

Hatschek's pit and origin of pituitary gland

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Abstract

Pituitary gland, or pituitary for short, is characteristic of all vertebrates. As a “master gland” controlling a multitude of important functions in the body, its evolutionary origin has been an object of investigations of evolutionary biology for two centuries. Previous morphological, ultrastructural and immunohistochemical studies suggested the homology of the Hatschek's pit of amphioxus and vertebrate pituitary. Developmental genetics study showed that the development of Hatschek's pit and vertebrate pituitary is both subject to regulation by the common genes such as *Pit1*, *Lhx3* and *BMP3b*. Our recent studies demonstrated that the Hatschek's pit is able to secrete growth hormone (GH)-like hormone and thyroid-stimulating hormone (TSH)-like hormone that both play functions similar to vertebrate GH and TSH. We thus think that the emergence of Hatschek's pit represents one of important events during endocrine network evolution, which laid a foundation for the subsequent formation of a hypothalamic-pituitary system in vertebrates.

Key words: protochordate, amphioxus, brain, pituitary, evolution

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1 Introduction

The term hypophysis is derived from *hypo*, under (the brain), + *physis*, growth. Its alternate name, pituitary gland or just pituitary for short, is derived from *pittuita*, meaning slime or phlegm. The pituitary, located behind the nose and above the nasal and oral cavities in humans, was believed to be the source of phlegm, one of the four humors of the body proposed by Caludius Galen (he was a noted Greek physician and medical writer, AD 130–200) about 1 900 years ago. The other humors were blood, black bile, and yellow bile. The doctrine of the Four Humours had a special relationship with Chinese Five Elements, with phlegm, blood, black bile and yellow bile equivalent to water, fire, earth and air, respectively (Singer, 1931). For centuries, it was thought that the vertebrate pituitary secreted mucus externally or cerebrospinal fluid internally.

The pituitary gland is now known as an endocrine gland located downstream of the hypothalamus at the base of the brain, and composed of an anterior, an intermediate and a posterior lobes. Pituitary endocrine functions were elucidated only during the first half of the 20th century (Haller, 1897; Candiani et al., 2008). It secretes pivotal hormones such as growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which regulate functions including growth, metabolism, reproduction and homeostasis. The acquisition of pituitary was considered to be a seminal event that led to physiological divergence (growth, metabolism, reproduction, homeostasis and stress) during animal evolution, and hence its origin has long been an object of investiga-

tions of evolutionary biology.

Amphioxus, a basal chordate, is the best available stand-in for the proximate invertebrate ancestor of vertebrates (Fig. 1), and thus is an excellent model for study of the origin of vertebrates and their structures. We have investigated the developmental and evolutionary biology of this evolutionarily important animal for more than 30 years (Zhang, 2020). Below we will discuss the state-of-the-art understanding of the evolutionary origin of vertebrate pituitary.

2 Developmental and morphological homology

Studies on the morphogenesis of vertebrate pituitary dated from the early 19th century (Haller, 1897), while studies on the genetic controls of pituitary development commenced only about 30 years ago (Simmons et al., 1990). The embryonic vertebrate brain develops as three distinct swelling known, from the anterior to the posterior, as the prosencephalon (forebrain), the mesencephalon (midbrain), and the rhombencephalon (hind-brain). With time, this initial simple segmentation of the brain becomes further subdivided. The prosencephalon becomes subdivided into the anterior telecephalon, and the posterior diencephalon; the mesencephalon remains undivided, and the rhombencephalon gives rise to the anterior metencephalon and the posterior myelencephalon. During subsequent development, these regions differentiate into the major components of the adult brain. The pituitary develops through fusion of a ventral growth or evagination from the diencephalon, the infundibulum, with an ectodermal sac known as the hypophyseal pouch or Rathke's pouch (RP) (Schwind, 1928; Kaufman, 1992).

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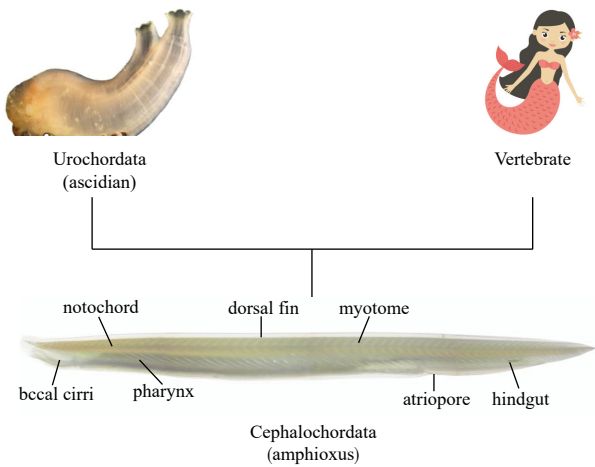


Fig. 1. Chordate phylogeny, showing the current understanding of chordate relationship. Phylogenomic studies suggest that the ascidian (Urochordate) is the sister group to the vertebrate and that amphioxus (Cephalochordate) is the basal group of chordates.

The development of vertebrate pituitary has been studied extensively in mouse, and it seems that, to a large degree, pituitary development in humans mirrors that in rodents. Developmentally the pituitary has a dual embryonic origin: the anterior and intermediate lobes (adenohypophysis) are derived from the oral ectoderm, while the posterior lobe (neurohypophysis) originates from the neural ectoderm. The adenohypophysis contains six distinct cell types, each of them defined by the hormone(s) they produce. How are these cell types formed? Soon after gastrulation, complex interactions between the head mesoderm and the neural and non-neural ectoderm define a domain at the border

of the neural plate. These involve fibroblast growth factor (FGF) signalling inducing neural fate, bone morphogenetic proteins (BMPs) and wingless-related integration site (WNT) activities inducing future epidermal identity in non-neural ectoderm, and active inhibition of BMP and WNT signalling pathways by the neural ectoderm and head mesoderm (Saint-Jeannet and Moody, 2014; Alatzoglou et al., 2020). Opposing activities of BMP4 and Sonic hedgehog (SHH) within the ventral diencephalon pattern this region and participate in correct morphogenesis of the infundibulum and in consequence positioning of RP. Within the infundibulum, BMP4 and FGF8, FGF10 and FGF18 are required for development of RP. In RP at 10.5 days post coitum (dpc), BMP2 is present and there is a uniform suppressor of mothers against decapentaplegic homolog (SMAD) activation profile, while the FGF pathway is only activated dorsally (Davis et al., 2011; Rizzoti, 2015). At this stage, different transcription factors required for RP progenitor proliferation and/or maintenance are ubiquitously expressed. Later, at 14.5 dpc, progenitors are confined around the RP lumen, while differentiating and differentiated cells away from the lumen define the developing anterior pituitary. The terminal differentiation of the anterior pituitary cell types is the result of complex interactions between extrinsic signaling molecules and transcription factors (Hesx1, Sox2, Sox3, Otx2, Lhx3, Lhx4, Gata2, Isl1, Prop1 and Pou1f1) (Rizzoti, 2015; Fig. 2). For example, the first identified homeodomain transcription factor Pou1f1 (initially known as Pit1) was shown to play a crucial role in the determination and differentiation of three anterior pituitary cell types representing the somatotroph, lactotroph and thyrotroph lineages (Kelberman et al., 2009).

Several structures in invertebrate deuterostomes have been proposed to be homologous to vertebrate pituitary (most often to the adenohypophysis). The earliest proposed homologs were the neural gland/duct complex of ascidian tunicates (Julin, 1881), the preoral organ/Hatschek's pit of amphioxus (Cattie, 1882) and the

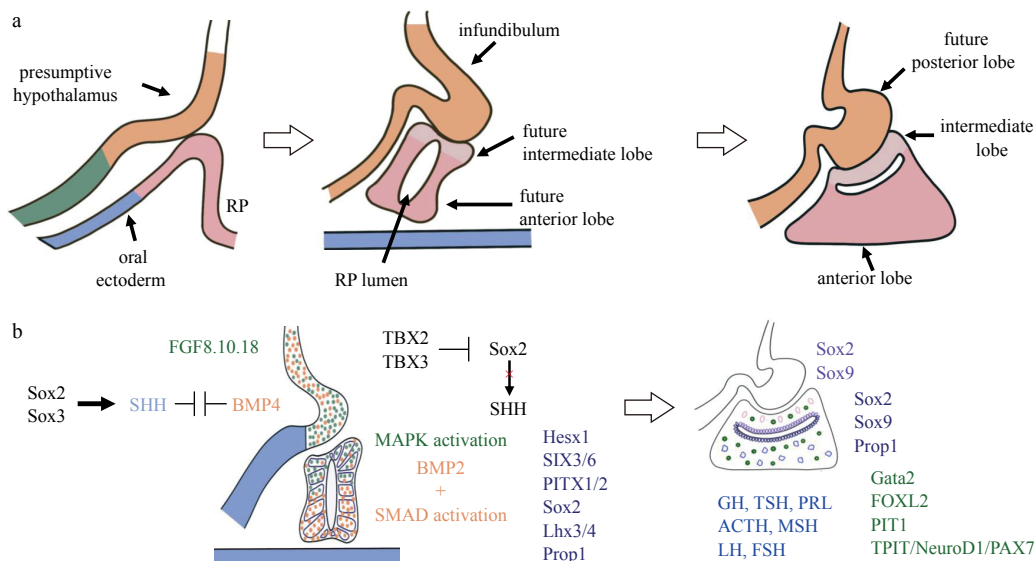


Fig. 2. Development of vertebrate pituitary (a) and main signalling pathways and factors controlling pituitary development (b). a. Pituitary development starts with the formation of the hypophyseal placode, apposed to the future ventral diencephalon. The placode later invaginates to form Rathke's pouch (RP), still in contact in its dorsal most part with the infundibulum, which lies within the ventral diencephalon. The infundibulum then evaginates towards RP. The anterior and intermediate lobes originate from RP. b. During pituitary development, the temporal and spatial expression of a cascade of signaling molecules and transcription factors plays a crucial role in organ commitment, cell proliferation, patterning, and terminal differentiation.

protoceol and its duct in hemichordates (Bateson, 1885; Cunningham, 1887). Subsequent proposals were Kölliker's pit (Hatschek, 1892) and Hatschek's nephridium (Legros, 1898) for amphioxus and the stomochord (Komai, 1951) and preoral ciliary organ (Barrington, 1963) for hemichordates. The most recent addition to the list was the ciliated funnel of appendicularian tunicates (Holmberg, 1982). Most structures proposed as homologs of vertebrate pituitary have long been discounted, and accumulating data tend to bolster the idea of a homology of Hatschek's pit and vertebrate adenohypophysis.

The neural tube of amphioxus is slightly enlarged rostrally to form the cerebral vesicle (Lacalli, 1996; Ruppert, 1997; Holland et al., 1999; Holland and Holland, 2001). There are two ciliated pits in the head region of amphioxus: Kölliker's pit, an epidermal invagination on the left outer side of the rostral area of the animal, and Hatschek's pit, a dorsal evagination of tall cells from the mouth epithelium which fuses with the adjacent part of preoral ectoderm (Hatschek, 1881, 1884; Glardon et al., 1998; Holland and Holland, 2001). As mentioned above, Hatschek's pit has been suggested on morphological grounds to be a homolog of vertebrate adenohypophysis more than a century ago (Cattie, 1882; Legros, 1898; Drach, 1948). This homology was later strengthened by ultrastructural and immunohistochemical examinations that showed the presence of granulated secretory cells in the epithelium lining this pit, together with ciliated cells (Tjoa and Welsch, 1974; Welsch and Welsch, 1978; Sahlin and Olsson, 1986), the production of vertebrate-like gonadotropic hormones in the secretory cells (Zhang et al., 1982; Chang et al., 1985; Nozaki and Gorbman, 1992) and the discovery of an infundibulum-like extension from the cerebral vesicle that ends at Hatschek's pit (Lacalli, 1996, 2004, 2008, 2017, 2018).

The homology of Hatschek's pit and adenohypophysis was also supported by the studies of developmental genetics, which is less prone to false negatives and false positives than immunocytochemistry. Pit1 or Pou1f1, a member of the POU protein family that belongs to the helix-turn-helix superfamily, is an anterior pituitary-specific transcription factor. In vertebrates, the adenohypophysis is chief site of *Pit1* expression in the embryo and the sole site of expression in postembryonic stages (Simmons et al., 1990; Treacy and Rosenfeld, 1992). Pit1 acts as developmental regulator of the anterior pituitary, and it is responsible for GH and PRL cell commitment, differentiation, and gene expression. Furthermore, Pit1 is necessary for the expression of the human and mouse thyrotropin β (TSH β) gene together with other factors (Rhodes et al., 1994; Cohen et al., 1996). It was found that the amphioxus Pit1, which is only one copy in the draft genome sequence of the Florida amphioxus (*Branchiostoma floridae*), was expressed solely in the nascent and definitive preoral organ (=Hatschek's pit in adult) of amphioxus embryos and larvae, respectively (Candiani et al., 2008). In accordance with this, Pit1 was also found to be localized in two groups of cells lining Hatschek's pit (Candiani and Pestarino, 1998), which correspond exactly to the granulated endocrine or type 1 cells previously described by Sahlin and Olsson (1986). Additionally, in vertebrates, *Lhx3*, a LIM-homeobox gene, is expressed after but not during gastrulation in special subsets of neuronal cells of the central nervous system and pituitary gland cells (Dawid et al., 1995; Zhadanov et al., 1995). Similarly, amphioxus *Lhx3* was expressed in the preoral pit of larvae (Wang et al., 2002), from which the wheel organ and Hatschek's pit develop (Hatschek, 1892; Conklin, 1932). BMP3b is a cytokine belonging to the Transforming Growth Factor β (TGF- β) superfamily. In *Xenopus*, *BMP3b* was expressed in the precursors of adenohypophysis

(Hino et al., 2003). Interestingly, the transcripts of amphioxus homolog *AmphiBMP3/3b* were also detected in the preoral organ during embryonic development (Sun et al., 2010).

3 Functional homology

Searching for evidence for pituitary hormones in invertebrate chordates started about four decades ago. Immunocytochemistry using antibodies raised against vertebrate pituitary hormones generated positive reactions in the neural gland/duct complex of ascidian tunicates (Pestarino, 1984, 1985) and in Hatschek's pit of amphioxus (Chang et al., 1985; Fang, 1993; Fang et al., 1999; Nozaki and Gorbman, 1992). However, earlier works to clone pituitary hormone genes such GH gene from amphioxus were not successful. GH is one of the most important hormones secreted by vertebrate pituitary. GH binds to the GH receptor (GHR) on the plasma membrane of hepatocytes, resulting in the production of insulin-like growth factor I (IGF-I), which plays a key role in the regulation of proliferation, differentiation and anti-apoptosis of bone and muscle cells. Through repeated attempts and experiments, we have cloned a cDNA encoding a protein of 208 amino acids, which belongs to class-I cytokine superfamily, and bears a 3-dimensional structure closely similar to that of human GH, and found that this GH-like polypeptide (GHI) was predominantly distributed in the Hatschek's pit (Li et al., 2014). Both amphioxus GHI and human GH share some conserved amino acids, such as R70 and R175, which are critical for the interaction with GHRs. We then showed that recombinant amphioxus GHI (rGHI) as well as zebrafish GH (rGH) exhibited a similar capacity to bind to the GHR prepared and partially purified through a wheat-germ agglutinin (WGA)-agarose column from the livers of zebrafish in a dose-dependent manner. We also demonstrated that rGHI stimulated the expression of *igf-i* in the liver of zebrafish, as rGH did so. As GH functions by binding to GHR, we thus set out to explore its receptor. We identified a peptide, called GH/PRLBP, consisting of 224 amino acids, from amphioxus by cDNA cloning. GH/PRLBP contains the entire hormone binding domain of GH/PRL receptors. It was widely expressed among the different tissues of amphioxus, including the hepatic caecum, gill, hing-gut, notochord and testis. Such wide expression pattern is consistent with the pleiotropic actions of GH peptides in vertebrates. Interestingly, we showed that recombinant GH/PRLBP (rGH/PRLBP) specifically interacted with amphioxus rGHI as well as zebrafish rGH, and the interaction of rGH/PRLBP with rGHI effectively suppressed the expression of *igf-i* stimulated by rGHI in the liver. Importantly, we found that rGHI, like zebrafish rGH, was able to promote the growth of zebrafish, and supplementation of amphioxus rGHI as well as zebrafish rGH could both similarly rescue the abnormal morphogenesis caused by GH shortage via *tbx5*-MO knockdown in zebrafish embryos. These indicate for the first time that there exists a functional GH-like hormone in the basal chordate amphioxus (Fig. 3).

In addition to growth promotion, GH is known to play an important role in osmoregulation in vertebrates. As GHI is the only and sole functional protein capable of promoting growth in amphioxus, we thus tested if it can mediate osmoregulation. We demonstrated clearly that rGHI increased not only the survival rate of amphioxus but also the muscle moisture under high salinity (Li et al., 2017). Moreover, rGHI induced the expression of the ion transporter Na⁺-K⁺-ATPase (NKA) and Na⁺-K⁺-2Cl⁻ cotransporter (NKCC) in the gill as well as the expression of IGF1 in the hepatic caecum, indicating that GHI fulfills the osmoregulatory activity through the same mechanisms of vertebrate GH. These

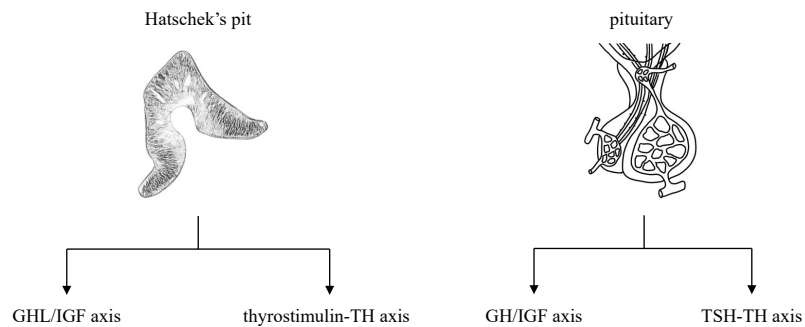


Fig. 3. Diagram showing the functional homology of Hatschek's pit and vertebrate pituitary.

suggested that the osmoregulatory activity of GH had emerged in the basal chordate amphioxus.

TSH is another important pituitary hormone which stimulates the production and secretion of the thyroid hormones (THs) T_4 and T_3 via binding to specific membrane TSH receptor (TSHR). TSH, FSH and LH form a family of glycoprotein hormones (GpHs) that have carbohydrate side chains. GpHs are heterodimers of two noncovalently associated α and β subunits. The α subunits (TSH α , FSH α and LH α) of these hormones are common; however, their β subunits (TSH β , FSH β and LH β) are unique and grant specific bioactivity. About two decades ago, a new GpH was identified and named thyrostimulin because it activated TSHR (Nakabayashi et al., 2002). Compared with other GpHs, thyrostimulin is believed to consist of two distinct subunits known as GpA2 (α subunit) and GpB5 (β subunit), forming a heterodimer based on the colocalization of its subunits in the pituitary in vertebrates (Park et al., 2005; Sudo et al., 2005; Veenstra, 2010). It was thus thought to act mostly as a regulator with autocrine and paracrine functions rather than as a systemic hormone.

In addition to thyrostimulin, all jawed vertebrates have three heterodimeric pituitary GpHs: TSH α /TSH β , FSH α /FSH β and LH α /LH β . These classical TSH, FSH and LH subunits in jawed vertebrates are not found in jawless vertebrates, such as the lamprey, and primitive chordates including amphioxus. It was found that the lamprey has one heterodimeric pituitary GpH consisting of GpA2/GpH β as well as thyrostimulin (Sower et al., 2006; Sower, 2015; Sower and Hausken, 2017), and amphioxus possesses only thyrostimulin consisting of GpA2/GpB5 as its sole GpH (Holland et al., 2008; Dos Santos et al., 2009; Tando and Kubokawa, 2009a, b; Sower, 2015). Previous studies showed that in amphioxus, the gene *gpa2* is expressed mainly in the anterior part of the nerve cord and the left side of the central canal (Tando and Kubokawa, 2009a), whereas the gene *gpb5* is expressed predominantly in the dorsal part of the nerve cord, the atrial cells of the gill, and the previtellogenic oocytes (Tando and Kubokawa, 2009b). Both phylogenetic and synteny analyses revealed that the thyrostimulin subunits are ancestral to the vertebrate GpH subunits (Sudo et al., 2005; Dos Santos et al., 2009; Tando and Kubokawa, 2009b), but their function remains poorly defined.

Recently, we have cloned both GpA2 and GpB5 as well as TSHR genes from amphioxus *Branchiostoma japonicum*, and clearly showed that amphioxus GpA2, GpB5, and TSHR represented the archetypes of vertebrate TSH α , TSH β and TSHR, respectively. In contrast to the different tissue expression reported by Tando and Kubokawa (2009b), we found that *gpa2* and *gpb5* were coexpressed in the Hatschek pit, a homolog of the vertebrate pituitary (Wang et al., 2018). We also demonstrated that recombinant amphioxus GpA2 and GpB5, like zebrafish TSH α and

TSH β , were able to bind to both amphioxus and zebrafish TSHRs and that tethered amphioxus thyrostimulin, like vertebrate TSH, activated both protein kinase A and protein kinase C pathways in the cells expressing amphioxus TSHR. Additionally, we showed that recombinant amphioxus thyrostimulin induced the production of thyroid hormone T_4 (Fig. 3). Because genuine TSH is absent in amphioxus and thyrostimulin is the only and sole glycoprotein hormone, thus our data suggest that amphioxus thyrostimulin is a functional glycoprotein hormone that plays a role as TSH does in vertebrates. These data also expand our understanding of the ancient origin of TSH and TSHR within the basal chordate amphioxus.

4 Conclusions and prospects

Our understanding of the Hatschek's pit of amphioxus has undergone a tremendous transformation over the past decade. Previously, morphological, ultrastructural and immunohistochemical examinations suggested the homology of Hatschek's pit and vertebrate pituitary. Developmental genetics study revealed that the development of Hatschek's pit and vertebrate pituitary is both subject to regulation by the common genes such as *Pit1*, *Lhx3* and *BMP3b*. Our recent studies have shown that the Hatschek's pit can produce GH-like hormone and TSH-like hormone, and both the hormones play physiological activities typical of vertebrate GH and TSH, providing functional evidences for a link between Hatschek's pit and vertebrate pituitary.

All vertebrate brains contain a hypothalamus, which forms the ventral part of the diencephalon. One of the most important functions of the hypothalamus is to link the nervous system to the endocrine system via the pituitary gland, which is called the hypothalamic-pituitary system. For example, the hypothalamus secretes GH-releasing hormone (GHRH), which triggers the production of GH. Of note, a brain-Hatschek's pit connection has been suggested in amphioxus by Gorbman et al. (1999). Additionally, we have identified a GHRH-like gene (Bb_267100R; <http://genome.bucm.edu.cn/lancelet/>) and its receptor gene (XP_019638674.1; <https://www.ncbi.nlm.nih.gov/protein/>) from amphioxus genome data (unpublished data). These suggest that an ancient form of the vertebrate-like hypothalamic-pituitary system might have emerged in amphioxus. However, this demands further study.

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