Pituitary Development: Regulatory Codes in Mammalian Organogenesis

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During mammalian pituitary gland development, distinct cell types emerge from a common primordium. Appearance of specific cell types occurs in response to opposing signaling gradients that emanate from distinct organizing centers. These signals induce expression of interacting transcriptional regulators, including DNA binding–dependent activators and DNA binding–independent transrepressors, in temporally and spatially overlapping patterns. Together they synergistically regulate precursor proliferation and induction of distinct cell types. Terminal cell type differentiation requires selective gene activation strategies and long-term active repression, mediated by cell type–specific and promoter-specific recruitment of coregulatory complexes. These mechanisms imply the potential for flexibility in the ultimate identity of differentiated cell types.

The pituitary gland is a critical component of the neuroendocrine system that is present in all vertebrates. It is essential for the maintenance of homeostasis, metabolism, reproduction, growth, and lactation. The synthesis and secretion of trophic hormones from distinct endocrine cell types in the pituitary gland is controlled by the central nervous system (via neuropeptides from the hypothalamus) and by positive and negative feedback loops from peripheral organs. The six endocrine cell types of the anterior pituitary gland elaborate proopiomelanocortin (POMC) [which is proteolytically cleaved to adrenocorticotropic hormone (ACTH) in corticotropes and melanocyte-stimulating hormone (MSHα) in melanotropes], growth hormone (GH) in somatotropes, prolactin (Prl) in lactotropes, thyroid-stimulating hormone (TSH) in thyrotropes, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in gonadotropes (Fig. 1). There is also an embryonic cell type referred to as the rostral tip "thyrotrope" (Fig. 1).

The pituitary gland develops in tandem with the specific hypothalamic nuclei that ultimately regulate homeostatic responses in the mature organism [reviewed in (*1–3*)]. Ablation and fate mapping experiments in frogs, chicks, and mice localized the pituitary anlage to the midline portion of the anterior neural ridge (ANR), immediately anterior to the cells in the neural plate that give rise to the endocrine hypothalamic components of the ventral diencephalon (*4–7*) (Fig. 1). A broad region of the ANR is competent to adopt a pituitary fate if subsequently induced by the ventral diencephalon (*4, 6*).

After displacement of the midline ANR cells by growth of the forebrain (*4*), overt organogenesis of the anterior pituitary gland begins at embryonic day 8.5 (E8.5) as the cells of the anterior pituitary placode in the oral ectoderm thicken and invaginate to form the nascent pituitary referred to as Rathke's pouch (*4–7*). Because of the absence of intervening mesenchyme, the dorsal portion of Rathke's pouch directly contacts the midline ventral diencephalon, which evaginates on E10 and acts as a key organizing center for the patterning and commitment of Rathke's pouch (Fig. 2). The six mature endocrine cell types emerge in a temporally and spatially specific fashion, as schematically indicated in Fig. 1, from E12.5 to E17.5. A neural component of the pituitary gland, the posterior pituitary, consists of axons and nerve endings of neurosecretory neurons.

In this review, we discuss genetic and biochemical studies that have elucidated the molecular mechanisms by which specific cell types arise from a common primordium (*1–3*).

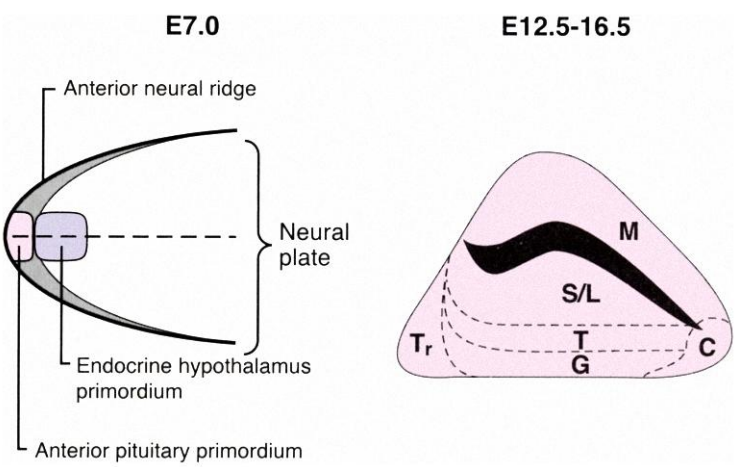

Fig. 1. Development of the hypothalamic-pituitary axis. The midline embryological primordia of the pituitary in the anterior neural ridge and of the endocrine hypothalamus in the neural plate are indicated. Cell types later arise in a temporally and spatially specific fashion: E12.5 corticotrope (C), E12.5 rostral tip thyrotropes (Tr), and subsequently, from E15.5 to E17.5, from somatotropes (S), lactotropes (L), thyrotropes (T), gonadotropes (G), and melanotropes (M).

Signaling Regulation

Initial proliferation and determination is controlled by sequential exogenous and endogenous combinatorial signaling. Subsequent attenuation of specific signals permits terminal differentiation (Fig. 2).

Extrinsic signals. The initial "extrinsic" signaling phase of murine pituitary development requires signals from both the ventral diencephalon and oral ectoderm (Fig. 2). The ventral diencephalic signals include members of the bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and *Wnt* gene families. The onset of expression of these factors coincides with the initial development of Rathke's pouch. Their expression differentially attenuates as specific cell lineages emerge (*8–10*). Another signal, Sonic Hedgehog (Shh), emanates from the oral ectoderm (*8*) (Fig. 2).

FGF signaling (*8, 9*) plays an instructive role by inducing the gene encoding the LIM homeodomain transcription factor Lhx3/P-Lim, which is required for progression of pituitary development beyond the initial invagination of Rathke's pouch (*11, 12*). Deletion of the gene encoding FGF10 or the FGF receptor type 2

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(FGFR2) IIIb isoform, an FGF10 receptor (*13*), results in failure to proliferate and rapid apoptosis after formation of Rathke's pouch. High levels of either FGF2 or FGF8 inhibit expression of cell determination markers such as POMC in organ culture (*9*), whereas sustained overexpression of FGF8 in vivo within the gland results in a marked overproliferation and loss of pituitary cell type–specific markers other than POMC (*8*).

BMP4 is also required for continued organ development after pouch formation (Fig. 2), because targeted expression of the BMP2/4 antagonist Noggin in vivo results in the arrest of pituitary development at E10 (after invagination) and the absence of all endocrine cell types except for a few POMC-expressing cells (*8*). Deletion of BMP4 causes embryonic death at ~E10 and failure of invagination of Rathke's pouch (*14*), although conditional gene deletion of *BMP4* will be required to establish direct causality. These phenotypes indicate that ventral diencephalic FGFs and BMP4 are required for initial organ commitment, proliferation, and progression.

Shh is expressed throughout the oral ectoderm on E8 but is excluded from the invaginating Rathke's pouch, thereby creating a clear boundary of Shh expression within the oral ectoderm. Targeted expression of the Hedgehog inhibitor HIP (Hedgehog interacting protein) in mice (*15*) and analysis of *you-too* mutants (homolog of the Shh-induced mediator Gli) in zebrafish [reviewed in (*3, 15*)] have shown that Shh is required for pituitary proliferation and patterning after E10—acting with FGFs to sustain ventral expression of Lhx3 (*12, 13, 15*)—and inducing of intrinsic BMP2 expression in the ventral pouch. This is analogous to the sequential and cooperative roles of BMPs and Hedgehog in limb and neural tube development and in organizing anterior-posterior patterning in the *Drosophila* wing (*3, 16–18*). Conversely, overexpression of Shh causes hyperproliferation of ventral gonadotropes and thyrotropes (*15*). Wnt factors, such as Wnt4, also regulate proliferation of anterior pituitary cell types (*8*).

Intrinsic signaling. Subsequent patterning of Rathke's pouch is governed by intrinsic and ventral mesenchymal signals, including BMP2 and Wnt4 expressed in the developing gland, which establish positional identity and stimulate proliferation of specific ventral cell types. *BMP2* is initially expressed in a ventral-dorsal gradient at E10.5, but by E12.5 its expression has expanded throughout the pouch (*8, 9*). Extrinsic signals emanating from ventral condensing mesenchyme beneath the developing pituitary gland include Indian Hedgehog (IHH), Wnt4, and BMP2. Caudal mesenchyme is a source of a Chordin signal that can oppose the function of BMP2 (*8*) (Fig. 2). In vivo inhibition of BMP2/4 actions causes loss of the Pit-1 lineage (see below) and gonadotropes, but not of POMC-expressing cells.

Signal attenuation. Although opposing dorsal → ventral FGF8/10/18 and ventral → dorsal BMP2 gradients appear to be associated with the positional determination of specific cell types (Fig. 2), attenuation of BMP signaling is also required for progression to terminal differentiation of pituitary cell types (*8*). Together, the combinatorial signal regulation of pituitary is analogous to that in many organs, including spinal cord, lung, and tooth [e.g., (*17, 18*)].

Combinatorial Transcriptional Regulation of Cell Type Determination

The transient signaling gradients result in the induction of expression of transcription factors in spatially overlapping patterns, as diagrammed in Fig. 2, which are proposed to be cell-autonomous determinants of pituitary cell fate. These transcription factors may be considered to act as a molecular memory of prior signals in the positional determination of specific cell types. They include classes of factors linked to development of other organs, including LIM homeodomain, paired-like homeodomain, bicoid-like homeodomain, and sine oculus–related factors (Fig. 3). Many of the initially expressed factors appear to be required to regulate expansion of the multipotent precursor cell population.

LIM homeodomain factors. Multiple members of the LIM homeodomain family of transcription factors are expressed at various stages in the developing Rathke's pouch, including Lhx3 (P-Lim/mLim3), Lhx4 (Gsh4), Lhx2, and Isl-1 [reviewed in (*1–3*)]. However, there is as yet no evidence of a combinatorial LIM homeodomain factor code in pituitary development analogous to that specifying distinct spinal cord motor neuron types (*19*). In *Lhx3* mutant mice, development ceases after the rudiment of Rathke's pouch forms and only a few corticotropes are present, hinting that specification of this cell type is Lhx3/4-independent (*11, 12*). In the absence of Isl-1, which is initially expressed throughout the pouch (perhaps under BMP regulation) and then restricted to the ventral region as the ventral

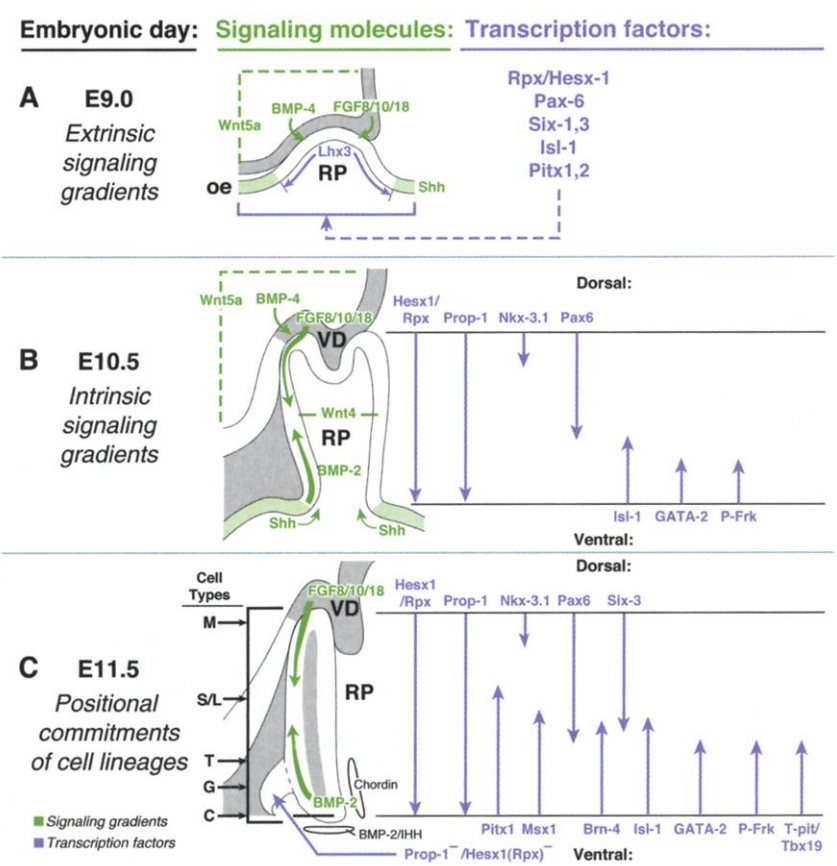

Fig. 2. Signaling and transcriptional strategies in pituitary development. Diagrams show exogenous and endogenous signaling gradients involved in pituitary organogenesis, and the spatially overlapping distribution of induced transcription factors. (**A** and **B**) Signals from the ventral diencephalon include Wnt5a, BMP4, FGF8, and FGF10. The ectoderm provides a ventral Shh signal. By E10.5, the ventral → dorsal BMP2 gradient in Rathke's pouch, as well as signals from ventral condensing mesenchyme (BMP2, IHH) and from caudal condensing mesenchyme (Chordin), are hypothesized to induce a series of transcription factors. (**C**) These signaling gradients result in positional commitment of cell lineages by E11.5 in a dorsal → ventral fashion, schematically diagrammed as putative regions from which mature cell types will ultimately emerge.

→ dorsal BMP2 gradient appears (*8, 9*), there is a failure of proliferation after invagination of Rathke's pouch (*19*).

Paired-like homeodomain factors. One potent strategy seen in temporal steps of organogenesis uses opposing actions of related repressors and activators capable of binding to similar DNA sequences (Figs. 2 and 3). In the case of pituitary development, two related paired-like homeodomain proteins, which exert opposing functions, are both required (Fig. 3). The repressor Rpx/Hesx1 (*20, 21*) is expressed only in early pituitary development, before the appearance of all terminal differentiation markers except POMC (*22*). *Prophet of Pit-1* (*Prop-1*), which encodes a structurally related paired-like homeodomain activator, is initially detected at E10 to E10.5, with robust expression continuing until E13.5 to E14.5 (Fig. 2). Analyses of the murine *Prop-1* hypomorphic mutation (*22, 23*) and studies of families with combined pituitary hormone deficiency involving more severe mutations in the human *PROP1* gene (*24*) suggest that the *Prop-1*$^{-/-}$ genotype causes failure of initial proliferation of the three Pit-1–dependent cell types and of gonadotropes (*22, 23*).

The Rpx/Hesx1 (*20, 25*) repressor can heterodimerize with Prop-1 and inhibit its gene activation properties (*26*), analogous to antagonism by Mix1/Siamois paired-like homeodomain factors in *Xenopus* development (*3*). *Rpx/Hesx1* appears to be important for initial progression and proliferation of the pituitary gland, whereas its subsequent down-regulation appears to permit a temporal switch that leads to emergence of Prop-1–dependent lineages at E13.5 (*22, 23, 26*). Using a conserved domain (*27*), Rpx/Hesx1 interacts with a member of the groucho/transducin-like enhancer of split family (TLE), which exhibits similar spatial and temporal patterns of expression in the pituitary gland, and is required to mediate the strong repressor actions of Rpx/Hesx1 (*26*).

Additional homeodomain regulators. Additional transcription factors are required to modulate ventral/dorsal determination. For example, Pax6 exhibits a dorsal → ventral gradient of expression, and *Pax6* mutant mice exhibit an increased number of ventral thyrotropes and gonadotropes, at the expense of the more dorsal somatotropes and lactotrope cell types (*27, 28*), analogous to the ventral neural tube phenotype of *Pax6* mutant mice (*29*). Thus, Pax6 may functionally oppose Shh signaling to specify a dorsal rather than ventral cell fate. Deletion of a genomic area that encompasses multiple transcription units including several *sine oculus* and *optix*–related genes, including *Six6*, is reported to cause human pituitary anomalies (*30*).

Pitx factors. Two bicoid-related Pitx homeodomain factors, Pitx1 and Pitx2, display distinct but overlapping patterns of expression and exert roles in the development of several organs, including pituitary [reviewed in (*1–3*)]. Targeted disruption of the *Pitx1* gene leads to diminished expression of terminal differentiation markers for gonadotrope and thyrotrope cells, and increased *POMC* gene expression, as well as craniofacial and hindlimb morphogenesis defects (*31, 32*). In *Pitx2*$^{-/-}$ mice, the pituitary gland fails to progress beyond E10.5 and is characterized by defects in early proliferation and patterning (*25, 33*); these findings imply that Pitx factors may serve as cell-specific components of signaling pathways that regulate cell proliferation. Deletion of the *Pitx2* gene also results in more severe developmental defects, including block of tooth development, failure of ventral body wall closure, and right lung isomerism (*25, 33, 34*).

Determination and Terminal Differentiation of Specific Cell Types

What are the subsequent molecular mechanisms by which the six mature endocrine cell types emerge from the patterned organ? Anterior pituitary cell types are initially positionally determined as they emerge from proliferation zones (*8, 9*), with the somatotrope/lactotrope cells arising caudomedially and gonadotropes more rostroventrally, corticotropes ventrally, and melanotropes dorsally (Fig. 2). For each cell type to progress beyond initial patterning by transient signaling gradients (*8*), induction of additional specific transcription factors is required (*36–39*). These transcription factors include Pit-1 (somatotropes, lactotropes, thyrotropes) (*36*); the orphan nuclear receptor SF1 (*37*) and Egr-1 (*38*) (gonadotropes); and T-pit and possibly STAT3 (*POMC* gene-expressing cells) (*39, 40*).

Pit-1 lineage. Pit-1, a POU domain protein (*34*), is required for terminal differentiation of the somatotrope, lactotrope, and thyrotrope cell types, as established by genetic analysis of Snell and Jackson dwarf mice (*36*). Pit-1 directly controls regulatory genes, including receptors involved in growth and homeostatic control (*41, 42*). The Pit-1 lineage has provided a model to investigate a key issue in organogenesis—the molecular mechanisms underlying transition from positional identity to cell-autonomous commitment. The Pit-1 lineage can be converted to alternative fates before E17.5 but exhibits a permanent cell-autonomous commitment after E17.5, when *Pit-1* gene regulation shifts from a Pit-1–independent to a Pit-1–autoregulated enhancer.

Thyrotropes and gonadotropes. One cell type in the Pit-1 lineage, the thyrotrope, shares molecular markers, including the α

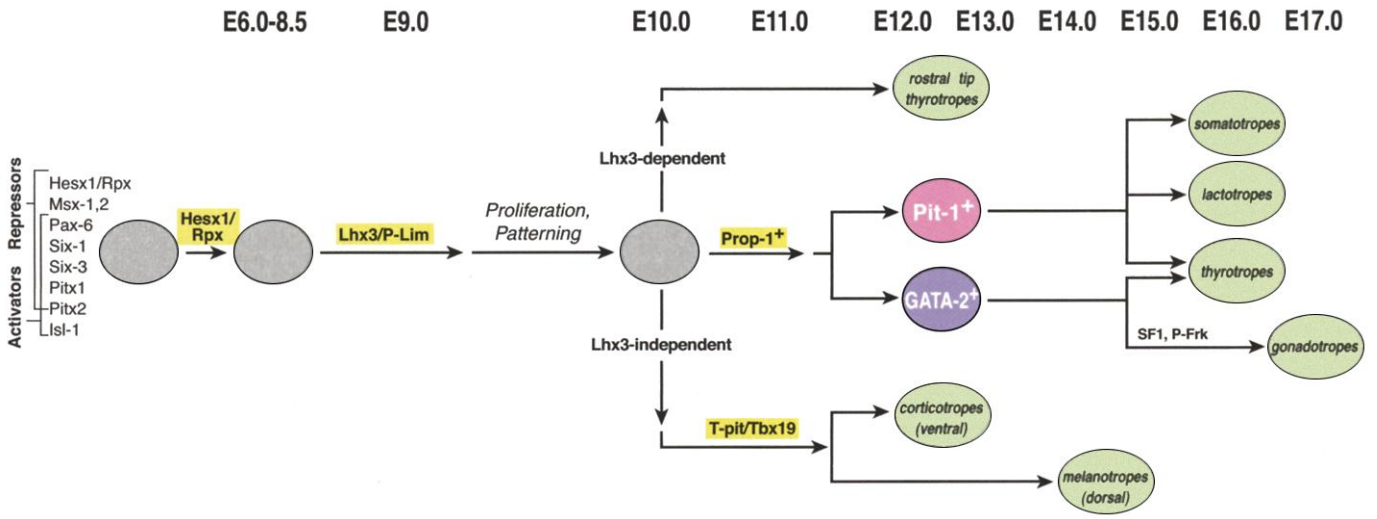

Fig. 3. Transcriptional regulators and cell lineage commitments. Several of the transcription factors that are critical for determination and differentiation, with the temporal aspect of cell type appearance, are indicated.

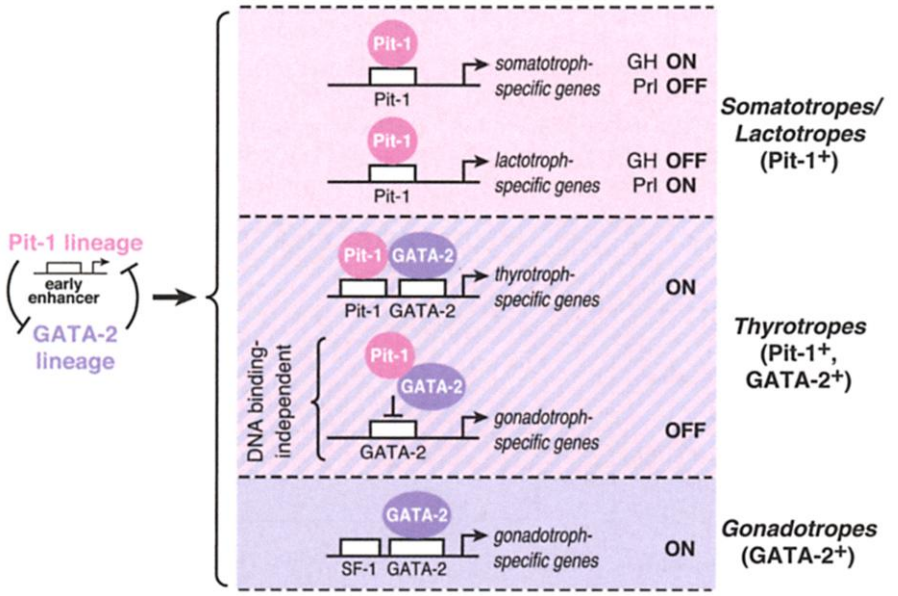

Fig. 4. A model of a proposed role of a BMP2-induced gradient of GATA-2 expression, acting with Pit-1, in determination of gonadotrope and thyrotrope lineages. In thyrotropes, Pit-1 exerts a DNA binding–independent transrepression to inhibit GATA-2–dependent activation of gonadotrope cell type–specific promoters; Pit-1 and GATA-2 synergistically activate TSHβ. GATA-2 may directly or indirectly inhibit activity of the Pit-1 early enhancer. Pit-1 but not GATA-2 is expressed and required in somatotropes and lactotropes. White boxes denote response elements.

glycoprotein subunit (αGSU), with the Pit-1–independent gonadotrope lineage. One ventrally induced factor, GATA-2 (Fig. 2), originally identified as a factor required for hematopoietic system development, has proven to be induced by the ventral → dorsal BMP2 signal (*8, 43*) and appears to be an important component of the gonadotrope and thyrotrope developmental programs. In gonadotropes, GATA-2 functions epistatically to other required factors, including SF1 (*43*) (Fig. 3). Plasticity in the determination of the Pit-1 lineages and gonadotropes is suggested by the observation that targeted expression of Pit-1 ventrally in vivo is sufficient to convert gonadotropes to thyrotropes, whereas dorsal expression of GATA-2 is sufficient to convert all of the Pit-1–dependent cell types to gonadotropes through activation of factors such as SF1 (Fig. 4).

At the highest levels of expression in the presumptive gonadotropes, GATA-2 appears to inhibit initial activation of the *Pit-1* gene (*43*), but at lower levels in thyrotropes, both Pit-1 and GATA-2 are expressed (diagrammed in Fig. 4). Inhibition of gonadotrope-specific genes by Pit-1 in thyrotropes is suggested to occur in part because Pit-1, expressed at very high levels, can inhibit GATA-2 binding to promoters that do not contain an adjacent Pit-1 site. This effect occurs independently of Pit-1 DNA binding and reflects specific interactions between the POU domain of Pit-1 and a critical DNA binding zinc finger of GATA-2 (*43*). In contrast, on specific genes that contain adjacent Pit-1 and GATA-2 binding sites such as the TSHβ promoter (*43, 44*), Pit-1 and GATA-2 act synergistically. Thus, Pit-1 functions as a DNA binding–independent transrepressor and a DNA binding–dependent activator, and both functions are based on conserved sequences in its DNA binding domain (Fig. 4). This is likely to be a general strategy in organogenesis.

Somatotropes and lactotropes. Multiple Pit-1 DNA binding sites are present in the cis-acting sequences required for cell type–specific expression of the rat growth hormone and prolactin genes, and binding of Pit-1 is required for their activation (*1–3*). The minimal growth hormone gene information required for selective expression in somatotropes, but not lactotropes, resides in the proximal promoter, which contains evolutionarily well-conserved sequences, including two Pit-1 binding sites (*3, 45*). On the basis of in vivo evidence of an allosteric effect of a Pit-1 DNA site, cocrystals of the Pit-1 POU domain dimer bound to growth hormone and prolactin promoter cognate sites were analyzed. The data revealed that the spacing between the DNA contacts made by the POU-specific domain (POU_S) and the POU homeodomain (POU_H) of each monomer is increased by two base pairs on the growth hormone element (Fig. 5). Deletion of these two base pairs results in a failure to effectively restrict reporter gene expression from lactotropes, suggesting that a critical transcriptional activator of cell type–specific gene targets is also involved in active repression of the same gene targets in other cell types (*45*).

Pit-1 can associate, directly or indirectly, with either coactivators or corepressors, including N-CoR and histone deacetylases (*46*). Indeed, the required coactivator components have proved to be signaling pathway dependent (*46*), as exemplified by the Ras-dependent activation of ets/Pit-1 synergy in prolactin gene activation (*47, 48*). Whereas both Pit-1 and thyroid hormone receptor β ($T_3R\beta$) are present on the growth hormone gene promoter, when it is transcribed (in somatotropes) and when it is not transcribed (as in lactotropes), the corepressor N-CoR is associated only with the nontranscribed promoter and it appears to be required in vivo for cell type–specific restriction (*45*). Active repression of the growth hormone gene requires the actions of at least two other factors, consistent with assembly of a "repressosome" complex that interacts with components of the corepressor machinery in the appropriate cellular context (Fig. 5). Regulation of expression or modification of any of these factors may underlie cell type specification. Together, these data suggest a potentially flexible commitment to terminally differentiated cell types that might be prototypic of events in many organs.

The human growth hormone gene, present

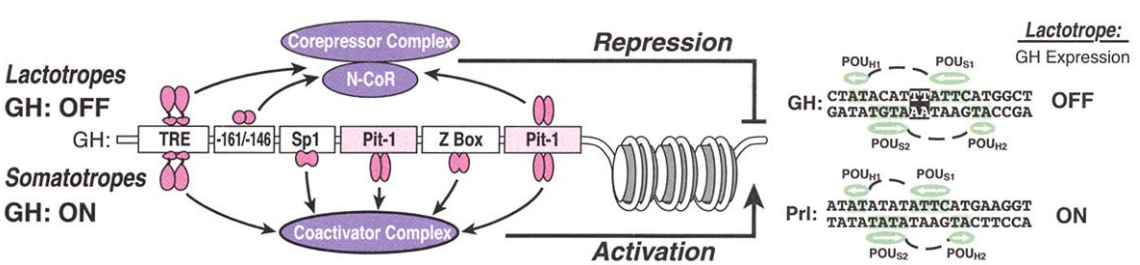

Fig. 5. Model of "repressosome"-based recruitment of a corepressor complex in cell type–specific long-term active repression of growth hormone gene expression in lactotropes. The altered spacing of the Pit-1 POU_S and POU_H on growth hormone and prolactin sites, in concert with additional DNA binding factors, dictates recruitment of an N-CoR–containing corepressor complex, causing restriction of growth hormone gene expression in the lactotrope.

in a cluster of five related genes, requires additional regulation by a Pit-1–dependent locus control region (LCR), located 14 to 16 kb upstream, for cell type–specific expression (*49*), which is itself dependent on Pit-1 binding sites.

POMC lineages. Cytokines such as LIF, acting via STAT3, can induce POMC gene expression and expand the POMC population, suppress Lhx3 expression, and decrease the number of gonadotropes, somatotropes, and lactotropes (*40, 50*). On the basis of the ventral pattern of initial POMC gene activation in the most peripheral aspects of the developing gland, it is possible that microvasculature invading from surrounding mesenchyme provides a source of cytokines or other critical signaling molecules. Recently, a T-box factor, T-pit, first identified in humans as Tbx19, has been shown to be selectively expressed in POMC-producing cells and to be capable of activating the *POMC* gene promoter (*39*). Misexpression of this T-box factor in vivo, under αGSU regulatory sequences, results in expression of ACTH in normally nonexpressing rostral tip cells (*39*) and a decrease in αGSU and TSHβ expression (*51*). In human kindreds, defective ACTH production is clearly correlated with mutations of the human *Tbx19/T-pit* gene (*39*), indicating that T-pit is an important component of the corticotrope developmental program. Many other factors, including Pitx1/Pitx2, Nur77, and Neuro D1 (*39, 50, 52–54*), regulate *POMC* gene expression in model cell lines, but their exact biological roles in *POMC* gene expression remain to be determined.

Conclusions

The development of specific cell types in the pituitary is directed by transient signaling gradients that induce nuclear mediators of cell type commitment, including transcription factors acting as repressors or activators, and their associated coregulators. These factors integrate, at the level of gene expression, the output in response to multiple signaling pathways. A more comprehensive understanding of the molecular strategies that underlie cell determination and proliferation in the pituitary can be expected with the availability of ever more powerful cell biological, genomic, and genetic screening approaches.

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