Modeling Rhythms on Differents Levels: Cells, Tissues, and Organisms

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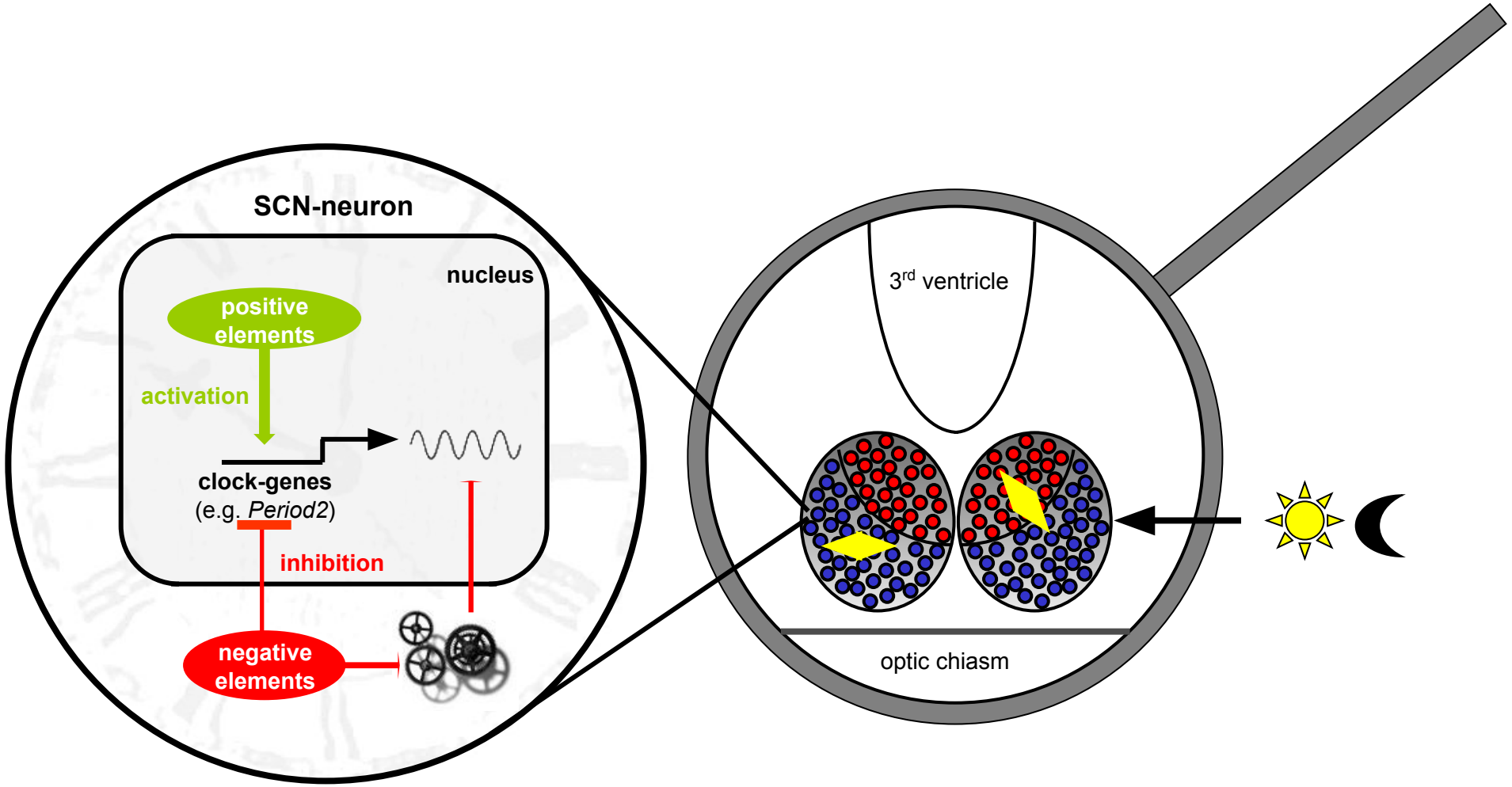
Molecular Chronobiology



Oscillation

Synchronization

Clock genes and feedback loops

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 9, pp. 2112–2116, September 1971

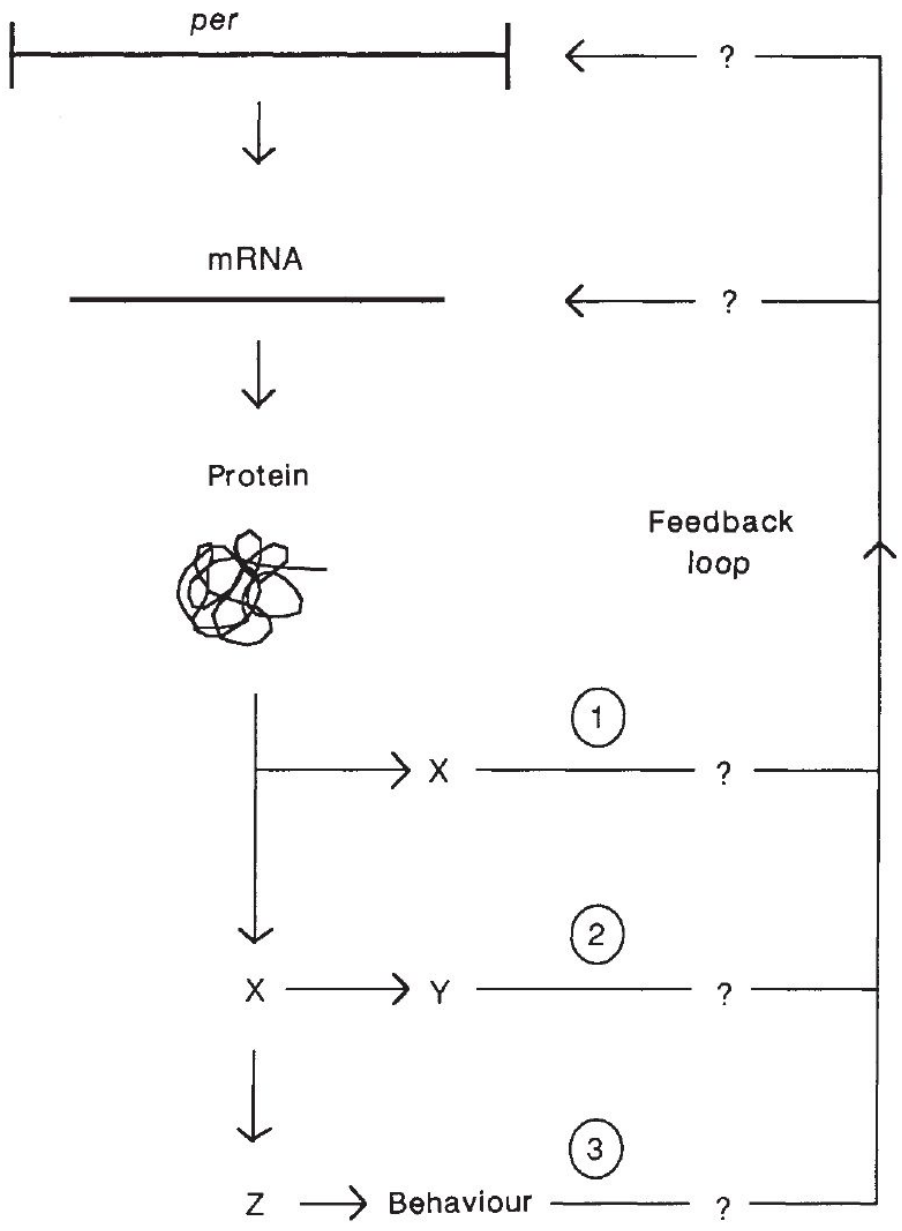
Clock Mutants of *Drosophila melanogaster*

(eclosion/circadian/rhythms/X chromosome)

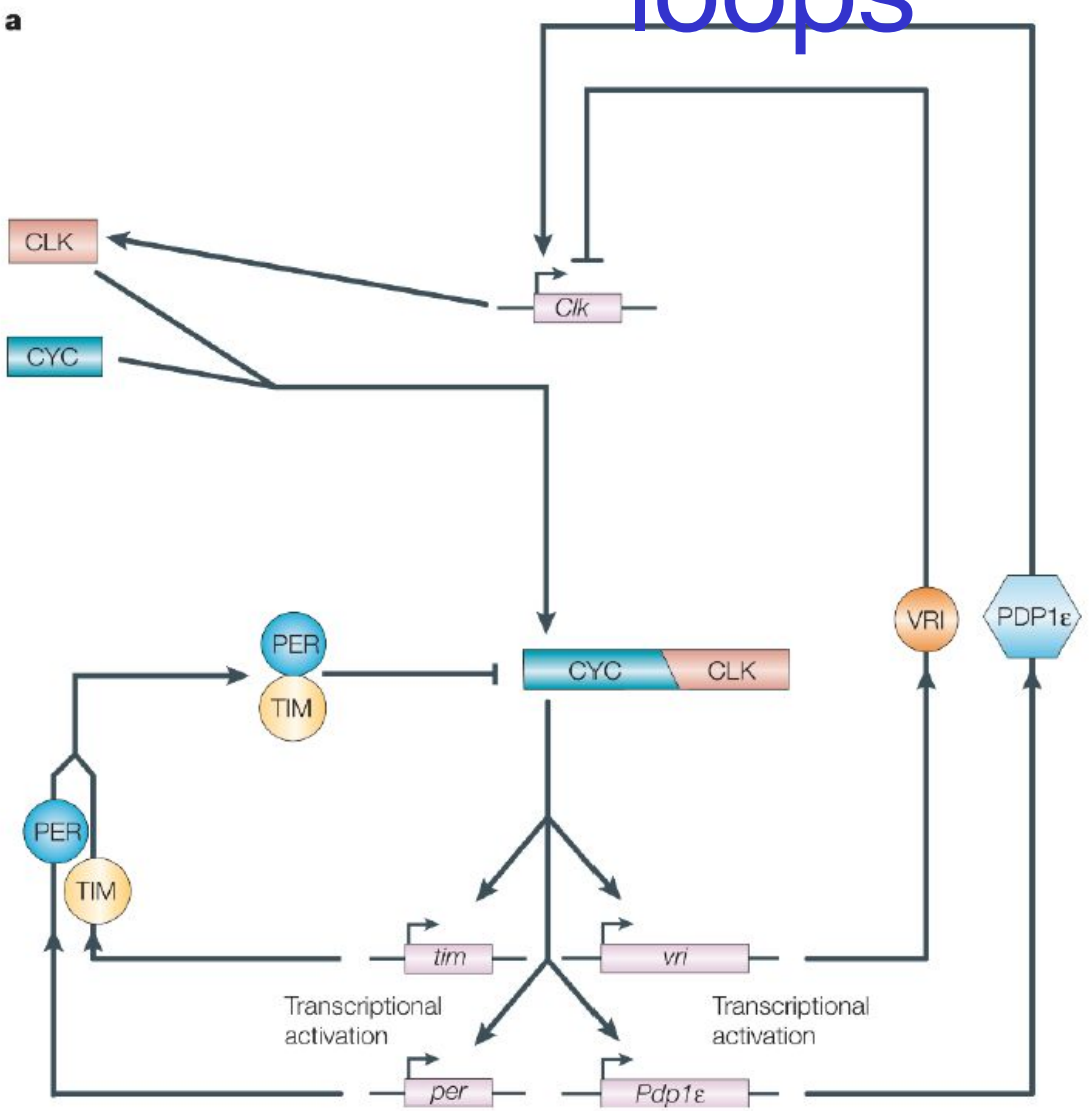
RONALD J. KONOPKA AND SEYMOUR BENZER

Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels

Paul E. Hardin*†, Jeffrey C. Hall† & Michael Rosbash*†

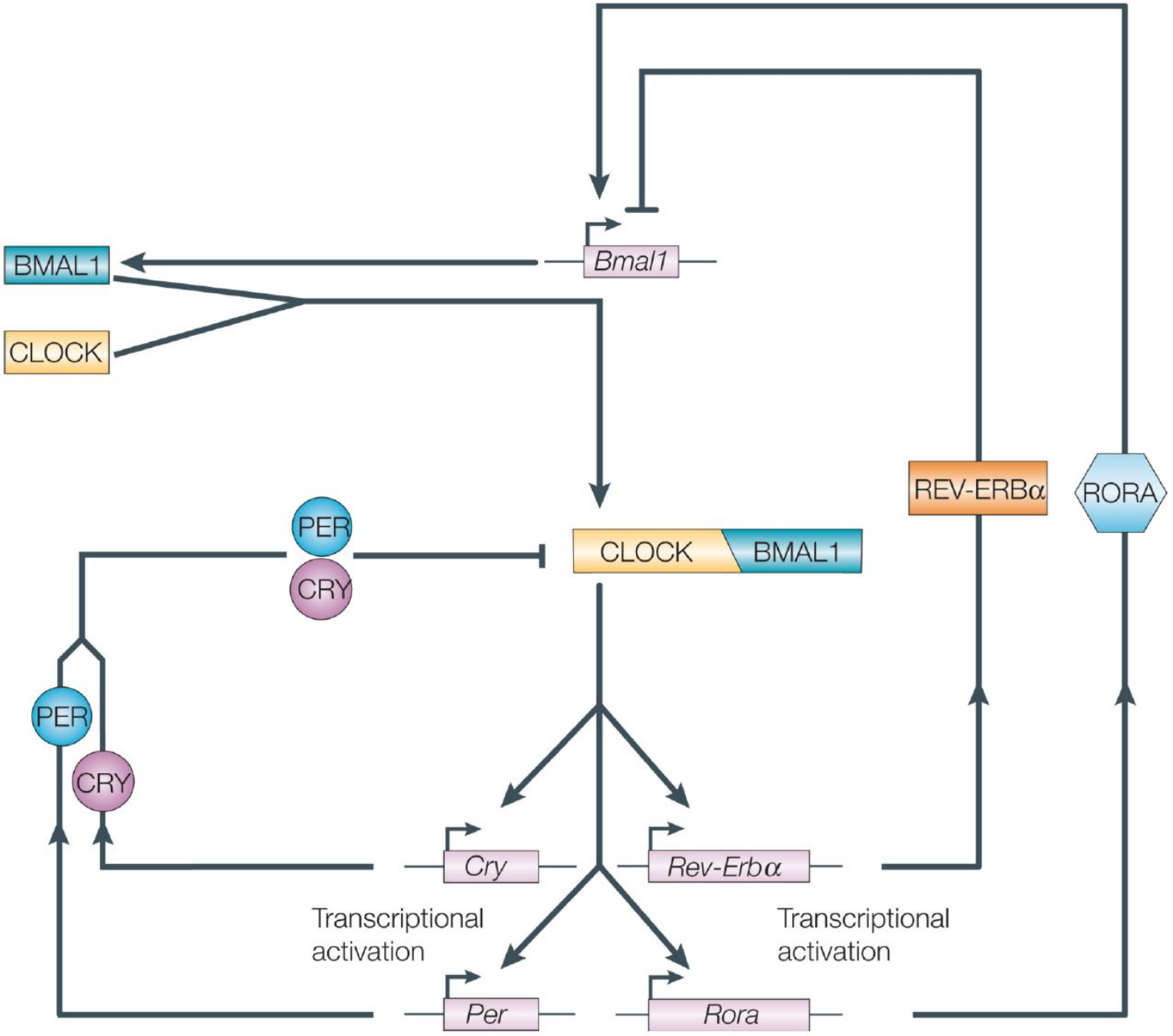


The core clock in Drosophila – negative and positive feedback loops



D. Bell-Pedersen et al. 2005

The core clock in mammals



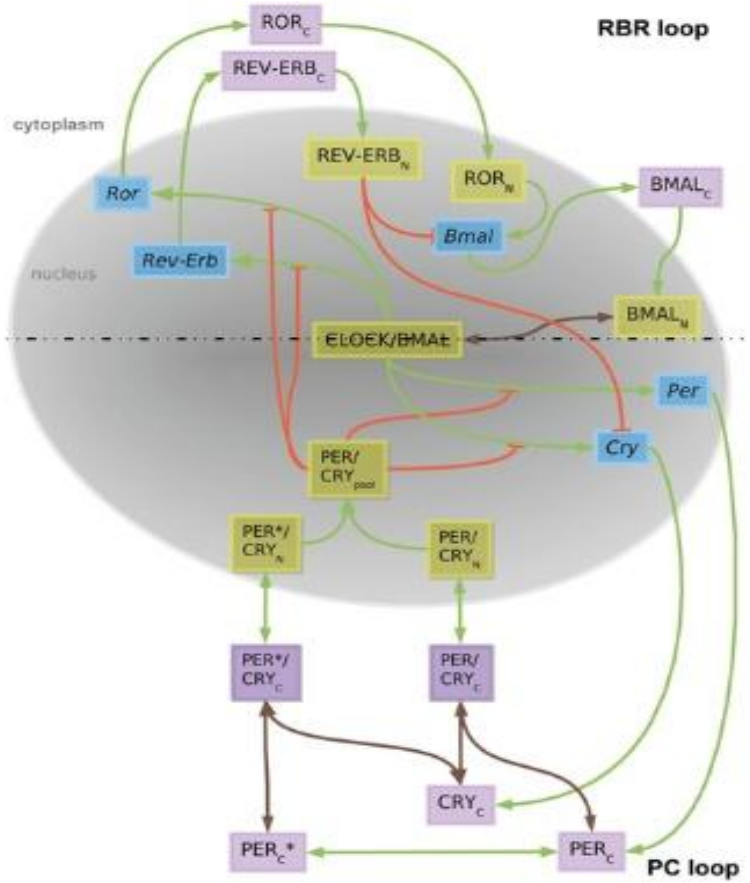
D. Bell-Pedersen et al. 2005

Limits of quantitative models

single cells

models

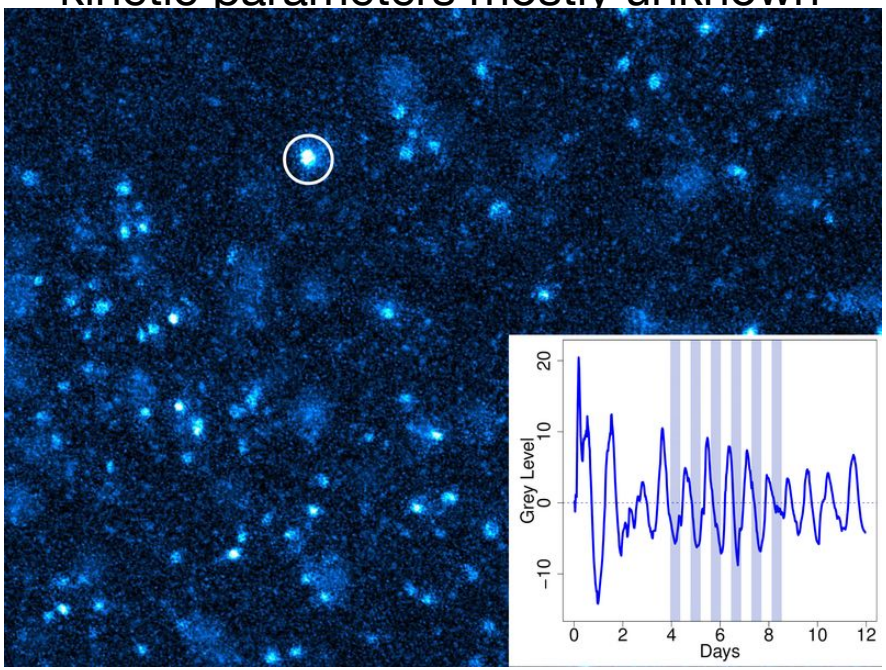
advanced models of feedback loops incl. PER/CRY loops and RORE-loops



Relogio et al. PLoS Comp. Biol. 2011

data

- noisy reporter signals of at most 44 days
- few PRCs (mostly phase resetting)
- kinetic parameters mostly unknown



Abraham, Schlichting et al., in revision

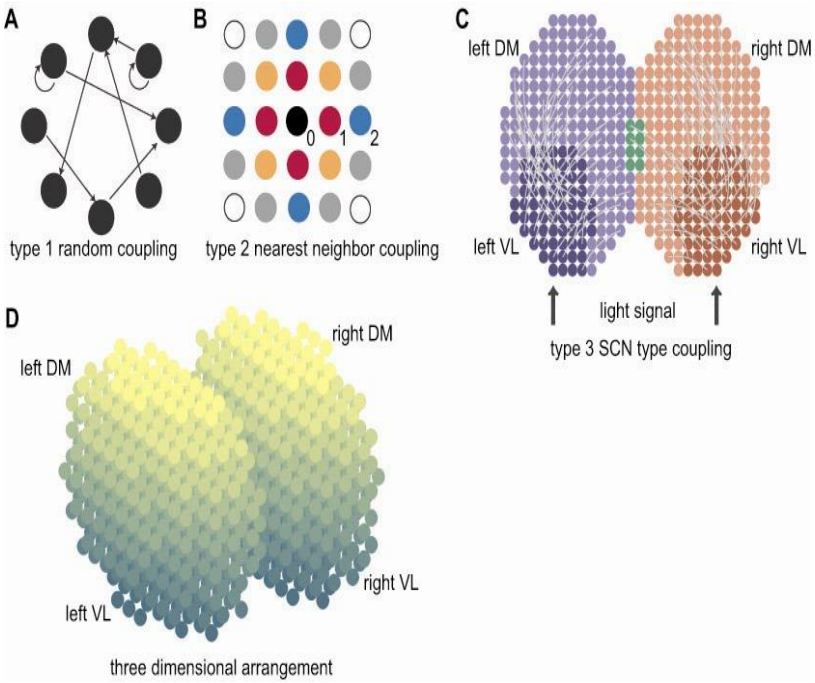
Limits of quantitative models

models **neuronal networks** **data**

network models with heterogeneity

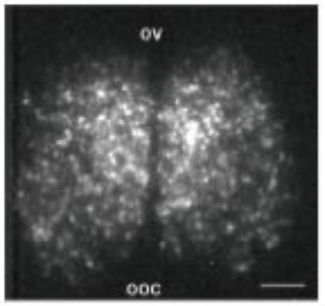
,

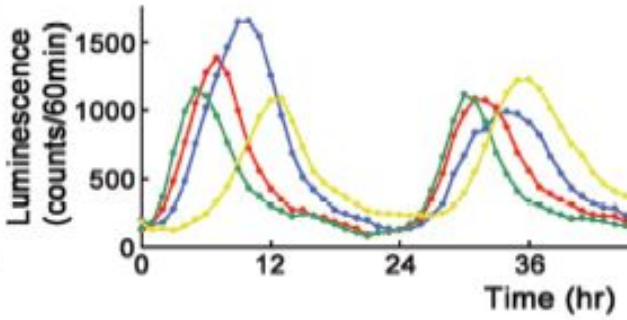
different coupling schemes etc.



Bernard et al. PLoS Comp. Biol. 2007

- movies with waves and tides
- multiple coupling mechanisms debated
- some PRCs (VIP, AVP, optogenetics…)
- splitting rare

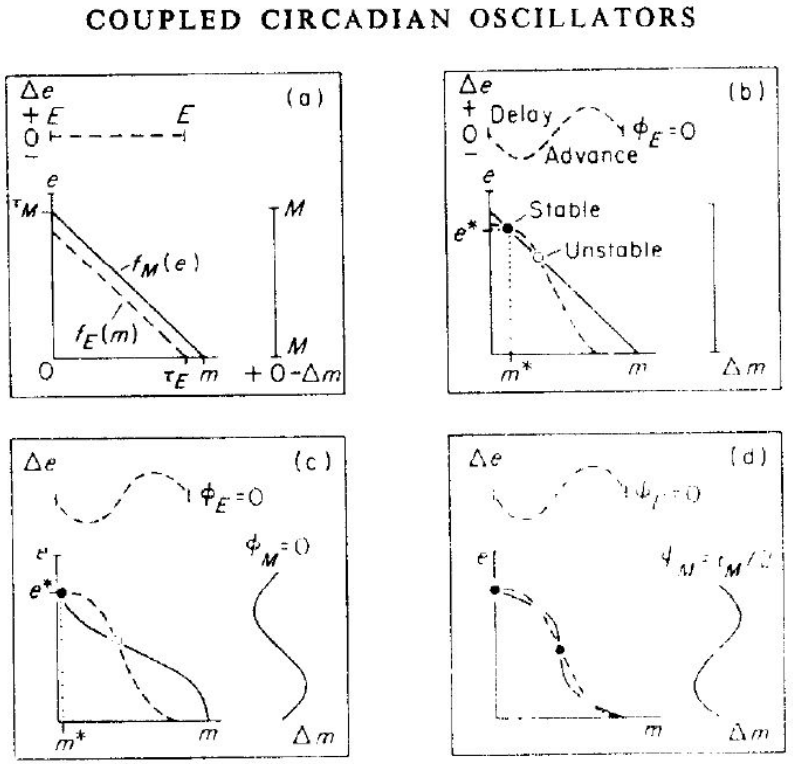




Yamaguchi et al., Science 2003

Limits of quantitative models

models **on organismic level** **data**

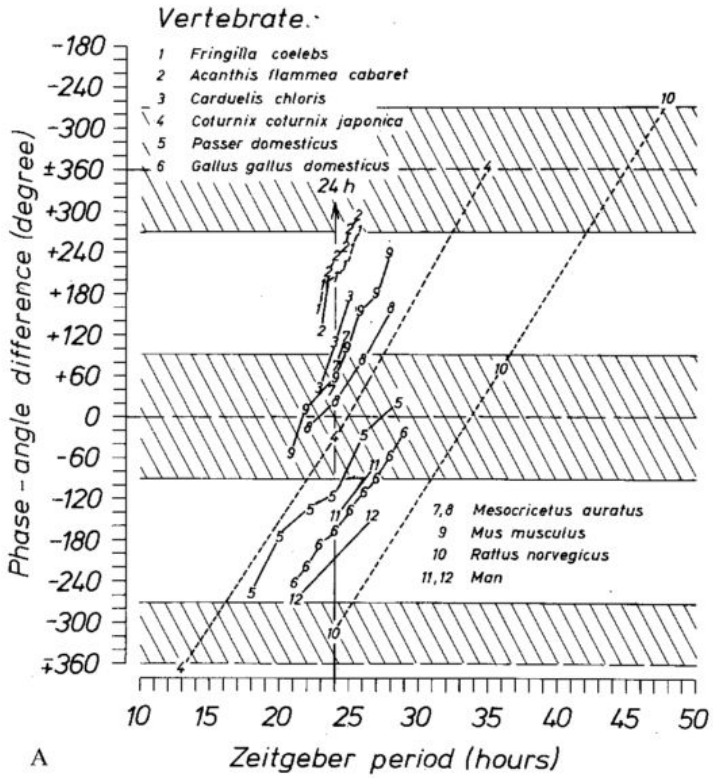


J. theor. Biol. (1978) **70**, 297–313

Two Coupled Oscillators: Simulations of the Circadian Pacemaker in Mammalian Activity Rhythms

SERGE DAAN† AND CHARLES BERDE

- long actograms
- many PRCs incl. dead zones
- entrainment/jetlag protocols
- saisonal variations



Aschoff and Pohl, 1978

Challenge

genetic feedback models + coupling ⟶ network models

Challenge

genetic feedback models + coupling → network models

Example of complexity:

single cell oscillator: amplitude, phase, +-4h PRC

↓

resonant coupling

↓

strong, rigid network oscillator with +-1h PRC

regarding cells versus ensembles see also:
Rougemont & Naef 2007; vanderLeest…Meijer 2009;
Bordyugov et al. 2011; John,Taylor et al. 2014; Pia Rose 2015

Complex network dynamics in insects as well

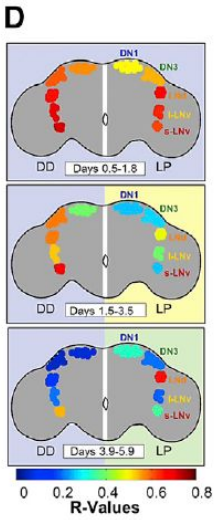


Figure 4. Alignment of Neuronal Subgroup Responses to a Light Pulse Reveals Temporally Distinct Kinetic Signatures of Phase Retuning

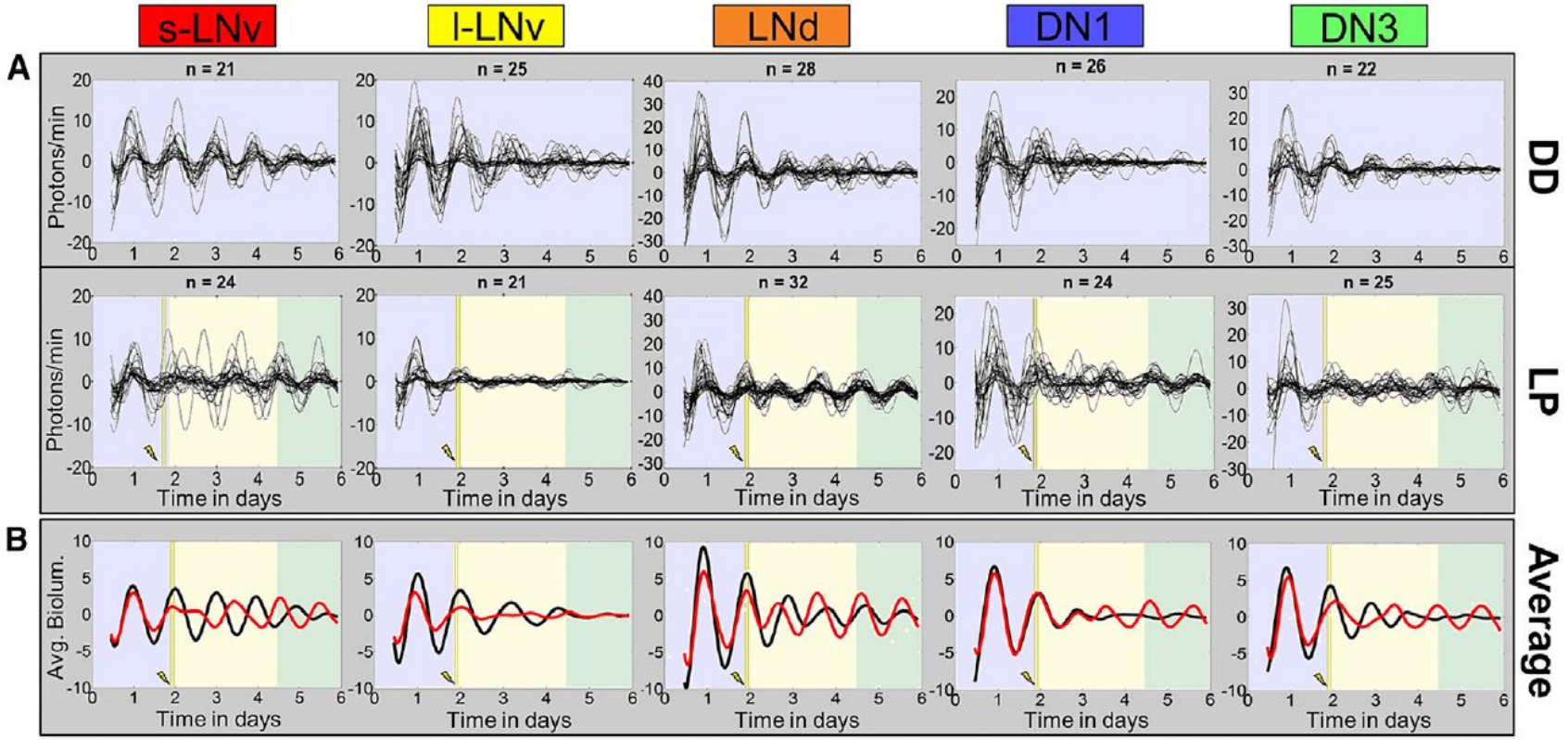
In (A)–(C), plots of neuronal subgroup data are coded by color: s-LNv (red), l-LNv (yellow), LNd (orange), DN1 (blue), and DN3 (green).

(A) Top: average bioluminescence traces for subgroups maintained in DD exhibit a progressive and monotonic loss of rhythmicity and inter-subgroup synchrony over time. Bottom: after a LP, average bioluminescence traces for subgroups exhibit a transient reduction in rhythmic amplitude and inter-subgroup synchrony, followed by a general strengthening of rhythmic amplitude and inter-subgroup synchrony over time relative to corresponding neurons in DD.

Light Evokes Rapid Circadian Network Oscillator Desynchrony Followed by Gradual Phase Retuning of Synchrony

Logan Roberts,[1] Tanya L. Leise,[2] Takako Noguchi,[3] Alexis M. Galschiodt,[1] Jerry H. Houl,[1] David K. Welsh,[3,4] and Todd C. Holmes[1,*]

Roberts et al., 2015, Current Biology *25*, 858–867



Exposure of Cultured Brain Explants to a Light Pulse Reveals Qualitatively Distinct Dynamic Signatures of Neuronal Subgroups

From cells to networks to organisms

1. Single cell rhythms: many detailed models but limited single cell data

2. Synchronization: sloppy oscillators useful, coupling phase matters

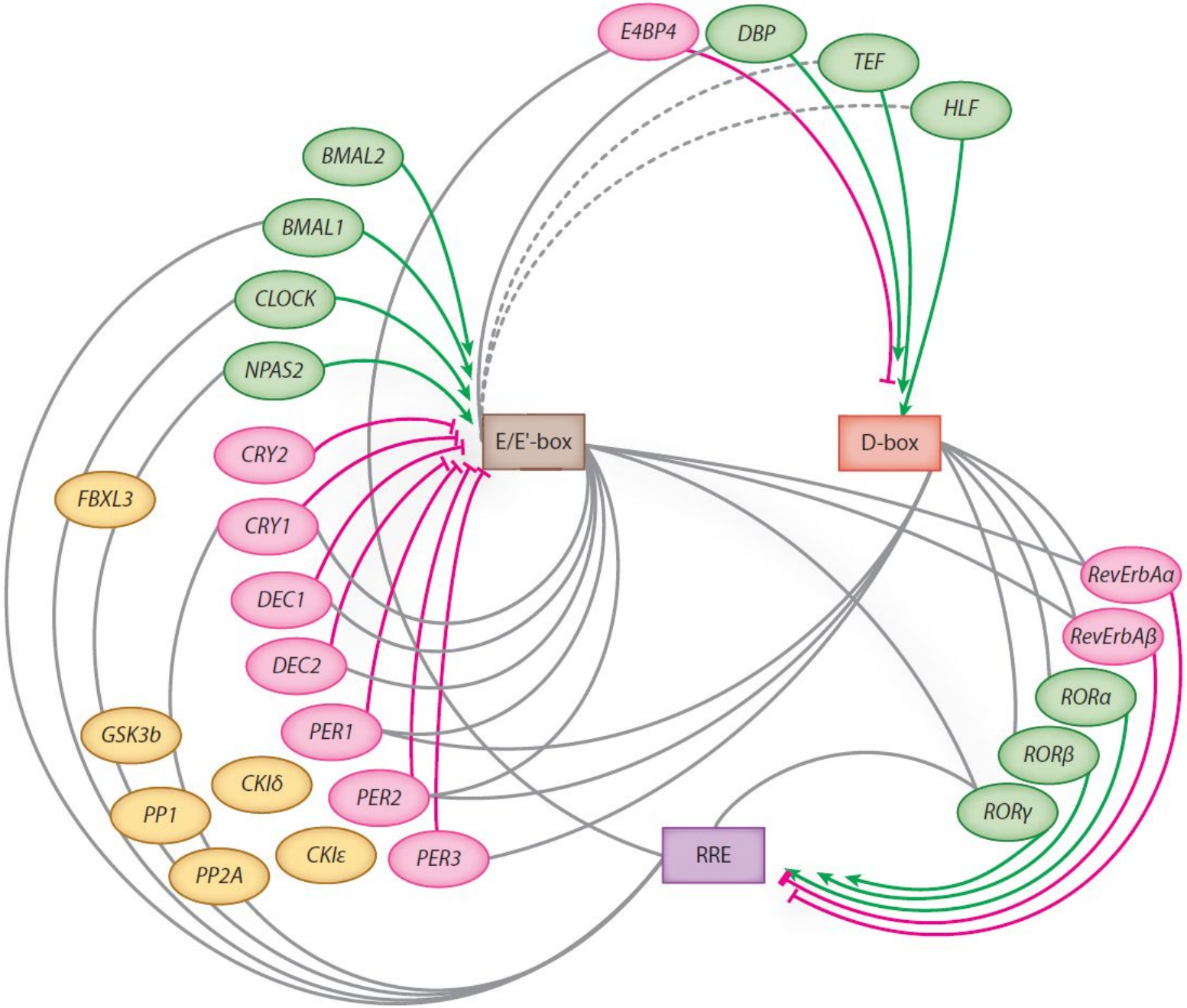
3. Coupling makes oscillators “strong” (small PRCs, narrow entrainment range)

4. Strong oscillators have flexible entrainment phases

Some open questions

- How is fine-tuning of periods possible despite molecular noise (e.g. transcriptional bursts)?
- How long delays (6-12 hours needed) are realized?
- What are the most essential switches?
- How do transcriptional feedback loops interact with other oscillators (cell cycle, metabolism, peroxiredoxins)?
- Where are the feedbacks in the cyanobacterial clock?
- How to get robust synchronization and entrainment instead of splitting and chaos?

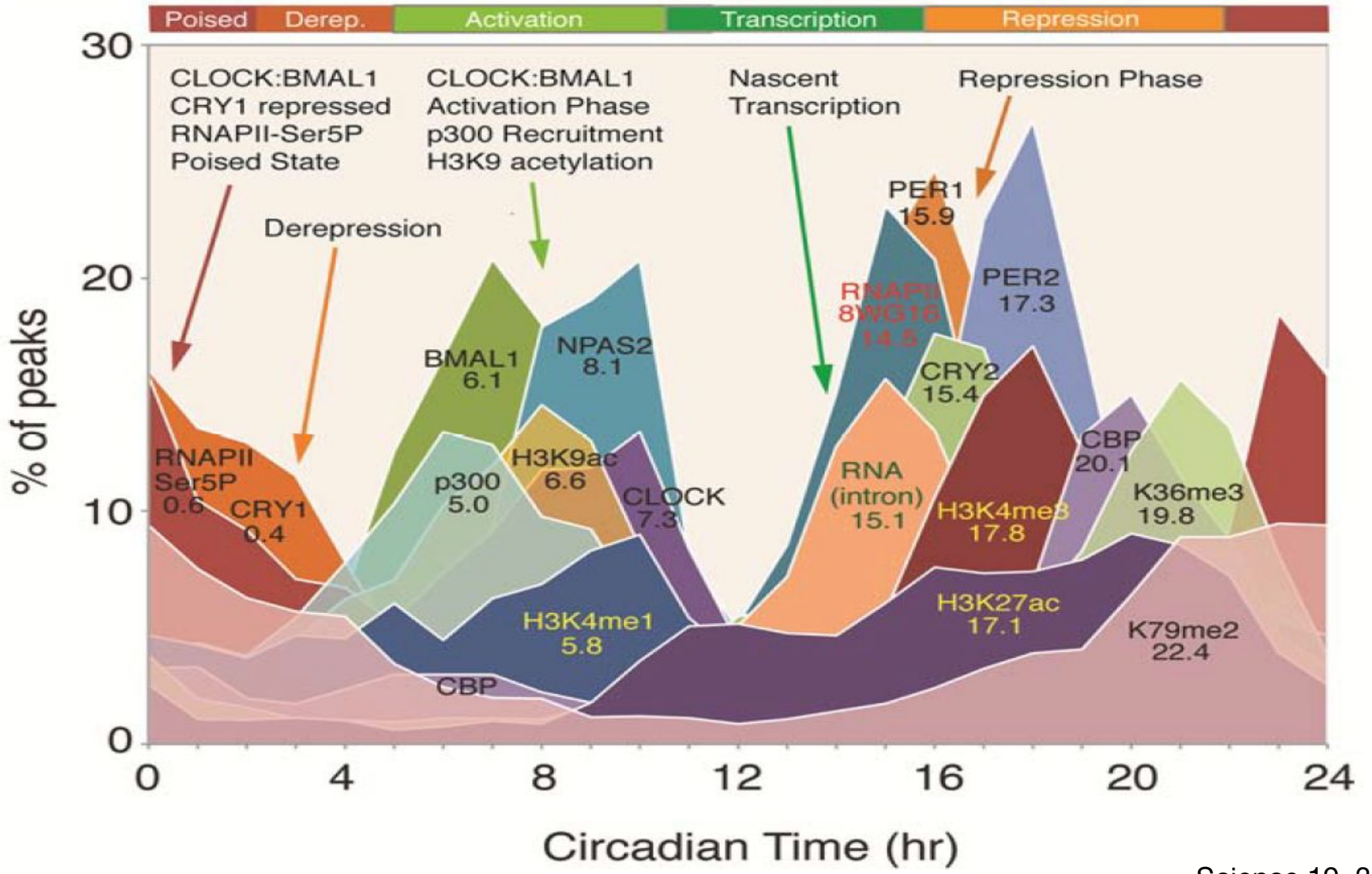
E-boxes, ROR-elements and D-boxes drive clock genes



H Ukai, HR Ueda: Annu Rev Physiol 72: 579-603 (2010)

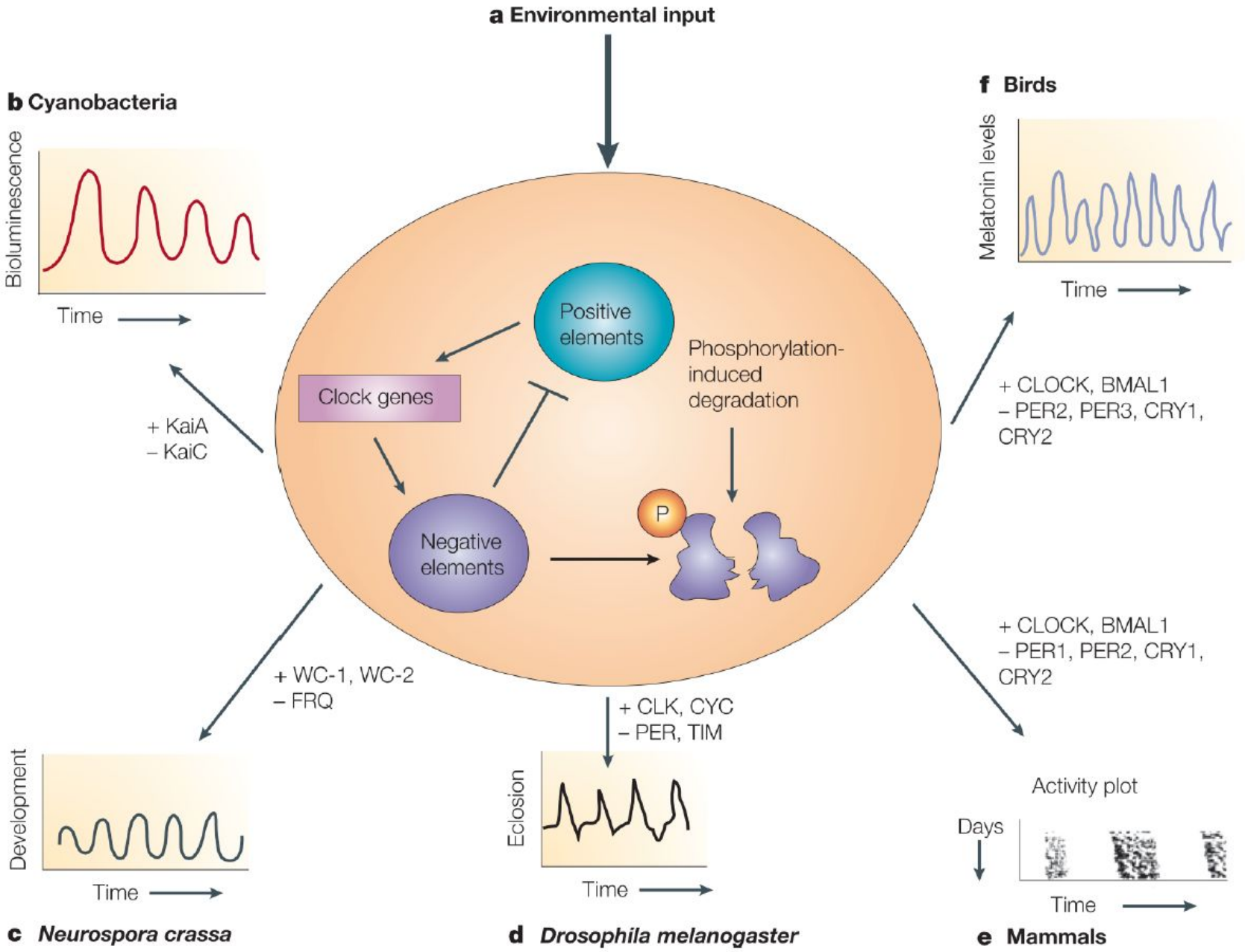
Transcriptional Architecture and Chromatin Landscape of the Core Circadian Clock in Mammals

Nobuya Koike,[1] Seung-Hee Yoo,[1] Hung-Chung Huang,[1] Vivek Kumar,[1] Choogon Lee,[2] Tae-Kyung Kim,[1] Joseph S. Takahashi[1,3]*



Science 19, 349-354,

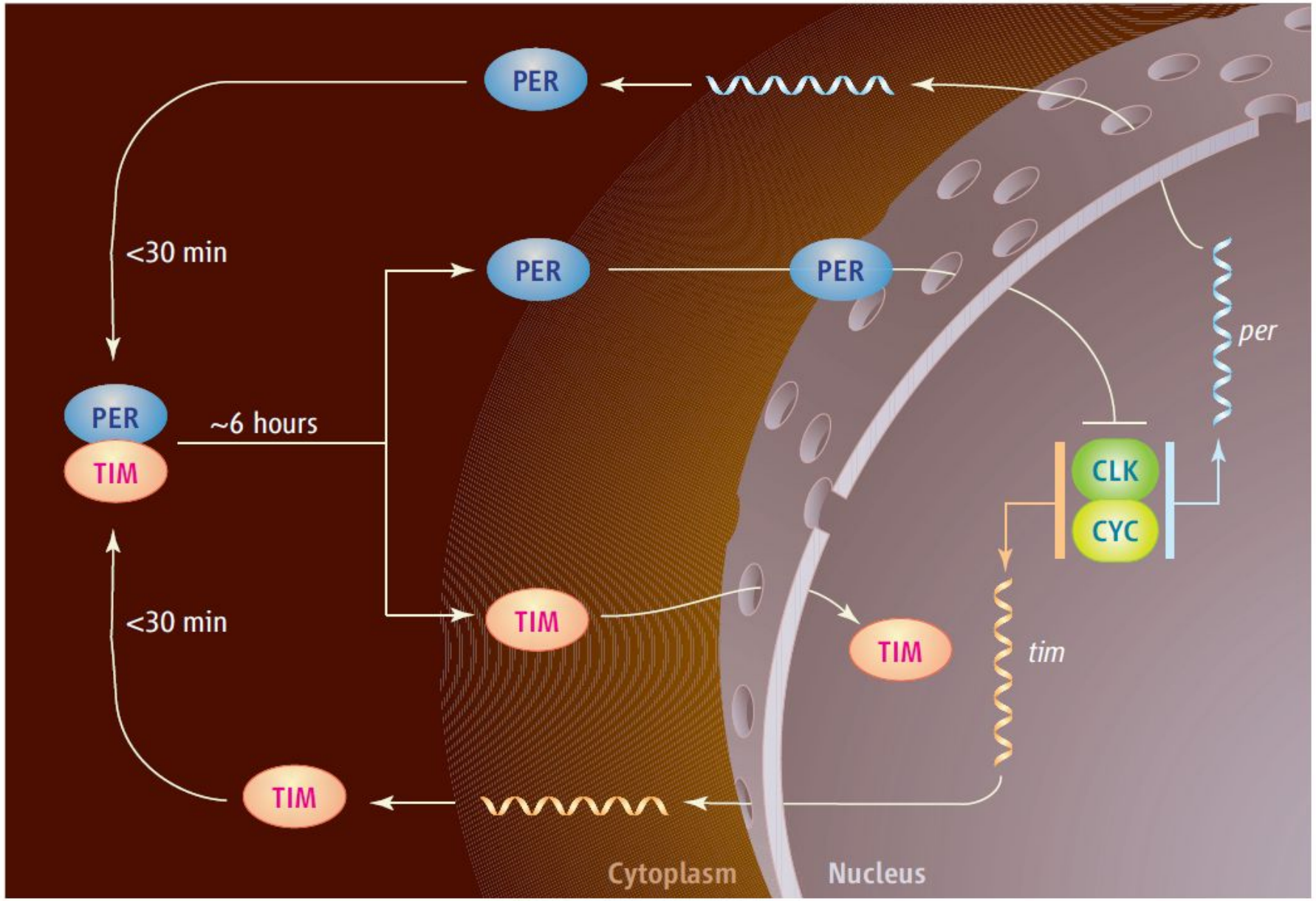
Negative feedbacks as a common design principle



D. Bell-Pedersen et al. 2005

Delayed nuclear import in Drosophila

P. Meyer, L. Saez, M. W. Young, Science 2006

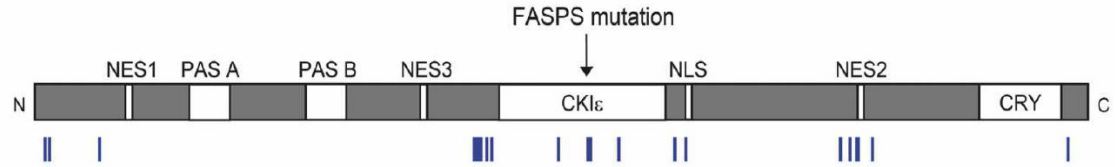


J.C. Dunlap 2006

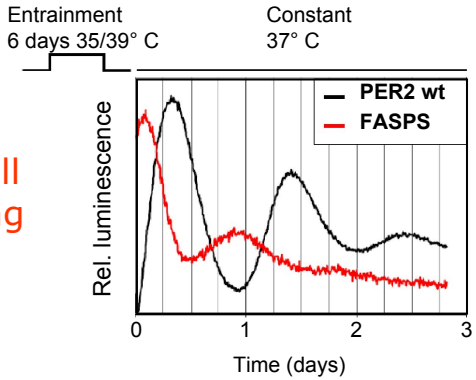
FASPS: Per phosphorylation controls delay

Measurements

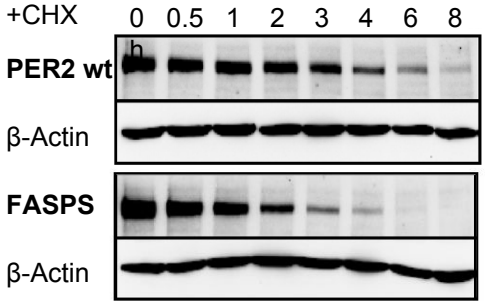
mass spec.



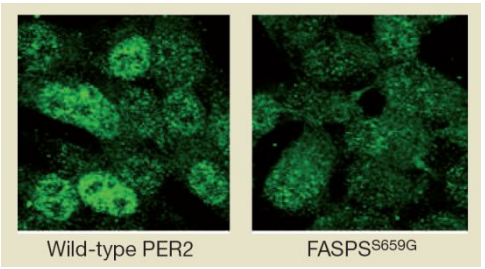
live cell imaging



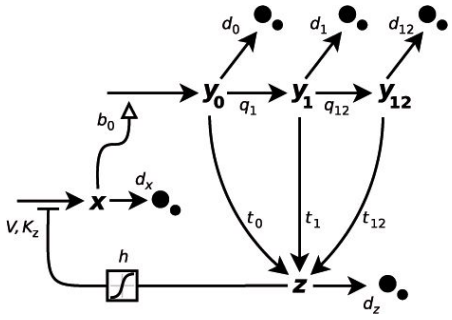
bio-chemistry



cell biology



Mathematical Model



$$\dot{x} = \frac{V}{1 + \left(\frac{z}{K_z}\right)^h} - d_x x$$

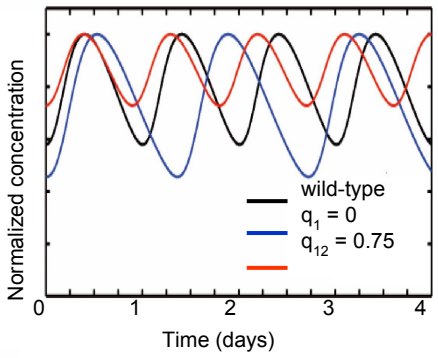
$$\dot{y}_0 = b_0 x - (d_0 + t_0 + q_1)\, y_0$$

$$\dot{y}_1 = q_1 y_0 - (d_1 + t_1 + q_{12})\, y_1$$

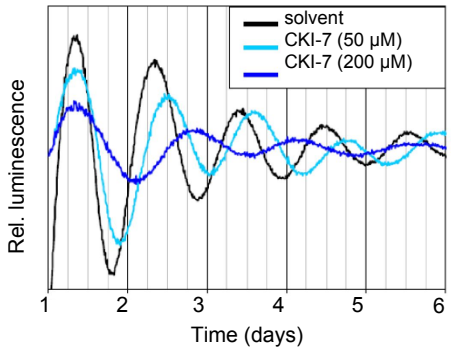
$$\dot{y}_{12} = q_{12} y_1 - (d_{12} + t_{12})\, y_{12}$$

$$\dot{z} = t_0 y_0 + t_1 y_1 + t_{12} y_{12} - d_z z$$

Model prediction

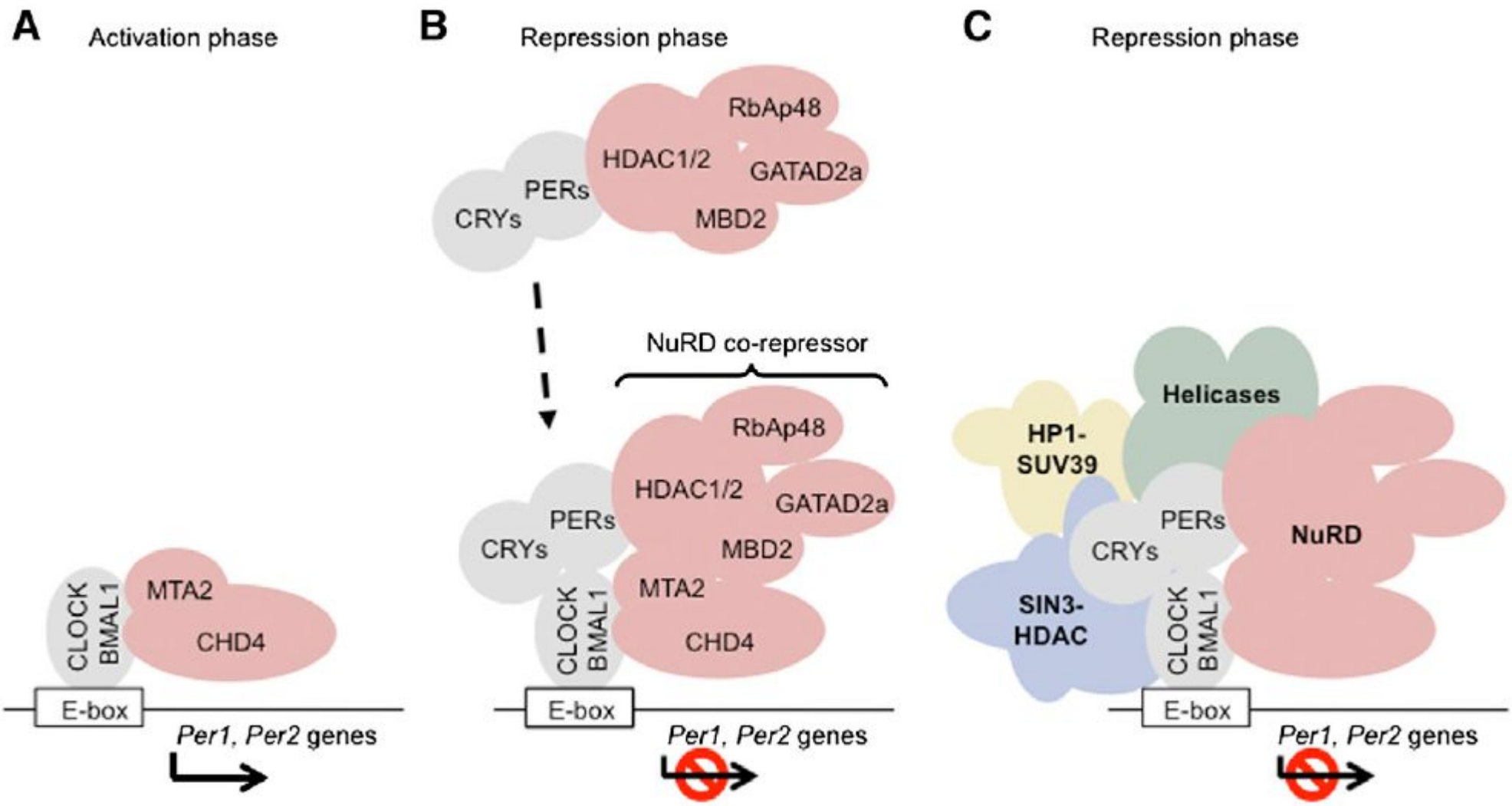


Test of prediction



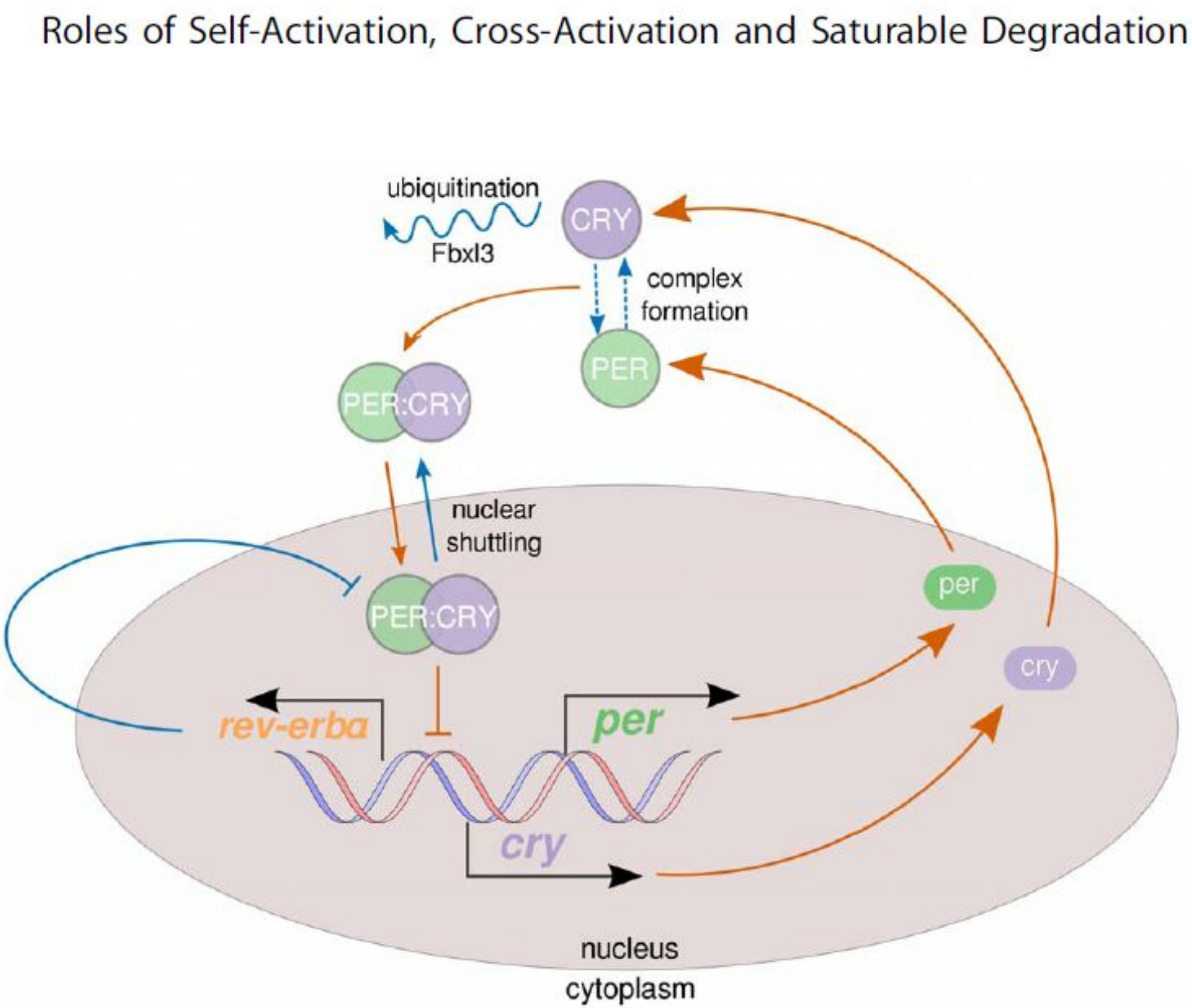
Vanselow et al., Genes & Dev, 2006

Huge protein complexes regulate transcription



Jin Young Kim et al.

Synergy of negative (orange) and positive (blue) regulations



B. Ananthasubramaniam et al. 2014

Take home messages

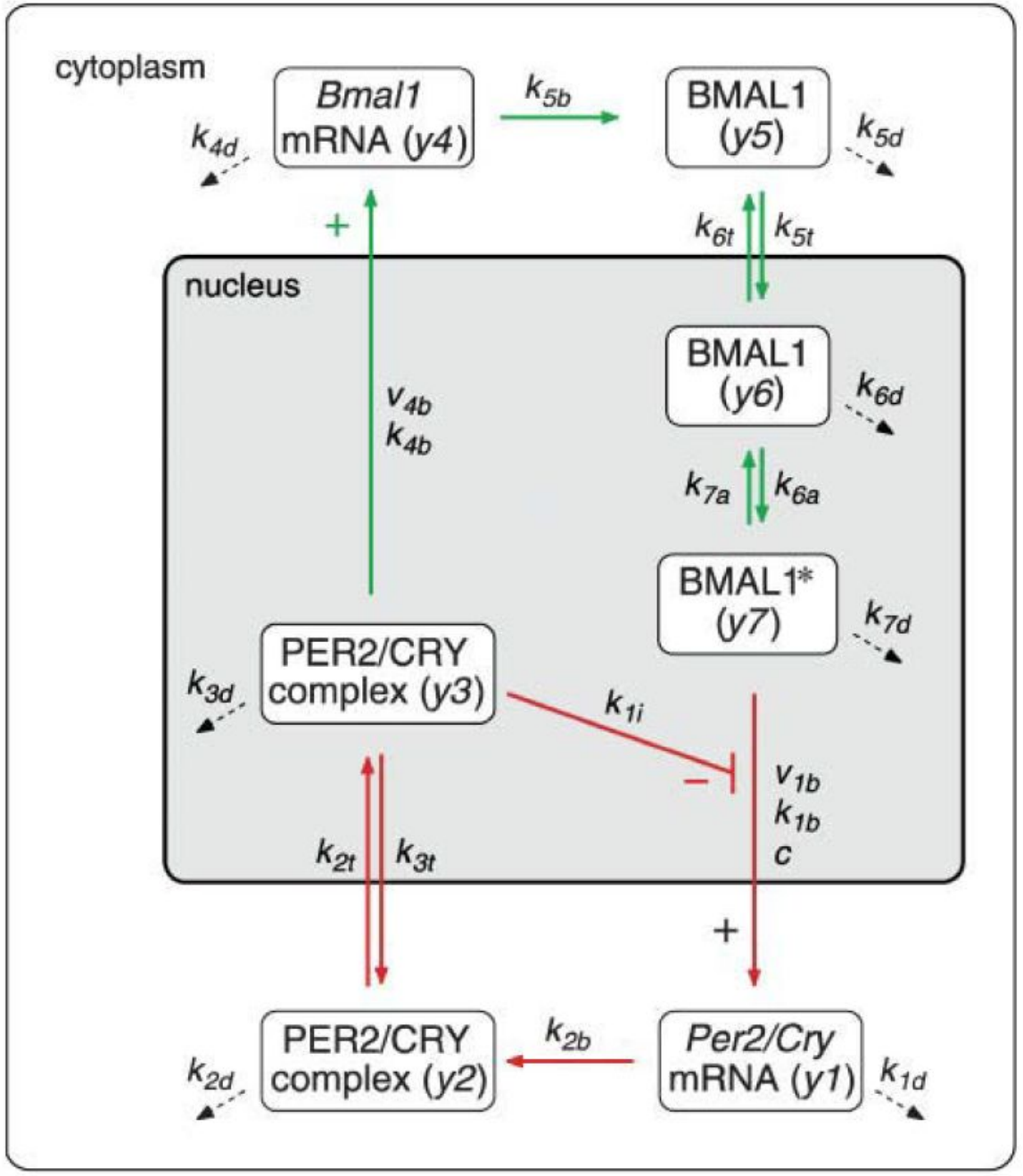
- Delayed **negative feedback** are necessary but not sufficient for self-sustained oscillation

- **Delays** are tuned by transcription, translation, complex formation, phosphorylation, nuclear translocation, stability, epigenetics, …

- Nonlinearities (**switches**) can result from cooperativity, positive feedbacks and sequestration

- Understanding **synchronization** and **entrainment** requires oscillator theory (PRCs, limit cycles, Arnold tongues….)

Negative PER/CRY Loop in Early Models

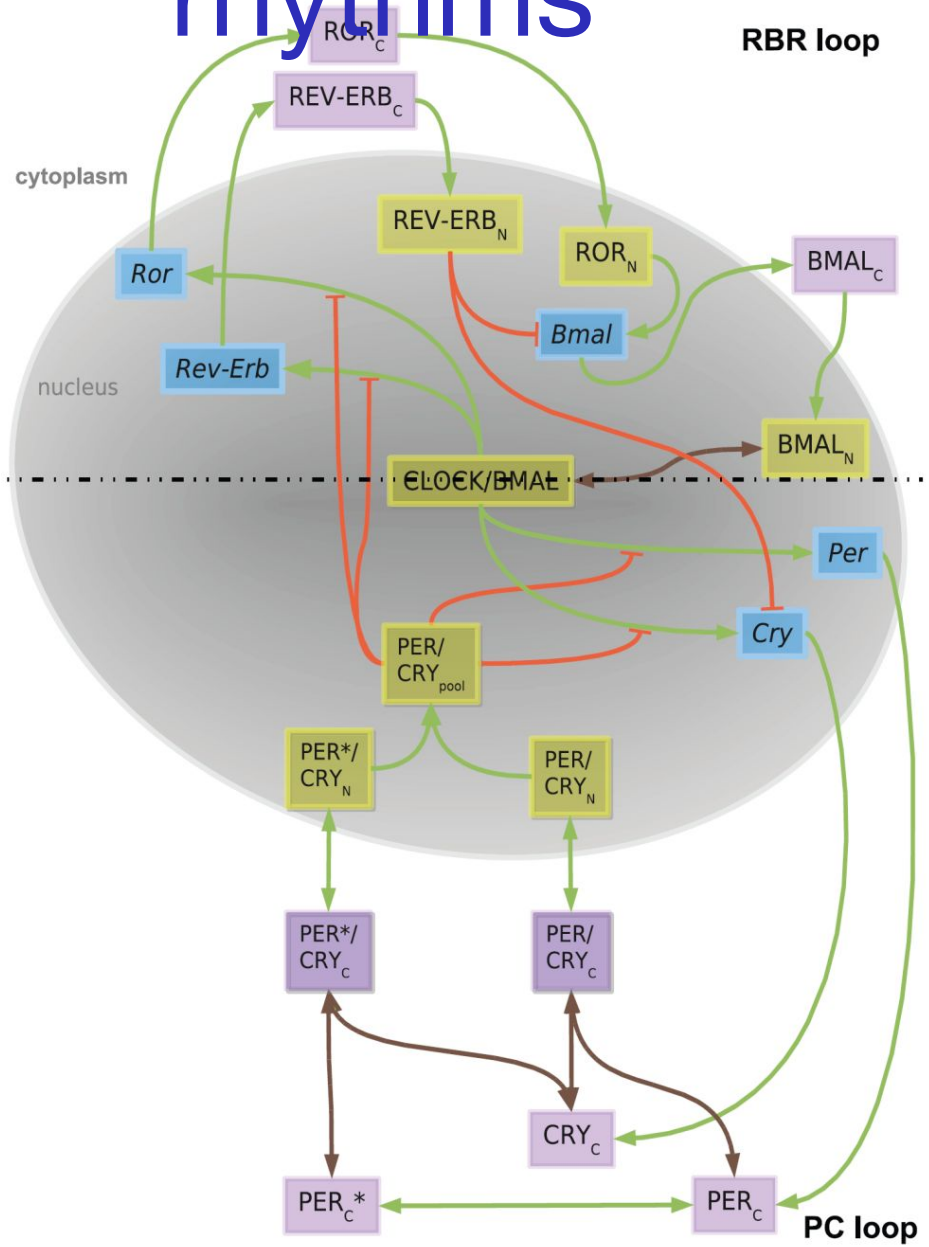


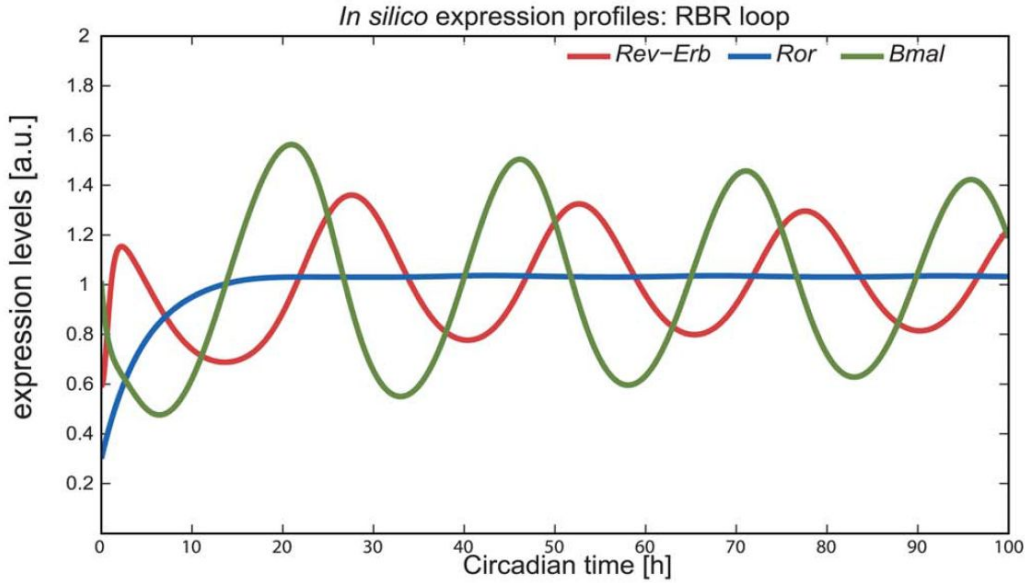
Forger, Peskin
PNAS 2003

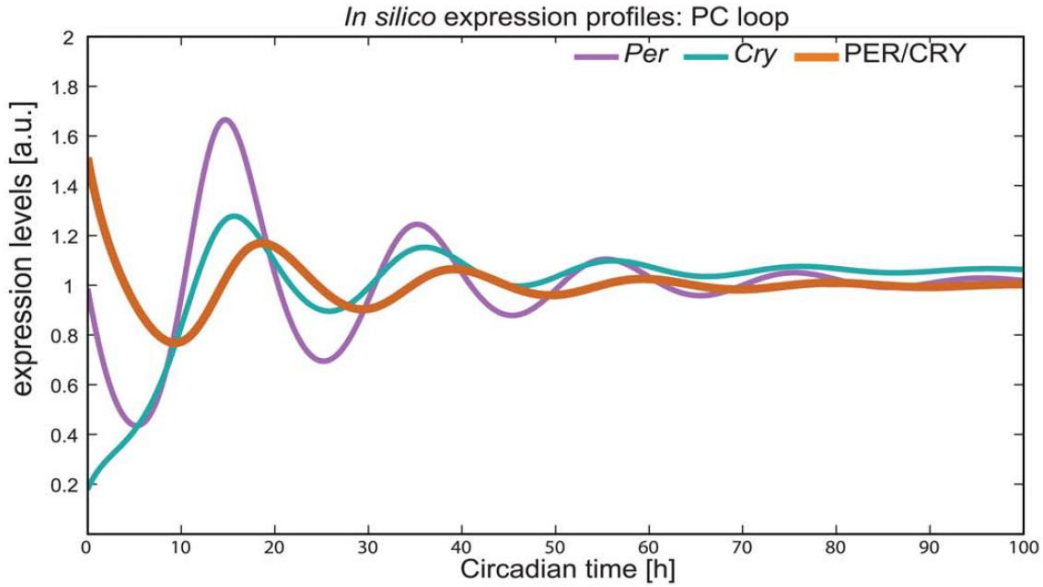
Leloup, Goldbeter
PNAS 2003

Becker-Weimann
Biophys. J. 2004

Both loops can generate rhythms







Relogio et al. PLoS Comp. Biol. 2011

Key role of the Rev-Erb loop

OPEN ACCESS Freely available online PLOS COMPUTATIONAL BIOLOGY

Tuning the Mammalian Circadian Clock: Robust Synergy of Two Loops

Angela Relógio[1]*, Pal O. Westermark[1], Thomas Wallach[2], Katja Schellenberg[2], Achim Kramer[2], Hanspeter Herzel[1]

1 Institute for Theoretical Biology, Humboldt University, Berlin, Germany, 2 Laboratory of Chronobiology, Institute of Medical Immunology Charité - Universitätsmedizin Berlin, Berlin, Germany

Rev-erbα and Rev-erbβ coordinately protect the circadian clock and normal metabolic function

Anne Bugge, Dan Feng, Logan J. Everett, Erika R. Briggs, Shannon E. Mullican, Fenfen Wang, Jennifer Jager, and Mitchell A. Lazar[1]

LETTER

doi:10.1038/nature11048

Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β

Han Cho[1], Xuan Zhao[1], Megumi Hatori[2], Ruth T. Yu[1], Grant D. Barish[1], Michael T. Lam[3], Ling-Wa Chong[1], Luciano DiTacchio[2], Annette R. Atkins[1], Christopher K. Glass[3], Christopher Liddle[4], Johan Auwerx[5], Michael Downes[1], Satchidananda Panda[2] & Ronald M. Evans[1,6]

ARTICLE

doi:10.1038/nature11030

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

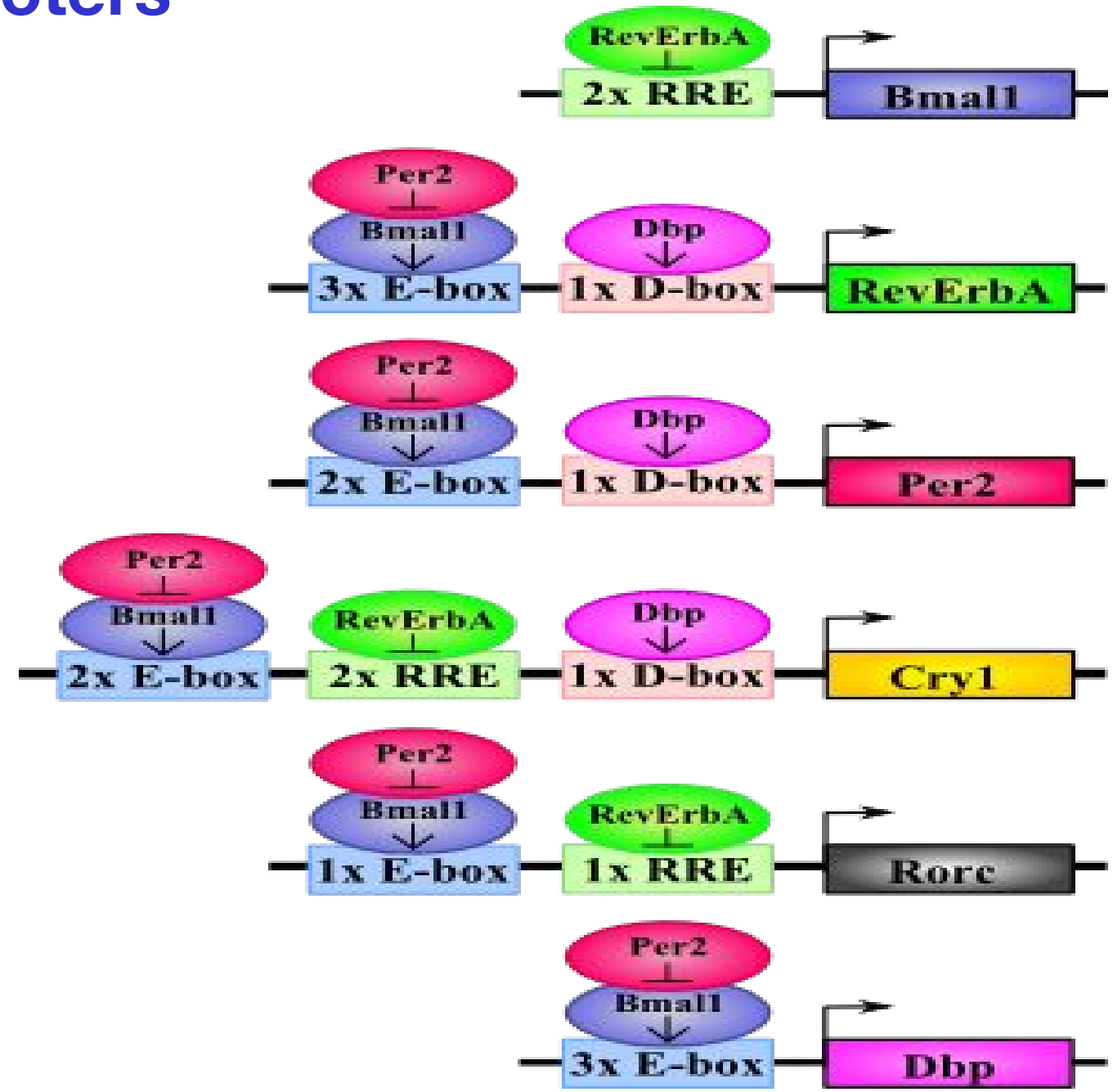
Laura A. Solt[1]*, Yongjun Wang[1]*, Subhashis Banerjee[1], Travis Hughes[1], Douglas J. Kojetin[1], Thomas Lundasen[1], Youseung Shin[2], Jin Liu[1], Michael D. Cameron[2], Romain Noel[2], Seung-Hee Yoo[3], Joseph S. Takahashi[3], Andrew A. Butler[4], Theodore M. Kamenecka[2] & Thomas P. Burris[1,5]

by courtesy of Marc Lefranc

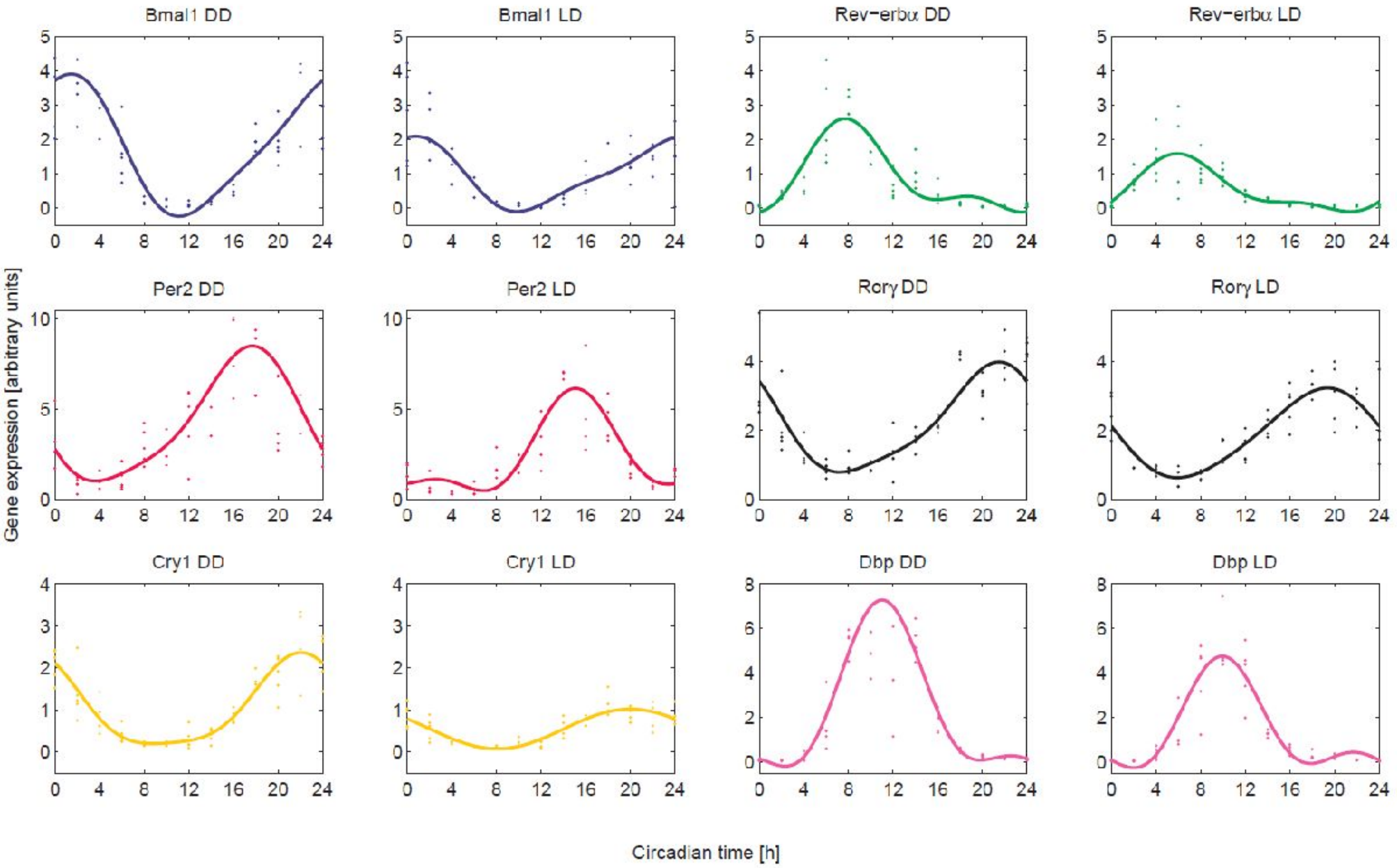
Construction of a core clock model using

- representative genes: activators (Bmal1, Dbp)+ early inhibitors (Per2, Rev-Erb)+late inhibitor (Cry1)
- experimentally verified binding sites
- known degradation rates
- reasonable delays
- fitted transcriptional parameters

Regulatory elements in clock gene promoters

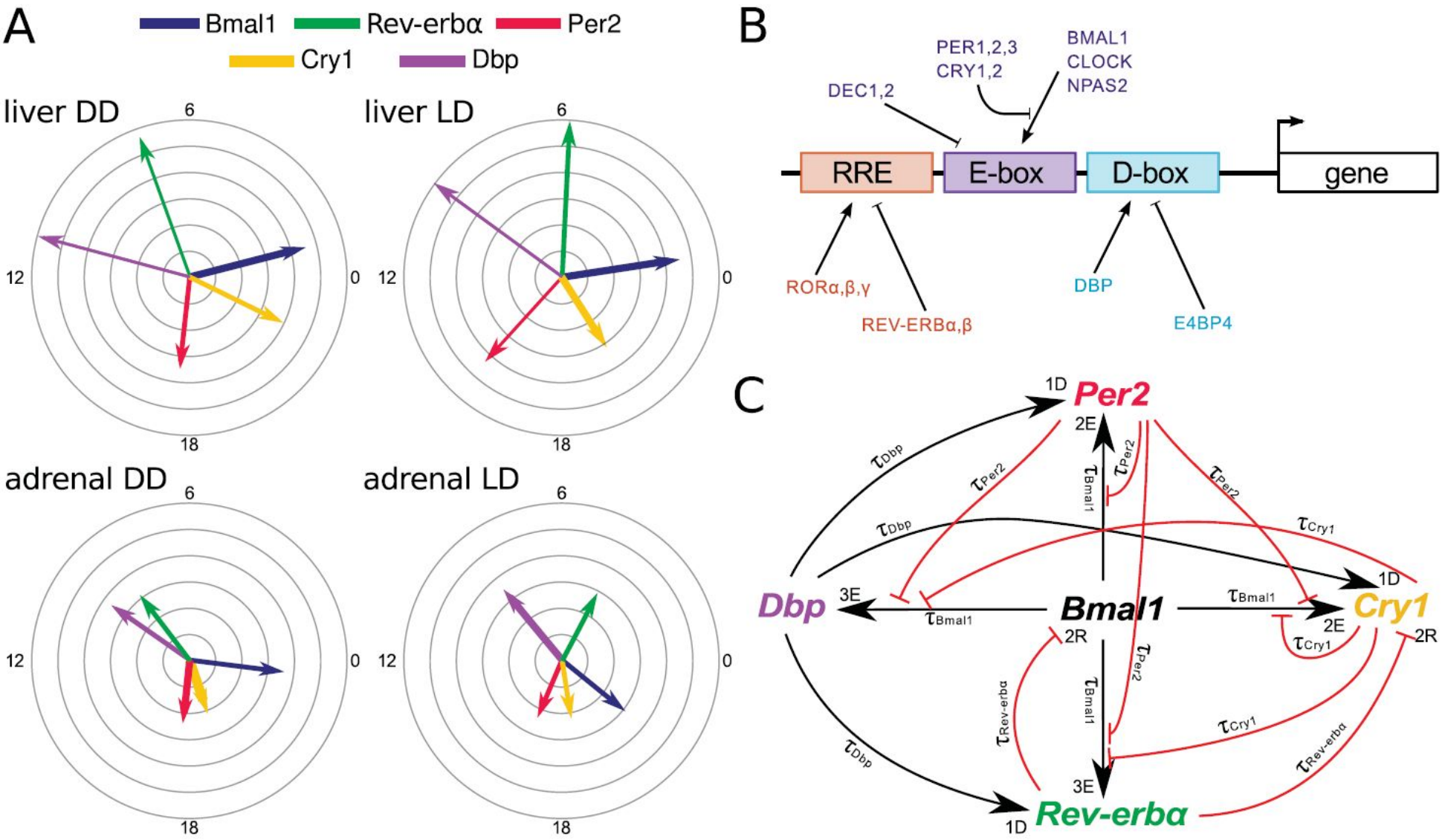


Expression profiles in liver & adrenal gland



A. Korencic et al., Scientific Reports 4:5782 (2014)

Core-clock model from expression profiles and promoters



Anja Korencic et al., Scientific Reports 2014

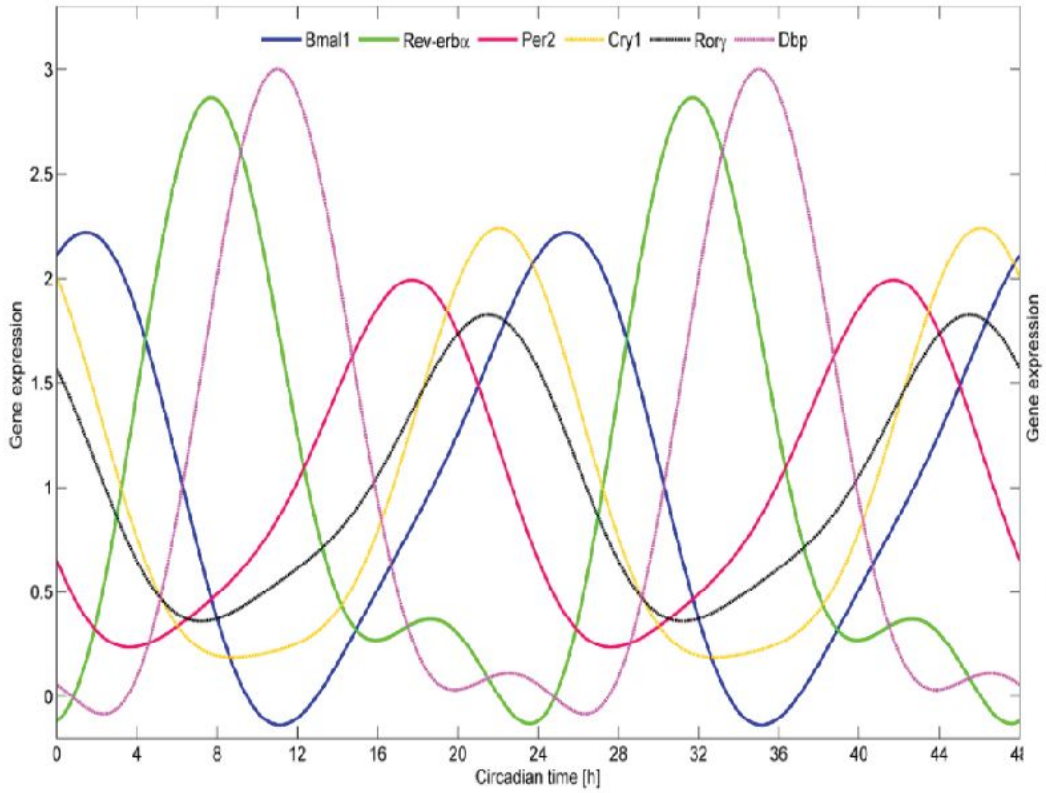
Quantitative agreement of experimental data and simulations with DDEs

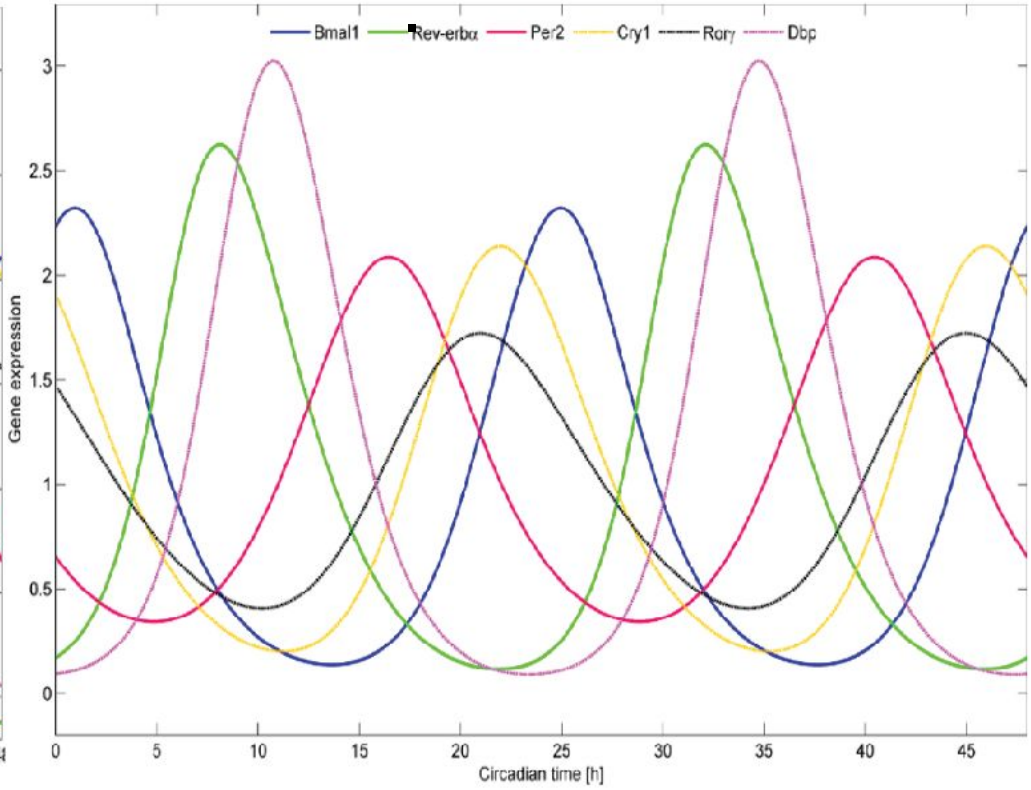
Expression profiles were fitted by sinusoidal functions with harmonics

Simulations

$$\frac{\mathrm{d}[Bmal1]}{\mathrm{d}t} = \left(\frac{a}{ak + [Rev-erba]_{\tau_{Reverb\alpha}}} \right)^2 - d_{Bmal1} \cdot [Bmal1]$$

etc





Multiple loops can generate sustained rhythms

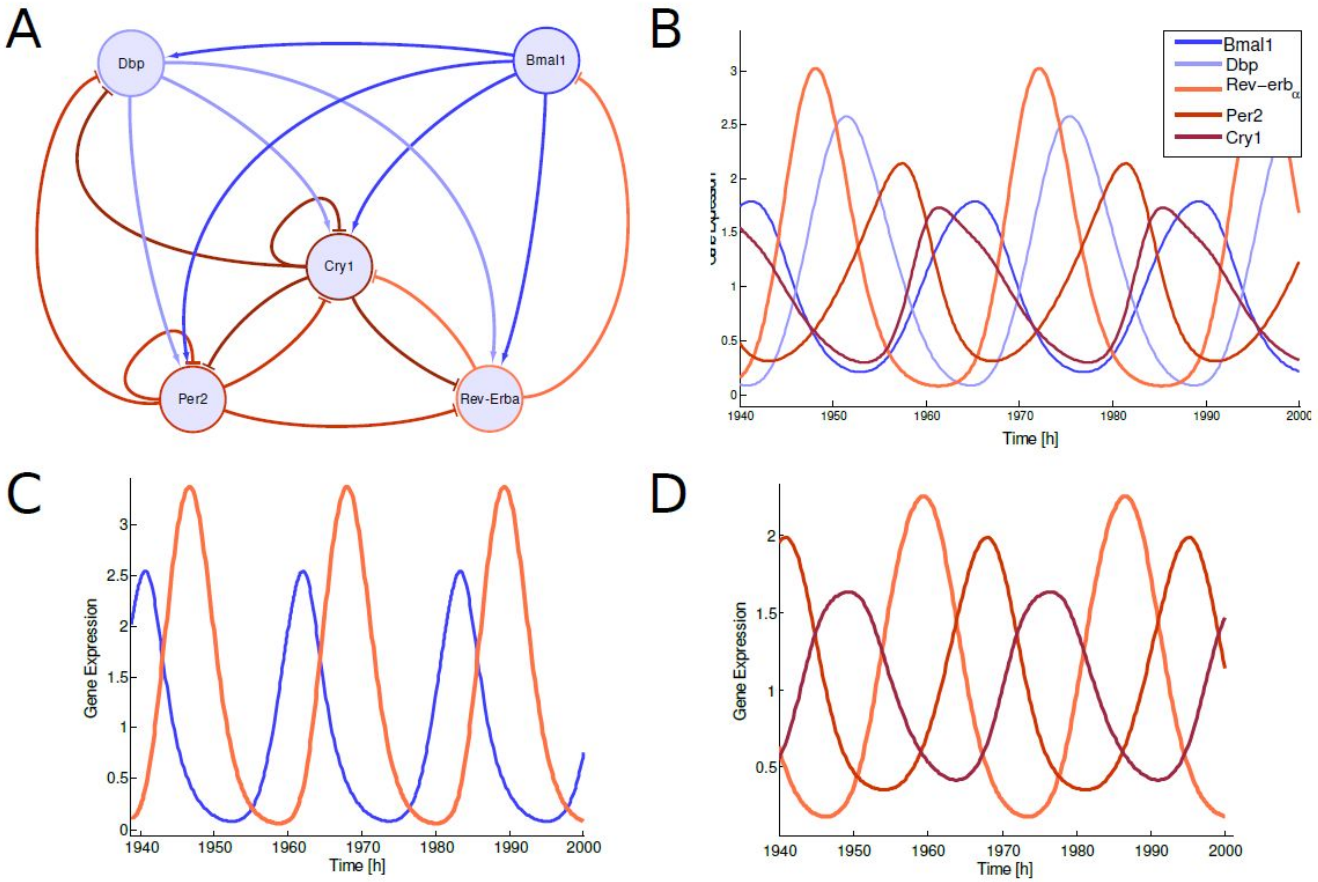


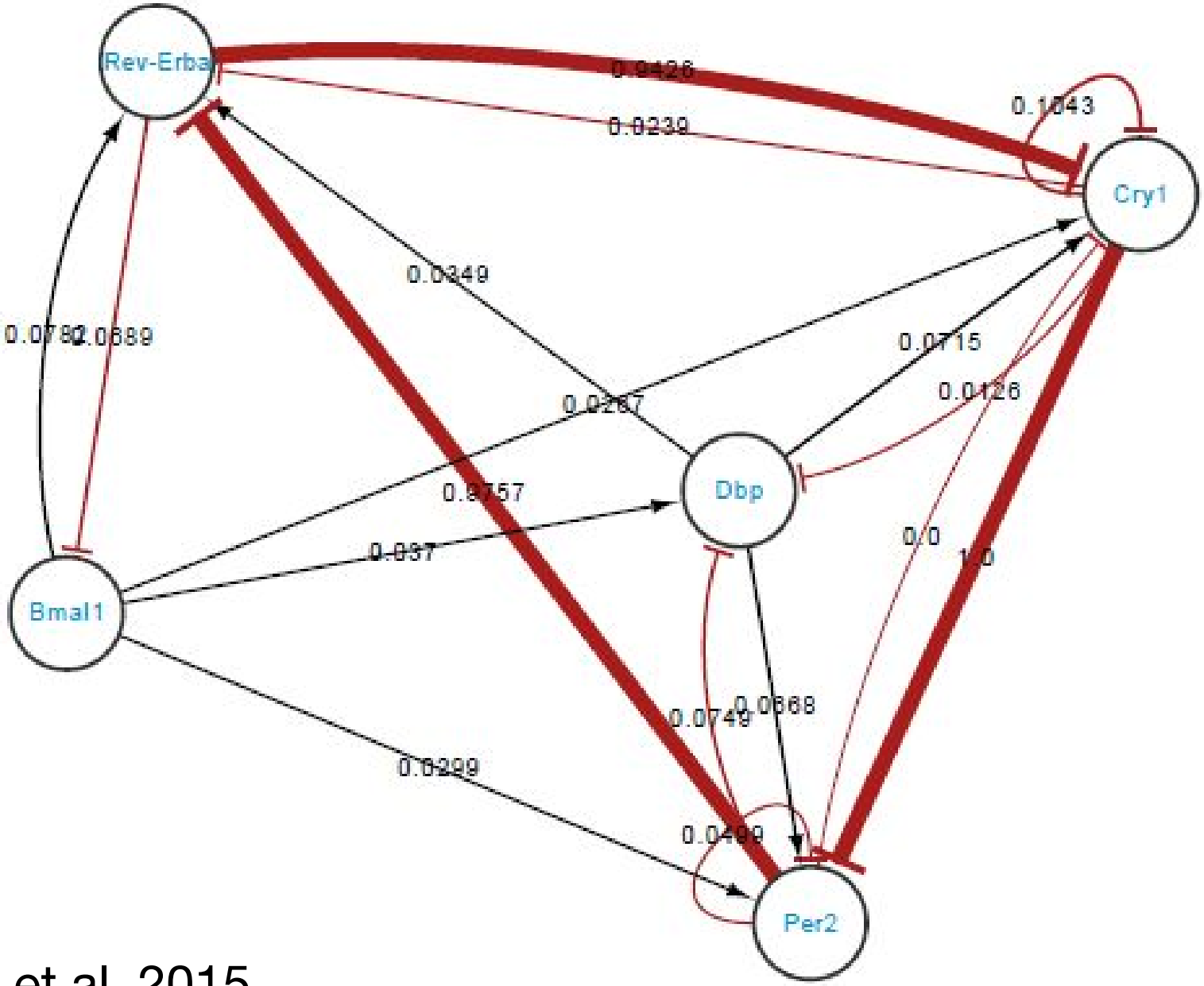
Figure 1: (A) Network graph of the model. Activating and inhibiting influences between genes are colored in blue and red respectively. (B) Simulation of gene expression of all 5 genes. (C) Simulation of gene expression of *Rev-Erba* and *Bmal1*, with other genes fixed to their constant mean value from figure A. After a slight increase of the activation strengh of *Bmal1* to *Rev-Erba*, oscillations are regained with a period of 24h. (D) Simulation of gene expression of *Rev-Erba*, *Per2* and *Cry1*, with other genes fixed to their constant mean value from figure A. The period increases to 27h, but oscillations are retained.

Patrick Pett

What are the most essential regulations?

- we test ON/OFF configurations of 17 regulations
- OFF: regulatory term is clamped to its mean
- out of 131072 configurations 14125 are rhythmic
- percentage of ON relates to relevance of regulation
- e.g. cyclic activation of Per2 is ON for only 2.99%
  but Rev-Erb inhibits Cry1 in 94.26%

Frequencies of Cyclic Regulatory Interactions



Patrick Pett et al. 2015

„Repressilator genes“ are essential for clock

LETTER

doi:10.1038/nature11048

Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β

Han Cho[1], Xuan Zhao[1], Megumi Hatori[2], Ruth T. Yu[1], Grant D. Barish[1], Michael T. Lam[3], Ling-Wa Chong[1], Luciano DiTacchio[2], Annette R. Atkins[1], Christopher K. Glass[3], Christopher Liddle[4], Johan Auwerx[5], Michael Downes[1], Satchidananda Panda[2] & Ronald M. Evans[1,6]

The circadian clock acts at the genomic level to coordinate internal behavioural and physiological rhythms via the CLOCK–BMAL1 transcriptional heterodimer. Although the nuclear receptors REV-ERB-α and REV-ERB-β have been proposed to form an accessory feedback loop that contributes to clock function[1,2], their precise roles and importance remain unresolved. To establish their regulatory potential, we determined the genome-wide *cis*-acting targets (cistromes) of both REV-ERB isoforms in murine liver, which revealed shared recognition at over 50% of their total DNA binding sites and extensive overlap with the master circadian regulator BMAL1. Although REV-ERB-α has been shown to regulate *Bmal1* expression directly[1,2], our cistromic analysis reveals a more profound connection between BMAL1 and the REV-ERB-α and REV-ERB-β genomic regulatory circuits than was previously suspected. Genes within the intersection of the BMAL1, REV-ERB-α and REV-ERB-β cistromes are highly enriched for both clock and metabolic functions. As predicted by the cistromic analysis, dual depletion of *Rev-erb-α* and *Rev-erb-β* function by creating double-knockout mice profoundly disrupted circadian expression of core circadian clock and lipid homeostatic gene networks. As a result, double-knockout mice show markedly altered circadian wheel-running behaviour and deregulated lipid metabolism. These data

Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms

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Many biochemical, physiological and behavioural processes show circadian rhythms which are generated by an internal time-keeping mechanism referred to as the biological clock. According to rapidly developing models, the core oscillator driving this clock is composed of an autoregulatory transcription–(post) translation-based feedback loop involving a set of ‘clock’ genes[1–6]. Molecular clocks do not oscillate with an exact 24-hour rhythmicity but are entrained to solar day/night rhythms by light. The mammalian proteins Cry1 and Cry2, which are members of the family of plant blue-light receptors (cryptochromes) and photolyases, have been proposed as candidate light receptors for photoentrainment of the biological clock[7–10]. Here we show that mice lacking the Cry1 or Cry2 protein display accelerated and delayed free-running periodicity of locomotor activity, respectively. Strikingly, in the absence of both proteins, an instantaneous and complete loss of free-running rhythmicity is observed. This suggests that, in addition to a possible photoreceptor and antagonistic clock-adjusting function, both proteins are essential for the maintenance of circadian rhythmicity.

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Nonredundant Roles of the *mPer1* and *mPer2* Genes in the Mammalian Circadian Clock

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Summary

Mice carrying a null mutation in the *Period 1* (*mPer1*) gene were generated using embryonic stem cell technology. Homozygous *mPer1* mutants display a shorter circadian period with reduced precision and stability. Mice deficient in both *mPer1* and *mPer2* do not express circadian rhythms. While mPER2 regulates clock gene expression at the transcriptional level, mPER1 is dispensable for the rhythmic RNA expression of *mPer1* and *mPer2* and may instead regulate mPER2 at a posttranscriptional level. Studies of clock-controlled genes (CCGs) reveal a complex pattern of regulation by mPER1 and mPER2, suggesting independent controls by the two proteins over some output pathways. Genes encoding key enzymes in heme biosynthesis are under circadian control and are regulated by mPER1 and mPER2. Together, our studies show that mPER1 and mPER2 have distinct and complementary roles in the mouse clock mechanism.

pervasive role in temporal organization of biochemical and physiological functions (Young, 2000).

The first clock mutants were isolated in *Drosophila* (Konopka and Benzer, 1971) and the corresponding molecular defects were later identified in the *Period* (*per*) gene (Bargiello et al., 1984). Since then, a number of additional clock genes, including *timeless* (*tim*), *clock* (*clk*), *cycle* (*cyc*), *doubletime* (*dbt*), *cryptochrome* (*cry*), and recently *vrille* (*vri*), have been identified in this model organism (Allada et al., 1998; Blau and Young, 1999; Kloss et al., 1998; Myers et al., 1995; Rutila et al., 1998). Mutations in these genes alter the period length and/or lead to behavioral arrhythmicity. At the molecular level, the clock is characterized by the oscillating expression of specific clock genes including *per* and *tim* (Young, 2000). This oscillating expression is driven by an autoregulatory negative feedback loop where CLK and CYC act positively to drive the expression of *per* and *tim*, while PER and TIM act as a complex to regulate their own transcription negatively by inhibiting CLK/CYC (Allada et al., 1998; Darlington et al., 1998; Rutila et al., 1998). The cyclical activity of PER and TIM is also controlled posttranslationally by regulation of phosphorylation that involves the kinase DBT and nuclear entry (Kloss et al., 1998).

Orthologs of most *Drosophila* circadian clock genes have been identified in mammals, highlighting a general conservation in the clock mechanism between insects and mammals. In particular, three mammalian *Period* genes (*mPer1*, *mPer2*, and *mPer3*), two *Cryptochrome* genes (*mCry1* and *mCry2*), as well as *Clock*, *Bmal1* (ortholog to *Cyc*), and *CK1ε* (ortholog to *Dbt*) have been identified (Albrecht et al., 1997a; Bunger et al., 2000;

Mammalian Period represses and de-represses transcription by displacing CLOCK–BMAL1 from promoters in a Cryptochrome-dependent manner

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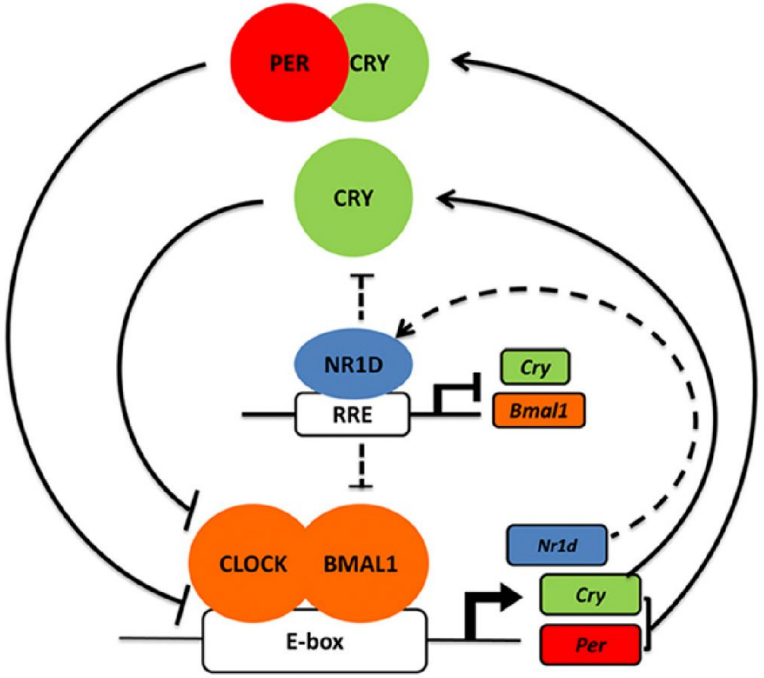
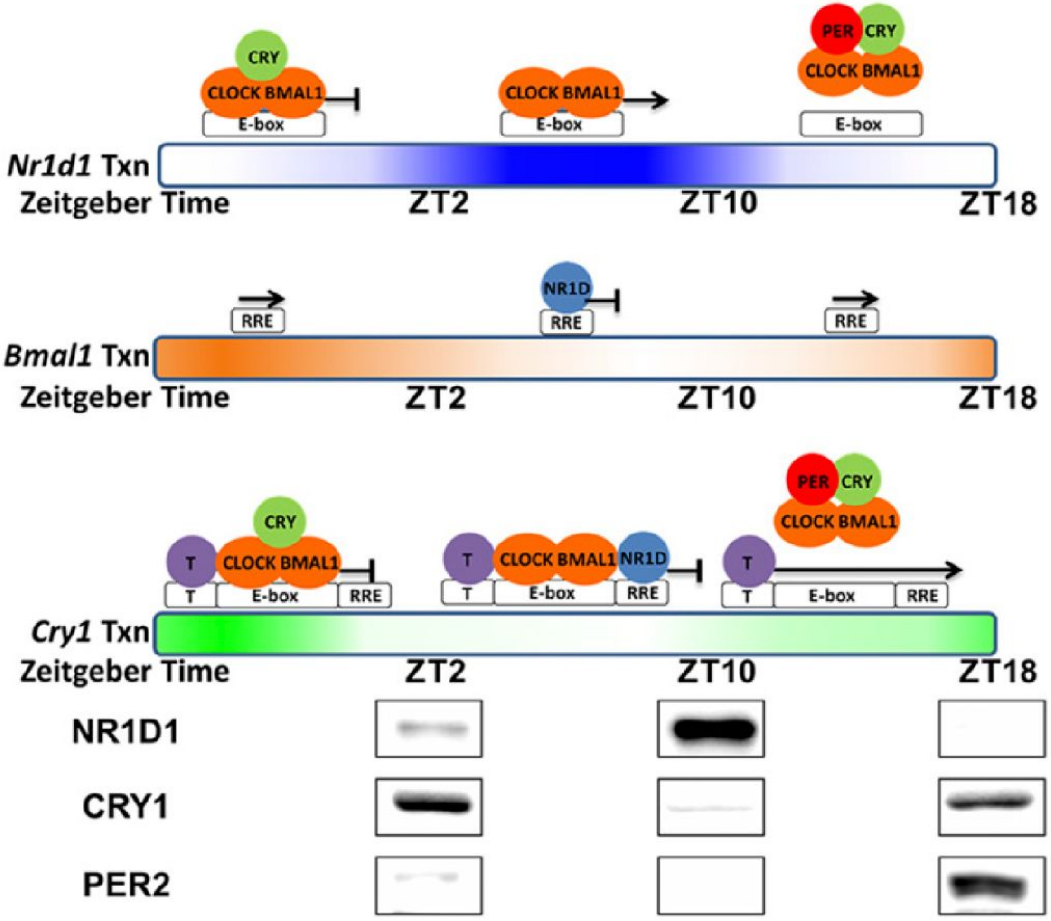




Serial repression via Cry1, Rev-Erba, and Per2 confirmed

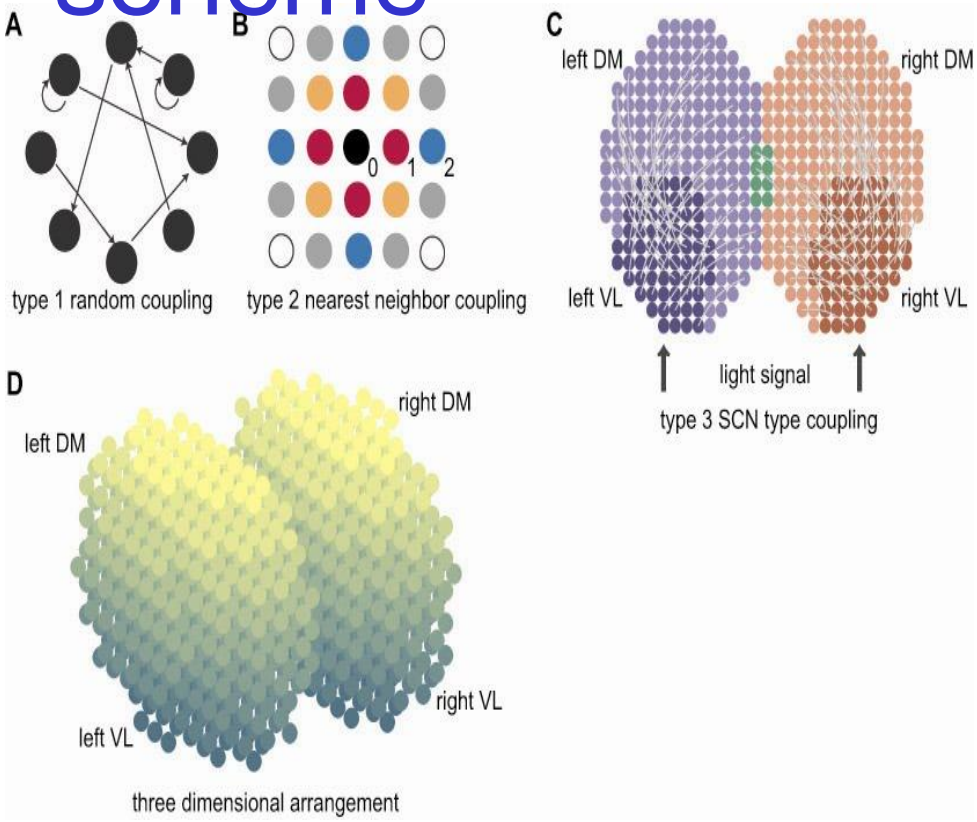
Lessons from Modeling

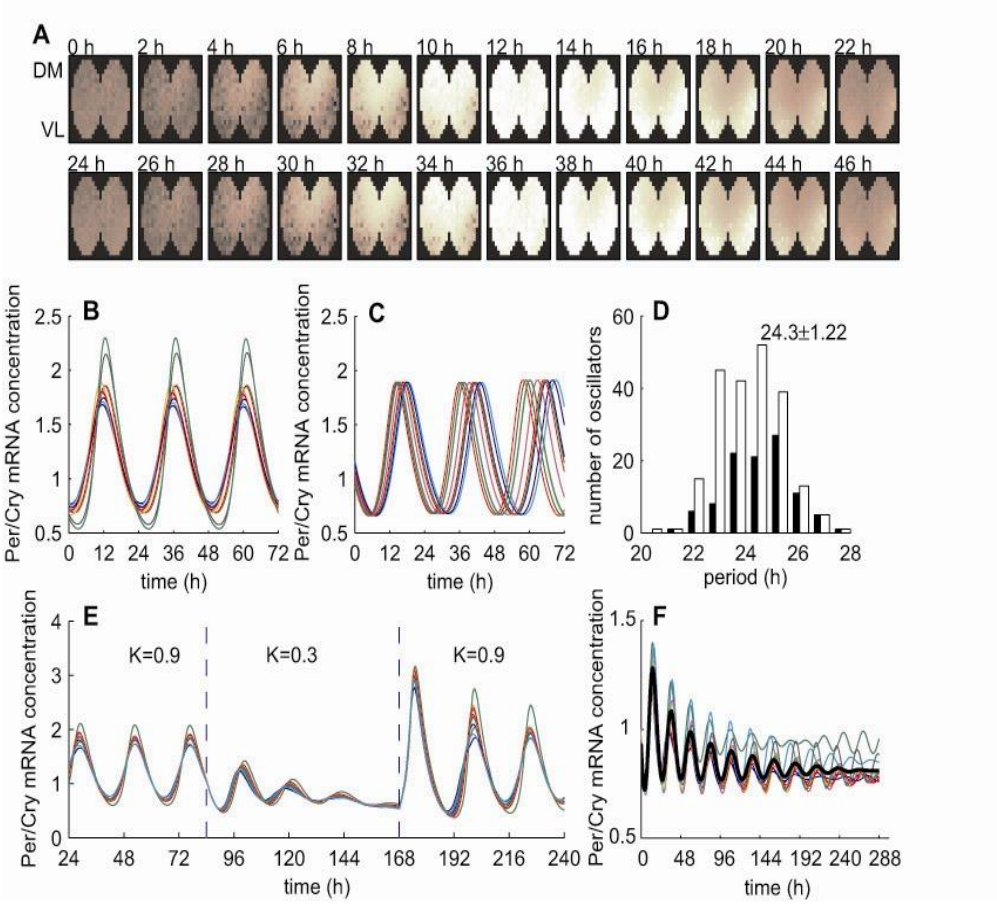
How to design a minimal core clock model?

- Five genes represent most regulations
- DDEs require few parameters
- Transcriptional regulations remain heuristic
- Per/Cry loop, Rev-Erba loop and repressilator possible

Synchronization of sloppy oscillators

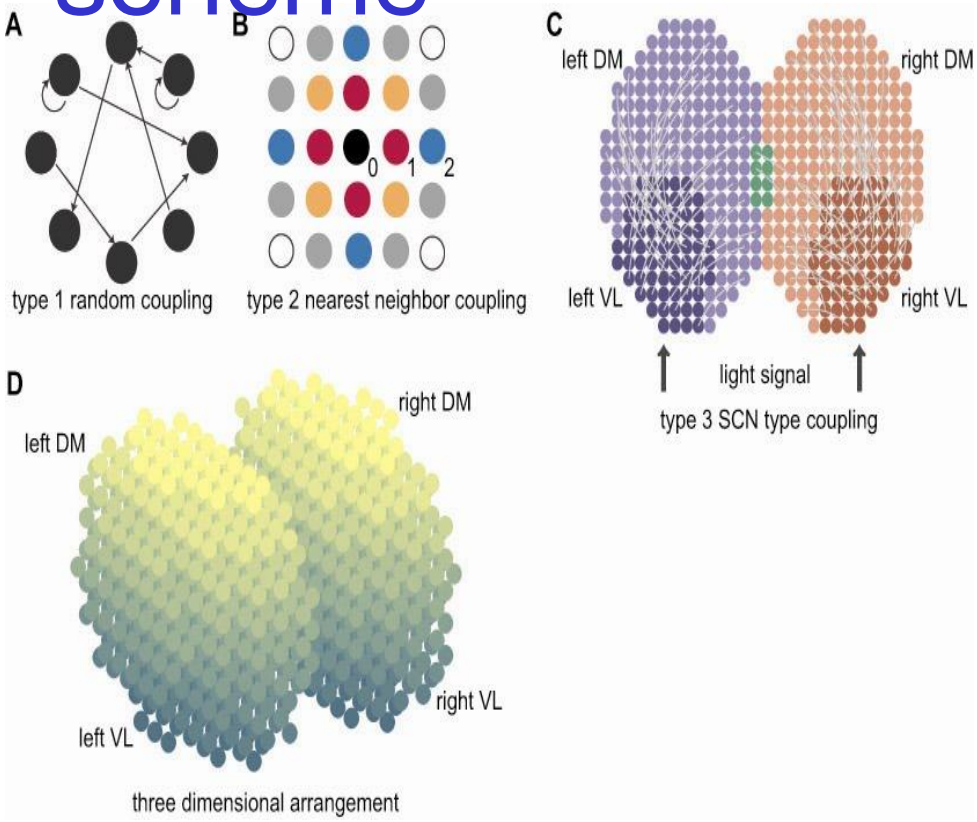
Sloppy oscillators synchronize well independent of specific coupling scheme

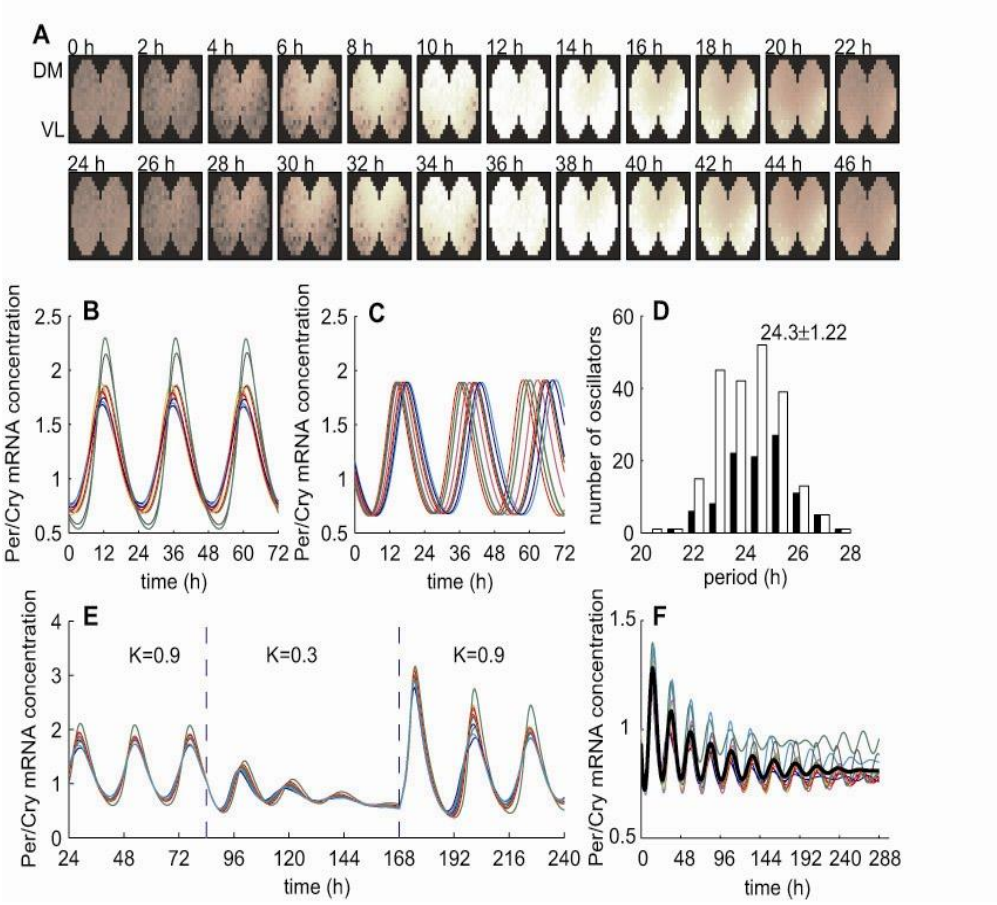




S. Bernard, D. Gonze, B. Cajavec, H. Herzel, and A. Kramer: Synchronization-Induced Rhythmicity of Circadian Oscillators in the Suprachiasmatic Nucleus, PLoS Comp. Biol. (2007) 3:e68.

Damped oscillators synchronize well independent of specific coupling scheme





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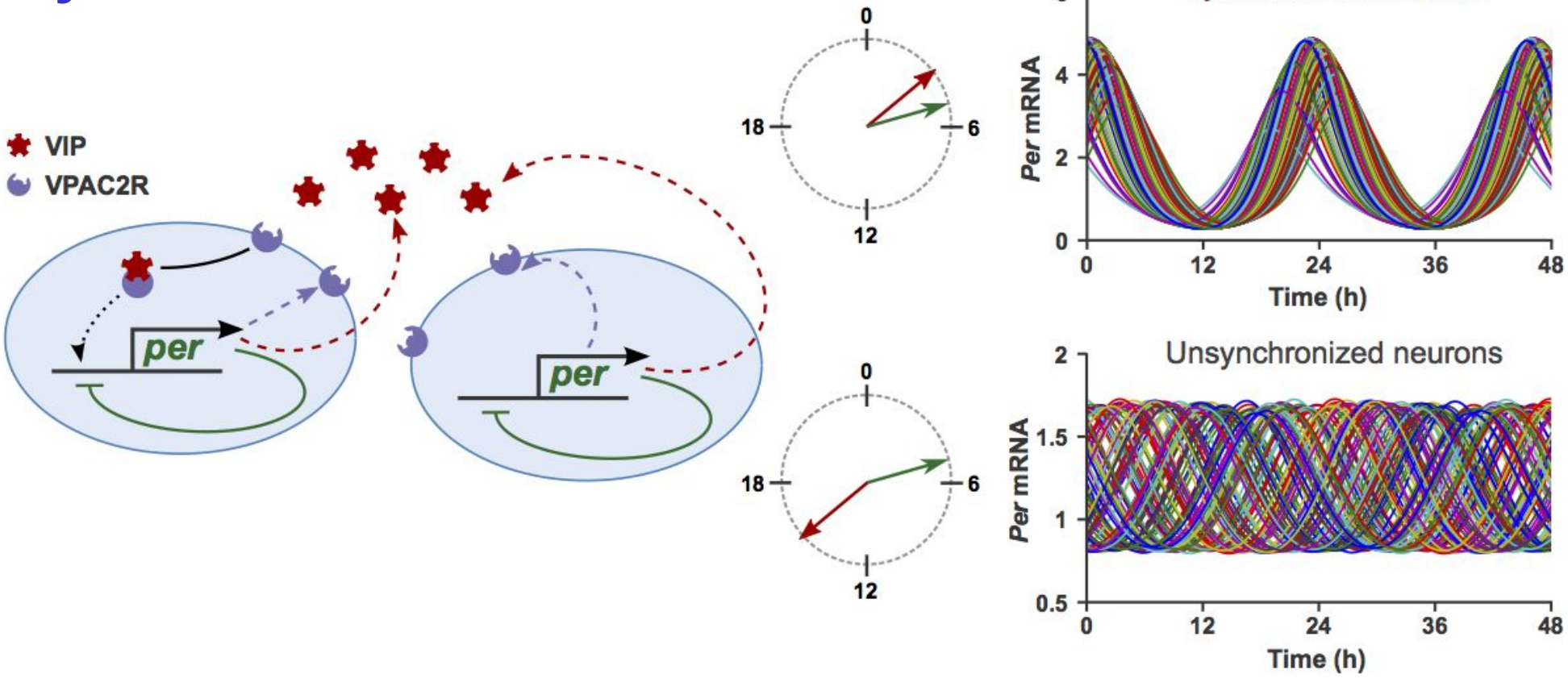
Intrinsic, nondeterministic circadian rhythm generation in identified mammalian neurons

Alexis B. Webb[a], Nikhil Angelo[a], James E. Huettner[b], and Erik D. Herzog[a,1]

makers. Instead, these results indicate that AVP, VIP, and other SCN neurons are intrinsic but unstable circadian oscillators that rely on network interactions to stabilize their otherwise noisy cycling.

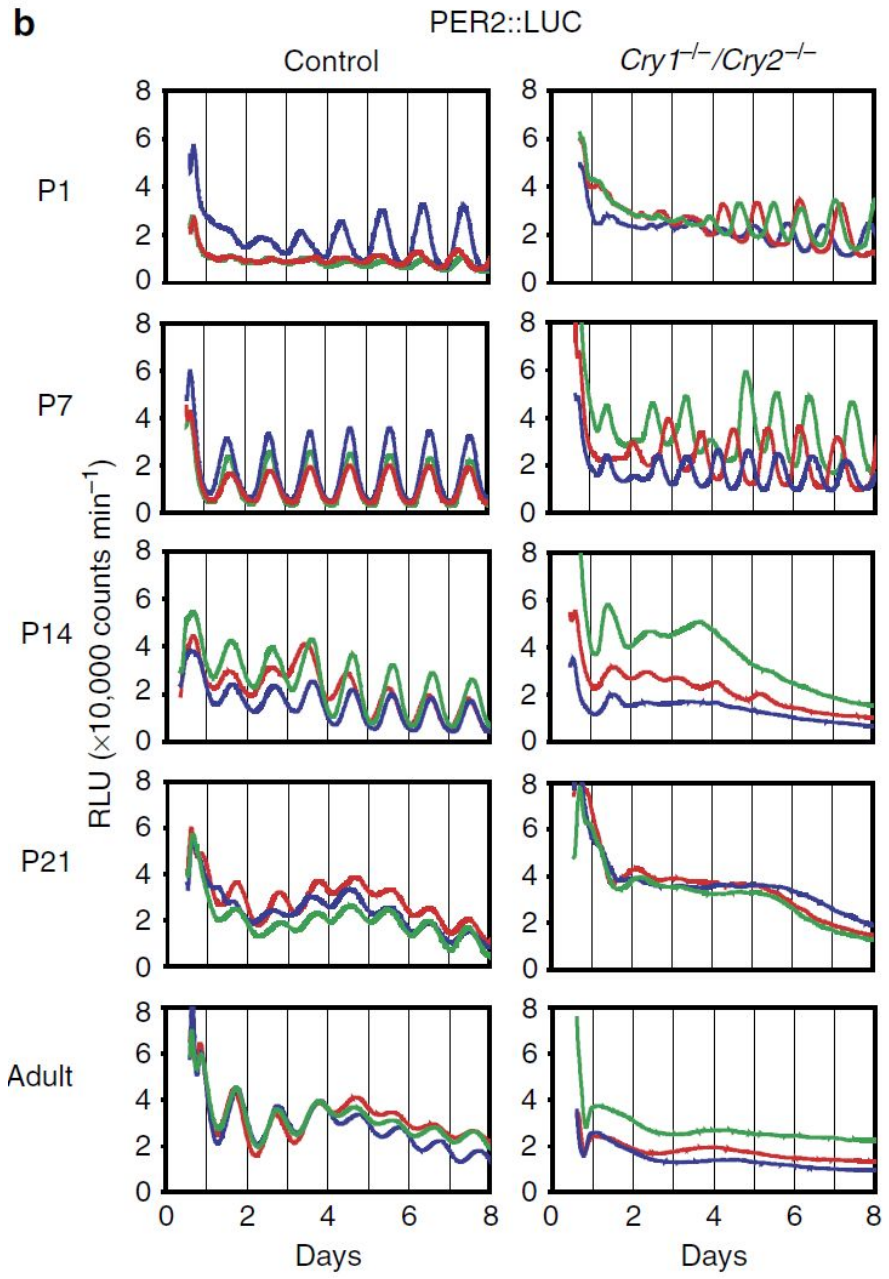
Coupling **phase** controls synchronization



Implications for dual role of GABA (J. Evans, Neuron 2013) and synchrony of neonatal versus adult SCN slices (Honma, Nature Communications 2013)

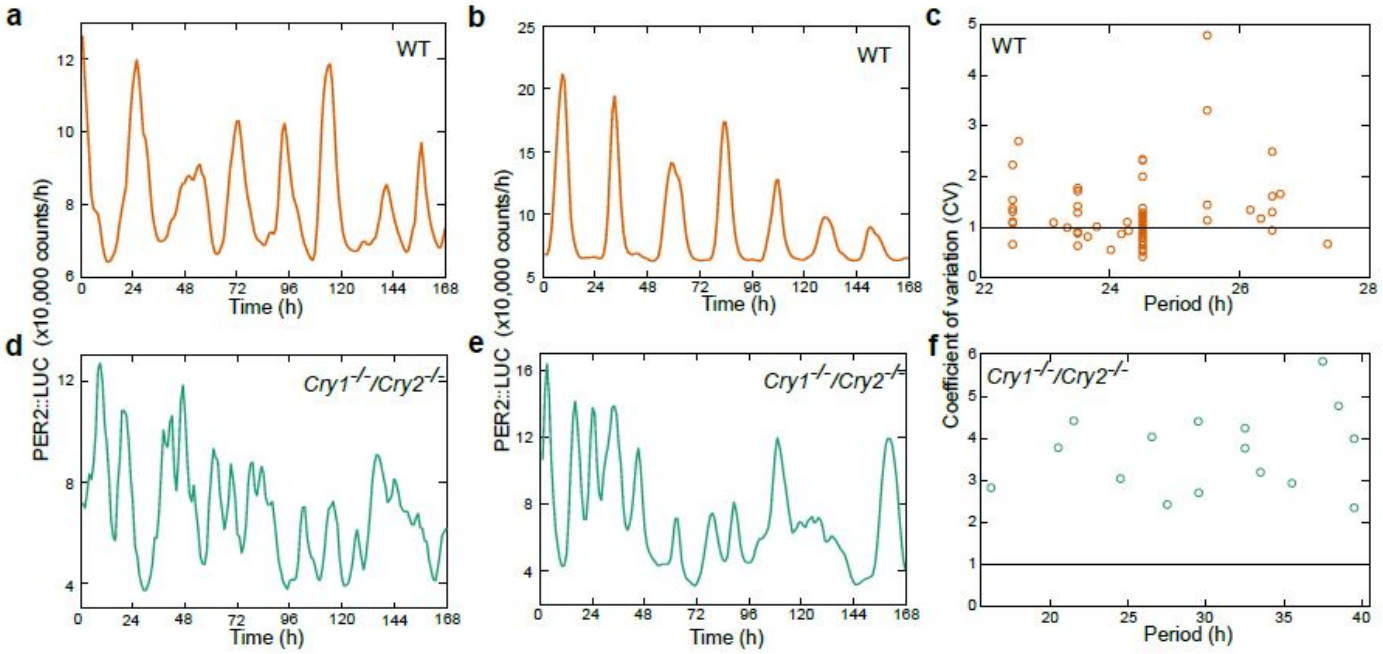
B. Ananthasubramaniam, E. D. Herzog, H. Herzel. PLoS Comp. Biol. 2014

Coupling can even synchronize Cry-DKO neonatal SCN



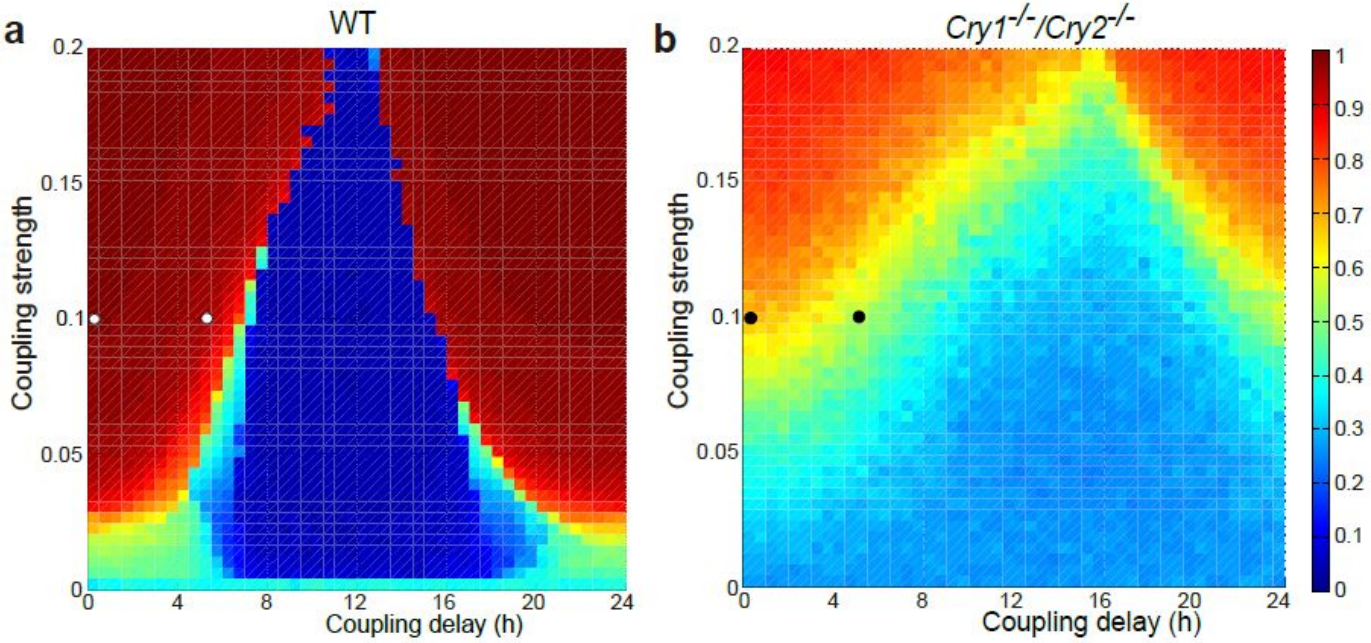
D. Ono, S. Honma, K. Honma
Nature Communications 2013

Synchronization of noisy oscillators (WT and DKO)



CV about 1

CV above 1



Better sync for WT

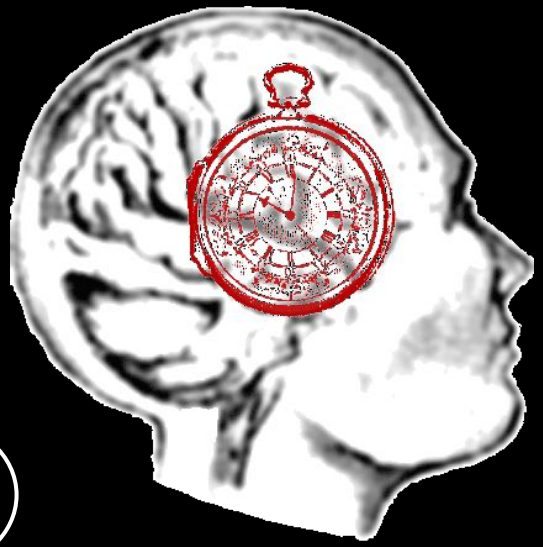
Coupling strength enhances sync

Coupling phase matters strongly

I.T.Tokuda et al. Biophys J.

Zeitgeber

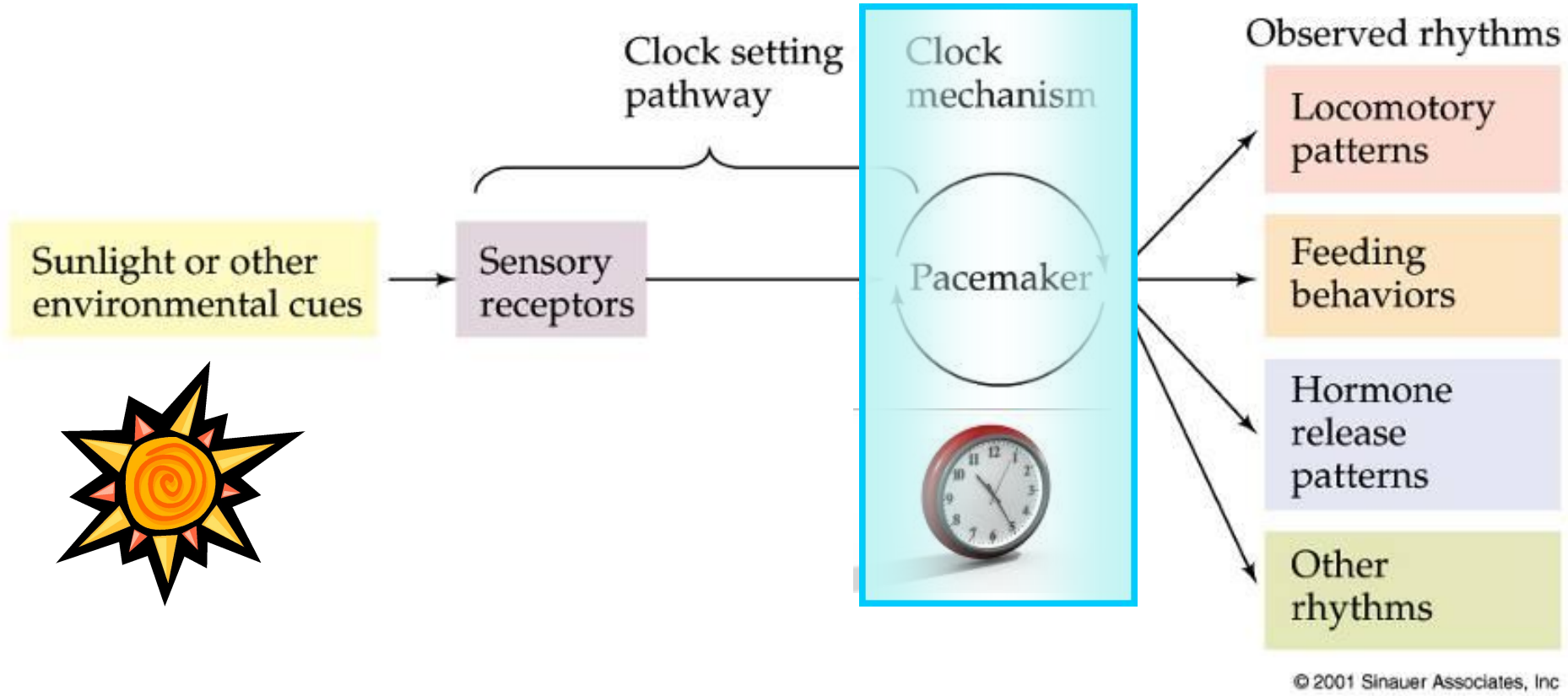
Light
Temperatur
e
Food
…



The environmental external oscillator entrains our internal oscillator

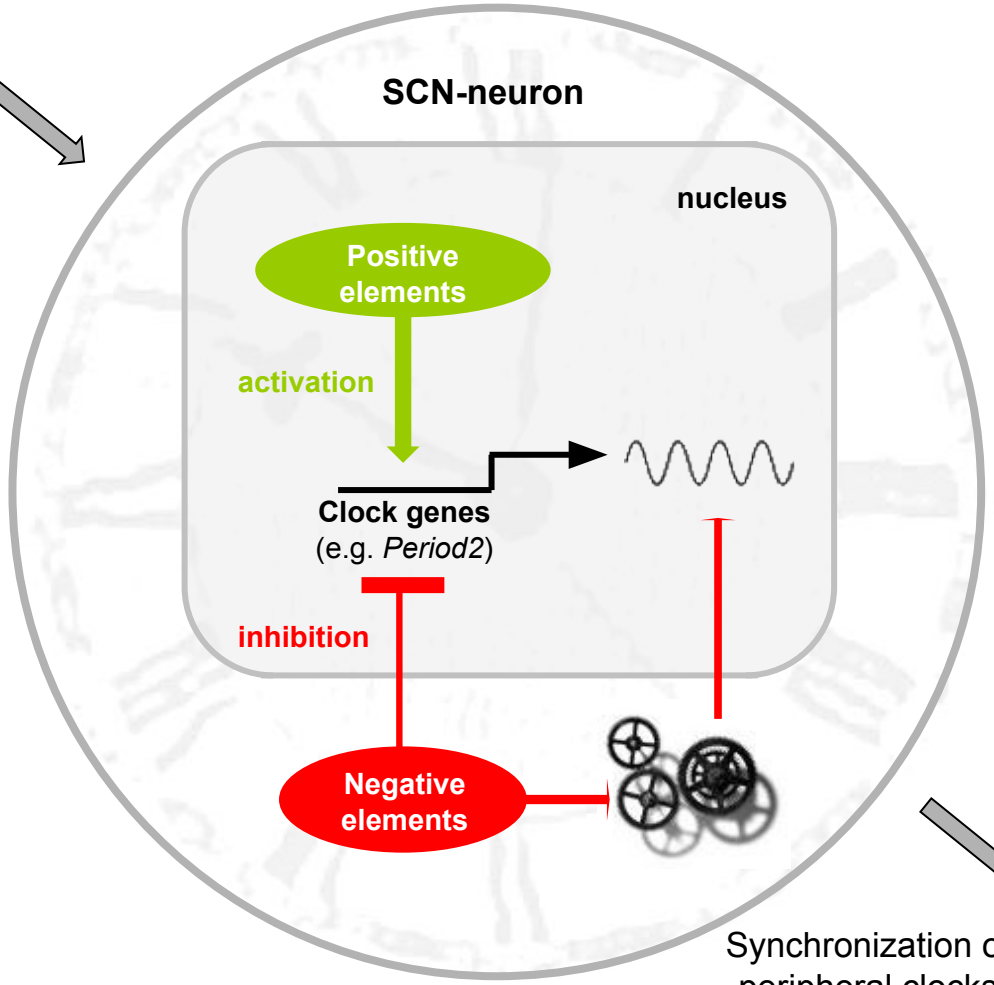
(the circadian clock)

The organization of the circadian system



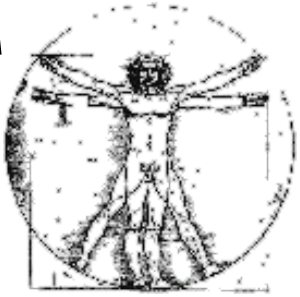
The system

Light synchronizes
the clock



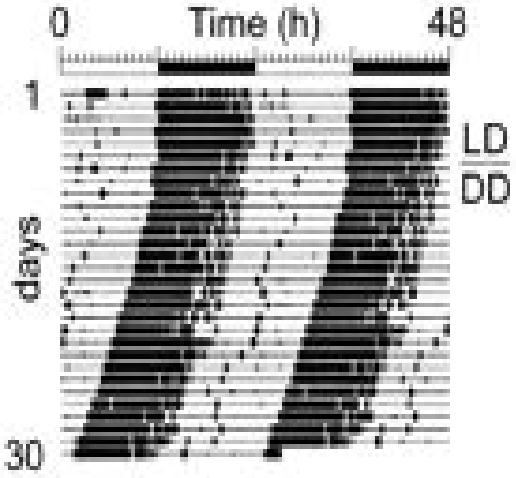
Regulation of
physiology and behavior

Synchronization of
peripheral clocks

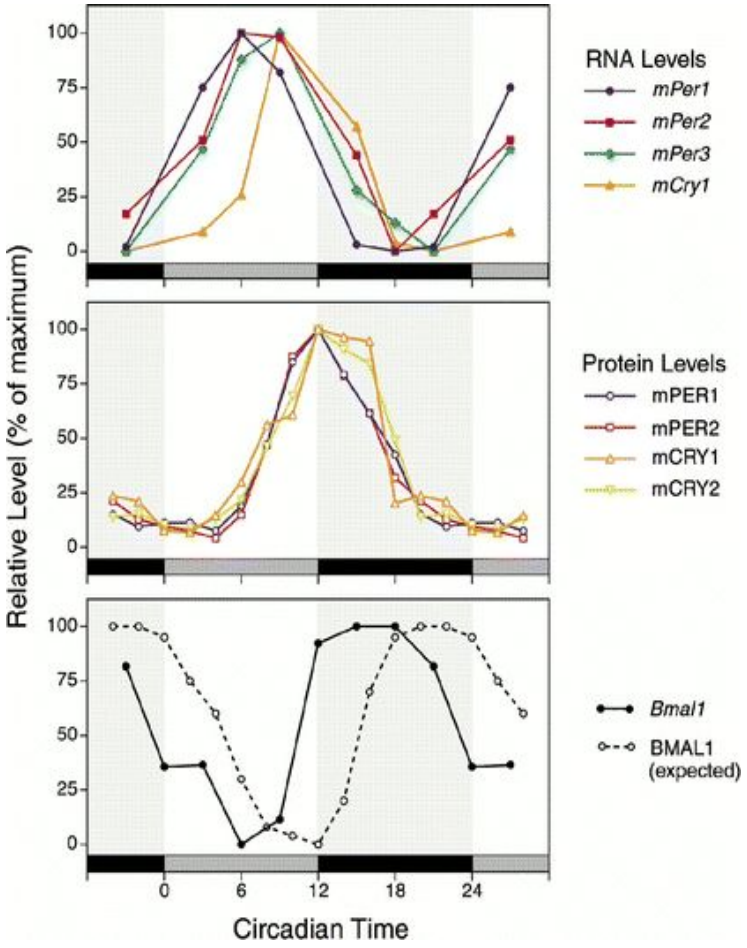


The circadian oscillator

Circadian rhythm ⟵ Oscillations ⟵ Feedback loops



Oster et al., 2002



Reppert and Weaver, 2001