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Genetic determinants of mammalian pituitary morphogenesis

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1. ABSTRACT

The anterior pituitary contains five trophic (hormone-secreting) cell types which are defined by their hormone products. During pituitary organogenesis, these lineages emerge in a stereotypical spatio-temporal pattern from a common ectodermal primordium, Rathke's Pouch (RP), thereby providing an excellent model system to address key developmental processes such as pattern formation, cell specification and differentiation. Genetic studies performed in mice have revealed that secreted factors released from neighbouring tissues are critical for the formation of RP and appear to establish positional identity within RP through regionally-restricted induction of transcription factor gene expression. Together, these transcription factors coordinate progenitor cell proliferation, specification and differentiation via a variety of mechanisms that include the recruitment of cell type specific co-activator and co-repressor complexes. Herein we discuss the roles of key components in the pituitary developmental program with particular focus on functionally conserved genes which are associated with various forms of pituitary hormone deficiency in humans.

2. INTRODUCTION

During development of the pituitary gland, five hormone-secreting cell types emerge from a common ectodermal primordium, Rathke's Pouch. Recently, significant advances in the understanding of the genetic program which orchestrates pituitary development have been gained, principally through analysis of targeted and spontaneous mouse mutants. Here we review the key genetic determinants of pituitary morphogenesis in the murine embryo and focus on examples of genes which are functionally conserved in humans.

3. FUNCTION AND DEVELOPMENT OF THE PITUITARY GLAND

The pituitary is a tripartite endocrine gland located at the base of the brain which functions as a central regulator of growth, fertility, pubertal development, metabolism, stress response and lactation. The anterior pituitary contains five hormone-secreting cell types which are defined by their hormonal products: Growth Hormone (GH)-secreting somatotropes, Prolactin (PRL)-secreting

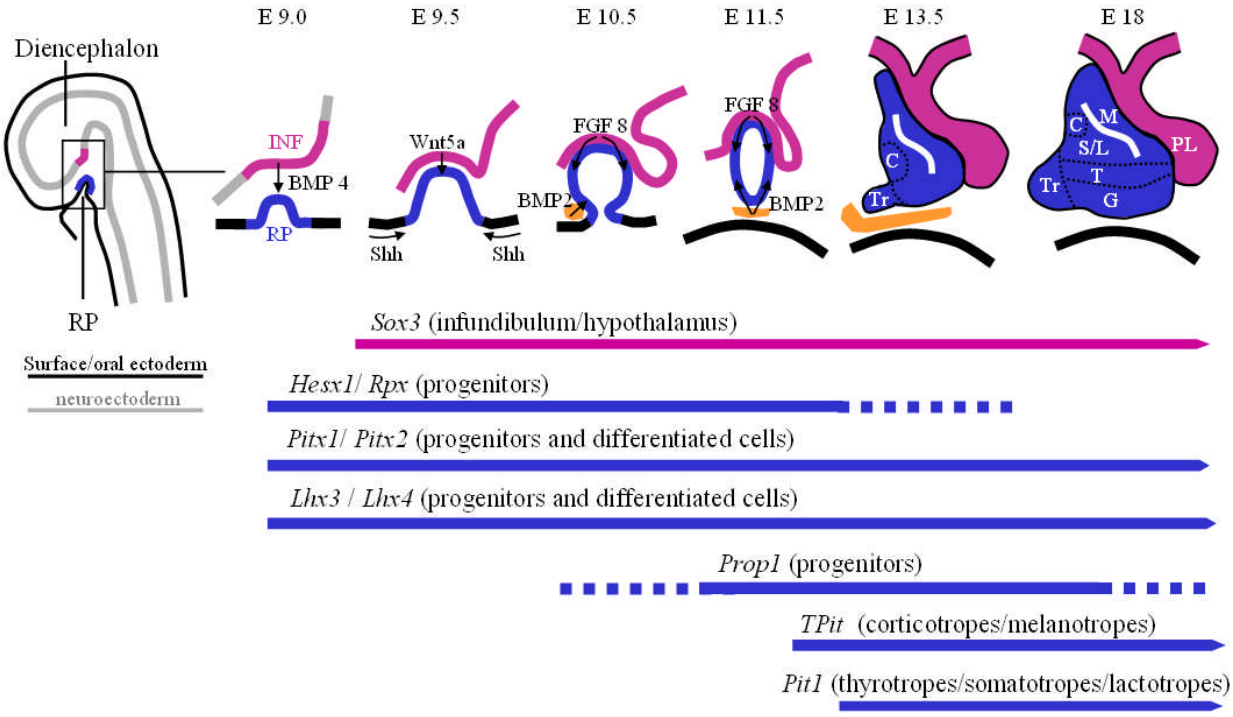

Figure 1. Schematic representation of pituitary morphogenesis. Rathke's Pouch (RP), the primordium of the anterior and intermediate pituitary lobes, is generated from an invagination of midline oral ectoderm (blue). Signaling factors released from the ventral diencephalon/infundibulum (pink), oral ectoderm (black) and ventral juxtapituitary mesenchyme (yellow) induce RP and establish regionally-restricted domains of transcription factor expression (indicated by the coloured bars at the bottom of the figure). Together, these factors are essential for progenitor cell commitment and differentiation, coordinating the sequential appearance of the trophic cell lineages in the anterior and intermediate lobes. Approximate developmental timepoints for each stage are shown at the top of the figure. See text for further details. Abbreviations: E, Embryonic day; Tr, Rostral tip thyrotropes; S, somatotropes; L, lactotropes; T, Thyrotropes; G, Gonadotropes; C, Corticotropes; PL, Posterior Lobe.

lactotropes, Thyroid Stimulating Hormone (TSH)-secreting thyrotropes, Follicle Stimulating Hormone (FSH)- and Luteinising Hormone (LH)-secreting gonadotropes and Adrenocorticotropic Hormone (ACTH)-secreting corticotropes. Synthesis and secretion of these hormones is regulated by hypothalamic peptides which are transported to the anterior lobe (AL) via a specialised portal vasculature system. The AL also contains significant numbers of Folliculo-Stellate (FS) cells which are generally found surrounding colloid–filled follicles. In contrast to the five trophic lineages, FS cells do not have a cognate hormonal product and may function as mediators of cell-cell communication and/or trophic progenitor cells (1, 2). The intermediate lobe (IL) contains melanotropes which secrete alpha-Melanocyte Stimulating Hormone (alphaMSH). The posterior (or neural) lobe contains axon terminals which release oxytocin and vasopressin which are synthesised by cell bodies located in the hypothalamus.

3.1. Murine pituitary development

The pituitary is generated via the confluence of two primordia derived from distinct ectodermal lineages (Figure 1). The anterior and intermediate lobes (adenohypophysis) are generated from an invagination of the midline oral ectoderm termed Rathke's Pouch (RP) while the posterior pituitary (neurohypophysis) is generated from the infundibular evagination of ventral diencephalon. In mice, RP forms at 9.0 days post coitum (dpc) and makes direct contact along its dorsal surface with the developing infundibulum (INF) which secretes signaling factors that are critical for induction and expansion of RP (see below). At 9.5-11.5 dpc, RP expands rostro-caudally and buds off from the oral ectoderm to generate a flattened epithelium of proliferating cells. At 12.5 dpc, progenitor cells with a high proliferative index line the luminal surface and retain an epithelial conformation. This "growth plate" is maintained until at least 17.5 dpc. Cell fate studies suggest that committed progenitor cells migrate ventrally from the periluminal proliferative zone to the anterior lobe (3) although it is not yet clear whether this cell movement is an active or passive process. The first hormone-secreting lineage to appear is the rostral tip thyrotrope, a transient population of ventral cells which are marked by alpha-GSU expression at 12.5 dpc. Corticotropes appear at 13.5 dpc, followed by thyrotropes at 14.5 dpc and somatotropes, lactotropes and gonadotropes between 15.5-17.5 dpc. Pituitary cell differentiation therefore resembles neural differentiation where differentiated cells types are derived from proliferating epithelial progenitors associated with the ventricular lumen. However, unlike neurons and glia, the hormone secreting lineages are not post-mitotic and continue to divide after birth resulting in a 14-fold increase in pituitary volume by adulthood (3).

3.2. Pituitary development in humans

Morphogenesis and terminal differentiation of the trophic cell types in human and murine pituitary glands is similar, although this process occurs over a significantly longer period in humans. RP is first evident in human foetuses at around 3.5 weeks of gestation (4). Morphogenesis of the anterior lobe begins with the appearance of "buds" in the rostral wall of RP which extend antero-laterally and engulf the vascularised mesenchymal tissue (5). Thickening of the RP epithelium also occurs at this time and at 5 weeks of gestation RP makes contact with the infundibulum (6-9). At 6 weeks of gestation, RP buds off from the oral ectoderm and subsequently undergoes massive expansion resulting in an 377-fold increase in total free surface area between week 5 and week 21 (10). During this period, the portal vasculature is established (20 weeks), the hypothalamic nuclei appear in the ventral diencephalon (weeks 14-15) and the hormone lineage appear in the anterior lobe (7-13 weeks). GH-positive cells are one of the first trophic cell lineages to appear in the embryonic pituitary and are first detectable at 7-8 weeks of gestation (6-9). ACTH–positive cells also appear in the AL around this time and subsequently in the IL at week 13 (10). AlphaGSU+ cells appear at 9 weeks followed by TSH-Beta, FSH-Beta and LH-Beta expression at 12 weeks. PRL+ lactotropes also appear for the first time around 12 weeks. Therefore, apart from the relatively early appearance of the somatotropes, the order in which the hormone-secreting lineages appear in mice and humans appears to be relatively well conserved.

4. INDUCTION AND MORPHOGENESIS OF RATHKE'S POUCH

4.1. Origin of the pituitary primordium

The adenohypophysis, hypothalamus and neurohypophysis are derived from the rostro-medial extreme of the anterior neural ridge and maintain a close topographical relationship throughout pituitary morphogenesis (11, 12). Experimental manipulation and explant culture experiments in frog, chick and rat embryos have demonstrated that signaling from the diencephalon is essential for the differentiation and expansion of hormone secreting lineages (13-17). This has been confirmed by genetic analysis using *Nkx2.1* (*T/Ebp*) null embryos. *Nkx2.1* is expressed in the ventral diencephalon during forebrain development but is not expressed in the developing anterior pituitary. RP morphogenesis in *Nkx2.1* mutants is arrested after the development of a rudimentary thickened placode which is subsequently eliminated via apoptosis (18). More recently, targeted mutagenesis, transgenesis and explant culture experiments in mice have revealed that the induction, proliferation and positional specification of RP requires input from several neighbouring signaling centres. As is the case for many embryonic tissues, signaling proteins from the BMP, FGF, hedgehog and Wnt families are key determinants of RP morphogenesis.

4.2. BMP4 is essential for induction of RP

Bone Morphogenetic Proteins (BMP) belong to the TGF-Beta superclass of signaling molecules and have extensive roles in embryonic patterning (19). BMP4 is expressed in the midline ventral diencephalon at 8.5 dpc, prior to the formation of RP, and is maintained in the presumptive infundibular region until 10.5 dpc (12, 20). BMP null mutants typically die around 9.0 dpc thereby precluding analysis of RP morphogenesis. However, on a mixed genetic background, some embryos survive to 10.0 dpc and display normal anterior structures (18). Histological analysis of these mutant embryos revealed agenesis of RP indicating that BMP4 is essential for the induction of RP. Early arrest of pituitary development was also observed in transgenic embryos which ectopically expressed the BMP4 inhibitor noggin in the RP (20). RP development in these embryos did not proceed beyond the formation of an epithelial rudiment. Together, these data indicate that BMP4 activity is required for the initial morphogenesis of RP.

4.3. Opposing BMP and FGF gradients specify pituitary progenitors and pattern RP

Elegant organ culture experiments have been used to investigate the timing of pituitary cell specification and the influence of neighbouring signaling centres in this process. Culture of 10.0 dpc RP explants results in near uniform expression of the ventral prospective thyrotrope markers *Isl1* and alpha-GSU (12). Therefore, in the absence of external signals, it appears that RP cells are initially specified to adopt a ventral cell fate. In contrast, 10.5 dpc RP explants generate ACTH-, alpha-GSU- and Pit1-positive cells (20) indicating that at least some pituitary progenitors are committed to the five principle trophic cell lineages between 10.0 and 10.5 dpc. Dorsalisation of the RP progenitors requires signaling from the INF which promotes expression of the dorsal marker *Lhx3* and suppresses ventral cell fate (12). One of the key signaling factors in this process is FGF8 which is expressed in the INF from 9.5 dpc until at least 14.5 dpc (12, 20). Maintenance of dorsal INF/FGF8 signaling inhibits corticotrope differentiation and appears to be required for the expansion of uncommitted progenitors in the dorsal region of RP (12, 20). An opposing gradient of BMP2 signaling from the ventral juxtapituitary mesenchyme promotes ventral cell fate through maintenance/upregulation of *Isl1* and alpha-GSU and inhibition of ACTH+ cells. Unlike FGF8, however, BMP2 does not exert a proliferative effect on RP progenitors and cannot override FGF-induced proliferation (12).

4.4. Sonic hedgehog signalling promotes proliferation of RP progenitors

Sonic hedgehog (*shh*) is expressed extensively throughout in the oral ectoderm but is excluded from the nascent and definitive RP (20, 21). *Shh* -/- embryos exhibit agenesis of RP, although this is likely to be due to dysmorphogenesis of the ventral forebrain which includes absence of the *Nkx2.1* expression domain (22). To determine the impact of reduced shh signaling on RP development, Treier and colleagues expressed a secreted form of the hedgehog binding protein HIP (Hedgehog inhibitor protein) throughout the oral ectoderm using Pitx-1 regulatory sequences (21). Transgenic embryos displayed marked hypoplasia of RP at 11.0 dpc and 13.0 dpc and

exhibit cell type-specific marker expression profiles that reflect an early arrest in development. Overexpression of *shh* in RP progenitors resulted in pituitary hyperplasia and expansion of ventral cell types, possibly due to upregulation of BMP2 in the AP. Therefore, shh stimulates proliferation of RP progenitors and may also, directly or indirectly, promote expansion of ventral cell types.

4.5. Wnt Signalling is essential for RP development

The canonical Wnt signaling pathway is activated through binding of secreted Wnt ligands to the frizzled/LRP coreceptor complex and ultimately results in translocation of Beta-catenin to the nucleus where it interacts with TCF/LEF family cofactors and modifies expression of target genes (23). Multiple components of the canonical Wnt signaling pathway have been identified in the 14.5 dpc pituitary including *frizzled2*, *Apc*, *Beta-catenin*, *Aes* and *Tcf7l2* (24), suggesting a role for this pathway in pituitary development. This is supported by the experiments of Kioussi and colleagues who activated the canonical signaling pathway in embryos using LiCl and showed that this results in direct activation of *Pitx2* which in turn regulates cell-type specific proliferation via induction of Cyclin D2 (25). Further, two members of the Wnt signaling family have been shown to be directly required for normal pituitary development. *Wnt4* is expressed in the oral ectoderm and RP throughout pituitary gland development (20) and has been reported to be the only Wnt that is expressed at detectable levels in the developing AL. Analysis of *Wnt4* null pituitary tissues at 17.5 dpc revealed moderate hypoplasia of the anterior lobe and decreased numbers of GH+, TSH+ and alpha-GSU+ cells supporting a role for *Wnt4* in expansion or differentiation of committed precursors (20). *Wnt5a* is expressed throughout the ventral diencephalon from 9.5 dpc and is maintained in the infundibular region until at least 14.5 dpc (20). Explant culture experiments suggest that this factor acts synergistically with BMP4 to promote the differentiation of early alpha-GSU+ lineages (20). *Wnt5a* null embryos exhibit outgrowth defects in the developing limbs, ears, face and genitals due to reduced proliferation of putative progenitor cells (26). Morphogenesis of the developing pituitary is also affected resulting in marked bifurcation of the dorsal RP (27). However, this defect does not appear to result from abnormal proliferation or programmed cell death in the INF or RP. Further, the expression of known genetic determinants of pituitary morphogenesis and cell differentiation are unaffected in the mutants and the five hormone-secreting lineages are specified normally. Therefore, the mechanism by which Wnt5a mediates morphogenesis of RP remains to be determined but appears likely to involve the non-canonical Wnt/Ca^{2+} signaling pathway (27, 28).

5. TRANSCRIPTIONAL CONTROL OF PROGENITOR CELL DIFFERENTIATION

From the studies detailed above, it is clear that factors secreted from neighbouring signaling centres are important for positional specification of RP progenitor cells. This is evidenced by the spatio-temporally regulated expression of a host of transcription factors, many of which belong to the homeodomain family. Genetic studies in mice have shown that these factors regulate lineage-specific commitment and differentiation through a variety of mechanisms including the recruitment of cell-type specific co-activator and co-repressor complexes. Below, we discuss the functions of conserved transcriptional components of the trophic cell differentiation program.

5.1. *Lhx3* and *Lhx4*

Lhx3 and *Lhx4* encode LIM class homeodomain proteins and have partially overlapping roles in RP development. *Lhx3* is expressed in RP from 9.5 dpc and is maintained in the anterior and intermediate lobes throughout embryogenesis and into adulthood (29, 30). In *Lhx3* null mutant mice, RP develops relatively normally as an epithelial rudiment but fails to expand and to generate hormone secreting cells from all lineages, apart from a few corticotropes (31). Marker expression analysis in *Lhx3* null embryos has revealed that *Hesx1* (*Rpx*) is present in the RP at 10.5dpc, indicating that progenitors are initially specified to a pituitary cell fate ((31). However, by 12.5 dpc, *Hesx1* expression is prematurely downregulated, suggesting that *Lhx3* is required for the commitment of progenitors for all of the hormone-secreting lineages, with the possible exception of corticotropes. *Lhx4* is also expressed throughout RP at 9.5 dpc and becomes restricted to the presumptive AL at 12.5 dpc (30). At 15.5 dpc, *Lhx4* is downregulated and is present at low levels in the adult anterior and intermediate lobes. The proliferation of AL progenitor cells is defective in *Lhx4* mutants, resulting in hypoplasia of RP, similar to *Lhx3* mutants (30). However, in contrast to *Lhx3*, the AL of *Lhx4* null pituitaries contains alpha-GSU, Pit-1, GH and TSH-BETA positive cells, indicating that *Lhx4* is not absolutely required for pituitary cell commitment. Analysis of *Lhx3* and *Lhx4* compound and double mutants indicate that these genes are also required for progression from a rudimentary pouch to a definitive pouch with *Lhx3* acting as the dominant partner (30).

LHX3 and *LHX4* mutations have been identified as rare causes of pituitary hormone deficiency in humans. Patients with homozygous mutations in *LHX3* phenotypically resemble *Lhx3* null mice and present with deficiencies in all AP hormones apart from ACTH. These patients also exhibit rigid cervical spine and limited head rotation, establishing a requirement for *LHX3* in extrapituitary sites (32). Only one family with a *LHX4* mutation has been reported (33). Affected members have anterior pituitary hypoplasia, variable pituitary hormone deficiencies and neural abnormalities. These patients harbour a dominant intronic mutation which activates two cryptic exonic splice sites and generates transcripts which lack the homeobox sequence. Interestingly, this mutation does not exhibit dominant negative activity in vitro suggesting that, unlike mice, humans with *LHX4* haploinsufficiency can have compromised pituitary function (34).

5.2. *Pitx1* and *Pitx2*

PITX1 and PITX2 belong to the Pitx family of homeodomain transcription factors and are expressed

throughout pituitary development in closely overlapping cell populations (35). These proteins have been shown to activate transcription of many hormone and cell cycle genes including POMC, GH, PRL, alpha-GSU, LH-Beta, FSH-Beta, Cyclin D1 and Cyclin D2 (25, 36-39). *PITX2* was originally identified as the causative gene for Rieger Syndrome, a dominant disorder which is characterised by defects in the eyes, teeth, umbilicus and heart and incompletely penetrant GH deficiency (40). The generation of null and hypomorphic targeted alleles has shown that Pitx2 function is broadly conserved in mammals and the developing eyes and teeth are particularly sensitive to *Pitx2* dosage (41, 42). *Pitx2* is critical for pituitary development; *Pitx2* null embryos arrest at the definitive pouch stage, resulting in massive hypoplasia and a lack of all hormone secreting cells apart from a few ACTH and alpha-GSU positive cells (41, 42). In contrast, pituitary morphology in *Pitx1* null embryos is normal, and only modest changes in the gonadotrope, thyrotrope and corticotrope populations are apparent (43). However, embryos lacking *Pitx1* and *Pitx2* fail to activate *Lhx3*, suggesting that these factors are required for establishment of pituitary cell fate (35).

5.3. *Hesx1*

Hesx1 (*Rpx*) encodes a paired class homeorepressor protein that is expressed during gastrulation in the presumptive forebrain and adjacent visceral endoderm (44, 45). In the developing pituitary, *Hesx1* is expressed throughout RP from 9.0 dpc-12.5 dpc and is progressively downregulated in a ventral to dorsal direction as the anterior lobe terminally differentiated cell types appear (46, 47). The restricted expression of *Hesx1* expression to the progenitor cell population and the repressor activity of Hesx1 protein in vitro suggests that this protein may inhibit progenitor differentiation via repression of prodifferentiation genes. This is supported by the work of Dasen and colleagues (47) who performed extensive biochemical and cell culture analyses which indicated that HESX1 repressor activity is mediated by direct interaction with the groucho family corepressor TLE1 via a conserved N-terminal domain. Temporally extended coexpression of HESX1 and TLE1 in pituitary progenitors resulted in the loss of all hormone secreting lineages apart from ACTH+ cells. This phenotype was not observed when TLE1 or HESX1 were misexpressed independently or when an N-terminal domain mutant of HESX1 (which is required for repressor activity in vitro) and TLE1 were co-expressed. Furthermore, these experiments, coupled with transcription reporter assays in heterologous cell lines, suggest that HESX1 functions by antagonising the prodifferentiation activity of the paired class homeoactivator Prop1 (see below) by competing for common binding sites (47, 48).

Targeted mutagenesis of *Hesx1* in mice has confirmed that this factor is functionally required for pituitary and CNS development. *Hesx1* null embryos exhibit variable deficiencies of anterior neuroectoderm tissue which results in a variety of CNS defects affecting the commissures, eyes and olfactory bulb (49). RP development is also compromised and in the most severe cases (approximately 5% of embryos), no discernible pituitary tissue is generated (47). More commonly, mutant embryos generate multiple/bifurcated pouches and/or hyperplasia of the AP which is most pronounced during differentiation of the hormone secreting lineages. Variable pituitary dysfunction and CNS defects are also associated with *HESX1* mutations in humans. This was first shown by Dattani and colleagues (49) who identified homozygous R160C mutations in two siblings with panhypopituitarism and agenesis of the corpus callosum. A homozygous I26T mutation was also identified in a girl with evolving combined pituitary hormone deficiency and ectopic posterior pituitary (50). Interestingly, this mutation lies within the conserved N-terminal repressor domain and inhibits interaction with TLE1. Rare, heterozygous mutations have also been identified in patients with variable hypopituitarism and midline defects (51-53). Given that a small percentage of *Hesx1* heterozygous mice also exhibit defects (49), mutations in these patients suggest that, in some cases, haploinsufficiency of *HESX1* results in pituitary and CNS defects.

5.4. *Prop1*

Prop-1 (*Prophet of Pit1*) was initially identified as the causative gene for the *Ames (df)* dwarf mouse, a spontaneous recessive mutant with massively reduced numbers of thyrotropes, somatotrope and lactotropes, and a 90% reduction in serum gonadotropins (48, 54, 55). *Prop1* expression is specific to the embryonic pituitary and is initiated in the dorsal RP around the time of pituitary cell fate determination (10.0-10.5 dpc; (48)). By 12.0 dpc, *Prop1* is expressed throughout RP and subsequently becomes restricted to the periluminal progenitors during terminal differentiation of AL lineages (48, 56). Abnormalities in pituitary development in *Prop1df/df* embryos are clearly evident from 14.5 dpc and are characterised by dorsal overgrowth, a convoluted lumen and hypoplasia of the AL. Elegant cell labelling studies indicate that this defect is due to the failure of 12.5 dpc RP periluminal cells to move ventrally into the AL, resulting in the abnormal collection of post-mitotic precursors in the growth plate (3). These cells continue to express progenitor cell markers, fail to differentiate and ultimately appear to be cleared by apoptosis.

PROP1 belongs to the paired-like family of homeoproteins and functions as a transcriptional activator in cell culture assays (48). Ames dwarf mice, which harbour a homozygous S83P homeodomain mutation with significantly reduced DNA binding activity, do not activate *Pit1* expression and consequently fail to generate thyrotropes, somatotropes and lactotropes (48). It has also recently been shown that *df/df* mice fail to activate normal levels of *Notch2* in the *Prop1*-positive periluminal progenitors, suggesting that Notch signaling is important for progenitor cell differentiation and that *Prop1* deficiency results in perturbation of this pathway (56). However, enforced expression of *Prop1* does not result in ectopic activation of *Notch2*, suggesting that additional factors are required for *Notch2* expression. Pituitary-specific deletion of Notch signaling components, many of which are active in the embryonic pituitary (56), will be required to definitively address the role of Notch signaling in pituitary development.

PROP1 mutations are the most common known cause of hypopituitarism in humans. In general, the phenotype of patients with *PROP1* mutations closely resembles *Prop1* deficient mice, although there is variability in the timing and degree of GH, FSH/LH, TSH and PRL deficiencies. Some patients also develop ACTH deficiency (57, 58). Eleven *PROP1* mutations have been identified in patients with CPHD, the most common of which is a recurring 2 bp A301G302 deletion which results in a premature truncation of the homeodomain (59, 60). There is no obvious phenotype-genotype correlation in patients with *PROP1* mutations indicating that genetic modifiers may influence the disease phenotype. This is supported by a recent study using *df/df* and *Prop1* null mice which indicated that phenotype strongly influenced by the genetic background (61).

5.5. *Pit1*

PIT1 is a pituitary-specific POU-domain transcription factor which is expressed in the caudomedial region AL from 13.5 dpc and is maintained in the somatotrope, lactotrope and thyrotrope lineages throughout adulthood (62). Snell (*dw/dw*) and *Jackson* (dw^J) dwarf mice, which carry recessive mutations/rearrangements in *Pit1*, lack somatotropes, lactotropes and thyrotropes, demonstrating an absolute requirement for *Pit1* in the establishment of these lineages. *Pit1* function is broadly conserved in vertebrates as mutations in the human (*POU1F1*) and zebrafish orthologs produce similar phenotypes (63, 64). At least 12 *PIT1* mutations have been identified in patients with CPHD (65). Most of these occur in the DNA binding domains and are autosomal recessive, although rare cases of autosomal dominant mutations with dominant negative activity have been reported (63). PIT1 functions as a context-dependent transcriptional regulator and is required for cell type-specific expression of GH, PRL, Growth Hormone Releasing Hormone Receptor (GHRHR), TSH-Beta and Pit1 (66). In thyrotropes, PIT1 and GATA2 bind to the TSH-Beta promoter and function synergistically to activate TSH-Beta expression (67). PIT1 also acts as a DNA binding-independent transrepressor in thyrotropes, thereby inhibiting the GATA2-mediated activation of gonadotrope-specific genes. In somatotropes, PIT1 activates GH through binding a conserved element in the proximal promoter and subsequent recruitment of a co-activator complex (68). However, the altered spacing of the PIT1 binding sites in the PRL promoter and associated conformation change in bound PIT1 results in recruitment of a corepressor complex containing N-CoR and repression of PRL gene expression in somatotropes. *Pit1* is therefore a key component of the developmental program that controls somatotrope, lactotrope and thyrotropes differentiation and analysis of Pit1 function has provided unique insight into the mechanism by which cell fate is established and maintained in the pituitary. It seems likely that the proposed mechanisms are also utilised by related transcription factors in other developmental contexts.

5.6. *TPit*

TPit (*Tbx19*) is a member of the T-box family of transcription factors and is expressed exclusively in the corticotrope and melanotrope lineages where is required for activation of *Pomc* (69, 70). *Tpit* null embryos generate corticotrope precursors, which in most cases fail to differentiate into *Pomc*-expressing corticotropes, suggesting that *Tpit* is required for terminal differentiation but not lineage commitment (71). In the IL, *Tpit* deficiency results in hypoplasia and a near total lack of melanotropes, which appear to switch fate and adopt gonadotrope and rostral tip thyrotrope phenotypes. Therefore, *Tpit* is required it the developing IL to suppress ventral cell fate, although the underlying mechanism is unclear. Eleven coding mutations in *TPIT* have been identified in patients with recessive isolated ACTH deficiency (IAD) indicating that the function of TPIT is conserved in humans (72, 73).

5.7. *Sox3*

Sox3 in an X-linked gene that belongs to the SOX (Sry-related HMG box containing genes) family of transcription factors and is expressed in the developing CNS and gonads (74). *Sox3* does not appear to be transcribed in RP but is highly expressed in the ventral diencephalon including the INF and presumptive hypothalamus (75). *SOX3* was first implicated in hypothalamic-pituitary axis function from the identification of duplications and polyalanine expansion mutations in male patients with variable pituitary hormone deficiencies and mental retardation (76-78). Subsequent studies using mice demonstrated that hemizygous null males have abnormal clefting and hypoplasia of the pituitary, variable CPHD and CNS defects which include hypocellularity of the hypothalamus (79). The pituitary abnormalities stem from abnormal morphogenesis/bifurcation of RP which is clearly evident from 11.5 dpc and is associated with altered expression of BMP4 and FGF8 in the overlying neuroectoderm. Very similar RP bifurcations occur in Wnt5a null embryos (27), suggesting that Wnt5a may be a downstream target of *Sox3* in the ventral diencephalon. Together, these data suggest that *Sox3* is required for the establishment and/or maintenance of the dorsal neuroectodermal signals that regulate morphogenesis of RP. Interestingly, RP bifurcation is also a feature of female heterozygous embryos (likely due to the enrichment of mutant cells in the INF region), but not dwarfism or GH deficiency, implicating postnatal hypothalamic dysfunction as the major contributor to CPHD in male mice (79). Supporting this, MRI analysis of males with *SOX3* duplications and mutations has revealed hypothalamic/infundibular abnormalities, indicating that hypothalamic dysfunction, as apposed to AP malformation, is the primary cause of CPHD in these patients ((78); PQT unpublished data).

6. FOLLICULO-STELLATE CELLS AND ADULT PROGENITORS

While the genetic program that controls the development of the trophic lineages is well understood, relatively little is known about the origin and function of folliculo-stellate (FS) cells. Agranular FS cells were originally identified by electron microscopical examination of the adult rat pituitary (80) and have since been shown to be present in various mammals, birds and reptiles, constituting 5-10% of the AL cells (reviewed in (81, 82)).

FS cells exhibit elongated processes and are frequently located in the lining of colloid-filled follicles. While the exact role of FS cells in the pituitary has not been defined, this lineage has been implicated in a variety of processes including the regulation of hormone secretion and long-distance communication in the AL (1, 82). FS cells have also been proposed to function as stem or progenitor cells, which may serve to provide trophic derivatives in the adult gland (2, 83). While there is evidence to suggest that the adult pituitary does contain a stem/progenitor for at least the somatotrope and lactotrope lineages (84), is it not yet clear whether this corresponds to the FS cell population, a subset of FS cells or recently identified cell populations with progenitor hallmarks (85-87).

Developmental studies in rat indicate that FS cells are generated postnatally and appear to be derived from the lateral margins of the epithelial cells that line the residual RP lumen (88). At present, there is no information in the literature about the impact of mutations in known pituitary development genes on the specification and/or differentiation of the FS cell lineage. Through careful examination of existing pituitary mutants and the generation of mouse models with mutations in FS cell "specific" genes, it should be possible to gain insight into the genetic determinants of FS cell development and function.

7. SUMMARY AND PERSPECTIVE

Genetic studies in mice have identified a host of transcription factors and secreted molecules that control the morphogenesis and trophic cell content of the pituitary gland. This knowledge base has proven to be an invaluable resource for the identification of genes that cause hypopituitarism in humans, underlining the high degree of functional conservation in the genetic program that controls vertebrate pituitary development. For the future, it appears likely that parallel studies in mice and humans will continue to contribute to our knowledge of pituitary development and further assist in identifying the aetiology of hypopituitarism, which, in the majority of cases, is idiopathic. Large scale mutagenesis screens using other vertebrate species such as zebrafish is also emerging as a powerful approach for the identification of novel and conserved genetic determinants of pituitary development (64, 89). Finally, the continued identification and functional analysis of pituitary development genes should lead to more definitive diagnosis of patients with pituitary hormone deficiency(ies) and is likely to assist efforts to derive trophic lineages from mouse and human Embryonic Stem cells.

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Abbreviations: RP: Rathke's Pouch; AL: Anterior Lobe; IL: Intermediate lobe; INF: Infundibulum; GH: Growth Hormone; PRL: Prolactin; TSH: Thyroid Stimulating Hormone; FSH: Follicle Stimulating Hormone; LH: Luteinising Hormone: ACTH: Adrenocorticotropic Hormone: FS: Folliculo-stellate; .BMP: Bone Morphogenetic Protein: FGF: Fibroblast Growth Factor; CNS: Central Nervous System

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