



Introduction to Chronobiology

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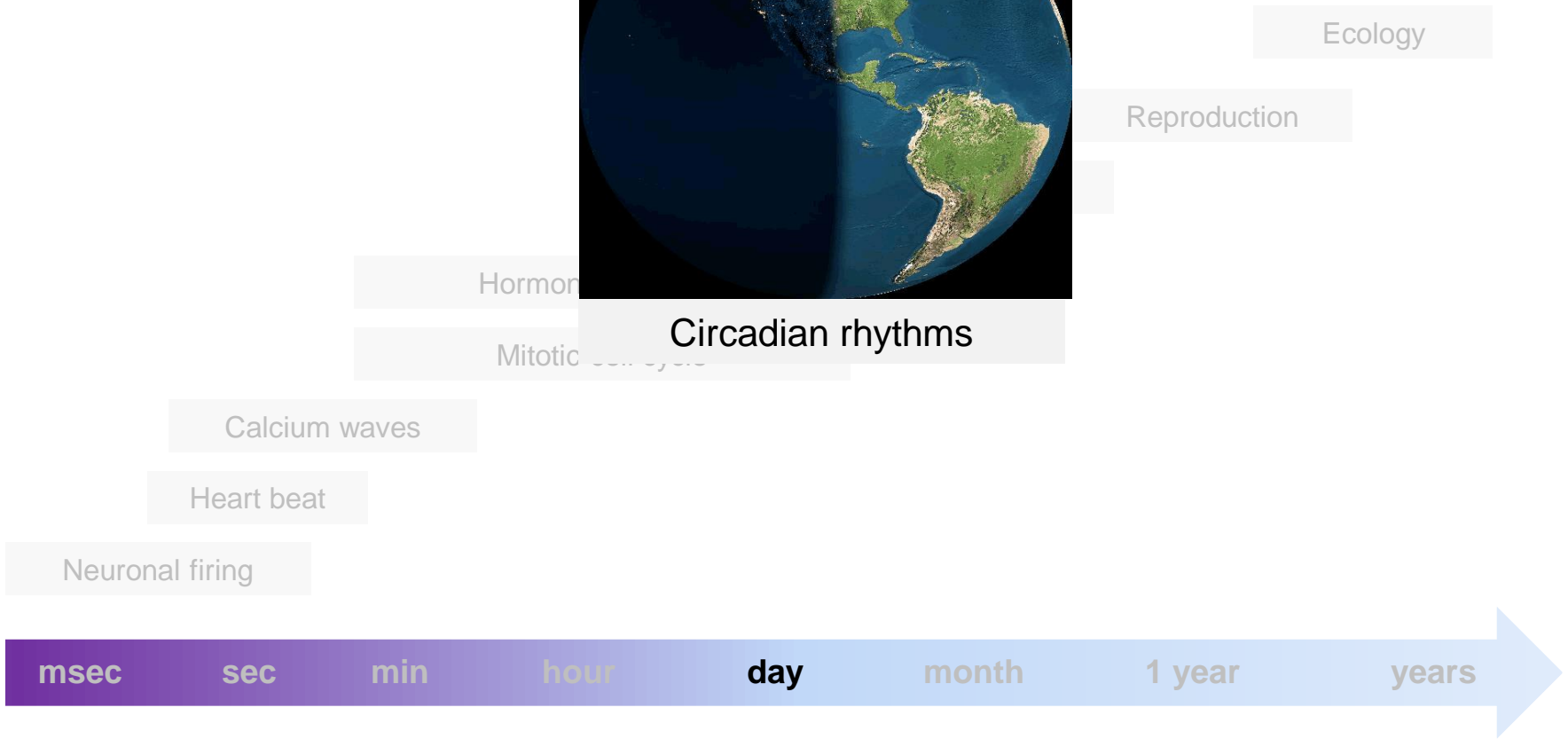
Ecole thématique Biorégul, 5-9 juin 2023

Outline

- Basic concepts
- Molecular biology of the circadian clock
- Circadian timing in health and diseases

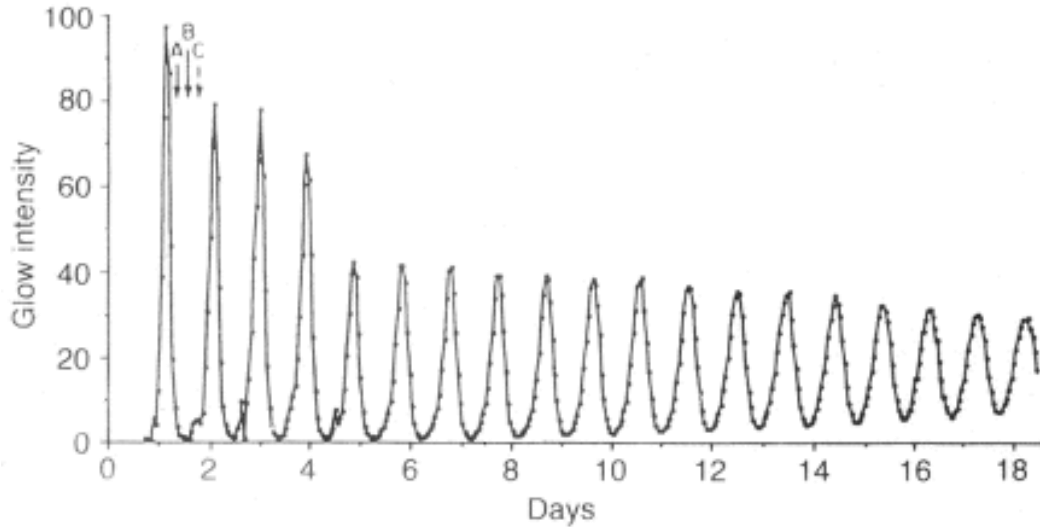


Biological oscillations

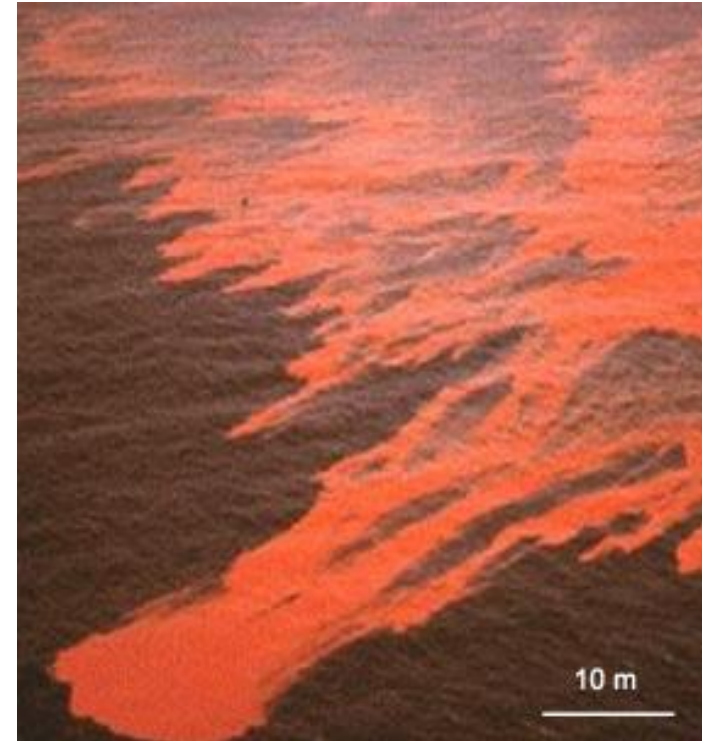


10 Logs !

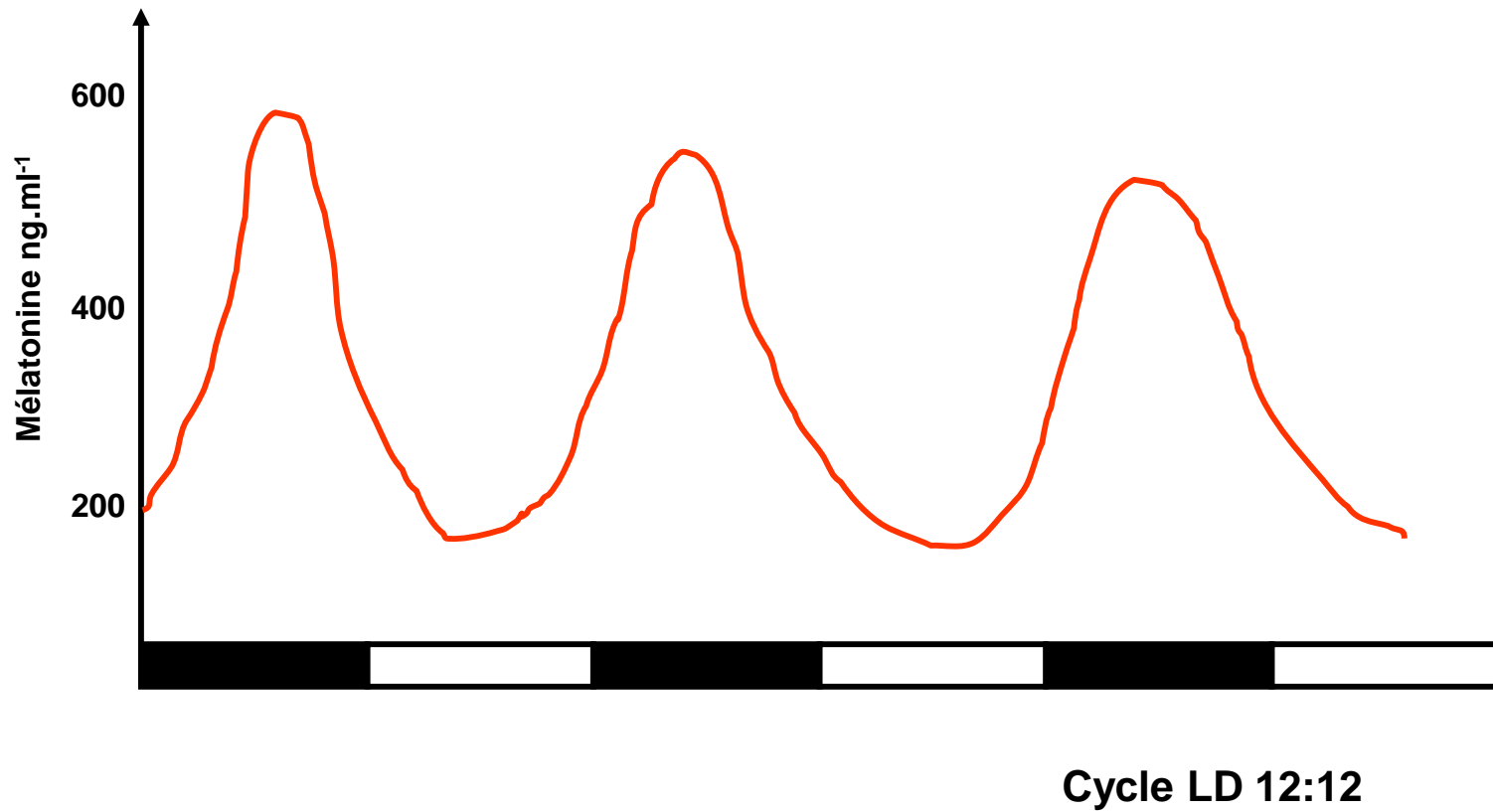
Circadian rhythm of bioluminescence in a dinoflagellate (*Lingulodinium polyedrum*)



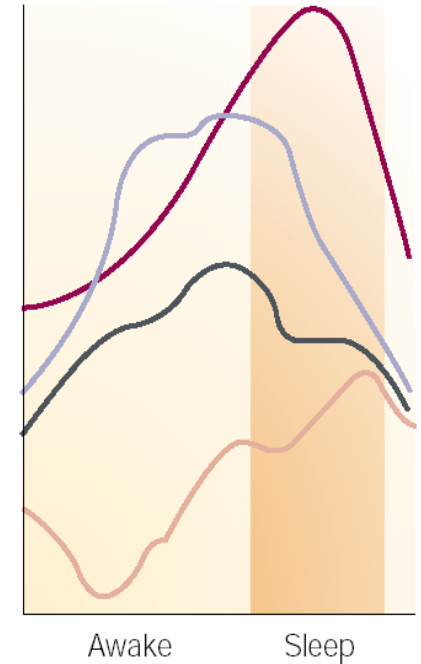
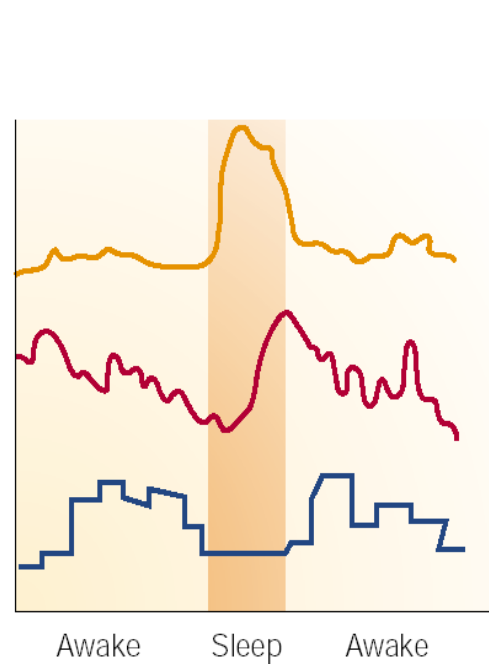
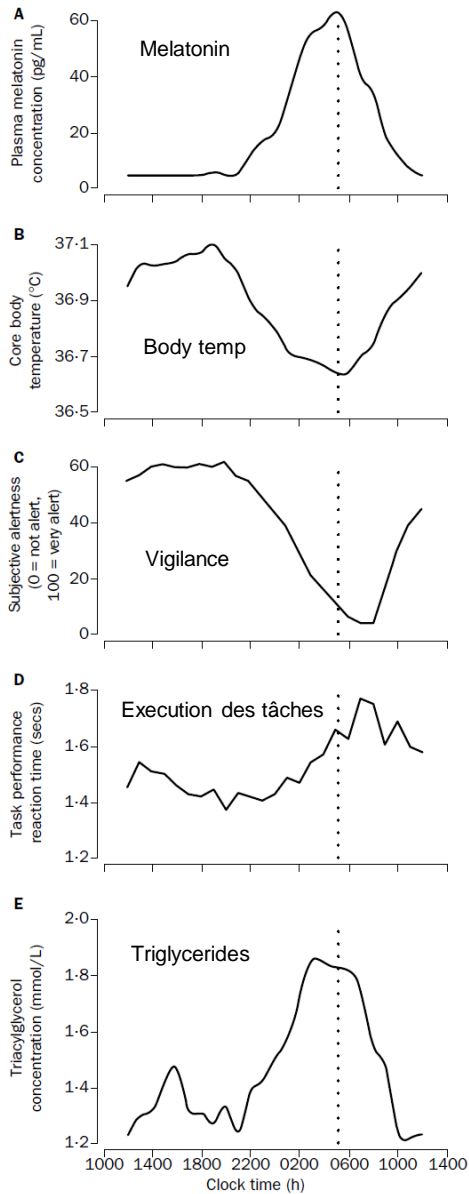
20 days in LL



Melatonin secretion from avian pineal gland *in vitro*



Examples of daily rhythms in human



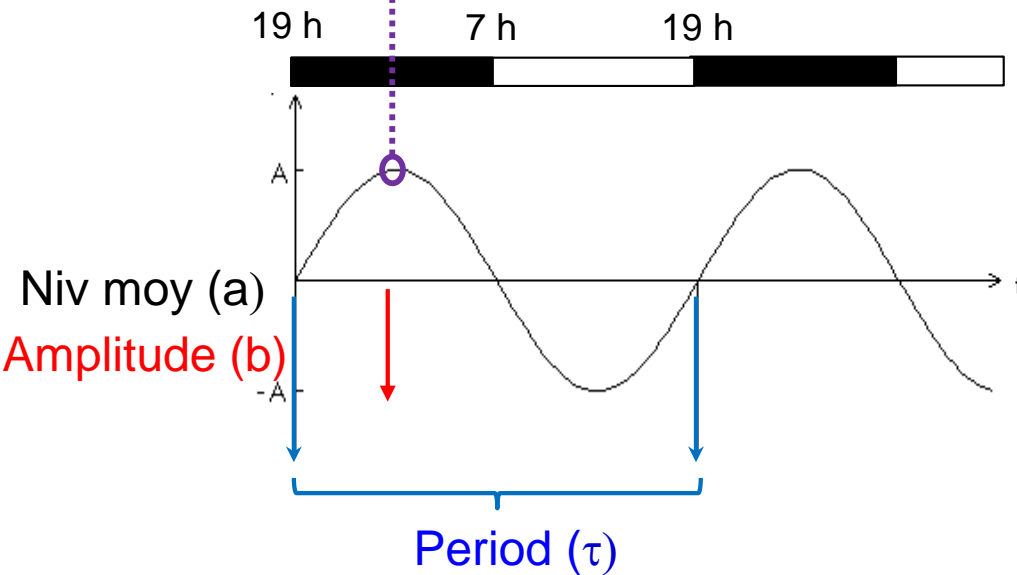
Fu & Lee, Nature 2003

Rajaratnam & Arendt, Lancet 2001

Some definitions for biological rhythms

Synchronizer = zeitgeber (ZT)

Phase $\Phi = ZT18$



Biological rhythm $\rightarrow Y = a + b \cos (2\pi (\tau - \Phi))$

$\tau < 20 h$	ultradian
$20 h < \tau < 28 h$	circadian
$\tau > 28 h$	infradian

The pioneer of modern chronobiology

Jean d'Ortous de Mairan
(1678-1771)



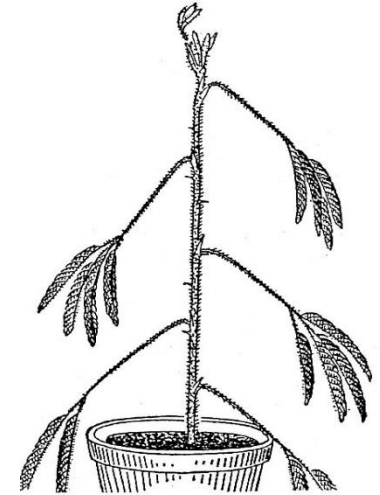
1729



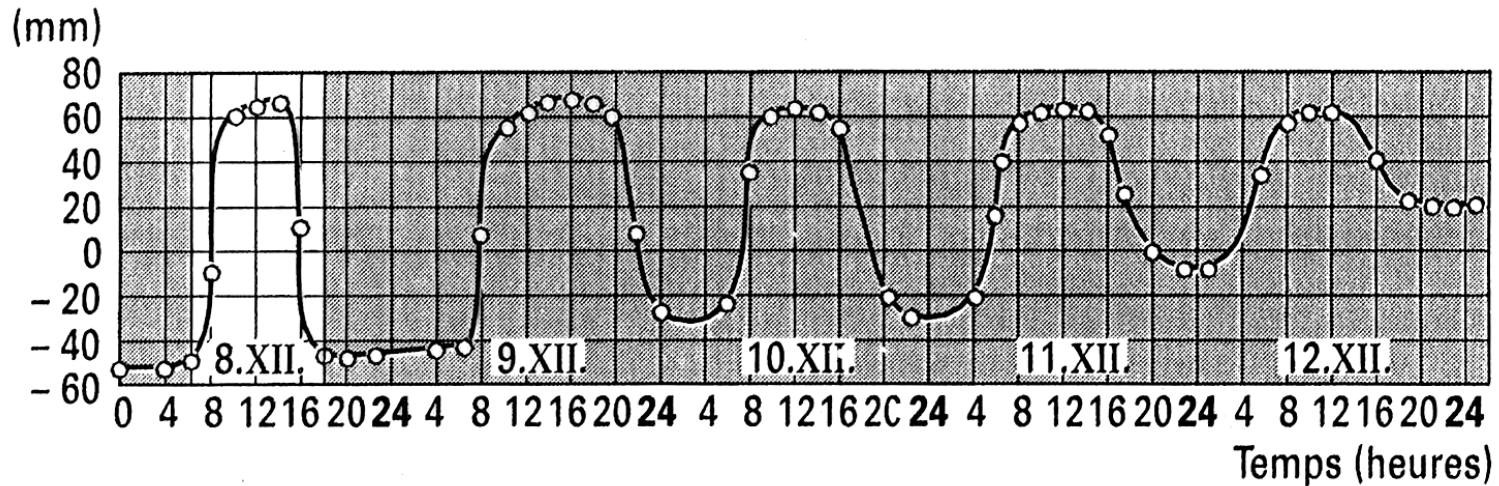
Jour



Mimosa pudica (sensitive)

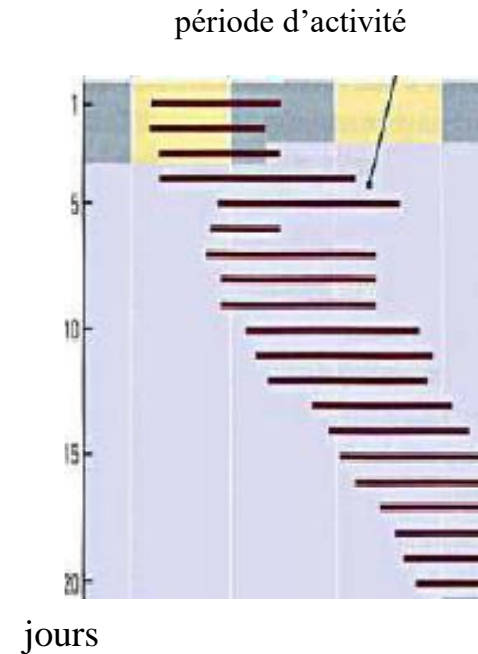


Nuit



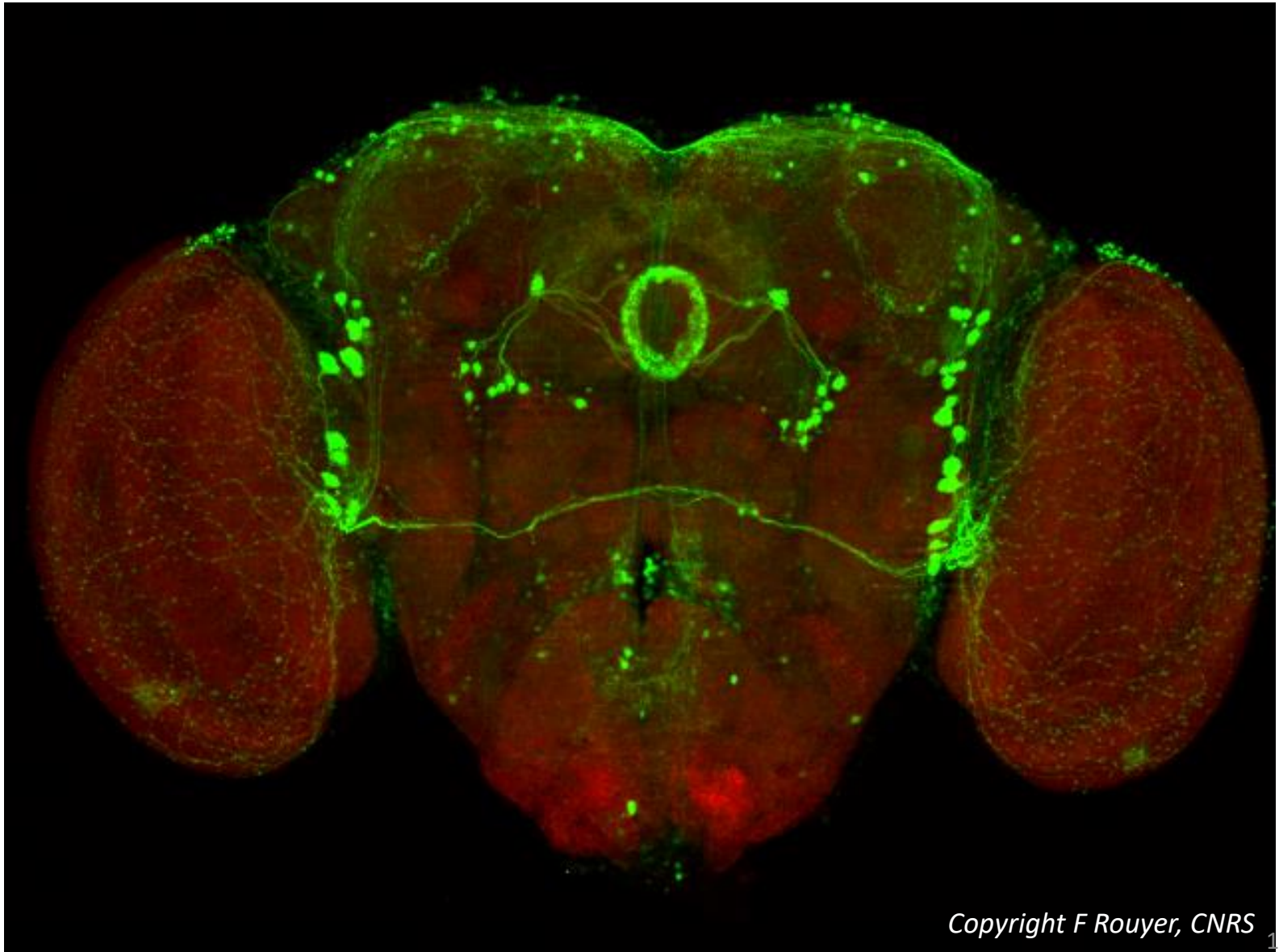
Free-running in human

1962, Michel Siffre stays 58 days in-free running conditions (gouffre de Scarasson)



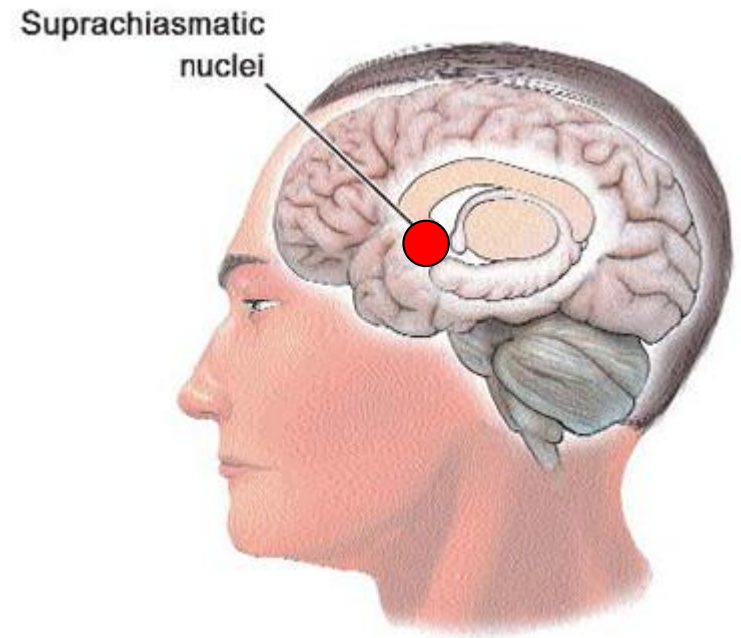
Many physiological processes remained rhythmic

The central clock



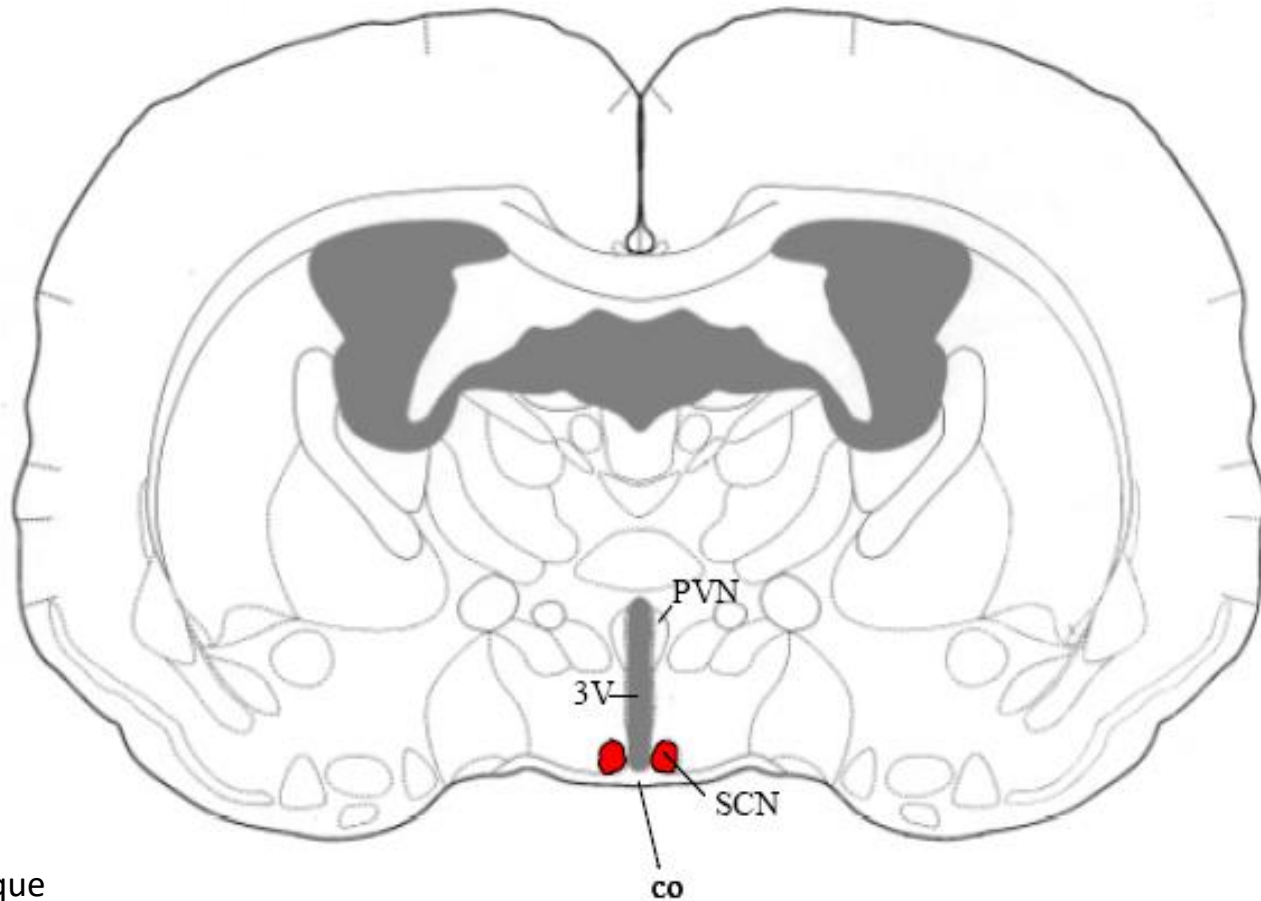
Localisation of the clock in mammals

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





SCN neurons are the only clock neurons

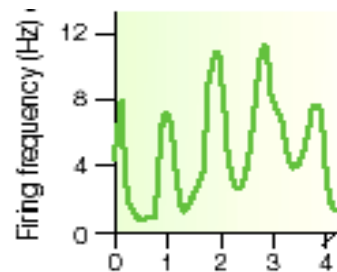


Co: chiasma optique

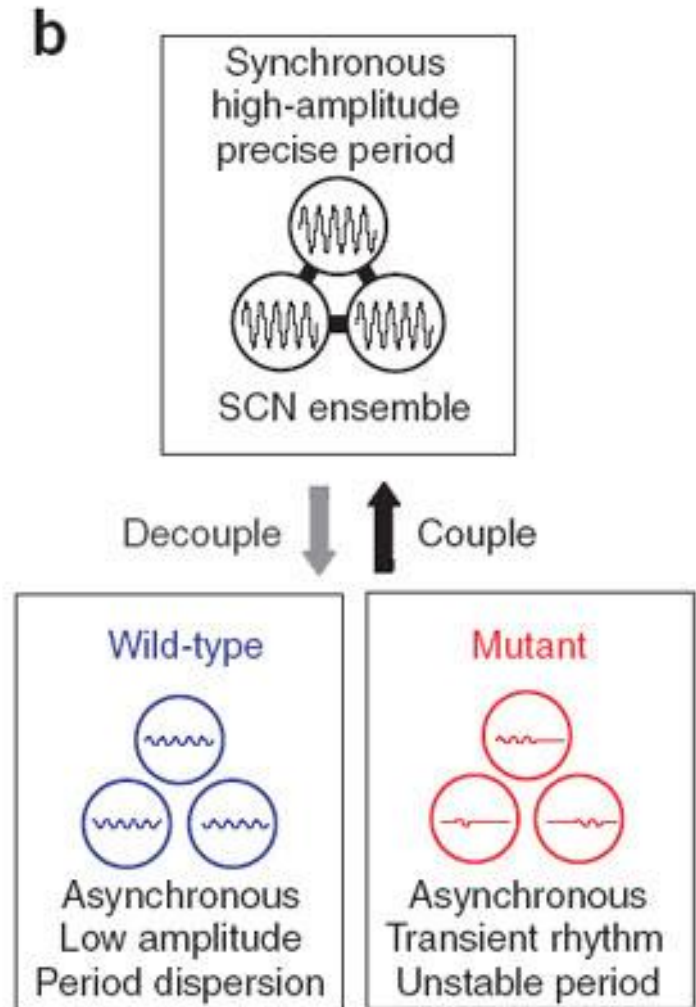
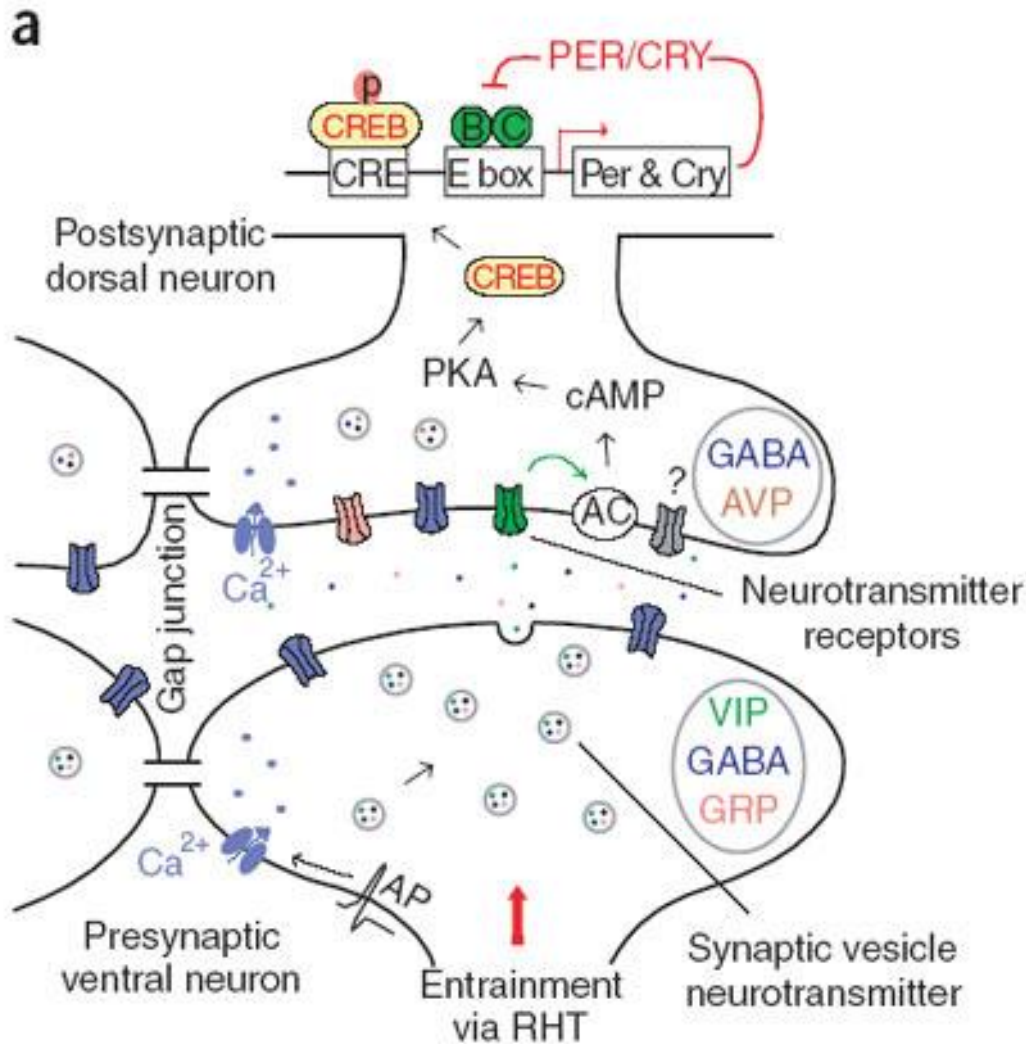
3V: 3ème ventricule

SCN: noyaux suprachiasmatiques

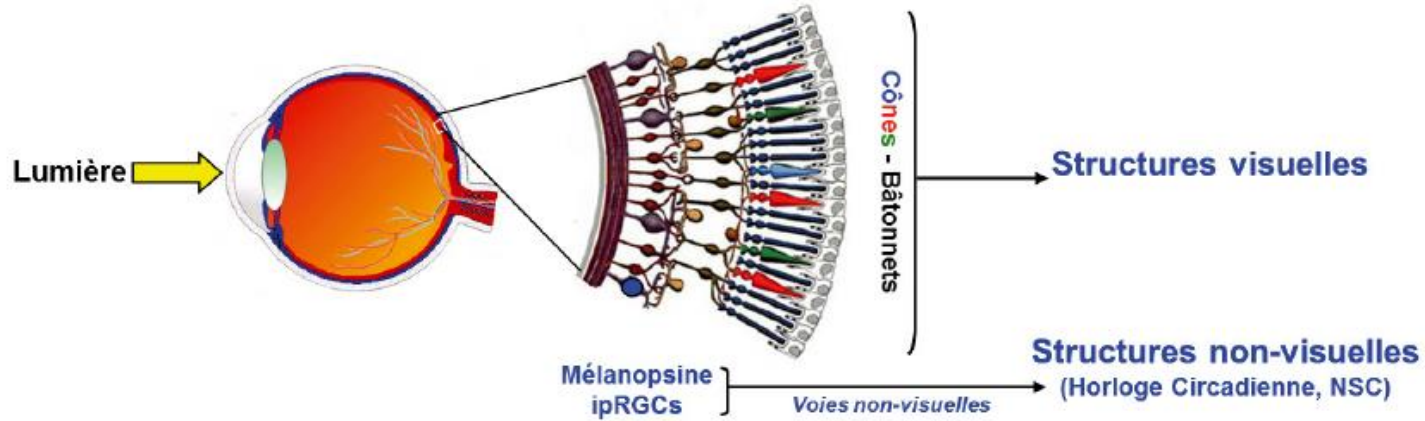
PVN: noyaux paraventriculaires



Intercellular coupling between SCN neurons



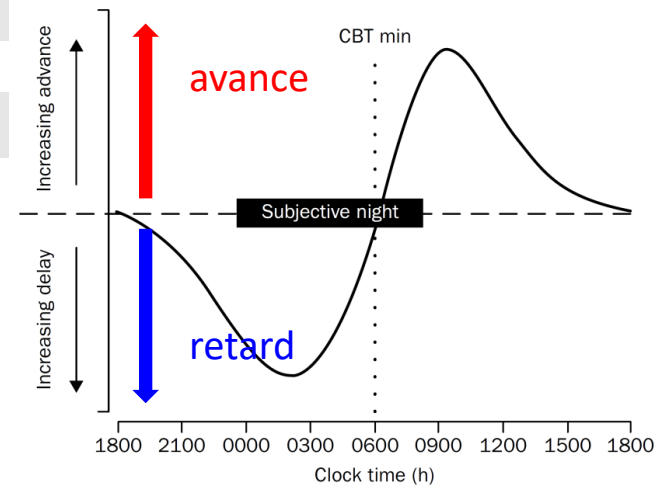
Synchronisation de l'hologe



Gronfier, *Biologie d'Aujourd'hui* 2014

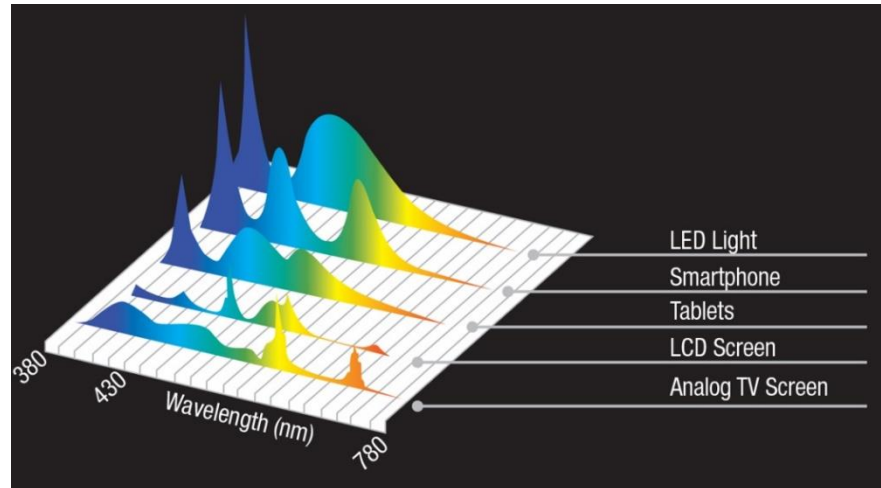
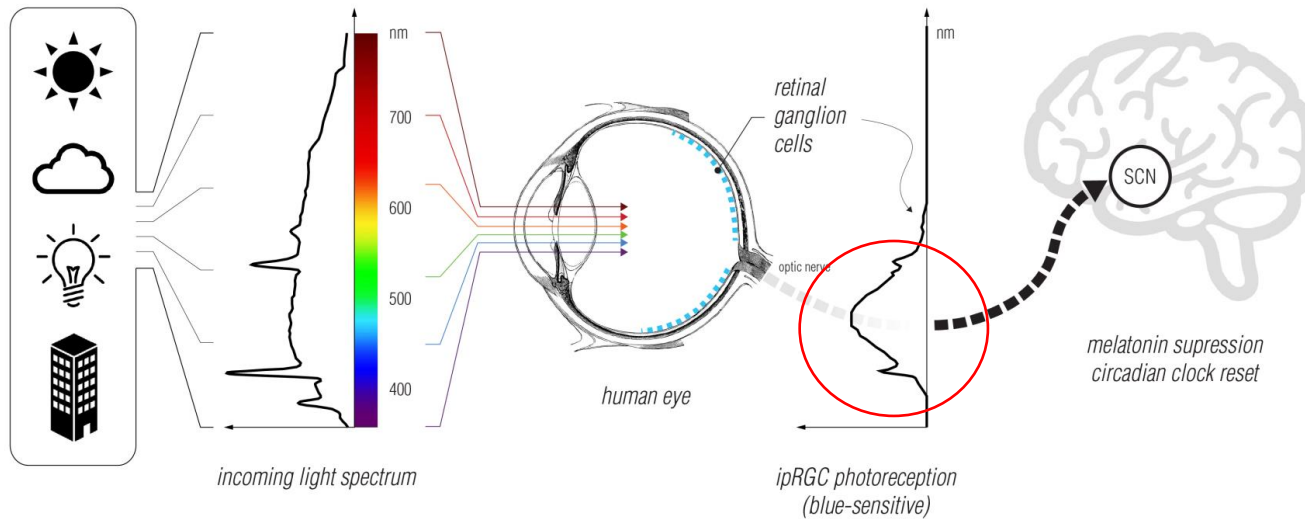
Lumière en **début** de nuit : **retard** de phase (voyage vers l'ouest)

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

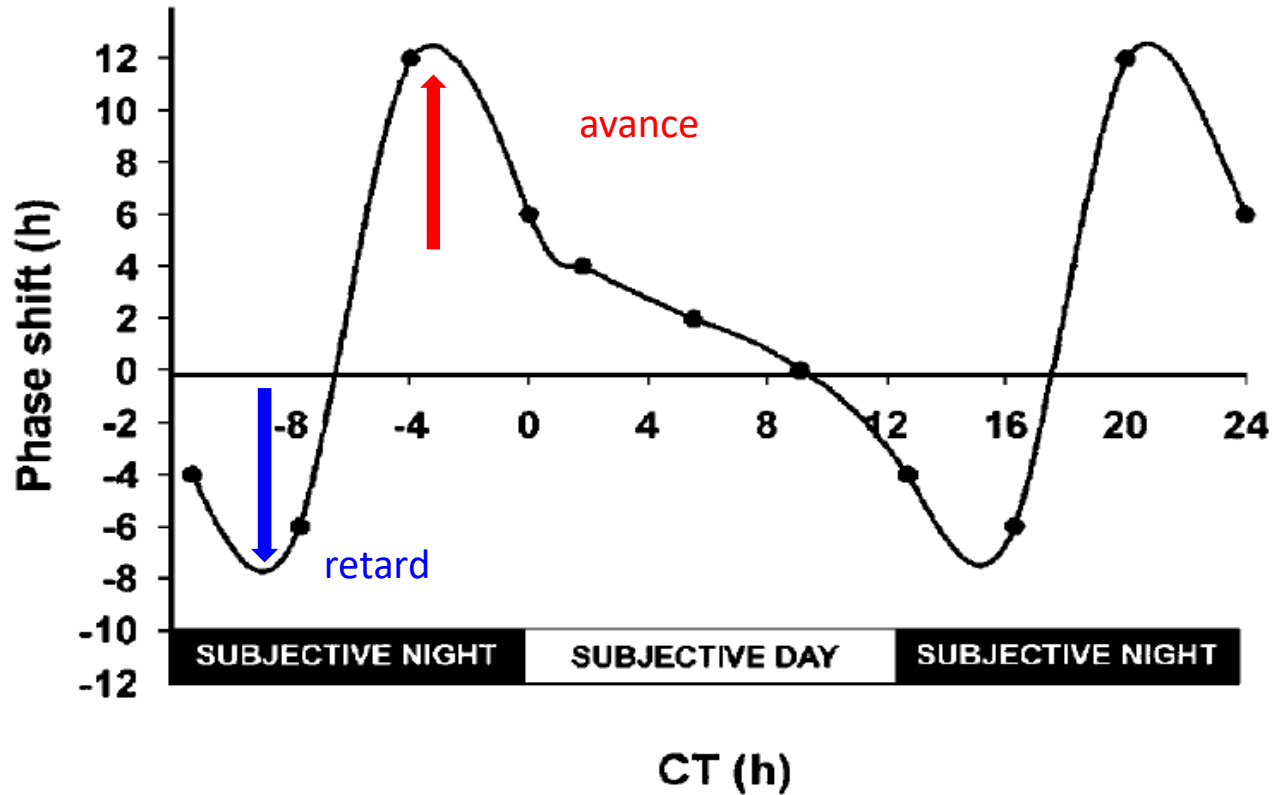


Rajaratnam & Arendt, *Lancet* 2001

La lumière bleue est la plus efficace pour resynchroniser notre horloge

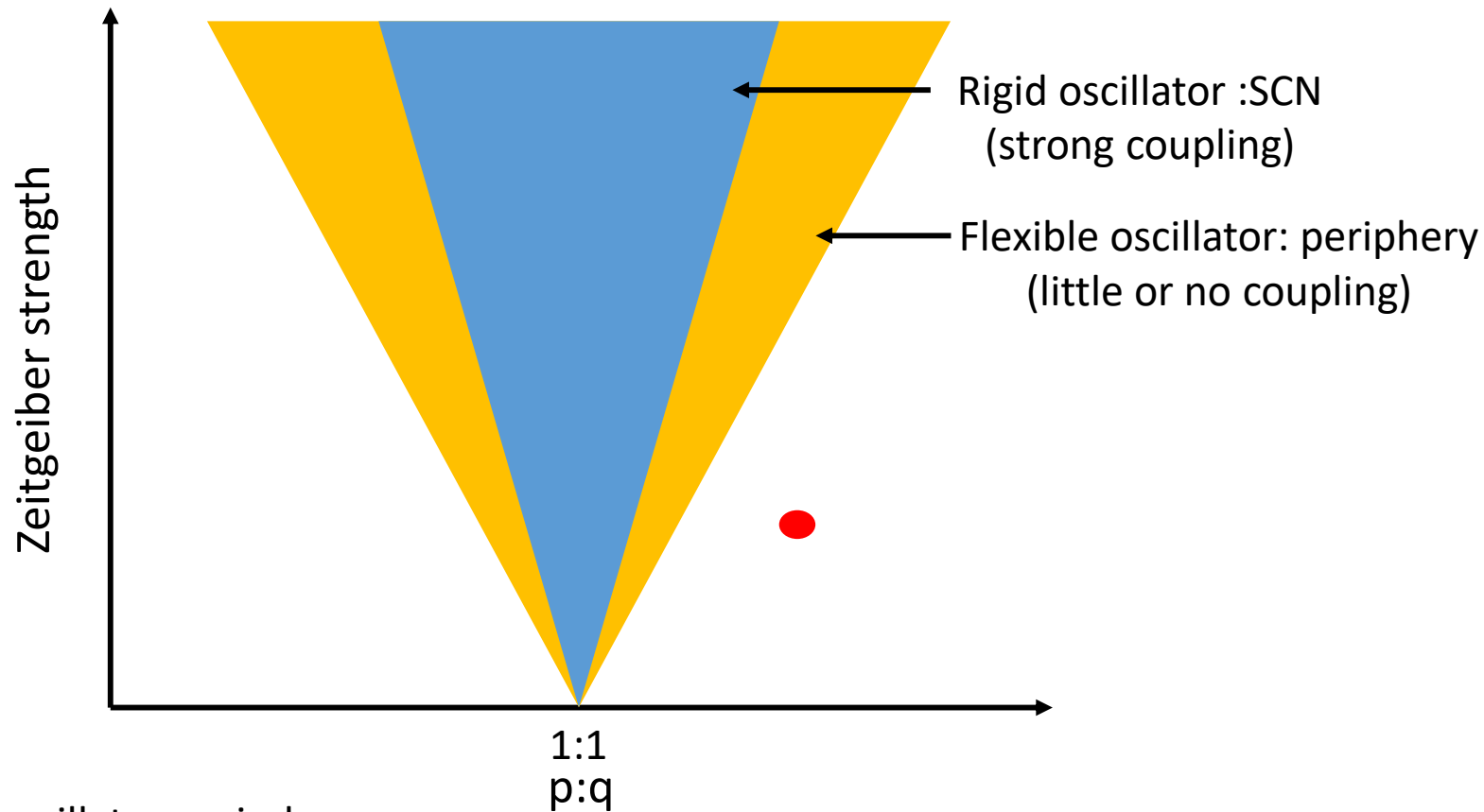


Light a universal synchronizer of circadian clocks



Phase response curve in *Arabidopsis thaliana*

The entrainment range depends on the coupling



P: clock oscillator period
q: zeitgeber period

The circadian clock is temperature compensated



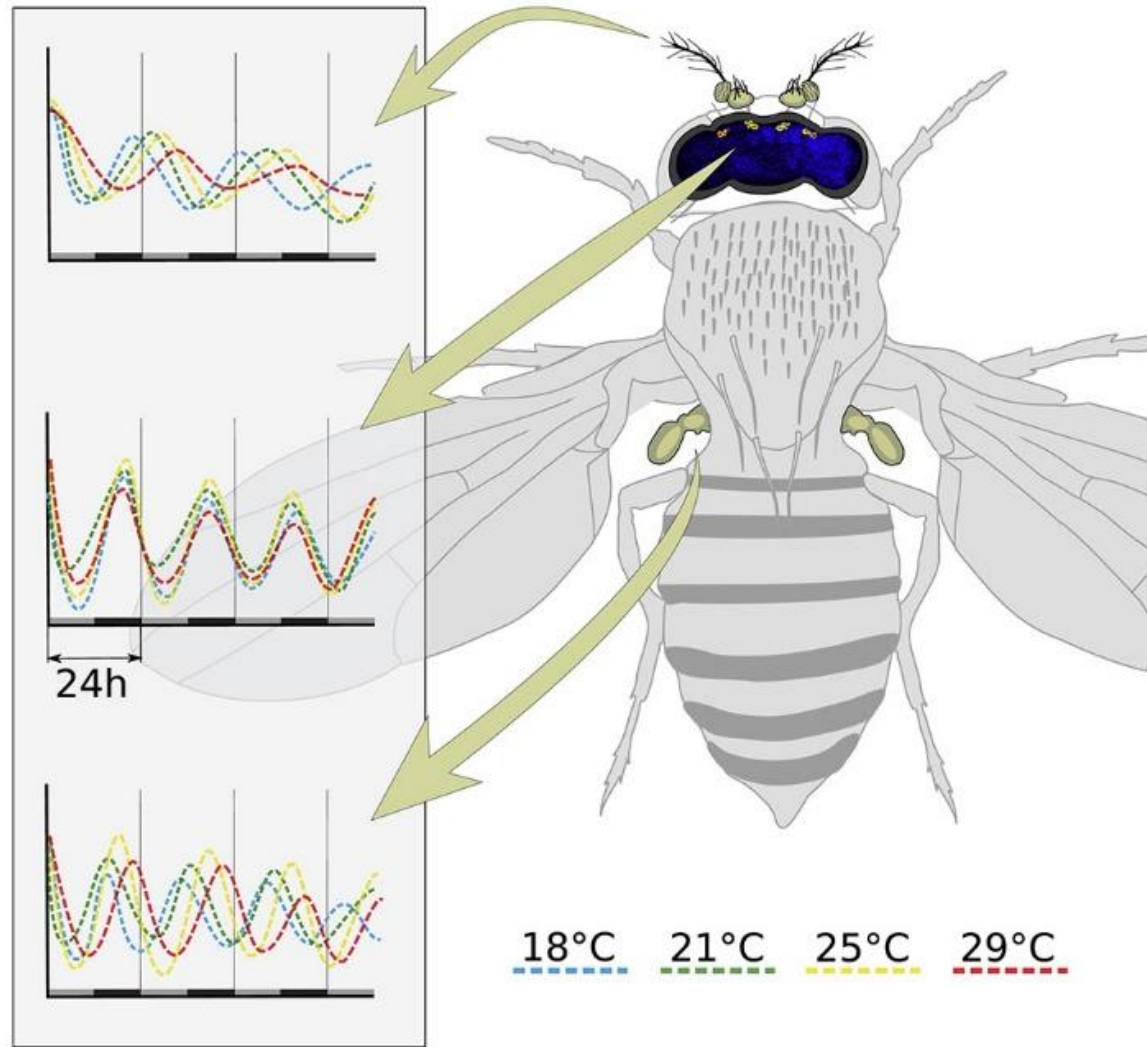
1859-1927

- 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).

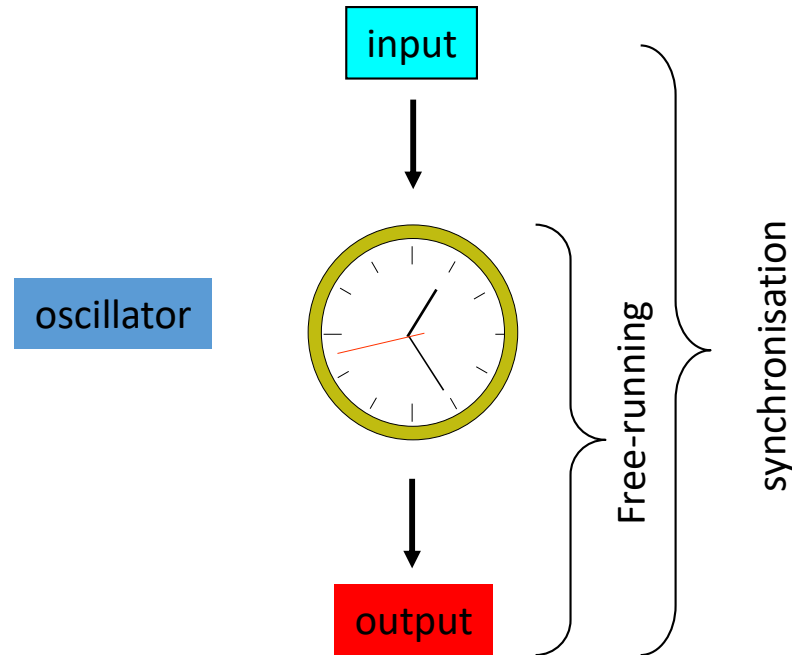
$$Q_{10} = \left[\frac{R_2}{R_1} \right]^{\frac{10}{(t_2 - t_1)}}$$

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

Thermal compensation of the circadian clock



The 3 components of the circadian clock



Summary 1

- Many biological processes oscillate with a circadian period
- The circadian clock is an endogenous mechanism
- The central pacemaker is localized in specialized neurons
- The circadian clock is synchronized by external cues
- The circadian clock is temperature compensated



Nobel 2017 Physiology and Medicine



JC Hall



M Rosbash

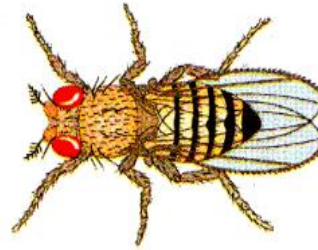


MW Young

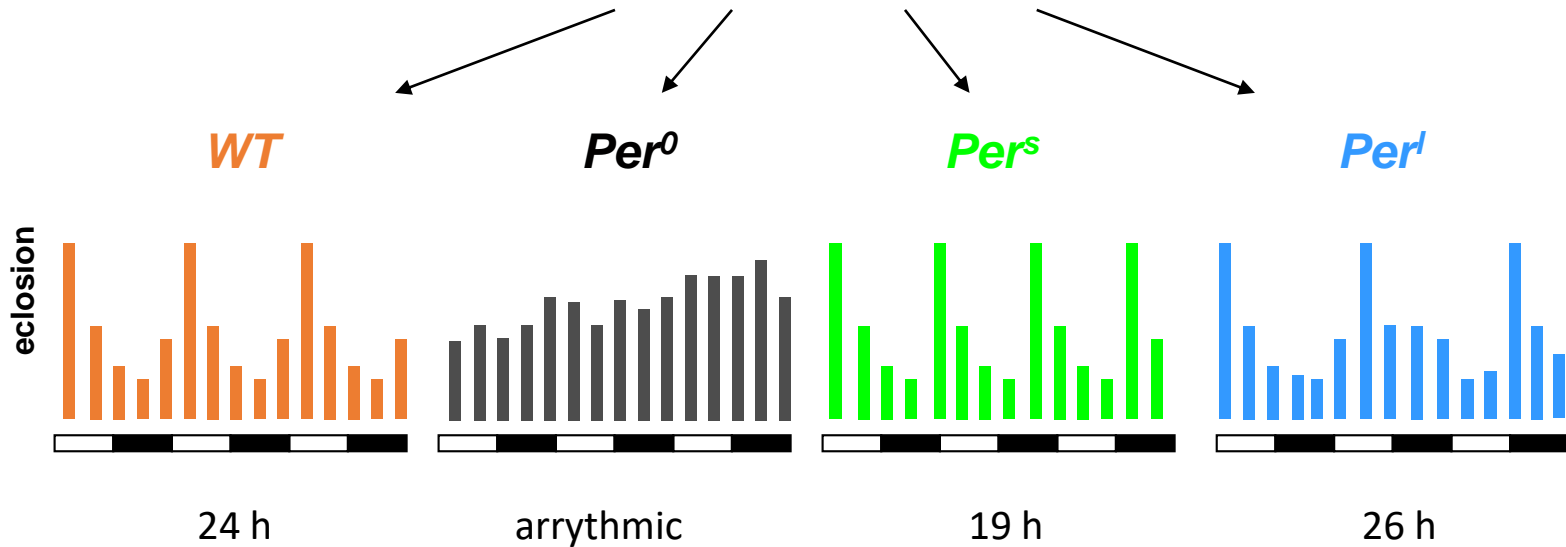
The genetic origin of circadian rhythms



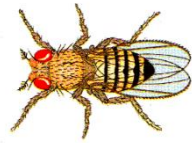
S Benzer (1921-2007)



Mutatagenesis



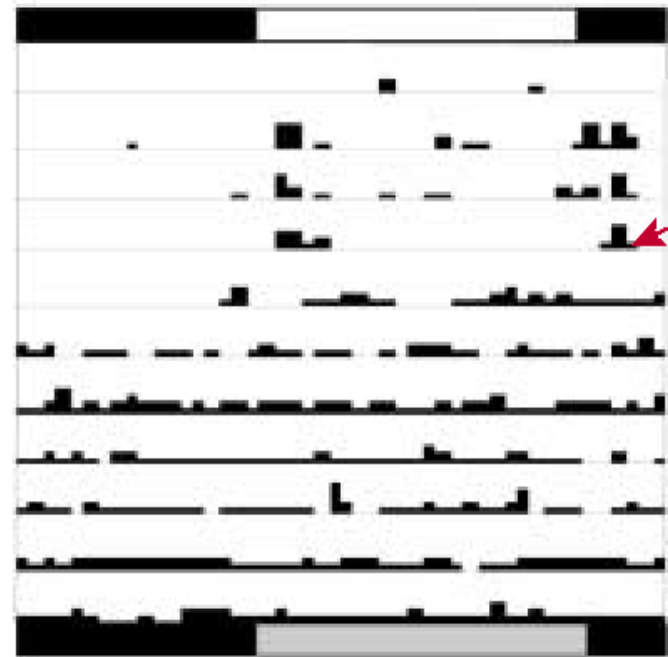
*Per*⁰ flies are arrhythmic



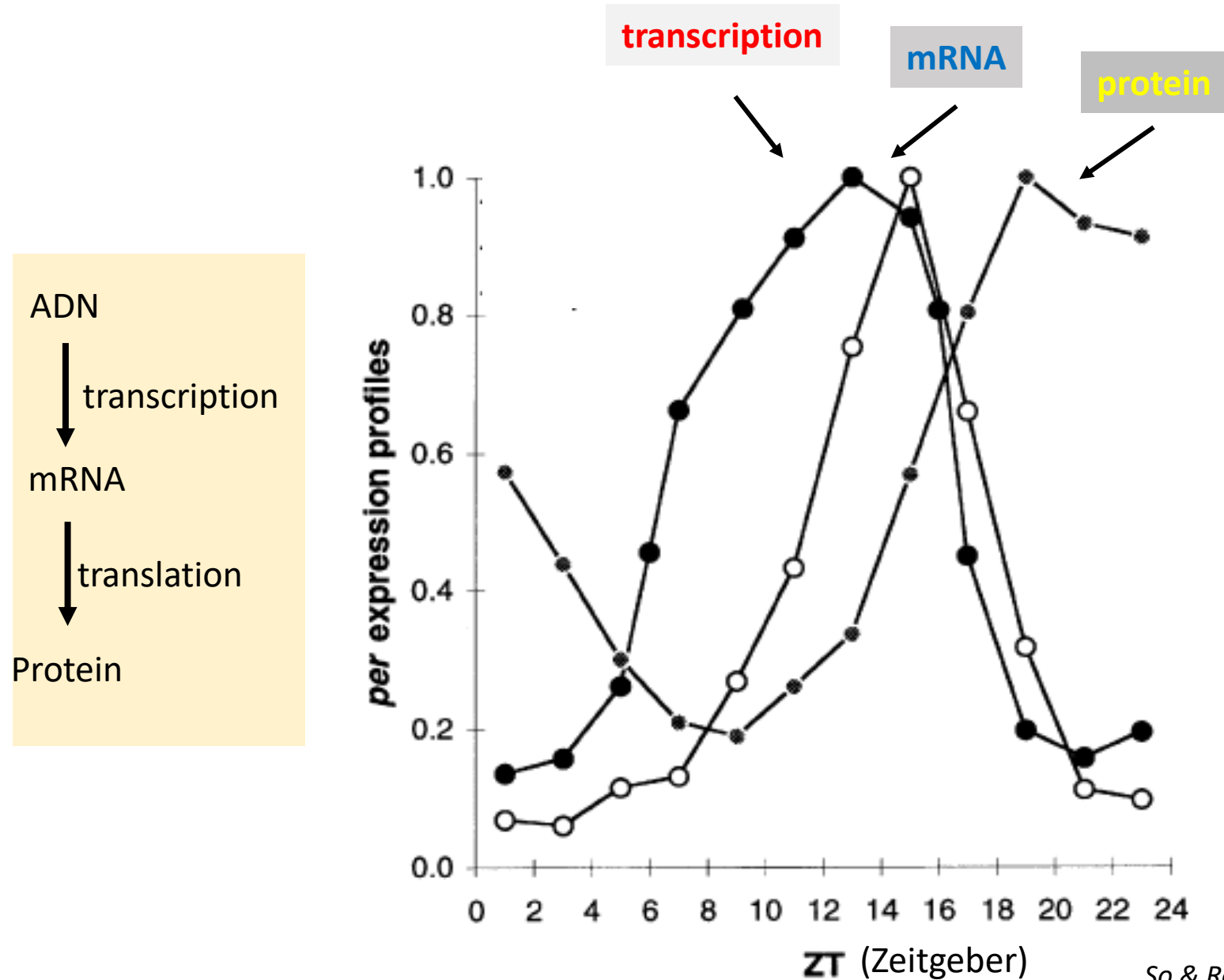
WT



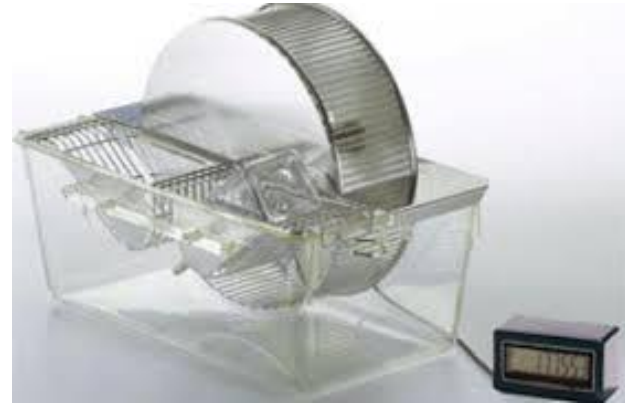
*per*⁰



The *Period* gene is rhythmically expressed

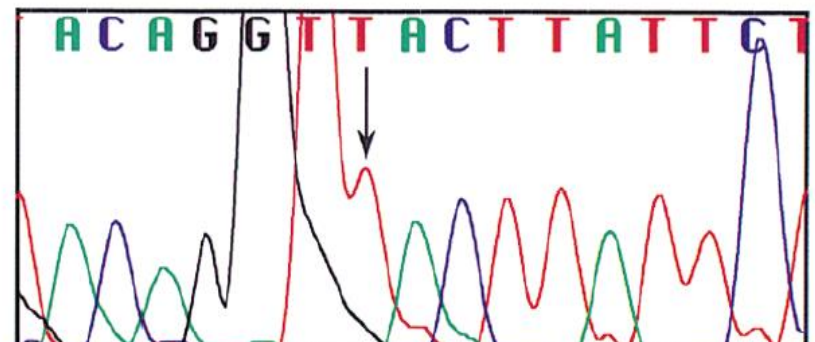
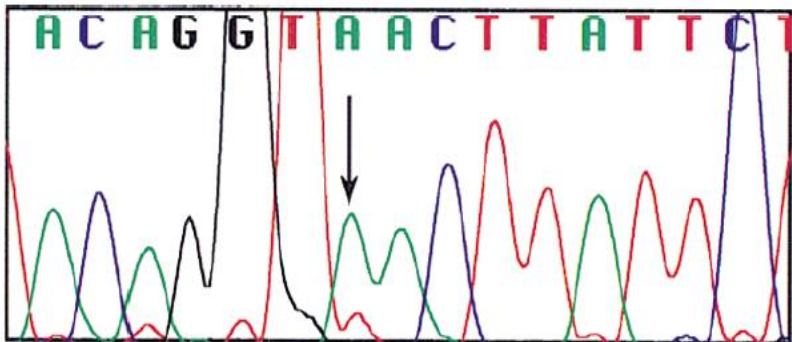
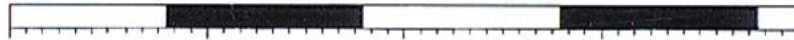


The *Clock* mutant mouse



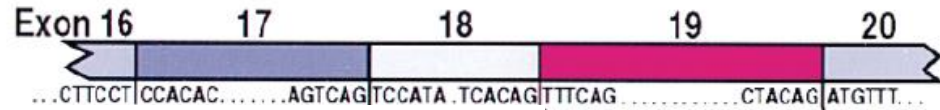
Wild-type

Clock/Clock

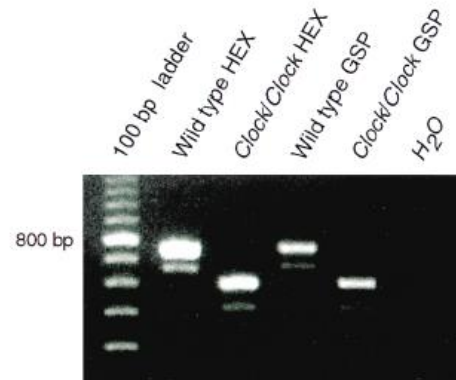
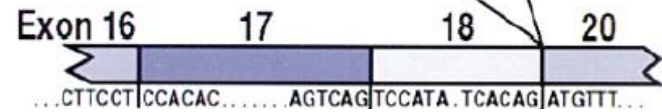


The Clock mutation *in the mouse*

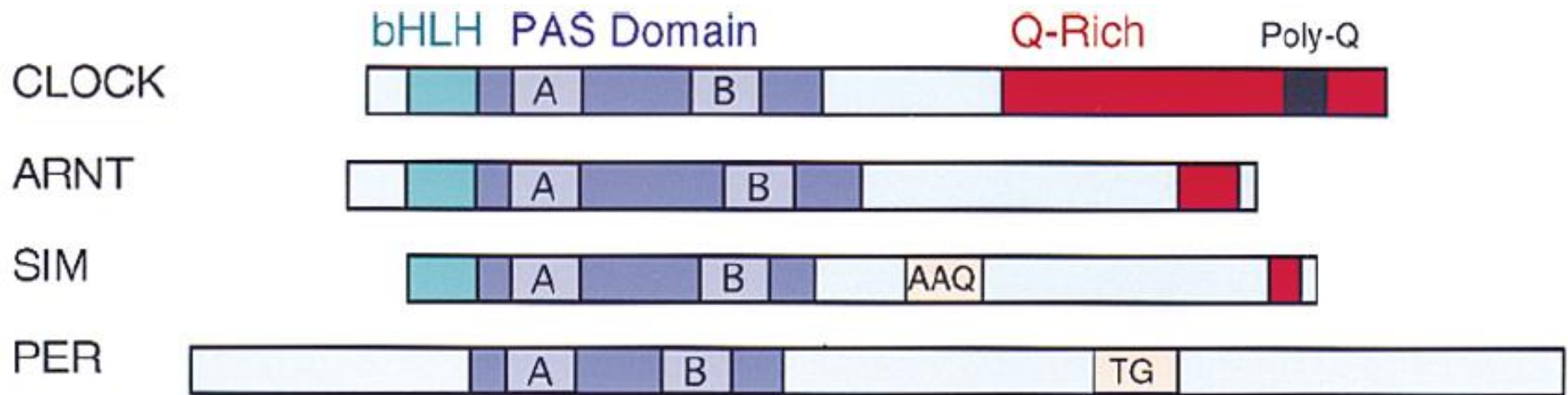
Wild type



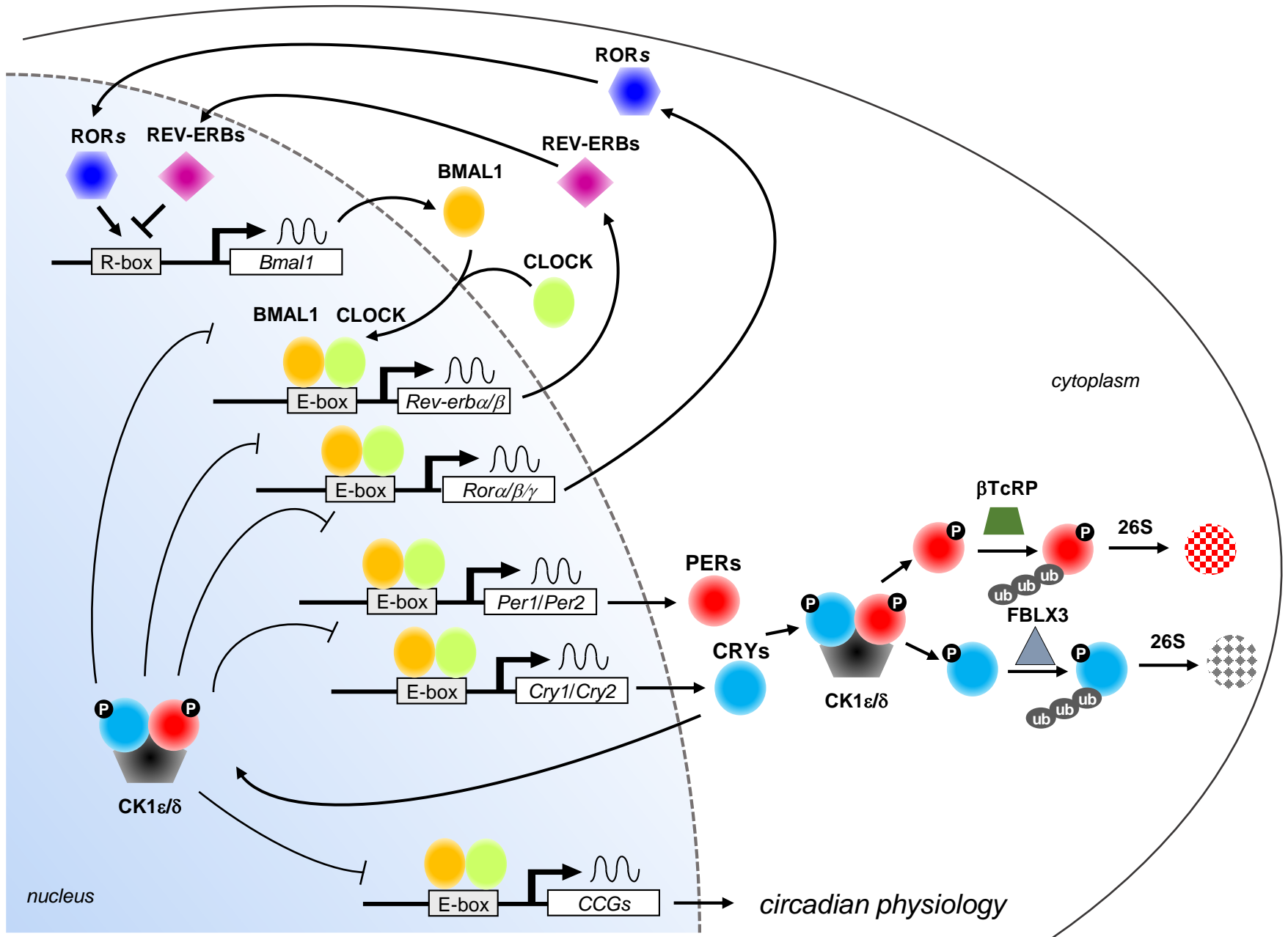
Clock



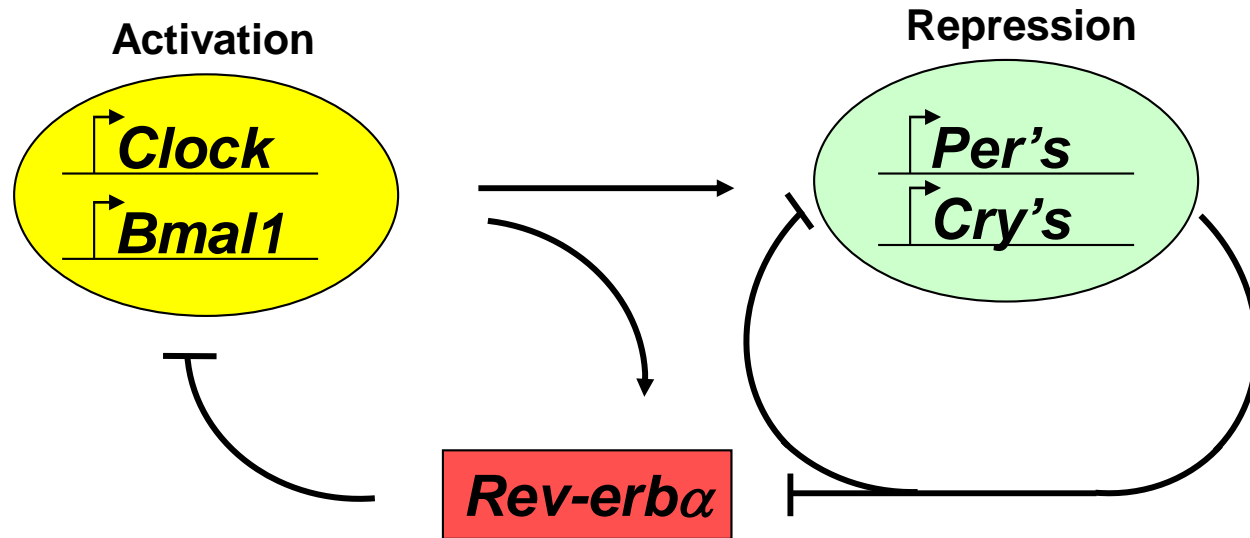
The CLOCK protein is a PAS domain transcription factor



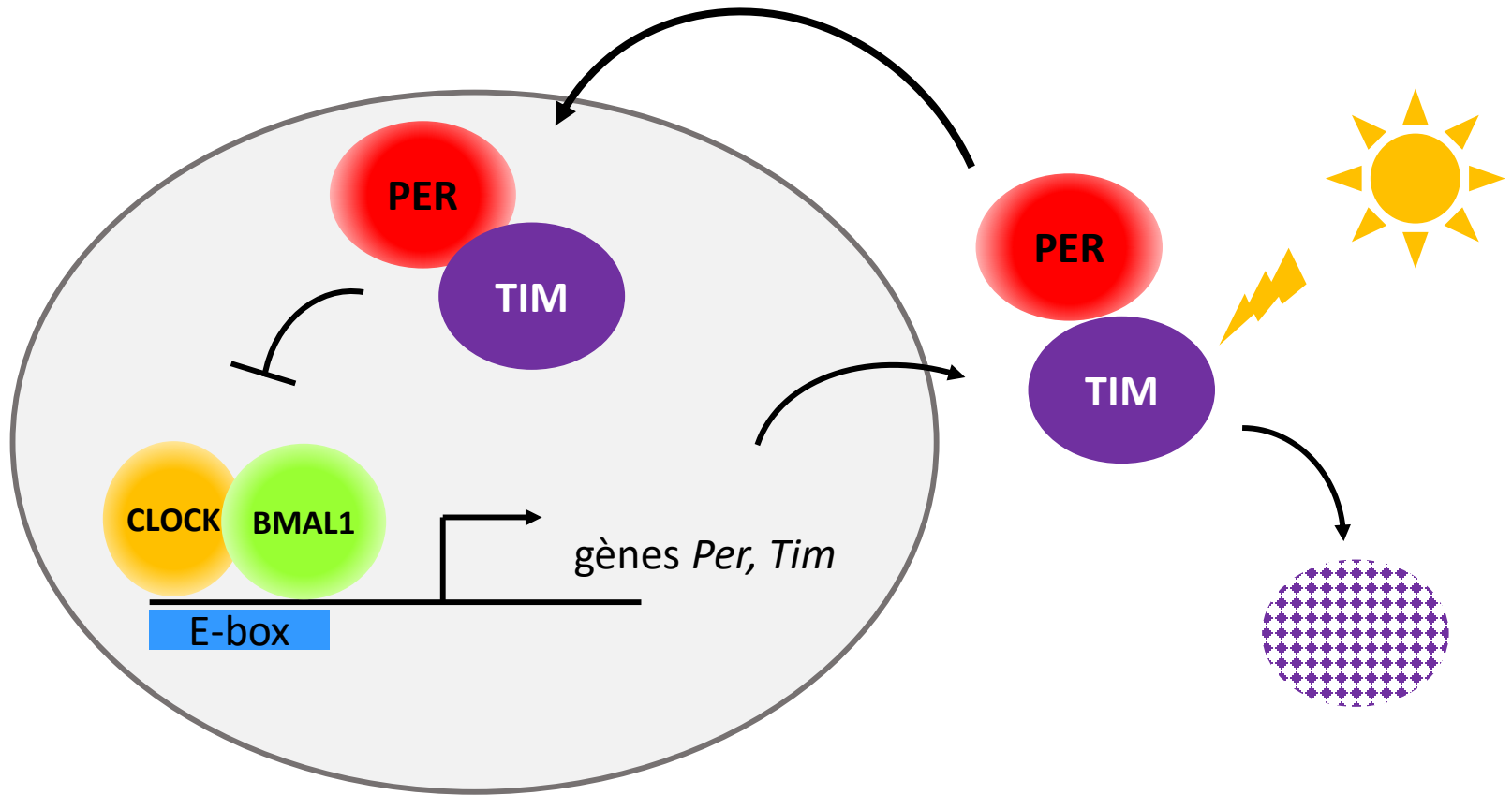
The circadian oscillator is a genetic network



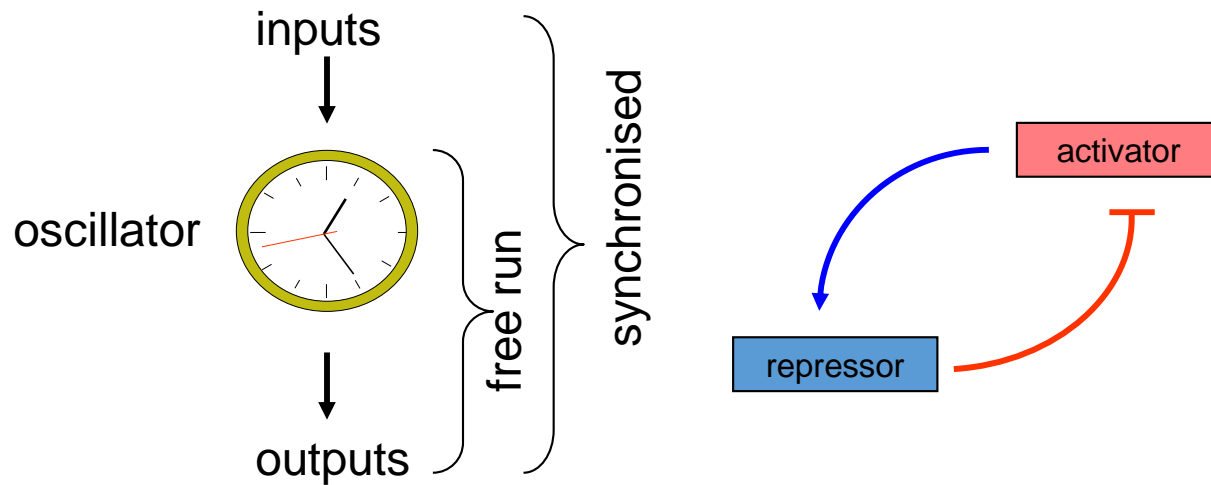
Simplified model of the mammalian circadian clock



Simplified model of the *Drosophila* circadian clock



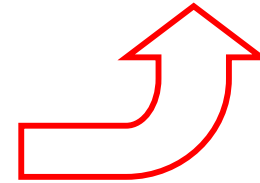
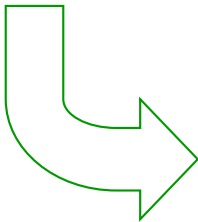
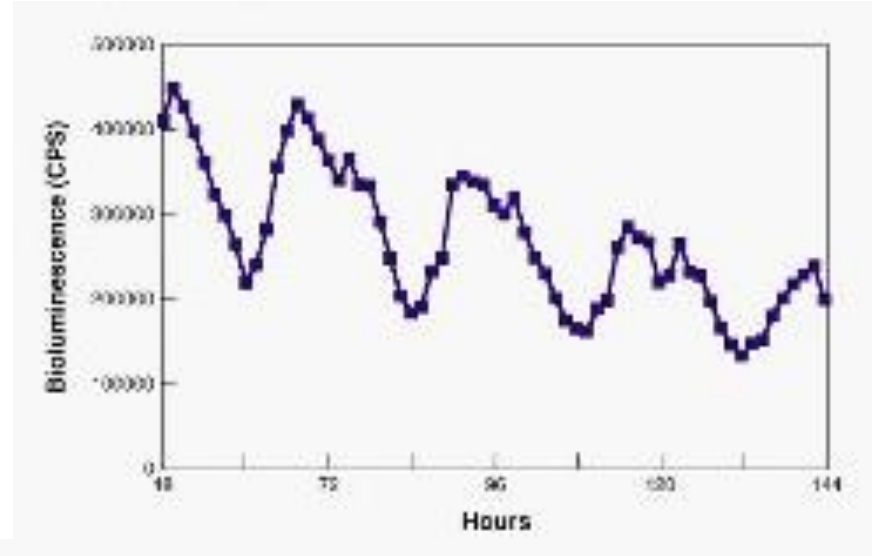
The same basic mechanistic principles govern all circadian clocks



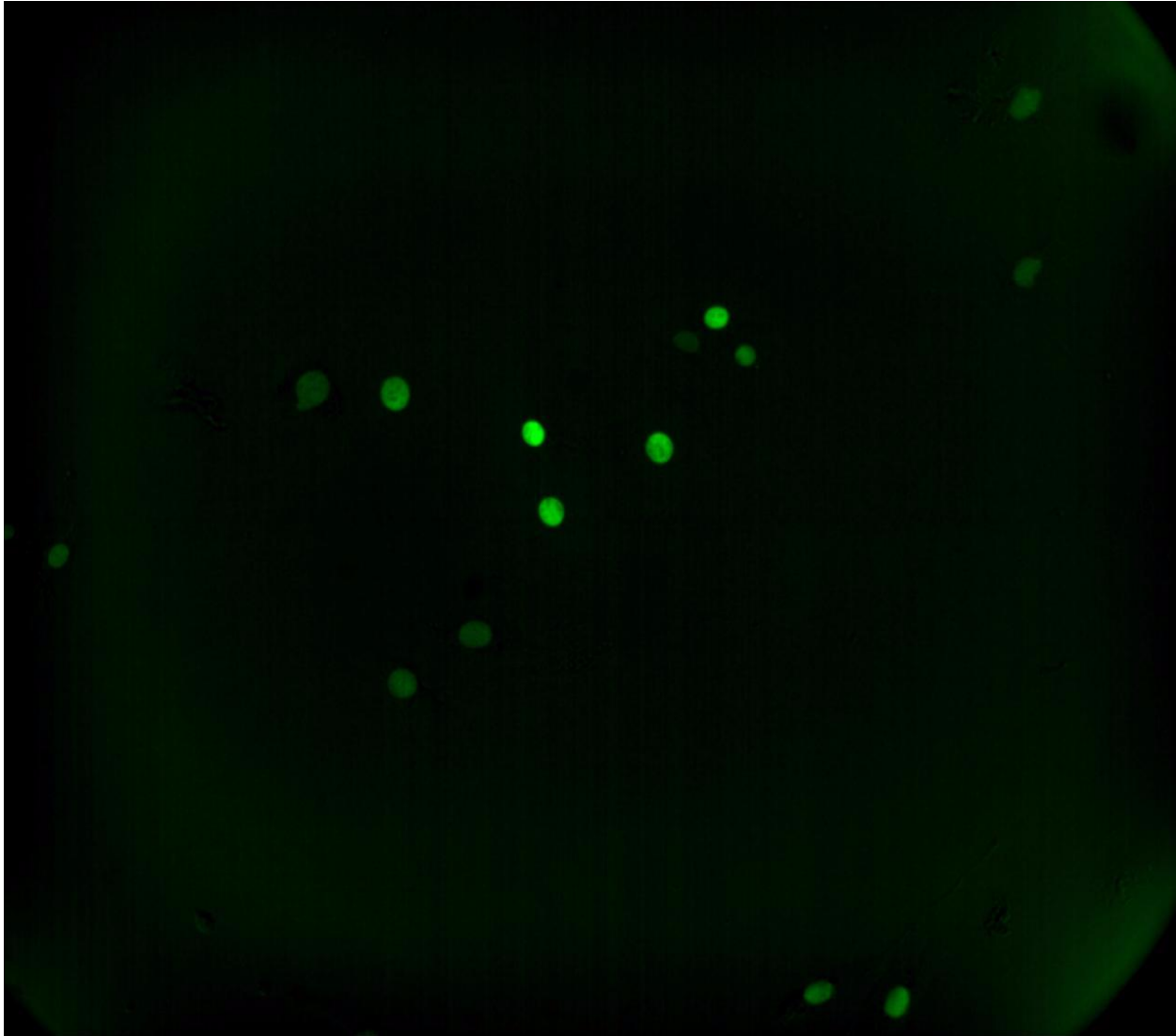
Biochemical / genetic oscillators

Function	Components	Period	Class
Metabolism	Glucose, 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

Peripheral clocks



A clock in every cell



Circadian Omics

- Methylome
- Acetylome
- 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

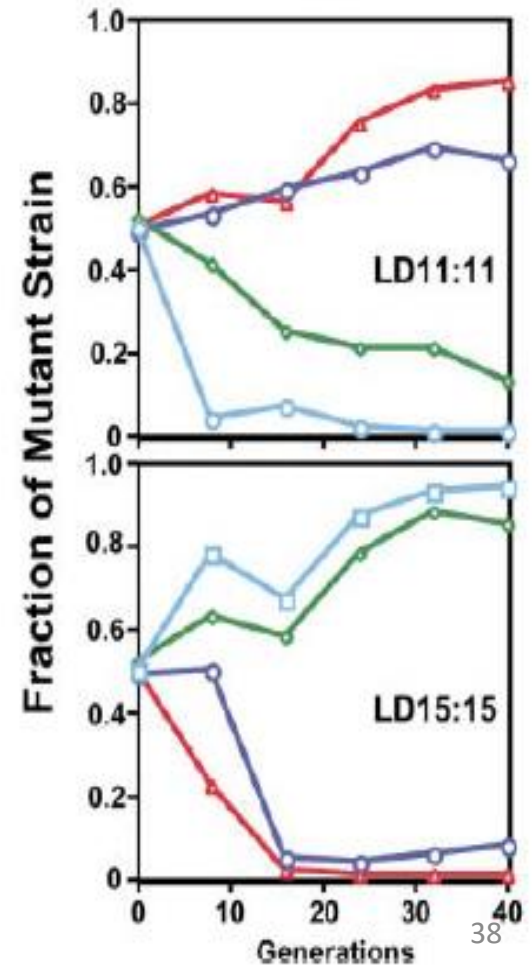
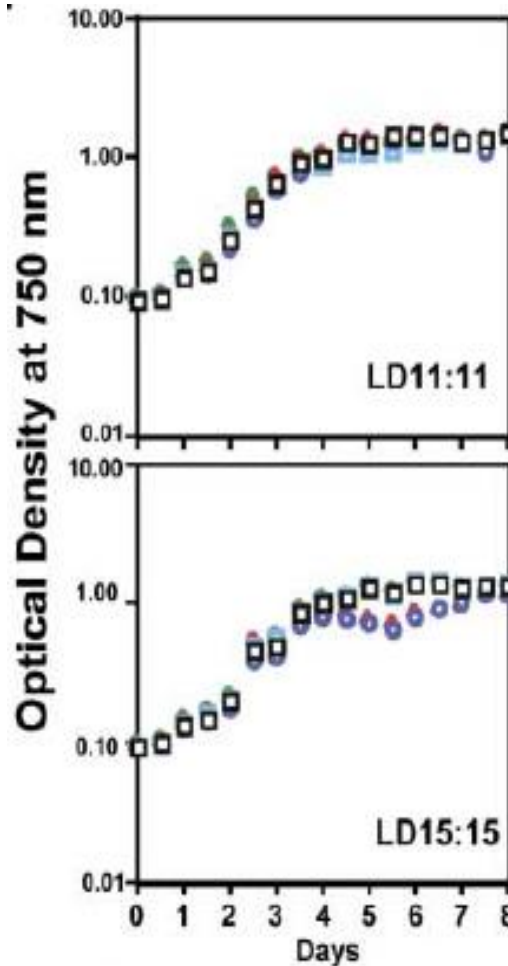
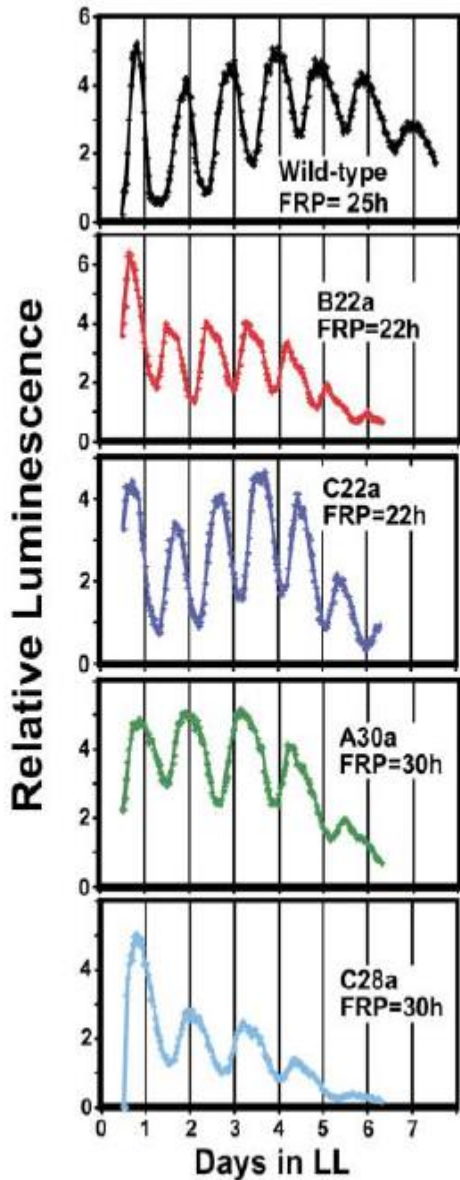
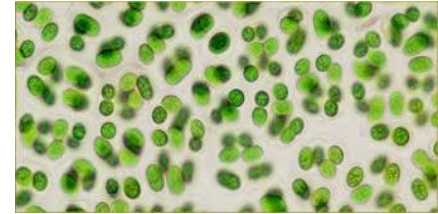
<http://circadiomics.ics.uci.edu/>

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

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

The adaptive value of circadian clocks

Synechococcus elongatus



Summary 2

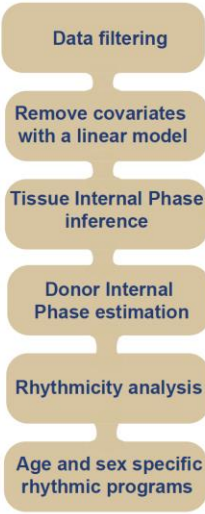
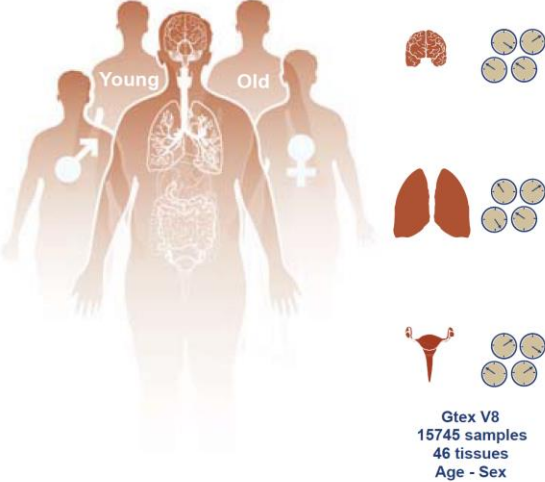
- Clock genes govern circadian rhythms
- A delayed negative feedback loop drives all circadian clock oscillator
- Circadian oscillators are present in virtually every cell
- Circadian gene expression is extensive and highly tissue-specific
- The clock has an adaptative value

CIRCADIAN RHYTHMS

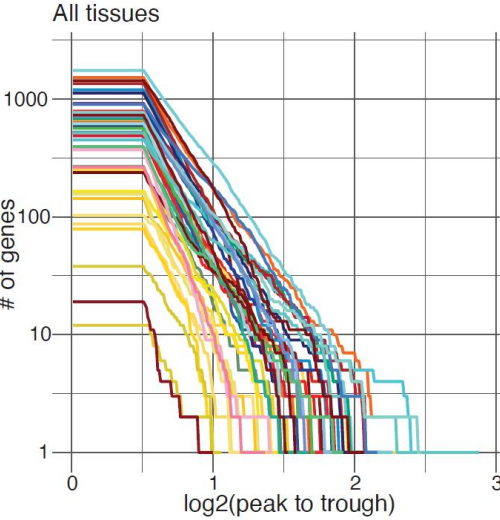
Sex-dimorphic and age-dependent organization of 24-hour gene expression rhythms in human

Lorenzo Talamanca†, Cédric Gobet†, Felix Naef*

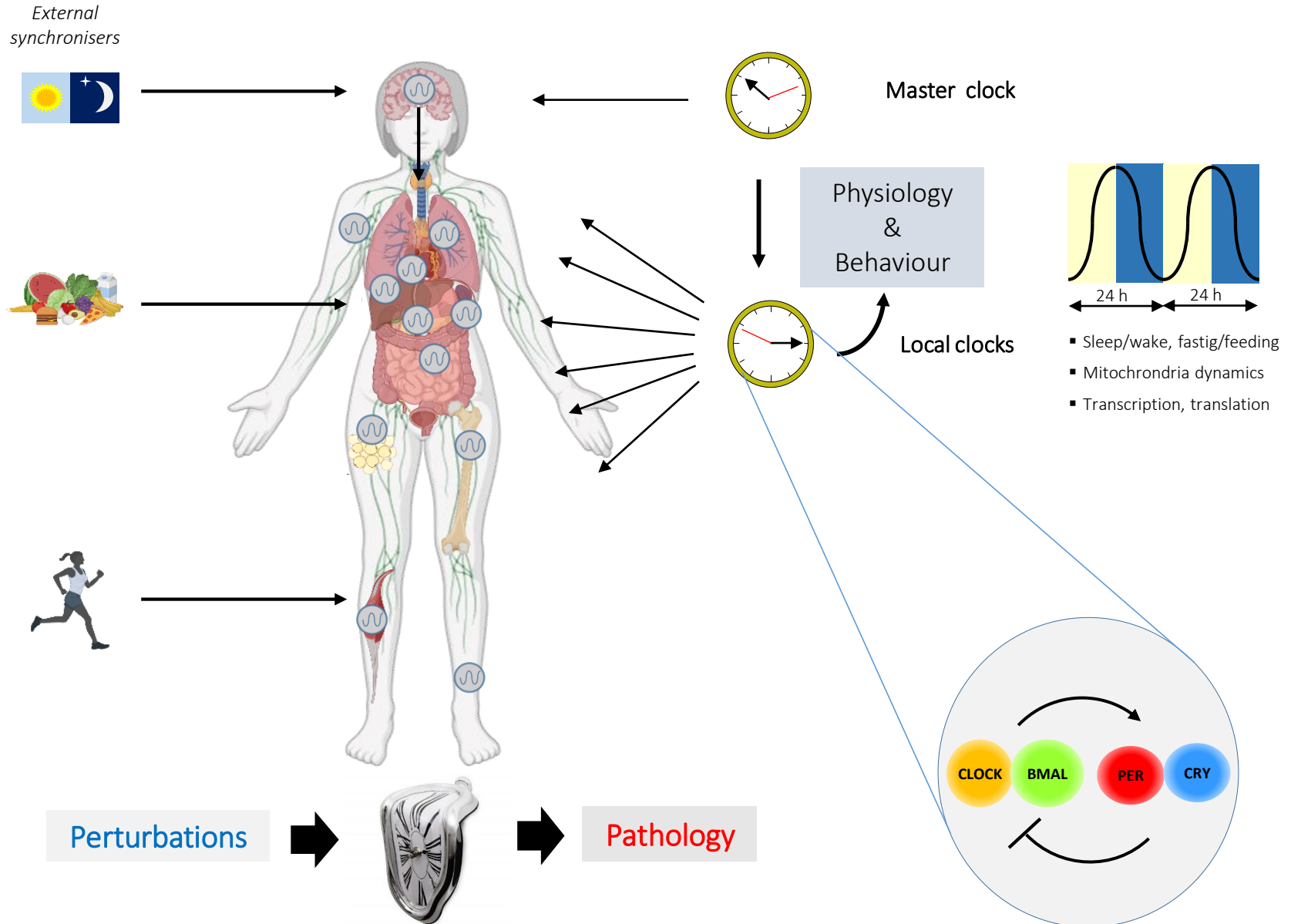
<p>Brain</p> <ul style="list-style-type: none"> Amygdala Anterior cing. cortex Caudate Cerebellar Hem. Cerebellum Cortex Frontal Cortex Hippocampus Hypothalamus Nucleus accumbens Putamen Spinal cord Substantia Nigra 	<p>Esophagus</p> <ul style="list-style-type: none"> Gastro. Junction Mucosal Muscularis <p>Colon</p> <ul style="list-style-type: none"> Sigmoid Transverse <p>Small intestine</p> <ul style="list-style-type: none"> Stomach
<p>Artery</p> <ul style="list-style-type: none"> Aorta Coronary Tibial <p>Heart</p> <ul style="list-style-type: none"> Atrial Appendage Left Ventricle 	<p>Kidney</p> <ul style="list-style-type: none"> Liver Lung Muscle Pancreas Spleen
<p>Adipose</p> <ul style="list-style-type: none"> Subcutaneous Visceral <p>Skin</p> <ul style="list-style-type: none"> Not Sun Exposed Sun Exposed 	<p>Adrenal Gland</p> <ul style="list-style-type: none"> Min. Salivary Gland Nerve - Tibial <p>Pituitary</p> <ul style="list-style-type: none"> Thyroid
<p>Breast</p> <ul style="list-style-type: none"> Ovary <p>Prostate</p> <ul style="list-style-type: none"> Testis Uterus Vagina 	



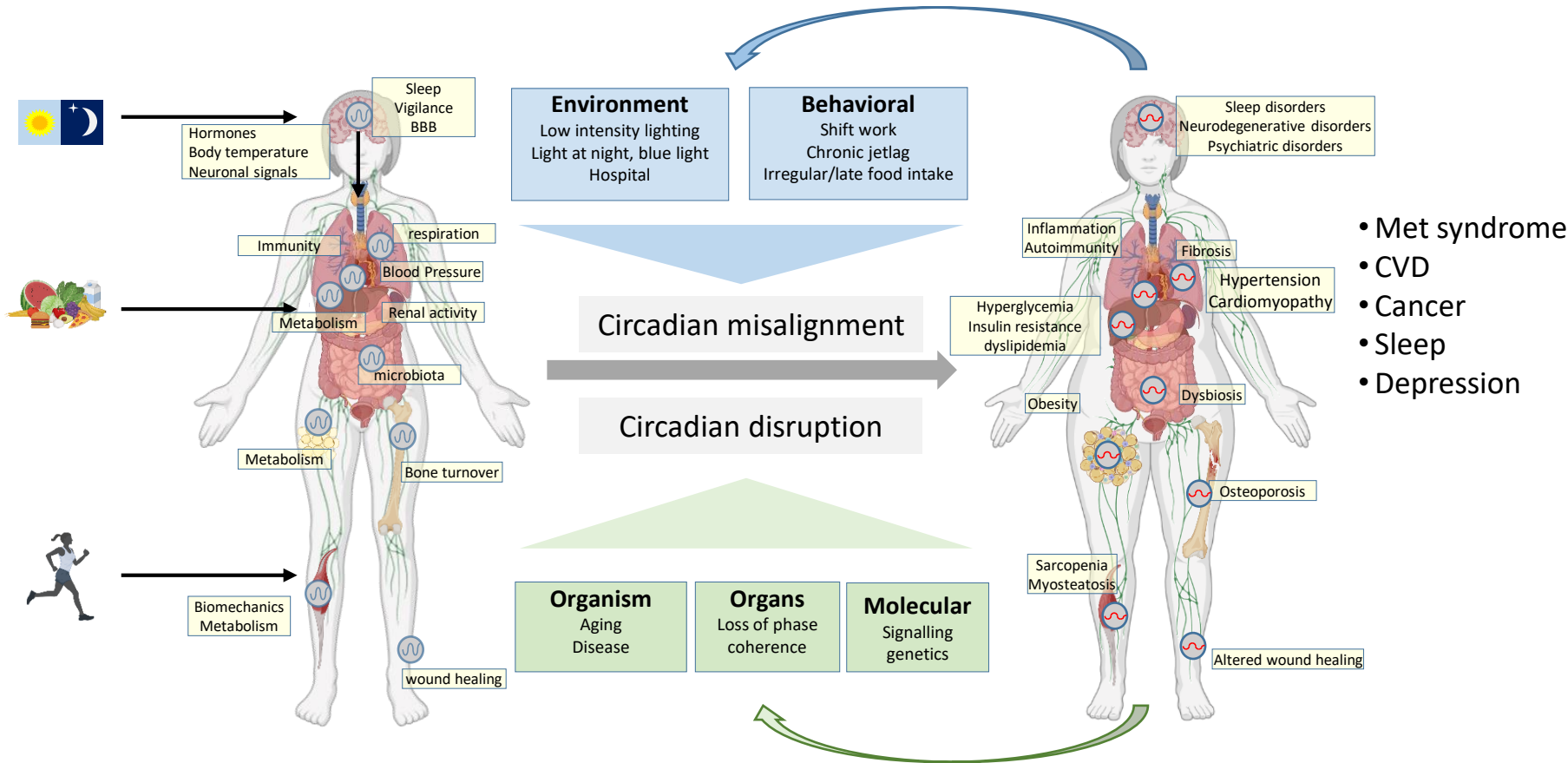
- Clock Reference Gen
- ARNTL
 - CIART
 - CRY1
 - CRY2
 - DBP
 - NPAS2
 - NR1D1
 - NR1D2
 - PER1
 - PER2
 - PER3
 - TEF



The circadian timing system



Biomedical implications of chronobiology research

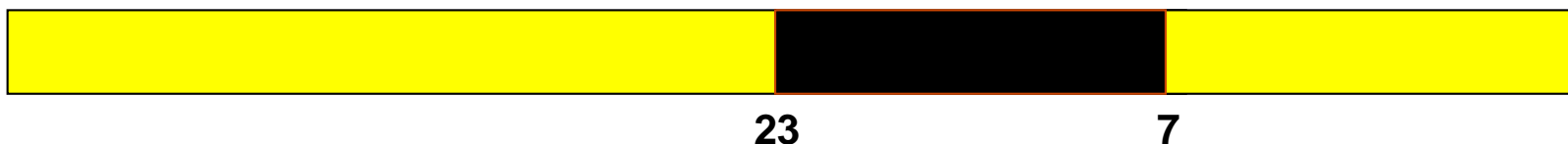


Les désordres du rythme circadien du sommeil (11% des consultations pour insomnie)

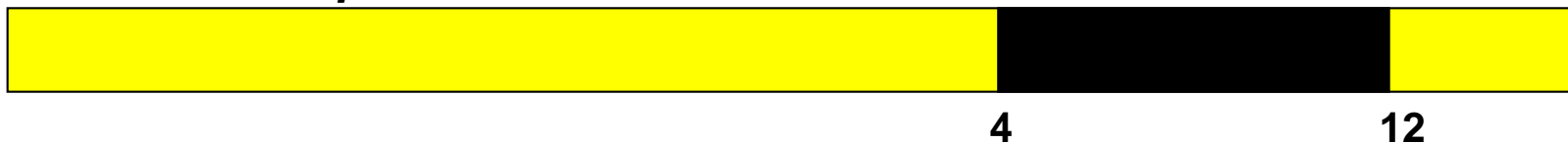
Syndrome d'avance de phase du sommeil : 13.5%



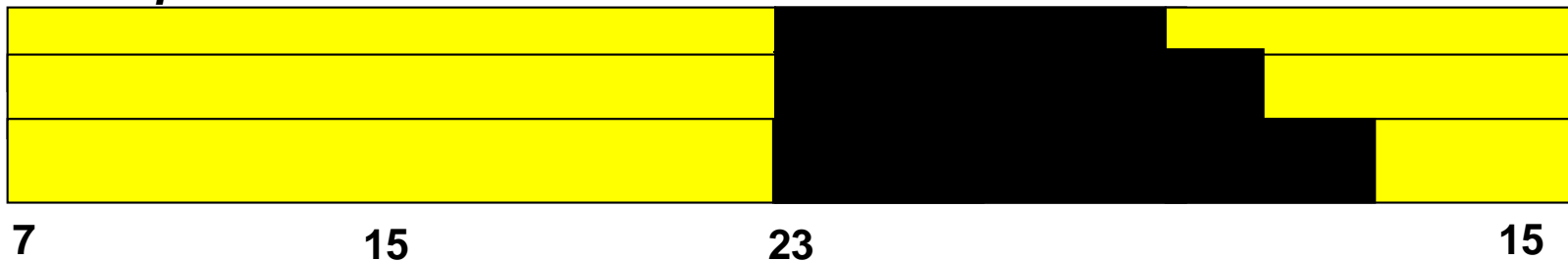
Sommeil normal



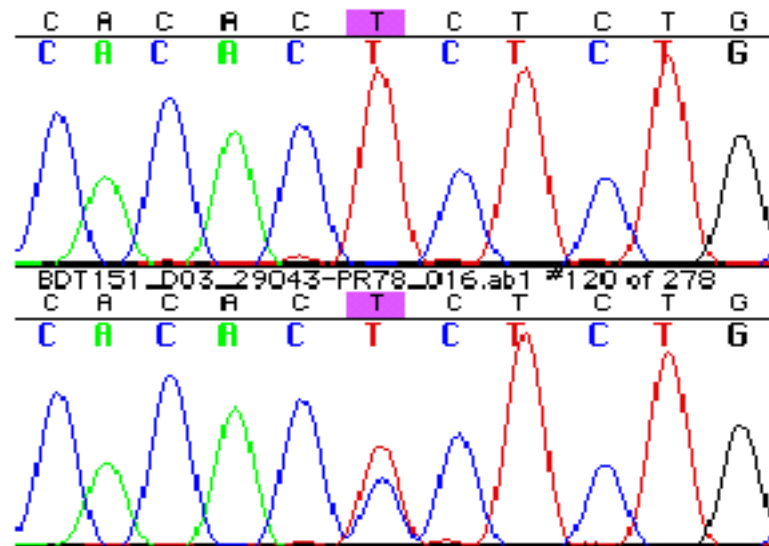
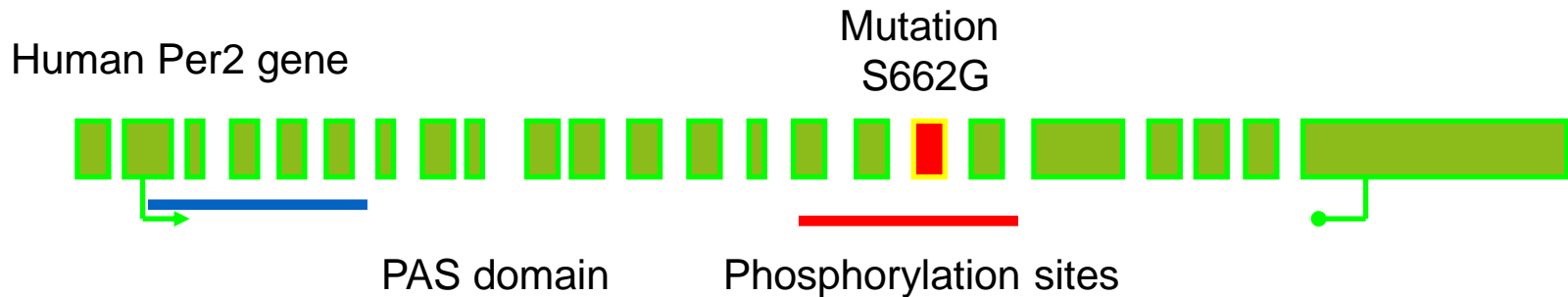
Syndrome de retard de phase du sommeil : 82.7%



Anomalie de la période du sommeil : 3.8%

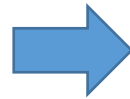


Une mutation dans le gène *Per2* humain est la cause d'une maladie du sommeil FASPS



Impact of circadian misalignment on metabolic homeostasis

Karlsson et al 2003 (WOLF study)
Scheer et al 2009



- Triglycerides ↑
- HDL-Cholesterol ↑
- Post-prandial glucose ↑
- Post-prandial insulin ↑
- Waist/hip ratio ↑

Feeding pattern in humans

- Healthy adults non shift workers (n = 156)

- Prediabetic men (n=6)

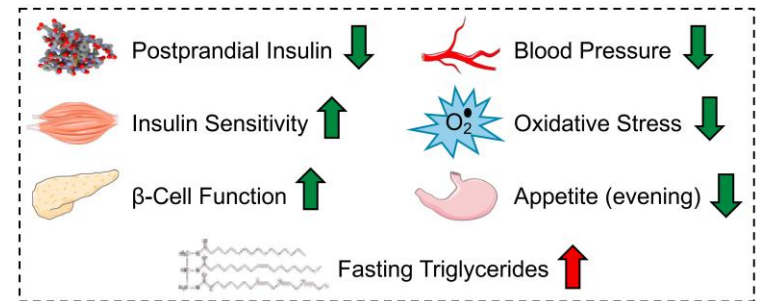
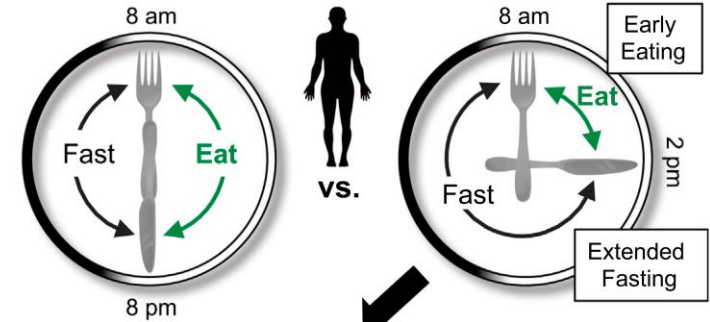
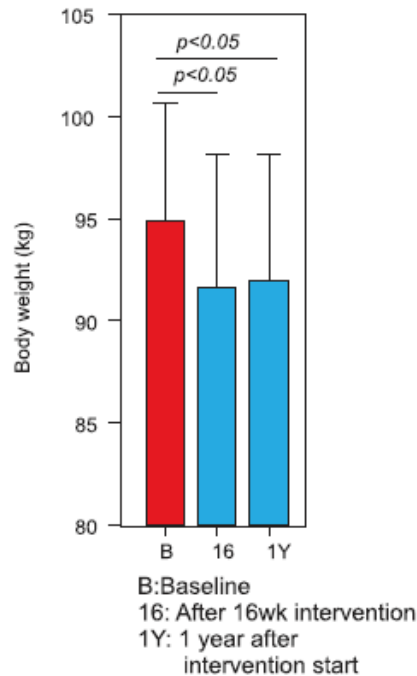
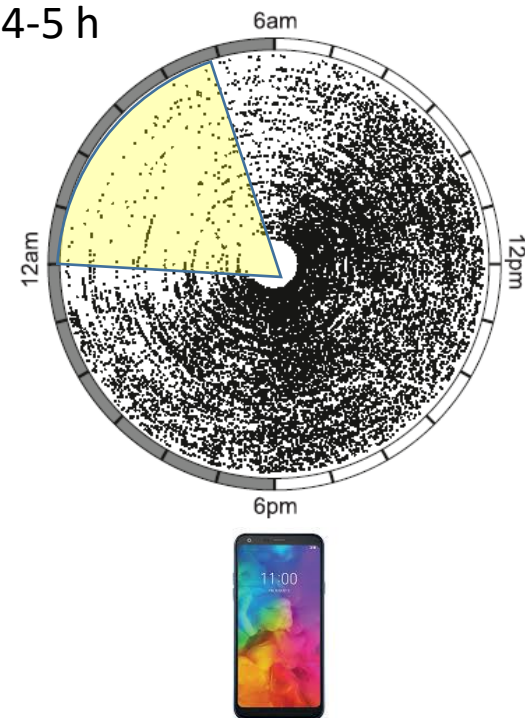
Baseline

Intervention

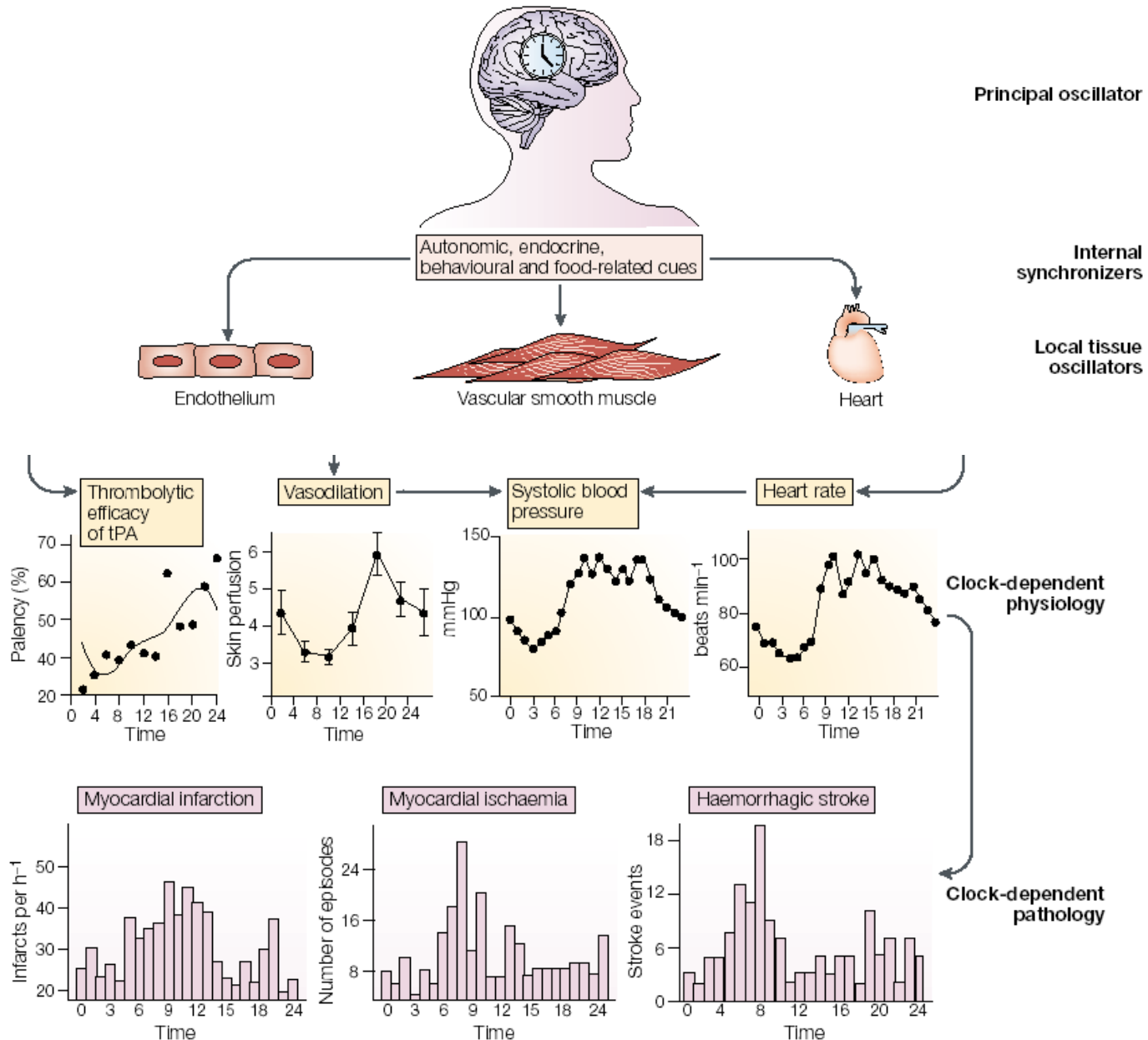
(> 25 BMI, > 14 h feeding duration → 12 h, n=8)

Median American Eating Patterns

Early Time-Restricted Feeding



Circadian rhythms and cardiovascular pathologies



Cardiac surgery

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

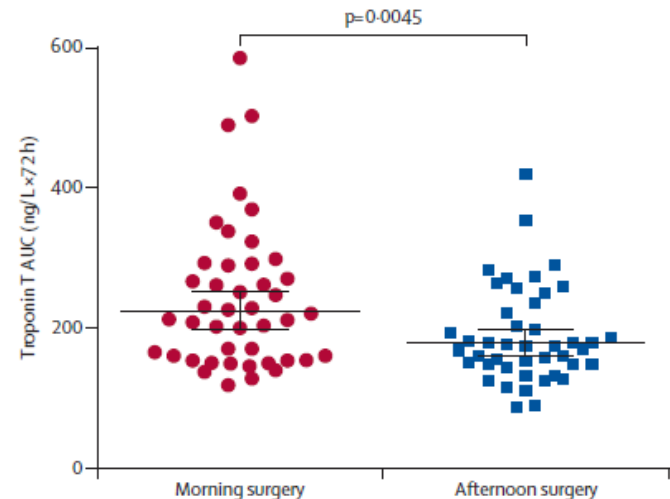
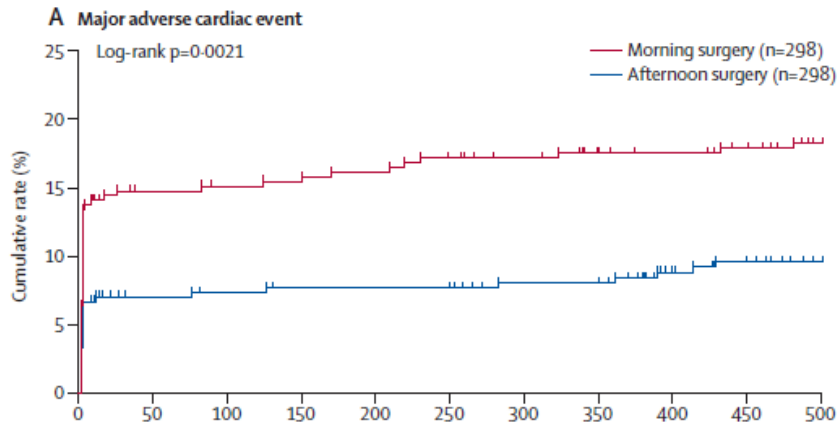


Lancet 2018; 391: 59-69

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

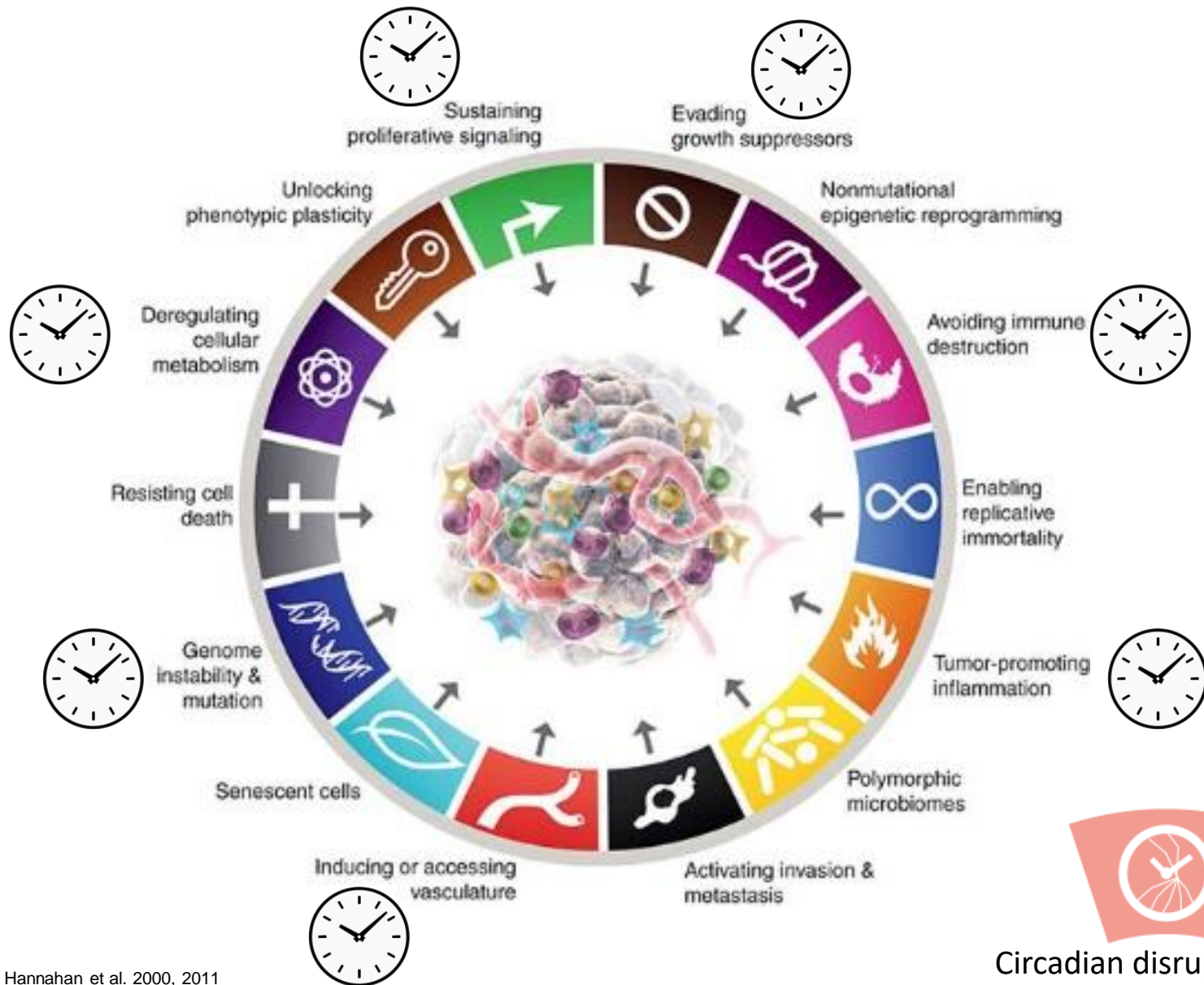


- Remplacement de la valve aortique
- Suivi de cohorte (298 + 298) + essai randomisé (44 + 44)



Biopsies myocarde \rightarrow Bmal1/Rev-erb α \rightarrow p21 \rightarrow tolérance à l'hypoxie

The circadian clock controls several cancer hallmarks



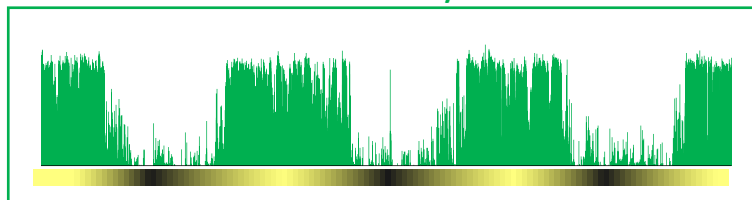
adapted from Hannahan et al. 2000, 2011

Circadian disruption as a new cancer hallmark

Circadian disruption and cancer

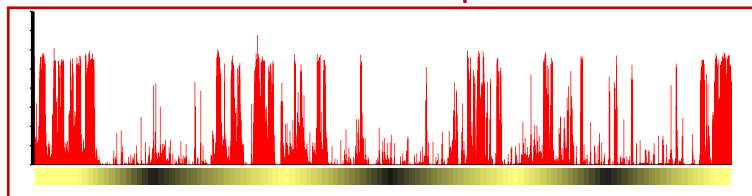
Patients with metastatic colorectal cancer

Circadian rhythm



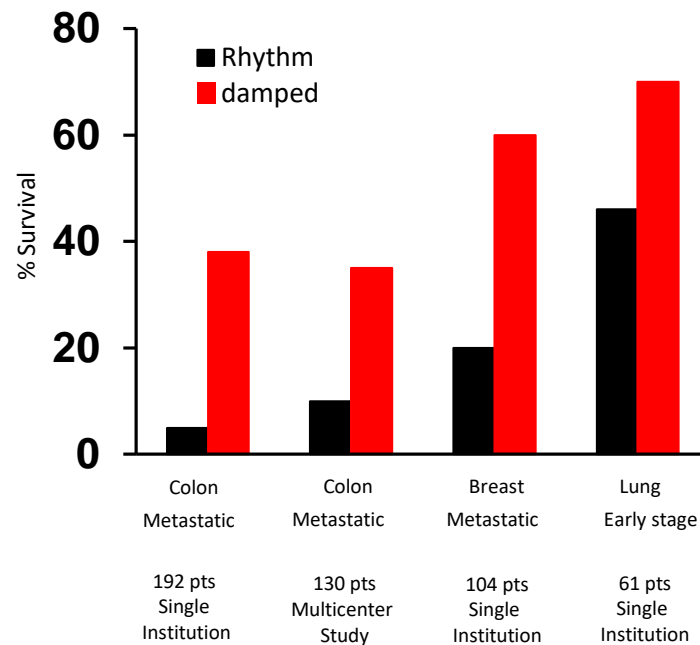
N= 67 patients

Circadian disruption



N= 68 patients

3 year survival rates

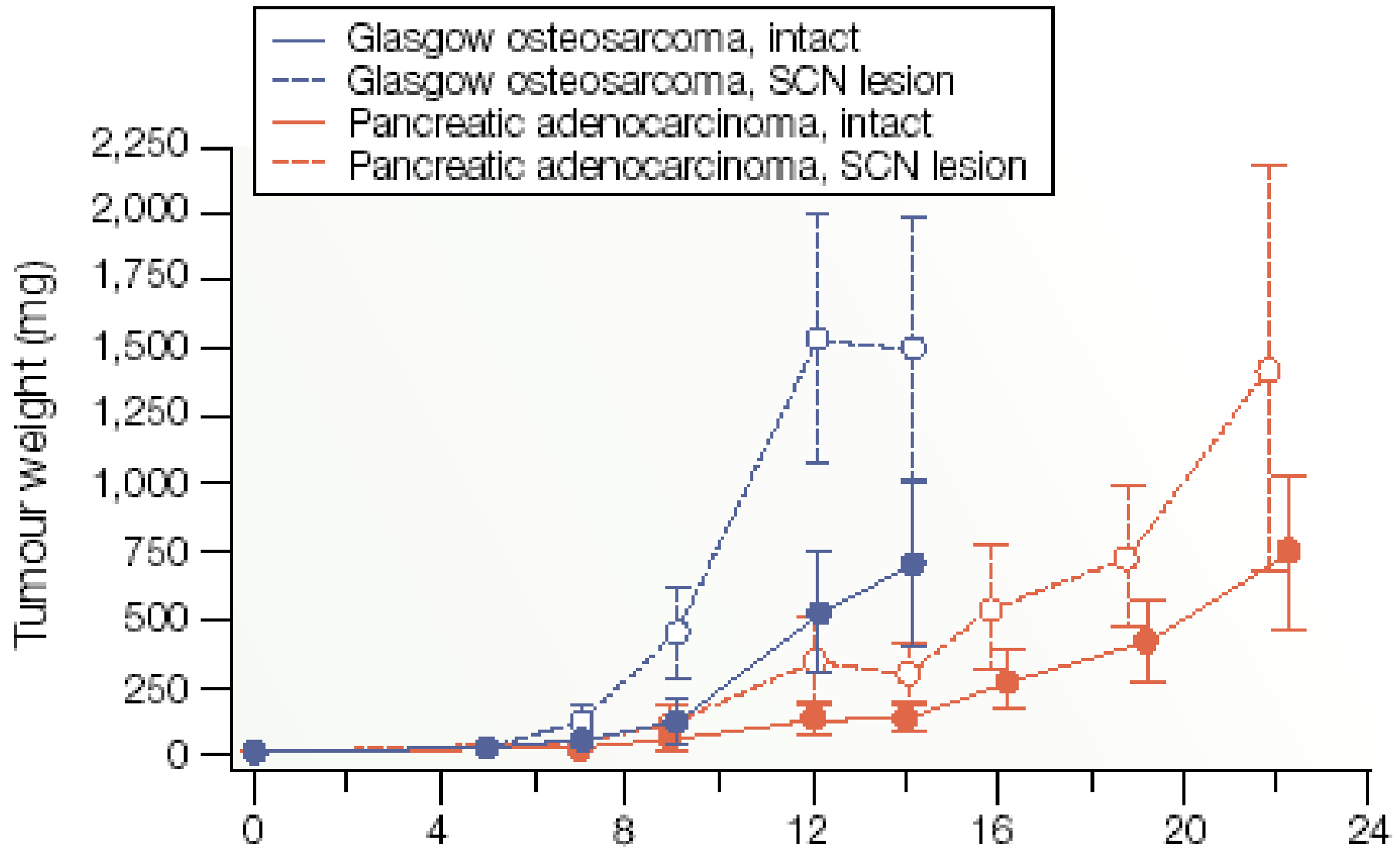


Rich, Innominato et al.
Clin Cancer Res 2005

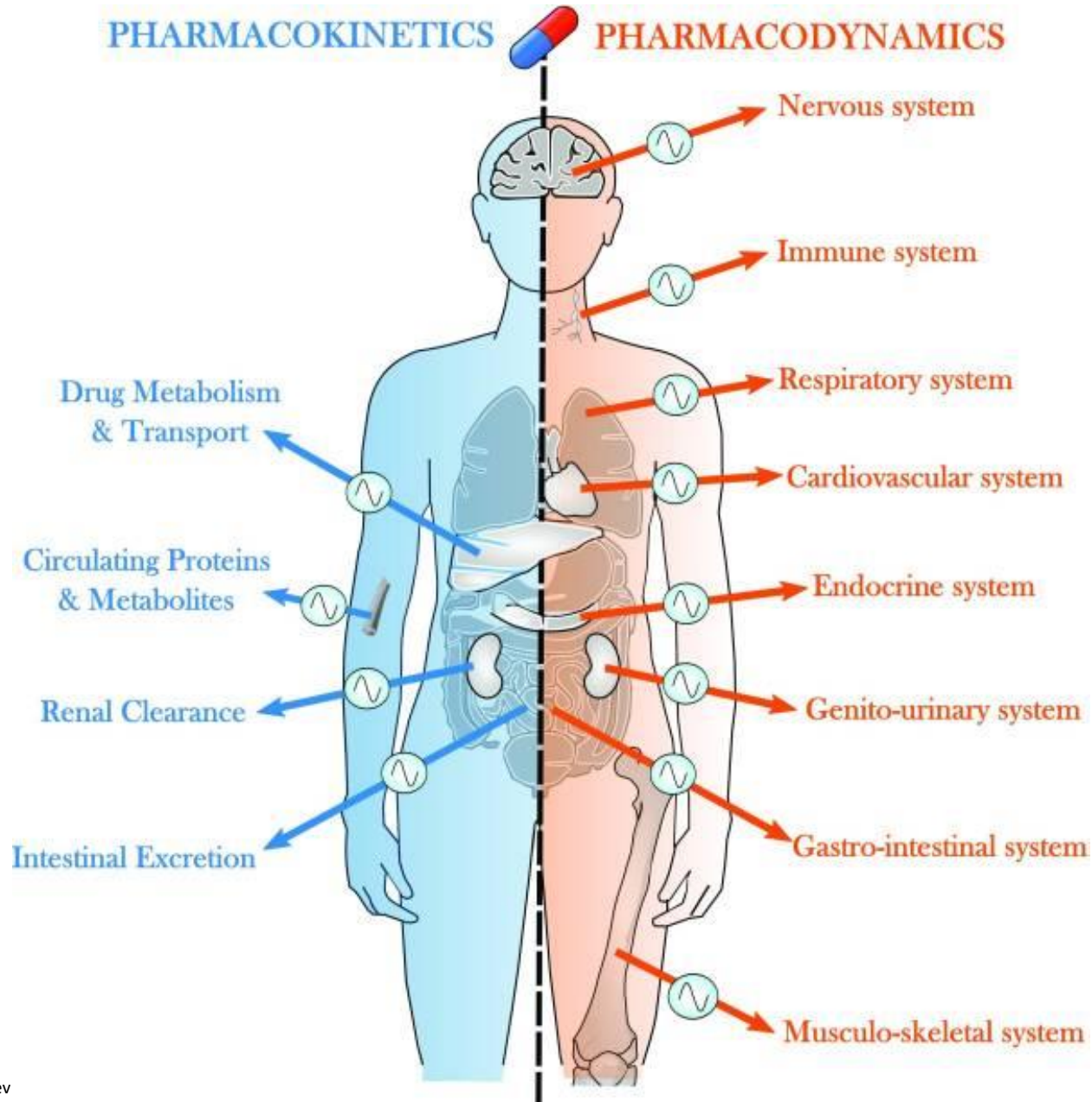
Innominato et al.
EBRS 2009

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

Circadian disruption accelerates tumour growth



From chronobiology to chronopharmacology



Tolerability of anticancer drugs in mice

Antimetabolites

- ▲ Gemcitabine
- L-alanosine

Topoisomerase inhibitors

- ◆ Irinotecan
- ▲ Mitoxantrone
- Etoposide

Intercalating agents

- Theprubicin

Alkylating agents

- ▲ Peptichemio
- Cisplatin
- ◆ Carboplatin
- Oxaliplatin

Nitrosoureas or related

- Mitomycin-C
- ◆ Cystemustin

Mitotic spindle poisons

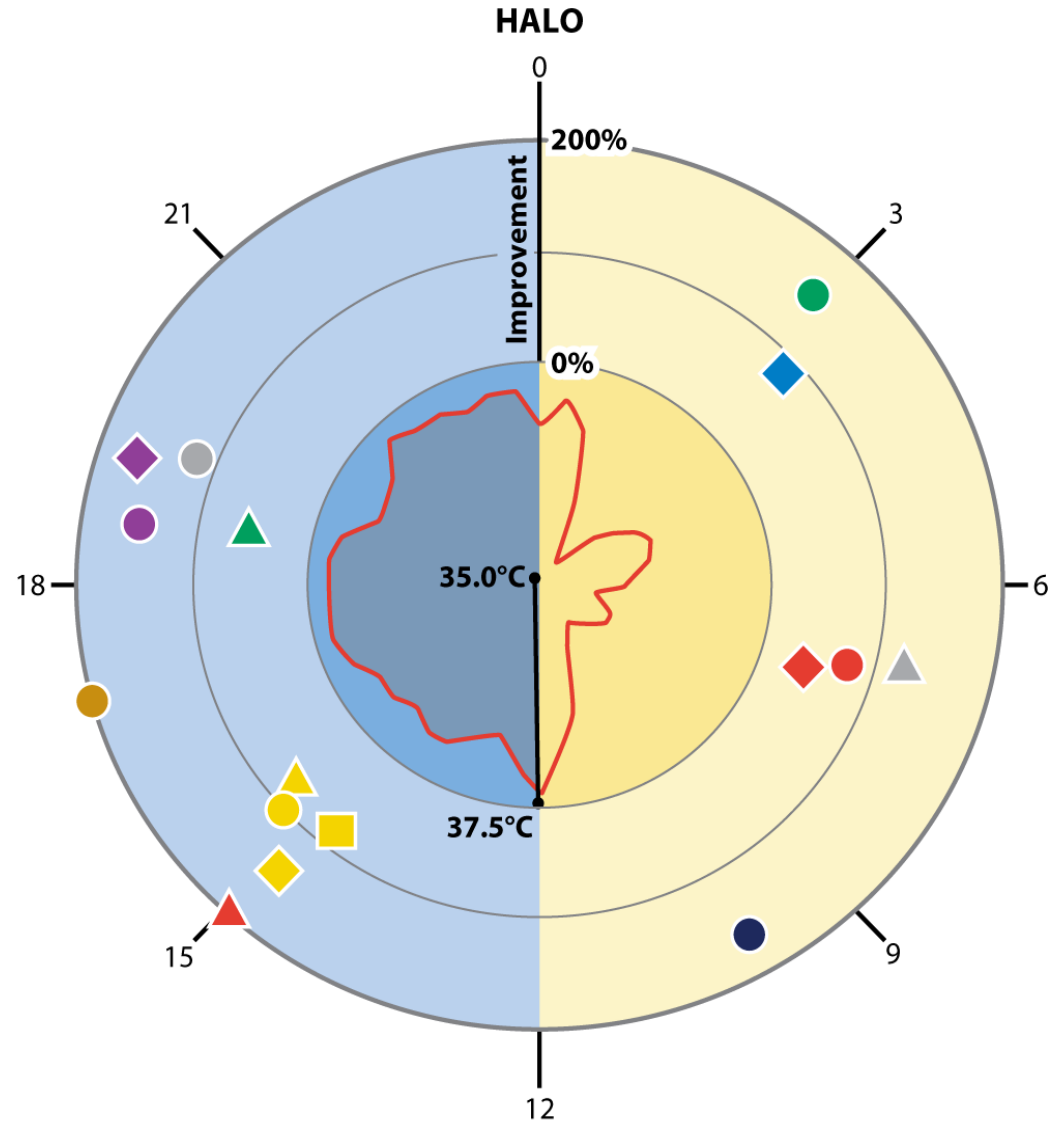
- Docetaxel
- ▲ Vinorelbine

Cytokines

- Interleukin-2

Small kinase inhibitors

- ◆ Seliciclib



The paradigm shift of anticancer chronochemotherapy

Current paradigm : the dose makes the poison (*Paracelese*)

Chronotherapy paradigm : dosing time makes the poison

Anticancer chronochemistry:

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

Chronotherapy: technological implementation



Time-scheduled delivery regimen



Multichannel pump for chronotherapy

- Centralized programming
- Any modulation of delivery rate
- 4 reservoirs (100-2000 ml)
- 2 independent channels
- Rates from 1 to 3000 ml/h



Summary 3

- Many diseases have a circadian component
- Circadian disruption is a risk factor for many pathologies
- Diseases alters the circadian coordination
- Human chronobiology is emerging
- Chronotherapy works but is not much implemented in the clinic