0163-769X/05/$20.00/0
Printed in U.S.A.

Endocrine Reviews 26(2):283–306

doi: 10.1210/er.2003-0039

Molecular Biology of Gonadotropin-Releasing Hormone (GnRH)-I, GnRH-II, and Their Receptors in Humans

Chi Keung Cheng and Peter C. K. Leung

Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada V6H 3V5

In human beings, two forms of GnRH, termed GnRH-I and GnRH-II, encoded by separate genes have been identified. Although these hormones share comparable cDNA and genomic structures, their tissue distribution and regulation of gene expression are significantly dissimilar. The actions of GnRH are mediated by the GnRH receptor, which belongs to a member of the rhodopsin-like G protein-coupled receptor superfamily. However, to date, only one conventional GnRH receptor subtype (type I GnRH receptor) uniquely lacking a carboxyl-terminal tail has been found in the human body. Studies on the transcriptional regulation of the human GnRH receptor gene have indicated that tissue-specific gene expression is mediated by differential promoter usage in various cell types. Functionally, there is growing evidence showing that both GnRH-I and GnRH-II are potentially important autocrine and/or paracrine regulators in some extrapituitary compartments. Recent cloning of a second GnRH receptor subtype (type II GnRH receptor) in nonhuman primates revealed that it is structurally and functionally distinct from the mammalian type I receptor. However, the human type II receptor gene homolog carries a frameshift and a premature stop codon, suggesting that a full-length type II receptor does not exist in humans. (*Endocrine Reviews* 26: 283–306, 2005)

First Published Online November 23, 2004

Abbreviations: AP-1, Activator protein-1; CRE, cAMP-responsive element; CREB, CRE-binding protein; E_2, 17β-estradiol; ER, estrogen receptor; GAP, GnRH-associated peptide; GL, granulosa-luteal; GPCR, G protein-coupled receptor; GSE, gonadotrope-specific element; hCG, human chorionic gonadotropin; IHH, idiopathic hypogonadotropic hypogonadism; JNK, c-Jun amino-terminal kinase; MEK, MAPK kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NRE, negative regulatory element; OSE, ovarian surface epithelial; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PR, progesterone receptor; SF-1, steroidogenic factor-1; TM, transmembrane; UTR, untranslated region.

Endocrine Reviews **is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

I. Introduction

MAMMALIAN GnRH (termed GnRH-I) is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) that plays a key role in the process of reproduction. It is produced by hypothalamic neurosecretory cells and released in a pulsatile manner into the hypothalamo-hypophyseal portal circulation, through which the hormone is transported to the anterior pituitary gland. After binding to its cognate receptor (type I GnRH receptor) on pituitary gonadotropes, the hormone stimulates the biosynthesis and secretion of LH and FSH, which in turn regulate gonadal steroidogenesis and gametogenesis in both sexes (1). In addition to this well-known endocrine function, it has become evident that GnRH-I is a potentially important autocrine and/or paracrine regulator in some extrapituitary compart-

ments such as the ovary, placenta, uterus, and immune system (2–10). Since its discovery some 30 yr ago, many GnRH-I analogs with enhanced biological potency have been developed and studied extensively (11). Clinically, some of these synthetic analogs have been used as an effective treatment for a variety of reproductive endocrinopathies, whereas others have been widely adopted in controlled ovarian hyperstimulation regimens for assisted reproductive techniques (12, 13).

Until now, more than a dozen isoforms of GnRH sharing 10–50% amino acid identity have been found in vertebrates (14). It is generally thought that most vertebrate species possess at least two, and usually three, forms of GnRH, which differ in their amino acid sequences, localizations, and embryonic origins. In addition to GnRH-I, a second GnRH subtype (termed GnRH-II) that was originally identified from chicken hypothalamus has been found in humans (15, 16). This second GnRH form differs from GnRH-I by three amino acid residues at positions 5, 7, and 8 (His^{5}Trp^{7}Tyr^{8}GnRH-I) and is conserved from primitive fish to humans (16, 17). One of the established biological functions specific to GnRH-II is to serve as a potent inhibitor of K^+ channels in the amphibian sympathetic ganglion (17). Inhibition of these ion channels facilitates rapid excitatory transmission by conventional neurotransmitters and may provide a general neuromodulatory mechanism for GnRH-II in the nervous system. Recently, Temple *et al.* (18) have shown that GnRH-II, but not GnRH-I, activates mating in energetically challenged musk shrews, suggesting a role of the evolutionarily conserved GnRH form in coordinating energy and reproductive behavior. In humans, a growing number of extrapituitary GnRH-II actions, such as suppressing tumor proliferation (3, 7, 8, 19–23), have been demonstrated although a full-length type II GnRH receptor transcript has not yet been identified in any of the human tissues or cell types.

II. GnRH Isoforms in Humans: GnRH-I and GnRH-II

A. cDNA and genomic structures

Cloning of the GnRH-I cDNA from human hypothalamus and placenta revealed that they possess identical coding and 3′-untranslated regions (3′-UTRs) (24, 25). However, the placental cDNA has a much longer 5′-UTR because of the inclusion of the first intron in the transcript (24, 26). The coding region of the GnRH-I cDNA contains an open reading frame of 276 bp encoding a precursor protein of 92 amino acids. The reading frame is followed by a 160-bp 3′-UTR, which contains an AATAAA sequence for polyadenylation shortly upstream of a polyadenylated tail. The first 23 amino acids of the precursor form the signal sequence and are separated by the GnRH decapeptide by two serine residues. The decapeptide, in turn, is followed by a GKR sequence as well as a 56-amino acid peptide termed GnRH-associated peptide (GAP). The GKR sequence serves to signal amidation of the carboxyl terminus and enzymatic cleavage of the decapeptide from the precursor.

The human GnRH-I gene is composed of four exons separated by three introns and is present as a single gene copy on chromosome 8p11.2-p21 (Fig. 1) (26, 27). The first exon of the gene is untranslated and consists of 61 bp in mRNA expressed in the hypothalamus. The second exon encodes the signal sequence, the GnRH decapeptide, the GKR processing signal, and the first 11 GAP residues. The third exon codes for the next 32 GAP residues. The fourth exon encodes the remaining GAP residues and contains the translation termination codon as well as the entire 3′-UTR (24, 26).

The human GnRH-II gene has been cloned and mapped to chromosome 20p13 by fluorescence *in situ* hybridization (16). It also comprises four exons interrupted by three introns, and the predicted GnRH-II preprohormone is organized identically to the GnRH-I precursor (Fig. 1). However, the human GnRH-II gene (2.1 kb) is shorter than the GnRH-I gene (5 kb)

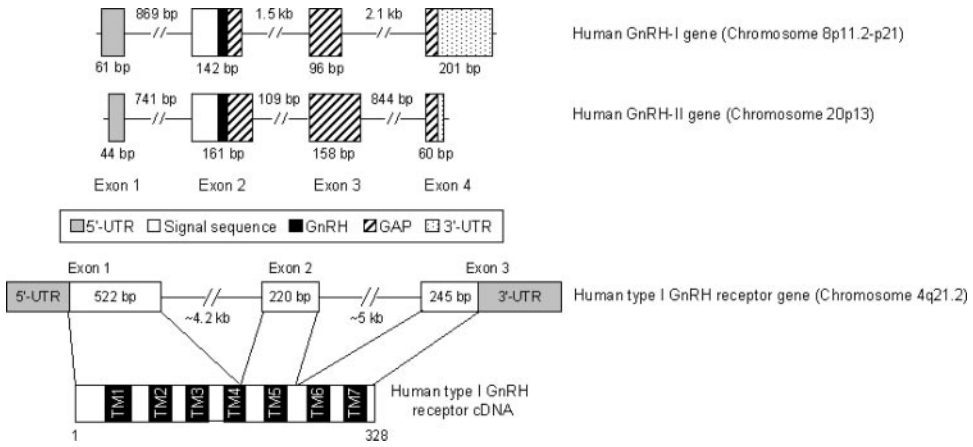

FIG. 1. cDNA and genomic structures of human GnRH and GnRH receptor genes. In humans, two forms of GnRH, termed GnRH-I and GnRH-II, encoded by separate genes on chromosome 8p11.2-p21 and 20p13 are identified. Both genes are composed of four exons (*boxes*) interrupted by three introns (*thin lines*), and their encoded preprohormones are organized identically such that they all have a signal sequence, followed by a GnRH decapeptide, a conserved GKR cleavage site, and a GAP. In contrast, only one conventional GnRH receptor subtype (termed type I GnRH receptor) is found in the human body. The gene coding for the type I GnRH receptor lies on chromosome 4q21.2 and consists of three exons separated by two introns. Exon 1 contains the 5′-UTR and encodes the first three TM domains and a portion of the fourth TM domain. Exon 2 is 220 bp in length and encodes the remainder of the fourth TM domain, the fifth TM domain, and part of the third intracellular loop. Exon 3 encodes the rest of the open reading frame and contains the 3′-UTR.

because introns 2 and 3 of the latter are much larger (16). Moreover, although their corresponding precursor proteins are quite similar in length, the GAP is 50% longer in the GnRH-II precursor (84 *vs.* 56 amino acids). In fact, a similar disparity in GAP has also been reported in the placental mammal, tree shrew (76 *vs.* 56 amino acids) (28), suggesting that a relatively larger GAP may be a common characteristic among mammalian GnRH-II precursors.

B. Tissue distribution in humans

1. Brain. The most prominent difference in the tissue distribution of GnRH-I and GnRH-II in humans is that the latter isoform is expressed at the highest level outside the brain (16). The levels of GnRH-II mRNA in the kidney are approximately 30-fold higher than in any brain region, whereas the expression in the bone marrow and prostate is about 4-fold greater than in the brain (16). Conversely, GnRH-I expression was not observed at a high level outside the brain (16). In humans, cell bodies of GnRH-I neurons are concentrated in the preoptic area and basal hypothalamus. However, they can also be found in the septal region and anterior olfactory area, as well as the cortical and medial amygdaloid nuclei (29). On the other hand, immunoreactive GnRH-I fibers are localized predominantly in the median eminence and infundibular stalk although substantial projections to the neurohypophysis can be detected (30, 31). Using *in situ* RT-PCR, expression of GnRH-I mRNA has been demonstrated in normal human pituitary and various types of pituitary adenomas (32, 33).

In human brain, immunopositive GnRH-II signals localize mainly in the periaqueductal region of the midbrain (34). However, expression of GnRH-II mRNA in the human brain was found to be most abundant in the caudate nucleus and, to a lesser extent, in the hippocampus and amygdala (16). Using RT-PCR and Southern blot analysis, two GnRH-II mRNA variants were identified in human fetal brain and adult thalamus but not in adult kidney. These transcripts differ in the size of their GAPs, which are predicted to contain 77 and 84 amino acid residues (16).

Coexpression of GnRH-I and GnRH-II has been demonstrated at both the mRNA and protein levels in certain human neuronal cell lines in which where the concentration of GnRH-I is 10- to 40-fold higher than that of GnRH-II (35).

2. Placenta. It has long been shown that human placenta *in vitro* synthesizes and secretes GnRH-I that is immunologically indistinguishable from its hypothalamic counterpart (36, 37). Likewise, Siler-Khodr and Grayson (38) have shown that GnRH-II is released from human placenta *in vitro* in a pulsatile fashion and that this second GnRH form is more resistant than GnRH-I to degradation by placental enzymes. Examination of the spatiotemporal distribution of these hormones revealed that both GnRH-I and GnRH-II mRNAs are expressed in human first-trimester placenta (39). However, only GnRH-I is also expressed in tissues obtained at term (39). Using immunohistochemistry, both hormones were found to localize in the mononucleate villus and in distinct subpopulations of the extravillous cytotrophoblast. However, GnRH-I is also present in the outer multinucleated syncytiotrophoblast layer and in cultures of cytotrophoblasts allowed to undergo differentiation and fusion *in vitro* (39).

3. Uterus. Expression of GnRH-I mRNA has been demonstrated in virtually all human uterine compartments (40–45). Interestingly, a dynamic expression pattern is observed in the endometrium as well as in isolated endometrial cells such that a significant increase in transcript levels is detected in the secretory phase of the menstrual cycle (44, 45). In support of these observations, GnRH-I immunoreactivity has been found in all endometrial cell types, with the most intense staining being observed in the luteal phase (45).

The spatiotemporal expression of GnRH-II has also been investigated in human endometrium. Throughout the entire reproductive cycle, two splice variants of GnRH-II mRNA are expressed, with the shorter transcript carrying a 21-bp deletion, which reduces the length of GAP from 77 to 70 amino acids (46). Like GnRH-I, GnRH-II immunoreactivity is dynamically expressed in stromal and epithelial cells such that stronger signals are detected in the early and midsecretory phases than in the proliferative and late-secretory phases (46).

4. Ovary. Expression of GnRH-I and GnRH-II mRNAs identical to their brain counterparts has been demonstrated in various human ovarian tissues including granulosa-luteal (GL) cells, ovarian surface epithelial (OSE) cells, and ovarian carcinoma (19, 20, 47, 48). In addition, expression of GnRH-I mRNA and protein has been found in the tubal epithelium of the fallopian tube during the luteal phase of the reproductive cycle (49).

5. Other tissues. Both forms of GnRH are expressed in normal human breast tissue and are overexpressed in breast cancer (50, 51). Moreover, certain immune cell lineages such as T lymphocytes and peripheral blood mononuclear cells have been found to produce GnRH-I or both GnRH-I and GnRH-II (7, 52, 53). In Jurkat leukemic T cells, the concentration of GnRH-I is higher than that of GnRH-II, as determined by RIAs (7). Expression of GnRH-I protein has also been demonstrated in human seminiferous tubular cells (54) and preimplantation embryos, in which immunoreactive signals are localized in all the blastomeres as well as the trophectoderm and inner cell mass of the blastocyst (55).

C. Regulation of gene expression in humans

1. GnRH-I and GnRH-II. In human OSE, GL, and OVCAR-3 ovarian cancer cells, treatment with GnRH-I analogs produces a biphasic effect on its mRNA levels such that high concentrations decrease whereas low concentrations increase the expression (20, 47, 48). In contrast, GnRH-I suppresses its mRNA levels in peripheral blood mononuclear cells in a dose-dependent manner (53). Homologous down-regulation of GnRH-I mRNA levels has also been demonstrated, in a dose- and time-dependent fashion, in rat hypothalamus *in vivo* and in GT1–1 cells (56, 57). On the other hand, heterologous regulation of GnRH-I expression has been studied only in human GL cells, in which GnRH-II or its analog causes down-regulation of GnRH-I mRNA levels at a wide range of concentrations (20).

2. Gonadal steroid hormones. There are substantial lines of evidence indicating that the expression of GnRH-I and GnRH-II is differentially regulated by gonadal steroids. In human GL, OVCAR-3, and TE-671 neuronal cells, treatment with 17β-estradiol (E_2) down-regulates the steady-state GnRH-I mRNA levels (58–61). This E_2 action is believed to be mediated via the nuclear estrogen receptor (ER) because cotreatment with the antiestrogen tamoxifen can abolish the inhibitory effect. Using the ER-negative Chinese hamster ovary-K1 cell line as a model, Chen *et al.* (62) demonstrated that E_2 can repress the human GnRH-I promoter when ERα is overexpressed. Also, they found that the estrogen response area lies between 169 and 548 bp 5′ of the upstream transcription start site of the GnRH-I gene. Similarly, E_2 has been found to suppress the mRNA expression and promoter activity of the GnRH-I gene in mouse GT1–7 neurons, possibly via an ERα-mediated mechanism (63). On the contrary, our laboratory has recently shown that E_2 increases GnRH-II mRNA levels in a dose- and time-dependent manner in human GL cells (61). Likewise, a stimulatory effect of E_2 on GnRH-II expression has been reported in TE-671 cells (60).

The role of progesterone in regulating GnRH-I and GnRH-II expression has been investigated in human GL cells. Whereas treatment with the progesterone receptor (PR) antagonist RU486 does not affect GnRH-I mRNA levels, the levels of GnRH-II transcript are stimulated by the antagonist in a dose and time-dependent manner (61), suggesting that the gonadal steroid has an inhibitory role in GnRH-II expression in the ovary.

Regulation of GnRH-I gene expression by androgen has been examined in the androgen receptor-expressing GT1–7 cell line. In these cells, treatment with 5α-dihydrotestosterone causes a time-dependent reduction in GnRH-I mRNA levels, and this repression can be blocked by the androgen receptor antagonist hydroxyflutamide (64). However, no significant changes in GnRH-I expression can be observed when the hypothalamic neurons are treated with cell-impermeable testosterone-BSA conjugates (65), indicating that the androgen action is mediated via classical nuclear receptor activation.

3. Gonadotropins. Further evidence that the expression of the two forms of GnRH is differentially modulated comes from studies on their regulation by gonadotropins, which mediate their actions by stimulating intracellular cAMP production and activating the protein kinase A signaling pathway. In human GL cells, treatment with FSH or human (h) chorionic gonadotropin (CG) up-regulates the mRNA levels of GnRH-II but down-regulates those of GnRH-I in a dose-dependent manner (20). Consistently, an increase in GnRH-II mRNA and protein levels in response to cAMP has been observed in TE-671 cells (66). This cAMP-activated GnRH-II gene expression is thought to occur at the transcriptional level because mutation of a putative cAMP-responsive element (CRE) in the human GnRH-II 5′-flanking region causes a reduction in both the cAMP-induced and basal promoter activities (66).

4. Other physiological regulators. It has been shown recently that IL-1β can up-regulate GnRH-I mRNA levels in human endometrial stromal cells *in vitro* in a dose-dependent manner (67). In addition, an increase in human GnRH-I gene transcription has been observed in NLT neuronal cells following IGF-I treatment (68). This stimulation is likely mediated via a consensus activator protein-1 (AP-1) motif in the proximal promoter region of the gene (68). Moreover, certain odorants have been found to induce a dramatic increase in GnRH-I mRNA levels and protein release in human olfactory cells (69), which share a common origin with GnRH-I neurons during organogenesis (70, 71). Using the immortalized GT1–7 neurons as a model, Roy *et al.* (72) and Roy and Belsham (73) have demonstrated that melatonin significantly down-regulates GnRH-I mRNA levels in a 24-h cyclical manner and that this regulation may involve the protein kinase C (PKC) and the ERK1/2 pathways.

5. Basal transcriptional regulation. The molecular mechanism underlying neuron-specific expression of the human GnRH-I gene has been explored. By means of deletion analysis, a region between −992 and −795 of the human GnRH-I 5′-flanking region was found to be essential and sufficient for targeting luciferase expression in the hypothalamus and olfactory tissue *in vivo* (74). This region contains two specific DNA-binding sites for the POU homeodomain transcription factors Brn-2 and Oct-1. Functional studies revealed that overexpression of Brn-2, but not Oct-1, can transactivate both the human and mouse GnRH-I promoters (74). These findings thus indicate a role of Brn-2 or Brn-2-related proteins in regulating neuron-specific GnRH-I gene transcription.

In addition to the putative CRE described (66), we have recently uncovered a novel function of the untranslated first exon of the human GnRH-II gene in mediating full expression of GnRH-II promoter activity (75). Although this exon can work as a stand-alone enhancer element, its enhancer activity is strictly dependent on its position and orientation relative to the target sequence (75). Two putative E box binding sites and one Ets-like element are present juxtaposed to each other within the exon, and site-directed mutagenesis indicated that these motifs function in a cooperative manner to stimulate basal GnRH-II gene transcription (75). Detailed characterization of the E box binding factors revealed that the basic helix-loop-helix transcription factor AP-4 (76), the expression of which correlates well with that of GnRH-II, is an enhancer protein for the human GnRH-II promoter (75).

III. Molecular Characterization of Human Type I GnRH Receptor Gene

A. cDNA cloning

The GnRH receptor belongs to a member of the rhodopsin-like G protein-coupled receptor (GPCR) superfamily, which contains a characteristic seven-transmembrane (TM)-domain structure (77–79). However, unlike other members of the GPCR family, the mammalian GnRH receptor lacks the entire carboxyl-terminal tail (77, 78), which is known to participate in various aspects of GPCR regulation through interaction with a network of receptor-associated proteins (80, 81). A number of amino acid residues of critical importance for receptor function have been identified in the human

GnRH receptor. For instance, Ala (261) in the third intracellular loop is crucial for G protein coupling and receptor internalization (82), whereas Asp (98), Trp (101), Asn (102), Lys (121), Asn (212), and Asp (302) are important for ligand binding (83–87). In addition, the extra species-specific Lys (191) residue has been shown to be a significant determinant of the expression and internalization of the human GnRH receptor (88).

B. Genomic organization and chromosomal localization

In contrast to the genes of many other GPCRs, which are intronless and believed to have arisen by retroposition (89), the human GnRH receptor gene is composed of three exons separated by two introns and spans more than 15 kb along the chromosome (Fig. 1) (90, 91). Exon 1 contains the 5′-UTR and the first 522 nucleotides of the open reading frame, which encode the first three TM domains and a portion of the fourth TM domain. Exon 2 encodes the next 220 nucleotides of the reading frame, which encompass the remainder of the fourth TM domain, the fifth TM domain, as well as part of the third intracellular loop. Exon 3 contains the rest of the coding sequence and the 3′-UTR. Although the location of all the exon-intron boundaries of the human GnRH receptor gene is perfectly conserved in the rodent and ovine sequences, the first intron of the human gene is comparatively much smaller (92–94). Using genomic Southern blot and chromosomal in situ hybridization, the human GnRH receptor gene has been identified as a single copy on chromosome 4q21.2 (91, 95).

C. Untranslated and 5′-flanking regions

Five and 18 transcription start sites have been identified for the GnRH receptor gene in human brain and pituitary, respectively (90, 91). All these start sites are clustered into two regions, which are 579–819 and 1348–1751 bp upstream of the ATG initiation codon. Five typical polyadenylation signals residing within an 800-bp area in a cluster-like format are present in the 3′-UTR of the human GnRH receptor gene (90). Also, the 3′-UTR contains several ATTTA motifs, which are implicated in mRNA instability and are notably present in many RNAs that are rapidly degraded (96, 97). The size of the GnRH receptor mRNA predicted from the length of the 5′- and 3′-UTRs is about 5.5 kb, which is in close agreement with the reported size of the major transcript (4.7–5 kb) expressed in human pituitary.

Although the proximal 5′-flanking region of the human GnRH receptor gene shares a substantial homology with that of the rodent and ovine sequences (90, 92–94), the human gene possesses some distinctive features that are not observed in other species. One significant difference between the human and rodent genes is the location of their transcription start sites. Thus, whereas the start sites for the rodent genes are within 100 nucleotides from the initiation codon (92, 94), those for the human gene are no less than 703 bp (90). Another difference is that the human sequence contains multiple canonical TATA and CAAT boxes residing in close proximity to each other near the transcription start sites (90, 91). The presence of consensus TATA boxes is unusual among all the GPCRs sequenced to date, as many of these genes contain GC-rich promoter regions (98–101).

D. Tissue distribution in humans

1. Pituitary. Northern blot analysis has revealed a predominant GnRH receptor transcript of 4.7–5 kb as well as two fainter bands of 2.5 and 1.5 kb in human pituitary (91, 102). All these mRNA species contain the full-length coding sequence and are correctly spliced (91). Additionally, two splice variants of the human GnRH receptor, termed sb2 and sb3, have been identified in normal pituitary and pituitary adenoma (103, 104). The shorter transcript sb3 contains a 220-bp deletion in exon 2 such that it codes for a protein of only 177 amino acids, lacking the last four TM domains, the second and third extracellular loops, as well as the third intracellular loop. On the other hand, the sb2 variant carries a shorter deletion of 128 bp and arises from alternative splicing by accepting a cryptic acceptor site in exon 2. This deletion generates a truncated protein *in which* the glutamine residue at position 174 is followed by a stretch of 75 new amino acids (104). Interestingly, when coexpressed with the full-length receptor cDNA, the sb2 variant exhibits a dominant-negative action on GnRH receptor signaling, potentially by impairing insertion of the wild-type receptor protein into the plasma membrane. This inhibitory effect is highly specific for the GnRH receptor as signaling via other GPCRs is not affected (103).

The distribution of GnRH receptor immunoreactivity in normal and tumorous human pituitary has also been determined. In normal adenohypophysis, immunopositive signals colocalize with α-subunit-, FSHβ-, LHβ-, TSH β-, and GH-producing cells (105), suggesting that the receptor is expressed in gonadotropes, thyrotropes, as well as somatotropes. Consistent with the mRNA expression pattern in tumorous pituitary, immunoreactive GnRH receptor signals are frequently detected in adenomas derived from gonadotropes, somatotropes, and α-subunit/null-cells (33, 105).

2. Placenta. Using *in situ* hybridization, expression of GnRH receptor has been demonstrated in the cytotrophoblast and syncytiotrophoblast cell layers of human placenta (106). The temporal expression of the placental receptor parallels with the time course of hCG secretion and peaks at 9 wk (106). The full-length GnRH receptor cDNA has been cloned from various human placental cell types, and their nucleotide sequences are identical to that of the pituitary receptor (107). Northern blot hybridization indicated that a 2.5- and 1.2-kb transcript, but not the major 4.7–5-kb one found in the pituitary, are expressed in the placental cells (107).

3. Ovary. High-affinity binding sites specific for GnRH-I have been detected in human corpus luteum, luteinized granulosa cells, epithelial ovarian carcinoma, and a number of ovarian cancer cell lines (108–111). Interestingly, an additional type of GnRH-I binding site, which is of lower affinity but higher capacity, is found in EFO-21 and EFO-27 ovarian cancer cells (110). Using RT-PCR and Southern blot analysis, expression of GnRH receptor mRNA indistinguishable from its pituitary counterpart has been demonstrated in various ovarian compartments (Fig. 2) (19, 48, 112, 113).

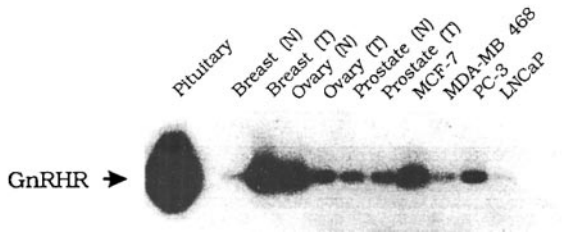

FIG. 2. PCR amplification and Southern blot analysis of the type I GnRH receptor cDNA from various human tissues and tumor cell lines. N, Normal; T, tumor. MCF-7 and MDA-MB 468 are breast tumor cell lines, whereas PC-3 and LnCAP are prostate tumor cell lines. [Reproduced with permission from S. S. Kakar *et al.*: *Mol Cell Endocrinol* 106:145–149, 1994 (112). © 1994 Elsevier.]

4. Uterus. Similar to EFO-21 and EFO-27 cells, two types of GnRH-I binding sites exist in HEC-1A and Ishikawa endometrial carcinoma cell lines (114). However, only one class of high-affinity binding site was found in normal endometrial tissue, endometrial carcinoma, and certain endometrial cancer cells (115, 116). Using immunohistochemistry, membrane receptors specific for GnRH-I have been found in myometrial and leiomyomal cells (43). On the other hand, expression of GnRH receptor mRNA has been demonstrated in both normal and neoplastic uterine cells including those derived from stromal and ectopic endometrial tissues (41, 43, 67, 117, 118). Like the placental and ovarian receptors, sequence analysis revealed that the entire coding region of the endometrial GnRH receptor cDNA contains neither mutations nor alternative splicing patterns (118).

5. Prostate gland. The presence of specific binding sites for GnRH-I has been demonstrated in human prostate cancer and certain prostatic cancer cell lines (119–121). However, the affinity of these sites is generally lower than that of the pituitary receptor (120, 121). PCR products of the expected size for the GnRH receptor cDNA have been obtained from both normal and neoplastic prostate samples (Fig. 2) (112, 122–125), whereas immunopositive signals have been detected in tumorous prostate tissue as well as intraprostatic lymphocytes (125). Expression of GnRH receptor in the prostate is further supported by the detection of a 64-kDa band, which corresponds well to the molecular mass of the pituitary GnRH receptor, in LnCAP and DU 145 cells (123).

6. Breast. The existence of specific GnRH-I binding sites has been reported in breast carcinoma and MCF-7 mammary cancer cells (126, 127). Interestingly, the MCF-7 cells express two distinct types of binding sites, one of high affinity, which is specific for GnRH-I, and the other, which is only recognizable by GnRH-I antagonists (127). Expression of GnRH receptor immunoreactivity and mRNA with sequence identical to the pituitary counterpart has been demonstrated in both normal and malignant breast tissues (Fig. 2) (112, 128, 129). However, unlike its ligands (51), expression of GnRH receptor is not up-regulated in breast cancer cells (128).

7. Other extrapituitary tissues. Multiple lines of evidence indicate that the expression of extrapituitary GnRH receptor is not limited to reproductive tissues. For instance, it has been demonstrated by RT-PCR and Southern blot hybridization that the receptor is also expressed in the liver, heart, skeletal muscle, kidney, and peripheral blood mononuclear cells (53, 130). Moreover, the receptor is expressed in melanoma cells at both the RNA and protein levels (131).

E. Regulation of gene expression in humans

1. GnRH-I and GnRH-II. It has been well documented that pituitary GnRH receptor expression is dynamically regulated by GnRH-I such that subnanomolar concentrations up-regulate whereas high concentrations down-regulate receptor expression (132–135). The extent of this up-regulation, however, is differentially controlled by varying GnRH-I pulse frequencies such that maximal stimulation is achieved at a frequency of every 30 min in cultured rat pituitary cells (136). Similarly, a biphasic effect of GnRH-I on GnRH receptor expression has been demonstrated in human GL, OSE, ovarian cancer, and peripheral blood mononuclear cells (20, 47, 48, 53). Conversely, a significant increase instead of decrease in receptor mRNA levels is observed in JEG-3 choriocarcinoma and extravillous trophoblast cells after chronic GnRH-I stimulation (107). The effect of GnRH-II on GnRH receptor expression has also been investigated in human GL cells. In contrast to the biphasic response induced by GnRH-I, treatment with GnRH-II or its analog significantly inhibits the mRNA levels of the receptor in the steroidogenic cells irrespective of the concentration used (20).

2. Gonadal steroid hormones. The role of E_2 in regulating GnRH receptor expression has been extensively studied at the pituitary level, where the gonadal effect is dynamic and apparently depends on the administration pattern (137–140). In humans, modulation of GnRH receptor expression by E_2 has been examined in extrapituitary tissues. Using semiquantitative RT-PCR, the steady-state mRNA levels of the receptor were found to be suppressed by E_2 in GL and OVCAR-3 cells in a dose- and time-dependent manner (58, 59). This inhibitory effect can be abolished by cotreatment with tamoxifen, suggesting the mediation through the classical ER. Accordingly, E_2 has been demonstrated to repress the human GnRH receptor promoter in ovarian cancer cells via an ERα-dependent mechanism (141). In addition to modulating gene transcription, prolonged E_2 treatment has been shown to increase glycosylation of the ovine GnRH receptor to generate a 43-kDa protein (142, 143). Although the biological significance of this estrogen-induced hyperglycosylation is unclear, it appears that this posttranslational modification is not associated with pituitary desensitization of LH response to GnRH-I (143).

Several lines of evidence indicate that progesterone directly inhibits GnRH receptor expression in the pituitary (144–147). Intriguingly, our colleagues have revealed that the gonadal steroid has a dual role in controlling human GnRH receptor gene transcription such that the hormone suppresses the GnRH receptor promoter in gonadotropes but stimulates it in placental cells (148). The molecular mechanism underlying these opposing effects of progesterone will be discussed in detail below.

3. Gonadotropins. In human GL cells, treatment with hCG for 24 h induces a dose-dependent inhibition of GnRH receptor mRNA levels (113). Accordingly, a similar effect has also

been demonstrated in rat granulosa cells, rat testis, and GT1–7 neurons (149–151). However, contradictory results have been obtained from JEG-3 cells, in which the gonadotropin stimulates the receptor expression at the transcriptional level (107, 152). Thus, it is conceivable that the effect of gonadotropins on GnRH receptor gene expression may be tissue specific.

4. Melatonin. It has become increasingly evident that melatonin can modulate ovarian functions in an autocrine manner (153–157). In human GL cells, transcripts encoding two melatonin receptor subtypes MT_1 and MT_2, which are homologous to their brain counterparts, have been identified (154, 157). Accordingly, treatment of the steroidogenic cells with melatonin significantly decreases the steady-state mRNA levels of the GnRH receptor and GnRH-I but increases those of the LH receptor in a dose-dependent manner (157). Because GnRH-I has been implicated as a luteolytic factor in the ovary (5), it is postulated that this melatonin-induced down-regulation of GnRH receptor expression may interfere with corpus luteum regression during the mid- and late luteal phases of the reproductive cycle (157).

5. Activin. It has been demonstrated that activin A can stimulate the synthesis of GnRH receptor in rat pituitary cells (158). In αT3–1 cells expressing the inhibin βB-subunit, activin A increases GnRH receptor mRNA levels and promoter activity in a dose- and time-dependent manner, and these effects can be abolished by the activin antagonist follistatin (159). On the contrary, treatment with follistatin alone decreases the basal transcription of the gene, suggesting a potential autocrine and/or paracrine role of endogenous activin B in GnRH receptor expression in the gonadotropes (159). The biological significance of this activin-stimulated GnRH receptor gene transcription is confirmed by the observation that activin A pretreatment can enhance the GnRH-I responsiveness of the human glycoprotein α-subunit promoter (159).

F. Pathophysiology of human GnRH receptor mutations

Idiopathic hypogonadotropic hypogonadism (IHH) is a clinical disorder characterized by delayed sexual development and inappropriately low gonadotropin and sex steroid levels in the absence of any anatomical or functional abnormalities of the hypothalamic-pituitary axis (160). Patients with IHH exhibit a wide spectrum of phenotypes, ranging from partial to complete hypogonadism even among affected kindred. In addition, this disorder is genetically heterogeneous and may be sporadic or familial. Mutations of two distinct genes located at the short arm of the X chromosome, *KAL-1* and *DAX-1*, are responsible for the X-linked forms of IHH, which are accompanied by anosmia and adrenal insufficiency, respectively (161–163). In contrast, mutations of the GnRH receptor gene cause IHH without anosmia or adrenal failure and are responsible for autosomal inheritance of the disorder. To date, a total of 15 naturally occurring mutations have been identified along the entire sequence of the human GnRH receptor gene. Of these, one is a truncation mutant, nine are compound heterozygotes (164–172), and five are compound homozygotes (168, 173, 174). Functional studies in heterologous cell systems demonstrated that the naturally occurring GnRH receptor mutants have impaired cellular expression, ligand binding, and/or signal transduction such that 10 of them are totally nonfunctional ($E^{90}K$, $A^{129}D$, $R^{139}H$, $S^{168}R$, $A^{171}T$, $C^{200}Y$, $S^{217}R$, $L^{266}R$, $C^{279}Y$, and $L^{314}X$), whereas others retain a modest degree of receptor function ($N^{10}K$, $T^{32}I$, $Q^{106}R$, $R^{262}Q$, and $Y^{284}C$). However, there are emerging data suggesting that misrouting of these mutant receptors contributes to the molecular etiology of normosomic, adrenal-sufficient IHH (175–177).

IV. Transcriptional Regulation of Human Type I GnRH Receptor Gene

A. Cell-specific promoters

The isolation of the human GnRH receptor 5′-flanking sequence has led to an intensive research on the transcriptional regulation of the gene (Fig. 3). Using αT3–1 cells as a model, the proximal 173-bp flanking region was found to be important for directing GnRH receptor gene expression in gonadotropes (178). This regulatory region contains two putative gonadotrope-specific elements (GSEs) with the core sequence 5′-TGA/TCC-3′ at −143/−135 and −13/−5. Such regulatory elements have been shown to confer cell-specific expression of the glycoprotein hormone α-subunit (179, 180) and LHβ (181) genes in pituitary gonadotropes. Site-directed mutagenesis revealed that the upstream GSE (*i.e.*, at −143/−135) is essential for gonadotrope-specific transcription of the GnRH receptor gene because mutation of this element selectively impairs the promoter function in αT3–1 cells (178). EMSAs indicated that the orphan nuclear receptor steroidogenic factor-1 (SF-1) binds specifically to the upstream GSE, of which the second, fifth, sixth, and the ninth nucleotides are crucial for the interaction (178). The functional significance of SF-1 in regulating human GnRH receptor gene transcription in gonadotropes is confirmed by the findings that overexpression of sense and antisense SF-1 mRNAs can stimulate and repress the native promoter, respectively (178).

Because SF-1 is also expressed in extrapituitary sites, most prominently in steroidogenic tissues (182, 183), it has been suggested that GSEs may not be the sole mediator in conferring gonadotrope-specific gene expression. In support of this view, an earlier study on the transcriptional regulation of the mouse GnRH receptor gene has revealed the importance of a tripartite enhancer element in targeting gonadotrope-specific GnRH receptor expression (184). This enhancer consists of a SF-1 binding site, a canonical AP-1 site, and a novel element termed GRAS. Interestingly, the GRAS motif can function as a stand-alone enhancer to stimulate a heterologous promoter selectively in *αT3–1 cells* (184). Despite sharing a high degree of homology with the mouse sequence, the human gene possesses neither the AP-1 nor the GRAS site in the corresponding positions along the proximal promoter region (178). These observations thus indicate that differential transcriptional apparatus may be involved in gonadotrope-specific expression of the human and rodent GnRH receptor genes.

Many studies suggest that tissue-specific gene expression

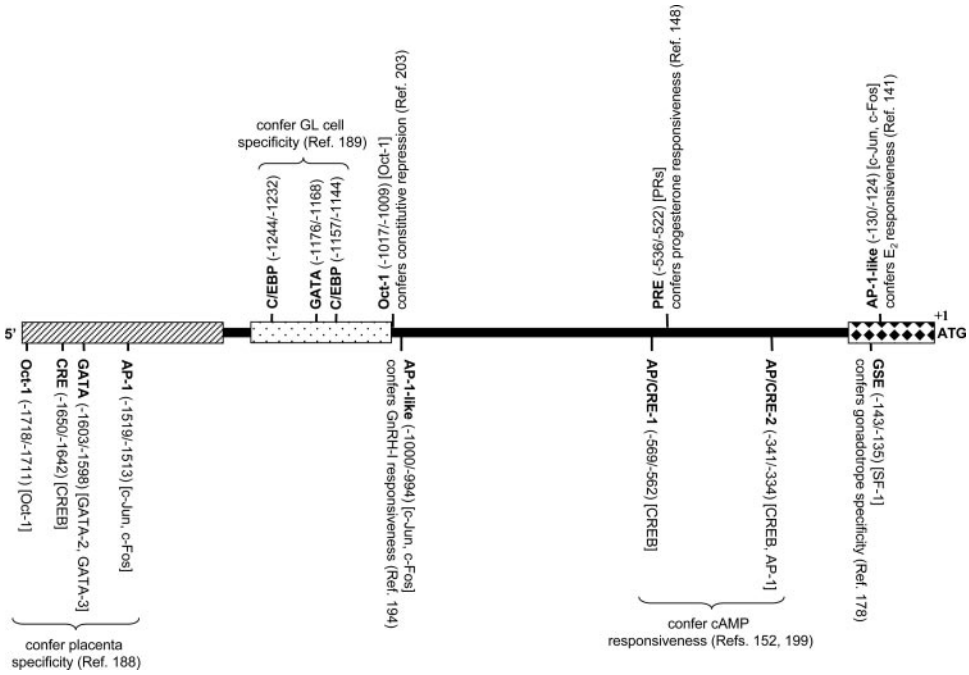

FIG. 3. Summary of transcriptional regulation of the human type I GnRH receptor gene. A diagrammatic representation of the human type I GnRH receptor 5′-flanking region. The location of key regulatory motifs (all locations are relative to the ATG start codon, of which the position is assigned as +1) and their functional significance are indicated. Transcription factors that interact specifically with the corresponding *cis*-acting motifs are listed in *square brackets*. It is noteworthy that tissue-specific gene expression of the GnRH receptor is mediated, at least partly, by differential usage of various promoter elements (shown as *shaded boxes)* in different cell types.

can be mediated via differential promoter usage in various cell types (185–187). Accordingly, Cheng *et al.* (188) have identified a novel human GnRH receptor promoter residing between −1737 and −1346, which is highly active in JEG-3 and extravillous trophoblast cells. The usage of this distal promoter is supported by the identification of five transcription start sites at −1629, −1608, −1416, −1391, and −1379 in the placental cells. Four putative *cis*-acting regulatory motifs termed human (h) hGR-Oct-1 (−1718/−1711), hGR-CRE (−1650/−1642), hGR-GATA (−1603/−1598), and hGR-AP-1 (−1519/−1513), which can interact specifically with transcription factors Oct-1, CRE-binding protein (CREB), GATA-2, GATA-3, and c-Jun/c-Fos heterodimer, were identified in the distal promoter (188). Mutational analysis indicated that the hGR-Oct-1 and hGR-AP-1 motifs act in a ubiquitous manner. Conversely, the hGR-CRE and hGR-GATA motifs appear to play a role in placenta-specific gene transcription because mutations of these elements result in a selective loss of promoter activity in JEG-3 cells (188).

Using a similar deletion approach, we have identified a new upstream GnRH receptor promoter that is primarily used by human GL cells (189). This novel promoter resides between −1300 and −1018 and contains two putative CCAAT/enhancer binding protein motifs and one GATA motif. The usage of this promoter in the GL cells is confirmed by the detection of a major transcription start site at −769, which is shortly downstream of a canonical TATA and CAAT box (189). Site-directed mutagenesis revealed that the CCAAT/enhancer binding protein and GATA binding sites work cooperatively to regulate the GnRH receptor promoter in the GL cells because simultaneous mutations of all these elements are required to cause a drastic abolishment of promoter function (189). Most importantly, these observations strengthen the notion that tissue-specific expression of the human GnRH receptor gene is mediated, at least partly, via differential promoter usage in various cell types.

B. Transcriptional regulation by GnRH-I

Previous studies on homologous activation of the mouse GnRH receptor promoter in αT3–1 cells have revealed an integral role of a consensus AP-1 motif as well as the PKC and ERK1/2 signaling pathways (190, 191). Interestingly, this GnRH-I-stimulated effect can be augmented by activin A pretreatment, which is inhibited by follistatin (192). Deletion analysis of the mouse GnRH receptor promoter indicated that the region between −387 and −308, which contains two overlapping *cis*-acting regulatory motifs (the GRAS at −329/−318 and a SMAD-binding element at −331/−324), is responsible for the augmented response (192, 193). Competitive EMSAs showed that AP-1 and SMAD protein complexes bind respectively to −327/−322 and −329/−328, and disruption of either motif can eliminate both the GnRH-I and activin A responsiveness of the mouse GnRH receptor promoter (193). The functional significance of the SMAD-binding element is further supported by the observation that overexpression of SMAD2 and SMAD3 along with SMAD4 can increase the transcription of the GnRH receptor gene (192).

Transcriptional regulation of the human GnRH receptor gene by GnRH-I has also been investigated. In αT3–1 cells, continuous administration of [D-Ala^6]-GnRH-I represses the human GnRH receptor promoter in a dose- and time-dependent manner via a PKC-dependent pathway (194). Subsequent experiments indicated that a 248-bp region between −1018 and −771 is sufficient for mediating the suppression and that mutation of an AP-1-like motif (−1000/−994) can abolish the sensitivity of the promoter to both the GnRH-I analog and phorbol ester (194). EMSAs revealed that the AP-1-like motif binds c-Jun homodimer under nonstimulated conditions. However, an additional complex that is recognized by both anti-c-Jun and anti-c-Fos is formed when nuclear extracts from GnRH-I-stimulated cells are used (194).

Therefore, it is apparent that homologous repression of the human GnRH receptor promoter may involve induction of c-Fos DNA binding activity at the AP-1-like site. Significantly, this GnRH-I-mediated down-regulation of GnRH receptor gene transcription may serve as a putative mechanism for pituitary desensitization to prolonged ligand stimulation. However, it is important to note that under conditions that can produce the maximal stimulatory response of the rodent GnRH receptor promoter (190, 191), a significant inhibition is observed for the human counterpart (194). These results thus further highlight the potential existence of species-specific mechanisms in transcriptional regulation among the GnRH receptor genes.

C. Transcriptional regulation by the cAMP-dependent signal transduction pathway

The cAMP signaling pathway is known to enhance the responsiveness of gonadotropes to GnRH-I by up-regulating GnRH receptor expression (195–198). Moreover, an increase in GnRH receptor mRNA levels has been demonstrated in placental cells after forskolin treatment (107). These stimulatory effects are thought to occur at the transcriptional level because forskolin can activate the human GnRH receptor promoter in a dose- and time-dependent manner in αT3–1 and JEG-3 cells (152, 199). Similar responses are also observed with other physiological regulators that activate the cAMP-dependent signaling pathway (152, 199). Using progressive deletion analysis, the forskolin response area has been mapped to a region between −577 and −167, within which two potential AP-1/CREB binding sites termed hGR-AP/CRE-1 (−569/−562) and hGR-AP/CRE-2 (−341/−334) partly contributing to the forskolin effect were identified (152, 199). Although both the hGR-AP/CRE-1 and hGR-AP/CRE-2 sites interact specifically with CREB in forskolin-stimulated cells, a differential binding of transcription factors to hGR-AP/CRE-2 was observed such that the motif interacts primarily with AP-1 when nuclear extracts from nonstimulated αT3–1 cells were used (152, 199).

D. Transcriptional regulation by gonadal steroid hormones

A study from Cheng *et al.* (148) has shown that progesterone can repress the human GnRH receptor promoter in αT3–1 cells in a dose- and time-dependent fashion. In contrast, the steroid exerts a stimulatory effect in JEG-3 cells as blockade of endogenous progesterone production silences the GnRH receptor promoter. Deletion and mutational analysis indicated that an imperfect progesterone-response element at −536/−522 is responsible for mediating the responses in both the αT3–1 and JEG-3 cells (148). Using EMSAs, a specific binding of PRs to the response element has been demonstrated (148), thus indicating a direct involvement of the nuclear receptors in conferring the transcriptional effects. Overexpression of the two human PR isoforms (PR-A and PR-B) indicated that PR-B plays a predominant role in mediating the down-regulatory effect in αT3–1 cells. On the contrary, a differential action of PR-A and PR-B is observed in JEG-3 cells such that PR-B stimulates whereas PR-A suppresses the GnRH receptor promoter (148). In concert with these findings, PR-B has been identified as the major PR subtype in the placental cells (148), thus supporting a positive role of progesterone in controlling human GnRH receptor gene transcription in the placenta.

The mechanism by which estrogen regulates GnRH receptor gene transcription in ovarian and breast cancer cells has only been recently elucidated. In these cells, E_2 can repress the human GnRH receptor promoter via a nonconsensus AP-1 motif and ERα, of which the DNA-binding domain and the ligand-binding domain are indispensable for the repression (141). Interestingly, the same *cis*-acting motif is also important for both the basal activity as well as phorbol 12-myristate 13-acetate (PMA) responsiveness of the GnRH receptor promoter. Multiple transcription factors including c-Jun and c-Fos, but not ERα, bind to the AP-1 site, indicating that the E_2-induced repression occurs independently of direct ER binding to the promoter (141). This observation may be supported by the fact that no estrogen-response elements can be identified in GnRH receptor 5′-flanking regions sequenced so far (90, 92–94). Intriguingly, the repressive effect of E_2 on the human GnRH receptor promoter can be antagonized by cotreatment with PMA, which stimulates c-Jun phosphorylation at serine 63 (141), a process prerequisite for recruitment of the transcriptional coactivator CREB-binding protein (200, 201). Concomitantly, overexpression of the coactivator can reverse the suppression in a dose-dependent manner (141), suggesting that E_2-bound ERα represses human GnRH receptor gene transcription via an indirect mechanism involving competition for a limiting amount of CREB-binding protein.

E. Transcriptional repression

Several reports from our laboratory have consistently suggested the presence of a very strong negative regulatory element (NRE) (−1017/−771) in the human GnRH receptor 5′-flanking region (188, 189, 202). Although this repressive element can work ubiquitously in a heterologous environment, its silencing activity is dependent on its orientation relative to the target promoter sequence (203). Progressive deletion analysis revealed that most of the NRE silencing effect resides in an evolutionarily conserved octamer sequence (−1017/−1009), which can suppress the native promoter activity by almost 90% in JEG-3 cells (203). Results from EMSAs and Southwestern blot analysis have convincingly shown that the ubiquitously expressed POU homeodomain transcription factor Oct-1 is the repressor protein binding to the powerful NRE (203).

It is important to point out that the mouse gonadotrope-derived αT3–1 cell line is employed as the model system, in most if not all studies, to investigate the transcriptional regulation of the human GnRH receptor gene in the pituitary (148, 178, 188, 194, 202, 203). Such cross-species studies may not be capable of accurately reflecting the mechanisms operating in the human counterpart, and the observable differences in transcriptional control between the human and rodent GnRH receptor genes may be due to the absence of certain species-specific transcription factor(s) in the mouse gonadotropes.

V. Signal Transduction Mechanism of the Mammalian Type I GnRH Receptor

A. G protein coupling

The nature of G protein-coupled signaling initiated by the GnRH receptor depends largely on the cellular context. For instance, it has been demonstrated that the human receptor couples to $G\alpha_{q/11}$ in heterologous Chinese hamster ovary-K1 and COS-7 cells (204) but to $G\alpha_s$ in the placenta (107). In contrast, others have reported that the receptor couples selectively to $G\alpha_i$ in some reproductive tract tumors and their derived cell lines (2, 118, 123, 205). Interestingly, there is evidence showing that the rodent GnRH receptor couples to multiple G proteins in a single cell type (206–208). In GT1–7 neurons, high GnRH-I analog concentrations induce a ligand-dependent switch of G protein coupling from $G\alpha_s$ to $G\alpha_i$, the activation of which inhibits episodic GnRH-I release, possibly via regulation of membrane ion channels (208). Such negative feedback action serves as an autocrine mechanism for the genesis of pulsatile GnRH-I secretion that is essential for the maintenance of normal gonadotropin release profiles and gonadal functions.

B. MAPKs

The MAPKs play an integral role in GPCR-mediated intracellular signaling (209, 210). In mouse pituitary gonadotropes, the GnRH receptor activates four MAPK cascades including the ERK1/2, the c-Jun amino-terminal kinase (JNK), the p38 MAPK, and the big MAPK (BMK1/ERK5) (211–213) to various extents by a PKC-, Ca^{2+}-, and tyrosine kinase-dependent mechanism (214, 215). For ERK1/2, the activation is primarily PKC dependent and involves two distinct pathways that converge at Raf-1 (216–218). Also, this process requires Ca^{2+} elevation (216, 219) and sublocalization of the receptor to low-density membrane microdomains (220). On the other hand, activation of JNK is highly dependent on cytosolic Ca^{2+} and is mediated via a pathway requiring sequential stimulation of PKC, c-Src, CDC42/Rac1, and MAPK kinase (MEK)K1 (221, 222). Although the signaling pathways leading to p38 MAPK and BMK1 activation are less clear, it appears that the activation involves a PKC-dependent cascade (214, 218, 223). Stimulation of MAPK cascades by the GnRH receptor has also been investigated in other cell types, in which the intracellular mechanisms mainly involve transactivation of the epidermal growth factor receptor (224, 225).

C. Receptor desensitization and internalization

Activation of GPCRs is typically followed by their desensitization and internalization, and these processes involve rapid agonist-induced receptor phosphorylation by both second messenger-dependent protein kinases and G protein-coupled receptor kinases (81). Because the serine and threonine residues that are phosphorylated by G protein-coupled receptor kinases are often located in the carboxyl-terminal tail (81), which is uniquely absent in the mammalian GnRH receptor, a number of studies have revealed that the tailless GnRH receptor neither undergoes rapid homologous desensitization nor exhibits agonist-induced receptor phosphorylation (226–230). In addition, the receptor internalizes slowly via clathrin-coated vesicles, and this process occurs independently of β-arrestin and dynamin (226, 229–231). The unusual resistance of the mammalian GnRH receptor to desensitization may be essential for mediating its direct antiproliferative effect (will be discussed in detail below), which requires sustained ligand stimulation and is shown to be ineffective by receptors having a carboxyl-terminal tail (230, 232).

VI. Biological Actions of GnRH-I and GnRH-II in Humans

In addition to its pivotal role in stimulating gonadotropin synthesis and secretion, GnRH-I functions as an autocrine and/or paracrine factor in a number of extrapituitary compartments, where it regulates steroidogenesis, cell proliferation, apoptosis, and embryo implantation (Table 1). In tumors derived from various reproductive tissues, there is solid evidence showing that the GnRH receptor couples to a pertussis toxin-sensitive Gα protein (most probably $G\alpha_i$) and mediates its biological effects via pathways that are distinct from the classical cascade operated in gonadotropes. Extrapituitary actions elicited by GnRH-II have also been demonstrated in certain human peripheral tissues.

A. Gonadotropin subunit gene transcription and secretion

GnRH-I plays a key role in the mammalian reproductive process by stimulating the synthesis and release of FSH and LH, which are heterodimeric glycoprotein hormones composed of a common α-subunit noncovalently bound to a specific β-subunit (FSHβ and LHβ) (233, 234). Activation of the human α-subunit gene transcription by GnRH-I is primarily Ca^{2+} dependent. Also, this process can be augmented by PKC and requires ERK1/2 and c-Src (235, 236). Although the nonreceptor tyrosine kinase c-Src has been demonstrated to mediate ERK stimulation by the GnRH receptor (217), the ERK and c-Src-response areas are located at different regions on the α-subunit promoter (236), indicating that c-Src contribution is independent of ERK activation.

The role of PKC, Ca^{2+}, and MAPK signaling cascades in mediating GnRH-I stimulation of LHβ gene transcription has not been clearly addressed. Although it was shown that the activation is Ca^{2+} dependent (237), others reported that PKC is mainly responsible for the effect (238, 239). Similarly, whereas it was found that both PKC and ERK1/2 are required for the stimulation (240), others demonstrated an essential role of JNK (216). These discrepancies are likely due to different experimental paradigms such as the use of different cell models or promoters from different species. Several *cis*-acting regulatory elements such as Sp1, CArG, and early growth response 1 transcription factor binding sites have been identified in the rat LHβ promoter (241–243). It is suggested that these motifs may act in concert with different signal transduction cascades to confer GnRH-I sensitivity to the LHβ promoter (237, 240, 244, 245).

Activation of FSHβ gene transcription by GnRH-I requires the Ca^{2+}, PKC, and MAPK signaling pathways in mouse LβT2 gonadotropes (246). The GnRH-I responsiveness of the ovine FSHβ promoter involves at least two elements at the distal region, in association with one or several motifs within

TABLE 1. Summary of extrapituitary actions of GnRH-I (I) and GnRH-II (II) in humans[a]

Target site	Extrapituitary action	Effect	Effective dose and % change (I)	Nature of the GnRH-I analog	Effective dose and % change (II)	Nature of the GnRH-II analog	Ref.
Ovarian cancer cell	Proliferation (I, II)	Decrease	10^{-6}, 10^{-5} M (15–33%)	Triptorelin/ Leuprolide	10^{-5} M (25–58%)	Native GnRII-II	19, 21, 270, 272
Endometrial cancer cell	Proliferation (I, II)	Decrease	10^{-11} to 10^{-5} M (13.5–47%)	Triptorelin/ Cetrorelix	10^{-9} to 10^{-5} M (25–50%)	Native GnRH-II	21, 118, 269, 278
Breast cancer cell	Proliferation (I)	Decrease	10^{-9} to 10^{-5} M (11–92%)	Native GnRH-I/ Buserelin			2, 268
Prostate cancer cell	Proliferation (I)	Decrease	10^{-6}, 10^{-5} M (46–53%)	Zoladex			271, 273, 279
Melanoma cell	Proliferation (I)	Decrease	10^{-9} to 10^{-6} M (25–43%)	Zoladex			131, 271
Epidermoid cancer cell	Proliferation (I)	Decrease	10^{-7} M (21–29%)	Triptorelin			280
Uterine leiomyoma cell	Proliferation (I)	Decrease	10^{-6}, 10^{-5} M (16–36%)	Leuprolide			6, 288, 289
Endometriotic cell	Proliferation (I)	Decrease	10^{-9} to 10^{-7} M (35–64%)	Leuprolide			10, 291
Ovarian OSE cell	Proliferation (I, II)	Decrease	10^{-9} to 10^{-7} M (20–33%)	[D-Ala^6]-GnRH-I	10^{-9} to 10^{-7} M (17–35%)	Native GnRH-II	19
T cell	Proliferation (I)	Increase	10^{-7} M (220%)	Deslorelin			52, 315
Ovarian GL cell	Apoptosis (I)	Increase	10^{-12} to 10^{-9} M (154–664%)	Buserelin			5
Endometriotic cell	Apoptosis (I)	Increase	10^{-8} to 10^{-6} M (220–297%)	Buserelin			291, 307, 311
Ovarian GL cell	Steroidogenesis (I, II)	Decrease	10^{-9} to 10^{-6} M (28–44%)	[D-Ala^6]-GnRH-I	10^{-10} to 10^{-7} M (16–32%)	Native GnRH-II/ GnRH-II agonist	20, 265, 266, 267
Placenta	hCG release (I, II)	Biphasic	10^{-11} to 10^{-8} M (36–56% ↓); 10^{-8} to 10^{-6} M (150–320% ↑)	Native GnRH-I	10^{-7}, 10^{-6} M (205–213% ↑)	Native GnRH-II	3, 38
Cytotrophoblast	uPA expression (I, II)	Increase	10^{-8}, 10^{-7} M (200–783%)	Native GnRH-I	10^{-8}, 10^{-7} M (198–625%)	Native GnRH-II	22
Decidual stromal cell	uPA expression (I, II)	Increase	10^{-9} to 10^{-7} M (176–400%)	Native GnRH-I	10^{-10} to 10^{-7} M (145–356%)	Native GnRH-II	8
Cytotrophoblast	PAI-1 expression (I, II)	Decrease	10^{-7} M (46–49%)	Native GnRH-I	10^{-8}, 10^{-7} M (42–87%)	Native GnRH-II	22
Decidual stromal cell	PAI-1 expression (I)	Increase	10^{-9} to 10^{-7} M (127–460%)	Native GnRH-I			8
Decidual stromal cell	PAI-1 expression (II)	Decrease			10^{-9} to 10^{-7} M (18–50%)	Native GnRH-II	8
Cytotrophoblast	MMP-2 and MMP-9 expression (I, II)	Increase	MMP-2: 10^{-7} M (144–178%) MMP-9; 10^{-7} M (188–338%)	Native GnRH-I	MMP-2: 10^{-11} to 10^{-7} M (153–323%) MMP-9: 10^{-7} M (227–355%)	Native GnRH-II	23
Decidual stromal cell	MMP-2 and MMP-9 expression (I)	Increase	MMP-2: 10^{-9} to 10^{-7} M (158–254%) MMP-9: 10^{-7} M (475%)	Native GnRH-I			9
Cytotrophoblast	TIMP-1 expression (I, II)	Decrease	10^{-9}, 10^{-7} M (27–67%)	Native GnRH-I	10^{-7} M (48–55%)	Native GnRH-II	23
T cell	Laminin receptor expression (I, II)	Increase	10^{-8} M (130–270%)	Native GnRH-I	10^{-8} M (310–990%)	Native GnRH-II	7
T cell	Adhesion, chemotaxis, and homing (I, II)	Increase	10^{-8} M (adhesion: 320%, chemotaxis: 250%, *in vivo*)	Native GnRH-I	10^{-8} M (adhesion: 300%, chemotaxis: 230%, *in vivo* homing)	Native GnRH-II	7
Olfactory neuron	Migration (I)	Increase	10^{-9} to 10^{-7} M (330–475%)	Native GnRH-I/ Buserelin			318
Sperm	Zona pellucida binding (I)	Increase	7.5×10^{-8} M (289%)	Native GnRH-I			316, 317

uPA, Urokinase-type plasminogen activator; PAI, plasminogen activator inhibitor; TIMP, tissue inhibitor of metalloproteinase; ↑, increase; ↓, decrease.
[a] Changes in gene expression refer to the steady-state mRNA levels.

the proximal promoter sequence (246). Although previous studies have shown that two downstream AP-1-like elements are important for mediating the GnRH-I response in heterologous HeLa cells (247), these motifs are not functionally equivalent in the gonadotropes (246). On the other hand, Pernasetti *et al.* (248) have shown that GnRH-I stimulation of β-subunit gene transcription in LβT2 cells is also dependent on an endogenous activin autocrine loop as follistatin treatment can block the GnRH-I induction.

An essential role of intracellular Ca^{2+} in mediating GnRH-I-stimulated gonadotropin secretion has been established (77, 249, 250). In contrast, the initial phase of gonadotropin release is apparently independent of extracellular Ca^{2+} (249, 251, 252). The role of PKC activation in mediating the GnRH-I effect is less clear (77, 250). Whereas phorbol ester can stimulate LH secretion, GnRH-I-induced LH release is impaired but not abolished in PKC-depleted gonadotropes (253, 254). These observations thus indicate that PKC activation is not an absolute requirement for exocytosis. It is believed that PKC participates in the control of gonadotropin secretion through its actions on cytoskeleton elements, Rab proteins, and other elements involved in the exocytotic process (255–257). Although there is no evidence for rapid desensitization of the mammalian GnRH receptor, sustained ligand exposure causes down-regulation of inositol (1,4,5) triphosphate receptor in αT3–1 cells (258), leading to a marked reduction in GnRH-I-stimulated Ca^{2+} mobilization and gonadotropin secretion (259, 260). This form of desensitization underlies the basis of hypothalamic-pituitary-gonadal axis suppression that is exploited in the major clinical applications of GnRH-I analogs (11).

Recently, GnRH-II has also been shown to be capable of stimulating LH and FSH release both *in vivo* (261) and in cultured pituitary cells (262). This stimulation is mediated via activation of the type I GnRH receptor because the effects can be blocked by antide (261, 262).

B. Ovarian steroidogenesis

There is a general consensus that GnRH-I analog treatment *in vivo* or *in vitro* exerts an inhibitory effect on gonadotropin-regulated steroidogenesis in human GL cells (20, 263–267). Exposure of the steroidogenic cells to [D-Ala^6]-GnRH-I rapidly activates ERK1/2 and causes a drastic increase in c-Fos mRNA levels (4). This GnRH-I-induced ERK activation is mediated via $G\alpha_{q/11}$ and requires PKC because the effect can be mimicked by PMA and abolished by the PKC inhibitor GF109203X (4). Interestingly, pretreatment of GL cells with the MEK inhibitor PD98059 completely abrogates the down-regulatory effect of GnRH-I on steroidogenesis (267), suggesting that a PKC- and ERK-dependent cascade is involved in mediating the antisteroidogenic response.

Recent findings from our laboratory have revealed that treatment of human GL cells with GnRH-II or its analog *in vitro* can also suppress hCG-stimulated progesterone production (20). This inhibition can be blocked by antide, indicating the mediation via the type I receptor (20). Similar to the effects produced by GnRH-I, GnRH-II does not interfere with hCG-stimulated cAMP generation. Instead, these hormones down-regulate the steady-state mRNA levels of both the FSH and LH receptors in the steroidogenic cells (20). These observations thus support a notion that GnRH-I and GnRH-II exert their antigonadotropic effects at the receptor level but not at the cAMP level.

C. Cell proliferation

The role of GnRH-I as a negative autocrine growth factor has been well reported in cell lines derived from human malignant tumors including those of the ovary, endometrium, breast, prostate gland, and melanoma cells (2, 268–271). It is generally thought that this antiproliferative action is mediated via high-affinity GnRH-I binding sites, as supported by the notion that the nucleotide sequence of the GnRH receptor is identical in tumor and pituitary cells (112, 118, 202). Nonetheless, in some systems, high doses of GnRH-I analogs (1–10 μM) are sometimes needed to demonstrate a significant but modest growth-inhibitory response (6, 21, 131, 272, 273). Although the intracellular mechanisms mediating the antiproliferative effect of GnRH-I analogs are not fully understood, several lines of evidence have suggested a role of the ERK1/2 signaling pathway. In ovarian carcinoma Caov-3 cells, the GnRH-I analog leuprolide induces phosphorylation of son of sevenless and Shc and causes a sustained stimulation of the MEK-ERK cascade (272). This process is mediated via a pertussis toxin-sensitive Gα protein and the Gβγ dimer and occurs independently of PKC and extracellular Ca^{2+}. Consequently, the prolonged ERK activation leads to hypophosphorylation of the retinoblastoma protein (272), a process known to prevent cell cycle progression from G_1 to S phase. Similar observations have also been reported in other gynecological cancer cell lines, in which GnRH-I analog treatment *in vitro* blocks cell cycle transition and decreases DNA synthesis (274, 275). These growth-inhibitory effects of GnRH-I analogs may be mediated via stimulation of the DNA binding activity of JunD (275), which has been suggested as a negative regulator of cell proliferation (276).

The involvement of the ERK1/2 cascade in mediating the antitumor effect of GnRH-I analogs is further supported by the observation that the analogs can antagonize growth factor-induced mitogenic signaling via coupling to $G\alpha_i$ proteins (2, 118, 123, 205). In primary ovarian and endometrial carcinomas as well as certain cancer cell lines, treatment with GnRH-I analogs *in vitro* activates phosphotyrosine phosphatase and causes a substantial loss of phosphotyrosines from cellular proteins such as the epidermal growth factor receptor (118, 277–280). These effects are associated with a significant reduction in growth factor-induced ERK1/2 activation, c-Fos gene expression, matrix metalloproteinase (MMP) secretion, and cell proliferation (280–282). In some prostate cancer cells, GnRH-I analog treatment may reduce cellular tyrosine phosphorylation and proliferation index via down-regulation of growth factor receptor expression (279, 283). Thus, depending on the cell context, GnRH-I analogs may attenuate the mitogenic action of growth factors and suppress the ERK cascade to mediate their antitumor effects.

In addition to MAPK regulation, several potential mechanisms have been suggested to account for the growth-inhibitory effect of GnRH-I analogs in cancer cells. These putative mechanisms include inhibition of phosphatidylinositol kinase activity (284) and stimulation of lysophosphatidic acid hydrolysis

(285), as well as down-regulation of telomerase reverse transcriptase, acidic ribosomal phosphoprotein, and prostate-specific antigen expression (51, 286, 287).

There are many reports showing that GnRH-I analogs can also inhibit cell proliferation in human uterine leiomyoma and endometriosis. Although GnRH-I-induced leiomyoma regression appears to occur predominantly through inhibition of gonadotropins and gonadal steroids (11), the suppression may involve alteration of growth factor (6, 288), cytokine (289), cell cycle regulator (43), and steroid hormone receptor (290) expression. Similarly, in endometriotic stromal cells, GnRH-I analog treatment *in vivo* reduces tumor necrosis factor α-induced nuclear factor-κB (NF-κB) activation and interleukin-8 expression (10, 291), which has been reported to promote endometriosis (292, 293).

The role of GnRH-II as an autocrine growth inhibitor has also been demonstrated. Like GnRH-I, treatment with GnRH-II *in vitro* inhibits the proliferation of both nontumorigenic and tumorigenic ovarian surface epithelial cells in a dose-dependent manner (19). In accord with the presence of two types of GnRH binding sites (110, 114), mRNA expression of a second GnRH receptor subtype (type II GnRH receptor) has been demonstrated in several endometrial and ovarian cancer cell lines (21). The proliferation of these cells can be dose- and time-dependently suppressed by GnRH-II, the antitumor effect of which is significantly stronger than that produced by equimolar concentrations of triptorelin (21). It should be emphasized that in type II GnRH receptor-positive but type I receptor-negative SK-OV-3 ovarian cancer cells, treatment with triptorelin has no effect on cell proliferation (282, 294), suggesting that the growth-inhibitory action of GnRH-II is mediated via a GnRH-II-specific receptor.

D. Apoptosis

It is well established that GnRH-I analogs promote apoptotic cell death in ovarian granulosa cells. In rat, treatment with GnRH-I analogs *in vivo* or *in vitro* induces a definitive ladder pattern of oligonucleosomal length DNA fragments in granulosa cells isolated from preovulatory follicles (295, 296). Likewise, continuous treatment of corpus luteum with a GnRH-I analog stimulates apoptosis *in vivo.* This stimulation is associated with an up-regulation of Bax gene expression and may involve the mitochondrial cytochrome *c* pathway (297). Consistent with the findings in rat, treatment with buserelin *in vitro* increases the incidence of apoptosis in human GL cells (5) although the mechanism underlying the proapoptotic effect of the analog in the human counterpart is not fully known.

The role of GnRH-I in regulating apoptosis, however, remains controversial in cancer cells. In some gynecological cancer cell lines and cells isolated from GnRH receptor-bearing tumors, buserelin treatment *in vitro* induces a dose-dependent stimulation of Fas ligand expression (298). Because Fas is frequently expressed in GnRH receptor-positive tumors (299), it is speculated that GnRH-I may function as an autocrine proapoptotic factor in Fas-positive tumors and that this proapoptotic action may account, in part, for its antitumor effect. In stark contrast, an earlier finding has shown that triptorelin treatment does not produce any morphological signs of programmed cell death in EFO-21 and EFO-27 ovarian cancer cells. Rather, the GnRH-I analog inhibits cytotoxin-induced apoptosis via activation of the NF-κB signal transduction cascade (300). NF-κB has been implicated as an antiapoptotic transcription factor by activating multiple genes, the products of which can block apoptosis triggered by either death receptors or the mitochondrial pathway (301–305). Although activation of NF-κB by triptorelin is also mediated by $G\alpha_i$ in EFO-21 and EFO-27 cells, the mechanism leading to NF-κB stimulation is apparently independent of interference with growth factor-induced mitogenic signaling (300). Thus, in some ovarian cancer cells, GnRH-I analogs may possess two counteracting activities (antiproliferative *vs.* antiapoptotic) that are mediated by two distinct signaling cascades but triggered by the same Gα protein. The balance of these two activities may be a critical factor in controlling ovarian tumorigenesis.

Contradictory results also exist regarding the role of GnRH-I in inducing apoptosis in uterine leiomyoma. Whereas GnRH-I analogs *in vivo* or *in vitro* can inhibit leiomyoma cell growth partly by stimulating apoptotic cell death (306–308), others have reported that the analog can decrease the expression of certain proapoptotic factors but increase that of the antiapoptotic Bcl-2 protein *in vivo* (309, 310). The latter findings suggest that GnRH-I analog therapy is ineffective in promoting apoptosis in uterine leiomyoma. Conversely, a series of studies have consistently shown that GnRH-I analogs can increase apoptotic cell death in endometriotic cells *in vitro* (291, 311, 312).

E. Embryo implantation

Recently, both forms of GnRH have been demonstrated to stimulate the mRNA and protein levels of urokinase-type plasminogen activator in human extravillous cytotrophoblasts and decidual stromal cells *in vitro* (8, 22). These findings suggest that the hormones play regulatory roles in proteolytic degradation of the extracellular matrix of the endometrial stroma, a process prerequisite for decidualization and trophoblast invasion (313, 314). This notion is supported by the observation that the hormones can also up-regulate the expression of MMP-2 and MMP-9, which are two other proteases operating actively at the maternal-fetal interface during early pregnancy, in trophoblast and stromal cells (9, 23). Concomitantly, both GnRH-I and GnRH-II have been found to suppress the trophoblastic expression of plasminogen activator inhibitor 1 and tissue inhibitor of metalloproteinase-1, the physiological inhibitors of urokinase-type plasminogen activator and MMPs, respectively, in a dose- and time-dependent manner (22, 23).

F. Other extrapituitary actions

In normal and cancerous T cells, both GnRH-I and GnRH-II induce the cell-surface expression of a 67-kDa nonintegrin laminin receptor, leading to stimulation of T cell adhesion, chemotaxis, and *in vivo* homing to specific organs (7). In addition, endogenous or exogenous GnRH-I can increase the proliferative activity of Jurkat cells via a cAMP-dependent mechanism (52, 315). On the other hand, both GnRH-I and GnRH-II have been shown to rapidly induce hCG secretion from human cytotrophoblasts and placental

explants *in vitro* (3, 38). Moreover, through a direct action on the locally expressed GnRH receptor, GnRH-I has been found to increase zona pellucida-sperm binding as well as to stimulate axon growth, actin cytoskeleton remodeling, and migration of olfactory neurons (316–318).

VII. Type II GnRH Receptor: Existence in Humans?

A. Nonhuman primate type II GnRH receptors

Molecular cloning of the marmoset, macaque, and green monkey type II GnRH receptor cDNAs revealed that they code for a typical seven-TM-domain GPCR (Table 2) (319, 320). Strikingly, the primate type II receptors, like all nonmammalian GnRH receptors cloned to date (321–326), possess a carboxyl-terminal tail, which confers the receptors susceptibility to rapid desensitization and internalization (226, 230, 231, 327–330). Also, the tail plays a role in agonist binding as well as receptor expression and regulation (331, 332). Other noteworthy features of the type II GnRH receptors are the presence of a Asp/Asp microdomain in the second and seventh TM helices and the substitution of VPPS for the LSD/EP sequence in the third extracellular loop (78, 319, 333, 334).

The type II GnRH receptor mRNA is expressed throughout the marmoset and human brains (319). Pharmacological characterization revealed that the marmoset type II receptor is highly selective for GnRH-II, and to a less extent, for salmon GnRH and [D-Arg^6]-GnRH-II (319, 320). Although both the marmoset type II and human type I receptors couple to $G\alpha_{q/11}$ and activate ERK1/2, they differ in stimulating the p38 MAPK pathway (319). Furthermore, the marmoset type II receptor can be activated by the GnRH-I antagonist 135–18, which is a full antagonist of the human type I receptor (319). This observation may help explain why certain GnRH-I antagonists exhibit agonistic activities in some gynecological cancer cells expressing the type II receptor (114, 319, 335, 336).

B. Putative type II GnRH receptor genes in the human genome

A search of the human genome database has revealed a putative type II GnRH receptor gene on chromosome 1q12 (329, 337, 338). This gene shares a 40% sequence identity with the type I receptor and is composed of three exons spanning approximately 7.5 kb along the chromosome. Intriguingly, the reading frame of this putative type II receptor gene is disrupted by a −1 frameshift and contains a cytosine to thymine substitution that changes the codon for Arg^{179} in the marmoset and frog sequences to an in-frame UGA premature stop codon (338). Disruption or silencing of the type II GnRH receptor gene has also been noted in the chimp, cow, sheep, and rat genomes (338–341).

In addition to the putative gene on chromosome 1, a truncated copy of the human gene lacking the first exon and part of the first intron is present on chromosome 14q22 (329, 338). This locus contains in the opposite direction an intronless RBM8A sequence, which is likely a pseudogene and originated from the chromosome 1 locus by reverse transcription and insertion into the genome (338, 342, 343).

C. Tissue distribution of human type II GnRH receptor mRNA

Expression of type II GnRH receptor mRNA has been demonstrated in human heart, pancreas, skeletal muscle, mature sperm, and postmeiotic testicular cells (319, 344). In addition, the mRNA is expressed in certain gynecological cancer cell lines as well as in pituitary adenoma and neuroblastoma cells (21, 338). Although these findings suggest that the human type II receptor gene is transcriptionally active, all the mRNAs identified have no alteration of the premature stop codon, although in some instances, alternative splicing of part of the exon 1 to circumvent the frameshift to encode a two-TM-domain protein is observed (338, 344).

TABLE 2. Summary of structural and functional differences of the marmoset type II and mammalian type I GnRH receptor

	Type II GnRH Receptor	Type I GnRH Receptor	Ref.
No. of amino acid	380	328/327	77, 78, 319
Carboxyl-terminal tail	Yes	No	77, 78, 319
Conserved Asp in the second TM domain of GPCRs	Yes	Replaced with Asn	77, 78, 319
Third extracellular loop	VPPS motif	LSD/EP motif	77, 78, 319
Ligand selectivity	Highly selective for GnRH-II, 50-fold less selective for GnRH-I	Highly selective for GnRH-I, 10-fold less selective for GnRH-II	17, 319
Antagonist 135-18	Full agonist	Full antagonist	17, 319
Rapid desensitization	Yes	No	17
Receptor internalization	Rapid	Slow	17
Activation of intracellular signaling components			
Ca^{2+}	Yes	Yes	17, 77, 319
PKC	Yes	Yes	17, 77, 319
ERK1/2	Sustained	Transient	17, 214, 319
JNK	No	Yes	17, 214, 319
P38 MAPK	Yes	Yes	17, 214, 319
BMK1	Not determined	Yes	214
Epidermal growth factor receptor	Not determined	Yes	224, 225
c-Src	Not determined	Yes	17, 214
Proline-rich tyrosine kinase 2	Not determined	Yes	351
Focal adhesion kinase	Not determined	Yes	352
Diacylglycerol kinase-ζ	Not determined	Yes	353

Several translation mechanisms have been proposed to explain how a functional receptor may be produced from the disrupted human type II receptor gene (338). One possibility is that translation begins at the second ATG initiation codon (situated at the end of the second TM domain) to generate a truncated five-TM-domain receptor missing the first two helices (338). Indeed, chemokine receptors with only five TM domains have been shown to behave in many aspects indistinguishably from the wild-type receptors, suggesting that a five-TM core structure is sufficient for normal GPCR functioning (345). Additionally, a functional receptor may be generated by lateral interaction or domain swapping of the truncated gene products with the type I receptor or other GPCRs, as hypothesized for some receptors (346, 347). Although a functional receptor may also be produced by incorporating a selenocysteine at the premature UGA codon (338, 348–350), no selenocysteine incorporation can be observed in cells transfected with the receptor cDNA (338). Also, insertion of a guanine residue to correct the frameshift is still required if a full-length receptor is to be expressed. Taken altogether, these observations thus rule out the potential existence of a conventional seven-TM-domain type II GnRH receptor in humans.

VIII. Conclusions

The findings that the tissue distribution patterns of GnRH-I and GnRH-II are dissimilar and that their expressions are differentially regulated indicate a distinct role of these decapeptides in our body. Whereas it is becoming evident that humans do not have a conventional type II receptor system, specific GnRH-II responses have been observed in certain cell types (21). Thus, one of the major challenges in the future is whether we can demonstrate functional type II receptor protein expression in cells lacking the type I receptor. Results from such investigations should facilitate functional dissection of the two GnRH hormones in controlling the reproductive and other cellular processes.

Acknowledgments

Address all correspondence and requests for reprints to: Peter C. K. Leung, Ph.D., Department of Obstetrics and Gynecology, University of British Columbia, 2H30–4490 Oak Street, British Columbia Women's Hospital, Vancouver, British Columbia, Canada V6H 3V5. E-mail: peleung@interchange.ubc.ca

This work was supported by grants from the Canadian Institutes of Health Research. C.K.C. is a recipient of the British Columbia Research Institute of Children's and Women's Health Studentship Award. P.C.K.L. is a Distinguished Scholar of the Michael Smith Foundation for Health Research.

Current address for C.K.C.: Department of Medicine, The University of Hong Kong, Hong Kong Special Administration Region, China.

References

1. **Fink G** 1988 Gonadotropin secretion and its control. In: Knobil E, Neill JD, eds. The physiology of reproduction. New York: Raven Press; 1349–1377
2. **Emons G, Muller V, Ortmann O, Schulz KD** 1998 Effects of LHRH-analogues on mitogenic signal transduction in cancer cells. J Steroid Biochem Mol Biol 65:199–206
3. **Islami D, Chardonnens D, Campana A, Bischof P** 2001 Comparison of the effects of GnRH-I and GnRH-II on HCG synthesis and secretion by first trimester trophoblast. Mol Hum Reprod 7:3–9
4. **Kang SK, Tai CJ, Nathwani PS, Choi KC, Leung PC** 2001 Stimulation of mitogen-activated protein kinase by gonadotropin-releasing hormone in human granulosa-luteal cells. Endocrinology 142:671–679
5. **Zhao S, Saito H, Wang X, Saito T, Kaneko T, Hiroi M** 2000 Effects of gonadotropin-releasing hormone agonist on the incidence of apoptosis in porcine and human granulosa cells. Gynecol Obstet Invest 49:52–56
6. **Chegini N, Ma C, Tang XM, Williams RS** 2002 Effects of GnRH analogues, 'add-back' steroid therapy, antiestrogen and antiprogestins on leiomyoma and myometrial smooth muscle cell growth and transforming growth factor-β expression. Mol Hum Reprod 8:1071–1078
7. **Chen A, Ganor Y, Rahimipour S, Ben-Aroya N, Koch Y, Levite M** 2002 The neuropeptides GnRH-II and GnRH-I are produced by human T cells and trigger laminin receptor gene expression, adhesion, chemotaxis and homing to specific organs. Nat Med 8:1421–1426
8. **Chou CS, MacCalman CD, Leung PC** 2003 Differential effects of gonadotropin-releasing hormone I and II on the urokinase-type plasminogen activator/plasminogen activator inhibitor system in human decidual stromal cells *in vitro*. J Clin Endocrinol Metab 88:3806–3815
9. **Chou CS, Tai CJ, MacCalman CD, Leung PC** 2003 Dose-dependent effects of gonadotropin-releasing hormone on matrix metalloproteinase (MMP)-2, and MMP-9 and tissue specific inhibitor of metalloproteinases-1 messenger ribonucleic acid levels in human decidual stromal cells *in vitro*. J Clin Endocrinol Metab 88:680–688
10. **Sakamoto Y, Harada T, Horie S, Iba Y, Taniguchi F, Yoshida S, Iwabe T, Terakawa N** 2003 Tumor necrosis factor-α-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-κB activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. J Clin Endocrinol Metab 88:730–735
11. **Conn PM, Crowley Jr WF** 1994 Gonadotropin-releasing hormone and its analogs. Annu Rev Med 45:391–405
12. **Kiesel LA, Rody A, Greb RR, Szilagyi A** 2002 Clinical use of GnRH analogues. Clin Endocrinol (Oxf) 56:677–687
13. **Shalev E, Leung PC** 2003 Gonadotropin-releasing hormone and reproductive medicine. J Obstet Gynaecol Can 25:98–113
14. **Dubois EA, Zandbergen MA, Peute J, Goos HJ** 2002 Evolutionary development of three gonadotropin-releasing hormone (GnRH) systems in vertebrates. Brain Res Bull 57:413–418
15. **Miyamoto K, Hasegawa Y, Nomura M, Igarashi M, Kangawa K, Matsuo H** 1984 Identification of the second gonadotropin-releasing hormone in chicken hypothalamus: evidence that gonadotropin secretion is probably controlled by two distinct gonadotropin-releasing hormone in avian species. Proc Natl Acad Sci USA 81:3874–3878
16. **White RB, Eisen JA, Kasten TL, Fernald RD** 1998 Second form of gonadotropin-releasing hormone in humans. Proc Natl Acad Sci USA 95:305–309
17. **Millar RP** 2003 GnRH II and type II GnRH receptors. Trends Endocrinol Metab 14:35–43
18. **Temple JL, Millar RP, Rissman EF** 2003 An evolutionarily conserved form of gonadotropin-releasing hormone coordinates energy and reproductive behavior. Endocrinology 144:13–19
19. **Choi KC, Auersperg N, Leung PC** 2001 Expression and antiproliferative effect of a second form of gonadotropin-releasing hormone in normal and neoplastic ovarian surface epithelial cells. J Clin Endocrinol Metab 86:5075–5078
20. **Kang SK, Tai CJ, Nathwani PS, Leung PC** 2001 Differential regulation of two forms of gonadotropin-releasing hormone messenger ribonucleic acid in human granulosa-luteal cells. Endocrinology 142:182–192
21. **Grundker C, Gunthert AR, Millar RP, Emons G** 2002 Expression of gonadotropin-releasing hormone II (GnRH-II) receptor in human endometrial and ovarian cells and effects of GnRH-II on tumor cell proliferation. J Clin Endocrinol Metab 87:1427–1430
22. **Chou CS, Zhu H, Shalev E, MacCalman CD, Leung PC** 2002 The effects of gonadotropin-releasing hormone (GnRH) I and GnRH II

on the urokinase-type plasminogen activator/plasminogen activator inhibitor system in human extravillous cytotrophoblasts *in vitro*. J Clin Endocrinol Metab 87:5594–5603
23. **Chou CS, Zhu H, MacCalman CD, Leung PC** 2003 Regulatory effects of gonadotropin-releasing hormone (GnRH) I and GnRH II on the levels of matrix metalloproteinase (MMP)-2, MMP-9, and tissue inhibitor of metalloproteinases-1 in primary cultures of human extravillous cytotrophoblasts. J Clin Endocrinol Metab 88: 4781–4790
24. **Adelman JP, Mason AJ, Hayflick JS, Seeburg PH** 1986 Isolation of the gene and hypothalamic cDNA for the common precursor of gonadotropin-releasing hormone and prolactin release-inhibiting factor in human and rat. Proc Natl Acad Sci USA 83:179–183
25. **Seeburg PH, Adelman JP** 1984 Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. Nature 311:666–668
26. **Radovick S, Wondisford FE, Nakayama Y, Yamada M, Cutler GB, Weintraub BD** 1990 Isolation and characterization of the human gonadotropin-releasing hormone gene in the hypothalamus and placenta. Mol Endocrinol 4:476–480
27. **Yang-Feng TL, Seeburg PH, Francke U** 1986 Human luteinizing hormone-releasing hormone gene (LHRH) is located on short arm of chromosome 8 (region8p11.2–p21). Somat Cell Mol Genet 12: 95–100
28. **Kasten TL, White SA, Norton TT, Bond CT, Adelman KP, Fernald RD** 1996 Characterization of two new preproGnRH mRNAs in the tree shrew: first direct evidence for mesencephalic GnRH gene expression in a placental mammal. Gen Comp Endocrinol 104:7–19
29. **Stopa EG, Koh ET, Svendsen CN, Rogers WT, Schwaber JS, King JC** 1991 Computer-assisted mapping of immunoreactive mammalian gonadotropin-releasing hormone in adult human basal forebrain and amygdala. Endocrinology 128:3199–3207
30. **Anthony EL, King JC, Stopa EG** 1984 Immunocytochemical localization of LHRH in the median eminence, infundibular stalk, and neurohypophysis. Evidence for multiple sites of releasing hormone secretion in humans and other mammals. Cell Tissue Res 236:5–14
31. **King JC, Anthony EL** 1984 LHRH neurons and their projections in humans and other mammals: species comparisons. Peptides 5:195–207
32. **Miller GM, Alexander JM, Klibanski A** 1996 Gonadotropin-releasing hormone messenger RNA expression in gonadotroph tumors and normal human pituitary. J Clin Endocrinol Metab 81:80–83
33. **Sanno N, Jin L, Qian X, Osamura RY, Scheithauer BW, Kovacs K, Lloyd RV** 1997 Gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor messenger ribonucleic acids expression in nontumorous and neoplastic pituitaries. J Clin Endocrinol Metab 82:1974–1982
34. **Chen A, Yahalom D, Ben-Aroya N, Kaganovsky E, Okon E, Koch Y** 1998 A second isoform of gonadotropin-releasing hormone is present in the brain of human and rodents. FEBS Lett 435:199–203
35. **Chen A, Yahalom D, Laskar-Levy O, Rahimipour S, Ben-Aroya N, Koch Y** 2001 Two isoforms of gonadotropin-releasing hormone are coexpressed in neuronal cell lines. Endocrinology 142:830–837
36. **Siler-Khodr TM, Khodr GS** 1979 Extrahypothalamic luteinizing hormone-releasing factor (LRF): release of immunoreactive LRF in vitro. Fertil Steril 32:294–296
37. **Khodr GS, Siler-Khodr TM** 1980 Placental luteinizing hormone-releasing factor and its synthesis. Science 207:315–317
38. **Siler-Khodr TM, Grayson M** 2001 Action of chicken II GnRH on the human placenta. J Clin Endocrinol Metab 86:804–810
39. **Chou CS, Beristain AG, MacCalman CD, Leung PC** 2004 Cellular localization of gonadotropin-releasing hormone (GnRH) I and GnRH II in first-trimester human placenta and deciduas. J Clin Endocrinol Metab 89:1459–1466
40. **Irmer G, Burger C, Ortmann O, Schulz KD, Emons G** 1994 Expression of luteinizing hormone releasing hormone and its mRNA in human endometrial cancer cell lines. J Clin Endocrinol Metab 79:916–919
41. **Chatzaki E, Bax CM, Eidne KA, Anderson L, Grudzinskas JG, Gallagher CJ** 1996 The expression of gonadotropin-releasing hormone and its receptor in endometrial cancer, and its relevance as an autocrine growth factor. Cancer Res 56:2059–2065
42. **Chegini N, Rong H, Dou Q, Kipersztok S, Williams RS** 1996 Gonadotropin-releasing hormone (GnRH) and GnRH receptor gene expression in human myometrium and leiomyomata and the direct action of GnRH analogs on myometrial smooth muscle cells and interaction with ovarian steroids *in vitro*. J Clin Endocrinol Metab 81:3215–3221
43. **Kobayashi Y, Zhai YL, Iinuma M, Horiuchi A, Nikaido T, Fujii S** 1997 Effects of GnRH analogue on human smooth muscle cells cultured from normal myometrial and from uterine leiomyomal tissues. Mol Hum Reprod 3:91–99
44. **Dong KW, Marcelin K, Hsu MI, Chiang CM, Hoffman G, Roberts JL** 1998 Expression of gonadotropin-releasing hormone (GnRH) gene in human uterine endometrial tissue. Mol Hum Reprod 4:893–898
45. **Raga F, Casan EM, Krussel JS, Wen Y, Huang HY, Nezhat C, Polan ML** 1998 Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. Biol Reprod 59:661–669
46. **Cheon KW, Lee HS, Parhar IS, Kang IS** 2001 Expression of the second isoform of gonadotropin-releasing hormone (GnRH-II) in human endometrium throughout the menstrual cycle. Mol Hum Reprod 7:447–452
47. **Kang SK, Cheng KW, Nathwani PS, Choi KC, Leung PC** 2000 Autocrine role of gonadotropin-releasing hormone and its receptor in ovarian cancer cell growth. Endocrine 13:297–304
48. **Kang SK, Choi KC, Cheng KW, Nathwani PS, Auersperg N, Leung PC** 2000 Role of gonadotropin-releasing hormone as an autocrine growth factor in human ovarian surface epithelium. Endocrinology 141:72–80
49. **Casan EM, Raga F, Bonilla-Musoles F, Polan ML** 2000 Human oviduct gonadotropin-releasing hormone: possible implications in fertilization, early embryonic development, and implantation. J Clin Endocrinol Metab 85:1377–1380
50. **Harris N, Dutlow C, Eidne K, Dong KW, Roberts J, Millar R** 1991 Gonadotropin-releasing hormone gene expression in MDA-MB-231 and ZR-75–1 breast carcinoma cell lines. Cancer Res 51:2577–2581
51. **Chen A, Kaganovsky E, Rahimipour S, Ben-Aroya N, Okon E, Koch Y** 2002 Two forms of gonadotropin-releasing hormone (GnRH) are expressed in human breast tissue and overexpressed in breast cancer: a putative mechanism for the antiproliferative effect of GnRH by down-regulation of acidic ribosomal phosphoproteins P1 and P2. Cancer Res 62:1036–1044
52. **Azad N, LaPaglia N, Kirsteins L, Uddin S, Steiner J, Williams DW, Lawrence AM, Emanuele NV** 1997 Jurkat cell proliferative activity is increased by luteinizing hormone-releasing hormone. J Endocrinol 153:241–249
53. **Chen HF, Jeung EB, Stephenson M, Leung PC** 1999 Human peripheral blood mononuclear cells express gonadotropin-releasing hormone (GnRH), GnRH receptor, and interleukin-2 receptor γ-chain messenger ribonucleic acids that are regulated by GnRH *in vitro*. J Clin Endocrinol Metab 84:743–750
54. **Bahk JY, Hyun JS, Chung SH, Lee H, Kim MO, Lee BH, Choi WS** 1995 Stage specific identification of the expression of GnRH mRNA and localization of the GnRH receptor in mature rat and adult human testis. J Urol 154:1958–1961
55. **Casan EM, Raga F, Polan ML** 1999 GnRH mRNA and protein expression in human preimplantation embryos. Mol Hum Reprod 5:234–239
56. **Cho S, Han J, Sun W, Choi D, Kwon HB, Jarry H, Wuttke W, Kim K** 1997 Evidence for autocrine inhibition of gonadotropin-releasing hormone (GnRH) gene transcription by GnRH in hypothalamic GT1–1 neuronal cells. Brain Res Mol Brain Res 50:51–58
57. **Han YG, Kang SS, Seong JY, Geum D, Suh YH, Kim K** 1999 Negative regulation of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor gene expression by a gonadotropin-releasing hormone agonist in the rat hypothalamus. J Neuroendocrinol 11:195–201
58. **Nathwani PS, Kang SK, Cheng KW, Choi KC, Leung PC** 2000 Regulation of gonadotropin-releasing hormone and its receptor

gene expression by 17β-estradiol in cultured human granulosa-luteal cells. Endocrinology 141:1754–1763
59. **Kang SK, Choi KC, Tai CJ, Auersperg N, Leung PC** 2001 Estradiol regulates gonadotropin-releasing hormone (GnRH) and its receptor gene expression and antagonizes the growth inhibitory effects of GnRH in human ovarian surface epithelial and ovarian cancer cells. Endocrinology 142:580–588
60. **Chen A, Ziv K, Laskar-Levy O, Koch Y** 2002 The transcription of the hGnRH-I and hGnRH-II genes in human neuronal cells is differentially regulated by estrogen. J Mol Neurosci 18:67–76
61. **Khosravi S, Leung PC** 2003 Differential regulation of gonadotropin-releasing hormone (GnRH)I and GnRHII messenger ribonucleic acid by gonadal steroids in human granulosa-luteal cells. J Clin Endocrinol Metab 88:663–672
62. **Chen ZG, Yu KL, Zheng HM, Dong KW** 1999 Estrogen receptor-mediated repression of gonadotropin-releasing hormone (GnRH) promoter activity in transfected CHO-K1 cells. Mol Cell Endocrinol 158:131–142
63. **Roy D, Angelini NL, Belsham DD** 1999 Estrogen directly represses gonadotropin-releasing hormone (GnRH) gene expression in estrogen receptor-α (ERα)- and ERβ-expressing GT1–7 GnRH neurons. Endocrinology 140:5045–5053
64. **Belsham DD, Evangelou A, Roy D, Duc VL, Brown TJ** 1998 Regulation of gonadotropin-releasing hormone (GnRH) gene expression by 5α-dihydrotestosterone in GnRH-secreting GT1–7 hypothalamic neurons. Endocrinology 139:1108–1114
65. **Shakil T, Hoque AN, Husain M, Belsham DD** 2002 Differential regulation of gonadotropin-releasing hormone secretion and gene expression by androgen: membrane versus nuclear receptor activation. Mol Endocrinol 16:2592–2602
66. **Chen A, Laskar-Levy O, Ben-Aroya N, Koch Y** 2001 Transcriptional regulation of the human GnRH II gene is mediated by a putative cAMP response element. Endocrinology 142:3483–3492
67. **Huang HY, Raga F, Wen Y, Kruessel JS, Soong YK, Polan ML** 2003 Interleukin-1β regulation of gonadotropin-releasing hormone messenger ribonucleic acid in cultured human endometrial stromal cells. Fertil Steril 79:399–406
68. **Zhen S, Zakaria M, Wolfe A, Radovick S** 1997 Regulation of gonadotropin-releasing hormone (GnRH) gene expression by insulin-like growth factor I in a cultured GnRH-expressing neuronal cell line. Mol Endocrinol 11:1145–1155
69. **Barni T, Maggi M, Fantoni G, Granchi S, Mancina R, Gulisano M, Marra F, Macorsini E, Luconi M, Rotella C, Serio M, Balboni GC, Vannelli G** 1999 Sex steroids and odorants modulate gonadotropin-releasing hormone secretion in primary cultures of human olfactory cells. J Clin Endocrinol Metab 84:4266–4273
70. **Schwanzel-Fukuda M, Pfaff DW** 1989 Origin of luteinizing hormone-releasing hormone neurons. Nature 338:161–164
71. **Wray S, Grant P, Gainer H** 1989 Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci USA 86:8132–8136
72. **Roy D, Angelini NL, Fujieda H, Brown GM, Belsham DD** 2001 Cyclical regulation of GnRH gene expression in GT1–7 GnRH-secreting neurons by melatonin. Endocrinology 142:4711–4720
73. **Roy D, Belsham DD** 2002 Melatonin receptor activation regulates GnRH gene expression and secretion in GT1–7 GnRH neurons. Signal transduction mechanisms. J Biol Chem 277:251–258
74. **Wolfe A, Kim HH, Tobet S, Stafford DJ, Radovick S** 2002 Identification of a discrete promoter region of the human GnRH gene that is sufficient for directing neuron-specific expression: a role for POU homeodomain transcription factors. Mol Endocrinol 16:435–449
75. **Cheng CK, Hoo RL, Chow BK, Leung PC** 2003 Functional cooperation between multiple regulatory elements in the untranslated exon 1 stimulates the basal transcription of the human GnRH-II gene. Mol Endocrinol 17:1175–1191
76. **Hu YF, Luscher B, Admon A, Mermod N, Tjian R** 1990 Transcription factor AP-4 contains multiple dimerization domains that regulate dimer specificity. Genes Dev 4:1741–1752
77. **Stojilkovic SS, Reinhart J, Catt KJ** 1994 Gonadotropin-releasing hormone receptors: structure and signal transduction pathways. Endocr Rev 15:462–499
78. **Sealfon SC, Weinstein H, Millar RP** 1997 Molecular mechanism of ligand interaction with gonadotropin-releasing hormone receptor. Endocr Rev 18:180–205
79. **Cui J, Smith RG, Mount GR, Lo JL, Yu J, Walsh TF, Singh SB, DeVita RJ, Goulet MT, Schaeffer JM, Cheng K** 2000 Identification of Phe313 of the gonadotropin-releasing hormone (GnRH) receptor as a site critical for the binding of nonpeptide GnRH antagonists. Mol Endocrinol 14:671–681
80. **Bockaert J, Marin P, Dumuis A, Fagni L** 2003 The 'magic tail' of G protein-coupled receptors: an anchorage for functional protein networks. FEBS Lett 546:65–72
81. **Ferguson SS** 2001 Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 53:1–24
82. **Myburgh DB, Millar RP, Hapgood JP** 1998 Alanine-261 in intracellular loop III of the human gonadotropin-releasing hormone receptor is crucial for G-protein coupling and receptor internalization. Biochem J 331:893–896
83. **Zhou W, Rodic V, Kitanovic S, Flanagan CA, Chi L, Weinstein H, Maayani S, Millar RP, Sealfon SC** 1995 A locus of the gonadotropin-releasing hormone receptor that differentiates agonist and antagonist binding sites. J Biol Chem 270:18853–18857
84. **Davidson JS, McArdle CA, Davies P, Elario R, Flanagan CA, Millar RP** 1996 Asn102 of the gonadotropin-releasing hormone receptor is a critical determinant of potency for agonists containing C-terminal glycinamide. J Biol Chem 271:15510–15514
85. **Flanagan CA, Rodic V, Konvicka K, Yuen T, Chi L, Rivier JE, Millar RP, Weinstein H, Sealfon SC** 2000 Multiple interactions of the $Asp^{2.61(98)}$ side chain of the gonadotropin-releasing hormone receptor contribute differentially to ligand interaction. Biochemistry 39:8133–8141
86. **Hoffmann SH, ter Laak T, Kuhne R, Reilander H, Beckers T** 2000 Residues within transmembrane helices 2 and 5 of the human gonadotropin-releasing hormone receptor contribute to agonist and antagonist binding. Mol Endocrinol 14:1099–1115
87. **Fromme BJ, Katz AA, Roeske RW, Millar RP, Flanagan CA** 2001 Roles of aspartate7.32(302) of the human gonadotropin-releasing hormone receptor in stabilizing a high-affinity ligand conformation. Mol Pharmacol 60:1280–1287
88. **Arora KK, Chung HO, Catt KJ** 1999 Influence of a species-specific extracellular amino acid on expression and function of the human gonadotropin-releasing hormone receptor. Mol Endocrinol 13:890–896
89. **Brosius J** 1991 Retroposons—seed of evolution. Science 251:753
90. **Fan NC, Peng C, Krisinger J, Leung PC** 1995 The human gonadotropin-releasing hormone receptor gene: complete structure including multiple promoters, transcription initiation sites, and polyadenylation signals. Mol Cell Endocrinol 107:R1–R8
91. **Kakar SS** 1997 Molecular structure of the human gonadotropin-releasing hormone receptor gene. Eur J Endocrinol 137:183–192
92. **Albarracin CT, Kaiser UB, Chin WW** 1994 Isolation and characterization of the 5′-flanking region of the mouse gonadotropin-releasing hormone receptor gene. Endocrinology 135:2300–2306
93. **Campion CE, Turzillo AM, Clay CM** 1996 The gene encoding the ovine gonadotropin-releasing hormone (GnRH) receptor: cloning and initial characterization. Gene 170:277–280
94. **Reinhart J, Xiao S, Arora KK, Catt KJ** 1997 Structural organization and characterization of the promoter region of the rat gonadotropin-releasing hormone receptor gene. Mol Cell Endocrinol 130: 1–12
95. **Leung PC, Squire J, Peng C, Fan N, Hayden MR, Olofsson JI** 1995 Mapping of the gonadotropin-releasing hormone (GnRH) receptor gene to human chromosome 4q21.2 by fluorescence in situ hybridization. Mamm Genome 6:309–310
96. **Ross J** 1996 Control of messenger RNA instability in higher eukaryotes. Trends Genet 12:171–175
97. **Gay E, Babajko S** 2000 AUUUA sequences compromise human insulin-like growth factor binding protein-1 mRNA instability. Biochem Biophys Res Commun 267:509–515
98. **Kamura T, Handa H, Hamasaki N, Kitajima S** 1997 Characterization of the human thrombopoietin gene promoter. A possible role of an Ets transcription factor, E4TF1/GABP. J Biol Chem 272: 11361–11368

99. **Pepitoni S, Wood IC, Buckley NJ** 1997 Structure of the m1 muscarinic acetylcholine receptor gene and its promoter. J Biol Chem 272:17112–17117
100. **Schaak S, Devedjian JC, Cayla C, Sender Y, Paris H** 1997 Molecular cloning, sequencing and functional study of the promoter region of the human α2C4-adrenergic receptor gene. Biochem J 328:431–438
101. **Moro O, Ideta R, Ifuku O** 1999 Characterization of the promoter region of the human melanocortin-1 receptor (MC1R) gene. Biochem Biophys Res Commun 262:452–460
102. **Chi L, Zhou W, Prikhozhan A, Flanagan C, Davidson JS, Golembo M, Illing N, Millar RP, Sealfon SC** 1993 Isolation and characterization of the human GnRH receptor. Mol Cell Endocrinol 91:R1–R6
103. **Grosse R, Schoneberg T, Schultz G, Gudermann T** 1997 Inhibition of gonadotropin-releasing hormone receptor signaling by expression of a splice variant of the human receptor. Mol Endocrinol 11:1305–1318
104. **Kottler ML, Bergametti F, Carre MC, Morice S, Decoret E, Lagarde JP, Starzec A, Counis R** 1999 Tissue-specific pattern of variant transcripts of the human gonadotropin-releasing hormone receptor gene. Eur J Endocrinol 140:561–569
105. **La Rosa S, Celato N, Uccella S, Capella C** 2000 Detection of gonadotropin-releasing hormone receptor in normal human pituitary cells and pituitary adenomas using immunohistochemistry. Virchows Arch 437:264–269
106. **Lin LS, Roberts VJ, Yen SS** 1995 Expression of human gonadotropin-releasing hormone receptor gene in the placenta and its functional relationship to human chorionic gonadotropin secretion. J Clin Endocrinol Metab 80:580–585
107. **Cheng KW, Nathwani PS, Leung PC** 2000 Regulation of human gonadotropin-releasing hormone receptor gene expression in placental cells. Endocrinology 141:2340–2349
108. **Bramley TA, Stirling D, Swanston IA, Menzies GS, McNeilly AS, Baird DT** 1987 Specific binding sites for gonadotropin-releasing hormone, LH/chorionic gonadotropin, low-density lipoprotein, prolactin and FSH in homogenates of human corpus luteum. II. Concentrations throughout the luteal phase of the menstrual cycle and early pregnancy. J Endocrinol 113:317–327
109. **Emons G, Pahwa GS, Brack C, Sturm R, Oberheuser F, Knuppen R** 1989 Gonadotropin releasing hormone binding sites in human epithelial ovarian carcinomata. Eur J Cancer Clin Oncol 25:215–221
110. **Emons G, Ortmann O, Becker M, Irmer G, Springer B, Laun R, Holzel F, Schulz KD, Schally AV** 1993 High affinity binding and antiproliferative effects of LHRH analogues in human ovarian cancer cell lines. Cancer Res 54:5439–5446
111. **Brus L, Lambalk CB, de Koning J, Helder MN, Janssens RM, Schoemaker J** 1997 Specific gonadotropin-releasing hormone analogue binding predominantly in human luteinized follicular aspirates and not in human pre-ovulatory follicles. Hum Reprod 12:769–773
112. **Kakar SS, Grizzle WE, Neill JD** 1994 The nucleotide sequences of human GnRH receptors in breast and ovarian tumors are identical with that found in pituitary. Mol Cell Endocrinol 106:145–149
113. **Peng C, Fan NC, Ligier M, Vaananen J, Leung PC** 1994 Expression and regulation of gonadotropin-releasing hormone (GnRH) and GnRH receptor messenger ribonucleic acids in human granulosa-luteal cells. Endocrinology 135:1740–1746
114. **Emons G, Schroder B, Ortmann O, Westphalen S, Schulz KD, Schally AV** 1993 High affinity and direct antiproliferative effects of luteinizing hormone-releasing hormone analogs in human endometrial cancer cell lines. J Clin Endocrinol Metab 77:1458–1464
115. **Srkalovic G, Wittliff JL, Schally AV** 1990 Detection and partial characterization of receptors for [D-Trp6]-luteinizing hormone-releasing hormone and epidermal growth factor in human endometrial carcinoma. Cancer Res 50:1841–1846
116. **Imai A, Ohno T, Iida K, Fuseya T, Furui T, Tamaya T** 1994 Presence of gonadotropin-releasing hormone receptor and its messenger ribonucleic acid in endometrial carcinoma and endometrium. Gynecol Oncol 55:144–148
117. **Borroni R, Di Blasio AM, Gaffuri B, Santorsola R, Busacca M, Vigano P, Vignali M** 2000 Expression of GnRH receptor in human ectopic endometrial cells and inhibition of their proliferation by leuprolide acetate. Mol Cell Endocrinol 159:37–43
118. **Grundker C, Volker P, Emons G** 2001 Antiproliferative signaling of luteinizing hormone-releasing hormone in human endometrial and ovarian cancer cells through G protein αi-mediated activation of phosphotyrosine phosphatase. Endocrinology 142:2369–2380
119. **Fekete A, Redding TW, Comaru-Schally AM, Pontes JE, Connelly RW, Srkalovic G, Schally AV** 1989 Receptors for luteinizing hormone-releasing hormone, somatostatin, prolactin, and epidermal growth factor in rat and human prostate cancers and in benign prostate hyperplasia. Prostate 14:191–208
120. **Limonta P, Dondi D, Moretti RM, Maggi R, Motta M** 1992 Antiproliferative effects of luteinizing hormone-releasing hormone agonists on the human prostatic cancer cell line LNCaP. J Clin Endocrinol Metab 75:207–212
121. **Ravenna L, Salvatori L, Morrone S, Lubrano C, Cardillo MR, Sciarra F, Frati L, Di Silverio F, Petrangeli E** 2000 Effects of triptorelin, a gonadotropin-releasing hormone agonist, on the human prostatic cell lines PC3 and LNCaP. J Androl 21:549–557
122. **Bahk JY, Hyun JS, Lee H, Kim MO, Cho GJ, Lee BH, Choi WS** 1998 Expression of gonadotropin-releasing hormone (GnRH) and GnRH receptor mRNA in prostate cancer cells and effect of GnRH on the proliferation of prostate cancer cells. Urol Res 26:259–264
123. **Limonta P, Moretti RM, Marelli MM, Dondi D, Parenti M, Motta M** 1999 The luteinizing hormone-releasing hormone receptor in human prostate cancer cells: messenger ribonucleic acid expression, molecular size, and signal transduction pathway. Endocrinology 140:5250–5256
124. **Halmos G, Arencibia JM, Schally AV, Davis R, Bostwick DG** 2000 High incidence of receptors for luteinizing hormone-releasing hormone (LHRH) and LHRH receptor gene expression in human prostate cancers. J Urol 163:623–629
125. **Tieva A, Stattin P, Wikstrom P, Bergh A, Damber JE** 2001 Gonadotropin-releasing hormone receptor expression in the human prostate. Prostate 47:276–284
126. **Eidne KA, Flanagan CA, Millar RP** 1985 Gonadotropin-releasing hormone binding sites in human breast carcinoma. Science 229: 989–991
127. **Segal-Abramson T, Kitroser H, Levy J, Schally AV, Sharoni Y** 1992 Direct effects of luteinizing hormone-releasing hormone agonists and antagonists on MCF-7 mammary cancer cells. Proc Natl Acad Sci USA 89:2336–2339
128. **Kottler ML, Starzec A, Carre MC, Lagarde JP, Martin A, Counis R** 1997 The genes for gonadotropin-releasing hormone and its receptor are expressed in human breast with fibrocystic disease and cancer. Int J Cancer 71:595–599
129. **Moriya T, Suzuki T, Pilichowska M, Ariga N, Kimura N, Ouchi N, Nagura H, Sasano H** 2001 Immunohistochemical expression of gonadotropin releasing hormone receptor in human breast carcinoma. Pathol Int 51:333–337
130. **Kakar SS, Jennes L** 1995 Expression of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor mRNAs in various non-reproductive human tissues. Cancer Lett 98:57–62
131. **Moretti RM, Montagnani Marelli M, Van Groeninghen JC, Limonta P** 2002 Locally expressed LHRH receptors mediate the oncostatic and antimetastatic activity of LHRH agonists on melanoma cells. J Clin Endocrinol Metab 87:3791–3797
132. **Loumaye E, Catt KJ** 1982 Homologous regulation of gonadotropin-releasing hormone receptors in cultured pituitary cells. Science 215:983–985
133. **McArdle CA, Gorospe WC, Huckle WR, Conn PM** 1987 Homologous down-regulation of gonadotropin-releasing hormone receptor and desensitization of gonadotropes: lack of dependence on protein kinase C. Mol Endocrinol 1:420–429
134. **Tsutsumi M, Laws SC, Sealfon SC** 1993 Homologous up-regulation of the gonadotropin-releasing hormone receptor in αT3–1 cells is associated with unchanged receptor messenger RNA (mRNA) levels and altered mRNA activity. Mol Endocrinol 7:1625–1633
135. **Tsutsumi M, Laws SC, Rodic V, Sealfon SC** 1995 Translational regulation of the gonadotropin-releasing hormone receptor in αT3–1 cells. Endocrinology 136:1128–1136
136. **Kaiser UB, Jakubowiak A, Steinberger A, Chin WW** 1997 Differential effects of gonadotropin-releasing hormone (GnRH) pulse

frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels *in vitro.* Endocrinology 138:1224–1231
137. **Menon M, Peegel H, Katta V** 1985 Estradiol potentiation of gonadotropin-releasing hormone responsiveness in the anterior pituitary is mediated by an increase in GnRH receptor. Am J Obstet Gynecol 151:534–540
138. **Emons G, Hoffman HG, Brack C, Ortmann O, Strum R, Ball P, Knuppen R** 1988 Modulation of gonadotropin-releasing hormone receptor concentration in cultured female rat pituitary cells by estradiol treatment. J Steroid Biochem 31:751–756
139. **Kaiser UB, Jakubowiak A, Steinberger A, Chin WW** 1993 Regulation of rat pituitary gonadotropin-releasing hormone receptor mRNA levels *in vivo* and *in vitro.* Endocrinology 133:931–934
140. **Quinones-Jenab V, Jenab S, Ogawa S, Funabashi T, Weesner GD, Pfaff DW** 1996 Estrogen regulation of gonadotropin-releasing hormone receptor messenger RNA in female rat pituitary tissue. Mol Brain Res 38:243–250
141. **Cheng CK, Chow BK, Leung PC** 2003 An AP-1-like motif mediates 17β-estradiol repression of GnRH receptor promoter via an estrogen receptor α-dependent mechanism in ovarian and breast cancer cells. Mol Endocrinol 17:2613–2629
142. **Gardner DB, Miller WL,** Estradiol and inhibin increase a single polypeptide GnRH receptor in ovine pituitary cultures. Program of the 77th Annual Meeting of The Endocrine Society, Washington, DC, 1995, p 140 (Abstract P1-110)
143. **Gardner DB, Sebastian J, Miller WL** 2000 Estradiol induces and hyperglycosylates the receptor for ovine gonadotropin-releasing hormone. Endocrinology 141:91–99
144. **Laws SC, Beggs JM, Webster JC, Miller WL** 1990 Inhibin increases and progesterone decrease receptor for gonadotropin-releasing hormone in ovine pituitary cultures. Endocrinology 127:373–380
145. **Wu JC, Sealfon SC, Miller WL** 1994 Gonadal hormones and gonadotropin-releasing hormone (GnRH) alter messenger ribonucleic acid levels for GnRH receptors in sheep. Endocrinology 134: 1846–1850
146. **Sakurai H, Adams BM, Adems TE** 1997 Concentrations of GnRH receptor and GnRH receptor mRNA in pituitary tissue of orchidectomized sheep: effect of oestradiol, progesterone, and progesterone withdrawal. J Endocrinol 152:91–98
147. **Kirkpatrick BL, Esquivel E, Moss GE, Hamernik DL, Wise ME** 1998 Estradiol and gonadotropin-releasing hormone (GnRH) interact to increase GnRH receptor expression in ovariectomized ewes after hypothalamic-pituitary disconnection. Endocrine 8:225–229
148. **Cheng KW, Cheng CK, Leung PC** 2001 Differential role of PR-A and -B isoforms in transcription regulation of human GnRH receptor gene. Mol Endocrinol 15:2078–2092
149. **Olofsson JI, Conti CC, Leung PC** 1995 Homologous and heterologous regulation of gonadotropin-releasing hormone receptor gene expression in preovulatory rat granulosa cells. Endocrinology 136:974–980
150. **Li X, Lei ZM, Rao CV** 1996 Human chorionic gonadotropin down-regulates the expression of gonadotropin-releasing hormone receptor gene in GT1–7 neurons. Endocrinology 137:899–904
151. **Botte MC, Lerrant Y, Lozach A, Berault A, Counis R, Kottler ML** 1999 LH down-regulates gonadotropin-releasing hormone (GnRH) receptor, but not GnRH, mRNA levels in the rat testis. J Endocrinol 162:409–415
152. **Cheng KW, Leung PC** 2002 Human chorionic gonadotropin-activated cAMP pathway regulates human placental GnRH receptor gene transcription in choriocarcinoma JEG-3 cells. J Clin Endocrinol Metab 87:3291–3299
153. **Tamura H, Nakamura Y, Takiguchi S, Kashida S, Yamagata Y, Sugino N, Kato H** 1998 Melatonin directly suppresses steroid production by preovulatory follicles in the cyclic hamster. J Pineal Res 25:135–141
154. **Niles LP, Wang J, Shen L, Lobb DK, Younglai EV** 1999 Melatonin receptor mRNA expression in human granulosa cells. Mol Cell Endocrinol 56:107–110
155. **Bodis J, Koppan M, Kornya L, Tinneberg HR, Torok A** 2001 Influence of melatonin on basal and gonadotropin-stimulated progesterone and estradiol secretion of cultured human granulosa cells and in the superfused granulosa cell system. Gynecol Obstet Invest 52:198–202
156. **Lee CJ, Do BR, Lee YH, Park JH, Kim SJ, Roh SI, Toon YD, Yoon HS** 2001 Ovarian expression of melatonin Mel(1a) receptor mRNA during mouse development. Mol Reprod Dev 59:126–132
157. **Woo MM, Tai CJ, Kang SK, Nathwani PS, Pang SF, Leung PC** 2001 Direct action of melatonin in human granulosa-luteal cells. J Clin Endocrinol Metab 86:4789–4797
158. **Braden TD, Conn PM** 1992 Activin-A stimulates the synthesis of gonadotropin-releasing hormone receptors. Endocrinology 130: 2101–2105
159. **Fernandez-Vazquez G, Kaiser UB, Albarracin CT, Chin WW** 1996 Transcriptional activation of the gonadotropin-releasing hormone receptor gene by activin A. Mol Endocrinol 10:356–366
160. **Seminara SB, Hayes FJ, Crowley Jr WF** 1998 Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. Endocr Rev 19:521–539
161. **Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, Brown CJ, Willard HF, Lawrence C, Persico MG, Camerino G, Ballabio A** 1991 A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 353:529–536
162. **Bick D, Franco B, Sherins RJ, Heye B, Pike L, Crawford J, Maddalena A, Incerti B, Pragliola A, Meitinger T, Ballabio A** 1992 Brief report: intragenic deletion of the KALIG-1 gene in Kallmann's syndrome. N Engl J Med 326:1752–1755
163. **Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER, Meitinger T, Monaco AP, Sassone-Corsi P, Camerino G** 1994 An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenital. Nature 372:635–641
164. **de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E** 1997 A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 337:1597–1602
165. **Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S** 1998 Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. Nat Genet 18:14–15
166. **Caron P, Chauvin S, Christin-Maitre S, Bennet A, Lahlou N, Counis R, Bouchard P, Kottler ML** 1999 Resistance of hypogonadic patients with mutated GnRH receptor genes to pulsatile GnRH administration. J Clin Endocrinol Metab 84:990–996
167. **de Roux N, Young J, Brailly-Tabard S, Misrhai M, Milgrom E, Schaison G** 1999 The same molecular defects of the gonadotropin-releasing hormone receptor determine variable degree of hypogonadism in affected kindred. J Clin Endocrinol Metab 84:567–572
168. **Pralong FP, Gomez F, Castillo E, Cotecchia S, Abuin L, Aubert ML, Portmann L** 1999 Complete hypogonadotropic hypogonadism associated with a novel inactivating mutation of the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab 84:3811–3816
169. **Kottler ML, Chauvin S, Lahlou N, Harris CE, Johnston CJ, Lagarde JP, Bouchard P, Farid N, Counis R** 2000 A new compound heterozygous mutation of the gonadotropin-releasing hormone receptor (L314X, Q106R) in a woman with complete hypogonadotropic hypogonadism: chronic estrogen administration amplifies the gonadotropin defect. J Clin Endocrinol Metab 85:3002–3008
170. **Beranova M, Oliveira LM, Bedecarrats GY, Schipani E, Vallejo M, Ammini AC, Quintos JB, Hall JE, Martin KA, Hayes FJ, Pitteloud N, Kaiser UB, Crowley Jr WF, Seminara SB** 2001 Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 86:1580–1588
171. **Costa EM, Bedecarrats GY, Mendonca BB, Arnhold IJ, Kaiser UB, Latronico AC** 2001 Two novel mutations in the gonadotropin-releasing hormone receptor gene in Brazilian patients with hypogonadotropic hypogonadism and normal olfaction. J Clin Endocrinol Metab 86:2680–2686
172. **Karges B, Karges W, Mine M, Ludwig L, Kuhne R, Milgrom E, de Roux N** 2003 Mutation of Ala(171)Thr stabilizes the gonadotropin-releasing hormone receptor in its inactive conformation, causing familial hypogonadotropic hypogonadism. J Clin Endocrinol Metab 88:1873–1879

173. **Pitteloud N, Boepple PA, DeCruz S, Valkenburgh SB, Crowley Jr WF, Hayes FJ** 2001 The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab 86:2470–2475
174. **Soderlund D, Canto P, de la Chesnaye E, Ulloa-Aguirre A, Mendez JP** 2001 A novel homozygous mutation in the second transmembrane domain of gonadotropin-releasing hormone receptor gene. Clin Endocrinol (Oxf) 54:493–498
175. **Brothers SP, Janovick J, Conn PM** 2003 Unexpected effects of epitope and chimeric tags on gonadotropin-releasing hormone receptors: implications for understanding the molecular etiology of hypogonadotropic hypogonadism. J Clin Endocrinol Metab 88: 6107–6112
176. **Janovick JA, Goulet M, Bush E, Greer J, Wettlaufer DG, Conn PM** 2003 Structure-activity relations of successful pharmacologic chaperones for rescue of naturally occurring and manufactured mutants of the gonadotropin-releasing hormone receptor. J Pharmacol Exp Ther 305:608–614
177. **Ulloa-Aguirre A, Janovick J, Leanos-Miranda A, Conn PM** 2003 Misrouted cell surface receptors as a novel disease etiology and potential therapeutic target: the case of hypogonadotropic hypogonadism due to gonadotropin-releasing hormone resistance. Expert Opin Ther Targets 7:175–185
178. **Ngan ES, Cheng PK, Leung PC, Chow BK** 1999 Steroidogenic factor-1 interacts with a gonadotrope-specific element within the first exon of the human gonadotropin-releasing hormone receptor gene to mediate gonadotrope-specific expression. Endocrinology 140:2452–2462
179. **Fowkes RC, Desclozeaux M, Patel MV, Aylwin SJ, King P, Ingraham HA, Burrin JM** 2003 Steroidogenic factor-1 (SF-1) and the gonadotrope-specific element (GSE) enhance basal and pituitary adenylate cyclase-activating polypeptide (PACAP)-stimulated transcription of the human glycoprotein hormone α-subunit gene (αGSU) in gonadotropes. Mol Endocrinol 17:2177–2188
180. **Heckert LL, Schultz K, Nilson JH** 1995 Different composite regulatory elements direct expression of the human α subunit gene to pituitary and placenta. J Biol Chem 270:26497–26504
181. **Halvorson LM, Kaiser UB, Chin WW** 1996 Stimulation of luteinizing hormone β gene promoter activity by the orphan nuclear receptor, steroidogenic factor-1. J Biol Chem 271:6645–6650
182. **Hanley NA, Ikeda Y, Luo X, Parker KL** 2000 Steroidogenic factor (SF-1) is essential for ovarian development and function. Mol Cell Endocrinol 163:27–32
183. **Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, Bakke M, Zhao L, Frigeri C, Hanley NA, Stallings N, Schimmer BP** 2002 Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog Horm Res 57:19–36
184. **Duval DL, Nelson SE, Clay CM** 1997 The tripartite basal enhancer of the gonadotropin-releasing hormone (GnRH) receptor gene promoter regulates cell-specific expression through a novel GnRH receptor activating sequence. Mol Endocrinol 11:1814–1821
185. **Flouriot G, Griffin C, Kenealy M, Sonntag-Buck V, Gannon F** 1998 Differentially expressed messenger RNA isoforms of the human estrogen receptor-α gene are generated by alternative splicing and promoter usage. Mol Endocrinol 12:1939–1954
186. **McCormick JA, Lyons V, Jacobson MD, Noble J, Diorio J, Nyirenda M, Weaver S, Ester W, Yau JL, Meaney MJ, Sekl JR, Chapman KE** 2000 5′-heterogeneity of glucocorticoid receptor messenger RNA is tissue specific: differential regulation of variant transcripts by early-life events. Mol Endocrinol 14:506–517
187. **Shields DJ, Agellon LB, Vance DE** 2001 Structure, expression profile and alternative processing of the human phosphatidylethanolamine N-methyltransferase (PEMT) gene. Biochim Biophys Acta 1532:105–114
188. **Cheng KW, Chow BK, Leung PC** 2001 Functional mapping of a placenta-specific upstream promoter for human gonadotropin-releasing hormone receptor gene. Endocrinology 142:1506–1516
189. **Cheng CK, Yeung CM, Chow BK, Leung PC** 2002 Characterization of a new upstream gonadotropin-releasing hormone receptor promoter in human ovarian granulosa-luteal cells. Mol Endocrinol 16:1552–1564
190. **Norwitz ER, Cardona GR, Jeong KH, Chin WW** 1999 Identification and characterization of the gonadotropin-releasing hormone response elements in the mouse gonadotropin-releasing hormone receptor gene. J Biol Chem 274:867–880
191. **White BR, Duval DL, Mulvaney JM, Roberson MS, Clay CM** 1999 Homologous regulation of the gonadotropin-releasing hormone receptor gene is partially mediated by protein kinase C activation of an activator protein-1 element. Mol Endocrinol 13:566–577
192. **Norwitz ER, Xu S, Jeong KH, Bedecarrats GY, Winebrenner LD, Chin WW, Kaiser UB** 2002 Activin A augments GnRH-mediated transcriptional activation of the mouse GnRH receptor gene. Endocrinology 143:985–997
193. **Norwitz ER, Xu S, Xu J, Spiryda LB, Park JS, Jeong KH, McGee EA, Kaiser UB** 2002 Direct binding of AP-1 (Fos/Jun) proteins to a SMAD binding element facilitates both gonadotropin-releasing hormone (GnRH)- and activin-mediated transcriptional activation of the mouse GnRH receptor gene. J Biol Chem 277:37469–37479
194. **Cheng KW, Ngan ES, Kang SK, Chow BK, Leung PC** 2000 Transcriptional down-regulation of human gonadotropin-releasing hormone (GnRH) receptor gene by GnRH: role of protein kinase C and activating protein 1. Endocrinology 141:3611–3622
195. **Young LS, Naik SI, Clayton RN** 1984 Adenosine 3′,5′-monophosphate derivatives increase gonadotropin-releasing hormone receptors in cultured pituitary cells. Endocrinology 114:2114–2122
196. **Abdilnour G, Bourne GA** 1995 Adenosine 3′,5′-cyclic monophosphate and the self-priming effect of gonadotropin-releasing hormone. Mol Cell Endocrinol 107:1–7
197. **Cassina P, Sellers J, Neill JD** 1995 Effect of cAMP on GnRH stimulated LH secretion from individual pituitary gonadotropes. Mol Cell Endocrinol 114:127–135
198. **Lin X, Conn PM** 1998 Transcriptional activation of gonadotropin-releasing hormone (GnRH) receptor gene by GnRH and cyclic adenosine monophosphate. Endocrinology 139:3896–3902
199. **Cheng KW, Leung PC** 2001 Human gonadotropin-releasing hormone receptor gene transcription: up-regulation by 3′,5′-cyclic adenosine monophosphate/protein kinase A pathway. Mol Cell Endocrinol 181:15–26
200. **Pulverer BJ, Kyriakis JM, Avurch J, Nikolakaki E, Woodgett JR** 1991 Phosphorylation of c-jun mediated by MAP kinases. Nature 353:670–674
201. **Smeal T, Binetruy B, Mercola DA, Birrer M, Karin M** 1991 Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. Nature 354:494–496
202. **Kang SK, Cheng KW, Ngan ES, Chow BK, Choi KC, Leung PC** 2000 Differential expression of human gonadotropin-releasing hormone receptor gene in pituitary and ovarian cells. Mol Cell Endocrinol 162:157–166
203. **Cheng CK, Yeung CM, Hoo RL, Chow BK, Leung PC** 2002 Oct-1 is involved in the transcriptional repression of the gonadotropin-releasing hormone receptor gene. Endocrinology 143:4693–4701
204. **Grosse R, Schmid A, Schoneberg T, Herrlich A, Muhn P, Schultz G, Gudermann T** 2000 Gonadotropin-releasing hormone receptor initiates multiple signaling pathways by exclusively coupling to $G_{q/11}$ proteins. J Biol Chem 275:9193–9200
205. **Imai A, Takagi H, Horibe S, Fuseya T, Tamaya T** 1996 Coupling of gonadotropin-releasing hormone receptor to Gi protein in human reproductive tract tumors. J Clin Endocrinol Metab 81:3249–3253
206. **Liu F, Usui I, Evans LG, Austin DA, Mellon PL, Olefsky JM, Webster NJ** 2002 Involvement of both G(q/11) and G(s) proteins in gonadotropin-releasing hormone receptor-mediated signaling in LβT2 cells. J Biol Chem 277:32099–32108
207. **Stanislaus D, Ponder S, Ji TH, Conn PM** 1998 Gonadotropin-releasing hormone receptor couples to multiple G proteins in rat gonadotrophs and in GGH_3 cells: evidence from palmitoylation and overexpression of G proteins. Biol Reprod 59:579–586
208. **Krsmanovic LZ, Mores N, Navarro CE, Arora KK, Catt KJ** 2003 An agonist-induced switch in G protein coupling of the gonadotropin-releasing hormone receptor regulates pulsatile neuropeptide secretion. Proc Natl Acad Sci USA 100:2969–2974
209. **Luttrell LM** 2002 Activation and targeting of mitogen-activated protein kinases by G-protein-coupled receptors. Can J Physiol Pharmacol 80:375–382
210. **Pierce KL, Luttrell LM, Lefkowitz RJ** 2001 New mechanisms in

heptahelical receptor signaling to mitogen-activated protein kinase cascades. Oncogene 20:1532–1539
211. **Widmann C, Gibson S, Jarpe MB, Johnson GL** 1999 Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 79:143–180
212. **Chang L, Karin M** 2001 Mammalian MAP kinase signalling cascades. Nature 410:37–40
213. **Johnson GL, Lapadat R** 2002 Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298:1911–1912
214. **Naor Z, Benard O, Seger R** 2000 Activation of MAPK cascades by G-protein-coupled receptors: the case of gonadotropin-releasing hormone receptor. Trends Endocrinol Metab 11:91–99
215. **Kraus S, Naor Z, Seger R** 2001 Intracellular signaling pathways mediated by the gonadotropin-releasing hormone (GnRH) receptor. Arch Med Res 32:499–509
216. **Yokoi T, Ohmichi M, Tasaka K, Kimura A, Kanda Y, Hayakawa J, Tahara M, Hisamoto K, Kurachi H, Murata Y** 2000 Activation of the luteinizing hormone β promoter by gonadotropin-releasing hormone requires c-Jun N-terminal protein kinase. J Biol Chem 275:21639–21647
217. **Benard O, Naor Z, Seger R** 2001 Role of dynamin, Src, and Ras in the protein kinase C-mediated activation of ERK by gonadotropin-releasing hormone. J Biol Chem 276:4554–4563
218. **Liu F, Austin DA, Mellon PL, Olefsky JM, Webster NJ** 2002 GnRH activates ERK1/2 leading to the induction of *c-fos* and LHβ protein expression in LβT2 cells. Mol Endocrinol 16:419–434
219. **Mulvaney JM, Zhang T, Fewtrell C, Roberson MS** 1999 Calcium influx through L-type channels is required for selective activation of extracellular signal-regulated kinase by gonadotropin-releasing hormone. J Biol Chem 274:29796–29804
220. **Navratil AM, Bliss SP, Berghorn KA, Haughian JM, Farmerie TA, Graham JK, Clay CM, Roberson MS** 2003 Constitutive localization of the gonadotropin-releasing hormone (GnRH) receptor to low density membrane microdomains is necessary for GnRH signaling to ERK. J Biol Chem 278:31593–31602
221. **Levi NL, Hanoch T, Benard O, Rozenblat M, Harris D, Reiss N, Naor Z, Seger R** 1998 Stimulation of Jun N-terminal kinase (JNK) by gonadotropin-releasing hormone in pituitary αT3–1 cell line is mediated by protein kinase C, c-Src, and CDC42. Mol Endocrinol 12:815–824
222. **Mulvaney JM, Roberson MS** 2000 Divergent signaling pathways requiring discrete calcium signals mediate concurrent activation of two mitogen-activated protein kinases by gonadotropin-releasing hormone. J Biol Chem 275:14182–14189
223. **Roberson MS, Zhang T, Li HL, Mulvaney JM** 1999 Activation of the p38 mitogen-activated protein kinase pathway by gonadotropin-releasing hormone. Endocrinology 140:1310–1318
224. **Kraus S, Benard O, Naor Z, Seger R** 2003 c-Src is activated by EGF-receptor in a pathway that mediates JNK and ERK activation by gonadotropin-releasing hormone in COS7 cells. J Biol Chem 278:32618–32630
225. **Shah BH, Soh JW, Catt KJ** 2003 Dependence of gonadotropin-releasing hormone-induced neuronal MAPK signaling on epidermal growth factor receptor transactivation. J Biol Chem 278:2866–2875
226. **Heding A, Vrecl M, Bogerd J, McGregor A, Sellar R, Taylor PL, Eidne KA** 1998 Gonadotropin-releasing hormone receptors with intracellular carboxyl-terminal tails undergo acute desensitization of total inositol phosphate production and exhibit accelerated internalization kinetics. J Biol Chem 273:11472–11477
227. **Willars GB, Heding A, Vrecl M, Sellar R, Blomenrohr M, Nahorski SR, Eidne KA** 1999 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 274:30146–30153
228. **Davidson JS, Wakefield IK, Millar RP** 1994 Absence of rapid desensitization of the mouse gonadotropin-releasing hormone receptor. Biochem J 300:299–302
229. **Vrecl M, Anderson L, Hanyaloglu A, McGregor AM, Groarke AD, Milligan G, Taylor PL, Eidne KA** 1998 Agonist-induced endocytosis and recycling of the gonadotropin-releasing hormone receptor: effect of β-arrestin on internalization kinetics. Mol Endocrinol 12:1818–1829
230. **McArdle CA, Franklin J, Green L, Hislop JN** 2002 Signaling, cycling and desensitization of gonadotropin-releasing hormone receptors. J Endocrinol 173:1–11
231. **Hislop JL, Everest HM, Flynn A, Harding T, Uney JB, Troskie BE, Millar RP, McArdle CA** 2001 Differential internalization of mammalian and non-mammalian gonadotropin-releasing hormone receptors. Uncoupling of dynamin-dependent internalization from mitogen-activated protein kinase signaling. J Biol Chem 276:39685–39694
232. **Everest HM, Hislop JN, Harding T, Uney JB, Flynn A, Millar RP, McArdle CA** 2001 Signaling and anti-proliferative effects mediated by gonadotropin-releasing hormone receptors after expression in breast cancer cells using recombinant adenovirus. Endocrinology 142:4663–4672
233. **Gharib SD, Wierman ME, Shupnik MA, Chin WW** 1990 Molecular biology of the pituitary gonadotropins. Endocr Rev 11:177–199
234. **Hamernik DL** 1995 Molecular biology of gonadotropins. J Reprod Fertil Suppl 49:257–269
235. **Holdstock JG, Aylwin SJ, Burrin JM** 1996 Calcium and glycoprotein hormone α-subunit gene expression and secretion in αT3–1 gonadotropes. Mol Endocrinol 10:1308–1317
236. **Harris D, Chuderland D, Bonfil D, Kraus S, Seger R, Naor Z** 2003 Extracellular signal-regulated kinase and c-Src, but not Jun N-terminal kinase, are involved in basal and gonadotropin-releasing hormone-stimulated activity of the glycoprotein hormone α-subunit promoter. Endocrinology 144:612–622
237. **Weck J, Fallest PC, Pitt LK, Shupnik MA** 1998 Differential gonadotropin-releasing hormone stimulation of rat luteinizing hormone subunit gene transcription by calcium influx and mitogen-activated protein kinase-signaling pathways. Mol Endocrinol 12:451–457
238. **Saunders BD, Sabbagh E, Chin WW, Kaiser UB** 1998 Differential use of signal transduction pathways in the gonadotropin-releasing hormone-mediated regulation of gonadotropin subunit gene expression. Endocrinology 139:1835–1843
239. **Vasilyev VV, Lawson MA, Dipaolo D, Webster NJ, Mellon PL** 2002 Different signaling pathways control acute induction *versus* long-term repression of LHβ transcription by GnRH. Endocrinology 143:3414–3426
240. **Call GB, Wolfe MW** 1999 Gonadotropin releasing hormone activates the equine luteinizing hormone β promoter through a protein kinase C/mitogen activated protein kinase pathway. Biol Reprod 61:715–723
241. **Kaiser UB, Sabbagh E, Chen MT, Chin WW, Saunders BD** 1998 Sp1 binds to the rat luteinizing hormone β (LHβ) gene promoter and mediates gonadotropin-releasing hormone-stimulated expression of the LHβ subunit gene. J Biol Chem 273:12943–12951
242. **Kaiser UB, Sabbagh E, Saunders BD, Chin WW** 1998 Identification of cis-acting deoxyribonucleic acid elements that mediate gonadotropin-releasing hormone stimulation of the rat luteinizing hormone β-subunit gene. Endocrinology 139:2443–2451
243. **Weck J, Anderson AC, Jenkins S, Fallest PC, Shupnik MA** 2000 Divergent and composite gonadotropin-releasing hormone responsive elements in the rat luteinizing hormone subunit genes. Mol Endocrinol 14:472–485
244. **Halvorson LM, Kaiser UB, Chin WW** 1999 The protein kinase C system acts through the early growth response protein 1 to increase LHβ gene expression in synergy with steroidogenic factor-1. Mol Endocrinol 13:106–116
245. **Tremblay JJ, 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. Mol Cell Biol 19:2567–2576
246. **Vasilyev VV, Pernasetti F, Rosenberg SB, Barsoum MJ, Austin DA, Webster NJ, Mellon PL** 2002 Transcriptional activation of the ovine follicle-stimulating hormone-β gene by gonadotropin-releasing hormone involves multiple signal transduction pathways. Endocrinology 143:1651–1659
247. **Strahl BD, Huang HJ, Sebastian J, Ghosh BR, Miller WL** 1998 Transcriptional activation of the ovine follicle-stimulating hormone β-subunit gene by gonadotropin-releasing hormone: in-

volvement of two activating protein-1 sites and protein kinase C. Endocrinology 139:4455–4465
248. **Pernasetti F, Vasilyev VV, Rosenberg SB, Bailey JS, Huang HJ, Miller WL, Mellon PL** 2001 Cell-specific transcriptional regulation of follicle-stimulating hormone-β by activin and gonadotropin-releasing hormone in the LβT2 pituitary gonadotrope cell model. Endocrinology 142:2284–2295
249. **Tse A, Tse FW, Almers W, Hille B** 1993 Rhythmic exocytosis stimulated by GnRH-induced calcium oscillations in rat gonadotropes. Science 260:82–84
250. **Stojilkovic SS, Catt KJ** 1995 Novel aspects of GnRH-induced intracellular signaling and secretion in pituitary gonadotrophs. J Neuroendocrinol 7:739–757
251. **Chang JP, Stojilkovic SS, Graeter JS, Catt KJ** 1988 Gonadotropin-releasing hormone stimulates luteinizing hormone secretion by extracellular calcium-dependent and independent mechanisms. Endocrinology 123:87–97
252. **Naor Z, Capponi AM, Rossier MF, Ayalon D, Limor R** 1988 Gonadotropin-releasing hormone-induced rise in cytosolic free Ca^{2+} levels: mobilization of cellular and extracellular Ca^{2+} pools and relationship to gonadotropin secretion. Mol Endocrinol 2:512–520
253. **Stojilkovic SS, Chang JP, Ngo D, Catt KJ** 1988 Evidence for the role of protein kinase C in luteinizing hormone synthesis and secretion. J Biol Chem 263:17307–17311
254. **Beggs MJ, Miller WL** 1989 Gonadotropin-releasing hormone-stimulated luteinizing hormone (LH) release from ovine gonadotrophs in culture is separate from phorbol ester-stimulated LH release. Endocrinology 124:667–674
255. **Strulovici B, Tahilramani R, Nestor JJ** 1987 Phosphorylation substrates for protein kinase C in intact pituitary cells: characterization of a receptor-mediated event using novel gonadotropin-releasing hormone analogues. Biochemistry 26:6005–6011
256. **Stojilkovic SS, Chang JP, Izumi SI, Tsaka K, Catt KJ** 1988 Mechanisms of secretory responses to gonadotropin-releasing hormone and phorbol esters in cultured pituitary cells. J Biol Chem 263: 17301–17306
257. **Kiley SC, Parker PJ, Fabbro D, Jaken S** 1992 Hormone- and phorbol ester-activated protein kinase C isozymes mediated a reorganization of the actin cytoskeleton associated with prolactin secretion in GH_4C_1 cells. Mol Endocrinol 6:120–131
258. **Willars GB, Royall JE, Nahorski SR, El-Gehani F, Everest H, McArdle CA** 2001 Rapid downregulation of the type I inositol 1,4,5-triphosphate receptor and desensitization of gonadotropin-releasing hormone-mediated Ca^{2+} responses in αT3–1 gonadotropes. J Biol Chem 276:3123–3129
259. **Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E** 1978 Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 202:631–633
260. **McArdle CA, Forrest-Owen W, Willars G, Davidson J, Poch A, Kratzmeier M** 1995 Desensitization of gonadotropin-releasing hormone action in the gonadotrope-derived αT3–1 cell line. Endocrinology 136:4864–4871
261. **Densmore VS, Urbanski HF** 2003 Relative effect of gonadotropin-releasing hormone (GnRH)-I and GnRH-II on gonadotropin release. J Clin Endocrinol Metab 88:2126–2134
262. **Okada Y, Murota-Kawano A, Kakar SS, Winters SJ** 2003 Evidence that gonadotropin-releasing hormone (GnRH) II stimulates luteinizing hormone and follicle-stimulating hormone secretion from monkey pituitary cultures by activating the GnRH I receptor. Biol Reprod 69:1356–1361
263. **Pellicer A, Miro F** 1990 Steroidogenesis in vitro of human granulosa-luteal cells pretreated in vivo with gonadotropin-releasing hormone analogs. Fertil Steril 54:590–596
264. **Guerrero HE, Stein P, Asch RH, de Fried EP, Tesone M** 1993 Effect of a gonadotropin-releasing hormone agonist on luteinizing hormone receptors and steroidogenesis in ovarian cells. Fertil Steril 59:803–808
265. **Gaetje R** 1994 Influence of gonadotropin releasing hormone (GnRH) and a GnRH-agonist on granulosa cell steroidogenesis. Clin Exp Obstet Gynecol 21:164–169
266. **Dor J, Bider D, Shulman A, Levron JL, Shine S, Mashiach S, Rabinovici J** 2000 Effects of gonadotropin-releasing hormone agonists on human ovarian steroid secretion in vivo and in vitro-results of a perspective, randomized in-vitro fertilization study. Hum Reprod 15:1225–1230
267. **Kang SK, Tai CJ, Cheng KW, Leung PC** 2000 Gonadotropin-releasing hormone activates mitogen-activated protein kinase in human ovarian and placental cells. Mol Cell Endocrinol 170:143–151
268. **Miller WR, Scott WN, Morris R, Fraser HM, Sharpe RM** 1985 Growth of human breast cancer cells inhibited by a luteinizing hormone-releasing hormone agonist. Nature 313:231–233
269. **Grundker C, Gunthert AR, Westphalen S, Emons G** 2002 Biology of the gonadotropin-releasing hormone system in gynecological cancers. Eur J Endocrinol 146:1–14
270. **Kang SK, Choi KC, Yang HS, Leung PC** 2003 Potential role of gonadotropin-releasing hormone (GnRH)-I and GnRH-II in the ovary and ovarian cancer. Endocr Relat Cancer 10:169–177
271. **Moretti RM, Marelli MM, van Groeninghen JC, Motta M, Limonta P** 2003 Inhibitory activity of luteinizing hormone-releasing hormone on tumor growth and progression. Endocr Relat Cancer 10:161–167
272. **Kimura A, Ohmichi M, Kurachi H, Ikegami H, Hayakawa J, Tasaka K, Kanda Y, Nishio Y, Jikihara H, Matsuura N, Murata Y** 1999 Role of mitogen-activated protein kinase/extracellular signal-regulated kinase cascade in gonadotropin-releasing hormone-induced growth inhibition of a human ovarian cancer cell line. Cancer Res 59:5133–5142
273. **Wells A, Souto JC, Solava J, Kassis J, Bailey KJ, Turner T** 2002 Luteinizing hormone-releasing hormone agonist limits DU-145 prostate cancer growth by attenuating epidermal growth factor signaling. Clin Cancer Res 8:1251–1257
274. **Kim JW, Lee YS, Kim BK, Park DC, Lee JM, Kim IK, Namkoong SE** 1999 Cell cycle arrest in endometrial carcinoma cells exposed to gonadotropin-releasing hormone analog. Gynecol Oncol 73:368–371
275. **Gunthert AR, Grundker C, Hollmann K, Emons G** 2002 Luteinizing hormone-releasing hormone induces JunD-DNA binding and extends cell cycle in human ovarian cancer cells. Biochem Biophys Res Commun 294:11–15
276. **Pfarr CM, Mechta F, Spyrou G, Lallemand D, Carillo S, Yaniv M** 1994 Mouse JunD negatively regulates fibroblast growth and antagonizes transformation by ras. Cell 76:747–760
277. **Imai A, Takagi H, Furui T, Horibe S, Fuseya T, Tamaya T** 1996 Evidence for coupling of phosphotyrosine phosphatase to gonadotropin-releasing hormone receptor in ovarian carcinoma membrane. Cancer 77:132–137
278. **Imai A, Horibe S, Takagi A, Tamaya T** 1997 Gi protein activation of gonadotropin-releasing hormone-mediated protein dephosphorylation in human endometrial carcinoma. Am J Obstet Gynecol 176:371–376
279. **Marelli MM, Moretti RM, Dondi D, Motta M, Limonta P** 1999 Luteinizing hormone-releasing hormone agonists interfere with the mitogenic activity of the insulin-like growth factor system in androgen-independent prostate cancer cells. Endocrinology 140: 329–334
280. **Huang YT, Hwang JJ, Lee LT, Liebow C, Lee PP, Ke FC, Lo TB, Schally AV, Lee MT** 2002 Inhibitory effects of a luteinizing hormone-releasing hormone agonist on basal and epidermal growth factor-induced cell proliferation and metastasis-associated properties in human epidermoid carcinoma A431 cells. Int J Cancer 99:505–513
281. **Emons G, Muller V, Ortmann O, Grossmann G, Trautner U, von Stuckrad B, Schulz KD, Schally AV** 1996 Luteinizing hormone-releasing hormone agonist triptorelin antagonizes signal transduction and mitogenic activity of epidermal growth factor in human ovarian and endometrial cancer cell lines. Int J Oncol 9:1129–1137
282. **Grundker C, Volker P, Schulz KD, Emons G** 2000 Luteinizing hormone-releasing hormone (LHRH) agonist triptorelin and antagonist cetrorelix inhibit EGF-induced *c-fos* expression in human gynecological cancers. Gynecol Oncol 78:194–202
283. **Moretti RM, Marelli MM, Dondi D, Poletti A, Martini L, Motta M, Limonta P** 1996 Luteinizing hormone-releasing hormone agonists interfere with the stimulatory actions of epidermal growth

factor in human prostatic cancer cell lines, LNCaP and DU 145. J Clin Endocrinol Metab 81:3930–3937
284. **Takagi H, Imai A, Furui T, Horibe S, Fuseya T, Tamaya T** 1995 Evidence for tight coupling of gonadotropin-releasing hormone receptors to phosphatidylinositol kinase in plasma membrane from ovarian carcinomas. Gynecol Oncol 58:110–115
285. **Imai A, Furui T, Tamaya T, Mills GB** 2000 A gonadotropin-releasing hormone-responsive phosphatase hydrolyses lysophosphatidic acid within the plasma membrane of ovarian cancer cells. J Clin Endocrinol Metab 85:3370–3375
286. **Nagai N, Oshita T, Mukai K, Shiroyama Y, Shigemasa K, Ohama K** 2002 GnRH agonist inhibits human telomerase reverse transcriptase mRNA expression in endometrial cancer cells. Int J Mol Med 10:593–597
287. **Sica G, Zelano G, Settesoldi D, Iacopino F** 2003 Regulation of prostate-specific antigen gene expression by an LH-RH analogue in human prostatic cells. Anticancer Res 22:1283–1287
288. **Di Lieto A, De Rosa G, De Falco M, Iannotti F, Staibano S, Pollio F, Scaramellino M, Salvatore G** 2002 Relationship between platelet-derived growth factor expression in leiomyomas and uterine volume changes after gonadotropin-releasing hormone agonist treatment. Hum Pathol 33:220–224
289. **Senturk LM, Sozen I, Gutierrez L, Arici A** 2001 Interleukin 8 production and interleukin 8 receptor expression in human myometrium and leiomyoma. Am J Obstet Gynecol 184:559–566
290. **Vu K, Greenspan DL, Wu TC, Zacur HA, Kurman RJ** 1998 Cellular proliferation, estrogen receptor, progesterone receptor, and bcl-2 expression in GnRH agonist-treated uterine leiomyomas. Hum Pathol 29:359–363
291. **Meresman GF, Bilotas M, Buquet RA, Baranao RI, Sueldo C, Tesone M** 2003 Gonadotropin-releasing hormone agonist induces apoptosis and reduces cell proliferation in eutopic endometrial cultures from women with endometriosis. Fertil Steril 80:702–707
292. **Iwabe T, Harada T, Tsudo T, Tanikawa M, Onohara Y, Terakawa N** 1998 Pathogenetic significance of increased levels of interleukin-8 in the peritoneal fluid of patients with endometriosis. Fertil Steril 69:924–930
293. **Iwabe T, Harada T, Tsudo T, Nagano Y, Yoshida S, Tanikawa M, Terakawa N** 2000 Tumor necrosis factor-α promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. J Clin Endocrinol Metab 85:824–829
294. **Volker P, Grundker C, Schmidt O, Schulz KD, Emons G** 2002 Expression of receptors for luteinizing hormone-releasing hormone in human ovarian and endometrial cancers: frequency, autoregulation, and correlation with direct antiproliferative activity of luteinizing hormone-releasing hormone analogues. Am J Obstet Gynecol 186:171–179
295. **Billig H, Furuta I, Hsueh AJ** 1994 Gonadotropin-releasing hormone directly induces apoptotic cell death in the rat ovary: biochemical and *in situ* detection of deoxyribonucleic acid fragmentation in granulosa cells. Endocrinology 134:245–252
296. **Yano T, Yano N, Matsumi H, Morita Y, Tsutsumi O, Schally AV, Taketani Y** 1997 Effect of luteinizing hormone-releasing hormone analogs on the rat ovarian follicle development. Horm Res 48:35–41
297. **Papadopoulos V, Dharmarajan AM, Li H, Culty M, Lemay M, Sridaran R** 1999 Mitochondrial peripheral-type benzodiazepine receptor expression. Correlation with gonadotropin-releasing hormone (GnRH) agonist-induced apoptosis in the corpus luteum. Biochem Pharmacol 58:1389–1393
298. **Imai A, Takagi A, Horibe S, Takagi H, Tamaya T** 1998 Evidence for tight coupling of gonadotropin-releasing hormone receptor to stimulated Fas ligand expression in reproductive tract tumors: possible mechanism for hormonal control of apoptotic cell death. J Clin Endocrinol Metab 83:427–431
299. **Imai A, Horibe S, Takagi A, Ohno T, Tamaya T** 1997 Frequent expression of Fas in gonadotropin-releasing hormone receptor-bearing tumors. Eur J Obstet Gynecol Reprod Biol 74:73–78
300. **Grundker C, Schulz K, Gunthert AR, Emons G** 2000 Luteinizing hormone-releasing hormone induces nuclear factor κB-activation and inhibits apoptosis in ovarian cancer cells. J Clin Endocrinol Metab 85:3815–3820
301. **Chu ZL, McKinsey TA, Liu L, Gentry JJ, Malim MH, Ballard DW** 1997 Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control. Proc Natl Acad Sci USA 94:10057–10062
302. **You M, Ku PT, Hrdlickova R, Bose Jr HR** 1997 ch-IAP1, a member of the inhibitor-of-apoptosis protein family, is a mediator of the antiapoptotic activity of the v-Rel oncoprotein. Mol Cell Biol 17: 7328–7341
303. **Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin Jr AS** 1998 NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680–1683
304. **Zong WX, Edelstein LC, Chen C, Bash J, Gelinas C** 1999 The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-κB that blocks TNFα-induced apoptosis. Genes Dev 13:382–387
305. **Kreuz S, Siegmund D, Scheurich P, Wajant H** 2001 NF-κB inducers upregulates cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. Mol Cell Biol 21:3964–3973
306. **Higashijima T, Kataoka A, Nishida T, Yakushiji M** 1996 Gonadotropin-releasing hormone agonist therapy induces apoptosis in uterine leiomyoma. Eur J Obstet Gynecol Reprod Biol 68:169–173
307. **Mizutani T, Sugihara A, Nakamuro K, Terada N** 1998 Suppression of cell proliferation and induction of apoptosis in uterine leiomyoma by gonadotropin-releasing hormone agonist (leuprolide acetate). J Clin Endocrinol Metab 83:1253–1255
308. **Wang Y, Matsuo H, Kurachi O, Maruo T** 2002 Down-regulation of proliferation and up-regulation of apoptosis by gonadotropin-releasing hormone agonist in cultured uterine leiomyoma cells. Eur J Endocrinol 146:447–456
309. **Huang SC, Chou CY, Lin YS, Tsai YC, Hsu KF, Liu CH, Huang KE** 1997 Enhanced deoxyribonucleic acid damage and repair but unchanged apoptosis in uterine leiomyomas treated with gonadotropin-releasing hormone agonist. Am J Obstet Gynecol 177:417–424
310. **Huang SC, Tang MJ, Hsu KF, Cheng YM, Chou CY** 2002 Fas and its ligand, caspases, and bcl-2 expression in gonadotropin-releasing hormone agonist-treated uterine leiomyoma. J Clin Endocrinol Metab 87:4580–4586
311. **Imai A, Takagi A, Tamaya T** 2000 Gonadotropin-releasing hormone analog repairs reduced endometrial cell apoptosis in endometriosis in vitro. Am J Obstet Gynecol 182:1142–1146
312. **Meresman GF, Bilotas MA, Lombardi E, Tesone M, Sueldo C, Baranao RI** 2003 Effect of GnRH analogues on apoptosis and release of interleukin-1β and vascular endothelial growth factor in endometrial cell cultures from patients with endometriosis. Hum Reprod 18:1767–1771
313. **Tabibzadeh S, Babaknia A** 1995 The signals and molecular pathways involved in implantation, a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. Hum Reprod 10:1579–1602
314. **Paria BC, Reese J, Das SK, Dey SK** 2002 Deciphering the cross-talk of implantation: advances and challenges. Science 296:2185–2188
315. **Enomoto M, Mori T, Park MK** 2001 GnRH agonist Buserelin affects colony-forming efficiency of HHUA and Jurkat cells. Biochem Biophys Res Commun 289:1180–1187
316. **Lee CY, Ho J, Chow SN, Yasojima K, Schwab C, McGeer PL** 2000 Immunoidentification of gonadotropin releasing hormone receptor in human sperm, pituitary and cancer cells. Am J Reprod Immunol 44:170–177
317. **Morales P, Pizarro E, Kong M, Kerr B, Ceric F, Vigil P** 2000 Gonadotropin-releasing hormone-stimulated sperm binding to the human zona is mediated by a calcium influx. Biol Reprod 63:635–642
318. **Romanelli RG, Barni T, Maggi M, Luconi M, Failli P, Pezzatini A, Pelo E, Torricelli F, Crescioli C, Ferruzzi P, Salerno R, Marini M, Rotella CM, Vannelli GB** 2004 Expression and function of gonadotropin-releasing hormone (GnRH) receptor in human olfactory GnRH-secreting neurons. An autocrine GnRH loop underlies neuronal migration. J Biol Chem 279:117–126
319. **Millar RP, Lowe S, Conklin D, Pawson A, Maudsley S, Troskie B, Ott T, Millar M, Lincoln G, Sellar R, Faurholm B, Graeme S, Kuestner R, Teresawa E, Katz A** 2001 A novel mammalian receptor for the evolutionarily conserved type II GnRH. Proc Natl Acad Sci USA 98:9636–9641
320. **Neill JD, Duck LW, Sellers JC, Musgrove LC** 2001 A gonado-

tropin-releasing hormone (GnRH) receptor specific for GnRH II in primates. Biochem Biophys Res Commun 282:1012–1018
321. **Tensen C, Okuzawa K, Blomenrohr M, Rebers F, Leurs R, Bogerd J, Schulz R, Goos H** 1997 Distinct efficacies for two endogenous ligands on a single cognate gonadoliberin receptor. Eur J Endocrinol 243:134–140
322. **Troskie BE, Illing N, Rumbak E, Sun YM, Sealfon SC, Conklin D, Millar RP** 1998 Identification of three putative GnRH receptor subtypes in vertebrates. Gen Comp Endocrinol 112:296–302
323. **Illing N, Troskie BE, Nahorniak CS, Hapgood JP, Peter RE, Millar RP** 1999 Two gonadotropin-releasing hormone receptor subtypes with distinct ligand selectivity and differential distribution in brain and pituitary in the goldfish (*Carassius auratus*). Proc Natl Acad Sci USA 96:2526–2531
324. **Troskie BE, Hapgood JP, Millar RP, Illing N** 2000 Complementary deoxyribonucleic acid cloning, gene expression, and ligand selectivity of a novel gonadotropin-releasing hormone receptor expressed in the pituitary and midbrain of *Xenopus laevis*. Endocrinology 141:1764–1771
325. **Okubo K, Nagata S, Ko R, Kataoka H, Yoshiura Y, Mitani H, Kondo M, Naruse K, Shima A, Aida K** 2001 Identification and characterization of two distinct GnRH receptor subtypes in a teleost, the medaka *Oryzias latipes*. Endocrinology 142:4729–4739
326. **Wang L, Bogerd J, Choi HS, Seong JY, Soh JM, Chun SY, Blomenrohr M, Troskie BE, Millar RP, Yu MH, McCann SM, Kwon HB** 2001 Three distinct types of GnRH receptor characterized in the bullfrog. Proc Natl Acad Sci USA 95:361–366
327. **King JA, Davidson JS, Millar RP** 1986 Desensitization to gonadotropin-releasing hormone in perifused chicken anterior pituitary cells. Endocrinology 119:1510–1518
328. **Hislop JN, Madziva MT, Everest HM, Harding T, Uney JB, Willars GB, Millar RP, Troskie BE, Davidson JS, McArdle CA** 2000 Desensitization and internalization of human and *Xenopus* gonadotropin-releasing hormone receptors expressed in αT4 pituitary cells using recombinant adenovirus. Endocrinology 141:4564–4575
329. **Neill JD** 2002 GnRH and GnRH receptor genes in the human genome. Endocrinology 143:737–743
330. **Pawson AJ, Maudsley SR, Lopes J, Katz AA, Sun YM, Davidson JS, Millar RP** 2003 Multiple determinants for rapid agonist-induced internalization of a nonmammalian gonadotropin-releasing hormone receptor: a putative palmitoylation site and threonine doublet within the carboxyl-terminal tail are critical. Endocrinology 144:3860–3871
331. **Lin X, Janovick JA, Brothers S, Blomenrohr M, Bogerd J, Conn PM** 1998 Addition of catfish gonadotropin-releasing hormone (GnRH) receptor intracellular carboxyl-terminal tail to rat GnRH receptor alters receptor expression and regulation. Mol Endocrinol 12:161–171
332. **Blomenrohr M, Heding A, Sellar R, Leurs R, Bogerd J, Eidne KA, Willars GB** 1999 Pivotal role for the cytoplasmic carboxyl-terminal tail of a nonmammalian gonadotropin-releasing hormone receptor in cell surface expression, ligand binding, and receptor phosphorylation and internalization. Mol Pharmacol 56:12229–12237
333. **Flanagan CA, Zhou W, Chi L, Yuen T, Rodic V, Robertson D, Johnson M, Holland P, Millar RP, Weinstein H, Mitchell R, Sealfon SC** 1999 The functional microdomain in transmembrane helices 2 and 7 regulates expression, activation, and coupling pathways of the gonadotropin-releasing hormone receptor. J Biol Chem 274:28880–28886
334. **Flanagan CA, Becker II, Davidson JS, Wakefield IK, Zhou W, Sealfon SC, Millar RP** 1994 Glutamate 301 of the mouse gonadotropin-releasing hormone receptor confers specificity for arginine 8 of mammalian gonadotropin-releasing hormone. J Biol Chem 269:22636–22641
335. **Yano T, Pinski J, Radulovic S, Schally AV** 1994 Inhibition of human epithelial ovarian cancer cell growth in vitro by agonistic and antagonistic analogues of luteinizing hormone-releasing hormone. Proc Natl Acad Sci USA 91:1701–1704
336. **Tang X, Yano T, Osuga Y, Matsumi H, Yano N, Xu J, Wada O, Koga K, Kugu K, Tsutsumi O, Schally AV, Taketani Y** 2002 Cellular mechanisms of growth inhibition of human epithelial ovarian cancer cell line by LH-releasing hormone antagonist Cetrorelix. J Clin Endocrinol Metab 87:3721–3727
337. **Millar R, Conklin D, Lofton-Day C, Hutchinson E, Troskie B, Illing N, Sealfon SC, Hapgood J** 1999 A novel human GnRH receptor homolog gene: abundant and wide tissue distribution of the antisense transcript. J Endocrinol 162:117–126
338. **Morgan K, Conklin D, Pawson AJ, Sellar R, Ott TR, Millar RP** 2003 A transcriptionally active human type II gonadotropin-releasing hormone receptor gene homolog overlaps two genes in the antisense orientation on chromosome 1q. 12. Endocrinology 144:423–436
339. **Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan K, Maudsley S** Gonadotropin-releasing hormone receptors. Endocr Rev 25:235–275.
340. **Pawson AJ, Morgan K, Maudsley SR, Millar RP** 2003 Type II gonadotropin-releasing hormone (GnRH-II) in reproductive biology. Reproduction 126:271–278
341. **Gault PM, Morgan K, Pawson AJ, Millar RP, Lincoln GA** 2004 Sheep exhibit novel variations in the organization of the mammalian type II gonadotropin-releasing hormone receptor gene. Endocrinology 145:2362–2374
342. **Faurholm B, Millar RP, Katz AA** 2001 The genes encoding the type II gonadotropin-releasing hormone receptor and the ribonucleoprotein RBM8A in humans overlap in two genomic loci. Genomics 78:15–18
343. **Salicioni AM, Xi M, Vanderveer LA, Balsara B, Testa JR, Dunbrack Jr RL, Godwin AK** 2000 Identification and structural analysis of human RBM8A and RBM8B: two highly conserved RNA-binding motif proteins that interact with OVCA1, a candidate tumor suppressor. Genomics 69:54–62
344. **van Biljon W, Wykes S, Scherer S, Krawetz SA, Hapgood J** 2002 Type II gonadotropin-releasing hormone receptor transcripts in human sperm. Biol Reprod 67:1741–1749
345. **Ling K, Wang P, Zhao J, Wu YL, Cheng ZJ, Ubi GX, Hu W, Ma L, Pei G** 1999 Five-transmembrane domains appear sufficient for a G protein-coupled receptor: functional five-transmembrane domain chemokine receptors. Proc Natl Acad Sci USA 96:7922–7927
346. **Gouldson PR, Snell CR, Bywater RP, Higgs C, Reynolds CA** 1998 Domain swapping in G-coupled receptor dimers. Protein Eng 11: 1181–1193
347. **Schultz A, Groose R, Schultz G, Gudermann T, Schoneberg T** 2000 Structural implication for receptor oligomerization from functional reconstitution studies of mutant V2 vasopressin receptors. J Biol Chem 275:2381–2389
348. **Behne D, Kyriakopoulos A** 2001 Mammalian selenium-containing proteins. Annu Rev Nutr 21:453–473
349. **Copeland PR** 2003 Regulation of gene expression by stop codon recoding: selenocysteine. Gene 312:17–25
350. **Wen W, Weiss SL, Sunde RA** 1998 UGA codon position affects the efficiency of selenocysteine incorporation into glutathione peroxidase-1. J Biol Chem 273:28533–28541
351. **Farshori PQ, Shah BH, Arora KK, Martinez-Fuentes A, Catt KJ** 2003 Activation and nuclear translocation of PKCδ, Pyk2 and ERK1/2 by gonadotropin-releasing hormone in HEK293 cells. J Steroid Biochem Mol Biol 85:337–347
352. **Davidson L, Pawson AJ, Millar RP, Maudsley S** 2004 Cytoskeleton reorganization dependence of signaling by the gonadotropin-releasing hormone receptor. J Biol Chem 279:1980–1993
353. **Davidson L, Pawson AJ, De Maturana RL, Freestone SH, Barran P, Millar RP, Maudsley S** 2004 Gonadotropin-releasing hormone-induced activation of diacylglycerol kinase-ζ and its association with active c-src. J Biol Chem 279:11906–11916

Endocrine Reviews **is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**