Circadian Rhythms

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What are Circadian Rhythms?
Circadian Rhythms

- A circadian rhythm is an endogenously driven roughly 24-hour cycle in biochemical, physiological, or behavioural processes.

- Although circadian rhythms are endogenous, they are adjusted (entrained) to the environment by external cues called zeitgebers, the primary one of which is daylight.

- Circadian rhythms can change sleep-wake cycles, hormone release, body temperature and other important bodily functions.
Fig. 3 An illustration of commonly-used circadian terminology. Depicted on top is a simple sinusoidal model of a subject’s behavioral or physiological rhythm (e.g., daily activity), and depicted below is the environmental cycle to which the subject was exposed...

Megan Hastings Hagenauer, Theresa M. Lee

The neuroendocrine control of the circadian system: Adolescent chronotype


http://dx.doi.org/10.1016/j.yfrne.2012.04.003
Right timing is in all things the most important factor.

It is well to be up before daybreak, for such habits contribute to health, wealth, and wisdom.

...not only do [animals] rest at night-time from dimness of vision...but even if a lighted candle be presented they continue sleeping quite as soundly.

Citing Androstenes of Thasos, trierarch to Alexander (324 BCE):

...there is another tree [on Tylos] “with many leaves”...that closes at night, but opens at sunrise, and by noon is completely unfolded; and at evening – again it closes by degrees and remains shut at night, and the natives say that it goes to sleep.
Animals Express Endogenous Rest-Activity Rhythms

Curt P. Richter (1894 – 1989)

Comp Psychol Monographs, 1922
Mammoth Cave, 75 Years Ago

“The Father of Modern Sleep Research”

Kleitman and his assistant Bruce Richardson attempted to adjust their routines to a 28-h day (19 h awake with lights-on and 9 h in bed with lights-off) in the absence of sunlight and a constant temperature of 54° F. They recorded their cycles of wakefulness and body temperature for about a month.
McCarthy et al., 2012
Localization of the Circadian Pacemaker: The Suprachiasmatic Nucleus (SCN)
The SCN is Composed of Many Single-Cell Circadian Oscillators


Mouse *mPer1::luc* (hypothalamic slice)

Rat isolated single units

Schaap et al., *PNAS* 100:15994, 2003
Effects of SCN Lesions
SCN Afferent and Efferent Pathways
A Distributed Network of Circadian Clocks
Circadian Photoreception Occurs in the Eye

- In mammals circadian phototransduction occurs in the eye and neural signals are conducted along the Retino-Hypothalamic Tract to the biological clock nucleus of the brain, the suprachiasmatic nucleus (SCN).
Melanopsin cells use glutamate and PACAP as neurotransmitters.

Ca++ influx leads to CREB phosphorylation.

CREB activation acts on CRE elements in *Per* gene promoters.
What else can entrain rhythms?

• Food
• Drugs of abuse
• Social cues

Methamphetamine 100mg/l
Peripheral Organ Entrainment by Temperature

$mPer^{2\text{Luc}}$ mice

Effects of behavioral state on the circadian pacemaker

- Stimuli evoking acute behavioral arousal and/or locomotor activity induce circadian phase shifting
- Feedback from spontaneous locomotor activity modulates free-running period
- Scheduled daily activity (either ‘voluntary’ or forced) entrains free-running rhythms
- Activity-induced phase shifting may be mimicked by simple sleep deprivation, even without intense locomotor activity
Not a Rigid Clock, but a Flexible Temporal Program

In the Laboratory

Indic, Schwartz, & Paydarfar,
*J Royal Soc Interface*, 6 August 2008

In the Wild

courtesy Dr. Bob Johnston, Cornell University
(near Kilis, Turkey, and the Syrian border)

Neural Pathways of the SCN for nonphotic entrainment

From Morin & Allen, 2006
Multiple Signaling Pathways Mediate Photic Entrainment
The *Period* Gene

• The story of specific “clock genes” begins with the work of Ron Konopka and Seymour Benzer at Cal Tech (1971) describing the *Period* locus.....

• Their basic design was to mutagenize flies and then screen for inherited changes in eclosion timing.
The *Period* Gene

A. normal

B. arrhythmic mutant

C. short-period mutant

D. long-period mutant
The *Period* Gene

**Summary of Early Findings**

- *Per* is the first single gene shown to control behavior.
- *PER* protein is located in the nuclei of brain and visual neurons.
- *Per* transcription, translation and nuclear localization is rhythmic.
- Accumulation of *PER* suppresses its own transcription.

**Later**

- The *PER* sequence contains a PAS protein-protein interaction domain.
- Homologs of *Per* (*Per1, Per2, Per3*) exist in mammals.
Mouse Period Genes

- The mouse *period1* gene (*mPer1*) was cloned simultaneously by two groups- Sun as well as Tei and Okamura in 1997.
Photic regulation of IEG and clock gene expression in the SCN

Albrecht et al., 1997
The *Clock* Gene

- *Clock* was identified in a screen of mutagenized mice done in the lab of J. Takahashi (Vitaterna et al., 1994).
The *Clock* Gene

- (Vitaterna et al., 1994).
The Clock Gene

- King et al., 1997
The *Clock* Gene

- The first mammalian single gene shown to control a complex behavior.
- Has PAS domains, like Per.
- Has a partner, Bmal1.
- A transcription factor that acts to increase Per transcription.
- Binds to E-box sequences in the Per gene promoter.
Plant TTFL

Nagel and Kay 2012
Drosophila TTFL

Core Loop
(negative feedback)

Accessory Loop
(regulation of + elements)

Modified from Hardin 2011
The molecular clock

Modified from Logan & Sarkar, 2012 and Gustafson & Partch, 2014
Do Bacteria have TTFLs?

Dong and Golden 2008
Oxidative stress/mitochondrial respiration

- **Redox state**: The process of cell respiration depends heavily on the reduction of NAD⁺ to NADH and the reverse reaction (the oxidation of NADH to NAD⁺). This ratio is a reflection of the redox state of the cell.

- **Oxidative stress** reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.
Interactions between mitochondrial and nuclear rhythms

McClung, J. Clinical Invest, 2013
NPAS2: A Gas-Responsive Transcription Factor

Elhadji M. Dioum,1 Jared Rutter,2 Jason R. Tuckerman,1 Gonzalo Gonzalez,1 Marie-Alda Gilles-Gonzalez,1* Steven L. McKnight2*

Neuronal PAS domain protein 2 (NPAS2) is a mammalian transcription factor that binds DNA as an obligate dimeric partner of BMAL1 and is implicated in the regulation of circadian rhythm. Here we show that both PAS domains of NPAS2 bind heme as a prosthetic group and that the heme status controls DNA binding in vitro. NPAS2-BMAL1 heterodimers, existing in either the apo (heme-free) or holo (heme-loaded) state, bind DNA avidly under favorably reducing ratios of the reduced and oxidized forms of nicotinamide adenine dinucleotide phosphate. Low micromolar concentrations of carbon monoxide inhibited the DNA binding activity of holo-NPAS2 but not that of apo-NPAS2. Upon exposure to carbon monoxide, inactive BMAL1 homodimers were formed at the expense of NPAS2-BMAL1 heterodimers. These results indicate that the heterodimerization of NPAS2, and presumably the expression of its target genes, are regulated by a gas through the heme-based sensor described here.

PAS domains are independently folding modules of ~130 amino acids that detect diverse environmental signals, including oxygen, light, voltage, redox potential, and many small aromatic molecules (I–7). Although these domains have modest sequence similarity, they share strikingly similar three-dimensional folds (8–12). Two groups of bacterial proteins—the FixL proteins of *Rhizobia* and the PDEA1 phosphodiesterases of *Acetobacter*—use heme bound within a PAS domain to sense oxygen (13). In FixL, binding of oxygen to the heme controls a kinase domain that phosphorylates a whether NPAS2 might represent yet another heme-based mode of signal transduction by PAS domains.

Overexpression of a fragment of NPAS2 containing its bHLH DNA binding domain and both PAS domains in bacteria yielded amber-colored cells. The absorption spectra of liquid cultures containing those cells revealed a correlation between NPAS2 expression and heme protein absorption (Fig. 1A). Obvious peaks of absorption for the intact living cells were observed at 426 nm (Soret or gamma) and 561 nm (alpha). Upon cen-
Schematic model of interaction of NPAS with BMAL1 in the presence of NAD(P)H and CO.

(a) inactive

\[ \text{bHLH} \quad \text{PAS-A} \quad \text{PAS-B} \quad \text{H} \quad \text{H} \]

NPAS2

NAD(P)H \downarrow \uparrow \text{NAD(P)⁺}

(b) active

\[ \text{bHLH} \quad \text{BMAL1-A} \quad \text{BMAL1-B} \quad \text{H} \quad \text{H} \]

\[ \text{bHLH} \quad \text{PAS-A} \quad \text{PAS-B} \]

NPAS2-BMAL1 heterodimer \rightarrow \text{can bind to E-box}

\[ \downarrow \text{CO} \]

(c) inactive

\[ \text{bHLH} \quad \text{PAS-A} \quad \text{PAS-B} \quad \text{H} \quad \text{H} \]

\[ \text{bHLH} \quad \text{PAS-A} \quad \text{PAS-B} \quad \text{H} \quad \text{H} \]

CO-bound NPAS2

BMAL1 homodimer

\[ \text{bHLH} \quad \text{BMAL1-A} \quad \text{BMAL1-B} \quad \text{H} \quad \text{H} \]

\[ \text{bHLH} \quad \text{BMAL1-A} \quad \text{BMAL1-B} \quad \text{H} \quad \text{H} \]

CO binding is very rare

“Up to now, only two heme-containing CO sensor proteins are known. In those bacteria that metabolize CO as a sole energy source, the coo operons that encode for the required proteins are under the control of the heme-based CO-sensing homo-dimeric transcriptional activator CooA. CO binding to the heme-PAS domain of CooA renders this protein competent for the transcriptional activation (see refs 28, 33, 56 – 58).

Neuronal PAS domain protein 2 (NPAS2) is a mammalian transcriptional factor implicated in the regulation of the circadian rhythm. NPAS2 binds DNA as an obligate dimeric partner of BMAL1, the brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1. CO binding to the heme-PAS domain of NPAS2 inhibits the transcriptional activator activity induced by NADPH (see refs 34, 59, 60).”

“Moreover, the cellular redox balance influences the circadian clock. In fact, oxidative conditions reduce melatonin and 5-methoxytrypta-mine levels, which are restored by NAD(P)H (87, 88). If so, the role of the NPAS2-BMAL1 heterodimer in sensing circadian oscillation of heme/CO metabolic pathways and the cellular redox balance might be pivotal (59, 87, 88).”

• Ascenzi et al., Life 2004.
“Transducer” model of astroglial HO-1 in chronic CNS disorders.

Melatonin: an ancient molecule that makes oxygen metabolically tolerable
Loss of BMAL1 or CLOCK/NPAS2 leads to massive increases in oxidative stress and neurodegeneration

- Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration.
Schizophrenia-like features in transgenic mice overexpressing human HO-1 in the astrocytic compartment.

The *Clock* mutant mice are very similar to bipolar patients in the manic state

<table>
<thead>
<tr>
<th>Bipolar patients</th>
<th>Clock mutant mice</th>
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</thead>
<tbody>
<tr>
<td>Hyperactivity</td>
<td>Hyperactivity</td>
</tr>
<tr>
<td>Decreased need for sleep</td>
<td>Sleep less than wild type mice</td>
</tr>
<tr>
<td>Feelings of euphoria</td>
<td>Are less depressed in behavioral models</td>
</tr>
<tr>
<td>Excessive involvement in activities that have a high potential for painful consequences.</td>
<td>Have lowered anxiety (increased risky behavior) in behavioral models</td>
</tr>
<tr>
<td>Propensity towards drug use and abuse</td>
<td>Are more sensitive to the rewarding effects of cocaine, sucrose, and brain stimulation</td>
</tr>
</tbody>
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Lithium or VPA treatment reverses these phenotypes

Circadian Clocks in Health and Disease
The health consequences of shortened or reduced sleep and desynchronized circadian rhythms

**Emotional responses**
- Feeling states
  - Exhaustion
  - Increased irritability
  - Mood fluctuations
  - Depressed mood
- Stress
  - Disorders of the hypothalamo-pituitary adrenal axis
- Overt behaviour
  - Frustration, anger
  - Increased impulsivity
  - Mania and increased risk taking
  - Decreased motor performance
  - Increased stimulant and sedative use
  - Alcohol use and misuse

**Cognitive responses**
- Attention
  - Reduced ability to concentrate
  - Difficulties sustaining attention and alertness
- Memory
  - Decreased working memory capacity
  - Reduced memory of facts
  - Reduced recall of events or episodes
- Executive functions
  - Reduced ability to multi task
  - Reduced decision making
  - Reduced creativity and productivity

**Somatic responses**
- Drowsiness
- Microsleep
- Bodily sensations of pain and being chilled
- Cardiovascular disease
- Risk of diabetes
- Reduced immunity to disease and viral infection
- Risk of cancer
- Metabolic problems
- Risk of cancer