REVIEW

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.

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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

While the neurohypophysis originates from the neural ectoderm, the adenohypophysis is derived from Rathke's pouch, which is an invagination of the oral ectoderm in response to neural epithelium signals (Watanabe 1982, Treier & Rosenfeld 1996). Pituitary cell proliferation and differentiation are regulated by transcriptional activators and repressors and by signalling molecules from adjacent regions (Roessler *et al.* 1996, 1997, Treier *et al.* 1998, 2001, Tremblay *et al.* 1998, Nanni *et al.* 1999, Roessler *et al.* 2003, Woods *et al.* 2005).

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 (Rizzoti & 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.*

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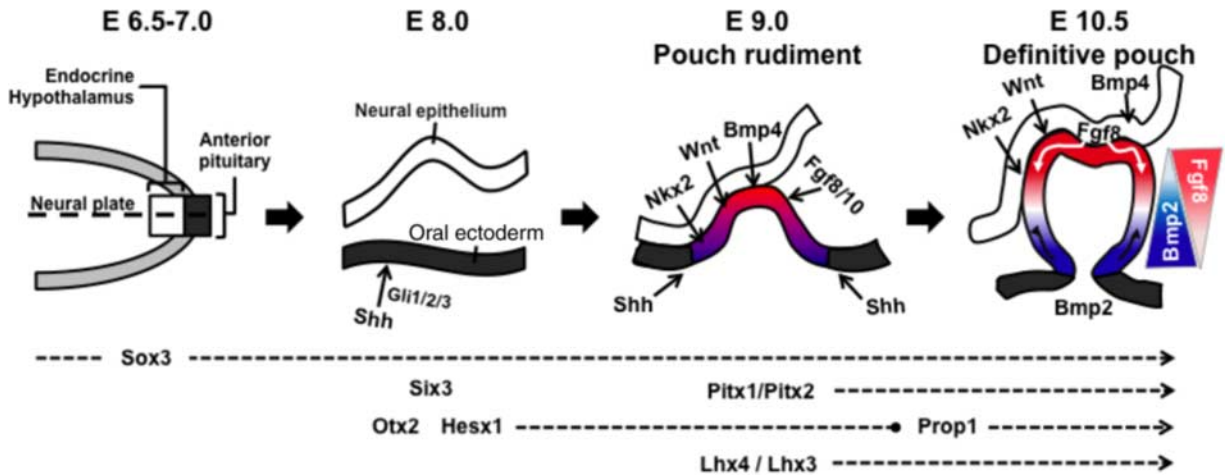

Figure 1 Early pituitary development. The most anterior portion of the neural plate gives rise to the anterior pituitary and the adjacent midline region forms the endocrine hypothalamus. In mice, at approximately E8, the oral ectoderm proliferates in response to SHH. SIX3, OTX2, HEX1 and SHH participate 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 e 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, Lamolet *et al.* 2001).

Other molecules play relevant roles in the development of the CNS, including the SOXB1 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 *Pax6* (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).

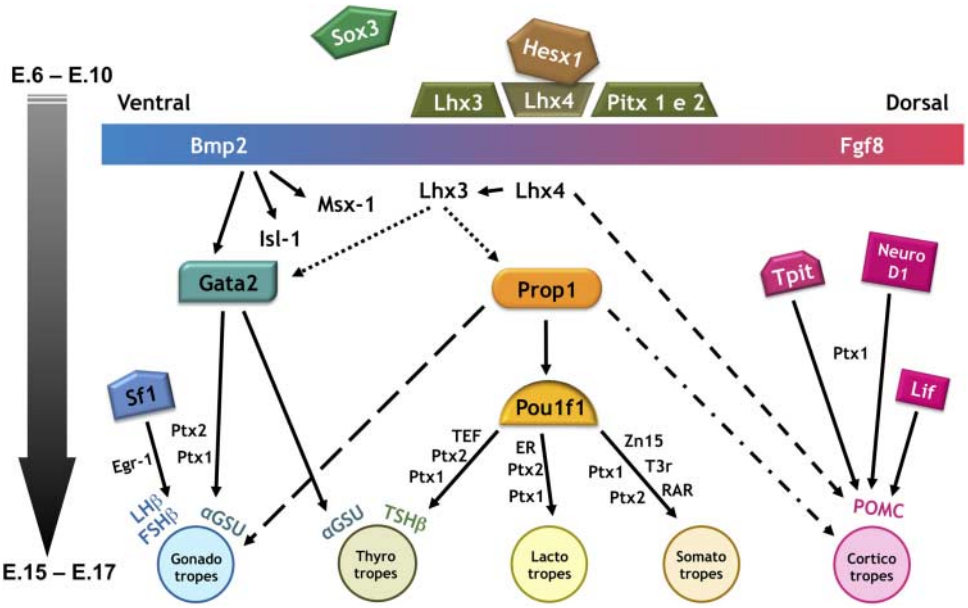

Figure 2 Temporal and spatial activation of pituitary transcription factors. In response to the BMP2–FGF8 ventral–dorsal gradient, pituitary cell lineages are determined by the activation or repression of each TF. Solid arrows indicate the activation of expression, dotted arrows indicate an unknown role 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; TPIT, 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 *Isl1*. The most ventral cells maintain the expression of this TF in response to BMP2, while FGF8 blocks *Isl1* expression in the more dorsal cells. The presence of ISL1 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 *FSH*β expression (Brown & McNeilly 1999). However, a recent study showed that GATA2 and GATA4 increase *FSH*β expression *in vitro* (Lo *et al.* 2011). Although SF1 contributes to gonadotropic 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*, *FSH*β and *LH*β (Tremblay *et al.* 1998), while Lhx3 up-regulates *αGSU* and *FSH*β (Bach *et al.* 1995, West *et al.* 2004), and HESX1 stimulates *LH*β expression (Brown & McNeilly 1999). Animals and humans with *Prop1* mutations usually have gonadotrophin deficiency. Functional studies suggest that PROP1 is important for *FSH*β 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 thyrotropic 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/lactotropic 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 *TSH*β (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 *TSH*β promoter, which leads to its effective transactivation (Drolet *et al.* 1991). PITX1 and Pitx2 also collaborate in thyrotrope differentiation by acting synergistically with *αGSU* and *TSH*β 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 (Schaufele *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 formation 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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