Pituitary development: a complex, temporal regulated process dependent on specific transcriptional factors

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Abstract

Pituitary organogenesis is a highly complex and tightly regulated process that depends on several transcription factors (TFs), such as PROP1, PIT1 (POU1F1), HESX1, LHX3 and LHX4. Normal pituitary development requires the temporally and spatially organised expression of TFs and interactions between different TFs, DNA and TF co-activators. Mutations in these genes result in different combinations of hypopituitarism that can be associated with structural alterations of the central nervous system, causing the congenital form of panhypopituitarism. This review aims to elucidate the complex process of pituitary organogenesis, to clarify the role of the major TFs, and to compile the lessons learned from functional studies of TF mutations in panhypopituitarism patients and TF deletions or mutations in transgenic animals.


Introduction

The pituitary gland comprises two parts: posterior and anterior. The posterior pituitary, or neurohypophysis, has a neuronal origin and is responsible for storing and secreting antidiuretic hormone and oxytocin, which are produced by neurons in the paraventricular and supraoptic nuclei of the hypothalamus. The anterior pituitary, or adenohypophysis, consists of five distinct cell types. These cells and their specific hormones are lactotropes, which produce prolactin (PRL); somatotropes, which produce GH; gonadotropes, which produce LH and FSH; corticotropes, which produce ACTH; and thyrotropes, which produce TSH. LH, FSH and TSH are called pituitary glycoproteins and consist of two subunits. The alpha-glycoprotein subunit (αGSU) is common to the three hormones and the beta subunit is specific to each of the hormones (β-FSH, β-LH and β-TSH).

When two or more pituitary cell types are impaired, panhypopituitarism results (Romero et al. 2009). Mutations in several transcription factors (TFs) can lead to impaired pituitary formation (Fernandez–Rodriguez et al. 2011, Mortensen et al. 2011). Recently, the increase in the number of identified mutations, functional studies and experiments using transgenic animals have helped us understand TF interactions and clarified the multiple steps of pituitary organogenesis.

Early organogenesis: Rathke’s pouch invagination


In the early stage of pituitary development, which corresponds to embryonic days (E) 6.5–10.5 in mice (Fig. 1), the extrinsic signalling pathways are activated, including the sonic hedgehog (SHH; Treier et al. 2001), bone morphogenetic proteins (BMPs; Ericson et al. 1998), fibroblast growth factor (FGF; Ericson et al. 1998) and wingless (WNT) pathways (Rizzotti & Lovell-Badge 2005).

SHH is not directly involved in Rathke’s pouch formation; however, it is required for midline formation, forebrain development, brain lobe determination, eye formation (Roessler et al. 2003, Ericson et al. 1998, Franca et al. 2010, Zhao et al. 2012) and Bmp2 expression induction (Ericson et al. 1998, Kato et al. 2010). Mouse embryos that lack Shh have pituitary hypoplasia and the optic disc is absent (Zhao et al. 2012).
Figure 1 Early pituitary development. The most anterior portion of the neural plate gives rise to the anterior pituitary and the adjacent midline region forms the endocrine hypothalamus. In mice, at approximately E8, the oral ectoderm proliferates in response to SHH. SIX3, OTX2, HES1 and SHH participate in the CNS and midline formation. Proliferation continues at approximately E9 in response to neural epithelium signalisation with the expression of BMP4, FGF8, WNT2 and NKX2. At this point, oral ectoderm begins to invaginate to form a rudimental pouch, which expresses LHX3/4 and PITX1/2. BMP2 is expressed at the edge of Rathke’s pouch that is in contact with the oral ectoderm and antagonises the FGF8 expressed by the neural epithelium. Thus, an BMP2–FGF8 ventral-dorsal gradient is set, which determines the activation of specific genes in each cell group according to their position in Rathke’s pouch. Full colour version of this figure available via http://dx.doi.org/10.1530/JOE-12-0229.

2012). The SHH pathway depends on zinc finger factors, such as GLI1, GLI2 and GLI3 (Treier et al. 2001). Although Shh is not expressed in Rathke’s pouch, GLI factors are found in the precursor structures of the pituitary. Therefore, it is possible that in response to SHH signalling, GLI proteins activate other target genes directly involved in pituitary organogenesis (Franca et al. 2010). Otx2 is another TF that is not expressed in the pituitary tissues themselves (Diaczok et al. 2008, Gorbenko Del Blanco et al. 2012). This TF encodes a bicoid protein that is important for eye and forebrain formation (Schilter et al. 2011, Gorbenko Del Blanco et al. 2012). OTX2 is also responsible for Hesx1 expression regulation (Diaczok et al. 2008). Hesx1 is the first pituitary-specific TF to be expressed at or before E6.5. (Hermesz et al. 1996, Brickman et al. 2001). Hesx1 expression begins in the rostral region and progresses dorsally; the restricted expression of this TF is responsible for Rathke’s pouch formation (Hermesz et al. 1996). HESX1 is important for midline formation and regulates the expression of other TFs (Hermesz et al. 1996, Diaczok et al. 2008, 2011, Reynaud et al. 2011; Fig. 1).

The Pitx1 and Pitx2 genes are expressed at approximately E9 and participate in the different steps of central nervous system (CNS) organogenesis. Pitx1 is initially expressed in the first branchial arch, then in the oral cavity, and next in Rathke’s pouch (Drouin et al. 1998). Pitx1 continues to be expressed in the latter stages of pituitary embryogenesis and participates in cellular differentiation (Drouin et al. 1998, Tremblay et al. 1998). Pitx2 is expressed in several organs, including the CNS, forelimbs, lungs, kidneys and tongue. In addition to its role in CNS formation, PTX2 appears to be important in the determination of the left–right axis. Similar to Pitx1, Pitx2 continues to be expressed during pituitary cell differentiation and acts synergistically with other TFs to determine pituitary cell types, primarily PIT1 (Pou1f1)-specific cells (Drouin et al. 1998, Tremblay et al. 1998, Lamotte et al. 2001).

Other molecules play relevant roles in the development of the CNS, including the SOX81 TFs (SOX1, SOX2 (Yako et al. 2011) and SOX3 (Woods et al. 2005)). SOX3 expression begins during early embryogenesis; recent studies have suggested that this gene must be expressed at a constant level because both increases and decreases in its expression are related to pituitary deficiencies and CNS malformations (Woods et al. 2005). Some signalling molecules expressed in the infundibulum directly contribute to the induction of pouch invagination, among which BMP4 (Ericson et al. 1998) and NKX2 are key (Kimura et al. 1996). Mutant animals lacking any of these factors may develop pituitary absence, malformation or even embryonic lethality (Sussel et al. 1998, Nasonkin et al. 2011).

In parallel with the invagination of oral ectoderm, the pituitary precursor cells proliferate and migrate. The WNT (Yako et al. 2011) and SHH pathways (Fernandez-Rodriguez et al. 2011) are important for proliferation regulation, while the BMP and FGF pathways are required for proliferation and for determining cellular migration (Kato et al. 2010). Rathke’s pouch formation is complete at approximately E10.5, and the pituitary precursor cells begin to express specific factors that determine their differentiation patterns (Yako et al. 2011). This activation of distinct target genes occurs in response to the establishment of a dorsal–ventral gradient of FGF8 and a ventral–dorsal gradient of BMP2 (Ericson et al. 1998). Thus, depending on its location, each
cell has a distinct starting point within the differentiation process (Fig. 2). For example, ventral cells express the TFs *Isl1* and *Gata2* (Dasen et al. 1999) and dorsal cells express *Ptx6* (Kioussi et al. 1999), *Tpit* (Lamolet et al. 2001) and *Prop1* (Sornson et al. 1996).

**Pituitary-specific factors**

*Lhx3* and *Lhx4* are predominantly expressed in Rathke’s pouch (Mullen et al. 2007) at approximately E9 (Bach et al. 1995), and the activation of these factors is essential for proper pituitary formation (Sloop et al. 2000, West et al. 2004, Machinis & Amselem 2005, Mullen et al. 2007, Pfaeffle et al. 2008). Although *LHX3* participates in the pituitary differentiation and maturation process (West et al. 2004, Mullen et al. 2007), *LHX4* is more important for cellular proliferation (Machinis & Amselem 2005). *LHX3* appears to play a role in the maintenance of some pituitary cellular strains because it is expressed in the adult pituitary gland (Sloop et al. 2000).

When the expression of *Hesx1* begins to fall by E10, *Prop1* expression progressively increases and reaches maximal expression at E12 (Sornson et al. 1996). These homeodomain factor pairs play distinct roles. *HESX1* is primarily a transcriptional repressor, while *PROP1* is an activator. When *Hesx1* expression is high, *HESX1* homodimers are formed and bind the promoter site, which leads to the recruitment of co-repressor elements. As *Prop1* expression increases, the *PROP1* homodimers predominate and bind to regulatory sites, recruiting co-activator complexes, and *PROP1*-dependent gene transcription increases (Dasen & Rosenfeld 2001). This mechanism is essential for determining the *Pit1*-specific cells and the gonadotropic lineages (Simmons et al. 1990, Drolet et al. 1991, Steger et al. 1994, Dasen & Rosenfeld 2001, Zhao et al. 2005). After *Pit1* activation, *Prop1* expression decreases rapidly, and it is not expressed in the adult gland (Cohen & Radovick 2002).

*Pit1* expression is first noticeable by E12, and it is necessary for the differentiation of thyrotropes, lactotropes and somatotropes, which are known as the pituitary-specific cell types (Simmons et al. 1990). It is well known that *Pit1* expression requires *PROP1* activation. *LHX4* also up-regulates *Pit1* expression by binding to its transactivation domain (Machinis & Amselem 2005).

**Figure 2** Temporal and spatial activation of pituitary transcription factors. In response to the BMP2–FGF8 ventral–dorsal gradient, pituitary cell lineages are determined by the activation or repression of each TF. Solid arrows indicate the activation of expression, dotted arrows indicate an unknown role in the activation of expression, dashed arrows indicate an undefined role and dash–dot arrows indicate an action of an important factor in the maintenance of long-term cell function. BMP2, bone morphogenic protein 2; EGR1, early growth response 1; ER, oestrogen receptor; FGF8, fibroblast growth factor 8; GATA2, GATA-binding protein 2; HESX1, HESX homeobox 1; ISL1, ISL LIM homeobox 1; LHX3, LIM homeobox 3; LHX4, LIM homeobox 4; LIF, leukaemia inhibitory factor; MSX1, msh homeobox 1; NeuroD1, neurogenic differentiation 1; Pit1, POU class 1 homeobox 1; PITX1, paired-like homeodomain 1; PITX2, paired-like homeodomain 2; POMC, pro-opiomelanocortin; PROP1, prophet of Pit-1; RAR, retinoic acid receptor; SF1, steroidogenic factor 1; T3r, thyroid hormone nuclear receptor; TEF, thyrotrope embryonic factor; TPT1, T-box 19; Zn15, zinc finger protein Zn15. Full colour version of this figure available via http://dx.doi.org/10.1530/JOE-12-0229.
Cellular differentiation

Initially, all Rathke’s pouch cells express *Isll*. The most ventral cells maintain the expression of this TF in response to BMP2, while FGF8 blocks *Isll* expression in the more dorsal cells. The presence of *Isll* activates αGSU, the common subunit of the heterodimeric hormones TSH, LH and FSH (Ericson 1998).

**Gonadotropes**

*Gata2* is another important TF that is expressed by ventral cells. This TF is necessary to restrict PIT activation in these cells and to ensure that a PIT-independent cell lineage is established. *GATA2* activates the expression of steroidogenic factor 1 (*Sf1*; Steger et al. 1994, Zhao et al. 2005), which in turn stimulates αGSU and LHβ gene expression; however, *GATA2* does not significantly influence FSHβ expression (Brown & McNeilly 1999). However, a recent study showed that *GATA2* and *GATA4* increase FSHβ expression in vitro (Lo et al. 2011). Although *SF1* contributes to gonadotropin differentiation, the treatment of *Sf1* knockout mice with GNRH completely restores the expression of gonadotrophins, demonstrating that *SF1* is not the only TF involved (Ikeda et al. 1995).

**PITX1** transactivates αGSU, FSHβ and LHβ (Tremblay et al. 1998), while Lhx3 up-regulates αGSU and FSHβ (Bach et al. 1995, West et al. 2004), and HESX1 stimulates LHβ expression (Brown & McNeilly 1999). Animals and humans with *Prop1* mutations usually have gonadotrophin deficiency. Functional studies suggest that *PROP1* is important for FSHβ expression, even in adulthood (Aikawa et al. 2006). It is possible that *PROP1* participates in gonadotrope differentiation in a manner that is not well defined. In response to temporally and spatially organised TF expression, the gonadotropes complete differentiation by E17 (Brown & McNeilly 1999).

**PIT1-specific cells: thyrotropes, somatotropes and lactotropes**

In response to BMP2 signalling, *Gata2* is activated and determines the gonadotrope and thyrotrropic precursors. It has been suggested that *Gata2* expression in thyrotropes is below the threshold necessary to block Pit1 activation, allowing the emergence of Gata2+/Pit1+ cells (Dasen et al. 1999). The PAX6 ventral-dorsal gradient is important for distinguishing between the thyrotropic and somatotropic/lactotrope lineages (Kioussi et al. 1999). In the absence of PAX6, thyrotropes occupy a larger region at the expense of lactotropes and somatotropes, and PRL and GH deficiencies result (Simmons et al. 1990, Bentley et al. 1999, Kioussi et al. 1999).

Thyrotropes are derived from two different populations. The first population appears in the rostral tip of the developing gland by E12, and this population is transient and independent of *Pit1* expression (Turton et al. 2012). The other population arises by E15-5 and is PIT1 dependent. This second population corresponds to the thyrotropes found in adulthood, suggesting that PIT1 is important for transactivating TSHβ (Lin et al. 1994) and for maintaining this cellular lineage. Thyrotroph embryonic factor (TEF) is expressed exclusively in the rostral portion of the developing pituitary, where the thyrotropic precursors are located. TEF can bind to three different elements of the TSHβ promoter, which leads to its effective transactivation (Drouin et al. 1991). PITX1 and Pitx2 also collaborate in thyrotrope differentiation by acting synergistically with αGSU and TSHβ transactivation (Drouin et al. 1998).

Lactotrope and somatotrope differentiation are completely dependent on Pit1 activation. These two cell types appear to arise from the same precursor; thus, secondary TFs restrict GH and PRL expression to their corresponding cell lineages (Simmons et al. 1990). PTX1 and PTX2 synergise with Pit1 to transactivate GH and PRL (Tremblay et al. 1998). Among the elements that are important in determining somatotrope specificity, a small zinc finger protein, Zn-15, binds to the GH promoter, synergising with Pit1 (Lipkin et al. 1993). The retinoic acid receptor and the thyroid hormone nuclear receptor also cooperate with Pit1 in the regulation of GH gene expression (Schaféle et al. 1992, Palomino et al. 1998). However, in lactotrope differentiation, the oestrogen nuclear receptor synergistically partners with Pit1 (Simmons et al. 1990). Somatotrope and lactotrope differentiation finish at approximately E16 and E17 respectively (Simmons et al. 1990).

**Corticotropes**

The most dorsal cells differentiate into corticotropes. This cell lineage is the most distinct among the pituitary cells (Reynaud et al. 2004). In response to FGF8 signalling, corticotrope progenitors do not express any of the rostro-dorsal-specific TFs. Corticotrope differentiation depends on the interactions between PTX1, TPIT, NeuroD1 and LIF, which are all expressed just before pro-opiomelanocortin (POMC) expression is first detected and act synergistically at the level of the POMC promoter to transactivate this gene (Poulin et al. 1997, Tremblay et al. 1998, Yano et al. 1999, Lo et al. 2011). PITX1 is also necessary for maintaining corticotrope-specific transcription (Tremblay et al. 1998). The terminal differentiation of corticotropes depends on FGF8 down-regulation, which occurs by E14-5 (Ericson et al. 1998). Although *Prop1* is not expressed in corticotropes, *PROP1*-deficient individuals may develop ACTH deficiencies. It has also been suggested that *PROP1* is required for long-term maintenance of the corticotrope population; however, Nasonkin et al. (2011) have shown that aged *PROP1*-deficient mice maintain ACTH production.

**Final considerations**

Pituitary organogenesis during embryogenesis is a complex process that depends on both the activation and inactivation
of different TFs at the appropriate times. Moreover, correct cellular migration in response to dorsal–ventral gradients enables each cell group to receive signals from distinct pathways, depending on the cell location. This process induces different responses and allows the determination of the five cell lineages that constitute the pituitary. Thus, as shown in Fig. 2, pituitary organogenesis is a temporally and spatially sequenced and organised process.

We can thus expect that any mutation that alters the length, quality or quantity of TF gene expression will result in pituitary development failure. The integrity of TF co-activator or co-repressor recruitment is also critical for the components of this gland, and any changes in the components of these pathways may contribute to the development of hypopituitarism, which would explain the existence of different phenotypes for the same mutation.

Functional studies of known human mutations and the knowledge obtained from transgenic animals have enabled the discovery of several TFs as well as the timing of their appearance and a partial understanding of their role in pituitary development. These discoveries have shaped our current understanding of the process of pituitary organogenesis. However, there are still many questions to be answered, mainly regarding the interaction mechanisms of TFs and co-factors.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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