Encoding and Decoding Photoperiod in the Mammalian Pars Tuberalis

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Key Words

Photoperiodism • Melatonin • MT1 • Pars tuberalis • Prolactin

Abstract

In mammals, the nocturnal melatonin signal is well established as a key hormonal indicator of seasonal changes in day-length, providing the brain with an internal representation of the external photoperiod. The pars tuberalis (PT) of the pituitary gland is the major site of expression of the G-coupled receptor MT1 in the brain and is considered as the main site of integration of the photoperiodic melatonin signal. Recent studies have revealed how the photoperiodic melatonin signal is encoded and conveyed by the PT to the brain and the pituitary, but much remains to be resolved. The development of new animal models and techniques such as cDNA arrays or high throughput sequencing has recently shed the light onto the regulatory networks that might be involved. This review considers the current understanding of the mechanisms driving photoperiodism in the mammalian PT with a particular focus on the seasonal prolactin secretion.

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Introduction

Animals living in seasonal environments are faced with predictable environmental challenges of cycles of food availability and climatic variability, which require

that they adjust their physiology over the year in order to survive. As a consequence, seasons of birth are tightly constrained to the optimal time of year, and annual fattening, metabolism and cycles of growth are generally matched to annual changes in food availability. Such physiological processes require long-term predictive changes in neuroendocrine circuits. For instance, reactivation of the reproductive system is a process that takes weeks to months, and the timing of conception also needs to be adjusted to ensure birth patterns are matched to the optimal season, when environmental conditions are the most favourable to raise the offspring. Therefore, tracking but also anticipating changes in day length are important features of photoperiodic time measurement. Various models, including the external and the internal coincidence models (reviewed in [1, 2]), have been proposed to describe photoperiodic time measurement mechanisms. In the external coincidence model, light entrains the photoperiodic response and triggers seasonal responses only when present during a particular window of time (or photo-inductive phase). The internal coincidence model on the other hand infers the existence of two oscillators both entrained by light, tracking dawn and dusk; in this model photoperiod is represented by the interval between these two oscillators. Current knowledge on photoperiodic time measurement in mammals cannot rule out either of these models suggesting they are non-exclusive.

Photoperiodic responses were first observed in insects and birds in the 1920s and about a decade later in mam-

Fig. 1. Seasonal regulation of prolactin in mammals. In this simplified cartoon, light signalling through the eyes inhibits the activity of the pineal gland and resets the circadian activity of the SCN which in turn controls the activity of the pineal gland. This leads to an exclusive nocturnal secretion of melatonin which duration signal (short in LP and long in SP) is read by the PT. The

photoperiodic information is then conveyed to the AP to control the seasonal production and release of PRL. The PT is also known to convey retrograde photoperiodic signals towards the hypothalamic third ventricle to control T_3 -dependent photoperiodic reproductive responses [104].

mals (reviewed in [1]). These responses can vary among species and include, among others, seasonal regulation of reproduction, thermoregulation, hibernation, migration behaviour, fattening and metabolic cycles and pelage growth and development. Mechanisms underlying mammalian seasonal physiology are complex and diverse with different species using seasonal timing to their own advantage. This is exemplified by the distinction between photoperiodic and circannual species (reviewed in [3, 4]). In the later, an internal circannual timer allows the animals to successively go through periods of quiescent and active reproductive activity when exposed to regular photoperiodic conditions. On the other hand, short lived species do not need such a long term mechanism, they respond quickly to the external photoperiod which in turn resets their physiology. Photoperiodic species have the ability to revert to an active reproductive state when exposed for a long duration to short photoperiod (SP), a process called refractoriness and reviewed in Morgan et al. [5].

In mammals, the nocturnal secretion from the pineal gland of the hormone melatonin is tightly regulated by the external light-dark cycle. Melatonin provides the brain with an internal endocrine representation of the external photoperiod, accurately tracking seasonal changes in night length. This hormone of darkness is a major internal cue which acts on neuroendocrine circuits and its function in regulating seasonal physiology and

seasonal breeding has been extensively studied (reviewed in [6]). The description of sites of action of melatonin in the brain in the 1990s lead to the identification of a little studied proximal region of the pituitary gland, the pars tuberalis (PT) (reviewed in [7]). We now know that the PT is a major site of action of melatonin for the regulation of mammalian seasonal physiology [7, 8]. This review focuses on the role of the photoperiodic processes driven by melatonin with a particular emphasis on the seasonal regulation of prolactin (PRL) secretion (fig. 1).

Melatonin, an Endocrine Index for Day Length

Melatonin Synthesis and Mode of Action

The main source of circulating melatonin (5-methoxy N-acetyltryptamine) is the pineal gland, specialised to metabolise indoleamines from the essential amino-acid tryptophan. The uptake of tryptophan in pinealocytes leads to the formation of melatonin through a 4-step metabolic pathway involving arylalkylamine-N-acetyltransferase (AA-NAT) and N-acetylserotonin O-methyltransferase (ASMT, also known as HIOMT). AA-NAT activity is inhibited by direct light input from the retino-hypothalamic tract and regulated by the circadian control of the suprachiasmatic nuclei (SCN) via sympathetic outflow and controls the timing of melatonin production,

while ASMT controls the absolute levels of melatonin produced during the night. Melatonin production is therefore under tight regulation, and its release at night, in both nocturnal and diurnal species, provides an indication of the time of the day to the different organs/tissues in the body, including the SCN (reviewed in [9]) (fig. 1). With a secretion pattern inverse to the duration of the light phase, melatonin also provides a good endocrine index of the external photoperiod [1] with long or short duration signals in SP or long photoperiods (LP), respectively, and has a major role in regulating seasonal physiology (reviewed in [6, 10]). The release of melatonin in the bloodstream and the cerebro-spinal fluid (CSF) [11], and its lipophilic nature are responsible for the pleiotropic effects of melatonin in diverse areas of the brain and peripheral tissues [12].

Three melatonin receptors have been identified: MT1, MT2 and Mel1c, all belonging to the GPCR family. MT1 and MT2 are both found in vertebrates although MT2 isn't functional in some species [13]. Mel1c is found in chicken, fish and xenopus only [14]. GPR50, an orphan GPCR has recently been characterised as the mammalian ortholog of Mel1c [15].

Melatonin and Photoperiodism

The importance of melatonin in the seasonal regulation of animal physiology and behaviour has been extensively reviewed [6, 16, 17]. The role of melatonin as a neuroendocrine timing signal was first defined in experiments which involved pinealectomy and timed melatonin replacement in hamsters and sheep (reviewed in [1]), showing that the duration of the melatonin signal is a photoperiodic cue, with long duration signal providing a SP-like response (reviewed in [18]). Studies involving targeted administration of melatonin in diverse areas of the brain determined that these effects were through a region within or surrounding the hypothalamus; however, the exact target of melatonin in the brain responsible for its seasonal effect was first unravelled with the description of a peculiar pattern in melatonin binding sites. 2-[¹²⁵I]-iodomelatonin binding sites were characterised in the SCN, supporting the role of melatonin in resetting the circadian clock and other regions of the hypothalamus. However, the binding observed in these areas was much lower than that observed in a discrete region of the pituitary: the PT (reviewed in [7]). The cloning of melatonin receptors (described above) confirmed the confined expression of MT1 within the PT in mammals, suggesting the central role for the PT in relaying the melatonin photoperiodic signal.

PT, a Melatonin Target Tissue to Control Photoperiodic Responses

The PT takes its origin from the lateral lobes of the Rathke's pouch to form a distinct region from the PD and PI [19]. Many lines of evidences support now the concept that the PT is an entity of its own from a developmental, structural and functional point of view.

Development and Ultrastructure of the PT

Ultrastructural studies during development in rodents provided important insights in the now well-described characteristics of the PT such as the presence of glycogen-like particles, secretory cells and an early differentiation during neonatal life [20]. The formation of the PT has recently been shown to involve the bHLH transcription factor Hes1 (hairy enhancer of split), indeed the anterior pituitary of *Hes1*^{-/-} mice is only represented by the PD with the PT failing to develop from the primordium [21]. Moreover, the pituitary specific transcription factor, Pit-1, is not expressed in the PT-specific cells [22–24]. These data clearly demonstrate that the PT and PD are two distinct tissues early on during their ontogeny. During embryonic development, the PT already shows most of its adult cytological features at the time that the PD starts to show signs of secretory activity, and reaches its final cellular organisation around birth. This precocious secretory differentiation was described as a 'striking feature' of the PT [20] and the description of the PT as the most vascularised region of the pituitary already suggested at the time an important functional role for the PT.

In all species studied, three PT cells types have been distinguished: follicular cells, PT-specific secretory cells and PD-like cells (the later originating from the PD and found in the zona tuberalis) (see review in Wittkowski et al. [27]). Recent studies in the rat have characterised up to five different secretory cell types [25]. In the same studies, the authors described the follicular cells as cistern-like structures making close contact with the PT-specific cells, portal capillaries and tanocytes; this complex cellular network highly supports a central role for the PT in processing information to and from the brain and periphery. The characterisation of secretory cell types in the PT suggested that this role was of an endocrine type, a hypothesis supported by immuno-histological evidences in many animal species showing the presence of gonadotrophs and thyrotrophs, morphologically different from the PD cells. PT cells express many of the PD hormone subunits with variations among different species [26–29]. β TSH and the alpha common subunit (α GSU) are found

at the protein and mRNA levels in PT-specific cells of many species (reviewed in [29–31]), but unlike in the PD, they are not regulated by TRH or thyroid hormones primarily because of the lack of expression of their respective receptors [22].

MT1 Expression and Photoperiodic Response of the PT

The PT is a dynamic tissue responding to the external photoperiod relayed by melatonin. Ultra structural analyses of the PT have demonstrated a seasonal-dependent change in morphology and secretory activity [32–35]. Similarly, melatonin administration induces SP-like modifications in the PT [36, 37]. These anatomical modifications associated with changes in photoperiod and melatonin signal duration are paralleled with changes in immuno-reactivity and/or mRNA expression of β TSH and α GSU in PT specific cells (in Siberian hamsters [38–41], in European hamsters [42, 43], in sheep [29, 44], and in mice [45]).

The specific expression of the melatonin receptor, MT1, is another characteristic feature of the PT. Melatonin binding sites can be characterised in the PT before the development of the hypophyseal-portal system during ontogenesis (reviewed in [30]). MT1 is exclusively found in the adult PT-specific cells and zona tuberalis, in most species studied [31, 46]. However, during development, MT1 is transiently expressed in gonadotrophs, before the maturation of the GnRH system [47]. Based on this observation, it was proposed, even though the PT matures very quickly, that it might remain undifferentiated, offering greater plasticity and allowing rapid responses and adaptations to different stimuli such as a change in photoperiod (reviewed in [18]).

Studies in rats and hamsters show that MT1 is co-expressed with β TSH and α GSU in PT-specific cells while no co-expression has been revealed to β LH or β FSH expressing cells [24, 31, 42]. The above description of the suppression of MT1 expression in the embryonic gonadotrophs was shown to be mediated by the induction of the transcription factor Egr1 [47]. Interestingly, Egr1 was shown to repress MT1 and stimulate β LH expression in these cells [47], providing an explanation to the lack of expression β LH in the PT-specific cells which are defined as Pit-1-independent thyrotrophs [24]. MT1 expression is regulated by melatonin in the PT-specific cells [31, 42]. Melatonin can therefore self-regulate its actions by down-regulating MT1 at the mRNA and protein levels leading to a progressive loss of melatonin signalling during the dark phase [48].

First, the early development of the PT combined with the specific expression of MT1 suggests an important role for melatonin during neonatal life [49]. Indeed, a correlation between changes in prenatal photoperiodic exposure and gonadal development or gene expression in the PT have been described [50–54]. Second, the fact that Siberian hamsters, a highly photoperiodic species, do not have a functional MT2 [13] and observations from MT1 and MT2 knock-out mice, clearly emphasises the specific role of MT1 in mediating photoperiodic responses to melatonin [55].

The Seasonal Regulation of PRL Is Driven by PT PRL-Releasing Factor(s)

Melatonin was shown to regulate reproduction and PRL by targeting the hypothalamus and the PT (reviewed in [56]). Moreover, the seasonal changes in PRL secretion are unlikely to be directly responsible for the seasonal change in reproduction [57], further emphasising this dual site of action for melatonin in regulating photoperiodic physiology. The effect on the hypothalamus to regulate reproduction has been reviewed elsewhere [6], here is summarised the current knowledge on the seasonal regulation of the pituitary hormone PRL (fig. 1).

Rhythmic PRL secretion, with high and low circulating concentrations in the summer (LP) and winter (SP), respectively, is an important feature of seasonal physiology and is observed in both SP and LP breeders (reviewed in [58]). Strong evidence shows that the melatonin duration signal does not target the PD [59] or hypothalamic factors such as dopamine [60, 61] to regulate the seasonal PRL rhythm. Indeed the PT is the major tissue target in this process (reviewed in [56, 62]) (fig. 1). The central role of the PT was clearly demonstrated in vivo in sheep, by Lincoln and Clarke [60]. In their studies, they surgically disconnected the pituitary from the hypothalamus *via* the insertion of an aluminium sheet, abrogating neuronal input from the hypothalamus to the pituitary and leaving intact the pituitary and its blood supply. Alternative exposure to LP and SP of these hypothalamo-pituitary disconnected (HPD) rams lead to a seasonal PRL rhythm associated with seasonal moult. Moreover, melatonin implants in LP-exposed HPD rams suppressed PRL secretion as shown in the sham controls. This is compatible with the hypothesis that melatonin directly regulates the seasonal PRL rhythm at the level of the pituitary without action on hypothalamic circuits (reviewed in [56]).

The identification of factors, termed tuberalin(s) [63, 64], secreted by the PT and responsible for the seasonal stimulatory effect on PRL secretion from the PD has not

yet been fully determined (reviewed in [30]). Under LP conditions, secretion of many proteins from the ovine PT, ranging from 14 to 100 kDa, was enhanced by forskolin, an effect reversed by melatonin [65]. Further purifications of these products lead to the description of very small (as small as 1 kDa) uncharacterised PRL secretagogues produced in the PT (reviewed in [66]). It is noteworthy that actinomycin D does not affect the secretion of forskolin-stimulated PT-specific proteins, suggesting the involvement of post-translational events [67]. In vitro experiments confirmed that in LP conditions, the PT secretes products which can stimulate PRL secretion from PD cells [59, 64, 68]. Moreover, these experiments have also shown that the response of the PD to this LP signal is also photoperiodic, revealing the existence of a photoperiodic memory within the PD itself [59, 69, 70].

The nature of PRL-releasing factor(s) driving the photoperiodic response of PRL secretion in seasonal mammals has yet to be established. Free α GSU, known to stimulate PRL [71, 72] and shown to be seasonally regulated in the PT, has been considered as a potential candidate but differences in expression observed between sheep and hamsters (upregulated or downregulated protein expression in LP, respectively) do not support its role as tuberalin (reviewed in [30]). However, its implication can still be considered, as its potential dimerization with a PT-specific β subunit has not yet been fully investigated. This hypothesis can be supported by the description of a PT-specific secretory product: tuberalin II, characterised from bovine PT extracts and suggested to be a PT-specific β -glycoprotein [73]. Recently, cDNA array studies have been performed on PT collected from sheep maintained on LP or SP conditions and from these novel candidate PRL-releasing peptides identified. These include substance P and neurokinin A, members of the tachykinin family, both of which are known regulators of PRL secretion. Both have the correct molecular weight for proposed tuberalins and their expression have previously been characterised in the PT [74]. The expression of the *TAC1* gene encoding these peptides is progressively upregulated in the sheep PT following LP exposure [75], which parallels the progressive rise in PRL secretion. Intriguingly, the expression of the neurokinin receptors (NK1,2 and 3R) in the sheep PD is not observed in PRL expressing cells (lactotrophs), suggesting the involvement of indirect pathways perhaps via gonadotrophs or folliculo-stellate cells (FCS), as both of them express neurokinin receptors. The recent description of an endocannabinoid system in the PT in mammals showed that non-peptidergic candidate should also be considered [76]. In these studies, the

authors described an increased concentration of 2-arachidonoyl glycerol (2-AG) in the PT under LP conditions, and the ability of 2-AG to induce PRL secretion from cultures of PD cells. Here again, the cannabinoid receptor, CB1, is not found in the lactotrophs but mostly in the FCS. The implication of the endocannabinoids system in photoperiodism has been reviewed elsewhere [76]. In both this study and the work described above on tachykinins, intermediate cell types (such as gonadotrophs and FCS) within the PD are likely to be involved in mediating the seasonal PRL regulation. This hypothesis is supported by the description of seasonally regulated paracrine interactions between gonadotrophs and lactotrophs [77] and seasonal secretory activity of FCS [78] which are known to influence PRL secretion [79]. Thus, photoperiodic signalling to the lactotrophs may operate *via* intermediary pituitary cell types.

Molecular Photoperiodic Code in the PT

Melatonin Signal Transduction Pathways in the PT

A number of studies have shown that melatonin acts as a potent regulator of cAMP in the PT. Various in vitro studies using PT dispersed cells or explants revealed that melatonin does not affect basal cAMP concentrations but is however a strong inhibitor of forskolin-induced cAMP accumulation (reviewed in [80]). In the PT, melatonin was shown to inhibit the classic transduction cascades induced by the accumulation of cAMP including PKA activity, CREB phosphorylation and MAPK activity (reviewed in [66, 81]). This effect is mainly mediated via coupling with a $G\alpha_i$ protein; however, pertussis toxin treatment only partially suppresses melatonin effects, suggesting a coupling with other $G\alpha$ proteins such as $G\alpha_z$ (reviewed in [30, 66]).

The absence of direct effects on the activity of the adenylate cyclase (AC) suggests that melatonin acts indirectly to inhibit the effects of an unknown stimulatory factor, termed 'StimX' [7] (fig. 2). Forskolin is a common tool used to activate AC and the nature of the endogenous factor responsible for the stimulation of the AC in the PT still remains unclear. Many factors such as adenosine have been investigated; however, in most cases melatonin do not inhibit their stimulatory effects on cAMP accumulation in the PT (reviewed in [30]). The nature of 'StimX' has been proposed to be a protein over 10 kDa, found in serum composition as inferred by studies in ovine PT primary cultures; there, serum activates cAMP and enhances Ca^{2+} , an effect that was found to be both

Fig. 2. Integration of the photoperiodic melatonin signal in PT-specific cells. The stimulation of AC by an unknown factor (StimX) [7] leads to an increase of intracellular cAMP responsible for the induction of cAMP-dependent gene transcription at dawn. At the onset of the dark phase, melatonin binds to its receptor MT1 which triggers the release of its coupled $G\alpha_i$ subunit and the inhibition of AC. As a consequence of MT1 activation, unknown

events presumably occurring *via* cAMP-independent pathways, lead to the transcriptional activation of a subset of genes at dusk. The transcriptional activation of specific set of genes, such as clock genes, at dusk and at dawn provides a local indication of the photoperiod [94]. The expression of *Eya3* in LP is thought to be a major switch which drives an LP response among which is the activation of *βTSH* [75, 102, 103].

melatonin and heat sensitive [82]. One possible candidate was considered to be the pituitary adenylate cyclase-activating polypeptide (PACAP), but PACAP receptors have only been characterised in the median eminence and not the PT [83]. Nevertheless, PACAP-dependent cAMP induction is inhibited by melatonin suggesting that PACAP acts on neighbouring cells of the ME to release a factor which is able to stimulate cAMP release in melatonin responsive PT cells.

An important characteristic of melatonin regulation of cAMP in the PT is the sensitisation mechanism observed after prolonged melatonin treatment, leading to an increased AC activity upon melatonin withdrawal (reviewed in [84]). This phenomenon is a strong feature of the PT since it does not occur in every cell type. Indeed, in the PT, sensitisation requires at least 4 h of exposure to melatonin and is established by 8 h while in other cell

types, such as CHO cells, sensitisation was shown to occur with as little as 1 h exposure [85]. The development of the melatonin duration-dependent sensitisation mechanism in the PT does not require protein synthesis, and was shown to involve the coupling with $G\alpha_i$ and a change in Tyrosine phosphorylation of the AC [86, 87].

Molecular Encoding of the Photoperiodic Signal within the PT

The expression of clock genes in the PT was first demonstrated by the description of the mouse Period gene (*Per1*) [88]. This discovery was followed by studies in hamsters and sheep where the amplitude and timing of expression of *Per1* in the PT was shown to be dependent on the duration of the melatonin signal (reviewed in [89]). Most clock genes including *Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1* and *Cry2* have now been shown to be expressed in the PT,

but unlike in the SCN, their expression is not under strict circadian control [90]. In the SCN, the photoperiodic melatonin signal does not affect the phase of expression of clock genes but was shown to have an effect on the amplitude of *Per1* and *Per2* rhythms. In the PT, however, melatonin is essential for the expression clock genes and controls their relative phasing [91–93]. Melatonin, itself an output of the circadian clock, provides therefore a clock input to the PT [89]. A mechanism has been proposed whereby the duration of the melatonin signal encodes a photoperiodic information within the PT via phasing of clock gene expression and has been proposed to operate as an internal coincidence timer for photoperiodic time measurement (reviewed in [94]) (fig. 2). In this model, *Per1* expression in the PT peaks at the onset of the light phase while *Cry1* peaks at the onset of the dark phase, the interval separating the peak of expression of these two genes therefore varies according to the photoperiod. By this means, seasonal response *via* PER/CRY protein complexes has been proposed.

Per1 is an early response gene responding to the rise in cAMP (*via* CRE elements) in the morning when circulating melatonin concentration declines, de-repressing cAMP accumulation. This induction of *Per1* at dawn is concomitant to the cAMP-dependent rise of ICER (inducible cyclic AMP response element repressor) which itself acts as a repressor of *Per1* expression [95]; *Per1* expression profile is therefore characterised by a sharp increase at dawn followed by a quick decline and is a good molecular indicator of the onset of the light phase. The repressive effect of ICER on *Per1* has been proposed to be responsible for the unexpected differences in amplitude in *Per1* expression in SP and LP (reviewed in [66]). Indeed, *Per1* amplitude is found to be suppressed in SP compared to LP in the PT of different species studied [93, 95, 96], which is counterintuitive since the sensitisation of the AC in SP (described above) would be predicted to lead to higher cAMP accumulation at dawn in SP compared to LP; a paradox that can be explained by the repressive effect of ICER on *Per1* expression. Interestingly, melatonin was also shown to suppress gene expression *via* cAMP-independent pathways [48], suppressing in particular the expression of E-box containing genes, including *Per1* [96]. If melatonin is therefore an inhibitory signal to *Per1* expression in the PT, it is important to point out that an intact endogenous melatonin rhythm is essential for the expression of *Per1* in this tissue. Indeed, in the PT of MT1 KO mice or melatonin-deficient C57BL mice, *Per1* expression is abolished [46, 81]. Similar observations have been made in pinealectomised

hamsters [97] and melatonin injections can rescue *Per1* expression, if given after the surgery [46, 90]. Taken together, these observations on *Per1* expression show that, in the PT, melatonin acts as a regulator of a complex regulatory network.

In contrast to the activation of dawn-associated genes (*i.e.* *Per1*) by a cAMP-mediated mechanism, melatonin is also known to directly activate gene transcription at the onset of darkness. Melatonin-dependent transcriptional activation in the PT was shown in both sheep and melatonin-proficient C3H mice [98–100] with the best studied example being *Cry1*. This upregulation occurs very quickly at dusk and the pathways involved, presumably involving cAMP independent mechanisms, remain uncharacterised. It was recently shown that the transcription factor Egr1 (early growth response 1) contributes to the melatonin-dependent activation of *Cry1* transcription [99], but whether Egr1 contributes to the transcriptional activation of the other melatonin induced genes hasn't yet been determined.

Decoding the Photoperiodic Signal: The Long-Day Response in the PT

In sheep, the stimulation of the PRL secretion is initiated within 24 h after a switch from SP to LP and is accompanied by quick changes in clock gene expression [101]. When exposed to prolonged LP, they become refractory with PRL concentration progressively declining from 10 weeks onwards. In such conditions, however, clock gene expression in the PT still exhibits a LP-like pattern (including *Rev-erba*, *Per1*, *Per2* and *Bmal1*) [44]. Similar studies in hamsters have revealed that SP refractory and SP control animals express a similar temporal pattern of clock gene expression in the PT [92]. These observations, made in hamsters and sheep, show that the melatonin-dependent clock gene expression is a primary mechanism encoding photoperiodic information in the PT [44]. On the other hand, in LP-refractory sheep, β TSH and α GSU retain a SP-like pattern of expression, suggesting that these factors belong to a secondary mechanism, distinct from the internal coincidence read-out and driving the circannual clock (fig. 2). Therefore, the melatonin read-out in the PT de-couples from the neuroendocrine output.

We do not fully understand how the melatonin duration signal, encoded via regulation of clock genes and others, is decoded into photoperiodic output driving seasonal physiology, nor how this is linked to the operation of a circannual clock. Recent studies, using cDNA microarrays in PT from sheep culled after 8 weeks of SP expo-

sure or after one day of LP exposure, have identified *Eya3* as a LP-induced gene, activated immediately after switching to LP exposure [75]. *Eya3* is a transcription factor with multiple functions, the best described being its role in photoreceptor differentiation, suggesting that an ancient photoreceptor developmental pathway may be used in the PT to track the duration of the light/dark phase. *Eya3* has recently been characterised as a core component of the LP switch triggering photoperiodic response in sheep and mice [102, 103]. In these studies, *Eya3* was shown to complex with Six1 (Sine oculis homeobox homolog 1) to synergise the Tef (thyrotroph embryonic factor)-dependent activation of β TSH in the PT of both species. This finding formally links the described function for TSH with the regulation of the photoperiodic availability of thyroid hormones in the tanycytes, which in turn regulates the T₃-dependent activation of the reproductive axis [104]. How *Eya3* expression is regulated in the PT remains to be investigated, however the described repressive effect of melatonin [102] suggests that, in common with *Per1*, *Eya3* may be downregulated via regulation of circadian E-boxes and upregulated via action on CRE elements (following de-repression of cAMP concentrations). Interestingly, *Eya3* expression is de-repressed 12 h after the dark onset in both SP and LP, providing strong support for an external coincidence model, where concomitant de-repression and activation signals in LP would lead to a stronger transcriptional activation of *Eya3* in LP compared to SP [105]. The identification of *Eya3* thus opens the way to further investigation to understand how the LP response is decoded in the PT. For instance, it would be of major interest to identify other *Eya3* targets in the PT and investigate whether *Eya3* is also involved in the cascade of events leading the seasonal regulation of PRL and other neuroendocrine pathways in the PD (fig. 2).

Perspectives

The central role of the PT in photoperiodism is clearly established, however much remains unknown regarding the signal encoded and decoded within the PT to translate the melatonin photoperiodic signal. Our current knowledge on melatonin signalling in the PT clearly places intracellular cAMP as a key component integrating different signals (such as StimX and melatonin, see fig. 2). However, recent findings suggest the existence cAMP-independent pathways driven by melatonin important in the transcriptional regulation of many genes. Data so far have demonstrated that the original models proposed for

photoperiodic time measurement (i.e. the external and internal coincidence models) are not exclusive. The challenge is now to characterise the pathways triggered by melatonin and decipher their interplay to understand how the daily duration of the melatonin signal is translated into seasonal information within the PT.

Alternative Models to Investigate Mammalian PT Signalling – a Conserved Mechanism

The advent of molecular techniques to investigate gene regulatory networks has recently contributed to major advances in the field. Although photoperiodic species such as hamsters and sheep have so far proven to be the best models, the use of mice or birds offer some advantages since more molecular tools are available. Although they are not considered a photoperiodic species, mice have recently been shown to be a useful model to investigate PT signalling in mammals. They retain a photoperiodic-dependent expression of clock genes in the PT mediated by MT1 [106, 107] suggesting that the adaptation to photoperiod in animals lies in the output driven by the PT and not in the melatonin read-out. Similarly, birds do not employ melatonin for photoperiodic time measurement [108], and are known to decode the photoperiod signals by direct action of light on deep brain photoreceptors [109–112]. Despite this, clock genes remain under photoperiodic control in the avian PT [113] and the LP response is broadly similar to responses observed in mammals [114].

Modulators of MT1

As described above, the PT is a highly specialised tissue comprising different cell types, distinctive from the PD cells, and interacting with both the hypothalamus and the pituitary. To date, no cell lines have been recovered from this tissue which can recapitulate the function of the PT in culture. For this reason, PT primary cultures have been mainly used to study melatonin signalling pathways in the PT, and such experiments are slow and technically demanding. There is therefore a general lack of understanding of the function and of MT1 in the PT. Data so far show the complex nature of the melatonin signal in the PT, with both stimulatory and inhibitory effects on gene transcription via cAMP-dependent and independent pathways, strongly suggesting the involvement of yet unidentified factors specific to the PT (fig. 2).

Nuclear receptors have been proposed to play a role in melatonin signalling, with evidence that melatonin can bind members of the ROR/RZR family, some of which are known to be expressed in the PT [115]. Studies in MCF-7

human breast cancer cells have shown a role for melatonin-dependent transcriptional regulation of ER and ROR α [116]. Whether melatonin binds or affects the expression of nuclear receptors or whether it alters their function in the PT has yet to be investigated.

The fine tuning of MT1 signalling can be determined by many factors, including its putative heterodimerisation with other GPCR [117], its interactions with other intracellular proteins [117], its coupling with different heterotrimeric G proteins or its action on different AC substrates [87]. Although it is not functional in some seasonal species, MT2 has recently been characterised in the sheep PT, with expression levels lower than those of MT1 [118]. With its ability to form heterodimers with MT1 [117], the involvement of MT2 in melatonin signalling in the PT remains to be investigated. Members of the RGS (regulators of G-protein signalling) family are currently

being considered as important factors modulating GPCR function in many cell types. In terms of melatonin signalling, RGS20 was shown to form a complex with MT1/G α_i [119], similarly RGS4 was shown to be involved in the desensitisation of MT1 in CHO cells [85] and recently described as a potential modulator of MT1 function in the PT [120]. Here again, RGS are amongst a non-exhaustive lists of candidate factors which roles need to be investigated within the PT.

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