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Expression patterns of *Shh*, *Ptc2*, *Raldh3*, *Pitx2*, Isl1, Lim3 and Pax6 in the developing chick hypophyseal placode and Rathke's pouch

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ABSTRACT

The adenohypophysis is derived from a structure called the Rathke's pouch, which is an invagination of the hypophyseal placode. Hedgehog (Hh) and retinoic acid (RA) signals as well as several transcription factors have been suggested to play a role in the development of the adenohypophysis. We have therefore examined the expression pattern of *Sonic hedgehog* (*Shh*), the hedgehog receptor *Patched2* (*Ptc2*), the retinoic acid producing enzyme *Retinaldehyde dehydrogenase3* (*Raldh3*) and four transcription factors, *Pitx2*, Isl1, Lim3 and Pax6 in chick embryos from head fold stage to embryonic day (E) 4.5. We show that already at the head fold stage, *Ptc2* is expressed in prospective hypophyseal placodal cells and that *Shh* is expressed in the underlying mesoderm. Moreover, *Shh* continues to be expressed in tissues surrounding the prospective adenohypophysis, and *Ptc2* is expressed in prospective hypophyseal cells. Lim3 and Pax6 are expressed from stage 10 in the prospective hypophyseal placode, whereas *Pitx2* starts to be expressed before stage 10. *Pitx2* is together with Pax6 expressed in the entire domain of the Rathke's pouch. *Raldh3* is detected at the 20 somite stage and is together with Lim3 expressed in the anterior part of the Rathke's pouch. Isl1 is expressed in the most posterior part of the hypophyseal ectoderm in a complementary pattern to *Raldh3* and Lim3.

1. Results and discussion

The pituitary gland is the main executor of hypothalamic function in vertebrates and consists of several hormone producing cell types. The pituitary contains three lobes and is of dual embryonic origin. The posterior lobe originates from the neuroectoderm, while the anterior and intermediate lobes that give rise to the adenohypophysis are derived from the hypophyseal placode (Ooi et al., 2004). The cranial placodes arise from the ectoderm at characteristic positions at the border region in between the prospective neural plate and epidermis (Baker and Bronner-Fraser, 2001; Schlosser, 2006). Fate maps in chick and zebrafish at late gastrula to neural fold stages, have shown that progenitor cells of the hypophyseal placode are located at the most anterior part of the neural plate border region (Baker and Bronner-Fraser, 2001; Bhattacharyya et al., 2004; Toro and Varga, 2007). At the 15 somite stage, the hypophyseal placode is morphologically detectable by the thickening of the non-neural ectoderm underlying the prospective ventral diencephalon, and at the 20 somite stage the hypophyseal placode invaginates and forms a structure called the Rathke's pouch. The Rathke's pouch subsequently deepens and detaches from the overlying oral roof ectoderm resulting in the formation of the adenohypophysis at embryonic day (E) 3 in chick (E11.5 in mouse) (Rizzoti and Lovell-Badge, 2005). Soon thereafter, the hormone producing cell types of the adenohypophysis begin to differentiate in a defined spatial and temporal order (Asa and Ezzat, 2004; Ooi et al., 2004).

Hedgehog (Hh) and retinoic acid (RA) signals, as well as transcription factors including *Pitx2*, Isl1, Lim3 and Pax6 have been implicated to play a role in the development of the adenohypophysis, however, the gene expression patterns of these molecules during the early development of the hypophyseal placode and the Rathke's pouch have been poorly defined. In this study we have analysed the expression of *Sonic hedgehog* (*Shh*) and the hedgehog receptor *Patched2* (*Ptc2*) during the development of the hypophyseal placode and the early formed Rathke's pouch by analysing Hamilton and Hamburger stage 6 to E4.5 chick embryos. Moreover, between stage 10 and E4.5 the *Shh* and *Ptc2* expression were compared to the expression of the retinoic acid producing enzyme *Retinaldehyde dehydrogenase3* (*Raldh3*), *Pitx2*, Lim3, Isl1 and Pax6. Thus, we give an overview of the temporal and spatial expression patterns of *Shh*, *Ptc2*, *Raldh3*, *Pitx2*, Isl1, Lim3 and Pax6, all implicated in the development of the adenohypophysis, during the early development of the hypophyseal placode and the Rathke's pouch.

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1.1. Embryonic expression of Shh and Ptc2 in the developing hypophyseal placode and the Rathke's pouch

Hedgehog (Hh) signals have been suggested to play a major role in the development of the adenohypophysis. Hh signaling is regulated by the receptors Ptc1 and Ptc2 which maintain repression of the Hh pathway in the absence of stimulation, and *Ptc1* and *Ptc2* are expressed in a similar pattern during development (Pearse et al., 2001). *Talpid*^{3} chicken mutants, which have a defective activation of the Shh pathway, develop ectopic lenses usually located in the midline deriving from or connected to the hypophyseal duct (Buxton et al., 2004; Davey et al., 2006). Similar results have been described in zebrafish mutants, which have disturbed Hh-signaling due to a dominant negative form of the Gli2 protein, resulting in ectopic lenses at the expense of the adenohypophysis (Kondoh et al., 2000). In addition, transgenic mice that over-express the Shh inhibitor Hip (Huntingtin interacting protein) in the oral ectoderm and in prospective Rathke's pouch cells only develop a rudimentary pouch (Treier et al., 2001).

Already at the head fold stage, stage 6 in chick, *Shh* is expressed in the mesoderm underlying the prospective hypophyseal placode, which express *Ptc2* (Fig. 1A and B). At this stage, the mesoderm underlying the prospective lens placode does not express *Shh*, and prospective lens placodal cells do not express *Ptc2* (data not shown). These expression patterns are in agreement with previous results suggesting that Hh signals direct the choice between adenohypophyseal and lens fate (Buxton et al., 2004; Davey et al., 2006; Kondoh et al., 2000). At neural fold and early neural tube stages, stage 9–10, *Shh* expression is maintained in the mesoderm located in between the prospective hypophyseal placode and the ventral diencephalon (Fig. 1C and E). At these stages, *Ptc2* expression is detected in the prospective hypophyseal placode and in the ventral regions of the neural ectoderm (Fig. 1D and F). At stage 13 (20 somites), the hypophyseal placode starts to invaginate creating a structure called the Rathke's pouch, bringing the placodal ectoderm closer to the neuroectoderm of the ventral diencephalon. Between 20 somites and E3, *Shh* is expressed in the oral ectoderm, located anterior and posterior to the hypophyseal placode (Fig. 2A′–C′ and A–C). In addition, *Shh* is expressed at higher levels in the ventral neuroectoderm anterior and posterior of the pouch, but not in the region abutting the invagination of the Rathke's pouch (Fig. 2A′–C′ and A–C). At these stages, *Ptc2* is expressed in the Rathke's pouch, and also in the ventral part of the diencephalon and spotted *Ptc2* expression can be detected in the mesenchyme surrounding the Rathke's pouch (Fig. 2E–G). At E4.5, *Shh* is still expressed in the oral ectoderm anterior and posterior of the Rathke's pouch (Fig. 2D' and D), and *Ptc2* expression is maintained in the entire domain of the Rathke's pouch (Fig. 2H). Thus, during the development of the hypophyseal placode and the formation of the Rathke's pouch *Shh* is expressed in surrounding tissues, while *Ptc2* is expressed in the hypophyseal placode and subsequently in the Rathke's pouch.

1.2. Embryonic expression of Raldh3 in the developing hypophyseal placode and Rathke's pouch

Retinoic acid (RA) is a small signaling molecule converted from vitamin A by alcohol dehydrogenase (Adh) and retinaldehyde

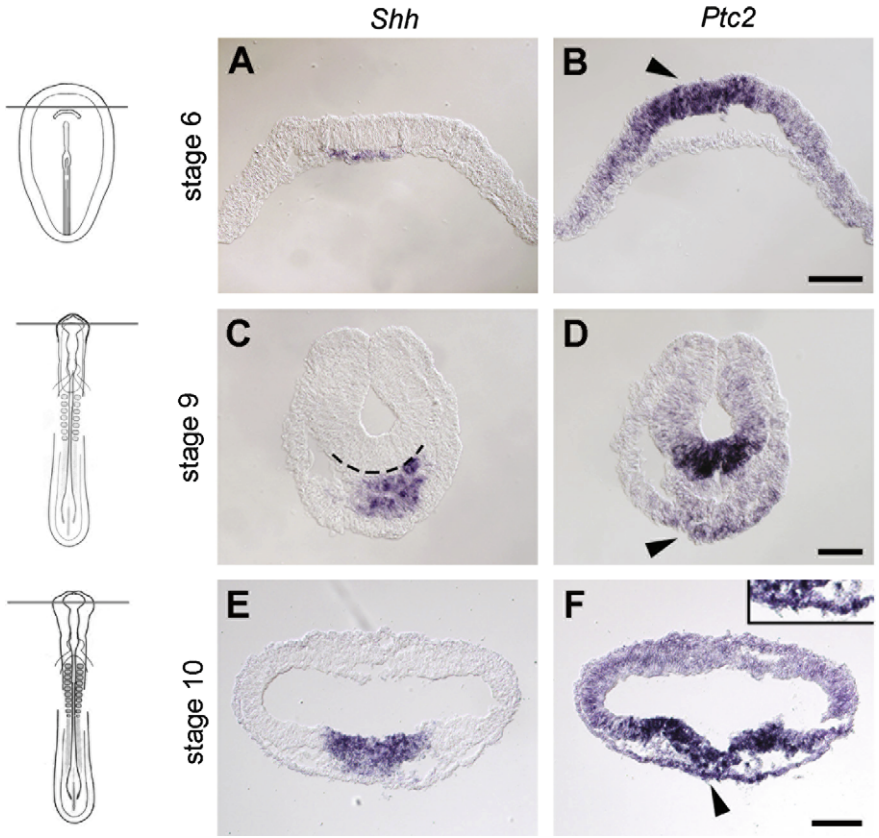

Fig. 1. Expression of *Shh* and *Ptc2* detected by *in situ* hybridization in the prospective hypophyseal placode in stage 6–10 chick embryos. Schematic chick embryos to the left indicate transversal planes of sections. At stage 6, expression of *Ptc2* is detected in the prospective hypophyseal placode and *Shh* in the underlying mesoderm (A and B). At stage 9 and 10, *Shh* expression is detected in the mesoderm close to the prospective hypophyseal placode and neuroectoderm (C and E). The broken line in (C) indicates the border between the neuroectoderm and the mesoderm. *Ptc2* is expressed in the prospective hypophyseal placode (arrowheads) and in the ventral neuroectoderm (D and F). The inset in (F) shows *Ptc2* expression in the prospective hypophyseal placode. Scale bars, 100 μm.