Chapter 12
Stem Cell Regulation of Circannual Rhythms

Gerald Lincoln and David Hazlerigg

Abstract Circannual rhythms depend on intrinsic timing mechanisms that operate over months and years. In this chapter, we briefly review the evidence that cyclical histogenesis, regulated by stem cells, underlies the generation of circannual rhythms. Three levels of circannual organization are considered: the concept of a "circannual clock shop" of multiple integrated timers; the concept of tissue-autonomous circannual oscillators dependent on cyclical regeneration; and, last, the remarkable possibility that circannual timing may actually be a cell-autonomous characteristic that first evolved in protists. We conjecture that all three mechanisms can contribute to the generation of the seasonal phenotype in complex organisms, different for each species, and that an evolutionally conserved, cell-autonomous mechanism may be expressed in stem cells to act as a pacemaker for circannual timing.

Keywords Canonical circannual genes • Clock shop • Endogenous clocks • Photoperiod • Seasonal rhythms • Stem cell niche • Tau

12.1 Introduction

The Earth's rotation about its axis and its orbit around the Sun give rise to two fundamental periodicities, daily and annual, to which life has evolved. Across taxa, this has led to the appearance of internal timing mechanisms, which permit physiological and behavioral changes to anticipate forthcoming changes in environmental conditions. That circadian clocks evolved to resonate with the daily periodicity generated by the Earth's rotation is now widely accepted, and much is now known about the structure and mechanism of circadian pacemakers (Reppert and Weaver 2002). Contrastingly, the genetic and cellular mechanisms underlying circannual clocks have received much less attention, and their fundamental nature remains enigmatic (Hazlerigg and Loudon 2008).

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The way the Earth's rotation and orbit have favored the evolution of endogenous circadian and circannual clocks is summarized in Fig. 12.1. In addition, the ability of the circadian system to measure daylength allows the precise synchronization of seasonal biology to the seasons. This synchronization permits organisms to optimize

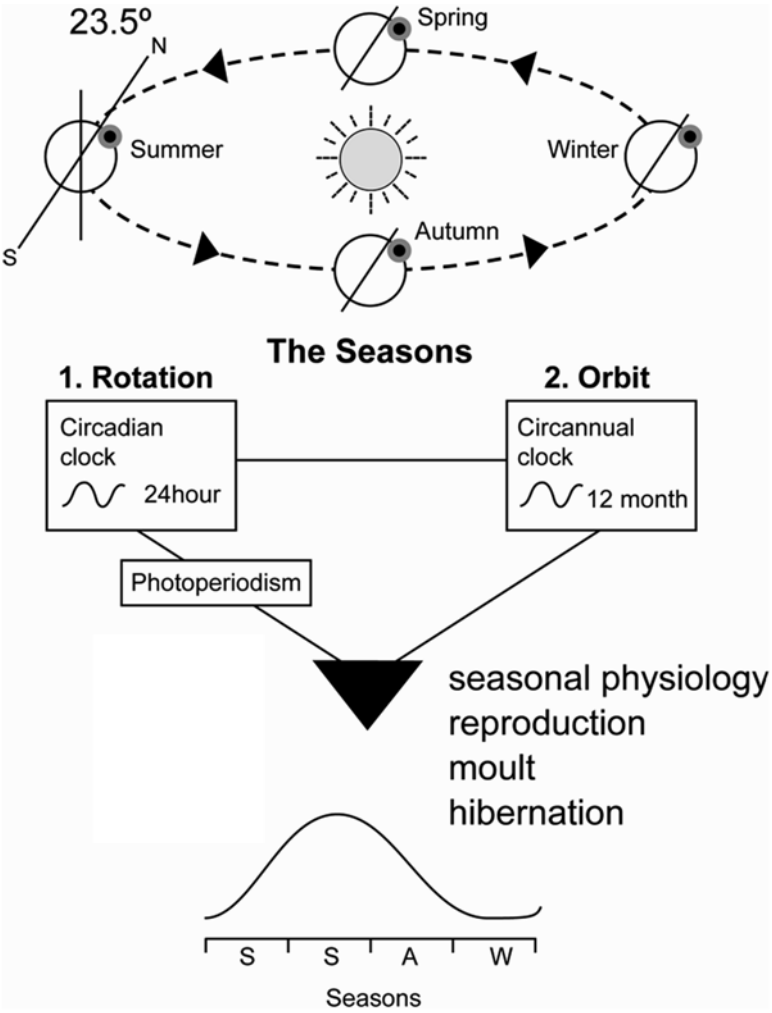

Fig. 12.1 Endogenous timers anticipate the Earth's periodicities. Schematic of the way the Earth's rotation on its axis every 24 h (*top panel*; line s/n across the earth indicates the rotational axis and the *filled circle* represent a UK northerly point of reference) and orbit around the sun every 12 months have favored the evolution of endogenous circadian clocks (based on about a dozen clock genes) and circannual clocks (based on stem cells and tissue regeneration: current chapter). The ability to respond to the annual cycle in daylength that varies with latitude (photoperiodic time measurement or photoperiodism) depends on the circadian system. Most long-lived organisms utilize such entrainment of a circannual pacemaker to precisely regulate long-term rhythms in reproduction, molt, hibernation, and other seasonal characteristics. (After Hazlerigg and Lincoln 2011)

their survival and reproduction, and is thus fixed by natural selection as an adaptive trait, accounting for the remarkable conservation of timing principles across taxa, from protists to vertebrates (Reppert and Weaver 2002; Bell-Pedersen et al. 2005).

Recently we have written two articles proposing that cyclical histogenesis is the basis of circannual rhythm generation (Lincoln and Hazlerigg 2010; Hazlerigg and Lincoln 2011). The long-time domains are the result of the protracted processes of cell differentiation, tissue remodeling, and feedback signaling from within and outside the tissue, as seen in developmental biology. These cyclical regeneration processes occur in multiple seasonally regulated tissues in the brain, pituitary gland, and peripheral tissues with variable phasing to produce each circannual rhythmic phenotype. In making this proposal, we were mindful of the cyclical regenerative biology seen in vertebrate hair follicles, testis tissue, blood-forming tissues, antlers, feathers, and pituitary gland. All these processes are dependent on stem cells that govern the period of the cycle and the morphology and phenotype of the regenerated cells and tissues.

Tissue-specific stem cell niches are viewed as reservoirs fueling cycles of tissue regeneration throughout the life of the organism. The property of circannual rhythmicity emerges from long-term feedback cycles that control stem cell reactivation after the phases of growth and quiescence and other potentially important innate timing processes. Because genes that regulate the development of an organ may also control cycles in the same tissue in the adult (e.g., hair follicle, Schneider et al. 2009; pituitary gland, Vankelecom 2009), it is evident that the adult's stem cells act to recapitulate events of ontogeny conferring cyclicity as a common feature of the adult phase of the life history sequence (Fig. 12.2). We propose that the seasonal transition is similar to a metamorphic event. It affects most tissues, radically changing physiology, but in contrast to the familiar metamorphosis of an amphibian, it is reversible and repeatable. The parallel role of thyroid hormone deiodinases in regulating seasonal transitions (Hanon et al. 2008) and in amphibian metamorphosis (Brown 2005) provides good evidence for the fundamental similarity of these processes.

In this chapter, we consider the basic question of where the long-time constants of circannual timing are generated. We summarize evidence that the adult stem cells residing in their tissue-specific stem cell niche may play a critically important role in circannual timing (Fig. 12.2). Three levels of circannual organisation are considered: (1) network, the concept of a "circannual clock shop" of multiple integrated timers (Hazlerigg and Lincoln 2011); (2) oscillator, the concept of tissue-autonomous circannual oscillators dependent on cyclical regeneration; and (3) basic, the remarkable possibility that circannual timing may actually be a cell-autonomous characteristic that first evolved in protists. It will be evident that there is a major overlap between categories 1 and 2 because a circannual clock shop depends on the presence of multiple circannual oscillators based on cyclical histogenesis, and these have tissue-autonomous characteristics (thus the overlap with level 2). However, we prefer to separate these categories because the special feature of a circannual clock shop is the network of histogenic sites hierarchically organized and networked together by hormonal mechanisms, whereas the key feature of tissue-autonomous control is the local regulation of long-term timing by stem cells potentially using autocrine/paracrine signaling.

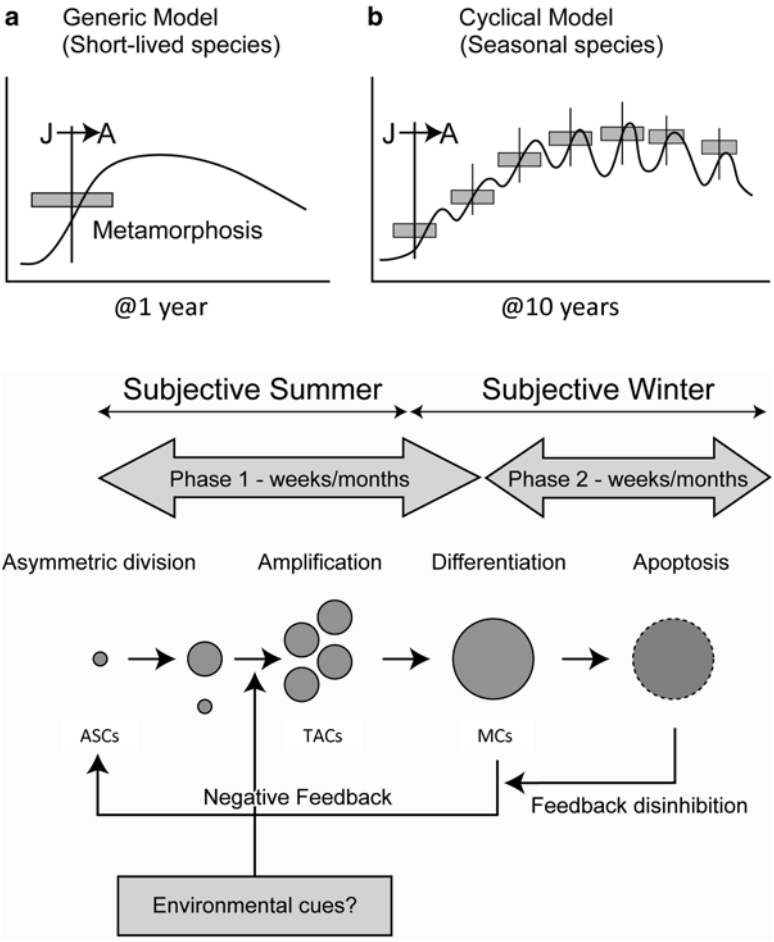

Fig. 12.2 Cyclical life histories. *Top panel*: Vertebrate life history models illustrating changes in body weight or testis size. (**a**) Generic model depicts a smooth change across the life history within a single juvenile–adult (J-A) transition (or metamorphosis). This model represents a short-lived species. (**b**) Cyclical model with a juvenile–adult transition (J-A) followed by repeated transitions throughout the life history each representing an endogenous circannual cycle: circannual cycles are an integral part of the life history program (Lincoln and Hazlerigg 2010). *Bottom panel*: Original generic model for circannual rhythm generation by cyclical histogenesis (Hazlerigg and Lincoln 2011). We proposed that circannual cycles oscillate between phases of subjective summer (*horizontal arrows*) with growth and maturation of tissues by histogenesis, and subjective winter (*dark bar*), during which tissues become quiescent. This is driven by pluripotent stem cells located in a tissue-specific stem cell niche. Stem cells undergo asymmetrical division as a rare event giving rise to populations of transient amplifying cells (TACs), which undergo repeated rounds of cell division to form a new cell population. Migration and differentiation then lead to the formation of new functional tissue, as well as providing feedback signals that arrest cell division in the stem cell niche. Withdrawal of negative feedback, possibly related to apoptosis, initiates a new circannual cycle. *ASC* adult stem cell, *MC* mature cell

12.2 Circannual Timing Based on a "Clock Shop"

Previously we presented a model for circannual organization in metazoan animals based on a coordinated "clock shop" of circannual oscillators residing in many tissues. We proposed that the oscillators are based on cyclical histogenesis controlled by resident stem cells, with coordination of the multiple oscillators achieved by peripheral hormonal signals (Fig. 12.3). The model proposes a hierarchical organization with oscillators in the hypothalamus and pituitary acting as primary pacemakers that respond to photoperiod and other environmental cues to ensure

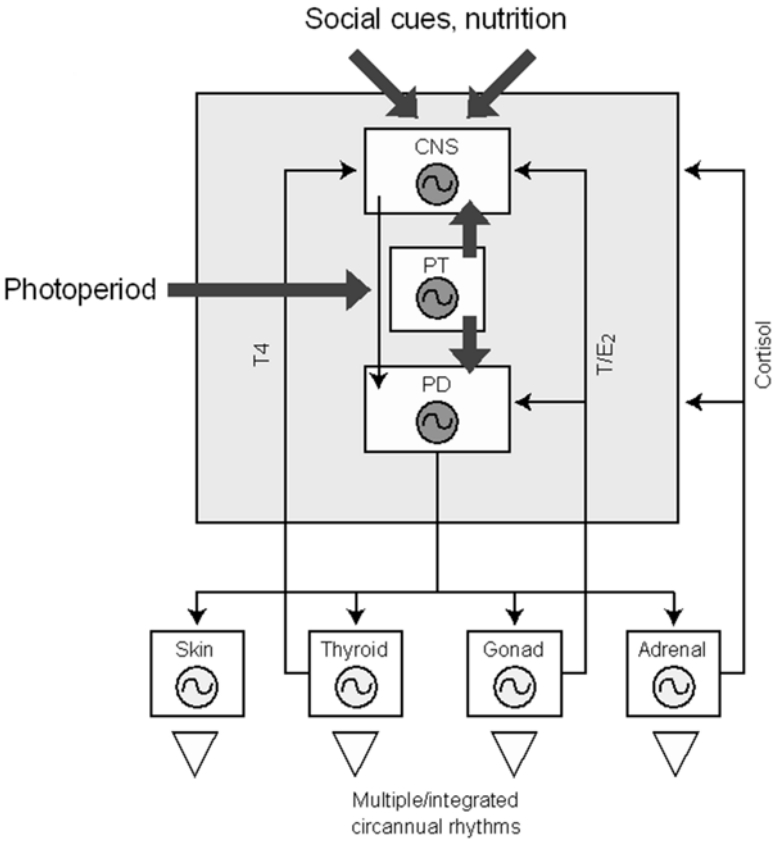

Fig. 12.3 Circannual clock shop. Organism-level circannual coordination is seen as the product of circannual cycles in multiple tissues (Hazlerigg and Lincoln 2011). The analogy to circadian organization can be drawn (Herzog and Tosini 2001). Multiple coordinating signals between pacemaker structures in the hypothalamus, pituitary, and peripheral organs including thyroxine (T_4), glucocorticoids (cortisol), and gonadal steroids (testosterone, *T*; estradiol, E_2) provide for feedback control. Circannual rhythms may be entrained by photoperiod acting at the level of the pars tuberalis (*PT*), as in mammals, or by other cues (social, nutritional, etc.) acting via the central nervous system (*CNS*). *PD* pars distalis, *circle/oscillator symbol* histogenic cycle

seasonal entrainment. Parallels can be drawn with the circadian system where the suprachiasmatic nucleus (SCN) acts as a central pacemaker for peripheral clocks, and also acts to relay effects of daylight for entrainment to the daily light–dark cycle.

Several lines of evidence suggest that multiple circannual rhythms can run in parallel in an organism, even in the absence of changes in feedback signals or effects of photoperiod. Sheep pinealectomized to remove melatonin as a mediator of photoperiodic input, castrated, and given constant-release implants of estradiol, still express circannual rhythms of gonadotropin secretion (Woodfill et al. 1994). These rhythms can be synchronized by seasonally appropriate programmed daily infusions of melatonin, but continue asynchronously in the absence of melatonin. Gonadectomized ground squirrels also express robust circannual rhythms, whose characteristics are modulated by gonadal steroid implants (Hiebert et al. 1998). In circannual rodent species there is also evidence that ablating major endocrine feedback pathways by thyroidectomy or castration fails to interfere with innate rhythms in hibernation, feed intake, and fat accumulation (Henderson and Demeneix 1981). Moreover, in hypothalamo-pituitary-disconnected (HPD) Soay rams, the surgical destruction of the descending hypothalamic control of the pituitary gland that blocks most seasonal rhythms (gonadal activity, food intake, body weight) fails to abolish circannual prolactin secretion (Lincoln and Clarke 1994; Lincoln et al. 2006). Pharmacological suppression of prolactin secretion in this model does not affect the phase of the circannual prolactin cycle (Lincoln et al. 2003), indicating that circannual rhythm generation is tissue localizable and independent of the output response.

Experimental manipulation of hormones known to control cell proliferation, differentiation, and organogenesis also has profound effects on the expression of seasonal rhythms, indicating the key role of histogenesis. Altering thyroid hormone (TH) status is particularly disruptive. Thyroidectomy blocks seasonal breeding cycles in birds and mammals (Follett and Potts 1990; Moenter et al. 1991; Webster et al. 1991; Nakao et al. 2008). In sheep, these effects represent a temporally specific and anatomically localized action of TH on the generation of the circannual reproductive rhythm. Detailed studies using thyroxine (T_4) replacement in thyroidectomized ewes have revealed a window of sensitivity to T_4, which opens in the spring and closes some 6 months later, as part of the circannual cycle (Thrun et al. 1997; Billings et al. 2002). Strikingly, micro-implants of T_4 placed in the histogenic regions of the mediobasal hypothalamus are sufficient to mimic the effects of systemic T_4 replacement (Anderson et al. 2003). Furthermore, central treatments with biologically active TH (tri-iodothryonine, T_3) promote a permanent summer breeding phenotype in quail and Siberian hamster (Follett and Potts 1990; Barrett et al. 2007).

Most recently, an endogenous control pathway regulating seasonal transitions has been characterized in the hypothalamus of mammals and birds (Hanon et al. 2008; Nakao et al. 2008; Ono et al. 2008). This pathway depends on the regulation of the expression of two enzymes, DIO2 and DIO3, which convert thyroid hormones between active and inactive forms, located within tanycytes in the ependymal cell layer lining the third ventricle in the mediobasal hypothalamus. Increased Dio2 gene expression (thus activating thyroid hormone) is seen following exposure to long days, with the converse sequence under short days, and spontaneous reversion occurs under prolonged photoperiod, indicating an innate timing mechanism.

Photoperiodic control of Dio3 (which inactivates thyroid hormone) has been less widely reported, but Dio3 expression increases markedly under a short photoperiod in the Siberian hamster, suggesting a key role in driving the summer-to-winter transition in physiology (Barrett et al. 2007). Studies in sheep, mice, and birds also demonstrate that it is thyroid-stimulating hormone (TSH) produced in the pars tuberalis (PT) of the pituitary stalk that acts locally in the adjacent hypothalamus to regulate Dio2 expression in the ependymal tanycytes. Thus, both inductive and entraining effects of photoperiod on seasonal physiology are regulated at the level of the PT through the control of thyroid hormone-dependent mechanisms in the brain (Dardente et al. 2010). These data are all consistent with a role for TH in supporting cyclical histogenesis in the hypothalamus as part of the long-term timing mechanism; tanycytes are the candidate stem cells for this oscillator (Lee et al. 2012).

The adrenal glucocorticoids also have far-reaching effects on histogenesis. Recently, we have investigated the impact of the glucocorticoid axis on circannual prolactin secretion in the HPD Soay sheep model by giving the long-acting ACTH secretagogue, synacthen-D (Syn-D), to chronically activate the adrenal axis (Hazlerigg and Lincoln 2011). The effect on the circannual prolactin rhythm was phase dependent, detectable immediately if the drug was given during the "subjective spring" phase of the circannual cycle when prolactin levels were increasing, but not detectable until some 3 months later (well after the end of adrenal activation) if Syn-D treatment was delayed until the peak of the prolactin cycle. Full recovery from the later Syn-D treatment was not seen until after 1 to 2 years.

Our interpretation of these results is that SynD causes a glucocorticoid-dependent suppression of cell division within the anterior pituitary (evidenced by the HPD procedure), possibly through induction of cell differentiation, and that the effect depends on the phase of the long-term cycle in pituitary histogenesis. Treatment in the proliferative phase (subjective spring) immediately delays the circannual cycle, whereas treatment later leads to markedly delayed responses. Again, this is strong evidence that histogenesis underlies the generation of circannual rhythms and that the major peripheral hormones act to coordinate the cycles in different physiological systems signals (Fig. 12.3).

12.3 Tissue-Autonomous Circannual Oscillators

Under this second scenario, circannual cycles are generated by a tissue-autonomous mechanism for each different physiological system rather than via a more global network as in a "clock shop." Adult stem cells residing in their tissue-specific niche are regulated locally by feed-forward and feedback signals from the progeny tissues, to produce a histogenic cycle of renewal. One or more such cycles may be required to generate the circannual rhythm. In developing this concept we have used the hair follicle as the prototype wherein the period of the tissue cycle, and the morphology of the new tissues, is regulated by a local population of stem cells. Other cyclical tissues including testis, blood-forming tissue, antler, and pituitary gland are also described.

12.3.1 Hair Follicle

The mammalian hair follicle acts as a semiautonomous miniorgan, capable of iterated cycles of growth and regeneration with timescales of months to years. The intrinsic cellular and molecular control of the hair follicle cycle is well understood (Schneider et al. 2009). The cycle is initiated by stem cell proliferation in the bulge region of the follicle (Fig. 12.4a). Progeny cells migrate to the dermal papilla (DP) and activate regrowth of a complete new hair fiber (anagen phase) and shedding of the old. During the cycle, paracrine signals are generated that exert autonomous feedback control as well acting on neighboring follicles, leading to synchronous cycles of hair growth (Plikus et al. 2008). The wnt/β-catenin signaling pathway, coupled with low levels of bone morphogenic proteins (BMPs), promote anagen whereas rising BMP levels cause growth arrest (catagen) and transition to the quiescent telogen phase. Return to anagen requires diminution of BMP levels, and so the hair follicle cycle can be thought of as a limit-cycle oscillation between the opposing forces of wnt/β-catenin and BMP signaling (Plikus et al. 2008). The stem cells of the individual follicles and tissue paracrine signaling govern both the follicle periodicity and the waves of synchronized hair growth within the skin (Plikus et al. 2008). The autonomy of the follicular cycles is demonstrated by their persistence in in vitro culture systems (Philpott and Kealey 2000).

In seasonal species, the hair follicle can produce more than one hair phenotype, generating distinctive summer and winter coats (Dicks et al. 1994). These phenotypes emerge from follicle growth cycles with different periodicities, leading to different fiber characteristics (Fig. 12.3b). Short cycles typically produce short summer hair fibers, and long cycles produce winter coats, which persist because all follicles enter an extended period of telogen over winter. The annual pelage cycle is thus the composite of more than one tissue cycle in the individual follicle,

Fig. 12.4 (continued) (Schneider et al. 2009). Once the growth of the new hair is complete, the germinal epithelial cells at the base of the hair shaft regress isolating the dermal papilla (DP) to produce the mature phase (catagen) and then the quiescent phase (telogen). The total period of the hair follicle cycle is 2 months to less than 2 years, and the relative time in anagen, catagen, and telogen varies markedly among species and between skin regions and seasons within species. (**b**) Model for seasonal changes in the hair follicle cycle in the sheep or red deer. Hair follicles (illustrated as *horizontal bars 1–8*) show limited local synchronization and cycle repeatedly during the summer; high prolactin levels are permissive to this state. Declining prolactin in the autumn synchronizes follicles throughout the pelage and leads to an extended anagen (*A*), producing a long winter coat. Follicles then remain quiescent in telogen (*T*) during the winter. Synchronous reactivation of the follicles produces the conspicuous spring body molt. The overall circannual period is a composite of multiple histogenic cycles in the hair follicle. (**c**) Experiment in which female red deer were given a small osmotic minipump implanted under the skin of the flank delivering prolactin for 28 days. Treatment in spring induced the local activation of anagen and the development of a skin patch with red summer coat (*S*), contrasting with the longer grey winter coat on the rest of the body. This skin patch developed a winter coat (*W*) some 5 months later, long after the end of prolactin treatment, demonstrating tissue-autonomous control of the cycling between the two pelage phenotypes. (After Loudon and Jabbour 1994)

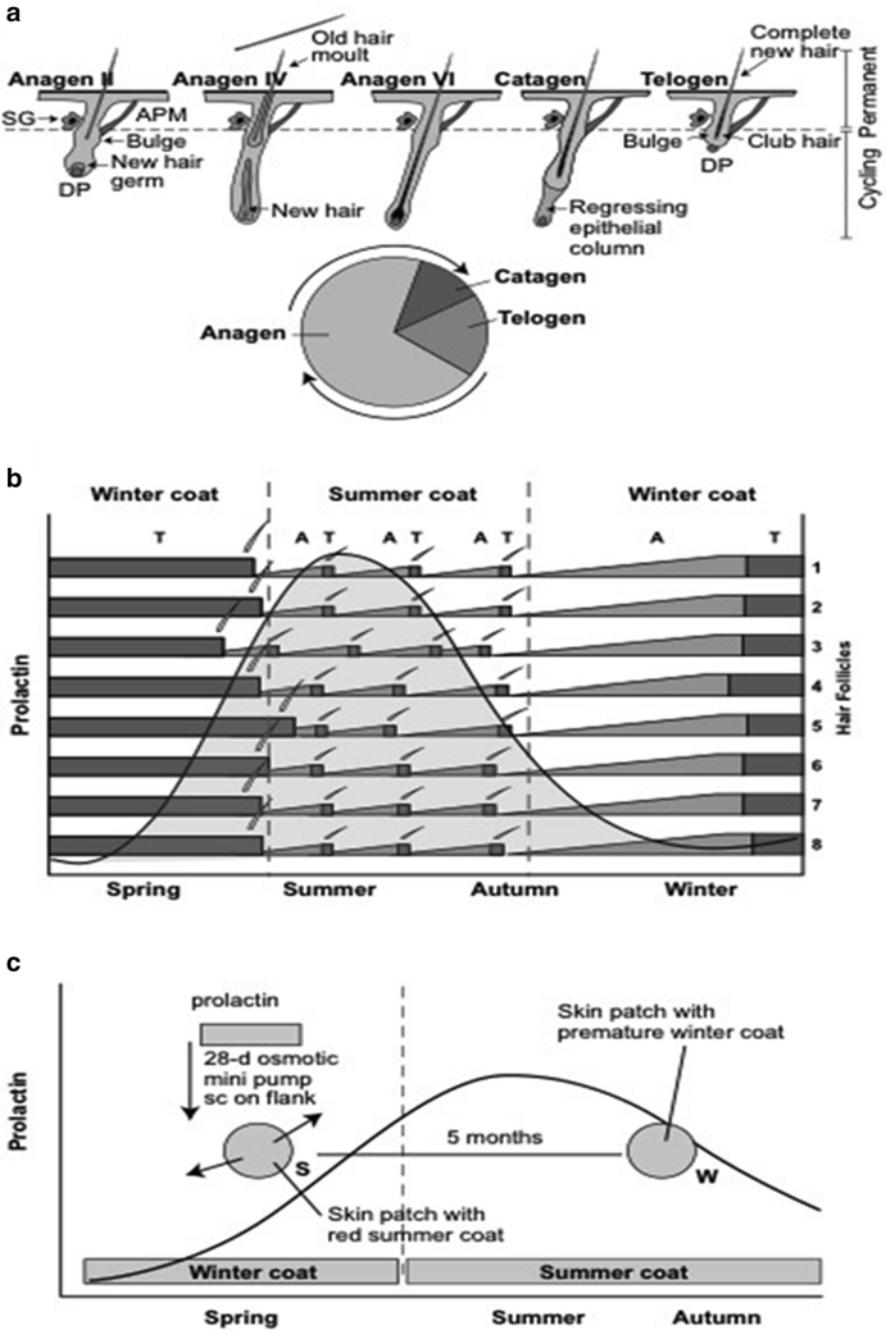

Fig. 12.4 Hair follicle as a tissue-autonomous rhythm generator governed by stem cells. (**a**) The growth phase of the follicular cycle (anagen) is initiated by stem cell activation in the bulge region at the base of the hair as a result of changes in the microenvironment of the bulge niche (*arrow*)

alternating between summer and winter programs. The transition between these summer and winter phases of follicle cycling can be synchronized by the hormone prolactin, in which secretion rises dramatically in spring, causing the conspicuous spring molt, and declines in the autumn, allowing growth of the winter coat (Dicks et al. 1994). Prolactin appears to trigger hair follicle reactivation and the seasonal molt by acting to curtail telogen and promoting a return to anagen across all hair follicles.

Importantly, the seasonal alternation of hair phenotype appears to be, in part, autonomously regulated within the skin, as has been elegantly demonstrated in red deer by the short-term administration of prolactin in the skin using osmotic minipumps to locally alter the hair follicle cycle (Loudon and Jabbour 1994; Fig. 12.4c). The treatment produced localized, premature growth of the red summer coat in winter animals with grey coats. The affected skin patch then remained out of phase with the remainder of the body's molt pattern for at least 5 months, long after the local hormonal manipulation, and in late summer prematurely developed a grey winter phenotype on animals with a natural red summer coat. This observation indicates that the skin tissue has intrinsic control of both hair follicle cycle and the switch between summer and winter phenotypes, striking evidence for a tissue-autonomous circannual clock. We are not aware of studies of these seasonal rhythms using in vitro organ culture: this is a major technical challenge because of the very long time frame required for such experiments.

In birds, the semiautonomous control of feather follicles, based upon intrinsic feedback cycles and communication between neighboring follicles in skin patches, accounts for the complexity in plumage (Mou et al. 2011). Seasonal control of plumage change appears to depend on the capacity of feather follicles for independent cyclicity, with wing flight feather and body feather molts often running asynchronously. In the migratory great knot, phase separation of these molts persists under constant photoperiod, reflecting intrinsic circannual control (Piersma et al. 2008).

12.3.2 Testis Tissue

One of the best examples of stem cell genetics controlling tissue cyclicity can be found in the mammalian testis. Xenotransplantation of spermatogonia (male germ stem cells) between laboratory strains of rats or mice have demonstrated that the period of the spermatogenic cycle (time taken from the differentiation division of spermatogonia until full maturation of spermatozoa within the seminiferous tubule) is regulated by the spermatogonia and not by the supporting somatic tissue of the host testis (Brinster and Zimmermann 1987). The full spermatogenic cycle involves multiple mitotic divisions of primary spermatocytes in the basal compartment of the tubules, differentiation of secondary spermatocytes through a meiotic reduction division, and final differentiation of spermatids into spermatozoa in the center of the tubules. The whole sequence takes 45–70 days, with the

duration being species specific. These cellular events are tightly synchronized and locally coordinated into defined cell associations in the seminiferous tubule, which allows division of tubules into defined stages and detailed quantitative analysis of the histogenic process (Hochereau de Reviers et al. 1985).

Seasonally breeding species express robust cycles in testis size and function with marked variation in the degree of involution during the nonsexual phase, and the pattern is circannually regulated under constant conditions (Lincoln 1989). This seasonal gonadal rhythm is primarily regulated through changes in gonadotropin secretion, governed by the circannual timer mechanisms of the hypothalamus (see Sect. 12.2). Interestingly, seasonal involution of the testis results from reduced efficiency in progeny cell production and survival caused by the action of reduced gonadotropins on the somatic cells of the testis (Sertoli and Leydig cells), with no fundamental change in the period of the spermatogenic cycle (Courot et al. 1979; Hochereau de Reviers and Lincoln 1978; Hochereau de Reviers et al. 1985). This finding indicates that the stem spermatogonia determine the period of the tissue cycle, despite major changes in any feedback signaling from the progeny cell population. There is also preliminary evidence that autonomous control of the long-term cycle in the efficiency of spermatogenesis is in part locally regulated. Thus, there can be a mismatch between the seasonal regression of the testis and the decline in gonadotropin secretion, variation in the efficiency of spermatogenesis in different parts of the testis, especially during the nonsexual phase, and a dissociation between cycles in the spermatogenic and androgenic functions of the testis in some seasonal species (Lincoln 1989). The overall story for the testis nicely illustrates autonomous control of tissue cyclicity, along with a global regulation by central circannual pacemakers that allows for the optimal timing of the different aspects of the seasonal sexual cycle.

12.3.3 Hematopoietic Tissues

Another compelling case of long-term cycles with stem cell origins comes from a family of hematological diseases (leukemias) in which long-term cyclical variations in the titers of different blood cell types are observed (Haurie et al. 1998). Periodic oscillations in numbers of granulocytes with period lengths of up to 100 days have been recorded in patients with periodic myelogenous leukemia, and of pancytopenia in which titers of all blood cell types are affected. Importantly for our concept of tissue autonomy, these diseases originate from abnormal bone stem cell function in lineages high in the hematopoietic cascade, thus producing life-threatening effects with periodic anemia, blood-clotting defects, and immunodeficiency. These effects are caused by specific gene defects that have been characterized by genetic analysis in human families with the rare disease; as well as in a breed of greyhound dogs that has been used as an animal model (Haurie et al. 1998). Experimental and modeling evidence indicate that the period length of these blood cell oscillations depends on the properties of bone marrow stem cells, progeny cells, and local signaling between

them, rather than feedback cycles involving systemic hormones (Haurie et al. 1998). We are unaware of any corresponding studies of the blood cell histogenesis in seasonal species to assess possible regulation on a circannual time scale.

12.3.4 Antler Tissue

The deer antler is a complete organ composed of multiple tissues that is regenerated annually, or circannually, under specific constant photoperiod regimens (Goss 1977, 1983, 1984). Antler regeneration is a wound-healing response within the permanent antler pedicles on the frontal bones of the skull that can only be triggered during the "summer phase" of the antler cycle (Lincoln 1984), similar to the seasonal window for limb regeneration in newts and other cold-blooded vertebrates in which there is intrinsic seasonal variation in the sensitivity of the wound site to neurotrophic signals (Singer 1974). The periodic antler growth persists in castrated deer where the dominant effect of sex hormones is removed (Goss 1983; Lincoln 1984). Moreover, double antler cycles with a large antler grown in summer and a small one in winter can occur notably in Pere David's deer where the seasonal reproductive cycle is phase advanced relative to the permissive window compared to the closely related red deer (Pocock 1923; Loudon and Brinklow 1992; Lincoln 1992). In male reindeer, the casting of the hard antlers occurs soon after the rut in winter, well in advance of the next cycle of antler regeneration, whereas in many deer species the initiation of antler growth in spring precedes casting of the old antlers (Goss 1983; Lincoln 1992). Thus, the mechanical events of antler casting (wound formation) are not an essential trigger for the start of next antler cycle. These observations are consistent with the idea of dual control of the antler: one mechanism governs the autonomous regulation of antler growth and maturation, and by the other the seasonal sex hormone cycle, itself governed by a brain pacemaker, "gates" the antler cycle to a particular phase (see Sect. 12.2).

Recently, the stem cells of the pedicle periosteum that regenerate the antler have been characterized in more detail using immunocytochemistry and tissue transplantation (Li et al. 2009; Li 2012) and the stem cells have been isolated (Seo et al. 2013). These cells express CD9 antigen, Oct4, and Nanog, and elevated levels of telomerase enzyme activity and nucleostemin, all biochemical markers of pluripotent stem cells (Li et al. 2009). In culture these cells can be triggered to differentiate into cartilage, bone, and fat cell lineages. The inner tissues of the antler (cartilage and bone) are derived from the pedicle periosteum stem cells, whereas the outer tissues (skin, nerves, and blood vessels) are derived from pedicle skin under the inductive influence of unknown factors released by the pedicle periosteum (Li 2012). Interestingly, numerous hair follicles form de novo in the antler velvet-like skin and express repeated hair follicle cycles and waves of synchronous molting as seen in normal skin (Lincoln 1984). Antlers developed in summer (e.g., red deer) produce short velvet hairs, whereas antlers developed in winter (e.g., roe deer) produce long velvet hair that may reduce heat loss and protect the growing tissue. Based on the

situation in normal skin, the prediction is that these features of the new antler hair are endogenously controlled both within the antler and by systemic seasonal hormones such as prolactin.

12.3.5 Pituitary Gland

The pituitary gland is one of the putative central circannual pacemakers (Hazlerigg and Lincoln 2011; see Sect. 12.2). Evidence that it may act as a tissue-autonomous pacemaker comes from detailed studies using the hypothalamo-pituitary-disconnected (HPD) Soay sheep model (Lincoln et al. 2006). The HPD operation permanently removes the neuroendocrine control of the pituitary but maintains its blood supply and tissue viability. This procedure should not be confused with hypophysectomy, which totally destroys the pituitary; HPD sheep still maintain some functional activities of the secretory cells that produce the classical anterior pituitary hormones, although no longer controlled by the brain.

Remarkably, HPD sheep express photoperiod-induced cycles in prolactin secretion similar to sham-operated controls, and the cyclicity free runs as a circannual rhythm under constant long days (Lincoln et al. 2006). Under such conditions the pattern persists for at least four cycles with a progressive desynchrony between adjacent animals, consistent with endogenous circannual control. The free-running period for the HPD sheep prolactin rhythm is close to 10 months and is significantly different from the Earth's 12-month periodicity, a temporal feature common to circannual rhythm generation in many different organisms (Gwinner 1986). Because the HPD surgery isolates the pituitary gland from the brain and removes most cyclical influences from other physiological systems (reproduction, food intake, body weight) (Lincoln et al. 2003), we infer that the pituitary gland itself acts as a circannual rhythm generator.

The marginal zone (MZ) of the pituitary cleft region in the anterior pituitary is the most likely site for a stem cell niche and cyclical histogenesis (Hazlerigg and Lincoln 2011). In the MZ there are polarized epithelial cells lining the cleft (remnant of the embryonic Rathke's pouch) that have the characteristics of pluripotent stem cells; these give rise to transient amplifying cells (TACs) that differentiate into new pituitary cells of different cell lineages (Vankelecom 2009). The prediction is that the "summer phase" of the circannual prolactin cycle is the time of proliferation of lactotrophs governed by the MZ stem cells. A second possible site for autonomous rhythm generation is the stalk region of the pituitary gland (PT, pars tuberalis) (Fig. 12.2); this is the location where the melatonin signal that relays the effects of photoperiod is transduced through a circadian clock gene-based mechanism (Dardente et al. 2010). Bromodeoxyuridine labeling experiments in sheep demonstrate that histogenesis in the PT is seasonally regulated (Migaud et al. 2010; Hazlerigg and Lincoln 2011; Hazlerigg et al. 2013). Thus, the PT may be a key convergence point between the photoperiodic input pathway and localized circannual rhythm generation.

12.4 Cell-Autonomous Circannual Time Keeping

If circannual oscillations are generated tissue autonomously, then how simple a level of cellular organization is required? Among higher plants, circannual rhythms of water uptake have been reported in bean seeds (Spruyt et al. 1987), and there are several reports of circannual rhythms in the transition between growth and resting phases in sea kelp (Dieck 1991; Schaffelke and Luning 1994) and in filamentous algae (Costa and Varela 1988; Costa and Lopez-Rodas 1991). For example, experimental cultures of *Spirogyra* maintained exponentially by serial transfer of vegetative cells, and held under constant light and temperature, exhibit progressive changes in phenotype (decrease in growth/cell division and increase in zygote formation) with a circannual pattern. There are parallel changes in cell morphology, notably affecting the spiraled chloroplast and loss of cell organization, features associated with cell aging. None of the plants survived without zygote formation (Costa and Lopez-Rodas 1991); thus, the circannual cycle requires the alternation between vegetative and reproductive phases, where both the aging of the photosynthetic cells and the hatching of the zygote are endogenously regulated.

Equally remarkable are the descriptions of circannual rhythms in a wide range of dinoflagellates, best studied in the genus *Alexandrium* (Andersen and Keafer 1987; Matrai et al. 2005). The life cycle of these complex unicellular organisms comprises asexual and sexual phases of propagation in the surface layers of the sea, as well as a diploid quiescent resting phase (hypnozygotes) during which cysts are formed and sink to the sediment layer on the seabed (Fig. 12.5). Reactivation of the cyst (hatching) is light- and temperature dependent, and in some strains is also subject to a strong circannual rhythm of sensitivity to these reactivating signals. There appears to be a strong ecological basis to whether a strain expresses circannual rhythmicity (Andersen and Keafer 1987). For strains in shallow coastal or estuarine waters, the seabed sediment layer is exposed to strong annual cycles of light and temperature, and emergence is environmentally driven, after an interval of 2–6 months. Hence, the encysted hypnozygote behaves as if it carries an hourglass-like interval timer mechanism to prevent winter emergence. By contrast, in strains found in deeper waters, where the seabed sediment layer is uniformly cool and dark, with only subtle temperature cycles, a robust, high-amplitude circannual rhythm of excystment potential is seen: the free-running period for this rhythm is close to 11 months, again less than the sidereal year.

The dinoflagellates provide the best evidence that a circannual rhythm can be generated within a single cell. In the dinoflagellate life history, it is the sexual diploid, encysted stage that expresses the rhythm, in some ways analogous to circannual

Fig. 12.5 (continued) no further temporal control, implying that an hourglass mechanism operates in the hypnozygote. Remarkably, in *A. tamarense* hypnozygotes collected from deep-water sediments, the temporal gate to excystment follows a circannual rhythm with a period length of approximately 11 months. (For further details, see Andersen and Keafer 1987 and Matrai et al. 2005

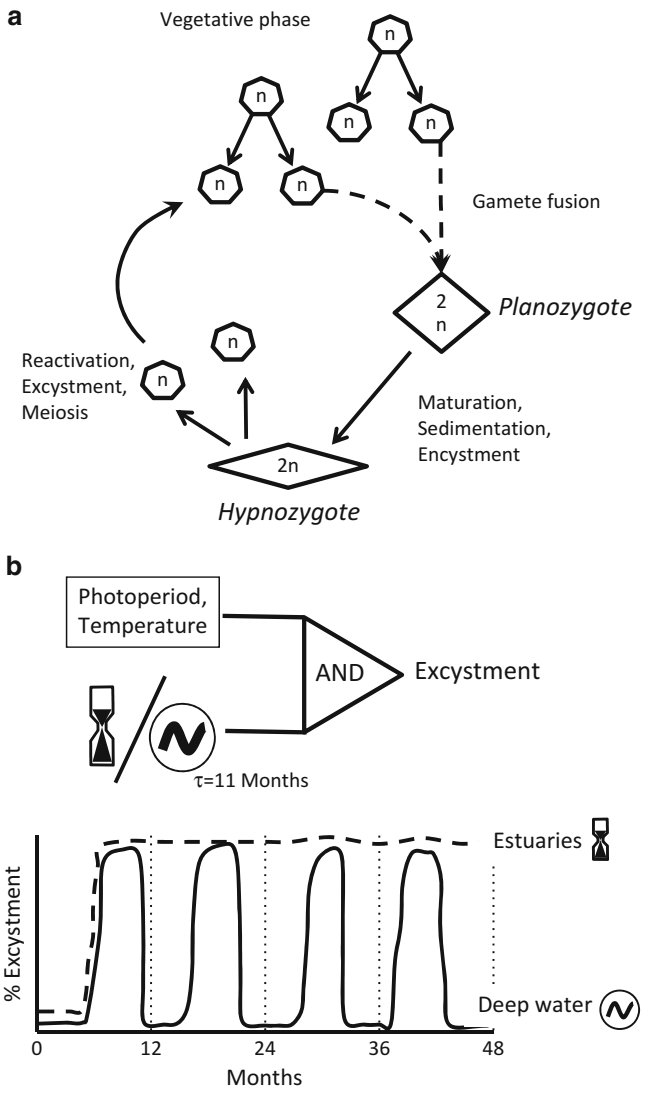

Fig. 12.5 Cell-autonomous circannual rhythms in dinoflagellates. (**a**) The simplified life cycle. Under favorable, typically summer, conditions, haploid ($n=1$) cells propagate vegetatively and are found in the sea surface water layers. A resting phase develops after fusion of two haploid cells, typically in the autumn months; this generates a diploid ($n=2$) "planozygote" that then develops into an encysted "hypnozygote" characterized by accumulation of storage vesicles, a thickened cell wall, and by increased cell density, which causes cysts to sink to the seabed. Reactivation in subsequent year(s) involves meiotic cell division and emergence of new vegetative cells from the cyst, a process known as excystment. (**b)** Long-term timing and circannual rhythms in the hypnozygote. The length of time spent in the hyponozygote phase is controlled by interactions between internal timing mechanisms and reactivating signals, particularly temperature and photoperiod. In *Alexandrium tamarense* strains living in shallow coastal waters and estuaries, excystment will not occur until a certain minimum length of time (2–6 months) has elapsed, but thereafter shows

rhythms in seeds in higher plants. The oscillation occurs without cell division or any overt changes in morphology in a seemingly quiescent encysted cell. It is clearly under genetic control because closely related strains of the same species vary in whether they do, or do not, express the circannual rhythm in cell responsiveness. Ticking in the cyst is a genetic timer. This raises the important question: could the rhythm expressed in single-celled organisms represent the very basic circannual clock that evolved at an early stage of eukaryotic evolution and is the mechanism conserved as a fundamental timer in more complex organisms?

Conclusions

In 1986, Ebo Gwinner published his seminal book on circannual rhythms (Gwinner 1986). He concludes: "Perhaps the most formidable physiological problem in circannual rhythm research arises from the extremely long duration of the processes involved. Some of the changes that occur within or between the various fractions of a circannual cycle have time constants that are way beyond the range of time constants known for any neuroendocrine feedback loop and are in many respects reminiscent of developmental processes occurring during ontogeny."

Decades later we can begin to confirm his vision. Circannual rhythm generation in birds and mammals depends upon reiterated cycles of histogenesis regulated as part of the life history program. Pluripotent, stem cells are set aside during development in defined stem cell niches, different for each tissue, and involve tissue repair and cycle generation over time intervals of months and years, thus accounting for the long-time domains for circannual timing.

The three levels of temporal organization summarized here ("clock-shop," tissue-autonomous, cell-autonomous) may all contribute to the regulation of circannual rhythmicity in complex organisms. It seems logical that the circannual timing mechanisms seen in single-celled organisms evolved first and are fundamental: they may provide the genetic program within the stem cell that regulates rhythmic activity over long time intervals. Tissue-autonomous timing mechanisms, wherein paracrine and autocrine feed-forward and feedback signaling between the stem cell and the progeny cells generate cyclicity, would be the next stage of complexity. And, finally, the "clock shop," with a hierarchy of oscillators from central to peripheral, is the best representation of the complexity of vertebrates.

The new concept that we wish to promote is that the circannual rhythmicity forms part of the life history program and is different for each organism depending on which life history phase requires to be timed predictively. This need may involve periods during the *adult* phase, as in most vertebrates, and affect different aspects of the phenotype (body weight, reproduction, pelage, migration, and hibernation behavior), each potentially timed independently through separate tissue oscillators. Alternatively, circannual rhythmicity may affect just *one part of the life cycle* as in insects (pupation) and dinoflagellates (hatch of the diploid cyst). Here intrinsic control is clearly adaptive because the organism is isolated from the environment.

In addition, circannual rhythmicity may operate *across generations* where the phase of the rhythm is transferred stepwise between generations as in short-lived organisms that alternate between asexual and sexual phenotypes.

Now, the number one challenge is to identify the canonical circannual genes that provide the core circannual timer. These genes are likely to be closely linked to the control of aging because circannual rhythms span long time periods as part of the cyclical life history program. They may also be part of the mechanism that governs cellular energy homeostasis and metabolism because long-term viability depends upon energy stores: thus, a long-term clock must register energy status to be adaptive.

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