韓國家畜繁殖學會誌 19(1) : 15~34(1995)
Korean J. Animal Reprod.

Gonadotropins: Basic View and Gene Expression

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성선자극호르몬 : 유전자 발현에 대한 고찰

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요 약

1970년말부터 뇌하수체 성선자극호르몬(gonadotropic hormone ;GTH)의 유전자 구조(FSHβ, LHβ 및 공통의 α鎖)가 다양한 종에서 밝혀지기 시작하였으나 이러한 유전자의 조직/세포 특이적 분비양식과 세포외 신호에 의한 조절양식은 정확히 밝혀져 있지 않다. 그러나 최근 들어 형질전환 마우스 제작기법에 의해 α鎖 유전자 上流에 세포특이적 발현을 조절하는 특이부위가 존재함이 보고됨을 시작, FSHβ 및 LHβ쇄 유전자발현을 조절하는 특이부위 또한 가까운 시기내 발견되리라 기대된다. 한편, 성선자극 호르몬 방출호르몬(GnRH), 스테로이드 호르몬 및 여러 결합단백질과 같은 세포외 신호는 각기 다른 신호전달체계를 통하여 GTH유전자 발현을 일으킨다. 또한 뇌하수체에서도 그 존재가 확인된 전사인자들 (cFos, cJun)과 未知의 인자들은 상호간에 다양한 二量體를 형성하여 유전자 발현을 조절하는 각 특이부위에 결합함으로써 전사단계에서의 다양한 제어가 존재함이 밝혀지고 있으며 이러한 유전자상의 특이발현영역과 세포외 신호별 전사인자에 관한 연구는 번식에 있어 중요한 성선자극호르몬에 관한 분자수준의 조절기전을 밝혀내리라 기대되어진다.

Ⅰ. INTRODUCTION

Two pituitary hormones, gonadotropins (follicle stimulating hormone, FSH and luteinizing hormone, LH) are composed of a common α subunit and distinct β subunit, which are produced in a subset of anterior pituitary cells (gonadotrophs) of all vertebrate (Fig. 1), except for GTH Ⅰ(corresponds to FSH) and GTH Ⅱ(corresponds to LH) of fishes (salmon) which are produced in different type of pituitary cells. Gonadotropins form a family of glycoprotein hormone, including placental gonadotropins (human chorionic gonadotropin, hCG, and equine chorionic gonadotropin or pregnant mare serum gonadotropin, eCG or PMSG) and thyroid stimulating hormone (TSH) that is structurally related composing common α and TSHβ subunit and produced in other type of pituitary cells (thyrotrophs). Pituitary gonadotropins exert divergent effects upon gonads for the development and the regulation and maintenance for essential reproductive processes such as gametogenesis, steroidogenesis, and ovulation. Abnormality of gonadotropins causes serial problems for development of gonads, ovulation and fertility. Since the studies on the structure of gonadotropins well summarize in the recent papers(Pierce and Parsons, 1981; Ryan et al.,

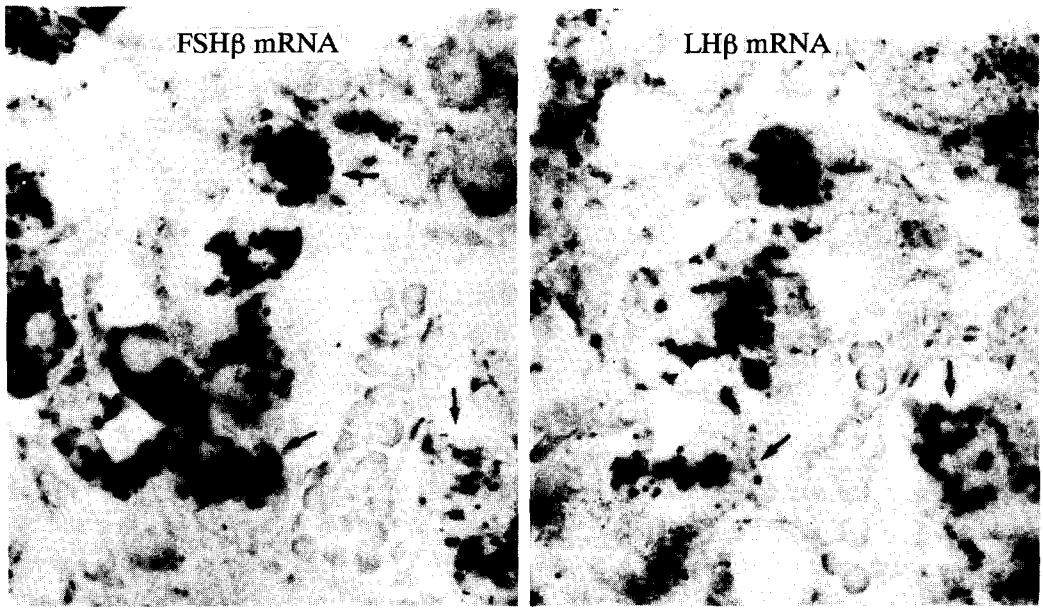


Fig. 1. *In situ* hybridization of FSHβ and LHβ cDNAs. *In situ* hybridization was performed with biotinylated porcine FSHβ and LHβ cDNAs for adjacent sections of porcine anterior pituitary (Liu et al., 1988). Cells containing both FSHβ and LHβ mRNAs are indicated by arrows.

1987), this review will only briefly touch on this aspect with recent topics and focus mainly on what is progressing in the field of gene regulation for gonadotropin.

Ⅱ. STRUCTURE OF GONADOTROPINS

Protein structure of FSH(210 amino acids and 34 kDa in human) and LH(204 amino acids and 28.5 kDa in human) has been determined for wide range species from fishes to mammals. The amino acid sequence of each subunit is mostly preserved throughout evolution (Pierce and Parsons, 1981; Ryan et al., 1987). Especially, the cysteinyl residues in each subunit molecule are well conserved and form intramolecular disulfide bridges (5 in the α subunit and 6 in the LHβ and FSHβ subunits). Each subunit has multiple sites for addition of N- and O-linked carbohydrates. Phylogenetic aspect of gonadotropin molecules indicates that two β subunits have diverged from the same ancestral gene (Fig. 2). A common α and a distinct β form a heterodimer with noncovalent linkage to play the biological functions. The three-dimensional structure confers the binding to own receptor and the biological specificity of FSH or LH. It is likely that β subunit largely determines the specificity.

Varied structures of subunit are known. Amino acid sequence of fish GTHs have considerably low homology with that of tetraploid gonado-

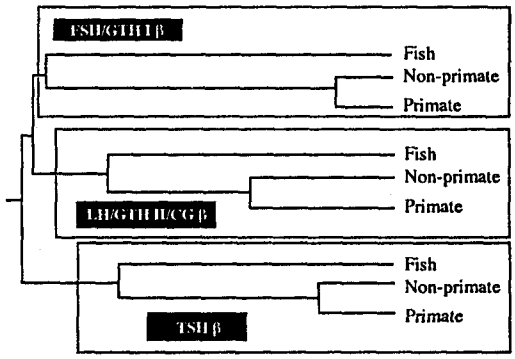


Fig. 2. Schematic draw of phylogenetic tree of amino acid sequence of β subunits of the vertebrate glycoprotein hormone family. The tree reported by Ito et al. (Ito et al., 1993) was modified.

tropins. Extreme diversity of GTHIβ subunit from other subunit suggests that it have diverged soon after the divergence of FSHβ and LHβ. The hCGβ subunit is similar to hLHβ but has an extended tail. The eCGβ subunit has also an extended tail but the sequence and functional role are different from hCGβ (Sugino et al., 1987). Interestingly, eLHβ subunit has sequence identical to that of eCGβ differing to LHβ subunits of other species(Bousfield et al., 1987).

Recently, several investigators reported successive results about the structure of gonadotropins and the binding to receptor. The three-dimensional structure of human CG demonstrated that the structures of α and β subunit are topologically similar and that β subunit wraps around the α subunit with a seat belt-like disulfide bridge between Cys26 and Cys110 (Lapthorn et al., 1994). Binding study of hCG /hFSH chimeras demonstrated that short independent sequences of the β subunit enable hCG to distinguish two distinct receptor for FSH and LH. Residues between the 11th and 12th cysteines conferred FSH receptor and residues between the 10th and 11th cysteines conferred LH receptor (Moyle et al., 1994). Valove et al. reported that the receptor binding and the signal transduction of FSH are involved in separate domains (Valove et al., 1994). The structure-function relationships of gonadotropins is progressing steadily.

Ⅲ. GONADOTROPIN GENES AND CONTROL OF mRNA LEVEL

Since early 1980's, cDNAs and genes encoding

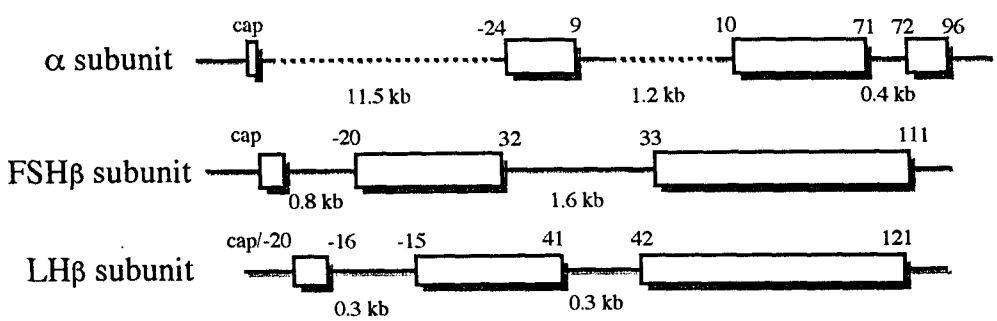


Fig. 3. Gene structure of porcine gonadotropin subunits (Kato et al., 1991; Hirai et al., 1990; Ezashi et al., 1990). Boxes indicate exons. Broken lines indicate regions whose nucleotide sequence were not determined. The amino acid numbers of both ends coded in exon and the size(kbp) of intron are indicated. Initiation start sites are indicated by cap.

gonadotropin subunit were determined for several animals such as man, cow, pig, sheep, rat, and mouse. Each of three subunits is encoded by a single gene on distinct chromosome. In human chromosome, the number 6, 11 and 19 carry α, FSHβ and LHβ subunit genes, respectively. TSHβ subunit gene locates on the chromosome 1. The structures of porcine gonadotropin subunit genes are shown in Fig. 3 (Kato et al., 1991; Hirai et al., 1990; Ezashi et al, 1990). Three gonadotropin genes consist of coding regions (exon) and disrupting non-coding region (intron). The α subunit gene has three introns and two β subunit genes have two introns. The positions of amino acids disrupted by intron are well preserved through species determined, while the length of intron and the nucleotide sequence are different in every species. The three subunit genes showed considerable differences in the nucleotide sequence of 5′ and 3′ flanking regions and introns. The α subunit gene contain-stetranuclotide(CTTT) and dinucleotide(CT) repeats in the first exon. The FSHβ subunit gene shows a high content of adenine and thymine and has SINES(short interspersed repeated sequence)-type retroposon sequence at four positions. The LHβ subunit gene is rich in GC content with clusters of GC boxes and CAC-CC sequence. Thus, even though the two β subunit genes are evolutionarily related, the definite similarity was absent in the putative regulatory region.

The hCG shares the same α subunit gene with gonadotropins, but the distinct β subunit has a multigene cluster tandemly repeated seven times (three are active and others are pseudogene) surround the LHβ subunit gene (Talamadge et al., 1984; Policastro, et al., 1986). The nucleotide sequence of the hCGβ gene revealed that the single nucleotide deletion in the carboxyl

Table 1. Extracellular signals that modulate levels of gonadotropin subunit mRNAs

Signals	Target gene	mRNA	Mechanism	Reference
GnRH	α, LHβ, FSHβ	↑	transcription	64
Pulsatile action[1]	LHβ	↑	transcription	17, 65
Desensitized[1]	LHβ	↓	transcription ?	66
cAMP /PK-A	α, FSHβ LHβ[2]	↑	transcription	49
Ca	α, FSHβ	↑	transcription	67
Estrogen	α	↓	transcription	55
	LHβ	↑	transcription	53
Androgen	FSHβ	↑	post-transcription	18
	hFSHβ[3]	↓	?	68
	LHβ	↓	transcription	18, 69
	GnRH receptor	↓	−	70, 70
Progesterone+E2	LHβ	↓	?	19, 20
Activin[4]	FSHβ	↑	not transcription	72~74
Inhibin[5]	FSHβ	↓	not transcription	74~75

1) different state of GnRH.

2) extension of mRNA length

3) response of human and rat FSHβ is different

4) membrane receptor mediates the signal and may induce the stability of FSHβ mRNA.

5) membrane receptor and intracellular signal are not yet clear.

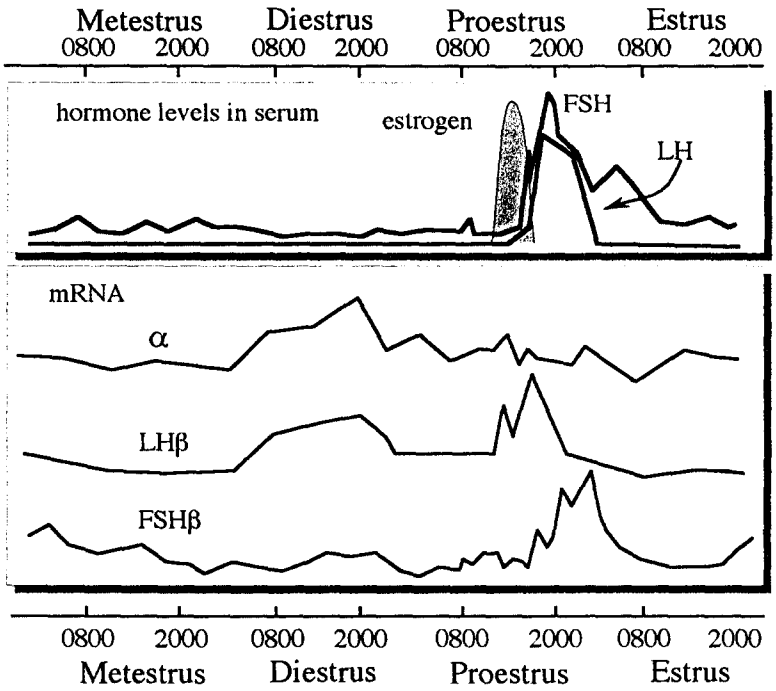


Fig. 4. Changes of gonadotropin subunit mRNAs in estrous cycle. Contents of gonadotropin subunit mRNAs in the rat anterior pituitary were measured throughout the 4 days estrous cycles (Zmeili et al., 1986; Ortoano et al., 1988).

terminal region made a frame shift and extended the tail. However, the extension of C-terminalp tail in eLHβ/eCGβ subunit might have evolved through somewhat different process in comparing with that in hCGβ subunit(Kato et al., 1991).

The transcription of FSH and LH are regulated with several hypothalamic factors and gonadal steroids (Table 1). They modulate the levels of each gonadotropin subunit mRNA with different manners. Gonadotropin releasing hormone (GnRH or LHRH) modulates the three mRNA species but affects differently (Papavasiliou et al., 1986; Kato et al., 1989; Gharib et al., 1990). On the other hand, polypeptides such as activin and inhibin, which specifically control the secretion of FSH, modulate only FSHβ subunit mRNA by indirect action to the gene. Androgen regulates the mRNA level of FSHβ subunit by post-transcriptional action (Gharib et al., 1987; Paul et al., 1990) and decreases the number of GnRH receptor (Giguere et al., 1981; Corbani et al., 1990). It is interesting whether the subunits are cooperatively transcribed during the estrus cycle. Measurements of gonadotropin subunit mRNAs demonstrated that the patterns did not couple with those of hormonal levels in blood nor the mRNA of counterpart (Fig. 4). LH is secreted at proestrus after the estrogen surge, while FSH is secreted just after the

LH secretion and continuously decreased in the late estrus. When the levels of gonadotropin subunit mRNAs were measured, their contents in pituitary are changed differently each other during the estrous cycle (Ortolano, 1988; Zmeili, 1986). The α subunit mRNA showed a single peak between 0800 to 2000h on diestrus, which synchronized to the first peak of LHβ mRNA, while it did not cooperate with the second peak of LHβ mRNA and the single peak of FSHβ at preestrus. These non-cooperative patterns may be caused by variation of GnRH signals and/or different effect by steroid hormones. Post-transcriptional regulation might balance the production of each subunit level to produce active dimer form. The molecular mechanism to regulate the different expression of the three gonadotropin subunit genes is greatly interesting. Besides, gonadotropins are produced only in gonadotroph but not in other pituitary cells, although these cells have been differentiated from the same origin. The transcription machinery to express the individual gonadotropin subunit genes has to be developed during the differentiation of the cells, indicating an importance to understand how the tissue/cell type specific system for expression of gonadotropin genes was developed.

Ⅳ. TISSUE/CELL TYPE SPECIFIC EXPRESSION OF PITUITARY HORMONES

Anterior pituitary is developed from ectodermal cells in Rathke's pouch. Five distinct cell types that produce definite pituitary hormones including gonadotropins are generated. The hormone producing cells are thought to be derived from a common lineage. The phenotype is acquired by the interaction between the *cis*-acting elements that are necessary for tissue/cell type specific expression and the transcription factors that bind to these elements. Ontogenetic analysis of gene expression of rat pituitary hormones (Fig. 5) demonstrated that transcripts encoding the common α subunit, but not any β subunit,

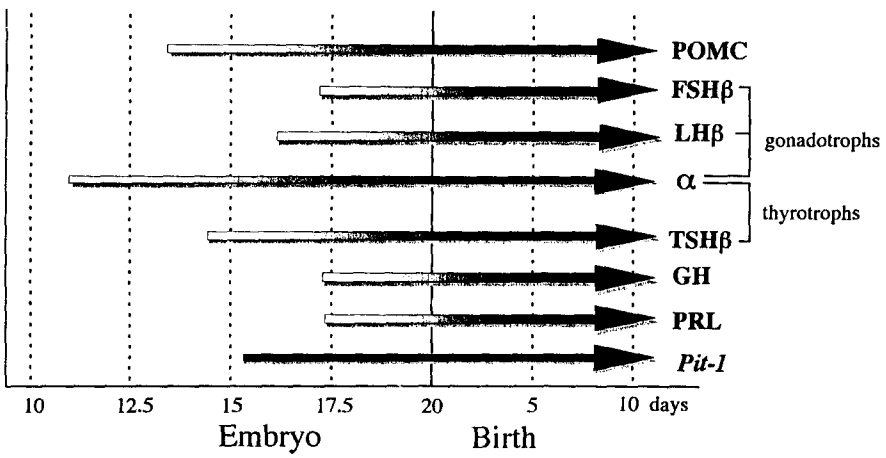


Fig. 5. Expression of rat anterior pituitary hormone genes during the fetal development. The data by *in situ* hybridization with cRNA encoding for pituitary hormones (Simmons et al., 1990) were modified.

appears on the embryonic day 11 (e11) prior to formation of a definitive Rathke's pouch (e12) (Simmons et al., 1990; Voss and Rosenfeld, 1992). The *in situ* hybridization study showed further initial appearance of five distinct hormone producing cells is in the order of corticotrophs(POMC) on e13, thyrotrophs(TSHβ) on e14, gonadotrophs producing LHβ on e16 and FSHβ on e17, and somatotrophs(GH) and lactotrophs(PRL) on e17. A part of the molecular basis for the differentiation of pituitary cells is clarified.

Genetic ablation experiments are feasible because regulatory sequences mostly present in the 5′ upstream region of the gene have been defined that they are sufficient to promote peculiar tissue/cell type specific gene expression. These studies used chimeric genes fused the promoter region of gonadotropin genes with reporter gene, such as diphtheria toxin or virus thymidine kinase(TK), which permit constitutive or inducible cytotoxicity. A construct of promoter of GH gene and TK gene provided definite result that tissue specific expression of TK gene under the administration of its substrate (acyclovir) produced cytotoxic metabolite and abolished somatotrophs (GH cells) as well as mammotrophs (PRL cells), presenting that the upstream region of GH gene contains regulatory element for tissue/cell type specific gene expression and the development of PRL cells are closely related with that of GH cells. After withdrawal of the substrate, GH cells were recovered followed by recovery of PRL cells. These data suggested that a stem cell for GH and PRL cells is present and a cell lineage arising PRL cells from GH cells is evident. Investigation of the transcription factors to regulate tissue/cell type specific expression of GH gene by binding to the promoter demonstrated that a pituitary specific factor, Pit-1/GHF-1, is a definite regulator for the differentiation of GH as well as PRL cells (Bodner et al., 1987; Ingraham, 1988). Interestingly, analysis of inheritable pituitary dwarfism (Li et al., 1990) and human cretinism with combined hormone deficiency (Tatsumi et al., 1992) which lack GH, PRL and TSH demonstrated that the abnormalities were caused by the point mutation in the Pit-1/GHF-1 gene. The correlation of Pit-1/GHF-1 gene with the differentiation of TSH cells is not yet clear, but a binding site of this regulatory factor was identified in the 5′ upstream of TSHβ gene(Steinfelder et al., 1992; Kim et al., 1993). Since GTH cells are appeared prior to that of GH and PRL cells, Pit-1/GHF-1 may not be involved in the differentiation of gonadotropin cells. Organogenesis of anterior pituitary may accomplish through several molecular events to produce distinct phenotypes of hormone producing cells.

Absence of appropriate gonadotroph cell lines hampers investigation of molecular mechanism of expression of gonadotropin genes until very recently. Instead, transgenic animals make us to search regulatory regions responsive for tissue/cell type specific expression of gonadotropin genes. Recently, transgenic mice transfected by the chimeric genes fused the gonadotropin subunit genes with reporter gene have been examined. The results demonstrated successfully that the 5′ upstream regions of gonadotropin subunit genes contain undoubtedly the elements that are responsive for the tissue/cell type specifc expression (Table 2). Upstream of human α subunit gene fused with β-galactosidase gene or CAT gene directed a specific expression of the reporter gene in gonadotrophs (Hamernik et al., 1992). The expression was controlled with GnRH but not T_3/thyroid stimulating hormone releasing hormone (TRH), suggesting that the cells expressed have phenotype of gonadotrophs

Table 2. Transgenic mouse transfected by gonadotropin gene and chimeric genes fused with gonadotropin gene and reporter gene

Gene	Origin	Length(bp)[1]	Reporter gene	Tissue expressed	Responsibility	Reference
α subunit	man	−1,600	CAT/β-gal	gonadotroph	GnRH(+), T3(−)	31
	cow	−315	CAT/β-gal	gonadotroph	GnRH(+), T3(−)	31
	man	−1,800	T-antigen	pituitary(adenoma)	GnRH(+), TRH(−)	32
	cow	−313	diphtheria toxin A	pituitary[2]	GnRH(+)	33
FSH β subunit	man	10,000[3]	none	gonadotroph	testosterone(+)	34
LH β subunit	man	?[4]	T-antigen	pituitary(adenoma)	?	32
	rat	?[4]	T-antigen	pituitary(adenoma)	?	32
	sheep	−1,900	CAT	gonadotroph	?	35

1) negative number indicate the nucleotide length from the transcription initiation site.

2) due to expression of diphtheria toxin, gonadotroph does not develop.

3) entire human FSHβ gene of 10 kbp covering 4 kbp and 2 kbp of 5' upstream and 3' downstream of the gene.

4) the results are described in the reference (Windle et al., 1990).

not thyrotrophs. Indeed, the definite responsiveness was directed by about 300 bp length region (Windle et al., 1990). A cell line, α-T3, was established from transgenic mouse by immortalization with expression of gonadotroph-directed SV 40 T-antigen. αT3 respond to GnRH but not to TRH, suggesting the gonadotroph lineage phenotype. αT3 produced only α subunit but not FSHβ nor LHβ. Since the expression of α subunit gene evidently preceeds that FSHβ and LHβ genes, the tumorigensis by T-antigen might block the development of expression of the β genes. Genetic ablation experiments of α subunit gene also demonstratedthat the expression of cytotoxic genes directed by at least about 300 bp upstream region is sufficient to ablate the gonadotropin cells not thyrotrophs (Windle et al., 1990; Kendall et al., 1991). Transgenic mouse transfected by human FSHβ gene (10 kbp) covering the entire coding region and 5'- and 3'-flanking region showed a specific expression of hFSHβ in the mouse gonadotrophs, while its responsiveness for testosterone was difference from that of endogenous mouse FSHβ gene (Kumar et al., 1992). Similar strategy was examined for sheep LHβ and demonstrated that the tissue/cell type specific expression of this gene is directed by a 1.9 kbp length of upstream sequence (Brown et al., 1993). Thus, each gonadotropin subunit gene has own regulatory element for the gonadotroph specific expression. On the other hand, searches for *trans*-acting factors that confer specific expression by interacting with the *cis*-acting element of the genes make progress not much, becacuse that the elements are yet elusive.

From the nuclear extracts of gonadotroph lineage cell line αT3, a *trans*-acting factor(s),

GSEB1, was purified (Horn et al., 1992). The protein of 54 kDa confers the binding of upstream region of α subunit gene which contains element sufficient for the gonadotroph specific expression. Ingraham *et al.* reported that ablation of gene encoding steroidogenic factor (SF1 or Ad4BP), which was originally characterized as a regulatory factor of 54 kDa for steroid hydroxylase (Honda et al., 1993; Lara et al., 1992), caused a selective loss of immunoreactive pituitary cells for anti-gonadotropin sera as well as lack of adrenal glands and gonads (Ingraham et al., 1994). In situ hybridization for pituitary gland showed that the transcripts of FSHβ, LHβ and GnRH receptor were absent and that of α subunit was present at detectable low level. Recently, an identity between GSEB1 and SF1/Ad4BP was confirmed (Barnhart and Mellon, 1994). On the other hand, since the primate α subunit gene is expressed both in placenta and anterior pituitary, the regulatory mechanism is examined as suitable model for tissue/cell type specific gene expression. A differences of regions respond for expression in placenta or pituitary and of *trans*-acting factors are observed (Schoderbek et al., 1992) but the details are remained to be clarified. Steger *et al.* reported that GATA-binding proteins bind to four upstream elements of α subunit and modulate the specific expression in placenta and pituitary (Steger et al., 1994). GATA binding proteins form a gene family and are identified as factors controlling erythroid cell-specific transcription of the chicken β-globin gene cluster (Yamamoto et al., 1990; Mitchell and Tjian, 1990). The factors described above are mostly relevant to the expression of α subunit gene. Though much less is known about two gonadotropin β genes, we observed that multiple proteins bind to a limited region of porcine FSHβ subunit gene (Gen et al., in preparation). Some of them show tissue specific localization, when the Southwestern blotting analysis was employed with the fragment of FSHβ gene. Whether these proteins are involved in the tissue/cell type specific expression of gonadotropin genes might be clarified in near future.

Table 3. *Trans*-acting factor and *cis*-acting element

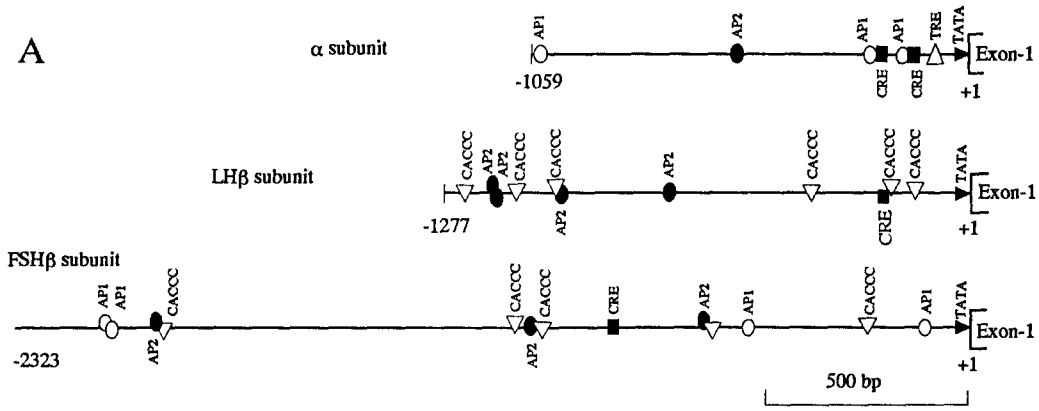
trans-acting factor	*cis*-acting element	Abbreviation
AP-1 (cJun)	TGAC/GTCA	AT-1/TRE[1]
AP2[2]	CCCCAGGC	AP-2
CREB	TGACGTCA	CRE
Estrogen receptor	AGGTCANNNTGACCT	ERE
Androgen receptor[3]	AGAACANNNTGTTCT	GRE
Progesterone receptor[3]	AGAACANNNTGTTCT	GRE
Thyroid hormone recptor	AGGTCAT - - - GACCT	TRE

N means any nucleotides. The sequence of TRE was aligned to match the sequence of ERE and GRE by hyphen.

1) AP-1 is also called as TRE (phorbor ester (TPA) responsive element), but TRE is used for thyroid hormone responsive element (TRE). In this review, TRE means the latter element.

2) Activator protein-2 responsive element (AP-2) is responsive both for signals of CRE and AP-1. Several responsive sequences are known as tissue/cell specific manner.

3) Receptors for progensterone and androgen bind to the same nucleotide sequence.



B

consensus-CRE	TGACGTCA	consensus-AP1	TGA G/C TCA
pα-CRE-1	---G----	pα-AP1-1	--T T ---
pα-CRE-2	---T----	pα-AP1-2	--A A ---
pLβ-CRE	---A----	pFβ-AP1-1	--- A ---
pFβ-CRE	----A---	pFβ-AP1-2	--- T ---

Fig. 6. Schematic draws of presence of putative regulatory elements in the 5' upstream region of gonadotropin subunit genes (A) and nucleotide sequences of variant elements (B). Typical regulatory elements of TATA box, AP-1, AP-2, CRE, TRE (thyroid hormone responsive element) and CACCC box are indicated. The sequence of variants of CRE and AP1 are shown. Numbers of two variants in the same gene are numbered in order from distal position to the transscription initiation site.

V. REGULATORY REGIONS AND TRANSCRIPTION FACTOR RESPONSIVE FOR EXTRACELLULAR SIGNALS

Generally, several *cis*-acting elements and respective *trans*-acting factor are known to regulate the expression of target genes that response to several extracellular signals (Table 3) (Mitchell and Tjian, 1989). The expression of three gonadotropin subunit genes are also modulated by peptide hormones, steroid hormones and others as described above (Table 1). Survey of consensus regulatory elements in short sequence demonstrated the presence of several regulatory sequence (Fig. 6B) and the homologus variants in each gonadotropin gene (Kato et al., 1991; Jameson et al., 1988). Human α subunit gene has a uniqul tandem repeat of cyclic AMP responsive element (CRE; TGACGTCA) composing of perfect palindromic structure (De-

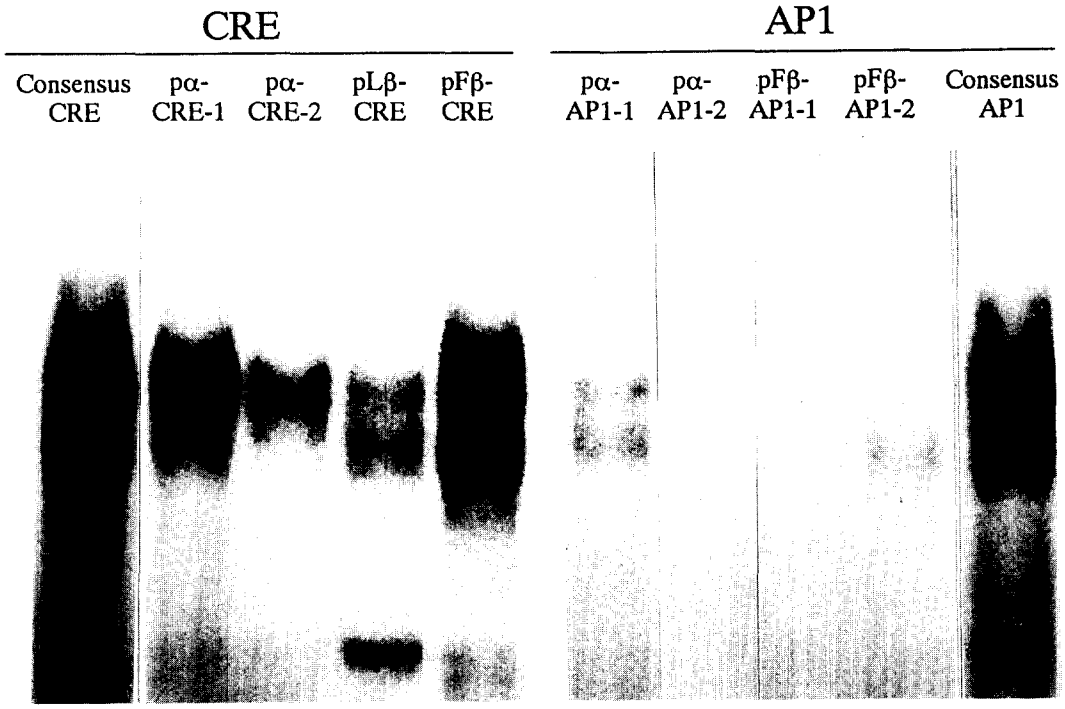


Fig. 7. Gel mobility shift assay of variants sequences of CRE and AP-1 found in the porcine gonadotropin subunit genes. The variants sequences were assayed by gel mobility shift against the nuclear extracts of porcine anterior pituitary (Kato et al., in preparation).

utsch et al., 1987). There are CATT-box and TATA-box necessary for basic transcription machinery and homologous sequences to CRE and/or activator protein 1 responsive element (AP-1: TGA(C or G)TCA; also called as TRE) in each GTH gene. The latter two elements show one or two nucleotide variations in comparison with that of consensus elements (Fig. 6B). CRE and AP-1 are responsive for intracellularly activated-transcription factors (CRE-binding proteins for CRE and cJun/cFos heterodimer for AP-1) mediated by two distinct membrane signal cascades. These sequences found in GTH genes are able to be recognized by pituitary nuclear proteins as shown in Fig. 7(Drust et al., 1991; Kato *et al.*, in preparation). Cyclic AMP (cAMP) stimulates the transcription of α subunit gene by transmitting the signal to CRE. However, most of the observation for α subunit gene is performed in placenta or choriocarcinoma cell lines, where CG gene is expressing. Though the regulation of α subunit gene in the pituitary is not clear for a long time, it is evident that the cAMP/protein kinase-A pathway is involved in regulation of the gonadotropin subunit messenger ribonucleic acids (Kay and Iameson, 1992; Ishizaki et al., 1993). In the gonadotrophs, hypothalamic factor such as pituitary adenyl cyclase activating polypeptide (PACAP), at least, may increase intracellular level

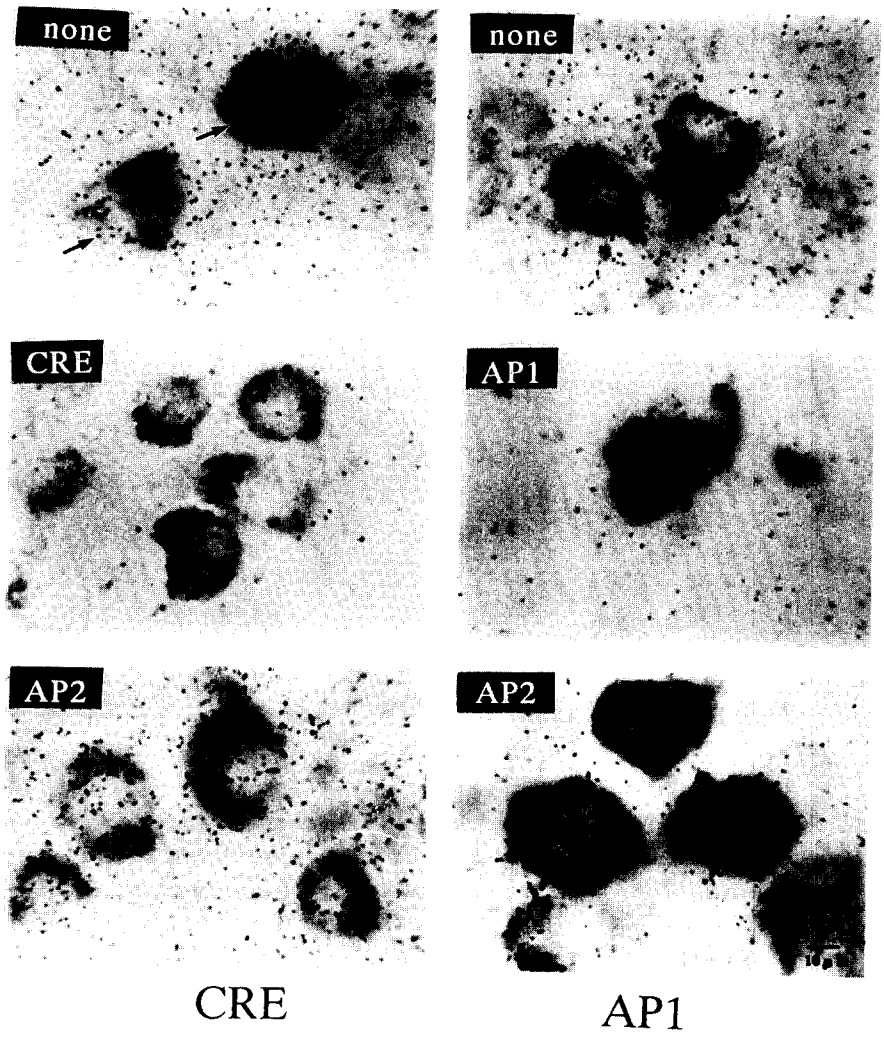
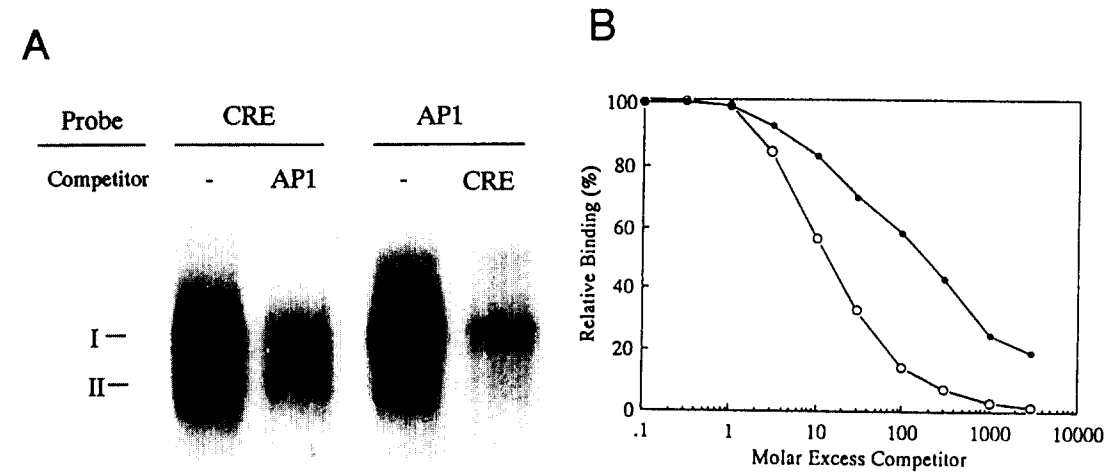


Fig. 8. *In situ* binding of CRE and AP-1 in porcine anterior pituitary (Kato et al., 1993). Oligonucleotide of ^{35}S-labeled consensus CRE (left) and AP1 (right) were incubated with porcine anterior pituitary slices previously developed color reaction by enzymatic immunohistochemistry using anti ovine LHβ antiserum. The incubation with labeled probe was performed with probe alone (top panel), probe and unlabeled CRE or AP-1 (second panel) and probe and AP-2 composing of unrelated sequence to CRE or AP-1. Arrows indicate gonadotrophs.

of cAMP. However, we could not obtain cDNA encoding CRE-binding protein cognates from porcine anterior pituitary cDNA library using CREB-341 cDNA (Kato et al., unpublished observation).

GnRH is a key signal among several extracellular stimuli to regulate gonadotropin genes. The signal mediated with intracellular protein



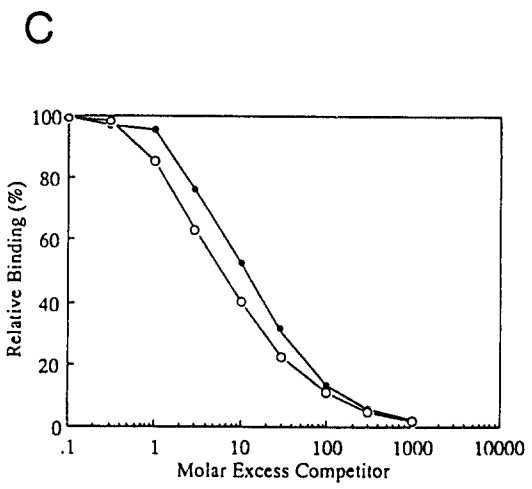


Fig. 9. Competitive binding of CRE and AP1 by gel mobility shift assay (Ezashi et al., 1992). A. Autoradiography of competitive binding of labeled CRE with unlabeled CRE duplex using nuclear extracts from porcine anterior pituitary. The binding of labeled CRE or AP1 was competed for with excess amount of unlabeled AP1 and CRE. Reciprocal competition of each ^{32}P labeled CRE- (B) and AP1-binding (C) was achieved with the amounts indicated. Competitive binding with CRE and AP1 is indicated by clear and solid circles, respectively.

ding to 20%, suggesting that approximately 80% of the total CRE-binding is caused by factors which bind to a common site shared by both CRE and AP-1. Relative binding affinities of AP-1 against CRE estimated from the reciprocal competition data were 0.17 for CRE-binding and 0.56 for AP-1-binding (Fig. 9B and C). Different molecular size of binding proteins for CRE and AP-1 was observed. Form the inconsistency of the relative binding affinities and the multiple molecular size of binding factors, we have claimed that the CRE / AP-1-binding factors alter the dimer form by changing each respective partner to bind CRE and / or AP-1. It is postulated that CRE- and / or AP-1-binding protein recognize their ligand by formation of alternative dimers with different partner and distinguish their specific target gene (Habener, 1990). Indeed, several CRE- and AP-1-binding proteins form heterodimers between members of the family (Lamb and McKnight, 1991; Hoeffler et al., 1989; Meyer and Haber, 1993). Based on *in vitro* data,

kinase C that activates transcription factor, such as cJun/cFos heterodimer. The dimer recognizes a consensus AP-1 sequence. Kay and Jameson reported that an element for GnRH stimulation was located in the 5'-upstream region of human α subunit gene between -346 and -244 (Kay and Jameson, 1992). This region was separated from the region containing CRE and did not contain any AP-1 related sequence. Absence of consensus regulatory element does not exclude the possibility of degenerating binding sites, since unique sequence conferring transcription factors might be present as observe previously in the hCGβ subunit gene (Albanese et al., 1991). Indeed, we found by *in situ* binding method, which can detect DNA binding proteins in the fixed tissue slice (Fig. 8), that DNA binding proteins for oligonucleotide of consensus AP-1 as well as that of CRE are present in the gonadotrophs (Kato et al., 1993). Furthermore, we cloned cDNAs encoding cJun and cFos from anterior pituitary cDNA library and observed that their gene expression is likely regulated by GnRH (Chung et al., in preparation). Taken together, interaction between several variant sequences homologous with AP-1 or CRE and typical transcription factors may play a role in a fundamental regulation of gonadotropin genes.

Generally, decrease of steroid hormones by castration increases the level of gonadotropin mRNAs as well as the serum level of gonadotropins. The steroidal regulation is another key role to modulate gonadotropin gene. However, we did not find any consensus steroid hormone responsive element (SRE) in the entire 10 kbp length (including about 5 kbp of 5' flanking region) of porcine FSHβ subunit gene as well as in α and LHβ subunit genes (Hirai et al., 1990). Only rat LHβ subunit gene contains an estrogen-responsive element (ERE), which is positively regulated by estrogen, at about 1.1 kbp upstream of the transcription initiation site, while negative ERE was absent (Shupnik et al., 1989). In the fish GTH IIβ (corresponding to tetrapod LHβ) gene, two EREs were identified (Xiong et al., 1994). One is located at proximal of the transcription start site with typical ERE, and another is at about 2700 bp upsteam with sequence comprised of three tandemly linked half-ERE. The two EREs were also positive regulator. On the contrary to the observation that steroid homones decreased the transcriptional level of gonadotropin genes, any negative ERE was reported (Keri et al., 1991). Cotransfection of α subunit gene promoter fused with CAT gene and estrogen receptor cDNA in human choriocarcinoma cells showed an estrogen directed suppression for the transcription of CAT gene. The data demonstrated that the response was forced without a high affinity binding site for estrogen receptor, though the molecular mechanism remains to be studied. Co-trans activators, which interact with DNA-bound transcription factor without direct binding to DNA, might concern with non-binding modulation of estrogen. Meanwhile, several regulatory elements show some similarity with their nucleotide sequence, therefore, some transcription factors binds to the cognate sequences make possible to communicate different signals at the same target site comprehensively.

Actual nuclear factors controlling gonadotropin genes are known scarcely at present, in contrast with the responsiveness of genes for extracellular signals which has been frequently examined. We have reported previously that most of anterior pituitary DNA binding proteins are indistinguishable from CRE- or AP-1-binding proteins (Ezashi et al., 1992). By reciprocal competition for the AP-1- and CRE-binding, CRE prevented AP-1-binding completely (Fig. 9A). On the other hand, AP-1 decreased the CRE-bin-

members of the AP-1 family form a heterodimer among their cognate molecules and have distinct relative binding affinities for CRE and AP-1 (Hirai and Yaniv, 1989). Some of them are known to modulate specific gene by forming heterodimer with a member of the family. The heterodimer may provide unique binding specificity and novel regulatory efficiency for each respective gene (Jones, 1990). Existence of a large number of heterodimer that recognize peculiar gene will preserve the transmission of specific extracellular signal on the target gene, while cooperative specific intracellular signal is also required. In addition, the fact that similar sequence is recognized by distinct factors including heterodimer with several combinations suggests that transcriptional cross-talk (Masquilier and Sassone, 1992) may exist and control the complex signals sharing with the same regulatroy element. Understanding of these types of regulatory factors can represent an answer to a question why and how the particular gene is regulated in correspond to the specific extracellular signals, though intracellular singal is thought to be trasmitted by universal signal cascades mediated by protein kinase A and C.

ACKNOWLEDGEMENTS

This study was supported in part by grants-in-aid from Ministry of Education, Science and Culture (No. 02640578 and 06454019) and the Agricultural Chemical Research Foundation of Japan.

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