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The 4th Dimension and Adult Stem Cells: Can Timing Be Everything?

Jeffrey M. Gimble[1], **Z. Elizabeth Floyd**[2], and **Bruce A. Bunnell**[3,4,5]

[1]Stem Cell Biology Laboratory, Pennington Biomedical ResearchCenter, Baton Rouge, LA, 70808

[2]Ubiquitin Laboratory, Pennington Biomedical ResearchCenter, Baton Rouge, LA, 70808

[3]Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA, 70112

[4]Center for Gene Therapy, Tulane University School of Medicine, New Orleans, LA, 70112

[5]Division of Gene Therapy, Tulane National Primate Research Center, Covington, LA, 70433

Abstract

The rotation of the earth on its axis influences the physiology of all organisms. A highly conserved set of genes encoding the “core circadian regulatory proteins” (CCRP) has evolved across species. The CCRP acts through transcriptional and post-transcriptional mechanisms to direct the oscillatory expression of genes essential for key metabolic events. In addition to the light:dark cycle, the CCRP expression can be entrained by changes in feeding and physical activity patterns. While mammalian CCRP were originally associated with the central clock located within the suprachiasmatic nucleus of the brain, there is a growing body of evidence documenting the presence of the CCRP in peripheral tissues. It is now evident that the CCRP play a role in regulating the proliferation, differentiation, and function of adult stem cells in multiple organs. This concise review highlights findings concerning the role of the CCRP in modulating the adult stem cell activities. Although the manuscript focuses on hematopoietic stem cells (HSCs), bone marrow-derived mesenchymal stem cells (BMSCs), adipose-derived stem cells (ASCs) and cancer stem cells, it is likely that the contribution of the CCRP merits consideration and evaluation in all stem cell pathways.

Keywords

Adipose-derived Stem Cell; Bone Marrow-derived Mesenchymal Stem Cell; Cancer Stem Cell; Circadian; Hematopoietic Stem Cell

Introduction

Why ask questions about the role of circadian biology in the regulation of adult stem cell function?

Biochemical and molecular tools, model organisms, and transgenic and knock out gene technology have promoted rapid gains in our understanding of adult stem cells. With these approaches, we have gained insight into fundamental questions relating to cell development, migration, and differentiation fate. Nevertheless, the majority of adult stem cell studies have been performed under static conditions. By necessity, cells are examined in a single point in

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time, rather than in a kinetic manner. As a result, it is possible that important information concerning stem cell dynamics and their regulatory pathways have been overlooked.

Advances from the field of circadian biology may provide the tools necessary to address this question. Using a variety of model organisms, sleep researchers and circadian biologists have defined a set of core circadian regulatory proteins (CCRP) that are highly conserved throughout the phylogenetic tree [Hirota and Fukada, 2004; Lowrey and Takahashi, 2004]. The levels of these proteins and their activities oscillate rhythmically over a 24 hour period [Lowrey and Takahashi, 2004]. Although the mammalian CCRPs were first defined in the context of the body's central clock, the suprachiasmic nucleus of brain, they have since been detected in peripheral tissues as well [Yoo et al., 2004]. This concise review highlights the growing body of literature linking the molecular components of the body's circadian clock to adult stem cell physiology and pathology. Currently, we focus on growth factors, receptors, and signal transduction pathways as mechanisms for manipulating adult stem cell proliferation, differentiation, and function; however, if timing really does matter, we may be able to exploit components of the body's own molecular clock to achieve these same goals.

Overview of Common Circadian Regulatory Proteins (CCRP)

What drives the circadian clock?

Molecular biological studies of circadian mutations in plants, Drosophila, and mammalian systems have identified the biochemical components of the central circadian oscillator (Figure 1) [Hirota and Fukada, 2004;Lowrey and Takahashi, 2004]. Transcriptional regulatory proteins belonging to the basic helix-loop-helix/Per-ARNT-Singleminded (bHLH-PAS) domain family interact with each other and downstream target proteins to create a self-perpetuating, rhythmic pattern of gene transcription (Table 1) [Hirota and Fukada, 2004;Lowrey and Takahashi, 2004]. The proteins CLOCK (or its ortholog, neural PAS domain 2 or NPAS2) and brain and muscle ARNT-like 1 (BMAL1) form heterodimers that act as positive transcriptional regulators. In contrast, heterodimers of the PERIOD (Per1, Per2, Per3) and CRYPTOCHROME (Cry1, Cry2) family members serve in a negative feedback capacity. As a result of these self-contained feedback loops, the circadian protein levels oscillate in a rhythmic manner.

The positive transcriptional regulators direct expression of immediate downstream targets, such as the albumin D site binding protein (DBP) which exerts a further transcriptional activating role. In contrast, related downstream targets such as Rev-erbα, Rev-erbβ, and Retinoid Orphan Receptor α (RORα), or other transcription factor families (E4BP4/NFIL3), repress transcription [Hirota and Fukada, 2004; Lowrey and Takahashi, 2004] [Duez and Staels, 2008; Yin and Lazar, 2005; Yin et al., 2006]. The serine/threonine kinases, casein kinase Iε (CK1 ε) and glycogen synthase kinase 3β (GSK3 β), phosphorylate BMAL1, PER, and other proteins. Once modified, the proteins are targeted to the ubiquitin/proteasomal pathway for degradation [Ko and Edery, 2005; Lee et al., 2008].

Circadian intersections with the stem cell regulatory highway

The CCRP intersects with a number of identified adult stem cell regulatory pathways. For example, the PAS domain family is notable for other protein members relevant to stem cell biology. The hypoxia inducible factor 1 (HIF-1) belongs to the PAS domain family and heterodimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) to form a transcriptional regulatory complex. Under hypoxic conditions, HIF-1 induces expression of vascular endothelial growth factor (VEGF), stromal derived factor-1, and related factors known to modulate adult stem cell migration and differentiation. There is a growing body of literature evaluating the relationship between tissue oxygen tension, stem cell number, and function. Indeed, it appears that hypoxic conditions within a stem cell's microenvironment

or niche may be critical to the preservation of the stem cell state [D'Ippolito et al., 2006; Fehrer et al., 2007; Grayson et al., 2006]. The shared protein structure between circadian and hypoxic transcriptional regulators, together with the recognized importance of hypoxia in stem cell biology, suggests that a similar link remains to be defined between circadian and stem cell biology.

It is well established that the nuclear hormone receptor family, acting through the glucocorticoid, retinoid, estrogen, and related receptors, plays an influential role in directing and determining adult stem cell fate. Furthermore, the expression of the mRNAs encoding these receptor exhibits circadian rhythmicity [Lemberger et al., 1996] [Yang et al., 2006]. The CCRP involves the NHR family members Rev-erb and ROR [Akashi and Takumi, 2005; Duez and Staels, 2008; Triqueneaux et al., 2004; Yin et al., 2006]. These proteins exert circadian modulating effects in what may prove to be a ligand dependent manner. Although Rev-erb had been characterized as an orphan receptor, recent studies have determined that heme is its natural ligand [Burris, 2008; Raghuram et al., 2007; Rogers et al., 2008; Yin et al., 2007]. This ubiquitous iron containing compound is capable of activating Rev-erb dependent transcription in *in vitro* models. Indeed, heme injection into mice has profound effects on the circadian biology of peripheral tissues, causing a phase shift in the expression of both the positive and negative CCRP genes in the liver [Kaasik and Lee, 2004].

The advent of induced pluripotent stem cells has underscored the importance of epigenetic mechanisms in adult stem cell biology. The introduction of transcription factors such as Oct 4, Sox2, Myc, and KLF4 have endowed adult stem cells with pluripotential properties similar to those demonstrated by embryonic stem cells [Takahashi et al., 2007; Wernig et al., 2007]. This has been associated with altered levels of histone acetyl transferase activity. Recently, valproic acid and related small molecule inhibitors of histone deacetylases (HDACs) have used to substitute for or complement these transgenic methods with success [Huangfu et al., 2008]. At least one CCRP protein, Clock, has been shown to possess histone acetyl transferase activity [Doi et al., 2006]. This chromatin modifying activity is an essential feature of the clock protein's circadian function [Doi et al., 2006]. Furthermore, recent studies have determined that the NAD+ dependent deacetylase, SIRT1, is responsible for the deacetylation of Period 2 [Asher et al., 2008]. This histone deacetylase enzyme plays a prominent role in regulating the oscillatory expression profile of multiple CCRP genes [Nakahata et al., 2008]. Likewise, the disruption of HDAC interaction with the nuclear receptor co-repressor (NCoR) has been found to disrupt circadian oscillations and metabolic events in murine models [Alenghat T, 2008]. Together, these studies demonstrate a close relationship between chromatin remodeling and circadian mechanisms.

Finally, GSK3β has profound effects on stem cell biology through its role in the Wnt signal transduction pathway [Baksh et al., 2007; Baksh and Tuan, 2007; Etheridge et al., 2004; Gregory et al., 2005; Nemeth and Bodine, 2007; Sato et al., 2004]. Studies have demonstrated that GSK3β inhibition and subsequent modification of β-catenin phosphorylation modulate bone marrow hematopoietic and mesenchymal stem cell differentiation and function [Trowbridge et al., 2006]. Likewise, GSK3β is responsible for phosphorylation and turnover of Period and related CCRP proteins [Akashi et al., 2002]. Inhibition of GSK3 using lithium chloride has been shown to lengthen the circadian period in animal studies [Iwahana et al., 2004; Padiath et al., 2004]. Thus, the CCRP intersects with multiple established adult stem cell regulatory pathways at the biochemical and protein level.

Stem Cell Dysfunction in CCRP Mutant Mice

Murine models with mutations or deficiencies in critical CCRP genes have revealed important insights into circadian biology [Antoch et al., 2008; King et al., 1997; Kondratov et al., 2006; Turek et al., 2005]. In many of these models, gene alterations are systemic and not limited to a single organ or tissue type. Consequently, they cannot always be used to distinguish between central versus peripheral circadian mechanisms. Nevertheless, these animals have provided valuable experimental tools. Among the best studied models are the Clock mutant mice which display arrhythmic circadian biology based on activity and biomarker evaluation [King et al., 1997; Turek et al., 2005]. These mice are prone to abnormalities directly or indirectly related to metabolism and adipose tissue function. Clock deficient mice are prone to hyperphagia, hyperinsulinemia, hyperglycemia, dyslipidemia, and obesity [Turek et al., 2005]. In response to radiation damage, the Clock mutant mice exhibit a phenotype of accelerated aging associated with reduced growth rates, cataract development, graying of the hair, and altered cell cycle dynamics [Antoch et al., 2008]. Bmal1 deficient mice exhibit a similar phenotype. Relative to wild type controls, these mice exhibit a shorter life span and increased atrophy of the bone, fat, and skeletal muscle with advancing age [Kondratov et al., 2006]. The mice show evidence of tendon calcification, consistent with abnormalities in the function and differentiation of the tendon stem cells [Bi et al., 2007; Kondratov et al., 2006]. Further evidence from Period deficient mice will be presented in the Cancer Stem Cell section below.

CCRP Regulation of Adult Stem Cell Models

Hematopoietic Stem Cells (HSC)

Studies from the 1950's and earlier first provided evidence of circadian rhythmicity in hematopoietic cell numbers [Halberg et al., 1953a; Halberg and Ulstrom, 1952; Halberg and Visscher, 1950; Halberg et al., 1953b; Halberg F, 1953]. Investigations by Halberg and others demonstrated that the number of circulating eosinophils in multiple murine strains, canine models, and human infants varied as a function of time of day. These findings were extended by Sharkis et al.[Sharkis et al., 1971; Sharkis et al., 1974], Aardal, Abrahamsen, Laerum, and their colleagues in Bergen, Norway [Aardal, 1984; Aardal and Laerum, 1983; Abrahamsen et al., 1997; Sletvold and Laerum, 1988a; Sletvold et al., 1991; Smaaland et al., 2002; Tsinkalovsky et al., 2006; Tsinkalovsky et al., 2005; Tsinkalovsky et al., 2007], Quesenberry [D'Hondt et al., 2004], and others [Baudoux et al., 1998; Bourin et al., 2002; Chen et al., 2000; Haus et al., 1983; Haus and Smolensky, 1999; Wood et al., 1998] who applied flow cytometric analyses, colony forming unit (CFU), and related assays to monitor circulating hematopoietic cell numbers and cell cycle. The circadian oscillation of hematopoietic stem cell-derived erythroid, myeloid, and lymphoid populations were demonstrated as well as those of circulating endothelial progenitor cells [Thomas et al., 2008]. Svetvold, Laerum, and colleagues demonstrated that the circadian oscillation of hematopoietic lineages was age dependent [Sletvold and Laerum, 1988b; Sletvold et al., 1988a; Sletvold et al., 1988b]. While young mice (3 months of age) displayed robust rhythmicity of splenic-CFU and granulocyte-monocyte-CFU, independent of season, the amplitude and peak levels declined in aged mice (26 months of age) [Sletvold and Laerum, 1988b; Sletvold et al., 1988a; Sletvold et al., 1988b]. Correlation between cell numbers and cell cycle indicated that common mechanisms regulated hematopoietic events in the marrow microenvironment. These observations were followed at the molecular level by direct measurements of CCRP gene expression profiles in circulating blood cells [Chen et al., 2000; Fukuya et al., 2007; Sun et al., 2006; Tsinkalovsky et al., 2006]. These studies indicated that the Cry and Per genes exhibited a robust circadian oscillation in peripheral blood cells. Analyses were extended to the examination of bone marrow $CD34^+$ cells obtained [Tsinkalovsky et al., 2005; Tsinkalovsky et al., 2007]. The $CD34^+$ cells displayed a

peak level in early morning hours and their levels of Cry and Per genes exhibited a robust circadian oscillation; in contrast, Bmal1 did not. A similar profile has been reported in peripheral blood leukocytes [Chen et al., 2000; Fukuya et al., 2007]. Consistent with these observations, circulating levels of hematopoietic growth factors such as Granulocyte-Colony Stimulating Factor, Granulocyte-Monocyte Colony Stimulating Factor, Tumor Necrosis Factor, and Interleukins 2, 6, and 10 were also observed to display circadian oscillations [Abdelaal et al., 2000; Dincol et al., 2000; Sothern et al., 1995; Young et al., 1995].

The circadian dynamics of bone marrow hematopoiesis have been linked to the signaling of neural and bone marrow cell derived catecholamines [Maestroni et al., 1998]. Recently, the circadian oscillation in circulating hematopoietic stem cell number have been correlated with circulating levels of stromal derived factor 1 (SDF-1 or CXCL12) in murine models (Figure 2) [Lucas et al., 2008; Mendez-Ferrer et al., 2008]. This dynamic oscillation in HSC levels was absent in Bmal1$^{-/-}$ mice [Mendez-Ferrer et al., 2008]. Based in part on this observation, Mendez-Ferrer et al. hypothesize that the suprachiasmatic nucleus transmit signals to the bone marrow microenvironment via the β-adrenergic system to directly regulate SDF-1 levels and subsequent HSC release [Mendez-Ferrer et al., 2008]. It is of interest that this phenomenon appears to be species dependent [Lucas et al., 2008]. In contrast to nocturnal mice, the timing of the HSC release and SDF-1 expression in humans is reversed relative to the light dark cycle [Lucas et al., 2008; Mendez-Ferrer et al., 2008]. Indeed, SDF-1 and its receptor CXCR4 are likely to play a critical role in determining the circadian dynamics of HSCs; however, in light of the fact that up to 25% of the transcriptome in peripheral tissues displays a circadian oscillation [Ptitysn AA, 2006; Zvonic et al., 2006], it is likely that the expression profile of multiple growth factors, receptors, and related metabolic enzymes will exhibit circadian characteristics.

Bone Marrow-derived Mesenchymal Stem Cells (BMSC)

Circadian mechanisms influence the other stem cell constituent of the bone marrow microenvironment, the bone mesenchymal stem cell (BMSC), and its progeny, the adipocytes, osteoblasts, and stromal cells. The BMSCs serve in multiple capacities. In the context of hematopoiesis, the BMSCs express surface adhesion proteins such as β_1 intergrin (CD29), hyaluronate receptor (CD44), and vascular cell adhesion molecule (VCAM-1) that sequester the HSCs and their lineage committed progeny [Kincade, 1991; Kincade et al., 1989; Simmons et al., 1992; Yin and Li, 2006]. By anchoring the HSCs, the BMSCs serve as a stromal feeder layer or nurse cell for hematopoietic events [Kincade et al., 1989]. Locally, the BMSCs release cytokines and growth factors that can maintain the HSCs in a stem-like state, stimulate proliferation, or promote differentiation along the various hematopoietic lineage pathways [Gimble et al., 1989; Kincade, 1991; Kincade et al., 1989; Yin and Li, 2006]. Systemically, the circulating levels of these growth factors fluctuate in a circadian manner. Well established examples include Granulocyte Colony Stimulating Factor and Interleukin 6 which oscillate in a time dependent manner [Abdelaal et al., 2000; Dincol et al., 2000; Sothern et al., 1995; Young et al., 1995]. Furthermore, the MSCs are themselves multipotent and can differentiate into adipocytes, chondrocytes, and osteoblasts as well as other lineages [Gimble et al., 1990; Gimble et al., 1996; Gimble et al., 2006].

Schibler and his colleagues performed pioneering work demonstrating that adherent rat fibroblast cells in culture contain an intact and operational molecular circadian clock [Balsalobre et al., 2000a; Balsalobre et al., 1998; Balsalobre et al., 2000b; Brown et al., 2005]. Their initial studies found that the CCRP in confluent and quiescent fibroblasts was synchronized following exposure to a 2 hr dexamethasone or 30% serum shock [Balsalobre et al., 1998]. Over the next 48 hrs, the levels of Cry, Per, DBP, and Rev-Erb mRNAs oscillated with a period of ~24 hrs. When human dermal fibroblasts were transduced with viral vectors containing a circadian gene promoter/luciferase reporter construct, the

luciferase activity levels could be entrained *in vitro* [Brown et al., 2005; Brown et al., 2008]. Following dexamethasone stimulation, the human cells expressed luciferase under the control of the murine Per2 promoter with a period of 23 to 26 hrs. Indeed, the period length could be correlated with the reported sleep/awakening patterns of the fibroblast cell donors [Brown et al., 2008].

The BMSCs display a pattern of CCRP synchronization similar to fibroblast models [Wu X, 2008]. When human or murine BMSCs were synchronized with dexamethasone, multiple CCRP genes displayed an oscillatory expression profile [Wu X, 2008]. These included Bmal1, Cry 1, DBP, Per 2/3, and Rev-erbα/β. Furthermore, the presence of the GSK3β inhibitor, lithium chloride, shifted the acrophase or time of peak expression for all genes by ~4 hrs [Wu X, 2008]. For BMSCs obtained from male mice, lithium chloride significantly increased the length of the period of gene expression; otherwise, no gender related differences were noted [Wu X, 2008]. *In vivo* studies of bone and related *in vitro* analyses of osteoblasts suggest that BMSCs and their progeny exhibit circadian dependent functionality [Fu et al., 2005; Meyer et al., 2000; Zvonic et al., 2007]. In transcriptomic analyses of the murine calvarial bone, ~25% of the expressed mRNAs exhibited a circadian oscillation [Zvonic et al., 2007]. This included a number of gene families directly associated with osteoblast function. Furthermore, murine models deficient in the Cry or Per genes exhibited increased bone mass relative to their controls [Fu et al., 2005]. This was attributed to centrally mediated clock effects on sympathetic signaling and osteoblast proliferation [Fu et al., 2005]. Likewise, mice deficient in RORα expression display skeletal abnormalities [Meyer et al., 2000]. Together, these studies indicate that both central and peripheral clocks directly regulate the biology of BMSCs.

Adipose-derived Stem Cells (ASC)

Adipose tissue contains adipose-derived stem cells (ASCs) that resemble BMSC with respect to differentiation potential, immunophenotype, immunogenicity, proteome, and transcriptome [Gimble and Guilak, 2003; Gimble et al., 2007]. In the undifferentiated state, the expression of CCRP genes by ASCs was similar to that of BMSCs [Wu X, 2007]. *In vitro*, the ASC profiles of Bmal1, Cry, Per, DBP, and Rev-erb were synchronized following exposure to dexamethasone, thiazolidinedione, or serum shock [Wu X, 2007]. Furthermore, the presence of lithium chloride increased the period of Rev-erbα and β gene expression by 4 hrs in the ASCs [Wu X, 2007]. While the adipocyte differentiated ASCs displayed an overall CCRP response profile similar to the undifferentiated cells, the period of expression was prolonged by 1 to 6 hrs, depending on the synchronizing agent [Wu X, 2007]. Consistent with these observations, individual CCRP genes have been implicated directly in the adipocyte transcriptional regulatory pathway [Shimba et al., 2005; Shimba et al., 2004]. In 3T3-L1 murine pre-adipocytes, knock down of the Bmal1 mRNAs by RNAi inhibited adipogenesis based on morphological criteria [Shimba et al., 2005]. Complimentary gain of function studies demonstrated that over-expression of Bmal1 increased expression of multiple adipogenic and lipogenic genes [Shimba et al., 2005]. Furthermore, transfection studies demonstrated that Bmal1 drove transcription from both an SREBP1α and Rev-erbα promoter construct [Shimba et al., 2005]. The related CCRP, EPAS1, displayed similar adipogenic regulatory function [Shimba et al., 2004]. Over expression of EPAS1 promotes adipogenesis in 3T3 fibroblasts which, unlike 3T3-L1 cells, are poorly able to differentiate along the adipocyte pathway [Shimba et al., 2004]. In contrast, the CCRP Dec 1 has been found to inhibit adipogenesis by modulating the association of the transcription factor, C/EBPβ, with a histone deacetylase [Gulbagci et al., 2008]. This , in turn, down regulates the expression of adipogenic master-regulators, C/EBPα and PPARγ2, which both lie downstream of C/EBPβ [Gulbagci et al., 2008]. Finally, both Rev-erbα and Rev-erbβ were initially identified as late markers gene markers of adipogenesis and have since been shown

to regulate adipogenesis at the transcriptional level [Chawla and Lazar, 1993; Yin and Lazar, 2005; Yin et al., 2006; Yin et al., 2007]. Recent studies have demonstrated that heme functions as Rev-erb ligand [Burris, 2008; Raghuram et al., 2007; Rogers et al., 2008; Yin et al., 2007]. In light of the fact that heme induces adipogenesis in the 3T3-L1 model, this suggests that Rev-erb acts as a ligand-dependent transcription factor controlling adipogenic differentiation while likewise serving as a CCRP [Chen and London, 1981].

In vivo findings complement these *in vitro* observations [Ando et al., 2005; Bray and Young, 2007; Ptitysn AA, 2006; Zvonic et al., 2006]. Transcriptomic studies have shown that between 20–25% of the mRNA transcripts in both white and brown murine adipose tissues exhibit a circadian expression profile [Ptitysn AA, 2006; Zvonic et al., 2006]. These include multiple genes induced with ASC adipogenesis, including those in the oxidative phosphorylation, glucose and lipid metabolic, steroidogenic, and heat shock/chaperone families [Zvonic et al., 2006]. *In vivo*, treatment of animals or human subjects with the lithium chloride leads to weight gain, increased adiposity, and obesity [Baptista et al., 1995; Zvonic S, 2006]. Paradoxically, the presence of lithium chloride inhibits adipogenesis in both human ASCs and murine 3T3-L1 cells *in vitro* [Aratani et al., 1987; Ross et al., 2000; Wu et al., 2007]. Despite this discrepancy, the weight of the evidence supports a direct role of CCRPs in regulating the ASC adipogenic program and lineage selection.

Cancer Derived Cell Models

There is a wealth of *in vivo* and *in vitro* evidence implicating the CCRP in the growth of tumors and, by extension, cancer stem/progenitor cells. The Period genes have been associated with a number of tumors, including myeloid leukemia, breast, and lung cancers [Fu et al., 2002; Gery et al., 2005; Gery and Koeffler, 2007; Gery et al., 2006]. In a number of human tumors, the levels of the Period genes were reduced [Gery et al., 2006]. In mice deficient for Per2, the animals showed an increased risk of tumor development following exposure to ionizing radiation [Fu et al., 2002]. This resulted in an increased incidence of salivary gland hyperplasia, lymphoma, angiosarcoma, and premature graying of the hair in $Per2^{-/-}$ mice relative to wild type controls [Fu et al., 2002]. Furthermore, thymocytes in the $Per2^{-/-}$ mice were relatively resistant to the apoptotic effects of ionizing radiation [Fu et al., 2002]. Consistent with this observation, over expression of Period in human cancer cell lines increased their sensitivity to DNA damage and apoptosis; in contrast, down regulation of Period was associated with protection against ionizing radiation induced apoptosis [Gery et al., 2006]. Both the CCRP proteins Period and Bmal1 regulate components of the cell cycle, such as the cell cycle inhibitor p21WAF1/CIP1 [Fu et al., 2002; Grechez-Cassiau et al., 2008]. The levels of p21WAF/CIP1 and related cell cycle genes displayed a robust circadian oscillation in the liver of wild type mice; however, in $Bmal1^{-/-}$ animals, all cell cycle genes were expressed at relatively constant levels [Grechez-Cassiau et al., 2008]. The transcription of the p21WAF/CIP1 promoter was positively regulated by expression of Clock and Bmal1 in co-transfection assay; the further addition of Rev-erbα/β or RORα/γ antagonized the actions of Clock and Bmal1 [Grechez-Cassiau et al., 2008]. It is noteworthy that disruption of Clock and Bmal1 genes in mice was not associated with increased carcinogenesis [Antoch et al., 2008; Kondratov et al., 2006]. When exposed to ionizing radiation, these mice show accelerated aging and increased evidence of lymphoid apoptosis but do not exhibit tumors [Antoch et al., 2008; Kondratov et al., 2006]. One interpretation of this finding is that the negative arm of the CCRP (Cry, Per), as opposed to the positive arm (Clock, Bmal1) plays a more direct role in suppressing tumor formation.

Dysregulation of the CCRP may account for related changes in downstream features of tumor cells. In murine breast cancer models, while the anti-apoptotic gene Bcl2 displayed a robust circadian oscillation in normal tissues, this rhythmicity was absent in the tumor itself [Granda et al., 2005]. The involvement of the CCRP in tumor development has substantial

implications with respect to chemotherapy [Gery and Koeffler, 2007; Gorbacheva et al., 2005; Granda et al., 2005; Levi, 2006; Levi et al., 2007]. The time of day when drugs are delivered to patients can profoundly impact both their benefits and adverse effects. Murine and human clinical studies have determined that there is an optimal time of day for the delivery of chemotherapy [Gery and Koeffler, 2007; Gorbacheva et al., 2005; Granda et al., 2005; Levi, 2006; Levi et al., 2007; Sahar and Sassone-Corsi, 2007]. For example, the administration of cyclin dependent kinase inhibitors to tumor bearing animals has been shown to enhance the rhythmicity of CCRP genes in the tumors themselves [Iurisci et al., 2006]. This has been associated with improved outcomes as evidenced by >50% reduction in the extent of tumor growth [Iurisci et al., 2006]. Oncologists have begun to incorporate the emerging concepts of chronotherapy into their strategies for dosage administration [Levi, 2006; Levi et al., 2007]. These findings merit continued evaluation and research to better define the underlying mechanisms of circadian biology in cancer and cell proliferation.

Future Directions

Again, why ask these questions? Because:

1. Timing is a variable that we can readily manipulate. As we develop cell based therapies that use exogenous, culture expanded stem cells or target the endogenous stem cells and their associated microenvironment, it is critical to maximize the returns to the patient. Studies of the CCRP in the context of stem cell biology will allow us to optimize the time when stem cell-based treatments are administered. Furthermore, this pathway will provide a molecular read out that can be readily monitored with appropriate diagnostic tools.
2. The CCRP provides an under-explored and novel regulatory pathway for potential direct interventions. The recent identification of heme as a direct ligand for Rev-erb proteins underscores this point. Since Rev-erb plays a significant role in adipogenic differentiation by ASCs, heme analogues have potential benefits for the prevention or treatment of obesity. Likewise, related nuclear hormone receptor ligands and substrates of critical rate limiting metabolic enzymes may also prove to be productive targets of opportunity. One example is the recent identification of cyclic AMP and its signaling pathway as a critical component of the circadian oscillatory network [O'Neill et al., 2008]. Furthermore, similar studies in model organisms have implicated cyclic adenosine diphosphate ribose and calcium as feedback components of the circadian oscillations [Dodd et al., 2007; O'Neill et al., 2008]. We hypothesize that these small molecules capable of entraining the CCRP apparatus can be used as drug discovery targets to manipulate adult stem cell lineage differentiation and function.

Conclusions

Modifying the period and amplitude of circadian oscillations in stem cell number and in stem cell metabolic activity is feasible using small molecule or genetic agents. Directing increased attention to these mechanisms has the potential to improve our understanding of stem cell biology and subsequently, our ability to modulate stem cell proliferation, differentiation, and function. The outcomes of such studies will directly impact our future capability to manipulate stem cell physiology and to prevent stem cell pathology.

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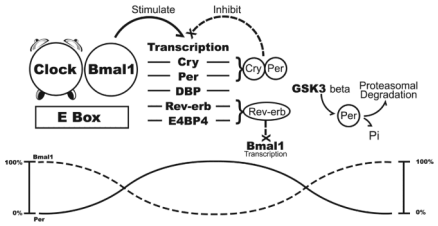

Figure 1.

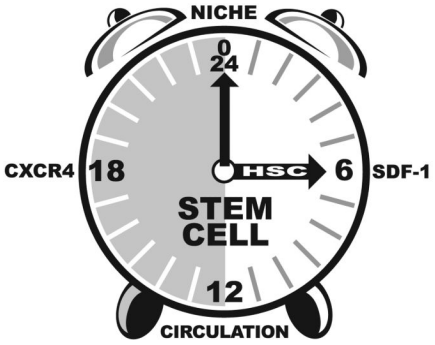

Figure 2.

Table 1

Circadian Rhythm Regulatory Genes/Proteins

bHLH-PAS Family Members (basic Helix Loop Helix – Period/Arnt/Simpleminded)	**bHLH-PAS Family Post-Translational Modifiers and/or Putative Downstream Targets**
BMAL 1 & 2 (Bone and Muscle Arnt-Like)	CK1ε (Casein Kinase 1 ε)
Clock	Cry 1,2 (Cryptochrome)
DEC 1 & 2 (Differentially Expressed in Chondrocytes)	DBP (Albumin D site binding protein)
EPAS 1 (Endothelial PAS)	E4BP4
NPAS1 & 2 (Neuronal PAS)	GSK3β (Glycogen synthase kinase 3β)
Per 1,2,3 (Period) (PAS domain only)	Rev-Erb α & β