Combinatorial codes in signaling and synergy: lessons from pituitary development
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The development of the hormone-secreting cell types in the pituitary gland provides an excellent model system in which to explore the complex transcriptional mechanisms underlying the specification and maintenance of differentiated cell types in mammalian organogenesis. Pituitary development is orchestrated through the combinatorial actions of a repertoire of signaling-gradient-induced transcription factors which, on the basis of their distinct and overlapping expression patterns, and functional interactions, ultimately has led to the generation of functionally distinct cell phenotypes from a common ectodermal primordium.

Introduction
The pituitary gland exerts critical roles in the homeostasis of vertebrates, integrating signals from the hypothalamus and periphery, regulating vital processes in metabolism, reproduction, and growth. The mature pituitary gland consists of six cell types — somatotropes, lactotropes, thyrotropes, gonadotropes, corticotropes, and melanotropes — characterized by the synthesis and secretion of cell type specific hormones (Figure 1). The expression of these distinct molecular markers, and the spatial and temporal pattern in which the determination of these cell types occurs, has made the study of the development of the anterior pituitary gland a favorable model system for exploring the molecular mechanisms of cell-type specification.

The pituitary is derived from the most anterior midline portion of the neural ridge; on head turn fold arising as an invagination of an initially uniform oral ectoderm, giving rise to a structure referred to as Rathke’s pouch appearing at embryonic stage 9.5 (e9.5) in mouse. Classic embryological and recent molecular studies have revealed that the early phases of pituitary development require direct contact of the overlying neural epithelium of the ventral diencephalon (the infundibulum) with Rathke’s pouch (reviewed in [1]). Subsequent to the initial commitment events, the pouch continues to grow and divide, with the cells that will eventually form the hormone-secreting cell types of the anterior pituitary appearing ventrally between e11.5–13.5. In the final stages, the pituitary cell types express a series of differentiation markers which appear in distinct temporal and spatial patterns between e14.5–16.5. Each of these stages is governed by the interplay of a series of signaling molecules with the subsequent induction of overlapping patterns of transcription-factor expression.

In this review, we focus on the aspects of pituitary cell type specification which are ultimately mediated through the interactions of a series of cell-type-specific or -restricted transcription factors that serve to establish a molecular memory of the transient signaling events. In many cases, the nature of these interactions are conserved across species and thus create a paradigm for understanding developmental events in other organ systems. Here, we focus on recent biochemical and genetic investigations into the development of anterior pituitary cell types, highlighting the combinatorial actions of several cell type restricted transcription factors.

Factors governing commitment and organogenesis of the pituitary
Signaling gradients establish overlapping patterns of transcription factor expression

Analogous to the well documented roles of morphogen gradients in Drosophila development, each stage of pituitary organogenesis is mediated by the actions of a series of intrinsic and extrinsic signaling molecules which act to positionally determine cell types (Figure 2). Several groups have recently investigated the involvement of signaling factors in pituitary development which include bone morphogenetic proteins (BMP4, BMP2), fibroblast growth factor 8 (FGF8), Sonic hedgehog (Shh) and Wnt5a [2**,3**,4]. BMP4 and FGF8 are expressed in distinct and overlapping patterns in the neuroepithelium of the ventral...
Multiple distinct cell types arise from a common primordium in pituitary organogenesis. The most anterior midline portion of the neural ridge gives rise to the primordia of the pituitary and endocrine hypothalamus. The cell types of the anterior and intermediate lobes of the pituitary arise in a distinct spatial and temporal pattern, with terminal differentiation markers for each hormone-secreting cell type appearing by e16.5 in mouse. These six cell types comprise somatotropes, lactotropes, thyrotropes, gonadotropes, corticotropes, and melanotropes — secreting growth hormone (GH), prolactin (Prl), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH)/luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and melanocyte-stimulating hormone (MSH), respectively. An embryonic ‘rostral tip’ thyrotrope (Tr) cell type also expresses TSH transiently. TSH, LH, and FSH are heterodimeric subunits containing a specific β subunit and a common α subunit (α-glycoprotein subunit or αGSU).

Pitx and Lhx interactions in pituitary organogenesis

The earliest phases of pituitary development require factors belonging to LIM and Otx classes of homeodomain factors. The LIM homeodomain factor Lhx3/P-Lim/Lim3 is initially expressed in Rathke’s pouch at e9.5 and is maintained throughout pituitary development [6,7], and appears to require dorsal→ventral FGF8 signaling from the infundibulum for its initial activation [4]. Another factor expressed in the early phases of pituitary organogenesis, the Otx-related homeodomain now referred to as Pitx1, was identified in screens for factors regulating expression of the pro-opio-melanocortin (POMC) gene, expressed in corticotropes and...
melanotropes [8], and for factors interacting with the amino terminus of the pituitary-specific POU homeodomain factor Pit-1 [9]. A second Pitx gene expressed in the developing pituitary, Pitx2 [10*,11,12], was first reported on the basis of genetic analysis of human patients with Rieger syndrome [13] and has been recently shown to be involved in pathways governing the establishment of asymmetry in the early embryo (reviewed in [14]). Both Pitx genes are expressed throughout the oral ectoderm early in development, becoming restricted oral ectoderm derivative including the developing tooth and nasal epithelium, as well as other discrete regions of the embryo.

Recently, it has been demonstrated that LIM-homeodomain factors can act synergistically with Pitx factors to activate expression of pituitary-specific genes such as αGSU [12,15], which is initially expressed at e10.5 and is later restricted to gonadotropes and thyrotropes. The synergy between Lhx and Pitx factors has been further shown to be mediated by the broadly expressed cofactors CLIM1/Lbd-2 and CLIM2/Lbd-1/nuclear NIM interactor [NLI]), which were identified in screens for proteins interacting with LIM domain zinc finger [15–17]. A Drosophila homologue of CLIM/Lbd factors, Chip, regulates the activity of the LIM homeodomain Apterous, suggesting that this interaction represents a conserved aspect of the function of LIM-homeodomain factors [18].

Genetic analyses of mice deleted for Lhx and Pitx gene family members confirm the critical roles these factors play in pituitary development. In Lhx3 and Pitx2 gene-deleted mice, pituitary development is arrested between e10.5–e12.5, the pouch fails to proliferate, and there is almost complete absence of anterior pituitary cells [19,20]. Disruption of the gene for a second LIM homeodomain factor expressed in the pituitary, Lhx4, also shows severe defects in pituitary development but only within the context of Lhx3 heterozygote mutants [20], suggesting that Lhx3 and Lhx4 serve redundant roles in governing the early phases of pituitary organogenesis. Pitx1, in contrast, exerts roles in later phases of pituitary cell type determination as Pitx1-deleted mice, along with defects in hindlimb morphogenesis [11,22*], show diminished expression in the terminal differentiation markers for gonadotrope and thyrotrope development and a mild increase in adrenocorticotropin (ACTH)-expressing cells [11]. Future genetic analysis of Pitx1 and Pitx2 double heterozygote mutant mice will allow determination of whether these factors also serve overlapping or distinct roles in the determination of pituitary cell types.

**Paired-like homeodomain interactions in pituitary development**

The progression and proliferation of anterior pituitary cell types from Rathke’s pouch requires the actions of another homeodomain factor identified through position cloning of the *Ames dwarf (df)* locus. Genetic analysis of *df* mice [23–25] deficient in at least three pituitary cell types, lead to the characterization of the pituitary-specific paired-like homeodomain Prophet of Pit-1 (Prop-1), whose expression begins in the pituitary coincident with closure of Rathke’s pouch at e10.5, and is epistatic to Pit-1 gene expression [26]. In *df* mice the epithelial cells surrounding the lumen of Rathke’s pouch fail to populate the anterior pituitary, generating expanded lumen and dysmorphogenesis of the pouch [26,27], suggesting the Prop-1 may function to allow cells to asymmetrically progress (Figure 2). In addition to its role in the determination of the three Pit-1-dependent cell lineages (somatotropes, lactotropes, and thyrotropes), Prop-1 also appears to be required for the generation of a
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Fourth anterior pituitary cell type, the gonadotrope, as humans with combined pituitary hormone deficiency (CPHD) bearing mutations in \textit{PROP1} show defects in response to gonadotropin-releasing hormone [28]\textsuperscript{*} and there is a delay in gonadotrope determination in \textit{df} mice.

Another paired-like homeodomain factor expressed in the pituitary, \textit{rpx}/\textit{Hesx}-1, belongs to a diverse family of homeodomain proteins containing a conserved amino terminal ‘eh-1’ repressor domain originally identified in the \textit{Drosophila} engrailed homeodomain [29]. Attenuation of \textit{rpx} expression occurs just prior to appearance of the terminal differentiation markers for anterior pituitary cell types [26,30], suggesting that its downregulation is required for their developmental progression. Consistent with this hypothesis, in Prop-1-defective mice there is an extension of \textit{rpx} expression beyond e13.5 [26,27] and \textit{rpx}/\textit{Hesx}-1-deleted mice often show pituitary dysmorphogenesis somewhat reminiscent of that in the hypomorphic \textit{df} Prop-1 mutant allele [31\textsuperscript{•}], although a detailed analysis of pituitary markers in \textit{rpx}/\textit{Hesx}-1 null mice has not yet been reported. \textit{Rpx} is capable of heterodimerization with Prop-1 on consensus binding sites and can inhibit Prop-1 activity [26], suggesting that \textit{rpx} acts to antagonize Prop-1 function \textit{in vivo}. A similar type of antagonism based on heterodimerization between two paired-like homeodomain factors has also been demonstrated in \textit{Xenopus} development where Mix.1 can block the axis duplication induced by the siamois homeodomain protein [32].

**Combinatorial control of pituitary cell type determination**

In the stages of pituitary development occurring subsequent to the initial patterning and proliferation events, additional factors required for the expression of terminal differentiation markers appear. Whereas ‘pan-pituitary’ transcriptional regulators such as Lhx3 and Pitx1/2 continue to exert critical roles in the direct regulation of target genes for terminal differentiation in pituitary cell types [6,9,12], other cell-type-restricted factors appear to act by establishing combinatorial codes which govern the specification of the distinct cell phenotypes. In many cases, this code involves reciprocal synergistic or inhibitory protein–protein interactions between two or more cell-type-restricted transcription factors.

Pit-1 is one of the most dynamic and promiscuous transcriptional regulators in pituitary development, governing the appearance of three pituitary cell types. Originally identified as a tissue-specific factor controlling expression of the growth hormone (\textit{GH}) and prolactin (\textit{Prl}) genes [33,34], genetic analysis of the Snell and Jackson dwarf mice established that Pit-1 is required for generation of three pituitary cell types: somatotropes, lactotropes and thyrotropes [35]. As Pit-1 is involved directly in the transcriptional regulation of the hormonal markers for terminal differentiation of pituitary cell types (e.g. \textit{GH} in somatotropes, \textit{Prl} in lactotropes, and \textit{TSHβ} in thyrotropes) a central issue is how Pit-1, in response to diverse signaling cascades and in collaboration with other factors, directs cell-specific expression of its multiple target genes.

**Somatotrope and lactotrope determination**

Unlike most cell types of the pituitary, in which hormone gene expression can be both positively and negatively regulated by releasing hormones secreted from the hypothalamus, prolactin gene expression is controlled primarily through the negative effects of dopamine on lactotropes. \textit{Prl} transcription is controlled through a series of distal and proximal enhancer elements containing multiple binding sites for Pit-1 which are both required and sufficient to direct cell-specific expression \textit{in vivo} [36]. Early evidence for a cell-type-specific partner for Pit-1 derived from studies on the prolactin promoter in which it was demonstrated that Pit-1 can activate \textit{Prl} expression in cooperation with the with the estrogen nuclear receptor (ER) at a distal enhancer site [37]. Consistent with a requirement for ER, it has been demonstrated recently that mice deleted for the α isoform of the ER gene show a dramatic decrease in \textit{Prl} gene expression and lactotrope cell types, with other pituitary cell types either unchanged or even expanded [38].

Other factors can cooperatively regulate \textit{Prl} gene expression with Pit-1 at its proximal enhancer elements. This regulation appears to involve the interplay between two ETS domain containing factors, Ets-1 and ERF (Ets-2 repressor factor). A pituitary-specific role for Ets-1 was initially identified through the characterization of a composite Pit-1/Ets-1 binding site in the \textit{Prl} gene, which confers synergy between these proteins [39,40] and is mediated through a mitogen-activated protein kinase (MAPK) pathway. The synergy between Ets-1 and Pit-1 can be abrogated by an Ets repressor factor, ERF, apparently by inhibition of Pit-1 binding to this site [41\textsuperscript{*}]. The interplay between Ets-1, ERF and Pit-1 thus may provide a component of the molecular mechanism for the inhibitory effects of dopamine on \textit{Prl} gene expression.

As with the control of \textit{Prl} gene expression, Pit-1 binding is required to direct pituitary-specific expression in the proximal promoter region of the growth hormone gene in somatotropes. As little as 320 bp of rat \textit{GH} promoter sequences are sufficient to target expression to somatotropes \textit{in vivo}, and contains binding sites for Pit-1, a novel zinc finger protein, and nuclear receptors [42]. Several groups have reported cooperatively between Pit-1 and the retinoic acid and thyroid hormone nuclear receptors (RAR and TR) in the control of \textit{GH} expression [43,44] and recent genetic studies have supported the proposed roles for these factors in somatotrope determination. Mice deleted for all known isofoms of TR show significant decreases in \textit{GH} expression and somatotrope cell numbers, with a reciprocal pronounced increase in \textit{TSHβ} expression and thyrotropes [45\textsuperscript{**}]. High levels of cell-specific expression of the human \textit{GH} gene also require a locus
control region located 14–16 kb upstream of the promoter, although the factors binding to this region have not yet been identified [46]. Additionally, the contribution of the structure of Pit-1 [47] bound to its distinct binding sites in the GH and Prl genes, and how these conformations effects Pit-1 interactions with other coregulators also remains an area for future exploration in understanding the determination of the somatotrope and lactotrope cell types.

**Thyrotrope and gonadotrope determination**

The conserved structure of the β subunits of FSH, LH (expressed in gonadotropes), and TSH (expressed in thyrotropes), and the shared common subunit αGSU suggests that the two most ventrally arising pituitary cell types evolved from a common ancestral origin. Both cell types express a series of ventrally-induced transcription factors, and are determined, in part, by the ventral→dorsal BMP2 gradient [2**,3**]. A central question has therefore been to address the mechanisms which govern the appearance of these two similar but distinct cell types.

An important distinction between these two cell types is the presence of Pit-1 in the thyrotrope and its absence in the more ventrally-arising gonadotrope. As ventralized expression of Pit-1 is sufficient to convert gonadotropes to thyrotropes in vivo [48**], it is likely that the factor(s)
regulating TSHβ expression in collaboration with Pit-1 are present in gonadotropes as well. One of these factors has proven to be the zinc finger protein GATA-2, originally identified to be critical for the development of the hematopoietic system [49], and later shown to be expressed in permanent pituitary cell lines derived from the αGSU lineage [50]. GATA-2 has been demonstrated recently to play an important role in the determination of both gonadotropes and thyrotropes and is a direct transcriptional target of the ventral BMP2 signal. Dorsal expression of GATA-2 is alone sufficient to convert all of the Pit-1-dependent lineages to the gonadotrope fate in vivo, whereas expression of a dominant-negative form of GATA-2 inhibits the terminal differentiation of both gonadotropes and thyrotropes [48**].

In thyrotropes, Pit-1 and GATA-2 can physically interact, leading to either synergistic activation of thyrotrope-specific genes, such as TSHβ, containing adjacent Pit-1 and GATA-2 binding sites [51], or inhibition of gonadotrope-specific genes [48**], as Pit-1 can inhibit GATA-2 binding to promoters not containing an adjacent Pit-1 site. The inhibition of GATA-2 binding accounts for the ability of Pit-1, independently of its DNA binding amino acid residues, to inhibit expression of gonadotrope-specific genes in thyrotropes. Similar context-dependent consequences of GATA/homeodomain interactions have been also observed in cardiac development, where the interaction between Nkx-2.5 and GATA-4 can have either synergistic [52] or inhibitory [53] effects on GATA-dependent transcription depending on whether there is an adjacent homeodomain-binding site, suggesting that promoter-dependent functional consequences of GATA-homeodomain or similar types of interactions between DNA binding proteins are important for the development of other organ systems. Additionally, the in vivo relevance of the DNA binding-independent actions of transcription factors have been reported recently, exemplified by the glucocorticoid receptor DNA-binding domain knockout mouse [54*], demonstrating that DNA-independent protein–protein interactions are an essential component of transcription factor function in cell-type determination.

The regulated control of the genes for the terminal differentiation markers for gonadotrope development, which include LHβ and FSHβ, require the activities of multiple factors including SF-1, Egr-1 and Pitx1 [10*,12,55]. Egr-1 (also referred to as NGFI-A, Krox-24, and Zif 268) is a zinc finger protein, homologous to the Wilms tumor [WT1]) gene product, which synergizes with the orphan nuclear receptor SF-1 on the LHβ promoter [56] and is rapidly induced in gonadotrope-derived pituitary cell lines treated with gonadotropin-releasing hormone (GnRH) [57*]. Mice disrupted in Egr-1 show specific defects in LHβ expression [56] and, surprisingly, reduced somatotrope numbers two [58], Pitx1 and SF-1 also cooperatively regulate LHβ, through direct interaction of the carboxyl terminus of Pitx1 with the amino terminus of SF-1. Defects in gonadotropin synthesis are also observed in Pitx1- and SF-1-deleted mice consistent with the critical roles these factors play in gonadotropin synthesis [10*,55].

Understanding the mechanisms controlling αGSU gene regulation has presented an interesting problem in pituitary development, as its expression can be controlled by distinct mechanisms within gonadotropes and thyrotropes, and is regulated by series of distinct cis-acting elements directing expression in a cell-restricted manner. As little as 313 bp of the proximal bovine promoter sequences, containing sites for Lhx/Pitx/CLIM synergy [15] as well as GATA-2/3 [50] and SF-1, are required to target αGSU expression to gonadotropes in transgenic mice [59]. Full activity of the murine gene requires a distal enhancer element [60,61] and this element is also apparently required for the restriction of αGSU expression from other pituitary cell types, as it can confer repression of basal αGSU promoter activity in somatotrope representative pituitary cell lines [62]. The regulatory region of the αGSU gene therefore presumably contains elements required for both its cell-specific activation in thyrotropes and gonadotropes and restriction from other pituitary cell types. This regulatory region contains potential binding sites for several classes of transcription factors including Ets, GATA, and HLH factors [62], although the contribution of these elements to the activation and restriction of αGSU expression in different pituitary cell types are as yet undetermined.

Control of Pit-1 gene expression
The regulation of Pit-1 expression also presents an interesting model for understanding the pathways leading to the serial activation of transcription factors in pituitary development, as its expression is initiated prior to the appearance of terminal differentiation markers for pituitary cell types and is temporally regulated by two distinct enhancers. Pit-1 expression is initiated at e13.5 by an early enhancer and subsequently switches to a late, autoregulatory enhancer between e16.5–P0 [63,64]. Although the factors which govern the initiation of Pit-1 expression are as yet unidentified, and may include Prop-1, a component of the regulation of Pit-1 gene expression appears to involve its restriction from the gonadotrope lineage by the ventral BMP2 signal [2**,48**]. As in the examples of the regulation of GH and Prl genes, a nuclear receptor/Pit-1 interaction has been demonstrated to be involved in the autoregulation of the Pit-1 gene, where RAR can act in a ligand-dependent manner to synergistically activate Pit-1 expression [63]. Recently, it has been demonstrated that human CPHD patients — who carry a mutation in the homeodomain of Pit-1 that fails to affect DNA binding — show attenuated synergy of Pit-1 and RAR, providing suggestive genetic evidence for this type of nuclear receptor–homeodomain interaction as critical for determination of the Pit-1-dependent cell lineages [65].

Conclusions and perspectives
Molecular and genetic approaches have proven to be powerful and complimentary tools in defining the combinatorial codes of transcription factors and signaling
molecules that determine cell types in mammalian organogenesis. In pituitary development, we can argue that cell-type determination occurs in part as a consequence of the combined actions of several morphogen-gradient-induced factors, and individual cell phenotypes are established as a result of the overlapping expression patterns of two or more factors. In part, this combinatorial code involves the induction of multiple homeodomain-containing factors including members of the Otx (Pitx1, Pitx2), LIM (Lhx3, Lhx4, Isl-1), Pax (Pax-6), Sin Oculis (Six-1, Six-3), paired-like (Prop-1, rpx) and POU (Pit-1, Brn-4) families.

A future goal will be to establish how the interactions of these and other factors are involved in establishing the genetic hierarchies in the determination of pituitary cell types. In some cases, however, as in the case of other developmental systems, a single induced factor (e.g. GATA-2 in gonadotropes) appears sufficient to define the cascade of factors which define a distinct cell phenotype in pituitary development. Furthermore, the contributions of other signaling molecules to the early phases of pituitary organogenesis — which may include, for example, leukemia inhibitory factor [66] — also remain to be fully understood. Thus, a complete understanding of the molecular details of how the overlapping morphogen gradients govern the appearance of a series of transcription factors will prove to be critical for elucidating further how the cell types of the anterior pituitary arise from a common primordium.

The direct protein–protein interactions of several factors have also proven to be a major contributor underlying the molecular memory of the transient signaling events. Future studies will allow exploration into the nature and regulation of these interactions, which, initially in response to early signaling gradients, establish early events in pituitary development, and later, are involved in the conversion of hypothalamic signaling into the regulated control of hormone synthesis and secretion. The recent connection of Pit-1 to the CBP coactivator/histone acetyltransferase [67,68] make it intriguing to speculate that part of the combinatorial codes are established by the modulation of activities and interactions between DNA-binding proteins and their coactivators. Indeed, it has been shown that Pit-1, the transcriptional activities of which are regulated by both cAMP and MAP kinase pathways, requires different domains of CBP to respond transcriptionally to different signals [67]. This is potentially representative of the intracellular signaling pathways predominating in different pituitary cell types. Additionally, the contribution of nuclear receptor coactivator complexes (reviewed in [69]) to the regulation of Pit-1/nuclear receptor synergy will also be an important area of future exploration in the cell-specific control of Pit-1 function.

Thus, it will informative to understand the detailed mechanisms of the regulated control of Pit-1 and other transcription factors activities by the pathways which modulate their functions in the cell types in which they are expressed. Then it will be possible to gain a more complete understanding of how transient signaling events establish ‘molecular memories’ in the transcriptional programs that govern cell-type determination in pituitary organogenesis.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


Using in vivo and explant culture systems, the importance of various signaling molecules expressed in the pituitary and ventral diencephalon are addressed in a series of misexpression and dominant negative experiments. Misexpression of FGF8 proves to inhibit the appearance of ventral pituitary cell types in vivo but not the dorsal POMC-expressing cell lineages. Expression of a dominant-negative BMP receptor inhibits the appearance of Pit-1 cell lineages, whereas overexpression of BMP2/4 proves to induce components of the ventral cell type determination program.


Using an explant culture system, the authors investigate the roles of various signaling molecules on pituitary development. They demonstrate that infundibulum can act to induce early markers for pituitary markers including Lhx3 and can inhibit the expression of ventral markers such as Isl-1. The induction of Lhx3 can be reconstituted with FGF8, which is also normally expressed in the infundibulum. BMP2 is shown to inhibit ventral markers such as Isl-1 and αGSU. From these and other data, the authors present a model of integrated ventral and dorsal signaling gradients for pituitary development.


Mice with a targeted deletion of the Pitx1 gene are shown to have defects in the expression of terminal differentiation markers for gonadotrope and thyrotrope development. In addition to its role in pituitary development, Pitx1 is also shown to be critical for hindlimb morphogenesis, as Pitx1-deleted mice show hindlimbs which appear similar in morphology to forelimbs.


The authors report hindlimb and craniofacial defects in Pitx1 gene deleted mice. Interestingly, the authors also find differences in the severity of the defect between the left and right hindlimbs, possibly as a result of compensation by Pitx2 on the left side.


The authors report mutations in the human PROPI gene which are associated with severe combined pituitary hormone deficiency (CPhD). PROPI mutations are also associated with defects in gonadotropin synthesis, suggesting PROPI is required for the determination of the gonadotrope cell type.


Mutations in the human HESX1 gene are shown to be associated with septo-optic dysplasia. In addition to variable defects in pituitary morphogenesis, mice disrupted in Hesx1 show defects in several areas including the forebrain commissures and optic vesicles, with most mice dying before weaning.


The authors extend previous studies showing that Ets-1 and Pit-1 can cooperate on the Prl promoter and show that this cooperativity can be abrogated by fusion of Ets, Ets-2 and Pit-1 promoters. They show that ERF interacts with Pit-1 binding to a composite Ets/Pit-1 site.


In this manuscript, the authors disrupt all known isoforms of the thyroid hormone receptor in mice. Consistent with the hypotheses that T3 acts to inhibit TSHβ expression and activate GH expression, TR knockout mice have expanded thyrotrope and diminished somatotrope populations. In addition, TR-deleted mice show defects in thyroid development and bone maturation.


The authors address the importance of signaling gradient-induced transcription factors in pituitary cell type determination, using an *in vivo* approach. Dorsalized expression of GATA-2 in transgenic mice is proven sufficient to convert the Pit-1 lineages to the gonadotrope fate. Ventral expression of Pit-1 can convert thyrotropes to gonadotropes, with Pit-1 acting to inhibit the appearance of gonadotrope markers by inhibition of GATA-2 binding to promoters not containing adjacent Pit-1 sites. The inhibition of GATA-2 by Pit-1 is shown to be independent of Pit-1 DNA binding *in vivo*, demonstrating a DNA binding-independent action of a transcription factor contributing to cell type specification.


Previous studies have demonstrated that the mouse knockout for the glucocorticoid receptor (GR) *die in uto*,. Here, the authors show that in mice deleted in the DNA binding domain of the GR gene, the mice are viable, establishing that the DNA binding function of GR is dispensable for survival.


The authors demonstrate that the immediate-early gene Egr-1 is rapidly induced in gonadotrope-derived cell lines treated with GnRH, activating a protein kinase C/MAP kinase pathway. They further show that Pit1 interacts with the orphan nuclear receptor SF-1 and Egr-1 through its carboxyl terminus.


