A mathematical model to understand how the liver tracks meal timing

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U1011 « Récepteurs nucléaires, maladies cardio-vasculaires et diabète »
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CEMPI scientific day,
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Lille1-Pasteur collaboration : coupling the clock to metabolism

PhLAM, Université Lille 1

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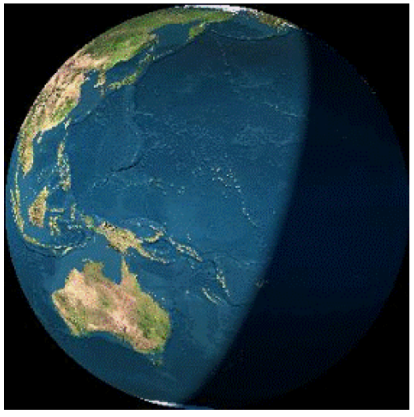
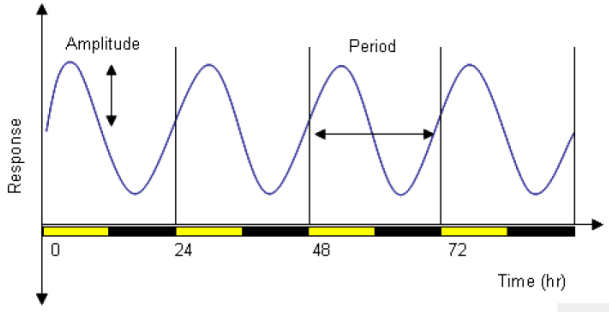
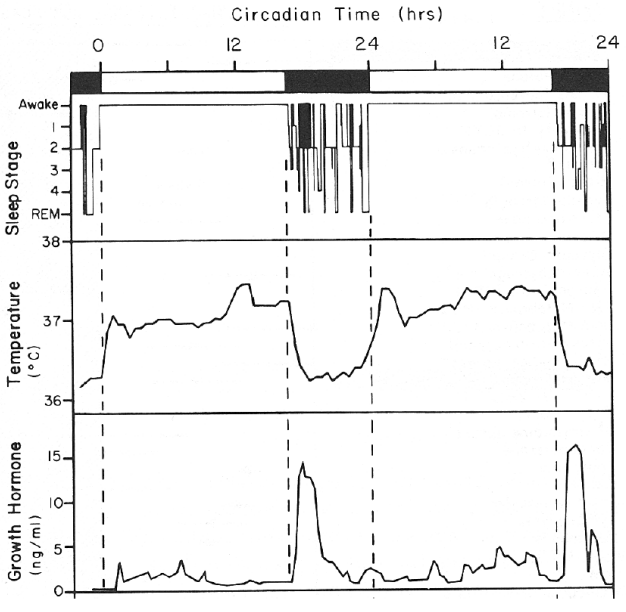
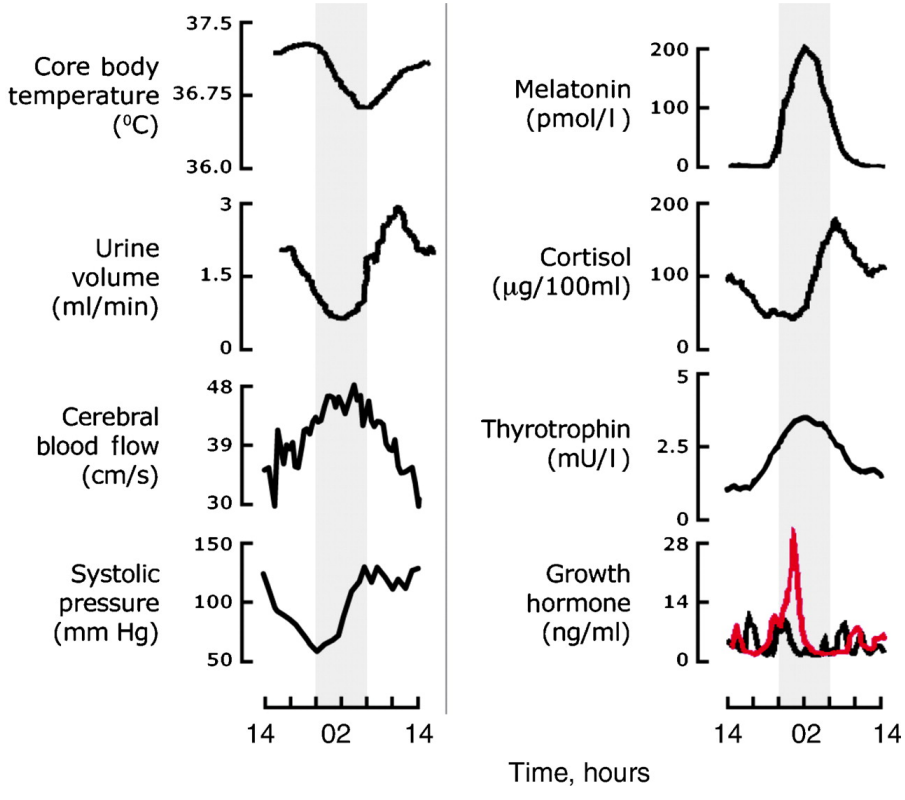
Circadian rhythms : adapting to the diurnal cycle

Circadian rhythms are physiological oscillations synchronized to day/night cycle which help organisms to anticipate daily changes in environment.



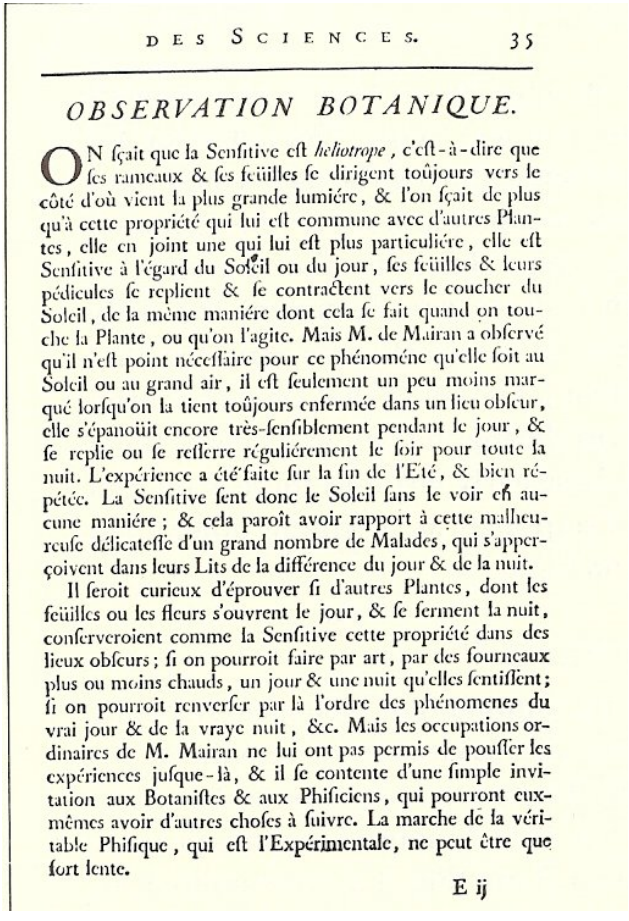






Circadian rhythms are generated by an internal clock

Circadian rythms are known since antiquity, however their endogeneous character was evidenced only in 1729 by J.-J. d'Ortous de Mairan, a french physicist and astronomer.



DES SCIENCES. 35

OBSERVATION BOTANIQUE.

ON ſçait que la Senſitive eſt *heliotrope*, c'eſt-à-dire que ſes rameaux & ſes feüilles ſe dirigent toûjours vers le côté d'où vient la plus grande lumiére, & l'on ſçait de plus qu'à cette propriété qui lui eſt commune avec d'autres Plantes, elle en joint une qui lui eſt plus particuliére, elle eſt Senſitive à l'égard du Soleil ou du jour, ſes feüilles & leurs pédicules ſe replient & ſe contractent vers le coucher du Soleil, de la même maniére dont cela ſe fait quand on touche la Plante, ou qu'on l'agite. Mais M. de Mairan a obſervé qu'il n'eſt point néceſſaire pour ce phénoméne qu'elle ſoit au Soleil ou au grand air, il eſt ſeulement un peu moins marqué lorſqu'on la tient toûjours enfermée dans un lieu obſcur, elle s'épanoüit encore très-ſenſiblement pendant le jour, & ſe replie ou ſe reſſerre réguliérement le ſoir pour toute la nuit. L'expérience a été faite ſur la fin de l'Eté, & bien répétée. La Senſitive ſent donc le Soleil ſans le voir en aucune maniére; & cela paroît avoir rapport à cette malheureuſe délicateſſe d'un grand nombre de Malades, qui s'apperçoivent dans leurs Lits de la différence du jour & de la nuit.

Il ſeroit curieux d'éprouver ſi d'autres Plantes, dont les feüilles ou les fleurs s'ouvrent le jour, & ſe ferment la nuit, conſerveroient comme la Senſitive cette propriété dans des lieux obſcurs; ſi on pourroit faire par art, par des fourneaux plus ou moins chauds, un jour & une nuit qu'elles ſentiſſent; ſi on pourroit renverſer par là l'ordre des phénomenes du vrai jour & de la vraye nuit, &c. Mais les occupations ordinaires de M. Mairan ne lui ont pas permis de pouſſer les expériences juſque-là, & il ſe contente d'une ſimple invitation aux Botaniſtes & aux Phiſiciens, qui pourront eux-mêmes avoir d'autres choſes à ſuivre. La marche de la véritable Phiſique, qui eſt l'Expérimentale, ne peut être que fort lente.

E ij

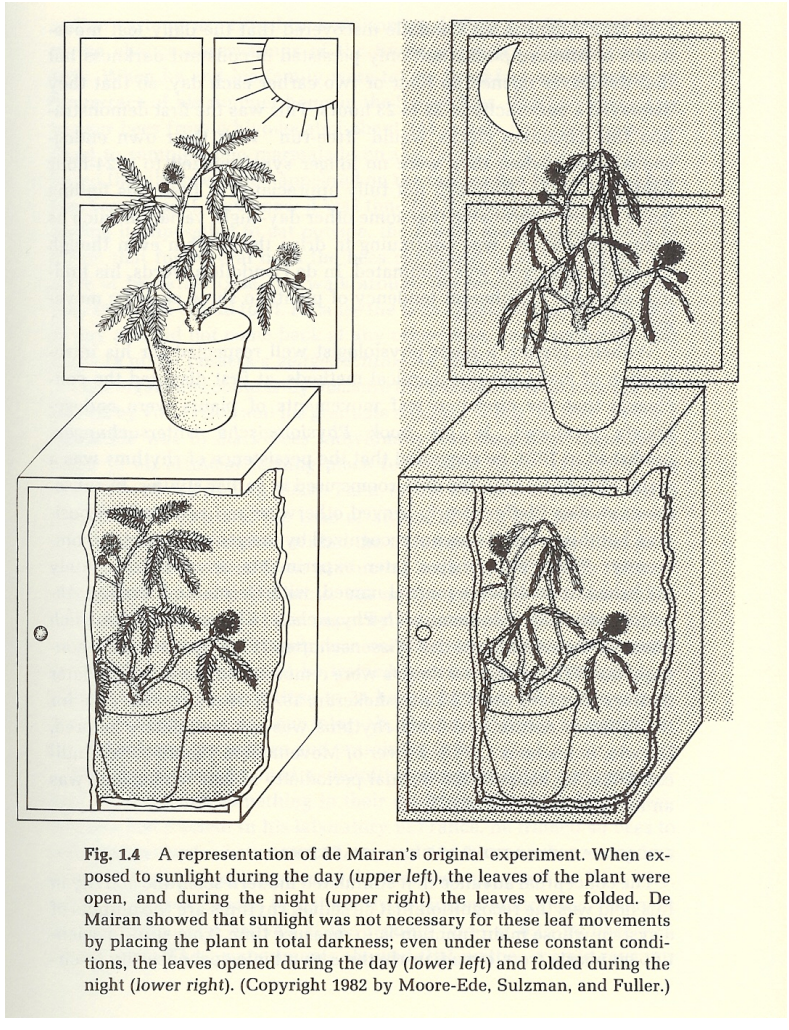


Fig. 1.4 A representation of de Mairan's original experiment. When exposed to sunlight during the day (*upper left*), the leaves of the plant were open, and during the night (*upper right*) the leaves were folded. De Mairan showed that sunlight was not necessary for these leaf movements by placing the plant in total darkness; even under these constant conditions, the leaves opened during the day (*lower left*) and folded during the night (*lower right*). (Copyright 1982 by Moore-Ede, Sulzman, and Fuller.)

Leaf oscillations of *Mimosa pudica* persist in the dark.

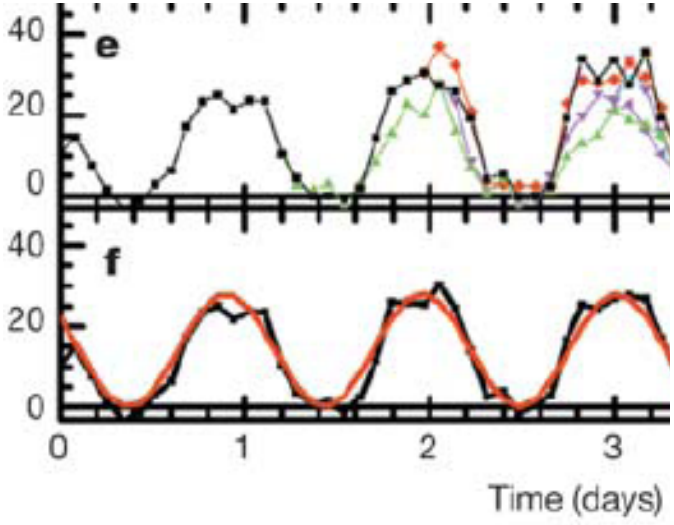
▸ Circadian oscillations of bean leaves

Circadian clocks are genetic oscillators

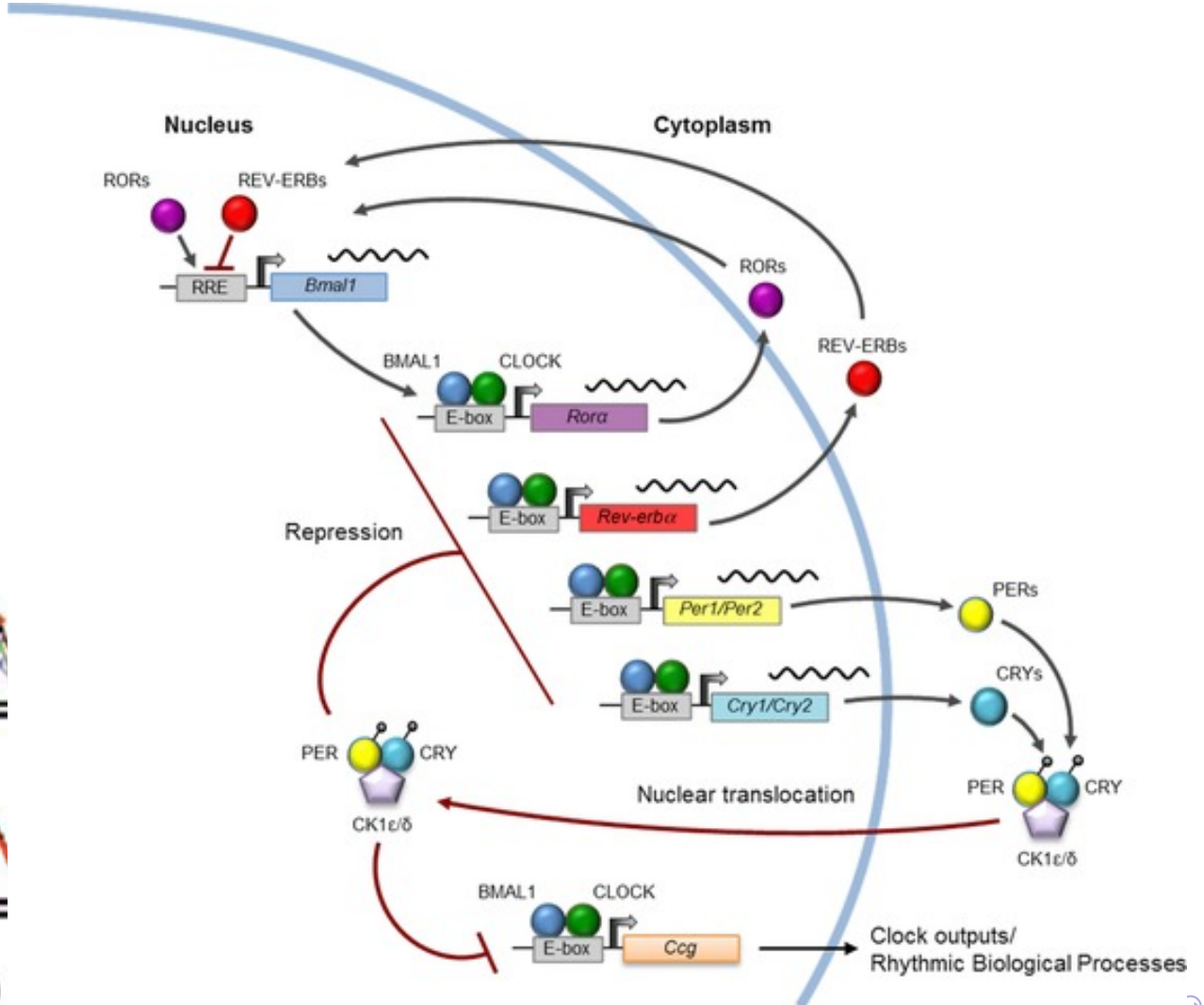
- **Bünning (1935)** Free-running period is an inherited property.
- **Konopka and Benzer (1971)** Mutation of *Per* gene in *Drosophila* induces variations in period or arrhythmia.

Circadian clocks are networks of interacting genes and proteins whose activities oscillate over a 24-hour interval.

Circadian oscillations in gene activity in cyanobacteria

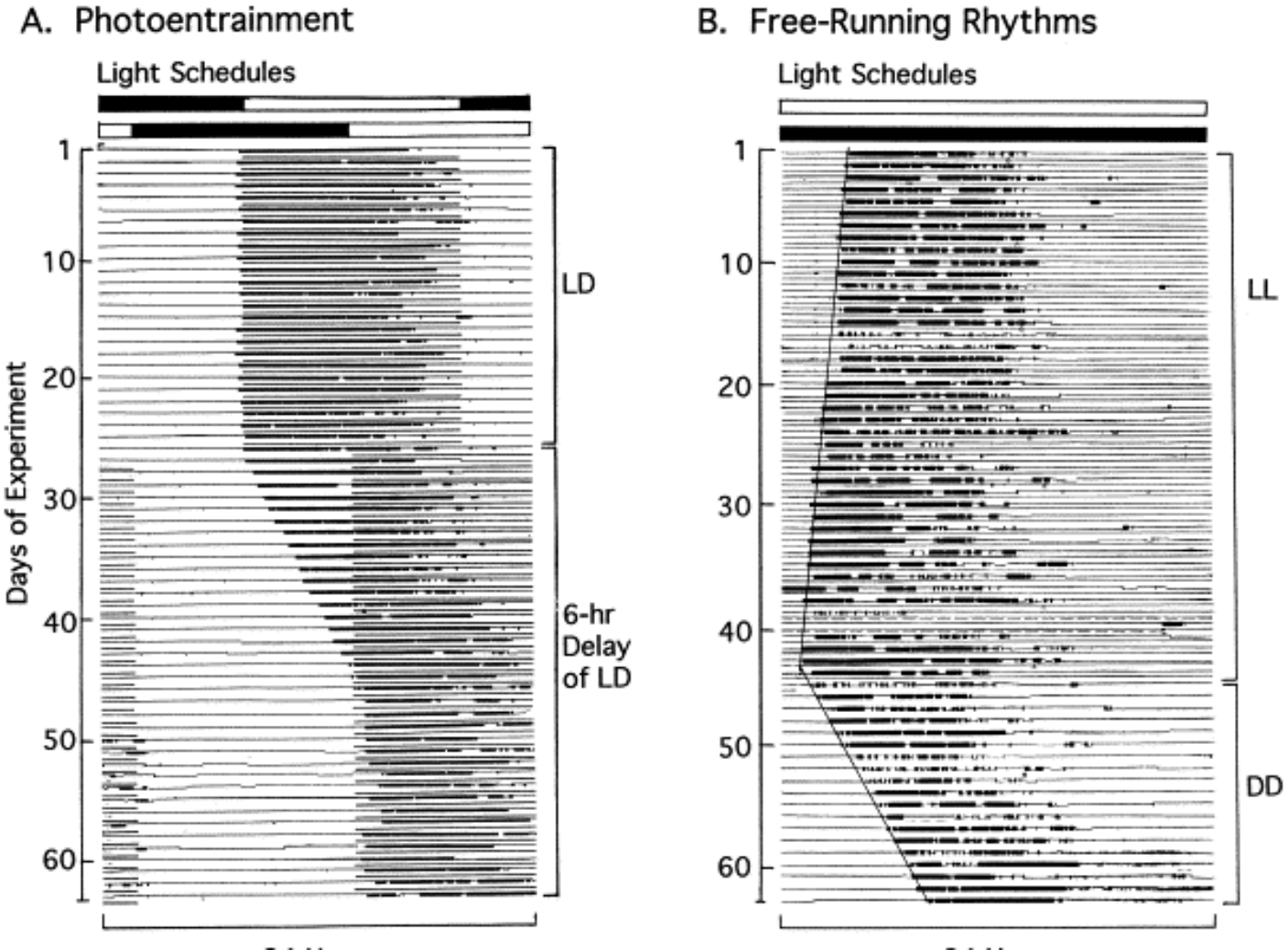


Core mammalian clock network



Time keeping requires entrainment by an external cycle

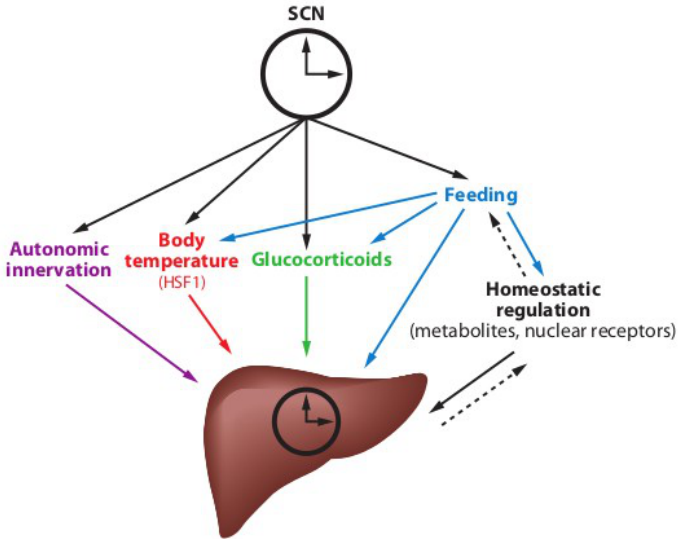
Wheel-running activity of squirrels

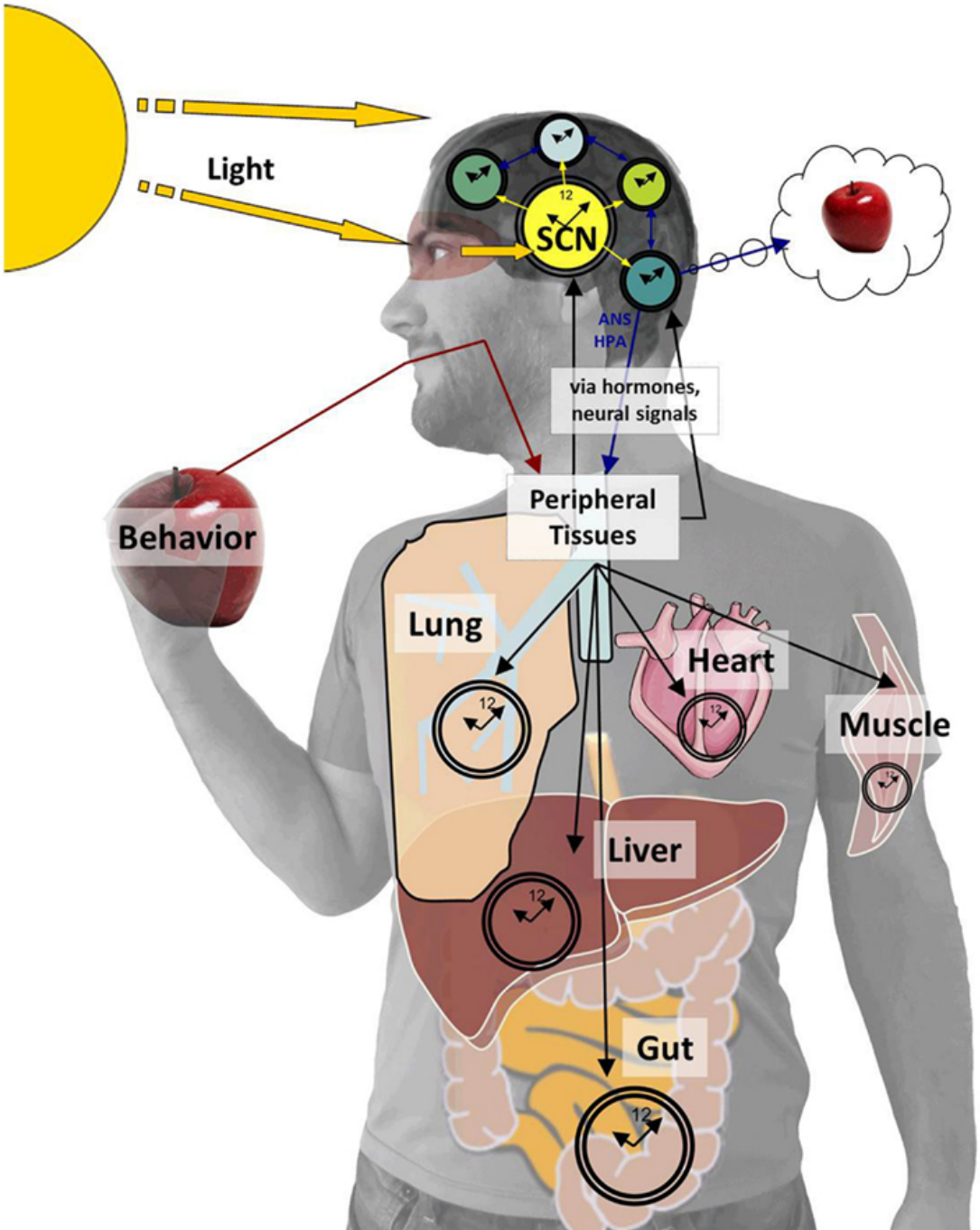


A network of circadian clocks

Our internal rhythms are governed a network of interconnected clocks in peripheral organs which synchronize to various signals

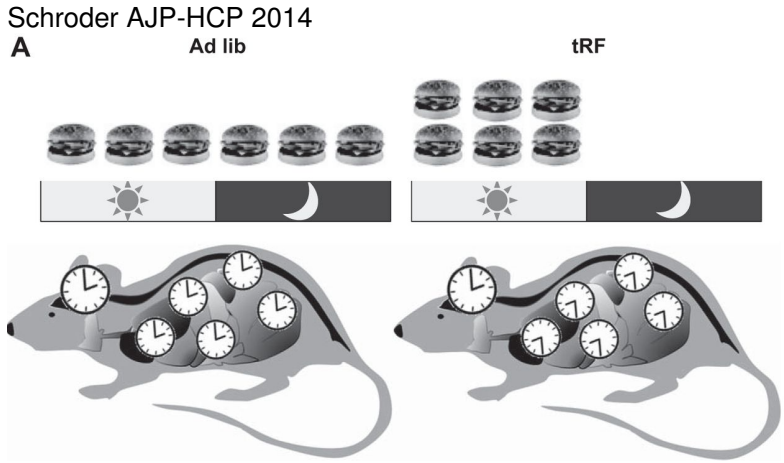
Only the master clock in the brain (SCN) sees the light directly

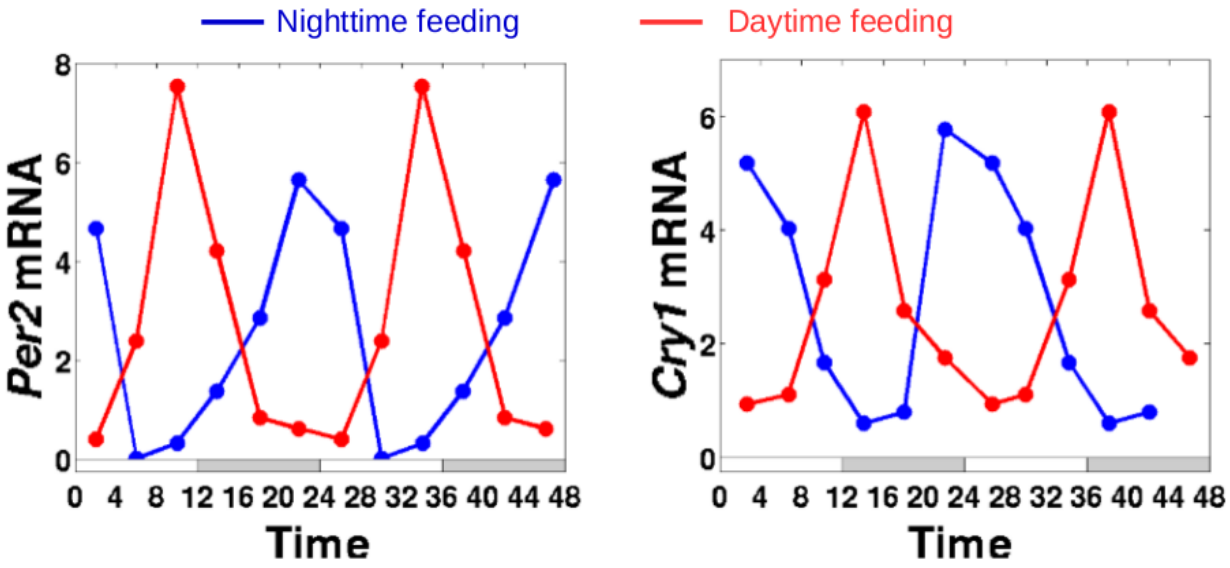




The liver clock is entrained by feeding/fasting cycles

Feed mice exclusively during the day, which is the normal rest period





Clock gene activity profiles in liver of mice fed during day vs during night

Damiola Genes Dev 2000

Daytime feeding changes the phase of clock gene expression in liver but not in the master clock

Circadian rhythms of metabolism

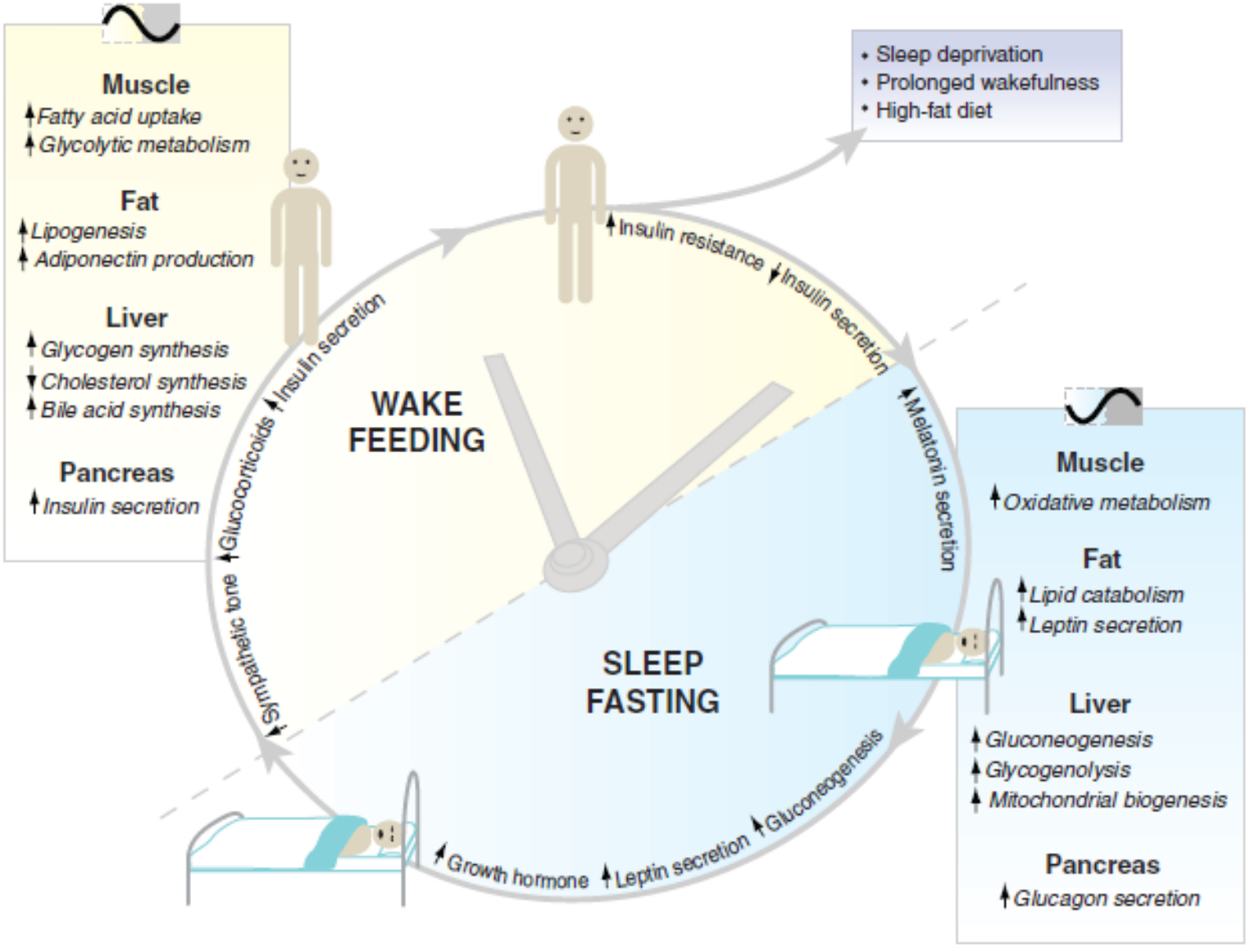
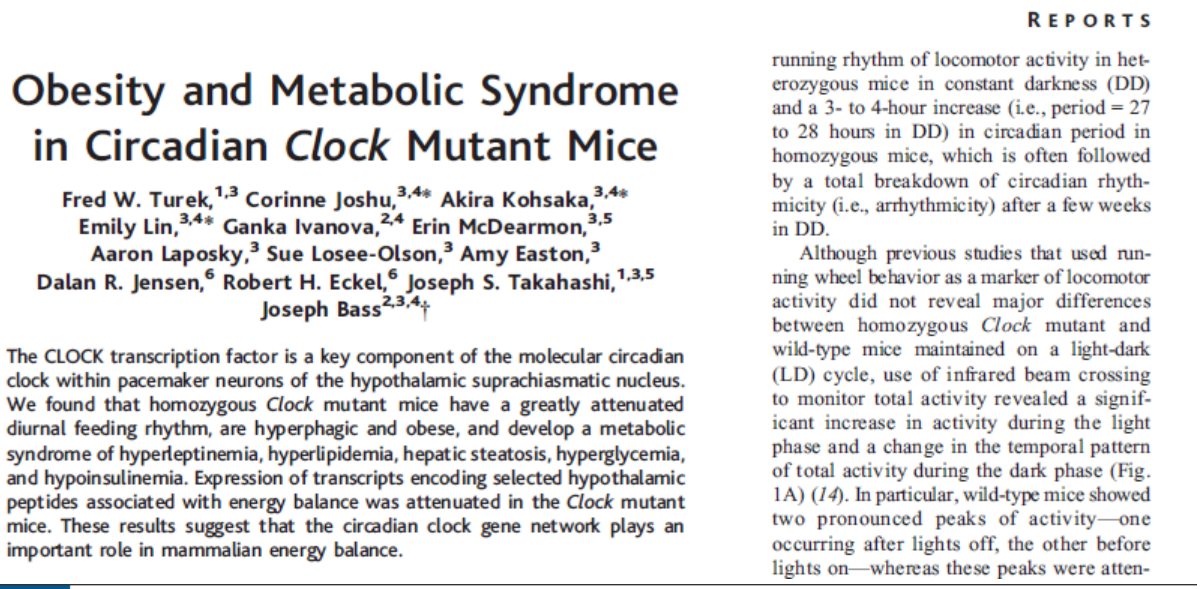


Fig. 3. The clock partitions behavioral and metabolic processes according to time of day. The clock coordinates appropriate metabolic responses within peripheral tissues with the light/dark cycle. For example, the liver clock promotes

Clock and metabolism interact strongly



Obesity and Metabolic Syndrome in Circadian *Clock* Mutant Mice

Fred W. Turek,[1,3] Corinne Joshu,[3,4*] Akira Kohsaka,[3,4*] Emily Lin,[3,4*] Ganka Ivanova,[2,4] Erin McDearmon,[3,5] Aaron Laposky,[3] Sue Losee-Olson,[3] Amy Easton,[3] Dalan R. Jensen,[6] Robert H. Eckel,[6] Joseph S. Takahashi,[1,3,5] Joseph Bass[2,3,4†]

The CLOCK transcription factor is a key component of the molecular circadian clock within pacemaker neurons of the hypothalamic suprachiasmatic nucleus. We found that homozygous *Clock* mutant mice have a greatly attenuated diurnal feeding rhythm, are hyperphagic and obese, and develop a metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia, and hypoinsulinemia. Expression of transcripts encoding selected hypothalamic peptides associated with energy balance was attenuated in the *Clock* mutant mice. These results suggest that the circadian clock gene network plays an important role in mammalian energy balance.

running rhythm of locomotor activity in heterozygous mice in constant darkness (DD) and a 3- to 4-hour increase (i.e., period = 27 to 28 hours in DD) in circadian period in homozygous mice, which is often followed by a total breakdown of circadian rhythmicity (i.e., arrhythmicity) after a few weeks in DD.

Although previous studies that used running wheel behavior as a marker of locomotor activity did not reveal major differences between homozygous *Clock* mutant and wild-type mice maintained on a light-dark (LD) cycle, use of infrared beam crossing to monitor total activity revealed a significant increase in activity during the light phase and a change in the temporal pattern of total activity during the dark phase (Fig. 1A) (*14*). In particular, wild-type mice showed two pronounced peaks of activity—one occurring after lights off, the other before lights on—whereas these peaks were atten-

Inactivating the clock leads to severe metabolic diseases

Cell Metabolism
Short Article

High-Fat Diet Disrupts Behavioral and Molecular Circadian Rhythms in Mice

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DOI 10.1016/j.cmet.2007.09.006

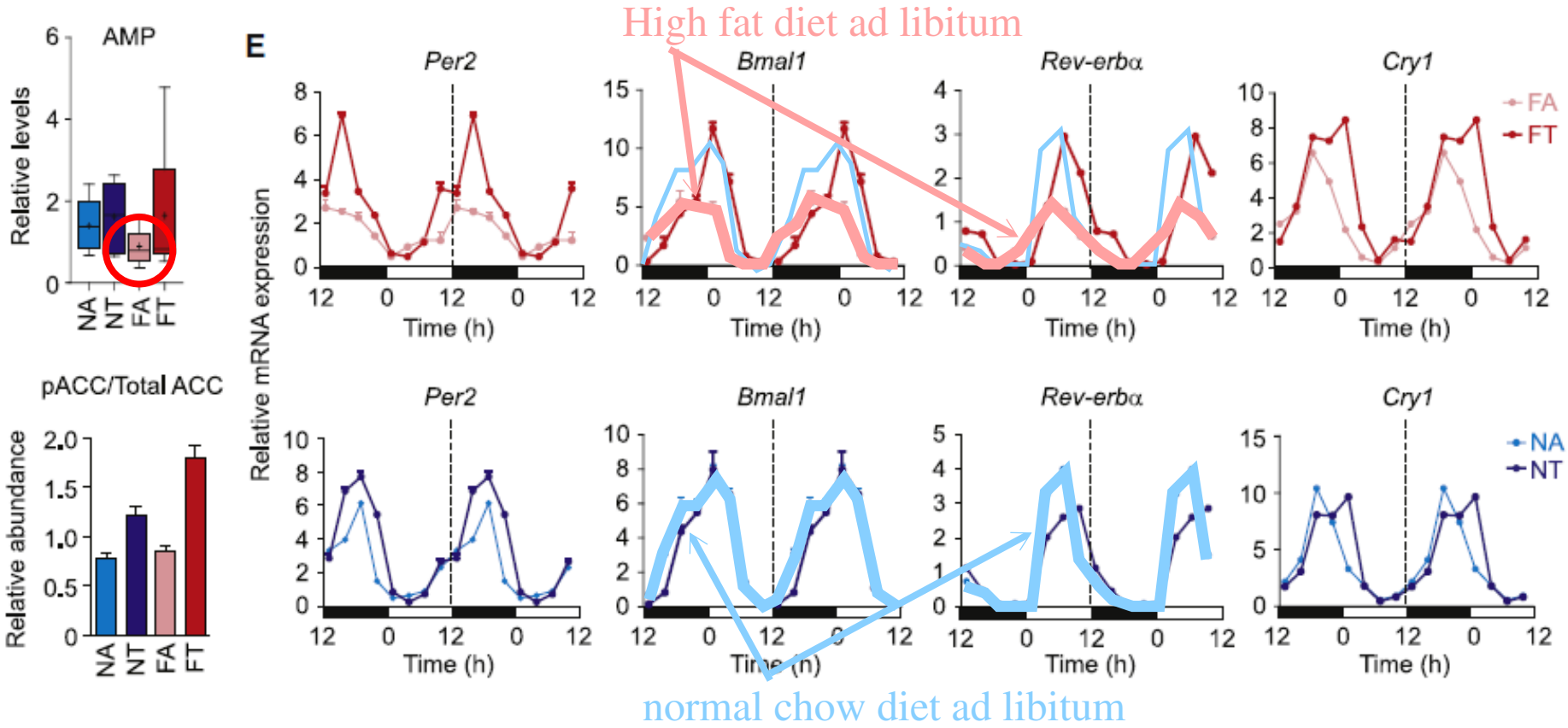
Metabolic stress disrupts circadian rhythms

How to describe mathematically the coupling of clock and metabolism ?

High fat diet (HFD) disrupts the clock

In HFD, the feeding/fasting cycles entraining the clock are perturbed and are associated with lower AMP levels

Hatori et al. Cell Metab 2012



Typically, the amplitude of gene activity oscillations is dampened.

Questions

How do feeding/fasting cycles entrain the clock ?

What are the metabolic sensors (“nutrireceptors”) ?

Can we build a mathematical model of the clock with these sensors ?

Can it explain how perturbations in feeding/fasting disrupt the clock ?

Can we design a pharmacological protocol to restore normal clock profiles ?

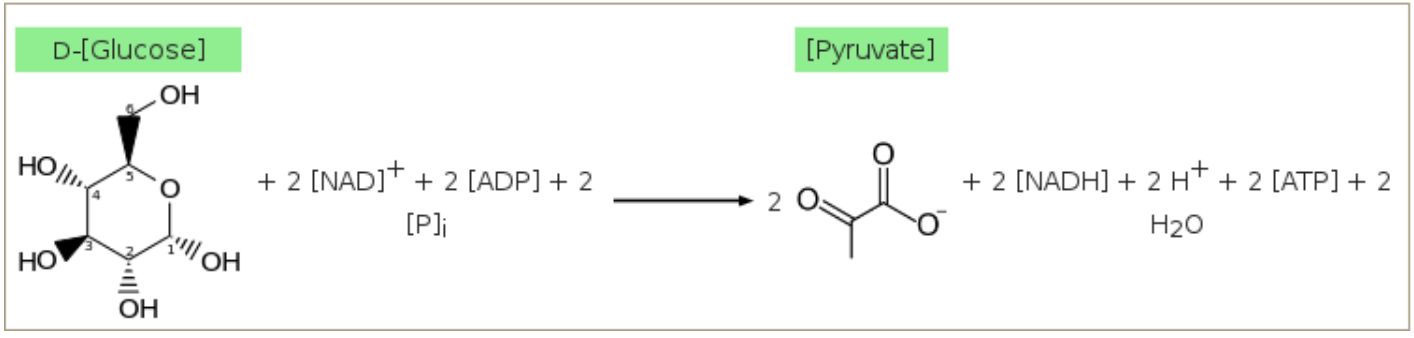
How can the clock sense metabolism ?

Main gauges of cellular metabolic state : NAD+/NADH, AMP/ADP/ATP

ATP is the cell fuel (e.g., muscle contraction)

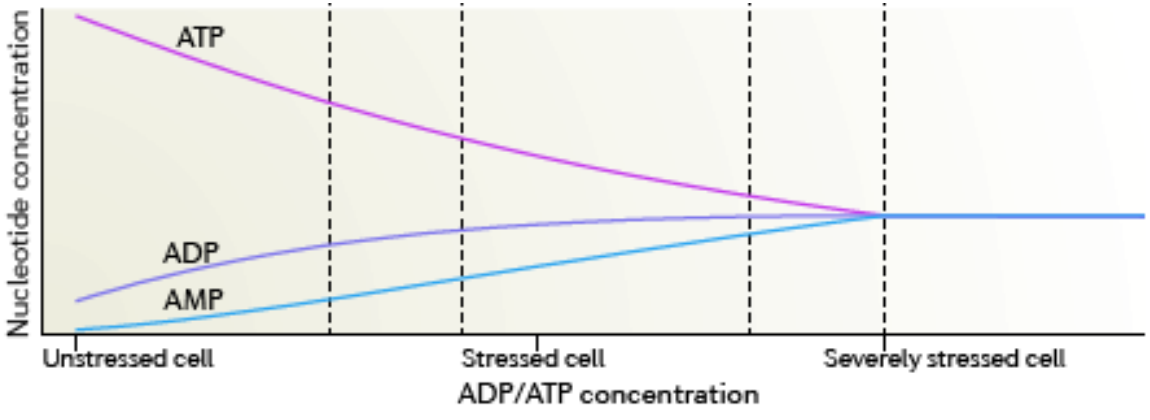
Metabolic reactions consume or produce ATP (ATP $\leftrightarrow$ ADP $\leftrightarrow$ AMP) and convert NAD+ to NADH or vice versa

Glycolysis

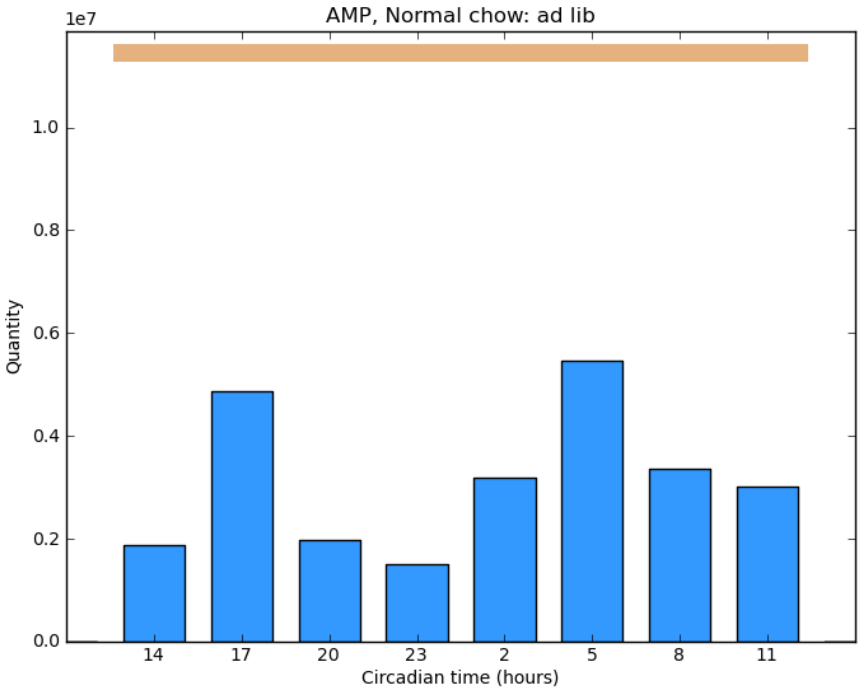


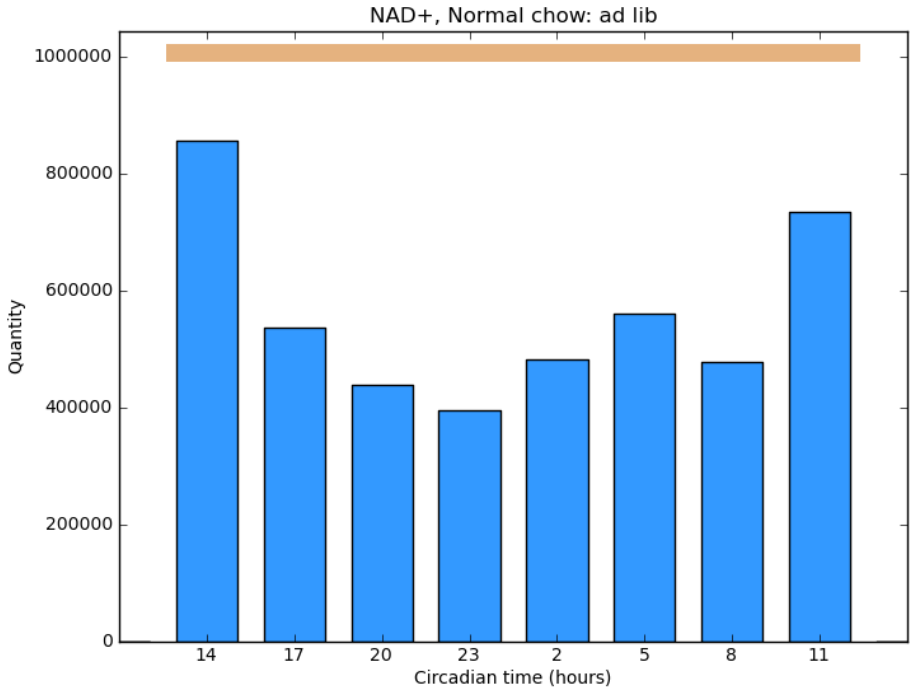
ADP+ADP $\longleftrightarrow$ ATP + AMP

Low AMP : energy-full
High AMP : energy-deprived



Nad+ and AMP display daily variations

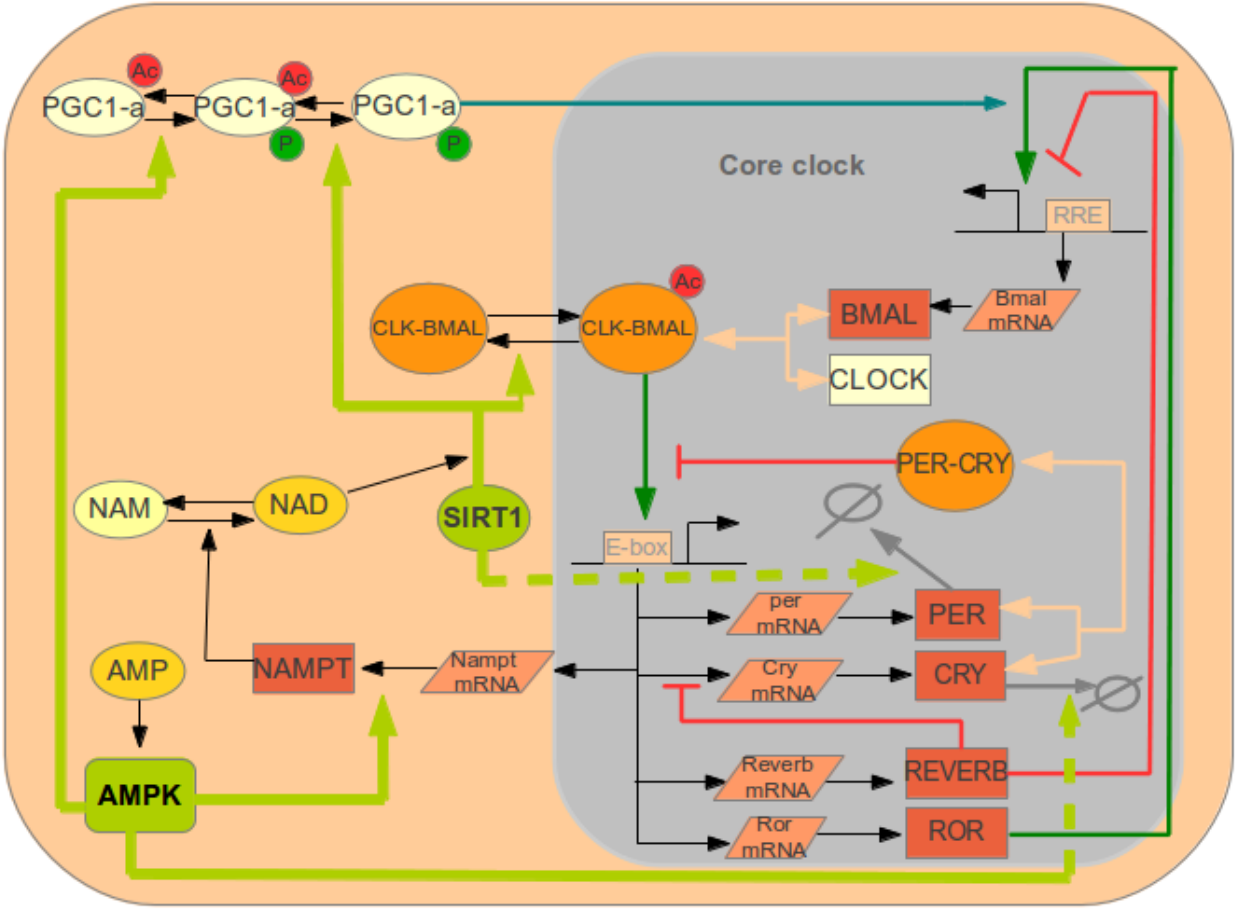




Note the presence of two peaks, including one at ZT5 and another one at ZT14-ZT17

Basic network coupling the clock to metabolism

NAD+ and AMP are important metabolites characterizing the cell metabolic state, and influence the circadian clock through SIRT1 (activated by NAD+), and AMPK (activated by AMP).



SIRT1 inhibits CLOCK/BMAL1 activity

SIRT1 deacetylates PGC1a which coactivates Bmal1 with RORa

SIRT1 deacetylates PER2 and destabilizes it

AMPK destabilizes CRY1 and indirectly PER,

AMPK enables deacetylation of PGC1a by SIRT1

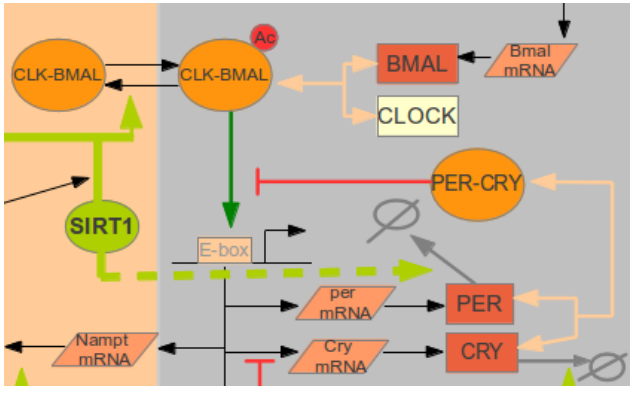
AMPK stabilizes NAMPT

Translating networks into differential equations

A gene is active when it synthesizes messenger RNA (transcription)

* The *Per* gene can be in 3 states, each with a different mRNA synthesis rate

1. bare DNA (low activity)
2. BMAL1-CLOCK protein complex bound to DNA (high activity)
3. PER-CRY complex bound to BMAL1-CLOCK bound to DNA (reduced activity)



* The fraction of time spent in each state is determined by the chemical equilibrium of

$$G \Longleftrightarrow G : CB \Longleftrightarrow G : CB : PC$$

* Affinity of CLOCK/BMAL1 to DNA is reduced by SIRT1

Rate of change of *Per* mRNA

Synthesis

Degradation

$$\frac{\mathrm{d}[Per]}{\mathrm{d}t} = \left(\frac{\mathrm{Vmax} \cdot \left(1 + \mathrm{fold} \cdot \left(\frac{[\mathrm{CB}]}{\mathrm{Ka} \cdot (1 + \mathrm{Act\_SIRT})}\right)^{\mathrm{hill\_cb}}\right)}{1 + \left(\frac{[\mathrm{CB}]}{\mathrm{Ka} \cdot (1 + \mathrm{Act\_SIRT})}\right)^{\mathrm{hill\_cb}} \cdot \left(1 + \left(\frac{[\mathrm{PC}]}{\mathrm{Ki}}\right)^{\mathrm{hill\_pc}}\right)} \right) - (\mathrm{dm} \cdot [Per])$$

Mathematical model

$$\frac{d([mPer]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dmper\cdot[mPer]) + V_{compartment}\cdot\left(\frac{basal\_mper\cdot\left(1+fold\_per\cdot\left(\frac{[CB]}{thr\_percb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_percb}\right)}{1+\left(1+\left(\frac{[PC]}{thr\_perpc}\right)^{hill\_perpc}\right)\cdot\left(\frac{[CB]}{thr\_percb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_percb}}\right)$$

$$\frac{d([mCry]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dmcry\cdot[mCry]) + V_{compartment}\cdot\left(\frac{basal\_mcry\cdot\left(1+fold\_cry\cdot\left(\frac{[CB]}{thr\_crycb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_crycb}\right)}{1+\left(1+\left(\frac{[PC]}{thr\_crypc}\right)^{hill\_crypc}\right)\cdot\left(\frac{[CB]}{thr\_crycb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_crycb}}\cdot\frac{1}{1+\left(\frac{[REVERB]}{thr\_cryrev}\right)^{hill\_cryrev}}\right)$$

$$\frac{d([mRevErb]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dmrev\cdot[mRevErb]) + V_{compartment}\cdot\left(\frac{basal\_mrev\cdot\left(1+fold\_rev\cdot\left(\frac{[CB]}{thr\_revcb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_revcb}\right)}{1+\left(1+\left(\frac{[PC]}{thr\_revpc}\right)^{hill\_revpc}\right)\cdot\left(\frac{[CB]}{thr\_revcb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_revcb}}\right)$$

$$\frac{d([mROR]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dmror\cdot[mROR]) + V_{compartment}\cdot\left(\frac{basal\_mror\cdot\left(1+fold\_ror\cdot\left(\frac{[CB]}{thr\_rorcb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_rorcb}\right)}{1+\left(1+\left(\frac{[PC]}{thr\_rorpc}\right)^{hill\_rorpc}\right)\cdot\left(\frac{[CB]}{thr\_rorcb\cdot(1+c\_srt\cdot[Sirt1])}\right)^{hill\_rorcb}}\cdot\frac{1}{1+\left(\frac{[REVERB]}{thr\_rorrev}\right)^{hill\_rorrev}}\right)$$

$$\frac{d([mBmal]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dmbmal\cdot[mBmal]) + V_{compartment}\cdot\left(\frac{basal\_mbmal\cdot\left(1+fold\_bmal\cdot(1+c\_pgc\cdot[PGC1])\cdot\left(\frac{[ROR]}{thr\_bmalror}\right)^{hill\_bmalror}\right)}{1+\left(\frac{[REVERB]}{thr\_bmalrev}\right)^{hill\_bmalrev}+\left(\frac{[ROR]}{thr\_bmalror}\right)^{hill\_bmalror}}\right)$$

$$\frac{d([PER]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dper\cdot[PER]) + V_{compartment}\cdot(trans\_per\cdot[mPer]) - V_{compartment}\cdot((kf\_pc\cdot[CRY]\cdot[PER]-kb\_pc\cdot[PC]))$$

$$\frac{d([CRY]\cdot V_{compartment})}{dt} = -V_{compartment}\cdot(dpcry\cdot[CRY]) + V_{compartment}\cdot(trans\_cry\cdot[mCry])$$

16 differential equations describe the time evolution of messenger RNA concentration (=gene activity) and protein concentration.

The model has 96 kinetic constants (mRNA and protein degradation rates, transcription and translation rates, ...) which are mostly unknown and must be estimated from experimental data.

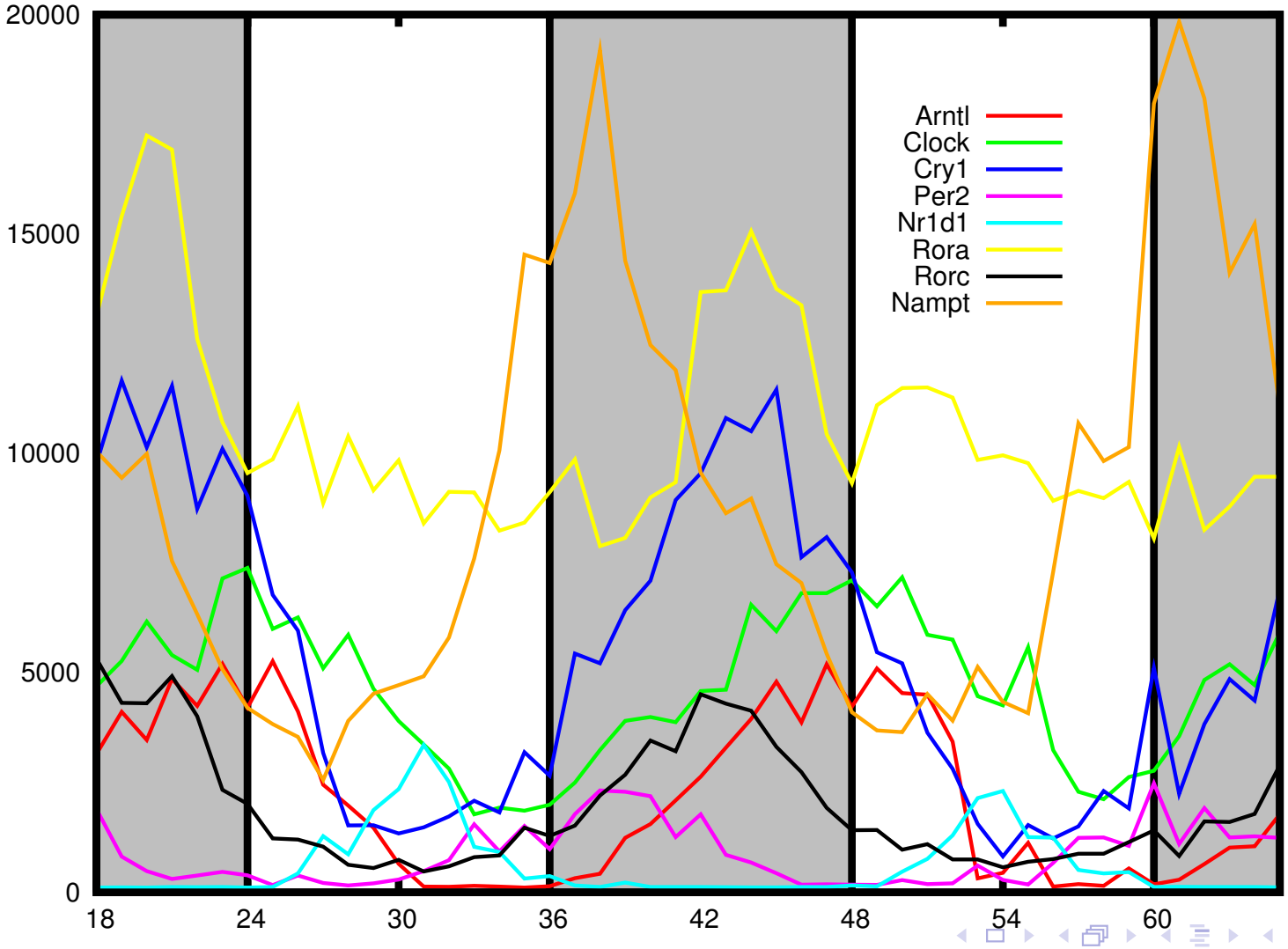
Expression data from mouse livers

OPEN ACCESS Freely available online PLOS GENETICS

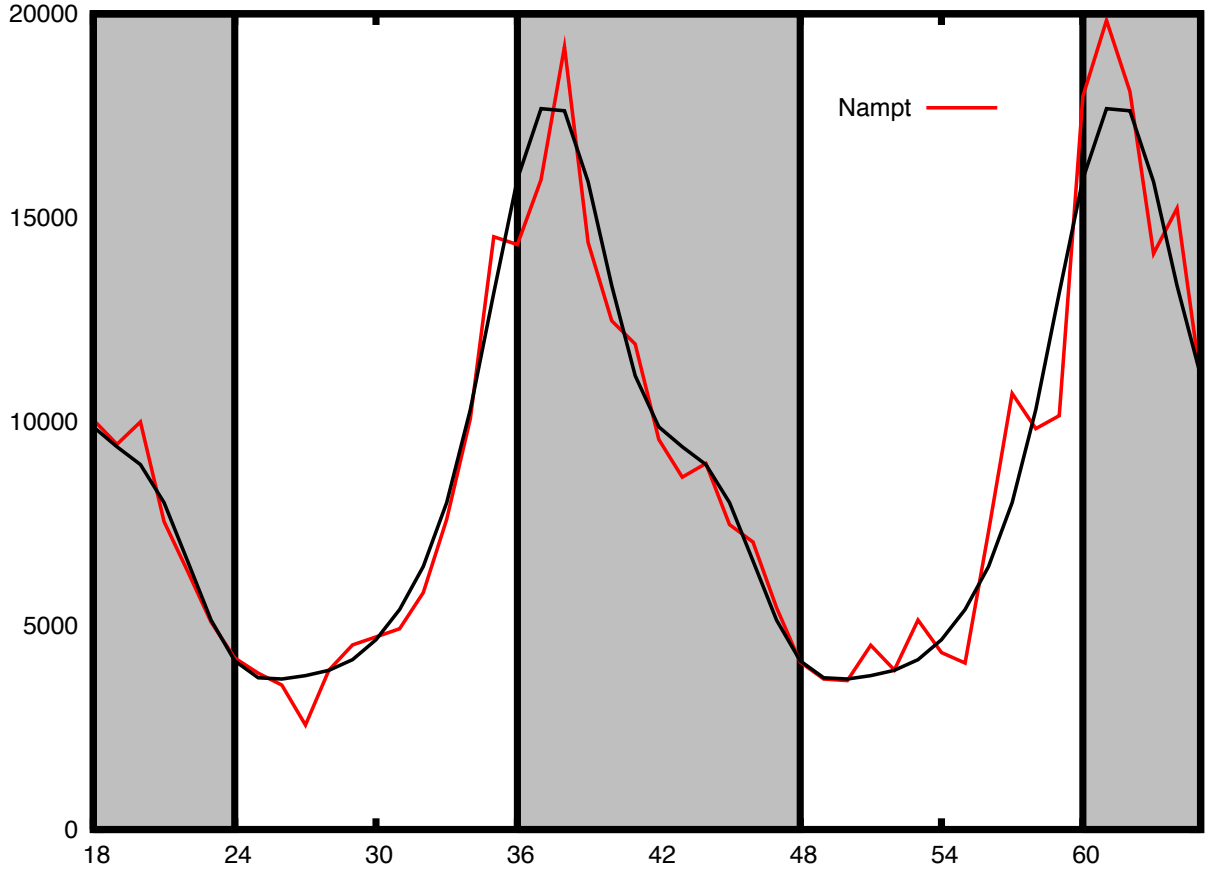
Harmonics of Circadian Gene Transcription in Mammals

Michael E. Hughes[1☯], Luciano DiTacchio[2☯], Kevin R. Hayes[1], Christopher Vollmers[2], S. Pulivarthy[2], Julie E. Baggs[1], Satchidananda Panda[2*], John B. Hogenesch[1*]

Hugues Plos Genet. 2009



Approximation of exp. data with Fourier Series



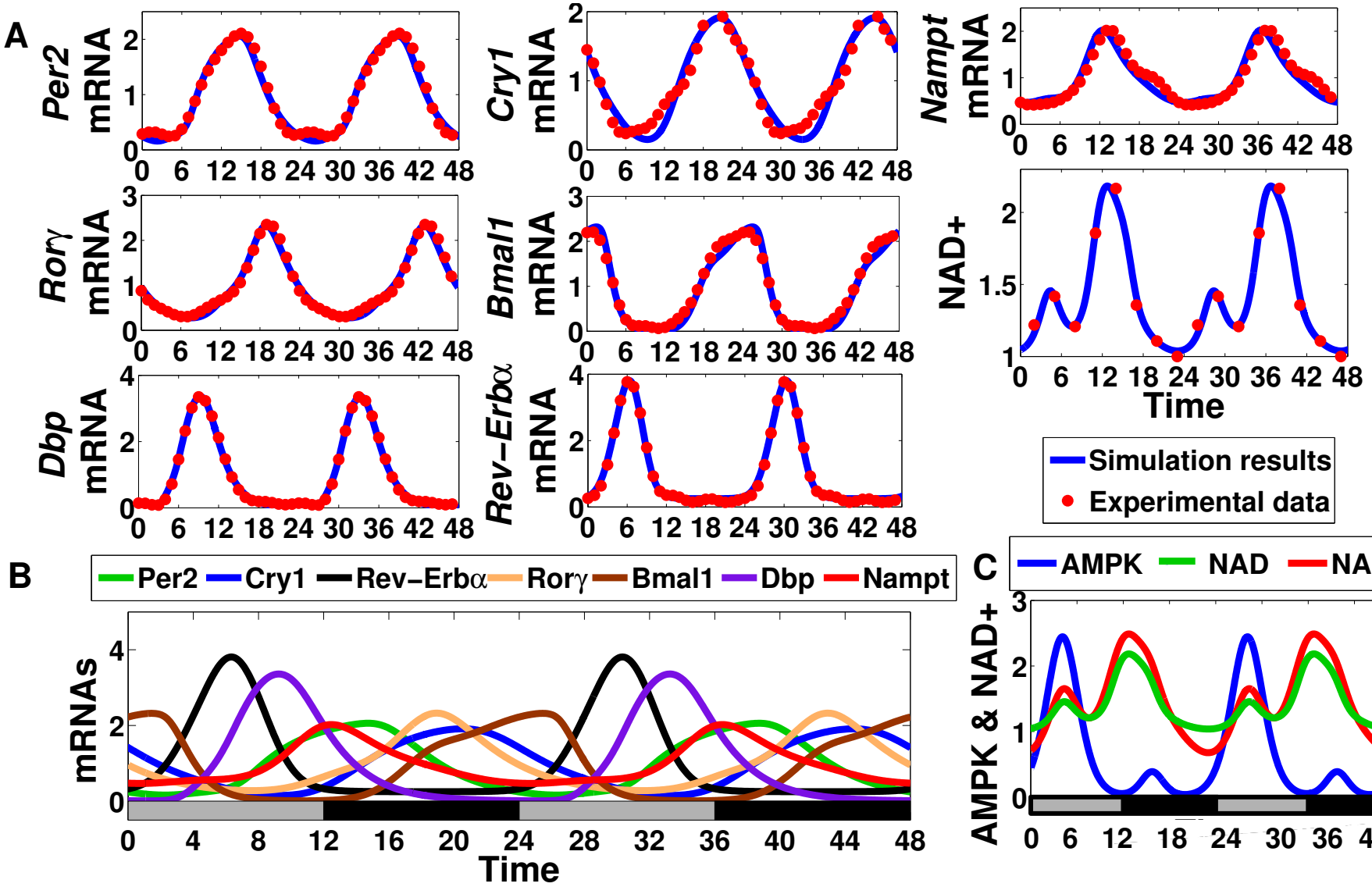
Mice put in DD and fed at libitum.

Fourier series and microarray expression time profile

Even though the data are obtained in vivo, they show a very good reproducibility from one day to the next

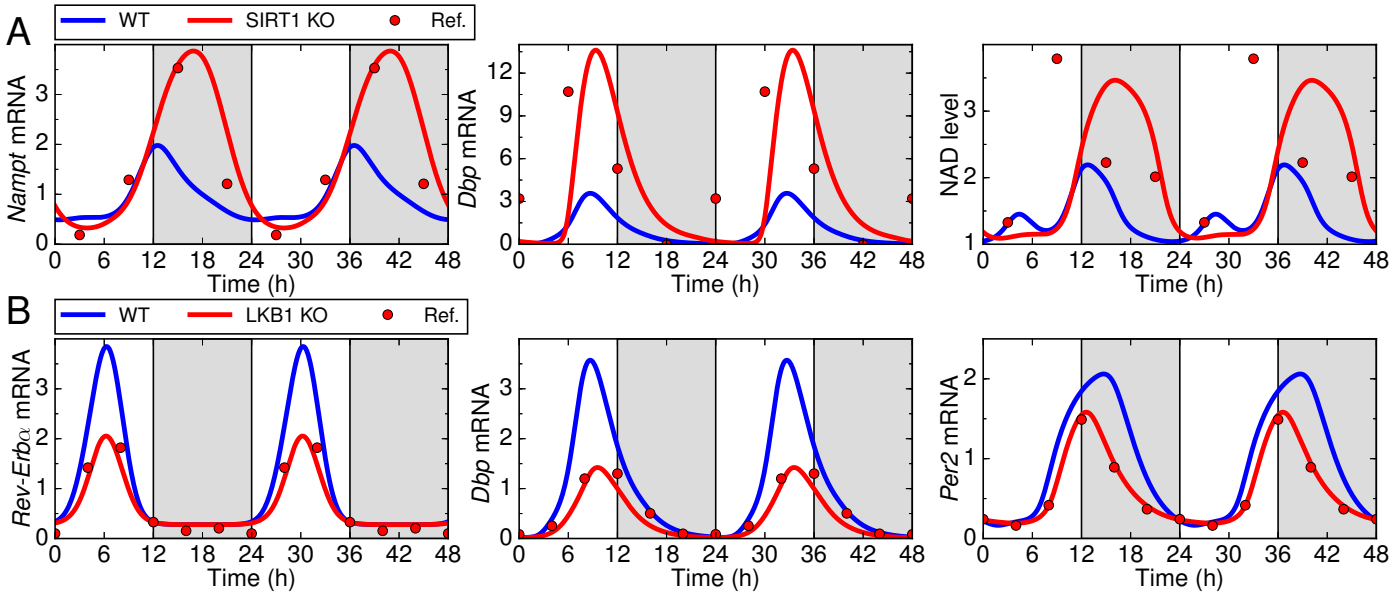
Adjustment of model to experimental data

Computationally intensive parameter estimation (96 kinetic constants)

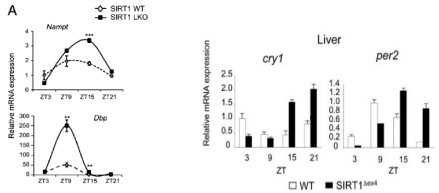


WT clock gene expression data and NAD+ profiles well reproduced

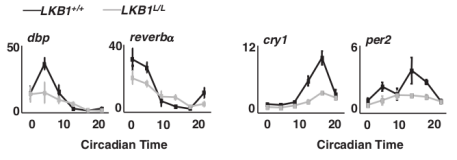
Reproducing Sirt1 and AMPK loss of function



Bellet PNAS 2013



Lamia Science 2009



Mutant phenotypes are well reproduced.

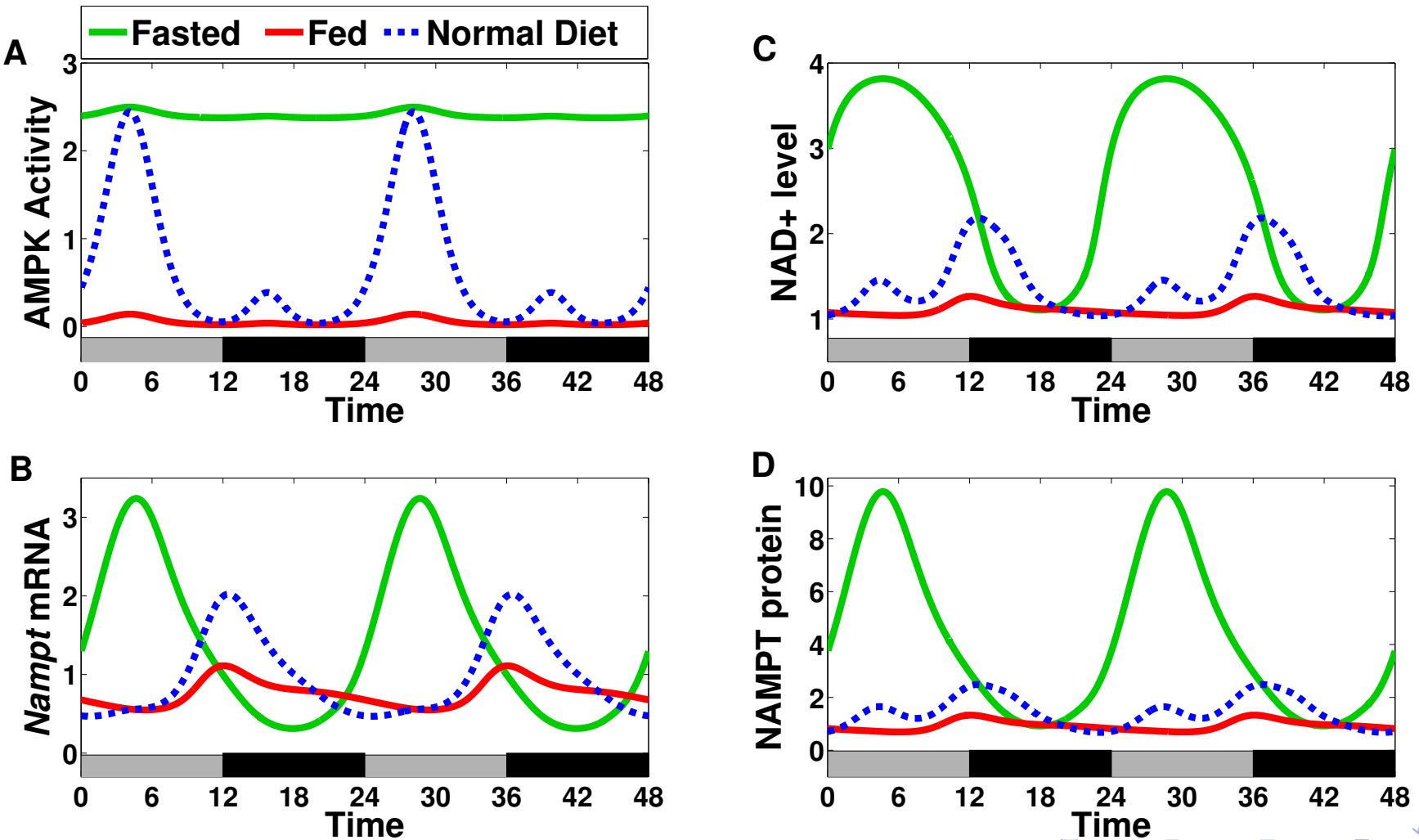
Knocking down Sirt1 generally amplifies oscillations in clock gene expression

Knocking down LKB1, hence disactivating AMPK, generally dampens oscillations in clock gene expression

Understanding the effect of AMPK rhythms

Simulate

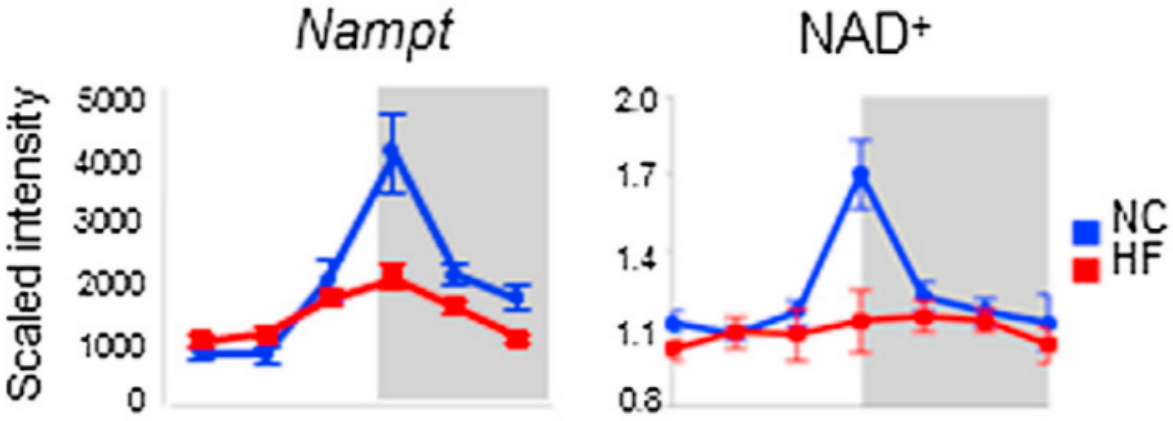
- A constantly fed-like state (AMPK activity constitutively low)
- Alternation of fasting and feeding (oscillating AMPK activity)
- A constantly fasting state (AMPK activity constitutively high)



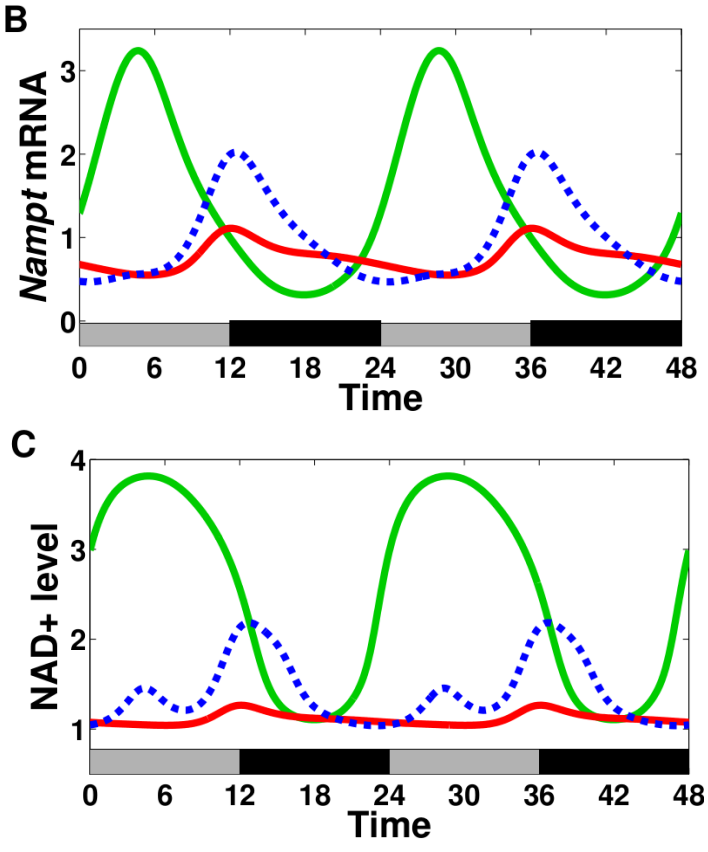
Model reproduces the loss of NAD+ oscillations

Loss of NAD+ oscillations as observed in a high-fat diet is potentially harmful

Eckel-Mahan et al. Cell 2013



Mathematical model



NAD+ peak essential for oxidative metabolism (Peek et al. Science 2013).

In obesity or type 2 diabetes, AMPK is systematically depressed regardless of the regimen.

A pharmacological approach is needed to restore clock function

Pharmacological action on the clock

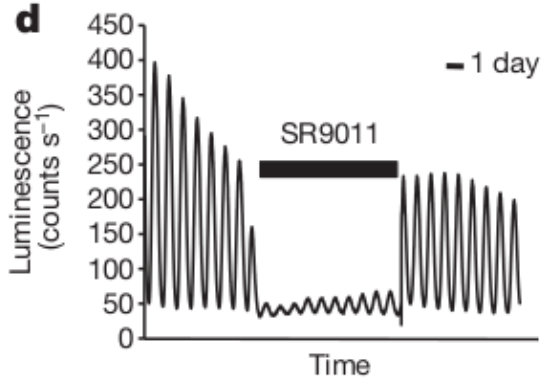
ARTICLE

doi:10.1038/nature11030

Regulation of circadian behaviour and metabolism by synthetic REV–ERB agonists

Laura A. Solt[1]*, Yongjun Wang[1]*, Subhashis Banerjee[1], Travis Hughes[1], Douglas J. Kojetin[1], Thomas Lundasen[1], Youseung Shin[2], Jin Liu[1], Michael D. Cameron[2], Romain Noel[2], Seung-Hee Yoo[3], Joseph S. Takahashi[3], Andrew A. Butler[4], Theodore M. Kamenecka[2] & Thomas P. Burris[1,5]

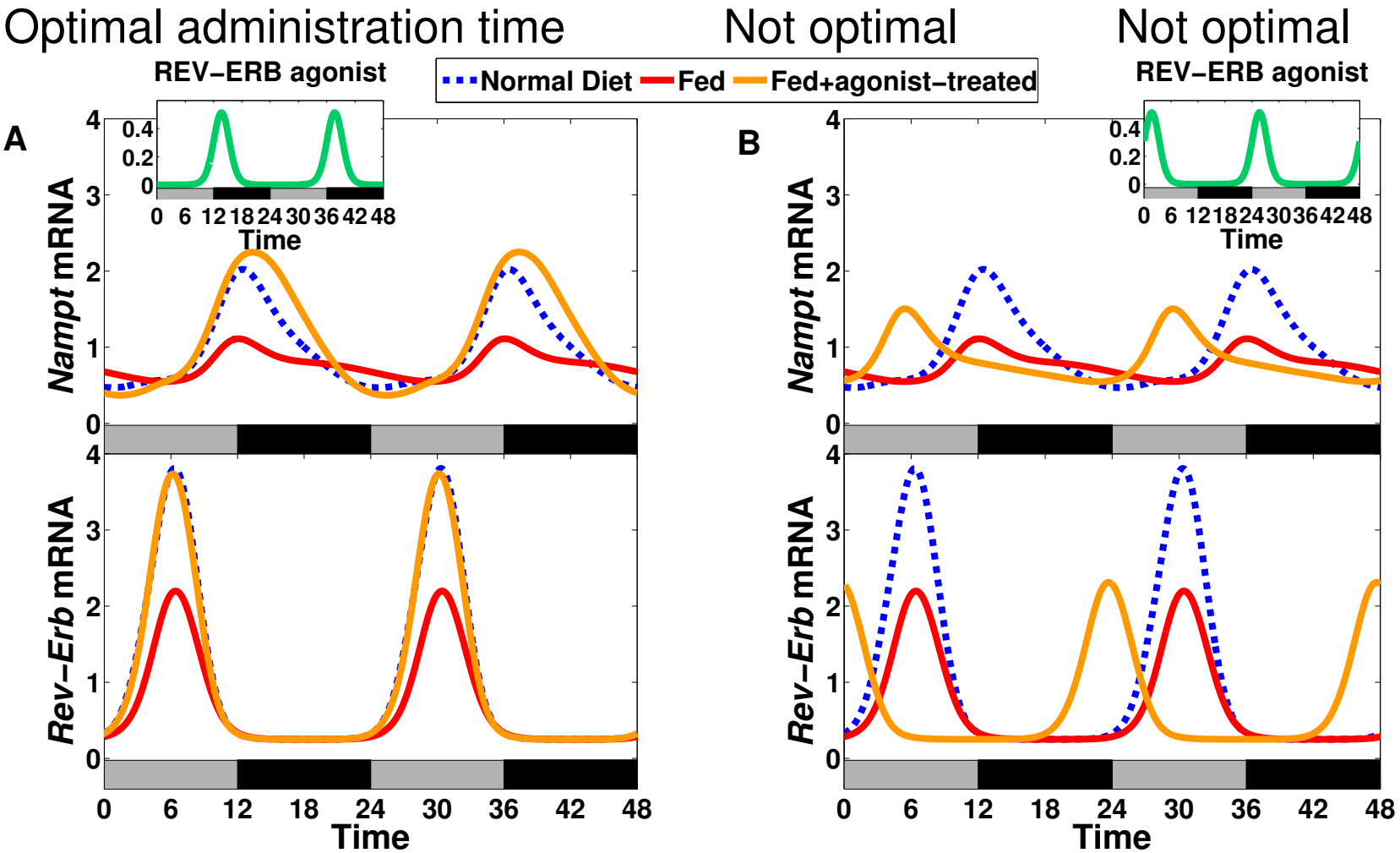
Drugs that can transiently modulate the activity of the core clock protein REV-ERBα have recently become available.



When a REV-ERB agonist is administered for several days, clock oscillations are abolished due to strong repression by REV-ERB.

Can we restore normal clock oscillations by administering a short REV-ERB agonist pulse at the right time ?

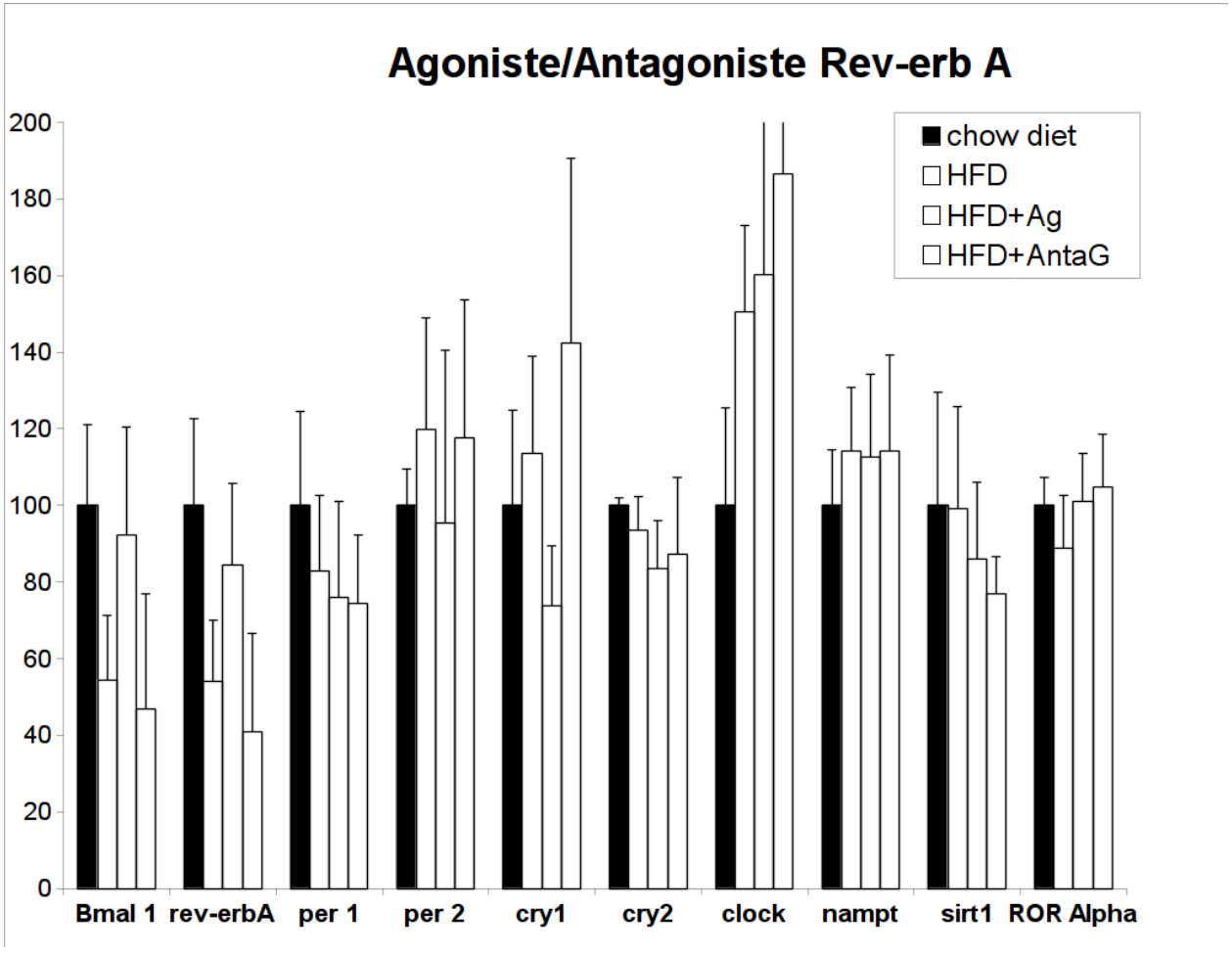
Rescue of clock gene oscillation amplitude in high-fat diet using a Rev-Erb agonist



Normal amplitude and phase are restored when the administration time of a REV-ERB agonist (green pulse) is carefully chosen.

First experiment in vivo

Mice at Institut Pasteur de Lille were fed a high fat diet and then administered a Rev-Erb agonist for 2 days before being sacrificed to analyse their livers 2 hours after beginning of the night



Encouraging but still much work is required to make the model more quantitative

Conclusion

- A mathematical model how the liver clock is entrained by feeding/fasting cycles had been designed, incorporating the metabolic sensors SIRT1 and AMPK.
- It agrees well with a number of WT and mutant phenotypes
- The mathematical model explains the daily patterns of NAD+ level.
- Adjusting the model to normal chow and high fat diet data may help to understand which actors are perturbed in nutritional stress. It seems important to have a long fasting period during the night to maintain high-amplitude rhythms
- Goal : deliver a drug affecting a clock gene at a precise timing, so as to restore normal clock rhythms.

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Article

Switch to Standard View

A Mathematical Model of the Liver Circadian Clock Linking Feeding and Fasting Cycles to Clock Function

Aurore Woller, Hélène Duez, Bart Staels, Marc Lefranc[3]
[3] Lead Contact

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DOI: http://dx.doi.org/10.1016/j.celrep.2016.09.060 | CrossMark

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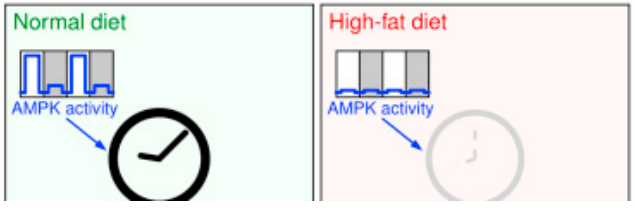
Highlights

- We construct a mathematical model of the mammalian liver clock and metabolic sensors
- The model integrates feeding and fasting cycles with the clock
- The model accurately reproduces high-fat-diet-induced loss of NAD^+ oscillations
- NAD^+ oscillations are predicted to be rescued by timed delivery of clock modifiers

Graphical Abstract



Mathematical modeling to understand the interplay of proliferation and differentiation in development

Benjamin Pfeuty, Development **142**, 477 (2015).

© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 477-485 doi:10.1242/dev.112649

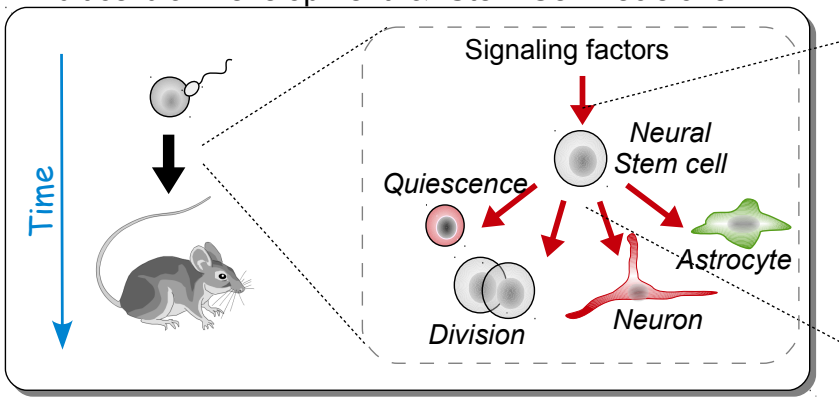
RESEARCH ARTICLE

STEM CELLS AND REGENERATION

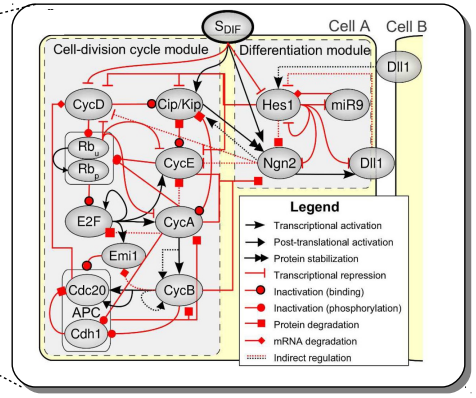
A computational model for the coordination of neural progenitor self-renewal and differentiation through Hes1 dynamics

Benjamin Pfeuty*

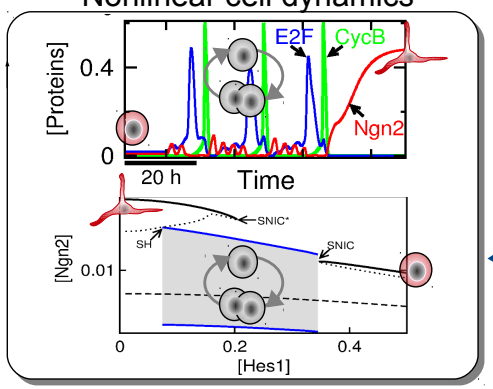
Multicellular Development & Stem Cell Decisions



Complex regulatory Network



Nonlinear cell dynamics



REGULATORY PRINCIPLES:

- Diverse and robust decisions driven by intracellular oscillations

MATHEMATICAL MODELLING:

- ODE simulations
- Bifurcation analysis
- Modular analysis

Biophotonics for testing cancer drug

L. Héliot and M. Gonzalez-Pisfil. Caracterizing molecular interactions with FRET, FCS, FLIM, etc...

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Article | Switch to Standard View

Compounds Triggering ER Stress Exert Anti-Melanoma Effects and Overcome BRAF Inhibitor Resistance

Michaël Cerezo[14], Abdelali Lehraiki[14], Antoine Millet[14], Florian Rouaud, Magali Plaisant, Emilie Jaune, Thomas Botton, Cyril Ronco, Patricia Abbe, Hella Amdouni, Thierry Passeron, Veronique Hofman, Baharia Mograbi, Anne-Sophie Dabert-Gay, Delphine Debayle, Damien Alcor, Nabil Rabhi, Jean-Sébastien Annicotte, Laurent Héliot, Mariano Gonzalez-Pisfil, Caroline Robert, Solange Moréra, Armelle Vigouroux, Philippe Gual, Maruf M.U. Ali, Corine Bertolotto, Paul Hofman, Robert Ballotti, Rachid Benhida, Stéphane Rocchi
[14] Co-first author

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Highlights

- HA15 is a molecule that targets specifically BiP/GRP78/HSPA5
- HA15 induces ER stress leading to cancer cell death in vitro and in vivo
- HA15 overcomes BRAF inhibitor resistance in melanoma cells

Graphical Abstract