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Genetic Regulation of the Embryology of the Pituitary Gland and Somatotrophs

Laurie E. Cohen

Division of Endocrinology, Children's Hospital, Boston, MA

Introduction

During embryogenesis, developmental factors are expressed in the region of the future anterior pituitary gland in overlapping, but distinct, spatial and temporal patterns. By interacting with each other, these factors control pituitary cell determination and specification. The primordium of the anterior pituitary, Rathke's pouch, is first detected at embryonic day (e) 8.5 in the mouse. It forms as an upward invagination of a single-cell-thick layer of ectoderm that contacts the neuroectoderm of the primordium of the ventral hypothalamus *(1,2)*. The anterior pituitary lobe separates from the oral ectoderm by e12.5 after intensified cell proliferation within Rathke's pouch *(2)*.

The *α-glycoprotein subunit* (α-GSU) gene, first detected on e11, is the first pituitary hormone transcript. α-GSU is a component of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyrotropin (TSH). α-GSU expression appears to be present in all of the cells of Rathke's pouch. However, targeted ablation of the α-GSU expressing cells results in selective loss of thyrotrophs and gonadotrophs, the cell types that express α-GSU in the mature pituitary *(3)*. Therefore, the α-GSU-expressing cell is not a common precursor. Eventually, the mature gland is populated by at least five highly differentiated cell types, with corticotrophs appearing to arise first. Thyrotrophs arise from two lineages, one related to somatotrophs and lactotrophs. Gonadotrophs also appear to be related to thyrotrophs, somatotrophs, and lactotrophs, because of dependence on common transcription factors. Ultimately, transcription factors are also involved in the cell-specific expression and regulation of the gene products of these pituitary cells, with corticotrophs producing adrenocorticotropin (ACTH), thyrotrophs producing TSH, gonadotrophs producing LH and FSH, somatotrophs producing growth hormone (GH), and lactotrophs producing prolactin (PRL).

Author to whom all correspondence and reprint requests should be addressed: Laurie E. Cohen, M.D., Division of Endocrinology, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, E-mail: cohen_l@a1.tch.harvard.edu

Extracellular Signaling (Fig. 1)

The initiation of anterior pituitary gland development depends on the competency of the oral ectoderm to respond to inducing factors from the ventral diencephalon *(4)*. Bone morphogenic protein (BMP) 4 signal from the ventral diencephalon is the critical dorsal neuroepithelial signal required for organ commitment of the anterior pituitary gland. Wnt5a and fibroblast growth factor 8 (FGF8) are also expressed in the diencephalon in distinct overlapping patterns with BMP4. Subsequently, a BMP2 signal arises from a ventral organizing center that forms at the boundary of a region of oral ectoderm in which Sonic hedgehog (Shh) expression, initially expressed uniformly in the oral ectoderm, is selectively excluded from the developing Rathke's pouch. The ventral BMP2 signal and the dorsal FGF8 signal appear to create opposing activity gradients that are suggested to dictate overlapping patterns of specific transcription factors underlying cell lineage specification events *(4)*. Dorsally-expressed transcription factors include Nkx-3.1 and Sine oculis-like homeobox 3 (Six-3). Ventrally-expressed transcription factors include P-Frk, GATA-2, Islet-1 (Isl-1), and Brn-4. The various extensions of these factors in their fields is suggested to combinatorally determine specific cell types. Temporally specific attenuation of the BMP2 signal is then required for terminal differentiation of the ventral cell types, with the gonadotrophs, thyrotrophs, somatotrophs, lactotrophs, and corticotrophs located ventrally to dorsally, respectively *(4)*. An additional population of thyrotrophs is found in the rostral tip and probably derives from Isl-1 expressing cells *(4)*.

Pituitary Transcription Factors (Fig. 2)

Several pituitary-specific transcription factors, Rpx, Pitx, Lhx3, Prop-1, and Pit-1, play a role in the determination of the pituitary cell lineages.

Rpx

Rpx (**R**athke's **p**ouch homeobo**x**), also known as Hesx1 (**h**omeobox gene e**x**pression in **e**mbryonic **s**tem cells), is a member of the paired-like class of homeobox genes origi-

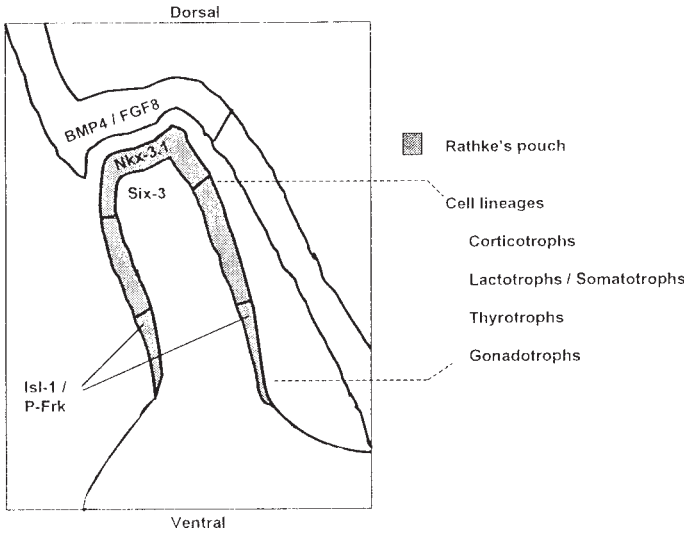

Fig. 1. Pituitary development in the mouse at e10.5. Dorsal signals from the ventral diencephalon (including BMP4 and FGF8) and ventral signals (including Nkx-3.1, Six-3, Isl-1, and P-Frk) form gradients that appear to determine overlapping patterns of specific transcription factors. The result is specific zones of the mature cell lineages (corticotrophs, lactotrophs, somatotrophs, thyrotrophs, and gonadotrophs, at the end of pituitary development (modified from ref. *4*).

nally described in *Drosophila melanogaster (5)*. It is the earliest known specific marker for the pituitary primordium, suggesting that it has a role in early determination or differentiation of the pituitary *(6)*, although no target genes for Rpx/Hesx1 have yet been identified *(7)*. In the mouse embryo, Rpx is first expressed in a small patch of cells in anterior midline visceral endoderm as gastrulation commences *(5)*. It is then found in the anterior midline ectoderm and prechordal plate precursor at e6.5–7, in the anterior neural plate at e7.5–8.5, and subsequently in the oral ectoderm that gives rise to Rathke's pouch at e8.5. By e9.5, Rpx expression is entirely restricted to Rathke's pouch *(5,6)*. From e11.5, Rpx is down-regulated, first in the rostral tip of the incipient anterior lobe and then progressing dorsally. Rpx transcripts are undetectable by e15.5, coinciding with activation of Pit-1 *(6)*. However, Pit-1 is not essential for Rpx regulation, as Rpx repression has normal kinetics in Pit-1-deficient mice. On the other hand, Lhx3 is required for maintenance of Rpx expression, as Rpx is extinguished early at e12.5 in Lhx3-/- mice *(8)*. Prop-1 is required for repression of Rpx transcription, as there is abnormal persistence of Rpx expression until e17.5 in the Ames dwarf mouse, which has a mutation in Prop-1 *(9)*. As Rpx expression is transient, it cannot be involved in maintenance of the anterior pituitary cell types.

Embryonic mice lacking Rpx have bifurcations in Rathke's pouch and pituitary dysplasia, reduced prosencephalon, anophthalmia or microophthalmia, defective olfactory development, and ventral midline defects in the hypothalamus. Neonatal mice have abnormalities in the corpus callosum, anterior and hippocampal commisures, and septum pellucidum, similar to the defects seen in septo-optic dysplasia in man *(5)*. Septo-optic dysplasia is a heterogeneous condition with any combination of optic nerve hypoplasia, pituitary gland hypoplasia, and midline abnormalities of the brain, such as absence of the corpus callosum and septum pellucidum. Two siblings with agenesis of the corpus callosum, optic nerve hypoplasia, and panhypopituitarism were found to have a homozygous mutation at codon 53 in the homeodomain of Hesx1, resulting in conversion of a highly conserved arginine to a cysteine. The result is a drastic reduction in DNA binding to a palindromic PIII sequence, to which mouse Hesx1 has been shown to bind *(5)*.

Pitx

Pitx 1 and 2 have been identified by several groups and have also been given the following names: Ptx1 and Ptx2, P-OTX1 and P-OTX2 (**p**ituitary **OTX**), and Otlx1 and Oltlx2. In addition, the human form of Pitx2 is also known as Rieg. They are paired-like homeodomain transcription factors closely related to the mammalian *Otx* genes that are expressed in the rostral brain during development and are homologous to the *Drosophila orthodenticle (otd)* gene, which is essential for the development of the head in *Drosophila melanogaster (10)*. Their homeobox is also highly homologous to that of *Caenorhabditis elegans* unc-30, which controls differentiation of GABAergic neurons *(11)*. Pitx1 and 2 are 97% identical at the homeodomain and 67% identical at the C-terminus *(9)*. Both are both present in the fetal pituitary and in most cells of the adult pituitary, albeit at different levels *(12)*, suggesting complementary functions in development and function *(9)*.

Pitx1

Pitx1 is first expressed on e8.5 in the mouse embryo in the region of the first brachial arch and in the ventral portion of the caudal-most region. On e9.5, it is expressed throughout the oral epithelium lining the roof of the buccal cavity and in the Rathke's pouch ectoderm. Expression continues throughout development in all regions of the anterior pituitary *(13)*. In adults, a high level of mRNA expression is maintained in the corticotrophs, whereas there is a low level of expression in the other cell types *(10)*. Pitx1 is also expressed in derivatives of the first brachial arch, the duodenum, and the hindlimbs *(13)*. Pitx1 expression overlaps temporally and spatially with Lhx3 *(13)* and is required for sustained expression of Lhx3 *(7)*.

Pitx1 directly activates the α-GSU promoter and is essential for its sustained expression *(7,13)*. Pitx1 is also important for maintenance of cell-specific transcription in corticotrophs (express Pitx1 exclusively) and gonadotrophs (expresses Pitx1 predominantly) *(7)*. It is synergistic with corticotroph upstream transcription factor

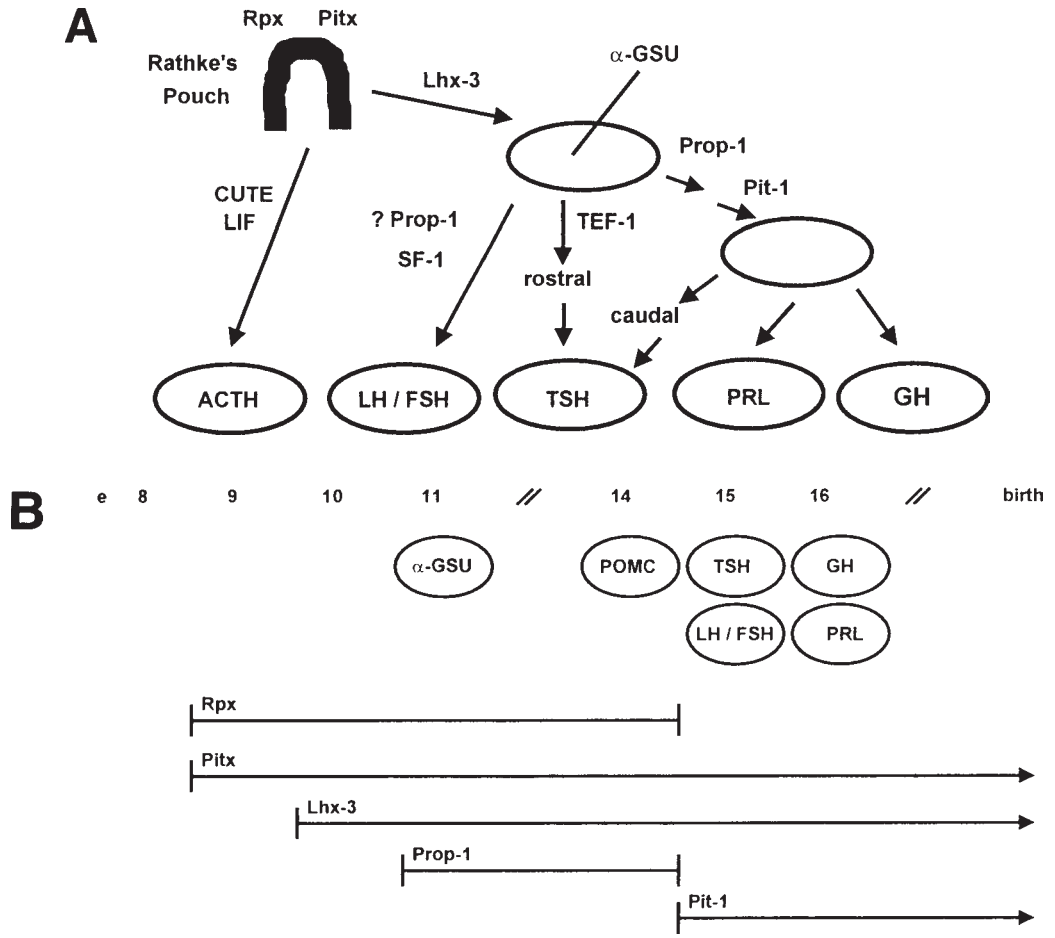

Fig. 2. Pituitary-specific transcription factors involved in anterior pituitary development. **(A)** Homeodomain factors, Rpx, Pitx, Lhx3, Prop-1, and Pit-1, play a role in the determination of specific pituitary cell lineages. **(B)** Time course of detection of pituitary transcription factors and cell lineages in the mouse. The rostral tip Pit-1-independent thyrotrophs appear earlier than the caudomedial Pit-1-dependent thyrotrophs and are not depicted.

(CUTE), a helix-loop-helix factor restricted to the corticotroph lineage, as an important determinant of POMC specificity *(10)*. POMC transcription is also enhanced by other factors, including leukemia inhibitory factor (LIF) *(14)*. Pitx1 is synergistic with steroidogenic factor-1 (SF-1, also known as Ftz-F1) on the *LH-β* promoter *(7)*. SF-1 is an orphan nuclear receptor transcription factor specifically expressed in gonadotroph cells and is required for the maintenance of the gonadotroph phenotype *(15)*. Pitx1 also transactivates the *GH* and *PRL* promoters, synergistically with Pit-1 on the latter *(9)*.

However, in vivo data do not support a role for Pitx1 in *GH* gene expression. In mice made deficient in Pitx1, GH expression in the somatotrophs appears unchanged *(16)*. In these mice, all known early developmental events, including invagination of Rathke's pouch, exclusion of *Shh* from the invaginating epithelium, and activation of *FfF8*, *Bmp2*, *Isl-1*, *Lhx3*, *α-GSU*, *Prop-1*, and *Pit-1* are normal. Evaluation of the pituitary gland from e15.5 through postpartum day 0 (p0) reveals a decrease in the number of gonadotrophs and thyrotrophs and diminished levels of *LH-β* and *TSH-β* transcripts and protein within individual cells. The *TSH-β* transcripts are reduced most severely in the Pit-1-independent rostral tip thyrotroph population. Interestingly, there is an increase, rather than a decrease, in the levels of ACTH transcripts and peptides in the corticotrophs *(16)*. The authors speculate that the altered phenotype may reflect the synergistic role of Pitx1 in target gene induction. These mice also have alterations of skeletal structures within a specific region of the hindlimb, as well as severe defects in the development of the palate and its derivatives. The human *Pitx1* gene maps to the same chromosomal region as Treacher–Collins Syndrome. As these patients have mandibulofacial dysostosis, mutations of the *Pitx1* gene may be responsible for a subset of this disorder.

Pitx2

Pitx2 transcripts are first detected at e8.5 in the mouse embryo in oral epithelium and oral ectoderm. At e9.5, there

is expression of Pitx2 in the nascent Rathke's pouch, as well as expression in the mesenchyme near the optic eminence, the basal plate of the central nervous system, the base of the forelimbs, and in domains of the abdominal cavity. At e16.5, there is a low level of expression in the intermediate lobe and the rostral tip of the anterior lobe of the pituitary gland in an apparently homogeneous pattern *(11)*. Pitx2 is expressed in the adult pituitary gland in the thyrotrophs, gonadotrophs, somatotrophs, and lactotrophs, but not in the corticotrophs, where Pitx1 is highly expressed. Thus, Pitx2 may be a determinant for one or more anterior pituitary cell types or function by acting in concert with other transcription factors. Unlike Pitx1, it is also expressed in the adult in kidney, lung, testis, and tongue *(9)*.

In the chick, *Xenopus*, and mouse, Pitx2 expression is on the left side of the embryo and then continues to be expressed asymmetrically in several organs that are asymmetric with respect to the left–right axis of the embryo. Ectopic expression of Pitx2 results in reversed looping of the heart and intestine and reversed body rotation in chick and *Xenopus* embryos, suggesting that Pitx2 may interpret and subsequently execute the left–right developmental program dictated by upstream signaling molecules *(17)*.

RIEG is the human homologue of Pitx2. In individuals with Reiger syndrome, an autosomal dominant condition with variable manifestations, including anomalies of the anterior chamber of the eye, dental hypoplasia, a protuberant umbilicus, mental retardation, and pituitary alterations, six mutations in one allele of RIEG have been found *(18)*. Five mutations were found to affect the homeobox region: three were missense mutations causing nonconsensus amino acid changes in the homeodomain, and two were splicing mutations in the intron dividing the homeobox sequence *(19)*. Patients with Rieger syndrome do not have an alteration in organ situs, which may be due to the presence of a wild type allele *(17)*.

Two of the missense mutations have been studied. One, a substitution of a glutamine for a highly conserved leucine at codon 54 (L54Q) in helix 1 of the homeodomain, leads to an unstable protein. The other is a nonconserved threonine altered to a proline at codon 68 (T68P) in helix 2 of the homeodomain. T68P binds to the DNA bicoid sequence, but with somewhat lower affinity than wild type Pitx2, and does not allow for the Pit-1 enhanced binding of Pitx2. Unlike wild type Pitx2, the T68P mutant does not transactivate the *PRL* promoter, nor does it yield synergistic activation with Pit-1 *(19)*. *GH* promoter activity was not evaluated, but since there is GH insufficiency in a subset of affected individuals with Rieger syndrome, Pitx2 may also have a role in activation of the *GH* gene *(18)*. Thus, as Pitx1 has a low level expression in lineages other than corticotrophs *(10)*, and Pitx1-deficient mice have a normal number of somatotrophs *(16)*, it is more likely that Pitx2 is the transactivator of the *GH* promoter *(9)*.

Lhx3

Lhx3 is a LIM-type homeodomain protein, where the acronyn LIM comes from the original members of the LIM homeobox genes, ***l****in-11*, ***i****sl-1*, and ***m****ec-3 (20)*. Lhx3 is also known as LIM-3 *(21)* and P-Lim (**p**ituitary **LIM**) *(22)*. The LIM proteins contain two tandomly repeated unique cysteine/histidine LIM domains located between the N-terminus and the homeodomain (the DNA binding domain) *(20)*. The LIM domains do not bind to DNA *(22)* but may be involved in transcriptional regulation *(20)*. There is weak expression of one isoform, Lhx3a, at e8.5 in the mouse embryo, while the other isoform, Lhx3b, does not appear until e9.5. At e9.5, Lhx3 is expressed in Rathke's pouch and the closing neural tube. During subsequent development, there is Lhx3 expression in the anterior and intermediate lobes of the pituitary gland, the ventral hindbrain, and the spinal cord. Lhx3 expression persists in the adult pituitary, suggesting a maintenance function in one or more of the anterior pituitary cell types *(20)*.

Lhx3 is expressed at high levels before the initial detection of α-GSU transcripts and binds to and activates the *α-GSU* promoter. Lhx3 and Pit-1 are synergistic in transcriptional activation of the *TSH-β* and *PRL* promoters and the *Pit-1* enhancer *(22)*. Lhx3 is expressed in GH1 cells, which secrete GH, GH3 cells which secrete GH and PRL, and α-TSH cells which express α-GSU *(20)*, suggesting a common cell precursor for gonadotrophs, thyrotrophs, somatotrophs, and lactotrophs *(8)*. Thus, Lhx3, with other proteins, may activate genes that define pituitary organ commitment. Lhx3 is not in found in AtT20 cells which secrete ACTH, so it is not required for POMC expression *(20)*.

In Lhx3-/- mice, where the two LIM domains and some of the homeodomain are deleted, Rathke's pouch is initially formed but fails to grow. There are also changes in the expression of pituitary specific markers. Rpx is detected at e10.5, suggesting that initial specification of Rathke's pouch occurs and initial expression of Rpx is independent of Lhx3. However, Rpx expression ceases early at e12.5. α-GSU is undetectable at e12.5 and e15.5. TSH-β and GH are undetectable at e16.5. There are no LH positive cells at e18.5, but as there is late activation of LH-β and small numbers of gonadotrophs in wild type mice, it is unclear whether LH-β is missing or decreased. There are also no Pit-1 transcripts, so Lhx3 must be required directly or indirectly for Pit-1 expression *(8)*.

In these mice, POMC is detected in the floor of the diencephalon and in a small cohort of cells at the ventral base of the Rathke's pouch remnant, with placement corresponding to the position of the first presumptive corticotroph cells to differentiate at e13. Specification of the corticotroph cell lineage occurs, confirming that the derivation of the corticotroph lineage must be distinct from that of the other anterior pituitary cell lineages. However, the POMC cells fail to proliferate, suggesting that there is

an intrinsic feature common to all Lhx3-/- pituitary cells, or failure of more than one pituitary cell type to differentiate and produce trophic factors that indirectly affect proliferation of other neighboring cells, or failure to respond to factors produced by adjacent structures or proliferative factors *(8)*.

Homozygotes are stillborn or die within 24 hr of birth. If they survive birth, they have normal size and color and are the same as wild type in their ability to breathe, suckle, and move. They have grossly normal hindbrains, spinal cords, and pineal glands. Deficits in brain stem function are suspected, because Lhx3 is expressed in putative precursor cells of Raphe nuclei and in part of the reticular formation. They have hypoplastic adrenal cortexes evidently secondary to pituitary hormone deficits. Lack of glucocorticoids may be a contributing factor in their inability to survive, but as dexamethasome fails to rescue them, it is not the only cause *(8)*.

Prop-1

Prop-1 (**pro**phet of **P**it-**1**) is a paired-like homeodomain transcription factor *(23)*. Prop-1 expression is restricted to the anterior pituitary. It is first detected at e10–10.5 in the mouse embryo, particularly over the dorsal portion of the gland, and there is maximal expression at e12 over the full caudomedial area in which Pit-1 is later expressed. Expression decreases after e14.5 *(1)*.

The Ames dwarf mouse has a homozygous mutation which involves a serine to proline substitution at amino acid 83 (S83P) in the α-1 helix of the homeodomain. These mice have deficiencies of GH, PRL, and TSH *(24)*. There is normal initial pituitary development, with normal patterns and levels of expression of Pitx and Lhx3 *(24)*. At e13, recently divided cells fail to accumulate in the caudomedial region, where Pit-1 is normally expressed *(25)*, and there is subsequent failure of determination of Pit-1 lineages, lack of *Pit-1* gene activation, and absence of progression to mature cells *(1)*. The size of the nascent pituitary gland is reduced by e14.5, with the adult pituitary size decreased by 85% *(2)*. The adult Ames dwarf mouse has less than 1% of the normal complement of somatotrophs, a scarcity of lactotrophs and thyrotrophs *(24)*, and reduced expression of gonadotropins *(26)*. In contrast to the Ames dwarf mouse, there is normal expression of gonadotropins in Pit-1-defective patients and Snell dwarf mice. Hence, Prop-1 may play a role in directing some of the precursors of the Pit-1 cell lineage into the gonadotroph lineage, prior to terminal differentiation events *(26)*.

The mutant S83P Prop-1 has an eightfold lower affinity DNA binding to two dimeric DNA sites that bind wild type Prop-1 in the *Pit-1* –5 to –10 kb early enhancer, an enhancer required for initial *Pit-1* gene activation. There is reduction in transcriptional activation of these two dimeric DNA binding sites. However, wild type Prop-1 alone fails to clearly activate the enhancer. Therefore, either additional factors or signals are required for a Prop-1 direct effect in the initial action of the *Pit-1* gene early enhancer, or the effects of Prop-1 are indirect *(24)*.

Several human mutations of Prop-1 resulting in combined pituitary hormone deficiency of GH, PRL, and TSH have also been described. Some subjects do not produce LH and FSH at a sufficient level to enter puberty spontaneously *(26)*, while others have a loss of gonadotropin secretion with age, but enter puberty spontaneously (albeit delayed), suggesting that Prop-1 is not needed for gonadotroph determination but may have a role in gonadotroph differentiation *(23)*. The mutant Prop-1 proteins studied have further decreases in binding to, and activation of, Prop-1 target genes relative to the Ames dwarf mouse mutant Prop-1 *(26)*.

Several nonconsanguineous patients from eight different countries have a documented recurring homozygous autosomal recessive mutation of Prop-1, delA301,G302 (also known as 296delGA) *(27)*. This mutation involves a 2 bp GA or AG deletion among three tandem GA repeats (295-CGAGAGAGT-303) of exon 2, which changes a serine to a stop codon at codon 109 (S109X). This results in a truncated gene product with only the N-terminus and first helix of the homeodomain *(27–29)*. This mutant lacks promoter binding and transcriptional activation *(26)*. delA301,G302 has been found in compound heterozygosity with 149delGA, a frame shift deletion causing divergence from wild type Prop-1 after amino acid 50 and resulting in the same S109X mutation as delA301,G302 *(29)*. In one patient, the delA301,G302 has also been found in compound heterozygosity with a phenylalanine converted to an isoleucine at amino acid 117 (F117I). This phenylalanine is part of the core DNA binding motif and almost invariant within the homeodomain family *(26)*. Several other families have a highly conserved arginine mutated to a cysteine at amino acid 120 (R120C), located in the third helix of the homeodomain *(23,26)*.

Pit-1

Pit-1 (official nomenclature now POU1F1), also known as GHF-1 (**G**rowth **H**ormone **F**actor-**1**), is a member of a family of transcription factors, POU, responsible for mammalian development. POU is an acronym for **P**it-1, **O**ct-1, which is widely expressed, **O**ct-2, which is expressed in B lymphocytes and in certain areas of the brain, and **U**nc-86, which functions in *Caenorhabditis elegans* neuronal cell development *(30)*. Pit-1 expression is restricted to the anterior pituitary lobe and was identified by its specific binding to AT-rich cell-specific elements in the rat *PRL* and *GH* genes *(32)*.

Pit-1 contains two protein domains, termed POU-specific (POU-S) and POU-homeo (POU-H), which are both

necessary for high-affinity DNA binding on the *GH* and *PRL* genes *(33,34)*. X-ray crystallographic evidence suggests there are four α-helices present in the POU-S and three α-helices present in the POU-H. Pit-1 usually binds to multiple sites on target genes, and dimerization of Pit-1 on DNA appears to be important for high-affinity DNA binding *(36–38)*. The third α-helices of the POU-S and POU-H make the majority of contacts with the major grooves of DNA, and Pit-1 forms dimers on DNA by interactions between the POU-S domain of one molecule and the C-terminus of the POU-H of the other *(35)*. When bound to DNA, Pit-1 activates *GH* and *PRL* gene expression, in part, through an N-terminal transactivation domain rich in hydroxylated amino acid residues (serine- and threonine-rich) *(32,36,37)*.

In addition to its role in cell-specific gene expression and regulation, Pit-1 has been shown to be essential for the development of certain anterior pituitary cells. Pit-1 is first detected on e13.5 in the mouse in cells occupying a central position in the primordial pars distalis, and then one day later in the entire pars distalis *(39)*. Pit-1 transcripts initially appear in cells of the caudomedial region of the anterior pituitary gland on e14.5 and are exclusively in these cell types by e15. Pit-1 protein is detected in the somatotrophs and lactotrophs, preceding *GH* and *PRL* gene expression on e16 and 17, respectively, suggesting that Pit-1 is the major cell-specific activator of hormone expression from these cell types *(40)*.

Pit-1 protein is also expressed in the thyrotrophs *(40)*. Thyrotrophs appear to arise from two independent cell populations in mice. The first population is Pit-1-independent and transient; it appears on e12 in the rostral tip of the developing anterior pituitary gland prior to the first detectable expression of Pit-1 on e14.5, but phenotypically disappears by the day of birth. The second population is Pit-1-dependent and arises subsequently in the caudomedial portion of the developing pituitary gland on e15.5, following the initial expression of Pit-1 in this area. Pit-1 appears necessary for the appearance of these precursors of the mature thyrotroph cell type based on the following observations: (1) caudomedial thyrotroph cells are not present in the Pit-1 defective Snell dwarf mouse, and (2) Pit-1 can bind to and transactivate the *TSH-β* promoter *(41)*. A PAR-bZIP protein, thyrotroph embryonic factor (TEF), may be involved in the initial expression of the mouse *TSH-β* promoter, as it is first selectively expressed in the rostral tip cells of the anterior pituitary concomitantly with the activation of *TSH-β* gene expression. TEF binds to and can effectively transactivate the *TSH-β* promoter *(42)*.

Pit-1 transcription is detected before the appearance of Pit-1 protein, and therefore, Pit-1 can not be responsible for developmental activation of its own gene *(43)*. In analysis of the Snell dwarf mouse (*see* below), Pit-1 transcripts appear at the normal time in the expected region of the pituitary gland, but by e18.5, there is a significantly decreased level of Pit-1 compared to wild type mice. Nevertheless, Pit-1 expression is detectable until postnatal days 0–5 *(44)*. These findings suggest that once Pit-1 protein has reached a critical threshold, autoregulation is subsequently required to sustain *Pit-1* gene expression. Additional transcriptional regulation likely maintains Pit-1 transcription as well. For example, retinoic acid (RA) induction of the *Pit-1* gene requires both the retinoic acid receptor (RAR) and Pit-1 *(44)*.

Naturally occurring mutations in the *Pit-1* gene have confirmed that Pit-1 is essential for the development of certain anterior pituitary cells. The Jackson dwarf mouse has a gross structural alteration of the *Pit-1* gene with either an inversion or insertion of a greater than 4 kb segment of DNA. These animals have hypoplastic anterior pituitaries, combined pituitary hormone deficiency (CPHD) of GH, PRL, and TSH, and no *Pit-1* gene expression *(45)*. Snell dwarf mice also have hypoplastic anterior pituitaries and CPHD, but they have a low level of Pit-1 expression. These mice have a tryptophan altered to a cysteine in codon 261 (W261C) in the putative recognition helix of the POU-H. This mutant Pit-1 does not bind a high affinity Pit-1 site in the *PRL* promoter, Prl-1P *(45)*.

A number of humans with CPHD and *Pit-1* gene mutations have also been described. The inheritance pattern and phenotypic presentation is quite different among these patients, reflecting the location of the mutation in Pit-1. A C to T sporadic mutation, changing an arginine to a tryptophan in codon 271 (R271W) in one allele of the *Pit-1* gene, is the most common mutation and has been described in several unrelated patients of different ethnic backgrounds *(46–53)*. The tryptophan substitution reduces the positive charge in a basic amino acid region of Pit-1. Mutant R271W Pit-1 binds normally to DNA, but the mutant protein acts as a dominant inhibitor of transcription *(46)*. Thus, the mutation need only be present in one allele to cause CPHD.

A patient with GH deficiency, but dysregulation of PRL and TSH has an amino acid change from a lysine to a glutamic acid at codon 216 (K216E). The mutant Pit-1 binds to DNA and does not inhibit basal activation of the *GH* and *PRL* genes. However, the mutant Pit-1 is unable to support RA induction of the *Pit-1* gene distal enhancer either alone or in combination with wild type Pit-1. Thus, the ability to selectively impair interaction with the superfamily of nuclear hormone receptors is another mechanism responsible for CPHD *(54)*.

Two Dutch kindreds have an alanine to proline conversion at codon 158 (A158P). One family has homozygous autosomal recessive inheritance, while members of the other family are compound heterozygotes with one absent Pit-1 allele. The A158P mutant Pit-1 has a minimal decrease in

DNA binding. However, it has no to low transcriptional activation of the *GH*, *PRL*, and *Pit-1* promoters *(55)*.

Seven Middle Eastern patients with hypopituitarism from three unrelated families have a homozygous proline to serine conversion at codon 239 (P239S) at the beginning of the second α-helix of the POU-H. The P239S Pit-1 mutant binds to DNA but has decreased *GH* promoter activation *(56)*.

Another patient has compound heterozygosity of mutations lying within exon 4 in a region that encodes the third α-helix of the POU-S. One mutation is a glutamate to glycine conversion at codon 174 (E174G) in the POU-H. The E174G Pit-1 mutant has 1% specific binding activity. The other mutation is a nonsense mutation at codon 172 (R172X) resulting in a truncated protein *(57)*. The R172X Pit-1 mutant should lose transcriptional activation, as well as DNA binding, as the third α-helix of the POU-S and the entire POU-H are lost. The R172X mutation is homozygous in another patient *(58)*.

Other *Pit-1* gene mutations have been identified but not characterized. Autosomal recessive mutations include (1) the conversion of an arginine to a glutamine in codon 143 (R143Q) *(48)*. This mutation might disrupt DNA binding by interfering with stabilization of Pit-1 on a negatively charged DNA backbone, as it is found in the first α-helix of the POU-S; (2) a phenylalanine to cysteine conversion at codon 135 (F135C) found in four siblings with complete GH deficiency, born to consanguineous parents, who only later developed central hypothyroidism and were found to have undetectable PRL levels. This mutation is found within the hydrophobic core of the POU-S, near the dimer interface of the POU-S, and might affect packing or stability of the protein domains *(59)*; (3) a nonsense mutation in codon 250 which alters a glutamate to a stop codon (E250X), with the complete loss of helix 3 of the POU-H and presumably loss of DNA binding *(60)*. Another patient has a proline converted to a leucine in codon 24 (P24L) in only one allele, which has been postulated to disrupt transactivation *(48)*.

Somatotroph Development

Additional factors have been identified that are important in the determination of the pituitary cell lineages. Somatotroph development, in particular, may be dependent on both CREB and the growth hormone releasing hormone (GHRH) receptor. Transgenic mice, overexpressing a transcriptionally inactive mutant form of CREB which can not be phosphorylated, have a dwarf phenotype. Their pituitary glands are atrophied and markedly deficient in somatotrophs, suggesting that transcriptional activation of CREB is necessary for the normal development of somatotrophs *(61)*. The *little* mouse has an autosomal recessive mutation of an aspartic acid to a glycine in codon 60 of the growth hormone releasing hormone (GHRH) receptor *(62)*. These mice have a reduced number of somatotrophs with reduced granules, resulting in a reduction of GH mRNA and GH protein levels *(63)*. These data indicate that the GHRH receptor may play a role in somatotroph proliferation.

Interestingly, ablating somatotrophs in transgenic mice results in the loss of lactotrophs as well, suggesting that both cell lines derive from a common precursor *(64)*. Indeed, simultaneous plaque assays and double-staining immunocytochemistry studies reveal some cells that secrete both GH and PRL *(65)*. Additional nuclear factors appear to be necessary for full expression of these target genes and may play a role in differentiation. For example, a zinc-finger transcription factor, Zn-15, synergizes with Pit-1 to activate the GH promoter *(66)*, with maximal levels of GH expression seen by e19–20. The estrogen receptor (ER), in a synergistic effect with Pit-1, appears to be capable of mediating the progressive increase in *PRL* gene expression characteristic of the mature lactotroph phenotype, with full PRL gene activation appearing developmentally after birth *(40)*.

Summary

Extrinsic and intrinsic signaling gradients determine expression patterns of pituitary-specific factors in the developing anterior pituitary gland. The temporal and spatial relations of these developmental factors are required for the determination of each of the pituitary cell lineages. Rpx is required for early differentiation of the anterior pituitary. The determination of the somatotroph cell line is dependent on the transcription factors Lhx3, Prop-1, and Pit-1. Pit-1 also plays a role in the activation and regulation of the somatotroph gene product, GH. Additional factors such as CREB and the GHRH receptor, may be involved in somatotroph determination, while Zn-15 and Pitx2 may be involved in *GH* gene activation.

Addendum

Since submission of this manuscript, Pitx2 knock-out mice have been generated *(67)*. These animals have normal formation of Rathke's pouch, but decreased cell content by e10.5. There is failure of organ progression with undetectable levels of Pit-1, TSH-β, and Lhx4, and only a few POMC-positive cells.

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