Society for Endocrinology
JOURNAL OF ENDOCRINOLOGY

How can a binary switch within the pars tuberalis control seasonal timing of reproduction?

Journal:	*Journal of Endocrinology*
Manuscript ID	JOE-18-0177.R1
Manuscript Type:	Invited Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Wood, Shona; Universitetet i Tromso Institut for arktisk og marin biologi, Arctic Chronobiology and Physiology; University of Manchester, Faculty of Biology, Medicine and Health
Keywords:	Development, Differentiation, Neuroendocrinology, pars tuberalis, pituitary

How can a binary switch within the pars tuberalis control seasonal timing of reproduction?

Shona Wood [1,2]

1. Department of Arctic and Marine Biology, UiT – The Arctic University of Norway, Tromsø NO-9037, Norway

2. Faculty of Biology, Medicine and Health, School of Medical Sciences, University of Manchester, A.V. Hill Building, Oxford Road, Manchester M13 9PT, UK

Short title: Seasonal Binary switches

Keywords: pars tuberalis, binary switches, seasonal, timing

Abstract

Life in seasonally changing environments is challenging. Biological systems not only have to respond directly to the environmental challenges, but schedule life history events in anticipation of seasonal changes. The cellular and molecular basis of how these events are scheduled is unknown. Cellular decision-making processes in response to signals above certain thresholds regularly occur i.e. cellular fate determination, apoptosis, firing of action potentials. Binary *switches,* the result of cellular decision-making processes, are defined as a change in phenotype between two stable states. A recent study presents evidence of a binary *switch* operating in the pars tuberalis (PT) of the pituitary, seemingly timing seasonal reproduction in sheep. Though, how, a binary switch would allow for anticipation of seasonal environmental changes, not just direct responsiveness, is unclear.

The purpose of this review is to assess the evidence for a binary switching mechanism timing seasonal reproduction, and to hypothesise how a binary switch would allow biological processes to be timed over weeks to years. I draw parallels with mechanisms used in development, cell fate determination, and seasonal timing in plants. I propose that the adult PT is a plastic tissue, showing a seasonal cycle of differentiation, and that the underlying processes are likely to be epigenetic. Therefore, considering the mechanisms behind adult cellular plasticity offers a framework to hypothesise how a long-term timer functions within the PT.

Introduction

Anticipation and responsiveness to environmental signals is key to adapting to a changing environment. Organisms can schedule life history events in order to maximise fitness. For example timing birth to the season of highest food availability. This is not solely a response to the prevailing environmental conditions but a scheduled/anticipated event, taking advantage of the predictability of seasonal cycles. These major physiological changes can take weeks to complete, thus, the organism must intrinsically know the time of year and anticipate upcoming conditions. Animals have evolved to use the annual variation in day length (photoperiod), a highly predictive signal, together with endogenous long-term timers ("circannual clock"). Therefore, they keep track of seasonal time, even in constant conditions (i.e. the hibernacula), and activate a seasonal adaptive programme. (Lincoln & Clarke 1994; Lincoln *et al.* 2005, 2006; Lincoln 2006; Wood & Loudon 2014, 2017; West & Wood 2018).

The cellular and molecular basis of how seasonal life-history events are scheduled is unknown. Binary decisions are at the core of many different cellular processes, such as cellular fate determination, apoptosis, and firing of action potentials. These are all regarded as decision-making processes in response to signals above certain thresholds, which leads to all-or-nothing activation of downstream pathways. Thresholding of responses is an important way to properly space biological events, and is seen during the accurately timed process of embryonic development (Ashe 2006; Chattwood *et al.* 2013). Developmental changes are characterised by binary switching of cellular phenotype from one stable state to another. A recent study presents evidence of a binary switch operating in the pars tuberalis (PT) of the pituitary, seemingly timing seasonal reproduction (Wood *et al.* 2015). The purpose of this review is not to extensively review the seasonal field, (for extensive reviews see: (Dardente *et al.* 2014; Nakane & Yoshimura 2014; Wood & Loudon 2014, 2017)), instead it will review the evidence of a binary switching mechanism in the PT. I shall draw parallels with mechanisms used in development, cell fate determination, and seasonal timing in plants. Finally, I will hypothesise how a binary switch would allow biological processes to be timed over weeks to years.

The photoperiodic response

Nocturnal secretion of pineal melatonin acts as a critical transducer of photoperiod change, providing the brain with an internal hormonal representation of external photoperiod (Fig. 1, reviewed in

(Hazlerigg & Loudon 2008; Dardente *et al.* 2014; Nakane & Yoshimura 2014; Wood & Loudon 2014, 2017; West & Wood 2018). The PT is the only consistent site of melatonin binding in a wide range of seasonally breeding mammalian species (Morgan *et al.* 1994). It impinges on the hypothalamo-pituitary axis and governs both an anterograde (prolactin) and retrograde (TH) pathway to the pars distalis and hypothalamus, driving seasonal physiological adaptations (Wood & Loudon 2017; Korf 2018). Here, I focus on the better characterised photoperiodic TH pathway relating to seasonal reproduction.

The PT-specific thyrotrophs contain receptors for melatonin (Klosen *et al.* 2002; Dardente *et al.* 2003; Johnston *et al.* 2006), which govern the local release of thyroid stimulating hormone (TSH) (Dardente *et al.* 2010). The photoperiodic control of TSH production, depends on up-regulation of TSHβ subunit by the transcriptional co-activator, EYA3 on long photoperiods ("LP induction") (Dardente *et al.* 2010; Masumoto *et al.* 2010). PT TSH signals to hypothalamic tanycytes, which in turn modulate the seasonal biological availability of thyroid hormone (TH), via the expression of deiodinase enzymes (DIOs). The DIOs convert T4 to active T3 (DIO2) and T4 to inactive reverse T3 (DIO3) (Dardente *et al.* 2014). Hypothalamic T3 status controls central structures involved in seasonal metabolic physiology and reproduction (Murphy *et al.* 2012; Klosen *et al.* 2013; Bank *et al.* 2017). Reproductive effects are mediated through alterations in the GnRH pulse generator, potentially involving kisspeptin and RFRP3 (Simonneaux *et al.* 2013; Hazlerigg & Simonneaux 2015; Beymer *et al.* 2016) (Fig. 2). The EYA3/TSH/DIO photoperiodic switch is highly conserved amongst vertebrates (Nakane & Yoshimura 2014) but PT tissue is not conserved in lower vertebrates i.e. fish (Lincoln *et al.* 2003; Wood & Loudon 2017).

Here, I focus on the PT-specific thyrotroph as point of initiation of the physiological change but also the site showing spontaneous reversion suggesting it is the site of endogenous long-term timekeeping (see below) (Lincoln *et al.* 2003, 2006; Wood & Loudon 2017). However, I note that in mammals photoperiodic information is transferred from mother to pup and that photoperiodic history dependence is seen in the hypothalamic tanycytes, not the PT (Sáenz de Miera *et al.* 2017). Therefore, both the PT and the hypothalamic tanycytes are critical sites of photoperiodic history, circannual cue integration and transmission to the endocrine and central nervous systems (Lincoln *et al.* 2006; Sáenz de Miera *et al.* 2014; Wood & Loudon 2014, 2017; Wood *et al.* 2015).

At a PT tissue level TSHβ progressively increases in RNA expression over the first 4 weeks of LP (Wood *et al.* 2015) (Fig. 3a), but the increase in expression is already beginning 3 days after the switch to LP in sheep (Dardente *et al.* 2010) and the F344 rat (Ross *et al.* 2011). In fact, from the first long day increased TSHβ is observed in quail (Nakao *et al.* 2008), chicken (Dunn *et al.* 2017) and the

bunting (Majumdar *et al.* 2014). This suggests a direct responsiveness and transcriptional activation, and is referred to as "LP induction", but it is important to remember this can only occur if the animal has experienced an appropriate duration of winter/short photoperiod signal and that the TSHβ signal is not maximal until 4 weeks of LP in sheep.

The tissue response of TSHβ in the PT, is seemingly consistent with progressive changes in sheep LH and FSH under natural photoperiods (Billings *et al.* 2002; Lomet *et al.* 2018) and square wave transitions (Bittman *et al.* 1983), leading to the downstream reproductive changes. However, the correlations between in DIO/TSH and LH/FSH in hamsters is not so clear, especially when considering refractory animals (see below), potentially implicating the tanycytes as important modulators of the refractory response in hamsters (Milesi *et al.* 2017). Though, a directly correlative approach to causality is not necessarily appropriate when dealing with auto-regulatory feedback loops across tissues. Therefore, I will focus on how these gradual tissue level responses may be generated in the PT to suggest how this tissue can act as an endogenous timer.

Endogenous timekeeping: scheduling and anticipation of events

The amount of light taken from a single day is indistinguishable from a matched point in autumn or spring, thus, for photoperiodic information to be meaningful, it must be registered within the context of recent preceding photoperiods (Butler *et al.* 2010; Sáenz de Miera *et al.* 2017; West & Wood 2018). Therefore, life history trajectories and seasonal programs are set depending on the prior photoperodic history. Importantly, the observed acute inductive effects of LP are only able to occur if the appropriate prior history has been experienced (reviewed in: (West & Wood 2018)). This demonstrates that the photoperiodic response is not a direct/immediate response to any photoperiod change but is a scheduled/anticipated responsiveness to ensure physiological changes occur at the appropriate time of year.

Changes in seasonal physiology can persist in constant conditions, and even in the absence of a pineal gland (Woodfill *et al.* 1994; Sáenz de Miera *et al.* 2014). These rhythms are no longer entrained to the solar year but proceed according to the prior photoperiodic history. In short lived species these rhythms only revert once and usually lead to the animal being locked on a reproductive phenotype (refractory). Long lived species show a persistence of rhythms for many years in constant conditions (circannual rhythms) (Gwinner 1986). In either case there is endogenous timing resulting in spontaneous changes in seasonal physiology.

To understand the mechanisms animals use to anticipate seasonal environmental changes and respond appropriately we must consider where in the photoperiodic TH pathway the endogenous timer acts. By placing sheep into constant LP we can reveal the circannual rhythm and assess the known photoperiodic circuits (Fig. 2). Here, both the melatonin signal and the local circadian clockwork in the PT does not change, continuing to reflect the prevailing photoperiod (Lincoln *et al.* 2002, 2005) but the EYA3/TSH/DIO does spontaneously revert in constant conditions tracking the cycles of seasonal physiology of the animal (Sáenz de Miera *et al.* 2013; Wood *et al.* 2015). DIO and TSH reversion have also been demonstrated in European hamsters (Sáenz de Miera *et al.* 2014).

PT TSHβ progressively declines on constant LP (Sáenz de Miera *et al.* 2013; Wood *et al.* 2015), tracking the circannual cycle of reproductive physiology, referred to as the "LP refractory" state (Fig. 2, Fig. 3b). This decline is also observed under natural photoperiod between May (~14.5hrs light) and August (~14.5hrs light), representing only an hour and a half decline from solstical photoperiod i.e. still a LP (Lomet *et al.* 2018). Importantly, TSHβ is not the only PT expressed gene to change after constant LP (Wood *et al.* 2015; Lomet *et al.* 2018) but it serves as a good marker for the LP physiological state and currently provides the best causal link to seasonal reproductive changes (Hanon *et al.* 2008).

LP induction appears to be well synchronised between individual animals, whereas the LP refractory decline (when animals are kept on constant LP) is variable amongst individual animals. This is unsurprising given the lack of a synchronising signal from a photoperiodic change. During LP induction at a cellular level, there may be greater synchronization potentially leading to a more consistent output. If the cells are synchronised two possibilities exist for how this gradual tissue response is generated during LP induction: 1. All the thyrotrophs within the tissue progressively increase the expression of TSHβ from the time of the LP switch. Or, 2. Individual thyrotrophs "switch on" TSHβ expression at different rates once exposed to LP. For option 2 variation in threshold of responses in individual cells would lead to the gradual accumulation in the number of TSHβ expressing cells after LP induction. These options represent the difference between a whole tissue response and a single cell response that accumulates into a tissue level output (Fig. 3c). But what evidence is there that there are individual cell switches in response to photoperiod?

Binary switch within the PT

Mathematical modeling of the photoperiodic response predicted a switching of cellular phenotype in the PT in response to photoperiod (Ebenhöh & Hazlerigg 2013) but without identification of a cellular marker of short photoperiod (SP – "winter") this could not be tested. A large transcriptomic dataset

on the sheep PT, allowed the identification of the SP marker, chromogranin A (CHGA) (Wood *et al.* 2015). CHGA, a hormone packaging molecule, strongly co-localises to the secretory granules in PT-specific thyrotrophs in SP, and displays the progressive tissue level response, contrasting to the long photoperiod signal TSHβ (Fig. 3a) (Wood *et al.* 2015). Unlike TSHβ, the role for CHGA in a seasonal context is unknown but the increase in expression corresponds to a decrease in rough endoplasmic reticulum (RER) and a decrease in secretion from these cells (Wood *et al.* 2015). However, prolactin was the hormone output monitored in this study, due to castrate male being used, therefore it cannot be directly linked to reproductive outputs (Wood *et al.* 2015).

Using CHGA as a marker for the "winter" state and TSHβ protein expression as a marker for the "summer" state, the distribution of individual PT thyrotroph cells were mapped at 4 weeks LP, SP, and 29 weeks LP (LP refractory). This revealed that virtually no PT-specific thyrotrophs (defined by αGSU expression) co-express both CHGA and TSHβ (0.01% - 2 cells out of 17,000)(Wood *et al.* 2015), indicating that PT-thyrotrophs can only exist in one of two binary states, winter-like (CHGA+ve) or summer-like (TSHβ+ve). Importantly, this switching occurred at 29 weeks LP (LP refractory), reverting to the winter like state, demonstrating that it occurs as an endogenously timed process (Wood *et al.* 2015)(Fig. 3b & d). The possibility that these observations were due to two different cell populations i.e. LP and SP specialised subsets of PT-specific thyrotrophs was considered. Cell counts revealed that in SP 4 weeks nearly all (91.6%) PT-specific thyrotrophs expressed CHGA and in LP 4 weeks 66.6% of PT-specific thryotrophs expressed TSHβ (approx. 5% still express CHGA and the remainder only expressing αGSU) therefore the most likely explanation is that within a population of PT-specific thyrotrophs cells switch from one phenotype to another (Wood *et al.* 2015). To add to that conclusion we and others have demonstrated that cell division in the PT is very low (<0.2%) and not within the PT-specific thyrotroph population (Migaud *et al.* 2011; Hazlerigg *et al.* 2013; Wood *et al.* 2015). Also the RNA expression of αGSU did not change, nor did the expression of established cell cycle genes (Wood *et al.* 2015). We cannot completely rule out that these observation are due to the presence of two different cell populations without cell fate mapping approaches, which are currently not tractable in seasonal model species. These observations have only been made in sheep, in this one study, and unfortunately, an individual cell level characterisation has not been made at different points of the seasonal cycle.

Nevertheless, a binary switch has good explanatory value for the a progressive tissue level changes observed and subsequent cycles of physiology that occur, and may provide a framework to understand seasonal timekeeping. To hypothesise how a binary switch could operate to time biological processes over weeks to years we would need to know: 1. What initiates the binary switch

(LP induction), 2. How the cycle is endogenously generated (LP refractory), 3. What is the basis of individual cell variability in response, and, 4. What is the role of input history (prior photoperiod).

Initiating the binary switch – A seasonal differentiation process?

The PT has a distinct developmental origin from the rest of the pituitary gland, it emerges from the rostral tip region of Rathke's pouch. The bHLH transcription factor hairy enhancer of split (HES1) is a PT-specific differentiating factor (Akimoto *et al.* 2010; Aizawa *et al.* 2016). All glycoprotein hormones comprise a common α subunit (αGSU) and a specific β subunit, (βFSH / βLH / βTSH). In embryological development αGSU is the first subunit gene expressed in the pituitary (Stoeckel *et al.* 1993; Kameda *et al.* 1998; Kita *et al.* 2007; Raetzman *et al.* 2007; Akimoto *et al.* 2010; Inoue *et al.* 2013). CHGA (with αGSU) appears just prior to TSHβ in embryonic development of the mouse pars distalis (Akimoto *et al.* 2010), and, is an early embryonic signal in zebrafish (Xie *et al.* 2008) and the chicken PT (Kameda *et al.* 1998). In the main pituitary upon differentiation to specific endocrine cell types, expression of the β subunit is gained in adult endocrine cells. In this context, adult PT specific thyrotrophs are unique since they persist as αGSU+ precursors, and only mature into αGSU/TSHβ expressing thryotrophs when exposed to LP. Therefore, by definition, the PT-specific thyrotroph appears to undergo a seasonal cycle of differentiation/maturation on exposure to LP, essentially undergoing a seasonal recapitulation of a developmental state, to drive seasonal physiological changes.

Differentiation dramatically changes a cells size, shape, metabolic activity and responsiveness to signals. Therefore, changes in gene expression and morphology are used to define differentiation. Within the sheep PT two large scale transcriptomic analysis clearly illustrate that genes related to development, cytoskeletal remodelling and tissue plasticity are enriched on LP (Wood *et al.* 2015; Lomet *et al.* 2018). PT thyrotrophs increase in size, increase rough endoplasmic recticulum (RER), gain a secretory phenotype and reorganise into networks on LP, presumably to coordinate the secretion of TSH (Wood *et al.* 2015)(Fig. 4a). Differentiation can occur without cell division (O'Neill & Stockdale 1972; Yang *et al.* 2007), which may be consistent with the lack of evidence for cell division in the PT thyrotroph (Migaud *et al.* 2011; Hazlerigg *et al.* 2013; Wood *et al.* 2015). This ability to differentiate without division has been suggested to allow for faster responses to signals (Yang *et al.* 2007). In the context of the PT, it is possible that over winter PT thyrotrophs are "primed" to respond to an LP signal, explaining the rapid production of TSHβ during LP induction. Therefore, I propose that the binary switch is initiated by a seasonal differentiation of PT thyrotrophs to a mature TSH secreting state.

A seasonal cycle of differentiation would require a differentiation signal/factor that is expressed on LP. A more detailed analysis of published data on the first, seventh and 28th day of LP, shows 24 genes changing within the PT on transition to LP were related cellular differentiation and cell cycle. KLF11, TRIM2, and EYA3 have a pattern of expression that would be consistent with a transient LP differentiation signal and a progressive accumulation of TSHβ positive cells (Fig. 4b). KLF11 has roles in the differentiation of adipocytes to brown fat (Loft *et al.* 2015) and the differentiation of pancreatic progenitor cells (Teo *et al.* 2018). TRIM2 regulates neuronal differentiation (Khazaei *et al.* 2011) and epidermal and hair follicle differentiation (Joost *et al.* 2016). Due to the difficultly in the dissection of the PT it is possible that some median eminence or pars nervosa was in the sample, therefore it remains to be shown if either TRIM2 or KLF11 have a PT specific expression. EYA3, does have specific PT expression (Fig. 2a), and, has an extensively documented role in retinal, pituitary and muscle development and differentiation (Xu *et al.* 1997; Jemc & Rebay 2007; Kozmik *et al.* 2007; Kumar 2009; Gordon *et al.* 2012; Tadjuidje & Hegde 2013). In the context of seasonal biology, EYA3 is emphasised to be a clock-dependent transcriptional co-activator of TSHβ (Dardente *et al.* 2010; Masumoto *et al.* 2010; Wood & Loudon 2014), not a cellular differentiation signal. However, after 4 weeks of LP in sheep only 38% of TSHβ expressing cells also express EYA3 (Wood *et al.* 2015), suggesting EYA3 is not the sustaining signal in TSHβ expression. Furthermore, the TSH-receptor is required to sustain TSHβ expression (Ono *et al.* 2008), suggesting positive feedback of TSH and transience of EYA3. A role for EYA3 as a seasonal differentiation signal is feasible, but remains to be demonstrated (Fig. 4c).

An endogenous cycle of binary switching – Seasonal de-differentiation or death?

If PT thyrotrophs do differentiate to a mature TSH producing cell type then an explanation for the reversion to a αGSU positive cell that is phenotypically characteristic of an immature endocrine cell during the LP refractory state and over a natural seasonal cycle is required. I suggest there are three possibilities; 1. A subsequent reversion to an immature/progenitor state (de-differentiation), 2. Trans-differentiation from a different mature cell type, or, 3. A cycle of death and replacement (Fig. 4c).

Traditionally, cell fate determination has been viewed as a process of progressively increasing restriction of cellular fate potential on the path to a fully mature differentiated cell. Recent studies have highlighted possibility that cells do not assume fixed final differentiated phenotypes in adult tissues. This plasticity of differentiated cells is likely to be central to survival through rapid response to injury or environmental changes (Paksa & Rajagopal 2017). The reversion of a PT-specific

thyrotroph to a progenitor or SP (winter like) state via de-differentiation is potentially the most parsimonious explanation in the PT (Fig. 4c). There is precedence for this, pancreatic β-cells in type 1 diabetes, de-differentiate (Talchai et al. 2012; Cinti et al. 2016) and insulin therapy re-differentiates these cells (Wang et al. 2014). Therefore, adult endocrine cells can undergo cycles of differentiation and de-differentiation.

Trans-differentiation (lineage reprogramming) suggests that a mature cell becomes a different cell type. For example, mature δ cells in the pancreas and hepatocytes in the liver converting to β-cells and biliary epithetial cells, respectively. The most common example of trans-differentiation is limb regeneration in amphibians (reviewed in: (Shen *et al.* 2004; Cai *et al.* 2007)). These examples indicate that by changing the environment of the cell there can be a reprogramming of fate (Paksa & Rajagopal 2017). However, the difference between de-differentiation and trans-differentiation becomes less clear when the majority of evidence points to differentiated cell, de-differentiating to a progenitor or stem cell phenotype, and then re-differentiating to a different mature cell type (Cai *et al.* 2007). In the context of the PT, and the strictest definition of trans-differentiation, the presence of an infiltrating (or resident) cell type that switches fate would be required. One study observed the presence of dividing CD45 positive cells with a microglia phenotype in the PT (Hazlerigg *et al.* 2013), there was no seasonal variation in the numbers of cells observed, but these could represent a candidate cell for trans-differentiation. SRRM4 shows transcriptional changes in the PT on LP (Wood *et al.* 2015) and is known to drive neuroendocrine trans-differentiation of prostate adenocarcinoma cells (Li *et al.* 2017), potentially providing a candidate gene, but it is difficult to draw conclusions from data on a mixed cell population.

Finally, a cycle of death and replacement (from a stem cell population) generating a circannual rhythm (seasonal histogenesis) was proposed before the observation of a binary switch (Hazlerigg and Lincoln, 2011). Seasonal histogenesis could provide a mechanism to explain loss and replacement of TSH positive cells within the PT. But there have been few studies of cell turnover in the PT, and these do not present a consistent picture as to the extent to which cell division varies with seasonal state (Migaud *et al.* 2011; Hazlerigg *et al.* 2013; Wood *et al.* 2015). In the context of a cycle of de-differentiation the capacity to gain proliferative function is a characteristic of Schwann cells, kidney cells and Sertoli cells following insult (Shen *et al.* 2004; Cai *et al.* 2007). Therefore, de-differentiation and histogenesis are not mutually exclusive explanations. Seasonal histogenesis remains to be adequately tested in the PT and would require assessment of cell death and division.

I have provided a potential framework to address how the seasonal cycle of binary switching may operate in the PT but I have not offered an explanation for how this would allow for long-term

timekeeping. I propose that individual cell variation in the threshold of responsiveness and variation in the subsequent reversion could generate a timer process.

The role of individual variability in accurate timing

Endocrine cells have acute, medium, and long-term outputs and these must be regulated across different time scales and in response to diverse environmental signals, while achieving accurate control of hormone expression. At first glance the idea of individual cell variability to environmental signals may seem to be contrary to accuracy and control of hormone expression. However, stochastic heterogeneity in single cell response can lead to a tissue progressive responses. Release of prolactin from the pituitary, for example, is based on heterogeneous patterns of gene expression at a single cell level but a well-regulated average level of gene expression is maintained (Featherstone *et al.* 2011; Harper *et al.* 2011). Prolactin expression follows a binary 'on-off' process, with activity of individual cells being unpredictable but the overall activity of a group of cells being predictable (Hey *et al.* 2015; Featherstone *et al.* 2016). The conclusions from these study were that heterogeneous single cells responses allowed the tissue to be more functionally adaptive to the environment and result in more robust tissue-level responses, avoiding inappropriate amplification of signals through feedback mechanisms (Featherstone *et al.* 2011, 2012, 2016; Harper *et al.* 2011; Hey *et al.* 2015).

During development, pluripotent cells differentiate and become restricted to specific lineages. Often, this depends on positional information and morphogenic gradients, the clear banding (position-effect variegation) seen in the *Drosophila* embryo is a good example, but there are also examples of position-independent patterning (Kay & Thompson 2009). Position independent patterning is characterised by cells switching or differentiating from one phenotype to another in an apparently stochastic manner, with variability in timing, across the tissue. Position independent patterning makes it appear as if two cells types are randomly "sprinkled" throughout a tissue, and for that reason it is referred to as, salt and pepper differentiation. Re-patterning is also a feature of this process, as seen in the PT (Fig. 4a). Examples of this include the early mouse embryo, and the R8 photoreceptor neurons in *Drosophila* that differentiate into rhodopsin 5 sensitive or rhodopsin 6 sensitive subtypes through stochastic expression of specific transcription factors determining cell (Eldar & Elowitz 2010; Jukam *et al.* 2013). This stochasticity is presumed to add robustness and accuracy to timing of differentiation during development (Kay & Thompson 2009; Eldar & Elowitz 2010; Jukam & Desplan 2010; Jukam *et al.* 2013). Importantly, many terminal differentiation processes show variability in the time from sensing the initial inducing signal to the final commitment to their new fate, this allows cells to defer commitment, which prevents responses to transient

322 signals, in the context of the PT this may be a mechanism to allow the long-term regulation of a
323 hormonal output.

324

325 **Input history - How is individual variability encoded?**

326 Within the PT we expect individual variability in the responsiveness to LP, whereby, PT thyrotrophs
327 differentiate into a mature TSH secreting state (LP induction), but, also in the reversion to winter
328 phenotype (LP refractory). Individual variability may arise through and be modulated by; 1. input
329 history to the cell effecting the threshold sensitvity of that cell, and, 2. individual differences in
330 sensitvity and therefore, the rate of commitment.

331 Vernalisation is the process of preparation for flowering, which depends on cold temperatures being
332 experienced for a suitable length of time to permit flowering on rewarming (spring). It is
333 characterised by a progressive binary switching of cells, in a salt and pepper fashion (see above)
334 based on individual variation in responsiveness. Cells progressively switch over weeks of cold
335 exposure, acting as a long-term timer, and only giving a full flowering response when the majority of
336 cells have flipped in state (Angel *et al.* 2011; Song *et al.* 2012). Importantly, the input history, in this
337 case low temperature, is key to "priming" cells to respond to a spring signal. This priming is achieved
338 by a long-term epigenetic repressive process is initiated at the beginning of winter. Prolonged cold in
339 the winter leads to the accumulation of repressive marks mediated by polycomb complex proteins
340 acting on the flowering control locus (FLC), which usually blocks the activation of flowering locus T
341 (FT). The longer the exposure to cold, the greater the number of cells entering an FLC-repressed
342 state, therefore upon re-warming FT is activated and flowering can occur, with the extent of
343 flowering based on the number cells in the FLC-repressed state (Angel *et al.* 2011; Andrés &
344 Coupland 2012; Song *et al.* 2013). Therefore, plants have a seasonal timer based on binary switching,
345 regulated by chromatin accessibility, which is dependent on duration of cold exposure.

346 As stated earlier, on natural photoperiod in sheep TSHβ begins to decline whilst still on LP (Lomet *et*
347 *al.* 2018), similar results have been seen in Siberian hamsters but only assessed at the DIO2 level
348 (Petri *et al.* 2016). Comparing the gonadal responses of quail and starlings, quail faithfully track the
349 natural photoperiod, but starlings begin to regress their gonads whilst on LP (Dawson 2015). This
350 suggests that LP induction leads to a simultaneous activation but also an initiation of long-term
351 repressive process leading to the LP refractory state (Dawson 2015). If this is the case we need to
352 consider how cells are primed to respond to an LP signal and how they are eventually "shut-down". I
353 propose two possibilities: 1. Chromatin is maintained in a permissive or primed state and the
354 thyrotrophs can always respond to an LP signal if the transcription factors are present, or, 2.

Chromatin accessibility is limited to prevent early responses to LP signals and/or to shut down responses after a prolonged period in LP.

Possibility 1 is based on cell fate the adult intestine. Here, the positioning of the cell in the crypt determine the cell fate and the cells exist as multipotent stem cells, with a broadly permissive chromatin state, meaning that there are multiple possible pathways of differentiation depending on the transcription factors present. Therefore, chromatin state is not the determining factor for cell fate, merely permissive to either fate depending on the transcription factors available (Paksa & Rajagopal 2017). If in the PT thyrotroph, the chromatin was maintained in a permissive state, as in the intestine, then you would expect all PT thyrotrophs to respond at the same time to any LP signal regardless of the circannual phase. This seems unlikely as there is a known requirement for a winter in order to respond appropriately to an LP signal and the observations of a progressive tissue level response do not support this.

Possibility 2 is based on a vernalisation model where there would be preparation or priming of PT thyrotrophs to respond to LP over winter, and/or, the initiation of a longer-term repressive process on LP leading to the disappearance of TSHβ cells. The targets of this proposed limited chromatin accessibility could be EYA3 or regulators of EYA3. Priming to over winter suggests a prolonged winter would synchronise all cells to respond immediately to LP. Given the earlier discussions of the importance of a robust, non-spurious response it seems possible that even when primed these cells will have different sensitivity thresholds and therefore individual variance in responsiveness, as demonstrated in cell fate determination in development (Eldar & Elowitz 2010). Differences in individual cell sensitivity to signals has also been shown in *Dictyostelium* (slime mould), here, "cells exhibit different intrinsic response biases or discrete transcriptional activation thresholds to signals" resulting in salt and pepper differentiation (Chattwood *et al.* 2013).

Whether timing is conferred by cyclical changes in chromatin accessibility remains to be demonstrated, and it is possible that, upstream regulators of specific transcription factors, not the known key circuits, are targets of this cyclical chromatin remodelling. Evidence for seasonal changes in epigenetic state within the PT are limited to the identification of differentially regulated transcripts of known epigenetic modulators (Wood *et al.* 2015; Lomet *et al.* 2018). EZH2, a member of the PRC2 complex that lays down the repressive H3K27me3 mark during development, is up-regulated in the PT in LP (Wood *et al.* 2015; Lomet *et al.* 2018)(Fig. 4d). EZH2 is required for proper differentiation of a mature lung secretory cell population during development (Snitow *et al.* 2015), potentially indicating a role for EZH2 in a seasonal cycle of differentiation through changes in chromatin state. 19 other chromatin/histone modifiers were also identified as differentially expressed in the PT on LP,

these include the histone methyltransferase; SUV39H2 (also identified by (Lomet *et al.* 2018)), and, histone acetyltransferase; JADE3 (Fig. 4d)(Wood *et al.* 2015). Furthermore, through its phosphatase activity EYA3 promotes DNA repair through post-translational modification of H2A.X to allow recruitment of DNA repair factors (Cook *et al.* 2009). Characterisation of the chromatin state in the PT remains to be investigated in order to test these hypothesises.

As a final point, the role for the tanycytes in the 3rd ventricle (ependymal zone) should not be over looked. As discussed above the mothers photoperiodic history programmed the trajectory of the offsprings reproductive development and sensitivity to photoperiodic signals, through changes in sensitivity to TSH in the tanycytes not the PT (Sáenz de Miera *et al.* 2017). In sheep, the reproductive switch-off at the end of the winter breeding season can be blocked by thyroidectomy (THX) with the greatest transcriptional effects in the ependymal zone (Lomet *et al.* 2018). Furthermore, the hypothalamic region is a known stem cell niche and therefore the hypotheses presented here, may also be relevant for timing circuits in the ependymal zone. Related to this SHH a known developmental morphogen is expressed by the tanycytes in a seasonal manner, suggesting a role for seasonal differentiation either in the median emenence (ME) and/or morphogenic signals from the ME that may signal to the PT. This highlights the potential importance of the interaction between tanycytes and the PT in the generation of a seasonal rhythm and that mathematical modelling to consider the complexity of interactions involving sensitivity, cellular plasticity, PT/tanycyte crosstalk and long-term timing is required.

Conclusions

The phase of the seasonal cycle is defined by a binary switching of cellular phenotype of the PT-specific thyrotroph to TSHβ positive and the proportion of cells in that state. Therefore, it is hypothesised that the basis of seasonal rhythm generation is a cellular population based timer within the PT. A cellular population based timer is used to time seasonal flowering in plants. I propose that the adult PT is a plastic tissue, showing a seasonal cycle of differentiation, and that the underlying processes are likely to be epigenetic. Considering the mechanisms behind adult cellular plasticity offers a framework to hypotheses how a long-term timer functions within the PT. Finally, the PT may offer a unique tissue to explore cellular plasticity in an adult mammal, as no injury or disease state is required to initiate this process.

Funding

SHW is supported by the Tromsø forskningsstiftelse (TFS) starter grant TFS2016SW.

Acknowledgements

Thanks to Andrew Loudon (University of Manchester) for his mentorship and support since I started in this exciting field of research. I thank David Hazlerigg (University of Tromsø, Norway), for lengthy discussion on this topic, and refinement of the hypotheses.

Figure Legends

Figure 1

Retrograde action of TSH on ependymal cells in the hypothalamus. Photoperiod is encoded by the nocturnal melatonin signal that is sculpted by day length, generating short-duration signals in response to long photoperiod (LP) conditions. The prime site of action is the pituitary pars tuberalis. LP activation of TSHβ leads to an increase in deiodinase 2 activity in adjacent ependymal cells (tanycytes), which express the TSH receptor. This in turn leads to LD augmentation of T_3, via conversion from T_4. Rfamides (including KISS-1) and RF-related peptides serve as neuroendocrine intermediates in the regulation of reproduction across taxa and their regulation is altered in response to photoperiod (reviewed in; (Kriegsfeld 2006; Simonneaux *et al.* 2013)). It has shown that T_3 regulates the expression of RFRP and KISS-1 (Henson *et al.* 2013), potentially via TSH (Klosen *et al.* 2013) but the mechanism of T_3 action is uncertain. RFRP has been noted to have high expression in LP and a low expression in Short photoperiod (SP), regardless of breeding season. It has been suggested that RFRP subsequently acts either directly on GnRH neurons or indirectly *via* kisspeptin (kp) neurons or other interneurons in the arcuate nucleus (ARC) to synchronize reproduction with season in a species dependent manner (reviewed in: (Henningsen *et al.* 2016)).

Figure 2

Schematic representations of the annual photoperiod, short photoperiod (SP), long photoperiod (LP) and long photoperiod refractory (LP-R). Bars to represent the daily dark and light cycle on those photoperiods, and daily melatonin. Schematic of the clock gene, CRY1, in the ovine pars tuberalis on

LP and LP refractory animals (LP-R). Adapted from data in Lincoln et al (2005). In-situ hybridization of the ovine pars tuberalis on LP (4 weeks) and LP-R (LP 29 weeks) for TSHβ and EYA3, adapted from Wood et al (2015), and DIO2 (with permission from David Hazlerigg).

Figure 3

A. RNA-seq normalised counts per million displaying the amount of TSHb (purple) and CHGA (green) transcript in the first 4 weeks of LP; SP- short photoperiod, LPD1 – 1st day of long photoperiod, LPD7 – 7th day of long photoperiod and LPD28 – 28th day of long photoperiod. Data from Wood (2015).
B. In-situ hybridization quantification for TSHb (purple) after 4 weeks (LPW4), 16 weeks (LPW16) and 29 weeks (LPW29) of long photoperiod. Data from Wood (2015).
C. Schematics showing the difference in a single cell binary response, which can occur at a variable length of time from the LP switch and a tissue progressive response, slowly accumulating over time in LP.
D. The model proposes that an endogenous timer switches TSHβ expression in the PT thyrotroph cells, driving TSH and hypothalamic thyroid hormone metabolism independently of photoperiod. Individual PT thyrotroph cells are either in a long (TSHβ+) or short (CHGA+) photoperiod state, and the relative proportion of these binary-state cells determines the phase of the circannual cycle and the subsequent reproductive physiology. Adapted from: data in Wood et al (2015) and a figure in (Dardente 2015).

Figure 4

A. Seasonal remodelling of the *pars tuberalis*. Diagrams representing the tissue level changes are below. SP and the LP-R state are characterised by a FS cell network, separating individual

PT-specific thyrotrophs. LP is characterised by larger PT-thyrotrophs remodelled into an integrated network. Adapted from: Wood et al (2015).

B. RNA-seq normalised counts per million displaying the amount of TRIM2, KLF11 and EYA3 transcript in the first 4 weeks of LP; SP- short photoperiod, LPD1 – 1st day of long photoperiod, LPD7 – 7th day of long photoperiod and LPD28 – 28th day of long photoperiod. * denotes FDR less than 0.01. ** = 0.001, ***= 0.0001, **** = <0.00001. Data from Wood (2015).

C. A model for the binary switch. Demonstrating the differences between differentiation, de-differentiation, transdifferentiation and death and replacement.

D. RNA-seq normalised counts per million displaying the amount of EZH2, SUV39H2 and JADE3 transcript in the first 4 weeks of LP; SP- short photoperiod, LPD1 – 1st day of long photoperiod, LPD7 – 7th day of long photoperiod and LPD28 – 28th day of long photoperiod. * denotes FDR less than 0.01. ** = 0.001, ***= 0.0001, **** = <0.00001. Data from Wood (2015).

Declaration of interest: No conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

Aizawa S, Higaki Y, Dudaui A, Nagasaka M, Takahashi S, Sakata I & Sakai T 2016 Identification of marker genes for pars tuberalis morphogenesis in chick embryo: expression of Cytokine-like 1 and Gap junction protein alpha 5 in pars tuberalis. *Cell and Tissue Research* **366** 721–731. (doi:10.1007/s00441-016-2484-9)

Akimoto M, Nishimaki T, Arai Y, Uchinuma E, Yamauchi H & Kameda Y 2010 Hes1 regulates formations of the hypophyseal pars tuberalis and the hypothalamus. *Cell and Tissue Research* **340** 509–521. (doi:10.1007/s00441-010-0951-2)

Andrés F & Coupland G 2012 The genetic basis of flowering responses to seasonal cues. *Nature Reviews. Genetics* **13** 627–639. (doi:10.1038/nrg3291)

497 Angel A, Song J, Dean C & Howard M 2011 A Polycomb-based switch underlying quantitative
498 epigenetic memory. *Nature* **476** 105–108. (doi:10.1038/nature10241)

499 Ashe HL 2006 The interpretation of morphogen gradients. *Development* **133** 385–394.
500 (doi:10.1242/dev.02238)

501 Bank JHH, Cubuk C, Wilson D, Rijntjes E, Kemmling J, Markovsky H, Barrett P & Herwig A 2017 Gene
502 expression analysis and microdialysis suggest hypothalamic triiodothyronine (T3) gates daily
503 torpor in Djungarian hamsters (Phodopus sungorus). *Journal of Comparative Physiology B* **187**
504 857–868. (doi:10.1007/s00360-017-1086-5)

505 Beymer M, Henningsen J, Bahougne T & Simonneaux V 2016 The role of kisspeptin and RFRP in the
506 circadian control of female reproduction. *Molecular and Cellular Endocrinology* **438** 89–99.
507 (doi:10.1016/j.mce.2016.06.026)

508 Billings HJ, Viguié C, Karsch FJ, Goodman RL, Connors JM & Anderson GM 2002 Temporal
509 requirements of thyroid hormones for seasonal changes in LH secretion. *Endocrinology* **143**
510 2618–2625. (doi:10.1210/endo.143.7.8924)

511 Bittman EL, Karsch FJ & Hopkins JW 1983 Role of the Pineal Gland in Ovine Photoperiodism:
512 Regulation of Seasonal Breeding and Negative Feedback Effects of Estradiol upon Luteinizing
513 Hormone Secretion *. *Endocrinology* **113** 329–336. (doi:10.1210/endo-113-1-329)

514 Butler MP, Turner KW, Park JH, Schoomer EE, Zucker I & Gorman MR 2010 Seasonal regulation of
515 reproduction: altered role of melatonin under naturalistic conditions in hamsters. *Proceedings.*
516 *Biological Sciences / The Royal Society* **277** 2867–2874. (doi:10.1098/rspb.2010.0396)

517 Cai S, Fu X & Sheng Z 2007 Dedifferentiation: A New Approach in Stem Cell Research. *BioScience* **57**
518 655–662. (doi:10.1641/B570805)

519 Chattwood A, Nagayama K, Bolourani P, Harkin L, Kamjoo M, Weeks G & Thompson CR 2013
520 Developmental lineage priming in Dictyostelium by heterogeneous Ras activation. *ELife* **2**
521 e01067. (doi:10.7554/eLife.01067)

522 Cook PJ, Ju BG, Telese F, Wang X, Glass CK & Rosenfeld MG 2009 Tyrosine dephosphorylation of
523 H2AX modulates apoptosis and survival decisions. *Nature* **458** 591–596.
524 (doi:10.1038/nature07849)

525 Dardente H 2015 Circannual Biology: The Double Life of the Seasonal Thyrotroph. *Current Biology :*
526 *CB* **25** R988-91. (doi:10.1016/j.cub.2015.09.002)

527 Dardente H, Klosen P, Pévet P & Masson-Pévet M 2003 MT1 melatonin receptor mRNA expressing
528 cells in the pars tuberalis of the European hamster: effect of photoperiod. *Journal of*
529 *Neuroendocrinology* **15** 778–786.

530 Dardente H, Wyse CA, Birnie MJ, Dupré SM, Loudon ASI, Lincoln GA & Hazlerigg DG 2010 A molecular
531 switch for photoperiod responsiveness in mammals. *Current Biology : CB* **20** 2193–2198.
532 (doi:10.1016/j.cub.2010.10.048)

533 Dardente H, Hazlerigg DG & Ebling FJP 2014 Thyroid hormone and seasonal rhythmicity. *Frontiers in*
534 *Endocrinology* **5** 19. (doi:10.3389/fendo.2014.00019)

535 Dawson A 2015 Annual gonadal cycles in birds: modeling the effects of photoperiod on seasonal
536 changes in GnRH-1 secretion. *Frontiers in Neuroendocrinology* **37** 52–64.
537 (doi:10.1016/j.yfrne.2014.08.004)

538 Dunn IC, Wilson PW, Shi Y, Burt DW, Loudon ASI & Sharp PJ 2017 Diurnal and photoperiodic changes
539 in thyrotrophin-stimulating hormone β expression and associated regulation of deiodinase
540 enzymes (DIO2, DIO3) in the female juvenile chicken hypothalamus. *Journal of*
541 *Neuroendocrinology* **29** e12554. (doi:10.1111/jne.12554)

542 Ebenhöh O & Hazlerigg D 2013 Modelling a molecular calendar: The seasonal photoperiodic response
543 in mammals. *Chaos, Solitons & Fractals* **50** 39–47.

544 Eldar A & Elowitz MB 2010 Functional roles for noise in genetic circuits. *Nature* **467** 167–173.
545 (doi:10.1038/nature09326)

546 Featherstone K, Harper C V, McNamara A, Semprini S, Spiller DG, McNeilly J, McNeilly AS, Mullins JJ,
547 White MRH & Davis JRE 2011 Pulsatile patterns of pituitary hormone gene expression change
548 during development. *Journal of Cell Science* **124** 3484–3491. (doi:10.1242/jcs.088500)

549 Featherstone K, White MRH & Davis JRE 2012 The prolactin gene: a paradigm of tissue-specific gene
550 regulation with complex temporal transcription dynamics. *Journal of Neuroendocrinology* **24**
551 977–990. (doi:10.1111/j.1365-2826.2012.02310.x)

552 Featherstone K, Hey K, Momiji H, McNamara A V, Patist AL, Woodburn J, Spiller DG, Christian HC,
553 McNeilly AS, Mullins JJ *et al.* 2016 Spatially coordinated dynamic gene transcription in living
554 pituitary tissue. *ELife* **5** e08494. (doi:10.7554/eLife.08494)

555 Gordon BS, Delgado Díaz DC, White JP, Carson JA & Kostek MC 2012 Six1 and Six1 cofactor
556 expression is altered during early skeletal muscle overload in mice. *The Journal of Physiological*
557 *Sciences : JPS* **62** 393–401. (doi:10.1007/s12576-012-0214-y)

Gwinner E 1986 *Circannual Rhythms*. Berlin Heidelberg: Springer Verlag.

Hanon EA, Lincoln GA, Fustin J-M, Dardente H, Masson-Pévet M, Morgan PJ & Hazlerigg DG 2008 Ancestral TSH mechanism signals summer in a photoperiodic mammal. *Current Biology : CB* **18** 1147–1152. (doi:10.1016/j.cub.2008.06.076)

Harper C V, Finkenstädt B, Woodcock DJ, Friedrichsen S, Semprini S, Ashall L, Spiller DG, Mullins JJ, Rand DA, Davis JRE *et al.* 2011 Dynamic analysis of stochastic transcription cycles. *PLoS Biology* **9** e1000607. (doi:10.1371/journal.pbio.1000607)

Hazlerigg D & Loudon A 2008 New insights into ancient seasonal life timers. *Current Biology : CB* **18** R795–R804. (doi:10.1016/j.cub.2008.07.040)

Hazlerigg D & Simonneaux V 2015 Seasonal reproduction in mammals. In *Knobil and Neill's Physiology and Reproduction*, 4th ed, pp 1575–1660. Ed TP& AZ Eds.

Hazlerigg DG, Wyse CA, Dardente H, Hanon EA & Lincoln GA 2013 Photoperiodic variation in CD45-positive cells and cell proliferation in the mediobasal hypothalamus of the Soay sheep. *Chronobiology International* **30** 548–558. (doi:10.3109/07420528.2012.754450)

Henningsen JB, Gauer F & Simonneaux V 2016 RFRP Neurons - The Doorway to Understanding Seasonal Reproduction in Mammals. *Frontiers in Endocrinology* **7** 36. (doi:10.3389/fendo.2016.00036)

Henson JR, Carter SN & Freeman DA 2013 Exogenous T_3 elicits long day-like alterations in testis size and the RFamides Kisspeptin and gonadotropin-inhibitory hormone in short-day Siberian hamsters. *Journal of Biological Rhythms* **28** 193–200. (doi:10.1177/0748730413487974)

Hey KL, Momiji H, Featherstone K, Davis JRE, White MRH, Rand DA & Finkenstädt B 2015 A stochastic transcriptional switch model for single cell imaging data. *Biostatistics (Oxford, England)*. (doi:10.1093/biostatistics/kxv010)

Inoue M, Aizawa S, Higaki Y, Kawashima A, Koike K, Takagi H, Sakai T & Sakata I 2013 Detailed morphogenetic analysis of the embryonic chicken pars tuberalis as glycoprotein alpha subunit positive region. *Journal of Molecular Histology* **44** 401–409. (doi:10.1007/s10735-012-9479-y)

Jemc J & Rebay I 2007 Identification of transcriptional targets of the dual-function transcription factor/phosphatase eyes absent. *Developmental Biology* **310** 416–429. (doi:10.1016/j.ydbio.2007.07.024)

Johnston JD, Klosen P, Barrett P & Hazlerigg DG 2006 Regulation of MT melatonin receptor

588 expression in the foetal rat pituitary. *Journal of Neuroendocrinology* **18** 50–56.
589 (doi:10.1111/j.1365-2826.2005.01389.x)

590 Joost S, Zeisel A, Jacob T, Lö P, Linnarsson S, Kasper M, Sun X & Manno G La 2016 Single-Cell
591 Transcriptomics Reveals that Differentiation and Spatial Signatures Shape Epidermal and Hair
592 Follicle Heterogeneity. *Cell Systems* **3** 221–237. (doi:10.1016/j.cels.2016.08.010)

593 Jukam D & Desplan C 2010 Binary fate decisions in differentiating neurons. *Current Opinion in*
594 *Neurobiology* **20** 6–13. (doi:10.1016/j.conb.2009.11.002)

595 Jukam D, Xie B, Rister J, Terrell D, Charlton-Perkins M, Pistillo D, Gebelein B, Desplan C & Cook T 2013
596 Opposite Feedbacks in the Hippo Pathway for Growth Control and Neural Fate. *Science* **342**
597 1238016–1238016. (doi:10.1126/science.1238016)

598 Kameda Y, Miura M & Ohno S 1998 Localization and development of chromogranin A and luteinizing
599 hormone immunoreactivities in the secretory-specific cells of the hypophyseal pars tuberalis of
600 the chicken. *Histochemistry and Cell Biology* **109** 211–222.

601 Kay RR & Thompson CRL 2009 Forming Patterns in Development without Morphogen Gradients:
602 Scattered Differentiation and Sorting Out. *Cold Spring Harbor Perspectives in Biology* **1**
603 a001503–a001503. (doi:10.1101/cshperspect.a001503)

604 Khazaei MR, Bunk EC, Hillje A-L, Jahn HM, Riegler EM, Knoblich JA, Young P & Schwamborn JC 2011
605 The E3-ubiquitin ligase TRIM2 regulates neuronal polarization. *Journal of Neurochemistry* **117**
606 29–37. (doi:10.1111/j.1471-4159.2010.06971.x)

607 Kita A, Imayoshi I, Hojo M, Kitagawa M, Kokubu H, Ohsawa R, Ohtsuka T, Kageyama R & Hashimoto N
608 2007 Hes1 and Hes5 Control the Progenitor Pool, Intermediate Lobe Specification, and Posterior
609 Lobe Formation in the Pituitary Development. *Molecular Endocrinology* **21** 1458–1466.
610 (doi:10.1210/me.2007-0039)

611 Klosen P, Bienvenu C, Demarteau O, Dardente H, Guerrero H, Pévet P & Masson-Pévet M 2002 The
612 mt1 melatonin receptor and RORbeta receptor are co-localized in specific TSH-immunoreactive
613 cells in the pars tuberalis of the rat pituitary. *The Journal of Histochemistry and Cytochemistry :*
614 *Official Journal of the Histochemistry Society* **50** 1647–1657.

615 Klosen P, Sébert M-E, Rasri K, Laran-Chich M-P & Simonneaux V 2013 TSH restores a summer
616 phenotype in photoinhibited mammals via the RF-amides RFRP3 and kisspeptin. *FASEB Journal :*
617 *Official Publication of the Federation of American Societies for Experimental Biology* **27** 2677–
618 2686. (doi:10.1096/fj.13-229559)

619 Korf H-W 2018 Signaling pathways to and from the hypophysial pars tuberalis, an important center
620 for the control of seasonal rhythms. *General and Comparative Endocrinology* **258** 236–243.
621 (doi:10.1016/j.ygcen.2017.05.011)

622 Kozmik Z, Holland ND, Kreslova J, Oliveri D, Schubert M, Jonasova K, Holland LZ, Pestarino M, Benes V
623 & Candiani S 2007 Pax-Six-Eya-Dach network during amphioxus development: conservation in
624 vitro but context specificity in vivo. *Developmental Biology* **306** 143–159.
625 (doi:10.1016/j.ydbio.2007.03.009)

626 Kriegsfeld LJ 2006 Driving reproduction: RFamide peptides behind the wheel. *Hormones and Behavior*
627 **50** 655–666. (doi:10.1016/j.yhbeh.2006.06.004)

628 Kumar JP 2009 The molecular circuitry governing retinal determination. *Biochimica et Biophysica*
629 *Acta (BBA) - Gene Regulatory Mechanisms* **1789** 306–314. (doi:10.1016/j.bbagrm.2008.10.001)

630 Li Y, Donmez N, Sahinalp C, Xie N, Wang Y, Xue H, Mo F, Beltran H, Gleave M, Wang Y *et al.* 2017
631 SRRM4 Drives Neuroendocrine Transdifferentiation of Prostate Adenocarcinoma Under
632 Androgen Receptor Pathway Inhibition. *European Urology* **71** 68–78.
633 (doi:10.1016/j.eururo.2016.04.028)

634 Lincoln GA 2006 Melatonin Entrainment of Circannual Rhythms. *Chronobiology International* **23** 301–
635 306. (doi:10.1080/07420520500464452)

636 Lincoln GA & Clarke IJ 1994 Photoperiodically-Induced Cycles in the Secretion of Prolactin in
637 Hypothalamo-Pituitary Disconnected Rams: Evidence for Translation of the Melatonin Signal in
638 the Pituitary Gland. *Journal of Neuroendocrinology* **6** 251–260. (doi:10.1111/j.1365-
639 2826.1994.tb00580.x)

640 Lincoln G, Messager S, Andersson H & Hazlerigg D 2002 Temporal expression of seven clock genes in
641 the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal
642 coincidence timer. *Proceedings of the National Academy of Sciences of the United States of*
643 *America* **99** 13890–13895. (doi:10.1073/pnas.212517599)

644 Lincoln GA, Andersson H & Loudon A 2003 Clock genes in calendar cells as the basis of annual
645 timekeeping in mammals--a unifying hypothesis. *The Journal of Endocrinology* **179** 1–13.

646 Lincoln GA, Johnston JD, Andersson H, Wagner G & Hazlerigg DG 2005 Photorefractoriness in
647 mammals: dissociating a seasonal timer from the circadian-based photoperiod response.
648 *Endocrinology* **146** 3782–3790. (doi:10.1210/en.2005-0132)

649 Lincoln GA, Clarke IJ, Hut RA & Hazlerigg DG 2006 Characterizing a mammalian circannual pacemaker.

650 *Science (New York, N.Y.)* **314** 1941–1944. (doi:10.1126/science.1132009)

651 Loft A, Forss I, Siersbæk MS, Schmidt SF, Larsen A-SB, Madsen JGS, Pisani DF, Nielsen R, Aagaard MM,
652 Mathison A *et al.* 2015 Browning of human adipocytes requires KLF11 and reprogramming of
653 PPARγ superenhancers. *Genes & Development* **29** 7–22. (doi:10.1101/gad.250829.114)

654 Lomet D, Cognié J, Chesneau D, Dubois E, Hazlerigg D & Dardente H 2018 The impact of thyroid
655 hormone in seasonal breeding has a restricted transcriptional signature. *Cellular and Molecular*
656 *Life Sciences* **75** 905–919. (doi:10.1007/s00018-017-2667-x)

657 Majumdar G, Yadav G, Rani S & Kumar V 2014 A photoperiodic molecular response in migratory
658 redheaded bunting exposed to a single long day. *General and Comparative Endocrinology*.
659 (doi:10.1016/j.ygcen.2014.04.013)

660 Masumoto K, Ukai-Tadenuma M, Kasukawa T, Nagano M, Uno KD, Tsujino K, Horikawa K, Shigeyoshi
661 Y & Ueda HR 2010 Acute Induction of Eya3 by Late-Night Light Stimulation Triggers TSHβ
662 Expression in Photoperiodism. *Current Biology : CB* **20** 2199–2206.
663 (doi:10.1016/j.cub.2010.11.038)

664 Migaud M, Batailler M, Pillon D, Franceschini I & Malpaux B 2011 Seasonal changes in cell
665 proliferation in the adult sheep brain and pars tuberalis. *Journal of Biological Rhythms* **26** 486–
666 496. (doi:10.1177/0748730411420062)

667 Milesi S, Simonneaux V & Klosen P 2017 Downregulation of Deiodinase 3 is the earliest event in
668 photoperiodic and photorefractory activation of the gonadotropic axis in seasonal hamsters.
669 *Scientific Reports* **7** 17739. (doi:10.1038/s41598-017-17920-y)

670 Morgan PJ, Barrett P, Howell HE & Helliwell R 1994 Melatonin receptors: localization, molecular
671 pharmacology and physiological significance. *Neurochemistry International* **24** 101–146.

672 Murphy M, Jethwa PH, Warner A, Barrett P, Nilaweera KN, Brameld JM & Ebling FJP 2012 Effects of
673 Manipulating Hypothalamic Triiodothyronine Concentrations on Seasonal Body Weight and
674 Torpor Cycles in Siberian Hamsters. *Endocrinology* **153** 101–112. (doi:10.1210/en.2011-1249)

675 Nakane Y & Yoshimura T 2014 Universality and diversity in the signal transduction pathway that
676 regulates seasonal reproduction in vertebrates. *Frontiers in Neuroscience* **8** 115.
677 (doi:10.3389/fnins.2014.00115)

678 Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, Yasuo S, Katou Y, Kageyama S, Uno Y *et*
679 *al.* 2008 Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature* **452** 317–
680 322. (doi:10.1038/nature06738)

O'Neill MC & Stockdale FE 1972 Differentiation without cell division in cultured skeletal muscle. *Developmental Biology* **29** 410–418.

Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, Ebihara S, Korf H-W & Yoshimura T 2008 Involvement of thyrotropin in photoperiodic signal transduction in mice. *Proceedings of the National Academy of Sciences of the United States of America* **105** 18238–18242. (doi:10.1073/pnas.0808952105)

Paksa A & Rajagopal J 2017 The epigenetic basis of cellular plasticity. *Current Opinion in Cell Biology* **49** 116–122. (doi:10.1016/J.CEB.2018.01.003)

Petri I, Diedrich V, Wilson D, Fernández-Calleja J, Herwig A, Steinlechner S & Barrett P 2016 Orchestration of gene expression across the seasons: Hypothalamic gene expression in natural photoperiod throughout the year in the Siberian hamster. *Scientific Reports* **6** 29689. (doi:10.1038/srep29689)

Raetzman LT, Cai JX & Camper SA 2007 Hes1 is required for pituitary growth and melanotrope specification. *Developmental Biology* **304** 455–466. (doi:10.1016/j.ydbio.2006.11.010)

Ross AW, Helfer G, Russell L, Darras VM & Morgan PJ 2011 Thyroid hormone signalling genes are regulated by photoperiod in the hypothalamus of F344 rats. *PloS One* **6** e21351. (doi:10.1371/journal.pone.0021351)

Sáenz de Miera C, Hanon EA, Dardente H, Birnie M, Simonneaux V, Lincoln GA & Hazlerigg DG 2013 Circannual variation in thyroid hormone deiodinases in a short-day breeder. *Journal of Neuroendocrinology* **25** 412–421. (doi:10.1111/jne.12013)

Sáenz de Miera C, Monecke S, Bartzen-Sprauer J, Laran-Chich M-P, Pévet P, Hazlerigg DG & Simonneaux V 2014 A circannual clock drives expression of genes central for seasonal reproduction. *Current Biology : CB* **24** 1500–1506. (doi:10.1016/j.cub.2014.05.024)

Sáenz de Miera C, Bothorel B, Jaeger C, Simonneaux V & Hazlerigg D 2017 Maternal photoperiod programs hypothalamic thyroid status via the fetal pituitary gland. *Proceedings of the National Academy of Sciences* **114** 8408–8413. (doi:10.1073/pnas.1702943114)

Shen C-N, Burke ZD & Tosh D 2004 Transdifferentiation, metaplasia and tissue regeneration. *Organogenesis* **1** 36–44.

Simonneaux V, Ancel C, Poirel VJ & Gauer F 2013 Kisspeptins and RFRP-3 Act in Concert to Synchronize Rodent Reproduction with Seasons. *Frontiers in Neuroscience* **7** 22. (doi:10.3389/fnins.2013.00022)

712 Snitow ME, Li S, Morley MP, Rathi K, Lu MM, Kadzik RS, Stewart KM & Morrisey EE 2015 Ezh2
713 represses the basal cell lineage during lung endoderm development. *Development* **142** 108–
714 117. (doi:10.1242/dev.116947)

715 Song J, Angel A, Howard M & Dean C 2012 Vernalization - a cold-induced epigenetic switch. *Journal*
716 *of Cell Science* **125** 3723–3731. (doi:10.1242/jcs.084764)

717 Song J, Irwin J & Dean C 2013 Remembering the prolonged cold of winter. *Current Biology : CB* **23**
718 R807-11. (doi:10.1016/j.cub.2013.07.027)

719 Stoeckel E, Hindelang C, Klein M-J, Poissonnier M & Félix J-M 1993 Early Expression of the
720 Glycoprotein Hormone Alpha-Subunit in the Pars Tuberalis of the Rat Pituitary Gland during
721 Ontogenesis. *Neuroendocrinology* **58** 616–624. (doi:10.1159/000126600)

722 Tadjuidje E & Hegde RS 2013 The Eyes Absent proteins in development and disease. *Cellular and*
723 *Molecular Life Sciences : CMLS* **70** 1897–1913. (doi:10.1007/s00018-012-1144-9)

724 Teo AKK, Lim CS, Cheow LF, Kin T, Shapiro JA, Kang N-Y, Burkholder W & Lau HH 2018 Single-cell
725 analyses of human islet cells reveal de-differentiation signatures. *Cell Death Discovery* **4** 14.
726 (doi:10.1038/s41420-017-0014-5)

727 West AC & Wood SH 2018 Seasonal physiology: making the future a thing of the past. *Current*
728 *Opinion in Physiology* **5** 1–8. (doi:10.1016/j.cophys.2018.04.006)

729 Wood S & Loudon A 2014 Clocks for all seasons: unwinding the roles and mechanisms of circadian
730 and interval timers in the hypothalamus and pituitary. *The Journal of Endocrinology* **222** R39-59.
731 (doi:10.1530/JOE-14-0141)

732 Wood S & Loudon A 2017 The pars tuberalis: The site of the circannual clock in mammals? *General*
733 *and Comparative Endocrinology* **258** 222–235. (doi:10.1016/j.ygcen.2017.06.029)

734 Wood SH, Christian HC, Miedzinska K, Saer BRC, Johnson M, Paton B, Yu L Le, McNeilly J, Davis JRE,
735 McNeilly AS *et al.* 2015 Binary switching of calender cells in the pituitary defines the phase of
736 the circannual cycle in mammals. *Current Biology* **25**. (doi:10.1016/j.cub.2015.09.014)

737 Woodfill CJ, Wayne NL, Moenter SM & Karsch FJ 1994 Photoperiodic synchronization of a circannual
738 reproductive rhythm in sheep: identification of season-specific time cues. *Biology of*
739 *Reproduction* **50** 965–976.

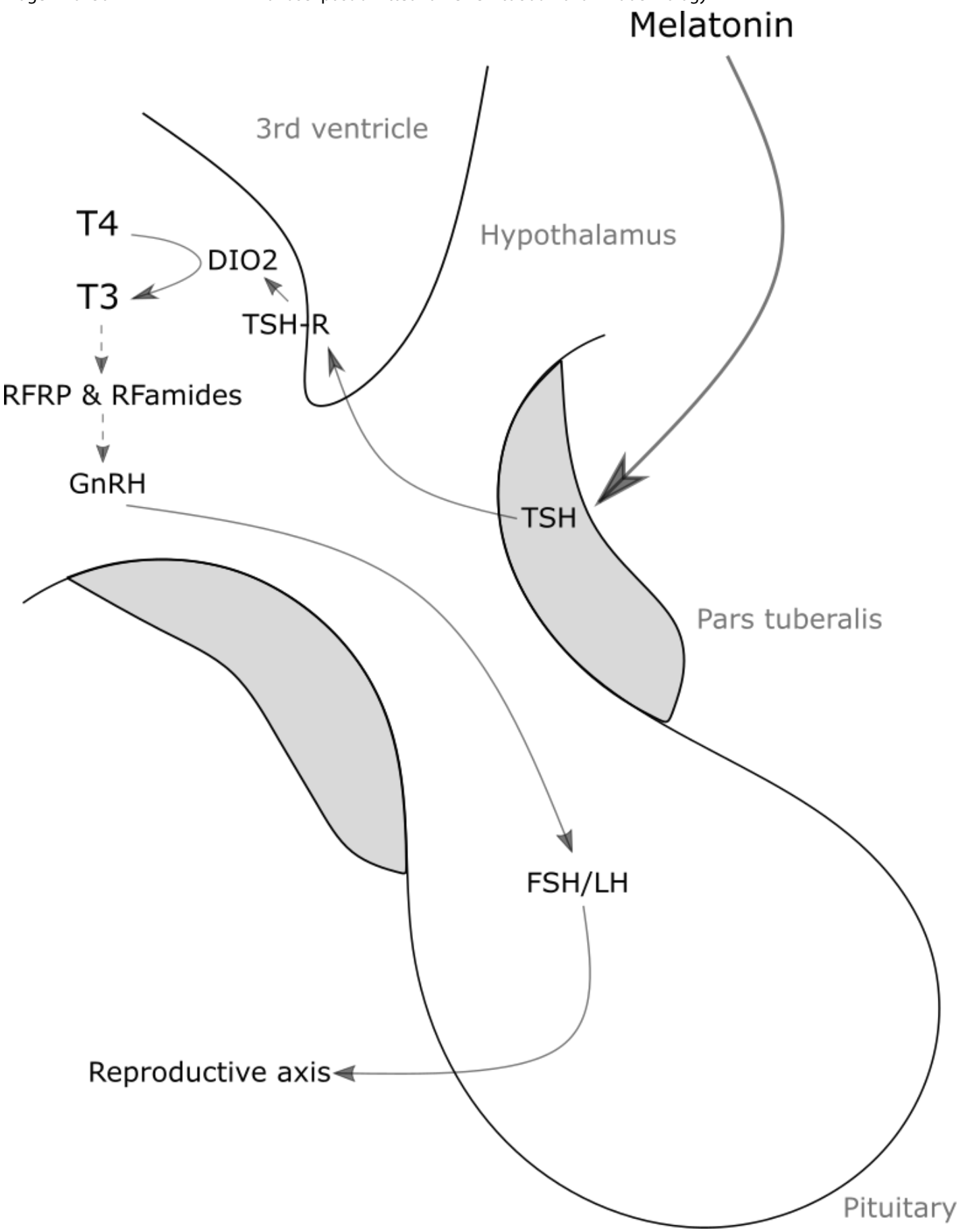
740 Xie J, Wang W-Q, Liu T-X, Deng M & Ning G 2008 Spatio-temporal expression of chromogranin A
741 during zebrafish embryogenesis. *Journal of Endocrinology* **198** 451–458. (doi:10.1677/JOE-08-

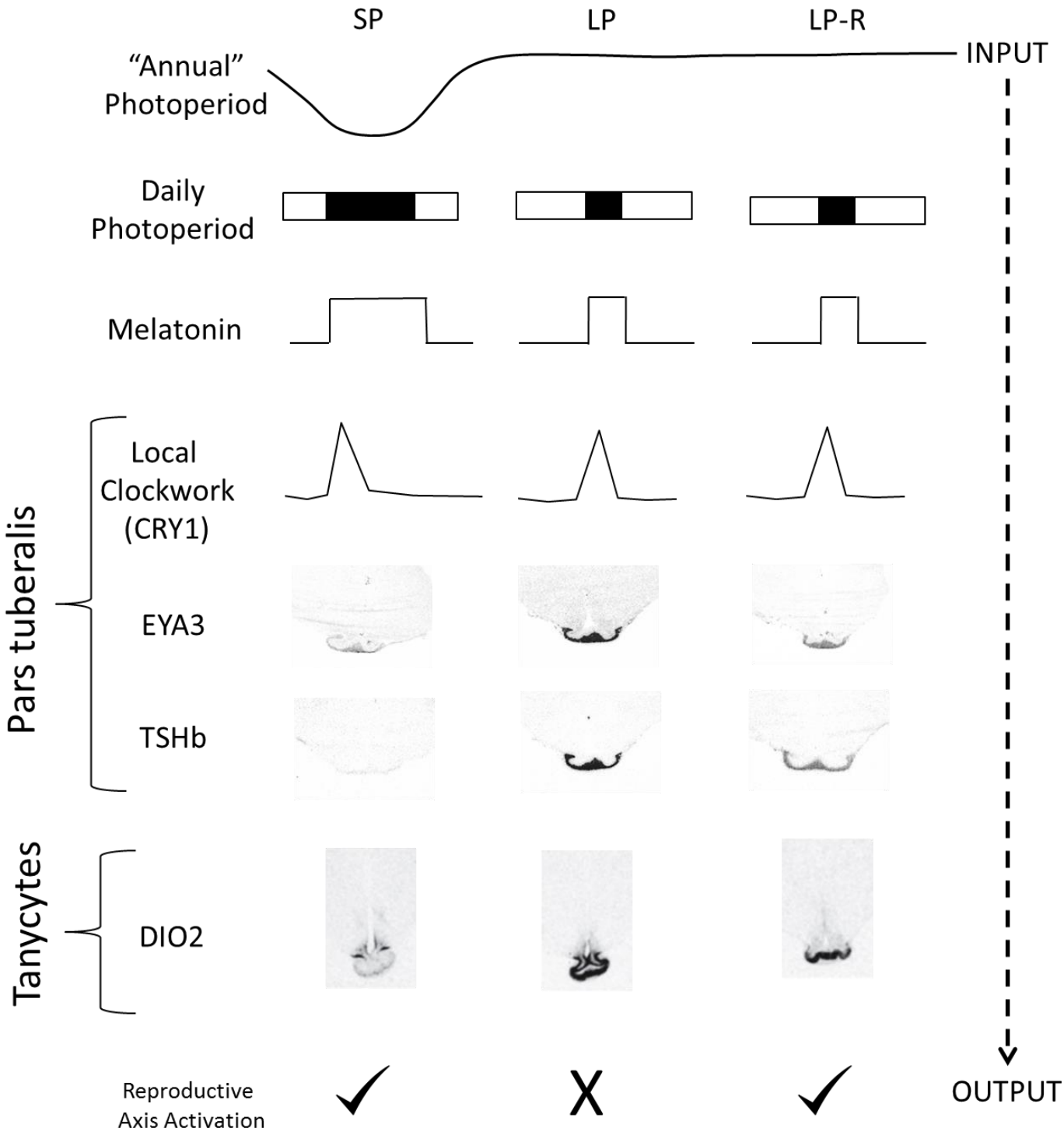
742 0221)

743 Xu PX, Cheng J, Epstein JA & Maas RL 1997 Mouse Eya genes are expressed during limb tendon
744 development and encode a transcriptional activation function. *Proceedings of the National*
745 *Academy of Sciences of the United States of America* **94** 11974–11979.

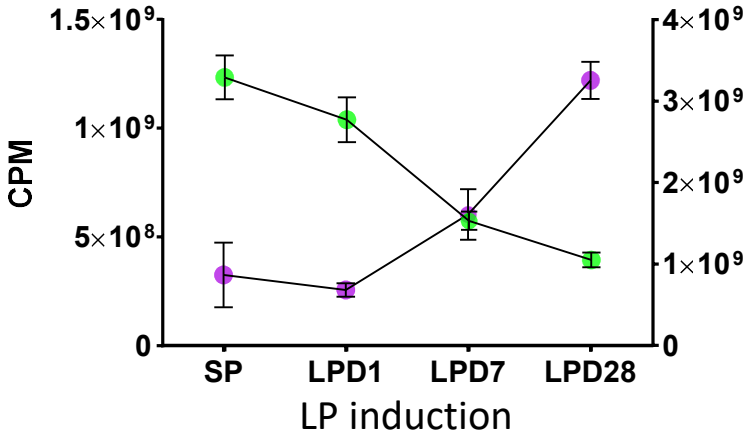
746 Yang Y, Tung JW, Ghosn EEB, Herzenberg LA & Herzenberg LA 2007 Division and differentiation of
747 natural antibody-producing cells in mouse spleen. *Proceedings of the National Academy of*
748 *Sciences* **104** 4542–4546. (doi:10.1073/pnas.0700001104)

749

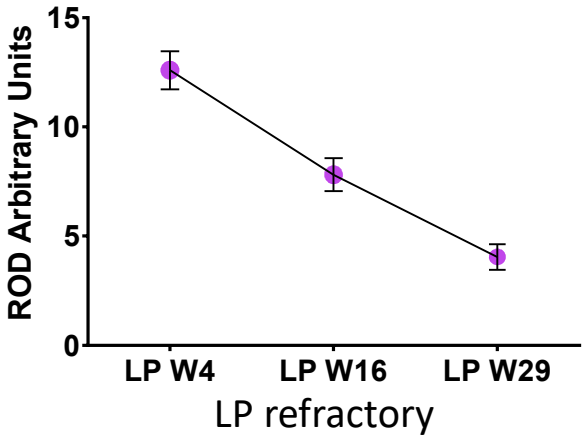

Melatonin
3rd ventricle
Hypothalamus
T4
DIO2
T3
TSH-R
RFRP & RFamides
GnRH
TSH
Pars tuberalis
FSH/LH
Reproductive axis
Pituitary


SP
LP
LP-R
INPUT
"Annual" Photoperiod
Daily Photoperiod
Melatonin
Pars tuberalis
Local Clockwork (CRY1)
EYA3
TSHb
Tanycytes
DIO2
Reproductive Axis Activation
OUTPUT

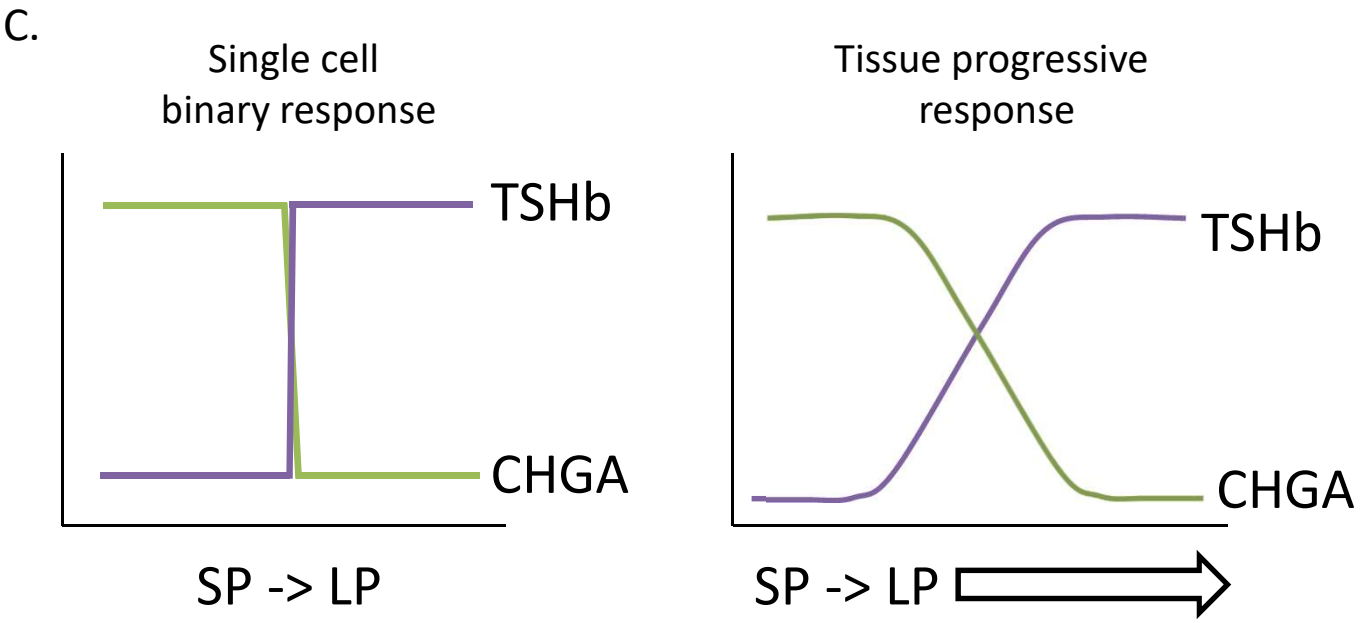
A.



B.



C.



D.