous cycle that is necessary for their synergistic action and the perturbation of which profoundly affects reproductive function (P. Brown, J. R. McNeilly, J. G. Evans, H. C. Christian and A. S. McNeilly, unpublished). Ultimately, regulatory signals are transduced into transcriptional activation or repression by interaction of transcription factors, with the specific transcription factor binding sites located on the gonadotrophin subunit gene promoters.

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Note added in proof:

Since submission of this manuscript, an additional article* detailing synergism between basal and GnRH-regulated factors has been published:

*Tremblay JJ and Drouin J (1999) Egr-1 is a downstream effector of GnRH and synergizes by direct interaction with Ptx1 and SF-1 to enhance luteinizing hormone β gene transcription *Molecular and Cellular Biology* **19** 2567–2576

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