

An introduction to chronobiology

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Ecole thématique “Modélisation formelle de réseaux de régulation biologique”

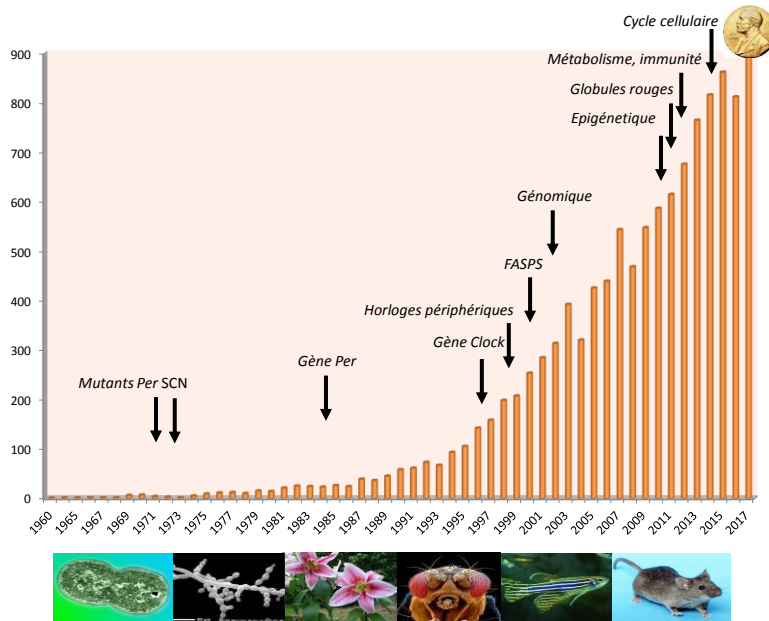
Porquerolles 24 juin 2019



Outline

- Circadian rhythms
- Circadian clocks (localisation, properties, mechanisms)
- Circadian clocks in health and diseases

A fast expanding field



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Nobel 2017 Physiology and Medicine



JC Hall



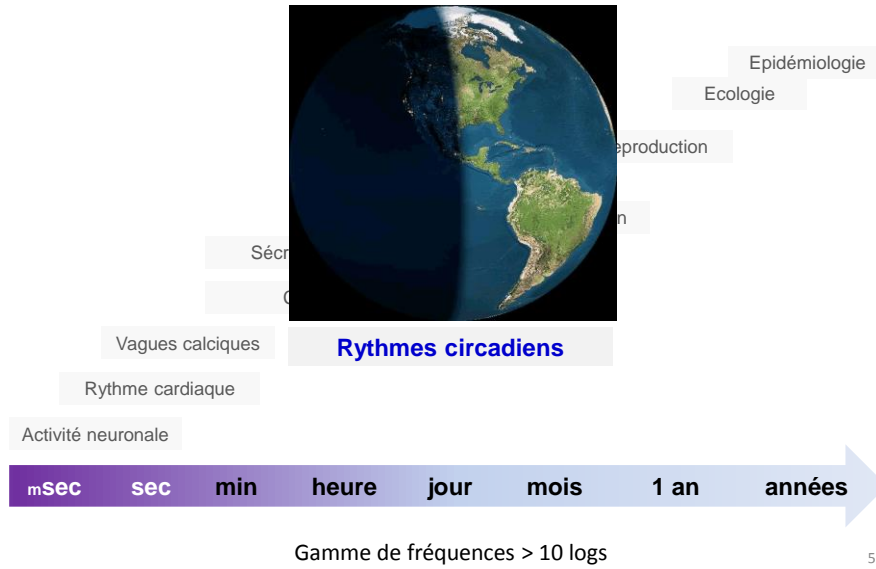
M Rosbash



MW Young

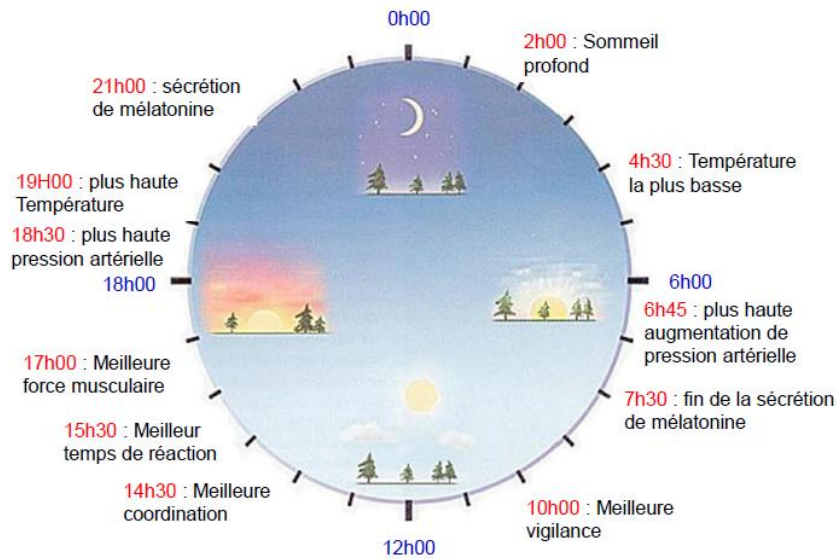
4

Biological oscillations



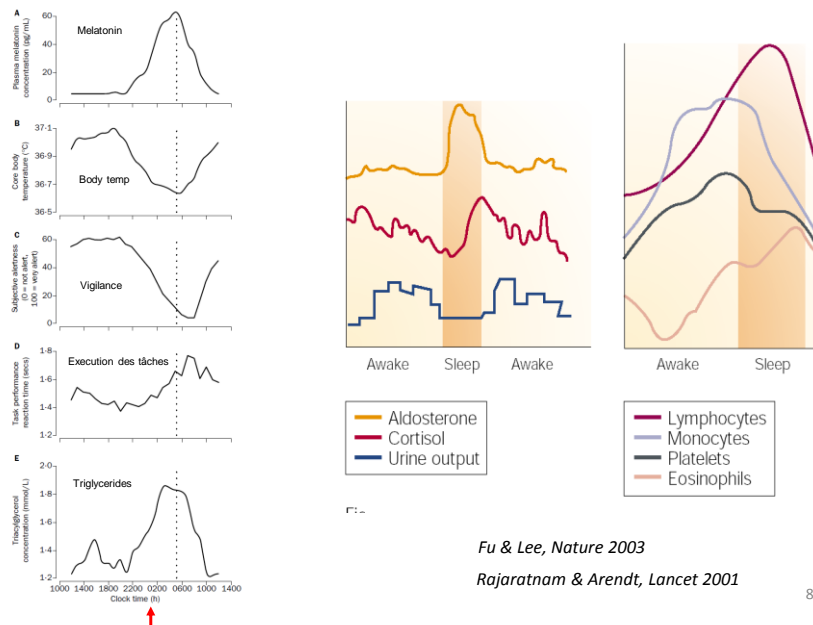
Anticipative adaptation





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Examples of daily rhythms in Human

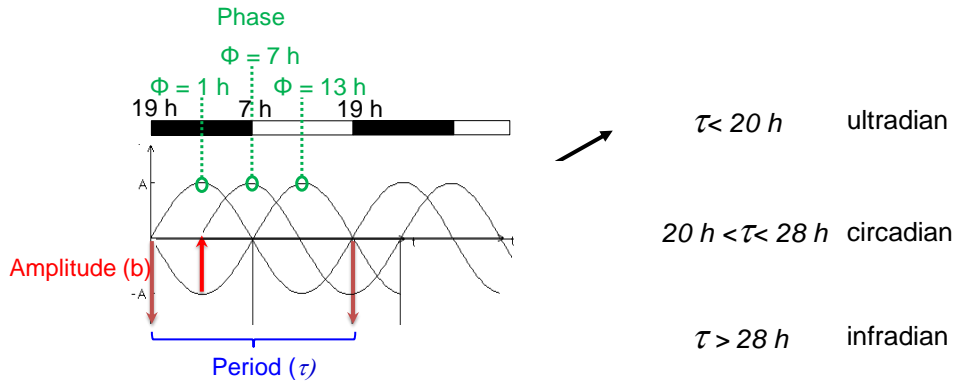


Fu & Lee, Nature 2003

Rajaratnam & Arendt, Lancet 2001

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Parameters that define a circadian rhythm



$$\text{Circadian rhythm} \rightarrow Y = a + b \cos(2\pi(\tau - \Phi))$$

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The father

Jean d'Ortois de Mairan
(1678-1771)

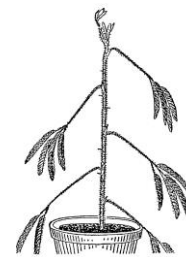


Jour

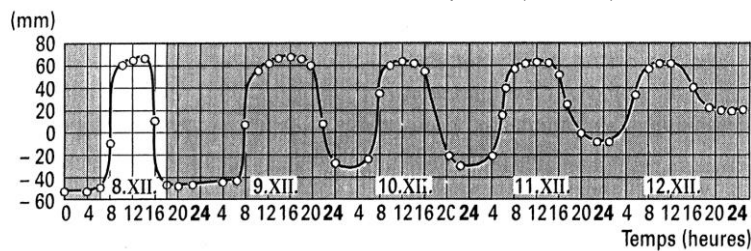
1729



Mimosa pudica (sensitive)



Nuit

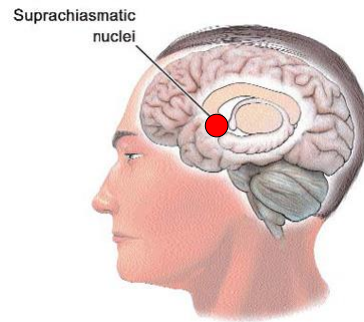


10

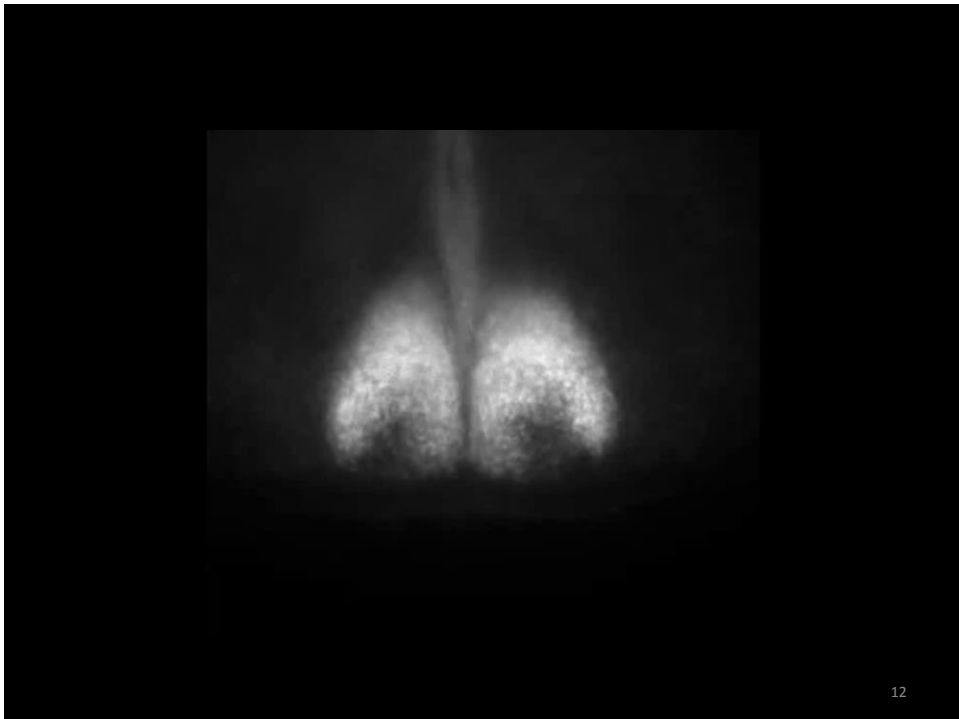


Localisation of the clock in mammals

1972 : Moore & Eichler and Stephan & Zucker locate the circadian Clock in the **suprachiasmatic nuclei** of mammals

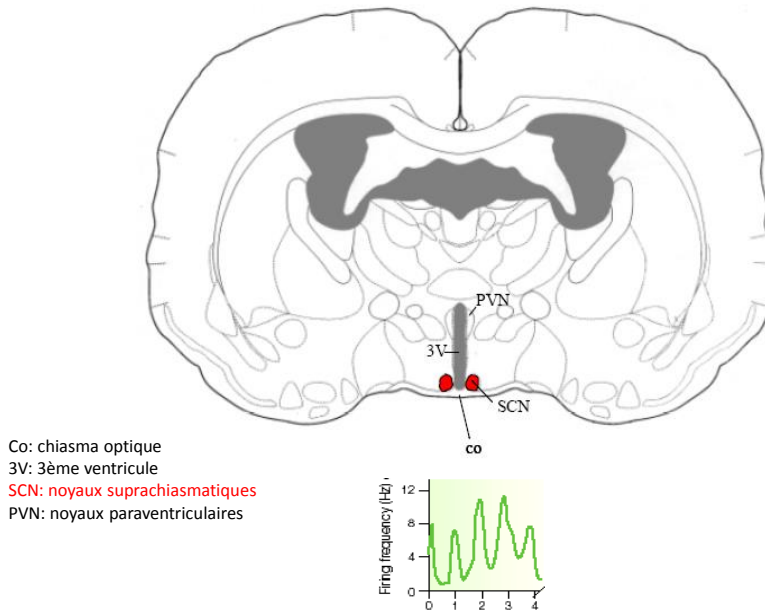


11



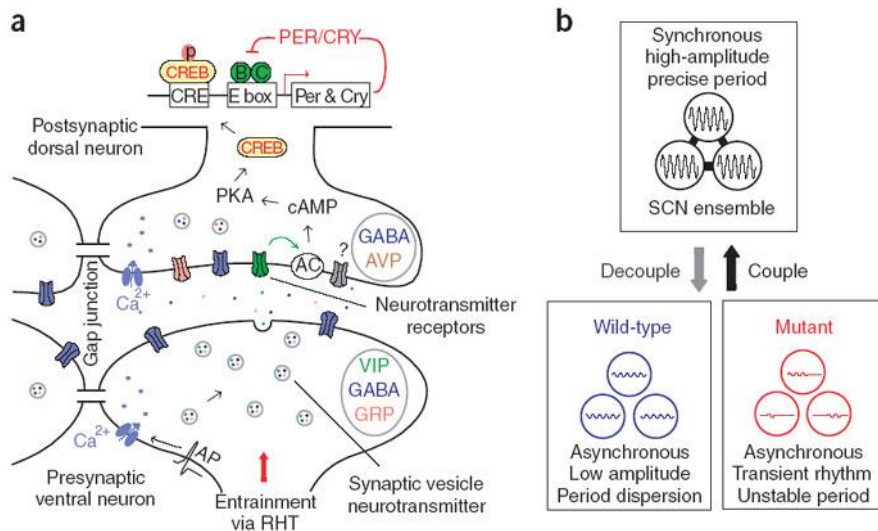
12

SCN neurons are the only clock neurons



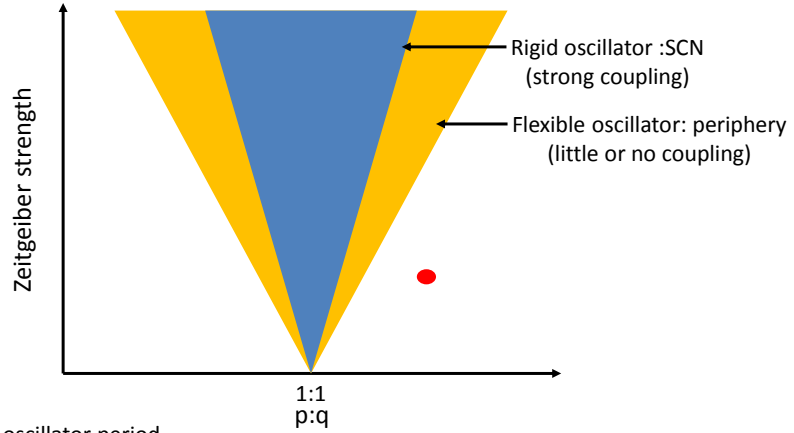
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Intercellular coupling between SCN neurons



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The entrainment range depends on the coupling

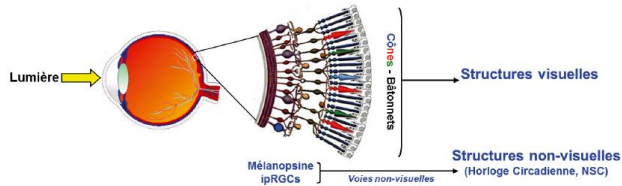


P: clock oscillator period
q: zeitgeber period

HP Herzel et al, Berlin

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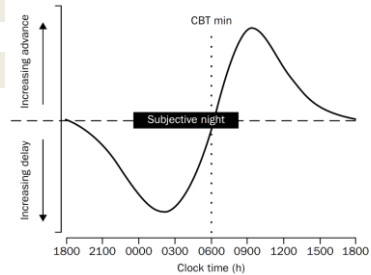
How too reset the brain clock?



Gronfier, *Biologie d'Aujourd' hui* 2014

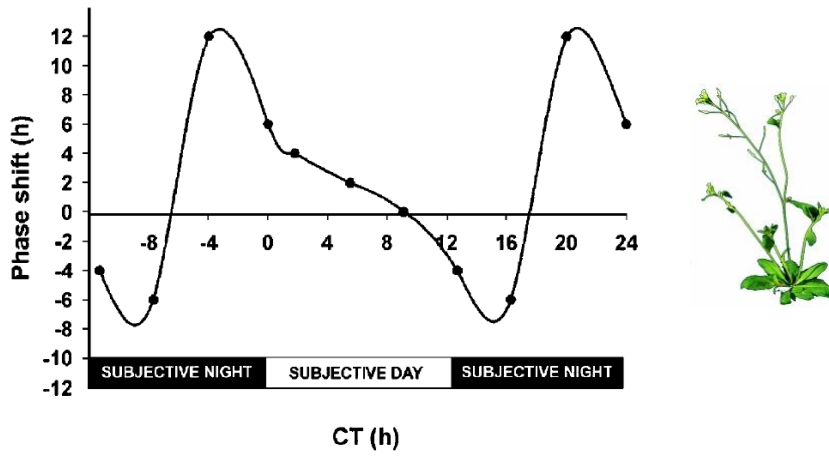
Lumière en **début** de nuit : **retard** de phase (voyage vers l'ouest)

Lumière en **fin** de nuit : **avance** de phase (voyage vers l'est)



Rajaratnam & Arendt, *Lancet* 2001

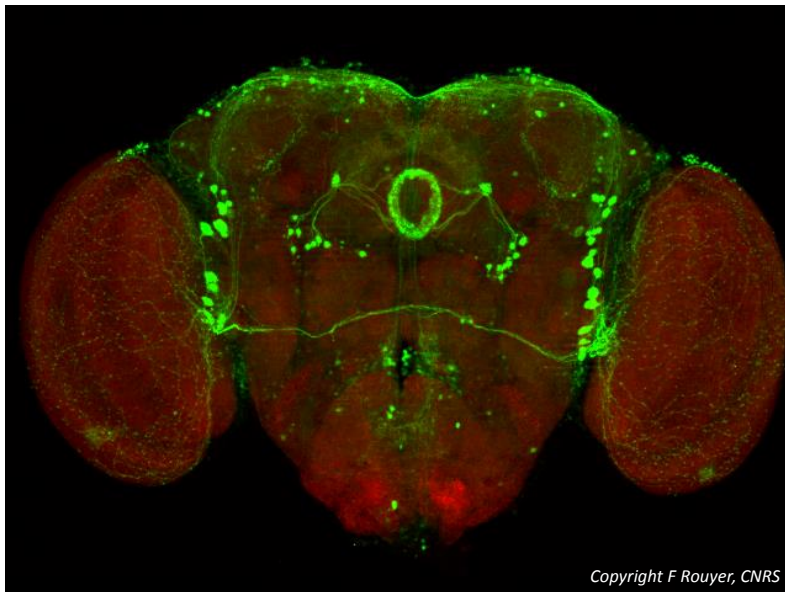
Circadian clock are synchronized by zeitgebers



Phase response curve in *Arabidopsis thaliana*

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If no hypothalamus?

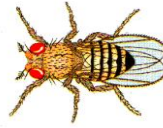


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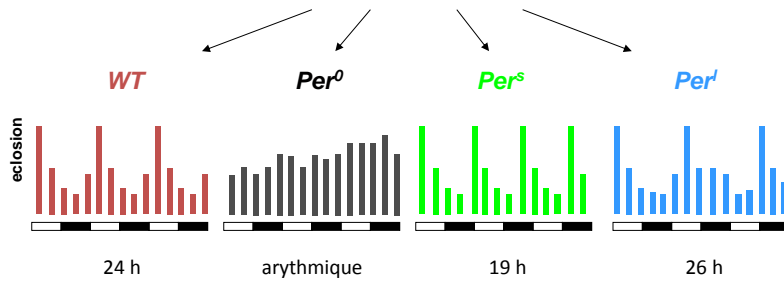
The genetic origin of circadian rhythms



S Benzer (1921-2007)



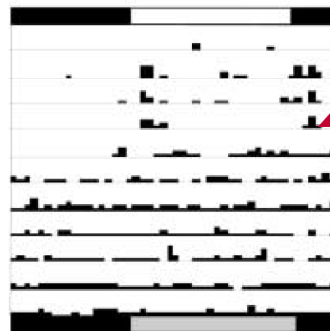
Mutagenèse

Kanopka & Benzer, PNAS 1971¹⁹

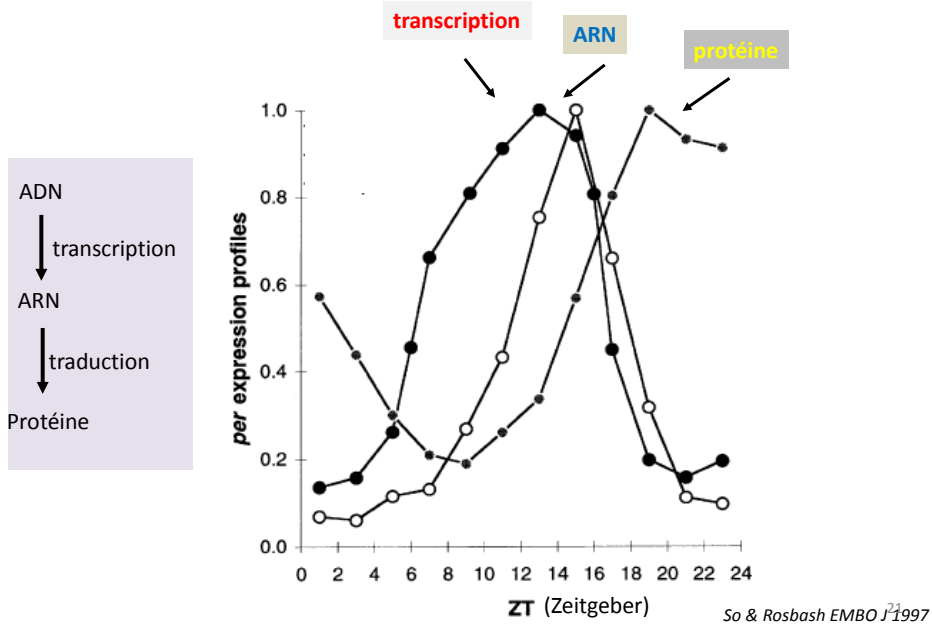
Per⁰ flies are arrhythmic



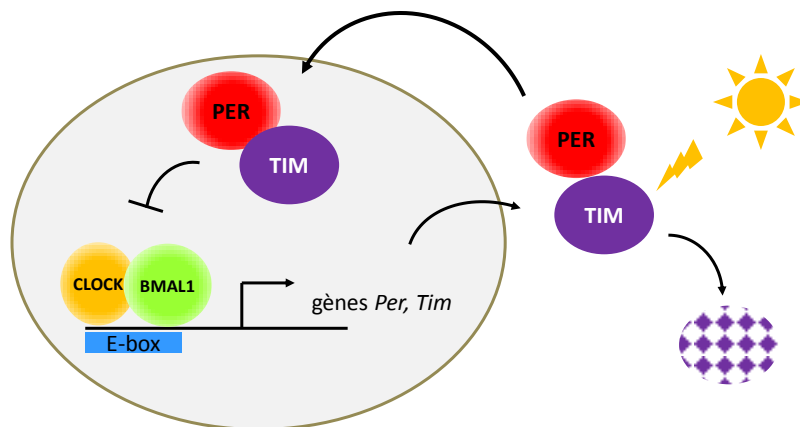
WT

*per⁰*

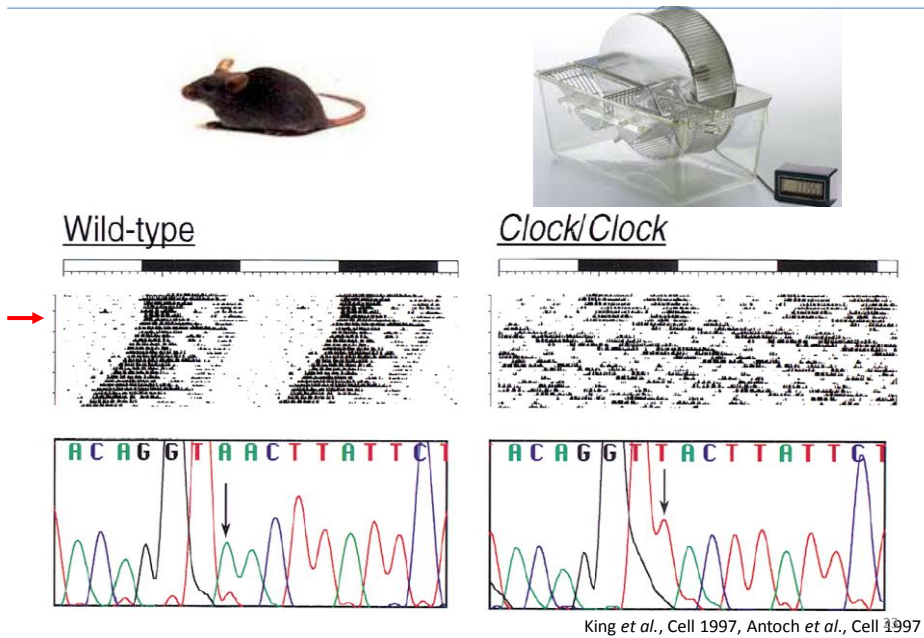
The *Period* gene is rhythmically expressed



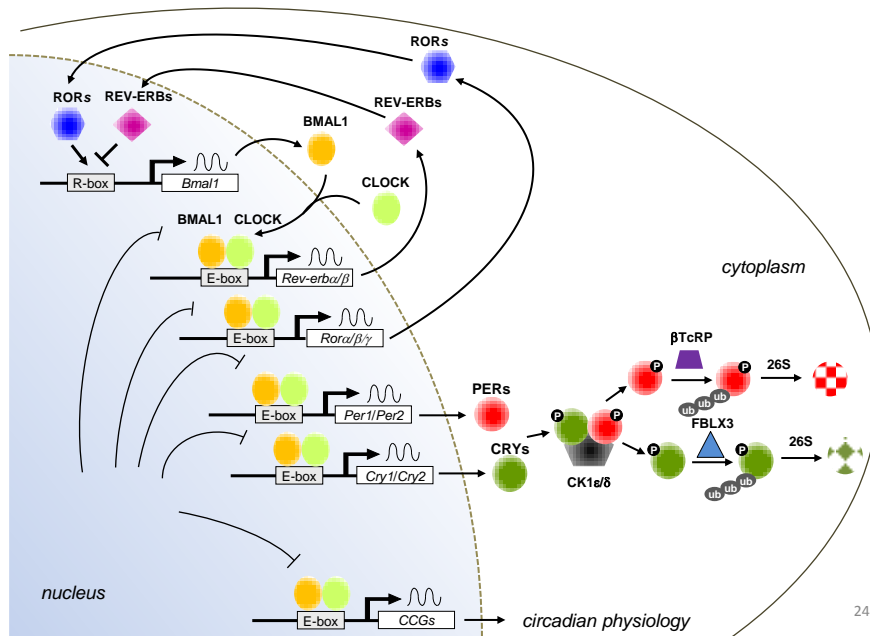
Simplified model of the *Drosophila* circadian oscillator



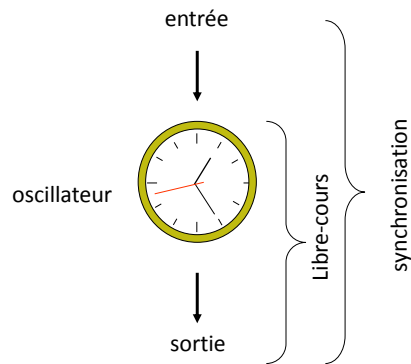
The Clock mouse



The mammalian circadian oscillator



Conceptualisation of the circadin clock

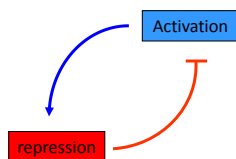


25

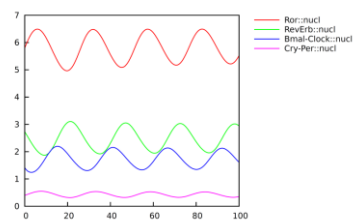
The same design principle governs all circadian clocks



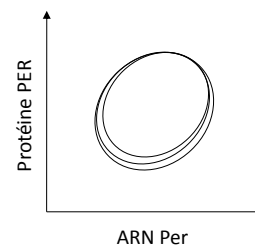
*Boucle de rétrocontrôle
négative + délai*



Modélisation



Cycle limite

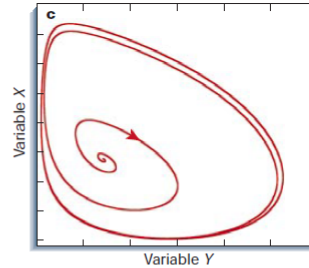
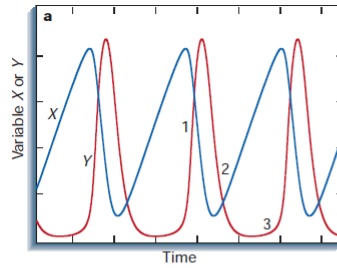
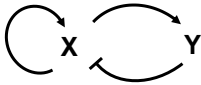


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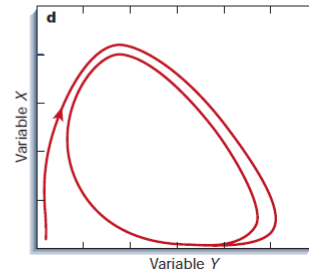
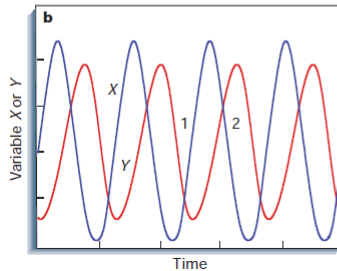
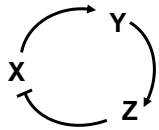
Limit cycle oscillator

Requirements : Feedback motif + Time delay + Non linearity

Amplified delayed feedback loop



Delayed feedback loop

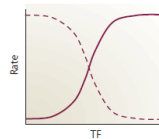
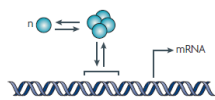


Goldbeter Nature 2002

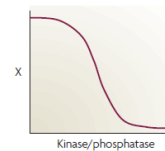
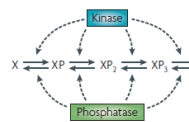
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Sources of non linearity

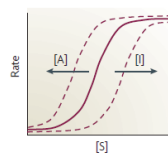
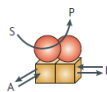
- Oligomer binding



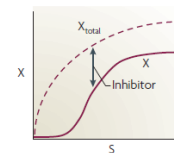
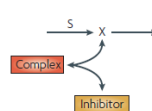
- Multisites phosphorylation



- Cooperativity and allostery

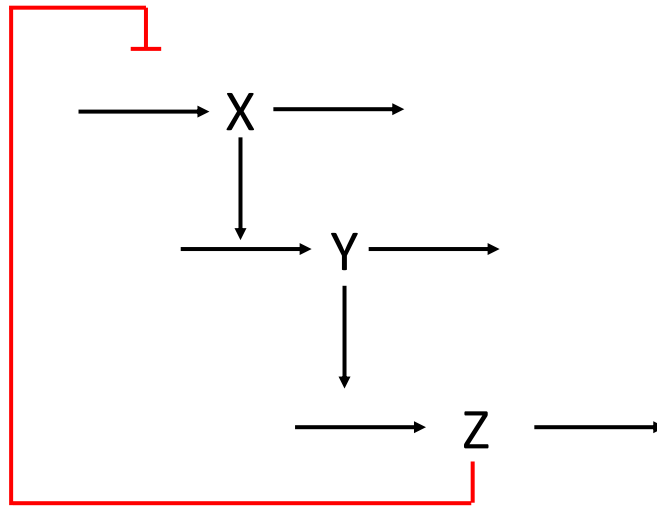


- Stoichiometric inhibition



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The Goodwin oscillator



Inhibition (Hill)

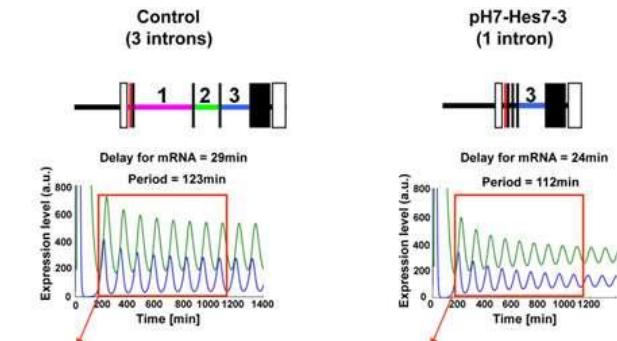
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Biochemical/genetic oscillators

| Function | Components | Period | Class |
|----------------------|-----------------------------------|-----------|----------------------------------|
| Metabolism | Gluucose, ATP, PFKase | 2 min | Amplified delayed feedback loop |
| Signaling | NF-kB, IKK | ~ 2 h | Delayed negative feedback loop |
| Signaling | P53, MDM2 | 5 h | Delayed negative feedback loop |
| Development | Her1, Her7, Notch | 30-90 min | Delayed negative feedback loop |
| Embryonic cell cycle | CDK1, CCNB, Wee1, Cdc25, Cdc20 | 30 min | Amplified negative feedback loop |
| Circadian rhythms | PER, CRY, CLOCK, BMAL1, CSNK1D... | 24 h | Delayed negative feedback loop |

Novak & Tyson Nat Rev Mol Cell Biol, 2008 ³⁰

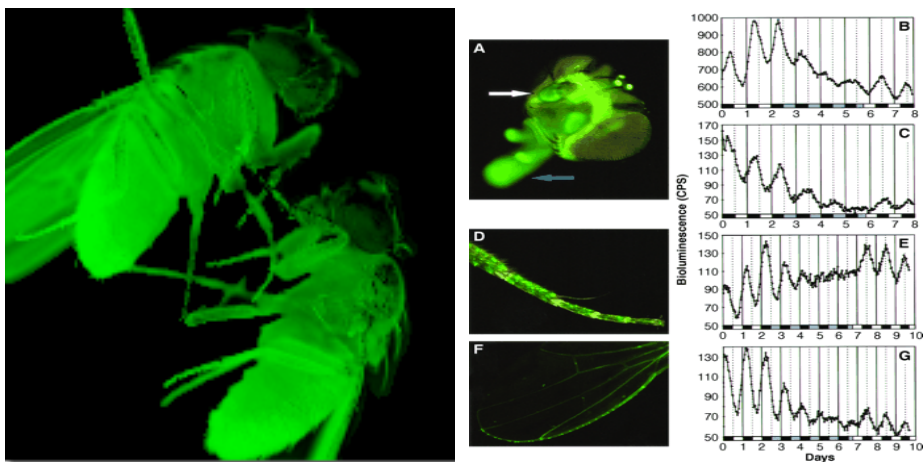
Impact of the intron length on the feedback delay



Harima Y et al. *Semin Cell Dev Biol* 2014

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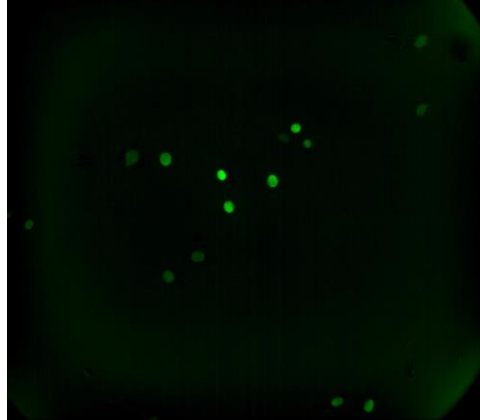
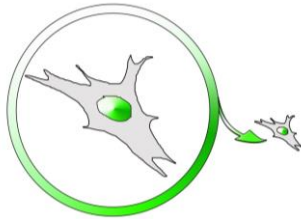
Clocks everywhere !



Plautz et al. *Science* 1997 32

Virtually every cell has a clock

Rapporteur d'horloge : **REVERB α ::VENUS**



(Nagoshi et al, Cell 2004)

Circadian clocks are temperature compensated



1859-1927

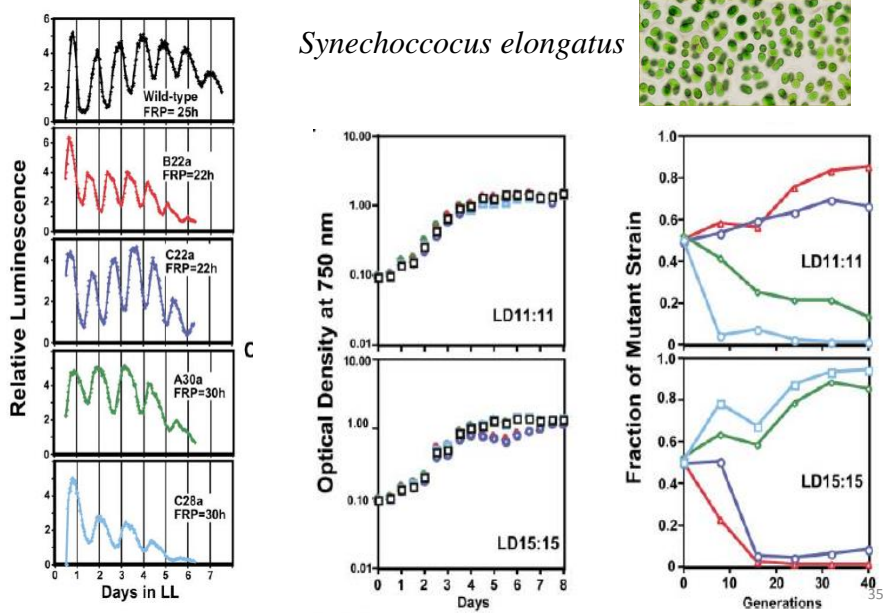
- Le coefficient thermique Q_{10} représente l'augmentation du taux d'une réaction pour un écart de température de 10°C.
- La vitesse de réaction peut être assimilée à n'importe quel processus (vitesse de production d'un composé chimique, vitesse de propagation d'un potentiel d'action, courant conduit à travers un canal ionique, rythme cardiaque, consommation d'oxygène).

$$Q_{10} = \left[\frac{R_2}{R_1} \right]^{\frac{10}{(t_2 - t_1)}}$$

Horloge circadienne: $Q_{10} \sim 1$

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The adaptive value of circadian clocks



The adaptive value of circadian clocks

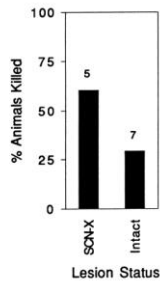


2 groups of spermophiles squirrels

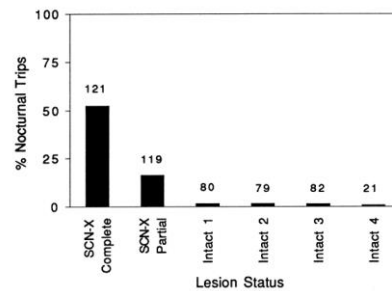
Intacts: diurnal

SCNx: increased nocturnal activity

A. Predation



B. Activity at Food Cache



CircadianOmics

- Methyloome
- Acetyloome
- Transcriptome
- Proteome
- Phosphoproteome
- Metabolome

> 400 datasets
> 50 tissues
> 10 species
> 10 condition types (KO, disease, diet, drugs)

- Circadian regulation operates at all levels
- Highly tissue specific
- Feeding behaviour which is controlled by the central clock has a prominent impact on rhythms in the periphery
- Many CCGs are involved in pathways targeted by drugs
- Emerging human circadian omics

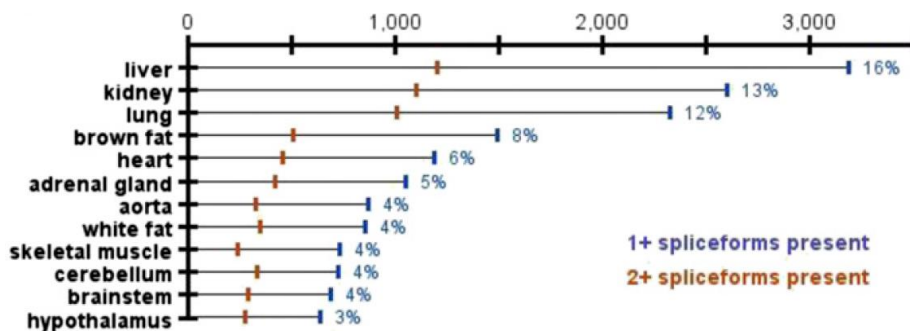
<http://circadiomics.ics.uci.edu/>

<http://circadb.hogenschlab.org/>

<http://cgdb.biocuckoo.org/index.php>

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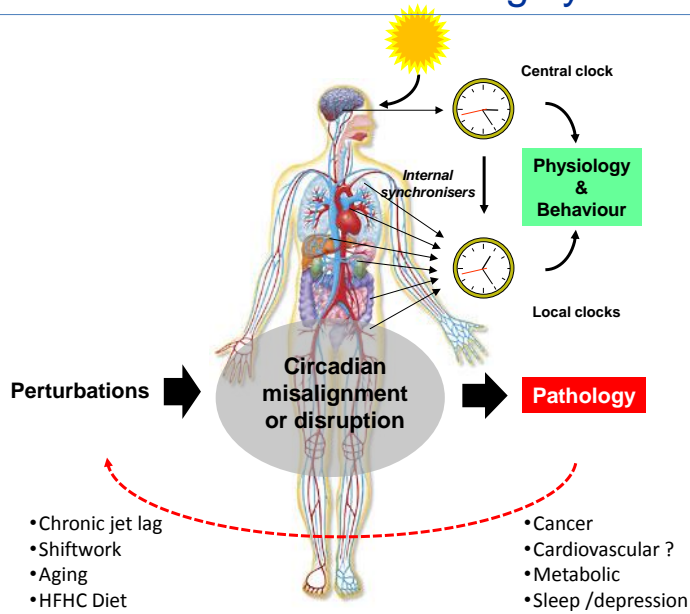
Circadian gene expression is extensive



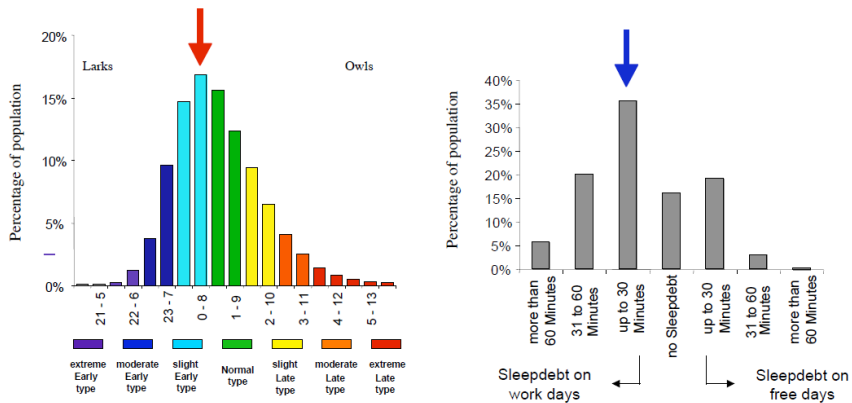
Zhang et al PNAS 2014

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The human circadian timing system

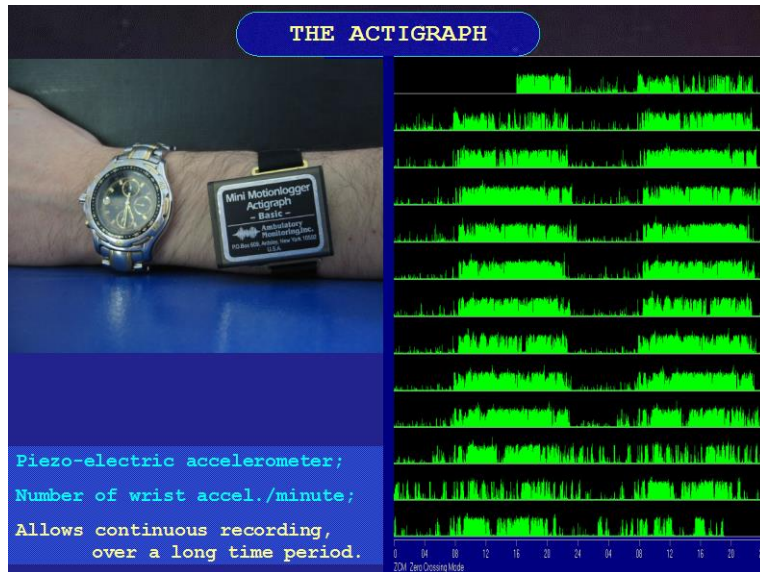


Chronotypes



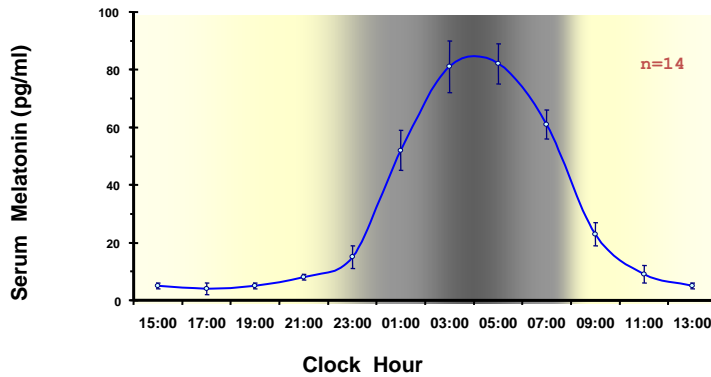
MCTQ, Till Roenneberg

Locomotor activity in humans



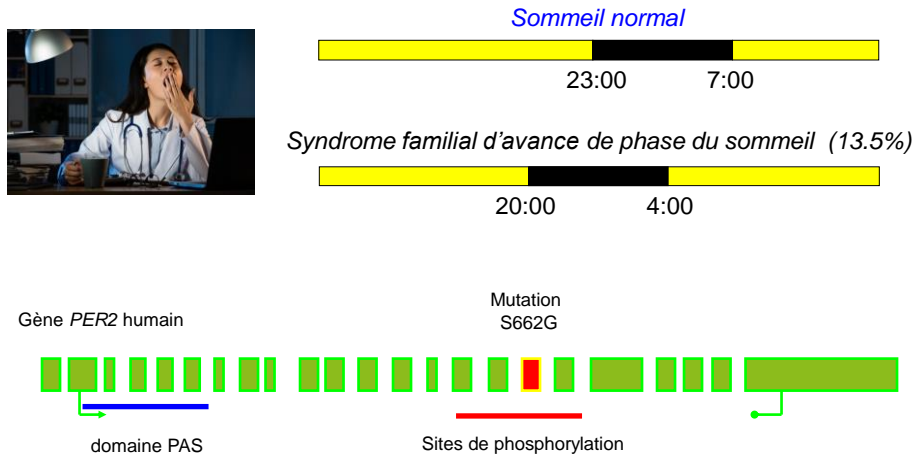
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The nocturnal serum melatonin peak



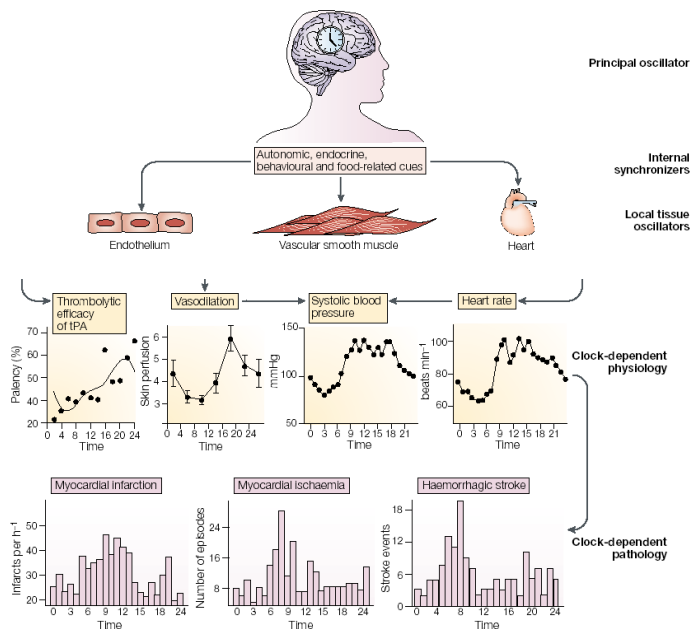
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Le gène *Per2* et le syndrome d'avance de phase



Patcek et al Science 2000
43

Circadian rhythms and cardiovascular pathologies



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Daytime variation of perioperative myocardial injury in cardiac surgery and its prevention by Rev-Erba antagonism: a single-centre propensity-matched cohort study and a randomised study

Prof David Montaigne, MD, Xavier Marechal, PhD, Thomas Modine, MD, Augustin Coisne, MD, Stéphanie Mouton, MD, Georges Fayad, MD, Sandro Ninni, MD, Cédric Klein, MD, Stanisl Ortmans, MD, Claire Seunes, MD, Charlotte Potelle, MD, Alexandre Berthier, MD, Celine Gheeraert, Eng, Catherine Piveteau, Eng, Rebecca Deprez, PhD, Jérôme Eeckhoutte, PhD, Héléne Duez, PhD, Prof Dominique Lacroix, MD, Prof Benoit Deprez, PhD, Bruno Jegou, MD, Mohamed Koussa, MD, Jean-Louis Edme, PhD, Philippe Lefebvre, PhD, Prof Bart Staels, PhD

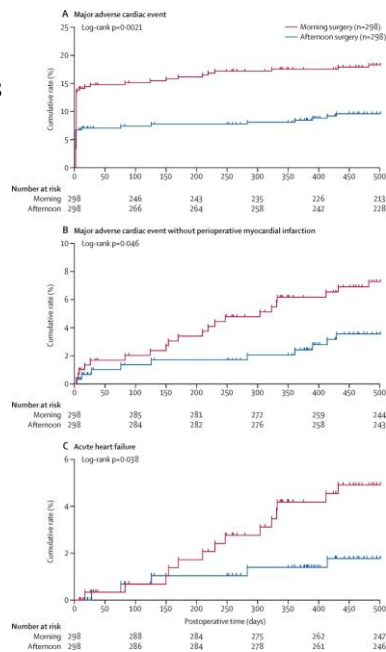
The Lancet

Volume 391, Issue 10115, Pages 59-69 (January 2018)



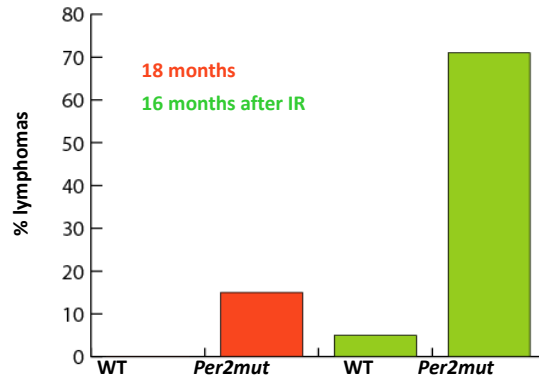
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Cohort study : n = 596
Randomised study : n=88



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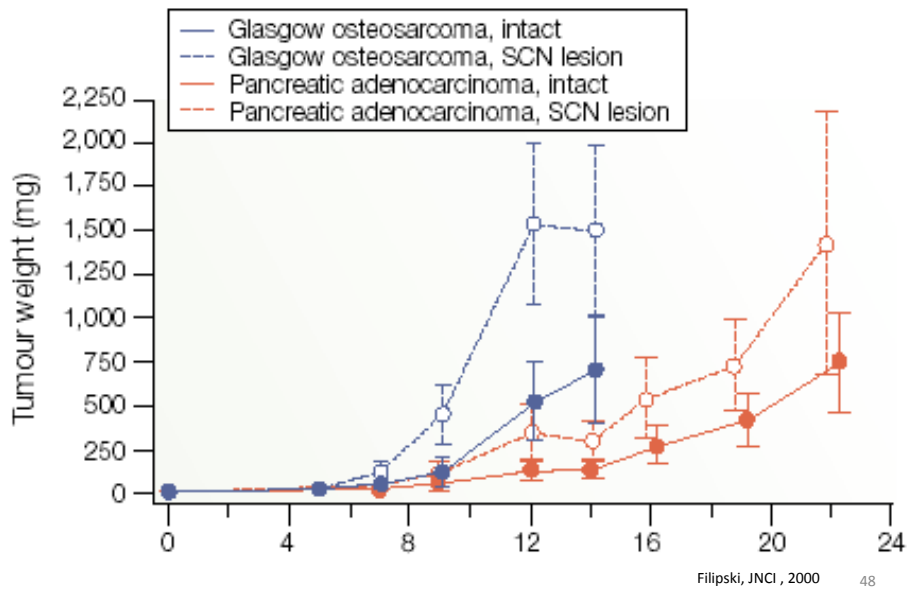
Per2 knockout mice are more prone to cancer



Fu et al., Cell 2002

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Circadian disruption accelerates tumour growth

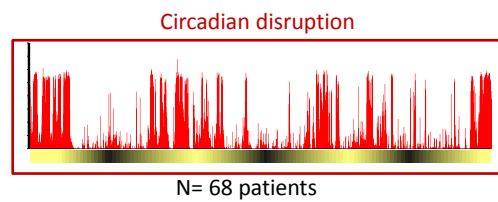
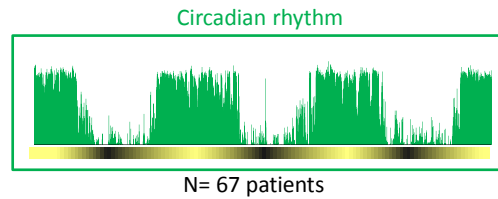


Filipski, JNCI, 2000

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Circadian disruption in cancer patients

135 patients with metastatic colorectal cancer



*Rich, Innominato et al.
Clin Cancer Res 2005*

*Innominato et al.
EBRS 2009*

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Chronobiology



Chronopharmacology

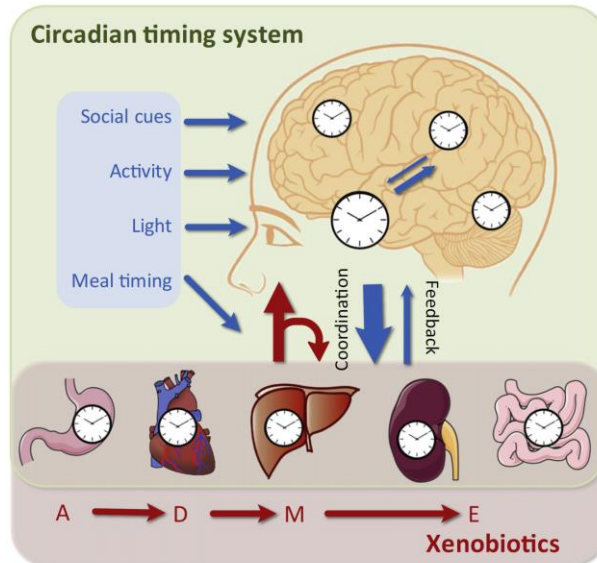


Chronotherapy

Only 0.016 % of clinical trials mention the time of treatment !

50

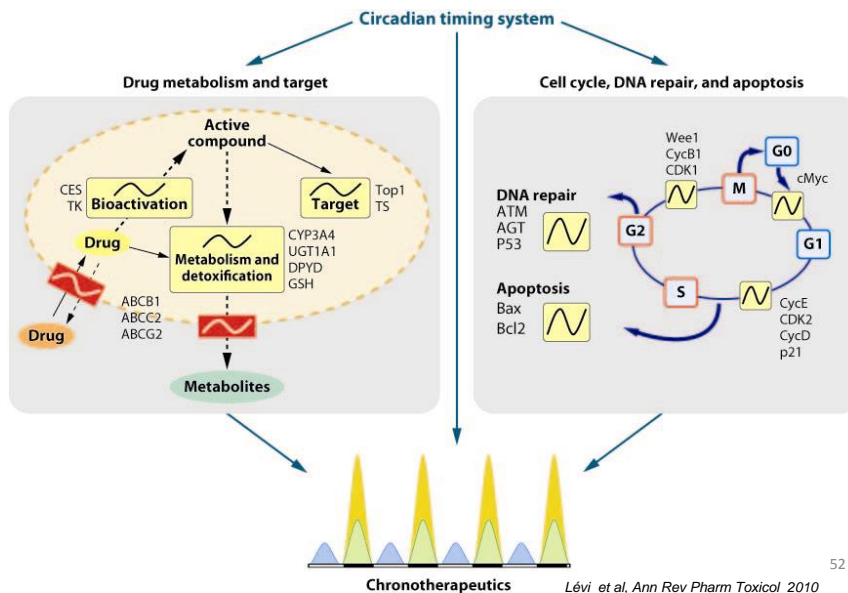
Chronopharmacology



Dallman et al Trends Mol Med 2016

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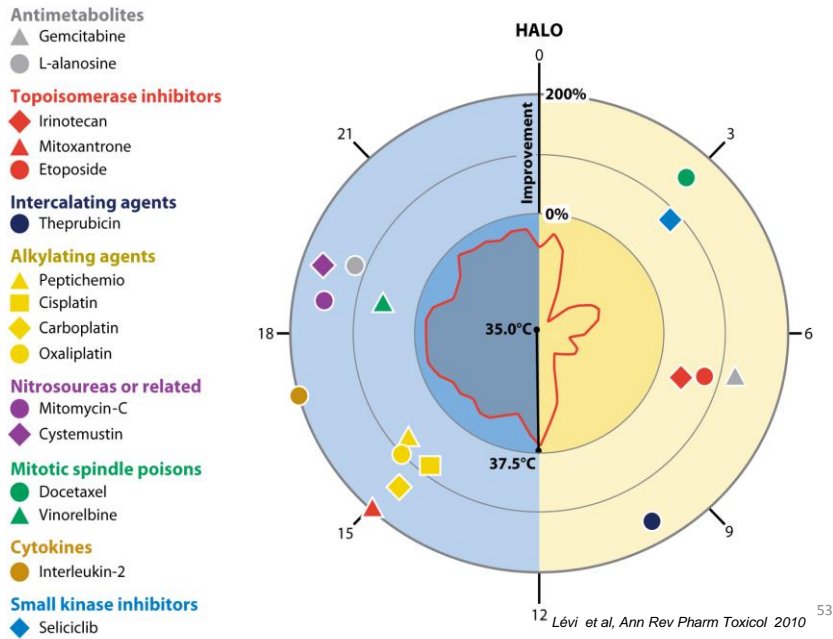
The circadian timing systems controls the chronopharmacology of anticancer drugs



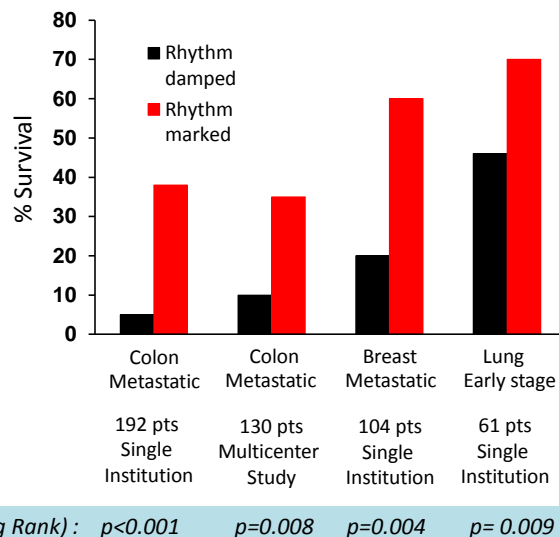
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Lévi et al, Ann Rev Pharm Toxicol 2010

Tolerability of anticancer drugs



3 year-survival rates according to circadian disruption in cancer patients



Mormont et al. Clin Cancer Res 2000; Innominato et al. Cancer Res 2009.; Sephton et al. JNCI 2000; Proust Conference, Torino 2008

Chronotherapy: technological implementation



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The paradigm shift of anticancer chronochemotherapy

Current paradigm : the dose makes the poison

Chronotherapy paradigm : dosing time makes the poison

Anticancer chronotherapy:

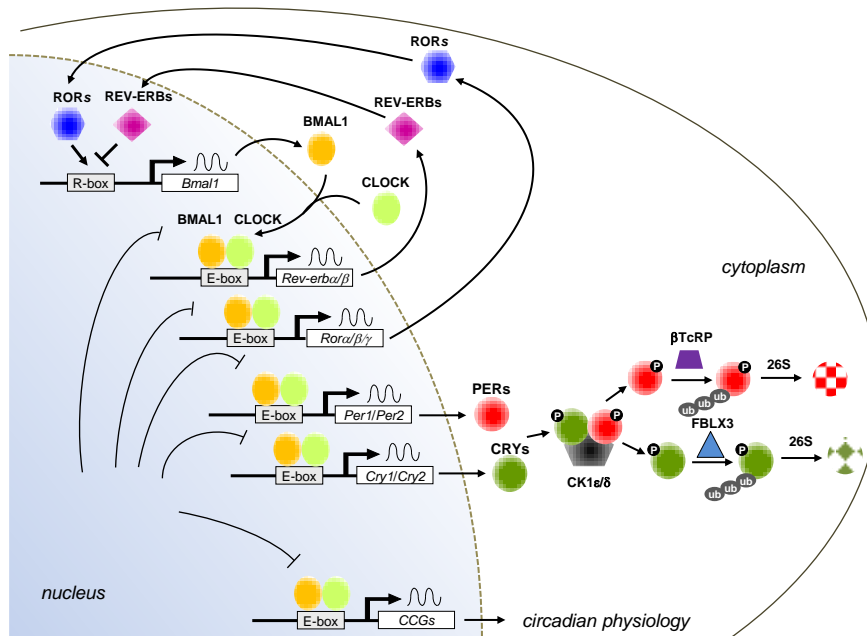
- Efficacy can be increased by 2
- Toxicity can be decreased by 2-10
- Improved therapeutic index

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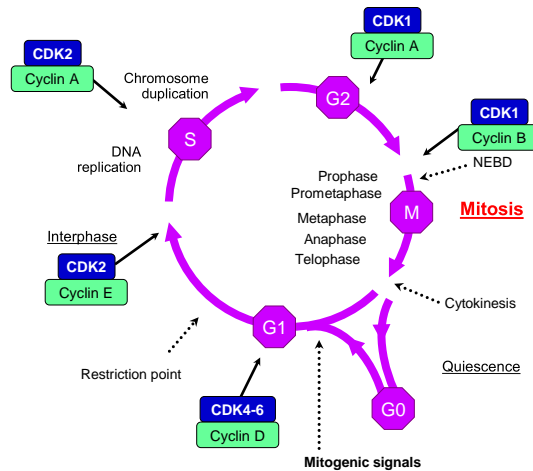
Interactions entre les gènes horloges et le cycle cellulaire: implications en cancérologie

Franck Delaunay
 Institut de Biologie Valrose
 Université Côte d'Azur -CNRS-INSERM

The mammalian circadian oscillator



The CDK oscillator driving the cell cycle



3

The circadian rhythm of cell division has been conserved during evolution



S elongatus



E gracilis



D melanogaster



D rerio



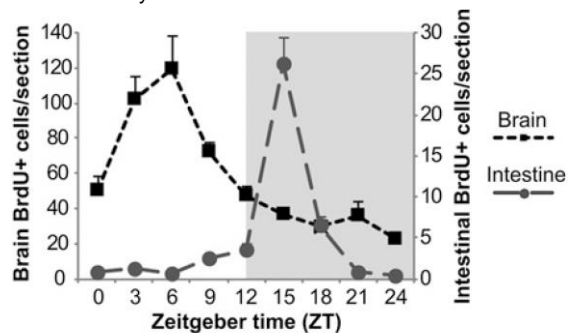
M musculus

• Kellicott, W. The daily periodicity of cell division and of elongation in the root of *Allium*. *Bull. Torrey Bot. Club*, 31: 1904

• Fortuyn-Van Leyden, Droogleeper. Some observations on periodic nuclear division in the cat. *Proc. Soc. of Sciences, Amsterdam*, 19: 38, 1916.

• Thuringer, J. M. Studies on cell division in the human epidermis. *Anat. Record*, 40: 1, 1928

DNA synthesis in zebrafish tissues



Laranjeiro et al, PNAS 2012

4

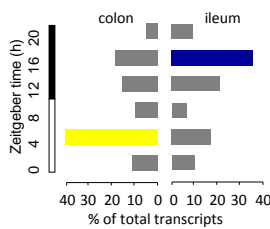
Cell cycle transcripts are enriched in the colon mucosa circadian transcriptome

Circadian transcriptomics

Cell cycle, microtubules, spindle assembly, apoptosis



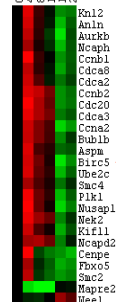
Colon mucosa



Siffroi-Fernandez et al, Cell Cycle 2014

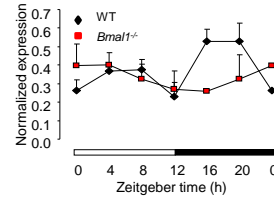
| GO term | Count | % | p-value |
|-------------------------------|-------|----|----------|
| GO:0022402~cell cycle process | 36 | 22 | 1.91E-24 |
| GO:0000279~M phase | 32 | 20 | 2.38E-24 |
| GO:0007049~cell cycle | 42 | 26 | 5.55E-24 |

Mitotic genes



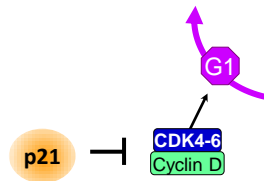
Mitotic genes

Wee1

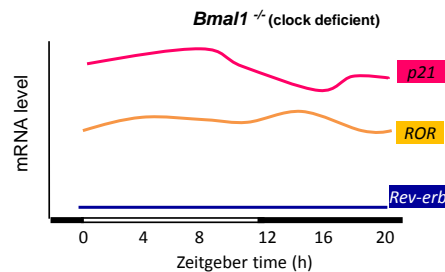
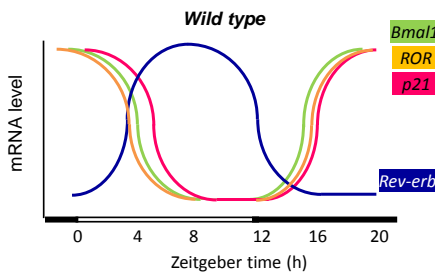
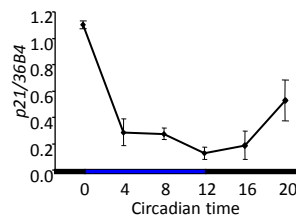


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The cell cycle gene p21 oscillates in the liver



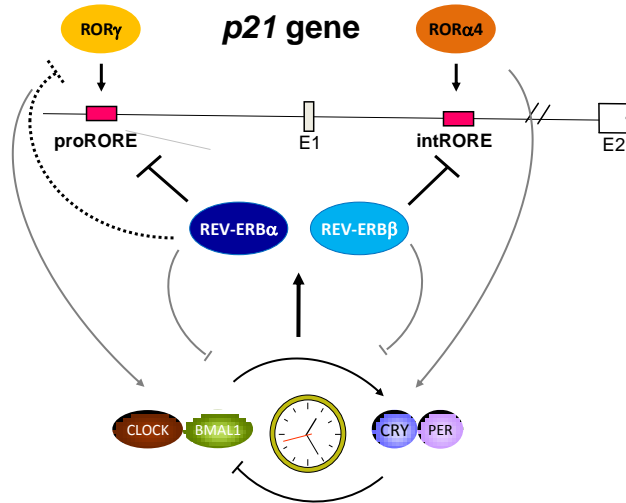
Constant darkness



Gréchez-Cassiau et al, JBC 2008

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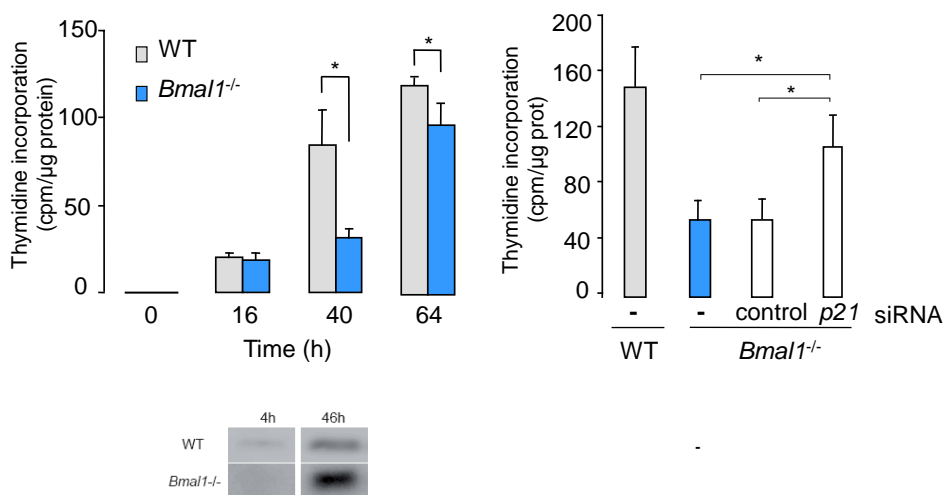
The REV-ERB/ROR loop drives the p21 oscillation



Gréchez-Cassiau et al, JBC 2008

7

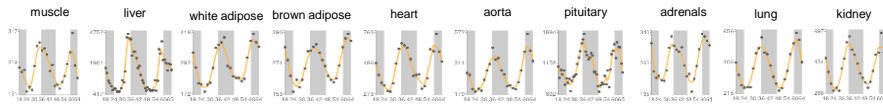
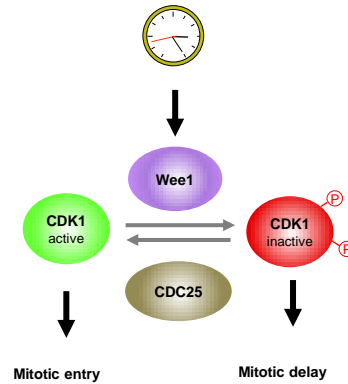
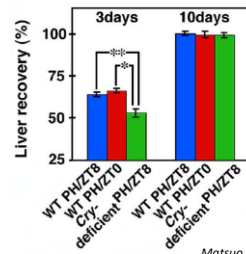
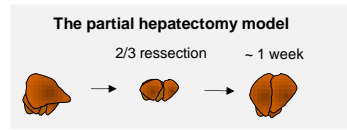
Impaired proliferation of *Bmal1*^{-/-} hepatocytes



Gréchez-Cassiau et al, JBC 2008

8

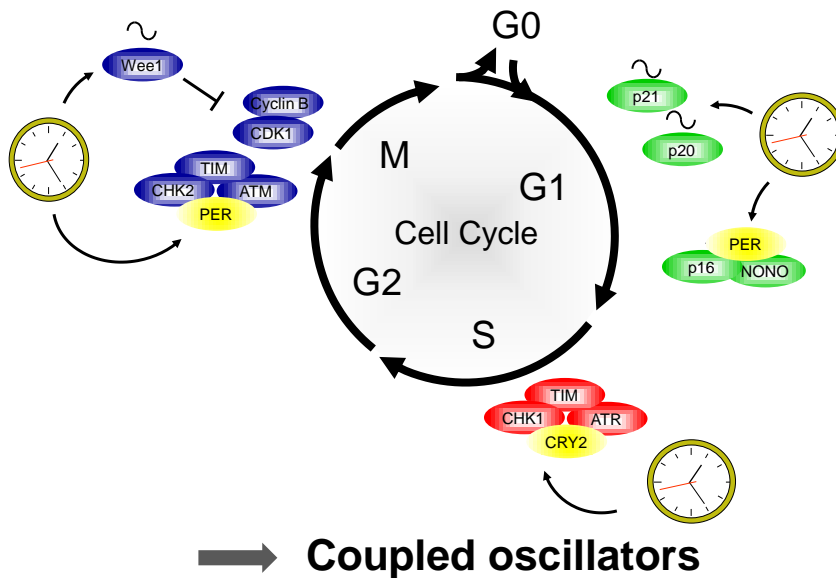
The G2/M kinase Wee1 is clock-controlled



<http://biogps.org/circadian/>

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Molecular links between the clock and the cell cycle



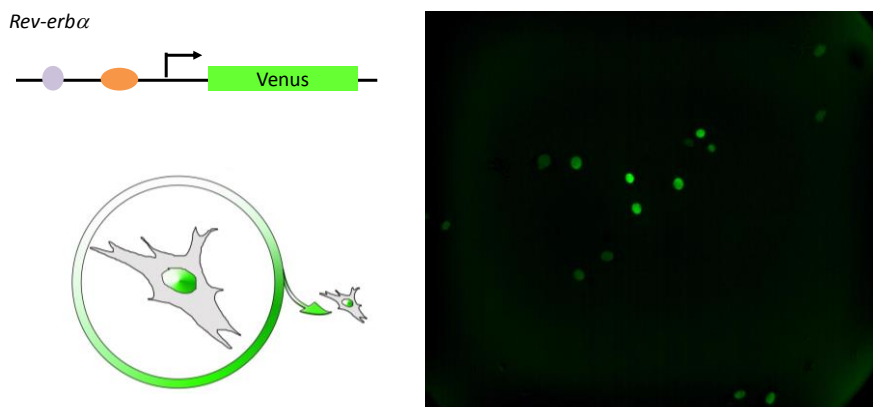
10

QUESTIONS

- Consequences of the coupling on the dynamics of the system ?
- How does the temporal organisation of the cell cycle at the single cell level produce daily rhythms at the tissue level ?
- Additional molecular links ?
- Relevance of the coupling in cancer cells ?

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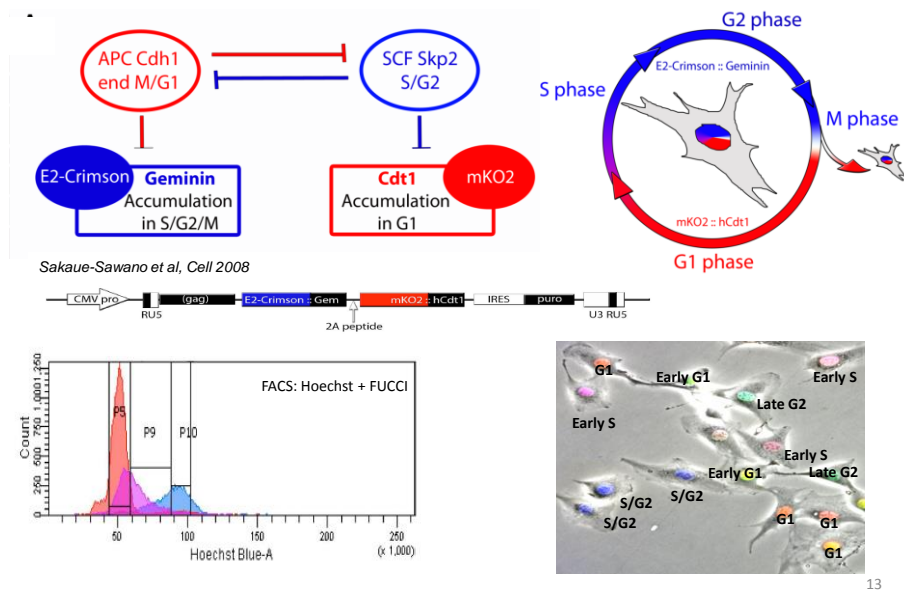
Monitoring the circadian clock in single cells



(Nagoshi et al, 2004)

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Monitoring of the entire cell cycle progression in single cells using **FUCCI**



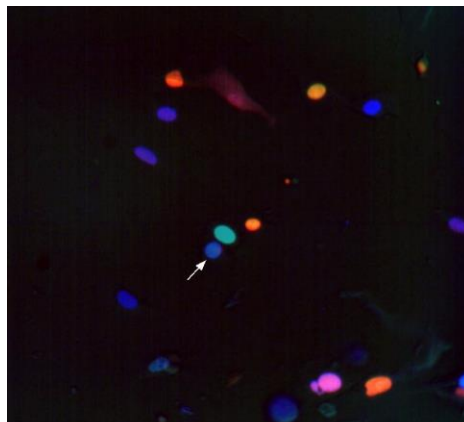
Visualizing the clock and the cell cycle in single cells



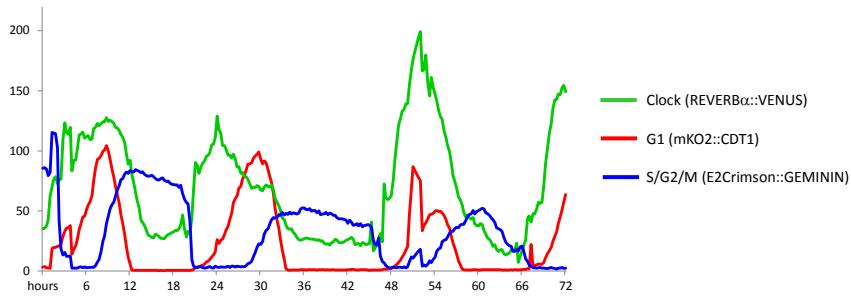
Céline Feillet

- Live cells
- Single cells
- Real time
- High temporal resolution (min)
- Spatial Information
- Variability

NIH3T3 cells + Fucci + Reverb::Venus



A lot of data

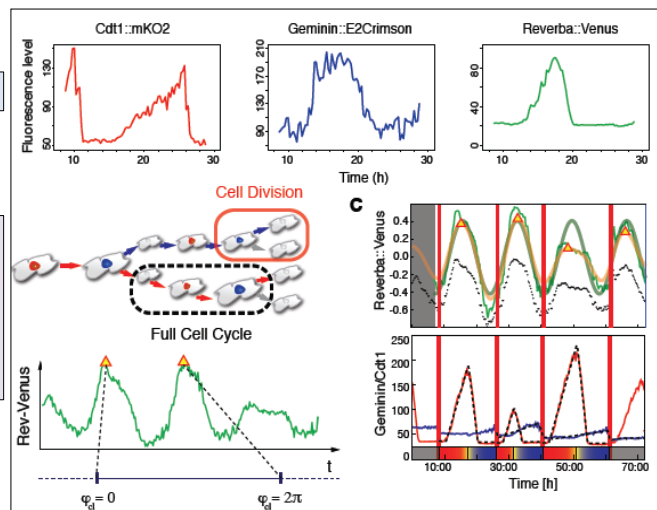


- 344 lineages
- 1709 cells
- 3551 cell cycles
- $> 10^6$ datapoints
- $> 2 T_0$

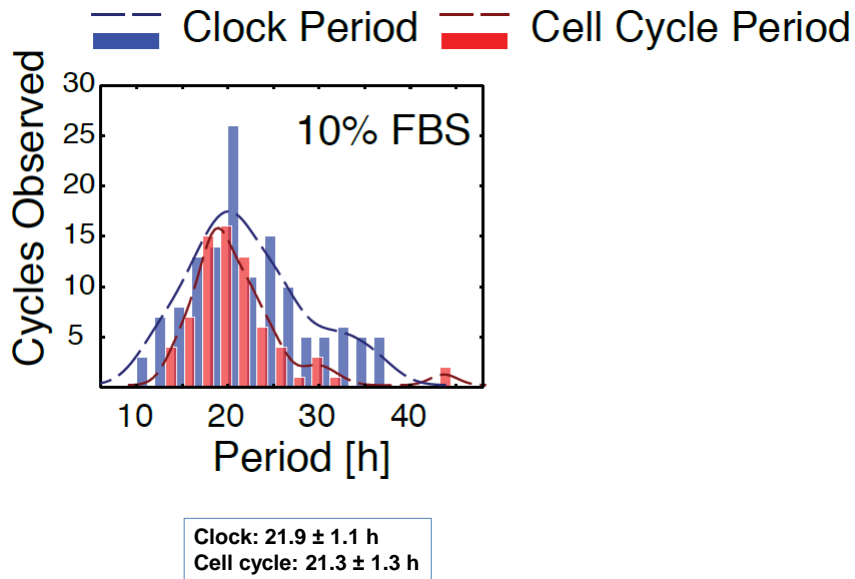
From images to global phase dynamics of lineages

Lineage tracker 2.0 (Fiji)

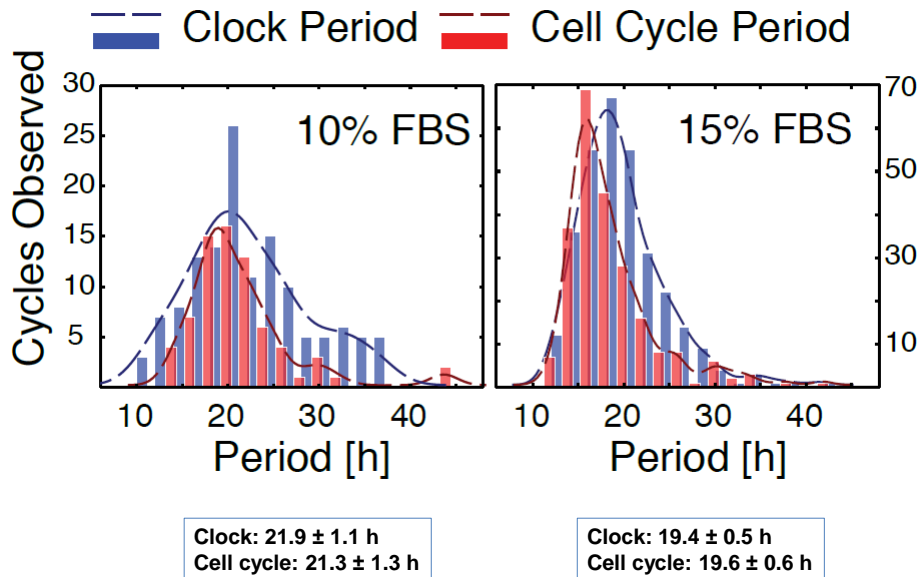
- Spectrum resampling (clock)
- Piecewise linear model (t_a, t_b, t_c) (cell cycle)



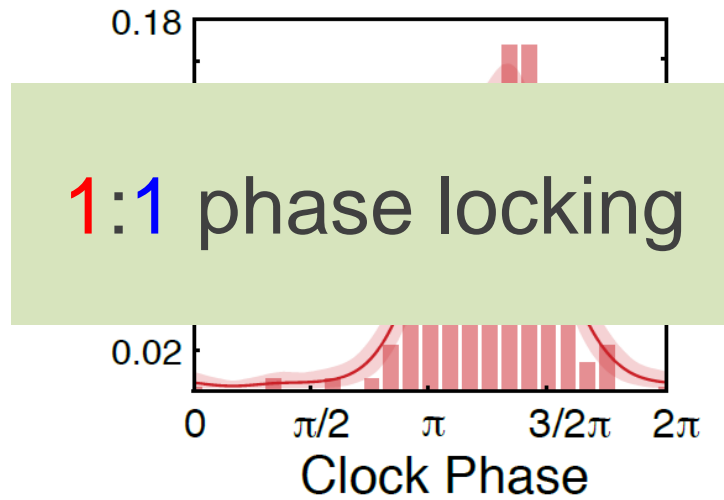
The clock and the cell cycle oscillate
at similar periods in unsynchronised cells



The clock and the cell cycle oscillate
at similar periods in unsynchronised cells

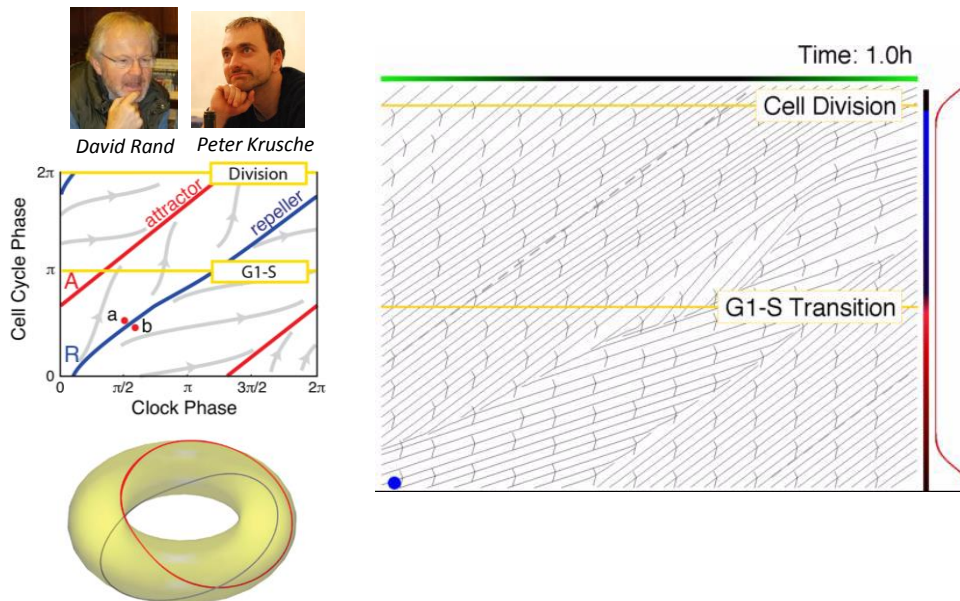


A fixed phase relationship between the clock and the cell cycle



Mean clock phase at division: 3.97 ± 0.14 radians

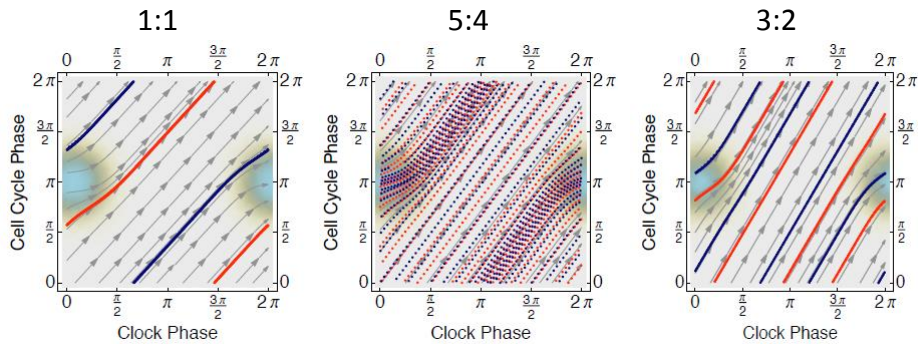
Joint trajectory for a 1:1 phase-locked system



One single coupling region can explain different regimen

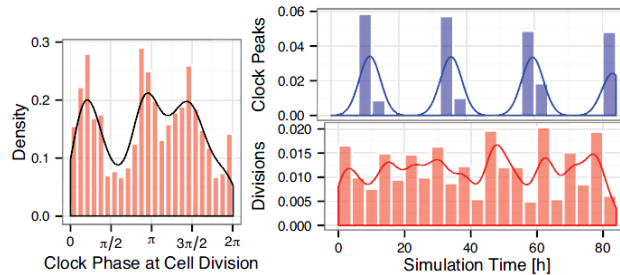
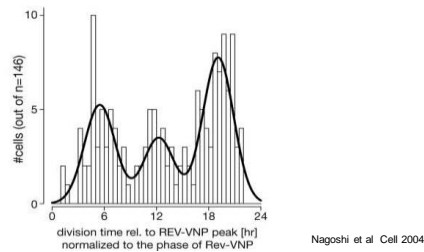
$$\varphi_{cl}' = v_1 + d\xi$$

$$\varphi_{cc}' = v_2 + f_{amp} \cdot Q(\varphi_{cl}, \varphi_{cc}) + d\xi$$



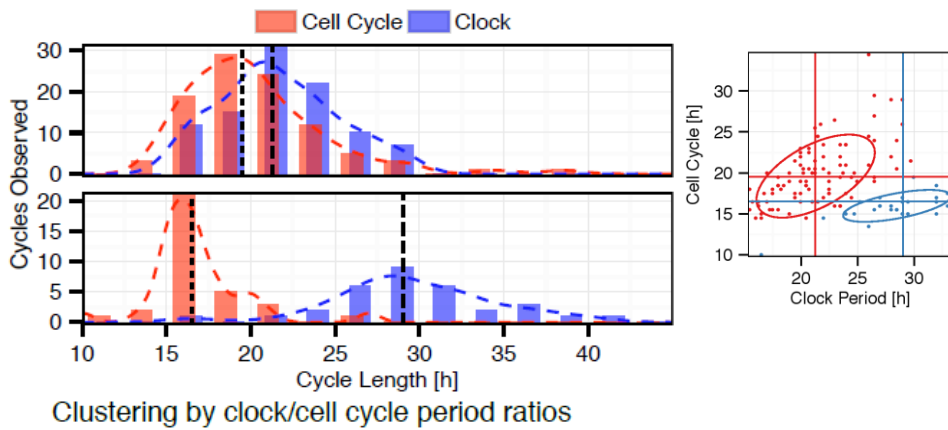
Simulation of a 3:2 coupling ratio

- Dex pulsed cells
- 20 % FBS



Two coexisting coupling regimes in synchronized cells cultured in 20 % FBS

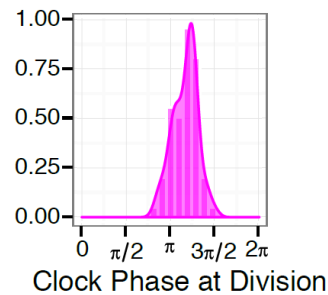
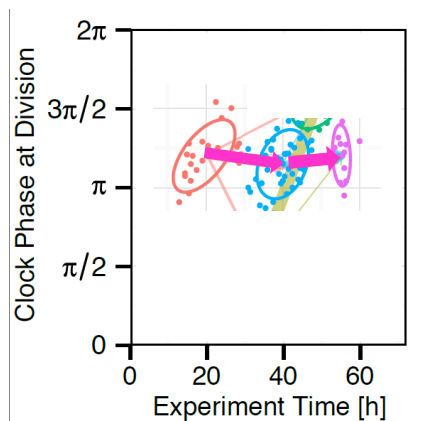
NIH3T3/20% FBS/Dex



Clustering analysis in 20 % FBS

Population A (1:1 ratio)

Clock phase at division vs Experiment time

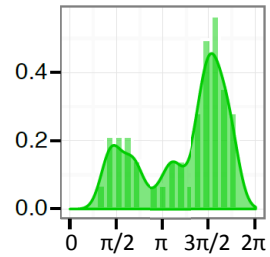
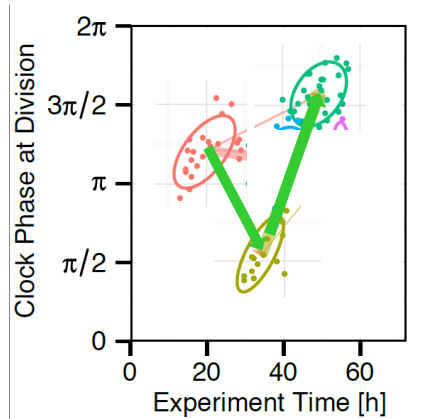


Clustering analysis in 20 % FBS

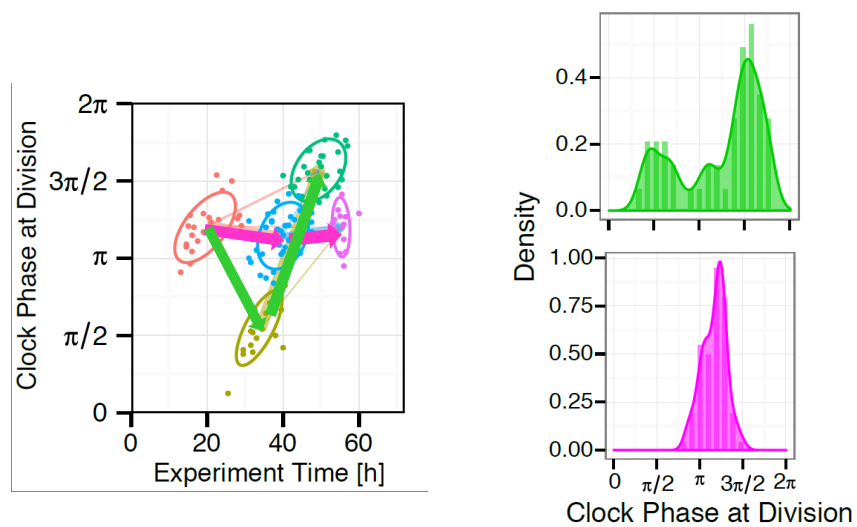
Population B (3:2 ratio)

Clock phase at division vs Experiment time

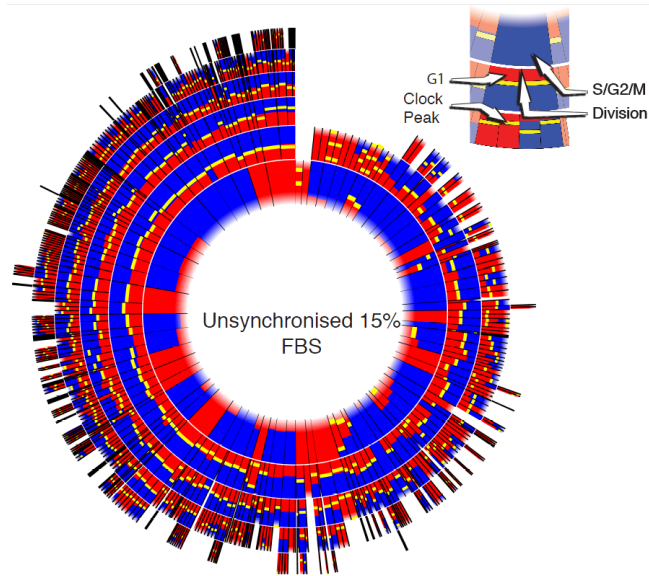
=> 3 clock phases at division



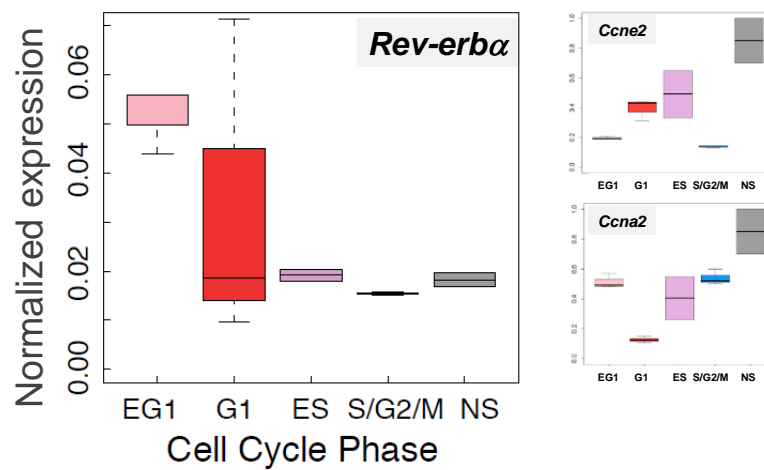
Clustering analysis in 20 % FBS



The clock and the cell cycle display a fixed phase relationship across generations



Cell cycle phase dependent *Rev-erba* mRNA expression



Conclusion

- Multiple molecular links
- A robust phase-locking mechanism
- Cell cycle progression has a significant influence on the clock in unperturbed cells
- Bidirectional coupling (*in vivo*)
- Results do not support the classical circadian gating model