Molecular Physiology of Pituitary Development: Signaling and Transcriptional Networks

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Zhu X, Gleiberman AS, Rosenfeld MG. Molecular Physiology of Pituitary Development: Signaling and Transcriptional Networks. Physiol Rev 87: 933–963, 2007; doi:10.1152/physrev.00006.2006.—The pituitary gland is a central endocrine organ regulating basic physiological functions, including growth, the stress response, reproduction, metabolic homeostasis, and lactation. Distinct hormone-producing cell types in the anterior pituitary arise from a common ectodermal primordium during development by extrinsic and intrinsic mechanisms, providing a powerful...
model system for elucidating general principles in mammalian organogenesis. The central purpose of this review is to inspect the integrated signaling and transcriptional events that affect precursor proliferation, cell lineage commitment, terminal differentiation, and physiological regulation by hypothalamic tropic factors.

I. INTRODUCTION

The pituitary gland functions as a relay between hypothalamus and peripheral target organs. It is composed of two anatomically and functionally distinct entities, the adenohypophysis, including the anterior and intermediate lobes, and neurohypophysis, also known as the posterior lobe. The adenohypophysis contains six different cell types characterized by different hormones they produce and secrete. Somatotropes secrete growth hormone (GH) and regulate linear growth and metabolism. Lactotropes produce prolactin (PRL), regulating milk production in females. Corticotropes secrete adrenocorticotropic hormone (ACTH), a prohormone of proopiomelanocortin (POMC), which regulates metabolic function through stimulation of glucocorticoid synthesis in the adrenal gland. Thyrotropes produce thyroid-stimulating hormone (TSH), which promotes thyroid follicle development and thyroid hormone (TH) secretion and modulates skeletal remodeling. Gonadotropes produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which act on the gonad to initiate sexual maturation and maintain reproductive function. TSH, LH, and FSH are heterodimeric glycoproteins composed of a common α-subunit (αGSU) and a hormone-specific β-subunit (TSHβ, LHβ, FSHβ). The intermediate lobe melanotropes secrete α melanocyte-stimulating hormone (α-MSH), a cleaved product of POMC, regulating production and distribution of melanin by melanocytes. The posterior lobe of the pituitary is composed of axonal terminals of the magnocellular neurons, surrounded by specialized astroglia known as pituicytes. The magnocellular neurons synthesize peptide hormones oxytocin and vasopressin and transport them to the axonal terminals located in the posterior lobe where they are secreted into the general circulation. The proper function of the pituitary is regulated by the hypothalamus, which secretes tropic factors modulating cell proliferation, hormone synthesis, and secretion (58, 245, 255, 258, 287, 302, 328, 329).

II. INITIAL STEPS OF THE ANTERIOR PITUITARY DEVELOPMENT

The pituitary gland of all vertebrates is an organ of dual origin. The posterior lobe of the gland derives from neuroectoderm while the anterior and intermediate lobes originate from the so-called hypophysial placode. These two parts of the gland closely interact both physiologically and developmentally. While morphological details as well as the organization of the gland vary in different taxa of vertebrates, the general principles of the gland organization, morphogenesis, and molecular machinery involved in the development and functions of terminally differentiated endocrine cell types are quite similar (Fig. 1).

A. Origin of the Ectodermal Pituitary Primordium

The ectodermal primordium of the anterior pituitary is considered to be a member of cranial placodes (reviewed in Refs. 15, 237). Fate map analysis in amphibians, birds, and mammals traced the origin of the anterior pituitary to the anterior neural ridge, the ectodermal midline structure located immediately anterior to the neural plate (51, 133, 150). The adjacent midline part of the neural plate is destined to become hypothalamus and the posterior lobe of pituitary (neuropituitary) (50).

In zebrafish as well as in amphibian embryos, the anterior pituitary starts to develop directly from the placode in close contact with the developing neural tissue (91, 135). In birds and mammals, two adjacent areas that are involved in the formation of the pituitary gland are displaced from their anterior position in the process of head morphogenesis. As a result, the pituitary primordium finally ends up as a part of the stomodeal ectoderm at the roof of the oral cavity; concurrently, the presumptive hypothalamus and neuropituitary that still keeps close contact with the hypophyseal placode move ventrally and become a part of ventral diencephalon. In birds and mammals, the development of the anterior pituitary per se starts from the formation of the Rathke’s pouch, a fingerlike invagination of the roof of oral cavity toward ventral diencephalon. Almost simultaneously, the ventral diencephalon of mammals generates an outgrowth termed infundibulum that is the primordium of the posterior lobe of pituitary. The infundibulum and Rathke’s pouch proceed to form a definite pituitary gland. In chick embryo, outgrowth of the infundibulum, as well as further development of the posterior lobe of the pituitary gland, is much less manifested morphologically than in mammals.

There are some disputes in the literature regarding neuroectodermal/placodal versus oral ectoderm origin of the anterior pituitary primordium. In a sense, these arguments are purely semantic. Fate map studies clearly indicate a placodal origin of the anterior pituitary in all vertebrates (135). In bird and mammals, transposition of the anterior pituitary primordium into the stomodeal area due to head morphogenesis partially obscures its placodal origin.
B. Initial Steps of Rathke’s Pouch Development

The initial step of Rathke’s pouch formation as an invagination of the oral ectoderm is apparently dependent on the bone morphogenetic protein (BMP)4, which is expressed in the overlying ventral diencephalon at E8.5 and later in the infundibulum and subsequently diminished. Deletion of \( \text{BMP}4 \) results in embryonic lethality around gastrulation (E6.5). In some genetic background, however, a proportion of the mutant embryos survive until E9.5. In these mutant animals, the initial ectodermal thickening and the invagination of Rathke’s pouch fail to occur (279). However, cautions should be taken given that these mutant animals are consistently developmentally delayed compared with their wild-type littermates (162).

It has also been suggested that the initial Rathke’s pouch and infundibulum formation as invaginations of oral ectoderm and ventral neuroectoderm, respectively, precedes their commitment to the pituitary fate and may be induced by morphogenetic signals from prechordal plate/notochord. Nonspecific Rathke’s pouchlike ectodermal invagination can be induced by heterotopic transplantation of prechordal plate/notochord at lateral head ectoderm of early chick embryo (92). This is in agreement with the known ability of notochord-derived signal(s), Shh in particular, to affect adjacent mesenchyme and the composition of extracellular matrix which in turn impacts the migration of placode-derived cells (76).

Ectodermal invagination brings primordium of the anterior pituitary in close physical contact with ventral diencephalon. It has been shown that the interaction between these tissues is absolutely essential for further pituitary development (reviewed in Ref. 134). Intriguingly, ventral diencephalon/infundibulum not only promotes further development of Rathke’s pouch and terminal differentiation of all endocrine cell types of the anterior pituitary but also provides positional cues by regulating combinatorial patterns of transcription factor gene expression. Pit1 is induced at e13.5 in the caudomedial region of the pituitary gland, which ultimately gives rise to somatotropes (S), lactotropes (L), and thyrotropes (T). Rostral tip thyrotropes (Tr) are Pit1 independent. Corticotropes (C) and gonadotropes (G) are differentiated in the most ventral part of the gland.

FIG. 1. Ontogeny of signaling molecules and selected transcriptional factors during mouse pituitary organogenesis. The most anterior neural ridge gives rise to primordium of the anterior and intermediate lobes of the pituitary. The adjacent neural plate develops into endocrine hypothalamus and the posterior lobe of the pituitary gland. Ventral diencephalon, which expresses BMP4, FGF8/10/18, and Wnt5, makes direct contact with oral ectoderm and induces the formation of Rathke’s pouch. Shh is expressed throughout the oral ectoderm except in the Rathke’s pouch, creating a boundary between two ectodermal domains of Shh-expressing and -nonexpressing cells. The opposing dorsal BMP4/FGF and ventral BMP2/Shh gradients convey proliferative and positional cues by regulating combinatorial patterns of transcription factor gene expression. Pit1 is induced at e13.5 in the caudomedial region of the pituitary gland, which ultimately gives rise to somatotropes (S), lactotropes (L), and thyrotropes (T). Rostral tip thyrotropes (Tr) are Pit1 independent. Corticotropes (C) and gonadotropes (G) are differentiated in the most ventral part of the gland. The dorsal region of the Rathke’s pouch becomes the intermediate lobe, containing melanotropes (M). The infundibulum grows downward and eventually becomes the posterior lobe (P). A number of transcription factors and cofactors regulating the lineage commitment and terminal differentiation of distinct cell types are illustrated in a genetic pathway. [Modified from Scully and Rosenfeld (255).]
III. SIGNALING MOLECULES THAT REGULATE STRATIFICATION OF RATHKE'S POUCH AND PITUITARY CELL TYPE DETERMINATION

As in many other organs, patterning of the anterior pituitary primordium is directed by a complex morphogenetic field that is composed of signals emanating both from ventral diencephalon/infundibulum and from Rathke's pouch itself. The signals that are essential for the stratification of Rathke's pouch and for the timely and spatially organized process of endocrine cell type appearance are the same familiar factors that are involved in the patterning of many other organs, namely, Shh and members of FGF, BMP, Notch, and Wnt families of growth factor/morphogens (58). The role of epidermal growth factor (EGF) signaling in pituitary development, while quite significant in the development of somatotrope and lactotrope cell lineages, is much less documented.

A. Shh

The secreted factor Shh plays an important role in embryo patterning, as well as in the specification of different cell types and in the control of proliferation of numerous cell types. In the early embryo, the major source of Shh production is the notochord (72). Both surgical ablation of notochord in chick embryos and deletion of the Shh gene in mouse result in similar profound changes in the embryo axis patterning and dramatic distortion of head morphogenesis accompanied by malformation of midline structures, cyclopia, and absence of Rathke’s pouch (44, 76, 77). Three related zinc finger transcription factors, Gli1, Gli2, and Gli3, acting downstream of the Shh pathway, are expressed in the ventral diencephalon and developing Rathke’s pouch (117). Inactivation of Gli2 causes variable loss of the pituitary, and deletion of both Gli1 and Gli2 results in complete absence of the pituitary (214). The effect on the pituitary development upon ablation of Shh signaling, however, seems to be a consequence of a general defect of midline structures including diencephalon/infundibulum rather than a defect in the anterior pituitary primordium itself. Furthermore, loss of either Shh or the notochord affects dramatically the formation of paired trigeminal ganglia that normally are located laterally to the pituitary gland. In the absence of Shh, a fused single trigeminal ganglion is formed in the place where the pituitary normally develops (76). In zebrafish, Shh produced by neuroectoderm instead of notochord or oral ectoderm is crucial for the initial patterning of the pituitary placode (246). Mutations that severely disrupt HH signaling, such as smu/smoothened and golt/Gli2, result in the development of lens tissue from the presumptive pituitary (149). Mutations that attenuate HH signaling, including syg/sonic hedgehog and dtr/Gli1, lead to hypoplasia of the pituitary gland (111, 246). Interestingly, lens tissue differentiation in naive head ectoderm of early chick embryo seems to be the default developmental pathway as opposed to the alternative anterior pituitary formation induced by ventral diencephalons and mesenchyme (16, 78, 79, 92). In mice during pituitary development, Shh is expressed in the ventral diencephalon and oral ectoderm but is excluded from the invaginating Rathke’s pouch. The HH downstream target gene Patched1 is expressed in the developing pituitary, indicating that pituitary progenitors respond to the HH signaling. Pituitary-specific blockade of Shh signaling by overexpressing the Shh antagonist Hip almost completely prevents Rathke’s pouch growth and appearance of endocrine cell lineages. In contrast, Shh overexpression in the developing pituitary under the control of αGSU regulatory element results in mild hyperplasia with expansion of ventral gonadotrope and thyrotrope cell lineages (286). This is consistent with the observation in zebrafish where overexpression of shh causes pituitary expansion (246), suggesting that Shh exerts effects on both proliferation and cell type determination.

B. FGFs

Multiple members of the FGF growth factor family play important roles in every known organogenesis and cell type differentiation, including anterior pituitary. Three members of this family, FGF8, -10, and -18, have been found in the ventral diencephalon and subsequently in the posterior lobe of pituitary during mouse pituitary development (75, 188, 285, 286, 316), and at least two of them, FGF8 and FGF10, have been shown to play important roles in the pituitary development. In mice lacking either FGF10 or the gene encoding its high-affinity receptor, FGF receptor 2 IIIb, the Rathke’s pouch initially forms but rapidly undergoes extensive apoptosis resulting in
agensis of the anterior pituitary by E14.5 (206, 234). A similar observation has been made in transgenic mice expressing a dominant negative form of FGFR2 (IIIb) (40), suggesting that FGF10 signaling is essential for cell survival. Inactivation of FGF8 in mice causes early lethality at E8.5 before pituitary development. The function of FGF8, which binds to a different set of FGFR receptors, in pituitary development, is largely drawn from studies of transgenic animals and in vitro organ culture. Overexpression of FGF8 under the control of αGSU regulatory element leads to ectopic Lhx3 induction and pituitary hyperplasia with dramatic expansion of POMC-producing cell lineages and simultaneous inhibition of other pituitary cell types (285, 286). When cocultured in vitro, the infundibulum can stimulate proliferation and induce Lhx3 expression, meanwhile antagonizing BMP2-induced expression of Isl1, αGSU in Rathke’s pouch explants. The patterning activity of the infundibulum can be mimicked at least in part by FGF8, and impeded by the FGFR antagonist SU5402, suggesting that FGF8 is an important component of signals emanated from ventral diencephalon. It functions in the patterning of Rathke’s pouch by maintaining proliferation and opposing the ventral BMP2 differentiation signal (75, 203). The function of FGF signaling in pituitary development has previously been implicated in mice lacking Titf1, where the entire pituitary is completely absent at birth. Titf1 is expressed in the ventral diencephalon. In Titf1−/− mice, the region that expresses FGF8 and FGF10 is deleted leading to a failure of Lhx3 and Lhx4 induction. The initial oral ectoderm invagination is ultimately eliminated by apoptosis (145, 279). In zebrafish, it has also been found that Fgf3, a ligand for FGFR2 (IIIb), is required for activation of genes regulating early steps of pituitary specification, including lim3/Lhx3 and pit1, and is essential for subsequent cell survival. Mutations in Fgf3 or blocking FGF signaling by the FGFR inhibitor SU5402, however, affect neither pituitary morphogenesis nor pituitary cell proliferation (110). Fgf3 is also required for pituitary-specific expression of ascl1a, which encodes a homolog of basic helix-loop-helix (bHLH) proteins of the achaete-scute subfamily. Similar to Fgf3/IIa mutants, ascl1a/pia mutants exhibit a failure of terminal differentiation of all pituitary cell types, lacking expression of pit1 and neurod. Interestingly, implanted Fgf3 beads can enhance pituitary ascl1a expression but fail to rescue pit1 expression and pituitary developmental defects of ascl1a/pia mutants, suggesting that ascl1a might act in parallel or downstream of Fgf3 signaling to mediate some effects of Fgf3 (219).

C. BMPs

BMP signaling is crucial for the anterior pituitary development. At least two members of the BMP family, BMP4 and BMP2, participate in the development of the anterior pituitary. As noted above, BMP4 is suggested to be essential for the invagination of Rathke’s pouch (279). Consistently, oral ectoderm invagination occurs in Titf1−/− mice, wherein expression domain of BMP4 is maintained at E9.5 despite lacking FGF8 and FGF10. Similarly, in transgenic mice intended to block early BMP signaling by overexpressing Noggin, a high-affinity BMP2/4 antagonist, in the oral ectoderm and Rathke’s pouch under the control of Pitx1 regulatory sequences, pituitary development is arrested at E10 after the pouch formation lacking all pituitary cell types except for a few corticotropes (285), suggesting that BMP4 signaling is critical for the continued progression of pituitary development. The pituitary phenotype of Pitx1-Noggin transgenic mice is almost identical to that of the Lhx3−/− mice (259). It remains to be determined whether BMP4, like FGF8, also regulates the expression or functions of Lhx3.

Subsequent to the onset expression of BMP4 in ventral diencephalon, BMP2 signal emanates from a ventral part of the anterior pituitary primordium at E10.5. In vitro culture of Rathke’s pouch in the presence of BMP2 has shown that BMP2 is capable of inducing the expression of Isl1 and αGSU, both of which are ventral markers of pituitary, and suppressing the expression of ACTH. Moreover, overexpression of BMP4 in the developing pituitary under the pituitary-specific promoter αGSU leads to mild hyperplasia of the anterior pituitary with overgrowth of ventral cell types as judged by their transcription factor profile. These data collectively suggest that BMP2 signaling provides a ventralizing positional cue for pituitary development (75, 285). This BMP2 signal together with a dorsal FGF8 signal appear to create opposing gradients that are suggested to generate temporally and spatially distinct patterns of transcription factors expression underlying cell lineage specification events (58, 75, 285). Interestingly, while BMP signaling is absolutely essential for the initiation of the cell type determination process, it has to be attenuated to achieve terminal differentiation. Normally, during anterior pituitary development the expression of BMP2 decreases dramatically after E14–15. Overriding this decline by sustained expression of BMP4 in αGSU-BMP4 transgenic mice leads to a failure of terminal differentiation as evidenced by lack of any terminal differentiation markers (285).

D. Notchs

Notch signaling is an evolutionally conserved mechanism that regulates proliferation, apoptosis, cell fate determination, and morphogenesis. Notch signaling is active during the early phases of pituitary development as indicated by expression of Dll1, Jag1, Notch2, Notch3, as well as the direct downstream targets Hes1 and Hey1. Their
expression becomes downregulated in the perspective anterior pituitary at E13.5 as cells undergo lineage commitment (226, 228, 330). Pituitary-specific inactivation of Rbp-J, which encodes a central mediator of the Notch signaling, using the transgenic Cre line under the control of Pitx1 regulatory sequences, leads to premature differentiation of progenitor cells as well as a conversion of the Pit1 lineage into corticotrope lineage. The former phenotype is recapitulated in mice deleted for the Hes1 gene, while the later phenotype is largely attributed to the significant downregulation of Prop1 at E12.5, which encodes the tissue-specific paired-like homeodomain transcription factor necessary for Pit1 expression. It has been shown that Rbp-J can bind to the evolutionally conserved recognition site in the first intron of the Prop1 gene and is required for terminal differentiation of distinct cell lineages (228, 330). Overexpression of the constitutively active form of Notch1 in Pit1+ cells under the control of Pit1 regulatory information (Pit1-NICD) completely blocks terminal differentiation of all three Pit1 lineages. Consistently, overexpression of the constitutively active form of Notch2 in thyrotropes and gonadotropes directed by the αGSU regulatory sequences leads to defects in thyrotrope and gonadotrope differentiation. Other components of the Notch pathway, including a subset of Notch-repressed neurogenic bHLH factors Mash1 and Math3, are expressed during pituitary development. It has also been shown that Mash1 and Math3 play critical roles in differentiation and functions of specific cell types (330; unpublished data).

E. Wnts

Wnt signaling plays an important role in the control of proliferation of many cell types as well as in the patterning and in the maintenance of stem cell fate. During pituitary development, the Wnt/β-catenin pathway is active between E11.5 and E15.5, as indicated by expression of a direct downstream target Axin2, and is functionally required for Pit1 lineage determination and pituitary gland growth. Targeted inactivation of the β-catenin gene in pituitary progenitors using the Pitx1-Cre transgenic line results in a smaller gland with no Pit1 expression, absence of three Pit1 lineages, and reduced number of gonadotropes. Unlike in most other developmental processes regulated by the canonical Wnt pathway where Wnt signaling is conveyed by association of β-catenin with the Lef/Tcf family of transcription factors, induction of Pit1 expression is mediated by direct interactions between β-catenin and the tissue-specific paired-like homeodomain transcription factor Prop1, through an evolutionarily conserved Pit1 early enhancer (66, 208, 273). The Prop1/β-catenin complex also acts as a transcriptional repressor for Hesx1, based on the recruitment of Groucho-related Tle, histone deacetylase (HDAC), and Reptin corepressors. Genetic studies have demonstrated that temporal control of the Wnt/β-catenin signaling is essential for proper pituitary development as premature activation of β-catenin leads to Hesx1 repression and pituitary gland agenesis by E13.5 (208).

Ten of 19 Wnt genes are detected in the developing E12.5 pituitary (208). So far, two of them, Wnt4 and Wnt5a, have been reported to be specifically associated with the developmental events in the anterior pituitary. Wnt5a is expressed in the ventral diencephalon and infundibulum of mouse embryo since at least E9.5, while Wnt4 expression is detected in Rathke’s pouch and sustained later through E14.5 (285). Surprisingly, ablation of either gene results in relatively mild phenotype in the developing anterior pituitary. Wnt4−/− mice have anterior pituitary hypoplasia with reduced number of gonadotropes, thyrotropes, and somatotropes, but not corticotropes (285). Wnt5a−/− mice have morphologically distorted pituitary with enlarged intermediate lobe and increased number of POMC+ in the anterior and intermediate lobes (41; unpublished observations). The double Wnt4/Wnt5a knockout mice display a combined phenotype with significantly abnormal shape of pituitary gland, hyperplasia of the intermediate lobe, and hypoplasia of the anterior lobe accompanied by an increased number of corticotropes and decreased number of all other endocrine cell types (unpublished observations). It seems that these two factors target independent, discrete, nonoverlapping populations of pituitary precursors, and they play a role not in the specification of particular cell lineages but rather in the expansion of endocrine cell types. Whether other members of the Wnt family in combination with Wnt4, -5a play a role in lineage specification, as implicated by the pituitary specific deletion of the β-catenin gene, awaits further investigation.

Other components of the Wnt/β-catenin signaling pathway, including frizzled2 (Fzd2), Lef1, Tcf3, and Tcf4, are expressed during pituitary development (69, 208). Fzd2 is detectable in Rathke’s pouch and infundibulum at E12.5 (69). Tcf3 is expressed from E9.0 to E14.5 but is restricted from the Pit1-expressing caudomedial region of the gland. Tcf4 is detectable in early pituitary as well as in surrounding tissues and is markedly diminished by E13.5. Lef1 exhibits biphasic expression, initial transiently at E9.0 in Rathke’s pouch and later reappearing at
E13.5 in anterior and intermediate lobes of the gland (208). Targeted inactivation of Tcf4 results in hyperplasia of the anterior lobe without affecting other aspects of pituitary development (30). Deletions of Lef1, on the other hand, lead to elevated expression of Pit1 as well as GH and TSHβ, consistent with a role of Lef1 in inhibiting Prop1/β-catenin-mediated Pit1 activation (208).

F. EGF

While EGF signaling plays a very important role in many developmental processes, its role in the development of the pituitary gland has not been studied in detail. Recently, it has been found that blocking EGF/transforming growth factor (TGF)-α signaling, by the expression of a dominant-negative form of EGF receptor lacking its intracellular protein kinase domain, has a profound stage-specific effect (240). Expression of mutated EGF receptor in dwarf-producing cells of embryonic pituitary results in dwarfism and pituitary hypoplasia with reduced numbers of both somatotropes and lactotropes. Delaying mutated receptor expression in somatotropes to the postnatal period or targeting its expression to lactotropes does not cause any discernable phenotype. These data demonstrate an essential role of EGF or TGF-α signaling in the early stages of development of somatotrope/lactotrope cell lineages. Whether this pathway is involved in any other developmental events in the pituitary gland remains to be addressed.

IV. TRANSCRIPTIONAL FACTORS THAT CONTROL THE EARLY PHASES OF PATTERNING

A. Pitx

Three bicoid-related Pitx transcription factors have been identified. Two of them, Pitx1 and Pitx2, exhibit overlapping and distinct expression patterns during pituitary development (reviewed in Ref. 89). Both transcription factors can recognize the same bicoid binding site and activate the promoters of multiple pituitary hormones including αGSU, TSHβ, LHβ, FSHβ, GnRHR, PRL, and GH (289). Genetic studies have demonstrated that they are required for cell proliferation, survival, and differentiation in a dosage-sensitive manner, with Pitx2 playing a more prominent role than Pitx1, and they function redundantly in controlling Lhx3 expression (43, 146, 274). Pitx1 was identified on the basis of its ability to interact with the NH2-terminal transactivation domain of Pit1 (278) and to bind a cis-acting element of the POMC promoter (159). Inactivation of Pitx1 results in defects in hindlimb development and craniofacial morphogenesis (160, 277). The anterior pituitaries of Pitx1−/− mice exhibit mild defects with increased expression of FSHβ, LHβ, and TSHβ and increased expression of POMC.

The Pitx2 gene was initially identified as the gene responsible for human Rieger syndrome type I, an autosomal dominant condition characterized by variable defects including anomalies of anterior chamber of the eye, dental hypoplasia, a proterbant umbilicus, mental retardation, and isolated growth hormone deficiency (256). Pitx2 knockout mice display multiple developmental defects including failure of body-wall closure, right pulmonary isomerism, and defects in heart, tooth, eye, and pituitary organogenesis (88, 148, 170, 181). In the pituitary, a definite pouch forms with induction of Lhx3, Hexx1, Pitx1, and αGSU. However, the gland fails to progress further with only a few POMC-positive corticocytes and no Pit1 induction. Decreased proliferation or reduced gland size has been observed in the pituitaries of Pitx2−/−, Pitx1+/− Pitx2+/− and Pitx1−/−Pitx2neo/neo (a hypomorphic allele of Pitx2) embryos, suggesting that Pitx1 and Pitx2 function in the same pathway to stimulate cell proliferation. Consistent with this view, overexpression of Pitx2 under the control of Pit1 or αGSU regulatory elements leads to an increase in the number of somatotropes and gonadotropes, respectively (43, 146, 274). Cell culture studies also show that the Wnt/β-catenin pathway can stimulate expression of Pitx2, which in turn regulates expression and mRNA stability of critical cell cycle regulators including Cyclin D1, D2 (13, 27, 146). It has also been demonstrated that Pitx2 is required for cell survival, and Pitx1 and Pitx2 together are obligatory for Lhx3 induction (43). In addition to the early roles of Pitx2 in pituitary development, studies of Pitx2neo/neo mice reveal that Pitx2 is necessary for expansion of the Pit1 lineages and differentiation of gonadotropes at later stages of pituitary organogenesis (274). The requirement of Pitx1 and Pitx2 for terminal differentiation in the ventral cell types maybe explained, at least in part by the fact that both Pitx1 and Pitx2 are preferentially expressed in these cells (43).

B. LIM Homeodomain Transcription Factors: Isl1, Lhx3, and Lhx4

LIM homeodomain proteins (LIM-HD) are characterized by two tandemly repeated LIM domains located NH2-terminally of the DNA binding homeodomain. The LIM domain provides a protein-protein interaction interface that recruits cofactors mediating LIM-HD functions (reviewed in Ref. 118). At least 12 members of the LIM-HD family have been identified in mammals, and several of them are expressed in Rathke’s pouch, including Isl1, Lhx3, and Lhx4. Expression of Isl1 in pituitary is dynamic. It is initially detected in the oral ectoderm at E8.5,
throughout Rathke’s pouch at E9.5, and then by E10.5 confined to the most ventral region of the pouch, apparently colocalized with \( a\GSU \) in the rostral tip thyrotropes. \( Isl1 \) expression is induced by BMP2 and repressed by FGF8/FGF2, the opposing ventral and dorsal signals critical for pituitary development (75). Inactivation of \( Isl1 \) results in embryonic lethality at approximately E10 with defects in heart, pancreas, and motor neuron development. In E9.5 \( Isl1^{-/-} \) embryos, the primitive pouch forms with aberrant thin epithelium, suggesting that \( Isl1 \) is necessary for proliferation and differentiation of pituitary progenitors (279). Similar functions of \( Isl1 \) have been elucidated in heart development, where \( Isl1 \) is expressed in precursors of the second heart field and essential for their proliferation, survival, and migration (34).

\( Lhx3 \) is expressed in the pituitary gland, motor neurons of the spinal cord and hindbrain, the retina, and the pineal gland. \( Lhx3 \) expression is first evident in Rathke’s pouch at E9.5 and persists predominantly in the anterior and intermediate lobes of the adult pituitary. Mice with disrupted \( Lhx3 \) are stillborn or die within 24 h lacking the anterior and intermediate lobes of the pituitary. In \( Lhx3^{-/-} \) mice, the pituitary gland is arrested after the formation of a definite Rathke’s pouch with a failure to maintain \( Hesx1 \) expression and to induce \( Pit1 \). Except for some residual corticotropes, all other cell types in the anterior and intermediate lobes are completely absent (259). A more extensive analysis of the \( Lhx3^{Cre/Cre} \) mutants, which carry a Cre recombinase expressing cassette in the 3’-UTR of the \( Lhx3 \) gene leading to reduced expression of \( Lhx3 \) protein and pituitary defects identical to those in \( Lhx3^{-/-} \) mutants, reveal that \( Lhx3 \) is required for cell survival, consistent with the view that \( Lhx3 \) acts downstream of \( Pitx1 \) and \( Pitx2 \) in preventing cell apoptosis (326). The function of \( LHX3 \) is conserved in humans. Patients with loss-of-function mutations in \( LHX3 \) have combined pituitary hormone deficiency (CPHD) (reviewed in Refs. 21, 46, 53, 193).

Because disruption of either \( Lhx3 \) or \( Isl1 \) results in an arrest of pituitary development at early stages, their functions in the later stages cannot be determined by analyzing the mutant mice. In vitro studies have shown that \( Lhx3 \) can interact with other transcription factors found in pituitary and synergistically regulate the promoters of pituitary hormones and transcription factor genes. For example, \( Lhx3 \) with \( Pitx1 \) synergistically activates the promoter of \( a\GSU \) and with \( Pit1 \) regulates the promoter of \( PRL \), \( Pit1 \) and \( TSH\beta \) (11, 271).

A closed related gene, \( Lhx4 \), is also expressed in the invaginating pouch at E9.5. In contrast to \( Lhx3 \) that is expressed throughout adulthood, \( Lhx4 \) expression becomes restricted to the future anterior lobe of pituitary and diminished at E15.5. Mice homozygous for the \( Lhx4 \) deletion die shortly after birth due to a failure of pulmonary maturation. In \( Lhx4^{-/-} \) mice, there is increased cell death in E12.5 and E14.5 pituitary, and by E18.5 different cell types, including somatotropes, corticotropes, thyrotropes, and gonadotropes, are present, but with markedly reduced numbers leading to a hypoplastic anterior lobe. In addition to distinct roles of \( Lhx3 \) and \( Lhx4 \) in pituitary organogenesis, \( Lhx3 \) and \( Lhx4 \) act redundantly in the formation of a definitive pouch. Inactivation of both \( Lhx3 \) and \( Lhx4 \) results in a more severe phenotype than either single mutant with an earlier developmental arrest in pituitary (227, 257).

C. \( Hesx1 \)

\( Hesx1 \) is a member of the paired-like class of homeodomain genes. It is first expressed in the anterior midline endoderm and prechordal plate precursor, subsequently activated in the overlying ectoderm of the cephalic neural plate, and ultimately restricted to the ventral diencephalon and Rathke’s pouch at E9.5 (108, 109, 280). Expression in the developing pituitary persists until E12, when \( Hesx1 \) is downregulated in a spatial and temporal order that coincides with the rise of \( Prop1 \) transcripts and progressive differentiation of pituitary specific cell types. \( Hesx1 \) expression is regulated by \( Lhx3 \) and \( Prop1 \). While \( Lhx3 \) is necessary to maintain \( Hesx1 \) expression, \( Prop1/\beta\)-catenin is required for \( Hesx1 \) repression (208, 259, 273). Inactivation of \( Hesx1 \) results in variable anterior central nervous system (CNS) defects, including a reduced prosencephalon, absence of developing optic vesicles, and defective olfactory development (59, 187). The phenotypes in the pituitary diverge greatly. Five percent of \( Hesx1^{-/-} \) embryos exhibit a complete lack of pituitary gland. The initial thickening of oral ectoderm and minimal induction of \( Lhx3 \) occur at E12.5; the pituitary gland however is absent at E18.5, probably owing to ectopic expression of \( FGF8 \) in the oral ectoderm. The majority of \( Hesx1^{-/-} \) mice are characterized by multiple oral ectoderm invagination and/or overproliferation of all pituitary cell types. In these mutants, the expression domain of \( FGF10 \) extends rostrally in the ventral diencephalon, leading to ectopic \( Lhx3 \) induction and formation of multiple pituitary glands, consistent with the role of \( FGF \) signaling in pituitary proliferation and development (56, 75, 206, 233, 285). The phenotype of \( Hesx1^{-/-} \) mice is similar to that of septo-optic dysplasia (SOD) in human, which is characterized by midline forebrain abnormalities, optic nerve hypoplasia, and hypopituitarism ranging from CPHD to isolated growth hormone deficiency (IGHD) (reviewed in Refs. 46, 53, 193, 328).

\( Hesx1 \) is a transcriptional repressor with two repressor domains located in the \( NH2 \) terminal and homeodomain regions, respectively (28, 56). The homeodomain recruits the \( NcoR/Sin3/HDAC \) corepressor complex. The \( NH2 \) terminus \( eh1 \) motif mediates the interaction with the
Groucho-related Tle corepressor, and their association is essential for Hesx1 function. Tle1 exhibits similar temporal and spatial patterns of expression to Hesx1 in Rathke’s pouch. Overproduction of both Hesx1 and Tle1, but not the mutant Hesx1 incapable of interacting with Tle1, in Rathke’s pouch results in near-complete absence of gonadotropes and three Pit1 lineages, probably by antagonizing the transactivation function of Prop1 on endogenous targets, suggesting that the interaction between Hesx1 and Tle is required for Hesx1 function and the temporal regulation of Hesx1/Tle complex is fundamental for pituitary organogenesis (56, 273). Recent identification of a homozygous mutation in the eh1 motif of human HESX1 in a patient with CPHD has further underlined that Tle association is an integral mechanism for Hesx1 function in vivo (38).

In addition to Tle1, other members of the Tle family, including Tle3, Tle4, and Aes, are expressed during pituitary development. Tle3 is first detectable in the ventral diencephalon, and by E14.5, Tle3 is localized in the dorsal region of Rathke’s pouch and later confined to the intermediate lobe. Tle4 is expressed in the developing infundibulum and posterior lobe and diminished by E16.5. Aes is transiently expressed in the dorsal region of Rathke’s pouch from E12.5 to E16.5. While the endogenous functions of Tle1, Tle3, and Tle4 during pituitary development are currently unknown, it has been shown that Aes is required for modeling the shape of pituitary gland. Inactivation of Aes leads to bifurcations of the pouch and severe pituitary dysmorphogenesis at birth (30).

D. Prop1

Prop1 is a paired-like homeodomain transcription factor essential for pituitary development and function. Prop1 can bind to its cognate site and activate target gene via the COOH-terminal transactivation domain, whereas the NH2 terminus and the homeodomain of Prop1 possess repression function (266, 273), suggesting that Prop1 can function as both a transcriptional activator and a repressor. Consistent with this notion, recent studies of the pituitary specific inactivation of the β-catenin gene reveal that Prop1/β-catenin complex acts as a transcriptional activator for Pit1 and a transcriptional repressor for Hesx1, depending on the associated cofactors (208). Expression of Prop1 is restricted in the developing pituitary. It is initially detected at E10 to E10.5, peaks at E12.5, and declines after E14.5. It has been shown recently that the Notch signaling is required for maintaining high levels of Prop1 expression at E12.5, a process mediated by an evolutionarily conserved Rbp-J binding site within the first intron of the Prop1 gene (330). A homozygous mutation in the Prop1 homeodomain in the Ames dwarf mice (df/df) or targeted deletion of the Prop1 gene leads to failure of Pit1 gene activation and delayed gonadotrope development. Proliferation of the pituitary progenitors surrounding the lumen is not affected. However, they fail to migrate to the caudomedial region of the developing gland where Pit1 is normally expressed, resulting in an expansion of luminal structure and dramatic dysmorphogenesis (8, 86, 87, 199, 208, 273, 299). Enhanced apoptosis is evident in the postnatal pituitaries of df/df mice, leading to a hypoplastic anterior pituitary (299). In addition to Pit1, both Wnt pathway and Notch pathway are apparently affected in the Ames mice (69, 226). Human PROP1 possesses similar function in pituitary development. Mutations in PROP1 are the leading cause of CPHD in humans (46, 53, 193, 315).

Genetic studies have demonstrated that temporal regulation of Prop1 expression is critical for proper pituitary development. Premature expression of Prop1 in Rathke’s pouch proves to be deleterious leading to agenesia of the anterior pituitary gland probably by inhibiting the endogenous function of Hesx1 (56). In contrast, persistent expression of Prop1 in thyrotropes and gonadotropes under the control of aGSU element delays gonadotrope differentiation and leads to transient hypogonadotropic hypogonadism and increased susceptibility to pituitary adenomas (54, 295).

E. Six Homeodomain Transcription Factors

Six genes are mammalian homologs of Drosophila melanogaster sine oculis (so) homeobox containing genes. Studies in Drosophila and mammalian systems reveal that an interactive network of the regulatory genes comprising eyegless (ey)/Pax6, soSix, eyes absent (eya)/Eya, and dachshund (dach)/Dach is required for the eye patterning (reviewed in Refs. 132, 268, 304). In mammals, six members of the Six family have been identified, which belong to three subfamilies based on sequence conservation: (so) Six1, 2; (six4) Six4, 5; (optix) Six3, 6. Four of the Six genes, Six1, Six3, Six4, and Six6, are expressed in the developing pituitary. Six1 and Six4 are coexpressed in many embryonic primordium structures including Rathke’s pouch (reviewed in Ref. 132). Six1 is required for the development of many organs and plays an important role in regulating cell proliferation. Loss of Six1 results in defects in most of the organs where Six1 is expressed, whereas inactivation of Six4 does not cause any embryonic defects (47, 154, 155, 167, 210, 211, 320, 327, 331). However, pituitary development is not affected in either Six1−/− or Six4−/− embryos. A recent screen for target genes of Six1 and Six4, respectively, reveal that they regulate overlapping and distinct sets of genes, implying that Six1 and Six4 may share overlapping functions (10). Indeed, inactivation of both Six1 and Six4 leads to more severe phenotypes than either single mutant with
craniofacial and rib defects and general muscle hypoplasia (99). Further analysis of *Six1<sup>−/−</sup>* and *Six4<sup>−/−</sup>* will facilitate our understanding of the roles of *Six1/4* in the process of pituitary organogenesis.

*Six1* regulates transcription of its target genes by recruiting cofactors via the Six domain. *Six1* can either function as a repressor by interaction with Dach or Tle family members, or as an activator by interaction with Eya proteins. Eya family proteins are characterized by a highly conserved COOH-terminal region known as the Eya domain, which mediates interactions with Six and Dach, as well as the less conserved NH<sub>2</sub>-terminal transactivation domain (31, 106, 167, 205, 267, 319). Recently, three laboratories have independently demonstrated that Eya domains possess intrinsic phosphatase activity that is required for lineage-specific differentiation. All the cell primordia; pituitary development is nonetheless not affected (167, 318, 331). However, in *Eya1<sup>−/−</sup>* embryos, the pituitary gland is ~5- to 10-fold smaller than the wild-type gland, suggesting that Six1 and Eya1 cooperate to regulate pituitary development. In zebrafish, *siz1* and *eya1* are coexpressed in all pituitary cell types. While consistent with findings in other systems, *siz1* plays a role in modulating proliferation of pituitary cells; *eya1* is required for lineage-specific differentiation. All the cell lineages except lactotropes are affected in *eya1* mutants. Differentiation of corticotropes, melanotropes, and gonadotropes is completely impaired, whereas differentiation of somatotropes and thyrotropes initiates; expression of cognate hormone genes *gh* and *tshβ*, however, fails to be maintained probably due to progress loss of *pit1* expression. Unlike in other systems or organs, *eya1* is not required for the survival of pituitary cells, thereby uncoupling the differentiation-promoting and antiapoptotic functions of Eya proteins (202).

*Six6* expression in pituitary exhibits a dorsal-ventral gradient at the early stage of pituitary development. At E13.5, the expression declines in the differentiated cells while persistent in periluminal cells. Deletion of *Six6* results in hypoplastic pituitary and retina with reduced number of terminal differentiated cells due to defects in precursor cell proliferation. In retina, *Six6* directly represses expression of *p27Kip1* by recruiting corepressor complexes (168). *Six3* is expressed in the most anterior part of the developing neural plate and later is restricted in retina precursor cells and pituitary. *Six3* promotes cell proliferation and counteracts Wnt and BMP signalings and therefore specifying anterior identity (90, 156). Because of anterior patterning defects associated with loss of *Six3*, the specific requirement of *Six3* in pituitary development remains to be determined. Intriguingly, *Six3* can also promote proliferation by sequestering Geminin from Cdt1, the key component for the assembly of the prereplication complex (64, 182).

F. *Pax6*

*Pax6* is a highly conserved member of a family of transcription factors containing a paired domain and a homeodomain. Studies of the mice carrying mutations in the *Pax6* gene and *Pax6*-deleted mice have established that *Pax6* is required for the development of the eye, olfactory system, brain, spinal cord, and pancreas. *Pax6* is initially expressed in the anterior neural plate that will become telencephalon, diencephalon, eyes, and pituitary. During pituitary development, *Pax6* is expressed in the oral ectoderm at E9.0, but is excluded from the *Shh* expressing region. By E10-E12, *Pax6* expression is detected in the Rathke’s pouch with an apparent dorsal/ventral gradient and becomes downregulated at E13.5 and diminished when cells reach terminal differentiation at E17.5. *Pax6* deficiency leads to a dorsal expansion of ventral &gsu;&expressing cell types, predominately thyrotropes, at the expense of dorsal cell types somatotropes and lactotropes. Thus the transient expression of *Pax6* is required to establish the boundary between dorsal somatotropes/lactotropes and ventral thyrotropes/gonadotropes territories (19, 147). The similar scheme of *Pax6* in the dorsal/ventral patterning and specification of cell types is reiterated in CNS forebrain and spinal cord development (reviewed in Ref. 270).

V. CORTICOTROPE DIFFERENTIATION

A. Corticotrope Lineage Specification

ACTH-producing corticotrope is the first pituitary cell type to reach terminal differentiation. The POMC-expressing corticotrophes occur at E12.5 followed by TSH-positive thyrotrophes at E14.5, GH-positive somatotrophes at E15.5, PRL-positive lactotrophes at E16.5, and finally FSH and LH expression gonadotrophes at E16.5 (124, 269). With the use of transgenic mice, it has been demonstrated that the 480-bp *POMC* promoter is sufficient to target expression in developing pituitary (103, 174, 291), whereas the distal enhancer region (~13 to ~9 kb) is required for expression in ventral diencephalon (63). Analyses of regulatory elements in the *POMC* promoter, using transgenic mice or the corticotrope cell line AtT20 as a model system, have revealed the binding sites for bHLH proteins, orphan nuclear receptors Nur, Pitx factors, and T-box factors (158, 159, 175, 185, 217, 218). The zebrafish *POMC* gene promoter −451 to +61, containing similar regulatory elements, can target expression in cor-
ticitrope, suggesting a conserved mechanism to regulate corticotrope differentiation (179).

NeuroD1 is a class B bHLH transcription factor. It is expressed in pancreatic endocrine cells, the intestine, developing CNS, and pituitary. In pituitary, NeuroD1 protein is detectable between E12 and E15.5. It has been shown that NeuroD1/E47 heterodimer can bind to an E-box in the promoter of POMC and activate transcription either alone or in synergy with Pitx1 and Tbx19 through direct interactions of E47/Pitx1 and E47/Tbx19 (157, 220, 221). Human and mice with NeuroD1 deficiency develop diabetes due to a failure to develop mature islets (200). Corticotrope differentiation is transiently delayed in NeuroD1+/− and NeuroD1−/− mice in a dosage-sensitive manner. However, corticotrope lineage commitment, indicated by Tbx19 expression, is not affected in the absence of NeuroD1, suggesting that NeuroD1 is required for the appropriate timing of corticotrope terminal differentiation (157, 176). Whether other members of bHLH family can function redundantly with NeuroD1 to control lineage commitment remains to be determined (176).

Tbx19, also known as Tpit, is a member of T-box transcription factors with a characteristic 180-amino acid DNA binding domain that plays a critical role in embryonic development (reviewed in Ref. 197). Mouse Tbx19 was identified in a screen for genes encoding T-box binding proteins present in POMC expressing AtT20 cells (158). It was also cloned as a homolog of the human TBX19 gene (176). Tbx19 expression is initiated at E11.5 in the most caudoventral region of the Rathke’s pouch before the onset of POMC expression. Tbx19 is also expressed in the ventral diencephalon, although wherein no protein is detected. In the pituitary, Tbx19 is restricted in the two POMC-expressing lineages, anterior lobe corticotropes and intermediate lobe melanotropes (158, 176). Tbx19 recognizes a T-box element located next to the Pitx1 binding site in the POMC promoter and synergizes with Pitx1 in activating the POMC promoter by recruiting SRC/p160 coactivators (158, 176, 184). The function of Tbx19 has been revealed by both loss-of-function and gain-of-function studies. Inactivation of Tbx19 leads to a failure of corticotrope and melanotrope terminal differentiation, although early corticotrope lineage commitment seems to be intact as indicated by NeuroD1 expression. Tbx19 is also required in POMC-expressing cells to repress alternate cell fates. In the absence of Tbx19, the intermediate lobe is hypoplastic with almost complete loss of the POMC-expressing melanotropes. The presumptive melanotropes as well as corticotropes in the ventrocaudal region of the anterior pituitary instead adopt cell fates of gonadotrope and Pit1-independent rostral tip thyrotrope (223). In contrast, overexpression of Tbx19 under the control of aGUS regulatory sequences results in ectopic activation of ACTH in the rostral tip and marked reduction of aGUS, TSHβ, LHβ, FSHβ, and SF1 expression in thyrotopes and/or gonadotropes (158, 176, 223). Thus Tbx19 promotes terminal differentiation of corticotropes and melanotropes by stimulating POMC expression and preventing differentiation of alternative cell fates (136, 223). Consistent with this notion, Tbx19 can antagonize Lhx3 and Pitx1 on the aGUS promoter and SF1 on an SF1-responsive promoter (176, 223). Interestingly, NeuroD1 is not expressed in the ectopically induced corticotropes in Tbx19 transgenic mice, implying that NeuroD1 is not obligatory for corticotrope differentiation and Tbx19 functions independent of NeuroD1 (158). Tbx19+/− mice have low plasma ACTH levels and impaired adrenal function (222). Similarly, mutations in human TBX19 gene are the leading cause for neonatal-onset isolated ACTH deficiency (222, 293).

B. Regulation of POMC Expression

The major role of corticotropes is their ability to respond rapidly to a variety of stress and inflammation stimuli. The POMC promoter is positively regulated by hypothalamic hormone corticotropin-releasing-hormone (CRH) through rapid and transient activation and induction of the three Nur subfamily transcription factors, including Nur77, Nurr1, and NOR1. Two Nur binding sites have been identified in the POMC promoter, the distal Nur response element (NurRE), which can be recognized by either homo- or heterodimers formed between subfamily members, and proximal monomer Nur77-binding response element (NBRE). However, only the NurRE site is necessary for CRH responsiveness in AtT20 cells. Additionally, a dominant negative mutant of Nur77 blocks the action of CRH on the POMC promoter, suggesting that the Nur factors are the major integrator of the CRH signal in pituitary corticotropes (185, 218). CRH stimulation activates Nur factors through the protein kinase A (PKA) pathway by increasing the DNA binding activity of Nur77 dimers and enhancing recruitment of p160/SRC transcription coactivators via the AF-1 domain of Nur77 (184).

ACTH regulates metabolic functions through stimulation of glucocorticoid synthesis in the adrenal gland. Glucocorticoids in turn mediate the feedback repression of the POMC expression by interaction with the glucocorticoid receptor (GR). Consistent with this notion, in GR−/− mice where the negative feedback is disrupted, there is a marked increase in POMC expression in corticotropes (232). DNA binding of GR is required for repression of the POMC gene in vivo as demonstrated in the mice carrying a point mutation in the dimerization domain of GR (GRdim, Ala458Thr) (231). In AtT20 corticotropes, glucocorticoids antagonize the actions of CRH by transrepression involving Swi/Snf chromatin remodeling protein Brg1-mediated recruitment of GR and corepressor HDAC2 to the Nur77-bound NurRE site (22, 186). Loss of
nuclear expression of Br γ1 or HDAC2 is associated with 50% glucocorticoid-resistant human and dog corticotrope adenomas (22).

Leukemia inhibitory factor (LIF) is a pleiotropic neuroimmune cytokine that promotes corticotrope differentiation and induces POMC expression and ACTH secretion. The role of LIF in corticotrope differentiation has been demonstrated by gain-of-function studies. Transgenic mice expressing LIF in somatotropes exhibit striking dwarfism with decreased number of somatotropes and lactotropes and increased number of corticotropes. Overexpressing LIF early during pituitary development under the control of aGSU regulatory sequences leads to a significant expansion of corticotropes and a dramatic decrease of somatotropes, lactotropes, and gonadotropes as well as a variably diminished thyrotropes. Lhx3 and Pit1 expression are decreased at E14.5 in transgenic mice. Thus LIF may repress Lhx3-dependent cell fates (somatotropes, lactotropes, thyrotropes, and gonadotropes) and drive pituitary progenitors differentiation toward corticotrope lineage (4, 321). In contrast, inactivating Pit1 gene results in a 30% decrease in pituitary POMC mRNA levels, consistent with the idea that LIF regulates POMC expression (300). LIF stimulates POMC expression by activating the JAK/STAT pathway. Two regions in the POMC promoter are responsive to LIF induction. A distal region harbors a STAT binding site, whereas a proximal site does not display STAT binding activity and may thus involve a DNA binding-independent mechanism (196).

VI. DIFFERENTIATION OF PIT1 LINEAGES: SOMATOTROPES, LACTOTROPES, AND THYROTROPES

A. Pit1

Pit1 is a member of POU domain containing transcription factors. The POU domain consists of a NH2-terminal POU-specific domain and a COOH-terminal POU homeodomain, which are both required for high-affinity DNA binding (reviewed in Ref. 9). Pit1 was identified by its specific binding to AT-rich elements in the rat PRL and GH genes. The expression of Pit1 is restricted to the caudomedial region of the pituitary gland. Its expression begins at E13.5 and continues in somatotropes, lactotropes, and thyrotropes throughout adult life. Genetics studies of the Snell (dw) and Jackson (dw3) mice, which carry nature mutations in the Pit1 gene, have established that Pit1 is essential for the terminal differentiation and expansion of three lineages: somatotrope, lactotrope, and thyrotrrope, as well as for repressing gonadotrope cell fate (36, 57, 166). Furthermore, Pit1 is necessary for the transcriptional regulation of genes encoding the hormone products of these cell types, including GH, PRL, TSHβ, and GHRHR (reviewed in Ref. 9). Recently, in zebrafish, two null alleles in the pit1 gene have been identified in a systematic screen for genes required for pituitary formation and patterning (110, 201). Somatotropes, lactotropes, and thyrotropes are completely absent in pit1 mutants, which also exhibit expansion of corticotropes and possibly gonadotropes, suggesting that Pit1 function is largely conserved throughout evolution. In humans, mutations in the PIT1 gene are associated with CPHD (reviewed in Refs. 46, 53, 193).

B. Regulation of Pit1 Expression

The initial activation of the Pit1 gene at E13.5 in anterior pituitary requires the concerted actions of Prop1 and the Wnt/β-catenin signaling and is mediated by an early enhancer located between −5.1 and −10.2 kb upstream of the transcription start site (66, 208). From E16.5, maintenance of Pit1 gene expression requires a distinct autoregulated distal enhancer located at −10 kb upstream of the transcription start site. The distal enhancer contains three functional Pit1 binding sites, a vitamin D receptor binding site, and a retinoic acid (RA) response element that confers Pit1-dependent RA induction (235). In the Snell (dw) mice, Pit1 expression is activated normally at E14.5, but because the Pit protein is defective and autoregulation is therefore nonfunctional, Pit1 expression declines and becomes extinguished in the perinatal period (235).

C. Regulation of Somatotrope/Lactotrope Determination

Studies of the cis-acting sequences of the rat growth hormone gene (rGH) have established that the minimal information required for selective expression in somatotropes but not lactotropes resides in the proximal 320 bp of the promoter, with as little as 180 bp being sufficient to target reporter in vivo (173). This region contains binding sites for Pit1, thyroid hormone receptor (TR), Sp1, a zinc finger protein Zn-15, and a yet unidentified protein. Extensive mutational analyses in transgenic mice have revealed that multiple elements within this region are essential to mediate GH gene activation in somatotropes and repression in lactotropes, respectively. Sp1 binding site and the zinc finger protein binding site (Z box) are apparently only required for GH activation in somatotropes while −161/−146 site is required only for GH restriction from lactotropes. On the other hand, thyroid hormone response element (TRE) and Pit1 binding sites (GH-1, 2) are required for both activation in somatotropes and repression in lactotropes. Interestingly, replacing the GH-1 site with the Pit1 binding site (Prl-1P) from the PRL gene results in a loss of restriction from the lactotropes.
without affecting expression in somatotropes. The allo-
steric effect of Pit1 sites is further supported by the cocrystals of the Pit1 POU domain dimer bound to either GH-1 or Prl-1P sites. The data reveal that the spacing between the DNA contacts made by the POU specific domain and the POU homeodomain of each monomer is increased by 2 bp on the GH-1 site. Deletion of these 2 bp in GH-1 leads to a failure to effectively repress reporter gene expression in lactotropes, suggesting that the configuration of Pit1 bound to the GH-1 site is critical for restriction of GH gene expression in lactotropes. The transcriptional corepressor N-CoR, which can interact with Pit1, has been found by chromatin immunoprecipitation (ChIP) assay to be associated with the nontranscribing GH promoter in nonsomatotrope cells. In addition, overexpression of a dominant negative form of N-CoR in lactotropes results in derepression of the endog-
enous GH gene. Thus cell type-specific restriction of GH requires combinatorial efforts of Pit1, TR, and an unknown factor that recognizes −161/−146 site together with the corepressor machinery including N-CoR (254).

The −500 bp promoter of human pituitary GH (hGH-N) gene contains binding sites for Pit1, Sp1, and zinc finger proteins. However, it is not sufficient for effi-
cient expression in transgenic mice and a locus control region (LCR), located 14.5 to 32 kb upstream, is required for efficient and position-independent expression of the hGH-N transgene (127). Further dissection of the hGH-N LCR reveals that a 1.6-kb region containing two pituitary specific DNase I hypersensitive sites (HSI and HSII) is sufficient to activate hGH transgene expression. The ma-
JOR functional activity within this region resides in a
404-bp fragment coincident with the HSI, which encompasses three closely spaced Pit1 binding sites (18, 260). All three Pit1 binding sites within the 404-bp fragment contribute to the LCR activity. Furthermore, these three Pit1 sites are sufficient to confer position-independent and somatotrope-restricted −0.5hGH-N transgene activa-
tion (261, 262). In pituitary gland of transgenic mice carrying the intact hGH locus, ChIP assays reveal a 32-kb domain of pituitary-specific histone hyperacetylation ex-
tending from the LCR to the hGH-N promoter with peaks at HSI and HSII sites. Targeted deletion of a region in HSI that contains two Pit1 sites results in a striking loss of the acetylated domain and a marked reduction of hGH-N transgene expression in the pituitary. Thus the Pit1 sites at HSI play an essential role in the establishment of acetylated active chromatin domain and in the transcriptional activation of the hGH-N gene (112). Interestingly, HSI is also required for the active intergenic Pol II tran-
scription in a domain that includes the hGH-N LCR and adjacent B lymphocyte-specific CD79b gene. Insertion of an exogenous transcriptional terminator within this do-
main blocks CD79b transcription and represses hGH-N expression without affecting histone acetylation within the hGH locus, suggesting that distal LCR transcription plays a critical role in long-range hGH-N activation (35, 113). It certainly will be interesting to appreciate the discrepancy between the rGH and hGH promoters and to determine whether the similar LCR sequence and function are conserved in rodents.

D. Regulation of Somatotrope-Specific Gene Expression

The rGH gene transcription is strongly stimulated by the TH and by RA via the TRE element that binds TR/ retinoid X receptor (TR/RXR) and RAR/RXR heterodimer. Pit1 interacts strongly with RXR and to a lesser extent with TR and RAR and synergistically regulates the GH promoter (212, 213, 247). The functional significance of the TRE in regulation of GH expression has been demonstrated in transgenic mice where TRE mutation results in lower reporter gene expression in somatotropes (254). Consistently, in TRα1−/−β−/− mutant mice which lack all known TRs, there are reduced numbers of somatotropes in anterior pituitary and reduced serum levels of insulin-
like growth factor (IGF) and pituitary GH leading to growth retardation (97). TH-mediated activation of the rGH promoter can be antagonized by a transcriptional repressor zinc finger homeodomain enhancer binding pro-
tein Zfhep through a mechanism other than simple competition for binding to the TRE (33).

GH expression is also tightly regulated by the hypo-
thalamic factor, growth hormone-releasing hormone (GHRH), through activating the GHRH receptor (GHRHR) expressed on the pituitary somatotropes. Binding of GHRH to its receptor elicits elevated intracellular cAMP levels and subsequently activates the PKA pathway (172). GHRH-stimulated activation of the GH promoter is medi-
ated by PKA-dependent phosphorylation of the transcrip-
tional coactivator CBP, which interacts and synergizes with Pit1 on the Pit1 binding sites (45, 317). The func-
tional significance of the GHRHR signaling pathway in somatotrope proliferation has been revealed in the little (GHRHRα+/GHRHRα−) mice which carry a single amino acid substitution in GHRHR leading to defective ligand binding, as well as in mice deleted for the GHRH gene. These mutant mice exhibit hypoplastic pituitary gland and GH deficiency, causing postnatal growth retardation (5, 93, 172). In humans, mutations in the GHRHR gene are associated with IGHD (reviewed in Ref. 193). Expression of GHRHR in pituitary is dependent on Pit1 as well as Math3, which encodes a bHLH transcription factor and itself is a downstream target of Pit1. Mice deficient in the Math3 gene lack GHRHR expression, exhibit defects in somatotropes maturation and proliferation, and are post-
natal dwarf (282, 330).
E. Regulation of Lactotrope-Specific Gene Expression

Analyses of the rat PRL (rPRL) promoter in transgenic mice have demonstrated that 3 kb of 5′-flanking region is sufficient to direct lactotrope specific transgene expression and a synergistic interaction between a distal enhancer (−1.8 to −1.5 kb), and a proximal promoter region (−422 to +33 bp) is required for high levels of expression (52). The distal enhancer contains four Pit1 binding sites and an estrogen response element (ERE) and has been shown to mediate the synergistic interaction between Pit1 and estrogen receptor (ER) and stimulate PRL transcription in response to estrogen (60, 204, 269). Estrogen-induced PRL expression also requires an intact mitogen-activated protein kinase (MAPK) signaling transduction pathway as interfering with MAPK activation ablates the ability of estrogen to induce PRL expression (303). The in vivo function of ER in regulating PRL expression has been demonstrated in mice deleted for the ERα gene. Specification of lactotrope lineage appears normal in these mutant animals; however, there is a marked reduction in PRL mRNA and a decrease in the number of lactotropes (253).

The proximal promoter of the PRL gene encompasses binding sites for Pit1, Ets, and Pitx factors and is sufficient to confer cell specific gene expression and mediate regulation by a variety of stimuli. Analyses of the RPL promoter in model cell lines have identified two critical Ets binding sites (EBS): a composite Ets-1/Pit1 binding site located at −212 and an EBS located at −96. The composite Ets-1/Pit1 binding site confers synergy between the two proteins and mediates stimulation by the Ras/MAPK signaling transduction pathway including FGF and thyrotropin-releasing hormone (TRH) (24–26, 116). Pit1 directly interacts with Ets-1. However, the synergy is independent of their physical interaction but requires respective DNA binding sites (71). The synergy between Pit1 and Ets-1 can be blocked by ETS-2 repressor factor (ERF) apparently by preventing Pit1 from binding to the composite site as well as other Pit1 sites (61). The more proximal EBS centered at −96 critical for several growth factor signaling pathways is recognized and activated by ETS factors GABPα and GABPβ (252).

Other transcription factors that have been implicated in regulation of the PRL promoter include Pitx factors and CCAAT/enhancer-binding protein (C/EBPα). Both Pitx1 and Pitx2 can interact and synergize with Pit1 in activating several pituitary-specific promoters (7, 278, 290). The synergy between Pitx2 and Pit1 is achieved by Pit1 binding to the COOH-terminal tail of Pitx2 and relieving the autorepression imposed by this region and thereby increasing DNA binding of Pitx2 to a canonical bicoid site (6). Two bicoid sites, B1 and B2, located at −27 and −110, respectively, have been identified in the human PRL proximal promoter with the B2 site and two Pit1 binding sites necessary for the synergistic interaction of Pitx2 and Pit1 (224). C/EBPα belongs to the bZIP family of transcription factors characterized by a conserved COOH-terminal domain containing a basic DNA-binding domain and a leucine zipper that mediates protein-protein interaction. C/EBPα can synergize with Pit1 to stimulate the rPRL promoter and the rGHRH promoter (122). The C/EBPα binding site in the PRL promoter overlaps with the proximal EBS which is recognized by GABPα/GABPβ (122). Interestingly, the physical interaction of Pit1 with C/EBPα leads to C/EBPα redistribution from otherwise centromeric heterochromatin region to nuclear regions occupied by Pit1 (62, 74).

PRL expression in pituitary is under the negative control of hypothalamic dopamine via the G i/o-coupled dopamine D2 receptor (D2R) expressed on lactotropes in pituitary. The inhibitory effects of dopamine on PRL transcription are mediated by antagonizing the elevation of intracellular cAMP or calcium. It has been shown that dopamine treatment leads to a rapid decrease in activated MAPKs, nuclear translocation of ERF, and recruitment of HDAC corepressors to the PRL promoter in cell lines or primary pituitary culture (177, 178). The physiological function of dopamine has been revealed in mice deficient in the D2R receptor (Drd2−/− mice). These mice have anterior lobe lactotrope hyperplasia and hyperprolactinemia that ultimately leads to lactotrope adenoma in aged animals (139, 242). Conversely, mice lacking the dopamine transporter (DAT), which mediates dopamine reuptake and thereby termination of dopamine action, display hypoplasia of lactotropes and somatotropes, with the latter attributed to the substantial decrease of GHRH expression in hypothalamus (23). Lactotropes are also subject to negative regulation mediated by prolactin receptor (PRLR) signaling via both dopamine-dependent and -independent mechanisms. Prlr−/− mice exhibit more profound hyperprolactinemia and large prolactinomas than Drd2−/− mice, and there are additive effects in compound homozygous mutant male mice. In addition, PRL treatment markedly inhibits lactotrope proliferation in primary mouse pituitary cultures, suggesting an autocrine/paracrine negative regulatory mechanism (249). Recent studies in transgenic zebrafish expressing red fluorescent protein directed by Prl regulatory elements demonstrate that dopamine-independent PRLR signaling exerts more robust inhibitory effects on embryonic lactotropes than dopaminergic signaling, highlighting the importance of dual peripheral and central interactions for lactotrope proliferation and PRL gene regulation during early pituitary development (180).
F. Specification of Thyrotrope Lineage

The third Pit1-dependent cell lineage, thyrotrpoe, shares some common features with the non-Pit1-dependent gonadotrope. They arise from the ventral portion of the gland and secrete heterodimeric hormones containing the common aGSU subunit and specific β-subunits. However, these two lineages appear to diverge at an early stage of pituitary development, such that transcription factors important for cell type-specific hormone expression are present in respective cell type, for example, Pit1 is present in thyrotropes and SF1 in gonadotropes. The plasticity of these two cell types has been demonstrated in several genetic models where gonadotropes can be converted into thyrotropes when Pit1 is ectopically expressed ventrally under the control of aGSU regulatory sequences and the Pit1 lineages including thyrotropes adopt the fate of gonadotropes in Snell mice, highlighting the critical function of Pit1 in lineage choice, and also implying that gonadotropes possess the necessary factor(s) for thyrotrpoe specification (57). The zinc finger transcription factor Gata2, expression of which is under the control of ventral to dorsal BMP2 gradient, is present in both thyrotropes and gonadotropes. Furthermore, Gata2 physically interacts and functionally cooperates with Pit1, leading to synergistic activation of the TSHβ promoter through adjacent Pit1 and Gata2 binding sites. Extensive transgenic studies have suggested that the unique combination of Pit1 and Gata2 in thyrotropes plays a critical role in TSHβ expression and thyrotrpoe specification (57, 95). The in vivo function of Gata2 in pituitary development has recently been investigated by targeted inactivation of Gata2 in the pituitary using a Cre line directed by the aGSU regulatory element (42). The mutant mice have fewer thyrotropes and gonadotropes at birth and exhibit reduced production of TSH and FSH in response to the loss of negative feedback by thyroid hormones and steroid hormones, respectively, suggesting that Gata2 is important for optimal thyrotrpoe and gonadotrope function but not for thyrotrpoe and gonadotrope cell fate specification, although it remains possible that the function of Gata2 is compensated by Gata3, as in the mutant pituitary gland Gata3 expression is elevated (42).

A second thyrotrpoe lineage, transiently residing in the rostral tip, is Pit1 independent (171). The ontogeny and function of this lineage is largely unknown. A member of the proline and acidic amino acid-rich basic leucine zipper (PAR bZip) transcription factor family, TEF, expressed in rostral tip, is a potent activator of the TSHβ promoter (70). Expression of all three PAR bZip factors is under the control of circadian rhythms. While deletion of a single factor does not have adverse effects, deletion of all three factors results in epilepsy due to neurotransmitter deficiencies, and dramatically shortened life span (85). The involvement of TEF and other family members in rostral thyrotrpoe specification remains to be defined.

G. Regulation of aGSU Gene Expression

aGSU is the first pituitary hormone transcript expressed during development. In mouse, it is first detected at E10.5 in the most ventral region of the Rathke’s pouch. Expression of aGSU gene is governed by a series of distinct elements that confer pituitary-specific expression as well as differential expression in thyrotropes and gonadotropes (reviewed in Ref. 128). Analyses of the mouse aGSU promoter in transgenic mice have revealed that 381 bp of the promoter is sufficient for expression of a reporter gene in both thyrotropes and gonadotropes, although hormonally and temporally regulated high levels of expression are achieved when 4.6 kb of 5′-flanking region is included (29, 140). However, 313 bp of the bovine aGSU promoter specifically directs expression to gonadotropes in transgenic mice (141), suggesting that the upstream elements confer aGSU expression in thyrotropes. The pituitary glycoprotein hormone basal element (PGBE) located from −337 to −330, which is recognized by a LIM-homeodomain transcription factor, such as Lhx3, is necessary for mouse aGSU promoter activity in both cell types in cell-based assay and critical for restricting expression to the anterior pituitary (12, 29, 239). Other transcription factors involved in transcriptional regulation of the aGSU promoter include the bHLH zipper protein USF, Pitx1/2, and Gata2 which are present in both cell types and interact with elements in the proximal promoter (121, 243, 278, 290). Gonadotrope specific expression of aGSU is regulated by SF1 via its cognate binding site, the gonadotrope-specific element (GSE), located at −208 (17, 120). The distal enhancer region located between −4.6 kb and −3.7 kb promotes reporter gene expression in both transgenic mice and in transient transfection assays. Its enhancer activity is dependent on the presence of GSE and PGBE in the proximal promoter in gonadotropes and PGBE in thyrotropes (312). The localized 125-bp enhancer element harbors consensus binding sites for GATA, SF1, Sp1, ETS, bHLH factors, and also mediates aGSU repression in cell types other than thyrotropes and gonadotropes, suggesting cooperative interactions between the enhancer and promoter (311).

In thyrotropes, expression of aGSU is stimulated by TRH and repressed by TH. In the anterior pituitary, binding of TRH to its receptor activates phospholipase C, leading to calcium mobilization and protein kinase C (PKC) activation. It has been shown that Lhx3 plays a pivotal role in mediating TRH signaling by recruiting transcriptional coactivator CBP to the aGSU promoter, and the PKC phosphorylation sites in the LIM1 domain of Lhx3 are essential for Lhx3/CBP binding and TRH re-
sponse (104). In gonadotropes, gonadotropin releasing hormone (GnRH) regulates expression of the \(\alpha\)GSU gene via two elements in the proximal promoter, PGBE and a second element recognized by an ETS factor which is activated by MAPK pathway in response to GnRH (238).

H. Regulation of TSHβ Gene Expression

Transient transfection experiments in thyrotrope cells have shown that the cell-specific activity of the mouse TSHβ promoter is localized between −270 and −80 of the 5′-flanking region (313). Pit1 binds to three sites within this region and is required for the TSHβ transcription in the caudomedial thyrotropes (171). Other transcription factors functionally cooperating with Pit1 in stimulating the TSHβ promoter include Lhx3, which is present in all cell types, and Gata2 found only in thyrotropes and gonadotropes (12, 42, 57, 95). Expression of TSHβ is positively regulated by hypothalamic TRH and inhibited by TH feedback regulation. Recent studies have shown that CBP and Pit1 act synergistically in TRH stimulation of the TSHβ promoter via Pit1 binding sites (105), and phosphorylation of CBP by PKC is critical for Pit1-dependent gene activation (323). TH-dependent negative regulation of TSHβ transcription is mediated by the β-isof orm of TR (TR-β) through an element downstream from the transcription start site via recruiting HDAC activity (1, 82, 244). DNA binding activity as well as intact coactivator-interacting surface of TR-β are both essential for the feedback regulation, as demonstrated in mice carrying the mutant form of TR-β (209, 263).

VII. GONADOTROPE DIFFERENTIATION

A. SF1 and Other Transcription Factors in Gonadotrope Lineage Differentiation and Function

Gonadotrope is the last cell type in the anterior pituitary to reach maturation with the expression of terminal differentiation markers LHβ, FSHβ, and GnRHR. The specification of gonadotrope cell fate, however, occurs a few days earlier with the onset of SF1 expression at E13.5 (120). Transgenic studies have localized an enhancer element in the sixth intron of SF1 conferring pituitary specific expression (264). SF1 is also expressed in developing gonads, adrenal glands, and ventromedial hypothalamic nucleus (VMH). It encodes a zinc finger nuclear receptor directly regulating a large number of genes involved in sex determination and differentiation, steroidogenesis, and reproduction including \(\alpha\)GSU, LHβ, FSHβ, and GnRHR. Recent studies have revealed that phospholipids bind to the ligand-binding pocket of SF1 with phosphatidylinositol 3-kinase-derived phosphatidinositolyls [PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃] delineated as high-affinity preferred ligands. Mutations intended to interfere with ligand binding abolish coactivator recruitment and impair SF1 transcriptional activity, implicating that the function of SF1 is likely to be modulated in vivo by endogenous ligands (153, 169, 298). While the regulation of SF1 activity is an exciting new area, the physiological function of SF1 has long been established by targeted disruption, which leads to adrenal and gonadal agenesis, defective differentiation of the VMH, and impaired expression of LHβ, FSHβ, and GnRHR in pituitary (120, 265; reviewed in Ref. 215). Pituitary-specific inactivation of SF1 recapitulates the SF1⁻/⁻ pituitary phenotype and results in sterile mice with hypoplastic gonads (324, 325). However, expression of LHβ and FSHβ in SF1⁻/⁻ mutant mice can be restored by GnRH treatment, arguing that SF1 is not necessary for gonadotrope cell fate specification (119, 325).

The GnRH compensation for the LHβ expression in SF1⁻/⁻ mice has been suggested to be mediated by Egr1, a GnRH-inducible zinc finger transcription factor that can synergize with SF1 to activate the LHβ promoter (163, 288). Egr1 is preferentially expressed in somatotropes, thyrotropes, and gonadotropes. Mice deficient for Egr1 exhibit reduced numbers of somatotropes and diminished expression of LHβ (163, 284). However, gonadotrope differentiation in Egr1⁻/⁻ mice occurs normally with FSHβ expression, consistent with a direct role of Egr1 in regulating LHβ transcription.

Extensive studies using transgenic mice have suggested that Gata factors, such as Gata2, expression of which precedes SF1, may play a role in gonadotrope differentiation. Ectopic expression of Gata2 in Pit1 lineages leads to repression of Pit1 and subsequent gonadotrope conversion while overexpression of dominant negative form of Gata2 blocks differentiation of both thyrotrope and gonadotrope (57). However, as noted above, pituitary-specific inactivation of Gata2 leads to reduced secretion of gonadotropins basically and in response to the loss of negative feedback by steroid hormones without affecting terminal differentiation of gonadotropes. The mild effect of deleting Gata2 is likely to be due to functional compensation by Gata3, although it remains to be determined whether Gata2 and Gata3 function together in lineage specification (42).

While no single transcription factor has been demonstrated to be necessary and sufficient for gonadotrope lineage commitment, other transcription factors, in addition to SF1, Egr1, and Gata2, have been implicated in regulating gonadotropes function, including Pitx1, Pitx2, Prop1, and Otx1. Otx1 is expressed in postnatal pituitary, and transient transfection experiments indicate that Otx1 can specifically activate promoters of \(\alpha\)GSU, LHβ, and FSHβ. Mice deficient for Otx1 exhibit transient dwarfism and hypogonadism at the prepubescent stage.
due to the selective and transient reduction of GH, FSH, and LH in pituitary, suggesting a differential requirement of transcription factors at different developmental stages (2).

B. Transcriptional Regulation of Gonadotrope Specific Genes

1. FSHβ gene regulation

FSH is a major regulator of gonadal development. It is required for oogenesis in females and is important for spermatogenesis in males. The basal expression of FSHβ is regulated by Lhx3, Pitx1, heterotrimeric nuclear factor-Y (NFY), and SF1. Lhx3 can activate the porcine FSHβ promoter by recognizing multiple elements within the promoter. These sites, however, are not required for the activin response in LβT2 gonadotrope cells (305). Pitx1 has been demonstrated to activate the rat FSHβ gene promoter both basally and in synergy with GnRH by binding to basal activity of the mouse FSHβ gene, which is conferred by SF1. It has been shown that SF1 physically and functionally interacts with ubiquitously expressed NFY contributing to basal activity of the mouse FSHβ promoter via the upstream elements (123).

Expression of FSHβ is primarily regulated by GnRH, gonadal steroids, and members of the TGF-β superfamily including activins (reviewed in Ref. 32). Mice with loss-of-function mutation in either Gnrh or Gnrhr gene have low levels of LH and FSH in pituitary (39, 216). Binding of GnRH to its receptor on the gonadotrope membrane activates the PKC and MAPK signaling pathways and induces transcription of the early response genes, c-fos, c-jun, and Egr1 (49). Two conserved AP1-like sites, located in the proximal promoter of the ovine FSHβ gene, are necessary to mediate GnRH responsiveness in heterologous cells. However, they are not required in LβT2 gonadotrope cell line and in transgenic mice in the context of −4741 bp to +753 bp of ovine FSHβ promoter (reviewed in Refs. 192, 294). In LβT2 cells, GnRH response is mediated by two distal elements (between −4152/−2878 and −2550/−1089 bp) in association with elements within the proximal region of the ovine FSHβ promoter (294). A recent study has identified a GnRH responsive element in the proximal promoter of mouse FSHβ gene consisting of an AP-1 half-site (−72/−69) and juxtaposed NFY binding site (−76).

Activin, a signaling protein secreted by the gonads and the pituitary, is a potent inducer of FSHβ transcription through binding to transmembrane serine/threonine kinase receptors. Mice deficient for Acvr2a (activin receptor type IIA) exhibit diminished levels of FSHβ in pituitary (189). Smad2 and Smad3 are the principal mediators of activin signaling. Upon phosphorylation induced by activin, Smad2 and Smad3 associate with common mediator Smad4 and translocate into nucleus to regulate gene transcription. Smad3 plays a key role in activin-induced FSHβ transcription (20, 275). A Smad-binding element (SBE) has been identified in the rat FSHβ promoter (−281/−253) to mediate Smad3-induced reporter activity (98, 276). However, the critical SBE is conserved only in rodent species. In the ovine FSHβ promoter, there are three regions (−973/−962, −167, and −134) required for full activin responsiveness (14). The distal site binds Smad4 protein, and the critical −134 site binds the TALE homeodomain proteins Pbx1 and Prep1 in association with Smad4, and the two proximal activin responsive elements are conserved across species and are bound by Pbx1 and Prep1 in the mouse gene (14). Pituitary-restricted regulation of FSHβ by activin can be conferred by Pitx2, which enhances both basal and activin/Smad3-induced activation of rat FSHβ promoter by interacting with a Pitx1 binding site (−230/−199) (276).

2. LHβ gene regulation

LH regulates folliculogenesis, ovulation, gametogenesis, and gonadal steroidogenesis. Transgenic studies have shown that 776 bp of bovine LHβ promoter is sufficient to direct gonadotrope-specific expression and confer regulation by GnRH and gonadal steroids (144). The proximal 140 bp of the LHβ promoter is remarkably conserved across species, whereas distal regions diverge depending on species. Within the conserved region, there are two SF1 binding sites (GSE, −58/−51, −127/−119), two Egr1 sites (−49/−41, −112/−104), and one Pitx site (−99/−96) critical for LHβ expression (100, 101, 143, 225, 288, 309, 310; reviewed in Ref. 128). Each of the transcription factors that recognize respective sites, SF1, Egr1, and Pitx1, can function alone or in synergy with others in activating the LHβ promoter through direct physical interactions (100, 288). The functional significance of the distal SF1 binding site and the Pitx1 binding site in vivo has been demonstrated in transgenic mice (143, 225). In the LβT2 gonadotrope cell line, the Pitx1 binding site is recognized by an unknown OTX-class homeodomain factor (241). A second putative Pitx1-binding site (−65/−60) in the rat LHβ promoter has recently been identified to mediate Pitx1-induced promoter activity and to contribute to the synergy between Pitx1 and SF1 (125). The distal region of the bovine LHβ promoter contains two NFY binding sites with different affinity. The more distal high-affinity NFY site is critical for conferring high basal activity of the LHβ promoter (142).

Expression of LHβ is stimulated by GnRH. GnRH responsiveness of the LHβ promoter is achieved in part by transient induction of Egr1 and the synergy between
Egr1, SF1, and Pitx1 (68, 102, 143, 225, 288, 310). The zinc-finger transcription factor Sp1 interacts with two GC-rich elements within the distal region of the rat LHβ promoter. Mutation of the Sp1-binding sites diminishes GnRH-induced activation of the promoter (130). Combined mutations in Sp1, SF1, and Egr1 binding sites have determined that these sites are required for full GnRH responsiveness, suggesting that communication exists between the distal and proximal regions of the rat LHβ promoter to mediate GnRH responsiveness (129).

VIII. THE HYPOTHALAMIC/PITUITARY REGULATORY SYSTEM

The physiological function of anterior pituitary is regulated by hypothalamic releasing hormones, which are secreted by the parvocellular neurons. CRH and TRH are synthesized by neurons in the paraventricular (PVN) nucleus. GHRH is synthesized by neurons of the arcuate nucleus and the adjacent ventromedial nucleus. Somatostatin (SS) is mainly synthesized by neurons in the anterior periventricular nucleus (aPV). GnRH is synthesized by neurons located in the preoptic region. The parvocellular neurons project to the median eminence where they release hormones that are conveyed to the anterior pituitary by the portal vascular system. Corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TRH) are synthesized by neurons in the PVN. Growth hormone releasing hormone (GHRH) is synthesized by neurons of the arcuate nucleus (ARN) and the adjacent ventromedial nucleus. Somatostatin (SS) is mainly synthesized by neurons in anterior periventricular nucleus (aPV). The parvocellular neurons project to the median eminence where they release hormones that are transported to the anterior pituitary by the portal vascular system.

Induction and patterning of the hypothalamus is regulated by HH, Nodal, BMP, and Wnt signals as well as unidentified stimulus from Rathke’s pouch (55, 131, 207; reviewed in Ref. 308). Functional studies have revealed that the ventral diencephalon is instrumental for the formation of Rathke’s pouch in part by producing signaling molecules including FGF, BMP, and Wnt. Here we discuss a number of hypothalamic specific factors that are re-
required for pituitary functions (Fig. 2). These factors, together with the signaling molecules and transcription factors critical for pituitary development, are summarized in Table 1.

A. Sox3 and the Morphogenesis of Pituitary

The HMG box transcription factor Sox3 and Sox2, members of the SoxB1 subfamily, are coexpressed in all neural precursors during CNS development. During pituitary development, they are expressed in the ventral diencephalon and throughout infundibulum. Sox2 is also expressed in Rathke’s pouch. Targeted deletion of Sox3 results in variable and pleiotropic defects, including craniofacial abnormalities, midline CNS defects, and hypopituitarism. In Sox3−/− mutant mice, expression domain of FGFS and BMP4 in the ventral diencephalon is expanded, which is accompanied by reduced proliferation and evagination of the infundibulum, leading to bifurcation of Rathke’s pouch and anterior pituitary dysmorphology with extra clefts. There is a reduction in the levels of GH, LH, FSH, and TSH in the pituitary. The pituitary of defect is suggested to result, at least in part, from a later GSH, LH, FSH, and TSH in the pituitary. There is a reduction in the levels of evagination of the infundibulum, leading to bifurcation of Rathke’s pouch and anterior pituitary dysmorphology with extra clefts. There is a reduction in the levels of GH, LH, FSH, and TSH in the pituitary. The pituitary defect is suggested to result, at least in part, from a later requirement for Sox3 within the hypothalamus neurons (236, 237). Inactivation of Sox2 leads to early lethality at peri-implantation stages precluding further analysis. Examinations of Sox2−/− mice have revealed bifurcation of Rathke’s pouch during pituitary development. The mutant mice also exhibit extra clefts in the pituitary with moderately reduced levels of pituitary LH and GH, implying a dosage-sensitive requirement for Sox3 in the pituitary development (138, 237). In humans, mutations in Sox3 are associated with X-linked mental retardation and GH deficiency, and mutations in Sox2 are associated with abnormalities in the hypothalamo-pituitary and reproductive axes (138, 161, 272).

B. Specification of GHRH Neurons: Hmx2/3 and Gsh-1

The homeodomain transcription factor Gsh-1 can bind to multiple sites in the rat GHRH promoter and stimulate promoter activity alone or in synergy with coactivator CBP (195). It is expressed in the neural tube, hindbrain, mesencephalon, and diencephalon of the developing CNS. Mice deficient for Gsh-1 exhibit significant postnatal dwarfism due to a failure of GHRH expression in the arcuate nucleus of the hypothalamus and subsequent GH deficiency. In the Gsh-1−/− mutant pituitary, in addition to reduced numbers of somatotropes as seen in the little mouse, the number of lactotropes is also decreased and the levels of LH are reduced, which may contribute to sexual infantilism of the Gsh-1−/− mutant, suggesting that Gsh-1 is required for multiple processes in addition to regulating GHRH expression (164).

Two members of the Hmx homeodomain transcription factors, Hmx2 and Hmx3, are required to maintain Gsh-1 expression in hypothalamus. They are coexpressed in developing inner ear, CNS including hypothalamus, and neural tube. They exert both overlapping and distinct functions in the development of the inner ear’s vestibular system, whereas their functions in hypothalamic/pituitary axis appear to be redundant. Deletion of either Hmx2 or Hmx3 results in defects in the inner ear without overt abnormalities in the nervous system. Inactivation of both Hmx2 and Hmx3 leads to progressive degeneration of the entire vestibular system, postnatal growth retardation, and early lethality. Expression of GHRH and galanin, a neuropeptide hormone regulating food intake, memory, learning, as well as sexual activity, in the arcuate nucleus is completely abolished. Secondary to the defects in the hypothalamus, the anterior pituitary of Hmx2−/− Hmx3−/− is hypoplastic with reduced expression of GH and progressive loss of Lhcr expression (296).

C. Genetic Hierarchy of Magnocellular Neuron Formation: Otp, Sim1/Arnt2, Sim2/Arnt2, and Brn2

The POU homeodomain transcription factor Brn2 is essential for the terminal differentiation and/or survival of the AVP- and OT-producing magnocellular neurosecretory cells in SON and PVN as well as CRH-producing parvocellular neurons in PVN. Given that Brn2 binds and activates the CRH promoter, it is suggested that Brn2 controls the development of these lineages at the terminal stage of their differentiation (165, 248). Loss of magnocellular neurons in Brn2−/− results in lack of axonal projections and progressive loss of pituicytes, the astroglial cells of the posterior lobe of the pituitary (198, 248).

The bHLH-PAS transcription factor Sim1 is expressed during the development of the hypothalamic-pituitary axis in three hypothalamic nuclei: PVN, aPV, and SON. In Sim1−/− mice, the entire magnocellular neurosecretory system, which secretes AVP and OT, and three major types of parvocellular neurosecretory cells, identified by the synthesis of TRH, CRH, and SS, fail to develop. Expression of Brn2 is downregulated in a region of the prospective PVN/SON, suggesting that Sim1 is required to maintain Brn2 expression, which in turn directs the terminal differentiation of neuroendocrine lineages within the PVN and SON (191).

Sim2, the homolog of Sim1, is coexpressed with Sim1 in dorsal preoptic area (dP), aPV and anterior and mid-PVN, where SS and TRH-expressing neurons reside. Targeted disruption of Sim2 leads to a reduction in the number of SS and TRH neurons, and this phenotype is
## Table 1. Signal pathways and transcription factors critical for pituitary and hypothalamus development and function

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression</th>
<th>Mouse/Zebrafish</th>
<th>Pituitary Phenotypes</th>
<th>Reference Nos.</th>
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<tr>
<td><strong>Signaling transduction pathways</strong></td>
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<td>KO</td>
<td>RP fails to form, embryonic lethal</td>
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<tr>
<td>FGFR10</td>
<td>VD</td>
<td>KO</td>
<td>RP arrested at e10 with loss of all cell types except corticotropes</td>
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<td>FGFR2-IIIb</td>
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<td>Anterior pituitary agenesis due to increased apoptosis</td>
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<tr>
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<td>KO</td>
<td>Increased apoptosis due to a complete loss of pituitary</td>
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<td>Pituitary dysmorphogenesis; cell differentiation occurs normally</td>
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<td>Decreased LHβ, FSHβ, ACTH, PRL in pituitary</td>
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<td>KO</td>
<td>Transient reduction of GH and PRL</td>
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<tr>
<td><strong>Transcription factors/cofactors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hesx1</td>
<td>VD and RP</td>
<td>KO</td>
<td>Absence of pituitary or multiple oral ectoderm invagination and cellular</td>
<td>59, 187</td>
</tr>
<tr>
<td>Pitx1</td>
<td>RP</td>
<td>KO</td>
<td>Decreased expression of LHβ, FSHβ, TSHβ; increased expression of POMC</td>
<td>277</td>
</tr>
<tr>
<td>Pitx2</td>
<td>RP</td>
<td>KO</td>
<td>RP forms but fails to proliferate and differentiate at e12.5; lacks all cell types</td>
<td>88, 170</td>
</tr>
<tr>
<td>Isl1</td>
<td>RP</td>
<td>KO</td>
<td>RP forms but remains primitive, thin pouch wall, embryonic lethal</td>
<td>279</td>
</tr>
<tr>
<td>Lhx3</td>
<td>RP</td>
<td>KO</td>
<td>Hypoplastic anterior pituitary with reduction of all cell types; increased apoptosis.</td>
<td>250, 326</td>
</tr>
<tr>
<td>Lhx4</td>
<td>RP</td>
<td>KO</td>
<td>Hypoplastic anterior pituitary with reduction of all cell types, increased apoposis.</td>
<td>227, 257</td>
</tr>
<tr>
<td>Six6</td>
<td>RP</td>
<td>KO</td>
<td>Hypoplastic pituitary</td>
<td>168</td>
</tr>
<tr>
<td>Six1</td>
<td>RP</td>
<td>KO</td>
<td>No pituitary phenotype. Six1 and Eya1 DKO have smaller pituitaries</td>
<td>167</td>
</tr>
<tr>
<td>eya1</td>
<td>Pituitary</td>
<td>aal, dog (zebrafish)</td>
<td>Impaired corticotrope, melanotrope and gonadotrope differentation; fail to maintain gh and tsh expression</td>
<td>202</td>
</tr>
<tr>
<td>Pax6</td>
<td>RP</td>
<td>KO</td>
<td>Dorsal expansion of ventral cell types at the expense of dorsal cell types.</td>
<td>19, 147</td>
</tr>
<tr>
<td>ascl1a</td>
<td>Pituitary</td>
<td>pia (zebrafish)</td>
<td>All pituitary cell types fail to differentiate; lack pit1 expression</td>
<td>219</td>
</tr>
<tr>
<td>NeuroD1</td>
<td>RP</td>
<td>KO</td>
<td>Delayed corticotropes differentiation</td>
<td>157</td>
</tr>
<tr>
<td>Tbx19</td>
<td>POMC precursors</td>
<td>KO</td>
<td>Reduced corticotropes and melanotropes; melanotropes transdifferentiate into gonadotropes and Pit1-independent thyrope</td>
<td>222, 223</td>
</tr>
<tr>
<td>Hes1</td>
<td>RP</td>
<td>KO</td>
<td>Reduced proliferation and premature corticotropes differentiation; intermediate lobe fails to develop; no posterior lobe</td>
<td>330</td>
</tr>
<tr>
<td>Rbp-J</td>
<td>RP</td>
<td>CKO</td>
<td>Markedly reduction of Prop1; Pit1 lineages adopt the corticotropes cell fate</td>
<td>330</td>
</tr>
<tr>
<td>Prop1</td>
<td>RP</td>
<td>KO, Ames</td>
<td>No Pit1 activation, lack three Pit1 lineages; reduced LH and FSH expression</td>
<td>199, 273, 290</td>
</tr>
<tr>
<td>β-catenin</td>
<td>RP</td>
<td>KO</td>
<td>Loss of Pit1 expression and Pit1 lineages; reduced LHβ expression</td>
<td>208</td>
</tr>
<tr>
<td>Lef1</td>
<td>RP</td>
<td>KO</td>
<td>Elevated Pit1, GH, and TSHβ expression</td>
<td>208</td>
</tr>
<tr>
<td>Tcf9</td>
<td>RP</td>
<td>KO</td>
<td>Hyperplastic anterior pituitary, prolonged Prop1 expression</td>
<td>30</td>
</tr>
<tr>
<td>Pit1</td>
<td>Pit1 lineages</td>
<td>Snell, Jackson</td>
<td>Loss of somatotropes, thyrotropes, and lactotropes and increased gonadotropes</td>
<td>36, 57, 166</td>
</tr>
<tr>
<td>Math3</td>
<td>Anterior pituitary</td>
<td>KO</td>
<td>Loss of Ghrhr expression, delayed somatotropes maturation</td>
<td>330</td>
</tr>
</tbody>
</table>
Sim2-dosage sensitive. The interplay between Sim1 and Sim2 is complex. Genetic analysis indicates that Sim2 acts downstream of Sim1, yet Sim1 can partially compensate for the loss of Sim2 (96).

Sim1 and Sim2 can form heterodimer with aryl hydrocarbon receptor nuclear translocator (Arnt), Arnt2, and Bmal1 (190). Arnt is broadly expressed in the mesoderm and endoderm, but only at a low level in the CNS. Disruption of Arnt leads to embryonic lethality at E10.5 associated with placental, vascular, and hematopoietic defects. Bmal1 expression is not detectable in the PVN and SON but instead is restricted to the SCN in this region of the hypothalamus (190). In contrast, Arnt2 is ubiquitously expressed in the hypothalamus with abundant expression in PVN and SON. Inactivation of Arnt2 results in a strikingly similar phenotype to that of Sim1−/−, suggesting that Arnt2 is the in vivo dimerization partner of Sim1 and they function together in controlling differentiation of aPV, PVN, and SON neurons (115, 137, 190). Arnt2 is also proposed to be the in vivo partner of Sim2 (96).

Otp, which encodes a homeodomain transcription factor, exhibits an overlapping pattern of expression with Sim1 except that Otp is also expressed in the ARN. Studies of the Otp−/− mice reveal that Otp, in parallel to Sim1, is required for both terminal differentiation of parvocellular and magnocellular neurons of aPV, PVN, and SON and for maintenance of Sim2 and Brn2 expression. Otp is also required for producing SS in ARH neurons. Before terminal differentiation impairments, Otp−/− mice display reduced cell proliferation of neuroblasts and abnormal migration of postmitotic neurons, suggesting that Otp may exert its functions at early stages leading to the establishment of the neuroendocrine hypothalamus (3, 297).

D. Specification and Migration of GnRH-1 Neurons

The GnRH-1 neurons control reproductive axis by projecting axons to the median eminence, where they secrete the GnRH-1 decapeptide in a pulsatile manner into the hypophysial blood system to regulate expression of LHβ and FSHβ. They are generated from the olfactory placodes, although recent studies in fish and chicken suggest that they originate from the neighboring domain where the anterior pituitary arises (reviewed by Ref. 306).
They migrate along vomeronasal nerves (VNN) across the cribriform plate to their ultimate destination scattered in the hypothalamic region of the basal forebrain late in embryonic development (reviewed in Refs. 183, 307, 314). Failure of GnRH-1 neurons to produce GnRH-1 or to migrate appropriately results in reduced levels of LH and FSH and subsequent reproductive dysfunction.

It has been shown that Pax6 is required for the generation of GnRH-1 neurons. In the small-eye mouse mutant (Sey/Sey), which results from a mutation in the Pax6 gene, both optical and olfactory placodes fail to develop (114). The pituitary forms, however, with defects in the dorsal-ventral patterning and cell type specification (19, 147). GnRH-1 neurons are absent in either the presumptive nasal area or in any region of brain during development (65).

GnRH-1 neurons must travel over long distances and various environments to reach their destination. Many in vivo and in vitro studies have revealed a number of molecules influencing their migration. Some of these molecules affect the migration of GnRH-1 neurons indirectly by altering the underlying migratory trajectory (reviewed in Refs. 281, 307). For example, axonal guidance molecule netrin 1 is expressed in the caudal olfactory epithelium and ventral forebrain, whereas DCC (deleted in colorectal cancer), a netrin 1 receptor, is present on vomeronasal and ventral forebrain, whereas DCC (deleted in colorectal cancer), a netrin 1 receptor, is present on vomeronasal nerves that extend toward forebrain. Inactivation of either netrin 1 or Dcc results in aberrant growth of caudal VNN and abnormal migration of GnRH-1 neurons (250, 251).

In humans, two genes have been identified as responsible for the Kallmann’s syndrome (KS), characterized by anosmia and hypogonadotropic hypogonadism. Anosmia is related to the absence or hypoplasia of the olfactory bulbs. Hypogonadism is due to GnRH deficiency and is likely to result from the impairment in the migration of GnRH-1 neurons. The gene underlying the X-linked form of this syndrome, KAL-1, is expressed in the anlagen of the olfactory bulbs. KAL-1 encodes a putative adhesion molecule named anosmin-1 that can induce the migration of immortalized GnRH neurons in vitro (37). More recently, loss-of-function mutations in FGFFR1 have been established to account for an autosomal dominant form of KS (reviewed in Ref. 67). Inactivation of Fgfr1 in the telencephalon in mice results in a failure in olfactory bulb formation (107). Although it is possible that the GnRH-1 neuron migration defect in KS could be secondary to olfactory abnormality, other studies have implicated a direct role of FGF signaling in maintaining GnRH-1 neurons. FGF receptors are expressed in a subpopulation of GnRH neurons in mouse, and expression of a dominant negative form of Fgfr1 in GnRH neurons leads to a reduction in the number of GnRH neurons. However, migration of the remaining GnRH-1 neurons is apparently not affected in the transgenic mice (292).

Gene targeting in mouse has revealed two classes of transcription factors that are critical for GnRH-1 neuron migration and survival. Ebf2, encoding a transcription factor with a zinc-finger DNA binding domain and a COOH-terminal HLH dimerization domain, is expressed both in GnRH and vomeronasal neurons. In Ebf2−/− mice, GnRH neurons migrate slowly out of the vomeronasal organ and are ectopically located in the forebrain at birth. The defect in GnRH neuron migration leads to impaired hypothalamus-pituitary axis and secondary hypogonadism (48). Nhlh1 and Nhlh2, members of the basic HLH transcription factors, are expressed in largely overlapping patterns in different areas of the central and peripheral nervous systems during the embryonic and perinatal stages, including hypothalamic GnRH-1 neurons. Nhlh2−/− mice are hypogonadal and infertile due to a reduction in the number of GnRH-1 neurons and projecting axons in the median eminence (94, 126, 152). In contrast, Nhlh1−/− mice are fertile and develop normally with no apparent morphological abnormality (151). Combined inactivation of Nhlh1 and Nhlh2 leads to a dramatic loss of GnRH-1 neurons and a complete absence of GnRH-1-positive fibers within the median eminence. Because there are no apparent defects in vomeronasal nerves, it is proposed that Nhlh1 and Nhlh2 control differentiation/migration of GnRH-1 neurons in a cell autonomous manner by regulating downstream target genes. One of the downstream targets is necdin, which is deleted in human Prader-Willi syndrome, characterized by obesity and infertility (152). Disruption of the mouse necdin gene results in a reduction of GnRH-1 neurons resembling the Nhlh2 mutation (194).

IX. CONCLUSIONS

Genetic studies (loss-of-function and gain-of-function), in combination with molecular and biochemical studies, have elucidated a primary sketch in which signaling molecules and transcription factors govern proper pituitary development and function. However, the large network required for these events remains incompletely defined, for example, what are the signals guiding the commitment of corticotropes, gonadotropes, and melanotropes? How do Pit1+ precursors embark on three different differentiation programs? Generation of temporally regulated tissue-specific deletion of genes or hypomorph- phic allele of genes that are necessary for early embryonic development will reveal new functions. Genome-wide mutagenesis screen in mice and model organisms, combined with integrated genomic, proteomic, and bioinformatics approaches, will certainly uncover novel players in this elaborately regulated genetic program. Identification of required cofactors as well as downstream target genes of the transcription factors and complementary studies in
epigenetic regulation of chromatin organization and nuclear architecture will delineate the molecular mechanisms underlying pituitary development, uncovering new principles of mammalian organogenesis.

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