Circadian rhythms allow living organisms to live in phase with the alternance of day and night...

Circadian rhythms in *Drosophila*

Expression of *per* gene

Molecular mechanism of circadian clocks

Core mechanism: negative feedback loop

- **Drosophila**
  - *per* (period), *tim* (timeless)
- **Mammals**
  - *mPer1-3* (period homologs)
- **Neurospora**
  - *frq* (frequency)

Goldbeter's 5-variable model

**CLOCK**
From Wikipedia, the free encyclopedia: Redirected from Clock genes

Circadian Locomotor Output Cycles Kaput, or Clock is a gene which encodes proteins regulating circadian rhythm. The CLOCK protein seems to affect both the persistence and length of the circadian cycle. CLOCK forms part of a basic-helix-loop-helix transcription factor, BMAL-1, dimerizes with CLOCK in vivo and transactivates gene expression of *Period* and *Timeless* in *drosophila* by binding to E-box elements in their promoters. BMAL-1·CLOCK also regulates Cryptochrome genes (*e.g.* Cry1, Cry2) and Period genes (*e.g.* Per1, Per2, Per3) in mammals. The BMAL/CLOCK complex itself is regulated by the expression of Per and Cry genes.

**Circadian rhythm**
From Wikipedia, the free encyclopedia
*Human clock* redirects here: *Humanclock*. A circadian rhythm is an approximate daily periodicity, a roughly-24-hour cycle in the biochemical, physiological or behavioural processes of living beings, including plants, animals, fungi and cyanobacteria. The term "circadian", coined by Franz Halberg, comes from the Latin *circa*, "around", and *diem* or *dies*, "day", meaning literally "approximately one day." The formal study of biological temporal rhythms such as daily, tidal, weekly, seasonal, and annual rhythms, is called chronobiology. Circadian rhythms are endogenously generated, and can be entrained by external cues, called Zeitgebers. The primary one is daylight. These rhythms allow organisms to anticipate and prepare for precise and regular environmental changes.

**Zeitgeber**
From Wikipedia, the free encyclopedia
Zeitgeber (from German for "time giver", synchrizer) is any exogenous (external) cue that entrains the endogenous (internal) time-keeping system of organisms. The strongest zeitgeber, for both plants and animals, is light. Other, non-photic, zeitgebers include temperature, social interactions, pharmacological manipulation and eating/drinking patterns. The German term Zeitgeber came into the English language when Jürgen Aschoff, one of the founders of the field of chronobiology, used it in the 1960s. It is now in common use in the scientific literature in this field.
Almost all organisms, from prokaryotes to humans, possess a biological clock that generates endogenous rhythms with precise circadian period. The three year record of the locomotor activity rhythm from a squirrel monkey shows the circadian rhythm of an organism.

At the core of the circadian clock is a autoregulatory molecular feedback loop associated with clock gene transcription. The motif is characterized by two α-helices connected by a loop. In general, transcription factors including this domain are dimeric, each with one helix containing basic amino acid residues that facilitate DNA binding. In general, one helix is smaller, and, due to the flexibility of the loop, allows dimerization by folding and packing against another helix. The larger helix typically contains the DNA-binding regions. BHLH proteins typically bind to a consensus sequence called an E-box, CANNTG, however some BHLH transcription factors bind to different sequences, which are often similar to the E-box.

**BMAL**

From Wikipedia, the free encyclopedia

Bmal (brain and muscle aroyl hydrocarbon receptor nuclear translocator (ARNT)-like) is a gene which encodes proteins regulating circadian rhythm. BMAL proteins form part of a basic-helix-loop-helix transcription factor. BMAL-1 dimerizes with CLOCK in vivo and transactivates gene expression of Period and Timeless in drosophila by binding to E-box elements in their promoters. BMAL1-CLOCK also regulates Cryptochrome genes (e.g. Cry1, Cry2) and Period genes (e.g. Per1, Per2, Per3).

A basic helix-loop-helix (bHLH) is a protein structural motif that characterizes a family of transcription factors. The motif is characterized by two α-helices connected by a loop. In general, transcription factors including this domain are dimeric, each with one helix containing basic amino acid residues that facilitate DNA binding. In general, one helix is smaller, and, due to the flexibility of the loop, allows dimerization by folding and packing against another helix. The larger helix typically contains the DNA-binding regions. BHLH proteins typically bind to a consensus sequence called an E-box, CANNTG. The canonical E-box is CACGTG (palindromic), however some bHLH transcription factors bind to different sequences, which are often similar to the E-box.

bHLH transcription factors are often important in development or cell activity. BMAL1-Clock is a core transcription complex in the molecular circadian clock. Other genes, like c-Myc and HIF-1, have been linked to cancer due to their effects on cell growth and metabolism.

**Basic-helix-loop-helix structural motif of ARNT. Two α-helices (blue) are connected by a short loop. (from [1])**

**Basic-helix-loop-helix DNA-binding domain**

**Molecular Control of Circadian Rhythm**
Period (gene)

Period (gen) is a gene in Drosophila which encodes a protein, PER, regulating circadian rhythm. There are some known alleles of the gene that make the circadian cycle longer or shorter than the usual cycle (which is around 24 hours). It is still uncertain how exactly this gene operates.

In humans, there are three known PER family genes: PER1, PER2, and PER3.

The Fruit Fly: Drosophila melanogaster

Period homolog 1 (Drosophila), also known as PER1, is a human gene. This gene is a member of the Period family of genes and is expressed in a circadian pattern in the suprachiasmatic nucleus, the primary circadian pacemaker in the mammalian brain. Genes in this family encode components of the circadian rhythms of locomotor activity, metabolism, and behavior. Circadian expression in the suprachiasmatic nucleus continues in constant darkness, and a shift in the light/dark cycle evokes a proportional shift of gene expression in the suprachiasmatic nucleus. The specific function of this gene is not yet known. Alternative splicing has been observed in this gene; however, these variants have not been fully described.

Period homolog 2 (Drosophila), also known as PER2, is a human gene. This gene is a member of the Period family of genes and is expressed in a circadian pattern in the suprachiasmatic nucleus, the primary circadian pacemaker in the mammalian brain. Genes in this family encode components of the circadian rhythms of locomotor activity, metabolism, and behavior. Circadian expression in the suprachiasmatic nucleus continues in constant darkness, and a shift in the light/dark cycle evokes a proportional shift of gene expression in the suprachiasmatic nucleus. The specific function of this gene is not yet known. A new genetic test from a cheek swab can use Per2 expression levels to tell whether a person is an early morning person or a “night owl”. It has also been shown that Per2 and Bmal1 work in opposition to each other.

Period homolog 3 (Drosophila), also known as PER3, is a human gene. This gene is a member of the Period family of genes and is expressed in a circadian pattern in the suprachiasmatic nucleus, the primary circadian pacemaker in the mammalian brain. Genes in this family encode components of the circadian rhythms of locomotor activity, metabolism, and behavior. Circadian expression in the suprachiasmatic nucleus continues in constant darkness, and a shift in the light/dark cycle evokes a proportional shift of gene expression in the suprachiasmatic nucleus. The specific function of this gene is not yet known.

Cryptochromes

Cryptochromes from the Greek κρυπτός κρυπτό χρώμα are a class of blue light photoreceptors of plants and animals. They form a family of flavoproteins that regulate germination, elongation, photoperiodism, and other responses in higher plants. Cryptochromes are involved in the circadian rhythm of plants and animals, and in the sensing of magnetic fields in a number of species.

Blue light also mediates phototropism, but this response is now known to have its own set of photoreceptors. The phototropins.

Cryptochromes are evolutionary very old and highly conserved molecules. They are derived from photolyase, a bacterial enzyme that is activated by light and participates in DNA damage repair. In eukaryotes the cryptochromes lost their original enzymatic activity. Cryptochromes possess two chromophores: pterin and flavin; a chemical relative of pterin. Pterin absorbs a photon, which causes it to emit energy; the latter is absorbed by flavin, which probably mediates the phosphorylation of a certain domain in cryptochrome. This triggers a signal transduction chain that affects gene regulation in the cell nucleus.

The genes coding for two cryptochromes, CRY1 and CRY2, are found in many species - including in humans on chromosomes 12 and 11. Studies in animals and plants suggest that cryptochromes play a pivotal role in the generation and maintenance of circadian rhythms. In fruit flies they are part of the mechanism that triggers coordinated spawning for a few nights after a full moon in the spring.

Cryptochromes in the photoreceptor neurons of the eyes of birds are involved in magnetic orientation during migration. Cryptochromes are also essential for the light-dependent ability of the fruit fly Drosophila melanogaster to sense magnetic fields. Furthermore, the cryptochromes in the plant Arabidopsis thaliana detect magnetic fields: growth behavior is affected by magnetic fields in the presence of blue (but not red) light. According to one model, cryptochrome when exposed to blue light can form a pair of two radicals (molecules with a single unpaired electron) where the spins of the two unpaired electrons are correlated, and the kind of this correlation is affected by the surrounding magnetic field; a subsequent reaction depends on spin. The overall result is that the molecule’s light-sensitivity depends on the magnetic field, so that the animal can “see” the magnetic field.
CRY1 Cryptochrome 1 (photolyase-like), also known as CRY1, is a protein which in humans is expressed by the CRY1 gene.

Structure
CRY proteins belong to a superfamily of flavoproteins that occurs in all kingdoms of life. All members of this superfamily have the characteristics of a N-terminal photolyase homology (PHR) domain. PHR domain can bind to FAD cofactor and a light-harvesting chromophore. The structure has a fold very similar to photolyase, with a single molecule of FAD noncovalently bound to the protein. These proteins have variable lengths and surfaces on the C-terminal end when compared to one another, due to the lack of DNA repair enzymes which ultimately results in changes in genome and appearance. The Ramachandran plot shows that the secondary structure of the CRY1 protein is primarily in a right-handed alpha helix conformation with little to no steric overlap. The structure of CRY1 is almost entirely made up of alpha helices, with remaining portions in loops and has few beta sheets present in the molecule. The molecule is arranged as an orthogonal bundle.

Function
In bacteria, photolyases are enzymes that mediate photoreactivation, a repair mechanism that removes UV-induced DNA damage. The homologous proteins in animals however are constitutively expressed which suggests that they may have functions other than DNA repair and may instead function as blue-light photoreceptors. In insects and plants, cryptochrome (CRY) functions as a photoreceptor for the circadian clock in a light-dependent fashion. In plants, the blue light photoreception can be used to cue developmental signals. The mammalian CRY1 and CRY2 act as light-independent inhibitors of CLOCK-BMAL1 components of the circadian clock.

Timeless (tim) is a gene in Drosophila which encodes a protein, TIM, that regulates circadian rhythm. The human timeless protein (hTIM) has been shown to be required for the production of electrical oscillations output by the suprachiasmatic nucleus (SCN), the major clock governing all tissue-specific circadian rhythms of the body. hTIM also interacts with the products of major clock genes CLOCK, BMAL, PER1, PER2 and PER3. A decrease in the expression of hTIM brings about a drastic decrease in the levels of the expression of the aforementioned canonical genes. The hTIM protein also exhibits a circadian regulation of expression. Interestingly, its closest phylogenetic relatives are cell-cycle related proteins and the hTIM protein itself has been shown to play an integral role in two cell cycle checkpoints: G2/M and intra-S checkpoints. A null mutation in the Timeless protein ortholog resulted in embryonic lethality in C. elegans and mice. The hTIM also has a cell cycle dependent oscillation that is low in G0, G1 phases and high in G2, S1 and M, with the highest expression occurring in S1 phase.

CRY2 Cryptochrome 2 (photolyase-like), also known asCRY2, is a human gene.

hTIM mediates the reaction between two proteins which subsequently results in an arrested phase in the cell cycle that allows DNA repair to occur. The G2/M checkpoint prevents cells from entering mitosis when DNA is damaged; thereby producing an opportunity for DNA repair and stopping the proliferation of damaged cells. HU (hydroxyurea) and UV light are both DNA replication inhibitors and when cells are exposed to either chemical, they produce breaks in the double stranded DNA. Within the nucleus there is a protein kinase called ATR (Ataxia-Telangiectasia mutated and Rad 3 related) that's recognizes damaged DNA. hTIM interacts with a subunit on the ATR known as ATRIP to bring about the phosphorylation of Checkpoint kinase protein 1 (Chk1). Chk1 is required for normal cell proliferation and survival. When this protein is phosphorylated, cell cycle is arrested at the G2/M phase which allows for DNA repair, or if the damage is too extensive, apoptosis. Hence, hTIM acts as an important mediator between ATR and Chk1 and helps to prevent the proliferation of cells with damaged DNA.
hTIM protein plays an essential yet undetermined role in the intra-S checkpoint system. In the intra-S checkpoint, stalled replication forks after dNTP pool depletion or DNA damage activates a signal transduction pathway that inhibits the firing of new origins of replications on DNA strand elsewhere. This checkpoint is essential in protecting stalled replication forks from pathological rearrangements that can result from unscheduled recombination. When hTIM is downregulated, the intra-S checkpoint is seriously compromised with continuous firing of replication origins in the presence of replication blocks, which results in unabated DNA synthesis. The timeless protein is thought to directly connect the cell cycle with the circadian rhythm in mammals. In this model called a “direct coupling” the two cycles share a key protein whose expression exhibits a circadian pattern. It should be noted that the circadian cycle operates normally in the absence of the cell cycle, such as the circadian cycle of non-dividing neural, muscle and liver cells.

Clock gene structure

basic Helix Loop Helix (bHLH) PAS family gene

Drosophila

- d Period
- d Timeless

Mammals

- m Clock
- m Per 1-3
- rBMAL1

The sequence of events that occur in certain cells of the Fruitfly that allow them to function as a clock.

Example: circadian clock in mammals

Core Components of the Circadian Clock

Input (e.g. light, neurotransmitters)
- Positive Feedback Loop
- Negative Feedback Loops

Output (e.g. metabolism, function)
Limit-cycle oscillations

- Mutants (long-period, short-period, arrhythmic)
- Entrainment by light-dark cycles
- Phase shift induced by light pulses
- Suppression of oscillations by a light pulse
- Temperature compensation

Goldbeter's 5-variable model

Molecular mechanism of circadian clocks

Example: circadian clock in mammals

Model for the mammalian circadian clock

Suprachiasmatic nucleus, or nuclei, (SCN), a tiny region on the brain's midline in a shallow impression of the optic chiasm, is responsible for controlling endogenous circadian rhythms. The neuronal and hormonal activities it generates regulate many different body functions over a 24-hour period. The SCN, pine cone shaped and smaller than a pea, interacts with many other regions of the brain. It contains several cell types and several different peptides (including vasopressin and vasoactive intestinal peptide) and neurotransmitters.
Circadian effects

The SCN receives inputs from specialized photoreceptive retinal ganglion cells via the retinohypothalamic tract. Destruction of the SCN leads to a complete loss of circadian rhythm. Rats with damage to the SCN have no circadian rhythms, i.e., they sleep the same total amount, but polyphasiaically for random lengths at a time.

The SCN also controls ‘slave oscillators’ in the peripheral tissues, which exhibit their own ~24 hour rhythms, but are crucially synchronized by the SCN.

The importance of entraining our bodies to an exogenous cue, such as daylight, is reflected by several circadian rhythm sleep disorders, where this process does not function normally.

Neurons in the ventrolateral SCN (vSCN) have the ability for light-induced gene expression. If light is turned on at night, the vSCN relays this information throughout the SCN in a process called entrainment.

Neurons in the dorsomedial SCN (dmsCN) are believed to make an endogenous 24-hour rhythm that can persist under constant darkness in humans averaging about 24h 11 min.

Melanopsin-containing ganglion cells in the retina have a direct connection to the ventrolateral SCN via the retinohypothalamic tract. A GABAergic mechanism couples the ventral and dorsal regions of the SCN.

The SCN sends information to other hypothalamic nuclei and the pineal gland to modulate body temperature and production of hormones such as cortisol and melatonin.
Entrainment (chronobiology)
Entrainment of a circadian system is the alignment of its own period and phase to the period and phase of an external rhythm. A common example is the entrainment of endogenous circadian rhythms, which in mammals are generated by the suprachiasmatic nuclei of the hypothalamus to the daily light-dark cycle. Of the several possible cues, called zeitgeber, German for time giver, synchronizer, which can contribute to entrainment, bright light is by far the most effective.

References
The SCN and circadian rhythms

A. Normal squirrel monkeys kept in a constant light environment.
B. Monkeys with lesions in both SCNs.

Adapted from Edlin et al., 1995

Transplantation of SCN to lesioned animals restores circadian rhythms

In mammals, a master clock is located in the hypothalamic suprachiasmatic nucleus (SCN), which conducts and coordinates the peripheral clocks in each organ and tissue.

The circadian oscillator

Circadian rhythm → Oscillations → Feedback loop

Structure of the SCN with input and output pathways

SCN is composed of single neuronal oscillators.

Multi-electrode array dishes

Slice culture

Dispersed cell culture

Electrode

Spine

Neuron
Continuous monitoring of spontaneous discharges of an SCN neuron

2. Molecular Circadian Clock
mammalian clock gene Clock was found from rhythm mutant mice

Wild type
Clock/Clock

Gene expression rhythms In the SCN

Networks of transcription-translation autorick feedforward loops
Rhythmic Per transcription
Positive elements: Clock, Bmal1 (bHLH-PAS transcription factor)
Negative elements: Per1, Per2, Cry1, Cry2

Rhythmic Bmal1 transcription
Positive elements: ROR, Per2
Negative elements: RevErb α

Binding to RevErb/ROR response element
3. Ontogeny of rat circadian system

Mother-Pup Interaction

Maternal cares are believed to have long-term effects on pups physiology and behavior

Growth
HPA Axis
Biological Clock

Body weight change in the first 2 days of life

Experimental paradigm

Maternal deprivation during the light phase

6 or 3h deprivation

6h
18h
6h

Eye enucleation

start daily maternal deprivation

in situ hybridization

24 h nursing

wean

Behavioral rhythm monitoring

Novelty exposure
Clock gene expression in pups' SCN (postnatal day 6)

**Per1**

- Control
- MD 12h

**Per2**

- Control
- MD 12h

Time of day:
- 8:00
- 12:00
- 20:00

**Per1 and Per2 expression rhythms in the pups' SCN**

**Per1**

- Control
- MD

**Per2**

- Control
- MD

Relative Optical Density [%]

Clock Time (hours)

<table>
<thead>
<tr>
<th>Clock Time (hours)</th>
<th>Control</th>
<th>MD</th>
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12h Maternal Deprivation phase reverses **Per1** Expression Rhythms in the rat SCN (postnatal day 6)

Light (6-18h, mother absent) Dark (18-6h)

Cont.

MD

Time of day (hours)

Control MD

Per1 and Per2 expression rhythms in the HIP and PVN (day 6)

**rPer1**

- Control
- MD

**rPer2**

- Control
- MD

Relative Optical Density [%]

Clock Time (hours)

<table>
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<tr>
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<th>MD</th>
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Behavioral rhythms after weaning

Control

Mother deprived (1-6 days)
Phase reversal of behavioral rhythms by maternal deprivation in the 1st W

Control

MD

Phase of activity offset

Activity onset

Activity offset

Results

1. Phase reversed the circadian Per1,Per2 expression rhythms in the SCN
2. Phase reversed the circadian AVP and GR expression rhythms in the PVN
3. Enhanced the rhythmic CRH expression in the PVN
4. Phase reversed the circadian behavioral rhythms after weaning
5. 24 h maternal nursing in postnatal day did not affect the pups’ clock
6. Even 3 h of maternal deprivation in the early light phase shifted the pups’ clock

Expression of Stress related genes in the PVN of rat pups

Results

1. Phase reversed the circadian Per1,Per2 expression rhythms in the SCN
2. Phase reversed the circadian AVP and GR expression rhythms in the PVN
3. Enhanced the rhythmic CRH expression in the PVN
4. Phase reversed the circadian behavioral rhythms after weaning
5. 24 h maternal nursing in postnatal day did not affect the pups’ clock
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Body weights of pups subjected to different MD conditions

<table>
<thead>
<tr>
<th>MD conditions</th>
<th>Body weight (g)</th>
<th>(mean±SE)</th>
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</thead>
<tbody>
<tr>
<td>MD12 (Warm)</td>
<td>12.34±0.50</td>
<td>48.03±1.18</td>
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<tr>
<td>MD12 (9-12h)</td>
<td>16.91±0.51</td>
<td>52.99±1.09</td>
</tr>
<tr>
<td>MD3 (9-12h)</td>
<td>17.26±0.69</td>
<td>51.35±1.36</td>
</tr>
<tr>
<td>MD3 (15-18h)</td>
<td>18.67±0.56</td>
<td>57.39±2.09</td>
</tr>
<tr>
<td>Control</td>
<td>19.10±0.48</td>
<td>54.42±1.55</td>
</tr>
</tbody>
</table>

*p<0.05 ** p<0.01 as compared with the control
**p<0.01 as compared with the same MD length but with a different temperature.
**Effects of Warming During MD on Stress Sensitivity**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Basal</th>
<th>Stress</th>
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<tr>
<td>Control</td>
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<tr>
<td>MD12h</td>
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<tr>
<td>MD12h Warm</td>
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**Per1 and Per2 expression in the SCN of mother rats**

In mammals, a master clock is located in the hypothalamic suprachiasmatic nucleus (SCN), which conducts and coordinates the peripheral clocks in each organ and tissue.
At the core of the circadian clock is a autoregulatory molecular feedback loop associated with clock gene transcription.

1st three clock genes cloned in of Mammals:
- basic Helix Loop Helix (bHLH) PAS family gene
- rBMAL1
- mClock

~260 AA

m Per 1-3

Q-rich

Circadian firing rhythms in single SCN neurons

Single neuronal rhythm of the SCN

Multi-electrode array disk (MEAD)

Dispersed cell culture on an MEAD

active phase

rest phase

0.2sec

20µV

Time of Day (hours)


Continuous monitoring of spontaneous discharges of an SCN neuron

Synchronized circadian firing rhythms in an organotypic slice culture of the rat SCN

Distribution of circadian periods depend on cell assemblage and tissue organization

Clock genes generate circadian rhythmicity in cells

Clock gene structure
basic Helix Loop Helix (bHLH) PAS family gene

Per1 and Bmal1 expression in the rat brain

Gene expression rhythms in the SCN
Networks of transcription-translation autofeedback loops

**Rhythmic Per transcription**

Positive elements: Clock, Bmal1 (bHLH-PAS transcription factor)
Negative elements: Per1, Per2, Cry1, Cry2

**DECs (Differentiating Embryonic Chondrocyte)** are bHLH proteins

- Dec1: induced by cAMP in human chondrocytes, kinin acid in rat hippocampus, serum starvation in NIH3T3 cells, NGF in PC12 cell, retinoic acid in P19 cell
- Dec2: similar gene to Dec1 sharing many properties with Dec1

Cycling transcripts in the SCN and liver

Dec expression in the rat (LD 12:12, ZT 6)
Dec expression in the rat brain in DD (day3)

Dec1

Dec2

rDec expression rhythms in the brain areas outside the SCN

Input pathway to the Clock

Involvement in the Light Entrainment

Light pulse phase-dependently induces Per1 in the SCN

Phase response curve of rat behavioral rhythm by light pulse
Light responsiveness of Dec1 and Dec2 in the rat SCN

Dec 1 was induced by phase-resetting light within 1 hour.

Roles of Decs in the core feedback loop

Examination using Reporter Construct E54-TK

5' flanking region of mPer1 gene

Roles of Decs in the core feedback loop

Comparison with other negative factors

DEC1
DEC2

Possible mechanisms of DEC action

1. Competitive binding to Per proximal E-boxes.
2. Interaction with CLOCK and/or BMAL1 which bind to E-box.

Specific binding of DEC1 and DEC2 to mPer1 proximal E-box sequence
Yeast two hybrid assay showing DEC interaction with BMAL1

**E-Box**

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CACGTG
```

**Presence of Dec loop interlocked with Per loop**

**Autoregulatory feedback loop of Dec1 expression**

Kawamoto et al. BBRC 2004

**Interlocked multiple molecular feedback loops**

Output signal

- Repression by DEC
  1. Competitive binding with E-box
  2. Interaction with BMAL1

Light entrainment

- Repression by PER and CRY
  1. Interaction with Clock/Bmal1

**Circadian rhythms mPer1 expression of the SCN and other tissues in vitro**

Yamazaki S. et al., Science 288:682,2000
Monitoring of clock gene expression rhythms in tissue explants

Luciferase activity (counts/min)

Time of day (hours)