V2: circadian clocks – Noble prize in physiology or medicine 2017

Jeffrey C. Hall
*1945

Michael Roshbash
*1944

Michael W. Young
*1949

„for their discoveries of molecular mechanisms controlling the circadian rhythm”

https://www.nobelprize.org/nobel_prizes

Noble prize in physiology or medicine 2017

During the 1970's, Seymour Benzer and Ronald Konopka tried 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 discovered 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?

https://www.nobelprize.org/nobel_prizes

Noble prize in physiology or medicine 2017

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

Noble prize story of Michael Roshbash

A 50-Year Personal Journey: Location, Gene Expression, and Circadian Rhythms

Michael Rosbash

Howard Hughes Medical Institute, National Center for Behavioral Genomics and Department of Biology, Brandeis University, Waltham, Massachusetts 02454

Correspondence: rosbash@brandeis.edu

"I worked almost exclusively on nucleic acids and gene expression from the age of 19 as an undergraduate until the age of 38 as an associate professor.

Mentors featured prominently in my choice of paths.

My friendship with influential Brandeis colleagues then persuaded me that genetics was an important tool for studying gene expression, and I switched my experimental organism to yeast for this reason.

Several years later, friendship also played a prominent role in my beginning work on circadian rhythms."

Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

Noble prize story of Michael Roshbash

„I graduated from Caltech in 1965 with a BS in Chemistry. There I worked on nucleic acids in the laboratories of Norman Davidson and then Robert Sinsheimer. ….

Then I attended graduate school at Massachusetts Institute of Technology (MIT). Although my PhD from there was officially in biophysics, I worked in the laboratory of Sheldon Penman; he was an ex-physicist turned cell physiologist with an intense interest in the messenger RNA (mRNA) of higher cells.

I then did a 3-year postdoc at the University of Edinburgh in the laboratory of John Bishop, who was a young faculty member in the Department of Epigenetics.

I arrived at Brandeis in the fall of 1974 as a newly minted assistant professor.

I was 30 years old, and 9 years had passed since I graduated from Caltech.

This was a standard trajectory in those days, when graduate work and postdocs were much shorter than they are today.”

Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

Noble prize story of Michael Roshbash

"In "the good old days", many prominent new professor instructors (PIs) had no publications during their postdocs, or their papers were published considerably after they took their first faculty jobs and often without the names of their postdoc mentors.

..

I was **denied tenure** in the Rosenstiel Center, where my laboratory was located in the 1970s and early 1980s. … my laboratory was forced to move to the only available Biology Department space, which was adjacent to Jeff 's laboratory.

… this proximity, including a shared conference room where we had joint laboratory meetings for many years, catalyzed our collaborative efforts.

… I had a serious health crisis in the summer of 1982. … this crisis lowered the energy barrier to making serious changes to my life. They included deciding to work on the cloning of *period* as soon as someone appeared who was interested."

Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

Noble prize story of Michael Roshbash

“I gave the *period* **cloning project** to the second-year graduate student Pranitha Reddy, and this is how my collaborative work with Jeff Hall on circadian rhythms began in the early fall of 1982.

We were locked in an intense battle for primacy with the Young laboratory at Rockefeller for the first few years, and the cloning and rescue of *period* was performed independently in both places.

Mike and his colleagues deserve high marks for their accomplishments. Although unpleasant, the competition contributed to a fast-paced focus, which probably contributed to some of our successes.”

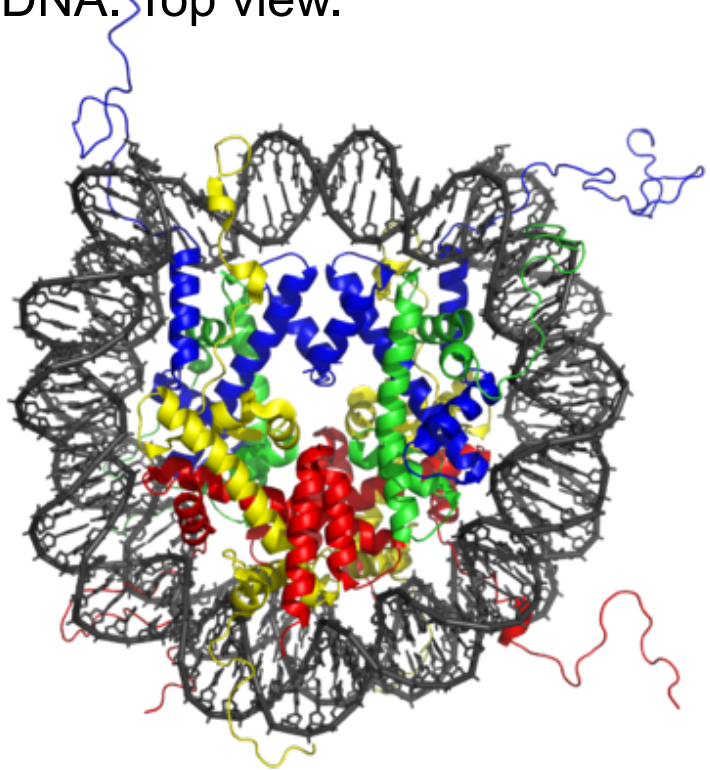
Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a032516 (2017)

The histone code

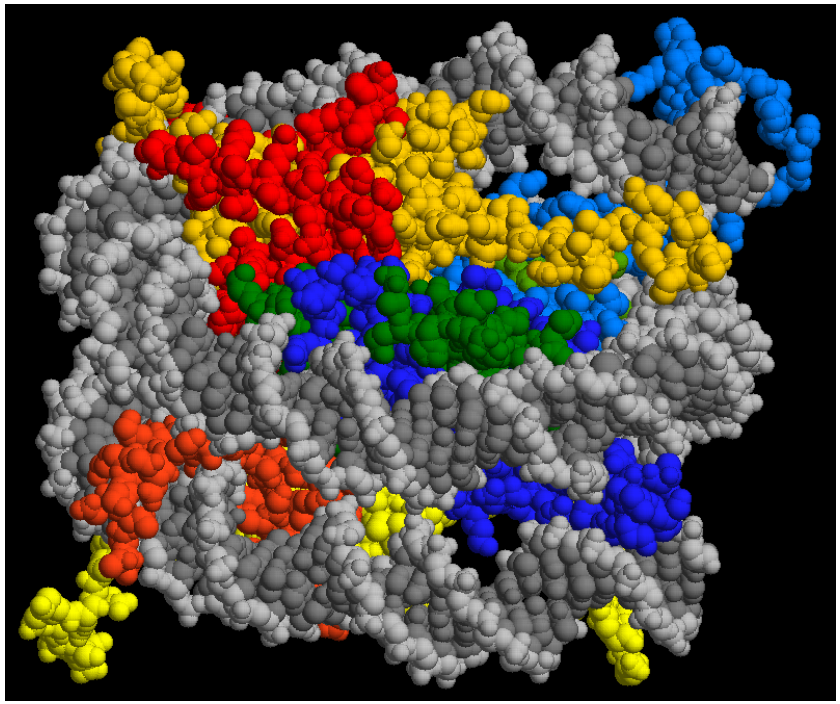
The DNA of eukaryotic organisms is packaged into chromatin, whose basic repeating unit is the **nucleosome**.

A nucleosome is formed by wrapping 147 base pairs of DNA twice around an octamer of four core histones, **H2A**, **H2B**, **H3** and **H4** (2 copies of each one).

X-ray structure of the nucleosome core particle consisting of core histones, and DNA. Top view.



Side view shows two windings of DNA and two histone layers



www.wikipedia.org

Post-translational modifications of histone tails

The disordered histone tails comprise 25-30% of the histone mass.

They extend from the compact histone multimer to provide a platform for various **post-translational modifications (PTMs)**.

These modifications affect the histones' ability to bind DNA and to other histones.

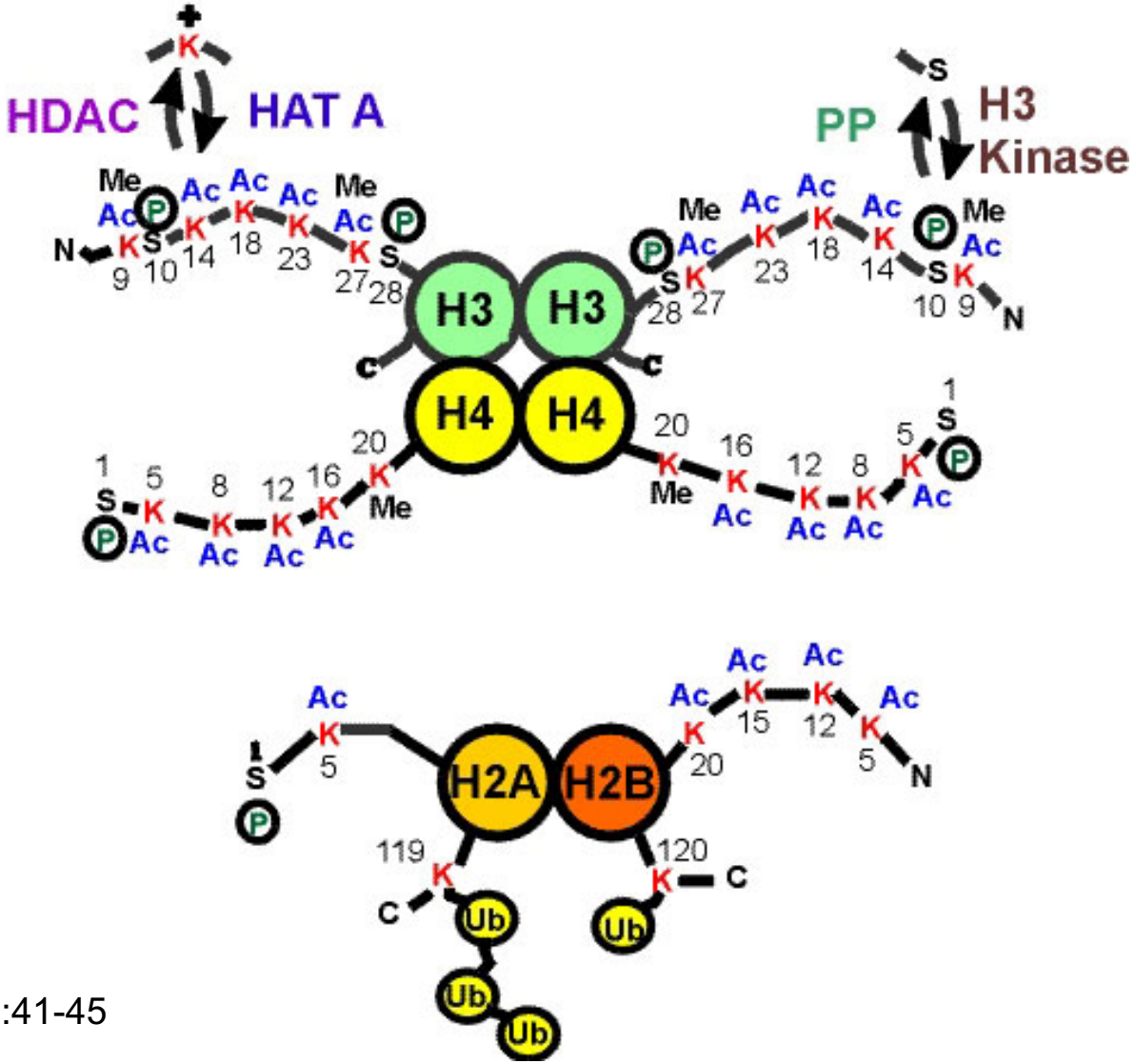
This, in turn, affects **gene expression.**

*ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS**

By V. G. Allfrey, R. Faulkner, and A. E. Mirsky

The Rockefeller Institute

PNAS 1964;51:786
First report on PTMs of histones



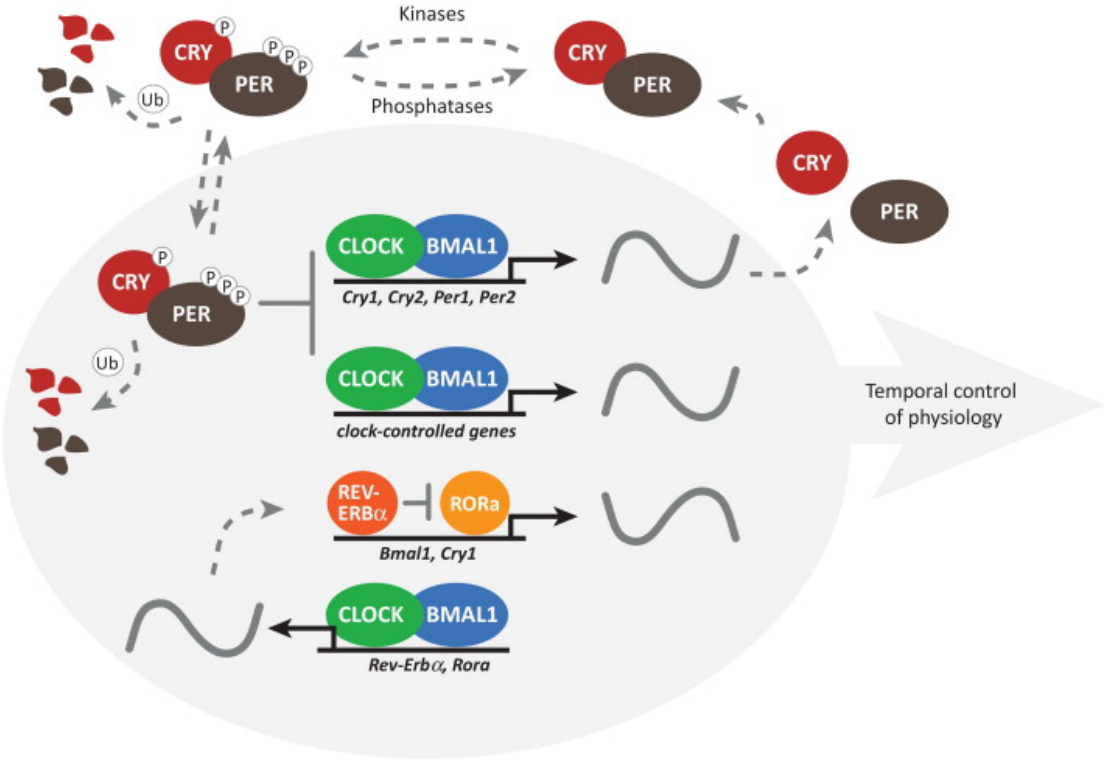
Strahl BD and Allis CD, 2000. Nature 403:41-45

Transcriptional effects of histone marks

Chromatin modification	Writers	Erasers	Location	Function
DNA methylation	DNMT1, DNMT3A and DNMT3B	TET1, TET2 and TET3	CpG dinucleotides	Silencing and others
H3K27me3	PRC2	• UTX1 • JMJD3	CpG-rich promoters and intergenic regions	Silencing
H3K9me2	G9A and GLP	• JMJD2A, JMJD2B, JMJD2C and JMJD2D • JMJD1A, JMJD1B and JMJD1C	Gene bodies, intergenic regions and enhancers	Silencing
H3K4me3	COMPASS-like proteins (SET1, MLL1–MLL2)	• JARID1A, JARID1B, JARID1C and JARID1D • KDM2B	Mainly promoters	Possibly activating
H3K27ac	HATs (including CBP/p300, GNATs and MYSTs)	HDACs and sirtuins	Promoters and enhancers	Activating
H3K4me1	COMPASS-like proteins (MLL3–MLL4)	LSD1 and LSD2	Promoters, enhancers and intergenic regions	Priming and/or activating

Atlasi & Stunnenberg, *Nature Rev Genet* **18**, 643–658 (2017)

Review (V1): The molecular circadian clock in mammals



The cell-autonomous molecular clock in mammals is generated by 2 interlocking transcription/translation feedback loops (TTFL) that function together to produce robust 24 h rhythms of gene expression.

The core TTFL is driven by 4 integral clock proteins:

2 activators (CLOCK and BMAL1) and 2 repressors (PER and CRY), as well as by kinases and phosphatases that regulate the phosphorylation (P) and thereby localization and stability of these integral clock proteins.

BMAL1, brain and muscle ARNT-like 1
CLOCK, circadian locomotor output cycles kaput
CKI: casein kinases I CKIα, CKIδ, and CKIε;
CRY: cryptochrome
PER: period
PP: protein phosphatases PP1, PP5.

Partch et al. Trends Cell Biol 24, 90 (2014)

Core clock proteins interact with chromatin and chromatin-modifying complexes

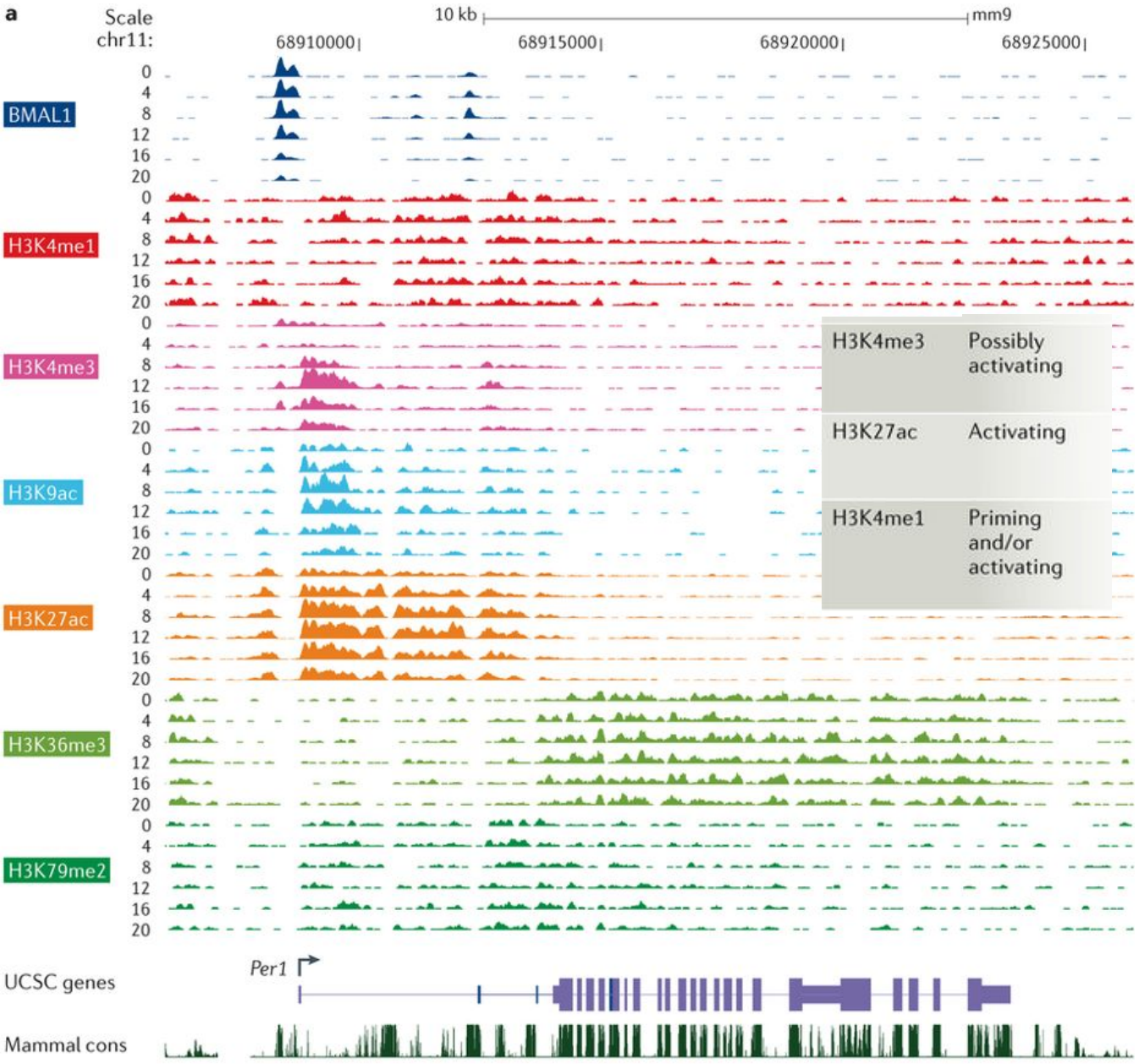
At the beginning of the transcription cycle, the activators CLOCK and BMAL1 interact with the **histone acetyltransferases** (HATs) p300 and CREB-binding protein (CBP), respectively, to acetylate histones and provide an accessible chromatin state for transcription.

CLOCK also has **intrinsic HAT activity** and acetylates histone H3 on Lys9 (H3K9) and Lys14 residues (H3K14).

The NAD^+-dependent histone deacetylase (HDAC) **sirtuin 1** (SIRT1) associates with CLOCK, BMAL1 and PER2, and a circadian rhythm in NAD^+ levels driven by the expression of the CLOCK–BMAL1 target gene *Nampt* in turn leads to a rhythm in SIRT1 activity that feeds back to inhibit the CLOCK–BMAL1 complex.

Takahashi
Nature Rev Genet
18, 164–179 (2017)

Circadian chromatin states in the mouse liver



UCSC genome browser view of histone methylation and acetylation at the *Per1* gene at 6 circadian times (CTs) of the day (0, 4, 8, 12, 16 and 20 hours).

The colours of the wiggle plots of chromatin immunopreci-pitation followed by sequencing (ChIP–seq) signal indicate the following: **BMAL1 occupancy**, **monomethylation of Lys4 at histone H3 (H3K4me1), H3K4me3**, etc.

Takahashi
Nature Rev Genet
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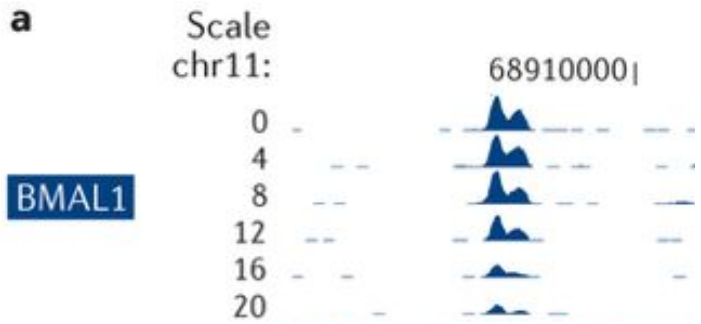
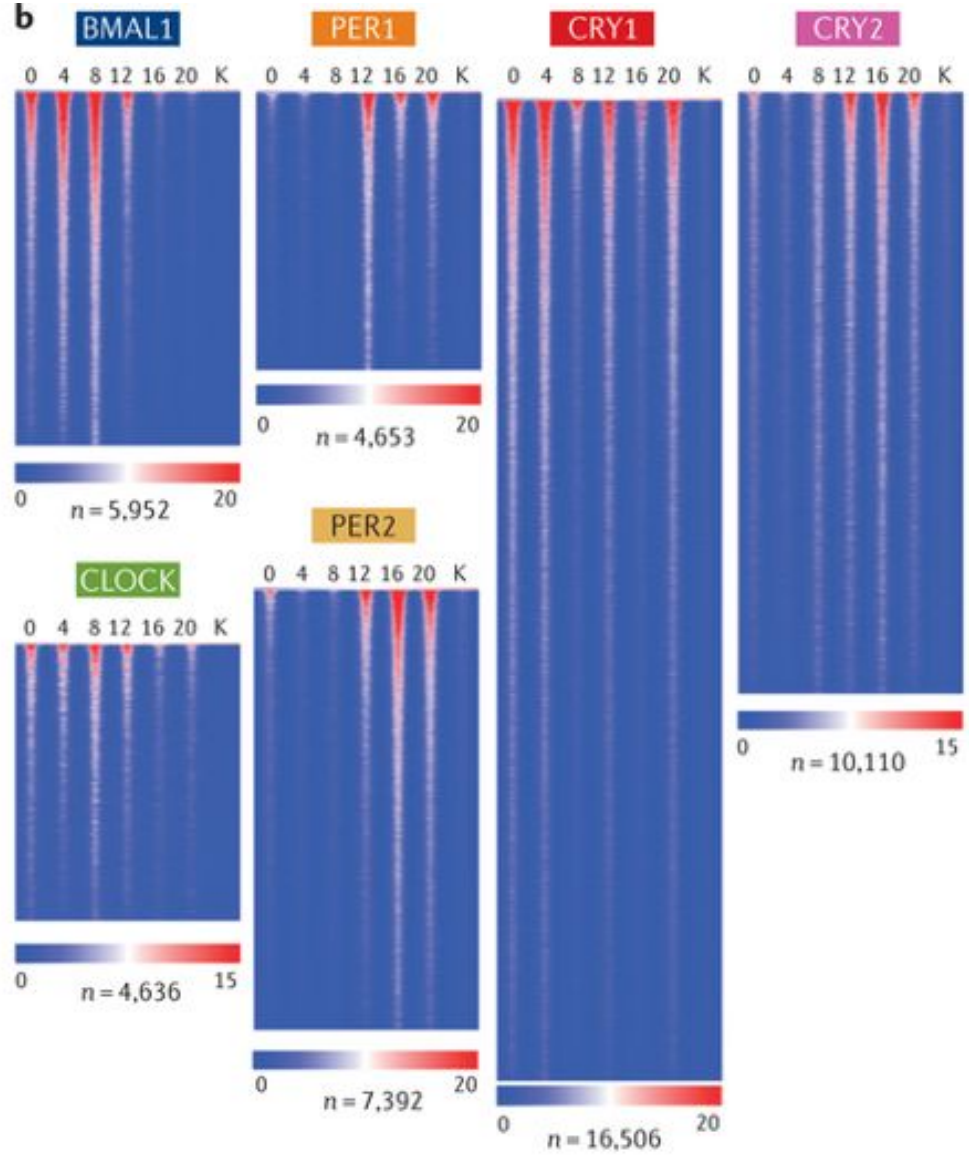
BMAL1:CLOCK activity in the mouse liver



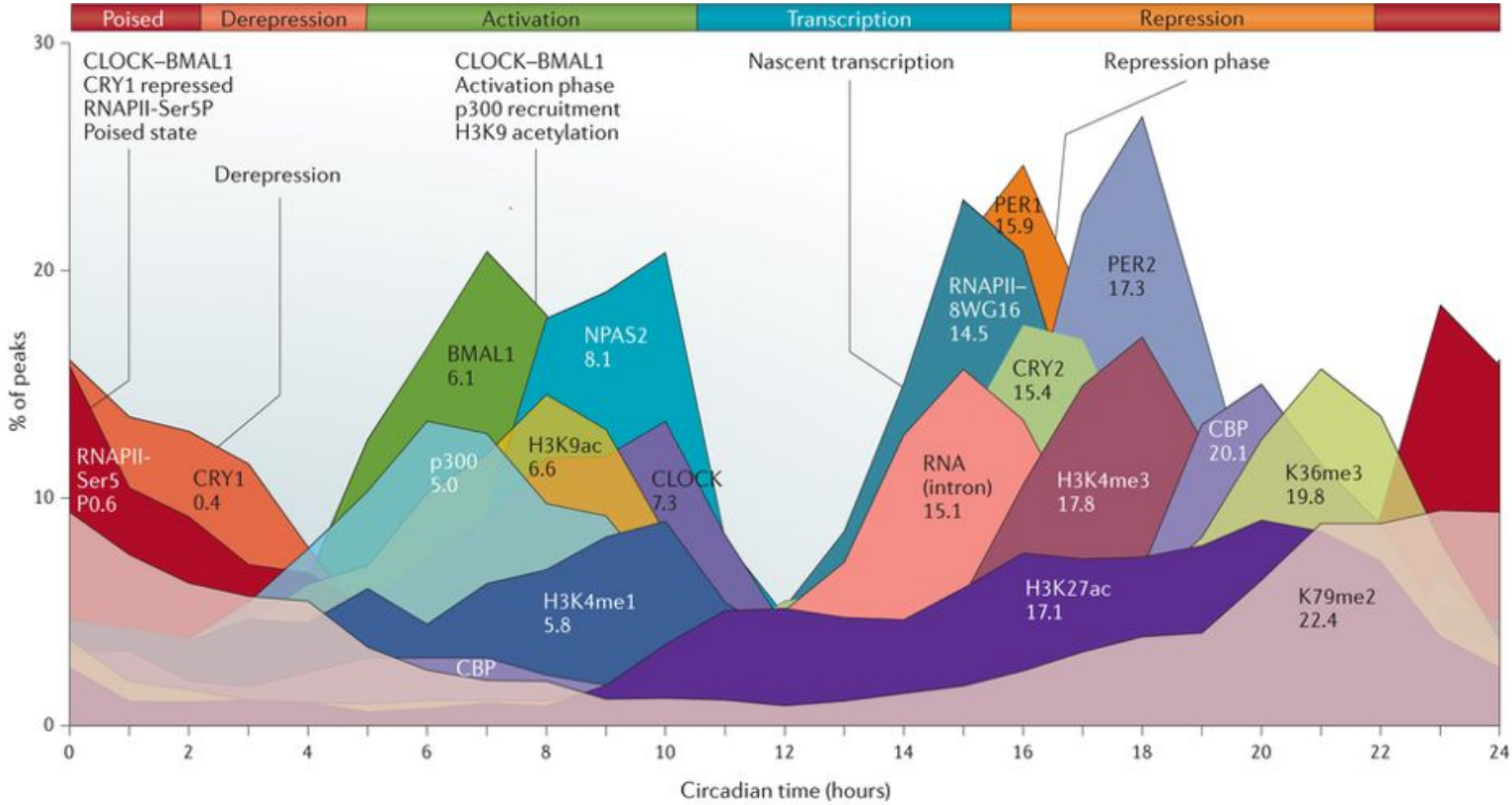
At Per1, the activators BMAl1 and CLOCK bind in a cyclic manner at the promoter between circadian time zero (CT0) and CT12, with maximal binding observed at CT8.

In genome-wide analyses, CLOCK and BMAL1 bind to more than 4,600 and 5,900 sites, respectively, corresponding to ca. 3000 unique genes in the liver.



Takahashi
Nature Rev Genet
18, 164–179 (2017)

Circadian cycle consists of 6 distinctive phases



Histograms showing the phase distributions of each factor as a function of time of day. ac, acetylation; CBP, CREB-binding protein; CRY, cryptochrome; me, methylation; NPAS2, paralogue of CLOCK; PER, period; RNAPII, RNA polymerase II; Ser5P, phosphorylation on Ser5.

Takahashi
Nature Rev Genet
18, 164–179 (2017)

Effect of sleep duration on humans?

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

reasons: work, habits, studies …

Importantly, **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?

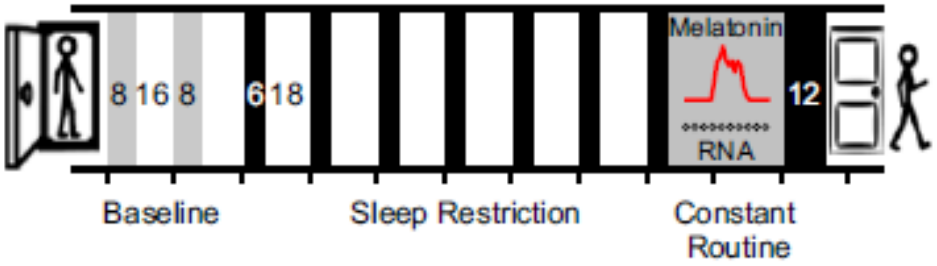
Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome

PNAS (2013) 110, E1132-E1141

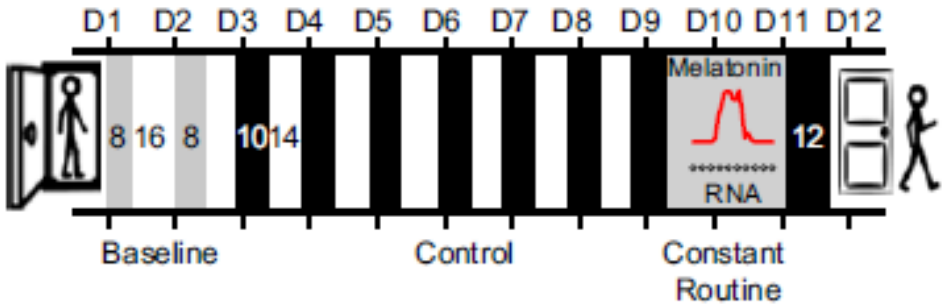
Carla S. Möller-Levet[1], Simon N. Archer[1], Giselda Bucca[1], Emma E. Laing, Ana Slak, Renata Kabiljo, June C. Y. Lo, Nayantara Santhi, Malcolm von Schantz, Colin P. Smith[1], and Derk-Jan Dijk[1,2]

Cross-over design study

26 participants were first put into **sleep-restricted conditions** with 6 hours of sleep opportunity per night (dark bars)



and then into conditions of **sufficient sleep** with 10 hours of sleep opportunity.
-> effects of genetic pre-disposition are mimimized by using „matched samples“



D1 to D12: day 1 to day 12

PNAS 110, E1132 (2013)

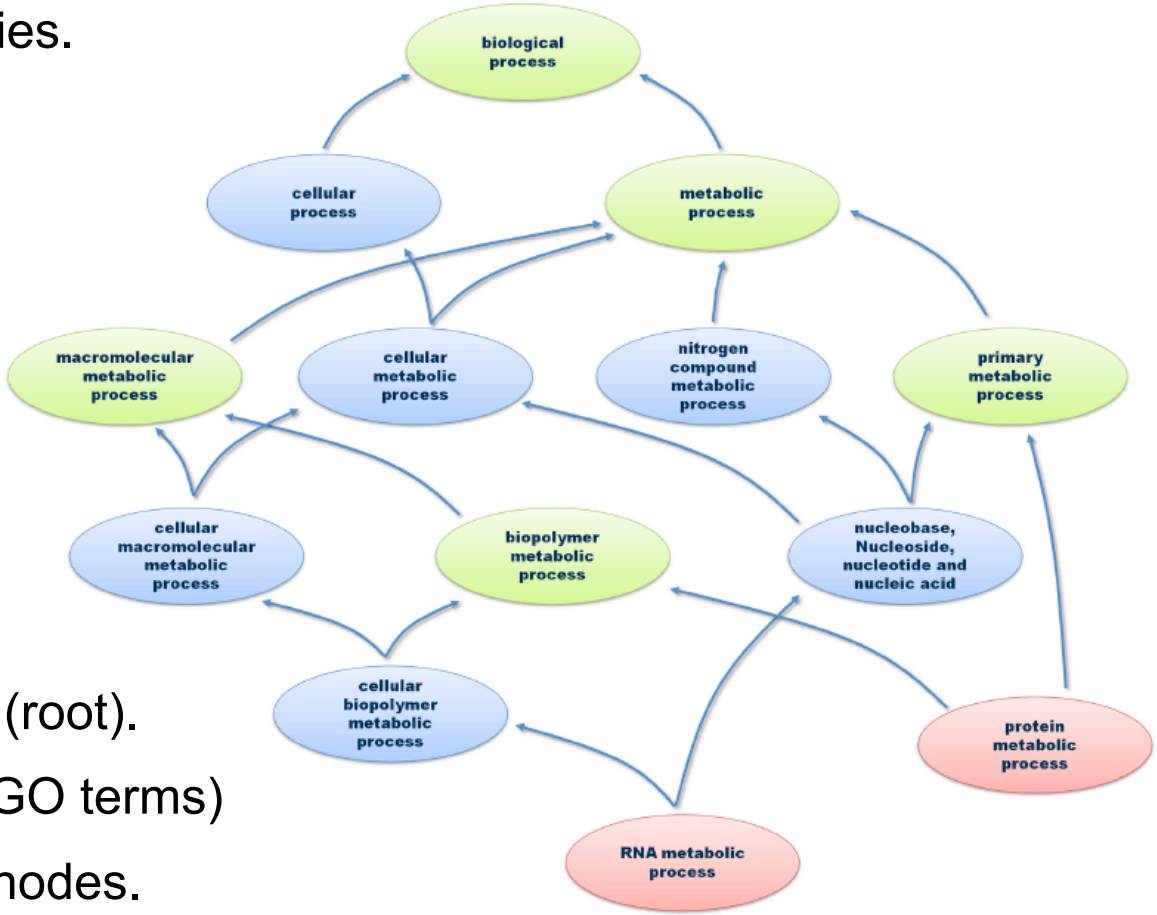
Gene Ontology (GO)

Ontologies are structured vocabularies.

The **Gene Ontology** has 3 tracks:

- biological process (BP)
- molecular function (MF)
- cellular component (lokalisation).

Shown here is a part of the BP tree.



At the top: most general expression (root).

Red: leafs of the tree (very specific GO terms)

Green: common ancestors of 2 red nodes.

Blue: other nodes.

Lines: „Y is contained in X“- relationships

Dissertation Andreas Schlicker (UdS, 2010)

Over-representation analysis (WebGestalt)

Suppose that we have *n* genes in a "**gene set of interest**" (A)
and *m* genes in the **reference gene set** (B).

Suppose further that there are *k* genes in A and *j* genes in B
that belong to a particular functional category (C)
(e.g. a GO category, a KEGG pathway, a BioCarta pathway etc.).

Based on the reference gene set, the expected proportion k_{exp} would be

$$k_{\text{exp}} = (n/m) \times j$$

If *k* exceeds the above expected value,
category C is said to be **enriched**,
with a **ratio of enrichment** (*r*) given by $r = k/k_{\text{exp}}$.

Zhang, Kirov, Snoddy (2013)
Nucl Ac Res *33: W741-W748*

Over-representation analysis (WebGestalt)

If B represents the population from which the genes in A are drawn, WebGestalt uses the **hypergeometric test** to evaluate the significance of enrichment for category C in gene set A,

$$P = \sum_{i=k}^{n} \frac{\binom{m-j}{n-i}\binom{j}{i}}{\binom{m}{n}}$$

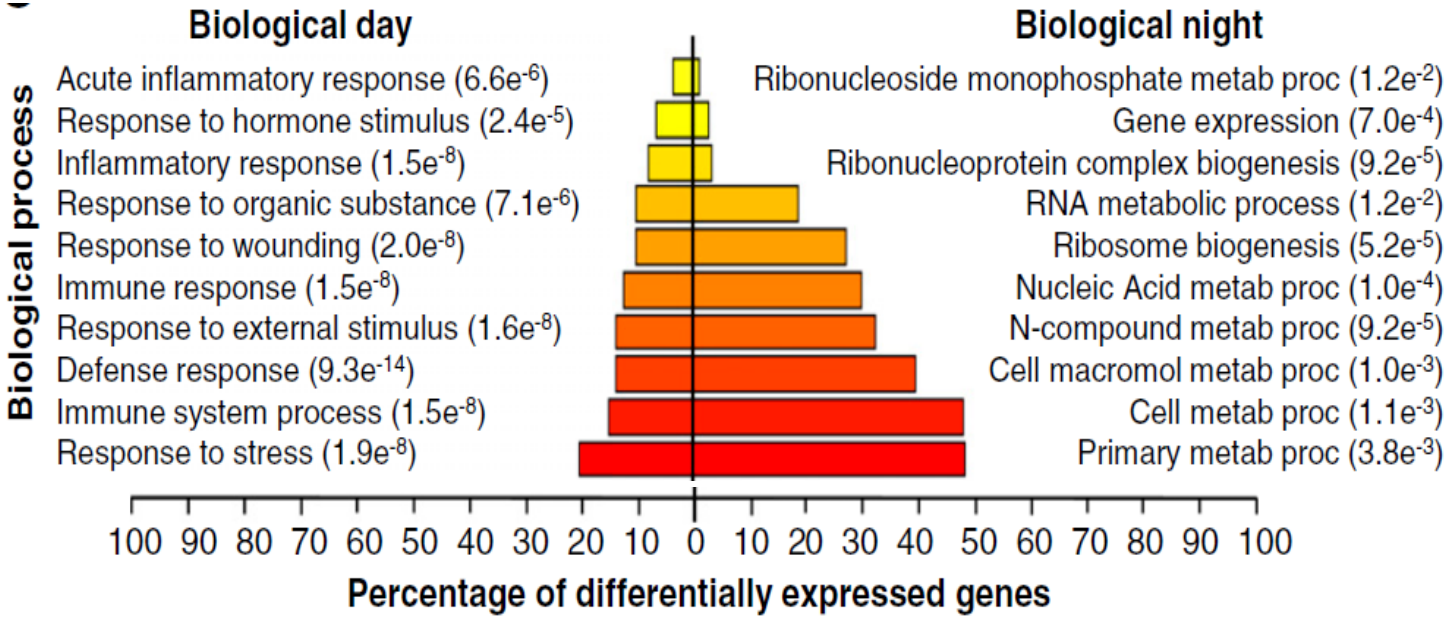
Interpretation: draw $i = k$ genes for A that belong to category C from the j genes from B that belong to C.
→ The other $n - i$ genes in A do not belong to C. They are drawn from the $m - j$ genes in B that do not belong to C.
Normalization is done by the total number of possibilities to draw n genes from m genes.

If A and B are two independent gene sets, WebGestalt uses **Fisher's exact test** instead,

$$P = \sum_{i=k}^{n} \frac{\binom{n}{i}\binom{m}{j+k-i}}{\binom{m+n}{j+k}}$$

Zhang, Kirov, Snoddy (2013)
Nucl Ac Res *33: W741-W748*

Gene functions of „normal“ circadian genes



Top 10 enriched GO BPs within the circadian gene list of the control condition using the human genome as a background

Enrichment p-values are given in brackets.

Immune, defense, stress and inflammatory responses, cytokine receptor activity, IL-1 receptor activity, NF-κB signaling are more prominent during day time.

(Also found for rodents, taking into account that they are night-active).

Night time processes: “normal” maintenance + growth processes …

Effects of sleep deprivation on melatonin (SCN marker)

Melatonin is a hormone that regulates sleep-wake cycles.

On D10 + D11, melatonin peaked significantly **later** after sleep restriction:

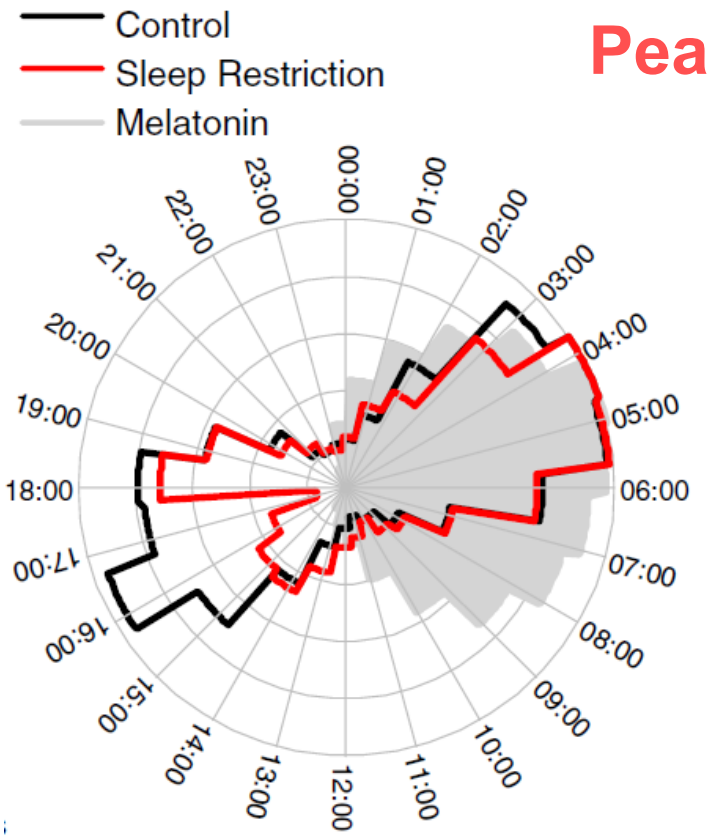
04:15 hours ± 19 min	→	05:01 hours ± 19 min
Control		sleep restriction

Duration of melatonin secretion was **insignificantly shortened:**

9:53 ± 12 min	→	9:35 ± 11 min

PNAS 110, E1132 (2013)

Peak times of expression

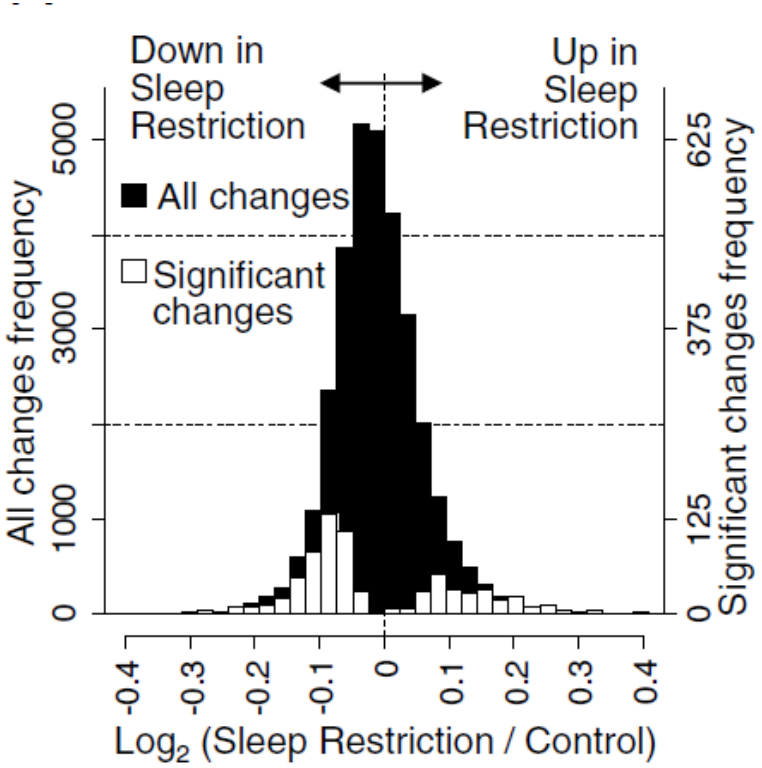


Shown are phase histogram of the peak times of prevalent circadian genes following sleep restriction or control.

The profiles of different individuals are aligned by their personal melatonin peaks.

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

Global overview: changes open sleep deprivation



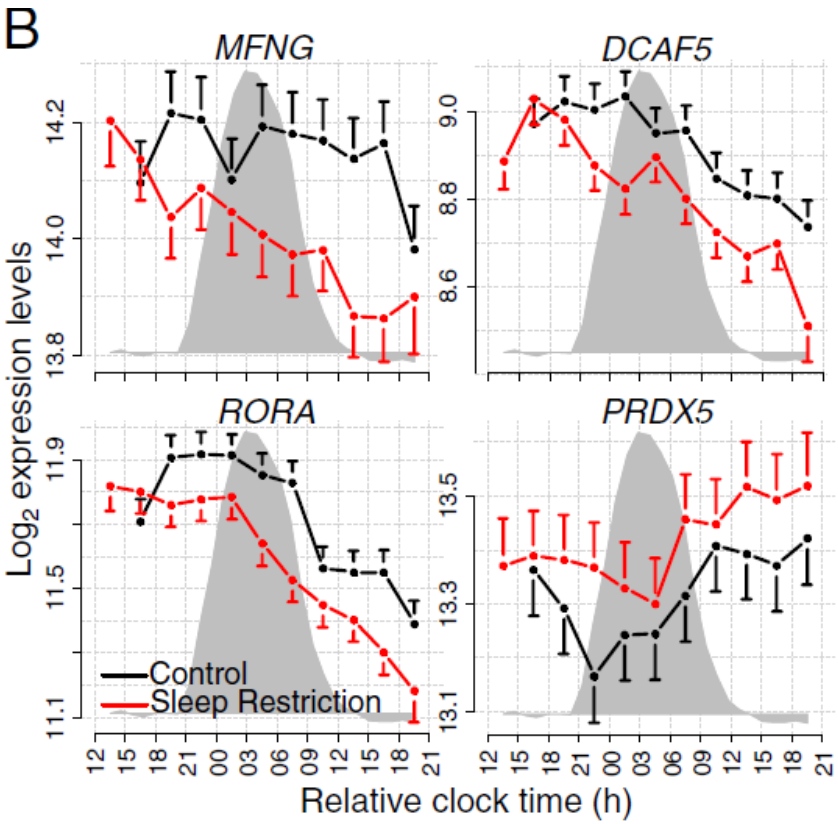
Frequency distribution of expression fold-changes after sleep restriction relative to control.
Filled area: Histogram of changes in all transcripts (31,685 probes that target 22,862 genes)

Open area: changes in transcripts identified as having a statistically significant (multiplicity corrected p-value < 0.05) main effect of sleep condition
(744 transcripts that target 711 genes).

444 genes are **down-regulated** upon sleep restriction (including the circadian rhythm related genes RORA, IL6, PER2, PER3, TIMELESS, CAMK2D)

267 genes are **up-regulated** (including several circadian-rhythm related genes)

Examples of genes with significant effect of Sleep Condition



Most affected genes: $p < 10^{-6}$

MFNG: O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase

DCAF5: is a protein-coding gene …

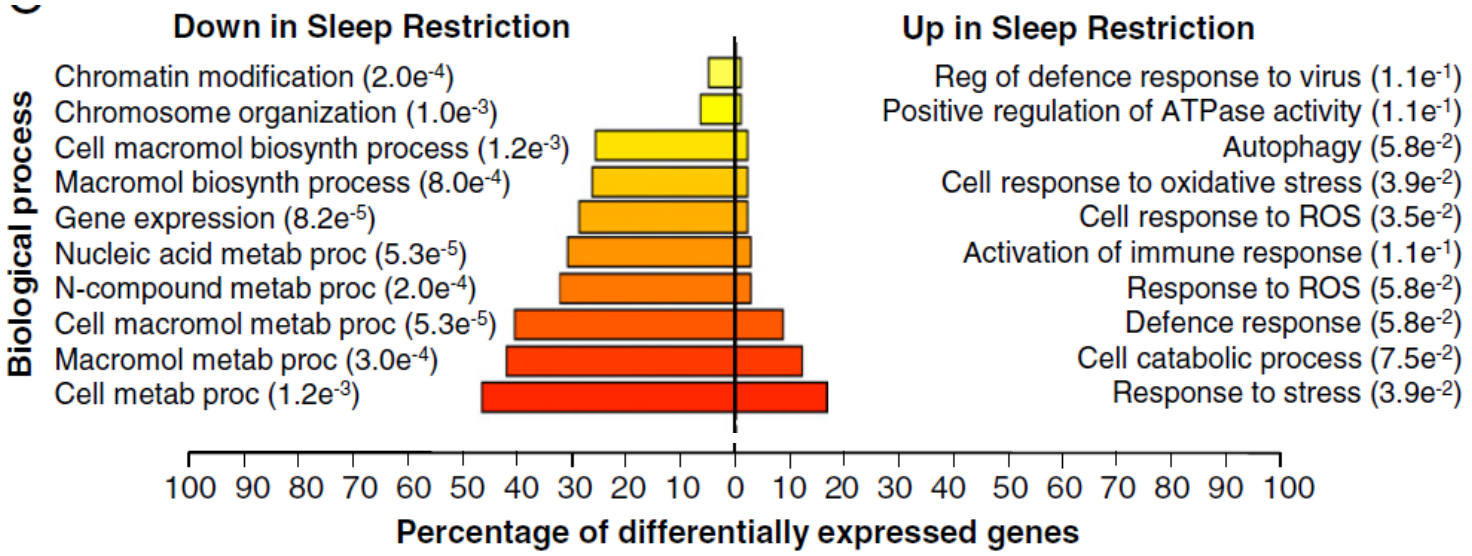
RORA: retinoic acid receptor-related orphan receptor alpha is a nuclear hormone receptor – associated with circadian rhythms

PRDX5: peroxiredoxin 5

Greyed areas: melatonin profile averaged for the two conditions.

Individual data were aligned relative to the individual melatonin rhythm and sorted into discrete circadian phase bins.

What sort of genes are differentially expressed upon sleep restriction?



Top 10 enriched GO biological processes within the statistically significant differentially expressed gene list as identified by WebGestalt when using the human genome as background.

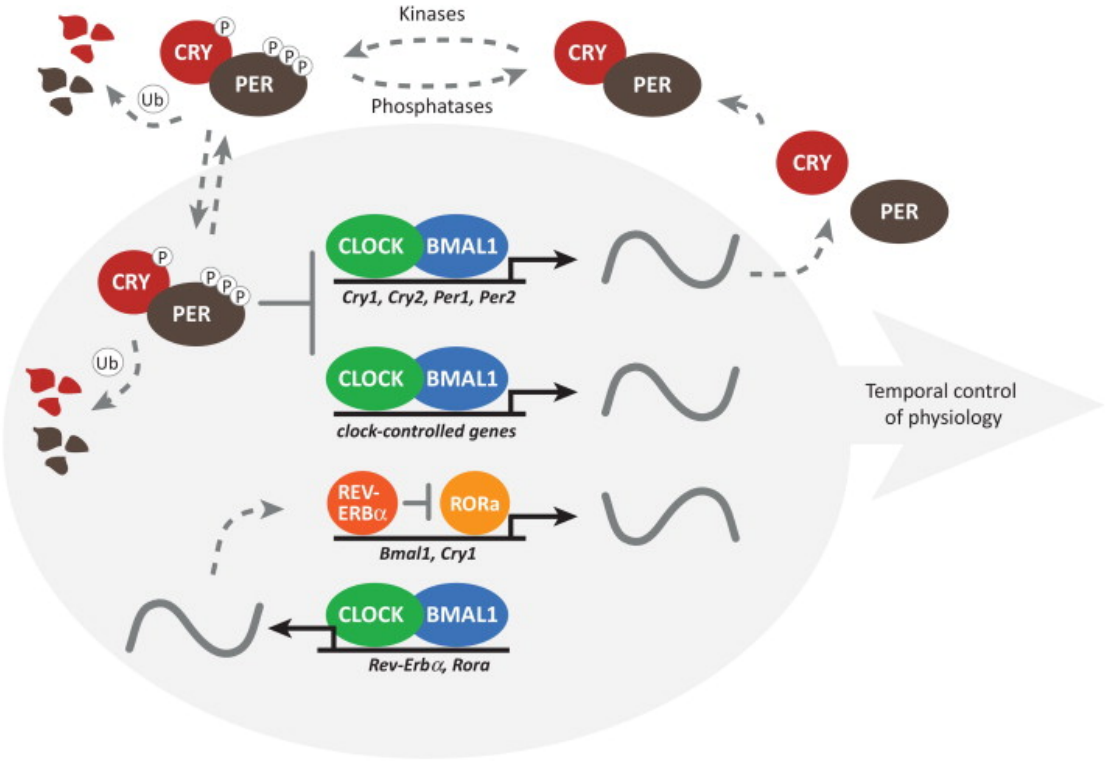
p-values are corrected by Benjamini-Hochberg method for multiple testing.

Down-regulation: chromatin modification and organization, metabolism

Up-regulation: cellular response to oxidative stress and reactive oxygen

This does not sound healthy!

Review (V1): The molecular circadian clock in mammals



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CLOCK, circadian locomotor output cycles kaput
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PER: period
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Partch et al. Trends Cell Biol 24, 90 (2014)

Next paper for you …

Crystal Structure of the Heterodimeric CLOCK:BMAL1 Transcriptional Activator Complex

Nian Huang,[1*] Yogarany Chelliah,[2,3*] Yongli Shan,[2] Clinton A. Taylor,[1,4] Seung-Hee Yoo,[2] Carrie Partch,[1,2,3†] Carla B. Green,[2] Hong Zhang,[1‡] Joseph S. Takahashi[2,3‡]

Introduction: 2 paragraphs

(1) Biological role of CLOCK and BMAL1

(2) Transcription factor family with bHLH and PAS domains

Methods section: 1 paragraph

(1) Strategy to determine structure

Results section:

(1) Overall structure

(2) Individual PAS-A and PAS-B domains

(3) CLOCK:BMAL1 heterodimer

(4) Effects of mutants

see https://science.sciencemag.org/content/337/6091/189.full