REVIEW

The role of SOX proteins in normal pituitary development

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Abstract

Pituitary development is a complex process that depends on the co-ordinated spatial and temporal expression of transcription factors and signalling molecules that culminates in the formation of a complex organ that secretes six hormones from five different cell types. Given the fact that all distinct hormone producing cells arise from a common ectodermal primordium, the patterning, architecture and plasticity of the gland is impressive. Among the transcription factors involved in the early steps of pituitary organogenesis are SOX2 and SOX3, members of the SOX family that are emerging as key players in many developmental processes. Studies *in vitro* and *in vivo* in transgenic animal models have helped to elucidate their expression patterns and roles in the developing hypothalamo–pituitary region. It has been demonstrated that they may be involved in pituitary development either directly, through shaping of Rathke's pouch, or indirectly affecting signalling from the diencephalon. Their role has been further underlined by the pleiotropic effects of their mutations in humans that range from isolated hormone deficiencies to panhypopituitarism and developmental abnormalities affecting many organ systems. However, the exact mechanism of action of SOX proteins, their downstream targets and their interplay within the extensive network that regulates pituitary development is still the subject of a growing number of studies. The elucidation of their role is crucial for the understanding of a number of processes that range from developmental mechanisms to disease phenotypes and tumorigenesis.

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Introduction

The pituitary gland is a central regulator of growth, metabolism, reproduction and homeostasis. It consists of three lobes: the anterior and the later-involuting intermediate lobe form the adenohypophysis, while the neurohypophysis consists of the posterior lobe. The anterior pituitary is populated by five distinct cell types producing six different hormones: somatotrophs (GH), thyrotrophs (TSH), lactotrophs (PRL), gonadotrophs (FSH and LH) and corticotrophs (ACTH). The posterior lobe of the pituitary consists of the axonal terminals of magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus, which secrete arginine vasopressin and oxytocin respectively.

Hypothalamic neuropeptides as well as positive and negative feedback loops from peripheral organs control the synthesis and secretion of pituitary hormones, and despite the low steady-state cell turnover, the number and types of hormone producing cells may vary depending on the needs at different stages of life. The doubling of the number of somatotrophs during puberty as well as the expansion and contraction of lactotrophs during lactation and weaning are examples of the plasticity of the gland (Sasaki 1988, Levy 2002).

The pituitary has a dual embryonic origin with the anterior and intermediate lobes deriving from the oral ectoderm while the posterior lobe originates from the neural ectoderm. The generation of the distinct hormone producing cell types that arise from a common ectodermal primordium and the correct morphogenesis of the gland require the sequential temporal and spatial expression of a cascade of signalling molecules and transcription factors that will eventually dictate organ commitment, cell proliferation, patterning and terminal differentiation (Sheng & Westphal 1999, Scully & Rosenfeld 2002, Zhu *et al.* 2007*a*).

Among them, SOX2 and SOX3, members of the SOX (SRY-related high mobility group (HMG) box) family of transcription factors are emerging as key players that control the early stages of pituitary development. SOX3 was the first member of this family to be associated with X-linked hypopituitarism in mice and humans (Laumonnier *et al.* 2002, Rizzoti *et al.* 2004, Solomon *et al.* 2004, Woods *et al.* 2005).

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Subsequently, SOX2 was also found to be critical for the development of the hypothalamo–pituitary axis (Kelberman *et al.* 2006, 2008).

In this review, we will focus on the role of SOX proteins as has been elucidated by transgenic animal studies and the pituitary phenotype resulting from their defects in humans.

Overview of normal pituitary development

The development of the pituitary gland has been studied extensively in mouse. Although relatively little is known about human pituitary development, it seems that it mirrors that in rodents (Sheng *et al.* 1997). Fate-map experiments have demonstrated that the origin of the anterior pituitary can be traced back to the hypophyseal or pituitary placode, one of the six cranial placodes that develop transiently as localised ectodermal thickenings in the prospective head of the developing embryo. The pituitary placode appears at 7·5 days post coitum (dpc); it is located ventrally in the midline of the anterior neural ridge and in the continuity with the future hypothalamo–infundibular region, which is located posteriorly in the rostral part of the neural plate. By 8·5 dpc the neural tube has bent at the cephalic end and the placode appears as a thickening of the roof of primitive oral cavity. From 9·0 dpc the placode invaginates dorsally to form a rudimentary Rathke's pouch, from which the anterior and intermediate lobes of the pituitary are derived. The definitive pouch is formed by 10·5 dpc, while the evagination of the neural ectoderm at the base of the developing diencephalon will give rise to the posterior pituitary. Between 10·5 and 12 dpc the pouch epithelium continues to proliferate as it closes and separates from the underlying oral ectoderm at 12·5 dpc. Subsequent to these initial patterning events, the progenitors of the hormone-secreting cell types proliferate ventrally from the pouch between 12·5 and 15·5 dpc to populate what will form the anterior lobe (Fig. 1). The remnants of the dorsal portion of the pouch will form the intermediate lobe, while the lumen of the pouch remains as the pituitary cleft, separating the intermediate from the anterior lobe (Scully & Rosenfeld 2002, Rizzoti & Lovell-Badge 2005).

The development of the anterior pituitary gland depends on extrinsic and intrinsic transcription factors and signalling molecules (Dasen & Rosenfeld 2001). At least two sequential inductive signals from the diencephalon are required for the induction and formation of Rathke's pouch (Takuma *et al.* 1998). Bone morphogenetic protein 4 (Bmp4) is the earliest secreted signalling molecule, detected at 8·5 dpc, followed by a second signal, fibroblast growth factor 8 (Fgf8) that activates the key regulatory genes *Lhx3* and *Lhx4*, essential for the development of the pouch rudiment into a definitive pouch structure (Treier & Rosenfeld 1996, Ericson *et al.* 1998, Sheng & Westphal 1999). Signalling molecules from the infundibular area (Bmp4, Fgf8, Fgf4, Nkx2.1, Wnt5α) as well as ventral signals from the oral ectoderm (Sonic Hedgehog, Shh), surrounding mesenchyme (Bmp2, Indian hedgehog IHH, Chordin) and the pouch itself (Bmp2, Wnt4) create a network of signalling gradients that is important for morphogenesis during early pituitary development (Dasen & Rosenfeld 2001). Within the pouch, this results in the induction of a cascade of transcription factors that are involved in patterning and terminal cell differentiation. Some (*Six3*, *Pax6*, *Hesx1*) are already expressed within the pituitary primordium and continue to be expressed in Rathke's pouch (Dasen & Rosenfeld 2001, Andoniadou *et al.* 2007, Zhu *et al.* 2007*b*). Concurrent with organ commitment, LIM-homeodomain factors (*Lhx3* and *Lhx4*) and OTX-related factors (*Ptx1/P-OTX*) are also expressed in the pouch. As endocrine cell types progressively differentiate in a temporally and spatially regulated manner, the process is dependent upon an increasing list of transcription factors (*Prop1*, *T-Pit*, *Pou1f1*), the contribution of which has been extensively reviewed (Dattani 2004, Kelberman & Dattani 2006, 2008, Zhu *et al.* 2007*a*, Mehta & Dattani 2008). Among them, the SOX family of transcription factors are emerging as major players controlling the early steps of pituitary development (Fig. 2).

SOX proteins

The SOX family of transcription factors are a group of proteins characterised by the presence of a 79-amino acid HMG DNA binding domain that is similar to the HMG domain of the mammalian sex determining gene *SRY* (OMIM 480000; Gubbay *et al.* 1990, Denny *et al.* 1992). They are widely expressed in many cell types at early stages of development and play critical roles in diverse developmental processes that range from maintenance of pluripotency and determination of cell fate to neurogenesis, sex development, chondrogenesis and haematopoiesis (Pevny & Lovell-Badge 1997, Kiefer 2007, Lefebvre *et al.* 2007).

SOX proteins are highly conserved across species and, based on sequence homology, more than 20 proteins and their genes have been identified in mammals and a variety of organisms ranging from birds to reptiles, insects and amphibians. Protein sequence comparison led to their subsequent classification into eight groups A–H. Group A was assigned to Sry and group B was further divided into subgroups B1 and B2 (Schepers *et al.* 2002). SOX proteins within the same group share 70–95% identity both within and outside the HMG domain, while proteins from different groups have only partial identity (Bowles *et al.* 2000).

SOX1 (OMIM 602148), *SOX2* (OMIM 184429) and *SOX3* (OMIM 313430) are all members of the SOXB1 subgroup, which exhibit the highest degree of similarity to SRY (Gubbay *et al.* 1990, Stevanovic *et al.* 1993, 1994, Bergstrom *et al.* 2000).

The HMG domains of SOX proteins have similar DNA binding properties and bind to DNA in a sequence-specific manner. They recognise a consensus 6–7 bp motif (A/T A/T

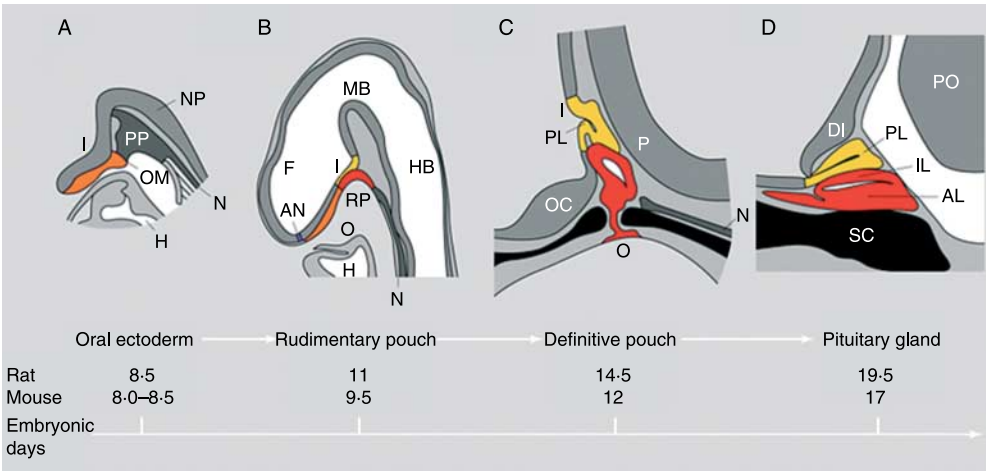

Figure 1 Stages of rodent pituitary development. (a) Oral ectoderm. (b) Rudimentary pouch. (c) Definitive pouch. (d) Adult pituitary gland. I, infundibulum; NP, neural plate; N, notochord; PP, pituitary placode; OM, oral membrane; H, heart; F, forebrain; MB, midbrain; HB, hindbrain; RP, Rathke's pouch; AN, anterior neural pore; O, oral cavity; PL, posterior lobe; OC, optic chiasm; P, pontine flexure; PO, pons; IL, intermediate lobe; AL, anterior lobe; DI, diencephalon; SC, sphenoid cartilage. Adapted from Sheng & Westphal (1999).

CAA A/T G) and interact with the minor groove of the DNA helix, inducing a sharp bend in the molecule that ranges between 30° and 113° depending on the protein and experimental conditions (Kamachi *et al.* 1999, Wegner 1999). Despite the similarity in DNA-binding and DNA-bending properties, there is evidence that SOX proteins have subtle preferences for nucleotides flanking the core sequence, thus contributing to target specificity (Mertin *et al.* 1999). Although, SOX proteins bind to DNA at low affinity, the induced DNA bending may facilitate binding of other transcription factors to adjoining DNA sites or facilitate protein–protein interactions (Weiss 2001).

SOXB1 proteins are widely expressed in the developing neural system and their expression patterns tend to overlap during development, supporting the notion that there is a degree of redundancy in their functions (Grindley *et al.* 1995, Uwanogho *et al.* 1995, Collignon *et al.* 1996). A common theme underlying the function of SOXB1 proteins is that they are expressed in pluripotent or undifferentiated cells, and their expression is downregulated once cells are committed to their

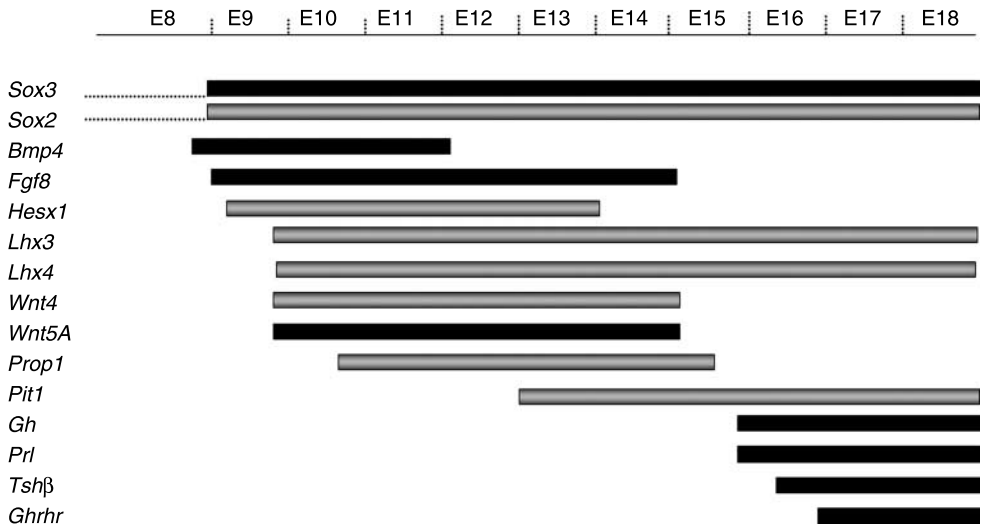

Figure 2 Diagram of the approximate timing of mRNA expression in the mouse for some of the transcription factors (TFs) and signalling molecules (SMs) that regulate anterior pituitary development. Black bars represent TFs and SMs expressed in the diencephalon and infundibulum, grey bars those expressed in Rathke's pouch and blue bars other marker genes. Broken line for *Sox2* and *Sox3* indicate their early expression in other parts of the developing CNS. Their expression persists in specific areas of the adult brain (see text). E, embryonic day.

cell fate (Karsten *et al.* 2003, Wegner & Stolt 2005). For instance, in the cascade of events that determine neuronal differentiation, suppression of *Sox1-3* by Neurogenin-2 (*Ngn2*) is essential for neurogenesis, while over-expression of *Sox3* blocks the activity of *Ngn2* to induce differentiation (Bylund *et al.* 2003). Despite the highly similar DNA binding properties, individual Sox proteins regulate a distinct array of genes and the same Sox protein appears to regulate different target genes, depending on the cell type and developmental stage. This selectivity is conferred by the temporal and spatial specific expression, which is conferred by distinct *cis*-regulatory elements (Zappone *et al.* 2000, Brunelli *et al.* 2003, Uchikawa *et al.* 2003, Miyagi *et al.* 2004, Rogers *et al.* 2008). In addition, the interaction of SOX proteins with cell specific partners is important for stable binding to DNA and target specificity (Kamachi *et al.* 2000, Wilson & Koopman 2002). For instance, the co-operative interaction between Sox2 and Pax6 is required for the activation of the enhancer of *δ-crystalline* during lens formation (Kondoh *et al.* 2004, Inoue *et al.* 2007). On the other hand, in embryonic stem cells, the interaction of Sox2 with class III POU proteins results in the activation of the *Nestin* enhancer (Tanaka *et al.* 2004).

SOX3

SOX3 (OMIM 313430) is a single exon gene spanning ~1·3 kb on chromosome Xq27. The encoded protein consists of a short 66-amino acid N-terminal domain of unknown function, the 79 amino acid DNA binding HMG domain and a longer C-terminal domain that contains four poly-alanine stretches involved in transcriptional transactivation (Stevanovic *et al.* 1993, Kamachi *et al.* 1998). Compared with the other SOXB1 proteins, SOX3 has the greatest sequence homology to SRY within the HMG domain, approaching 90%, thus leading to the assumption that *SRY* may have evolved from *SOX3* (Foster & Graves 1994).

Sox3 expression patterns and implications for hypothalamo–pituitary development

In the mouse, *Sox3* is expressed throughout the developing CNS, including the brain and spinal cord. Its expression is first detected at the earliest stages of development, prior to the appearance of the primitive streak, at 6·5 dpc throughout the epiblast and in a band of extraembryonic ectoderm at the boundary of the embryonic and extraembryonic tissues. After gastrulation, its expression is subsequently rapidly down-regulated in extraembryonic tissues and remains restricted to the anterior ectoderm of the epiblast and to a posterior domain adjacent to the primitive streak (Wood & Episkopou 1999). By 9·5 dpc *Sox3* is detected in the neuroectoderm while some expression is evident in the olfactory placode and optic vesicle. By 11·5 dpc its expression is maintained in the foetal brain, excluding the optic cup, and at 13·5 dpc, it is restricted to the ependymal layer where undifferentiated progenitor cells are still actively dividing (Collignon *et al.* 1996). In neonatal and adult mouse forebrain, *Sox3* expression persists in neurogenic regions that include the subventricular zone and the subgranular zone of the hippocampal dentate gyrus. Its expression is also maintained, though at lower levels, in specific non-neurogenic regions of the adult mouse brain (i.e in the cerebellar Purkinje cell layer, ventromedial hypothalamus and dorsolateral septum; Wang *et al.* 2006). These SOX3 positive cells may represent quiescent progenitors but the significance of this expression pattern remains unknown.

Targeted disruption of *Sox3* in mice has been performed by two independent groups and provided insight into its role in brain and pituitary development (Table 1). Weiss *et al.* (2003) reported that *Sox3* knock out mice did not show embryonic lethality and had variable growth and abnormal development of teeth. However, they neither report any pituitary abnormality, nor any evidence of GH deficiency. Their study focused mainly on gonadal phenotypes and reported that male knockouts were hypogonadal with a 42% reduction in testicular weight and abnormal germ cell development while severely affected heterozygous $Sox3^{-/+}$ females had multiple atretic follicles.

The role of *Sox3* in pituitary development was examined in detail by Rizzoti *et al.* (2004). Mutant mice did not show embryonic lethality, and were born at the expected sex ratio which suggests that *Sox3* is not required for sex determination and exhibited a variable phenotype with reduction in size and fertility. Almost one third of null males were normal but the more severely affected had poor growth, generalised weakness and did not survive to weaning (Camper 2004). They also showed craniofacial defects including overgrowth of teeth due to jaw misalignment and malformed, displaced or even absent pinna (Rizzoti & Lovell-Badge 2007). Heterozygous and obligatory mosaic females due to X-inactivation were mostly normal although some had mild craniofacial defects.

The endocrine deficit of mutants was variable and correlated with their body weight. The pituitary concentration of GH was almost three times lower than their wild type littermates and there was a reduction in LH, FSH and TSH at two months of post-natal age. On histological examination, *Sox3* mutants had a hypoplastic anterior pituitary lobe with disrupted distinction between the anterior and intermediate lobes and midline brain defects in the most severely affected mice at birth. These included dysgenesis of the corpus callosum, failure of the dorsal hippocampal commissure to cross the midline and apparent continuity of the intercerebral fissure within the third ventricle. Further examination of sections from mutant embryos between 11·5 and 16·5 dpc showed that Rathke's pouch was expanded and bifurcated, the evagination of the infundibulum was less pronounced and the presumptive hypothalamus appeared thinner and shorter. The floor plate of the diencephalon was expanded and at 16·5 dpc, mutant mice showed extra Rathke's lumens that resulted in the appearance of extra Rathke's clefts at birth (Rizzoti *et al.* 2004). The bifurcated Rathke's pouch in

Table 1 Phenotypes of *Sox3* transgenic mouse models

	Phenotype	Fertility & gonads	Pituitary morphology	Endocrinology	Reference
Mouse model					
Sox3 null	Misalignment and overgrowth of front teeth (50–70%)	XY: no reduction in fertility. Reduced size of testicles, abnormal germ cell development	Not examined	GH, LH & FSH, similar to Wt	Weiss *et al.* (2003)
	Sporadic defects in growth	XX: impaired fertility, ovaries with small atretic follicles			
(*Sox3* XY^{-}*Sox3* XX$^{-/-}$)	Normal 1/3		Extra Rathke's clefts		
	Severely affected had poor growth, generalised weakness, overgrowth of teeth, abnormal shape and position of pinna	XY: no reduction in fertility in less affected. Severely affected had testes with necrotic and empty tubules	Dysgenesis of corpus callosum	GH, LH & FSH, lower than Wt	Rizzoti *et al.* (2004)
		XX: small ovaries with atretic follicles	Thinner infundibulum		
Sox3 XX$^{+/-}$	Normal	No reduction in fertility	–	GH, LH & FSH, similar to Wt	Weiss *et al.* (2003)
	Normal or mild craniofacial defects	–	Abnormal in ¾, similar to null mice	–	Rizzoti *et al.* (2004)

Sox3 mutants was reminiscent of that in *Hesx1*-deficient mice (Dattani *et al.* 1998) and was subsequently reported in *Wnt5α* (Cha *et al.* 2004, Potok *et al.* 2008) and *Sox2* mutants (Kelberman *et al.* 2006).

Despite the abnormalities in Rathke's pouch morphogenesis, *Sox3* expression was not detected in the pouch, but was found at high levels in the ventral hypothalamus and infundibulum (Rizzoti *et al.* 2004). The ventral diencephalon provides the necessary inductive signals (i.e. Bmp4, Fgf8) for the invagination of the oral ectoderm and the maintenance of contact between the infundibulum and Rathke's pouch is required for the correct morphogenesis of the pouch, cell determination and differentiation, at least until 10·5 dpc (Takuma *et al.* 1998, Rizzoti & Lovell-Badge 2005). *Sox3* mutants showed flattened morphology of the ventral diencephalon and transient expansion of the expression domains of *Bmp4* and *Fgf8* (Rizzoti *et al.* 2004). The attenuation of Bmp signalling during normal pituitary induction is important for maintenance of the balance of signalling factors as was demonstrated in mice deficient in *Noggin*, a Bmp2/4 inhibitor, which show multiple invaginations of the pouch (Davis & Camper 2007).

These observations suggested that *Sox3* is essential for the formation and morphogenesis of the infundibulum and hypothalamus and is implicated in the formation of Rathke's pouch indirectly, by stimulating growth of the infundibulum and restricting signalling domains. Notably, expression of *Sox3* is maintained post-natally in a group of ventral hypothalamic cells and in the median eminence, a structure that conveys neural and vascular connections from the hypothalamus to the pituitary gland. It is conceivable that disruption of signalling in the absence of *Sox3* may also contribute to the pituitary dysfunction (Rizzoti *et al.* 2004, Rizzoti & Lovell-Badge 2005). Despite the fact that *Sox3* is expressed widely in neural progenitor cells in the developing CNS (Brunelli *et al.* 2003, Pevny & Placzek 2005) its deletion had a relatively mild effect, which may be explained by the adduced functional redundancy with other members of the SOXB1 group (Pevny *et al.* 1998, Bylund *et al.* 2003, Ekonomou *et al.* 2005). The concept of redundancy within this group is further supported by the fact that the phenotype from mutations in each of the SOXB1 genes is often limited to the site where only the mutant gene is expressed (Nishiguchi *et al.* 1998, Ekonomou *et al.* 2005).

SOX3 dosage and hypopituitarism in humans

Studies of *SOX3* alterations in humans provide compelling evidence that the normal development of the diencephalon, infundibulum and, consecutively, the anterior pituitary is sensitive to *SOX* dosage. Overdosage of *SOX3*, resulting from duplications in Xq26–27, has been identified in pedigrees with hypopituitarism and mental retardation (Hamel *et al.* 1996, Lagerstrom-Fermer *et al.* 1997, Hol *et al.* 2000, Solomon *et al.* 2002, 2004). Affected males had GH deficiency with a variable combination of ACTH, TSH or

gonadotrophin deficiency and a degree of mental retardation. A smaller duplication (685·6 kb in length) was described in two siblings by Woods *et al.* (2005) leading to variable hypopituitarism with anterior pituitary hypoplasia, a hypoplastic or absent infundibulum and a partially descended or undescended posterior pituitary. Conversely, loss-of-function *SOX3* mutations involving expansions within the polyalanine tract lead to impaired nuclear localisation of the mutant protein resulting in combined pituitary hormone deficiency, although the severity of the phenotype is variable (Laumonnier *et al.* 2002, Woods *et al.* 2005). The absence of mental retardation in some of the patients (Woods *et al.* 2005) cannot be explained solely by the redundant function of other SOXB1 proteins, and may result from different dosage effects.

SOX2

SOX2 (OMIM 184429) is a single exon gene located on chromosome 3q26.3–27 and encodes a 317 amino acid protein. Consistent with the structure of the other SOXB1 proteins, it consists of an N-terminal domain of unknown function, a DNA binding domain and a C-terminal transcriptional activation domain.

Sox2 expression in mouse

Sox2 expression in the mouse is detected before gastrulation in cells at the morula stage at 2·5 dpc and in the inner cell mass of the blastocysts by 3·5 dpc (Avilion *et al.* 2003). After implantation, *Sox2* expression becomes restricted to the presumptive anterior ectoderm and by 9·5 dpc it is expressed throughout the developing CNS, as well as sensory placodes, branchial arches and gut endoderm including the oesophagus, the trachea and the inner ear (Collignon *et al.* 1996, Wood & Episkopou 1999, Williamson *et al.* 2006, Hume *et al.* 2007). At 11·5 dpc, *Sox2* is expressed uniformly in Rathke's pouch (Kelberman *et al.* 2006) and by 18·5 dpc *Sox2* expression is detected in proliferating cells of the dorsal zone and in scattered cells of the anterior pituitary. The expression of *Sox2* in the adult pituitary gland is maintained in a small population of cells, lining the pituitary cleft or scattered in the parenchyma, that maintain their potential to differentiate into all pituitary cell types, representing progenitor cells (Fauquier *et al.* 2008). This persistence of the expression of *Sox2* in cells of the adult pituitary may be crucial for the plasticity and dynamic response of the gland to fluctuate endocrine demands, its capacity to regenerate after trauma or even to explain the potential for tumour formation (Vankelecom 2007, Fauquier *et al.* 2008).

Consistent with its expression at the earliest stages of development, homozygous $Sox2^{-/-}$ mice show early embryonal lethality, shortly after implantation. On the other hand, one third of heterozygous mice show perinatal lethality while others appear normal but show reduction in size and fertility (Avilion *et al.* 2003). Further insight into the role of *Sox2* in the developing CNS was obtained by Ferri *et al.* (2004) who introduced a regulatory mutation ($Sox2^{\Delta ENH}$) in addition to the $Sox2^{\beta geo}$ null allele. Compound heterozygotes had reduced *Sox2* expression (25–30%) compared with wild type and this resulted in reduced numbers born and reduced post-natal survival. Surviving mice were severely affected showing epileptic spikes in the cortex and hippocampus and dystonic movements with circling behaviour. Their neuroanatomical defects included a reduced cortex and corpus callosum, decreased anterior thalamus and enlarged ventricles. Although, they exhibited growth retardation, this was usually compensated by 6 weeks of age. These observations further support the central role of *Sox2* in the maintenance of neural progenitor cell identity (Graham *et al.* 2003, Pevny & Placzek 2005).

Given the reduced size and fertility in $Sox2^{\beta geo}$ heterozygotes, their hypothalamo–pituitary function was investigated in detail by our group (Kelberman *et al.* 2006). *Sox2* expression was detected in the infundibulum and Rathke's pouch at 11·5 dpc but, as cell differentiation occurred, expression was confined to proliferative zones. Morphogenesis of the gland was abnormal with bifurcation of Rathke's pouch in a third of mutants at 12·5 dpc and subsequent extra clefts in some of the adult pituitaries (Fig. 3). Embryonic pituitaries at 18·5 dpc were smaller and had significantly reduced numbers of somatotrophs and gonadotrophs, with reduced GH content. Evaluation of hormonal content showed that in 3-month old heterozygotes there was moderate reduction in GH and LH, which was significant for males. It seemed, however, that the reduction in cell numbers was not type specific, but reflected the generally reduced size of the gland. Alternatively, it is possible that the anterior pituitary hypoplasia was the result of hypothalamic dysregulation associated with *Sox2* disruption (Kelberman *et al.* 2006). The different phenotypes resulting from the targeted disruption of *Sox2* are summarised in Table 2.

SOX2 expression in human embryos

The critical role of SOX2 in the development of the hypothalamo–pituitary axis in humans was further elucidated by expression studies in embryos from Carnegie stage 14 to Fetal stage 2, corresponding to 4·5–9 weeks of development (Kelberman *et al.* 2008). Strong *SOX2* expression was detected within Rathke's pouch, which was maintained throughout the development of the anterior pituitary, as well as in the overlying hypothalamus but not in the infundibulum or posterior pituitary (Fig. 4). In the telencephalon, there was positive staining in the region of the dentate gyrus of the hippocampus while, in later stages of fetal development, SOX2 was expressed in the thalamus and hypothalamus. However, the expression of SOX2 in the hypothalamus was not uniform, suggesting that haploinsufficiency for *SOX2* may affect only certain populations of neuroendocrine neurons, or their afferent inputs.

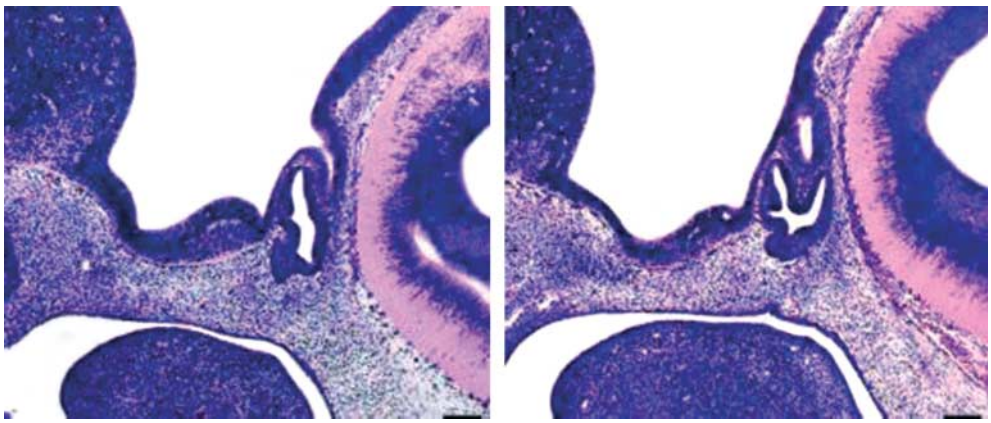

Figure 3 Abnormal morphogenesis of Rathke's pouch in *Sox2* heterozygous mouse embryos. Bifurcation of Rathke's pouch is seen in sagittal sections of 12·5 dpc mutant embryos (right) compared with wild-type embryos (left). Adapted from Kelberman *et al.* (2006).

Heterozygous SOX2 mutations are associated with a hypopituitary phenotype in humans

Heterozygous *de novo* mutations were initially described in patients with bilateral anophthalmia or severe microphthalmia (Fantes *et al.* 2003, Ragge *et al.* 2005, Bakrania *et al.* 2007). Other abnormalities included developmental delay, spastic diplegia, oesophageal atresia, sensorineural hearing loss and male genital abnormalities (Hagstrom *et al.* 2005, Zenteno *et al.* 2005, Faivre *et al.* 2006, Williamson *et al.* 2006, Chassaing *et al.* 2007).

The association of *SOX2* mutations with hypopituitarism was first reported by our group, underlining its critical role in pituitary development (Kelberman *et al.* 2006, 2008). However, despite the invariably hypoplastic anterior pituitary, all eight reported patients had isolated gonadotrophin deficiency, an observation which is unusual in the presence of a normal GH response (Dattani 2005, Kelberman *et al.* 2006). Conversely, Sato *et al.* (2007) reported a female patient with a heterozygous mutation affecting the HMG domain of *SOX2*, who also presented with isolated hypogonadotrophic hypogonadism, but a normal anterior pituitary and unilateral anophthalmia. This apparent selectivity for gonadotrophin deficiency in the face of anterior pituitary hypoplasia and sparing of other hormone axes, suggests that *SOX2* may be involved independently at multiple levels during the development of the hypothalamo–pituitary axis, and may be consistent with the non-uniform expression of *SOX2* in the hypothalamus.

Although, mice with haploinsufficiency of *Sox2* have more generalised pituitary deficits and severe brain abnormalities (Avilion *et al.* 2003, Ferri *et al.* 2004), not all patients with mutations in *SOX2* have developmental delay. Apart from anterior pituitary hypoplasia, forebrain defects in patients with *SOX2* mutations include hippocampal abnormalities, hypoplasia of the corpus callosum and hypothalamic hamartoma (Kelberman *et al.* 2006, Sisodiya *et al.* 2006). This apparent discrepancy could result from the redundant function of other SOXB1 factors or a differential sensitivity to the level of *SOX2* expression in different areas of the developing CNS (Miyagi *et al.* 2008). Sensitivity to dosage of *Sox2* has been noted in mice that develop an eye phenotype once *Sox2* expression is less than 40% of that of wild type (Taranova *et al.* 2006), while heterozygous mutants exhibit the hypopituitary phenotype.

Indications that SOX proteins cross-talk with other transcription factors and signalling molecules during early pituitary development

Pituitary development depends on a complex network of transcription factors and signalling molecules expressed in a spatially and temporally defined sequence, and a number of studies indicate that Sox proteins interact with members of this network during the early stages of pituitary development (Kelberman & Dattani 2007, Zhu *et al.* 2007*b*).

SOX proteins and the Wnt/β-catenin pathway

Wingless (Wnt) signalling, through the canonical and non-canonical pathway, is crucial for embryonic patterning, migration and cell fate determination and is highly conserved in many developmental processes (Salinas & Zou 2008). Activation of the canonical pathway leads to stabilisation of β-catenin that translocates to the nucleus and functions as a co-activator of Lef/Tcf transcription factors to stimulate target gene expression (Clevers 2006, Gordon & Nusse 2006). This action is mediated by the displacement of HDAC and TLE co-repressor complexes and the recruitment of co-activators p300/CBP and Brg1 for chromatin remodelling (Barker *et al.* 2001, Filali *et al.* 2002, Daniels & Weis 2005).

Knock-out mouse models for *Wnt5α* and *Wnt4* gave direct evidence for the involvement of Wnt signalling in pituitary organogenesis. Although, all hormone-producing cells are generated in *Wnt5α* mutants, the mutants exhibit abnormal branching of the gland which is mediated by the expansion of

Table 2 Phenotypes of various *Sox2* transgenic mouse models

Mouse model	Targeted disruption of *Sox2*	Phenotype	Pituitary & CNS	Reference
HM null $Sox2^{-/-}$ ($Sox2^{\beta\text{-}geo/\beta geo}$)	ESC *Sox2* ORF replaced by *βgeo*	Die at peri-implantation	–	Avilion *et al.* (2003)
HT	ESC	1/3 die between birth and weaning	Reduced size of anterior lobe	Avilion *et al.* (2003)
$Sox2^{-/+}$ ($Sox2^{\beta geo/+}$)	*Sox2* ORF replaced by *βgeo*	Surviving have normal appearance Reduced size (compensated by 6 weeks) Reduced fertility in males, small testes, sperm blockage in seminiferous tubules ↓LH, ↓GH; some have ↓TSH & ↓PRL	Extra pituitary cleft in adults	Kelberman *et al.* (2006)
Compound HT $Sox2^{\Delta ENH/\beta geo}$	Neural cell precursors	Dystonic movements, epileptic spikes, circling behaviour	Absent corpus callosum (15%) Enlarged ventricles	Ferri *et al.* (2004)
$Sox2^{\Delta ENH/\Delta ENH}$ $Sox2^{\Delta ENH/+}$	Neural cell precursors	Normal	–	Ferri *et al.* (2004)
Compound HT	Retinal progenitor cells	Variable eye phenotype: mild microphthalmia to severe bilateral anophthalmia (*Sox2* expression <40% of Wt)	Hypoplastic ON and OC	Taranova *et al.* (2006)
$Sox2^{EGFP/LP}$ $Sox2^{EGFP/IR}$ $Sox2^{+/EGFP}$ $Sox2^{+/LP}$, $Sox2^{+/IR}$ $Sox2^{LP/LP}$, $Sox2^{IR/IR}$	Retinal progenitor cells	Normal	Normal ON and OC	Taranova *et al.* (2006)

ESC, embryonic stem cells; ORF, open reading frame; HM, homozygous; HT, heterozygous; Wt, wild type; ON, optic nerve; OC, optic chiasm; *βgeo*, fusion gene of β-galactosidase and neomycin resistance genes; ΔENH, deletion of neural cell specific enhancer; EGFP, enhanced green fluorescent protein; LP & IR, hypomorphic alleles with activity <40%.

BMP and FGF signalling (Cha *et al.* 2004, Potok *et al.* 2008). Further evidence is provided by the study of downstream mediators of the Wnt signalling pathway Lef1, Tcf3 and Tcf4, which are expressed during pituitary development. *Tcf4* null embryos manifest pituitary overgrowth with increased volume of the anterior lobe (Brinkmeier *et al.* 2003), and show rostral expansion of the expression domains of *Fgf10* and *BMP4* and expansion of the *Six6* domain (Brinkmeier *et al.* 2007). These data suggested that Tcf4 regulates pituitary growth indirectly, by restricting BMP and FGF signalling in the ventral diencephalon, and directly through the restriction of Six6 expression within the pouch.

The similarity in the morphological abnormalities of Rathke's pouch between *Sox3* mutants and mutants involving the *Wnt* pathway suggest their interplay during pituitary organogenesis. An indication that SOX proteins can modulate the response to Wnt signalling came from studies in *Xenopus* which demonstrated that XSox3, XSox17α and XSox17β can bind to β-catenin and repress its Tcf mediated signalling (Zorn *et al.* 1999). This study also demonstrated that the negative effect of *XSox3* on *Wnt* downstream signalling was dose-dependent as cells expressing high levels of *XSox3* would not transcribe β-catenin target genes in response to Wnt.

Other members of the SOXB1 family have also been reported to suppress β-catenin mediated TCF/LEF signalling (Kan *et al.* 2004). In murine osteoblasts, Sox2 inhibits Wnt/β-catenin signalling by interfering with a β-catenin responsive promoter that contains Tcf/Lef binding sites.

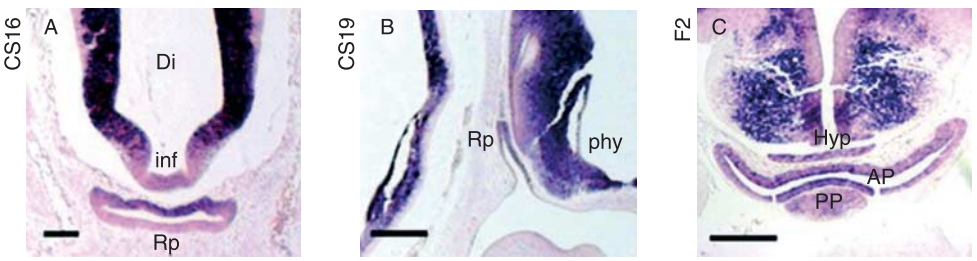

Figure 4 Expression pattern of SOX2 in human embryos. Transverse (A) and sagittal (B) sections show expression of SOX2 within Rathke's pouch (Rp) and overlying neural ectoderm at CS16 and CS19 respectively. At fetal stage 2 (C) SOX2 transcripts continue to be detected in the cells lining the lumen of the anterior pituitary (AP), but are absent in the hypothalamic area (Hyp) and posterior pituitary (PP). Adapted from Kelberman *et al.* (2008).

This inhibitory activity is mediated by the COOH-terminal domain of Sox2 and is independent of the DNA-binding ability (Mansukhani *et al.* 2005). Recently, Kelberman *et al.* (2008) demonstrated that human SOX2 inhibits β-catenin mediated reporter gene activation and that some naturally occurring *de novo SOX2* mutations (c.387-388delC and p.Q177X), although they retain their ability to bind DNA, are unable to repress β-catenin activity *in vitro*.

These experimental models suggest that SOX/β-catenin interactions may be important for pituitary development and that their disruption may result in a pleiotropic phenotype. The above hypothesis is consistent with the observation that, in addition to interactions mediated by Lef/Tcf factors, the direct interaction between β-catenin and transcription factors involved in pituitary development is crucial for cell fate determination. The direct interaction of β-catenin with Prop-1 is required for the induction of *Pou1f1* (*Pit-1*) expression. In addition, the Prop-1/β-catenin complex, through recruitment of co-repressors, results in repression of *Hesx1* expression; these processes are important for the determination of the specific hormone-producing cells (Olson *et al.* 2003, 2006).

SOX2 and LHX3 interaction

An indication for possible interaction between SOX2 and LHX3 during pituitary development came from the observation that SOX2 and LHX3 show consecutive and overlapping expression domains (Sheng *et al.* 1996, Sobrier *et al.* 2004, Hume *et al.* 2007, Kelberman *et al.* 2008). In the embryonic inner ear both genes are co-expressed with onset of expression of SOX2 occurring prior to that of LHX3 (Hume *et al.* 2007). *Lhx3* expression in the mouse is first detected at 9·5 dpc throughout Rathke's pouch; by 12·5 dpc there is a gradient of expression with higher protein levels found in the dorsal area of the pouch that persists in adulthood (Sheng *et al.* 1996, Raetzman *et al.* 2002). In the developing pituitary in human embryos, *LHX3* is expressed during the formation of Rathke's pouch at 5–6 weeks of development, although at 9 weeks, its expression is maintained in the anterior and intermediate lobes, but not in the posterior lobe of the pituitary (Sobrier *et al.* 2004). Recent *in vitro* studies demonstrated that SOX2 is capable of directly regulating and activating *LHX3* expression, mediated through binding to a site in the *LHX3* promoter at −2213 to −2224 bp relative to the *LHX3A* transcription start site (Rajab *et al.* 2008). Activation of *Lhx3* is important for the maintenance of dorsal–ventral patterning, cell survival and the commitment of the hormone-producing cells of the anterior pituitary (Sheng *et al.* 1996, 1997, Ellsworth *et al.* 2008). *Lhx3* null mice do not survive after birth, and although pituitary induction is normal, Rathke's pouch remains rudimentary and undifferentiated. Null embryos fail to express *Pit1*, have few corticotrophs and differentiated gonadotrophs that are not detected (Sheng *et al.* 1996).

Therefore, the genetic interaction between *SOX2/LHX3* may be important for normal anterior pituitary development. Additional studies in mice with conditional deletion of these genes may help to determine their role in detail.

The interactions of SOX proteins in other systems may provide clues for their role in pituitary development

The widespread expression of SOX proteins and their multiple roles have prompted research into their role in the development of many systems. It is not surprising that a large number of studies involve embryonic stem cells, as the identification of transcriptional targets of the SOX family and the sequence of the activation/repression cascade is the first step in the understanding of the networks that govern developmental processes (Boyer *et al.* 2005, Boer *et al.* 2008, Chakravarthy *et al.* 2008, Sharov *et al.* 2008). There has been evidence for the possible interaction of SOXB1 proteins with an array of early developmental transcription factors, some of which are also expressed during pituitary development. Although indirect, this evidence suggests that further studies are needed in order to understand the role that SOX proteins may play in the early morphogenesis, dorso-ventral patterning and cell specification in the developing pituitary. Furthermore, in the developing CNS, SOXB1 proteins control diverse processes, including neuronal migration, differentiation and connectivity. It is therefore conceivable that they may play a role in the development and maintenance of specific neuronal circuits, which in turn influence pituitary development. In this section, we will present the results of some of these studies and their implications in elucidating the role of SOX proteins in pituitary development.

SOX proteins interact with developmentally conserved transcription factors in different systems: possible implications for pituitary development

There has been recent evidence that in neural progenitor cells *Sox2* acts upstream of *Notch1* (Boyer *et al.* 2005). Although, little is known about the mechanisms underlying the *Sox2* mediated transcriptional activation of *Notch1*, it seems that its action upstream of the Notch signalling pathway is necessary for the maintenance of the proliferative potential of cells and the generation of sufficient cell numbers and phenotypes in the developing mouse neocortex (Bani-Yaghoub *et al.* 2006). Notch signalling is an evolutionarily conserved pathway implicated in pituitary development. During pituitary organogenesis, *Notch2* expression defines the boundary between the proliferative zone of Rathke's pouch and the developing anterior lobe (Ward *et al.* 2006). In the early phases, *Notch1* signalling is active and regulates directly the transcription of *Prop1*. During the later stages, however, the terminal differentiation

of gonadotrophs and post-mitotic *Pou1f1* (*Pit1*) positive cells requires the attenuation of *Notch1* signalling (Raetzman *et al.* 2006, Zhu *et al.* 2006). Therefore, a possible interaction between *Sox2* and *Notch1* may affect patterning and cell specification in the developing gland.

Studies in different organisms have demonstrated that the interaction of SOXB1 proteins with other transcription factors affects patterning of diverse organ systems; it is therefore possible that there may be an analogy with normal pituitary development. For instance in *Drosophila*, SoxNeuro and Dichaete, the two orthologs of the vertebrate SoxB1 group genes (Overton *et al.* 2002) interact genetically with the dorso-ventral patterning genes intermediate neuroblast defective (*ind*) and ventral nerve chord defective (*vnd*; Buescher *et al.* 2002, Zhao & Skeath 2002). Their orthologs in vertebrates are Gsh1/2 (Valerius *et al.* 1995) and Nkx2.2 (Pabst *et al.* 1998) respectively.

Evidence for the interaction between *Sox2* and *Nkx2.1* (*Titf1*) came from studies in embryonic stem cells which demonstrated that *Sox2* represses *Nkx2.1* (Boyer *et al.* 2005). In addition, during the development of the anterior foregut, *Sox2* is expressed in the endoderm suggesting that it plays a role in the correct morphogenesis and segmentation (Okubo *et al.* 2006, Williamson *et al.* 2006). Furthermore, there is evidence that the genetic interaction of *Sox2* and *Nkx2.1* in the developing foregut is crucial for the specification of dorsal–ventral patterning. According to the proposed model, this interaction is dose dependent and if the level of *Sox2* falls below a critical threshold, *Nkx2.1* is expressed ectopically, which in turn disturbs the normal separation of the tube (Que *et al.* 2007). It is not clear if an analogous interaction exists during early pituitary development, when *Nkx2.1* is expressed in the presumptive ventral diencephalon, and its mutation in mice may cause severe defects that include developmental disorders of the diencephalon and anterior pituitary (Takuma *et al.* 1998).

The synergy and redundancy between members of the SOXB1 family is important for organ patterning (Miyagi *et al.* 2008). Recent experiments in *Sox3* null mice demonstrated that *Sox3* interacts with *Sox2* and *Fgfr1* in the pharyngeal arch and this interaction is pivotal for the pharyngeal segmentation and neural crest cell migration in the developing pouches (Rizzoti & Lovell-Badge 2007).

Taken together, the above interactions further support the hypothesis that critical levels of SOX proteins play a role in determining the timing and expression domain of other transcription factors and signalling molecules and that their disturbance results in defects in patterning and a variable phenotype.

Interactions between SOX proteins and POU factors control developmental processes

The role of POU domain factors has been established in many developmental processes, including the regulation of gene expression in the hypothalamus and pituitary, as is the example of *Pou1f1* (*Pit1*) and *Pou3f2* (*Brn-2*; Andersen & Rosenfeld 2001). It has been demonstrated that Sox2 acts synergistically with Pou5f1 (Oct-3/4) to activate the *Fgf4* enhancer in embryonic stem cells, an interaction that depends on the spatial arrangement of the binding sites for these factors (Yuan *et al.* 1995, Ambrosetti *et al.* 1997, 2000). The Sox2–Pou5f1 complex is critical for the maintenance of the pluripotency of embryonic stem cells and the auto-regulatory control of *Sox2* expression (Zappone *et al.* 2000, Tomioka *et al.* 2002, Chew *et al.* 2005). Expression of *Pou5f1* has been detected in germ cells, embryonic stem cells and in early stages of neurogenesis (Nichols *et al.* 1998). Further experiments demonstrated that in the murine embryonic spinal cord the combinatorial action of SOX2 and POU3F2 results in the activation of the *Nestin* enhancer (Tanaka *et al.* 2004). POU3F2 has an established role in pituitary development and is required for the differentiation of the magnocellular and parvocellular neurons in the paraventricular and supraooptic nuclei of the hypothalamus (Schonemann *et al.* 1995, Andersen & Rosenfeld 2001). Whether these interactions have any role in hypothalamo–pituitary development remains to be established.

SOX proteins may regulate neuronal circuits involved in pituitary development

γ-aminobutyric acid (GABA)-mediated signalling is essential for the organisation and migratory pathway of the GnRH and olfactory neurons from the nasal placode to the olfactory bulbs and up to the arcuate nucleus of the basal hypothalamus (Tobet *et al.* 2001, Gonzalez-Martinez *et al.* 2004). Recent experiments *in vivo* and *in vitro* have demonstrated that *Sox2* is essential for the number and connectivity of the GABAergic neurons and that the olfactory bulbs of adult *Sox2* knock-down mice exhibit reduced numbers as well as abnormal morphology and connectivity of GABAergic neurons (Cavallaro *et al.* 2008). These observations suggest that *Sox2*, apart from having a direct effect on the formation of Rathke's pouch, may also be involved in neuronal migration and the establishment and maintenance of GnRH neurons in the hypothalamus.

Conclusion

SOX2 and *SOX3* are early developmental transcription factors that are expressed widely in the developing CNS and play an important role in the morphogenesis of the hypothalamo–pituitary axis. During pituitary development, *SOX2* is expressed in the diencephalon and Rathke's pouch while *SOX3* is expressed in the diencephalon and infundibulum, suggesting that their mechanism of action may be direct or indirect. Their disruption leads to variable phenotypes, indicating that their effects are dose sensitive and depend on their expression site. Although, the importance of their role is established, the mechanisms that regulate their expression and their multiple interactions within the extensive signalling network during pituitary

organogenesis are still unclear. The mechanisms whereby they lead to specific phenotypes are also unknown. Better understanding of these interactions and of their role in the maintenance of progenitor cells in the adult pituitary may help to elucidate mechanisms involved in processes that range from early development to tumorigenesis.

Declaration of interest

The authors declare they have no financial or other potential conflict of interest.

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