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Anterior pituitary development and Pit-1/GHF-1 transcription factor

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Abstract. Anterior pituitary differentiation is a well-established paradigm in mammalian organogenesis. *PIT1/GHF1*, a homoeotic gene, plays a key role in terminal pituitary differentiation. Recently, a new set of transcription factors involved in early pituitary differentiation have been identified: Lhx-3, Lhx-4, P-OTX and Prop1. A pituitary-specific transcriptional cascade regulating a developmental programme leads to the determination of the mature cell types.

Key words. Pituitary development; Pit-1; GHF-1; transcription factors; gene expression.

Introduction

The elucidation of mechanisms underlying tissue development, cell commitment and the continued fine-tuning of gene expression is a major challenge in biology and molecular genetics. Much of our understanding of these processes in mammalian cells originates from studies of a small number of selected model systems, for example pituitary-specific gene expression.

Pituitary organogenesis is driven by a series of developmental decisions controlled by a small number of genes coding for transcription factors. One of the most studied is pituitary transcription factor-1 (Pit-1)/growth-hormone factor-1 (GHF-1). This factor is responsible for the differentiation of three specific cell types (GH^+, PRL^+ and $TSH\beta^+$), transcriptional regulation of target promoters and for the proliferation and survival of cells within its lineage.

Recently, the in vivo functions of a new set of genes involved in anterior pituitary development (Lhx-3, Lhx-4, P-OTX and Prop1) have been examined through targeted gene disruption in the mouse.

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Anterior pituitary cell differentiation

Pituitary ontogeny gives rise to five different cell types as defined by the hormones these cells produce and secrete: corticotrophs, thyrotrophs, gonadotrophs, somatotrophs and lactotrophs [1–3]. These cells originate from an invagination of the neural ectoderm beneath the diencephalon called Rathke's pouch [1]. Both the combined pattern of expression of different transcription factors (mainly homeotic genes) and proliferative induction of signals from cells of the floor of the diencephalon (which will later give rise to the hypothalamus) account for the organogenesis of the anterior pituitary (fig. 1).

Formation of Rathke's pouch is a two-step process: a Rathke's pouch precursor arises from a neural ectoderm layer beneath the floor of the diencephalon; and Rathke's pouch develops from this precursor. No transcription factor has been directly related to the first step, but expression of the homoeotic genes *Rpx (Hesx1)*, *Six3*, *Pax6* and *Six1* overlaps in the neural ectoderm [4]. More interestingly, expression of *Rpx* becomes restricted to Rathke's pouch precursor cells, suggesting it is involved in the determination of the anterior pituitary by creating a $Six3^+$ compartment [5].

Signals from the diencephalon/hypothalamus are also involved in this step since suppression of the *T/EBP* gene impairs the formation of any Rathke's pouch precursor [6]. Loss-of-function experiments indicate that either Lim-3 or Lhx-4 (a LIM-related gene) are necessary to accomplish the second step. However, other experiments suggest a redundant role for these two factors [7].

The onset of the first pituitary-specific marker (αGSU) correlates with the expression of both transcription factors LIM-3 and P-OTX. Once the formation of Rathke's pouch has been accomplished, several developmental steps transform it into a mature pituitary. LIM-3 has proved to be a key regulator in pituitary organogenesis since no terminal differentiation marker except ACTH appears in a LIM-3-defective mouse [8]. Lhx-4 is not able to substitute this LIM-3 function. Since P-OTX expression becomes restricted to corticotrophs (the only developmental lineage which is not LIM-3-dependent) it may be that there are LIM-3- and P-OTX-dependent differentiation pathways in pituitary organogenesis [8]. Clearly, a P-OTX loss-of-function experiment is needed to confirm this hypothesis.

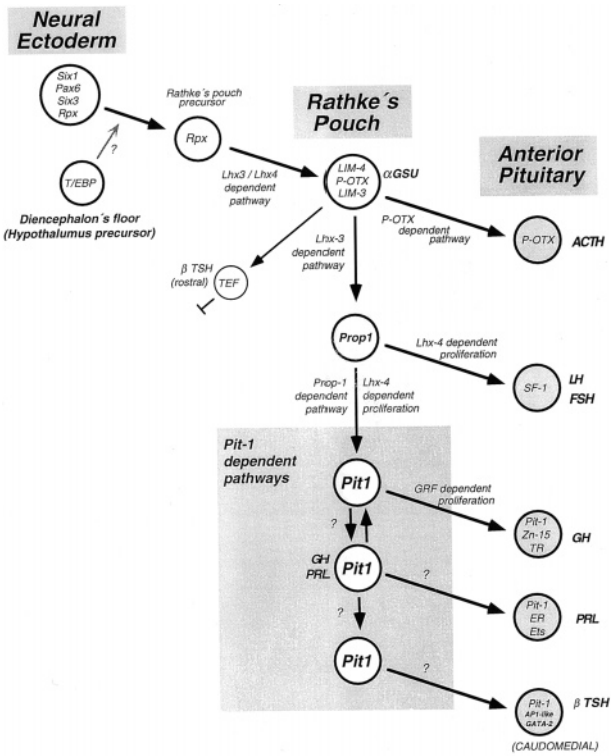


Figure 1. Development of anterior pituitary cell lineages. The products of transcription factors and peptide hormone genes that serve as phenotypic markers of each lineage are indicated. αGSU, α glycoprotein subunit; ACTH, adrenocorticotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; PRL, prolactin; TSHβ, β thyroid-stimulating hormone subunit.

The LIM-3 developmental pathway gives rise to gonadotrophs and Pit-1/GHF-1-dependent cell types. At present no transcription factor directly related with the onset of gonadotroph differentiation. Loss-of-function experiments show that nuclear receptor SF-1 is essential for gonadotroph development [9]. SF-1 is expressed in gonadotrophs but also in the gonads and ventral diencephalon. Treatment of SF-1-defective mice with gonadotropine-releasing hormone (GnRH) fully restores gonadotroph phenotype, suggesting that SF-1-defective mice have an impaired GnRH-dependent induction by the floor of the diencephalon. Loss-of-function experiments suggest that Lhx-4 is involved in the proliferation of the Pit-1/GHF-1-dependent cellular precursor which give rises to gonadotrophs, thyrotrophs and lactotrophs but also in the cellular precursor which gives rise to gonadotrophs [4]. *Prop-I* is a pairedlike homeotic gene. There are no *Prop-I* loss-of-function experiments, but several data suggest *Prop-I* is involved in the onset of *PIT1/GHF1* gene expression. Ames mice have *Prop-I* mutations, but no *PIT1/GHF1* mutation. They do not express Pit-1/GHF-1, but they have the same phenotype as Pit-1/GHF-1-defective Snell and Jackson mice. Prop-1 is expressed in the caudomedial area of the anterior pituitary, the same area where *PIT1/GHF1* gene expression is detected two days later. Moreover, Prop-I binds and transactivates sequences involved in activation of *PIT1/GHF1* gene [10]. Recent studies which correlate human Prop-1 mutations with familial combined pituitary hormone deficiency (CPHD) suggest that Prop-1 is critical not only for the *PIT1/GHF1* gene activation but also for gonadotroph lineage determination [11]. This raises the question whether Prop-1 is involved in anterior pituitary differentiation by creating a Lim-3$^+$ compartment or by cooperating with Lim-3 in two different compartments (fig. 1).

The Pit-1/GHF-1 developmental pathway gives rise to somatotroph, lactotroph and thyrotroph pituitary cell types [12, 13]. The mechanism underlying the differentiation of three cell lineages from a common Pit-1$^+$ progenitor cell is still not well understood, but activation of GH, PRL and TSHβ genes by Pit-1/GHF-1 in distinct cell types requires the coordinate action of additional factors. In this respect, Pit-1/GHF-1 cooperates with E twenty-six specific oncogene-related (Ets) transcription factors to regulate PRL gene expression [14], with Zn-15 to regulate GH gene expression [15], and with activator protein-1 (AP-1)-like (or GATA DNA sequence-specific factor-2 (GATA-2)) factors to regulate TSHβ gene expression in caudomedial thyrotrophs [16, 17]. It is worth noting that TSHβ is expressed in two different thyrotroph populations. A

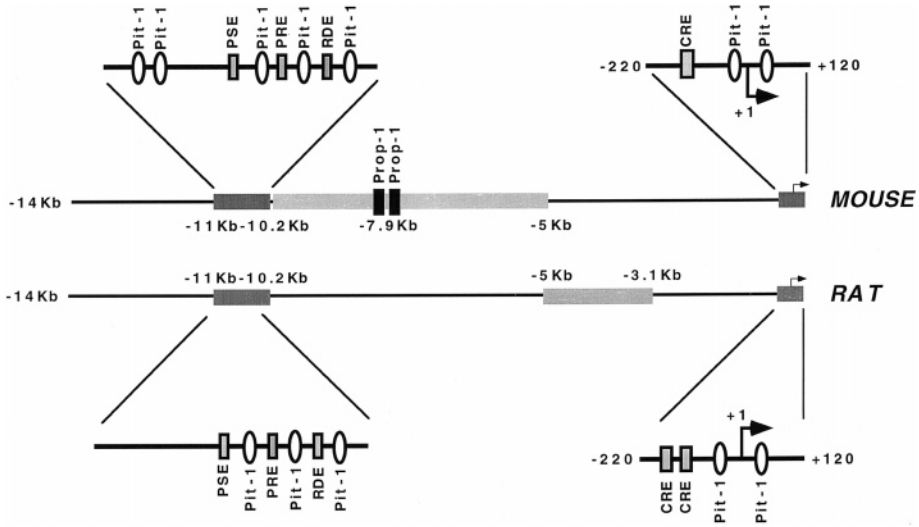


Figure 2. Structure of mouse and rat *PIT1/GHF1* promoter and enhancer regions. These promoters contain autoregulatory binding sites for Pit-1/GHF-1 factor and other transcriptional regulators. CRE, cAMP-responsive element; Prop-1, prophet-1-binding sites.

rostral population arises even in Pit-1/GHF-1-defective mice. Transcriptional enhancer factor (TEF), a bZip transcription factor expressed only in this cell population, accounts for the expression of TSHβ [18]. This rostral population completely disappears by the day of birth (fig. 1) [4].

Regulatory sequences in rodent and human *PIT1/GHF1* genes

The *PIT1/GHF1* gene was first characterized in rats and mice [19–23]. The 5′-flanking promoter is a 300-bp region surrounding the transcriptional start site (fig. 2). It includes two autoregulatory Pit-1/GHF-1-binding sites: an upstream positive autoregulatory site plus a downstream inhibitory site. There are several examples of positive autoregulatory sites in transcription factor genes [24–26], and these sites may be widespread among genes involved in determination of cell fate. In the case of factors involved in terminal cell differentiation, the role of this autoregulatory loop seems clear. Once a factor is turned on, it will remain active for the duration of the cell's life. By contrast, negative autoregulatory sites are not so common. In fact, *PIT1/GHF1* is the only known gene which includes these kinds of negative regulatory sites. The negative role of the Pit-1/GHF-1-binding site beyond the cap site is not sequence-dependent but position-dependent. It works as a positive site when placed 5′ of the TATA box [20]. In both rat and mouse genes the TATA box overlaps a perfect palindrome sequence. It has been suggested that a pituitary specific factor other than Pit-1/GHF-1 interacts in this site [21].

The rat *PIT1/GHF1* promoter has two cyclic adenosine monophosphate (cAMP) response elements (CRE) [19, 20], whereas the mouse promoter has only one CRE site [22]. The significance of this difference is not yet clear, since CRE sites in rat and mouse promoters seem to regulate *PIT1/GHF1* gene expression in the same way. An increase in intracellular cAMP levels upregulates *PIT1/GHF1* transcription. The physiological role of this behaviour is well understood at least in the case of somatotroph lineage. Growth hormone-releasing factor (GRF) stimulates GH secretion, both increasing GH gene expression through CRE sites in the GH promoter and increasing Pit-1/GHF-1 levels, which in turn also increase GH expression. The CREs in the rat *PIT1/GHF1* promoter are not only involved in cAMP activation but also in retinoic acid activation [27], glucocorticoid activation [23] and T3 downregulation [28].

The proximal promoter region by itself is not sufficient to regulate the onset of *PIT1/GHF1* expression in a developmental context [29–31]. Several 5′ upstream regulatory regions have been described both in rat and mouse *PIT1/GHF1* genes (fig. 2). A 390-bp distal enhancer has been identified in mouse (10 kb 5′ of the transcription start site). It includes five additional autoregulatory Pit-1/GHF-1-binding sites, a 1,25-dihydroxy-vitamin D_3 response element (VDRE), a retinoic acid response element (RARE) and a binding site for an

as yet unidentified factor which is essential for the function of this enhancer [29]. An intermediate enhancer involved in the initial onset of *PIT1/GHF1* expression is located at [−10/−5 kb] [31]. Two Prop-1-binding sites have been identified in this region at positions [−7.8/−7.9 kb] [10], which correlates with the proposed role of Prop-1 in Pit-1/GHF-1 lineage determination. A proximal enhancer located at [−5.1/−3.1 kb] regulates rat *PIT1/GHF1* expression in a developmentally restricted manner [30].

Recently, the human *PIT1/GHF1* gene was cloned, and the regulatory mechanisms controlling its promoter were characterized (fig. 3) [32]. A minimal promoter region [−102 to +1] contains the *cis*-acting elements which confer a high basal transcriptional activity, tissue-specific expression and autoregulation by its own Pit-1/GHF-1 protein. The nucleotide sequence comparison with the rat minimal promoter indicated an 88% similarity in this region.

The human distal promoter region [−0.8 kb] has no homology with the rodent sequences (fig. 3). It contains (i) six Pit-1/GHF-1-binding sites which do not show any synergistic interaction with the minimal promoter, (ii) an octamer-binding site (OTF) and (iii) a TPA-responsive element (TRE) which overlaps with a Pit-1/GHF-1-binding site. Oct-1 and AP-1 transcription factors downregulate the expression of the human *PIT1/GHF1* gene [32].

In contrast with the rat or mouse genes, the human *PIT1/GHF1* promoter has no CRE within the proximal or distal promoter regions. This indicates that besides a common autoregulatory mechanism controlling the basal expression of the *PIT1/GHF1* gene in both humans and rats, the mechanism of activation substantially differs between the two species (fig. 3). Transcription of the human *PIT1/GHF1* gene is not positively controlled by activators of the protein kinase A (PKA) pathway but is downregulated by AP-1 and by Oct-1 [32]. Further analysis is, however, required to better understand the functional significance of these interactions and to determine, for instance, the mechanism for GRF-dependent induction of the human *PIT1/GHF1* gene.

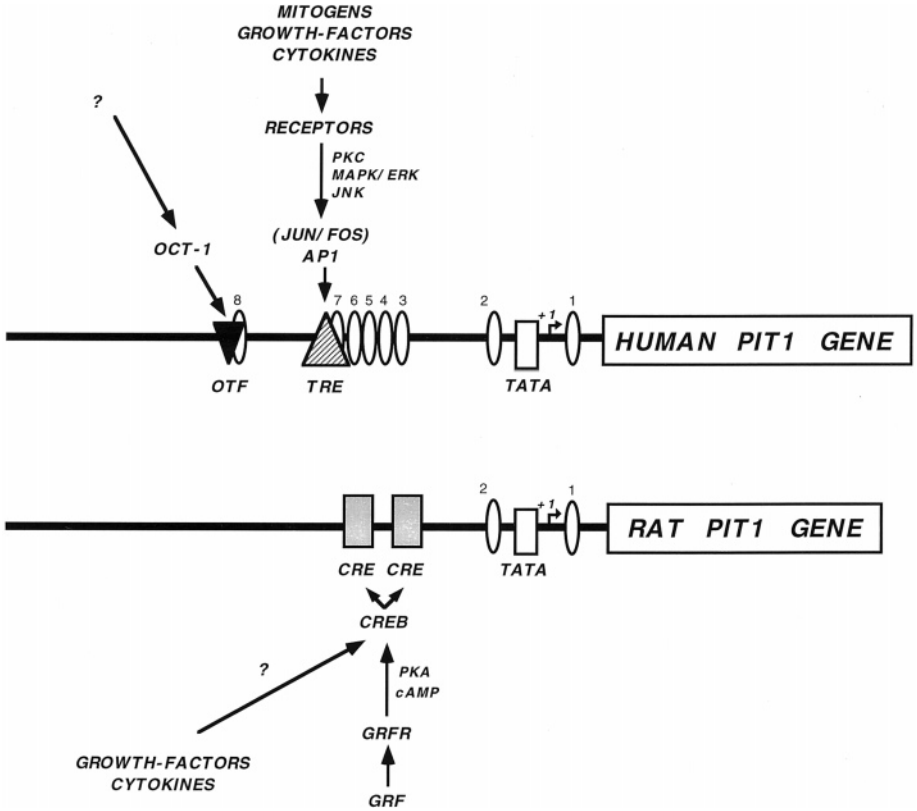
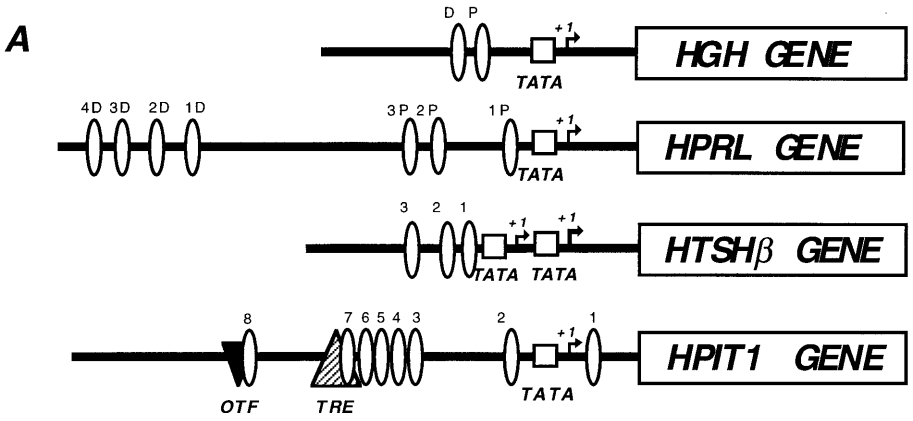


Figure 3. Comparison of human and rat *PIT1/GHF1* promoters and signal-transduction pathways regulating its gene expression. OTF, octamer factor binding site; TRE, TPA-responsive element; CRE, cAMP-responsive element; TATA, TBP-binding site.

A



B

GENE	SITE	ORIENT.	SEQUENCE	POSITION
HGH	P	s	ccc ATGCATAAA tgt	-86/-78
HGH	D	as	cta ATGGATAAT tta	-111/-119
HPRL	1P	as	ttc ATGAATATA atg	-45/-53
HPRL	2P	s	aat ATGAATAAg aaa	-154/-146
HPRL	3P	as	att tTGATTAAT tag	-201/-209
HPRL	1D	s	aag ATGAATTTT tgt	-1213/-1205
HPRL	2D	as	tat ATGAATAAg aac	-1272/-1280
HPRL	3D	s	tag ATGCATgTA ctt	-1327/-1319
HPRL	4D	s	ctg ATGAATgAg gta	-1389/-1381
HTSHβ	1	s	caa ATGCAATTg tat	-73/-65
HTSHβ	2	s	agt ATGAATTTT caa	-119/-111
HTSHβ	3	s	tgc ATGCTTTAA taa	-196/-188
HPIT1	1	s	ctg ATGTATATA tgc	+15/+23
HPIT1	2	as	aac ATGTATAAA ggg	-47/-55
HPIT1	3	as	caa ATGTATAAA ata	-323/-331
HPIT1	4	s	ttc ATGTTTTAT cgc	-378/-370
HPIT1	5	s	att ATGTATAAT act	-408/-400
HPIT1	6	s	ctc ATGTTTAgT att	-461/-453
HPIT1	7(TRE)	s	agt ATGAATcAT taa	-491/-483
HPIT1	8(OTF)	s	tgc ATGAcATAA ctg	-777/-769

Figure 4. (*A*) Structure of GH, PRL, TSHβ and *PIT1/GHF1* human promoters. Binding sites for Pit-1/GHF-1 factor are shown with open ellipses. (*B*) Table of Pit-1/GHF-1-binding sites present in these promoters. The orientation (sense or antisense) and the relative position to the transcriptional start site (+1) is shown. OTF, octamer factor-binding site; TRE, TPA-responsive element; TATA, TBP-binding site. P, proximal, and D, distal sites.

From a developmental point of view, the present data suggest a three-step model for *PIT1/GHF1* gene expression. First, the intermediate enhancer regulates the initial onset of *PIT1/GHF1* expression, and Prop-1 is involved in this process. Second, the proximal enhancer regulates *PIT1/GHF1* expression at a developmental stage where neither GH nor PRL are yet expressed and the level of Pit-1/GHF-1 protein is too low to autoregulate its own gene expression. Third, the proximal promoter and the distal enhancer cooperate to activate *PIT1/GHF1* transcription in the adult pituitary. At this step, the Pit-1/GHF-1 transcription factor would be a critical protein in regulation of its own gene expression.

The transcription factor Pit-1/GHF-1

Comparison of the 5′-flanking regions of human and rat growth hormone genes reveals extensive similarity over the first 500 bp of DNA, signalling the importance of these sequences in controlling GH expression. Indeed, a 300-bp promoter region is sufficient for conferring somatotroph-specific expression in cultured cells by transfection assays [33]. The location of protein-binding sites within the GH promoter was determined by DNaseI footprinting [33]. Although several DNA-binding positions were detected, only one was unique to GH-expressing cells [34]. The pituitary-restricted distribution of this protein, named growth hormone factor-1 (GHF-1), suggested that it plays a major role in the pituitary-specific expression of the GH gene [35].

GHF-1 was purified to near homogeneity from extracts of GC cells, a GH-expressing cell line derived from a rat pituitary tumour, and positively identified by elution from SDS-polyacrylamide gel and renaturation as a 33-kDa polypeptide [36]. Purified GHF-1 stimulates transcription from the GH promoter when added to nuclear extracts of cells which do not express GH or GHF-1 [34]. These findings indicate that, although GHF-1 is a pituitary-specific transcription factor, it is able to interact with the basic transcriptional machinery in both GH-expressing and nonexpressing cells. In fact, this was the first demonstration that a cell-type-specific transcription factor isolated from one cell type can be added to heterologous cell-type extracts and activate transcription.

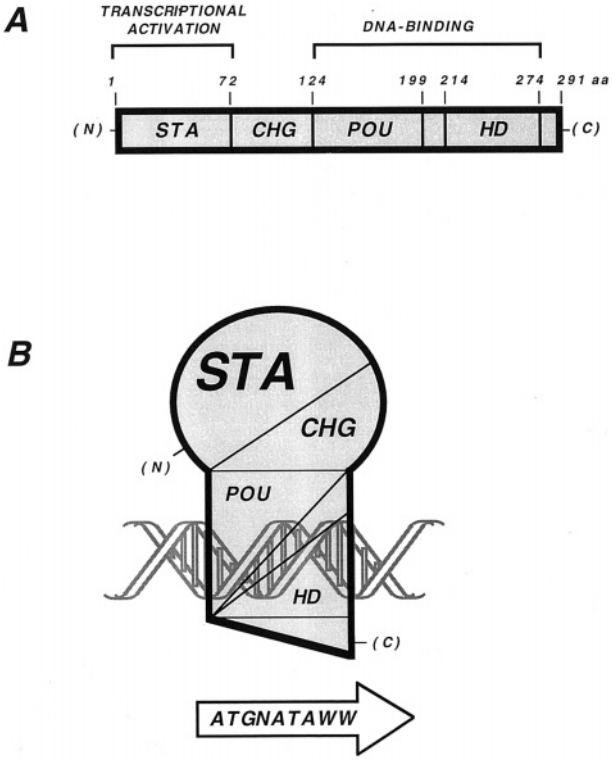


Figure 5. (*A*) Schematic diagram of the primary structure of Pit-1/GHF-1 protein. It contains a 60-aa POU homoeodomain (HD), a 75-aa POU-specific domain (POU) and a 71-aa serine/threonine-rich region or STA domain (serine-threonine activation domain). The CHG box indicates a region rich in charged amino acids. (*B*) Model for 3D structure. POU and HD domains interact with the consensus DNA sequence 5′-ATGNATAWW-3′. STA and CHG regions should have an interface in contact with the surfaces of coactivators (TAFs, USA or mediator) and general transcription factors.

A partial amino acid sequence of GHF-1 was obtained and used for development of oligodeoxynucleotide hybridization probes. Screening of rat and bovine pituitary complementary DNA (cDNA) libraries with these probes resulted in isolation of GHF-1 cDNA clones [37]. Antipeptide antibodies generated against this peptide reacted specifically with GHF-1 and confirmed that the sequence of the isolated peptide was correct and that it was indeed derived from GHF-1 [37]. Other investigators isolated an identical cDNA clone, which was called Pit-1, by screening of an expression library prepared from rat pituitary and using a labelled GHF-1-binding site [38].

In addition to specific interaction with the GH promoter, Pit-1/GHF-1 protein binds to PRL, TSHβ and its own *PIT1/GHF1* promoter [13]. Figure 4 shows the DNA sequences and their position relative to the transcriptional start site (+1) of the Pit-1/GHF-1 binding sites in the human promoters. The consensus sequence is 5′-ATGNATAWW-3′ [13].

The Pit-1/GHF-1 protein is composed of two functional domains. The C-terminal half contains its DNA-binding domain [amino acids (aa) 124–273], and the N-terminal half contains the transcriptional activation domain or STA domain (aa 1–71) (fig. 5A). The DNA-binding domain contains a 60-aa homoeodomain (HD) and a 75-aa POU-specific domain (POU) separated by a linker domain of 15 aa [12]. The identification of the first tissue-specific mammalian transcription factor as a homoeodomain protein was an important breakthrough that served to bridge the gap between two separate fields: transcriptional regulation and developmental biology. It provided the first conclusive proof that homoeodomain proteins, already known to be involved in developmental decisions, do indeed function as transcription factors.

The N-terminal half of Pit-1/GHF-1 is responsible for transcriptional activation [39]. This activity is destroyed by deletion of sequences within the first 72 aa, a region rich in serine, threonine and proline amino acids. This region can be fused to a heterologous DNA-binding

domain derived from cJun, to construct a chimerical protein capable of activating transcription from AP-1-dependent promoters [39]. Interestingly, this region contains only a few negatively charged amino acids or glutamines, the kind of residues shown to be associated with other known transcription factors.

Figure 5B represents a model of the three-dimensional (3D) structure of Pit-1/GHF-1. POU and HD domains interact with the consensus DNA sequence 5′-ATGNA-TAWW-3′. STA and CHG regions should have an interface for directing contacts with surfaces of coactivators (TBP-associated factors (TAFs), upstream stimulatory activity (USA) or mediator) and general transcription factors. The purification and cloning of specific pituitary coactivators will reveal a better understanding of the role of transcription factors in gene activation and cell differentiation.

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