Systems biology of circadian-immune interactions

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Abstract

There is increasing evidence that immune system is regulated by circadian rhythms. A wide range of immune parameters, such as the number of red blood cells and peripheral blood mononuclear cells as well as the level of critical immune mediators such as cytokines, undergo daily fluctuations. Current experimental data indicates that circadian information reaches immune tissues mainly through diurnal patterns of autonomic and endocrine rhythms. In addition, immune factors such as cytokines can also influence the phase of the circadian clock, providing bidirectional flow of circadian information between the neuroendocrine and immune system. This network of neuroendocrine-immune interactions consists of complexly integrated

molecular feedback and feedforward loops that function in synchrony in order to optimize immune response. Chronic stress can disrupt this intrinsic orchestration, as several endocrine signals of chronically stressed patients present blunted rhythmic characteristics. Reprogramming of biological rhythms has recently gained much attention as a potent method to leverage homeostatic circadian controls to ultimately improve clinical outcomes. Elucidation of the intrinsic properties of such complex systems and optimization of intervention strategies requires not only an accurate identification of the signaling pathways that mediate host's response, but also a systems-level description and evaluation.

Introduction

In order to cope with environmental challenges and optimize biological fitness, organisms adopt rhythmic variations in their physiological functions. In mammals, this intrinsic timing system is organized in a hierarchical manner where a light sensitive master pacemaker synchronizes a bodywide web of cell autonomous and self-sustained subsidiary clocks that are present in nearly every tissue of the body. The focal point of this system which is commonly referred as the master clock is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus of the brain. SCN neurons translate the photic signal of daily cycles to chemical information by altering the expression of various genes. Although SCN neurons adopt oscillatory behavior even in *in vitro* conditions independent of any external cues, the exogenous input of light-dark information ensures its synchronization to a 24 hour period. Similar behavior is observed in peripheral clocks which, in the absence of an entrainer, oscillate freely while falling out of sync, yet are synchronized *in vivo* by periodic physiological cues. Therefore, even considering the interactions between the central and peripheral clocks in isolation, requires a systems-level approach in order to gain understanding of the internal properties of the network. Obtaining fundamental and useful knowledge about these systems is even more difficult when one considers that the physiological systems regulated by circadian clocks also have their own complex internal dynamics.

One reason that understanding circadian rhythms is important is because they are associated with disease. Environmental desynchronization either by external stressors (e.g. shift work and jet lag) or by other genetic disorders may lead to vulnerabilities to infection and disease both in humans and in rodents. Abolishing master's clock rhythmicity in mice by surgical ablation of the SCN seriously alters the daily rhythms of corticosterone, disturbs the

rest-activity cycles, and ultimately leads to accelerated tumor growth. Furthermore, the LPS-induced inflammatory response is magnified in jet-lagged mice relative to control animals, further leading to hypothermia and death after a certain period of time [1]. On the other hand, disease itself can impact circadian rhythmicity. In particular, current experimental data show that systemic inflammatory diseases are associated with blunted rhythmicity of numerous intrinsic signals. For instance, sepsis has been associated with loss of diurnal rhythms of leptin and cortisol [2], and circadian rhythmicity of cortisol has been shown to be prognostic of longer survival in patients with metastatic breast cancer [3].

The intersection between circadian rhythms and the inflammatory response, both governed by complex signaling networks, truly necessitates systems biology-based investigation if we are to understand the relationships between these systems and leverage this knowledge towards practical ends. This requires both experimental and computational approaches aimed at understanding circadian rhythms, inflammation, and their interactions. In this review, we discuss knowledge acquired in recent years relative to the bidirectional links between circadian and immune response and the occasions where their rhythmic orchestration is disrupted, as well as current knowledge relative to the reprogramming of endogenous rhythms. We present system biology approaches that have been leveraged in order to gain insight in these networks.

Mechanistic insight to circadian entrainment of central and peripheral clocks

The core clock elements that give rise to circadian timekeeping in mammalian SCN and found in most, if not all peripheral cells, are a group of so-called clock genes. Mouse SCN involves three period genes (*Per1*, *Per2*, and *Per3*), two cryptochrome genes (*Cry1* and *Cry2*), two basic helix-loop-helix transcription factors (CLOCK and BMAL1), and two orphan nuclear

hormone receptors (REV-ERBα and RORα). Negative and positive feedback interactions among these clock genes lead to transcriptional oscillations that retain an approximate 24-h periodicity independent of any external entrainer. In order to stay in harmony with environmental changes, clock gene oscillations must be corrected on a daily basis by entraining signals. As such, SCN neurons after receiving the environmental input from light/dark cycles, deliver photic information to the periphery of the body via direct routes such as the circadian secretion of hormones and the neuronal activity of the autonomic nervous system, and indirect routes such as the daily rest/activity cycles that further control the feeding time. This network of interactions among SCN and peripheral tissues reveals a nested level of biological organization where circadian information is retained in various levels of mammalian physiology from rhythmic patterns of clock gene expression to behavioral rhythms of sleep/wake cycles. Systemic integrity is largely dependent on the coherent function of the sub-systems composing the network. Their circadian rhythmicity is further related to the integrity of humoral and neuronal entraining signals, further underscoring the need of a system-level approach in order understand the underlying properties and design principles.

Quantitative mathematical models have been applied to gain mechanistic insights into clock gene network function [4-9] (Table 1). Among them, Becker-Weinmann *et al.* using a reduced model to simulate clock genes network, denoted that the negative feedback loop among *Per* and *Cry* genes is critically important for the maintenance of clock gene oscillations. They showed that even if the positive feedback loop among *Bmal, Ror* and *Rev-erbα* genes is substituted by a constantly expressed activator, oscillations can still occur. These modeling results were supported by the experimental evidence showing that *Rev-erbα* $^{-/-}$ mutant mice have rhythmic behavior even though their positive feedback mechanism is not functional. More

recently, Mirsky *et al.* used a detailed model to predict the phenotype of 7 knockout and 2 double knockout mutations as well as concentration variations in clock genes in the respective scenarios. However, as it was noted in the work of Mirsky *et al.*, in order to describe phenotypes observed in tissue or organ levels, additional dynamics such as entrainment and synchronization must be taken into consideration.

Recently, the mechanism of peripheral entrainment by systemic cues has been further elucidated. Single cell experiments of Nagoshi *et al.* in rat and mouse fibroblasts showed that single peripheral cells even in *in vitro* conditions independent of any entraining cue retain robust rhythmicity similar to SCN cells [10]. As a result, the dampening rhythms they observed *in vitro* at the population level emerge from robust single cell oscillations that fall out of phase and desynchronize in the absence of an entraining force. Based on this, we recently studied the synchronization properties of cortisol in a population of peripheral clock genes. We found that cortisol entrains the peripheral clocks in an amplitude and frequency dependent manner [11] (Table 1). Chronically stressed conditions, represented by blunted cortisol circadian amplitudes, lead to loss of peripheral clock gene entrainment and synchronization. Similarly, cortisol oscillatory frequencies largely deviating from a 24-h period, which have been experimentally found in rodent models of jet lag, fail to synchronize peripheral clocks resulting in high level of desynchronization and weaker ensemble average rhythm. Furthermore, Abraham *et al.* showed that the oscillator qualities that greatly determine entrainment efficiency, are the ratio between entrainer coupling strength and oscillator amplitude, and the rigidity of the oscillatory system as defined by the relaxation rate upon perturbation [12] (Table 1). This result can explain the evidence that peripheral tissues such as lung, can be entrained by a wider range of entrainer's

frequencies whereas SCN do not, since SCN neurons due to their intercellular coupling retain characteristics of rigid oscillators.

Additionally, there has been a lot of interest relative to entrainment of central and peripheral clocks and several mathematical models have been constructed in order to characterize their underline dynamics [12-17] (Table 1). In particular, Abraham *et al.* showed that the oscillator qualities that greatly determine entrainment efficiency, are the ratio between entrainer coupling strength and oscillator amplitude, and the rigidity of the oscillatory system as defined by the relaxation rate upon perturbation [12] (Table 1). This result can explain the evidence that peripheral tissues such as lung, retain significantly different entrainment characteristics than the SCN since SCN neurons due to their intercellular coupling retain characteristics of rigid oscillatory dynamics.

Deciphering entrainment dynamics of peripheral cells is very important since several genes that are critically involved in the immune function are regulated by peripheral clock genes. These genes are commonly referred as clock controlled genes (CCGs). Transcription factors such as signal transducer and activator of transcription 3 and 5 (STAT3 and STAT5), as well as nuclear factor kappa B (NF-κB) are directly regulated by the molecular clock and exhibit circadian rhythmicity in humans and rodents. Further, these transcription factors participate in cytokine signaling pathways and as such they indirectly regulate innate and adaptive immune responses. As a result, a robust circadian signal of clock genes implies the efficient delivery of circadian information to the immune response of the body. In addition, circadian information through metabolic, neuronal, and humoral entraining signals reaches organs of the immune system such as lymph nodes and spleen. Outputs of this entrainment among others are the rhythmic variation of critical immune components such as natural killer cell (NK) levels, and

cytokine expression that ultimately control critical immune responses such NK cytotoxicity, phagocytosis, and the inflammatory response [18]. On the other hand, the temporal variations of immune mediators tune the central clock by affecting critical behavioral rhythms such as sleep/wake patterns forming a feedback interaction between circadian and immune systems.

Bidirectional communication between circadian clock and immune system

The complexity inherent in both the inflammatory response and the hierarchical system of circadian clocks necessitates a systems biology view to study how these systems interact. One useful experimental technique to study systemic inflammation is the human endotoxemia model. Human endotoxemia consists of the administration of low doses of endotoxin (lipopolysaccharides, LPS) to healthy human volunteers. Through the binding of LPS to its Toll-like receptor 4 (TLR4), endotoxemia provokes physiological changes that, in part, mimic those occurring in acute and chronic inflammation-linked diseases such as sepsis and trauma. Thus, human endotoxemia functions as a practical model of TLR4 agonist-induced systemic inflammation by eliciting neuroendocrine, hemodynamic, and leukocyte transcriptional responses [19]. Furthermore, circadian properties of the endotoxemia response have been studied both experimentally [20] and computationally [21].

Neuro-endocrine and autonomic circadian regulation of the immune response

The master clock in the SCN governs the central release of circadian hormones and signals which travel throughout the body and entrain the peripheral oscillators to a consistent phase. Interestingly, as summarized in Figure 1, many of these circadian signal transduction

mediators also play roles in regulating the immune response, such as cortisol, melatonin, and the autonomic nervous system.

One of the direct routes through which the central clock entrains peripheral tissues is by the production of glucocorticoids (cortisol, in humans) in the adrenal gland. In addition to being notable for its clear circadian pattern in homeostasis, cortisol is a key anti-inflammatory hormone. Balsalobre *et al.* showed that glucocorticoids can induce circadian gene transcription in rat-1 fibroblasts as well as phase shift clock gene expression in the liver, kidney, and heart without influencing clock gene expression in the central clock, as the glucocorticoid receptor is not expressed in the SCN [22]. In addition, experiments of Burioka *et al.* illustrated that administration of dexamethasone, a synthetic glucocorticoid, in human bronchial epithelium and peripheral blood mononuclear cells (PBMCs) *in vitro* and *in vivo* significantly influences the expression of the *PER1* clock gene [23]. Interactions between glucocorticoids and circadian rhythms have been studied through pharmacokinetic and pharmacodynamic modeling to understand the pharmacological implications of endogenous circadian rhythmicity. Glucocorticoids exert downstream effects through a signaling pathway that includes the binding of glucocorticoids to glucocorticoid receptor molecules, translocation of the activated receptor complex to the nucleus, and transcriptional regulation by binding to glucocorticoid responsive elements (GRE) in the promoter region of target genes. Experiments in mouse peripheral organs as well as mesenchyman stem cells (MSCs) indicated that *Per1* and *Per2* clock genes contain GRE elements in their promoter regions and therefore are directly regulated by glucocorticoid signaling pathways. Since glucocorticoids interact with clock genes and regulate their expression, they indirectly modulate clock mediated pathways and CCG (e.g. STAT3, STAT5,

NF-κB). This results in far-reaching effects, as glucocorticoids influence circadian cytokine production, leukocyte distribution, proliferation, and apoptosis.

The circadian secretion of melatonin may also regulate the expression of immune mediators such as cytokines. The primary mechanism of melatonin production is by the pineal gland of the brain, which is tightly regulated by the SCN. In addition to operating as a circadian entraining hormone, melatonin conveys a significant immunomodulatory effect. For instance, the peak of melatonin circadian rhythm at night has been correlated with the nocturnal rise of blood T lymphocytes. Pinealectomy on the other hand, is followed by an overall immunosuppression, likely mediated by the reduction in lymphocytes and other cytokines such as IL-2, IL-12, and TNF-α that naturally assist the host to mount humoral responses [24]. Furthermore, in murine bone marrow cells, administration of melatonin appears to induce immunity by inhibiting apoptosis during early B cell development [25]. Melatonin plays also a role in the development and growth of cancer since its production is correlated both with a reduction of IL-10 anti-inflammatory cytokine that has cancer growth promoting-activity and with an increase of human monocytes to produce IL-6 cytokine that has cancer-stimulatory activity.

The autonomic nervous system, through both its sympathetic and parasympathetic efferent arms, also conveys circadian information to the immune system. Light/dark information reaches autonomic system through inhibitory and excitatory inputs from the SCN to the paraventricular nucleus (PVN) that control pre-autonomic neurons and ultimately regulate sympathetic and parasympathetic activity. Autonomic activity is then mediated to the periphery of the body through autonomic innervation of various peripheral organs. The adrenal and pineal glands are innervated by autonomic projections and as such there is an indirect autonomic

regulation of immunity embedded in the secretion of cortisol and melatonin respectively. Additionally, primary and secondary lymphoid organs such as the spleen, and liver receive extensive autonomic input. Upon stimulation, sympathetic nerve terminals in the spleen secrete norepinephrine (NE) that ultimately mediates activity of NK cells, and macrophages. It has been recently shown that the NE input to the spleen retains diurnal rhythmicity further illustrating the role of autonomic nervous system as a conveyor of photic information [25]. Interestingly, in the same experiment, sympathetic denervation disrupted the diurnal variations of splenocyte cytokines and NK cells. Similarly, hepatic NK cells are also regulated by the circadian sympathetic input of the liver. The autonomic nervous system also mediates immunomodulatory effects by the cholinergic anti-inflammatory pathway through the release of acetylcholine (ACh) from reticuloendothelial organs such as the spleen, liver, and heart that further interact with ACh receptors on tissues macrophages and ultimately inhibit the release of TNF, IL-1, and other cytokines [26]. This, combined with the autonomic innervation of critical lymphoid and reticuloendothelial organs, allows for autonomic regulation of the inflammatory response.

These and other centrally-mediated dual circadian and inflammatory signals, impose a circadian character on the inflammatory response. Blood stimulated *ex vivo* with LPS at different times throughout the circadian cycle, results in significant circadian rhythms in the peak responsiveness of cytokines. *In vivo* human endotoxemia experiments showed that, when LPS is injected into healthy volunteers in the evening (when cortisol levels are low) versus in the morning (when cortisol levels are high), there is a significantly larger increase cortisol levels as well as in body temperature [27].

Herman *et al.* developed a mathematical model to evaluate the neuroendocrine-immune system interactions specifically in the context of rheumatoid arthritis [28] (Table 1). This model

describes mainly the measured circadian responses of plasma levels of TNF, noradrenaline (NA), and cortisol, making use of a set of ordinary differential equations. The model was calibrated with experimental data of healthy subjects and rheumatoid arthritis (RA) patients. Importantly, they predicted through mathematical modeling that treatment with glucocorticoids between 00:00 and 02:00 am induced the strongest inhibitory effect on TNF secretion. In chronic inflammatory diseases such as RA where patients are characterized by an overexpressed inflammatory response, reduce of pro-inflammatory mediators such as TNF is often a clinical target. Similary, Scheff *et al.* incorporated a mutli-level mathematical modeling scope based on which evaluated the interplay between inflammation and circadian rhythms [21] (Table 1). This model predicted that LPS administration during the night induces larger increases in inflammatory mediators and larger reductions in the heart rate variability (HRV) relative to administration in the morning. HRV, defined as the distribution of time intervals between successive heartbeats, is considered as a potential prognostic marker in systemic inflammatory diseases such as sepsis with lower values generally associated with poorer health outcomes. Relative to this, the modeling results of Scheff *et al.* lie in accordance with experimental data showing that septic patients have a significantly increased risk of mortality at night [29]. Extension of this model, further incorporating a mathematical description of the sympathovagal signals that give rise to heart beats and ultimately to heart rate variability [30] (Table 1), allowed for further investigation of the mechanistic underpinnings of the inflammatory response that ultimately lead to changes in HRV. The importance of this model lies in the fact that it incorporates a semi-mechanistic based representation of circadian heart rate dynamics that includes their derivation from cellular, molecular, and neural signals enabling the evaluation of multiple *in silico* scenarios relative to physiology underpinning changes in HRV.

Immune mediators regulate circadian clock

Just as the mechanisms described above convey circadian information to the immune system, mediators produced in the inflammatory response can in turn modulate the function of circadian clocks. It is well established *in vitro* that LPS-induced responses can exert significant effects on the circadian clock mechanism. Administration of LPS to SCN slices increases the secretion of arginine-vasopressin (AVP) neuropeptide. Originally, the rhythmic expression of AVP is directly regulated by SCN [31]. This suggests that the neuroendocrine output of SCN can be also modified by immune challenge and ultimately influence the behavioral rhythms of the host. In accordance with this, Marpegan *et al.* showed that intraperitoneal injection of LPS in mice induced phase delays to their locomotor activity [32]. These phase shifts to rodent's activity were present only if LPS was administered specific times of day illustrating a time of day dependency of inflammatory outcome. As it can be implied by the experiment of Marpegan *et al.*, inflammatory stimulus even at the periphery of the body can trigger alteration in the circadian clock of the organism. In particular, Okada *et al.* showed that intravenous injection of LPS to rats results in significant suppression of the clock gene *Per2* and the clock-controlled gene *Dbp* in the SCN underlining a direct effect of a peripheral inflammatory stimulus on the central circadian clock of the body [33].

It is likely that the precise mechanism through which LPS mediates its downstream effects on the SCN and peripheral tissues involves cytokines which are released in response to inflammatory stimuli (Figure 1). Relative to the interactions of cytokines and the central clock, Kwak *et al.* found that long term treatment of rat SCN cultures with interferon gamma (IFN-γ) blunts the diurnal rhythmicity of *Per1* even at the level of single cells [34]. Similarly, a cocktail of TNF-α, LPS and IFN-γ caused a decrease in the SCN neuronal firing rhythmicity. Beynon *et*

al. further showed that in rodent's SCN, the interleukin-1β (IL-1β) proinflammatory cytokine receptor (IL-1R1) is rhythmically expressed. In addition, a peripheral immune challenge by a large dose of LPS significantly up-regulated IL-1R1 along with critical components of IL-1β signaling pathway such as c-Fos and p65-NF-κB [35]. This suggests that the central clock is directly sensitive to immune challenge from peripheral tissues. Numerous experiments have shown that the brain receives inflammatory signals from the periphery of the body in response to injury/infection. This signaling has been implicated to exacerbate sickness, develop symptoms like depression, and impair numerous diurnal rhythms such as temperature and melatonin.

As it is the case for LPS, cytokines cause also alterations in the peripheral clock function. Treatment of human hepatocytes with IFN-α induces a downregulation in the expression of *CLOCK* and *BMAL1* genes in a STAT1 transcription factor dependent manner [36]. Furthermore, through both *in vitro* and *in vivo* studies, Cavadini *et al.* showed that TNF-α and Il-1β downregulated the expression of mouse *Per1-3,* and *Dbp* [37], clearly illustrating that also the output of the clock network is regulated by the cytokine signaling. However, our current mechanistic understanding of these interactions is not sufficient to explain the temporal dynamics observed in endotoxemia experiments, suggesting that there are likely more undiscovered links between circadian rhythms and the inflammatory response [20].

The extent by which LPS injection modulates the central clock *in vivo* in humans is still a topic of debate. An endotoxemia study done in humans [20] found that melatonin levels, a proxy for the function of the circadian clock, did not change in response to endotoxemia. However, unlike the rat endotoxemia study which directly analyzed gene expression in the SCN, transcriptional analysis was only done in peripheral blood leukocytes, which exhibited similar regulation of clock genes. Thus, it could be that like the effect of feeding on the central clock but

unlike the effect of light, endotoxemia primarily effects peripheral clock function. Further studies, including injections at more time points, will be required to further investigate the issue of central clock regulation by LPS, but it is clear that peripheral circadian clocks can be significantly perturbed in endotoxemia. This could indicate a loss of coupling between the central and peripheral clocks under stress.

Disruption of circadian rhythmicity of the body – Reprogramming of biological rhythms

There is a fair amount of evidence indicating that several chronically stressed conditions are correlated with disruption of biological rhythms such as sleep/wake cycles, immune mediators circadian rhythms, and hormone diurnal oscillations [3]. Chrousos *et al.* further showed that a chronically stressed HPA axis is characterized by a decreased variance of cortisol both due to evening nadir elevation and to morning zenith decreases [38]. In critical illness, this has been hypothesized to be driven by elevated levels of inflammatory mediators, as well as neural input to the adrenal, directly stimulating glucocorticoid secretion. Circadian rhythms in melatonin were observed to be suppressed in septic patients indicating a loss of central clock rhythmicity due to the tight link between SCN function and melatonin secretion [39]. However, non-septic patients in the ICU still had circadian rhythms in melatonin suggesting that there may be subtle disease-specific mechanisms driving the specific characteristics of the loss of circadian rhythmicity in critical illness.

Disruption of circadian rhythmicity may be also presented as a phase delay or advance in diurnal rhythmicity. In particular, Alesci *et al.* showed that patients suffering from major depression syndrome (MDD) exhibited a phase shifted profile of IL-6 expression by 12-h

compared to non-depressed healthy subjects [40]. Circadian disruption is also occasionally seen in cancer patients. Among patients with different cancer diseases, disruption of circadian rhythm has been noted in endocrine (e.g. cortisol, melatonin) metabolic (e.g. proteins and enzymes) and immunological components (e.g. cytokines) [41]. Whether the circadian dysregulation is a cause or a consequence of a stressful condition is a topic of ongoing research.

As was noted earlier in this review, disrupted biological rhythms are often associated with negative clinical outcomes. Studies in shift workers showed that night working is a risk factor in several types of cancers such as prostate, breast, endometrial, and colon. Furthermore, other diseases such as obesity, diabetes, and cardiovascular seem to be more common among shift workers. Mormont *et al.* showed that patients with poor circadian rhythmicity of sleep/activity cycles had a 5-fold higher risk of dying within 2 years than the patients with a better circadian rhythmicity [42]. As a result, there may be a relationship between circadian rhythmicity and disease development. This notion raises the possibility that reinforcement of disrupted biological rhythms may reset the circadian clock and further improve outcome. Recently, there have been efforts particularly in cancer treatment in order to experimentally investigate the concept of biological rhythm reprogramming. This approach may have a transformative impact on clinical care, given that current standard practices proceed with little regard for circadian rhythms. Incessant lighting, continuous feeding, and other processes of care remove the external cues that normally maintain circadian patterns. If recovery from cancer or critical illness is indeed linked to the recovery of endogenous biological rhythms, optimizing the timing of care to re-engage key immunoregulatory clock mechanisms could improve outcome for those patients. Preliminary results linking circadian rhythms and recovery are promising. Li *et al.* showed that intermittent feeding for 2-h/day induces 24-h rhythmic expression of critical

genes in clock-deficient tumors. Further, mice fed intermittently 2-h per day exhibit ~40% less tumor growth than mice fed *ad libitum* emphasizing that meal timing can not only enhance circadian rhythmicity but also improve survival [43]. Similar results have been obtained for mice with Glasgow osteosarcoma, where restricted feeding induced an increase in the amplitude of temperature and activity diurnal rhythms [44]. Importantly, both of these studies indicated that intermittent feeding reinforced endogenous rhythmicity independent of its caloric composition, which further underlines the specific importance of feeding time. Along the same lines, Filipski *et al.* showed that altering feeding time can counterbalance the detrimental effect of chronic jet lag on tumor growth [45]. In particular, restricted feeding prevented the circadian disruption originally induced by chronic jet lag in the liver and slowed cancer progression. Restricted feeding in arrhythmic (SCN ablated) rats also restores rhythmicity of the pineal gland's melatonin secretion [46], highlighting the possibility that melatonin may also play a role in the resetting of biological rhythms in chronically stressed patients. In fact, oral melatonin administration has been found to restore sleep efficiency in tracheostomy patients who had lost circadian rhythmicity in endogenous melatonin [47] . In addition, Blask *et al.* used a tumor perfusion model to show that rhythmic output of melatonin suppress breast cancer proliferation in rats [48]. Finally, melatonin regulation of cancer progression both in C57BI/6 null mice that exhibit lower levels of dark phase melatonin and in B6D2f1 jet lagged mice that exhibit atypical melatonin production mainly due to increased light phase production raises the possibility that rhythmicity of melatonin may play more important role than its absolute concentration [49].

Conclusions

Homeostasis is maintained in the body through a large number of bidirectional interactions among physiological systems. The circadian and immune systems play leading roles

in this dynamic behavior, and their effective regulation is of utmost importance. Experimental evidence shows that chronically stressful conditions often disturb the rhythmic orchestration of the body, resulting in blunted circadian rhythmicity of critical endocrine signals. Due to the highly complex, multi-level network of interactions between the circadian clock and the inflammatory response, a systems-level approach is attractive in order to elucidate the underlying dynamics and design potential clinical interventions.

Significant progress along these lines has been made through studying mathematical models that elucidate the properties of circadian clocks relative to its entrainment dynamics and interactions with inflammatory mediators. These models illustrate the predictive power of mathematical approaches that also enable broader comprehensiveness of the biological system. In addition, their application in the context of the immune response based on current *in silico* studies seems promising. Further steps forward will continue to narrow the gap between scientific knowledge and clinical practice by incorporating system biology approaches in real clinical intervention scenarios.

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Tables:

Table 1: Mathematical models relative to clock genes, entrainment of clock genes, and immune/clock dynamics. ODE=Ordinary Differential Equations, SDE=Stochastic Differential Equations.

Author / Ref.	Dynamics of interest	Mathematical formulation	Findings
Leloup and Golbeter / [4]	Clock gene dynamics	ODE	Autonomous oscillations with adverse phase of *Per* and *Bmal1* mRNAs in dark period.
Misrky *et al.* / [5]		ODE	Predicts phenotypes of 7 single knockouts and 2 double knockout mutations of clock genes.
Relogio *et al.* / [6]		ODE	Dependence of clock gene periodicity on *Per* mRNA degradation rate.
Weinmann *et al.* / [7]		ODE	Retaining of clock gene oscillatory behavior even when the positive feedback is replaced by a constant term (Rev-Erbα mutant).
Gallego *et al.* / [8]		ODE	The casein kinase mutant (CKI_{ε}^{tau}) increases kinase activity.
Westermark *et al.* / [9]		SDE	Predicts that robust oscillations in peripheral cells found experimentally may be in reality damped oscillations driven by noise.
Abraham *et al.* / [12]		ODE	Entrainment is regulated by i) the ratio between entrainment strength and oscillator amplitude ii) the rigidity of the oscillatory system.
Antle *et al.* / [13]		ODE	Rhythmic regulation in region of SCN involves arrhythmic (gate) and oscillatory cells.
Antle *et al.* / [14]		ODE	Reveals that the previous model [13] can be entrained by a circadian input and maintain phase response curve (PRC) similar to what observed experimentally.

Bernard *et al.* / [17]	Entrainment of clock gene dynamics	ODE	The number of oscillators and their connectivity are important for synchronization dynamics as well as their periodic behavior.
Geier *et al.* / [15]		ODE	Importance of PRC shape for entrainment dynamics.
Gonze *et al.* / [16]		ODE	Efficient synchronization of SCN cells is achieved when intracellular coupling dampens the individual cell oscillations.
Mavroudis *et al.* /[11]		SDE	Cortisol synchronizes peripheral clock genes in an amplitude and frequency dependent manner.
Meyer-Hermann *et al.* / [28]	Immune/clock dynamics	ODE	Treatment with glucocorticoids between 00:00 and 02:00 am was found to have the strongest inhibitory effect on TNF secretion
Scheff *et al.* / [21]		ODE	LPS administration at night induces higher reductions in HRV
Scheff *et al.* / [30]		ODE	Semi-mechanistic link between generation of heart beats and immune compartments.

Figures:

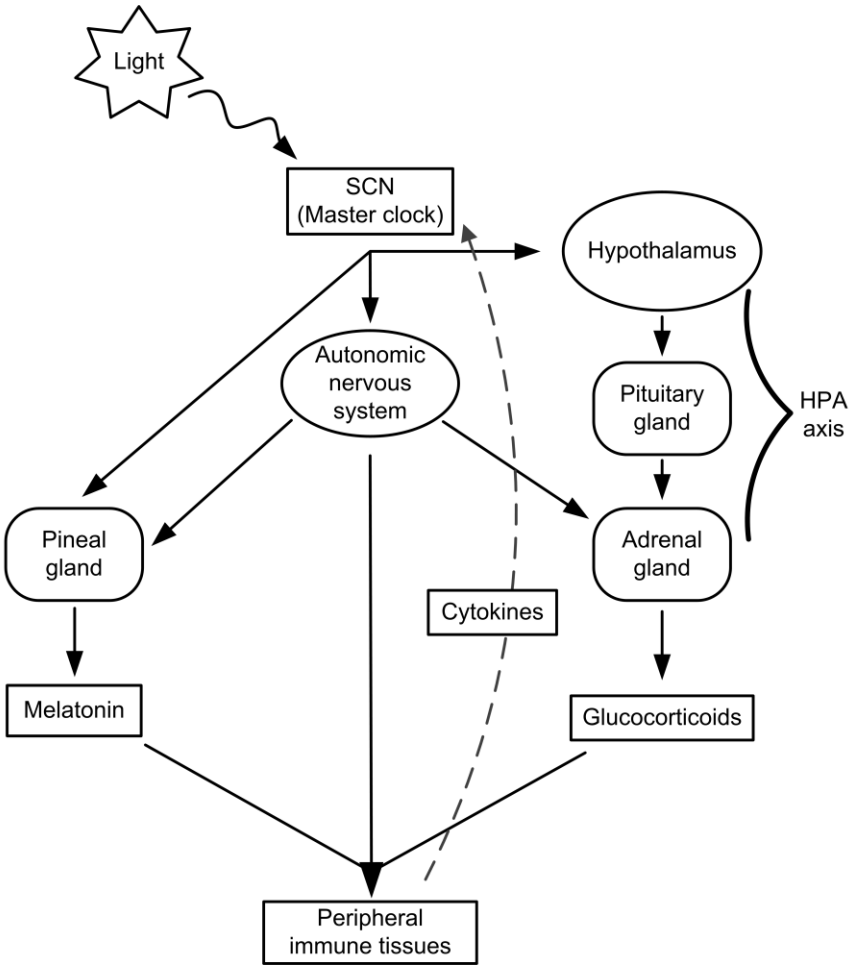

Figure 1: Schematic review of some components of the bidirectional communication between circadian clocks and inflammatory mediators.