How circadian clocks keep time: the discovery of slowness
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Circadian transcription activators
Circadian transcription activates heterodimerization via PAS domains, large intrinsically disordered regions

Phosphorylation sites:
Blue: priming-independent
Orange: potentially priming-dependent

Intrinsically disordered with multiple phosphorylation sites, dimerization via coiled-coils or PAS domains

Circadian inhibitors

KaiC hexamer
CI

Molecular basis of slowness
1) Slow ATPase of KaiC CI

fsKaiB binds to post ATP-hydrolysis or ADP-bound state of KaiC CI

Molecular basis of slowness
Slow hyperphosphorylation of low-affinity sites in disordered clock proteins
SLOW
Priming-independent phosphorylation:
Dependent on CK1 anchoring

Fast
Priming-dependent phosphorylation:
Direct recognition of primed site

KaiC CI
KaiB

Post-ATP Hydrolysis

KaiC CI-KaiB complex
KaiC CI light blue
KaiB: orange

SasA

KaiC CI

Pru

PAS A PAS B

PAS A PAS B

PAS A PAS B

COMPACT
EXTENDED

MC

FRQ/PERs

CRY, FRH, TIM

CRY, FRH, TIM

FRQ/PERs

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Circadian clocks are biological timing systems that synchronize physiology and behavior with the daily light-dark cycle. Circadian clock precision is based on autonomous clocks that communicate with the environment and with each other through feedback loops that are self-maintained even in the absence of transcription or translation. This ensures that the body keeps time closely synchronized with the environment, even after a 24-hour period.

Eukaryotic circadian clocks: The core circadian clock of fungi and animals relies on interlocked TFI proteins. The heterogeneous nuclear ribonucleoproteins TFI (TFI-C, TFI-D, and TFI-E) rhythmically control the expression of their inhibitors FRQ, TIM, and PER, as well as PER1/2 and CRY1/2 in Neurospora, Drosophila, and mammals, respectively. The association of FRQ with FRB, Drosophila TIM with PER, and mammalian TIM1-2 with CRY1/2 stabilizes the complexes [10-12]. Casein kinase 1 (CK1) is conserved in eukaryotic circadian clocks and anchored to FRQ and PERs. These large molecular complexes accumulate in the nucleus and inhibit their TFs through a phosphorylation-induced release from DNA [13-15]. In mammals, CRY association may also directly inhibit CLOCK-BMAL1 [16, 17]. Circadian pacemakers appear to be associated with highly tuned protein interactions [18], and the slow, progressive hyperphosphorylation of FRQ and PERs [19], followled by interaction with repressive complexes and degradation, is required to trigger the next cycle. Additional interlocking mechanisms associated with the core oscillator link the circadian pacemaker to cellular metabolism [20]. These loops are critical for robust circadian function under physiological conditions and are not discussed here.

Activators of circadian transcription: CK-CYC from Drosophila and CLOCK-BMAL1 from mouse are conserved orthologs that share a DNA-binding domain conserved to tandem PAS domains [21]. This structural architecture is conserved in the evolutionarily unrelated WC1-WC2 complex (from Neurospora) [22]. PAS domains are sensory and ligand binding domains found in all kingdoms of life [23]. CK-CYC is the circadian clock's molecular timer, mediating the clock's output by controlling transcript levels of many potential photophilic pathways [24]. Therefore, FRQ and PER, together with their major kinase, CK1, appear to play a major role in circadian timekeeping. Other biochemical steps control the pacemaker, such as how tightly CRY1/2 is bound to CLOCK-BMAL1 [18].

Casein kinase 1 and the sluggishness of circadian phosphorylation: The kinase domain of CK1 has several conserved anion binding sites that facilitate its activity in the slow-phosphorylated (primed) substrates [25]. However, the main feature that makes CK1 crucial for circadian function is its ability to control the phosphorylation of up-regulated (primed) sites with low affinity [19, 26]. Phosphorylation of such low-affinity sites in FRQ and PER relies on site-specific anchoring of CK1 to a structurally conserved binding domain (CKBD), increasing its local concentration and allowing the kinase to come into contact with sites throughout the protein via dynamic looping of the IDRs [19]. The regulation of progressive phosphorylation by substrate-looping may underlie some of the sluggishness and temporal precision of the circadian pacemaker. In mammalian PER2, phosphorylation of a series of sites within the FAS1 (Familial Advanced Sleep Phase) region [27], located within the CK1BD, constitutes a phospho-switch that further slows CK1 phosphorylation, including at a degree for β-TRCP-mediated degradation [25, 28]. A similar mechanism has been described for Drosophila PER2 [29] and proposed for Neurospora FRQ [19].

The IDRs of FRQ and PER contain several motifs which may surface function in phosphorylation, including NLSs, NESs, degrons, and intra- or intermolecular interaction motifs. Therefore, phosphorylation of a large number of sites in different regions of the protein may be functionally redundant, decreasing phosphorylation and eventually inactivating and degrading circadian repressor complexes through various mechanisms and pathways.