V4: Circadian rhythms – summary

(1) Look at some previous mini tests (lecture modelling cell fate – SS 2013)

(2) Schein conditions (V1)

(3) Content of minitest #1:

- Lectures V1, V2, V3 (today we will only review V1-V3)

- Papers 1 to 3

Conditions for certification

(1) There will be 6 biweekly **assignments**. Students need to write short essays about topics covered in the lecture and in assigned research papers.

There are three possible grades: excellent, pass, failed. Students need to get a "pass" grade on at least *5* assignments or *3* "pass" and one "excellent" grade.

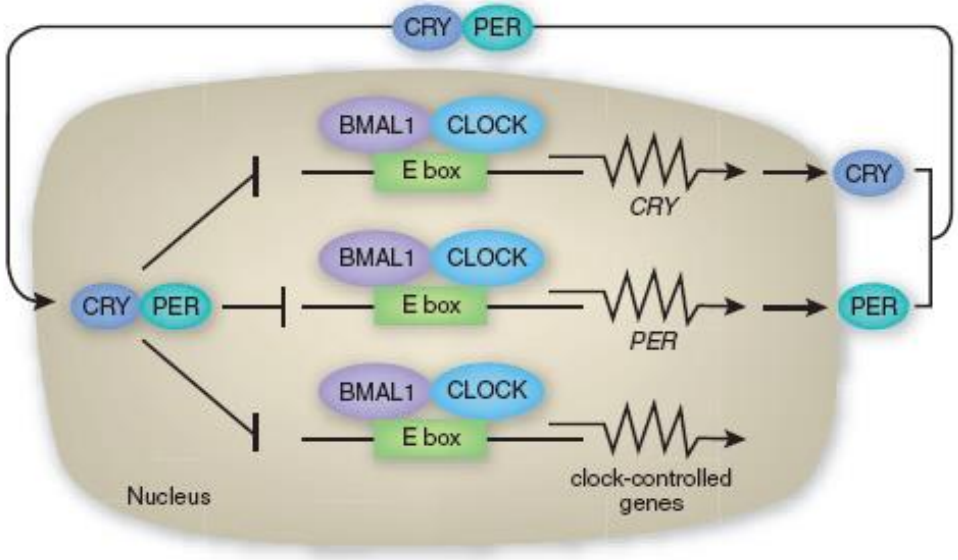
(2) There will be three 45-minutes **tests** on different parts of the lecture.

Students need to pass at least two out of the three tests.

Tests will cover the content of the lecture and of the assigned research papers.

(3) Students need to **present** at least once during the lecture on the content of an assigned research paper (**team work**, 20 min. powerpoint presentation and 10 min. discussion).

(V1) Basic molecular elements of the mammalian clock



This is the **minimal scheme** for the mammalian clock.

It requires several interconnecting transcriptional, translational and post-translational loops to achieve gene expression with circadian periodicity

(a) 2 TFs **CLOCK** and **BMAL1 heterodimerize**.

(b) BMA1:CLOCK binds to the **E-boxes** in the promoters of the *PER* and *CRY* genes, as well as in the clock-controlled genes, activating their transcription.

(c) Once translated, the PER and CRY proteins dimerize, enter the nucleus and **inhibit** CLOCK-BMAL1–activated transcription.

Sancar,
Nat. Struct. Mol. Biol. 15, 23 (2008)

Full (?) circuit of circadian rhythms in mammals

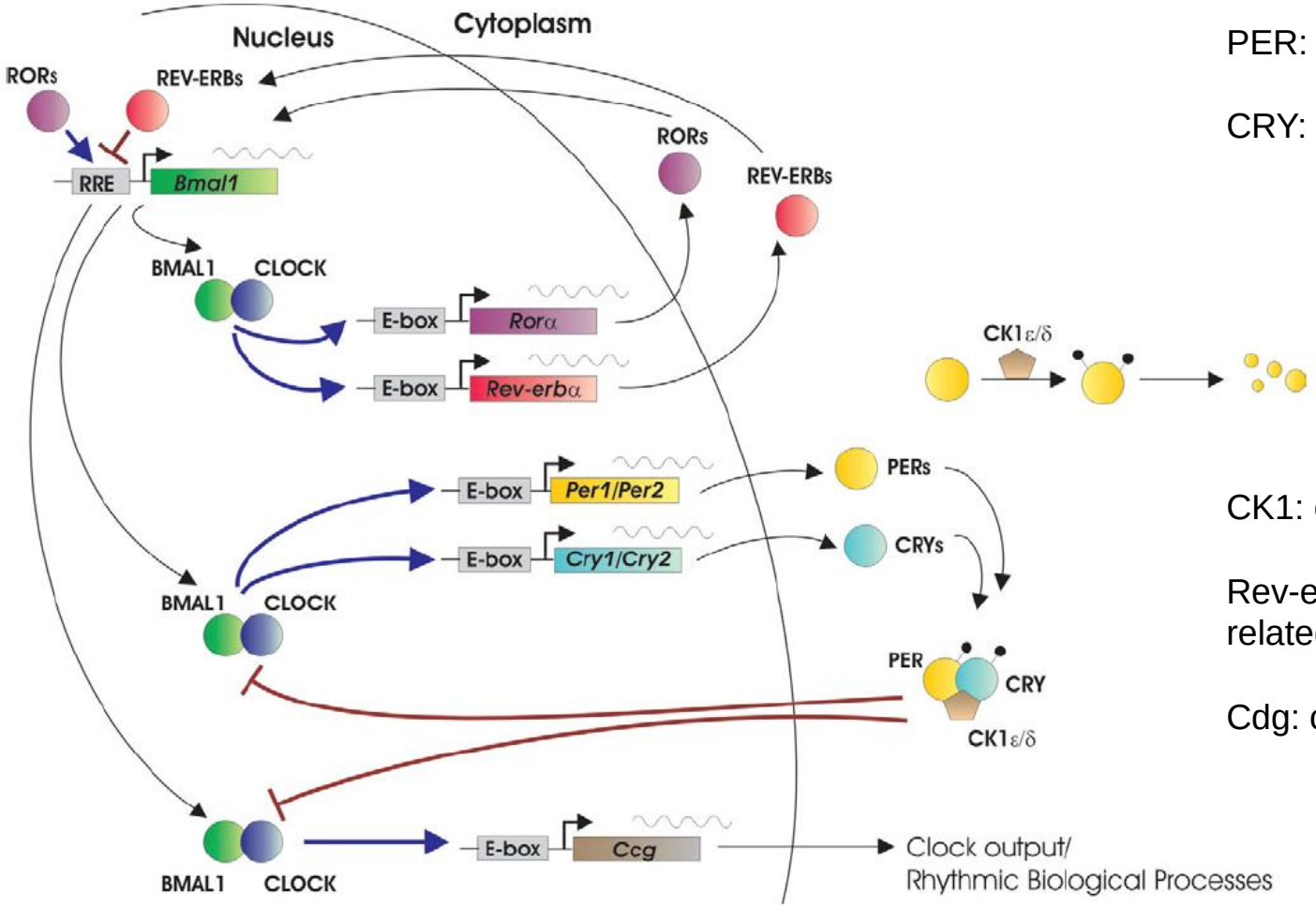


Figure 1. A network of transcriptional–translational feedback loops constitutes the mammalian circadian clock.

PER: period

CRY: cryptochrome

CK1: casein kinase

Rev-erb, ROR: retinoic acid-related orphan nuclear receptors

Cdg: clock-controlled gene(s)

Ko & Takahashi Hum Mol Genet 15, R271 (2006)

Detect unknown control mechanisms: Probe gene expression by microarrays

Harmer *et al.* used oligonucleotide-based arrays to determine steady-state mRNA levels in *Arabidopsis* at 4-hour intervals during the subjective day and night.

→ identify temporal patterns of gene expression in *Arabidopsis* plants under constant light conditions using GeneChip arrays representing about 8200 different genes.

Score all genes whether their expression is correlated with a **cosine** test wave with a period between 20 and 28 hours (probable correlation > 95%)
→ consider those genes as circadian-regulated.

→ 453 genes (6% of the genes on the chip) were classified as **cycling**.

Harmer et al. Science 290, 2110 (2000)

(V2) Noble prize in physiology or medicine 2017

During the 1970's, Seymour Benzer and his student Ronald Konopka asked whether it would be possible to identify genes that control the circadian rhythm in fruit flies. They demonstrated that mutations in an unknown gene disrupted the circadian clock of flies. They named this gene *period*. But how could this gene influence the circadian rhythm?

In 1984, Jeffrey Hall and Michael Rosbash, working in close collaboration at Brandeis University in Boston, and Michael Young at the Rockefeller University in New York, succeeded in isolating the *period* gene.

Jeffrey Hall and Michael Rosbash then went on to discover that PER, the protein encoded by *period,* accumulated during the night and was degraded during the day. Thus, PER protein levels oscillate over a 24-hour cycle, in synchrony with the circadian rhythm.

https://www.nobelprize.org/nobel_prizes

Noble prize in physiology or medicine 2017

The next key goal was to understand how such circadian oscillations could be generated and sustained. Jeffrey Hall and Michael Rosbash hypothesized that the PER protein blocked the activity of the *period* gene. They reasoned that by an inhibitory feedback loop, PER protein could prevent its own synthesis and thereby regulate its own level in a continuous, cyclic rhythm.

The model was tantalizing, but a few pieces of the puzzle were missing. To block the activity of the *period* gene, PER protein, which is produced in the cytoplasm, would have to reach the cell nucleus, where the genetic material is located. Jeffrey Hall and Michael Rosbash had shown that PER protein builds up in the nucleus during night, but how did it get there?

In 1994 Michael Young discovered a second clock gene, *timeless*, encoding the TIM protein that was required for a normal circadian rhythm. In elegant work, he showed that when TIM bound to PER, the two proteins were able to enter the cell nucleus where they blocked *period* gene activity to close the inhibitory feedback loop.

https://www.nobelprize.org/nobel_prizes

Effect of sleep duration on humans?

30% of civilian adults in the US sleep less than 6 hours per day …

However, **short sleep** duration (< 6 hours/day) has been associated with **negative health outcomes**!

Short sleep increases: overall mortality, obesity, diabetes, cardiovascular diseases …

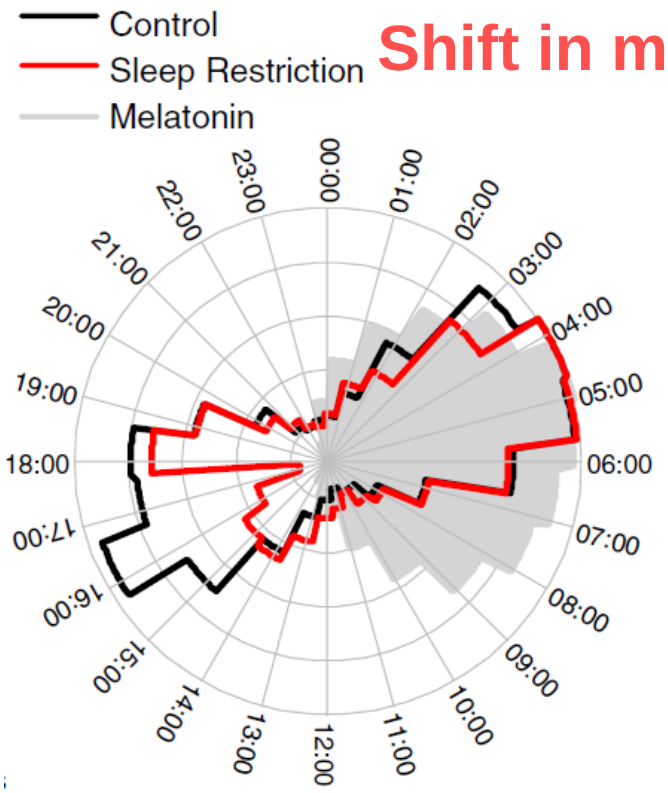
→ What happens on the molecular level?

Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome

PNAS (2013) 110, E1132-E1141

Carla S. Möller-Levet[1], Simon N. Archer[1], Giselda Bucca[1], Emma E. Laing, Ana Slak, Renata Kabiljo, June C. Y. Lo, Nayantara Santhi, Malcolm von Schantz, Colin P. Smith[1], and Derk-Jan Dijk[1,2]

Shift in melatonin-aligned peak times



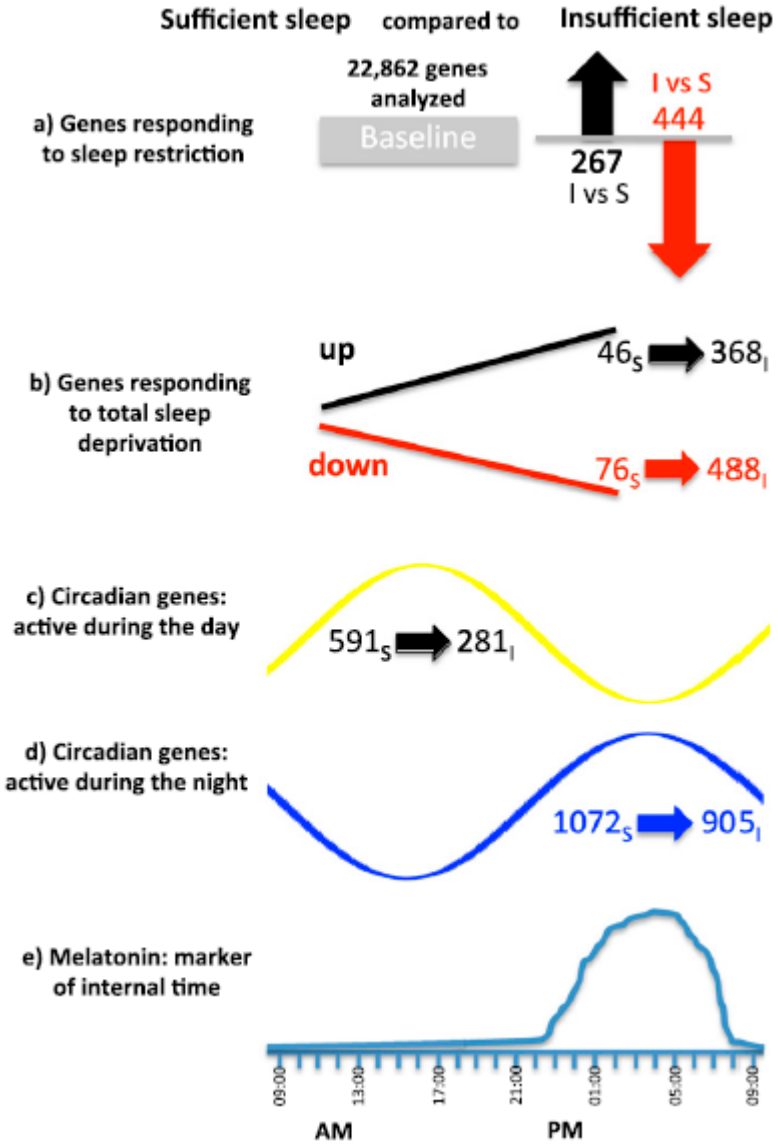
Phase histogram of melatonin-aligned peak times of prevalent circadian genes following and sleep restriction.

Clear reduction (> 50%) of the # of genes that peak during day time!

Genes with **night peaks** (control) are enriched in GO terms for:
- gene expression,
- RNA metabolic processes,
- cellular metabolic processes

Genes with **day peaks** (control) are enriched in:
- response to hormone and stress,
- inflammatory,
- immune and defense response,
- interleukin and cytokine activity.

Summary of results



Processes

Response to stress, response to oxidative stress, response to reactive oxygen species

Chromatin organization and modification, gene expression, RNA binding, nucleic acid binding and metabolism, cellular macromolecule metabolism

IL6 signaling, response to wounding, response to external stimuli, inflammatory response, phagocytosis

Chromosome organization, nucleic acid binding and metabolism, RNA processing, gene expression, protein binding, protein transport, cellular macromolecule metabolism

Hormone response, inflammatory response, immune response, defense response, stress response, interleukin & cytokine activity, protein dimerization

Gene expression, nucleic acid binding, RNA metabolism, cellular metabolism

Next paper (2) for you …

A circadian gene expression atlas in mammals: Implications for biology and medicine

Ray Zhang[a,1], Nicholas F. Lahens[a,1], Heather I. Ballance[a], Michael E. Hughes[b,2], and John B. Hogenesch[a,2]

[a]Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; and [b]Department of Biology, University of Missouri, St. Louis, MO 63121

Proc Natl Acad Sci USA (2014) 111:16219-24

Introduction: 3 paragraphs

(1) What are circadian rhythms? Biological/medical relevance

(2) Previous work, only single organs analyzed – here: profiling of 12 organs.

(3) What has been achieved in this study?

Methods section:

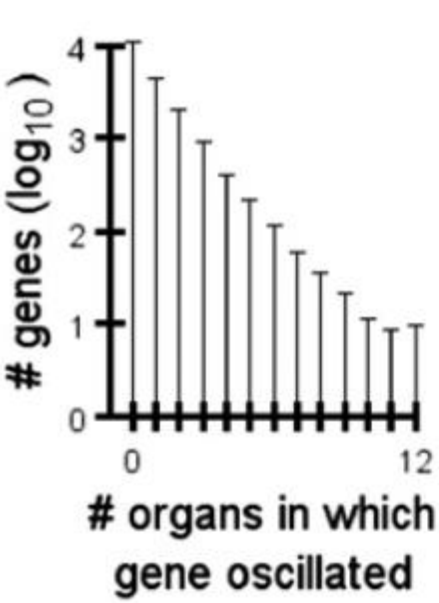
(1) Animal Preparation and Organ Collection

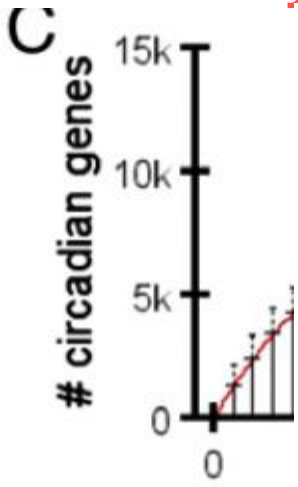
(2) Microarray Data

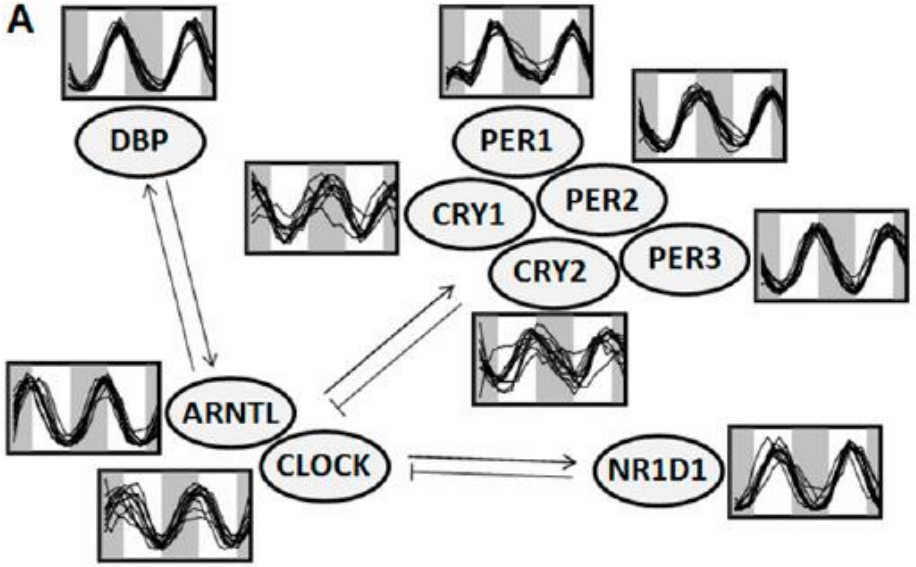
(3) RNA-seq Data

(4) Oscillation Detection

Globally oscillating genes







Only 10 genes oscillated in all organs:

Arntl, Dbp, Nr1d1, Nr1d2, Per1, Per2, and *Per3* (core clock factors – **as expected**), and *Usp2*, *Tsc22d3*, and *Tspan4*.

Usp2 - Ubiquitin carboxyl-terminal hydrolase 2

Tsc22d3 - TSC22 domain family protein 3

Tspan4 - The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family.

(V3) Circadian rhythms are coupled to metabolism

cAMP-Dependent Signaling as a Core Component of the Mammalian Circadian Pacemaker

John S. O'Neill,[1]* Elizabeth S. Maywood,[1] Johanna E. Chesham,[1] Joseph S. Takahashi,[2] Michael H. Hastings[1]†

O'Neill et al.
Science, 320, 949 (2008)

Review:

The suprachiasmatic nuclei (SCN) of the hypothalamus are the principal **circadian pacemaker** in mammals,

They drive the sleepwake cycle and coordinate peripheral clocks in other tissues.

Current understanding:

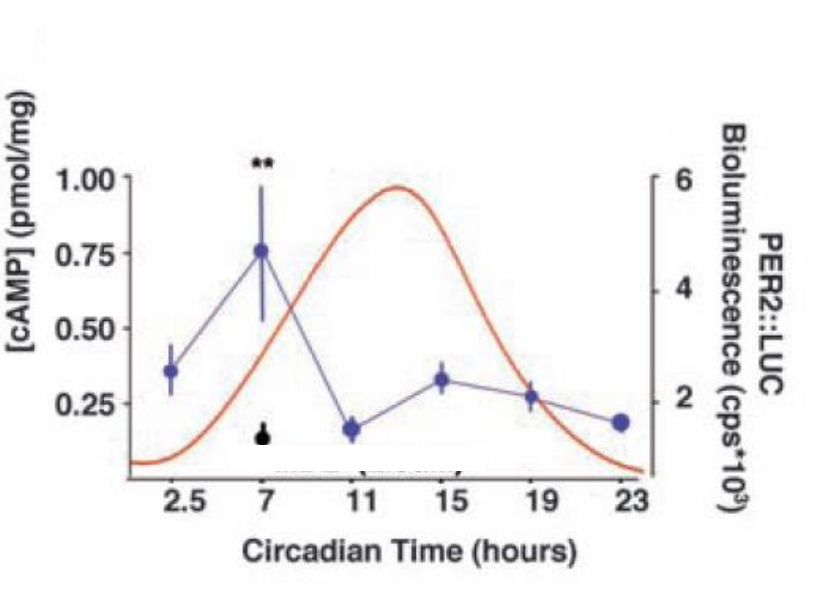
The molecular clockwork within the SCN is being modeled as a combination of **transcriptional** and **posttranslational negative feedback loops**.

Protein products of *Period* and *Cryptochrome* genes periodically suppress their own expression.

Cyclic cAMP levels in mouse brain

The molecular oscillations of the SCN were tracked as circadian emission of bioluminescence by organo-typical slices from transgenic mouse brain.

Picture: a fusion protein of mPER2 and LUCIFERASE (mPER2::LUC) reported circadian protein synthesis rhythms.



Circadian oscillation of cAMP concentration (blue) and PER2::LUC bioluminescence (red).

Interpretation: Under these conditions, the cAMP content of the SCN was circadian.

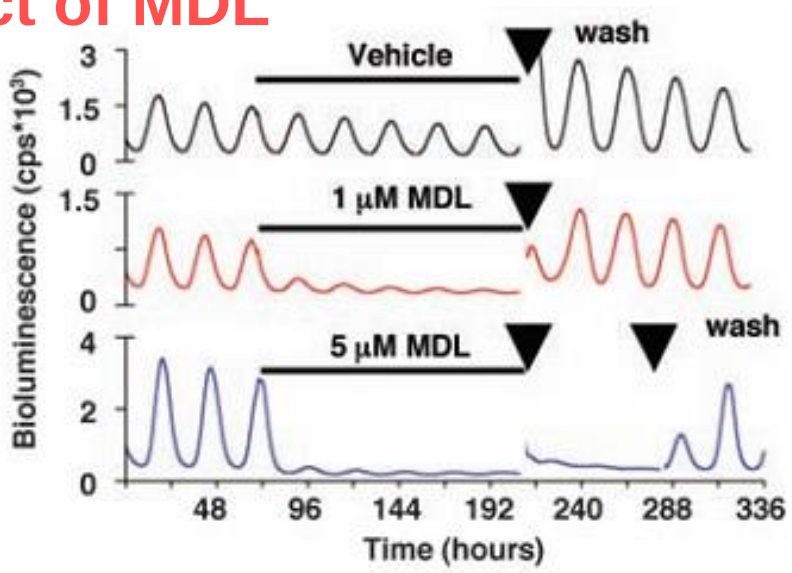
O'Neill et al.
Science, 320, 949 (2008)

Effect of MDL

Idea: can one show that cAMP is the reason for the oscillations?

Realization: need to suppress cAMP-production in the cell.

Experiment: treat SCN slices with MDL, a potent, irreversible inhibitor of the enzyme adenylyl cyclase (that synthesizes cAMP) to reduce concentrations of cAMP to basal levels.



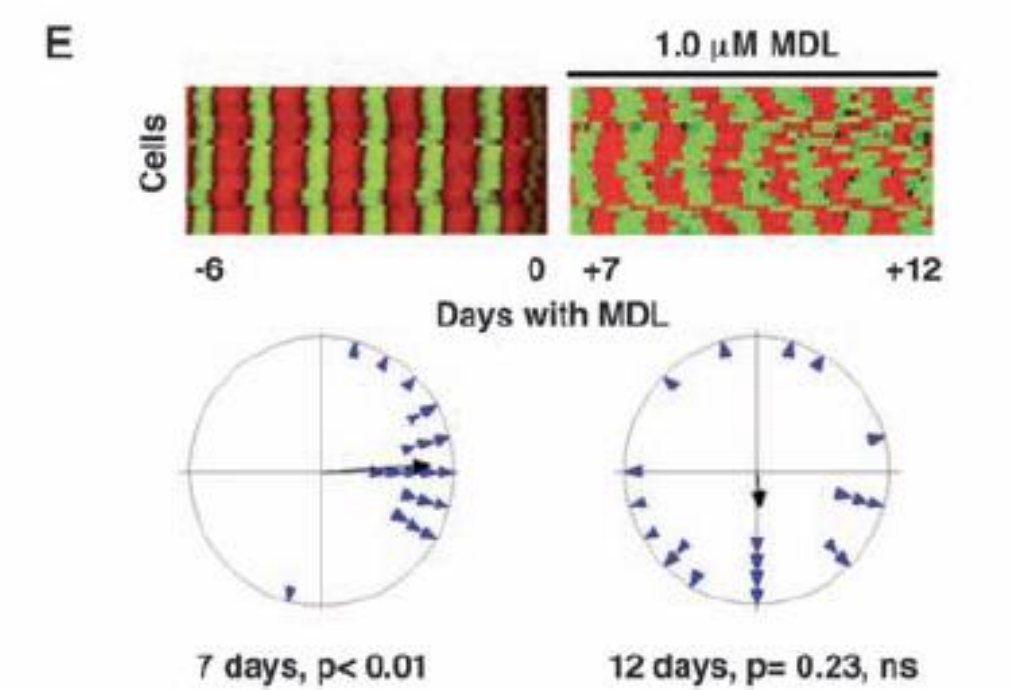
Interpretation: MDL rapidly suppressed circadian CRE:luciferase activity, presumably through loss of cAMP-dependent activation of CRE sequences.

This caused a dose-dependent **decrease** in the **amplitude** of cycles of circadian transcription and protein synthesis observed with mPer1::luciferase and mPER2::LUC.

O'Neill et al.
Science, 320, 949 (2008)

MDL also affects the synchronization of the clock

Prolonged exposure to mild levels of MDL (1.0 μM) suppressed and desynchro-nized the transcriptional cycles of SCN cells.



O'Neill et al.
Science, 320, 949 (2008)

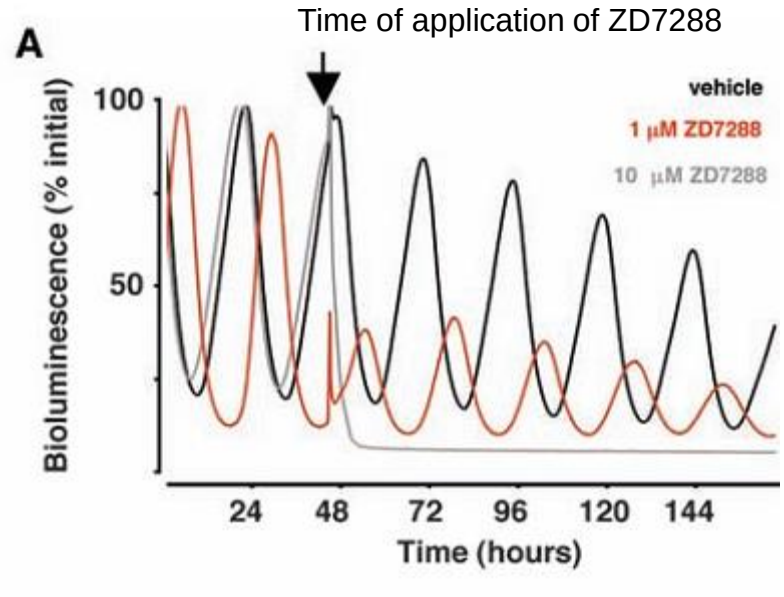
Can one block cAMP action?

Idea: If cAMP sustains the clock, interference with cAMP effectors should compromise pacemaking.

PlanA: treat brain slices with inhibitors of cAMP-dependent protein kinase. This had no effect, however, on circadian gene expression in the SCN.

PlanB: But cAMP also acts through hyperpolarizing cyclic nucleotide–gated ion (HCN) channels and through the guanine nucleotide–exchange factors Epac1 and Epac2 (Epac: exchange protein directly activated by cAMP).

O'Neill et al.
Science, 320, 949 (2008)



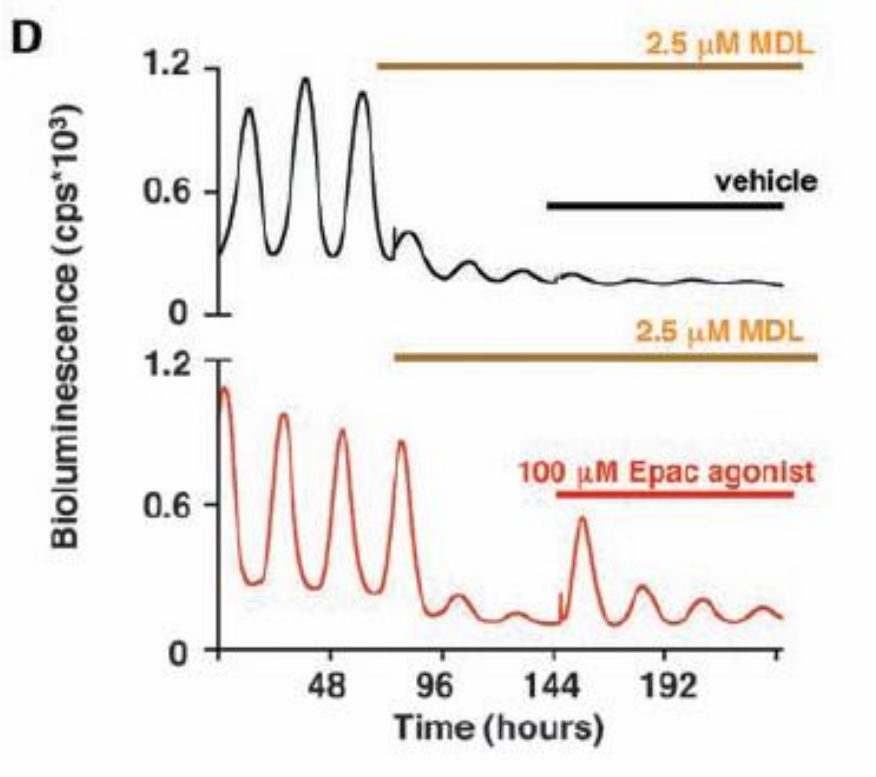
The irreversible HCN channel blocker ZD7288, which would be expected to hyperpolarize the neuronal membrane, dose-dependently damped circadian gene expression in the SCN.
This is consistent with disruption of transcriptional feedback rhythms.

Can cAMP stimulation be recoved?

Experimentalists typically interrupt a cellular process and then restore it by a side-process.

Idea: **Direct activation** of the **effectors** might compensate for inactivation of adenylate cyclase by MDL.

Observation: A hydrolysis-resistant Epac agonist (bottom plot) transiently activated oscillations in transcriptional activity in SCN treated with MDL.

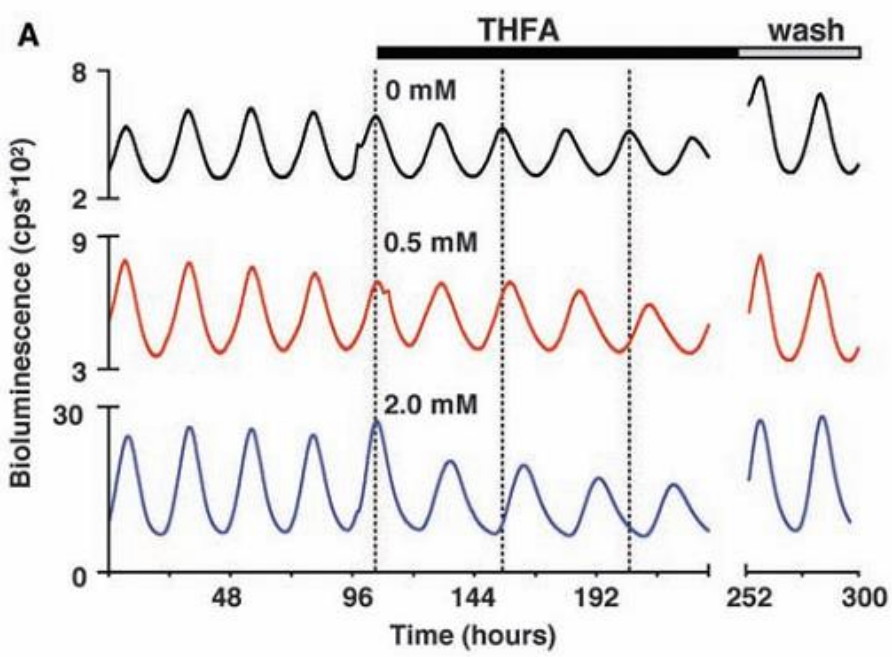


O'Neill et al.
Science, 320, 949 (2008)

slowing cAMP synthesis

Idea: if cAMP signaling is an integral component of the SCN pacemaker, altering the rate of cAMP synthesis should affect circadian period.

Experiment: 9-(tetrahydro-2-furyl)-adenine (THFA) is a noncompetitive inhibitor of adenylate cyclase that slows the rate of G_s-stimulated cAMP synthesis, which attenuates peak concentrations.



Interpretation: THFA dose-dependently increased the period of circadian pacemaking in the SCN, from 24 to 31 hours, with rapid reversal upon washout

O'Neill et al.
Science, 320, 949 (2008)

Circadian regulation of epigenetic chromatin

Circadian Regulator CLOCK Is a Histone Acetyltransferase

Doi, Hirayama, Sassone-Corsi,
Cell 125, 497 (2006)

Masao Doi,[1,3] Jun Hirayama,[1,2] and Paolo Sassone-Corsi[1,2,*]
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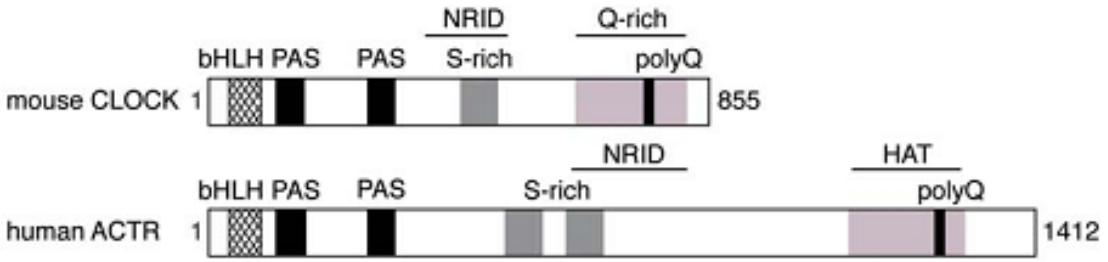
*Contact: paolosc@igbmc.u-strasbg.fr
DOI 10.1016/j.cell.2006.03.033



Schematic representation of the primary structures of mouse CLOCK and human ACTR with common features; a basic helix-loop-helix (bHLH) motif (bind to DNA), Per-Arnt-Sim (PAS) domains, serine-rich (S-rich) regions, a nuclear receptor interaction domain (NRID), a glutamine-rich (Q-rich) region containing a poly-glutamine (polyQ) stretch.
A horizontal line above hACTR indicates **a region known to have HAT activity**.

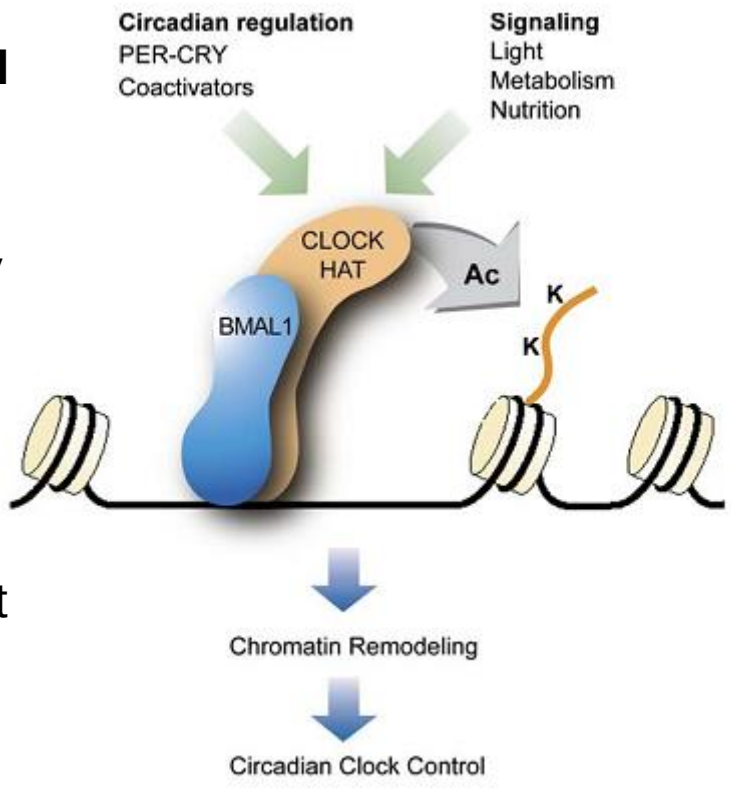
Schematic model

Schematic Model of CLOCK-Mediated Histone Acetylation and Its Role within the Physiological Pathways of Circadian Rhythmicity

The HAT function of CLOCK activity is enhanced by BMAL1, its natural heterodimerization partner, with which it binds to E box promoter elements within clock gene promoters (such as per1).

Acetylation by CLOCK, e.g. at H3 Lys-14, is thought to elicit chromatin remodeling by inducing a transcription-permissive state.

Metabolic, nutritional, and environmental circadian cues likely modulate the HAT function of CLOCK.



Doi, Hirayama, Sassone-Corsi, Cell 125, 497 (2006)

Next paper for V4

An elegant study by Ebert and co-workers (Puram et al., 2016) demonstrates that the core circadian TFs *Bmal1* and *Clock* are required for leukemia stem cell (LSC) growth and selfrenewal, establishing a novel pro-tumorigenic role for circadian clock genes in acute myeloid leukemia (AML).

Core Circadian Clock Genes Regulate Leukemia Stem Cells in AML
Rishi V. Puram, Monika S. Kowalczyk, Carl G. de Boer, ..., Fatima Al-Shahrour, Aviv Regev, Benjamin L. Ebert
Cell 165, 303–316 (2016)