Functional maturation of growth hormone cells in the anterior pituitary gland of the fetus

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Abstract

Recent studies have disclosed the molecular mechanisms responsible for the phenotype determination of the anterior pituitary cell types. However, as far as growth hormone (GH) cells are concerned, particular extra-cellular cues are required for the initiation of GH and GH-releasing hormone (GHRH)-receptor gene production in addition to the expression of the cell type specific transcription factor, pit-1. The glucocorticoids play a principal role in the functional maturation of nascent GH cells in the fetal pituitary glands in rodents, inducing GH and GHRH-receptor gene expression, and establish the GH secretory system regulated by the brain in late gestation. Research supporting this role for glucocorticoid in the development of GH cells is discussed.

Key words: Growth hormone; growth hormone-releasing hormone receptor; fetus; glucocorticoids, pituitary gland

1. Introduction

Growth hormone (GH) is secreted from the anterior pituitary gland, and plays principal roles not only in longitudinal bone growth at the pubertal growth spurt, but also in the regulation of carbohydrate and lipid

metabolism throughout life. While GH is produced in the extra-pituitary tissues including the immune system [1] and central nervous systems [2,3], the serum GH level is regulated by the anterior pituitary gland, and a deficiency in pituitary GH results in severe growth retardation [4]. GH-producing cells in the anterior pituitary gland develop on embryonic day 19 (E19) in rats [5-7], and accordingly, GH secretion starts before birth [8]. The expression of the GH-releasing hormone (GHRH)-receptor, another GH cell-specific gene, also occurs on E19 [9,10]. These data suggest that the functional maturation of GH cells in rats is achieved by E19. On the other hand, the onset of pit-1 [11] (also termed GHF-1 [12], or PUF-1 [13]), a pituitary specific transcription factor that is a prerequisite for GH gene transcription, occurs on E15 [14]. Thus, several days are required for the functional maturation of nascent GH cells after the onset of pit-1 expression. Little is known about the changes in the cellular function that occur in GH cells during these 4 days. We will discuss here the ontogeny of GH cells in the fetal pituitary gland, the factors that trigger the activation of GH cell-specific gene expression, such as the GH and GHRH-receptor genes in nascent GH cells.

2. Differentiation of the anterior pituitary cells

The mechanisms responsible for the phenotype determination of the anterior pituitary cells have been proposed by Rosenfeld and his co-workers [15,16]. Organogenesis of the pituitary gland starts with the

invagination of oral ectoderm to form Rathke's pouch. The cells that compose Rathke's pouch are the progenitors of anterior pituitary cells. The dorsoventral gradient of the concentration of FGF 8 produced by the ventral diencephalon and the opposing gradient of BMP2 emanates from the ventral pituitary organizing center results in the overlapping expression patterns of specific transcription factors, the dorsal pit-1 and the ventral GATA2. Pit-1-expressing cells differentiate into GH cells, some of which are believed to differentiate into prolactin (PRL) cells. The mechanisms for PRL cell differentiation have not yet been elucidated. The cells expressing GATA2 differentiate into gonadotropic hormone (GTH) cells that secrete both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The cells expressing both GATA2 and pit-1 differentiate into thyroid-stimulating hormone (TSH) cells. Adrenocorticotropic hormone (ACTH) cells belong to neither of the cell lineages that express pit-1 or GATA2, and are considered to be specified by the expression of T-pit [17,18].

3. Ontogeny of GH cells in embryonic pituitary gland

Early immunohistochemical studies [6,7] have revealed that the ACTH cells are those that appear first in the developing anterior pituitary gland in the rat fetus. ACTH cells are detected first on embryonic day 15 (E15) in rats, followed by TSH cells on E16. The LH cells appear on E17 and FSH cells develop on E19. PRL cells have been reported to appear only after birth. A small number of GH cells were detectable in the

anterior pituitary gland as early as E18 in rats, and they had increased remarkably in number by E19 (Fig. 1). The GH cell number showed a moderate increase by E20 but showed no marked increase thereafter. The age at which each pituitary cell type is first detectable differs to a certain extent among investigations, probably due to the sensitivity of the immunohistochemistry utilized [19,20]. However, the sequential order of the differentiation of pituitary cells is consistent. Using in situ hybridization, The GH mRNA has not been detected on E18 or earlier in rats, but a number of GH mRNA positive cells have been detected on E19 [21] (Fig. 1). By reverse hemolytic plaque assay, Frawley et al. [22] revealed that cells that secrete GH comprised less than 1% of pituitary cells at E18, but increased to 13.6% and 22.4% in cultures of E19 and E20, respectively. GH is detectable in the fetal circulation by radioimmunoassay as early as E19 [8].

In mice, GH cells are scarce until E15, increasing rapidly on E16 [23]. The number of GH cells increases sharply between E14 and E16 in chicken pituitary gland [24,25]. Thus, the rapid increase in the number of GH cells in the developing pituitary gland is seen in several species and occurs during the 24 h between E18 and E19 in rats (Fig. 1). Though it is hardly likely that such a rapid increase in GH cell number is brought about by the mitotic division of preexisting GH cells, it is highly conceivable that a number of premature GH cells are present in the fetal pituitary on E18 in rats, and they started in concert the production of GH in response to an extra-cellular signal.

4. Hormonal factors regulating GH cell development

Several reports have pointed out that autonomous GH cell development did not occur in a fetal pituitary explant taken before the onset of GH production [26-28], suggesting that the initiation of GH production in the fetal pituitary gland requires an extra-pituitary cue. Hemming et al. [26] first described that GH cells differentiate in the pituitary primordia of rats taken on E14 and kept for 5 days in serum-free medium containing cortisol. The GH cells did not appear when cultured in a medium without glucocorticoids, and the addition of a high dose of insulin suppressed their differentiation. The effect of cortisol was dose-dependent. Higher dose (250 nM) of cortisol showed a maximal effect, while 50 nM was ineffective. Though thyroid hormone has been known as a potent stimulator of GH-production and gene transcription in GH producing cell lines, such as GH3 cells [29,30], it failed to induce GH cells in the pituitary primordium in culture when used alone [27,31,32]. However, thyroid hormone did stimulate GH cell induction when used in conjunction with a low dose of glucocorticoids [27,32]. These results suggest that the principal inducer of GH production in the embryonic rat pituitary explant is the glucocorticoids.

We examined the effects of glucocorticoids on the induction of GH cells in the fetal pituitary gland of rats in vivo, and found that a single injection of synthetic glucocorticoid, dexamethasone (DEX), to a dam on the morning of day 17 of gestation induced a number of GH cells in the E18 pituitary gland, in which few GH cells are normally encountered [33]. This effect of DEX was not evident in the earlier stages, suggesting that the expression of glucocorticoid receptor would not take place in GH cells until E18.

Expression of glucocorticoid receptor mRNA in the anterior pituitary gland was noted as early as E15 in rats [34]. At E17, when no GH-producing cells have yet developed, the cells expressing glucocorticoid receptor were demonstrated by immunohistochemistry to be mostly ACTH cells [31]. Although mature GH cells express glucocorticoid receptors [35], their precursors do not produce the receptors at this stage. The number of cells positive for glucocorticoid receptor, but not for ACTH, increased on E18. Many GH cells appeared at E19, most of which being positive for glucocorticoid receptor. These data suggest that the expression of the glucocorticoid receptor is one of the important changes in immature GH cells that must be achieved before the onset of GH production.

The effect of glucocorticoids on the induction of GH gene expression was also observed in the embryonic chick pituitary gland. Porter et al. [24] demonstrated the presence of a factor which is able to induce the premature induction of GH in the embryonic pituitary gland. That factor was undetectable in the blood of E12 when no GH cells had differentiated, but had increased to relatively high levels by E16 when numerous GH cells are detectable in the pituitary gland. They identified this blood born factor to be corticosterone [36].

A synergistic effect between DEX and thyroid hormone was observed upon the induction of GH cells not only in vitro but also in vivo [28]. Again, thyroid hormone showed little effect when used alone, being in agreement with the results obtained in vitro [26]. However, the reduction of the thyroid hormone level in the fetal circulation by methimazole, an inhibitor of thyroid hormone biosynthesis, resulted in reduced GH

mRNA levels in the fetus on E19 and E21. The replacement of thyroxine to methimazole-treated rats restored GH mRNA in the fetus, suggesting that a rapid increase in the GH production rate in the fetus in late gestation was achieved by the synergy between glucocorticoids and thyroid hormone [28].

GH-releasing hormone (GHRH) is secreted from the hypothalamic arcuate nucleus, and stimulates the release of GH, GH gene transcription, and the proliferation of GH cells [37]. A defect in the functional receptor for GHRH in the pituitary GH cells results in a severe reduction of GH cells, which leads to dwarfism [38,39]. The effects of GHRH on GH gene expression in the embryonic pituitary gland have been studied in vitro, but no involvement of this hormone in the initiation of GH production has yet been observed. However, GHRH enhanced the size of GH cells and of GH release from the explant [26]. Therefore, it is conceivable that GHRH might stimulate secretory activity and the proliferation of functionally mature GH cells, while having little effect on the induction of GH production in immature GH cells. Similar results have been observed in cultures of chicken embryonic pituitary cells [40]

5. Other factors in GH cell development

Several important findings on the mechanisms of GH cell development have been reported using pituitary cell lines. GHF-T1 cells [41]are established from a pituitary tumor grown in a transgenic mouse that was generated by the targeted expression of SV40 T-antigen in the progenitors for GH and PRL cells

using the regulatory region of GHF-1 (pit-1) gene. These cells express GHF-1 but produce neither GH nor PRL, and are considered to be the precursors of GH and PRL cells. Schaufele et al. [42] successfully induced GH production in these cells by a transient expression of CCAAT-enhancer binding protein (C/EBP)α. A specific binding site for C/EBPα has been identified in the proximal region of the rat GH gene promoter. This study demonstrates that the expression of pit-1 is not sufficient for the initiation of GH production, and that the onset of the C/EBPα expression is an important developmental step occurring between pit-1 expression and functional maturation of GH cells.

From an estrogen-induced mammotrophic tumor of the pituitary gland, Inoue et al. [43] established several cell lines including MtT/E cells which are considered to be immature GH cells. The MtT/E cells express pit-1, but GH expression is not fully activated. Mogi et al. [44] demonstrated that retinoic acid induces the differentiation of these cells to GH-producing cells, suggesting that retinoic acid plays a role in the differentiation of GH cells. Retinoic acid is produced by the enzymatic oxidation of retinal. Recently, Fujiwara et al. [45] observed the increased expression of retinalaldehyde dehydrogenase 2 and 3 in the fetal pituitary gland on E15-E17, which falls within the developmental period of fetal GH cells between pit-1 expression and GH production. It is conceivable that the elevation of local retinoid acid concentration due to the increase in these enzymes may be implicated in the maturation of GH cells. Previous studies have revealed the transcriptional activation of rat GH gene by retinoic acid in clonal pituitary cell lines [46,47]. However, the effect of retinoic acid on GH cell development in vivo has not yet

been examined.

6. Mechanisms for inducing GH expression by glucocorticoids

As described above, several lines of evidence indicate that glucocorticoid triggers the functional maturation of GH cells and the onset of GH production in the fetal rat pituitary gland. The mechanisms behind the hormonal regulation of GH gene expression have been examined extensively using clonal GH cell lines such as GC cells [30] and MtT/S cells [47]. Although it is well established that glucocorticoids and thyroid hormone act synergistically to increase GH mRNA levels in GH cells, only thyroid hormone has been demonstrated to enhance the transcription of the GH gene. In most reports, the transcriptional effect of glucocorticoid was described as being very small or undetectable [30,47,48,49]. Therefore, it is considered that glucocorticoids stimulate GH mRNA expression by stabilizing the mRNA [49]. In the fetal rat pituitary gland, DEX induces GH mRNA expression both in vivo [33] and in vitro [28,31], at a stage in which normally GH mRNA is very low or undetectable. These data strongly suggest that glucocorticoids stimulate the transcription of GH gene at least at a particular stage of GH cell development, a finding inconsistent with the results obtained in the GH cell lines.

Further examination revealed that the effect of glucocorticoids on GH gene transcription in the fetal pituitary appears to be indirect, and is mediated by a certain protein produced de novo in response to

glucocorticoid stimulation, because glucocorticoid action on GH expression in the rat fetus requires ongoing protein synthesis [31] (Fig. 2). A similar result was observed in the GH3 cells [50] and the embryonic chicken pituitary gland [51]. The ligand-bound glucocorticoid receptor exerts its effects in 2 distinct ways [52]. One is characterized by the interaction of GR with other classes of transcription factors (such as AP-1 proteins) in the absence of specific DNA binding, and the other is based on the GR interaction with specific DNA elements (glucocorticoid response elements, GREs) of the promoter of the target gene [52]. The mode of glucocorticoid action on the GH gene seems to be of the later kind, but the target of the glucocorticoid receptor may not be the GH gene. The nature of the GR target gene remains to be elucidated. It is also obscure whether this gene is expressed in the GH cells or in other pituitary cells. The effect of glucocorticoid in the anterior pituitary gland may be mediated in part by annexin-1, a member of the annexin superfamily of Ca2+- or phospholipid binding proteins [53]. Annexin-1 is synthesized in the pituitary folliculo-stellate cells in response to glucocorticoids, and acts on GH, PRL and ACTH cells to modulate their hormone secretion [54-56]. It is possible that annexin-1 mediates glucocorticoid action in the fetal pituitary gland for the induction of GH production.

7. Ontogeny and regulation of GH-releasing hormone receptor (GHRH-receptor) gene expression

During the functional maturation of fetal GH cells, the onset of the expression of specific receptors for

GHRH is an important step, since that establishes pulsatile GH secretion under the functional correlation of the brain and GH cells in the anterior pituitary gland. Interestingly, GHRH-receptor mRNA expression occurs first at the same embryonic stage (E19) as that of the onset of GH gene expression [9,10], and increases with age during gestation. The expression level of the mRNA is developmentally regulated, being higher in the prenatal pituitary gland [9,57], and declining thereafter. The expression of GHRH-receptor gene is also induced by glucocorticoids in the fetal pituitary gland [10]. A number of humoral factors are reported to up-regulate GHRH-receptor mRNA expression in the pituitary gland of adult rats, such as thyroid hormone [58-61], glucocorticoids [10,62-64] and estrogens [62]. GHRH is also implicated in the regulation of its own receptor, and is reported to be either a stimulator [65,66] or a suppressor of GHRH-receptor mRNA expression [67]. The inconsistency of the previous results appears to be due to differences in experimental design, including the duration of treatment and the developmental stage of the animal [68]. The effects of GH on the GHRH-receptor expression have been reported to be either positive [69] or negative [65,70]. Insulin-like growth factor-I (IGF-I) suppresses GHRH-receptor expression in pituitary cell cultures [61,71].

Among these substances, only glucocorticoids have been shown to induce GHRH-receptor mRNA alone in the E18 pituitary gland, in which no GHRH-receptor mRNA is normally detectable [10,72]. Thyroid hormones and retinoic acid acted synergistically with glucocorticoid in the induction of the GHRH-receptor mRNA, whereas each of them had little effect when used alone [72]. The effect of

glucocorticoids and their synergy with thyroid hormones and retinoic acid were observed also in MtT/S cells that express the receptor. GHRH, estradiol, forskolin and cyclic AMP were all ineffective in in inducing GHRH-receptor gene expression in MtT/S cells [72]. It remains to be elucidated whether these factors are implicated in the induction of GHRH-receptor gene activation in the fetal rat pituitary gland in vivo. Although glucocorticoids induce both GH and GHRH-receptor gene expression in the fetal pituitary gland in rats on the same day of gestation (E19), the mechanisms of those inductions differ from each other significantly. The induction of GHRH-receptor mRNA occurs rapidly and is insensitive to a protein synthesis inhibitor [10] (Fig. 2), suggesting that, unlike GH gene activation, the ligand-bound glucocorticoid receptor directly stimulates GHRH-receptor gene transcription.

8. Molecular mechanism of glucocorticoid-induction of GHRH-receptor

The GHRH-receptor is a member of the family of the G-protein-coupled receptors with 7 transmembrane domains [38,73]. The promoter activity of the mouse GHRH-receptor increases markedly under the presence of pit-1 [39], and the receptor gene is not expressed in the dwarf mouse that does not express pit-1 [38]. Thus, similarly to GH, the transcription of the GHRH-receptor gene also depends on pit-1. The requirement of pit-1 for transcription makes the expression of this gene pituitary-specific. The cloning and analysis of the promoter region of the GHRH-receptor gene has been described in humans

[74,75], rats [76-78] (Fig. 3), and chickens [79]. High affinity binding sites for pit-1 (P1 and P2) were identified in the human GHRH-receptor gene by Iguchi et al. [75], and the mutation of these binding sites has resulted in the decreased transcription of GHRH-receptor [75,80]. In the rat, a pit-1 binding site was identified in the proximal region of the gene, and its sequence was almost identical to that of the P2 site found in the human gene [77]. The mutation of the pit-1 binding site in the rat GHRH-receptor gene reduced both basal and glucocorticoid stimulated gene transcriptions. A recent study by Mayo and his co-workers identified additional pit-1 binding sites in the 1.6kbp promoter region of the gene with a novel high-affinity pit-1 binding site located approximately 1kbp upstream of the proximal pit-1 site [78].

In the 5'-flanking sequences of the proximal pit-1 site, a composite hormone response element was found (Fig. 3) composed of two functional glucocorticoid response elements (GREs) and a thyroid hormone response element (TRE) [77]. These two GREs (proximal and distal GREs) and pit-1 site are 14bps equidistant from each other. The upstream hexamer motif of the distal GRE is also a downstream hexamer motif of the TRE. A response element for retinoic acid was found about 1.1kb upstream of the proximal pit-1 site. Two GREs were non-consensus but were able to bind glucocorticoid receptors, and a. mutation in either one resulted in a severe reduction in the promoter activity in a transient transfection study, suggesting that both elements contribute to the glucocorticoid-induced stimulation of gene transcription. A further study revealed that the activity of these GREs is very weak and neither one alone can increase promoter activity. However, if these GREs are combined, they exert stronger GRE activity. The combined

GRE mediates glucocorticoid action on GHRH-gene expression in the presence of pit-1 [81]. The molecular mechanisms of the pit-1-dependent and glucocorticoid-induced GHRH-receptor gene transcription have yet to be elucidated.

8. Endocrine interplay for development of GH cells

Since glucocorticoids pass through the placental barrier, the circulating glucocorticoid level in the fetus is considered to be in equilibrium with that of the maternal compartment. In fact, glucocorticoids are detectable in fetal circulation before the onset of glucocorticoid production in the fetal adrenal gland. As noted above, the differentiation of ACTH cells in the anterior pituitary gland and the secretion of ACTH occur earlier than that of GH in rats as well as in mice [82] and humans [83]. ACTH plays a pivotal role in maturation of the steroid genesis of the adrenal cortex [84]. In the rat fetus, the serum ACTH level increases in late gestation [85], and this change is considered to induce an increased corticosterone secretion from the fetal adrenal cortex with a peak on E19 [85-87]. The increased glucocorticoid, in turn, induces the functional maturation of GH cells in the pituitary gland. Thus, the functional maturation of the pituitary GH cells is brought about by maturation of the pituitary-adrenal axis. Experimental support for this concept has recently been provided by Jenkins et al. [88] who revealed that the administration of ACTH to chick embryos induces an increase in the circulating glucocorticoid levels and pituitary GH cells.

Furthermore, Boudouresque et al. [85] demonstrated that the passive immunization of pregnant rats with corticotropin-releasing hormone (CRH) results in a decrease in ACTH secretion from the fetal pituitary gland, demonstrating in vivo that ACTH secretion is under the regulation of CRH even during the fetal period. Based on these findings, we propose that the interplay of multiple endocrine organs, such as hypothalamus, pituitary gland, and adrenal gland, promotes the functional maturation of pituitary GH cells in fetal rats (Fig. 4), i.e., the development of a hypothalamic CRH neuron system stimulates pituitary ACTH cells which have developed by that time to enhance ACTH secretion. The ACTH stimulates maturation of the adrenal cortex to induce corticosterone secretion. The resultant increased corticosterone in turn induces the functional maturation of the pituitary GH cells. The development of pituitary PRL cells are, on the contrary, suppressed by glucocorticoids, and only after birth does the expansion of the population of PRL cells occur [89,90].

In most species studied, there is a distinct increase in the fetal plasma glucocorticoid concentration due to maturation of the hypothalamic-pituitary-adrenal (HPA) axis in late gestation. Glucocorticoids induce the functional maturation of a number of fetal organs such as the liver, kidney and lung in preparation for a transition from intra-uterine to extra-uterine life [91,92]. The maturation of GH cells may be one of those glucocorticoid activities.

9. Perspectives for future studies

The mechanisms underlying the glucocorticoid induction of GH gene transcription in the fetal pituitary gland are largely unknown, including the factor that mediates glucocorticoid action in this context. Identification of this factor is indispensable for our understanding of the developmental process of fetal GH cells. Another important issue is the development of the responsiveness of fetal GH cells to glucocorticoids. Currently, we hypothesize that the increased glucocorticoid availability on E18-19 induces GH cells in the E19 pituitary gland in rats, and that this induction is reproducible in vitro by incubating E18 pituitary gland for 24 h in a medium containing glucocorticoids as the sole hormonal factor, indicating that GH cells had already acquired glucocorticoid sensitivity by E18. However, the advanced GH cell induction in E18 pituitaries in vivo by DEX-treatment on day 17 of gestation was not reproducible in vitro; i.e., the incubation of E17 pituitaries with DEX in vitro induced only a few GH cells. These results suggest that the development of glucocorticoid responsiveness occurs between E17 and E18 in vivo but not in vitro serum-free culture condition. It is likely that the acquisition of the responsiveness to glucocorticoids reflects the production of functional glucocorticoid receptors, and that the factors that promote this process are available only in vivo. It is of particular importance to determine the mechanisms underlying the induction of glucocorticoid receptors in GH cells to enhance our understanding of the developmental processes of GH cells.

Not only GH cells in the anterior pituitary gland, but also hypothalamic GHRH and somatostatin

neurons develop during late gestation in rats. Furthermore, the specific receptors for GH are already expressed in the fetal period in a wide variety of tissues [93], suggesting that GH is required for the normal growth or metabolism of fetuses. What are the physiological roles of fetal GH? Our previous study revealed that the GH is implicated in the normal body growth of rat fetuses [94], but that the dependency of body growth on GH during fetal period was much less than that for postnatal growth. Further studies are required for better understanding the role of GH in fetal development.

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Lrgend for the figures

Fig. 1. Immunocytochemical staining for GH (A and C) and in situ hybridization for GH mRNA (B and D) of fetal rat pituitaries on E18 (A and B) or E19 (C and D). Only a few GH or GH mRNA positive cells are encountered in the E18 pituitary gland, but they rapidly increase in number by E19.

Fig. 2. Effects of protein synthesis inhibitor on glucocorticoid induction of mRNA for GH and GHRH-receptor.

Pituitary glands from E18 fetuses were incubated in serum-free medium containing dexamethasone (DEX, 50 nM) and/or puromycin (PM, 1μM)) as indicated. Total RNA was prepared from explants, and the GH (A) or GHRH-receptor (B) mRNA was determined by RNase protection assay. Probe (A, lanes 1 and 6, B, lane 1) represents undigested cRNA probe. Yeast tRNA (A, lane 13) was used as negative control.

A. Incubation of E18 pituitaries for 24 h in a medium containing DEX resulted in expression of GH mRNA in explants (lanes 2 and 3). In the experiment illustrated in the right panel, GH mRNA induction was observed after treatment of E18 pituitaries with DEX for 8 h followed by a subsequent 16 h chase incubation in DEX-free medium (lanes 7 and 8). When pituitaries were incubated with DEX and PM, GH mRNA induction was not observed (lanes 9 and 10), suggesting that induction of GH mRNA by DEX requires on-going protein synthesis. GH mRNA accumulation was observed after an 8 h DEX/PM treatment followed by an 16 h chase incubation with DEX, indicating that inhibition of GH mRNA induction by PM is not a result of cytotoxicity of PM. B. DEX-treatment of E18 pituitaries for 8 h induced GHRH-receptor mRNA (lane 3). No GHRH-receptor mRNA was detected in explants incubated without DEX (lane 2) or with PM (lane 4). Unlike GH mRNA induction, PM did not inhibit GHRH-receptor mRNA induction by DEX.

Fig. 3. Binding sites for transcription factors in the rat GH and GHRH-receptor promoters.

A 250bp region of rat GH promoter contains binding sites for most important transcription factors. Thyroid

hormone response element (TRE) of GH promoter also function as a retinoic acid response element (RARE) [46]. Distances from the transcription start site are indicated in GH promoter.

Because different transcription start sites were reported [76,77], distances from the translation initiation codon are indicated in the figure showing the GHRH-receptor promoter (bottom), We previously identified a single major transcription start site at -105 (b) [77], while Miller et al. [76] found four start sites within 286bp upstream of the translation initiation codon. One of those start sites located at most upstream position is shown in this figure (a). A high-affinity pit-1 binding site located at -150bp (pit-1) was identified and revealed to be required for glucocorticoid regulation of transcription of this gene [77]. A recent study by McElvaine et al. [78] found another high-affinity binding site at the distal portion of the promoter (pit-1*). They found 8 additional weak pit-1 binding sites (not shown in this figure) within a 1.6 kb promoter region. Single binding site for pit-1, a TRE and two GREs are identified within a proximal region of the promoter. TRE sequences partially overlap that of distal GRE. A RARE is found at a region more than 0.8kb upstream of the proximal promoter.

Fig. 4. Interplay of multiple endocrine organs that induce functional maturation of GH cells in fetal pituitary gland

During late gestation of rodents, increased release of hypothalamic CRH into pituitary portal circulation stimulates secretion of ACTH, which then stimulates fetal adrenal cortex to increase glucocorticoid

secretion. Increased serum glucocorticoids, in turn, act on pituitary gland to activate GH and GHRH-receptor genes, while suppressing ACTH and PRL expression. Functional maturation of GH cells is results from maturation of the hypothalamo-pituitary-adrenal axis.