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Protein Kinases in the Photic Signaling of the Mammalian Circadian Clock

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Circadian clocks drive biological rhythms in physiology and behavior, providing a selective advantage by enabling organisms to synchronize to the 24 h environmental day. This process depends on light-dark transitions as the main signal that shifts the phase of the clock. In mammals, the light input reaches the master circadian clock in the hypothalamic suprachiasmatic nucleus through glutamatergic afferents from the retina, resulting in phase-shifts of the overt rhythms which depend on the time of the day at which light is applied, leading to changes in the activity of circadian core clock genes (*i.e.*, *Per1*). This circadian gating of the synchronizing effect of light is dependent on the specific activation of signal transduction pathways involving several kinases acting on protein effectors. Protein phosphorylation is also an important regulatory mechanism essential for the generation and maintenance of circadian rhythms and plays a crucial role in the degradation and the appropriate turnover of PER proteins. In this work, we review the role of the main kinases implicated in the function of the master clock, with emphasis in those involved in circadian photic entrainment.

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†Abbreviations: 4EBP, 4E binding protein; AC, Adenylyl cyclase; BDNF, Brain-derived neurotrophic factor; BMAL1, Brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1; CaMK, Calcium/calmodulin-dependent protein kinase; cAMP, Adenosine 3',5'-monophosphate; cGMP, Cyclic guanosine-3',5'-monophosphate; CLOCK, Circadian locomotor output cycles kaput; CK1, Casein kinase 1; CREB, cAMP-responding element binding protein; CRY, Cryptochrome; EGF, Epidermal growth factor; Elk-1, ETS domain-containing protein; eIF4E, eukaryotic translation initiation factor 4E; ERK, Extracellular signal-regulated kinase; GC, Guanylyl cyclase; GRP, Gastrin-releasing peptide; IEGs, Immediate-early genes; ipRGCs, Intrinsically photosensitive melanopsin-containing retinal ganglion cells; JNK, c-Jun N-terminal kinase; mTOR, mammalian/mechanistic target of rapamycin; MAPK, Mitogen-activated protein kinases; MEK, MAPK/ERK kinase; MSK-1, Mitogen- and stress-activated protein kinase; NGF, Nerve growth factor; NMDAr, N-methyl-D-aspartate receptor; nNOS, Neuronal nitric oxide synthase; NO, Nitric oxide; PAC1, Pituitary adenylate cyclase-activating polypeptide type 1; PACAP, Pituitary-adenylate cyclase-activating polypeptide; PDE, Phosphodiesterase; PER, Period; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinases; RHT, Retinohypothalamic tract; RSK-1, 90 kDa Ribosomal S6 kinase; S6K, 70 kDa Ribosomal S6 kinase; SCN, Suprachiasmatic Nucleus; TrkB, Tropomyosin receptor kinase B; VIP, Vasoactive intestinal peptide; VPAC2, Vasoactive intestinal peptide receptor 2.

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INTRODUCTION

Most – if not all – organisms have endogenous circadian clocks that generate rhythms with periodicities of approximately 24 h in behavior and physiology. In mammals, the circadian system is formed by a hierarchical array of oscillators in which a master clock, located in the hypothalamic suprachiasmatic nuclei (SCN†), imposes temporal architecture by endocrine, autonomic, and behavioral outputs to peripheral oscillators in order to coordinate physiology [1,2]. The molecular mechanism of the endogenous circadian clock contains a feedback loop composed of cycling gene products that control their own synthesis by means of negative and positive regulation of clock proteins and mRNA. Briefly, the heterocomplex of Clock/BMAL1 proteins activate the transcription of three mammalian period genes (*Per1*, *Per2*, and *Per3*) and two cryptochrome genes (*Cry1* and *Cry2*) by binding to the E-box sites on their promoters. The three PER proteins accumulate in the cytoplasm to undergo interactions with themselves and with the two CRY proteins. Thereafter, the heteromultimeric complexes repress the transcription of *Per* genes by attenuating the activation potential of the Clock/BMAL1 dimer, thus forming a negative feedback loop. Additional complexity arises from supplemental loops and components linked to this central feedback loop.

Since the period of the master circadian clock is not exactly 24 h, it must be capable to be synchronized to the daily cycle by appropriate environmental cues. For most organisms, the most potent entraining cycle (*i.e.*, zeitgeber) is the light/dark alternation due to the rotation of the Earth, with social interaction, food availability, and temperature cycles acting as secondary zeitgebers. The mechanism of entrainment is mediated through daily shifts induced by light on the phase of the pacemaker at adequate circadian times [3]. Brief exposure to light during the early subjective night (*i.e.*, in nocturnal rodents, a time window of about 4 h after behavioral activity onset) induces phase delays, while exposure during the late subjective night (about 6-8 h after behavioral onset) generates phase advances of circadian rhythms. Intrinsically photosensitive retinal ganglion cells (ipRGCs) containing melanopsin receive circadian non-visual irradiance, which reaches the ventral retinorecipient SCN neurons through their retinohypothalamic tract afferents. Several neurotransmitters are implicated in photic communication, including glutamate, PACAP, and substance P [1,2]. Glutamate interacts with N-methyl-D-aspartate (NMDA) and non-NMDA receptors in the SCN, and several intracellular signaling pathways are activated leading to an increase in the expression of immediate-early genes, as well as an alteration in the expression of clock genes, thereby resetting the master clock [3].

Posttranslational modifications play a crucial role in the SCN to accomplish the generation of a 24 h oscillation. Among these, phosphorylation is so far the most widely identified modification in the circadian clock mechanism. Many clock components are rhythmically phosphorylated including CLOCK [4], CRYs [5], BMAL1 [6] and PERs [7]. The rhythmic phosphorylation of a clock protein is achieved by the regulated action of kinases and phosphatases that counterbalance their activities at different time windows. Indeed, levels of clock proteins forming heterodimers are essential to activate the transcriptional-translational feedback loops, and thus setting the period and phase of the circadian oscillator. For instance, it is known that PER phosphorylation by the casein kinase 1 (CK1) family prompts the ubiquitination-degradation of the protein to end negative feedback on their own transcription [8,9].

In this paper, we review the post-translational regulatory role of protein phosphorylation in the circadian clock, by analyzing kinase activity at the SCN level. First, we provide a regulatory framework for kinases controlling the proper circadian molecular clock; then, we describe the phospho-transduction systems implicated in the photic entrainment.

PROTEIN KINASES INVOLVED IN THE CIRCADIAN MOLECULAR CLOCK

Casein Kinases

The role of kinases in the circadian molecular clockwork is exemplified by the importance of casein kinases. Stability of clock proteins is of importance for establishing the rate of its cytosolic accumulation and nuclear import, which in turn determinates the circadian period. Characterization of circadian period mutants have revealed the role of phosphorylation in the clock mechanism. Alterations in the phosphorylation of PER determined period phenotypes in mice [10], hamsters [11], and humans [12]. The tau (τ) mutant hamster, displaying a short period behavioral rhythm, carries a missense mutation in the *CKIε* gene [11], and human familial advanced sleep phase syndrome (FASPS) with early sleep times is attributed to missense mutations of *Per2* and *CkIδ* genes [12,13].

Moreover, PER phosphorylation by CK1δ/ε regulates its stability and cellular localization [14,15]. The tau mutation in *CKIε* reduces kinase activity *in vitro* and determines a short period in hamster circadian rhythms [11]. A similar phenotype is found in humans with Familial Advanced Sleep Phase (FASP), with two different types of mutations [16]: one affects *hPer2* in a CKIε binding site, reducing its phosphorylation levels [12], and the other in the *hCKI*δ kinase gene, also reducing its

enzymatic activity [13]. The complexity of the system is exemplified by the fact that multisite phosphorylation of PER proteins can lead to different effects depending on the sites being phosphorylated (see [17]; [14]) leading to a composite picture of its dynamics and interactions [18]. Indeed, a tight control on PER2 accumulation/degradation is achieved by specific phosphosite activities for the different CKI members [19]. PER phosphorylation by CK1ε/δ is also essential to establish temperature compensation of the circadian period, a canonical property of the clock [20]. In addition, *CKI* mutations might lead to alterations in sensitivity to light, therefore adding an extra degree of regulation of circadian entrainment [21].

Casein kinase inhibitors such as PF670462, D4476, IC261, and longdaysin have showed to lengthen the circadian period by blocking CKIδ/ε-dependent phosphorylation and degradation of PER proteins [22-25] revealing the role of CKs in the maintenance of the circadian molecular oscillation. It is interesting that CKI activity in the SCN is rhythmic [21] and regulated by glutamate receptors [26], involved in the photic retinohypothalamic pathway responsible for entrainment.

PROTEIN KINASES INVOLVED IN PHOTIC TRANSDUCTION

Mitogen-Activated Protein Kinases (MAPKs)

MAPKs are serine-threonine protein kinases that regulate numerous cellular activities. In mammals, MAPKs include three families: c-Jun (JNK), p38, and ERK, each of which exhibiting several isoforms. All members of the MAPKs family are implicated in different signaling cascades [27]. They have been linked directly to the clock mechanism, playing a role in the photic entrainment of the circadian rhythms [28,29]. In agreement with this, we have reported that MAPKs are rhythmically activated in the hamster SCN at post-translational level, since no changes in the total amount of the proteins was determined [30]. Moreover, we found that MAPKs increased their activities after the photic induction during the late night, suggesting the role of the MAPKs in the entrainment of the master circadian clock.

In mammals, ERK1 and ERK2 [31] are involved in numerous cellular and physiological processes such as cell proliferation, differentiation, and synaptic plasticity. Moreover, ERKs were found to play a role in the photic resetting of the SCN [28,32]. In general, ERKs are activated through dual phosphorylation on threonine/tyrosine residues in response to various extracellular stimuli, including growth factors and stress. At the SCN, several peptides such as NGF [33], PACAP [34], EGF [35], and GRP [36] participate in photic activation. More recently, VIP has been found to regulate the circadian clock through ERK1/2 signaling [37].

Downstream targets of ERK1/2 include c-Fos and c-Myc, kinases as MSK-1 and RSK-1 and translational regulators that control the expression of target genes, including *Elk-1* and clock genes [31]. It was reported that ERK can directly interact with components of the circadian oscillator, and ERK-mediated phosphorylation of these proteins likely plays an important role in the maintenance of biological rhythms [38,39]. As mentioned above, the light input pathway is mediated through glutamatergic transmission from ipRGCs efferents interacting with NMDA receptors at the SCN. Glutamate increases the opening probability of receptors to generate a transient increase in intracellular Ca^{2+}, followed by an increase in cAMP that activates the ERK cascade [40,41]. In addition to the activation of ERK, increases in both intracellular Ca^{2+} and cAMP generate the phosphorylation and activation of CREB [42]. CREB phosphorylation is mediated through different kinases, including ERK [40,42]. The photic activation of CREB stimulates transcriptional activation through CRE region on the promoters of target genes [42,43]. The IEGs are targets of CREB, and are up-regulated in the SCN following photic stimulation [44]. Many of the IEGs, notably *c-Fos* and the clock gene *Per1*, require a functional ERK pathway for their light-induced expression [45].

In mice, the phosphorylation state of ERK, and thereby its activity, display a robust circadian rhythm. In addition, it is phosphorylated after a light-pulse, increasing its activity during the subjective night [43]. As mentioned above, we reported similar results in hamsters [30]: ERK was rhythmically phosphorylated under light-dark and constant conditions, with a maximal value during the day, increasing its phosphorylation and activity after a light-pulse during the night. In addition, p-ERK activation rhythms vary between different anatomical regions of the SCN. In the dorsomedial area of the SCN, known as the "shell," ERK activation cycles peaks during the subjective day [46]. In contrast, in the ventrolateral region of the SCN, known as the "core," p-ERK activity cycles show a peak during the subjective night [43,46] supporting the idea of functional compartmentalization within the SCN clock [47]. All these data support a role for the ERK pathway in photic circadian entrainment.

There are four known isoforms of p38 (α, β, γ, and δ), another well-known MAPK, that show tissue-specific expression, with p38α being the most ubiquitous. Like ERK, p38 is activated by dual phosphorylation of conserved Tyr-Gly-Thr residues. The classical activating signals of the p38 pathway are the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS), which leads to a p38-mediated inflammatory response [31]. In addition, p38 in mammals and lower eukaryotes is activated by many different stress

signals, including reactive oxygen species (ROS), osmotic stress, and heat shock.

In contrast to ERK, the role of p38 in the photic resetting of the circadian clock is less clear and seems to be dispensable. In hamsters, p38 is activated in response to light in the SCN during the subjective night, matching the phase gating of ERK [30]. p38 may phosphorylate clock oscillator proteins, affecting their stability and altering the cycling of the molecular oscillator [48]. However, no specific interactions between p38 and clock proteins have been described to date. This supports the idea that p38 does not directly participate of the SCN clock resetting, but rather that its function seems to couple humoral signals from the SCN to individual oscillators in peripheral tissues [49].

Mammalian JNK is encoded by three distinct, but closely related, JNK genes: *JNK1* and *JNK2* are expressed in most cells, whereas *JNK3* is expressed mainly in the brain [31]. Like other MAPKs, a variety of environmental signals activate the JNK pathway, such as hyperosmotic stimuli, ultraviolet radiation [50] stress signals, growth factors and inflammatory cytokines [51-53]. JNK activation requires dual phosphorylation of the motif Thr-Pro-Tyr [31,54]. As well as its role in cell apoptosis and proliferation, an important role for JNK was reported in the modulation the circadian oscillator by phosphorylating the clock proteins in both the positive and negative branches [55,56]. Activated JNK was shown to phosphorylate both CLOCK and BMAL1 [58] and, on the other hand, activated JNK is also able to phosphorylate PER2. Along with this data, light pulses during the subjective night induced rapid activation of JNK supporting the idea that JNK kinase is implicated in mammalian photic entrainment by phosphorylation of clock proteins, which in turn affects clock protein stability [31,58].

In summary, each MAPK pathway subfamily serves as input pathways to the clock in distinctly different ways. The ERK pathway facilitates clock resetting in the SCN in response to light via CREB induction of clock genes. On the other hand, p38 appears to signal through CREB-mediated clock resetting in peripheral tissues. Finally, the JNK pathway contributes to photic clock resetting in the SCN, but this action is facilitated through a CREB independent mechanism.

Calcium/Calmodulin-dependent Kinase (CAMK)

Another candidate pathway for the transmission of photic information to the core circadian clockwork is the one composed by the CaMK family. CaMKs belong to a group of kinases activated by an increase of cytosolic Ca^{2+} [57] and calmodulin, with multiple roles in neuronal transmission: synaptic plasticity, circuit development, and cognition. The CaMK family is classified into four groups: CaMKI, II, III, and IV. CaMKI, CaMKII, and CaMKIV phosphorylate serine/threonine residues in a broad range of substrates due to their low substrate specificity and affinity. As the circadian activity of CaMKII is the most reported in the SCN as compared to other CAMKs, this paper will focus in the main characteristics of this kinase in the entrainment of the circadian rhythms.

In mammals, CaMKII is encoded by four genes (α, β, γ, δ) among which α and β are expressed predominantly in the brain, while γ and δ are ubiquitously expressed [58]. CaMKII seems to regulate the circadian clock at multiple levels. We and others have shown that CaMKII plays an important role in the photic input pathway and the rhythmic gene expression of clock genes [59,60]. We also found that CaMKII is implicated in both regulation of neuronal nitric oxide (NO) synthase (nNOS) activity, and phosphorylation induced by light in the SCN [59]. Photic signals induce a cytoplasmatic Ca^{2+} increase in SCN neurons, which interact with CaMKII to induce nNOS activity, increasing NO synthesis by oxidizing arginine to citrulline. The pathway diverges downstream of nNOS to sustain the circadian gating of phase shifts through the induction of *Per1* gene expression [61]. Moreover, CaMKII activity is essential in the regulation of the cell-autonomous molecular clockwork, and in the synchronization of the circadian behavioral oscillators. Kon and colleagues [62] described a model in which CaMKII signaling couples Ca^{2+} dynamics with the E-box dependent transcriptional feedback mechanism, stabilizing self-sustaining cellular oscillations. They also reported that, at the behavioral level, a CaMKII knock-in mouse resulted in dissociation of the morning and evening activity rhythms in behavior causing the decoupling of oscillations between the left and right SCN.

CYCLIC NUCLEOTIDE DEPENDENT PROTEIN KINASES

cGMP-dependent Kinase (PKG)

Nitric oxide (NO) plays an important role in diverse physiological processes. NO is produced by NOS which are activated by an increase in intracellular Ca^{2+} levels. Most NO effects are mediated through direct activation of the soluble guanylyl cyclase (GC), an enzyme that produces cGMP. An intracellular cGMP rise can activate multiple pathways by interaction with different proteins targets, of which the main one appears to be the cGMP-dependent protein kinase (PKG). PKG are enzymes activated by cGMP, and act on serine/threonine residues in diverse substrates. PKGs are found in diverse organisms and tissues [63-65]. Mammals have two PKG genes coding for PKGI and PKGII [65,66]. PKGII appears to be widely distributed in several brain regions [67] and in the SCN [68]. Tischkau and colleagues described that PKG II activity exhibits an endogenous oscillation with maximal

values at the end of subjective night [69]. Activation of PKGII is critical for regulating the phase resetting induced by light and glutamate in the late subjective night. On the other hand, no effect was detected when light or glutamate were applied during the early night [3]. These data support the idea that the NO-cGMP-PKGII pathway is relevant in the photic entrainment by phase advances (but not delays) of the circadian clock. This resetting is presumably accomplished by the PKGII- mediated phosphorylation of the transcription factor CREB [70], which is also a *bona fide* substrate for the cAMP-dependent protein kinase (PKA) in the SCN. Transcription of several genes, including the immediate early genes c-fos and junB [71], and clock gene *Per1* [72,73], is initiated near dawn in the SCN, immediately after the activation of the NO-cGMP-PKGII pathway.

In summary, PKGII may influence core clock components to signal the conclusion of nighttime processes following the transit to the daytime domain. Thus, clock-controlled activation of PKGII may function as a critical checkpoint of temporal state at the night-to-day transition [69].

cAMP-dependent Kinase (PKA)

In mammals, cAMP is an intracellular second messenger stimulated by hormones and neurotransmitters interacting with G protein-coupled receptors. cAMP exerts many of its physiological effects by activating PKA, which in turn regulates the functions of downstream protein targets including enzymes and transcription factors. It has been demonstrated that the PKA signaling pathway is one of the main cascades involved in photic resetting [74] which leads to the phosphorylation of CREB and CRE-mediated transcriptional activation of *Per1* [43,44]. This, in turn, leads to clock-induced behavioral phase shifting [75]. In fact, several studies indicate that PKA is involved in the translocation of activated MAPK to the nucleus [76] regulating CREB-mediated gene expression in the SCN.

Blocking PKA activation in the early subjective night decreases phase delays in response to *in vitro* SCN administration of glutamate, as well as glutamate-induced *Per1* expression [77]. During the late subjective night, inhibition of PKA enhances glutamate-induced advances and *Per1* expression *in vitro*, and behavioral phase advances after light stimulation [77]. Together, these data suggest that PKA is an important component of the photic phase delay response, while phase advances are likely mediated through activation of cGMP and PKG [74].

OTHER PROTEIN KINASES INVOLVED IN CIRCADIAN ENTRAINMENT

Post-translational mechanisms modulate multiple molecular pathways of the circadian clock. Indeed, while several protein kinases are regulated by light, there is strong evidence indicating that at least some of them are also responsible for photic entrainment of the clock.

Protein kinase C. This kinase is rhythmic in the SCN, peaking at the end of the day or circadian day, and interacts with the *Per2* clock gene. Moreover, while pharmacological inhibition of this enzyme results in an attenuation of photic responses [78], mice deficient in the A subtype of protein kinase C have a ≈50 percent decrease in the maximal behavioral response to light [79].

Glycogen synthase kinase 3 (GSK3). This kinase has been identified as a potential target for lithium treatment in bipolar disorder, and also seems to be involved in the molecular circadian clock, through the phosphorylation of CLOCK [80] and, together with DDYRK1A, the regulation of CRY2 dynamics [81]. Both GSK3α and β are constitutively active in the SCN, and rhythmically phosphorylated for its inactivation [82] to also regulate BMAL1 [83], and its inhibition impairs light-induced phase shifts of the clock [84]. Although upstream GSK3 kinases are unknown at the SCN, a potential regulator of GSK3β activity is the small GTP-ase Ras, one of the main upstream regulators of ERK-CREB pathway. Mice with constitutively enhanced Ras activity show downregulated phosphorylation of GSK3β, as well as potentiation of both light-induced phase shifts and pERK1,2/pCREB and c-Fos pathway [85]. As several clock proteins such as BMAL1, CRY2, PER2, and REV-ERBα are phosphorylated by GSK3β leading to protein stability and degradation [86], the activity of this kinase could contribute to regulate both photic transduction and circadian period mechanism.

mTOR. The mammalian/mechanistic target of rapamycin complexes (mTORC) 1 and 2 are highly conserved nutrient sensors integrating metabolic activity of the cell. mTORC1 signaling pathway acts trough ribosomal protein S6 kinases (S6K) and eukaryotic translation initiation factor 4E (eIF4E) and its binding proteins (4EBPs) enhancing protein synthesis to regulate photic transduction and circadian oscillation at the SCN [87]. This pathway is rapidly activated by light in the SCN [88], while its inhibitor (rapamycin) blocks light induced phase-delays in mice [89]. mTORC1 regulates light-inducible mRNA translation at the SCN, although the mechanism is not completely elucidated [87]. The mTOR pathway regulates VIP expression in the SCN [90] and, more recently, it was reported that activation of the system increases levels of core clock proteins, including CRY1, CLOCK, and BMAL1 [91].

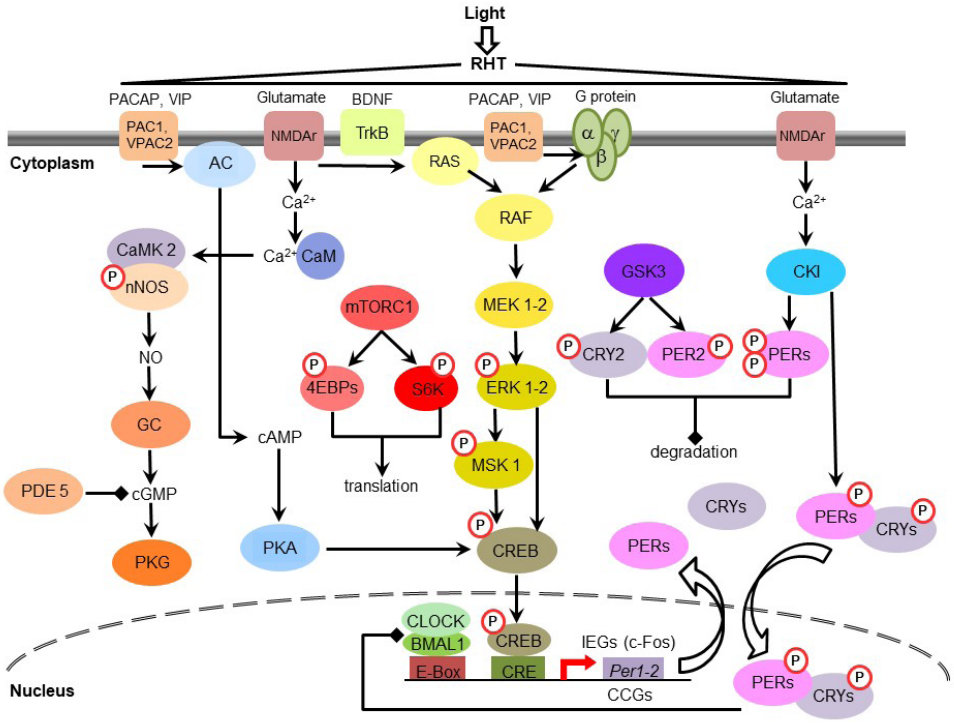

Figure 1. Photic signaling pathways involving main kinases activities in the circadian clock. The scheme depicts an SCN neuron of the retinorecipient region receiving retinohypothalamic (RHT) efferent photic stimulation. The neurotransmitters glutamate, BDNF and PACAP are released in response to light. Other neurotransmitters released from SCN neurons such as VIP, NGF, and GRP (not shown) also participate. Ca^{2+}/cAMP dependent pathways PKG, PKA, and ERK, requires convergent activation of their corresponding upstream receptors (NMDAr, PAC1, VPAC2, and TRKB. Except for PKG, whose downstream effectors are still unknown, the photic activation of these pathways converge to CREB phosphorylation for its nuclear translocation and interaction at CRE elements for the induction of immediate-early genes (*e.g.*, *c-Fos*, *c-Myc*) and clock genes *Per1-2* and *Cry1-2* (not shown). mTOR, GSK, and CKI signaling affect the circadian machinery by direct regulation on stability and half-life of clock proteins. mTORC1 signaling can enhance translation of CLOCK, CRY1, BMAL1, and SCN neurotransmitter VIP. Upstream mTOR regulators are unknown at the SCN. GSK-dependent phosphorylation of PER2, CRY2, BMAL1 and REV-ERB**α** (not shown) is constitutive, it is downregulated by a circadian phosphorylation/dephosphorylation cycle and contribute to degradation. CK1 activity respond to the circadian clock, as well as to glutamate-NMDAr photic pathway. Since the activity of CK1 is complex, the degradation pathway for PER was only included for the sake of simplicity. The figure also summarizes part of the molecular clockwork which generates the circadian output composed by interacting transcription/translation feedback loops of negative (PER:CRY) and positive (CLOCK:BMAL1) heterodimers inhibiting or activating transcription, respectively. Positive loop starts with CLOCK:BMAL1 heterodimer binding at E-boxes to promote transcription of *Per1-2* and *Cry1-2* clock genes, and also of the clock-controlled genes (CCGs) driving clock output. PERs and CRYs proteins accumulate in the cytoplasm and are phosphorylated at specific phosphosites. Then they dimerize and translocate to the nucleus, where they inhibit *Clock* and *Bmal1* transcription generating the negative feedback loop each circadian cycle. Half-life of clock proteins determined by the accumulation/degradation ratio, contribute to the circadian period regulation. Black arrows indicates alosteric activation/increased levels; red circle indicate phosphorylation; degradation is indicated with a diamond; white arrows indicate translocation; red arrow, transcription.

Additional kinases. Although the number of kinases involved in the regulation of the molecular circadian clock and/or circadian entrainment pathways is high and increasing, it is worth at least mentioning a couple of additional examples. The salt-inducible kinase 1 (SIK-1) inhibits the CREB-regulated transcription coactivator 1, which is light-regulated [92] and modulates phosphorylation/activation of CREB in the SCN [93]. As expected, SIK-1 inhibition increases the magnitude of light-induced phase shifts.

In addition, G-protein-coupled receptor kinase 2 (GRK2) modulates transcription and phosphorylation of Per2, altering its dynamics and, in turn, affecting light-induced phase shifts in a complex manner [94,95].

Again, this is just a sample of the complex network of kinases involved in both the molecular machinery of the mammalian circadian clock and, more specifically, its regulation and entrainment by environmental light.

CONCLUSIONS AND OUTLOOK

Posttranslational modifications play a crucial role in the generation of circadian rhythms. Among these, phosphorylation has been investigated extensively due to its importance in processes such as subcellular localization, transcriptional activity, and conformation of clock components over the course of a circadian day. In this paper, we have reviewed the functions of the main kinases involved in the generation and entrainment of circadian rhythms in mammals. As mentioned above, modifications by kinases are important to maintain the periodicity of the clock, because kinases influence directly or indirectly phosphorylation of clock proteins, leading to changes in their dynamics, proteasome-mediated degradation and an appropriate turnover, to maintain the 24 h periodicity. However, it should be mentioned that at physiological level, phosphorylation by kinases is as important as the de-phosphorylation by phosphatases [96]. In some cascades, phosphatase activity counteracts the effects of a kinase, modifying the activity, stability, and putative interactions of the protein involved. For this reason, both kinases and phosphatases should be considered when attempting a circadian-based approach of different diseases, such as depression, seasonal affective disorder or metabolic disorders [97,98]. Notably, despite the great advances in genetics and biological tools, hundreds of kinases need to be characterized to reveal the physiological function in the generation and maintenance of circadian periodicity. Figure 1 summarizes some of the kinase activities directly implied in the photic regulation of circadian entrainment.

High throughput analysis by existing molecular techniques such as RNAseq, or microarrays, provide a first step for the construction of a framework based on gene products activated downstream in the photic entrainment pathways at the SCN. However, phosphoproteomic analysis remains essential to uncover unknown effectors characterizing the specific transduction mechanisms involved. Indeed, high-throughput efforts began to identify putative modulators of kinase activity that are potent regulators of circadian rhythms [22,23,99,100], offering a unique and novel perspective for potential chronobiological treatments of disease.

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