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Invited Review

Complex integration of intrinsic and peripheral signaling is required for pituitary gland development†

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Abstract

The coordination of pituitary development is complicated and requires input from multiple cellular processes. Recent research has provided insight into key molecular determinants that govern cell fate specification in the pituitary. Moreover, increasing research aimed to identify, characterize, and functionally describe the presumptive pituitary stem cell population has allowed for a better understanding of the processes that govern endocrine cell differentiation in the developing pituitary. The culmination of this research has led to the ability of investigators to recapitulate some of embryonic pituitary development in vitro, the first steps to developing novel regenerative therapies for pituitary diseases. In this current review, we cover the major players in pituitary stem/progenitor cell function and maintenance, and the key molecular determinants of endocrine cell specification. In addition, we discuss the contribution of peripheral hormonal regulation of pituitary gland development, an understudied area of research.

Summary Sentence

Key transcription factors, extracellular molecular networks, and hormones work in concert to coordinate lineage specification and differentiation in the developing pituitary.

Key words: anterior pituitary, developmental biology, stem cells, hormones.

Introduction

The pituitary is an endocrine gland that dynamically regulates peripheral tissues to coordinate fundamental physiological functions such as growth, metabolism, sexual maturity and reproduction, pigmentation, the body's response to stress. The pituitary regulates these homeostatic processes by interpreting hypothalamic signals and, in response, releases hormones from specialized cells in the anterior pituitary. These cell types include somatotropes that produce growth hormone (GH), thyrotropes that produce thyroid stimulating hormone (TSH), lactotropes that produce prolactin (PRL), gonadotropes produce luteinizing hormone (LH) and

follicle-stimulating hormone (FSH), melanotropes that produce melanocyte-stimulating hormone (αMSH), and corticotropes that produce adrenocorticotropic hormone (ACTH).

The use of genetic mouse models has provided novel insight to the molecular mechanisms that govern pituitary development and maintenance. These models have identified key transcription factors and signaling pathways that are necessary for normal pituitary development in both mouse and humans. More recently, the identification of a presumptive stem cell/progenitor population in the pituitary gland has provided insight into processes that underlie endocrine cell specification and organ homeostasis. These cells, identified by
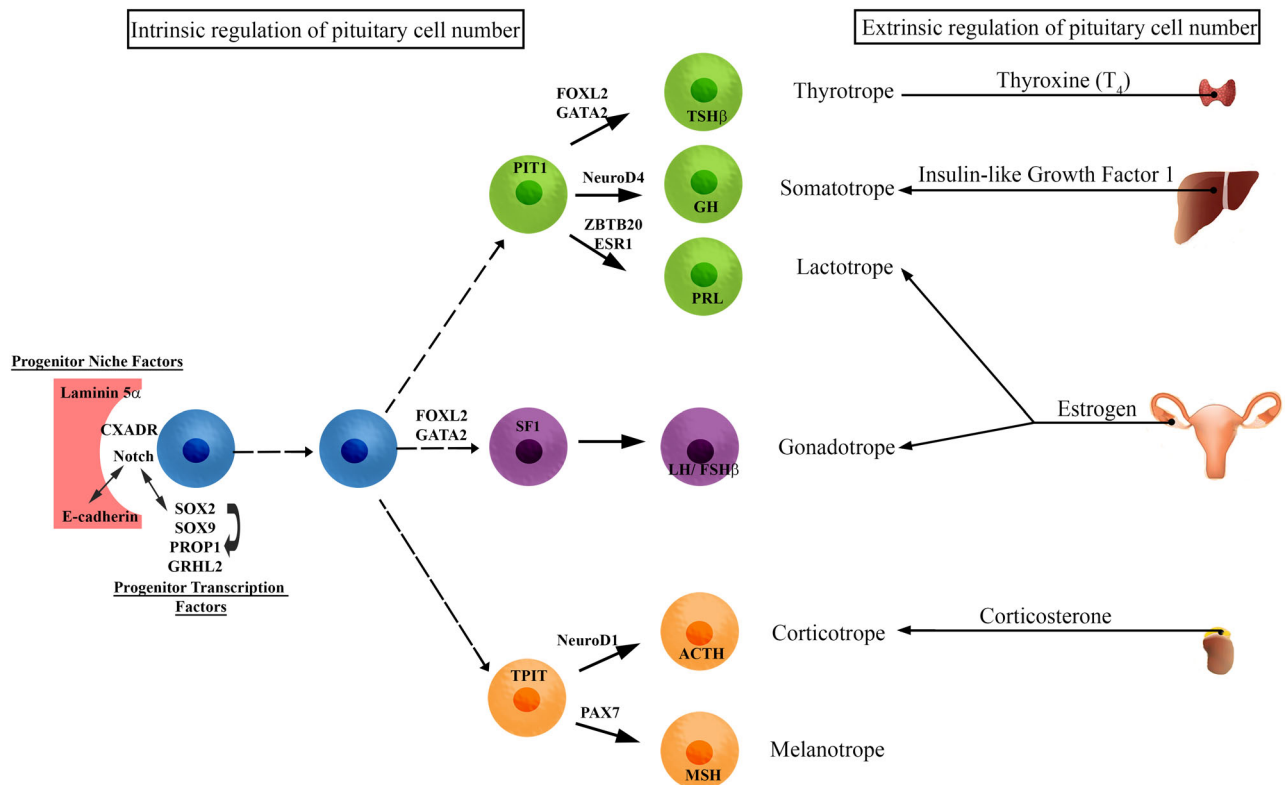
Figure 1. Coordination of pituitary progenitor differentiation into the endocrine cell types requires both intrinsic and extrinsic factors. During pituitary gland development, the hormone producing cell types are derived from a common progenitor cell. The balance of progenitor cell maintenance and differentiation is necessary for proper gland development. Maintenance of the progenitor cell compartment is dependent on transcription factors and niche components. Endocrine cell differentiation and cell number is coordinated by both transcription factors and peripheral hormones.

their expression of hallmark stemness markers, contribute to embryonic and postnatal development of the gland, as well as generation of endocrine cell types in response to physiological demands such as puberty, pregnancy, and injury. The current model of pituitary cell differentiation involves multipotent stem/progenitor cells giving rise to a progenitor population that functions as rapidly dividing transit-amplify cells. These cells then give rise to committed precursor cells that, under the influence of lineage-specific differentiation cues, will further mature into the hormone producing cells. The progression from stem/progenitor cell to a fully functional endocrine cell is dependent on a multifactorial process that integrates several factors including morphogenic factors, cell-to-cell signaling, key transcription factors, and hormonal influences (Figure 1).

The importance of pituitary stem cell research is to potentially develop cell-based treatments for pituitary diseases that result in the absence or decreased levels of pituitary hormones such as combined pituitary hormone deficiency (CPHD), traumatic brain injury, or after surgical or radiation treatment of pituitary tumors. The goal is to treat patients with stem cells that can regenerate the endocrine cells types and restore normal pituitary functions. Recent studies have provided evidence that this type of advanced treatment is possible; however, more knowledge about pituitary development is necessary to improve these types of medical technologies. In this review, we aim to discuss the major determinants of cell lineage specification in the developing pituitary. We will review the well-known as well as the most novel factors that are important for the function and maintenance of the pituitary stem/progenitor cells, intrinsic factors that are necessary for lineage

differentiation, and the extrinsic factors that contribute to pituitary expansion.

Identification, regulation, and function of the stem/progenitor cells population

Several groups have provided convincing evidence that stem/progenitor cells are present in the pituitary and are required for proper development and maintenance of the gland. Initial studies identifying this population of cells took advantage of inherent properties of stem cells including sphere-forming capabilities and side population isolation via verapamil sensitive dye efflux assays [1, 2]. These early studies identified a small number of cells that expressed a cohort of genes that are characteristic of stem cell function and maintenance in other organs [1, 2]. Subsequent research demonstrated that these populations of cells are capable of differentiating into all of the endocrine cell types during neonatal development of the pituitary and contribute to gland regeneration in the adult [3, 4] (Table 1).

TFs that identify the stem cell population

SOX2

One of the earliest markers of the stem/progenitor population in the developing pituitary is sex determining region Y-box 2 (SOX2), a high mobility group box transcription factor, known to regulate stem cell plasticity during embryonic development [5, 6]. In humans

Table 1. Transcription factors and niche components identified in the pituitary progenitor compartment.

Marker	Gene Name	Reference
Transcription factors		
Sox2	SRY-box containing gene 2	[3, 4, 9]
Sox9	SRY-box containing gene 9	[1, 2, 4, 9, 96]
Prop1	Prophet of Pit1, paired-like homeodomain transcription factor	[2, 12–16]
Grhl2	Grainyhead like transcription factor 2	[26]
Oct4 (Pou5f1)	Octamer-binding protein 4	[1, 2, 97]
Prrx1	Paired related homeobox protein 1	[98, 99]
Prrx2	Paired related homeobox protein 2	[98, 99]
Ctnnb1 (β- Catenin)	Cadherin-associated protein beta	[100, 101]
Notch signaling components		
Hes1	Hairy and enhancer of split 1	[22, 23, 28, 102]
Hey1	Hairy/enhancer-of-split related with YRPW motif 1	[28, 97]
Dll1	Delta like canonical Notch ligand 1	[15, 22, 23, 28]
Jag1	Jagged 1	[97, 102]
Notch1	Notch 1	[1, 97, 102]
Notch2	Notch 2	[15, 22, 23, 28, 97, 102]
Notch3	Notch 3	[23, 97, 102]
Extracellular receptors/cell surface antigens		
Ly6a (Sca1)	Stem cell antigen 1	[1, 97, 102]
SSEA-4	Stage-specific embryonic antigen-4	[2]
Cxadr	Coxsackievirus and adenovirus receptor	[2, 103]
Cxcr4	C-X-C motif chemokine receptor 4	[97, 103, 104]
Lgr5	Leucine rich repeat containing G protein-coupled receptor 5	[97]
CD44	CD44 antigen	[97]
Gfra2	GDNF family receptor alpha 2	[2]
Cellular adhesion molecules		
Cdh1 (E-cadherin)	Calcium-dependent adhesion protein	[2, 9, 26]
Lama5 (Laminin 5α)	Laminin subunit alpha 5	[39]
Itgb1 (Integrin 1β)	Integrin subunit beta 1	[104]
Other		
S100b	S-100 protein subunit beta	[1, 2, 9, 16, 20, 103]
Nestin	Nestin	[1, 97]

and mice, loss of SOX2 in the pituitary results in varying degrees of hypopituitarism, demonstrating its essential role in normal pituitary development [6–8]. SOX2 is ubiquitously expressed in proliferating epithelial progenitors throughout Rathke's pouch (RP) as early as embryonic day 11.5 (e11.5) [9]. In postnatal development and in the adult, SOX2 expression decreases and becomes restricted to the marginal zone cells that surround the lumen. Interestingly, during early-postnatal maturation, clusters of SOX2-positive cells can also be found in the anterior lobe parenchyma [10]. This population of stem/progenitor is regionally distinct but remains in a homotypic network with the niche located in the marginal zone [11]. Studies have shown that all of the hormone-producing cell types are derived from a SOX2-expressing precursor cell, providing convincing evidence that these cells are in fact stem/progenitors in the pituitary [3, 4, 9]. SOX2-positive cells also express additional progenitor transcription factors including sex determining region Y-box 9 (SOX9), a marker of multipotent progenitors in other tissues [1, 2, 4, 9]. During early embryonic development of the gland, the progenitor population weakly expresses SOX9 but during postnatal development and in the adult the majority of SOX2 cells are also positive for SOX9 [9].

PROP1

The molecular markers that define the stem/progenitor cell population do not appear to be constant throughout maturation of the pituitary, suggesting a sequential development of the progenitor population and potentially a heterogeneous population of these cells. This theory is further supported by the temporal and regionally specific expression of paired like homeodomain factor 1 (PROP1), a pituitary specific transcription factor that has also been identified as a marker of the stem/progenitor cell population. PROP1 is weakly detected in the progenitor cell niche of RP at e11.5, a time in which SOX2 is highly expressed. However, by e13.5 the majority of luminal progenitors in RP are positive for both PROP1 and SOX2 [12–14]. Beginning in the late embryonic and early postnatal development, PROP1-positive cells are excluded from the intermediate lobe and become restricted to marginal zone and parenchyma of anterior lobe. In the adult, PROP1 is maintained in the marginal zone albeit at a significantly lower level [2, 13–16]. PROP1 mutations are the most common identified cause of hypopituitarism in humans [17, 18]. This phenotype is recapitulated Ames dwarf mice (*Prop1^{df/df}*) that harbor a loss-of-function mutation in PROP1. Characterization of the progenitor cell population of these mice has clearly defined a role for PROP1 in maintenance and function of the stem/progenitor cell population in the pituitary. *Prop1^{df/df}* mice display an inability of progenitor cells to migrate away from the marginal zone indicative of impaired epithelial-to-mesenchymal transition [14]. This is coincident with decreased expression of proliferation markers and decreased expression of NOTCH2, an important regulator of stem

cell maintenance in the developing pituitary. Recently, lineage tracing studies have demonstrated that PROP1-expressing cells are able to differentiate into all of the anterior lobe cell types [19]. These data, in combination with previous studies that observed PROP1 in the adult pituitary stem cell niche, solidify PROP1 as a bona fide marker and regulator of the stem/progenitor population.

S100β

A subset of anterior lobe cells that express S100 protein, β polypeptide, neural (S100β) have been identified as folliculo-stellate cells. These cells are nongranular and display long cytoplasmic processes that aid in maintaining cellular networks. S100β-positive cells are not present during embryonic development and a subset of these cells are thought to function as a postnatal specific stem/progenitor cell population. It has been shown that a subpopulation of folliculo-stellate have colony-forming capacity and the ability to differentiate into endocrine cell types [20, 21]. In addition, multiple studies have demonstrated that S100β is detected in cells that express other stem cell makers including SOX2 and PROP1, although not exclusively. Interestingly, it appears that there is a progressive increase in SOX2/S100β-positive cells as the pituitary develops, with approximately 85% of SOX2 cells co-expressing S100β in the adult rat [2, 9, 16]. The fact that these cells are morphologically, temporally, and regionally distinct from other stem/progenitor cell populations in the pituitary may suggest that they constitute a specialized progenitor cell niche.

Stem cell niche factors

The maintenance of the stem/progenitor cell population is not only dependent on the expression of transcription factors that regulate cellular plasticity but also on the components of their specialized microenvironments. Factors that compose the stem cell niche are tissue specific and often include structural proteins that make up the extracellular matrix (ECM), cell surface receptors that function in cell-to-cell communication, soluble factors, supportive cells, and cell surface proteins. In the pituitary, relatively little is known about the niche components that contribute to stem/progenitor cell maintenance and function. However, a few factors that contribute to the structural integrity and cell–cell interactions in the pituitary stem/progenitor cell niche have been investigated.

Notch signaling

Notch signaling is a cell-to-cell contact-dependent pathway that regulates stem cell maintenance and cell fate choice in many organs including the developing pituitary. Activation of the pathway requires a cell expressing a Notch ligand to activate a Notch receptor on an adjacent cell. The interaction of the receptor and the ligand results in two successive proteolytic cleavages, the first by a disintegrin and metallopeptidase domain 10 (ADAM10) which releases the Notch extracellular domain of the receptor. Subsequently, the second cleavage by gamma secretase releases the Notch intracellular domain (NICD), which translocates to the nucleus and regulates transcription of target genes via binding of the co-factor protein recombination signal binding protein for immunoglobulin kappa J region (RBPJ). Core components of the Notch signaling pathway define the stem/progenitor cell compartment of the developing pituitary. The receptors *Notch2* and *Notch3*, the ligands Jagged 1 (*Jag1*) and delta like canonical Notch ligand 1 (*Dll1*), as well as the canonical downstream target genes hes family basic helix-loop-

helix factor 1 (*Hes1*) and hairy/enhancer-of-split related with YRPW motif 1 (*Hey1*) are detected in RP in proliferating progenitors during early pituitary development [22–24]. During postnatal development and in the adult, expression of these genes is downregulated but maintained in the presumptive stem cell compartment, following an expression pattern similar to that of other stem cell makers.

Genetic mouse models have provided useful insight into role of Notch signaling in the developing pituitary. Conditional loss of either the essential Notch cofactor *Rbpj* or the notch effector gene *Hes1* during embryonic pituitary development results in a hypoplastic pituitary, decreased proliferation of pituitary progenitors, and premature cell cycle exit [23, 25]. A similar progenitor phenotype was observed in mice with a conditional loss of the *Notch2* receptor (*Notch2* cKO) specifically during postnatal pituitary development. These mice display decreased progenitor proliferation, and a decrease in expression of the well-known progenitor cell markers SOX2 and SOX9 as well as the novel pituitary progenitor marker grainyhead like 2 (GRHL2) [26]. Additionally, pituitary stem cells in culture rely on Notch signaling because loss of *Rbpj* or treatment with DAPT, a gamma secretase inhibitor, results in the inability of progenitors to expand and form pituispheres [15]. These findings suggest that Notch may directly regulate the expression transcription factors necessary for stem cell plasticity in the pituitary, as all of these have been shown to be directly regulated by Notch in other systems [27]. Furthermore, both mice with a loss of *Notch2* or *Rbpj* have decreased expression of *Prop1*, which was also shown to be a direct Notch target in the pituitary [23, 28]. PROP1 is also thought to be a regulator of *Notch2* expression suggesting a reciprocal relationship between these two factors [14, 22, 29, 30]. In agreement, overexpression of Notch in the pituitary inhibits differentiation of the hormone producing cell types and results in ectopic expression of SOX2 [31].

Active Notch signaling may also regulate the expression of cell adhesion molecules that are crucial for the maintenance of the stem/progenitor cell niche in the pituitary. Gene expression studies have shown that the pituitary progenitor population is enriched for both Notch signaling components and cadherin 1 (*Cdh1*; also known as Ecad), a cellular adhesion molecule, known to be an important regulator of stem-cell niche adhesion [2, 9, 32]. Decreased expression of E-cadherin was observed in *Notch2* cKO pituitaries, specifically in the marginal zone of the intermediate lobe. Interestingly, an almost complete loss of SOX2-positive cells was observed in this region, indicating a severe disruption in the intermediate lobe progenitor niche [26]. Additional in vitro studies have established a link between Notch signaling and Ecad expression. Inhibition of cell-to-cell contact via downregulation of Ecad in pituitary monolayer cell culture results in decreased expression of the canonical Notch target, HES1 [33]. These findings may indicate a reciprocal relationship between pituitary niche adhesion molecules and Notch signaling activity.

Taken together, these studies demonstrate that Notch signaling is necessary for pituitary stem/progenitor maintenance and expansion. In particular, it appears that Notch signaling not only regulates the expression of transcription factors necessary for stem cell self-renewal but also adhesion molecules that modulate the stem cell microenvironment.

CXADR and ECM protein expression

Additional components that contribute to the stem cell niche have been relatively unexplored, but a series of studies have highlighted

novel factors that may be important in the developing pituitary. Expression of Coxsackievirus and adenovirus receptor (CXADR), a transmembrane protein that associates with tight junction complexes, was recently identified in the progenitor cell compartment throughout pituitary development [34]. CXADR expression has been observed in neuroepithelial cells of the central nervous system and it thought to facilitate maturation of tight junctions during development [35]. However, the function of CXADR in the developing pituitary stem/progenitor cells remains unknown.

Extracellular matrix proteins are important for the proper development, morphology, and tumorigeneses in the pituitary [36]. Little is known about the specific ECMs that contribute to the stem/progenitor cell niche in the pituitary, but a few studies have demonstrated that ECMs may contribute to the function and maintenance of these cells. In vitro studies show S100β-positive cells interact with various ECM proteins including laminins, collagens, and integrins, and these interactions regulate proliferation and morphological characteristics of these cells [37, 38]. In addition, the ontogeny of laminin chain expression was examined during neonatal pituitary development. Interestingly, Laminin 5α, which is present in pluripotent embryonic stem cells, was detected in RP as well as in the marginal zone during postnatal pituitary development [39]. These data suggest that ECM components are important regulators of the stem/progenitor compartment in the developing pituitary.

Transcription factors that drive differentiation and lineage specification in the developing pituitary

As pituitary development progresses, stem/progenitor cells must exit the cell cycle and differentiate into one of the hormone producing cell types. The process of adopting a specific cell fate is facilitated by the expression of transcription factors that promote lineage specification. The use of genetically engineered mouse models and naturally occurring spontaneous mutations in both mice and humans have led to the discovery of several genes that are crucial for endocrine cell specification and function.

Corticotropes and melanotropes

The terminal differentiation of the two proopiomelanocortin (POMC) expressing cell types, melanotropes which cleave POMC into melanocyte-stimulating hormone (αMSH) and corticotropes which cleave POMC into adrenocorticotrophin hormone (ACTH), is driven by the expression of T-box 19 (TBX19; also known as TPIT). In humans, inactivating mutations in *TPIT* are associated with isolated adrenocorticotropic hormone deficiency (IAD), eluding the importance of TPIT in the proper differentiation of the POMC lineage in the pituitary. TPIT expression is detected in the caudoventral region of RP as early as e12.5 before the onset of POMC expression [40, 41]. Studies have demonstrated that TPIT regulates POMC transcription in corticotropes through synergistic interactions with other transcription factors including Paired-like homeodomain transcription factor 1 (PITX1), neurogenic differentiation 1 (NeuroD1), and est variant 1 (ETV1) [42–44]. *Tpit* null mice display a decrease in the number of POMC-expressing corticotropes but do express other corticotrope-specific genes indicating a defect in the later stages of differentiation. In the absence of *Tpit*, the intermediate lobe is hypoplastic and melanotropes adopt an alternate cell fate indicated by the expression of glycoprotein hormones, α subunit (*Cga*; also known as αGSU) and nuclear receptor subfamily 5, group A, member 1 (NR5A1; also known as steroidogenic factor 1 (SF1)), markers of thyrotropes and gonadotropes [41]. *Tpit* overexpression in the αGSU-expressing cells initiates corticotrope differentiation in these cells and as a result expression of LHβ, FSHβ, and SF1 are decreased [40, 41]. These data demonstrate a role for TPIT not only in the promotion of corticotrope differentiation but also as an active suppressor of other lineage-specific makers.

Aside from the role of Notch signaling as regulator of stem/progenitor cell maintenance, it functions as a binary cell fate determinant in the developing pituitary. Specifically, it appears that Notch signaling regulates the balance between the TPIT and PIT1 lineages. Models of reduced Notch signaling in the pituitary show precocious differentiation of corticotrope cells [23, 28]. In addition, constitutive activation of Notch signaling in POMC-expressing cells prevents differentiation of melanotropes and corticotropes. This inability to differentiate is marked by increased expression of SOX2 in the intermediate lobe indicating these cells are confined to a progenitor like state. These studies demonstrate that Notch signaling is necessary to suppress aberrant corticotrope expansion and downregulation of this pathway is necessary for melanotrope and corticotrope differentiation.

Although TPIT is a common factor necessary for terminal differentiation in both corticotropes and melanotropes, additional lineage-specific factors such as NeuroD1 and paired box 7 (PAX7) have been shown to regulate development and maturation of these cell types. NeuroD1 is dynamically expressed in pituitary corticotropes and is not only a regulator of *Pomc* transcription but also is implicated in the differentiation of the corticotrope lineage. Loss of *Neurod1* results in a minor delay in corticotrope differentiation that is recovered by e16.5, indicating that it may contribute to early differentiation of this lineage [42]. PAX7, a pioneering transcription factor, is an important regulator of melanotrope identity and melanotrope-specific POMC transcription. PAX7 is exclusively detected in the intermediate lobe cells that co-express TPIT beginning at e15.5 [45]. In *Pax7* knockout mice pituitaries, melanotropes appear to adopt a corticotrope cell fate indicated by the downregulation of genes enriched in melanotropes, including proprotein convertase subtilisin/kexin type 2 (*Pcsk2*; also known as *Pc2*) and dopamine receptor D2 (*Drd2*), and a subsequent upregulation of genes specific to corticotropes including nuclear receptor subfamily 3, group C, member 1 (*Nr3c1*; also known as glucocorticoid receptor), *Neurod1*, and corticotropin releasing hormone receptor 1 (*Crhr1*). In addition, these studies demonstrate that PAX7 actually is not the main driver of melanotrope differentiation, rather is it necessary to allow accessibility of melanotrope-specific genes to other transcription factors that regulate differentiation such as TPIT [45, 46].

It is also important to note that cellular localization and cell network formation are important for the function and development of the POMC lineage. Loss of the cellular adhesion molecule Cadherin 2 (*Cdh2*; also known as Ncad) in POMC-expressing cells results in disrupted intermediate lobe boundaries, mis-localization of corticotropes, and an overall decrease in *Pomc* levels [47]. Additional studies have demonstrated the endocytic adaptor protein NUMB, known for its role in degradation of the Notch receptors and cell adhesion, is also necessary for intermediate lobe organization and progenitor cell localization [48]. Taken together, these studies identify additional regulatory proteins that contribute to the proper development of the POMC lineage.

PIT1 lineage: somatotropes, lactotropes, and thyrotropes

POU domain, class 1, transcription factor 1 (PIT1), is a major determinant of terminal differentiation of three hormone producing cell types: somatotropes, lactotropes, and thyrotropes. Mutations in *PIT1* in both humans and mice results in CPHD, establishing an important role of PIT1 in pituitary development [49]. Genetic mouse models of PIT1 deficiency, including the Snell's dwarf and Jackson dwarf, exhibit pituitary hypoplasia coincident with a decrease in the number somatotropes, lactotropes, and thyrotropes [50]. These phenotypic changes are observed only during postnatal pituitary development suggesting that PIT1 is not required for the initial differentiation of these linages but rather it is necessary for postnatal expansion. PIT1 is first detected at e13.5 and increases in expression into adulthood as endocrine cells undergo major waves of expansion [51]. In terminally differentiated cells, PIT1 transcriptionally regulates the expression of *Gh*, *Tshb,* and *Prl* as well as the hypothalamic signaling receptor, growth hormone releasing hormone receptor (*Ghrhr*) [52, 53]. The Notch signaling pathway and PROP1 are currently thought to be the primary regulators of *Pit1* expression. Ames dwarf mice and mouse models of reduced Notch signaling exhibit a similar decrease in the number of somatotropes, lactotropes, and thyrotropes in the developing pituitary [23, 28]. PROP1 is a direct transcriptional activator of *Pit1* expression while Notch is thought to exert its effects on *Pit1* indirectly through regulation of PROP1 expression [54]. Although it has been shown that PIT1 is necessary for terminal differentiation of somatotropes, lactotropes, and thyrotropes, the molecular mechanisms that govern specification of these different cells remain elusive.

Somatotropes

The bHLH factor neurogenic differentiation factor 4 (NeuroD4), a downstream target of PIT1 necessary for somatotrope differentiation, is expressed by e13.5 in the mouse. Somatotropes are severely decreased in *Neurod4* null mice embryonically. Although somatotrope differentiation mildly recovers during postnatal development, these mice fail to express GHRHR and display dwarfism [23]. The postnatal pituitary hypoplasia due to loss of somatotrope expansion in *Neurod4* null mice is likely due in part to loss of hypothalamic input in this cell type. This is supported by a similar hypoplasia phenotype observed in little mice (*Ghrhr^lit/lit^*) which harbor a point mutation in GHRHR [55]. More recently, it was demonstrated that forkhead box O1 (FOXO1), is also important for embryonic somatotrope differentiation. Conditional loss of *Foxo1* in the developing pituitary results in a decrease of *Gh* and *Ghrhr* mRNA levels, although adult animals appear unaffected. In addition, FOXO1 may be an upstream regulator of *Neurod4* as its expression is reduced embryonically in these mice [56].

Thyrotropes

Two populations of thyrotrope cells are present during pituitary organogenesis: the first to appear is a PIT1-independent lineage located in the rostral tip specifically during embryonic development and a second PIT1-dependent lineage. Little is known about the regulation and function of rostral tip thyrotropes; however, factors have been identified that regulate the PIT1-dependent lineage. During early gland specification events, a set of common genes are expressed that are necessary for both thyrotrope and gonadotrope specification. These include two transcription factors, forkhead box L2 (FOXL2) and GATA binding protein 2 (GATA2), as well as *Cga*, the common α-glycoprotein subunit of TSH and the gonadotropins LH and FSH. FOXL2 regulates expression of αGSU and is the earliest maker of thyrotrope and gonadotrope specification [57]. Its expression is observed ventrally in RP as early as e10.5 before the onset of hormone expression in these lineages at e12 [58]. Loss-of-function studies have identified GATA2 as a regulator of differentiation of both thyrotropes and gonadotropes. Expression of a dominant negative form of GATA2 or a pituitary-specific knockout of *Gata2* inhibits terminal differentiation of thyrotropes indicated by reduced express if *Tshb* [59–61]. In addition, *Pit1* is increased in these pituitaries suggesting that PIT1 alone is not sufficient for the proper expression of TSHβ. In support of this observation, these studies also demonstrated that GATA2 and PIT1 act synergistically to transcriptionally regulate *Tshb* expression.

Lactotropes

Terminally differentiated lactotropes marked by the expression of PRL are detected by e15.5 in mice [62, 63]. Although lactotropes are detected before birth, lactotrope cell number remains low during embryonic pituitary development, with the majority of lactotrope expansion occurring during postnatal development. Interestingly, studies using targeted ablation of GH-producing cells demonstrated a significant decrease in lactotrope cells as well, indicating that mature lactotropes may be derived from a precursor cell that expressed GH referred to as somatolactotropes [64, 65]. However, these studies are somewhat controversial as lineage tracing studies suggest that the majority of lactotropes are derived from pituitary progenitor cells [66, 67]. Relatively little is known about the factors driving further lactotrope specification. More recently, zinc finger and BTB domain containing 20 (ZBTB20), a zinc finger containing transcription factor, was shown to be important for lactotrope specification. ZBTB20 null mice exhibit postnatal pituitary hypoplasia due to an absence of terminally differentiated lactotropes and a decreased number of somatotropes. These studies also demonstrate ZBTB20 as a transcriptional regulator of *Prl* expression [68]. In addition, it has been shown that lactotrope terminal differentiation and proliferation are regulated by hormonal signals such as estrogens. The decrease in PRL expression and lactotrope cell number observed in estrogen receptor alpha (*Esr1*; also known as ERα) disrupted mice (*Esr1^−/−^*) suggests that estrogenic signaling is necessary for maturation of lactotrope cells and may provide a mechanism to devise terminally differentiated lactotropes from somatolactotrope precursor cells [69, 70]. It has also been demonstrated that shortly after pregnancy there are an increased number of proliferating lactotropes and increased levels of circulating PRL, thought to be triggered by estrogen. However, it is unclear if the primary mechanism of increased PRL in response to estrogen in this capacity is due to increased lactotrope number or hypertrophy [71].

Gonadotropes

As mentioned previously, transcription factors GATA2 and FOXL2 are early markers of gonadotrope cells. FOXL2 interacts with Smad proteins to directly regulate transcription of *Fshb* in gonadotropes [72–74]. The necessity of FOXL2 in FSH expression is exemplified by FSHβ deficiency and subsequent reduced fertility in mice with a conditional deletion of *Foxl2* in gonadotropes [57, 75]. GATA2 is not necessary for differentiation of this lineage but is important for

function as reduced GATA2 expression is also associated with reduced FSH levels [60]. GATA2 is also a positive regulator of gonadotrope-specific genes including the orphan nuclear receptor NR5A1 (also known as SF1) [59]. NR5A1 expression is detected starting at e13.5, and is one of the first gonadotrope-specific makers before the onset of LHβ and FSHβ [76]. The loss of *Nr5a1* globally or in the pituitary specifically results in decreased basal levels of gonadotropins; however, hormone expression can be induced in response to the hypothalamic stimulus gonadotropin releasing hormone (GnRH) [77]. It has also been demonstrated that GnRH-mediated transcriptional induction of *Lhb* requires synergism between SF1 and the transcription factors early growth response 1 (EGR1) and PITX1 both of which are known regulators of gonadotropins [78].

The Notch signaling pathway has been also implicated in gonadotrope differentiation. Overexpression of the Notch downstream target *Hes1* and constitutively active Notch in *Cga*-expressing cells results in delayed gonadotrope differentiation [24, 79]. *Notch2* cKO mice have increased expression of both *Nr5a1* and *Lhb* but with no increase in gonadotrope number [28]. In contrast, no apparent gonadotrope phenotype was observed in mice with conditional loss of *Rbpj*, the essential Notch cofactor [23]. These studies indicate that active Notch signaling is sufficient to suppress gonadotrope differentiation; however, it does not appear to affect gonadotrope specification.

Hormonal influence on pituitary differentiation

The pituitary gland is a major hub of endocrine function and as such it is under feedback regulation from peripheral tissues. The release of hormones, such as sex steroids, thyroid hormones, glucocorticoids (GCs), and insulin-like growth factor 1 (IGF-1), from the corresponding peripheral tissues is the primary mechanism of feedback to the pituitary to ensure homeostatic balance. The endocrine cells of the pituitary are therefore functionally controlled by peripheral hormones, but these hormones may also regulate the differentiation and specification of cells in the pituitary. Here we discuss the most well-studied examples of peripheral hormones influence on pituitary development.

In response to activation of hypothalamic-pituitary-adrenal axis (HPA), pituitary corticotropes release ACTH which acts on the adrenal gland and subsequently results in the release of GCs that regulate peripheral tissues and negatively feedback to both the pituitary and hypothalamus. It is well established that GCs regulate the response to stress in the adult animal, but evidence suggests GC signaling also regulates corticotrope differentiation. In adult mice, it has been demonstrated that corticotrope cell number is increased in response to adrenalectomy [80]. The increase in cell number and the ability of dexamethasone to attenuate this response indicate that GCs are capable of regulating corticotrope differentiation. Furthermore, mice with a conditional deletion of the GC receptor ($Nr3c1^{PomcCre}$) in the pituitary have increased *Pomc* mRNA levels and an apparent increase in corticotrope number at postnatal day 6 [81]. Taken together, these studies demonstrate a suppressive role of GCs in corticotrope differentiation in the adult and postnatal pituitary. In contrast, GCs have been shown to positively regulate somatotrope differentiation [82]. Studies in rat and chicken embryos demonstrate that in vivo exposure to GCs results in an increase in *Gh* levels and premature differentiation of somatotropes [83–85]. Coincidentally, terminal differentiation of somatotropes coincides with the onset of HPA axis activity during late embryonic development [86]. These studies indicate that GCs regulate differentiation of the both corticotropes and somatotropes during pituitary development.

Similar to the regulation of corticotrope number by GCs, peripheral hormones from target organs also regulate somatotrope differentiation. Secretion of GH from somatotropes stimulates the production of IGF-1 from the liver and feeds back negatively to hypothalamic-pituitary axis to suppress GH release. Studies in IGF-1 knockout mice demonstrate that loss of IGF-1 results in increased somatotrope number in adult mice, indicating IGF-1 is necessary to maintain the correct number of somatotropes [87].

As mentioned previously, the most well-known example of hormonal regulation on cellular differentiation in the pituitary is the effects of estrogen on lactotrope differentiation. However, other endocrine cells types in the pituitary are regulated by estrogenic signaling. In adults, it is well established that estrogens play a role in regulating gonadotropin secretion, but studies also indicate a role for estrogens during gonadotrope cell development. In the chick embryo, exposure to estradiol promotes an increase in gonadotrope differentiation and proliferation [88]. In support of this observation, adult rodents treated with tamoxifen, an estrogen receptor modulator, have an increased number of gonadotropes cells [89]. Furthermore, target organ ablation studies in which mice are gonadectomized show an increase in the gonadotrope population, most likely due to mobilization of the stem/progenitor cell population indicating that peripheral hormonal signals regulate cell number in the pituitary [80].

The influence of estrogens on pituitary development suggests that it may also be sensitive to exogenous estrogenic compounds. Specifically, exposure to the endocrine disrupting chemical (EDC) Bisphenol A (BPA) during critical windows of development affects gonadotrope differentiation. Female neonatal mice exposed to BPA or estradiol during gestation have increased gonadotrope cell numbers [90]. In addition, mice exposed to BPA and estradiol during postnatal development show decreased expression of sexually dichotomous genes in the pituitary, indicating postnatal sensitivity to hormonal regulation [91]. Several studies have also demonstrated that exposure to BPA during critical windows of development results in HPA axis dysfunction in adult mice [92–94]. The pituitary contribution to this dysfunction remains unclear but studies have reported changes in pituitary *Crhr1* and *Pomc* mRNA levels [95]. Changes in gene expression and cell number after exposure to EDCs further demonstrate that the pituitary is sensitive to hormonal regulation during early developmental periods. While the mechanism/contribution of hormonal regulation has been relatively unexplored in the developing pituitary, this mode of regulation is important for gland development.

Conclusion

Current research has provided a substantial basis for understanding the molecular mechanisms that dictate pituitary cell fate decisions and organ homeostasis. However, in order to achieve the goal of using stem cell-derived treatment for pituitary disease, the contributing players in the stem/progenitor cell niche and peripheral regulation of pituitary development will need to be further dissected. Future research using single cell genomic approaches will be helpful in the identification of transitional cell types and novel molecular determinants that coordinate differentiation of the endocrine cell types. These studies will aid the field in identifying the developmental progression of each of the endocrine cell types thus allowing researchers recapitulate pituitary development.

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