Circannual Clocks: Annual Timers Unraveled in Sheep

A recent study has revealed new insight into how the annual clock may drive seasonal hormone rhythms in mammals; the data suggest that melatonin-receptor-containing cells in the pituitary gland may operate as key calendar cells, transmitting seasonal temporal information to the endocrine system.

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In the past decade key features of the core molecular oscillators involved in the regulation of circadian (daily) clocks have been defined in mammals and flies, and elegant studies conducted to examine their role in neural and behavioural pathways, as well as peripheral tissue physiology. In contrast, we still have a very poor understanding of how animals tell time on an annual time-scale. That circannual clocks exist is not in doubt, but little is known about them, partly because of the extraordinary time scale required for their study. Research by Gwinner and colleagues (reviewed in [1]) on migratory birds has shown that long-term reproductive and migratory behavioural annual rhythms persist in individual birds kept for up to 12 years in artificial constant environments, while other researchers studying mammals have reported long-term cycles of metabolism, hibernation behaviour and reproduction following many years of exposure to constant conditions [2]. Such circannual cycles tend to run with slightly shorter periods than 12 months. A recent paper by Lincoln *et al.* [3] on sheep now offers new insight into this circannual timer, and suggests an anatomical substrate based in the pituitary gland (Figure 1).

Sheep are seasonally breeding mammals, and as such use seasonal changes in day-length to cue reproductive and hormonal rhythms. We have known for more than 20 years that photoperiod regulates the nocturnally secreted pineal melatonin signal, generating long-duration signals in winter and short-duration signals in summer, and this provides the brain with an accurate internal representation of external photoperiod, which drives seasonal physiology. Curiously, the brain is not rich in structures expressing melatonin receptors, and in seasonal mammals, a major site of receptor expression

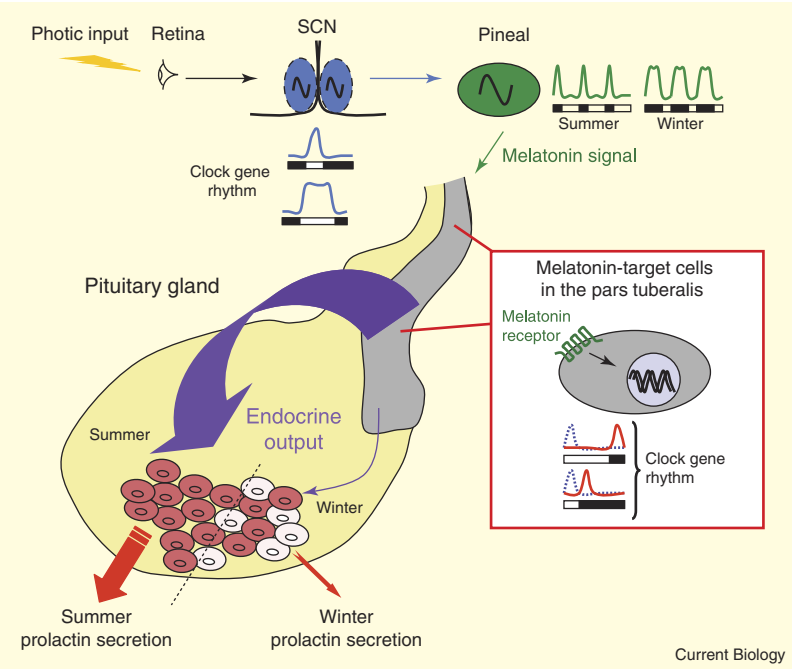

Figure 1. A speculative model of entrainment of a seasonal calendar cell system by the melatonin signal acting on pituitary gland target sites.

Light is perceived by the eyes and entrains the activity of circadian oscillators in the suprachiasmatic nucleus in the hypothalamus (SCN), where both the pattern of electrical firing rate and of circadian clock genes *Period* (*Per*) and *Cryptochrome* (*Cry*) are regulated by photoperiod [8]. PER–CRY protein dimers are central to the regulation of the circadian clock. Sympathetic output from the SCN, as well as input from the retinal hypothalamic tract, controls rhythmic activity of the pineal gland and duration of secretion of the nocturnal melatonin signal. This appears to be controlled by regulation in the pineal of the rate-limiting enzyme, arylalkylamine N-acetyltransferase (AANAT). The read-out for the melatonin signal in target tissues involves a G protein-coupled receptor (MT1), which is strongly expressed in the pars tuberalis of the pituitary gland. These pars tuberalis cells have many of the properties we might expect of a calendar cell, capable of recording seasonal time. Melatonin is known to induce pars tuberalis expression of *Cry1*, so that RNA levels rise at early night (red line). In contrast, the clock gene *Per1* is expressed in the pars tuberalis at the end of the night, co-incident with the decline in melatonin secretion, via a cAMP-mediated pathway (blue line) [7]. In this model, the phasing of these two elements in the pars tuberalis is dependent on duration of the melatonin signal: the durational melatonin signal generated by the circadian axis is thus translated to an internal co-incidence of two core clock genes, which exhibit altered seasonal phasing and presumably differential output on downstream targets. Nothing is yet known of the seasonal molecular machinery downstream of pars tuberalis clock genes. The pars tuberalis cells are thought to produce a prolactin-regulating signal, which acts on downstream lactotroph cells to stimulate synthesis and secretion of this important seasonal hormone [9]. A current model suggests lactotroph cells are recruited to the secreting population in a binary 'on–off' state, with many more cells active on long summer day-lengths. The new work of Lincoln *et al.* [3] suggests that prolactin-generating machinery of the pituitary may be capable of undergoing spontaneous rhythmical seasonal changes in activity in the face of an invariant (long-day like) photoperiod and can be re-set by melatonin. A clear implication is that the pars tuberalis cells may be the first identified cell types capable of generating an annual rhythm.

is in the proximal pituitary gland region, at the point where it joins the hypothalamus, the pars tuberalis [4] (Figure 1). An elegant surgical approach pioneered by Lincoln and Clarke [5] showed that in 'hypothalamo-disconnected' animals, in which the hypothalamus was surgically disconnected from the pituitary with the part tuberalis attached, the photoperiod-controlled melatonin signal could still regulate seasonal rhythms of the hormone prolactin. This demonstrated that blood borne melatonin signals must directly regulate this axis.

In the new study [3], the same group has now extended these earlier observations by studying a cohort of hypothalamo-disconnected sheep kept on constant long photoperiods for just under three years. Despite an invariant long-day melatonin signal, most of the animals underwent robust circannual rhythms of prolactin secretion with an average period for each cycle of 9 to 10 months. When exposed to short photoperiods, prolactin levels fell and the cycle was re-set. Together, these studies suggest that the circannual machinery driving prolactin rhythms can operate in the absence of direct neural links from the hypothalamus, that it is likely to be pituitary specific, and that it remains sensitive to changes in the melatonin signal.

In a further group of hypothalamo-disconnected animals, rhythmic production of the melatonin signal was blocked by removal of the superior cervical ganglia, thus inhibiting sympathetic drive to the pineal gland (blue arrow in Figure 1). Deprived of the melatonin signal, levels of prolactin fell and remained basal for just under three years, and were no longer sensitive to photoperiod change [3]. This suggests that, although the daily melatonin signal does not need to change in duration to drive the circannual oscillator, it nonetheless serves an important permissive function within the pituitary gland maintaining the circannual oscillation.

Other studies have investigated whether the local melatonin-regulated clock gene rhythm within the pituitary exhibits changes in phasing over the circannual cycle [6,7]. It appears that in both rodents and sheep, the clock gene rhythm faithfully reflects the ambient melatonin signal, and thus the circannual component probably lies downstream. The search is now on for molecular pathways involved in such seasonal timing and for this, the pars tuberalis is the ideal starting point.

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DOI: 10.1016/j.cub.2007.01.042

Plant Endocytosis: It Is Clathrin after All

Endocytosis occurs in plants, but the involvement of clathrin-coated vesicles has been unclear; a new study provides strong evidence that, as in animal cells, clathrin-coated vesicles are a major means of internalisation by plant cells.

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In eukaryotic cells, endocytosis is essential for regulation of the protein and lipid compositions of the plasma membrane and for acquisition or removal of material from the extracellular medium [1,2]. In animal cells, plasma membrane proteins are continuously internalised by a variety of mechanisms, some of which involve clathrin-coated vesicles. After internalisation, the resulting vesicles are transported to endosomes where proteins are sorted for traffic back to the plasma membrane or kept in the endocytic pathway to later endosomes and lysosomes. Similar sorting mechanisms exist in plant cells [2], but the mechanisms involved in internalisation have been unclear. New data, reported by Dhonukshe *et al.* [3] in this issue of *Current Biology*, show that clathrin is required for internalisation of a wide variety of plasma membrane markers, implicating