V3: Circadian rhythms – program for today

(1) Case study: circadian effects on **drug response**

(2) Circadian rhythms are closely connected to **metabolism**

(3) Circadian clock genes generate **epigenetic effects**

“Circadian rhythms in the absence of the clock gene Bmal1” (paper for V4)

(1) Case study: Circadian effects on drug response

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 32 No. 4, August 2017 345–358
DOI: 10.1177/0748730417710348

The Liver Circadian Clock Modulates Biochemical and Physiological Responses to Metformin

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Daily fluctuations in drug absorption, metabolism, and elimination can alter the effectiveness and toxicity of many pharmaceutical compounds.

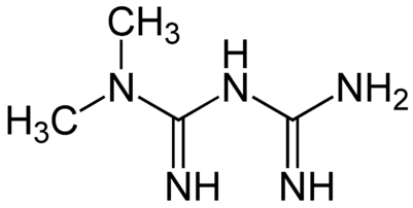
A **xenobiotic** is a chemical substance found within an organism that is not naturally produced or expected to be present within the organism.

The **xenobiotic metabolizing system** constitutes a series of biochemical reactions that enable the transport, solubilization, chemical conversion, and eventual elimination of a wide variety of environmental toxins and drug compounds.

Many enzymes and transporters involved in xenobiotic metabolism undergo **circadian oscillations** of expression at the mRNA and/or protein level.

Metformin – mechanism and uptake

Metformin is currently the most widely prescribed drug for type 2 **diabetes** worldwide.
It is also the treatment of choice for polycystic ovary syndrome (PCOS) and is being investigated as a treatment for certain types of cancer and even to delay aging.

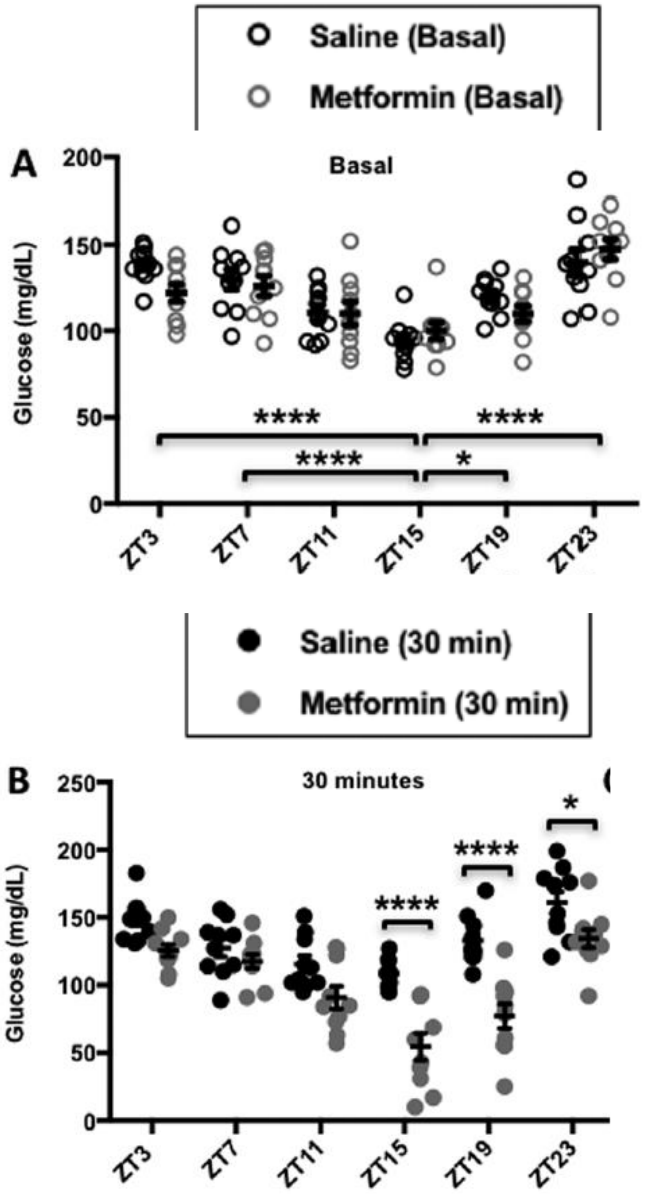


It is believed to exert its clinical effects by inhibiting **mitochondrial complex I**. The resulting reduced flux through the electron transport chain lowers cellular ATP production. This activates **AMP-activated protein kinase** (AMPK).

Metformin is most commonly prescribed as an immediate-release formula, which reaches a peak concentration in plasma within 1 to 3 h and is usually taken twice each day.

An extended-release formulation that peaks in circulation 4 to 8 h after delivery is recommended for single daily dosing.

Gluose level shows response to metformin



(A) Typical daily variation of **blood glucose level** in male mice **before** (basal) intraperitoneal **injection** (*injection into the body cavity/dt. Bauchfell*) of 250 mg of metformin per kilogram of bodyweight at the indicated zeitgeber times (ZT, hours after lights-on). Metformin is dissolved in salt solution (saline).

“Saline”: control group, injection without metformin, shows initially no noticeable difference.

(B) Same measurement 30 minutes **after** the **injection**. Metformin treatment resulted in a significant **reduction in blood glucose** compared to saline-treated mice.

Metformin response is greatest at ZT15 and ZT19, corresponding to the middle of the active phase for mice and likely similar to late morning in humans.

Metformin – mechanism and uptake

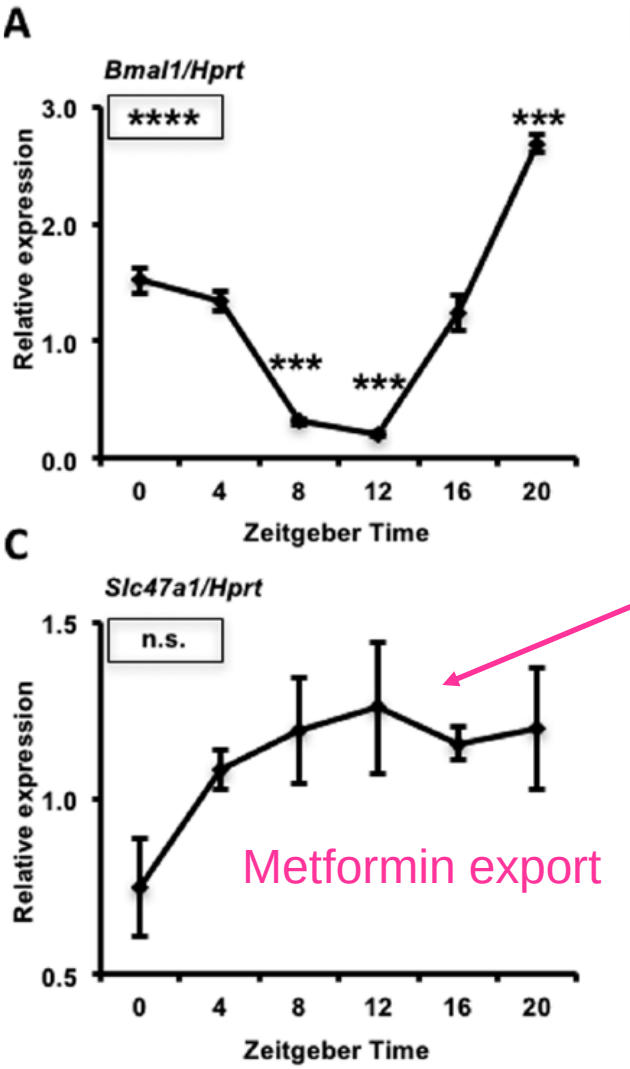
Metformin entry into hepatocytes is largely driven by the organic cation transporter 1 (OCT1) expressed from the gene ***Slc22a1***.

CLOCK, BMAL1, CRY1, CRY2, and PER2 all bind to the promoter region of *Slc22a1* in mouse liver, suggesting that the **hepatic circadian clock** could directly regulate the expression of *Slc22a1*.

Multidrug and toxin extrusion-1 (MATE-1) protein, expressed from the solute carrier ***Slc47a1***, enables the **export of metformin** from hepatocytes.

CRY1 and CRY2 bind to the promoter region of *Slc47a1* in mouse liver, suggesting that it could also be under clock control.

mRNA expression of Bmal1 and solute carriers



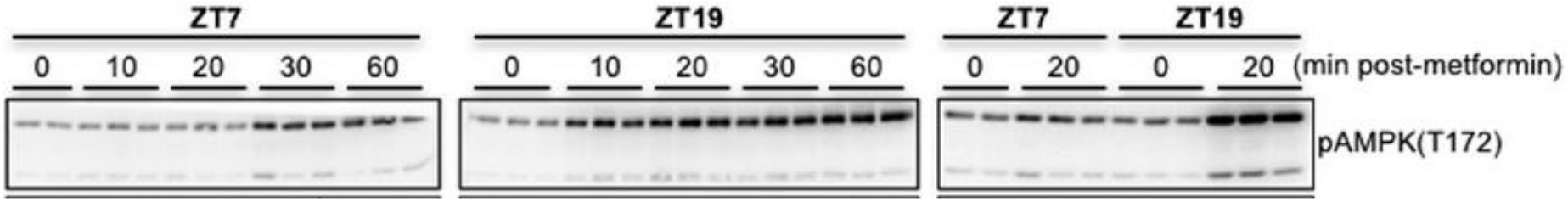
Slc47a1 tends to be more highly expressed at night, whereas no significant effect of zeitgeber time was observed on the mRNA expression of *Slc22a1*.

Activation of AMPK (AMP-activated protein kinase)

What downstream consequences may result from the observed differences in metformin effects on blood glucose?

→ measure the kinetics of the **signal transduction response** to metformin at ZT7 and ZT19. These 2 time points exhibit similar basal blood glucose levels but markedly different reductions in blood glucose in response to metformin.

Consistent with the observed enhanced reduction of blood glucose during the night, the activating phosphorylation of AMPK on threonine 172 (T172) occurred more quickly after metformin treatment at ZT19 (10 min) compared with treatment at ZT7 (30 min).



(2) 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)
https://science.sciencemag.org/content/320/5878/949.long

Background:

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.

Control of circadian rhythms?

Open question: how are long-term, high-amplitude oscillations with a daily period maintained?

Transcriptional feedback loops are typically less precise than the oscillation of the circadian clock and oscillate at a higher frequency than one cycle per day.

Possible explanations:
- Phosphorylation (e.g. casein kinase) causes delay (see V1),
- secondary loops give stabilization (see V1)

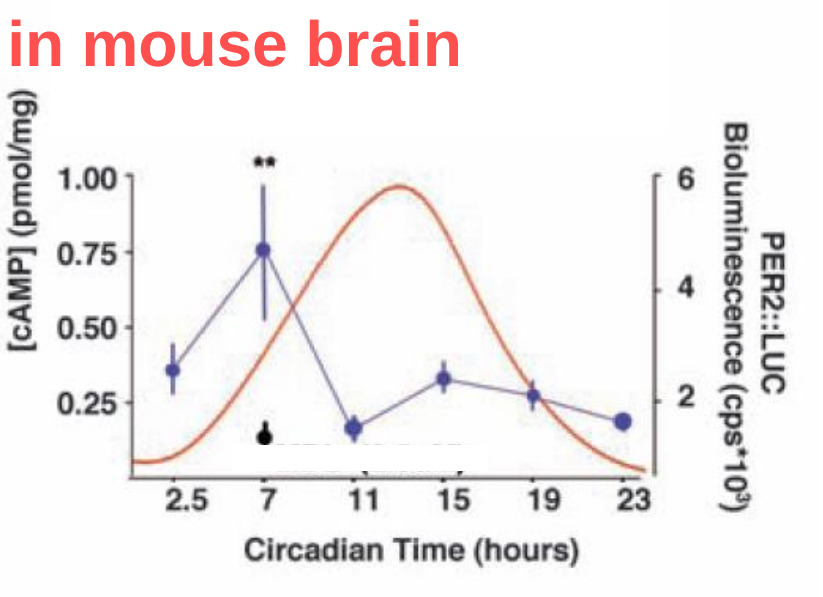
But transcriptional mechanisms may not be the sole, or principal, mediator of circadian pacemaking.

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

Cyclic cAMP levels in mouse brain

The molecular oscillations of the SCN were tracked as circadian emission of bioluminescence in slices from transgenic mouse brain.

As expected, a fusion protein of mPER2 and LUCIFERASE (mPER2::LUC) reported circadian protein synthesis rhythms (Fig).

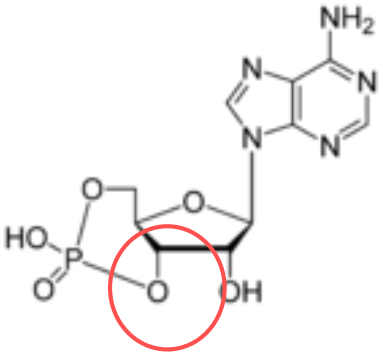


Circadian oscillation of cAMP concentration (blue) and PER2::LUC bioluminescence (red).

Novel finding: Under these conditions, the **cAMP** content of the SCN was also circadian.

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

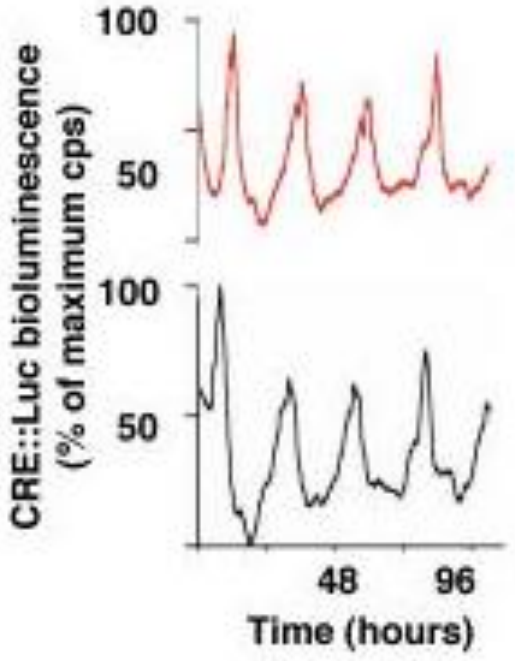
Cyclic adenosine monophosphate (**cAMP**) is a second messenger that is important in many biological processes.



„cyclic"

Cyclic cAMP levels in mouse brain

The circadian cAMP content of the SCN is accompanied by a circadian cycle in activity of cAMP response element sequences (CRE) reported by a *CRE::luciferase* adenovirus.



Circadian oscillation of CRE activity in two representative SCN slices (red and black) reported by *CRE:luciferase* adenovirus.

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

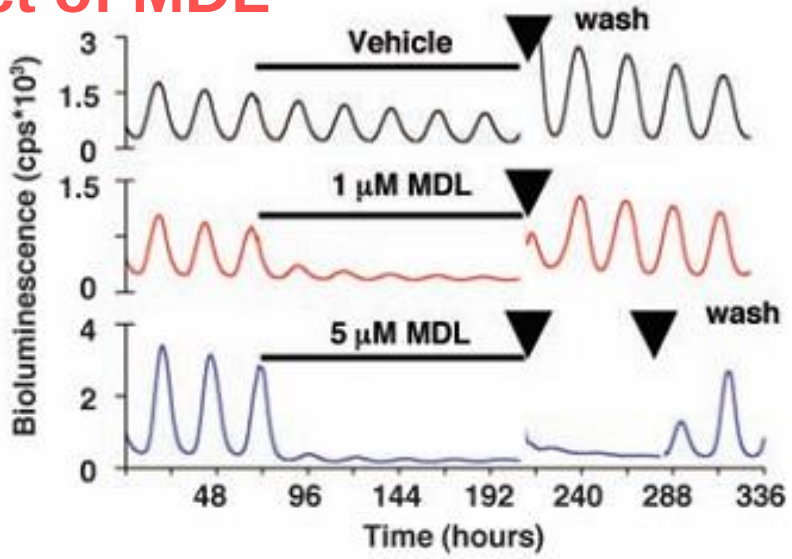
Effect of MDL

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.

“Vehicle” is a control experiment.



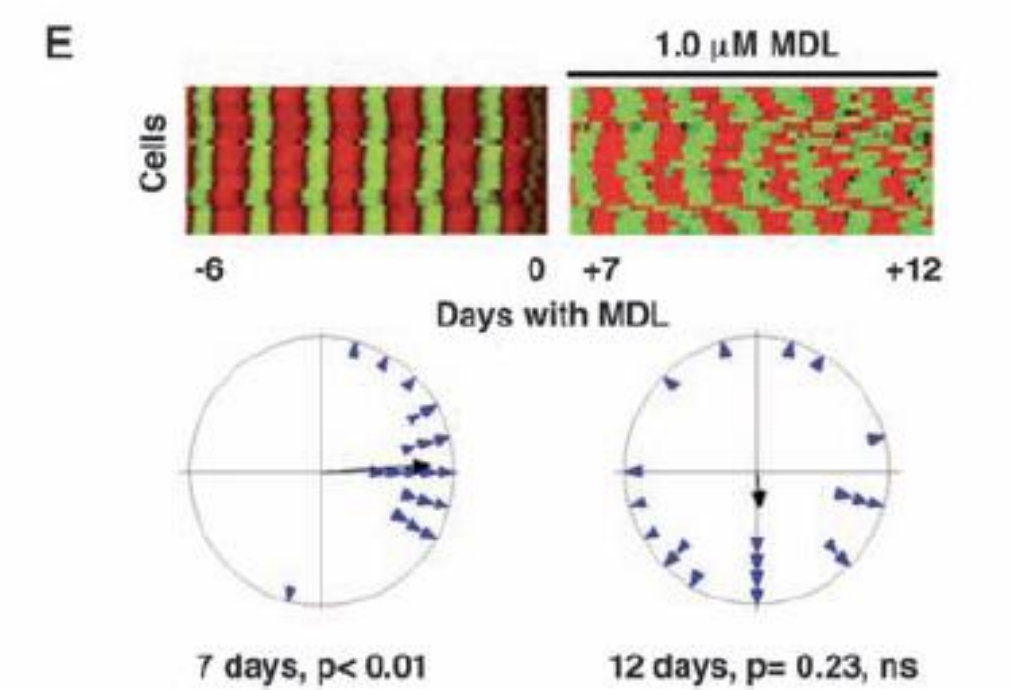
Interpretation: MDL rapidly suppressed circadian CRE:luciferase activity, presumably through loss of cAMP-dependent activation of CRE sequences.

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)

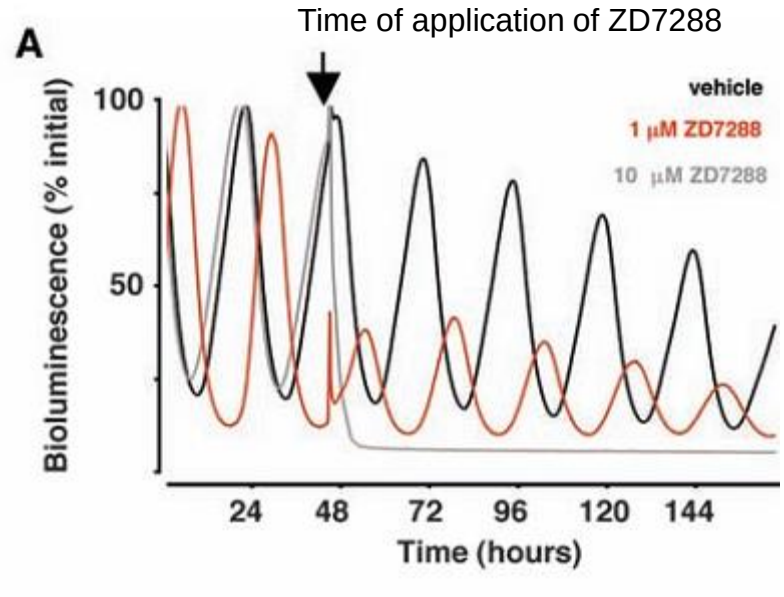
Can one block cAMP action?

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)



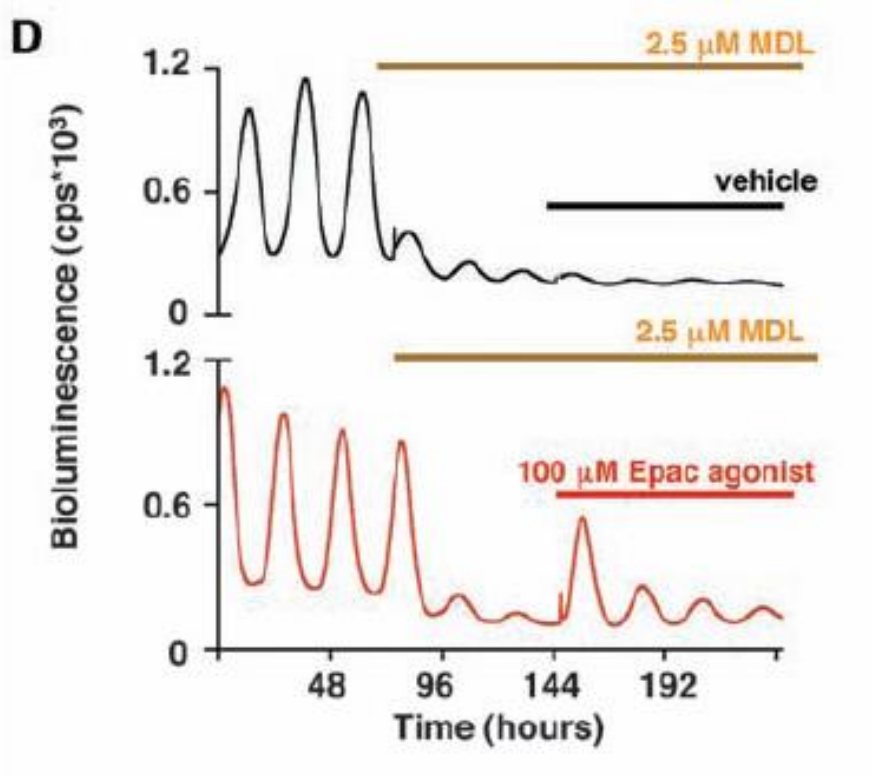
The irreversible HCN channel blocker ZD7288, which would be expected to hyperpolarize the neuronal membrane, dose-dependently damped circadian gene expression in the SCN.
This is consistent with disruption of trans-criptional feedback rhythms.

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.

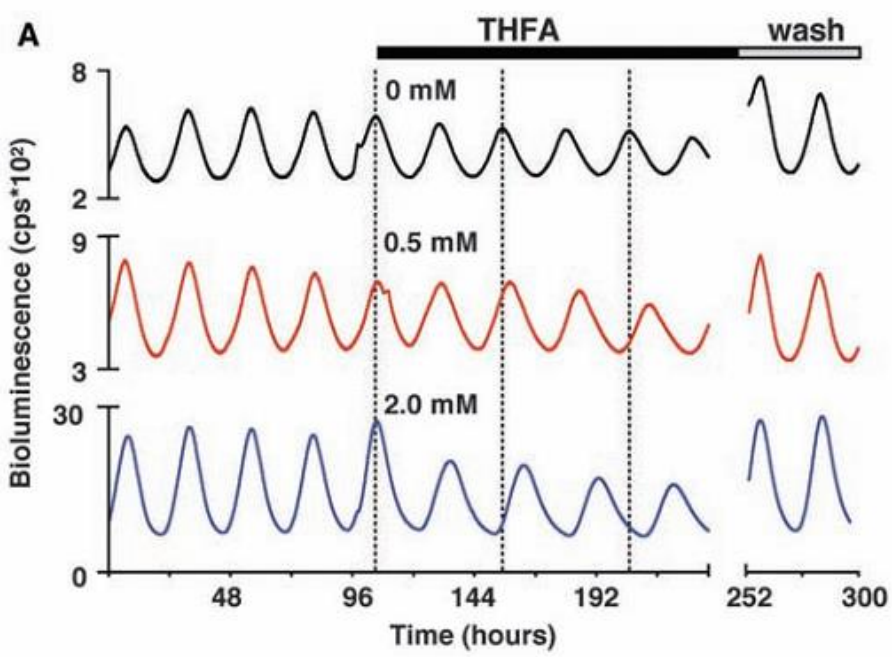


O'Neill et al.
Science, 320, 949 (2008)

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)

Conclusions on cAMP-coupling

Circadian pacemaking in mammals is **sustained**.

Its canonical properties of **amplitude**, **phase**, and **period** are determined by a reciprocal interplay in which transcriptional and posttranslational feedback loops drive rhythms of cAMP signaling.

Dynamic changes in cAMP signaling, in turn, regulate transcriptional cycles.

Thus, output from the current cycle constitutes an input into subsequent cycles.

The interdependence between nuclear and cytoplasmic oscillator elements we describe for cAMP also occurs in the case of Ca^{2+} and cADPR.

This highlights an important newly recognized common logic to circadian pacemaking in widely divergent taxa.

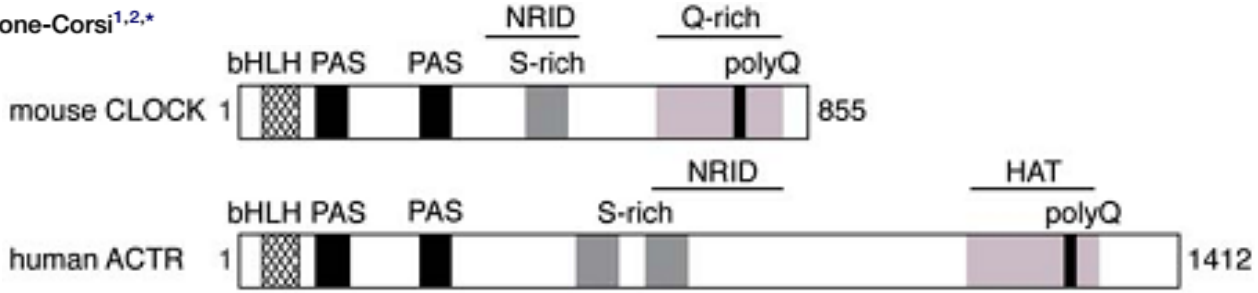
O'Neill et al.
Science, 320, 949 (2008)

(3) Circadian regulation of epigenetic chromatin

Circadian Regulator CLOCK Is a Histone Acetyltransferase

Masao Doi,[1,3] Jun Hirayama,[1,2] and Paolo Sassone-Corsi[1,2,*]

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



Mouse CLOCK and human ACTR have very similar organization:
a basic helix-loop-helix (bHLH) motif (binds to DNA), Per-Arnt-Sim (PAS) domains, serine-rich (S-rich) regions, a nuclear receptor interaction domain (NRID), and a glutamine-rich (Q-rich) region containing a poly-glutamine (polyQ) stretch.

The polyQ region of hACTR is known to have **HAT activity**.

Histone acetyltransferases (**HATs**) are enzymes that acetylate conserved lysines on histone proteins by transferring an acetyl group from acetyl-CoA to form ε-*N*-acetyllysine.

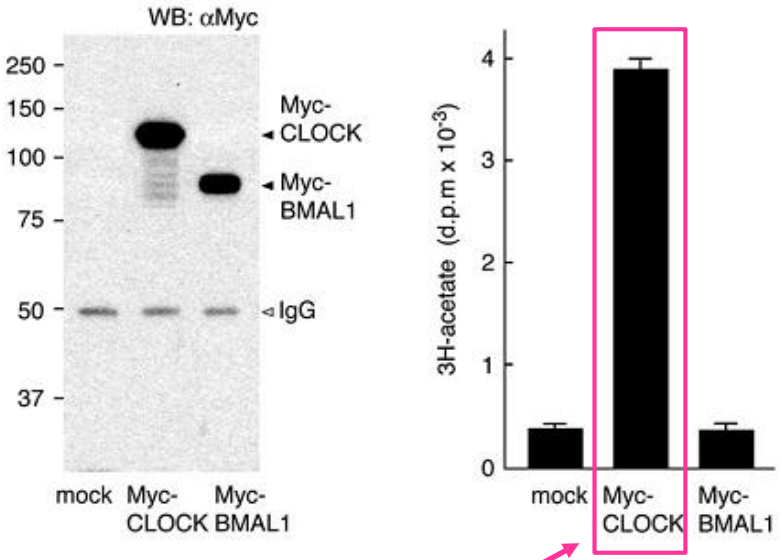
CLOCK is a histone acetyl transferase

Myc-mCLOCK or Myc-mBMAL1 were transientlv expressed in JEG3 cells and then immunoprecipitated with antiMyc 9E10 antibody

(Left) Western blot, illustrating similar protein levels of the immunoprecipitated Myc-tagged proteins CLOCK and BMAL1.

(Right) After extensive washing, the resulting immunoprecipitates were incubated with [^{3}H] acetyl-CoA and a mixture of histone H3 and H4 amino-terminal tail peptides.
The incorporated [^{3}H] acetate was detected by filter binding assays.

→ CLOCK has significant HAT activity.



As a **control**, cells transfected with an empty vector (**mock**) were also subjected to the immunoprecipitation-HAT assay.

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

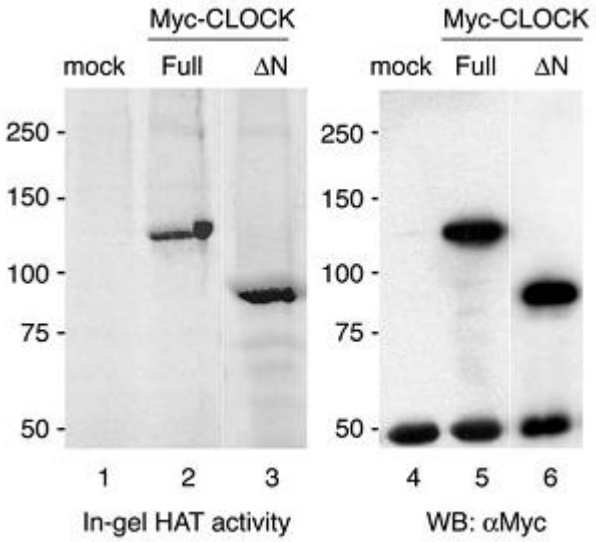
CLOCK is a histone acetyl transferase

In-gel HAT activities of Myc-CLOCK.
Either a full-length (Full) or an **N-terminally truncated (ΔN) mCLOCK protein** was expressed in JEG3 cells and immunoprecipitated as described on the previous slide.

(Left) The immunoprecipitates were resolved on a 7.5% SDS-PAGE gel containing core histones and processed to detect **acetyltransferase activity**.

The truncated CLOCK protein lacks N-terminal residues 1–242 but has an intact C-terminal region and still displays efficient HAT activity in the gel.

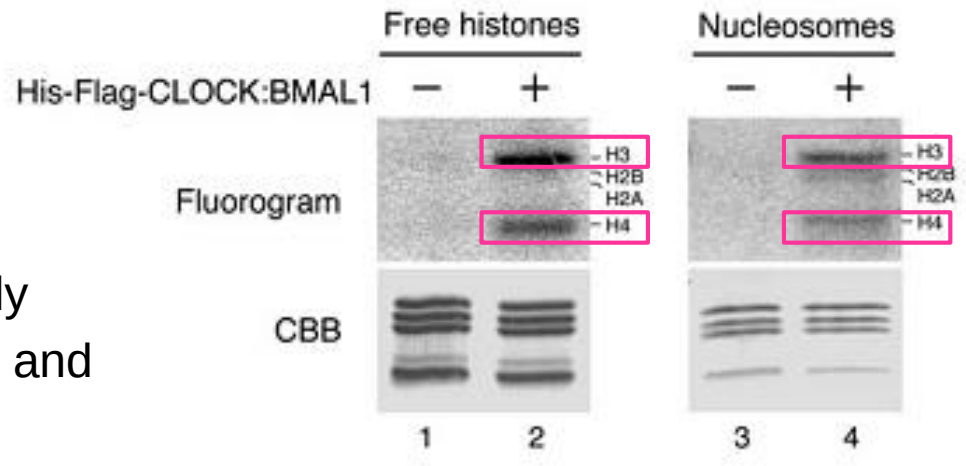
(Right) Identical immunoprecipitated samples were electrophoresed in a parallel SDS-PAGE gel and immunoblotted with antiMyc 9E10 antibody.



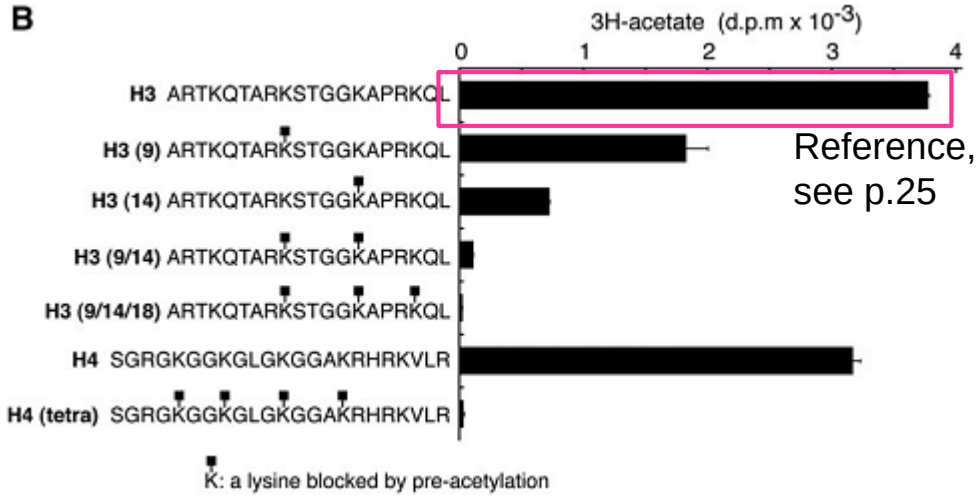
Doi, Hirayama, Sassone-Corsi,
Cell 125, 497 (2006)

CLOCK is a histone acetyl transferase

TOP: HAT assays using either free core histones or mononucleosomes were performed and the reaction products analyzed on SDSPAGE.
The mCLOCK protein acetylated primarily histones H3 and H4 on both free histone and mononucleosomes.



BOTTOM: Specificity of CLOCK enzymatic activity investigated by using **H3 and H4 tails** with **pre-acetylated lysines**.
In this approach, putative HAT substrate sites are occupied, resulting in a block of potential de novo acetylation.
→ H3 K14, and in a lesser extent K9, are the major sites acetylated by mCLOCK.



Reference, see p.25

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

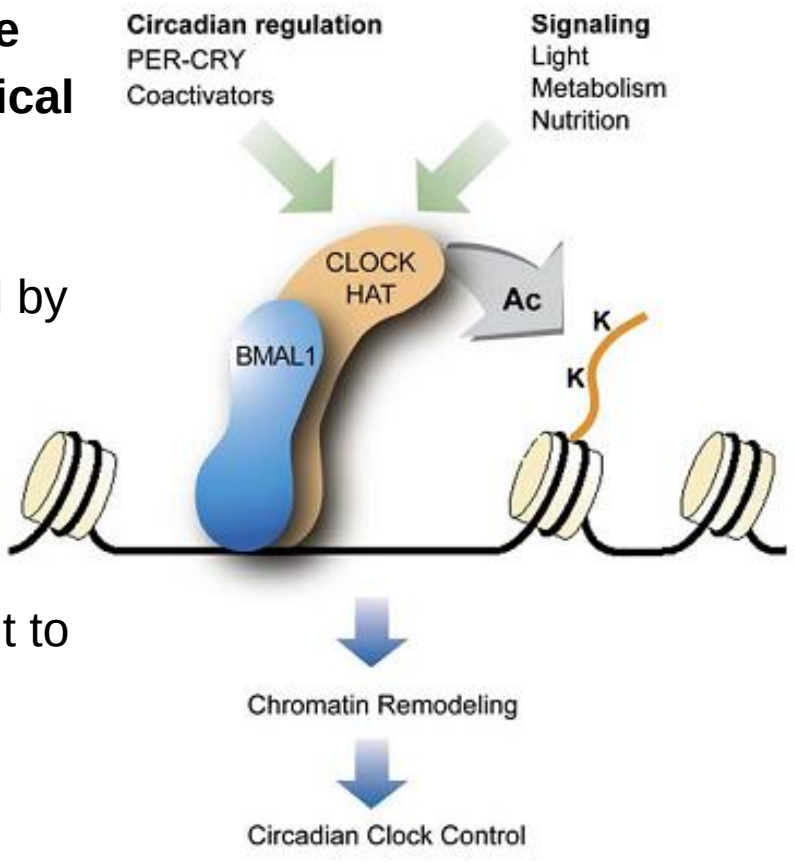
Schematic model

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 K14, 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.



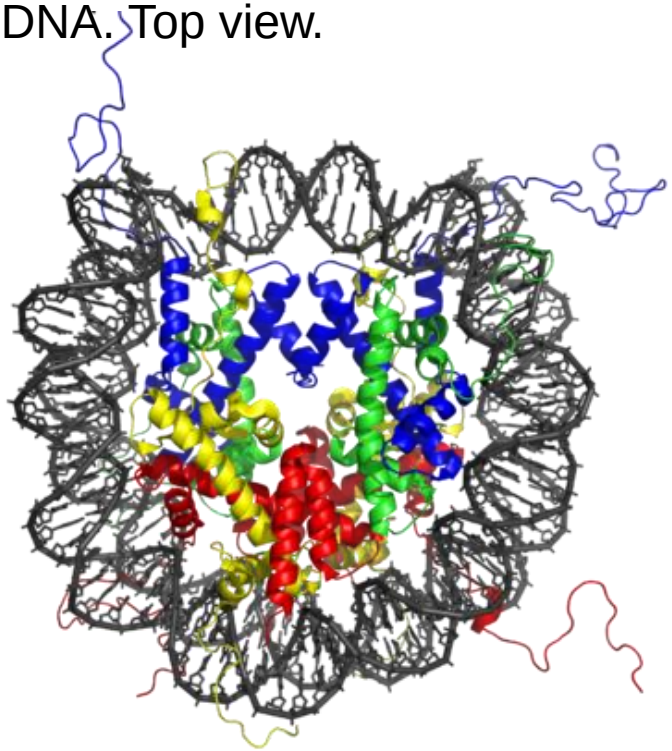
Doi, Hirayama, Sassone-Corsi, Cell 125, 497 (2006)

The histone code

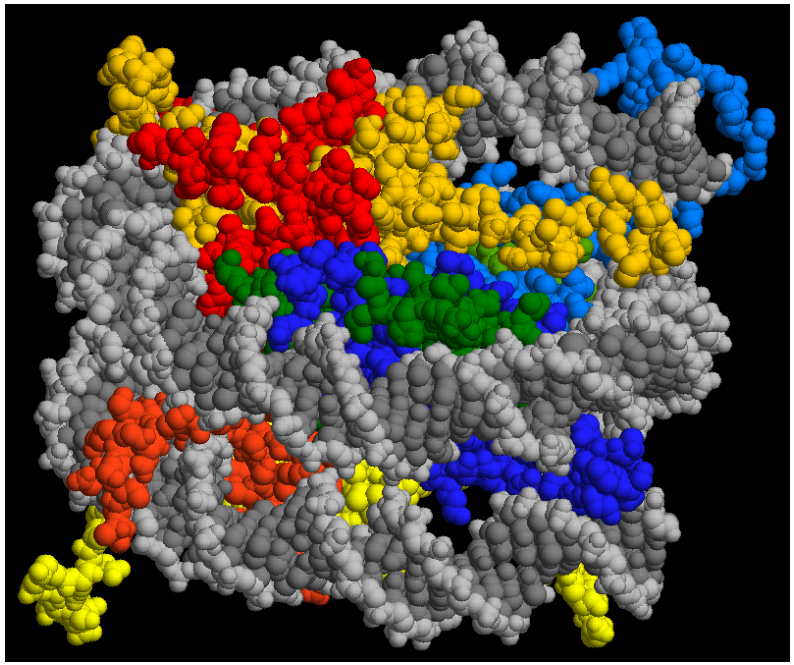
The DNA of eukaryotic organisms is packaged into chromatin, whose basic repeating unit is the **nucleosome**.

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.

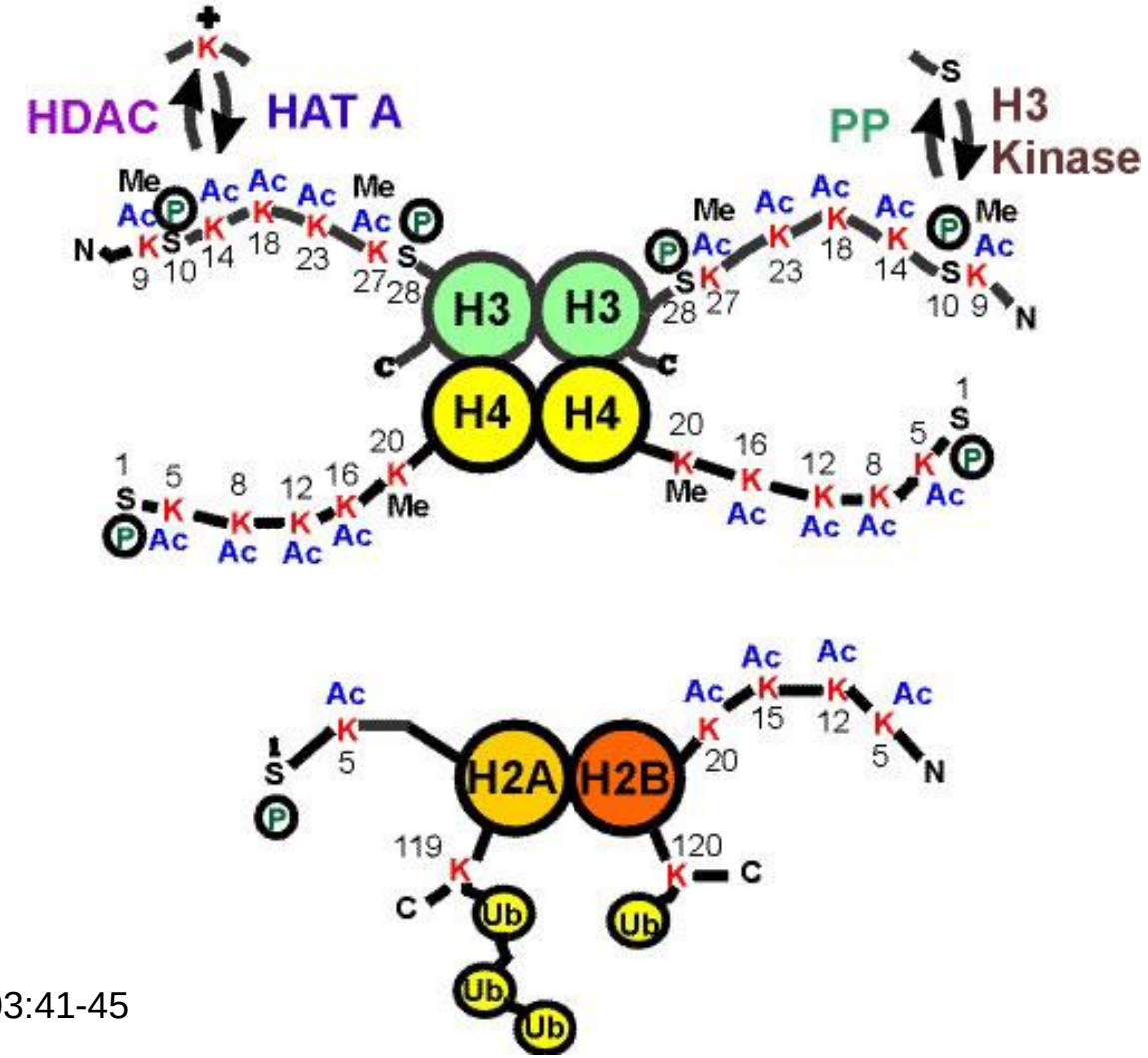
This, in turn, affects **gene expression.**

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)

Core clock proteins interact with chromatin and chromatin-modifying complexes

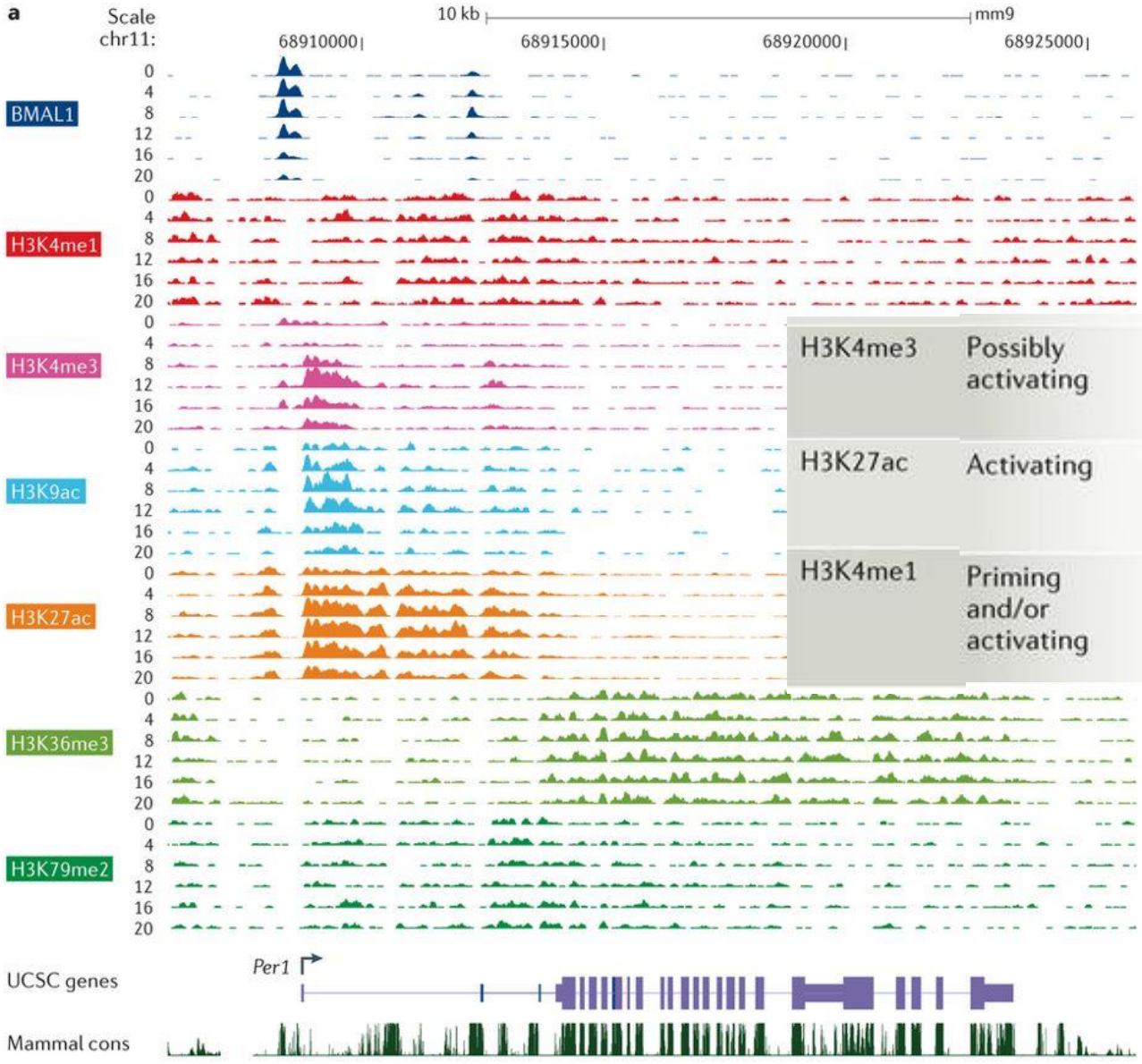
At the beginning of the transcription cycle, the activators CLOCK and BMAL1 interact with the **histone acetyltransferases** (HATs) p300 and CREB-binding protein (CBP), respectively, to acetylate histones and provide an accessible chromatin state for transcription.

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

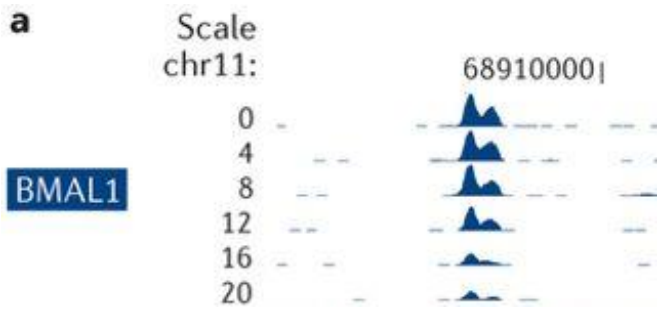


UCSC genome browser view of histone methylation and acetylation at the *Per1* gene at 6 circadian times (CTs) of the day (0, 4, 8, 12, 16 and 20 hours).

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

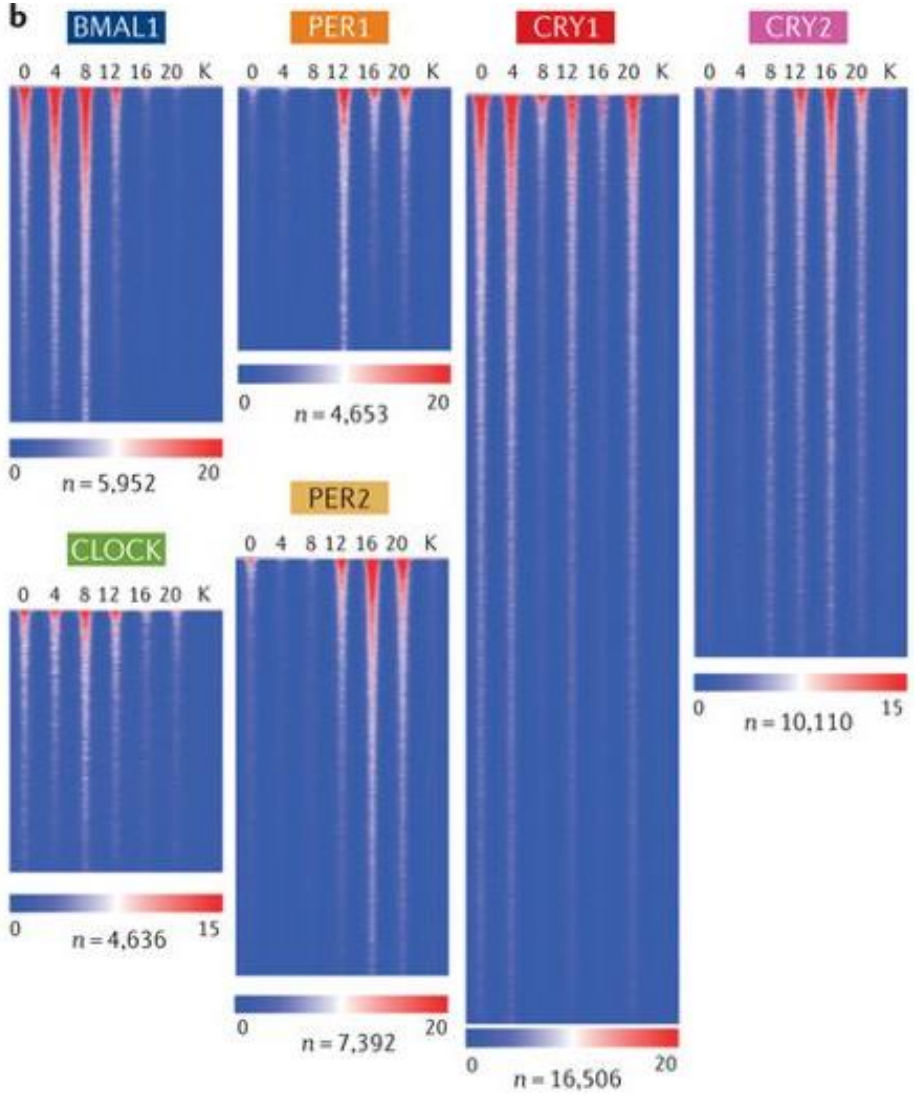
Takahashi
Nature Rev Genet
18, 164–179 (2017)

BMAL1:CLOCK activity in the mouse liver



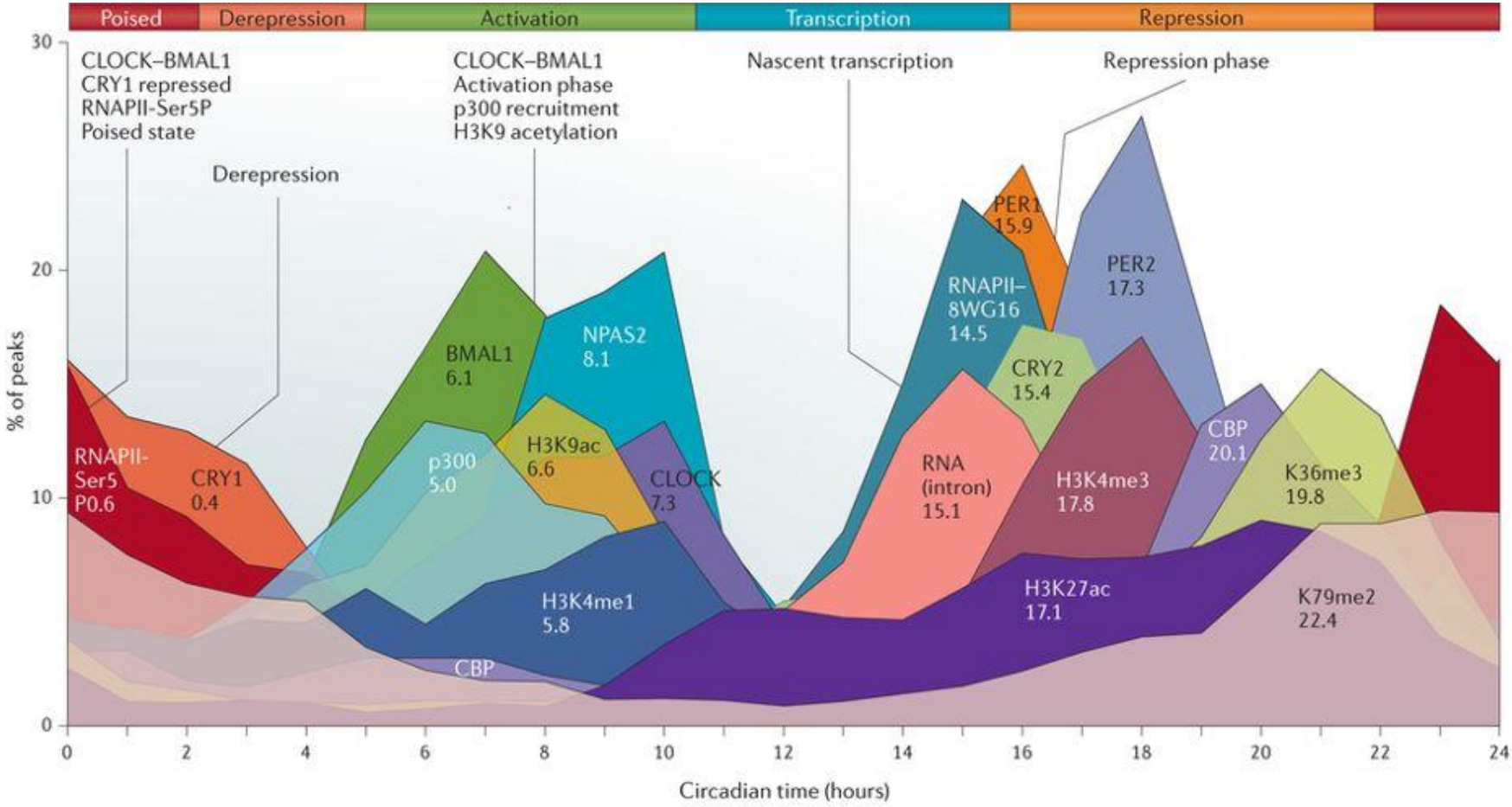
At Per1, the activators BMAl1 and CLOCK bind in a cyclic manner at the promoter between circadian time zero (CT0) and CT12, with maximal binding observed at CT8.

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)

Next paper for V4

Ray et al, Science 367, 800-806 (2020)

“Circadian rhythms in the absence of the clock gene Bmal1”
https://science.sciencemag.org/content/367/6479/800

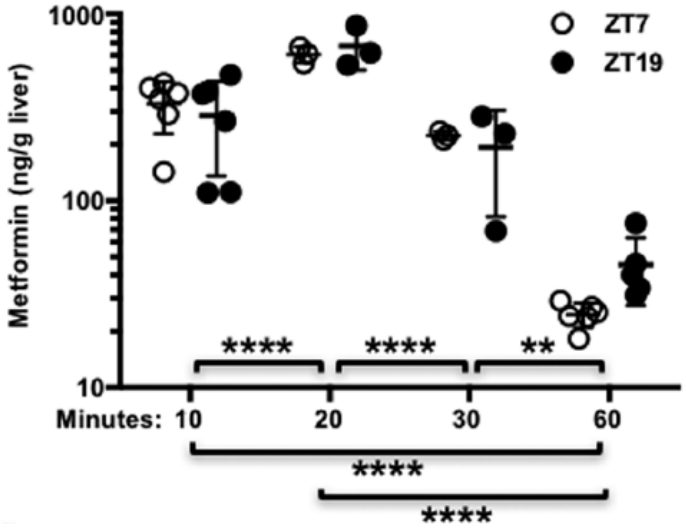
This study is somehow related to paper #1.

• Fig. 1 Rhythmic transcriptome of Bmal1−/− mouse liver tissues and skin fibroblasts.
Fig. 2 Temperature compensation of circadian transcriptional rhythms in the absence of BMAL1.
Fig. 3 Twenty-four-hour rhythmicity of ETS transcription factors and peroxiredoxin (PRDX) oxidation in Bmal1−/− tissues.
Fig. 4 Rhythmic proteome and phosphoproteome in the absence of BMAL1.

Finding: although Bmal1 is necessary for the expression of 24-hour behavioral cyclicity, it is not required for 24-hour molecular rhythms at the transcriptional, translational, and posttranslational levels.

Additional slides (not used)

Metformin level in liver



Metformin concentration in mouse liver, as detected by mass spectrometry.

Tissues were snap frozen in liquid nitrogen at the indicated **times after intraperitoneal injection** with metformin at ZT7 (open circles) or ZT19 (closed circles).

→ In both cases, metformin peaked in the liver 20 min after administration

Summary

Acute reduction in blood glucose in response to metformin depends on the time of day of treatment in mice.

The kinetics of metformin-induced activation of AMPK are dramatically different in the middle of the day (ZT7) compared with the middle of the night (ZT19, active phase for mice).

Thus, the timing of metformin treatment could affect its clinical efficacy

Evidence for coupling of circadian clocks with metabolism

(1) Recombinant cyanobacterial proteins can **sustain circadian cycles** of autophosphorylation in vitro, in the absence of transcription,

(2) The intracellular signaling molecules cyclic adenosine diphosphate–ribose (**cADPR**) and Ca^{2+} are essential **regulators** of circadian oscillation in *Arabidopsis* and *Drosophila*.

This indicates that transcriptional mechanisms may not be the sole, or principal, mediator of circadian pacemaking.

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

Example of a gene regulatory network

O’Neill and co-workers showed that the transcriptional feedback loops of the SCN are sustained by cytoplasmic cAMP signaling.

cAMP signaling determines their canonical properties (amplitude, phase, period).

Roles of cAMP?

In molluscs, birds, and the mammalian SCN, cAMP is implicated in entrainment or maintenance of clocks, or both, or mediation of clock output.

It was not considered as part of the core oscillator sofar.

These findings extend the concept of the mammalian pacemaker beyond transcriptional feedback to incorporate its integration with rhythmic cAMP-mediated cytoplasmic signaling.

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

What is cAMP

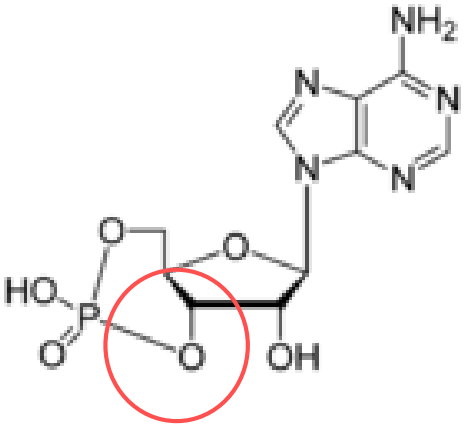
Cyclic adenosine monophosphate (**cAMP**) is a second messenger that is important in many biological processes.

cAMP is derived from ATP and used for intracellular signal transduction in many different organisms, conveying the cAMP dependent pathway.

In humans, cyclic AMP works by activating **cAMP-dependent protein kinase** (PKA)**.**

Cyclic AMP binds to specific locations on the regulatory units of the protein kinase, and causes dissociation between the regulatory and catalytic subunits

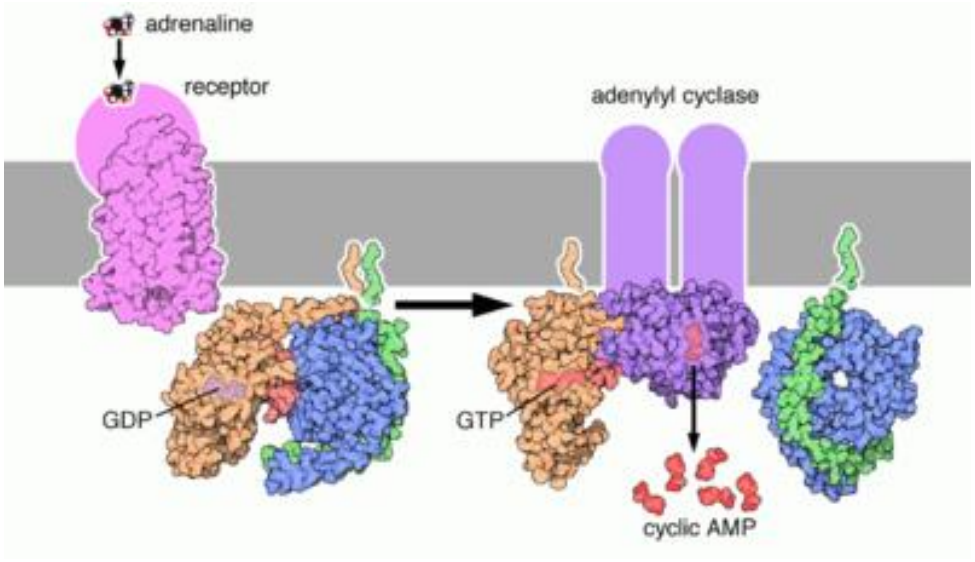
Thus it activates the catalytic units of PKA and enables them to phosphorylate substrate proteins.



„cyclic“

www.wikipedia.org

Side functions of cAMP



There are some minor PKA-independent functions of cAMP, e.g. activation of calcium channels.
This provides a minor pathway by which growth hormone is released.

Picture: Epinephrine (adrenaline) binds its receptor, that associates with an heterotrimeric G protein. The G protein associates with adenylyl cyclase that converts ATP to cAMP, spreading the signal.

www.wikipedia.org

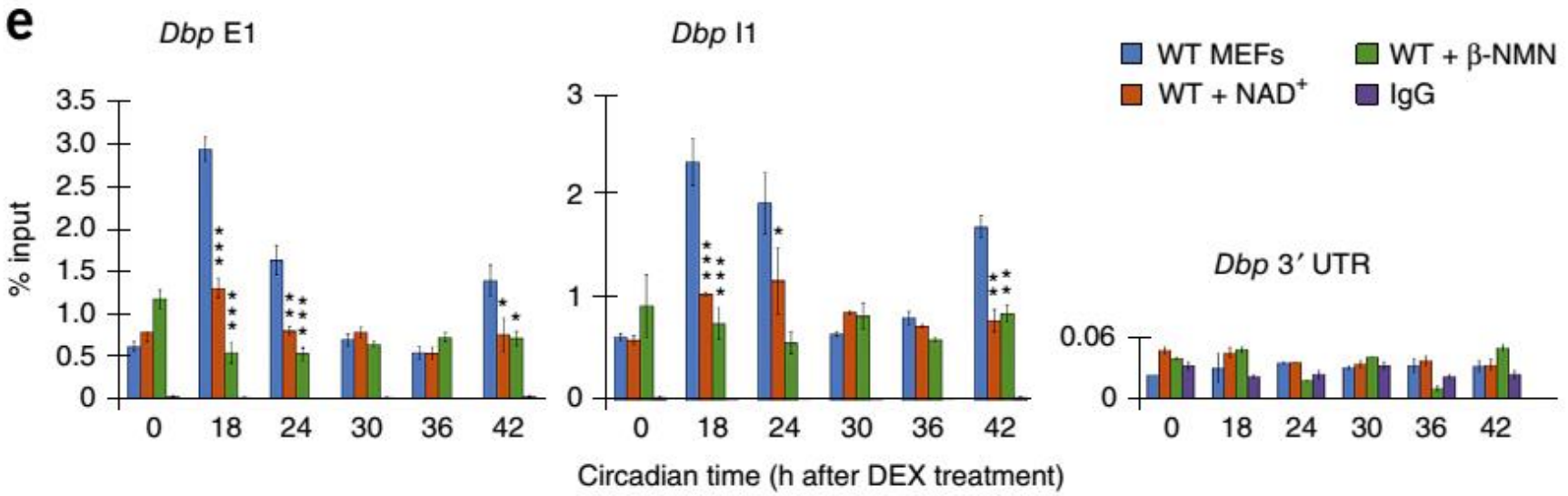
CLOCK is a histone acetyl transferase

NAD+-SIRT1 control of H3K4 trimethylation through circadian deacetylation of MLL1

Lorena Aguilar-Arnal[1], Sayako Katada[1,2], Ricardo Orozco-Solis[1] & Paolo Sassone-Corsi[1]

Fig. (e) shows H3K4 ChIP-data for the promoter of the circadian gene *Dbp*.

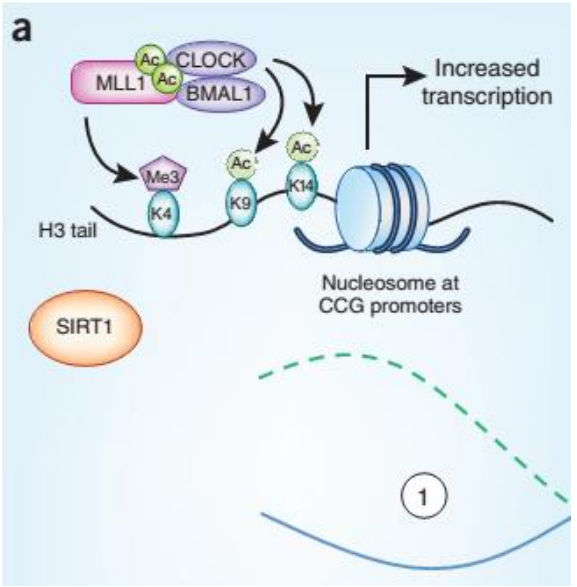
→ H3K4-methylation levels are modified by changing the NAD+ concentration.



Aguila-Arnal et al.
Nature Struct Mol Biol
22, 312 (2015)

Interpretation: Circadian regulation of epigenetic chromatin

Circadian fluctuations in NAD^+ levels and SIRT1 activity drive oscillations of the transcriptionally activating H3K4 trimethyl mark at promoters of clock-controlled genes (CCGs).



(**a**) At circadian times with low NAD^+ levels (1), SIRT1 deacetylase activity is low, and the histone methyltransferase MLL1 remains acetylated and active, increasing H3K4me3 levels at the promoters of CCGs.

Acetylated MLL1 also favors recruitment of the HAT complex, CLOCK–BMAL1, and acetylation of H3K9 and H3K14 at these promoters. Together, the activating methyl and acetyl histone marks **promote transcription** of CCGs.

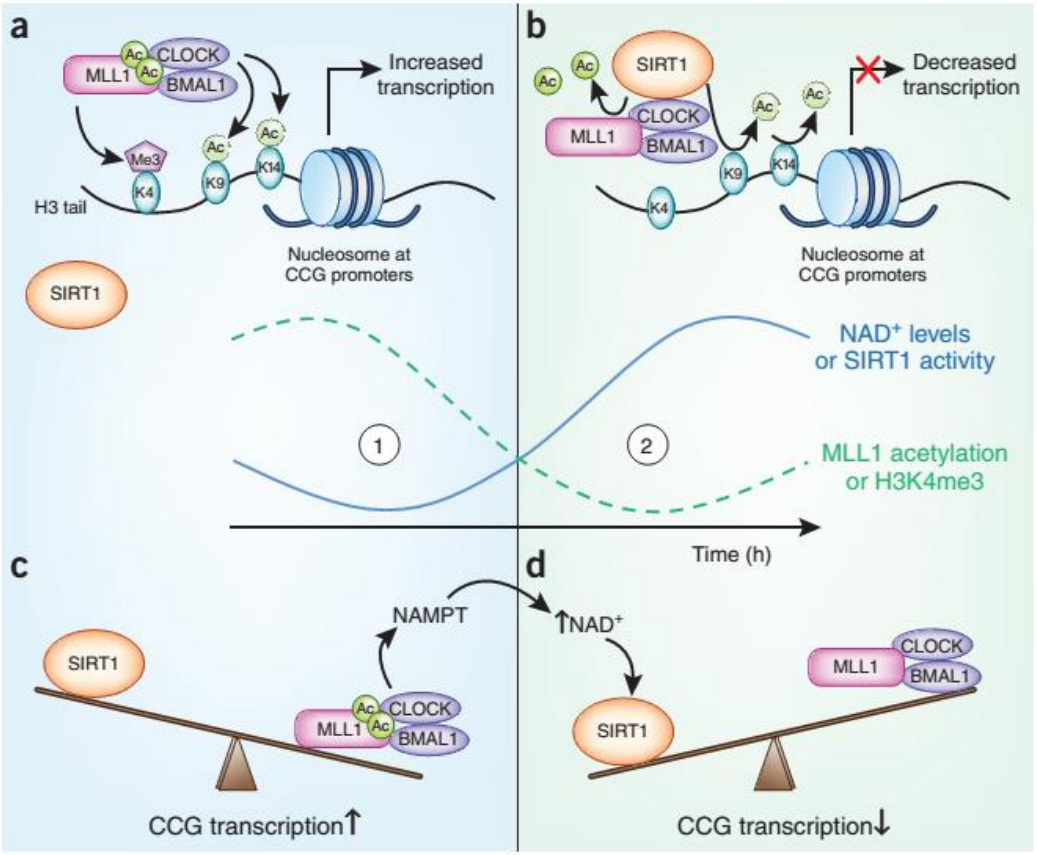
Tasselli & Chua,
Nat Struct Mol Biol 22, 275 (2015)

Interpretation: Circadian regulation of epigenetic chromatin

(**b**) As NAD^+ levels increase over time, the deacetylase SIRT1 is activated, and it deacetylates MLL1.

This reduces the methyltransferase activity of MLL1 and thus decreases H3K4me3 occupancy at CCG promoters.

This, together with SIRT1 deacetylation of H3K9 and H3K14, results in **reduced transcription** of CCGs.



Tasselli & Chua,
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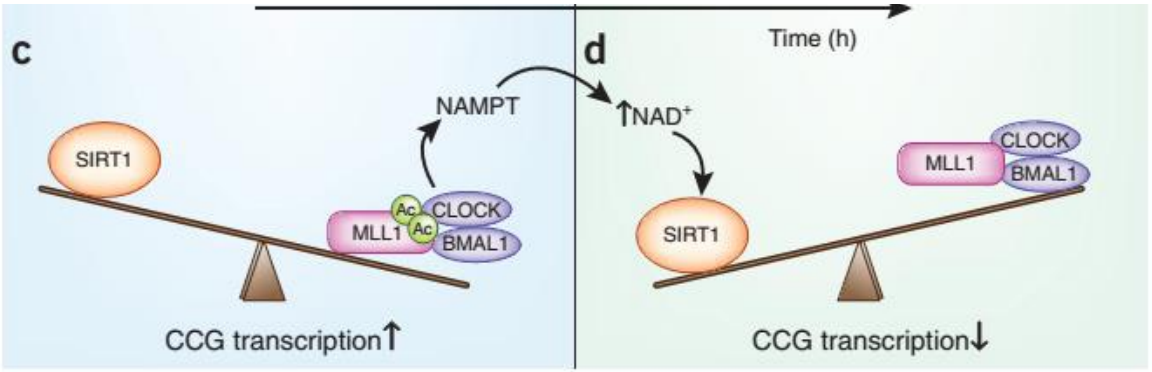
(**c**,**d**) Schematic illustrating the shifting balance between SIRT1 versus MLL1 activities over circadian time. The circadian oscillations in these activities are linked to each other and to the cellular bioenergetic state via feedback loops involving cyclic production of NAD^+.

In conditions of low cellular NAD^+ (**c**), the balance favors transcription dependent on MLL1 and CLOCK–BMAL1.
Among the CCGs is the *Nampt* gene, which encodes a key enzyme in NAD^+biosynthesis. Over time, as NAD^+ synthesis continues, rising NAD^+ levels tilt the balance back toward SIRT1 activity and transcriptional repression (**d**).



Nat Struct Mol Biol 22, 275 (2015)

Current understanding: clock – chromatin - metabolites

Circadian transcription is associated with **rhythmic changes in epigenetic marks** at circadian promoters such as H3K4 trimethylation and H3K9 and H3K14 acetylation.

The histone methyltransferase **MLL** contributes to the **recruitment** of **CLOCK-BMAL1** to chromatin and thereby to the expression of clock-controlled genes.

Sirtuins are a class of NAD^+-dependent deacetylases.
Circadian fluctuation of NAD^+-levels induce rhythmicity in SIRT1 enzymatic activity.

NAD^+-oscillation is dictated by CLOCK-BMAL1 which control the gene *Nampt*, encoding the nicotinamide phosphoribosyltransferase enzyme.

Aguila-Arnal et al. show that MLL1 is an acetylated protein and its enzymatic activity is controlled by SIRT1-dependent deacetylation.

Aguila-Arnal et al.
Nature Struct Mol Biol
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