# The hypothalamic-pituitary-thyroid axis and biological rhythms: the discovery of TSH's unexpected role using animal models

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**Abstract**

Thyroid hormones (TH) are important for development, growth, and metabolism. It is also clear that the synthesis and secretion of TH are regulated by the hypothalamic-pituitary-thyroid (HPT) axis. Animal models have helped advance our understanding of the roles and regulatory mechanisms of TH. The animals' bodies develop through coordinated timing of cell division and differentiation. Studies of frog metamorphosis led to the discovery of TH and their role in development. However, to adapt to rhythmic environmental changes, animals also developed various endocrine rhythms. Studies of rodents clarified the neural and molecular mechanisms underlying the circadian regulation of the HPT axis. Moreover, birds have a sophisticated seasonal adaptation mechanism, and recent studies of quail revealed unexpected roles for thyroid-stimulating hormone (TSH) and TH in the seasonal regulation of reproduction. Interestingly, this mechanism is conserved in mammals. Thus, we review how animal studies have shaped our general understanding of the HPT axis in relation to biological rhythms.

## Introduction

Thyroid hormones (THs: precursor thyroxine [$T_4$] and active triiodothyronine [$T_3$]) are important for metabolism, development, and growth. In mammals and birds, TH synthesis and secretion are regulated by thyroid-stimulating hormone (TSH) that is derived from the pars distalis (PD) of the anterior pituitary gland (Fig. 1). TSH production and secretion are regulated by hypothalamic thyrotropin-releasing hormone (TRH). Circulating TH control synthesis and release of TRH and PD-TSH as part of a classic negative feedback loop [1,2], which is called the hypothalamic-pituitary-thyroid (HPT) axis.

Animals are exposed to various rhythmic events, such as daily and seasonal cycles, and developed endocrine rhythms to adapt to these rhythmic environmental changes. Cell-autonomous rhythmicity in approximately 24-h cycles is called a circadian rhythm, which tightly regulates physiological functions and endocrine rhythms. The HPT axis is under circadian control at multiple levels. Recent comparative studies have revealed a critical role of TSH and TH also in the regulation of seasonal rhythmicity [3–6], as seasonal changes in physiological functions are triggered by TSH that is derived from the pars tuberalis (PT) of the anterior pituitary gland (Fig. 1) [4,7,8]. As the PT lacks TRH receptors (TRHR) and TH receptors (THRs), PT-TSH production does not depend on the HPT axis [5,9] and is instead regulated in mammals by melatonin from the pineal gland [7]. Activated hypothalamic TH can induce morphological changes between gonadotropin-releasing hormone (GnRH) neurons and glial endfeet [4].

This review examines how animal studies have shaped our understanding of the HPT axis, and describes the unexpected role of TSH and its HPT axis-independent regulatory mechanisms. We also describe the role of TH in the regulation of frog development.

**HPT axis**

TRH that is derived from the paraventricular nucleus (PVN) of the hypothalamus is a tripeptide (L-pyroglutamyl-L-histidyl-L-prolineamide), which is secreted from the hypothalamic median eminence to the anterior pituitary via the hypothalamic-hypophyseal portal system [10]. In mammals and birds, TRH stimulates thyrotrophs in the PD to synthesize TSH by increasing mRNA levels of *Tsha* and *Tshb*, which encode the TSH α and β subunits, respectively [11]. TSH is a noncovalently linked heterodimeric glycoprotein that consists of α and β subunits [12]. TSH directly stimulates the thyroid gland to produce TH ($T_3$ and $T_4$) via TSHR, which is a G protein-coupled receptor, on the thyroid follicle cell membrane [2]. Thyroglobulin (Tg) is a polypeptide backbone of tyrosine residues that is produced by thyroid epithelial cells, secreted and stored in the follicular lumen, and then iodinated. After degradation of iodinated Tg through the lysosomal pathway, $T_3$ and $T_4$ are released into the bloodstream. Similar to chemicals that bind liver-derived proteins, $T_3$ and $T_4$ in the bloodstream can reversibly bind to thyroid-binding proteins (thyroxine-binding globulin [TBG], transthyretin [TTR], and albumin). In humans, $T_3$ and $T_4$ have a higher affinity for TBG, compared to TTR or albumin, whereas most THs bind to TTR and albumin in adult rodents, which have very low circulating levels of TBG. Released $T_3$ and $T_4$ generate feedback to inhibit the production of hypothalamic TRH and PD-TSH, as THs act on nuclear hormone receptors and regulate target gene expressions. There are two THR isoforms (THRα and THRβ), and these receptors regulate gene expressions by binding to the TH response element (TRE) in DNA as either a THR/retinoid X receptor (RXR) heterodimer in the *Trh* promotor or as a THR monomer in the *Tshb* promotor [13]. However, the nature of the THR complex

on the *Tshb* promotor has not been fully characterized. The THR complex modulates gene expressions by interacting with various co-repressors, which include nuclear receptor corepressor (NCoR) [14], silencing mediator for retinoic acid and thyroid receptors (SMRT) [15], and histone deacetylase (HDAC) [16]. Furthermore, the THR complex interacts with various coactivators, which include steroid receptor coactivator (SRC), histone acetyltransferase (p300), coactivator associated arginine methyltransferase 1 (PRMT1), and protein arginine methyltransferase 1 (CARM1).

Local activation of $T_3$ at the tissue level is increasingly recognized as an important mechanism of TH action [17]. After cellular entry through TH transporters (e.g., Mct8 and Oatp1c1), THs are metabolized by deiodinase enzymes (Dios), with type 2 deiodinase (Dio2) transforming $T_4$ into $T_3$, type 3 deiodinase (Dio3) inactivating $T_4$ and $T_3$, and type 1 deiodinase (Dio1) performing both reactions. Deiodinase genes have been identified in a variety of vertebrate species, including fish, tadpoles, chickens, rats, mice, and humans [18–22]. However, their tissue distributions and functional significance varies according to species. *Dio2* mRNA is abundant in the human thyroid and much less abundant in the adult rat thyroid, which may be caused by the different responses of the *Dio2* promotor to thyroid transcriptional factor [23]. In contrast, Dio1 activity is high in both human and rat thyroids. This discrepancy is related to the fact that in the rat circulating $T_3$ is mainly generated in the thyroid gland through Dio1 activity, while in humans in extra-thyroid gland tissues (e.g., the skeletal muscle) through Dio2 activity [24–26].

**Discovery of TH using amphibian metamorphosis**

The animal body develops through coordinated timing of cell division, cell

differentiation, cell movement, and programed cell death during development [27]. Appropriate timing of TH signaling is crucial for normal perinatal development, and especially for fetal growth and neurogenesis. Although the involvement of TH in regulating perinatal development has been confirmed in rodents [28], access to the fetuses *in utero* is not easy. In addition, fetuses are constantly exposed to maternal hormones. However, TH is required for the metamorphosis of fish and amphibians [29], which makes them a valuable model for understanding the role of TH in development. In 1912, Gudernatsch served fresh extracts from newly killed animals (horse, calf, dog, cat, rabbit, and pig) to tadpoles of the local frog (*Rana temporaria*) and observed that thyroid gland extract induced metamorphosis [30]. Furthermore, removal of the tadpole's thyroid gland inhibited metamorphosis [31]. These results lead to the discovery of $T_4$ [32] and $T_3$ [33]. Furthermore, Smith et al. found that the administration of a bovine pituitary gland extract to tadpoles accelerated metamorphosis [34]. Those researchers also discovered the role of the pituitary gland in the thyroid function of frogs and mammals [35]. Thus, metamorphosis provides a highly robust and easily accessible model for understanding TH's function in development. Furthermore, *Xenopus* tadpoles have been used for high-throughput screening of chemicals that affect TH signaling [36]. Because THRα mediates the effects of TH on heart rate, and THRβ is dominantly expressed in the liver and mediates metabolism, a THRβ-selective analog was developed to reduce lipid levels in tadpoles [37]. Although tadpoles are useful for evaluating TH signaling, TRH does not appear to regulate TSH expression in amphibians and some reptiles. Interestingly, corticotrophin-releasing hormone (CRH), but not TRH from the same PVN, regulates TSH during metamorphosis (Fig. 2A) [38]. In contrast, TRH stimulates prolactin secretion from the pituitary gland, which suppresses the metamorphosis of tadpoles [38].

## Mechanisms of THs' effects on metamorphosis

Although humans and frogs are very different organisms, significant parallels between mammalian birth and metamorphosis have been proposed [39]. For example, humans and frogs exhibit peak plasma TH levels during the aquatic-to-terrestrial transitions at birth and metamorphosis (Fig. 2B). In addition, humans and frogs have conserved TH-target organs (e.g., the central nervous system and muscles), THR, TRE sequences, downstream genes, TH transporters, and deiodinase-mediated metabolisms [28,40-42].

In frogs, THR expression begins earlier in the developmental stage, compared to the increase in TH levels. Therefore, a dual function model of THR action during development (unliganded and liganded THR states) has been proposed (Fig. 2C). Frog studies have demonstrated that THR-mediated gene induction in response to TH binding is required for tissue differentiation [43]. Furthermore, transcription activator-like effector nuclease (TALEN)-mediated THRα knockout in frogs resulted in an earlier start of metamorphosis and accelerated development in response to higher TH levels, because of the lack of THRα-mediated repression [43]. Furthermore, several *in vivo* studies have demonstrated that liganded THRs induce chromatin remodeling (e.g., DNA methylation and post-translational histone modifications). Before metamorphosis, histones H3 and H4 are deacetylated around the TRE site during the pre-metamorphosis period (when TH is absent) and are later acetylated during metamorphosis when TH increases [44] through THR co-transcriptional factor HDAC or SRC/p300. Although amphibian studies have clarified the role of TH in metamorphosis, it remains unclear how the precise timing of morphological changes and the tissue specificities of THR responses are achieved.

Addressing these questions may improve our understanding of TH's action in other animals, including humans.

**Circadian regulation of the HPT axis**

Circadian rhythms are driven by the transcription-translation feedback of circadian clock genes (e.g., *Per*, *Cry*, *Bmal1*, and *Clock*) [45,46]. In mammals, the master circadian pacemaker is located in the suprachiasmatic nucleus (SCN) [47]. The HPT axis is also under circadian regulation [48]. The SCN neuron innervates the PVN and may be responsible for the circadian pattern of *Trh* expression [49]. In addition, SCN lesions in rats disrupt circulating TSH and TH rhythms [50]. In humans, circulating TSH and free $T_3$ levels exhibit a clear daily rhythm with a nocturnal peak, although it is unclear if there is oscillation in free $T_4$ levels [51]. Diurnal marmosets and nocturnal rats have antiphase rhythms in circulating TSH levels that are based on light-dark cycles, with marmosets having high levels at night (similar to humans) and rats having high levels during the day [52]. As the circadian rhythms in the PVN of diurnal grass rats is 180° out of phase with the rhythms of nocturnal rodents [53], anti-phase rhythms in the PVN appear to regulate the phase of circulating TSH rhythms. It is of note that the response of PVN to neurotransmitter from the SCN is opposite between diurnal grass rats and nocturnal rats [54]. Although PD-TSH is regulated by TRH, PD-TSH expression rhythms also appear to be controlled by an intra-pituitary circadian clock. For example, the circadian rhythm of *Tshb* expression is reportedly regulated by the circadian clock gene (Rev-Erbα/NR1D1) with NCoR1 [55]. In the pituitary glands of fish, expressions of *Tshb, Tsha,* and *Dio3* exhibit clear circadian rhythms, which suggests that intra-pituitary TH inactivation is involved in TSH negative feedback control [56]. Diurnal rhythms in the Dio2 activity

179 from rat PD have also been reported [57], and pituitary-specific Dio2 knockout mice
180 exhibit reduced *Tsha* mRNA levels in the PD [58]. Elimination of TSH through
181 hypophysectomy abolishes the circulating TH rhythm but not the circadian clock gene
182 (*Per1* and *Bmal1*) expression rhythms in the rat thyroid [59], which indicates that the
183 circulating TH rhythm may be regulated by rhythmically secreted TSH but not by a
184 thyroid circadian clock.
185
186 **TSH and TH in the regulation of seasonal rhythms**
187 Non-tropical animals experience seasonal changes in photoperiod, temperature, and
188 precipitation. These animals have adapted by changing their physiology and behaviors,
189 such as growth, migration, hibernation, molting, and reproduction. Among various
190 environmental cues, animals typically use the photoperiod (day length) to drive their
191 adaptation, which is called "photoperiodism" [60]. This approach appears reasonable, as
192 temperature and precipitation are unreliable parameters that could induce an inappropriate
193 change during a warm winter, dry rainy season, or cool summer. In contrast, solstices and
194 equinoxes occur at almost identical times every year. Although humans, mice, and rats
195 are generally considered non-seasonal breeders, most other animals are seasonal breeders.
196 For example, small mammals and birds breed during the spring and summer, and are
197 classified as long-day breeders. In contrast, relatively large mammals (e.g., goats and
198 sheep) breed during the fall and are classified as short-day breeders. This is because goats
199 and sheep have a 6-month gestational period. Thus, the offspring of both long day and
200 short day breeders are born during the spring and can grow when food is abundant and
201 temperatures are moderate.
202 It has been suggested for several decades that TH is somehow involved in

seasonal rhythms [61–63]. For example, thyroidectomy blocks seasonal reproduction in sheep and Japanese quail [64,65], and $T_4$ replacement therapy restored the seasonal response in quail [64]. Interestingly, birds have highly sophisticated photoperiodic mechanisms that allows them to decrease their gonadal weight during non-breeding short days, which minimizes their body weight and maximizes their flight performance. However, birds re-develop gonads within a few weeks after detecting long-day conditions, with >100-fold differences in testicular size. Recent functional genomic analysis of quail revealed the signal transduction pathway that regulates avian seasonal reproduction [3,4], and microarray analysis identified long-day induction of TSH in the PT of the anterior pituitary gland [4]. Furthermore, functional analysis unexpectedly revealed that PT-TSH acts on the ependymal cells (ECs) within the hypothalamus, which increases expression of *Dio2* and reduces expression of *Dio3* through the TSHR-Gsα-cAMP pathway [4]. This Dio2/Dio3 switching fine-tunes local bioactive TH concentrations within the hypothalamus. Because the thyroid gland is the only source of TH, $T_4$ in the cerebrospinal fluid is transported to the ECs by Oatp1c1 for conversion into $T_3$ by Dio2 in the hypothalamus [3,4,66]. TH is also crucial for the development and plasticity of the central nervous system. Electron microscopy has revealed that locally activated TH induces morphological changes between the gonadotropin-releasing hormone (GnRH) nerve terminals and glial endfeet, which controls seasonal GnRH secretion to regulate gonadal development [3,67].

Cold stimulation accelerates the short day-induced gonadal regression in birds [68]. Adaptive thermogenesis in homoeothermic animals is controlled by TH activation through the induction of *Dio2* expression in the brown adipose tissue of rodents and in the liver of birds [69]. When birds are transferred to winter condition, cold stimulation

leads to increase serum $T_3$ that triggers germ cell apoptosis by activating genes involved in amphibian metamorphosis [29,69]. Thus, TH has dual roles in the regulation of seasonal reproduction; central TH activation by a long day stimulus transmits a summer signal to the hypothalamus to trigger seasonal reproduction, whereas peripheral release of TH induced by a cold stimulus transmits a winter signal to the testes to shut off the gonadal activity.

**HPT axis-independent regulation of TSH in the PT**

After the discovery that PT-TSH was a "springtime hormone" in quail, a similar function was confirmed in mice and sheep [7,8]. However, unlike mammals, birds receive photoperiodic information directly through deep brain photoreceptors (e.g., Opsin 5 [OPN5]) [70,71]. Furthermore, OPN5-positive cerebrospinal fluid (CSF)-contacting neurons directly innervate the PT to transmit photoperiodic information. In contrast, the eyes are the only photoreceptor organ in mammals (Fig. 3A), where photoperiod information is received by the eye and transmitted to the pineal gland through the SCN. Melatonin is secreted from the pineal gland during the night with a secretion profile that reflects the length of night, which allows melatonin to regulate mammalian seasonal reproduction [72]. For example, pinealectomy abolishes seasonal reproduction in hamsters and sheep and melatonin replacement therapy restores this cycle [73,74]. However, the mode of melatonin action remained unclear for several decades. PT thyrotrophs lack TRHRs and TRs, which makes PT-TSH independent of the HPT axis [5,6]. Nevertheless, PT thyrotrophs densely express melatonin receptors [75,76], and studies using knockout mice revealed that PT thyrotrophs are the target of nocturnal melatonin, with PT-TSH being negatively regulated by melatonin via the MT1 melatonin

receptor (Fig. 3A) [7,77].

**Glycosylation of TSH**

TSH is a glycoprotein hormone, and the TSH glycans in the PD are mainly biantennary and sulfated complexes [5,78], although glycosylation influences the bioactivity and half-life of these glycoproteins [79,80]. TSH plays a pivotal role in controlling the HPT axis and serves as the most reliable physiological marker of TH action [81]. Circulating TSH heterogeneity has been reported in human patients, and our recent study using photoperiod-sensitive laboratory mice revealed that PT-TSH is secreted into the circulation at a similar level to PD-TSH, but has minimal effect on the thyroid gland. This is because PT-TSH forms macro-TSH complexes with immunoglobulin and albumin in the circulation [5], which is caused by different *N*-glycosylation patterns (branched and sialylated) (Fig. 3B). Thus, tissue-specific glycosylation in the PT and PD appear to compartmentalize TSH's function, which prevents functional crosstalk within the body. Humans and rats with hypothyroidism have increased macro-TSH levels, which has little bioactivity. Interestingly, the dynamics of circulating TSH in hypothalamic hypothyroid patients and rodents are similar to that of PT-TSH, with low bioactivity, longer half-life, increased macro-TSH, and multi-antennary and sialylated glycosylation [82–87]. These tissue-specific glycosylation features appear to be the result of tissue-specific expression of glycosyltransferases [5]. Although the mechanism how PT-TSH avoids affecting the thyroid gland became clear, it is still unclear how PD-TSH avoids affecting the hypothalamus. A recent study has indicated that increases in patients' serum macro-TSH levels appear to be related to low sleep quality, in a relationship that is independent of TRH treatment [88]. Although humans do not exhibit clear seasonality, including in their

circulating TSH levels [89], the functional significance of human PT-TSH is of interest [90].

**Regulation of PT-TSH expression by the circadian clock**

The circadian clock is thought to be involved in photoperiodic measurement. Recent reports have also indicated that long-day-induced transcriptional co-activator eyes absent 3 (Eya3) [4,91] may regulate *Tshb* expression in the PT by forming a complex with a circadian transcription factor (thyrotroph embryonic factor [TEF] and hepatic leukemia factor [HLF]) binding to D-box in mice and sheep [92,93]. Furthermore, the effect of melatonin on *Eya3* expression has been proposed to involve phase synchronization and direct suppression, which appear to trigger a morning peak of *Eya3* under long-day conditions that subsequently induces *Tshb* expression. This "external coincidence" mechanism [94] may link the circadian system to the photoperiodic response in mammals. In the mammalian SCN, temporal expression profiles of circadian clock genes are altered during different photoperiods [95]. As photoperiodic changes also affect the expression profiles of clock genes in the PT of birds and mammals [96,97], an intra-PT circadian clock may measure photoperiodic changes as an "internal coincidence" timer [98]. Nevertheless, it remains unclear how animals measure photoperiods

**Summary**

TH plays crucial roles in development, metabolism, and the seasonal regulation of reproduction. Research using mice and rat models have greatly contributed to our understanding of the functional significance and regulatory mechanisms of the HPT axis. However, mice and rats are not always optimal for understanding specific aspects of

physiology, and it is important to select the appropriate organism(s). This is Krogh's principle: "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied". Indeed, comparative studies have uncovered the unexpected role of TSH. The human body exhibits seasonal changes in metabolism, immune function, and mood despite generally being considered a non-seasonal mammal [99]. Therefore, comparative studies using unique animal models can be powerful tools to further understanding human health.

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The most important references are [3][4][5][7][14][15][30][32][92][93].

**Practice points**

- The HPT axis is controlled by circadian rhythms.
- Although humans are considered non-seasonal mammals, they exhibit seasonal changes in metabolism, immune function, and mood.
- PD-TSH stimulates the thyroid gland, whereas PT-TSH transmits springtime information to the brain.
- PT-TSH forms macro-TSH complexes in the circulation to avoid crosstalk with PD-TSH.

**Research agenda**

- Develop TH analogs.
- Investigate the underlying mechanism(s) of the human seasonal rhythm.
- Explore the functional significance of human PT-TSH.

## Figures

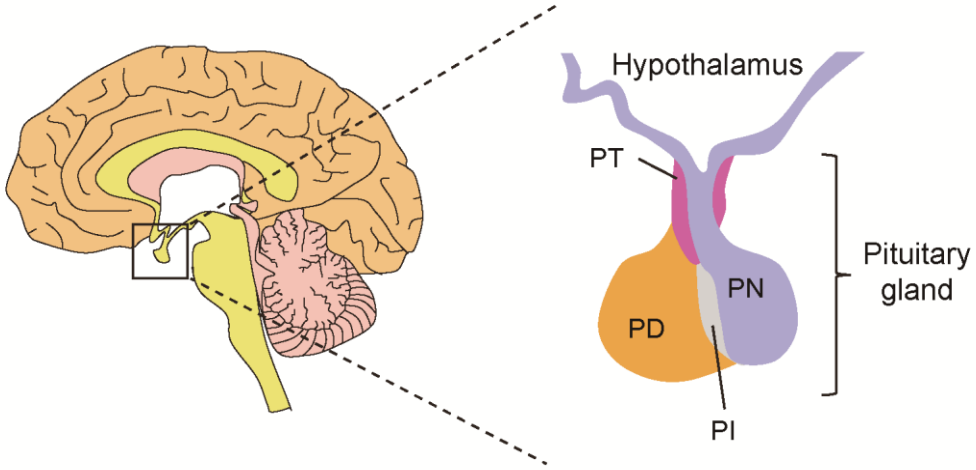
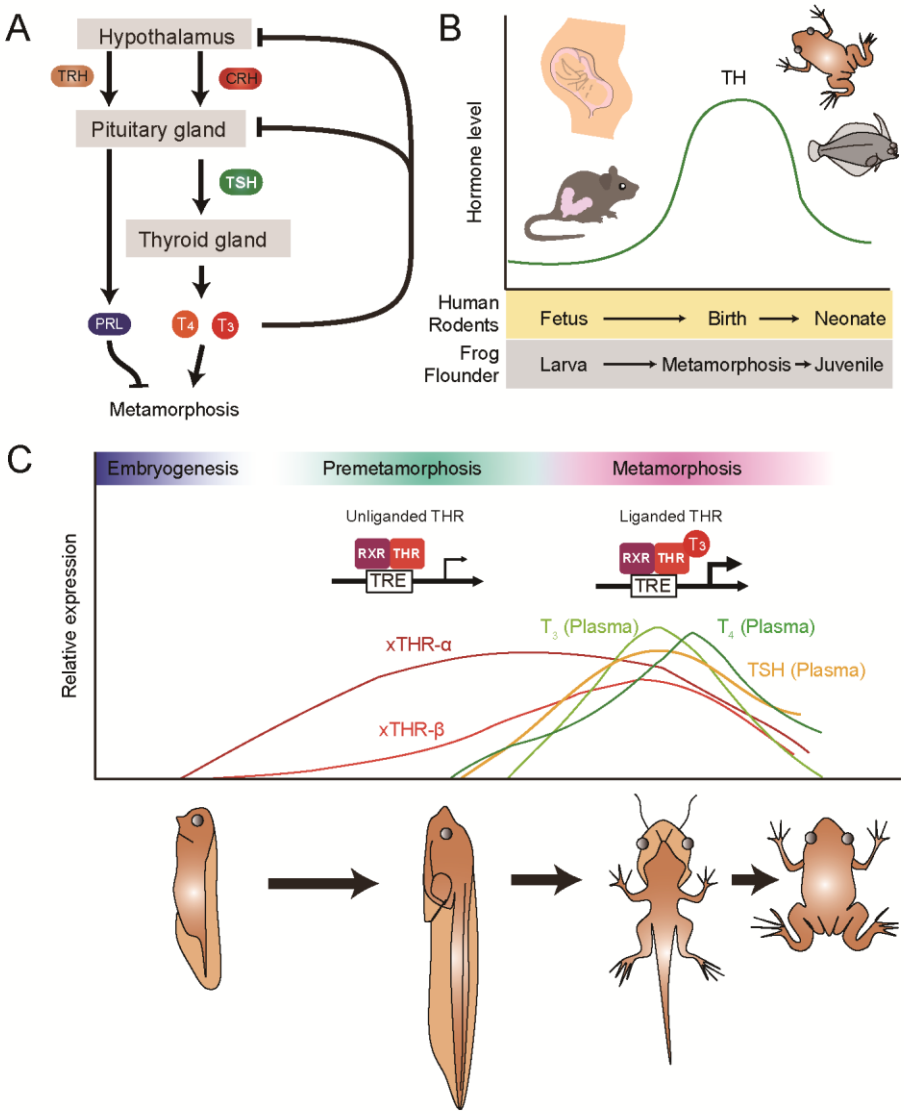



**Fig. 1** Human pituitary gland. PD, pars distalis; PT, pars tuberalis; PI, pars intermedia; PN, pars nervosa.




638

639 **Fig. 2** Thyroid hormones' action in metamorphosis. (A) The hypothalamic-pituitary-
640 thyroid (HPT) axis in the regulation of amphibian metamorphosis. Corticotrophin-
641 releasing hormone (CRH), but not thyrotropin-releasing hormone (TRH), from the
642 paraventricular nucleus (PVN) stimulates synthesis of thyroid-stimulating hormone
643 (TSH) in the pituitary gland. TSH directly stimulates the thyroid gland to produce thyroid
644 hormones (TH; $T_3$ and $T_4$). In turn, circulating TH negatively regulates TRH and PD-
645 TSH synthesis and release as part of a negative feedback loop. In frogs, TRH regulates
646 prolactin (PRL) secretion and PRL suppresses metamorphosis. (B) Conservation of TH

signaling in endocrine mechanisms during the development of mammals, amphibians, and fish. During birth and metamorphosis, organisms have increasing plasma TH levels that regulate neurogenesis, skeletal growth, and development towards the aquatic-to-terrestrial transition (mammals and amphibians) or towards the planktonic-to-benthic transition (flounders). (C) Differential expressions of HPT axis-related factors and the dual functions of THR during amphibian development. Relative THR levels in whole embryos and tadpoles are shown throughout their development to the end of metamorphosis. Plasma TH and TSH levels are only elevated during metamorphosis. Adapted from [39,42,100].

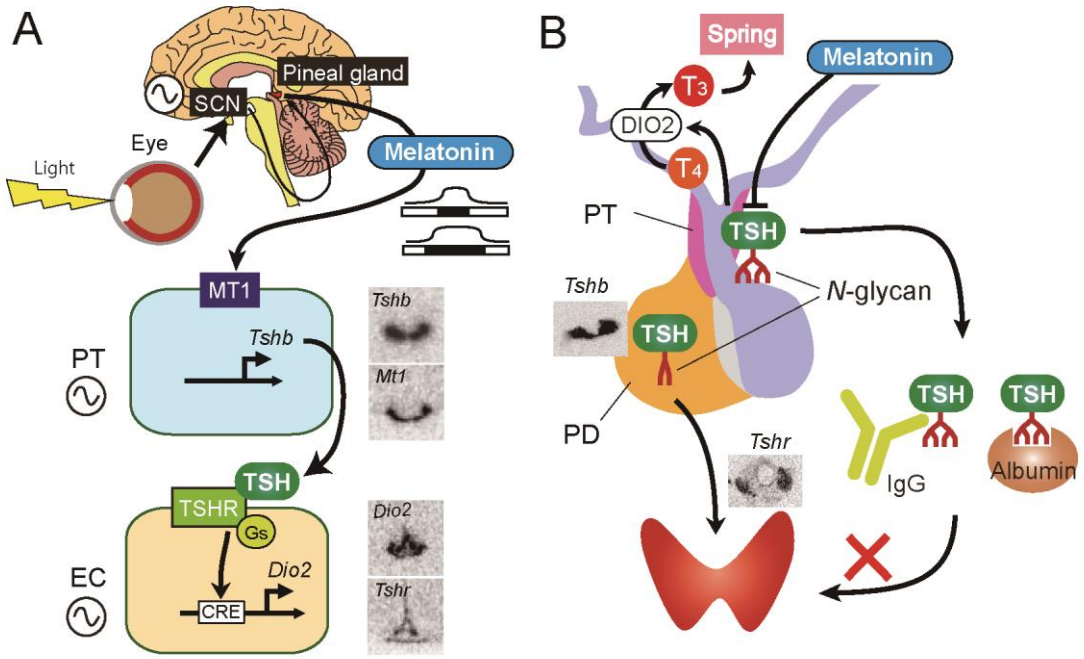



**Fig. 3** Pars tuberalis-derived thyroid-stimulating hormone (PT-TSH) regulates seasonal reproduction. (A) Light information is detected by the eyes and transmitted to the pineal gland via the circadian pacemaker suprachiasmatic nucleus (SCN). Pineal melatonin reflects the length of nights, and suppresses PT-TSH. Long days are associated with increased production of PT-TSH, which acts on ependymal cells (ECs) in the hypothalamus to induce Dio2 expression through the TSHR-Gsα-cAMP signaling pathway. Dio2-induced TH activation transmits the springtime signal. (B) Pars distalis (PD)-derived TSH (PD-TSH) stimulates the thyroid gland to produce TH, whereas PT-TSH activates hypothalamic TH through Dio2 expression. PT-TSH has tissue-specific *N*-glycans and forms macro-TSH complexes in the circulation with immunoglobulin (IgG) and albumin. The macro-TSH complexes cannot stimulate the thyroid gland, which prevents functional crosstalk between the two TSHs. The images are *in situ* hybridization autoradiographs from mice. MT1, melatonin receptor 1; CRE, cAMP responsive element.