Cell Cycle:
Runs like "Clock" Work

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«Η ολοκλήρωση της εργασίας αυτής έγινε στο πλαίσιο της υλοποίησης του μεταπτυχιακού προγράμματος συγχρηματοδοτήθηκε μέσω της Πράξης «Πρόγραμμα Χορήγησης Υποτροφιών Ι.Κ.Ι. με διαδικασία εξατομικευμένης αξιολόγησης ακαδ. έτους 2011-2012» από πόρους του Ε.Π. «Εκπαίδευση και Δια Βίου Μάθηση» του Ευρωπαϊκού Κοινωνικού Ταμείου (ΕΚΤ) και του ΕΣΠΑ, του 2007-2013»

CELL CYCLE: RUNS LIKE "CLOCK" WORK

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ABSTRACT

The circadian clock is the intrinsic timekeeping device that exists within all living organisms and whose function is to integrate information about the light and dark phases of a day into the biological processes that occur in order to sustain life. This integration manifests as oscillations in the expression of molecules or in the rhythmicity of certain processes with a period of ~24 hours. The master pacemaker resides in a region of the brain called the suprachiasmatic nucleus (SCN) and its job is to detect when it is light and dark and pass this information onto secondary oscillators that reside in almost all tissues of a body, which in turn modify the processes that take place in that tissue. On the molecular level, the circadian circuit consists of a network of transcriptional and translational autoregulatory feedback loops that result in the temporal expression of core clock proteins. Links have been found between the circadian clock and the progression of the cell cycle, indicating that cell division is, in part, regulated by day and night rhythms. Some of the proteins that mediate this connection have been identified. Due to this link, the deregulation of circadian rhythmicity may also lead to aberrant cell cycling and increased susceptibility to diseases such as cancer. On the other hand, this intertwinement of the two processes can be utilized in anticancer treatments, so that the efficacy can be maximized and the toxicity minimized by taking into account the time of day that they are administered.

INTRODUCTION

All living organisms need to regulate their biological processes in accordance with the environmental changes that occur around them in order to successfully adapt and survive[1]. Perhaps the most predictable and rhythmic environmental change is the daily rotation of the earth to which many organisms have adapted by means of an intrinsic timekeeping device dubbed the circadian clock, derived from the Latin *circa diem* which means "about a day"[2]. This endogenous timing system manifests as cycles of gene expression, metabolic flux, physiological processes and behavioral activities that display a periodicity of approximately 24 hours[3,4].

That same periodicity can be detected in another fundamental aspect of all life: cell division. In fact, vertebrate cell division occurs in ~24 hour cycles, which suggests the existence of a link between the two processes.

The aim of this review is to describe what is currently known about the circadian clock in mammals by gathering data about

the clock components and how they work together to establish the circadian timing system. Next is an attempt to find the associations between circadian timing and the cell cycle followed by a discussion on the implication of clock components in cancer and their potential value as targets in anti-cancer strategies. A reference is made to chronotherapy, an emerging theme in cancer treatment whereby aspects of circadian timing and cell cycle are taken into account and can have an effect in the way cancer treatments are administered.

ABOUT THE CLOCK

All circadian rhythms are defined by three fundamental properties: (1) daily oscillations are self-sustained and continue to be produced with high precision even in the absence of external cues, (2) they are temperature compensated meaning that the period of the rhythms remains the same during changes in temperature within the physiological range, (3) they are entrained (synchronized) each day to the cycle of light and dark[5]. Regardless of the phylum in which they were discovered, all circadian systems are comprised of three major components: (1) a light-input pathway through which the self-sustained master circadian pacemaker is entrained to the light and dark cycle, (2) the actual circadian pacemaker and (3) output pathways by which the circadian pacemaker affects a number of rhythmic biological processes[4,6].

Circadian rhythms have been identified in vastly diverse organisms such as cyanobacteria, fungi, algae, plants, flies, birds and mammals[7]. Within this assortment of life forms, many of the molecular pathways associated with the circadian clock are evolutionary conserved[8]. The presence of a circadian timing system across the tree of life suggests that entrainment of biological processes to the day and night cycle provides some kind of adaptive advantage[9]. This is empirically corroborated by experiments done with *S. elongatus* mutants, each of which had a different intrinsic circadian period. When grown under controlled light-dark cycles of different periods, the strain that had the highest reproductive success (a measure of adaptability) was always the one whose intrinsic rhythm was closest to the light-dark cycle that was artificially created[10,11].

Possibly the largest advantage provided by circadian timing, or any biological clock for that matter, is that environmental changes don't need to occur before a physiological reaction can be instigated; on the contrary, it can occur pre-emptively in anticipation of the environmental changes[12]. In the case of plants, this allows them to utilize light energy more efficiently since photo-system I and II components that are necessary for photosynthesis are produced already just before sunrise[13]. Nocturnal animals can, in the same fashion, anticipate dusk from their underground habitat, avoiding surfacing to check whether sunset has occurred and the risk of being preyed on by day-active animals[12]. In Cyanobacteria, circadian timing allows the temporal separation of nitrogen fixation and photosynthesis, chemical processes that if occurred simultaneously they would render each other inefficient[12,14]. In mammalian liver cells, circadian expression of cytochrome p450 genes that can generate harmful reactive oxygen species peaks during the absorptive phase, minimizing the risk of damage from the ROS[15–17].

Concerning the evolutionary pressures that gave rise to circadian timing systems, several theories have been postulated, usually regarding the cyanobacterial circadian clock since it is, evolutionary speaking, one of the oldest clocks that have been discovered. The most popular theory is the "Escape from light" hypothesis according to which organisms that restricted the S phase of the cell cycle, during which DNA replication occurs, at night had a selective

advantage due to less chances of DNA damage occurring because of ultraviolet (UV) light[18]. This is especially taking into account the fact that 3-4 billion years ago when the first Cyanobacteria came to existence, the composition of the earth's atmosphere was completely different and did not have the ability to filter out harmful radiation as it does currently[19].

Another hypothesis is that described by Hut and Beersma[9]. According to them, the primary function of the phosphorylation-dephosphorylation cycle of KaiC, a key component of the Cyanobacteria clock, which displays a circadian pattern was to store ATP during the day and to supply ATP at night when photosynthesis cannot occur. This was derived by the finding that KaiC forms hexamers by using ATP molecules not as an energy supply but as a building block needed for the stabilization of the hexamer[20]. During the day, KaiC hexamers in association with ATP are formed and their phosphorylation is promoted by KaiA, another clock component. The phosphorylated KaiC hexamers in a complex with ATP and KaiA can be bound by KaiB, yet another clock component, causing a change in conformation of the complex which inhibits further phosphorylation. During the night dephosphorylation occurs which makes the complex less stable and more likely to disintegrate, releasing ATP molecules which can be used as an energy source. This process is thought to be an ATP storage mechanism during a time when starch and sugar had not yet evolved, acting to provide the cells with ATP during the night when photosynthesis cannot occur and ensuring an even flow of ATP through the entire day[9].

About the Cell Cycle

One of the most fundamental processes that all cells go through at least at some stage of their development is the cell-division cycle. This term refers to the series of spatially and temporally regulated events that take place within a cell culminating in its division and duplication[21]. In eukaryotic cells, the cell cycle is divided into two basic parts, interphase and mitosis. Interphase accounts for approximately 95% percent of the time that an entire cell cycle lasts and entails cell growth and DNA replication in preparation for mitosis and cell division. Three main phases can be distinguished within interphase (Figure 1A):

- **G_1** (gap 1) phase separates the previous mitosis event from the subsequent DNA replication event. During this phase the cell is metabolically active and continuously grows.
- **S** (synthesis) phase is the phase during which DNA is replicated in order to ensure that the genome exists in 2 copies and can be equally divided during the cell division that is to follow.
- **G_2** (gap 2) phase separates the previous DNA replication event from the subsequent mitosis events. During this phase the cell continues to grow and simultaneously produces the proteins that are necessary for mitosis to occur.

Certain cell types that have completely stopped dividing or only divide to replace cells that were lost e.g. during an injury enter a phase of the cell cycle called **G_0** during which they remain metabolically active but do not proliferate unless they are instructed to do so by the appropriate extracellular signals.

The M (mitotic) phase accounts for 5% of the duration of a cell cycle and is the phase during which the duplicated DNA in each cell is equally separated between 2 daughter cells. It consists of the following sub-phases: prophase, prometaphase, metaphase, anaphase and telophase (Figure 1B).

All events of the cell cycle happen in a tightly regulated and sequential fashion, meaning that several safeguards have been installed to ensure that the transition from one phase to the next occurs only after certain requirements have been met. These

safeguards come mostly in the form of checkpoint protein complexes that monitor what is happening at each moment of the cell cycle and either promote or inhibit the transition from one phase to the next in a variety of ways. These complexes are themselves tightly regulated and are only expressed at certain stages during cell cycle progression.

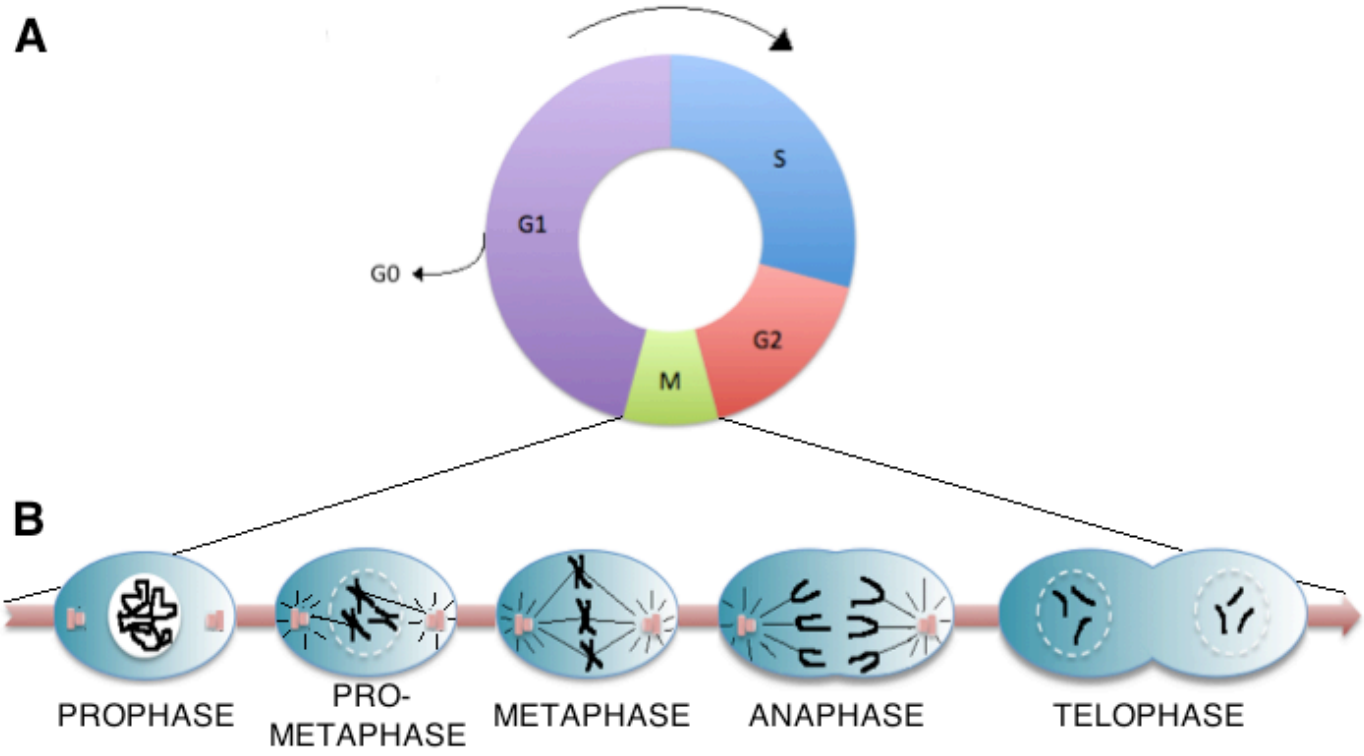

Figure 1. A) The phases of the cell cycle are depicted in different colours and the size of each section represents the respective percentage that each phase takes up in the cell cycle. Chromosome duplication during S phase is followed by equal separation of them into the new daughter cells in M phase (mitosis). The S and M phases are interjected by the two gap phases, G1 and G2. **B)** Mitosis can be further broken down into five distinct phases. During prophase, the duplicated DNA begins to condense. During prometaphase the nuclear envelope begins to break down and nucleosomal microtubules enter the nuclear space. In metaphase every chromosome is attached to microtubules of both nucleosomes and all chromosomes are aligned at the metaphase plate. In anaphase the sister chromatids are separated and microtubular depolymerization draws them to opposite sides of the cell. In telophase a new nuclear envelope begins to form around the 2 separated sets of chromosomes. During cytokinesis (not shown), the plasma membrane at the height of the metaphase plate begins to ingress until finally 2 new cells are formed.

What Makes the Mammalian Circadian Clock Tick

Primary and Secondary Oscillators

Almost all cell types of complex organisms such as mammals have been found to exhibit intrinsic circadian oscillations with phases that differ from one cell type to the other[22]. Expression profiling studies have shown that in any given tissue, up to 15% of the transcripts demonstrate patterns of circadian regulation[8,23]. Although all these cells are capable of exerting circadian functionality independently of each other, there needs to be some kind of higher order organization so that all tissues can work together in harmony to sustain an organism. In mammals, the coordination of these peripheral oscillators is the duty of the master pacemaker which is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus[24–26]. The SCN is a paired structure that contains approximately 8,000-10,000 neurons[27], each of which is capable of generating self-sustained circadian rhythms when dissociated from the SCN or when cultured as an immortalized cell line[22,28,29]. When examining electrical firing sequences of individually cultured neurons, circadian fluctuations can be detected but the period length displays a very wide range between different cells. The average period length of electrical activity however, almost completely matches the period length of the locomotor activity of the donor, indicating that the SCN neurons do not function independently of each other in the brain of living organisms[30]. Indeed, *in vivo,* SCN neurons couple to form a network that expresses synchronized rhythms[31]. This coupling is thought to involve neurotransmitters, neuropeptides, gap junctions and chemical synaptic mechanisms[31]. The master pacemaker function was conclusively attributed to the SCN after the conduction of transplantation experiments by several groups over the last few decades. In these experiments, mice with SCN lesions that had rendered them arrhythmic received SCN tissue transplants from mice harboring circadian clock gene mutations that resulted in different periods of circadian rhythmicity. Circadian rhythms were restored in the transplant recipients and the period length of the rescued rhythmicity was determined by the donor's genotype[32–34].

The circadian rhythm generated by the SCN is mainly determined through light entrainment[35]. In mammals, photic perception occurs through retinal photoreceptors in the eyes that include the rods and cones in combination with a distinct subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) that contain melanopsin, a novel photopigment[36,37]. The photic information gathered by the different retinal components is transmitted to the SCN via the retinohypothalamic tract (RHT). In addition to photic signals, the SCN also integrates signals from feeding, locomotor activity and photobiotic hormones creating a more extensive network of feedback interactions that can prevent the SCN neurons from reacting to sporadic photic stimuli[38]. After processing all the environmental data it received, the SCN in turn orchestrates a neuronal and humoral response that will coordinate the peripheral oscillators present in all parts of the body[39].

Apart from the direct timing cues that peripheral oscillators receive from the master oscillator of the SCN, entrainment of clocks in many organs can also occur directly by temperature changes and more importantly through the daily feeding and fasting cycles[40–42].

THE MOLECULAR CIRCADIAN CIRCUITRY

Major insights into the gene and protein composition of the circadian machinery have been provided by genetic studies performed with the fruit fly *Drosophila melanogaster*. These insights are likely to be relevant for mammalian circadian biology since orthologs of many of the Drosophila clock genes can be found in mammals too[12]. The current understanding of the molecular mechanism of circadian rhythms is that they rely on a network of autoregulatory transcriptional and translational positive and negative feedback loops that result in the approximate 24-hour cycling of key clock components[43]. At the core of this model lie two transcription factors, **BMAL1** (**B**rain and **M**uscle **A**ryl hydrocarbon receptor nuclear translocator-**l**ike **1**) and **CLOCK** (**C**ircadian **L**ocomotor **O**utput **C**ycles **K**aput) that contain a PAS (**P**eriod-**A**rnt-**S**ingle minded) domain in their amino acid sequence that allows them to interact with each other and form heterodimers[44–47]. The *Clock* gene was first identified by a forward genetic approach in mice[47]. The heterodimer formation is limited by transcript and protein levels of *Bmal1,* which display circadian cycling and peak during the middle of the circadian night whereas the *Clock* gene seems to be constitutively expressed[48]. Both transcription factor molecules also contain a bHLH domain (**b**asic **H**elix-**L**oop-**H**elix) through which they are able to bind DNA sequences[49]. BMAL1 and CLOCK are produced in the cytoplasm where they dimerize and subsequently translocate to the nucleus and bind to the promoter region of various genes harboring circadian E-box elements and activate their transcription[44,49] (Figure 2). The BMAL1/CLOCK dimer is directly or indirectly involved in all parts of the circadian circuitry by promoting the expression of genes whose protein products are part of the feedback loops that constitute the molecular circadian machinery. The BMAL1/CLOCK transcription-regulating dimer binds to the E-box elements in the promoter region of the following target-genes:

- ***Per1, Per2*** and ***Per3*** (**Per**iod genes)**,** paralogous members of the PAS protein family and ***Cry1*** and ***Cry2*** (**Cry**ptochrome genes), members of the vitamin B_2-based blue-light photoreceptor/photolyase family[35]. These two groups of genes encode the major players in the negative feedback loops of the circadian circuitry[50,51]. PER1, PER2, CRY1 and CRY2 are produced in the cytoplasm and are able to bind to each other creating heteropolymeric complexes of unknown stoichiometry[12]. Once these complexes have been formed, they translocate to the cell nucleus where they begin to accumulate. Once the concentration of these complexes in the nucleus reaches a critical concentration, they are able to interact with the BMAL1/CLOCK dimer and abolish its transcription activation potential, inhibiting among that of others their own expression[50,52]. As a result, mRNA and protein levels of the *Per* and *Cry* genes begin to decrease and once the concentration of PER-CRY complexes in the nucleus is not sufficient for autorepression, a new round of *Per/Cry* transcription initiates[53,54]. Null mutations of the *Per3* gene have almost no noticeable effects on the circadian period so it is considered as a non-essential component of the circadian core oscillatory mechanism. It may however play a role in clock output pathways[55,56] (Figure 2).
- ***Rev-Erbα*** and ***Rorα*** (**R**etinoic acid receptor-related **o**rphan **r**eceptor **alpha**). These protein members of the family of nuclear receptors function as transcription factors that compete with each other and exert opposite effects on the transcription of their target genes. Both REV-ERBα and RORα are able to bind to ROR elements present in the promoter region of the *Bmal1* gene and inhibit or promote its expression respectively[57,58]. It is also speculated that

they can have the same effect on the *Clock* and *Cry1* genes. This leads to a rhythmic expression of *Bmal1* and *Clock* mRNA that is antiphasic to the expression of *Rev-Erb*α[59]. By being involved in the negative feedback loop, REV-ERBα and RORα offer an additional point of regulation and add robustness to the system. Whereas the PER/CRY complexes directly cause their downregulation by interacting with the BMAL1/CLOCK heterodimers, REV-ERBα does this indirectly by inhibiting the expression of its own activator (Figure 2).

- ***Dec1*** and ***Dec2*** (**D**ifferentially **e**xpressed in **c**hondrocytes). *Dec1* and *Dec2* encode transcription factors that contain, like the positive regulators of the core circadian circuitry, a basic helix-loop-helix domain. This allows DEC1 and DEC2 to bind to E-box regulatory elements but in contrast to BMAL1/CLOCK, they repress the expression of their target genes, creating an additional regulatory loop[60].
- **CCG**s (**C**lock **C**ontrolled **G**enes). This term is used to collectively refer to all genes whose expression is regulated by components of the circadian machinery but are not themselves involved in the creation and maintenance of circadian rhythmicity. These genes may be involved in a multitude of other processes that go on in mammals such as metabolism, cell cycle, locomotor activity and body temperature, providing a link between them and the circadian clock[61] (Figure 2). The number of clock controlled genes has not yet been determined. This question has mainly been addressed with the conduction of microarray experiments that are, however, prone to underestimation of this number due to the inability of these methods to factor in transcript stability and transcripts that are periodically expressed but at very low levels.

Even though the above molecular mechanism stands true in cells of the SCN and most peripheral organs, nevertheless, tissue-specific differences can be detected. For example, in the forebrain, NPAS2 (Neuronal PAS domain protein 2), which is a paralog of *Clock* that contains both PAS and bHLH domains, appears to be the more relevant heterodimer partner for BMAL1[63]. The ability of NPAS2 to take over the function of CLOCK explains why *Clock*$^{-/-}$ mice continue to normally generate circadian rhythms of locomotor activity and display very little gene expression variation in SCN tissue when compared to wild-type animals[64]. This apparently does not apply to peripheral oscillators since the presence of CLOCK is imperative for normal circadian cycling to occur[65].

As mentioned earlier, light entrainment is the main way through which circadian rhythms can be influenced by the environment so that they can adapt to it. The mechanism that allows this to happen has also been discovered. When intrinsically photosensitive retinal ganglion cells (ipRGCs) are stimulated by light, glutamate and pituitary adenylate cyclase-activating polypeptide is released by their axon terminals onto post-synaptic SCN neurons[66–70]. This causes a large uptake of calcium by these neurons which leads to the activation of a number of protein kinase pathways. One of the final outcomes of the activated pathways is the phosphorylation of Ca^{2+}/cAMP-response element binding protein (CREB)[71]. Phosphorylated CREB molecules homodimerize and have the ability to bind to Ca^{2+}/cAMP-response elements (CREs) that are present in many gene promoters and activate their transcription[72]. *Per1* and *Per2*, which encode for proteins that are part of the negative feedback loops of the core circadian circuitry, are among the genes that have these CREs[73], thus providing the link between light and the molecular circadian mechanism. It has been shown, that the expression of *Per1* and *Per2* is rapidly induced in SCN neurons after exposure to nocturnal light[74–76] and depending on the

time of night that this occurs, mice display phase advanced or phase delayed circadian rhythms of locomotor activity[71].

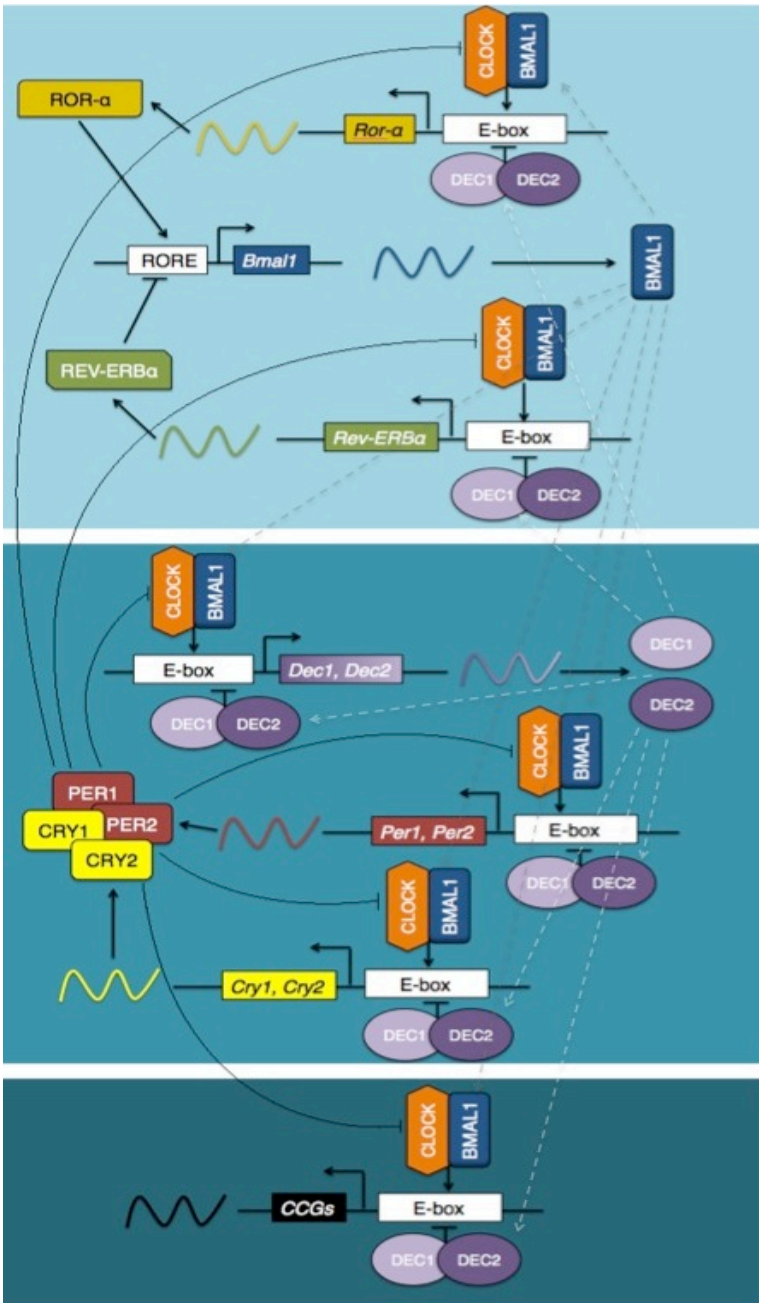

Figure 2. Model of the mammalian molecular circadian oscillator. The basic helix-loop-helix PAS-domain containing transcription factors BMAL1 and CLOCK form a heterodimer that is able to activate the transcription of a group of core clock genes (*Pers* and *Crys*) and two nuclear receptors (*Rev-Erbα* and *Rorα*) through an E-box element in their promoter region. CRY and PER proteins form heteropolymeric complexes that repress the transcriptional activity of the BMAL1/CLOCK complex, thus inhibiting their own expression as well as those of the other BMAL1/CLOCK targets. REV-ERBα and RORα are two nuclear receptors that compete for binding to the ROR response element (RORE) in the promoter of *Bmal1* and repress or activate its transcription, respectively. BMAL1/CLOCK also regulates the expression of multiple clock-controlled genes (CCGs). This can occur directly through E-box elements in the promoters of target genes or indirectly through the activity of other transcription factors that are under BMAL1/CLOCK regulation. DEC proteins bind to E-boxes and repress the expression of those genes. Their own expression is activated by the BMAL1/CLOCK complex.

POST-TRANSLATIONAL AND POST-TRANSCRIPTIONAL MECHANISMS IN CIRCADIAN REGULATION

Though the transcriptional-translational feedback loops described above compose the basic mechanism for circadian oscillations, alone they are insufficient to maintain them with the precision and sensitivity that they have *in vivo*. The importance of post-translational modifications of core clock components in the regulation of the circadian system is becoming increasingly more apparent with some of them being absolutely imperative for the 24-hour cycling whereas others are involved in the fine-tuning of the rhythm[2]. As usual, the most well studied post-translational modification in clock components is phosphorylation; however, the list has expanded greatly in recent years with dephosphorylation, ubiquitination, sumoylation and acetylation emerging as important modifications as well.

- **Phosphorylation:**

CK1ε (**C**asein **K**inase **1** **ε**), initially identified as an important circadian regulator in Drosophila[77,78], later proved to be the component responsible for the *Tau* mutant mice and human FASPS (**F**amilial **A**dvanced **S**leep **P**hase **S**yndrome) phenotype[79,80]. CK1ε and its close family member CK1δ recognize and phosphorylate sites on the PER proteins[81,82], regulating PER's subcellular localization and its repressive function on BMAL1/CLOCK-mediated transcription while at the same time promoting ubiquitin-degradation via the 26S proteasome[83,84]. CK1δ and ε therefore act as an additional point of regulation that co-determine the levels of PER proteins at each moment in the cell so that the period length of circadian rhythmicity is 24 hours. Both mice carrying the *Tau* mutation, which is essentially a mutation in CK1ε that significantly reduces its activity towards various substrates, and humans suffering from FASPS, where a CK1ε phosphoacceptor site on PER2 is lost, have hypophosphorylated PER2. In contrast to their hyperphosphorylated counterparts, hypophosphorylated PER proteins are more stable and are less susceptible to degradation, which leads to a faster accumulation of PER2 in cells. This means that the threshold of PER complex levels for auto-repression are reached faster, causing a decrease in the circadian period length in both of the cases mentioned above[12]. Another member of the CK1 family, CK1α, was recently found to be mammalian clock regulatory kinase in a chemical compound screen although not much is known about its function as of yet[85].

CK2 (**C**asein **K**inase **2**) is a serine-threonine kinase composed of two α catalytic and two β regulatory subunits[86]. Although its implication in the regulation of circadian rhythms was initially proposed by studies done in plants and insects[87], it is now thought to apply in mammals as well, owing to studies done by three groups that, however, produced controversial data. The first report of CK2α implication in the mammalian clock was the phosphorylation of BMAL1 on one site that causes nuclear localization of the protein as seen in CK2α knockdown experiments where this localization is lost[88]. CK2α was also found by another group to have two effects on PER2: a direct phosphorylation on Ser53 and a potentiation of CK1ε-dependent degradation of PER2, possibly indirectly due to it phosphorylating CK1ε and enhancing its activity towards PER2, an interaction that is only speculated and has not been confirmed[89]. The third study, which was an RNAi screen, provided evidence that downregulation of either CK2α or CK2β causes a lengthening of the circadian period while simultaneous knockdown of both lead to arrhythmicity[90]. This group also showed that PER2 is phosphorylated by CK2α but

the proposed sites don't match to the one mentioned previously. Additionaly, the effect that phosphorylation of PER2 by CK2α is proposed to have conflicts with the previous findings. Whereas before, CK2α was suggested to enhance degradation, here it supposedly stabilizes PER2. This controversy has yet to be resolved.

GSK-3 (**G**lycogen **s**ynthase **k**inase-**3**) is a serine-threonine, phosphate-directed protein kinase that has two isoforms in mammals, GSK-3α and GSK-3β, and has been shown to be involved in many intracellular pathways[91,92]. GSK-3$\beta^{-/-}$ mutant mice are not viable, so many of the results concerning the function of the GSK-3 proteins come from experiments using lithium, which apparenty competes for binding to GSK-3 with Mg^{2+}, a cofactor that is required for normal GSK-3 function[93–95]. Lithium treatment studies all seem to display the same effect, a lengthening of the period of behavioral rhythms as well as firing-rate rhythms in isolated SCN neurons[96–98]. A surprising finding was the identification of small molecule inhibitors of GSK-3β that shorten the circadian period[99]. Protein targets of GSK-3β include members in all loops of the core oscillatory circuitry and the effects that phosphorylation has on them vary widely. PER2 phosphorylation by GSK-3β promotes its nuclear localization[100], phosphorylation of CRY2 leads to its degradation by the proteasome[101] whereas it has the opposite effect on REV-ERBα and causes its stabilization[102]. BMAL1 is phosphorylated by GSK-3β on two sites and lead to its ubiquitination and subsequent degradation by the proteasome[103]. It is possible that the small molecule inhibitors only block specific GSK-3β interactions, possibly explaining the difference in phenotype between them and the lithium treatment where, in theory, all GSK-3β is abolished.

A number of other kinases are thought to be involved in phosphorylation of core clock components but either the effect that they have has not yet been determined or the interaction itself has still not been confirmed. There are also some known phosphorylation events that take place such as the rhythmic phosphorylation of CLOCK but the culprit remains elusive.

- **Dephosphorylation**:

Although little research has been done on the phosphatases involved in circadian timekeeping, there is some evidence suggesting their contribution to the complexity of the molecular clock that seems to greatly exceed what was originally speculated. If adding a modification to a circadian substrate can have an important impact on its localization or functionality, then reversal of the modification, which is what phosphatases do, can be just as important.

CK1ε and CK1δ, the major circadian kinases appear to undergo autophosphorylation, which causes a decrease in their activity. It has been found that at least eight autophosphorylation events need to be reversed in order for the two kinases to become active[104]. It has been proposed that the phosphatase responsible for this is PP5 and that it interacts with and is noncompetitively inhibited by the CRY proteins[105]. Thus, it appears that the CRY proteins indirectly regulate the activity of the circadian kinases by interacting with their activator. A role for PP1 has also been found in the molecular clock whereby PP1 can dephosphorylate CK1δ/ε-phosphorylated PER2 and reduce PER2's degradation rate by the proteasome[106].

- **Ubiquitination:**

One of the most well known pathways for protein degradation is the ubiquitin-mediated proteasomal system, its mechanism described in many reviews[107–109]

The positive and negative feedback loops described earlier rely on protein levels reaching and surpassing a threshold that will allow them to activate or repress some branch of the system. In this regard, it is

clear how crucial the process of protein degradation can be in circadian regulation since circadian protein half-life in combination with the mRNA and protein synthesis rates will determine how quickly those levels are reached. Studies by several groups have shown that many of the proteins that comprise the core circadian oscillator are indeed targeted for degradation by the 26S proteasome[83,84,87,110–112].

- **Sumoylation:**

Small ubiquitin-like modifier (SUMO) proteins become attached covalently at lysine residues of target proteins and can have various effects on their function[113]. It has been shown by independent groups that BMAL1 is polysumoylated in a rhythmical fashion at a conserved lysine residue in the PAS domain linker region by all three SUMO proteins that are present in mice and humans (SUMO1/2/3) and this process is dependent on CLOCK[114]. When BMAL1 is sumoylated, the BMAL1/CLOCK dimer transactivating capability is increased but at the same time BMAL1 ubiquitin-dependent proteasomal degradation is promoted[115].

- **Histone modifications:**

Epigenetics is the field that studies the modifications that chromatin is subject to, other than changes in nucleotide sequence, and which could have an effect in gene expression and/or cellular phenotypes. In order for them to be part of an epigenetic mechanism, these changes need to be heritable, self-perpetuating and reversible[116].

The first indication that chromatin remodeling could be one of the mechanisms utilized in circadian regulation was provided by an experiment that demonstrated in mice that light pulses during the subjective night lead to phosphorylation of serine 10 in histone H3[117]. It was later proposed that lysine 9 of histone H3 is rhythmically acetylated in the promoter of some circadian genes and it was even suggested that it is CLOCK and NPAS2 that recruits histone acetyltransferases (HATs) there[118,119]. CLOCK has also been found to exhibit intrinsic HAT activity towards lysine residues of histones H3 and H4, opening up the possibility that it may perform an acetylating function simultaneously as it activates transcription alongside its dimerization partner BMAL1[120].

Many of the core clock genes, together with the clock-controlled genes, display circadian cycling of their transcripts. This combined with the fact that in mice, only 33-50% of the genes that encode rhythmic proteins also exhibit rhythmicity at the transcript level, has raised the suspicion that there are also post-transcriptional mechanisms at play in circadian regulation[121,122]. Research in this field has only recently begun to receive attention, however, there are already some serious indications that confirm the suspicion. Indeed, both miRNAs (short, single-stranded RNA molecules that interact with the 3' untranslated regions of target transcripts to induce cleavage/destabilization of, or to repress translation of the target mRNA)[123] and RNA-binding proteins (proteins that interact with elements in the 3' UTR of many transcripts and regulate their splicing, transport, stability and translation)[124,125] have been proposed to specifically target core clock transcripts in the SCN and other tissues, contributing to the multitude of regulation mechanisms utilized in circadian timekeeping[125–132].

Circadian Gating of the Cell Cycle

Over the last few years, there has been an accumulation of evidence suggesting that the circadian clock and many other biological processes are interconnected. Originally, when circadian biology started to receive attention, it was speculated that the clock transcriptional machinery would only control the temporal expression of core clock genes. However, this quickly proved to not be the case. As mentioned previously, expression profiling studies demonstrated that up to 15% of the genes expressed in any given tissue are under circadian regulation and exhibit ~24 hour cycling patterns[8,23]. The expression of these genes can be clock-regulated directly by the BMAL1/CLOCK dimer or indirectly through circadian expression of other transcription factors. By means of these rhythmically expressed genes, circadian information can be integrated into almost all cellular processes and have some effect on them, larger or smaller depending on the case.

One of the most fundamental processes that is thought to be reciprocally linked to the circadian oscillator is the cell division cycle (CDC), whose regulation relies on sequential phases of transcription-translation, protein modification and degradation[133]. One of the initial reasons for suspecting that there is a link between the circadian clock and cell cycle was exactly this: genes known to be involved in the regulation of cell cycle and the progression from one phase to the next display consistent daily expression patterns in continuously proliferating cell populations. But probably one of the most obvious observations testifying to this connection is the fact that eukaryotic cells in many proliferating tissues divide once per 24 hours.

One of the experimental setups commonly used in studies related to cell division are mice that have undergone a partial liver hepatectomy. When partially excised, the liver of mammals exhibits a remarkable ability to regenerate and it has been observed that during this regeneration, hepatocytes enter the mitotic cycle in a synchronous manner[134]. Research utilizing this system showed that, following partial hepatectomy, hepatocytes entered the G2 phase of the cell cycle approximately at the same time every day, irrespective of the time of day that the hepatectomy took place[135]. This strict regulation of the G2/M transition, yet again provides a strong suggestion that the cell cycle is gated by the circadian clock.

More evidence towards this direction was provided by experiments using Cry-deficient mice. As described before, CRYs are the major components of the negative feedback loop of the core circadian machinery. Although this deficiency appears to not have an effect in the initial development of the liver (mean weights of the livers of wild type and mutant mice are the same), differences can be observed in liver regeneration following partial hepatectomy. Specifically, the mean weight of the liver 72 hours after PH in the *Cry*-deficient mice was significantly lower than that in the wild type mice. Even though the difference was not detectable anymore by day 10 after PH, still, this is an indication that proper circadian clock function is required for efficient cell cycling in vivo[135].

On the other hand, it appears that the opposite is not necessarily true, i.e. the circadian clock is not dependent on the cell cycle, since post-mitotic tissues such as adult SCN neurons that do not divide still exhibit robust circadian rhythms of gene expression. Additionally, when cell division is inhibited in cultured rat fibroblasts, gene expression displaying circadian rhythmicity persists[136].

Conceptually, there are two ways in which the coupling of these two processes could occur, without them being mutually

exclusive. The first mechanism is through serial coupling or what could be called a "two-process model", according to which the circadian clock machinery holds "hostage" the biochemical reactions necessary for the transition from one phase of the cell cycle to the other and only allows the concentration of certain components to cross a critical threshold with a periodicity of around 24 hours. This model implies the direct regulation of the expression of cell cycle components by the core circadian circuit, locking in this way the cell cycle to the period and phase of the circadian cycle.

The second model describes the direct or parallel coupling of the two processes that requires a key protein to be directly involved in both of them and exhibit a circadian expression pattern.

Therefore, the hunt for the identification of the gene(s) responsible for the interconnection of two of the most fundamental cellular processes began. Sure enough, gene candidates that support both of the proposed models were found.

Direct Circadian Regulation of Cell Cycle Genes – The Case of Wee1

Using the *Cry*-deficient mice once again as an animal model, Matsuo et al.[135] attempted to determine the underlying cause for their failure to progress normally through the cell cycle. They did this by comparing expression profiles of 68 cell cycle-related genes between wild-type and *Cry*-deficient mice and required a 2.7-fold change in expression between the two groups of animals in order for the expression of a gene to be considered significantly altered.

From this analysis, a few candidate genes emerged whose product is already known to be involved in the entry to mitosis and could explain the defect in the *Cry*-deficient mice. Of these genes, emphasis was given to *Wee1*, a gene that encodes for a key kinase in the G2/M transition.

Several studies had already drawn a connection between expression levels of WEE1 and the timing of entry into the M phase in mammalian cells[137–139]. The mechanism for this is now understood quite well. WEE1 is responsible for the inactivation of the CDC2-cyclin B1 complex, which is an initiator of mitosis. It does this by phosphorylating CDC2 on a conserved tyrosine residue. When the WEE1 kinase levels are low, CDC25 is able to remove the inhibitory phosphate group and thereby activate the CDC2-cyclin B1 complex and promote the transition from G2 into mitosis.

In the liver of wild-type mice, the *wee1* transcript displays a robust circadian oscillation with the mRNA levels peaking at around 8 hours after subjective dawn. This means that entry into mitosis is most effectively inhibited just after midday and is allowed to proceed later in the day. In effect, DNA replication will occur sometime in the night, supporting the "Escape from light" hypothesis described in the introduction. Moreover, *wee1* transcript levels are constitutively elevated in the regenerating liver of *Cry1/Cry2*-double mutant mice[140] that have undergone partial hepatectomy, causing a prolonged inactivation of the CDC2-cyclin B1 complex and hindered cell division cycles[135]. Conversely, in *Clock* mutant mice that carry dominant negative *Clock* mutations, *wee1* levels are constitutively low. In sum, this data suggests that the *wee1* gene is directly activated by the BMAL1/CLOCK complex and suppressed by the CRY proteins and is thus under direct circadian regulation.

When examining the architecture of the mouse *wee1* promoter, three E-box elements, the hallmark of clock-regulated genes, were found within 1.2 Kb of the 5'-upstream region. This fragment alone was enough to cause an increase in transcriptional activity in a luciferase assay

when cells were transfected with both CLOCK and BMAL1 constructs. The transcriptional activation was reduced when all three E-boxes were mutated whereas it was completely diminished when CRY1 and CRY2 was present in addition to CLOCK and BMAL1. These results once again suggest that *wee1* transcription is directly regulated by the core components of the feedback loop of the circadian oscillatory mechanism, providing a strong candidate that fits the model for serial coupling of circadian and cell cycle (Figure 3).

Although there are other cell cycle-related genes whose expression is suspected to be regulated by the circadian clock, such as cyclin D1[135], still, they have not been thoroughly investigated.

ON TIMELESS

Mammalian Tim (Timeless) was initially identified as a homologue of the *Drosophila* clock protein Tim[141] and was found to be required for robust circadian rhythmicity[142]. However, its closest phylogenetic relatives are not other clock proteins but cell cycle-related proteins such as budding yeast Tof1, and fission yeast Swi1. These findings were the first indications that suggested that mammalian Tim could have an additional function other than that of a clock component, possibly as a cell cycle checkpoint protein. Tim is not traditionally considered to be part of the core clock components and is therefore not included in most of the models that describe the core clock circuitry.

Despite being expressed in the mouse SCN, it was thought that mammalian Tim does not exhibit circadian oscillation[143]. This was disputed and attributed to the discovery of two splicing forms of mammalian Tim in the SCN and other tissues. While full-length Tim does exhibit circadian oscillation patterns, the more abundant shorter splice variant does not, creating the misconception that it is not clock-regulated.

Human Tim has been shown to specifically interact with CRY2 in co-immunoprecipitation assays[144] and yeast two hybrid assays[52], an interaction that is consistent with the role attributed to Tim as part of the core circadian clock[142].

In fission yeast, the Tim orthologue called SWI1 is responsible for the activation of CDS1, a signal transduction kinase. So, in order to determine whether Tim could have some role in the cell cycle too, the putative interaction between Tim and CHK1, the human CDS1 functional homologue was investigated by means of co-immunoprecipitation assays. Interestingly, it was discovered that Tim and CHK1 interact specifically and in a manner similar to the Tim-CRY2 interaction. This interaction was found to be stimulated and enhanced when cells were treated either with hydroxyurea (HU) or UV light. Both of these treatments cause some kind of stress to the cells, be it replication stress or DNA damage, and ultimately lead to the activation of the DNA damage checkpoint pathways. The fact that an interaction between what is considered a clock protein (Tim) and a checkpoint protein (CHK1) is enhanced in DNA-damaging situations suggests that Tim may play a role in the cell cycle checkpoint through the regulation of CHK1.

It has previously been shown that replication stress by HU leads to a phosphorylation event on Ser345 of CHK1 that is carried out by ATR[145,146]. This phosphorylation is imperative for CHK1 to become activated and stall the entry into mitosis until any DNA-damage issues have been resolved. Based on this report, the possibility of a Tim-ATR interaction was investigated. Co-immunoprecipitation experiments proved indeed that TIM also interacts specifically with a small ATR subunit called ATRIP and that this interaction is enhanced significantly by HU

treatment. Taken together, this data supports the concept that Tim functions as a mediator, facilitating the ATR-CHK1 interaction in checkpoint signaling[144].

When inducing the downregulation of Tim levels by siRNA, both basal and damage-induced CHK1 Ser345 phosphorylation is markedly reduced, indicating that, indeed, Tim mediates CHK1 activation during DNA damage events. The downregulation of Tim also had a noteworthy effect on core clock proteins, with PER2 levels being significantly decreased[144], confirming the effect report previously by Barnes et al.[142]. This single experiment provided evidence that Tim is critical for the function of both a circadian cycle and a cell cycle checkpoint protein.

Tim was also found to play a role in the intra-S checkpoint although the mechanism through which it accomplishes this has not been elucidated. Stalled replication forks induced by the depletion of the deoxynucleoside triphosphate pool or by DNA damage activate a signal transduction pathway that inhibits the firing of new replication origins and ultimately leads to a general repression of DNA synthesis[147–149]. Abolishment of the intra-S checkpoint results in the continuous firing of replication origins even in the presence of replication blocks, a phenomenon coined radioresistant DNA synthesis (RDS). Down-regulation of Tim results in the rise of an RDS phenotype, implicating it in more than one of the cell cycle checkpoints.

Collectively, these data show that Tim meets all the requirements for the direct coupling model of the circadian and cell cycle to hold true; it is a protein whose expression is under circadian control and specifically interacts with and has an effect on the function of components of the core circadian oscillator and cell cycle checkpoints (Figure 3).

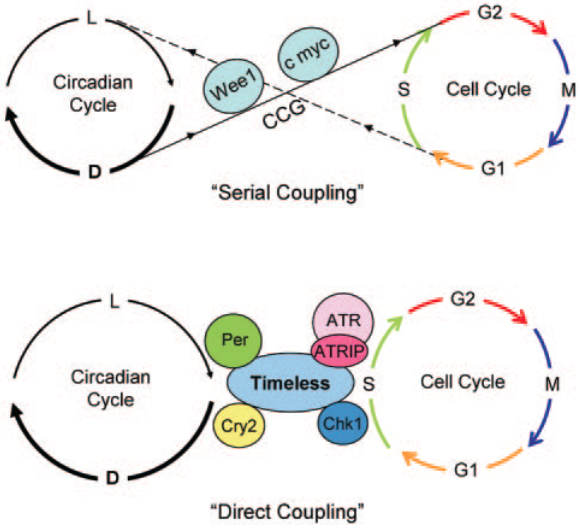

Figure 3. Two models for coupling circadian and cell cycles. In serial coupling (top), protein components of one cycle regulate the expression of genes involved in the other cycle(*Wee1, c-myc*). In direct coupling (bottom), a protein, such as Tim, directly participates in the molecular machineries of both cycles. The protein interactions shown are meant to point out Tim's putative functions as a checkpoint and clock protein and do not imply a defined biochemical pathway or the formation of a supramolecular cpmplex of clock-checkpoint proteins participating in two cycles simultaneously or alternately (adapted from Ünsal-Kaçmaz et al.[146]).

CANCER: THE CIRCADIAN ASPECT

The intertwinement of the circadian cycle with the cell cycle, integrating environmental cues with one of the most fundamental cellular processes, has been well established, as discussed in the previous section. Components of the circadian machinery are necessary for the normal progression of a number of pathways that take place during a cell's physiological health. It, therefore, makes sense that the aberrant function of circadian proteins could contribute to an organism's susceptibility to diseases that are characterized by the deregulation of cell cycle processes. The

most well studied case where cell division and cell proliferation occur aberrantly and lead to the rise of a pathological phenotype is cancer in its various forms.

Association studies have indeed provided data, albeit circumstantial, showing that there is an increased chance of cancer occurrence when normal circadian rhythmicity is disrupted. Night-time work can have at least two consequences: (1) disruption of the human circadian system frequently leading to jet-lag and/or (2) exposure to light at night preventing the nocturnal secretion of melatonin that normally happens. It has been found that night shift workers have an increased chance of developing breast cancer [150–152], endometrial cancer[153], colorectal cancer[154] and non-Hodgkin's lymphoma[155]. Additionally, prostate cancer has an increased prevalence in people that work rotating shifts, such as airline pilots, firefighters and police officers[156–159].

The above evidence made the search for possible mechanisms through which cancer can be related to clock components an interesting line of research.

PER1 AND PER2 HAVE A TUMOR-SUPPRESSIVE ROLE

The first indication that PER2 has other important regulatory functions was the observation that aged mice with a disruptive *Per2* gene mutation developed enlarged, hyperplastic salivary glands[160]. Hyperplasia usually occurs due to the combinatory effects of an enhanced cell proliferation rate and a reduced apoptosis rate.

When exposed to ionizing radiation (IR), the PER2-deficient mice were more prone to developing spontaneous lymphomas in comparison to their wild-type counterparts. The *Per2*-mutant mice also appear to be hypersensitive to genotoxic stress as seen by the accelerated graying of their coat after IR treatment, an effect also observed in heterozygous ATM-mutated mice, one of the kinases activated in response to DNA damage response.

Strikingly, the induction of *Per2* expression in cancer cells leads to inhibition of cell growth, causes cell cycle arrest, apoptosis and loss of clonogenic ability, some of the most common hallmarks of tumor cells[161].

These effects of *Per2* mutations have, in large, been attributed to the interaction between PER2 and the proto-oncogene *c-myc*. *c-myc* encodes for a bHLH-containing transcription factor that binds to E-box enhancers in target genes involved in cell proliferation, cell differentiation and apoptosis. Overxpression of *c-myc* has been reported in a large number of human cancers. This gene was suspected to be under circadian control from quite early on when it was observed that it displayed a temporal difference in expression levels that correlated to various diurnal time points[162]. This theory was further supported by the discovery of E-box sequences in the promoter of *c-myc*. The rhythmic expression of *c-myc* becomes shifted in *Per2* mutants and transcript levels are significantly elevated in comparison to wild-type mice. In contrast to the usual function of the BMAL1/NPAS2 complex, in the case of *c-myc* it appears that its promoter activity is inhibited by BMAL1/NPAS2 heterodimer in a dose-dependent manner in cell culture experiments. This repression is relieved in the presence of CRY1[160]. What is more, genes whose products participate in cell proliferation, such as *Cyclin D1* and *Gadd45α* and are themselves targeted by c-myc for activation, are rhythmically expressed in wild-type mice but display altered rhythms in the *Per2* mutants. In sum, the above data suggests that PER2 normally acts in a *c-myc* suppressive nature, although probably indirectly, through its stimulatory effect on

Bmal1 transcription[163]. In PER2-deficient mice, BMAL1 production is decreased and the reduced levels prevent the formation of BMAL1/NAPS2 or BMAL1/CLOCK complexes which, in turn, lead to the derepression of *c-myc*. The aberrant overexpression of *c-myc* subsequently results in DNA damage and tumor formation owing to the loss of cell cycle control[160].

More recently, PER1 has also been attributed a tumor-suppressive function. It was observed that the rate of cell apoptosis following irradiation was significantly higher in cancer cells overexpressing PER1 in comparison to control cells. The opposite can be observed when PER1 levels are reduced via RNA interference technology, i.e. the apoptotic rate of cancer cells after IR treatment was decreased.

Cryptochrome Loss Increases Apoptosis Rates of p53-Deficient Tumor Cells

When cells undergo extensive DNA damage after exposure to genotoxins such as UV radiation, p53 causes the expression of pre-apoptotic target genes that effectively kill the damaged cells and remove them from the organism. Consequently, the loss of p53 function allows damage cells to escape death and acquire new mutations necessary for the formation of tumors and the progression of cancer. Therefore, it is not surprising that many types of cancer that are resistant to chemotherapy are characterized by a deficiency in p53[164].

Research performed by Sancar et al. showed that chryptochrome disruption in the tumor prone p53 deficient mice increased their tumor-free survival. This observation was associated with an increase in DNA damage sensitivity in the mice lacking both the cryptochromes (CRY1 and CRY2) and p53 compared with those lacking only p53. This suggests that the loss of chryptochromes somehow reverses the DNA damage resistance conferred to the cells by the loss of p53.

The mechanism through which this effect is mediated is further investigated in a later paper by the same group and has to do with the p53-realted gene *p73.* Like p53, p73 also has tumor-suppressive properties and contributes to the maintenance of genomic integrity[165–167]. Expression of TAp73, a p73 isoform that is highly similar in structure and in function to p53, is elevated in *Cry1/Cry2*-deficient cells in response to DNA damage[168]. The *Cry1/Cry2* deficiency relieves the repression exercised on BMAL1/CLOCK and causes the increased activation of *Egr1* expression. EGR1 is a mammalian transcription factor that is specifically recruited to the *TAp73* promoter and stimulates its transcription. However, this activation can only occur when C-EBPα, a transcriptional repressor, is removed from the *TAp73* promoter, an event that is induced by the response to DNA damage. In this way, TAp73 levels are temporally regulated by the circadian clock and acutely regulated in response to DNA damage[164] (Figure 4).

This research provided insight into how components of the circadian clock can have an effect in the DNA damage response that is utilized in cancer treatment, highlighting possible novel targets for anti-cancer strategies.

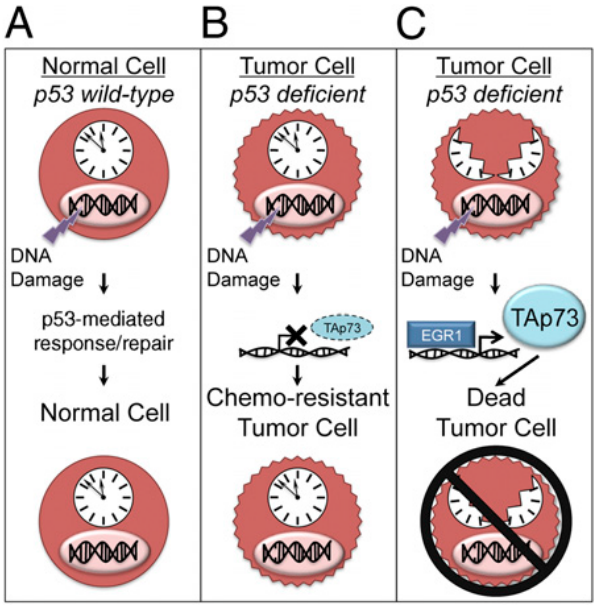

Figure 4. Circadian clock-dependent sensitization of p53-deficient cells to DNA damage. **A)** In normal cells that have a functional p53, the DNA damage response that either leads to apoptosis or DNA repair is primarily facilitated by p53. **B)** p53-deficient tumor cells have an acquired resistance to DNA-damaging chemotherapy. **C)** Tumor cells deficient in CRY1, CRY2 and p53 have high levels of EGR1. EGR1 binds to the TAp73 promoter and induces its activation after DNA damage, resulting in the death of tumor cells (adapted from Ramsey and Ellisen[164]).

CHRONOTHERAPY

It has been shown that circadian clock components affect the progression of diseases such as cancer. Based on this, an emerging theme in anti-cancer treatments is chronotherapy, a field that is attempting to determine and maximize the efficiency and tolerability of chemotherapy taking into account the time of day that the treatment is administered. Indeed, it has been found that when treatment is given according to a specific time schedule, it can influence the long-term survival and non-specific toxicity[169,170]. Circadian dosing time can have an effect on the extent of toxicity caused by at least 30 anti-cancer drugs and research using animal models has shown that the survival rate can vary by more than 50% depending on what time of day a "lethal dose" of drug is administered[171]. More importantly, the administration of a drug at a time of day when it is best tolerated by the patient usually achieves the most optimal anti-cancer activity[117]. The whole field of chronotherapy relies on asynchronies in cell proliferation and drug metabolic rhythms between the populations of healthy and malignant cells[118].

CONCLUDING REMARKS

Although the main components of the circadian clock have been identified and a current model exists of how these components work with each other to form the core circadian circuit, much work still remains to be done in order to uncover the full complexity of this molecular oscillator. As research progresses, it is becoming apparent that the pathways that comprise the circadian circuitry branch out a lot more than was originally expected, with proteins that have a role in circadian cycling, albeit less critical than those of the core loops, being constantly discovered. The role and function of circadian proteins has been studied through knockout experiments, but still not all functions have been uncovered.

Perhaps, experiments with more combinations of knockouts of circadian components would provide more insight to this end. As in many fields of research at the moment, the role of epigenetics has lately been receiving increasing attention in circadian biology as well. Experiments to uncover the full scope of chemical modifications that circadian proteins may have and their effect on them would be a fruitful path.

Circadian proteins have been shown to have an effect on the timing and the efficiency of cell cycle events. Many proteins of the cell cycle are thought to be under circadian control due to their transcript oscillations displaying a daily pattern. For some of these proteins, their linking function and the way that they do it has been extensively studied and confirmed. However, for the majority of the suspects, the linking of the two cycles is merely circumstantial and requires further investigation.

The involvement of circadian components in the progression of cell cycle processes in physiological health leads to the obvious assumption that they are also implicated in disease states that are defined by the deregulation of these same processes. Indeed, an association between circadian components and various types of cancer has been reported and in some cases the way that this is done has been discovered. However, it is difficult to try to speculate about the effect that the deregulation of each circadian component will have in regards to the rise of cancer, as results can be surprising. For example, the deficiency of PER2 and CRY proteins has been reported to have very opposing effects in cancer states, even though they are both part of the negative limb of the molecular circadian oscillator; PER2 deficiency results in the increase of spontaneous lymphomas in mutant mice whereas CRY deficiency in mice that also have a p53 deficiency leads to increased apoptosis rates of those cancer cells. This could possibly be a tissue-specific effect; not all clock-controlled genes are expressed in every tissue, one of the factors contributing to this being chromatin architecture. So, a deficiency in some part of the circadian circuitry will have a different effect in each tissue owing to the genes whose repression is being alleviated. It could of course also be that circadian proteins possess other properties and functions that have not yet been attributed to them.

Cancer is probably one of the most multi-factor diseases; deregulation of the function of a plethora of proteins and pathways can lead to the rise of cancer. Due to the links between circadian proteins and other processes, the effect that circadian deregulation will cause is dependent on the rest of the genetic background in each case. Perhaps expression profiling of cells from different types of cancer with a focus on the known deregulated proteins in each case and the circadian components will shed light on previously unknown interactions and produce novel anti-cancer therapies.

Chronotherapy could very well be a promising path for further research in clinical applications, but if the mechanisms through which the circadian clock exerts its function and affects other processes is better understood, it cannot be optimally applied. Apart from this, chronotherapy will have to face many bureaucratic and clinical regulatory issues that hamper its more extensive application.

In any case, circadian biology has proven to have a much larger role in almost all aspects of mammalian physiology than was originally thought and the elucidation of the mechanisms through which it does this is of great importance as it would lead to the better understanding of fundamental processes but could also contribute to the development of treatments for the diseases in which they are implicated such as cancer.

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LIST OF ABBREVIATIONS

bHLH: basic helix-loop-helix
BMAL1: Brain and muscle aryl hydrocarbon receptor nuclear transloctaor-like 1
CCGs: Clock controlled genes
CDC: Cell division cycle
CK1: Casein kinase 1
CLOCK: Circadian locomotor output cycled kaput
CRE: Ca^{2+}/cAMP-response element
CREB: Ca^{2+}/cAMP-response element binding protein
CRY: Chryptochrome
DEC: Differentially expressed in chondrocytes
FASPS: Familial advanced sleep phase syndrome
GSK-3: Glycogen synthase kinase 3
HATs: Histone acetyltransferases
HY: Hydroxyurea
iPRGCs: intrinsically photosensitive retinal ganglion cells
IR: Ionizing radiation
NPAS2: Neuronal PAS domain protein 2
PAS: Period-arnt-single minded
PER: period
PP1: protein phosphatase 1
RDS: Radioresistant DNA synthesis
RHT: retinohypothalamic tract
ROR: Retinoic acid receptor-related orphan receptor
ROS: reactive oxygen species
SCN: suprachiasmatic nucleus
SUMO: Small ubiquitin-like modifier
TIM: Timeless

SUMMARY

The key to survival for all living creatures is the ability to adapt the environment they live in. This adaptation does not only include modifications to behavioral traits but also to the way that biological processes occur inside each animal's body. One of the most pronounced and predictable environmental phenomenon that all organisms come in contact with is the day-night-day transition. The circadian clock is a system that exists in many organisms and can detect these transitions and then pass on the information to all parts of the body. The headquarters of the circadian clock in mammals is located in a part of the brain called the suprachiasmatic nucleus (SCN), but secondary branches of it are located in almost all cell types and they can effect the processes that take place in those cells. On the molecular level, the circadian circuit consists of a group of proteins whose amounts vary between different times of the day but have a consistent and rhythmic pattern of approximately 24 hours. Research has shown that the circadian clock is interconnected with many of the other biological processes that occur in a cell. This paper attempts to define the links between the circadian clock and the cell division cycle, showing that day and night rhythms can affect the way that cells divide. When circadian rhythmicity is deregulated, it is possible that through its connection to the cell cycle it can lead to diseases where cells divide uncontrollably, such as cancer. The paper discusses this possibility and mentions a way that this link can be used in a positive way, chronotherapy. Chronotherapy is the administration of anti-cancer drugs at specific times of the day, aiming to maximize the efficacy and minimize toxicity.