Circadian clocks as gene regulatory systems

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Topics and learning objectives

1) Impact of circadian clocks on physiology and health

2) Molecular bases of circadian clocks

3) Circadian epigenetic modifications

4) Examples of our work: structure-function analyses of circadian clock proteins
Circadian clocks/rhythms

circadian, *lat.* circa diem = about/around a day

Adapt activity cycles to light-dark changes of 24 h earth rotation
→ anticipate light-dark changes
Many physiological processes are circadianly regulated, e.g.

- behaviour
- sleep-wake cycle
- metabolism
- detoxification
- immune system
- nervous system
- blood pressure
- body temperature
- hormone production (e.g. melatonin, cortisol)
Stroke (Schlaganfall) and myocardial infarction (Herzinfarkt) occur mostly in the morning when blood pressure rises.
Living against our inner clock (excessive shiftwork, jetlag, overtime work) affects health.
Our physiology is circadianly regulated – master and peripheral clocks

**Master clock, brain**
→ **SCN** (suprachiasmatic nucleus)
→ set by **light**

**Peripheral clocks**
→ set by **SCN**
→ set by **food, i.e. metabolic, redox, energy** state of cell

Misalignment by irregular food intake (e.g. shift work, jetlag) affects human health.
Mutations of clock genes result in arrhythmic behavior

1971: Konopka & Benzer: *Drosophila* random mutagenesis → arhythmic, 29 h (long) or 19 h (short) days

1984: Identify **period** as first clock gene → per⁰, per⁻, per⁵

Nobel Prize 2017
About 10% of all genes are circadianly expressed, but in a tissue specific manner.

Clock controlled genes (ccgs) differ between tissues

- Tissue specific transcription factors (TF)
- Epigenetic regulation: tissue specific chromatin states
Molecular circadian oscillator in Mammals: Cell-autonomous gene regulatory feedback loop

- **Input:** Circadian Photoreceptor Melanopsin

- **Activators:** CLOCK, BMAL1

- **Repressors:** PER, CRY

- **Output:** 24 h rhythms in physiology, metabolism and behaviour

- **Clock controlled genes (CcgS):**

- **Endogenous**
- **Independent of external timing cues (Zeitgeber)**
Impact of light and feeding on the clock

SCN
master clock

Light

CLOCK + BMAL1

Activators

~24h

Repressors

PER ↔ CRY

Energy e.g. AMP/ATP

Redox e.g. ROS, Reduction-equivalents

Output: ccgs
24h activity cycle

Peripheral clocks

Food

Energy e.g. AMP/ATP

Redox e.g. ROS, Reduction-equivalents
CRY1- and CRY1-PER2 crystal structures revealed an intramolecular CRY1 disulfide bond and a Zinc ion in a CRY1-PER2 complex interface.
CRY as redox-dependent Linker between circadian Clock and cellular Metabolism? Roles of Zinc and Disulfide Bridge?
How is the endogenous ~ 24 h period generated?

- Transcriptional activity
- Posttranscriptional regulation
- Protein Synthesis (Translation)
- Protein Degradation (Proteasome)
- Protein interactions
- Post-translational modifications
- Cellular localization
- Stabilizing loops → robustness
- Small molecules e.g. heme, AMP/ATP
- Chromatin modifications (Epigenetics)

Output: ccgs
24h activity cycle
Epigenetic regulation of the mammalian circadian clock

- MLL1, EZH2, Jarid1a, LSD1, pCAF...
- Histone...
- Methyltransferase
- Demethylase
- Acetyltransferase

\[ \text{Deacetylase} \]

\[ \text{SIRT1} \]

\[ \text{p300/CBP; HAT} \]

\[ \text{Acetyl-dependent repressive BMAL1-CRY1 interaction} \]

\[ \text{CLOCK HAT} \]

\[ \text{BMAL1} \]

\[ \sim 24h \]

\[ \text{PER1/2} \]

\[ \text{CRY1/2} \]

\[ \text{e.g. HDAC1/2} \]

\[ \text{Output:} \]

\[ 24h \text{ activity cycle} \]
Circadian transcriptional regulation in mouse liver

1. Active Transcription
   - CT 4–12: "day"

2. Early transcriptional repression
   - CT 12–20: "night"

3. Late transcriptional Repression
   - CT 0–4: "morning"

CT = Circadian Time
CT 0: sunrise, activity onset
CT 12: sunset

MDa repressive complex, > 30 proteins
**Circadian transcriptional regulation in mouse liver**

1. **CBP/p300** and **MLL1** co-activate BMAL1/CLOCK
   - **CBP/p300**: acetylate H3 K9/K14
   - **MLL1**: H3 K4 di/tri-methylation

   CBP: e.g. Takahata et al, Genes Cells 2000
   MLL1: P. Sassone-Corsi group, NSMB 2010, 2015

2. MDa repressive PER/CRY containing complex, total > 30 proteins
   e.g. NONO, WDR5, Sin3-HDAC, NuRD (HDAC), HP1-SUV39-MT, Helicases, transcription termination factor
Circadian landscape of transcription regulators and epigenome in liver

Koike et al, Science 2012
Circadian landscape of transcription regulators and epigenome in liver

Chip-Seq
Analyse genome-wide circadian transcription
Circadian clock proteins
RNA-Pol II occupancy
Histone modifications

Koike et al, Science 2012
p300 HAT binds CLOCK during transcriptionally active phase

Co-Immunoprecipitation in mouse liver nuclei:
- p300 binds to CLOCK in activating phase (CT6, day)
- less binding at CT 18 (night, repressive phase)

Echegaray et al. Nature 2003
Circadian Histone H3 acetylation and RNA-Pol II binding in per1 and per2 clock gene promotor in liver

More H3 acetylation during day (CT 3–12) than at night (CT 15-24)

More RNA-Pol II binding to promotor during day

Consistent with open transcriptionally active chromatin during day.

RPB: RNA-Pol II-binding

Echegaray et al. Nature 2003
p300 HAT activates BMAL1/CLOCK (B/C)-dependant transcription of *per1* clock gene in mouse liver

Transcriptional activity of clock gene *per1* (*luciferase (luc) as reporter*):

- CRY1/2 repress B/C
- p300 activates B/C
- p300 cannot activate B/C in presence of CRY → competition?

Echegaray et al. Nature 2003
Daily rhythmic BMAL1-Lys537 acetylation enhances CRY1 binding and BMAL1/CLOCK transcriptional repression \textit{in vivo} \cite{Hirayama}

CRY1 and CBP/p300 compete for binding to C-terminal BMAL1-TAD. \cite{Xu, Gustafson}
Isothermal titration calorimetry (ITC) of purified CRY1 + purified Bmal1-TAD

$K_D \approx 2 \mu\text{M}$ for Bmal1-TAD with non-acetylated Lys537

$K_D \approx 1 \mu\text{M}$ for Bmal1-TAD K537Q (Glutamine as acetyl-Lysine-mimetic amino acid)

Czarna, Wolf et al, 2011 and 2013
Peptide scan: positively charged CRY residues are important for binding to the BMAL1 transactivation domain

CRY on BMAL1 - TAD

BMAL1 helix
BMAL1 C-term

C

BMAL1-TAD on CRY1 and CRY2

CRys

Ala scan

CCA Cp

CBP (KIX domain) binds to similar regions on BMAL1-TAD
(Gutafson et al, 2017; Xu et al, 2015; C. Partch, NMR)

→ Binding site competition of CRY1 and CBP
Model for acetylation dependent CRY1-binding to BMAL1-TAD

**Active state:**

Weaker CRY1 binding to non-acetylated BMAL1-TAD

Lys537 masks negative BMAL1 charges

**Repressed state:**

Enhanced CRY1 binding to acetylated BMAL1-TAD

Lys537-Ac cannot mask negative BMAL1 charges

Czarna, Wolf et al, JBC 2011
Reminiscent of chromatin opening by Histone acetylation …..

Inactive
Compact

Active
Open
Circadian gene regulation: BMAL1 acetylation and Chromatin remodeling

Activation via Chromatin:

- **Histone H3-K9/K14-Acetylation:**
  CLOCK, p300/CBP, other HAT

- **Histone H3-K4-Methylation:**
  Mixed Lineage Leukemia 1 (MLL1)

Repression via Chromatin

- **Histone H3-K9/K14-Deacetylation:**
  SIRT1 and other HDACs

- **Histone H3-K9-Di-Methylation →** bind heterochromatin protein Hp1

- **Histone H3-K27-Methylation:**
  EZH2 Polycomb group Methyltransferase
CRY dependent repression

Switch on BMAL1:

- **BMAL1-K537-Acetylation**: CLOCK or other acetylase, max. at CT15

→ **CRY binding to Bmal1-Ac**

→ transition to **CRY-repressed state**
Circadian gene regulation: BMAL1 acetylation and Chromatin remodeling

Activation via Chromatin

p300/CBP, MLL1

BMAL1-Deacetylation: Sirtuin1

p300/CBP displaces CRY

⇒ activation

Repression via Chromatin
(in addition to CRY repression):

SIRT1, HDACs, HP1, EZH2

L. Aguilar-Arnal, P. Sassone-Corsi;
The circadian epigenome

Clock-associated proteins
- CBP
- P300
- MLL
- DDB1–CUL4 complex
- WDR76
- Mediator complex
- TRAP150
- LSD1
- JARID1A

Clock-associated proteins
- NuRD complex (HDAC1/2)
- Sin3 complex (HDAC1/2)
- SIRT1
- SUV39

1. Active
   - BMAL1
   - CLOCK
   - E box
   - H2B ub

2. Repressed
   - PER
   - CRY
   - BMAL1
   - CLOCK
   - E box
   - H2B ub

Activation phase

Repression phase

4. Activation
   - CLOCK
   - BMAL1
   - HP1
   - HP1
   - HP1

3. Epigenetic silencing
   - HP1
   - HP1
   - HP1
   - HP1
   - H2A.Z

H3K4me
H3K9me
Acetyl group
H2A.Z

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Circadian gene regulation: BMAL1 acetylation and Chromatin remodeling

2 MDa repressive PER/CRY containing complex:
e.g. NONO (RNA binding), WDR5 (scaffold), Sin3-HDAC (Histone-Deacetylase), NuRD (HDAC), HP1-SUV39-Methyltransferase, Helicases
**The histone methyl transferase MLL1 in the circadian clock**

- MLL1 methylates histone H3 K4 circadianly
- MLL1 is activated by CBP acetylation
- MLL1 is inactivated by SIRT1 deacetylation

**Aguilar-Arnal et al., 2015:**

- MLL1 regulates circadian genes as co-activator of BMAL1/CLOCK
- Interdependance MLL1 and CBP
- SIRT1 activity is NAD+ dependent → link to metabolism?
Architecture of the early repressive complex

2. MDa repressive PER/CRY containing complex: > 30 additional proteins e.g. NONO (RNA binding), WDR5 (scaffold), Sin3-HDAC (Histone-Deacetylase), NuRD (HDAC), HP1-SUV39-Methyltransferase, Helicases
Known components of the nuclear repressive complex

Kim et al, Mol Cell, Dec 2014
Single particle negative stain EM of mammalian clock protein complexes

Mouse liver, PER2-FH mice (Flag, HA tag), BN-PAGE

- **two cytoplasmic PER/CRY-containing complexes** (0.9 and 1.1 MDa)
- **1.9 MDa repressive complex** with PER1-3, CRY1/2, CK1δ, CLOCK/BMAL1 (nuclear)
- **0.75 MDa CLOCK/BMAL1 activator complex** with BMAL1/CLOCK (nuclear)

Aryal et al, Mol Cell 2017
Protein interactions of the PERIOD2 clock protein

- Heterodimerization with CRY (C-terminal region)
- Homodimerization (PAS domains)
- CKI (middle region)

Additional protein interactions of PER2 e.g. within the repressive complex?
The CRY/PER containing repressive complex also includes WDR5

Pulldown, Mass spectrometry  Size exclusion chromatography

Nuclear extracts mouse liver

Brown et al., Science 2005

WDR5 - WD repeat-containing protein 5

Rat-1 fibroblast cell line

Co-Immunoprecipitation

At least indirect interaction of WDR5 with PER1/2

Brown et al., Science 2005

Odho et al. 2010, PDB 2XL2
Roles of WDR5 in circadian regulation

WDR5 downregulation (doxycyclin, siRNA)

→ no circadian H3 K4 (activating) and H3 K9 (repressing) methylation rhythms

→ WDR5 plays a role in circadian histone methylation
  (epigenetic circadian regulation, via MLL1 ?)

WDR5 helps repress BMAL1/CLOCK

But: WDR5 not required for circadian clock gene expression

→ no central clock function of WDR5

Brown et al., Science 2005
MLL1-WDR5-RbBP5-Ash2L-DPY30 complex enhances histone methylation activity of MLL1 SET domain

- MLL1 complex mono-, di- and tri-methylates H3K4
- Subunits MLL1, WDR5, RbBP5, Ash2L, 2 DPY-30
- MLL1 SET-domain activity increases in complex

→ WDR5 as scaffold in active histone methylation complex, binds MLL1 and RbBP5 in 2 binding sites
Role of WDR5 in circadian gene regulation in mammals?

WDR5 protein does not cycle much.

1. MLL1 co-activates BMAL1/CLOCK → WDR5 likely in activating MLL1 complex

PER2-WDR5 interaction in repressive complex?

PER2-WDR5 interaction within other cellular PER containing complexes?
Our work: Structural analyses of circadian clock mechanisms

X-ray crystallography of clock components

- > 95% pure Protein
- Protein crystal
- Rotating anode
- Synchrotron: high brilliance, variable λ X-rays

Biophysical and biochemical analyses

- e.g.
  - SDS-PAGE
  - X-rays in lab
  - FFT

- Protein crystallography pattern, “raw data”
- Electron density to build protein structure

- Protein activity, interactions, affinities, folding ...
- UV/VIS
- CD
- ITC

SDS-PAGE

Electron density to build protein structure
Our work: Structural analyses of circadian clock mechanisms