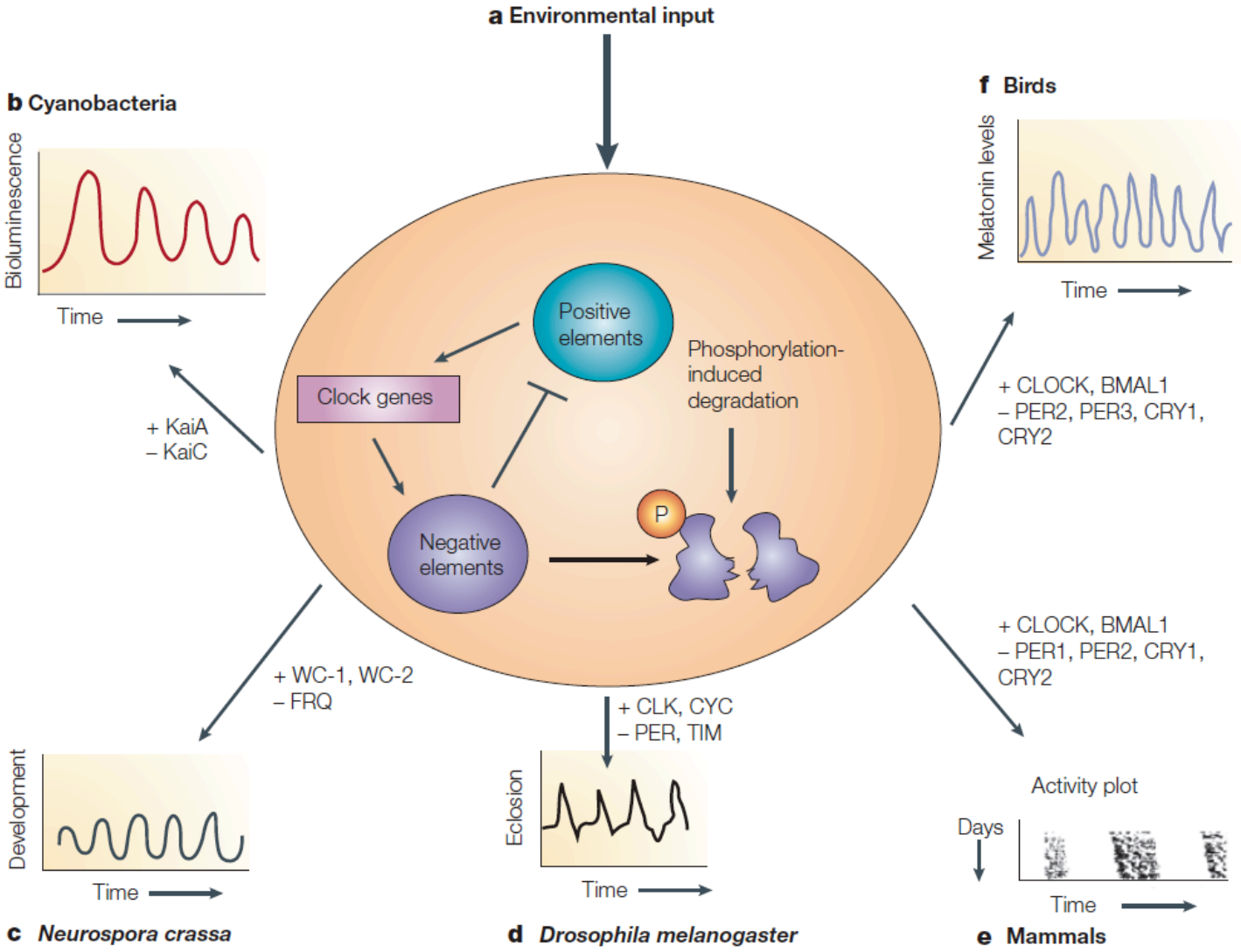
Mammalian circadian clock

Mammalian circadian clock



Bell-Pedersen et al. 2005

Mammalian circadian clock

Molecular mechanism

Interlocked feedback loops

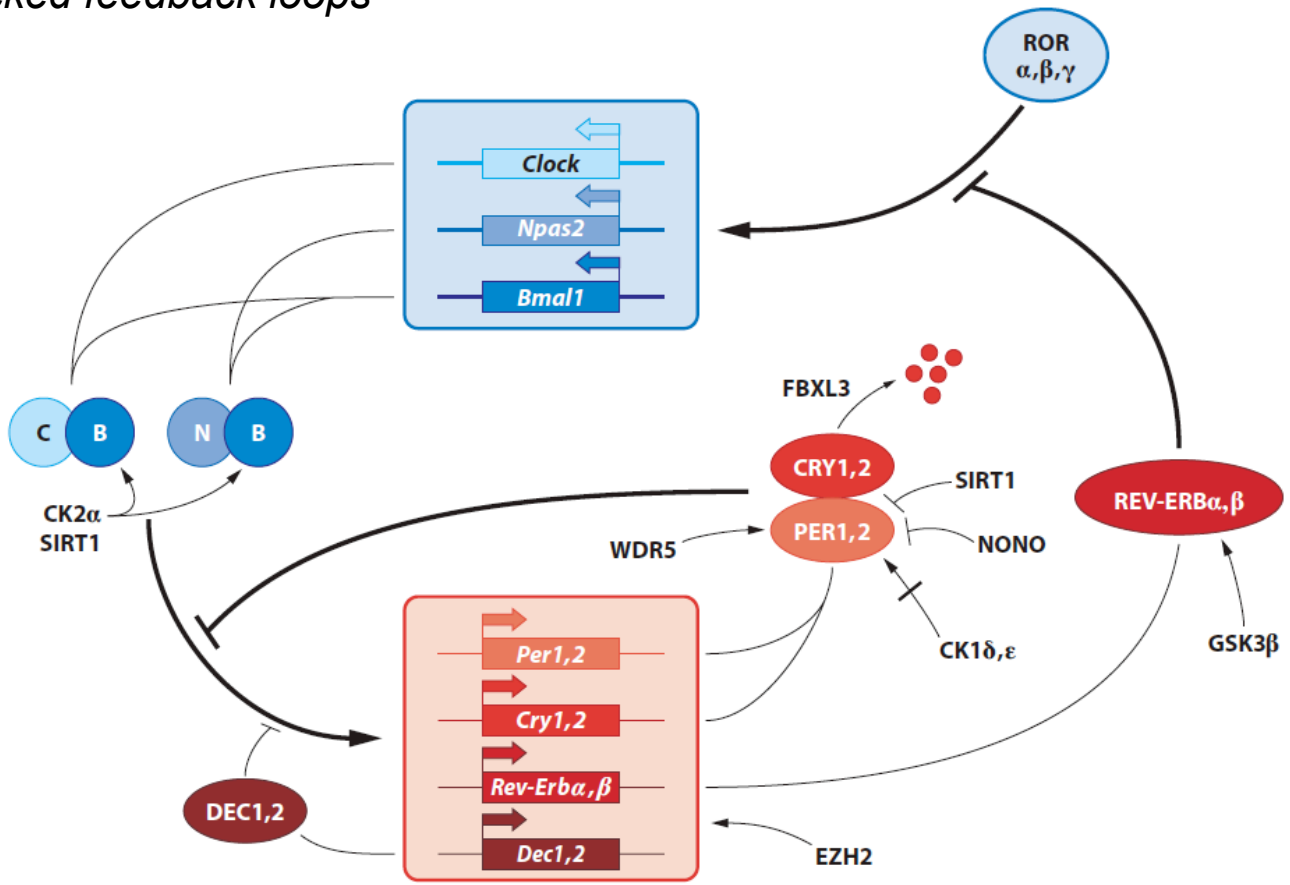
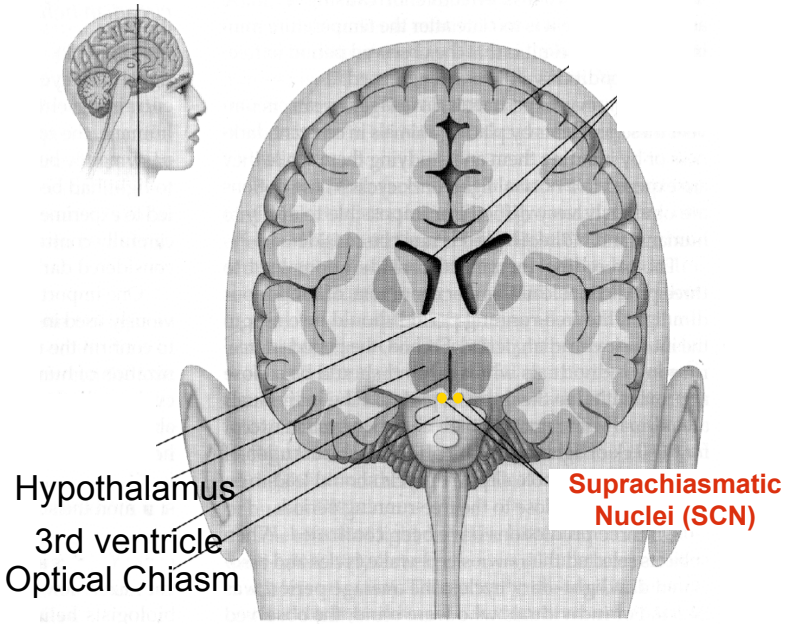


Figure from Lévi *et al* (2010) *Annu Rev Pharmacol Toxicol*. 50:377-421.

Mammalian circadian clock

Suprachiasmatic nucleus (SCN)



- SCN = circadian pacemaker
- Located in the hypothalamus
- Receives light signal from the retina
- Contains about 10000 neurons

Molecular mechanism

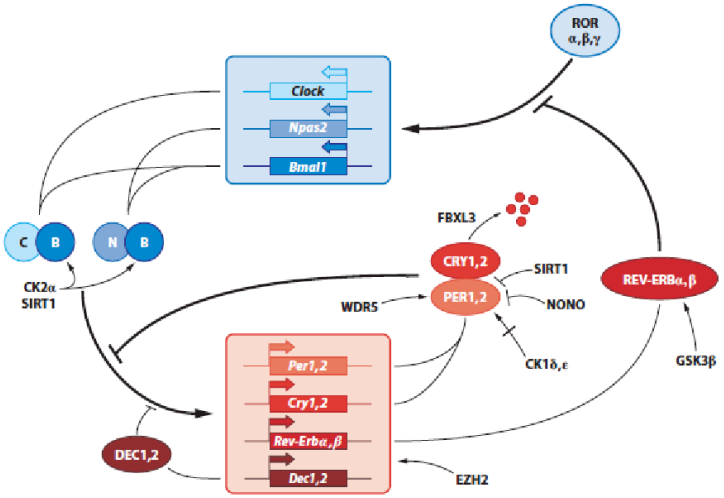


Figure from Lévi *et al* (2010) *Annu Rev Pharmacol Toxicol*. 50:377-421.

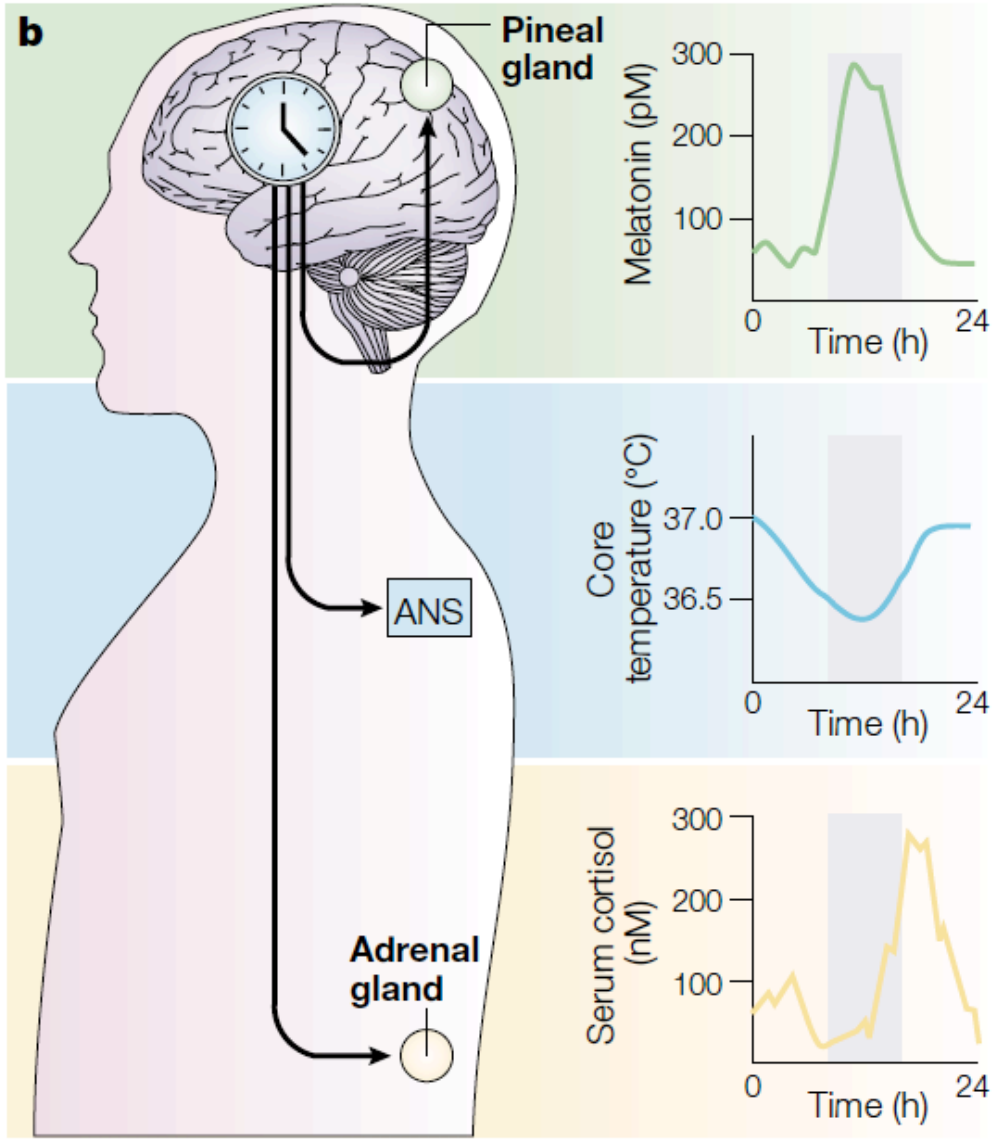
- Circadian oscillations are generated at the level of single SCN neuron
- Based on interlocked feedback loops
- Light activates *per/cry* expression
- Cells are coupled (and synchronized) by neurotransmitters

Mammalian circadian clock

View of the circadian organization in human:

The hypothalamic pacemaker (SCN) communicates through various neural and endocrine pathways to drive and/or synchronize rhythms in peripheral organs, thereby controlling physiological and behavioural outputs.

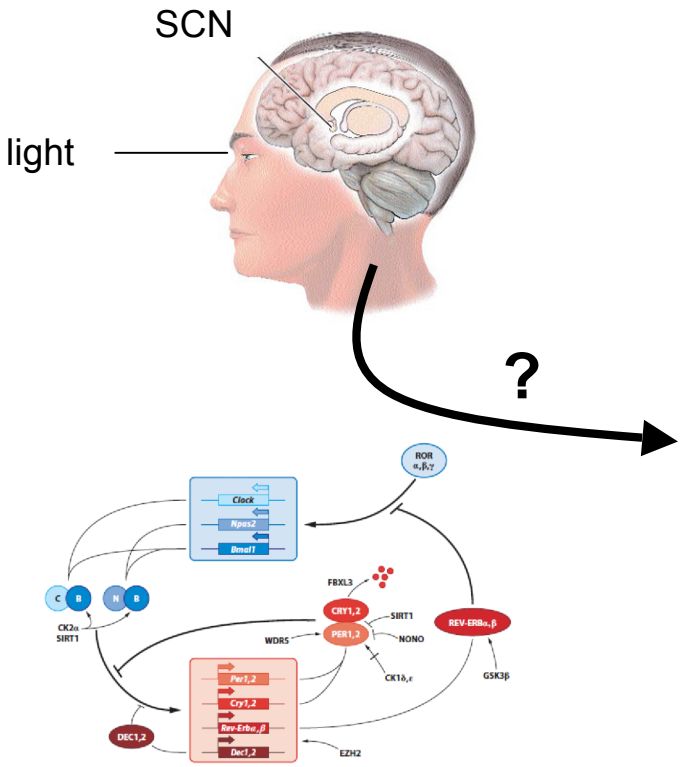
This ensures that as individuals progress through the regular 24h cycle of sleep/wakefulness their metabolism is adjusted accordingly to anticipate the demands and opportunities of the solar day.



Hastings (2003) *Nat Rev Neurosci 4*: 649-61.

Mammalian circadian clock

Central clock (pacemaker): SCN



Molecular mechanism of the circadian clock

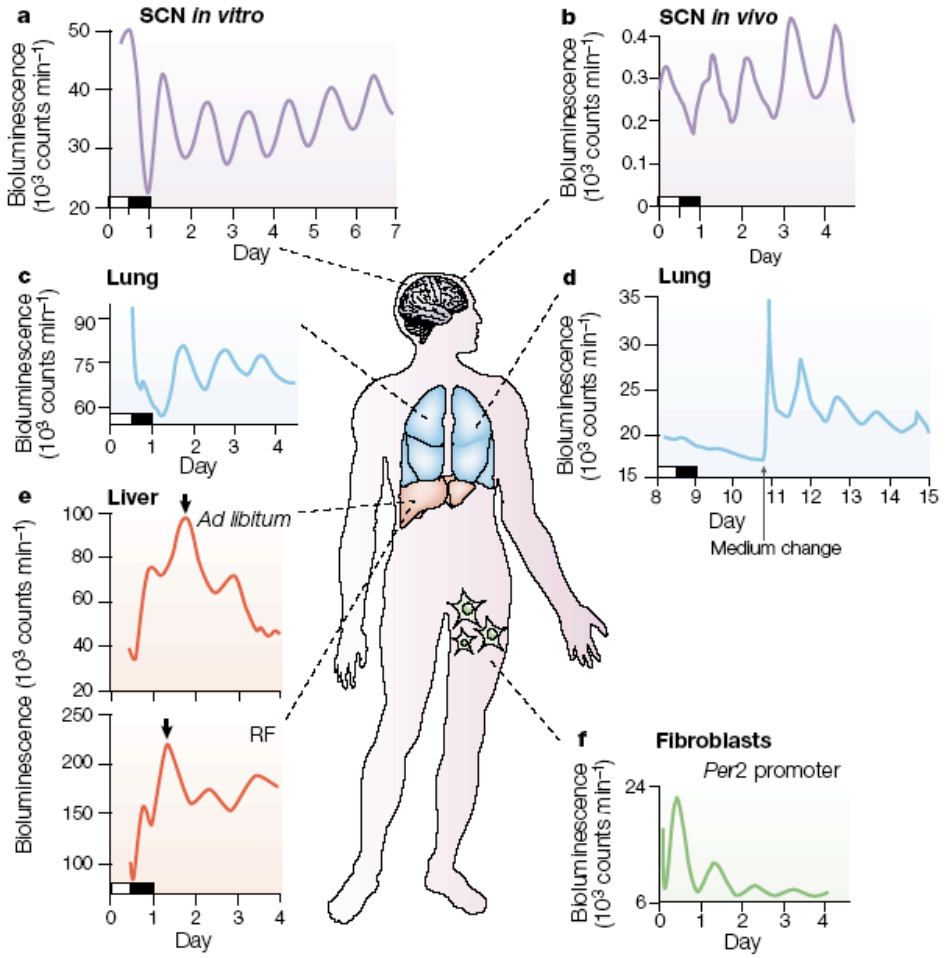
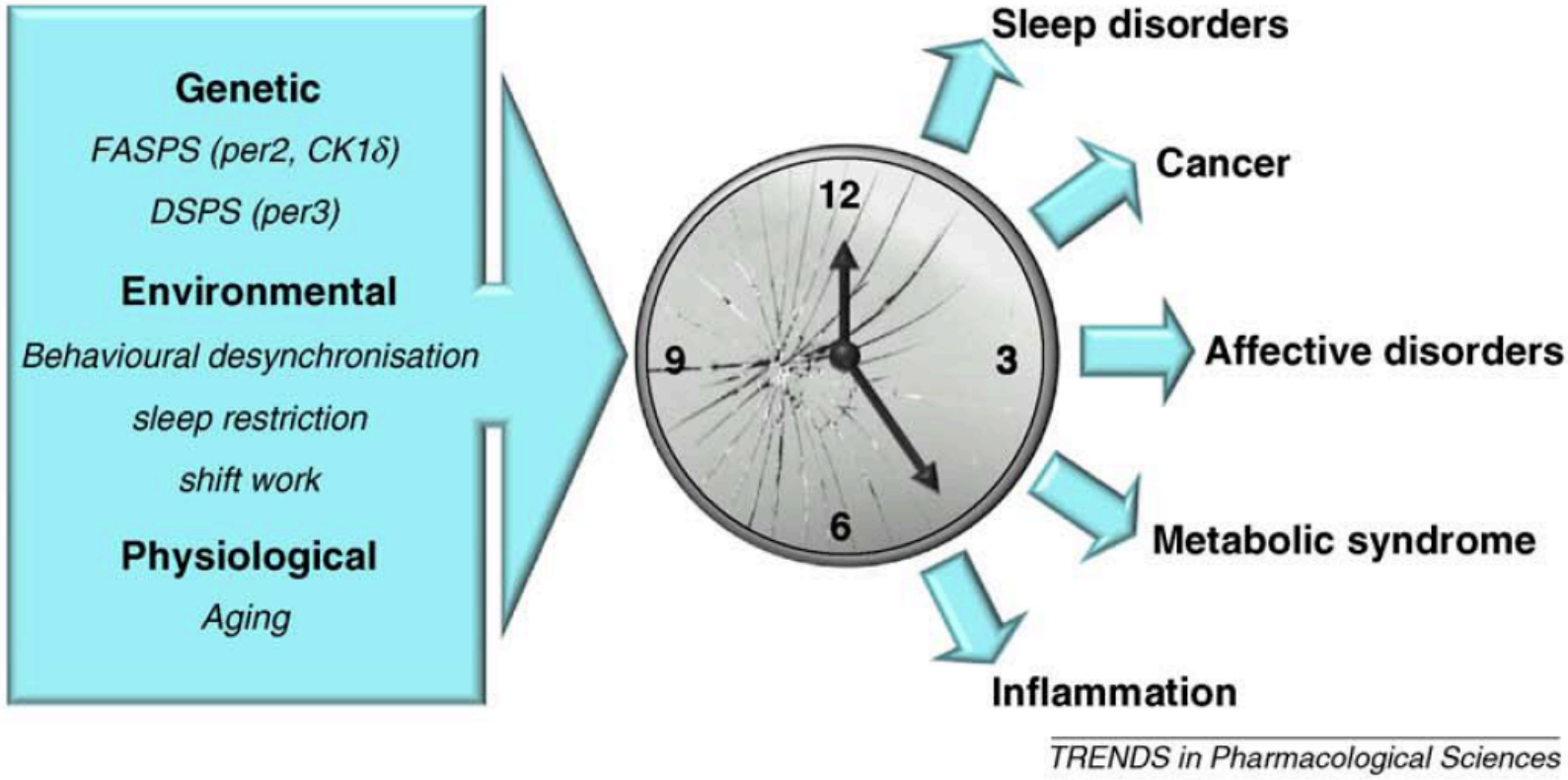


Figure 3 | ***Per::luciferase*** **transgenes reveal a diversity of tissue-based circadian oscillators.**

Hastings (2003) *Nat Rev Neurosci 4*: 649-61.

Mammalian circadian clock & diseases

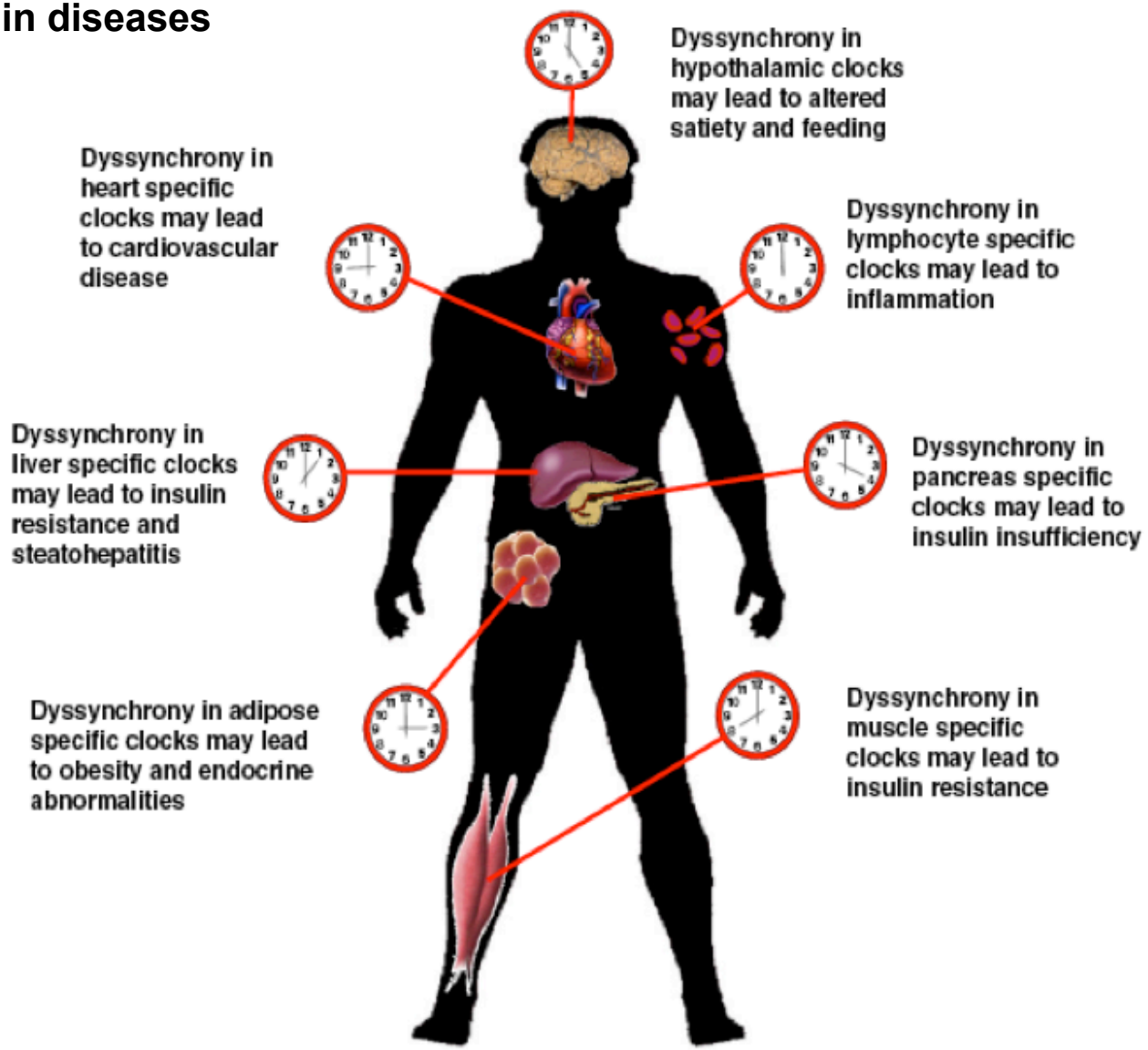
Circadian dysfunction in diseases



Bechtold, 2010

Mammalian circadian clock & diseases

Circadian dysfunction in diseases



Young, 2007

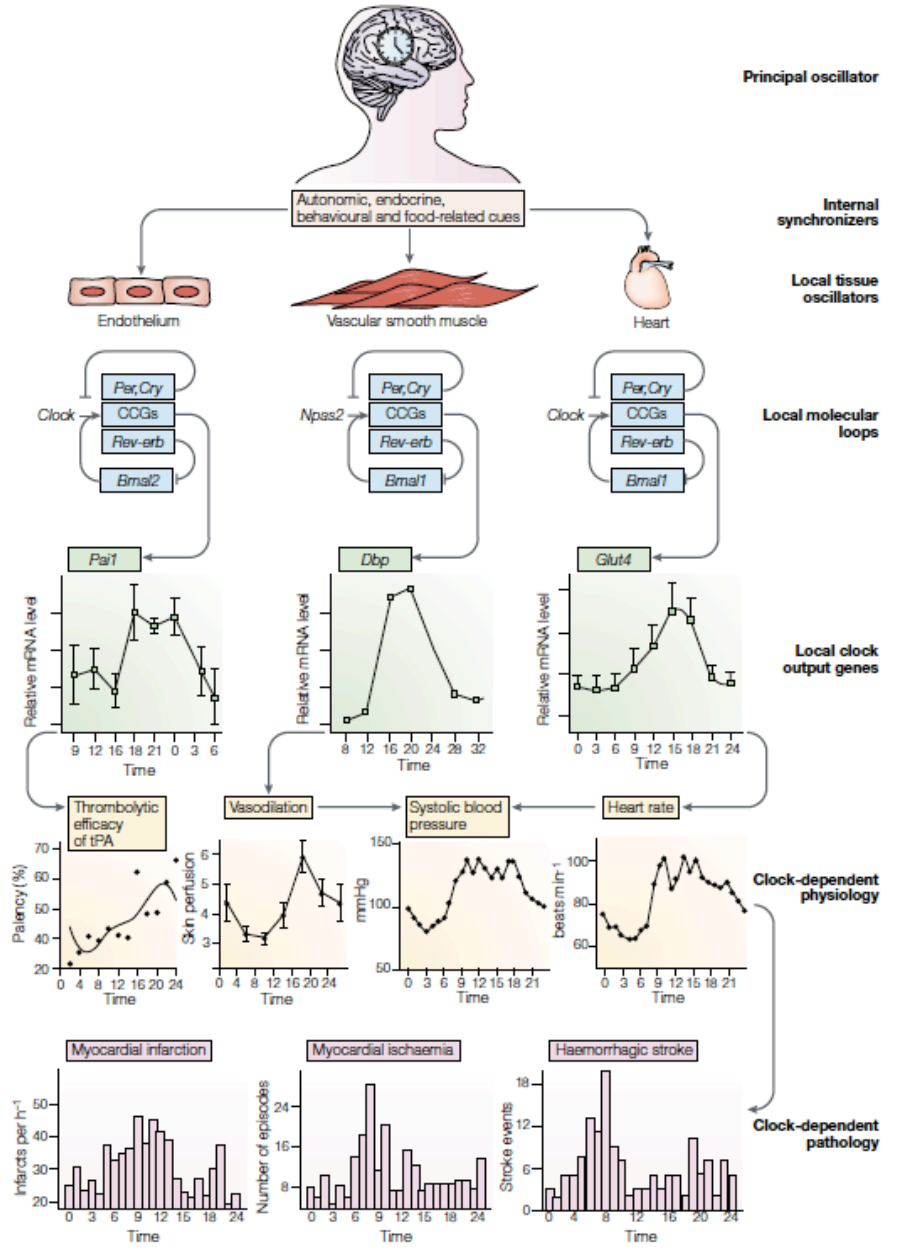
Mammalian circadian clock & diseases

Circadian clocks and cardiovascular diseases.

Local circadian oscillators in the vascular endothelium, smooth muscle and myocardium, based on clock feedback loops, are synchronized by signals from the SCN.

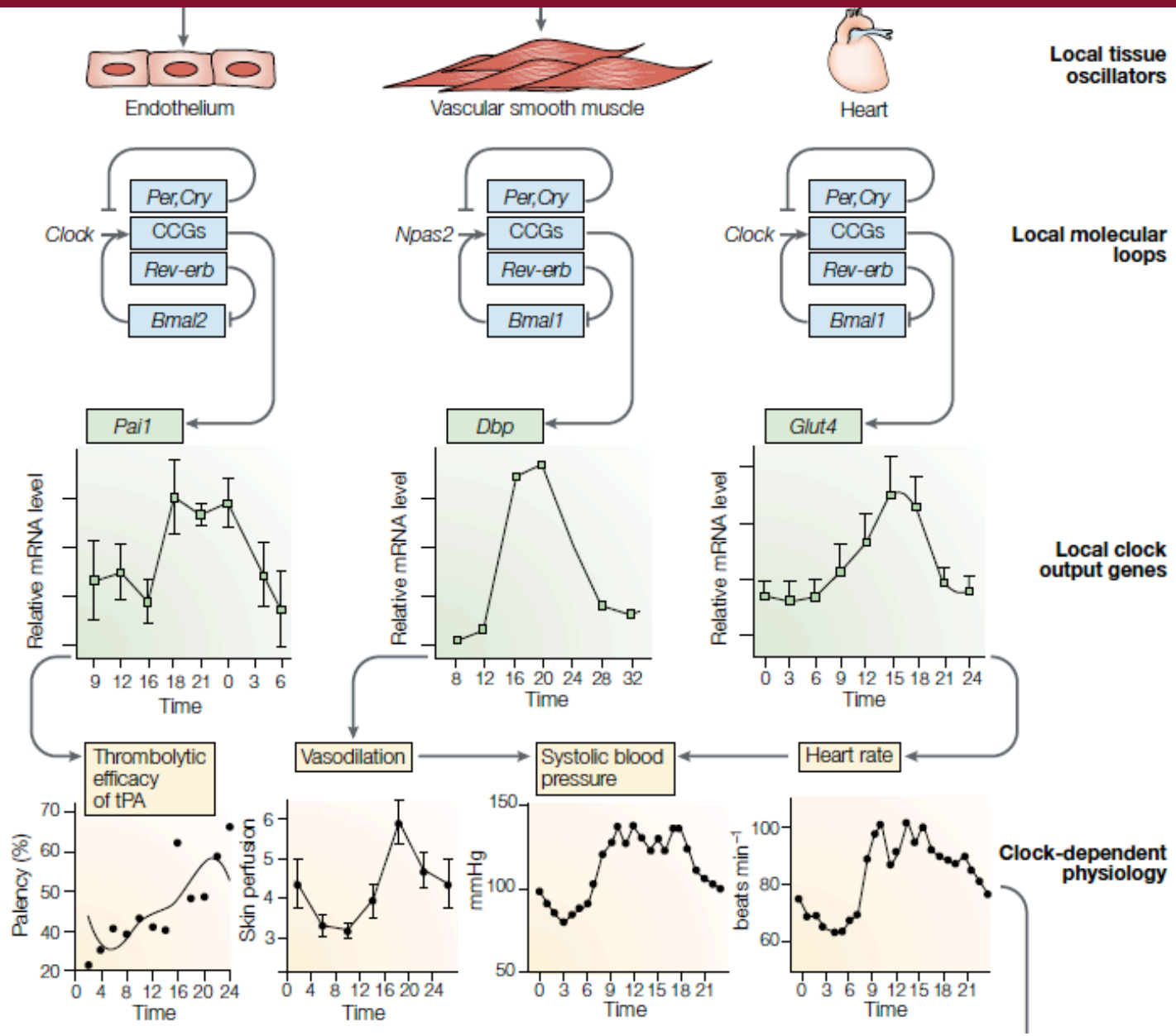
They drive tissues-specific circadian patterns of clock-controlled genes (CCG, i.e. *Pai1*, *Dbp*, *Glut4*).

In turn these molecular cycles sustain local clock-dependent physiologies, including circadian cycle in thrombolytic activity, vasodilatation, blood pressure and heart rate.

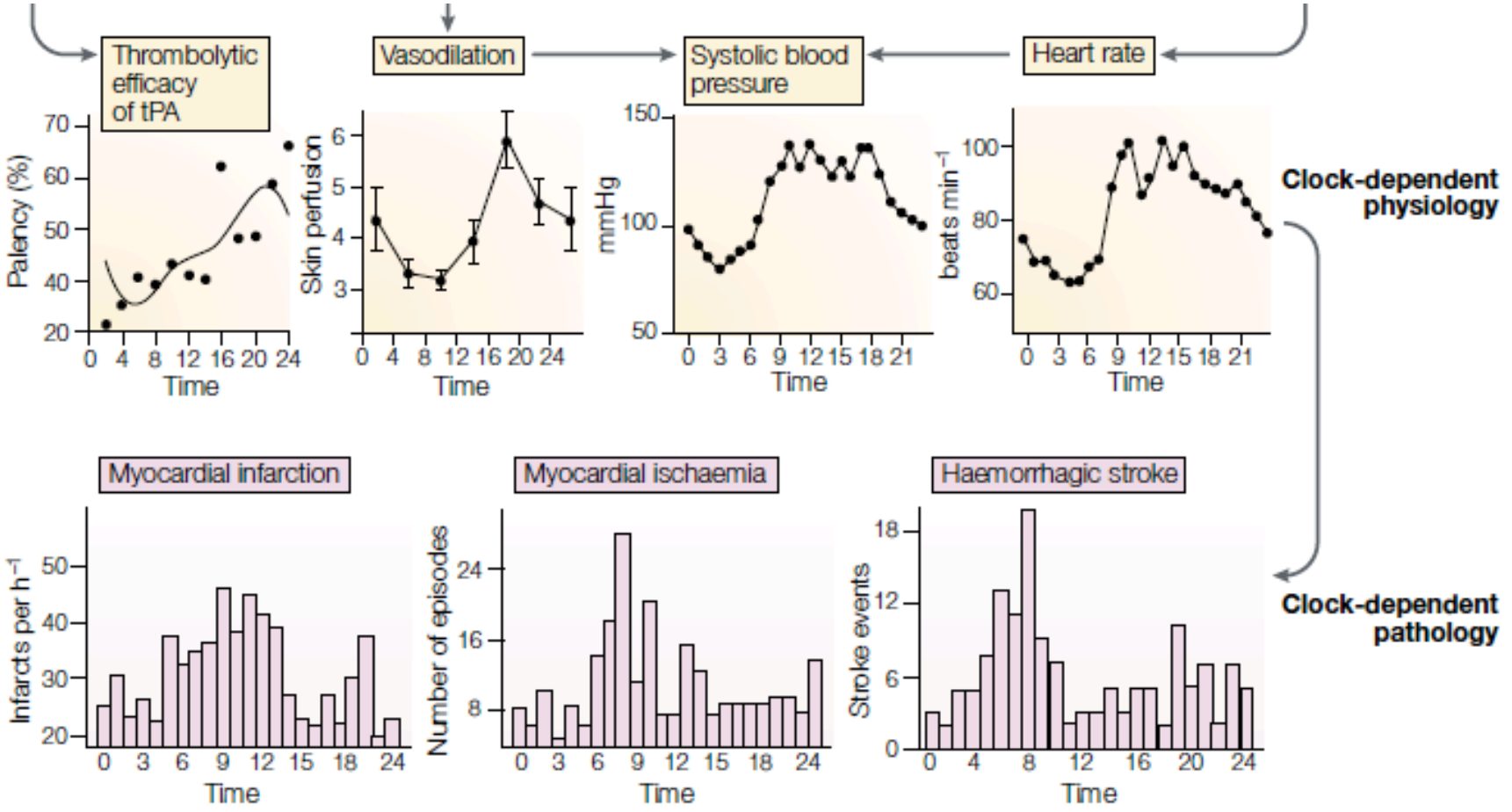


Hastings (2003) *Nat Rev Neurosci 4*: 649-61.

Mammalian circadian clock & diseases



Mammalian circadian clock & diseases



Clock-dependent cardiovascular diseases pathologies include ischaemia (lack of blood supply in tissues), and stroke. Those diseases occur preferentially at some time during the day. Understanding the origin of these diseases will help to develop new therapeutic interventions.

Mammalian circadian clock & diseases

Circadian rhythms and cancer

Clock perturbation (through genetic defects, chronic jet lags or shift work) has been shown to increase the risk of cancer, to accelerated tumor growth and to reduce survival time.

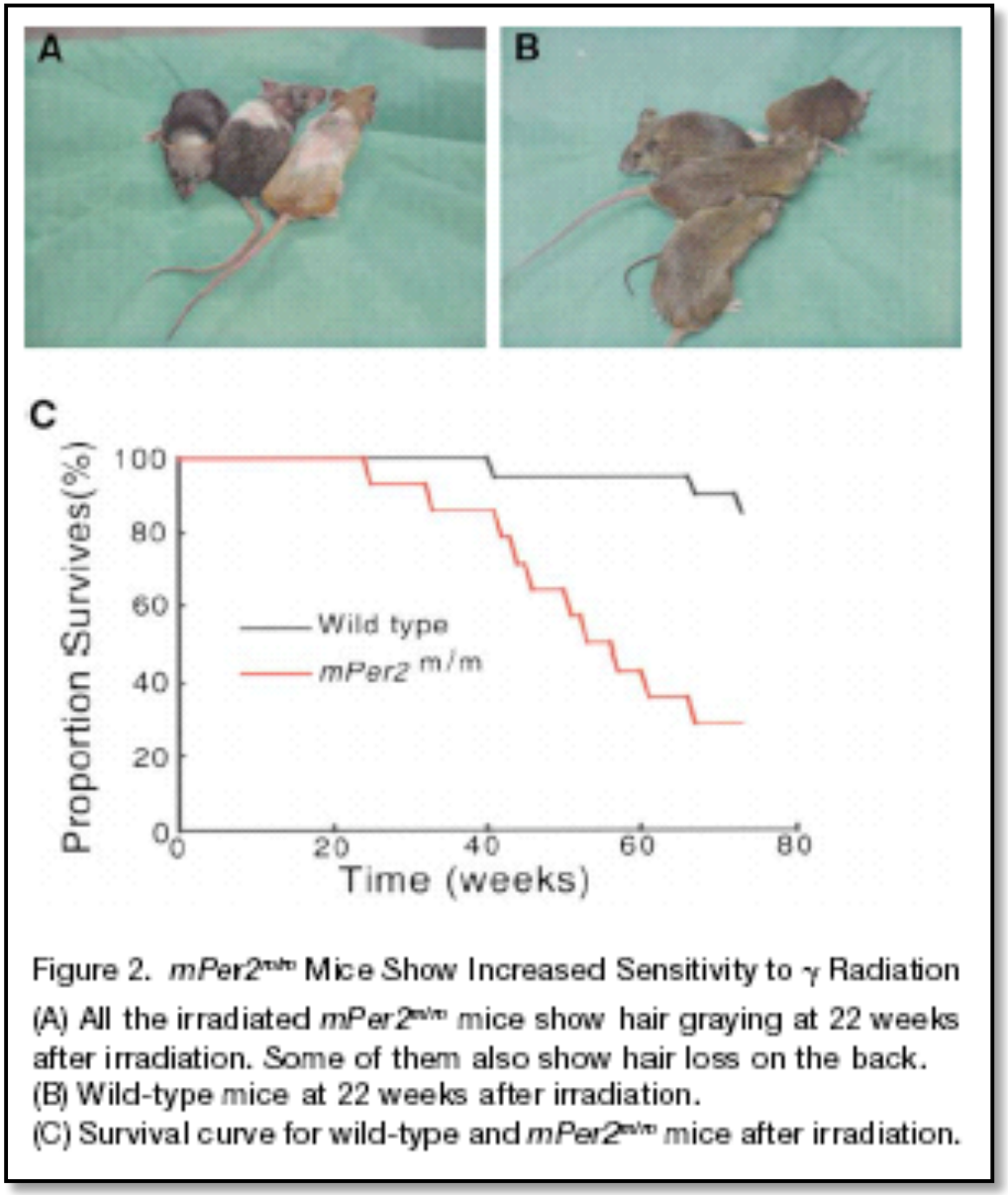


Figure 2. *mPer2*$^{m/m}$ Mice Show Increased Sensitivity to γ Radiation

(A) All the irradiated *mPer2*$^{m/m}$ mice show hair graying at 22 weeks after irradiation. Some of them also show hair loss on the back.

(B) Wild-type mice at 22 weeks after irradiation.

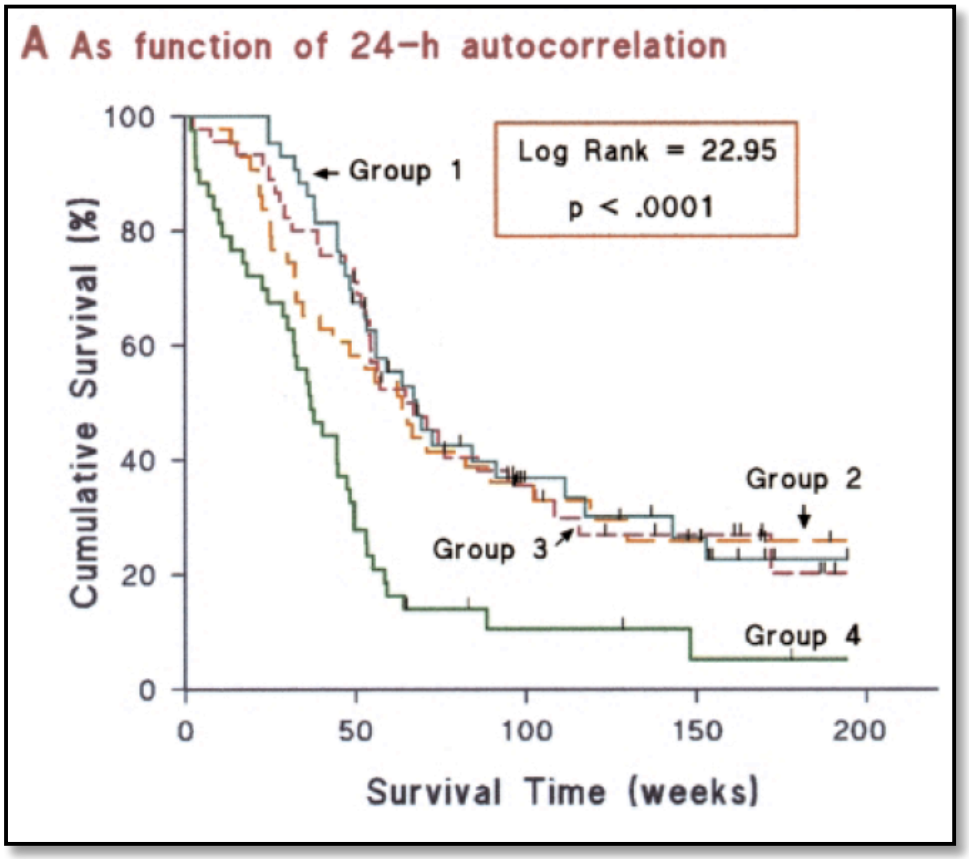
(C) Survival curve for wild-type and *mPer2*$^{m/m}$ mice after irradiation.

Fu et al. Cell 2002

Mammalian circadian clock & diseases

Circadian rhythms and cancer

The patients with poor circadian rhythmicity (i.e., with r24 [Fig. 5A] or I<O [Fig. 5B] in the lowest quartile had a five-fold higher risk of dying within 2 years than the patients with better circadian rhythmicity.



Groups: according to the auto-correlation of the circadian rhythmicity:

Group 1: >75% quartile (very good rhythmicity)
Group 2: 50%-75% quartile (good rhythmicity)
Group 3: 25%-50% quartile (bad rhythmicity)
Group 4: <25% quartile (very bad rhythmicity)

Mormont & Levi (2003) Cancer Chronotherapy: Principles, Applications, and Perspectives

Mammalian circadian clock & diseases

Circadian rhythms and cancer

Chronic jet lag

LD 12:12
Entrainment
(Circadian behaviour)

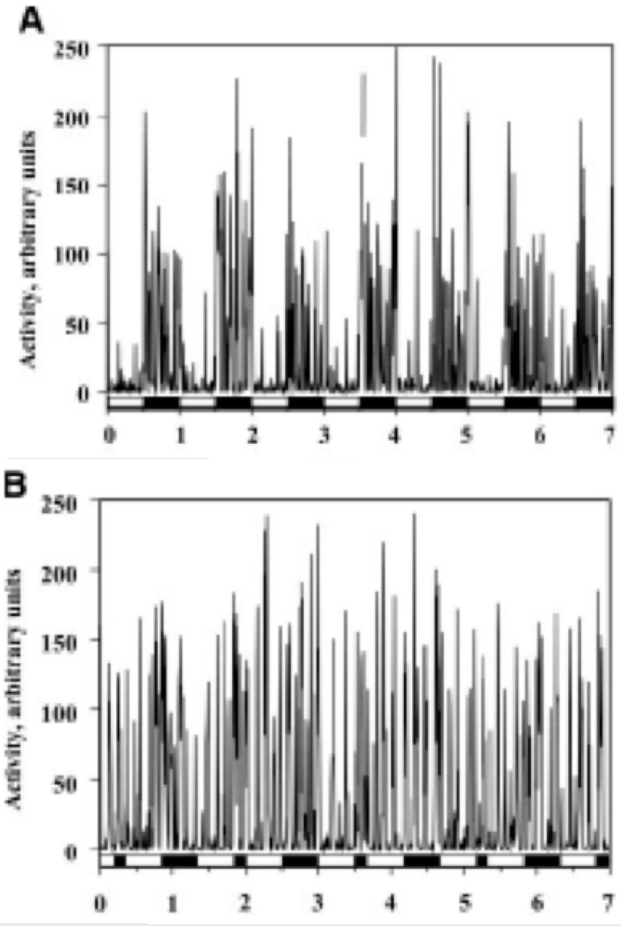
Chronic jet lag
No entrainment
(Irregular behaviour)

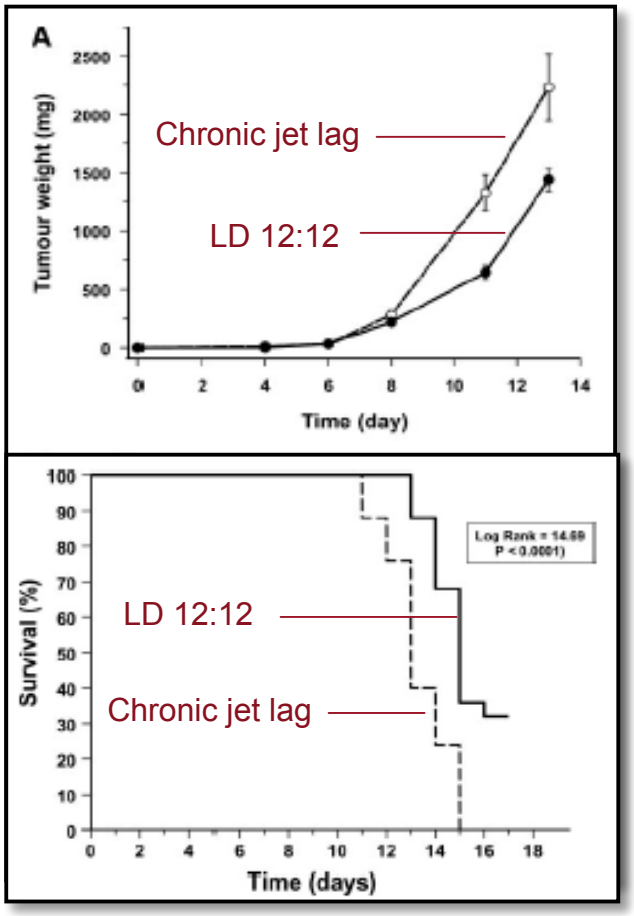
[CANCER RESEARCH 64, 7879–7885, November 1, 2004]

Effects of Chronic Jet Lag on Tumor Progression in Mice

Elisabeth Filipski,[1] Franck Delaunay,[2] Verdun M. King,[3] Ming-Wei Wu,[1] Bruno Claustrat,[4] Aline Gréchez-Cassiau,[2] Catherine Guettier,[5] Michael H. Hastings,[6] and Lévi Francis[1]

[1]INSERM E 0354 Cancer chronotherapeutics, Hôpital Paul Brousse, Villejuif Cedex, France; [2]CNRS UMR 6078 Université de Nice-Sophia Antipolis, Villefranche sur mer, France; [3]Department of Anatomy, University of Cambridge, Cambridge, United Kingdom; [4]Service de Radioanalyse, Hôpital Neurocardiologique, Lyon, France; [5]Laboratory of Anatomy and Pathologic Cytology, Hôpital Paul Brousse, Villejuif, France; and [6]Laboratory of Molecular Biology, MRC Centre, Cambridge, United Kingdom





Mammalian circadian clock & diseases

Circadian rhythms and cancer

Chronotherapy

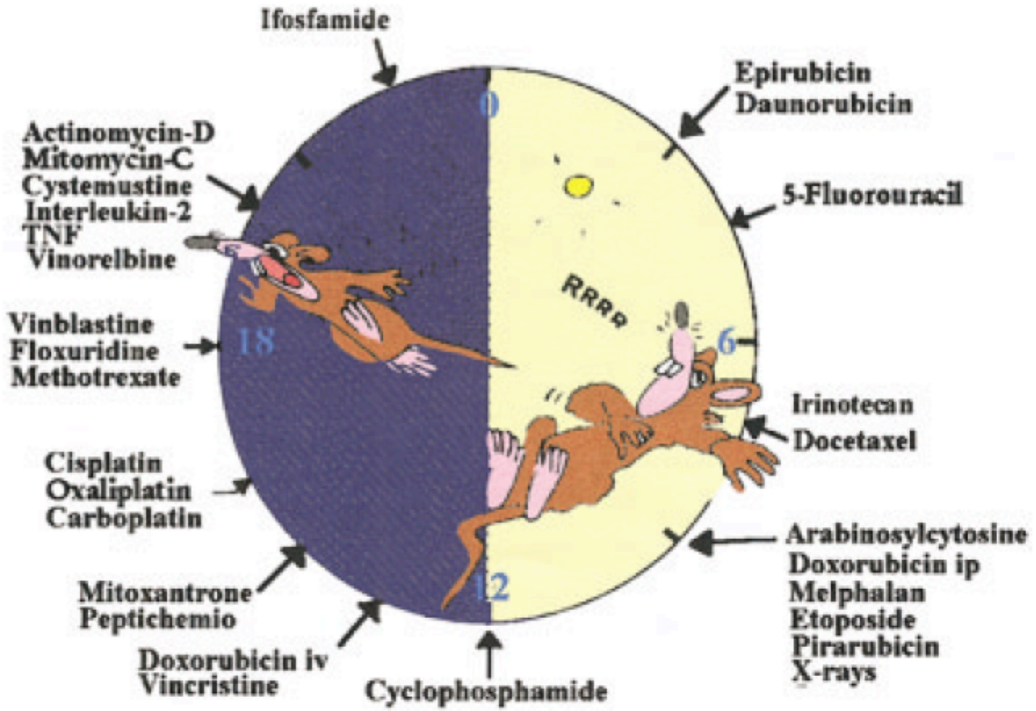


FIGURE 6. Circadian rhythms in anticancer drug tolerability in laboratory mice or rats. The least toxic dosing time is indicated for each cytostatic or immunologic agent as a function of the rest-activity cycle. iv: intravenous; TNF: tumor necrosis factor.

Mormont & Levi (2003) Cancer Chronotherapy: Principles, Applications, and Perspectives

Mammalian circadian clock

Towards a detailed molecular model of the mammalian circadian clock...

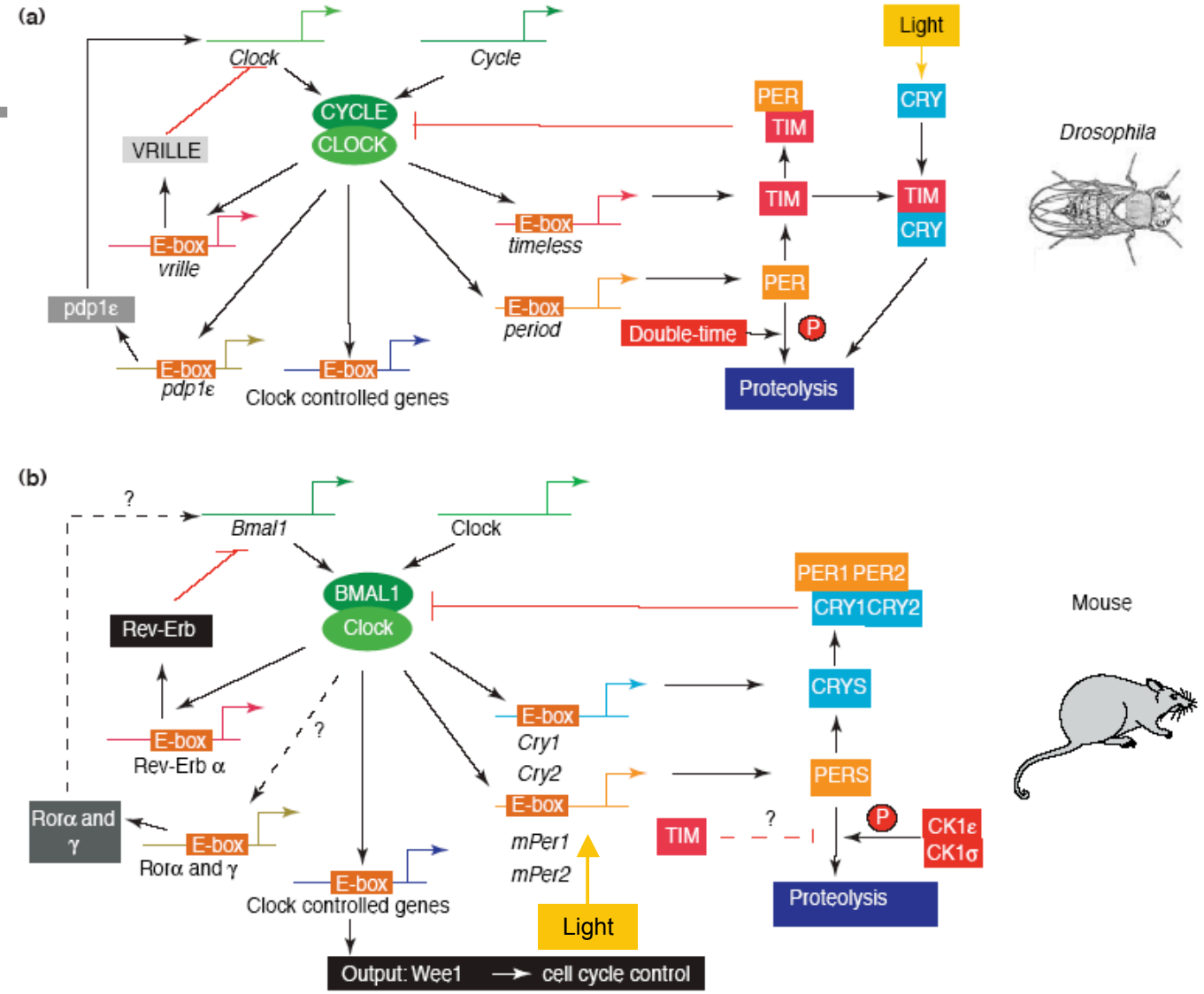
Mammalian circadian clock

Many similarities:

- homologous genes (per, cry, tim, clock)
- clock architecture (feedback loops)

Some differences:

- different genes (cyc -> bmal1, reverbα)
- different roles for some genes (tim, cry)
- different effect of light



Mammalian circadian clock

Circadian oscillation of BMAL1, a partner of a mammalian clock gene Clock, in rat suprachiasmatic nucleus.
Honma S, Ikeda M, Abe H, Tanahashi Y, Namihira M, Honma K, Nomura M. *Biochem Biophys Res Commun* (1998) 250:83-7.

Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain.
Takumi T, Taguchi K, Miyake S, Sakakida Y, Takashima N, Matsubara C, Maebayashi Y, Okumura K, Takekida S, Yamamoto S, Yagita K, Yan L, Young MW, Okamura H. *Neuron* (1998) 20:1103-10.

A new mammalian period gene predominantly expressed in the suprachiasmatic nucleus.
Takumi T, Matsubara C, Shigeyoshi Y, Taguchi K, Yagita K, Maebayashi Y, Sakakida Y, Okumura K, Takashima N, Okamura H. *Genes Cells* (1998) 3:167-76.

A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light.
Albrecht U, Sun ZS, Eichele G, Lee CC. *Cell* (1997) 91:1055-64.

Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript.
Shigeyoshi Y, Taguchi K, Yamamoto S, Takekida S, Yan L, Tei H, Moriya T, Shibata S, Loros JJ, Dunlap JC, Okamura H. *Cell* (1997) 91:1043-53.

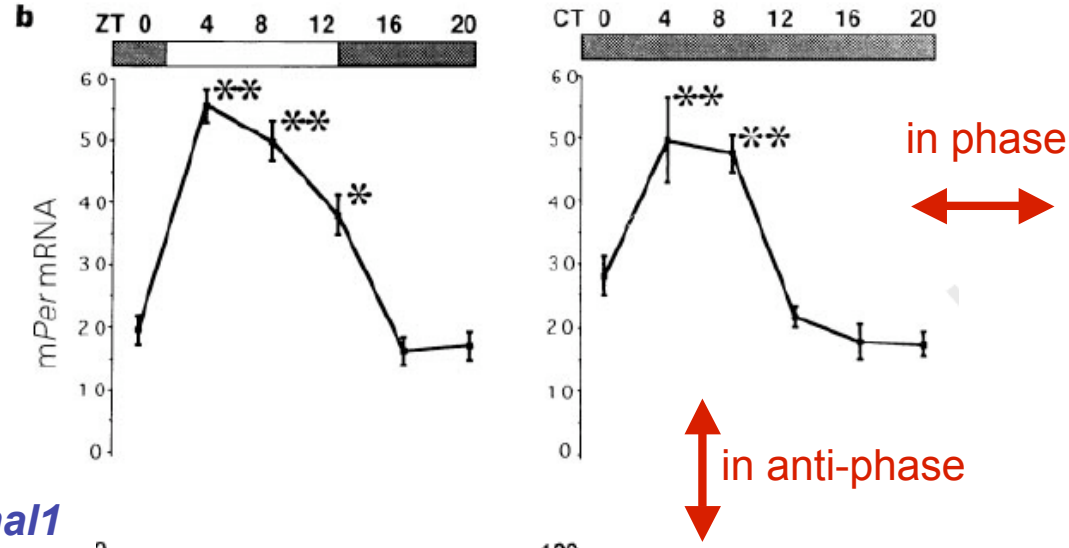
Mammalian circadian autoregulatory loop: a timeless ortholog and mPer1 interact and negatively regulate CLOCK-BMAL1-induced transcription.
Sangoram AM, Saez L, Antoch MP, Gekakis N, Staknis D, Whiteley A, Fruechte EM, Vitaterna MH, Shimomura K, King DP, Young MW, Weitz CJ, Takahashi JS. *Neuron* (1998) 21:1101-13.

Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses.
Thresher RJ, Vitaterna MH, Miyamoto Y, Kazantsev A, Hsu DS, Petit C, Selby CP, Dawut L, Smithies O, Takahashi JS, Sancar A. *Science* (1998) 282:1490-4

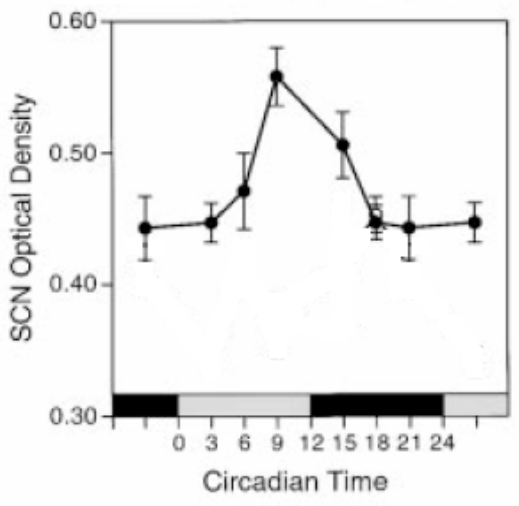
Mammalian circadian clock

Circadian oscillation of *mper1, cry1* and Bmal1 in mouse

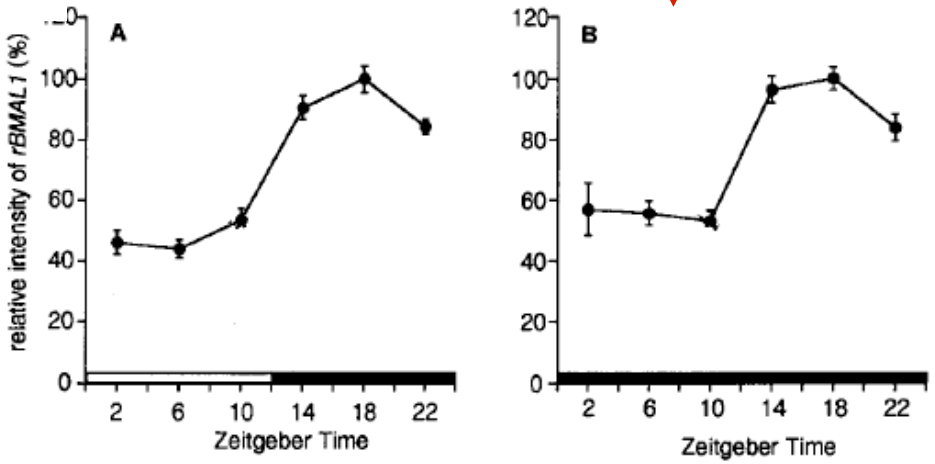
mper1



cry1

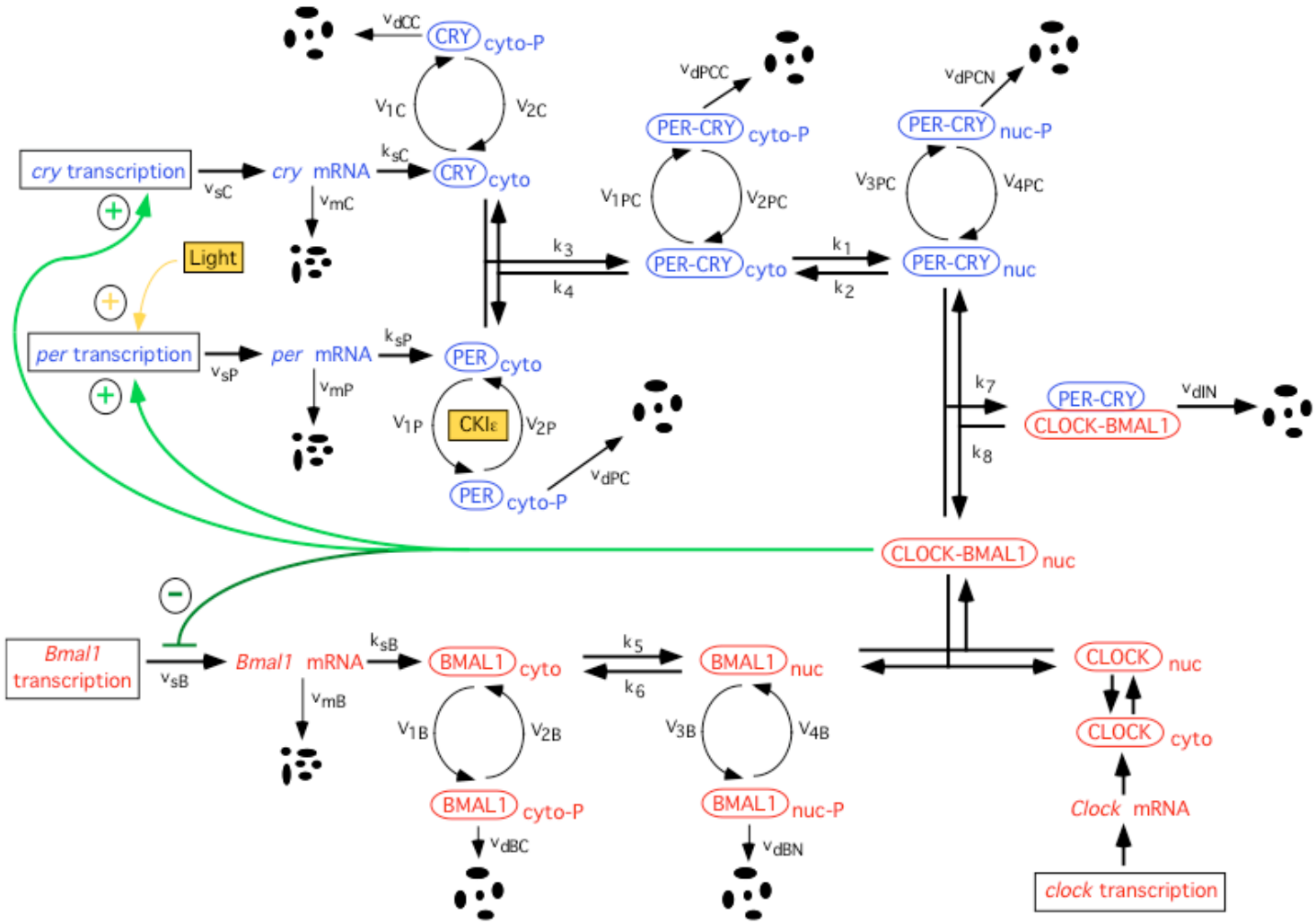


bmal1



- Tei *et al* (1997) Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. *Nature*. 389:512-6
- Honma *et al* (1998) Circadian oscillation of BMAL1, a partner of a mammalian clock gene Clock, in rat suprachiasmatic nucleus. Biochem Biophys Res Commun. 250:83-7.
- Kume *et al* (1999) mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98:193-205.

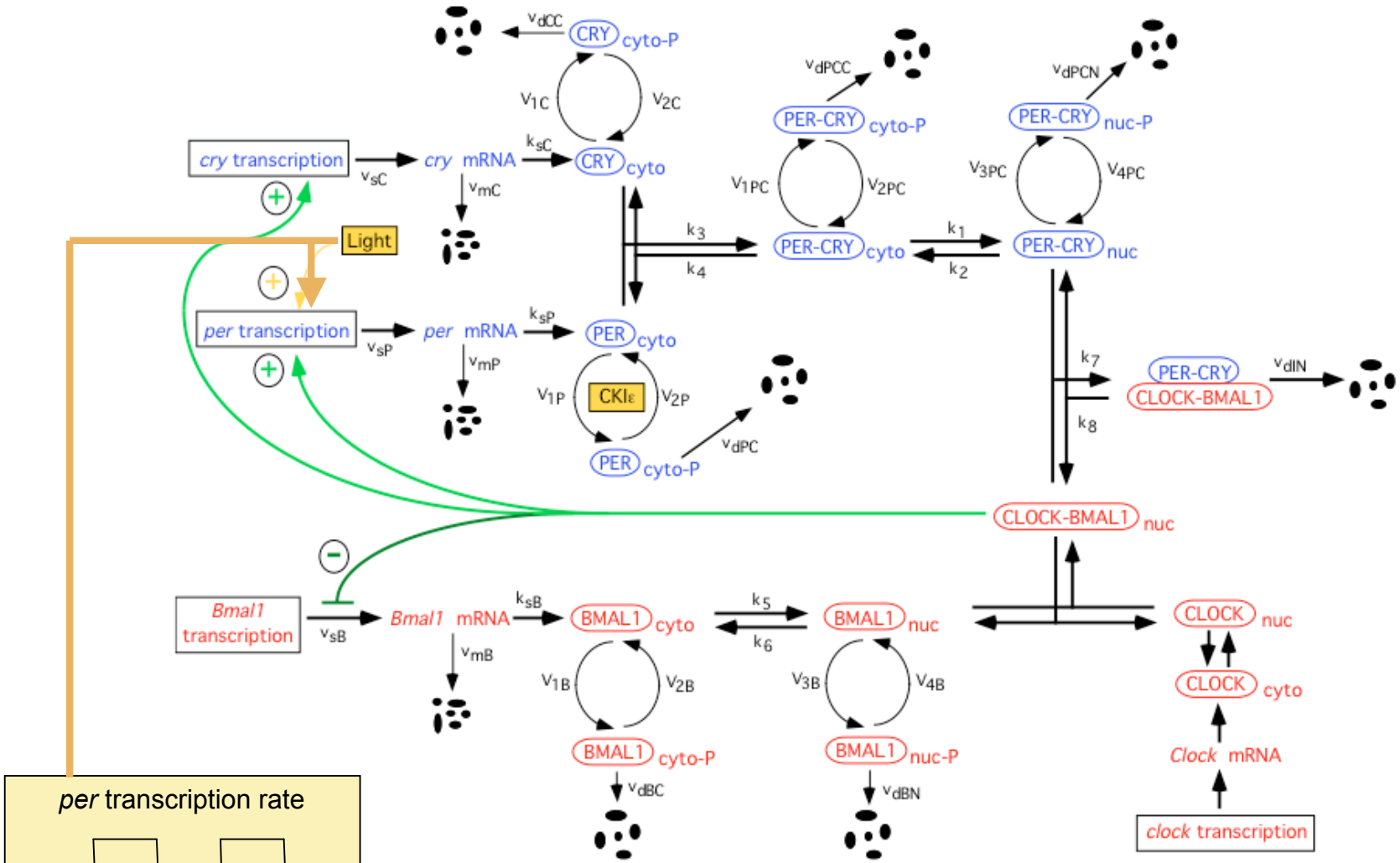
Mammalian circadian clock



16-variable model including *per, cry, bmal1, clock*
(18 if *rev-erbα* also included)

Leloup J-C & Goldbeter A (2003) Toward a detailed computational model for the mammalian circadian clock. *Proc Natl Acad Sci USA*. 100: 7051-7056.

Mammalian circadian clock



Light is modeled by an increase of the rate of *per* transcription

Mammalian circadian clock

mRNAs of *Per*, *Cry* and *Bmal1*:

$$\frac{dM_P}{dt} = v_{sP}\frac{B_N^{\,n}}{K_{AP}^{\,n} + B_N^{\,n}} - v_{mP}\frac{M_P}{K_{mP} + M_P} - k_{dn}M_P$$

$$\frac{dM_C}{dt} = v_{sC}\frac{B_N^{\,n}}{K_{AC}^{\,n} + B_N^{\,n}} - v_{mC}\frac{M_C}{K_{mC} + M_C} - k_{dn}M_C$$

$$\frac{dM_B}{dt} = v_{sB}\frac{K_{IB}^{\,n}}{K_{IB}^{\,n} + B_N^{\,n}} - v_{mB}\frac{M_B}{K_{mB} + M_B} - k_{dn}M_B$$

PER-CRY complexes :

$$\frac{dPC_C}{dt} = -V_{1PC}\frac{PC_C}{K_p + PC_C} + V_{2PC}\frac{PC_{CP}}{K_{dp} + PC_{CP}} - k_4PC_C + k_3P_CC_C + k_2PC_N - k_1PC_C - k_{dn}PC_C$$

$$\frac{dPC_N}{dt} = -V_{3PC}\frac{PC_N}{K_p + PC_N} + V_{4PC}\frac{PC_{NP}}{K_{dp} + PC_{NP}} - k_2PC_N + k_1PC_C - k_7B_NPC_N + k_8I_N - k_{dn}PC_N$$

$$\frac{dPC_{CP}}{dt} = V_{1PC}\frac{PC_C}{K_p + PC_C} - V_{2PC}\frac{PC_{CP}}{K_{dp} + PC_{CP}} - v_{dPCC}\frac{PC_{CP}}{K_d + PC_{CP}} - k_{dn}PC_{CP}$$

$$\frac{dPC_{NP}}{dt} = V_{3PC}\frac{PC_N}{K_p + PC_N} - V_{4PC}\frac{PC_{NP}}{K_{dp} + PC_{NP}} - v_{dPCN}\frac{PC_{NP}}{K_d + PC_{NP}} - k_{dn}PC_{NP}$$

PER and CRY proteins :

$$\frac{dP_C}{dt} = k_{sP}M_P - V_{1P}\frac{P_C}{K_p + P_C} + V_{2P}\frac{P_{CP}}{K_{dp} + P_{CP}} + k_4PC_C - k_3P_CC_C - k_{dn}P_C$$

$$\frac{dC_C}{dt} = k_{sC}M_C - V_{1C}\frac{C_C}{K_p + C_C} + V_{2C}\frac{C_{CP}}{K_{dp} + C_{CP}} + k_4PC_C - k_3P_CC_C - k_{dn}C_C$$

$$\frac{dP_{CP}}{dt} = V_{1P}\frac{P_C}{K_p + P_C} - V_{2P}\frac{P_{CP}}{K_{dp} + P_{CP}} - v_{dPC}\frac{P_{CP}}{K_d + P_{CP}} - k_{dn}P_{CP}$$

$$\frac{dC_{CP}}{dt} = V_{1C}\frac{C_C}{K_p + C_C} - V_{2C}\frac{C_{CP}}{K_{dp} + C_{CP}} - v_{dCC}\frac{C_{CP}}{K_d + C_{CP}} - k_{dn}C_{CP}$$

BMAL1 proteins:

$$\frac{dB_C}{dt} = k_{sB}M_B - V_{1B}\frac{B_C}{K_p + B_C} + V_{2B}\frac{B_{CP}}{K_{dp} + B_{CP}} - k_5B_C + k_6B_N - k_{dn}B_C$$

$$\frac{dB_{CP}}{dt} = V_{1B}\frac{B_C}{K_p + B_C} - V_{2B}\frac{B_{CP}}{K_{dp} + B_{CP}} - v_{dBC}\frac{B_{CP}}{K_d + B_{CP}} - k_{dn}B_{CP}$$

$$\frac{dB_N}{dt} = -V_{3B}\frac{B_N}{K_p + B_N} + V_{4B}\frac{B_{NP}}{K_{dp} + B_{NP}} + k_5B_C - k_6B_N - k_7B_NPC_N + k_8I_N - k_{dn}B_N$$

$$\frac{dB_{NP}}{dt} = V_{3B}\frac{B_N}{K_p + B_N} - V_{4B}\frac{B_{NP}}{K_{dp} + B_{NP}} - v_{dBN}\frac{B_{NP}}{K_d + B_{NP}} - k_{dn}B_{NP}$$

PER-CRY-CLOCK-BMAL1 Inactive complex :

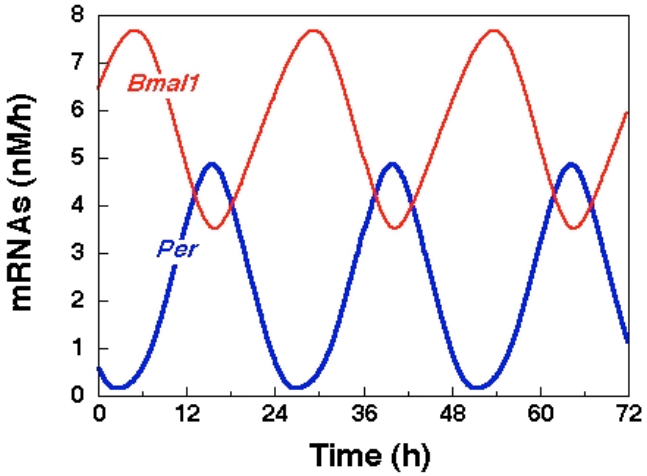
$$\frac{dI_N}{dt} = -k_8I_N + k_7B_NPC_N - v_{dIN}\frac{I_N}{K_d + I_N} - k_{dn}I_N$$

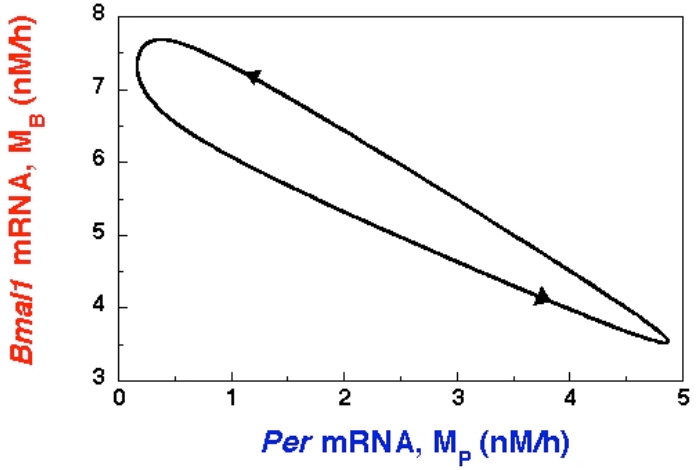
16-variable model

Leloup J-C & Goldbeter A (2003) *Proc Natl Acad Sci USA.* 100: 7051-7056.

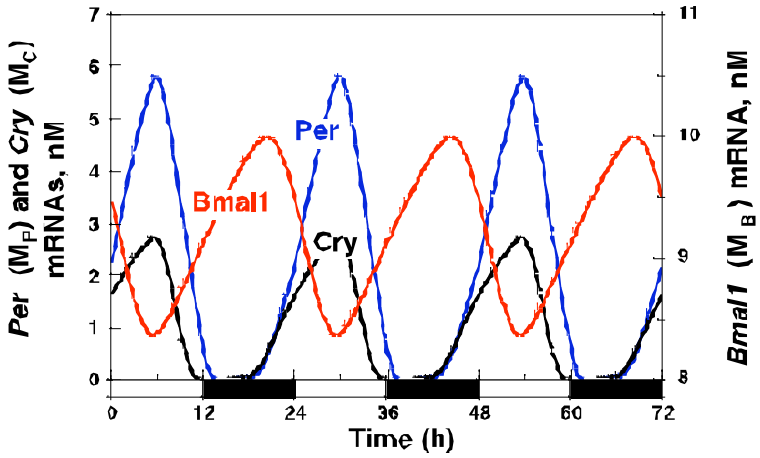
Mammalian circadian clock

Limit-cycle oscillations (in constant conditions, DD)

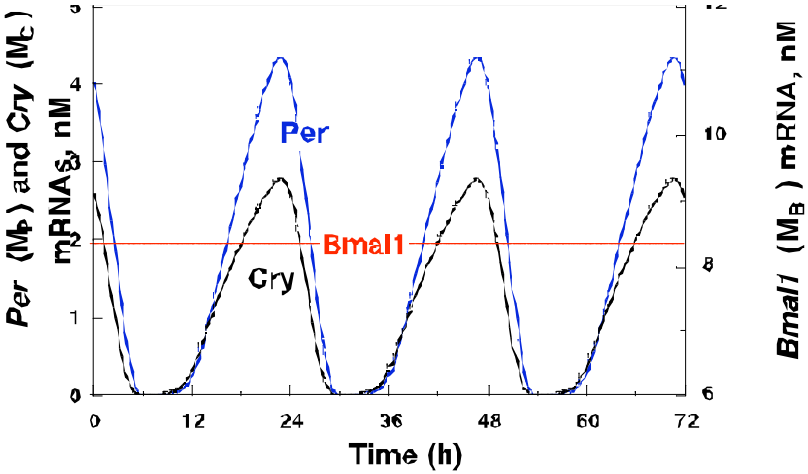




Oscillations in LD conditions



Oscillations of Per/Cry with Bmal1 mRNA maintained constant



Mammalian circadian clock

Physiological properties studied with the model:

Sleep phase disorders

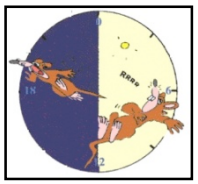
The model can be used to understand the links between the mutation of clock genes and their impact on the period and entrainment phase of the circadian oscillator.

Jet lag and chronic jet lag

The model can be used to predict the resynchronization time after jet lags (i.e. shift in the LD cycle). The effect of pre-jet-lag treatment (e.g. by the light) can also be simulated.

Shift work

The model can be used to assess the impact of various shift work timings on the circadian clocks.



Link with cell cycle and cancer (chronotherapy)

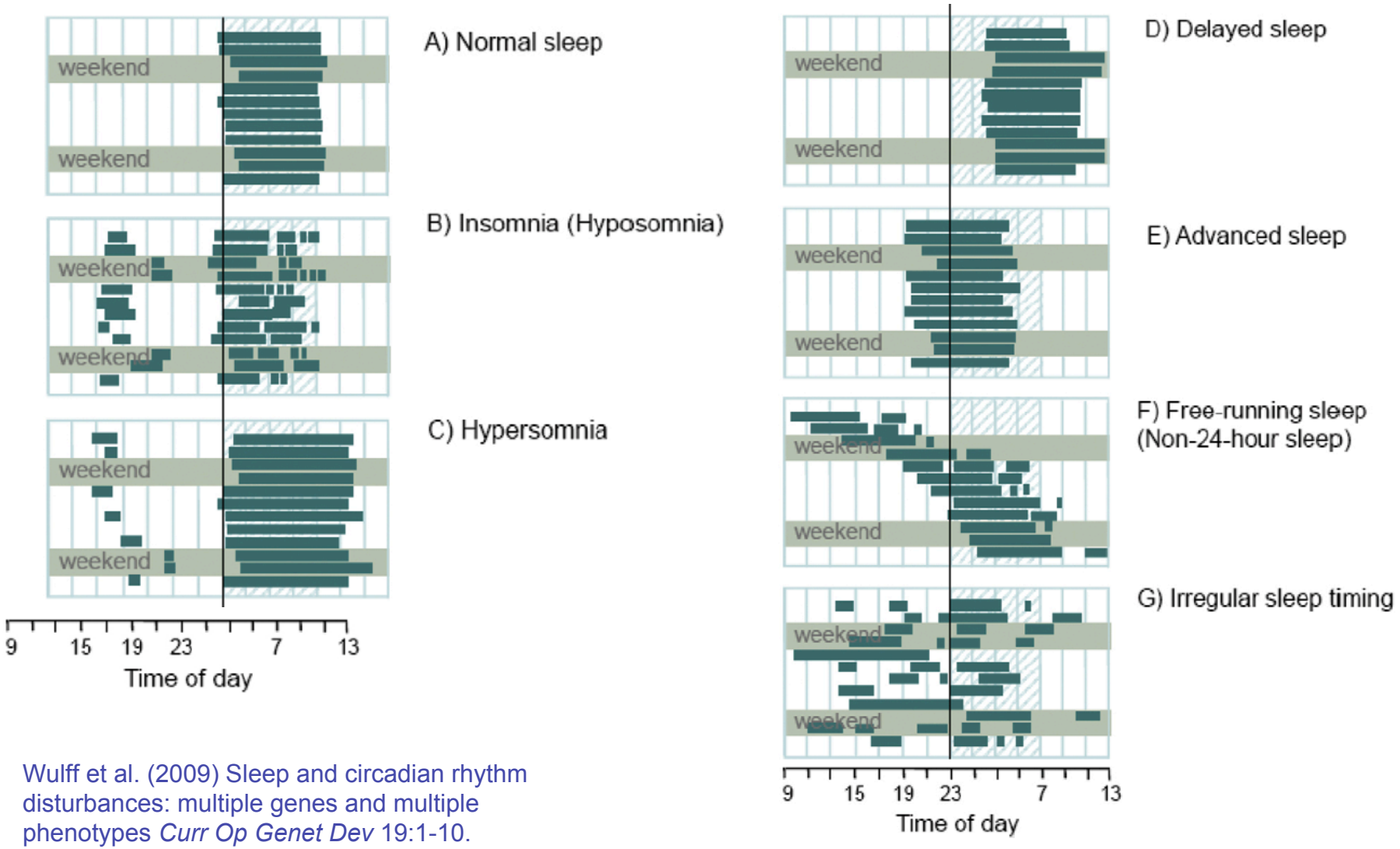
The model, in combination with a model for the cell cycle, can be used to assess the effect of anti-cancer drugs (and of their administration profiles) on the cell cycle.

Sleep phase disorders

Sleep-wake disorders

Sleep phase disorders

Different types of sleep phase syndromes



Wulff et al. (2009) Sleep and circadian rhythm disturbances: multiple genes and multiple phenotypes *Curr Op Genet Dev* 19:1-10.

Sleep phase disorders

An h*Per2* Phosphorylation Site Mutation in Familial Advanced Sleep Phase Syndrome

Kong L. Toh,[1*] Christopher R. Jones,[2,3*] Yan He,[4] Erik J. Eide,[5] William A. Hinz,[5] David M. Virshup,[5,6] Louis J. Ptáček[2,7†] Ying-Hui Fu[4]

Familial advanced sleep phase syndrome (FASPS) is an autosomal dominant circadian rhythm variant; affected individuals are "morning larks" with a 4-hour advance of the sleep, temperature, and melatonin rhythms. Here we report localization of the FASPS gene near the telomere of chromosome 2q. A strong candidate gene (h*Per2*), a human homolog of the *period* gene in *Drosophila*, maps to the same locus. Affected individuals have a serine to glycine mutation within the casein kinase Iε (CKIε) binding region of hPER2, which causes hypophosphorylation by CKIε in vitro. Thus, a variant in human sleep behavior can be attributed to a missense mutation in a clock component, hPER2, which alters the circadian period.

FASPS <-> Mutation in PER2 (in the CKIε binding region) <-> hypophosphorylation

Toh KL *et al.* (2001) *Science* 291:1040-3.

Sleep phase disorders

An *hPer2* phosphorylation site mutation in familial advanced sleep-phase syndrome (FASPS).

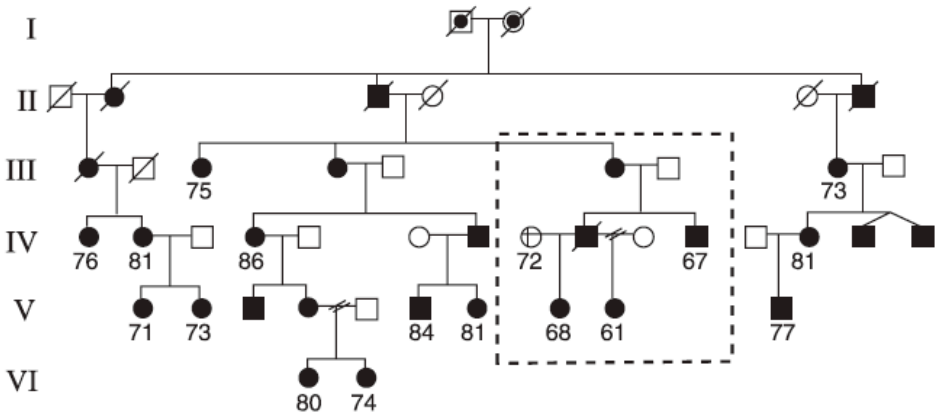
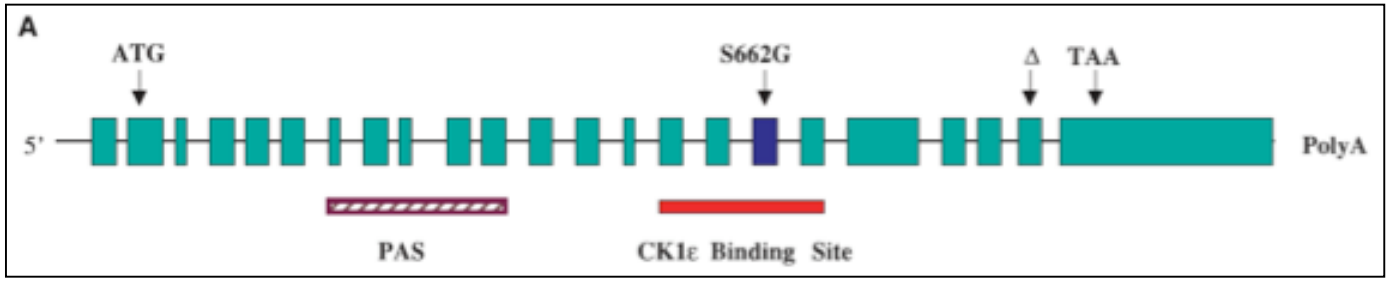


Fig. 1. ASPS kindred 2174. Horne-Östberg scores are shown below individuals. The dotted line marks a branch (branch 3) where the ASPS phenotype does not cosegregate with the mutation. Circles, women; squares, men; filled circles and squares, affected individuals; empty circles and squares, unaffected individuals. Unknown individuals (not meeting strict criteria for being "affected" or "unaffected") were eliminated from this pedigree for the sake of simplicity.



- The FASPS mutation (S662G), occurs in exon 17 of the Per2 gene (which has 23 exons, shown in green).
- This site is located in the phosphorylation site of the protein (red bar).
- The mutation S662G results in the hypophosphorylation of the protein.

Sleep phase disorders

Functional consequences of a *CKIδ* mutation causing familial advanced sleep phase syndrome

**Ying Xu[1]*, Quasar S. Padiath[1]*, Robert E. Shapiro[2], Christopher R. Jone
Susan C. Wu[1], Noriko Saigoh[1], Kazumasa Saigoh[1]†, Louis J. Ptáček[1]
& Ying-Hui Fu[1]**

Familial advanced sleep phase syndrome (FASPS) is a human behavioural phenotype characterized by early sleep times and early-morning awakening[1]. It was the first human, mendelian circadian rhythm variant to be well-characterized, and was shown to result from a mutation in a phosphorylation site within the casein kinase I (CKI)-binding domain of the human *PER2* gene. To gain a deeper understanding of the mechanisms of circadian rhythm regulation in humans, we set out to identify mutations in human subjects leading to FASPS. We report here the identification of a missense mutation (T44A) in the human *CKIδ* gene, which results in FASPS. This mutant kinase has decreased enzymatic activity *in vitro*. Transgenic *Drosophila* carrying the human *CKIδ-T44A* gene showed a phenotype with lengthened circadian period. In contrast, transgenic mice carrying the same mutation have a shorter circadian period, a phenotype mimicking human FASPS. These results show that CKIδ is a central component in the mammalian clock, and suggest that mammalian and fly clocks might have different regulatory mechanisms despite the highly conserved nature of their individual components.

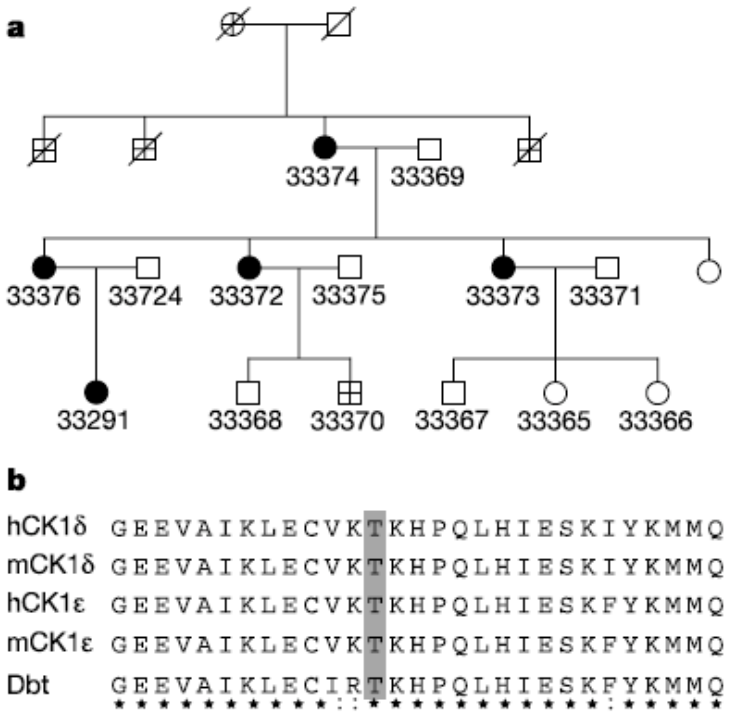


Figure 1 CKIδ-T44A FASPS pedigree and the amino acid alignment around the mutation. **a**, FASPS kindred 5231. Circles represent women, squares denote men, filled circles and squares show affected individuals; empty circles and squares show unaffected individuals. The individual marked with a cross is 'probably affected' but was conservatively classified as unknown. Diagonal lines across symbols indicate deceased individuals. **b**, Alignments for *Drosophila* Dbt and mouse (m) and human (h) CKIδ and CKIε proteins. The T44A mutation is highlighted.

NATURE | VOL 434 | 31 MARCH 2005 | www.nature.com/nature

Mutation in CKIδ <-> FASPS & period decrease (in mice/human)

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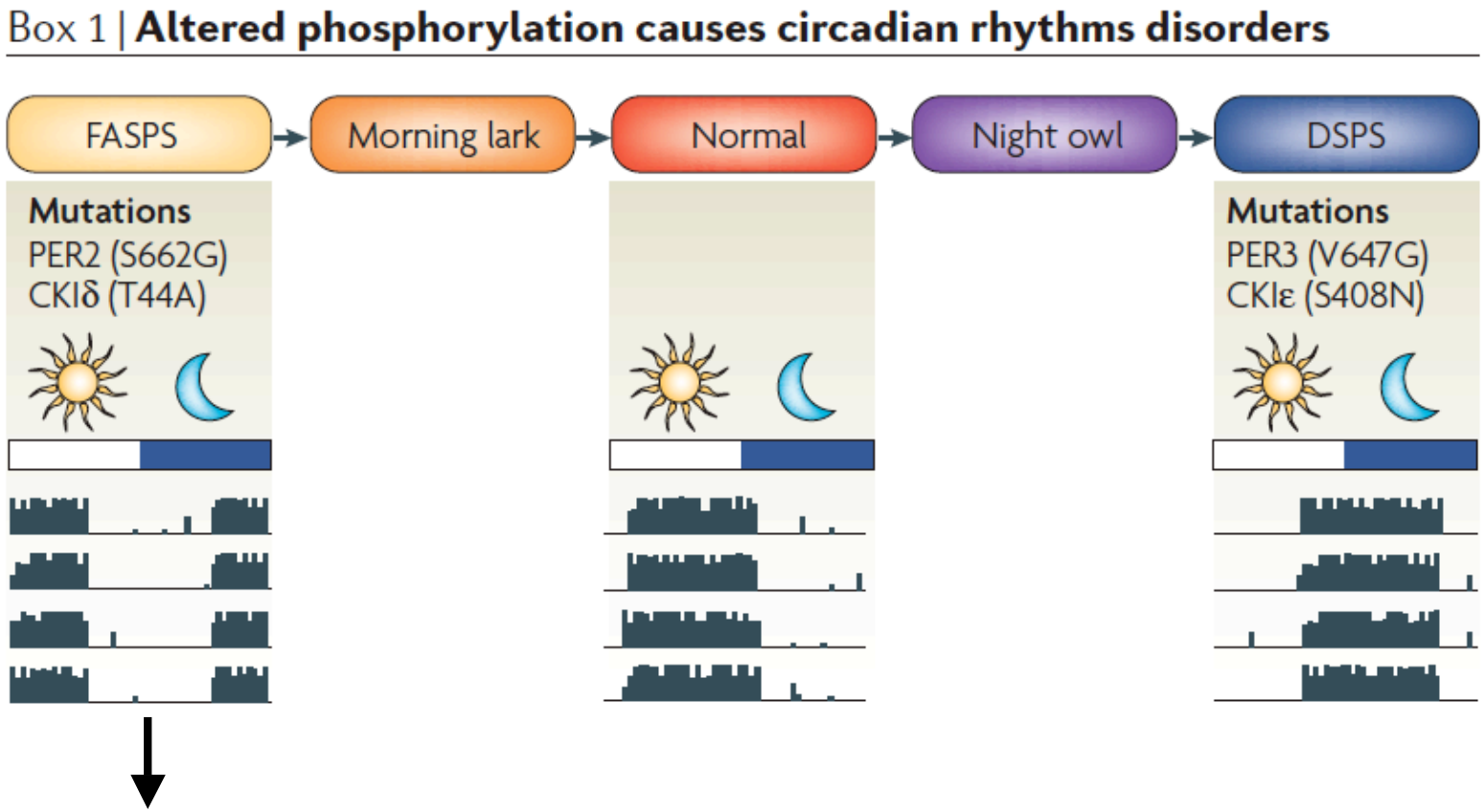
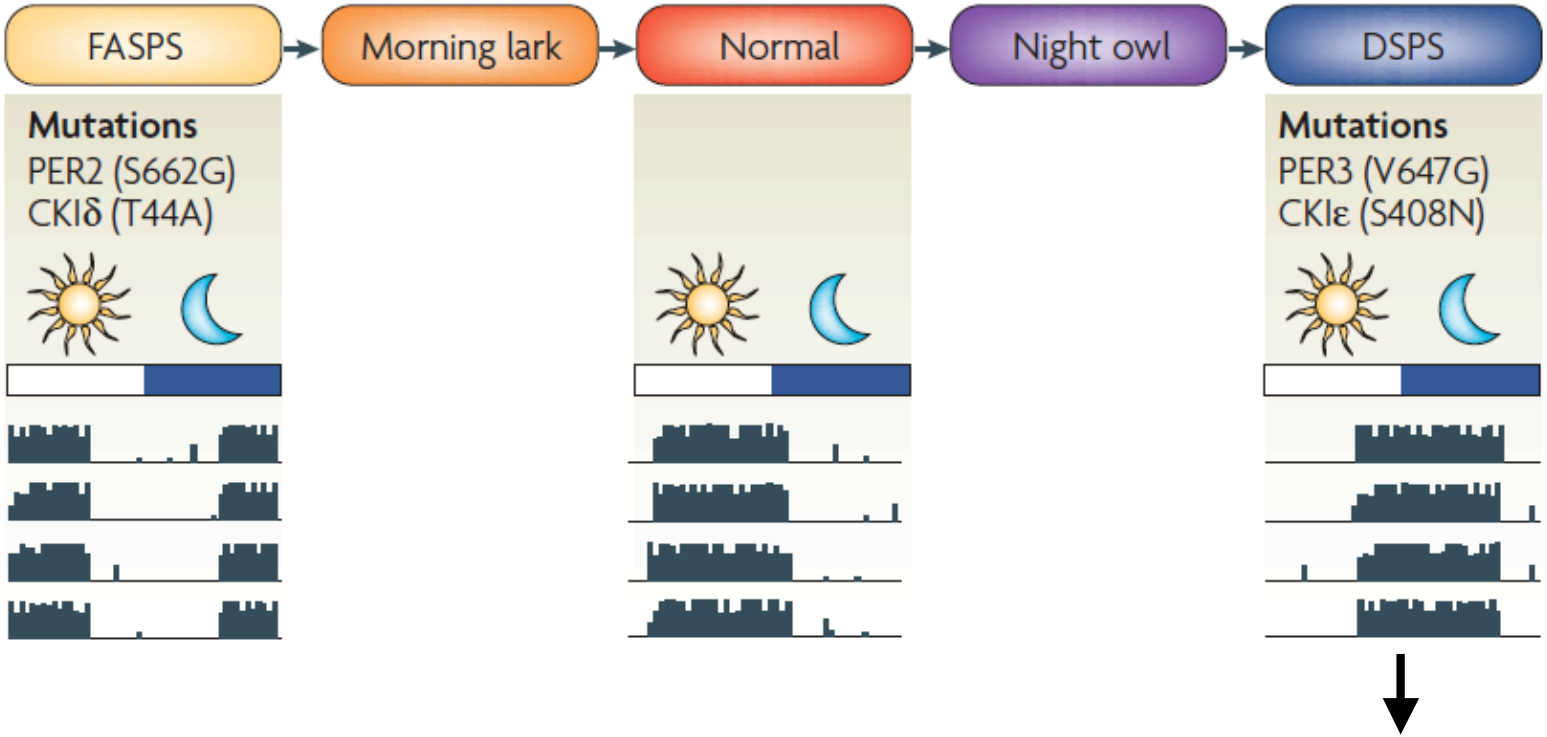


- **FASPS** patients have a **mutation in Per2 gene**, within the casein kinase ε (CKIε) binding region, which causes hypophosphorylation of PER2.
- A **mutation in the CKIδ gene** was found in another family with FASPS, leading to a decrease of the enzymatic activity of the kinase.

Gallego & Virshup (2007) *Nature Reviews Mol Cell Biol* **8**:139-148

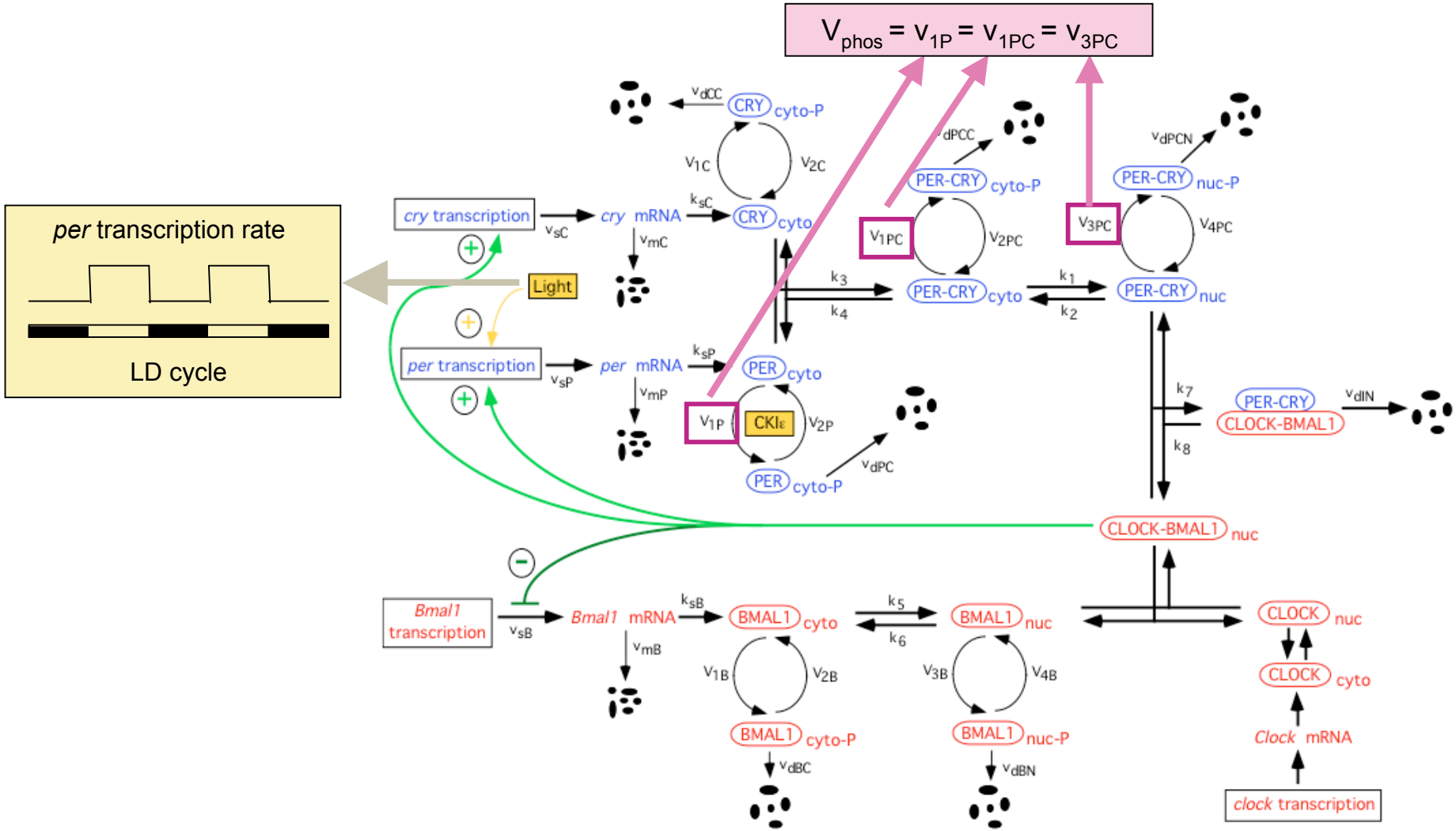
Sleep phase disorders

Box 1 | **Altered phosphorylation causes circadian rhythms disorders**



- A polymorphism in the **PER3 gene** (V647G) has been linked to **DSPS**. Residue 647 locates in a region similar to the CKIε-binding region of PER1 and PER2, close to the serine residue in PER2 that is disrupted by the FASPS mutation. Therefore, this polymorphism might alter the CKIε-dependent phosphorylation of human PER3.
- Another polymorphism leading to DSPS is in CKIε (S408N), but this variant is significantly less common.

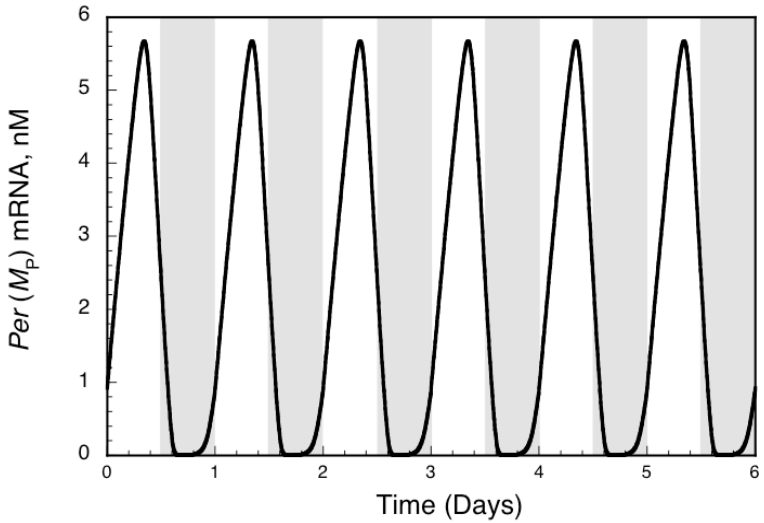
Sleep phase disorders

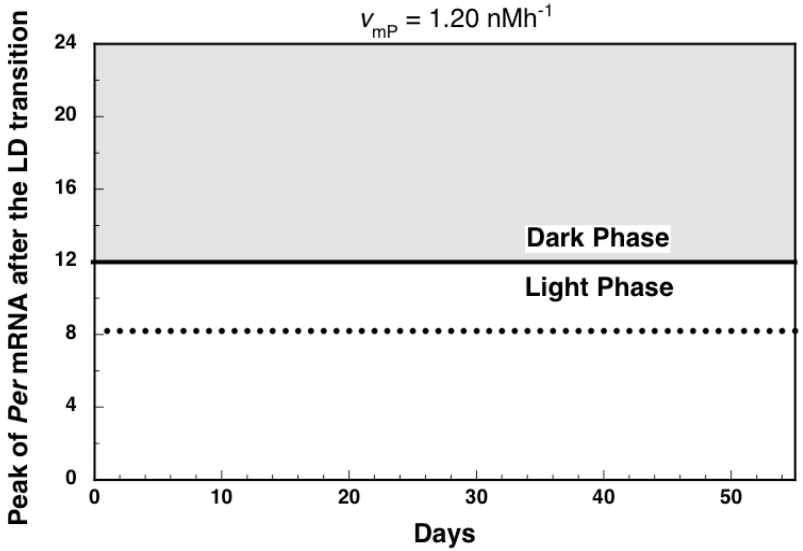


A mutation in the PER phosphorylation binding site or in the kinase that phosphorylates PER is simulated by changing the phosphorylation rate constant (V_{phos}).

Sleep phase disorders

“Normal” Case: entrainment and phase locking





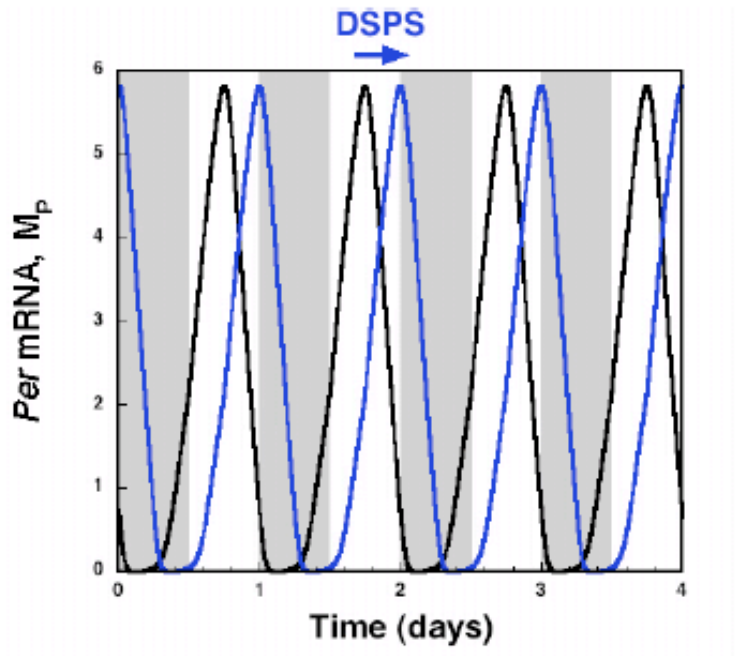
In normal conditions, under the periodic forcing by the light-dark cycle (LD) the circadian rhythm is **entrained** and the phase of the oscillations (with respect to the LD cycle) is **locked** (i.e. constant).

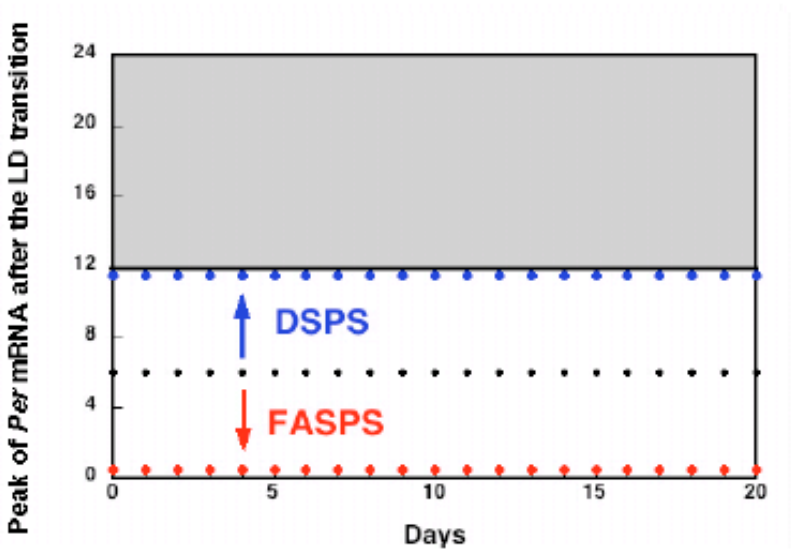
As a function of the conditions (parameter values, strength of the forcing <-> light intensity), several pathological cases can be observed:

- FASPS & DSPS
- Non 24h Sleep Phase Syndrom (SPS) + Non 24h SPS with jump
- Irregular SPS

Sleep phase disorders

FASPS & DSPS



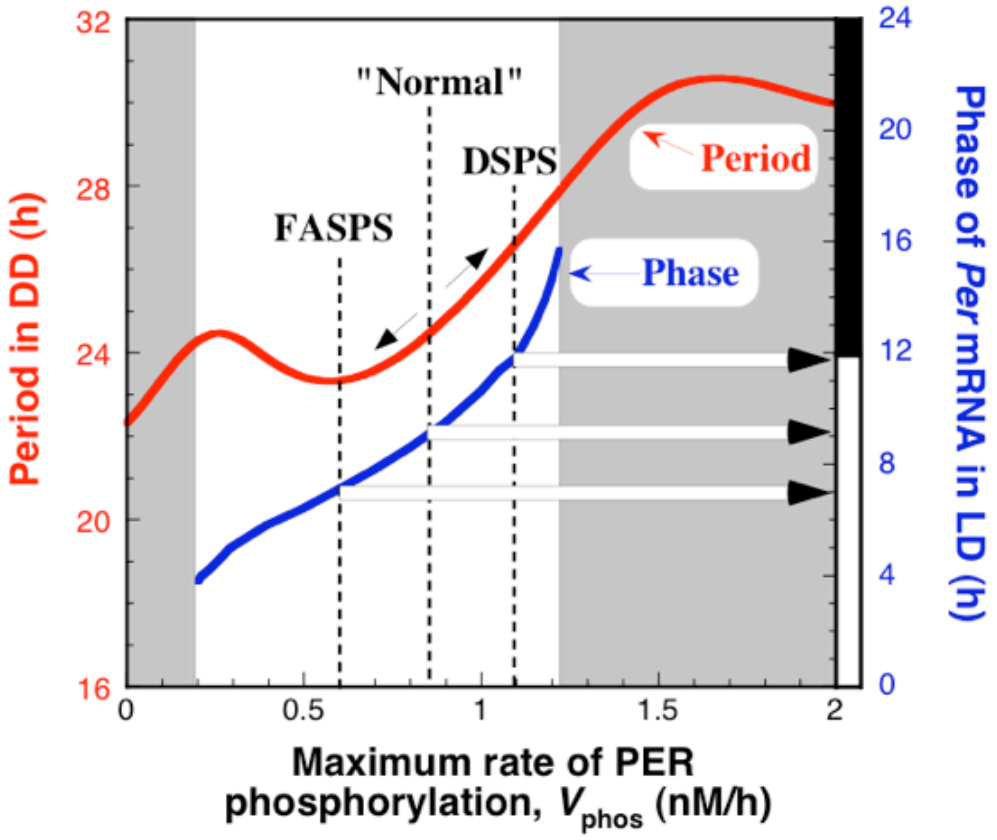


FASPS is simulated by decreasing the phosphorylation rate of PER (V_{phos}) while DSPS is simulated by increasing the phosphorylation rate of PER (V_{phos}).

FASPS & DSPS are characterized by a "proper" entrainment (in the sense that the phase is locked), but with a shift in the phase of the oscillations with respect to the LD cycle.

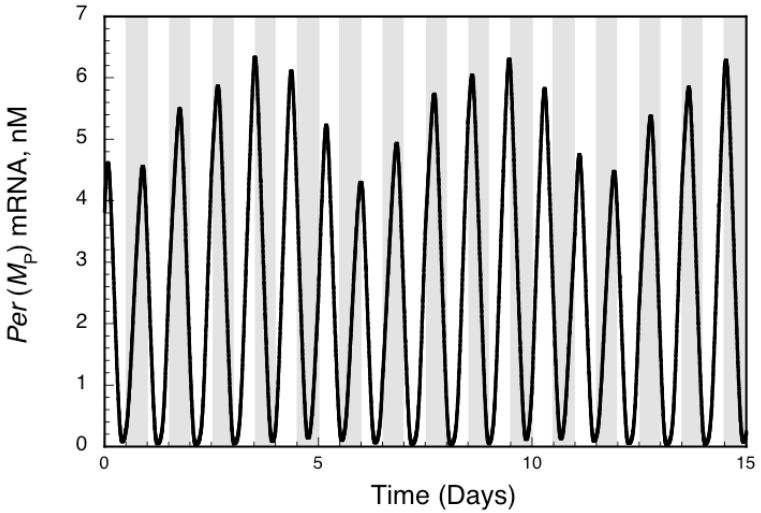
Sleep phase disorders

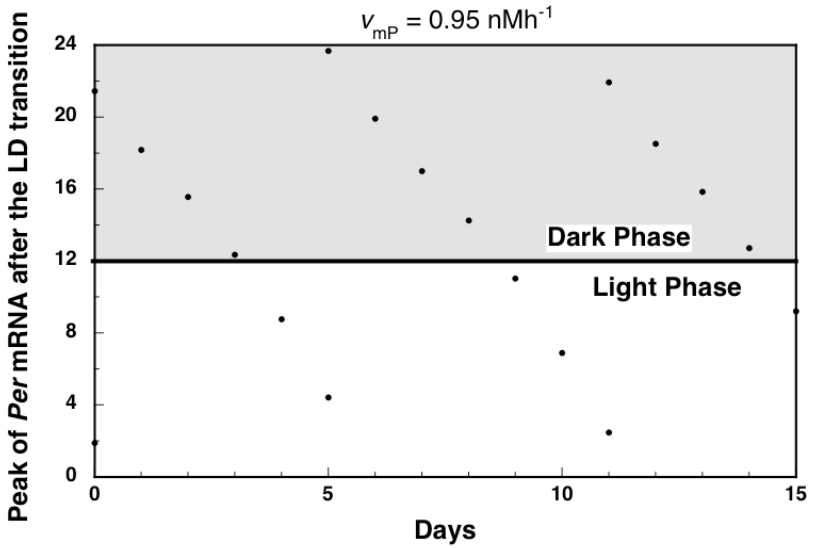
An *hPer2* phosphorylation site mutation in familial advanced sleep-phase syndrome (FASPS).



Sleep phase disorders

Non-24h Sleep Phase Syndrom



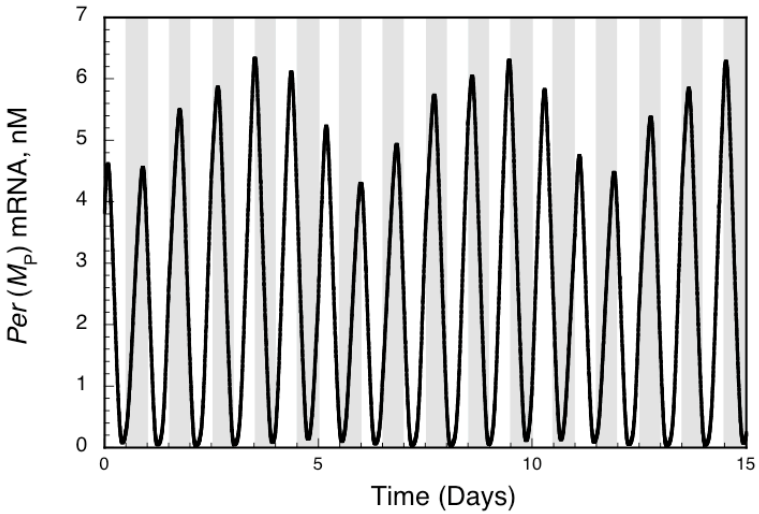


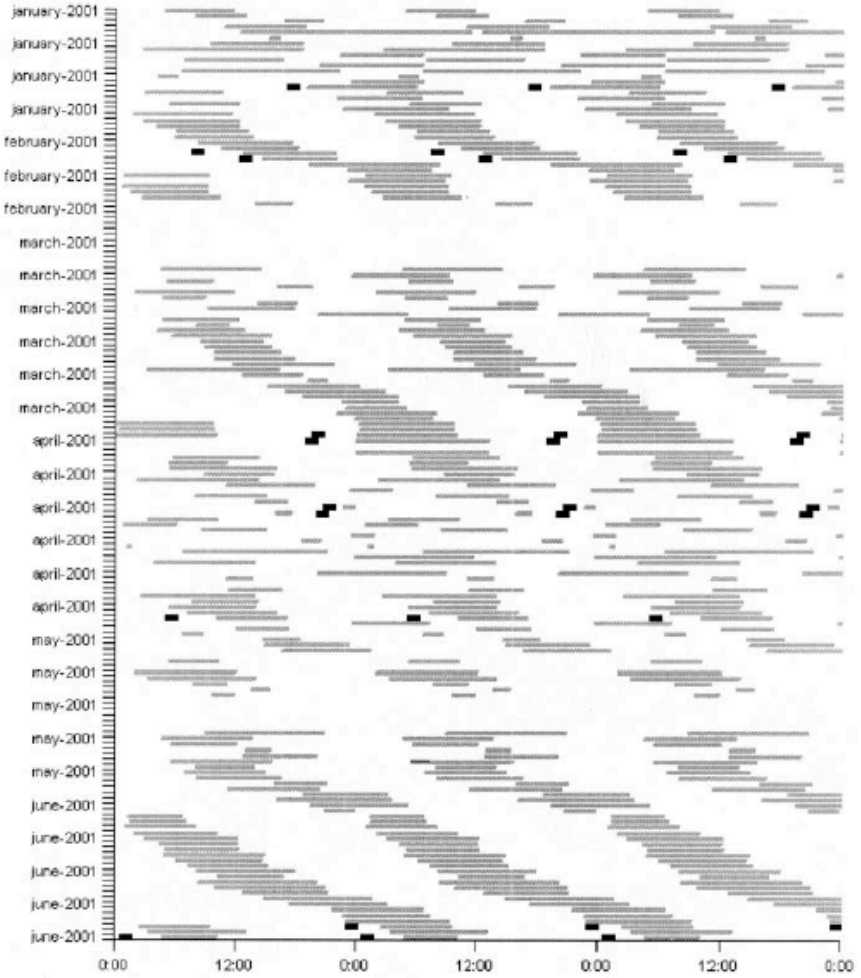
In some conditions, the oscillations are not entrained by the light-dark cycle. In this case, we may observe a **quasi-periodic behavior**: the oscillations look relatively regular, but there are some beats in their amplitude and their phase is not locked (i.e. the period is not precisely 24h). As a consequence, the rhythm (and thus the sleeping phase) is shifted more and more every day. This so-called **non-24h syndrom** was reported in blind patients, but also, rarely, in sighted patients.

NB: this syndrome is sometimes also called **free-running syndrom.** It should be noted that from the theoretical point of view the 2 types of dynamics are different (beats is a consequence of the periodic forcing and would not appear in free-run).

Sleep phase disorders

Non-24h Sleep Phase Syndrom



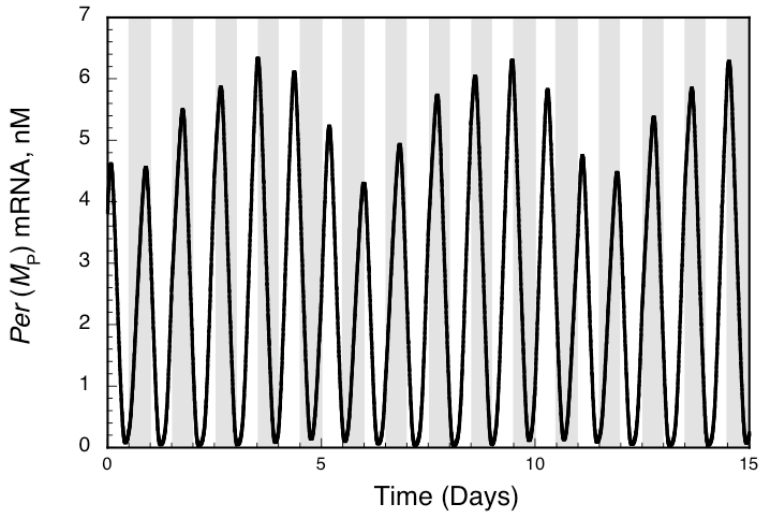


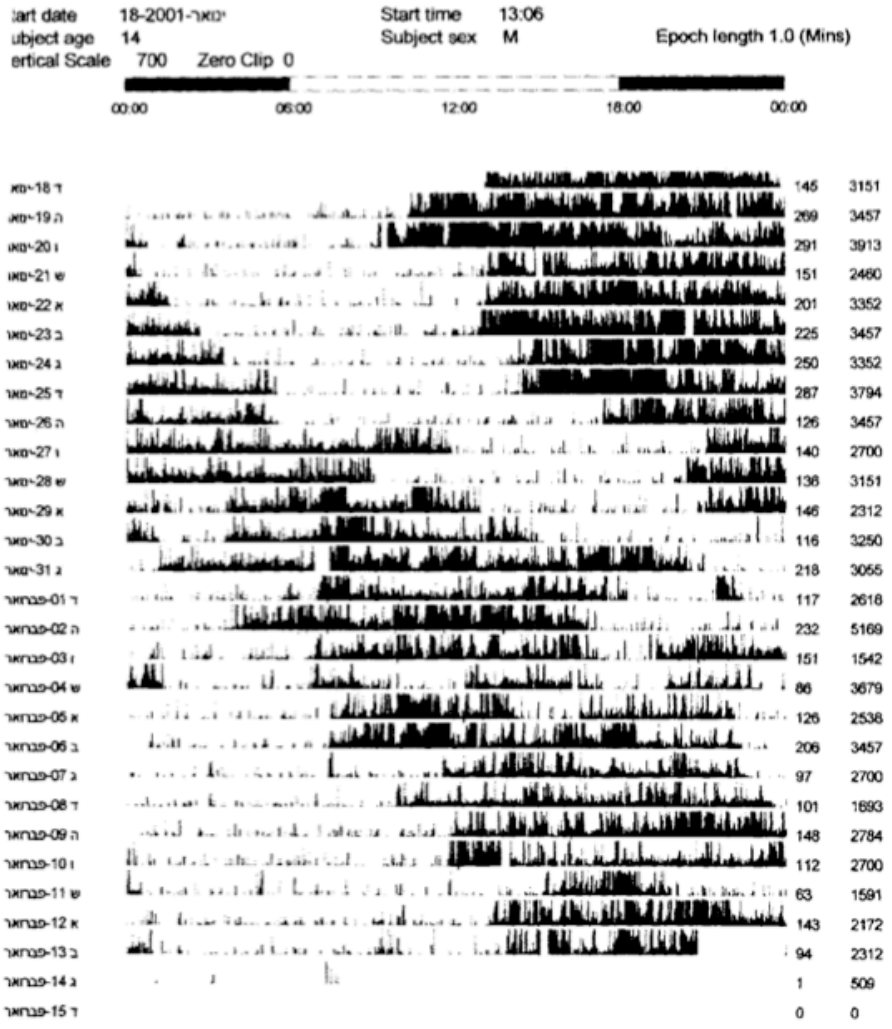
The authors report the case of a 39-year-old sighted woman who displayed non-24-hour sleep–wake cycles following a car accident.

Boivin *et al.* (1992) Non-24-hour sleep-wake syndrome following a car accident. *Neurology* 60:1841-3.

Sleep phase disorders

Non-24h Sleep Phase Syndrom

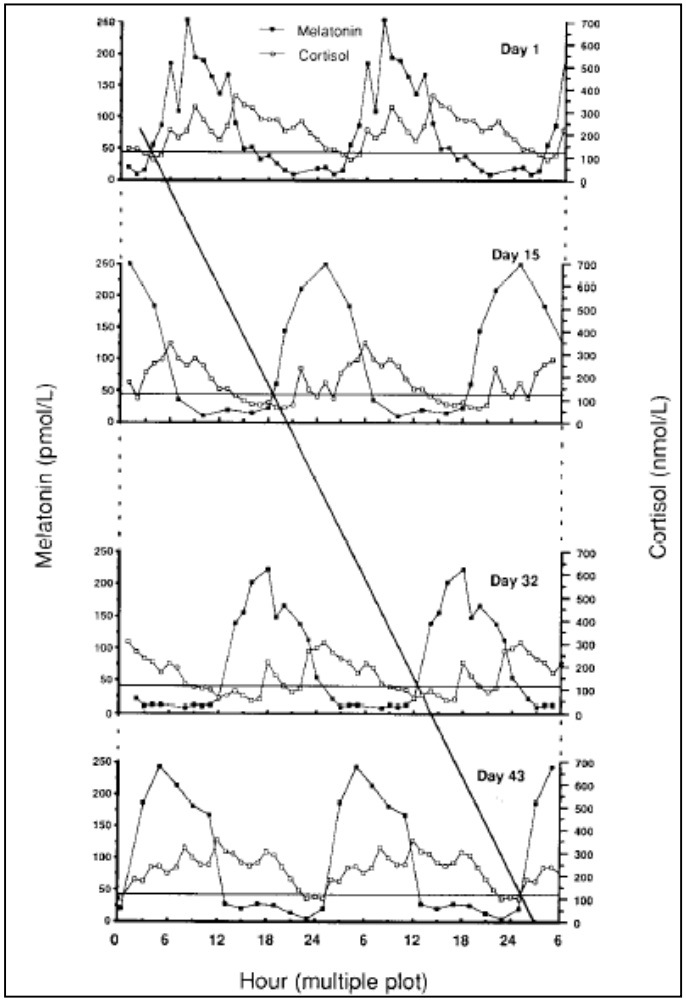


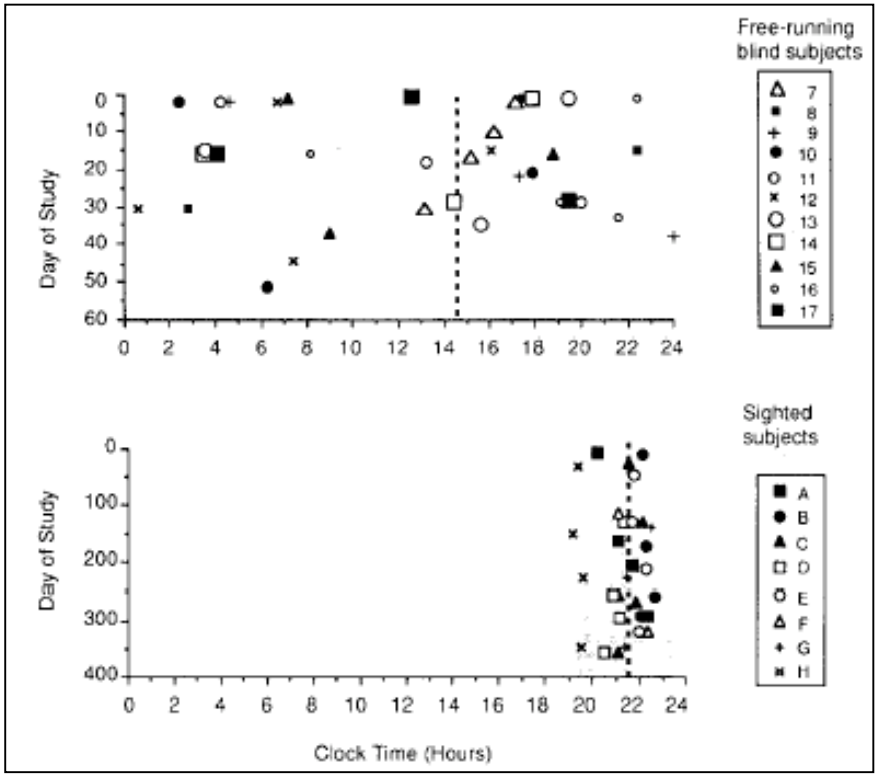


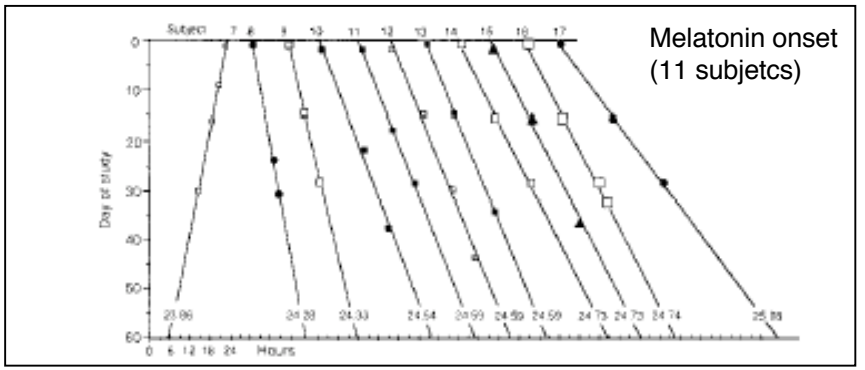
Dagan Y (2002) Circadian rhythm sleep disorders (CRSD), *Sleep Medicine Reviews* 6:45–55.

Sleep phase disorders

Non-24h SPS
(free running syndrom?)



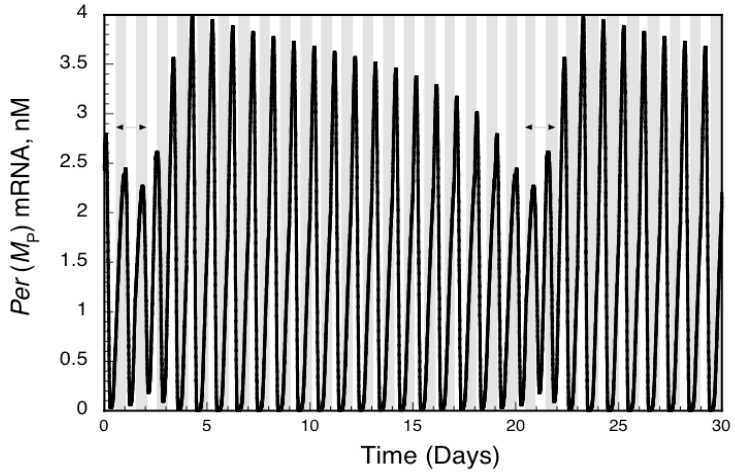


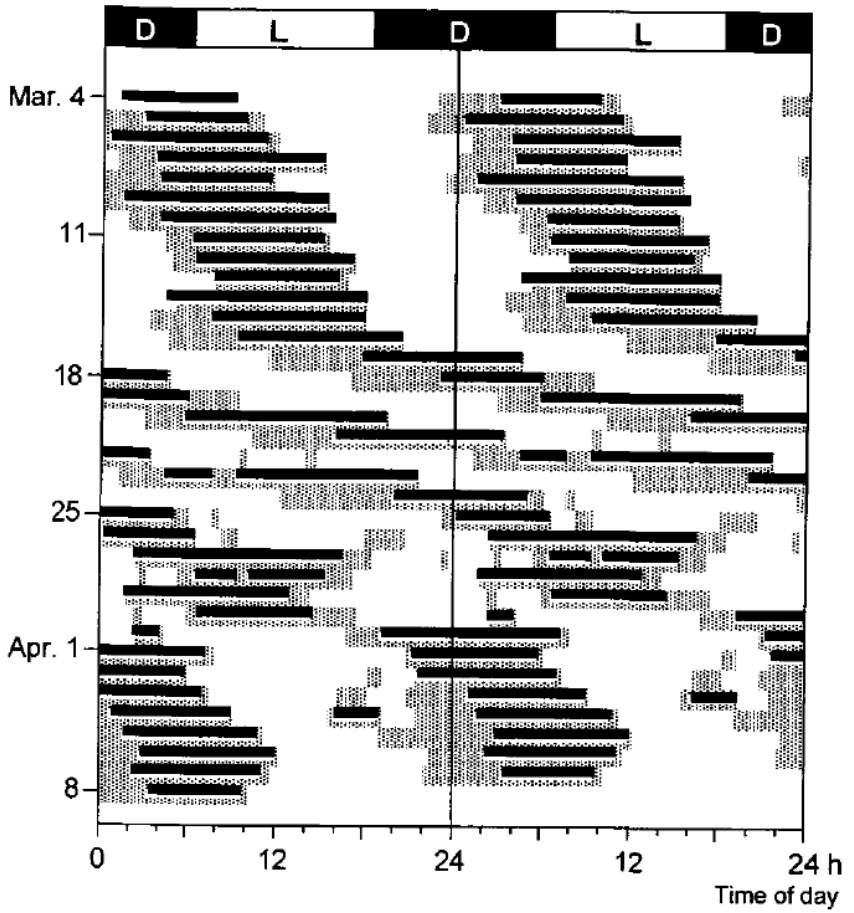


Sack RL *et al.* (1992) Circadian rhythm abnormalities in totally blind people. *J Clin Endocrinol Metab* 75:127-34.

Sleep phase disorders

Non-24 h SPS (with phase jumps)

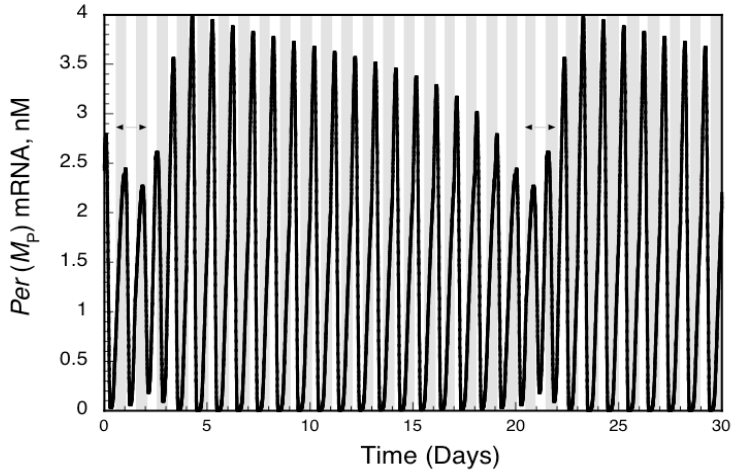


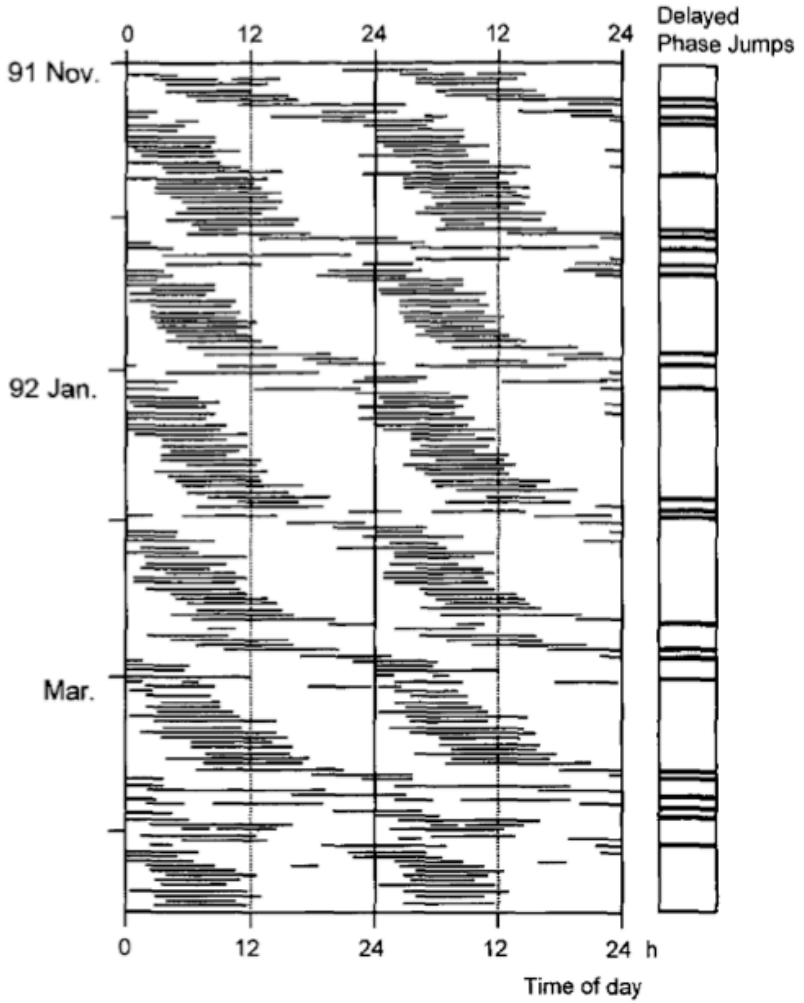


Uchiyama *et al.* (1996) *Sleep* 19:637-40.

Sleep phase disorders

Non-24 h SPS (with phase jumps)

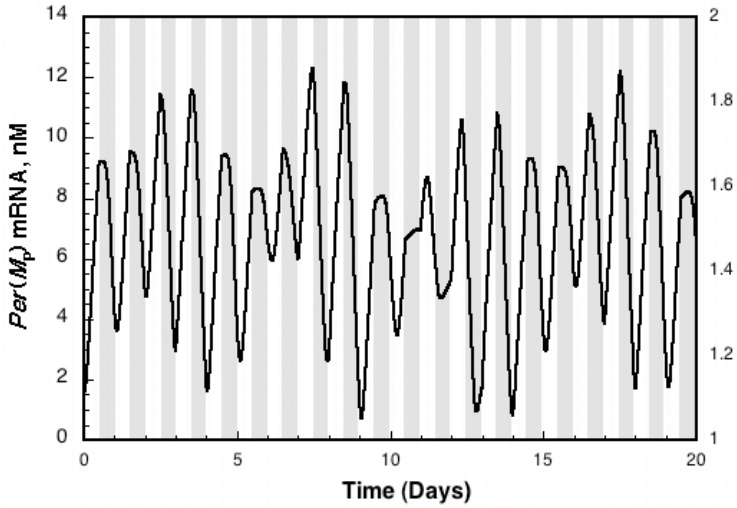


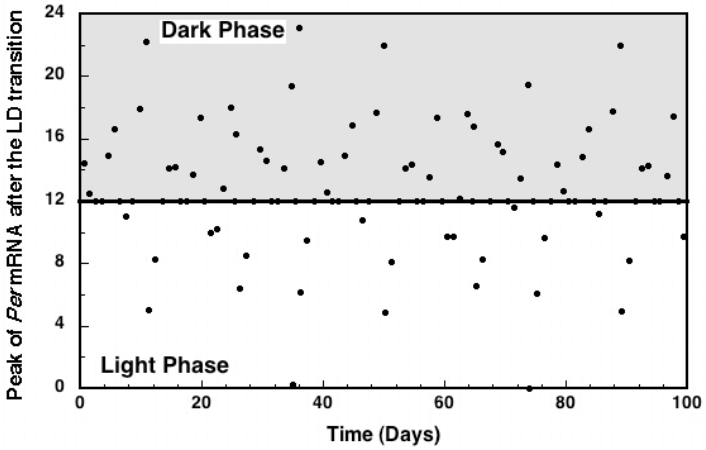


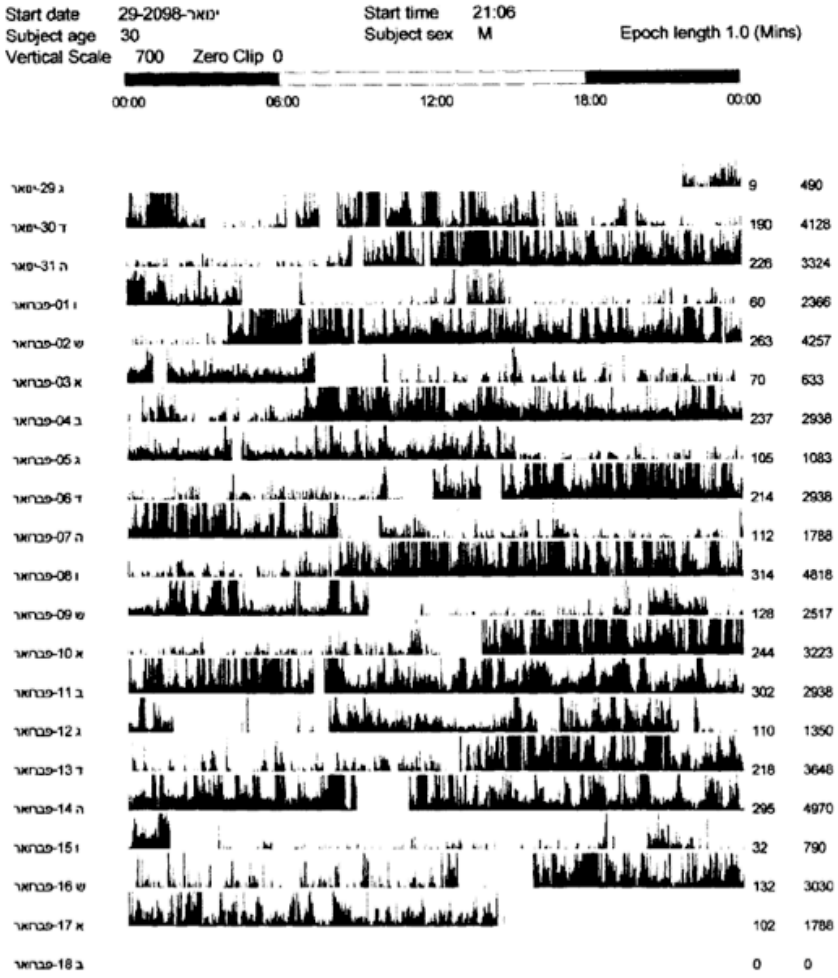
Uchiyama *et al.* (1996) *Sleep* 19:637-40.

Sleep phase disorders

Irregular Sleep Phase Syndrom



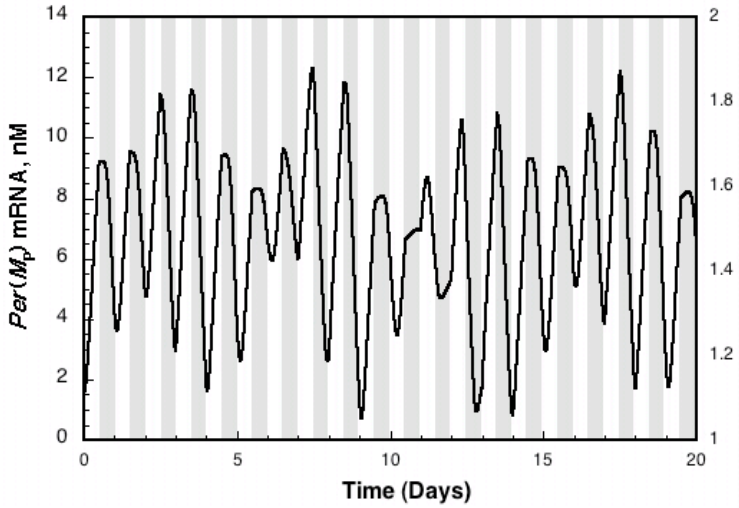


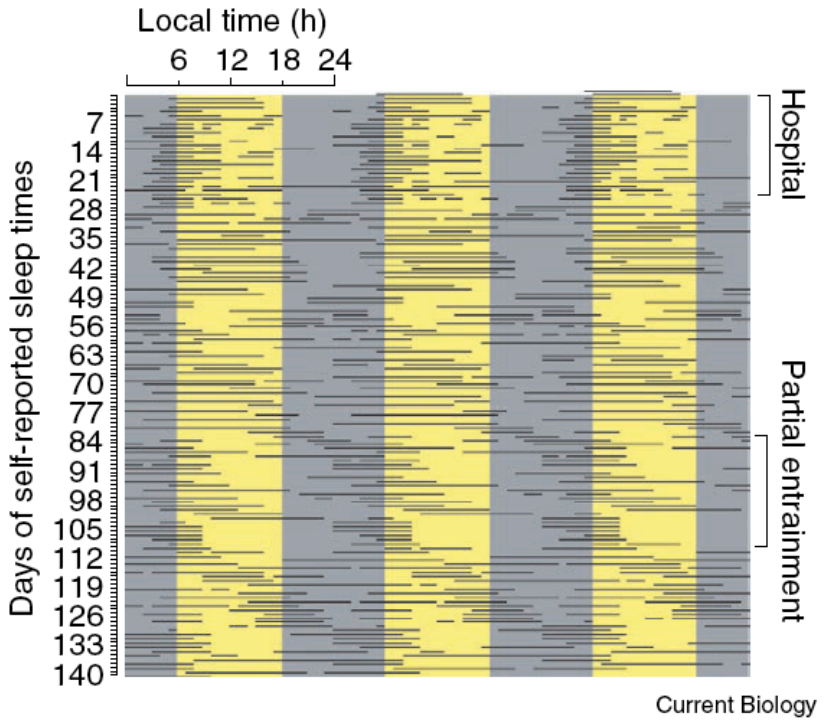


Dagan, 2002

Sleep phase disorders

Irregular Sleep Phase Syndrom



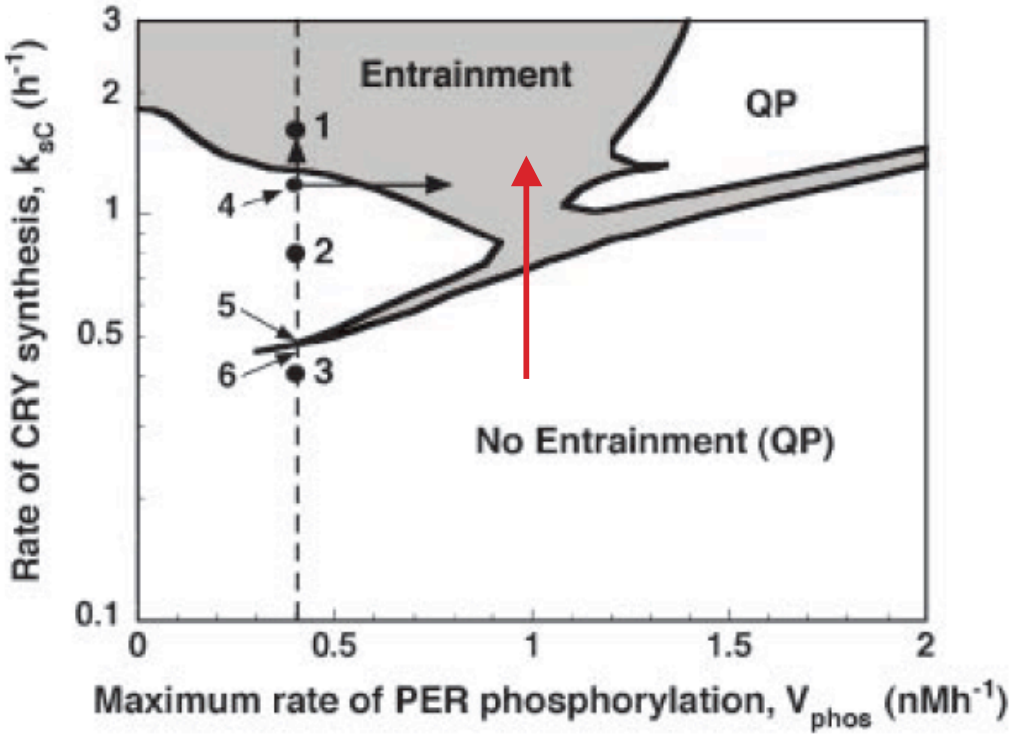


Roenneberg & Merrow (2003)
Curr Biol 13:R198-R207.

Sleep phase disorders

How to restore circadian entrainment?

The model can be used to identify the disregulation responsible for the lack of entrainment and to suggest ways to restore a proper entrainment.



The lack of entrainment in the model was shown to be due to an inappropriate balance between PER and CRY levels.

Thus, by tuning parameters such as the CRY synthesis rate (or PER degradation rate), it is possible to restore entrainment (see red arrow).

Leloup JC, Goldbeter A (2008) *Bioessays* 30:590-600.

Sleep phase disorders

How to restore circadian entrainment?

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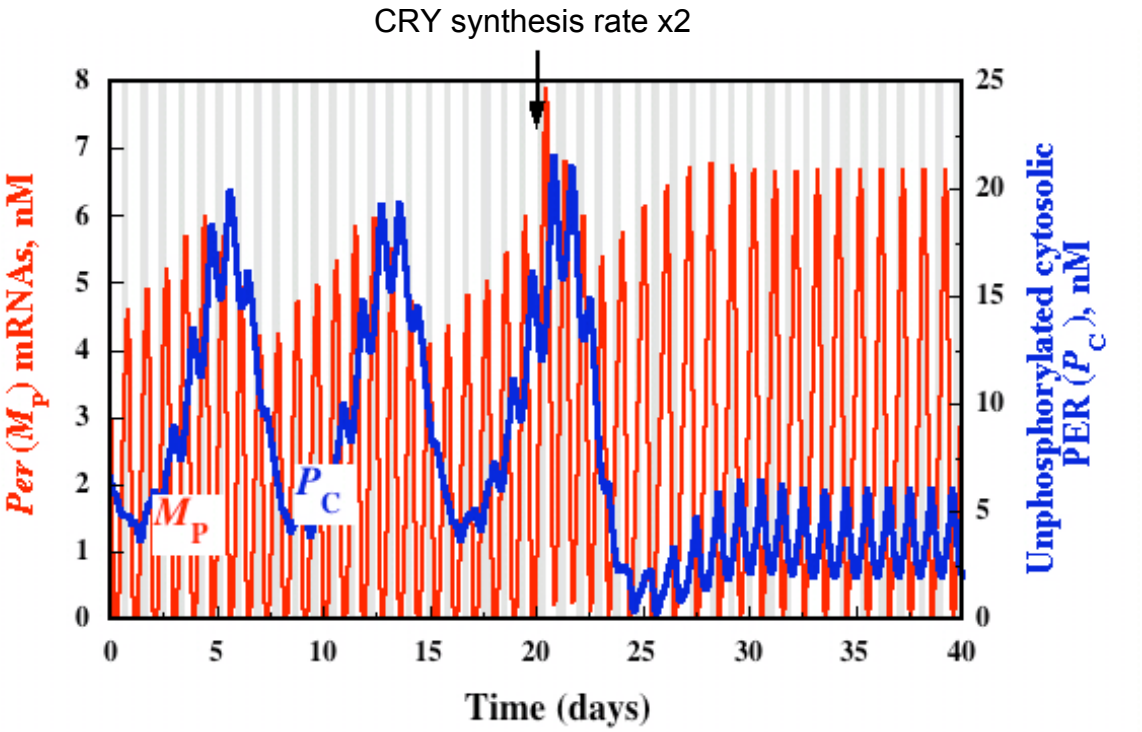


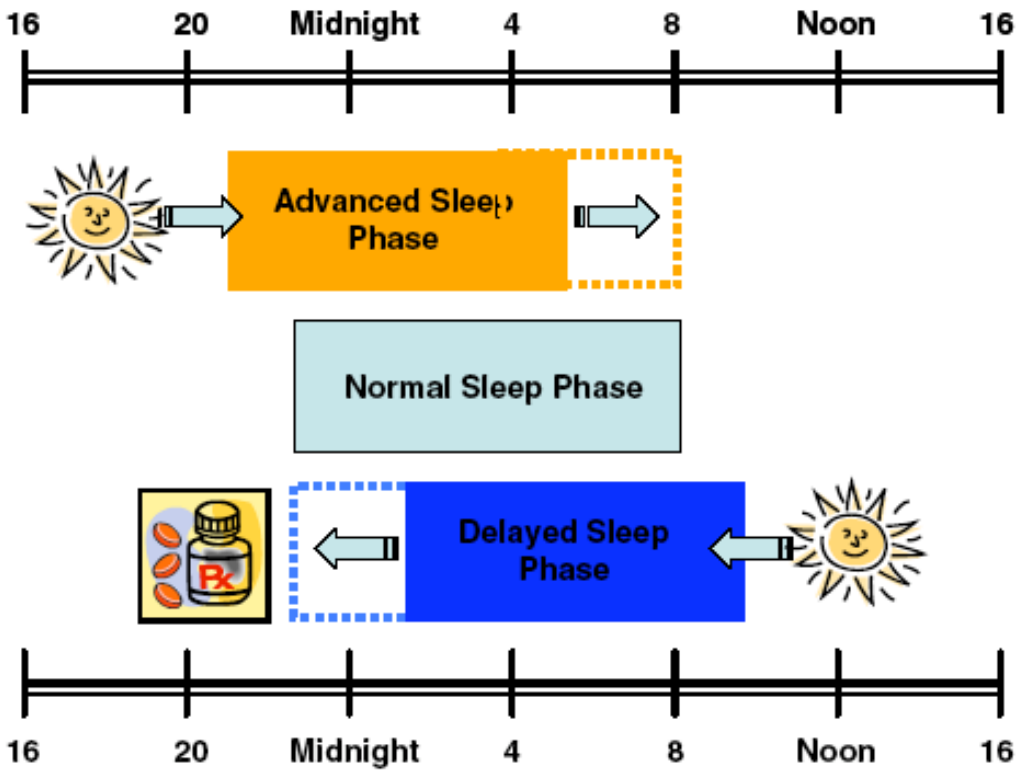
At time *t*=20, the CRY synthesis rate is doubled.

Recovery of a proper entrainment by increasing the CRY synthesis rate.

Leloup JC, Goldbeter A (2008) *Bioessays* 30:590-600.

Sleep phase disorders

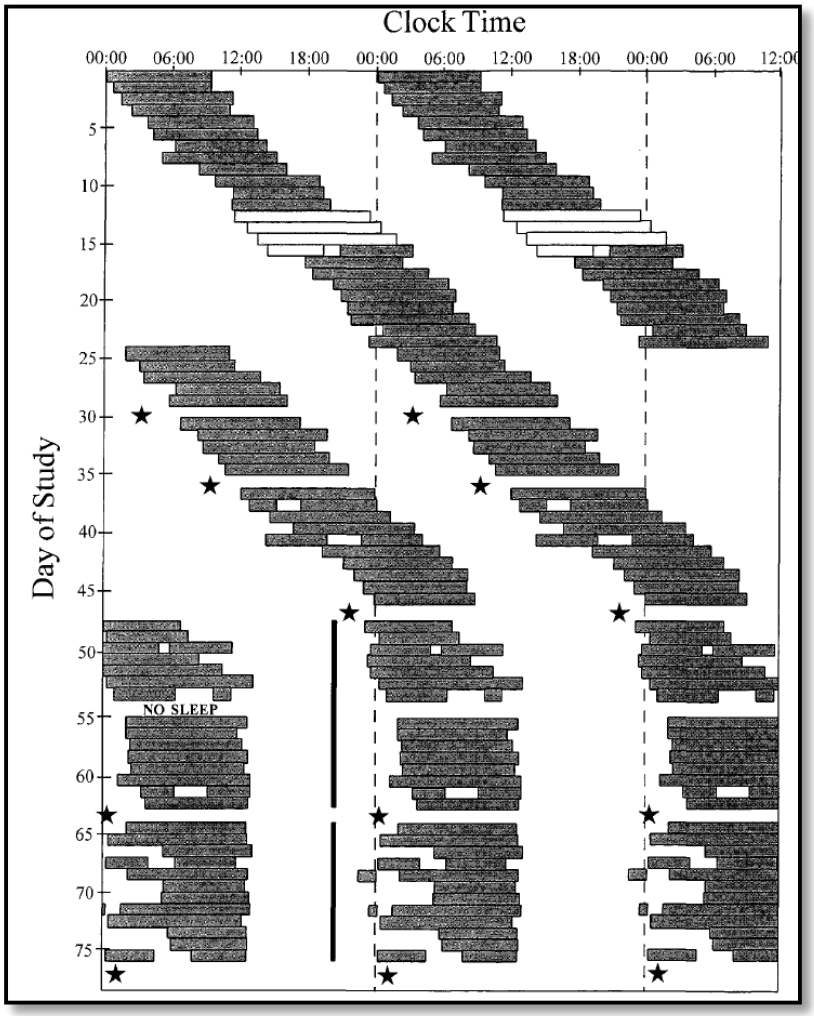
How to restore circadian entrainment in practice?



Barion & Zee, 2007

Sleep phase disorders

How to restore circadian entrainment in practice?



τ = 25.1 h

Non-25 SPS

Melatonin treatment
τ = 24 h

Entrainment

McArthur et al, 1996

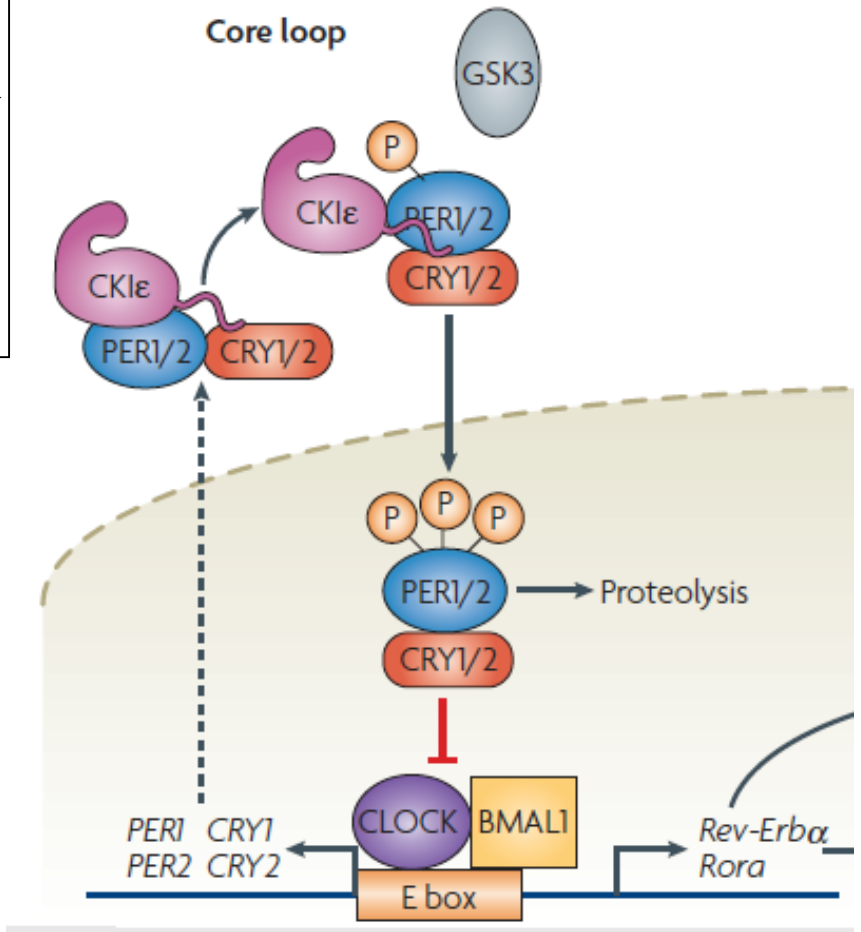
Circadian clock & phosphorylations

Post-translational modifications regulate the ticking of the circadian clock

Monica Gallego and David M. Virshup*‡*

Abstract | Getting a good night's sleep is on everyone's to-do list. So is, no doubt, staying awake during late afternoon seminars. Our internal clocks control these and many more workings of the body, and disruptions of the circadian clocks predispose individuals to depression, obesity and cancer. Mutations in kinases and phosphatases in hamsters, flies, fungi and humans highlight how our timepieces are regulated and provide clues as to how we might be able to manipulate them.

- PER proteins undergo multiple phosphorylation/dephosphorylation.
- These post-translational modifications allow a tight(?) regulation of the circadian clock.
- Mutations in kinases and phosphatases deregulate the circadian clocks and can affect their period and entrainment properties.



Circadian clock & phosphorylations

PER phosphorylation

- Phosphorylation of the PER proteins can affect various functions :
  - (**a**) nuclear localization
  - (**b**) regulatory/binding activity
  - (**c**) stability (degradation)

- In mutants, the altered function depends on which kinase / which phosphorylation site is affected.

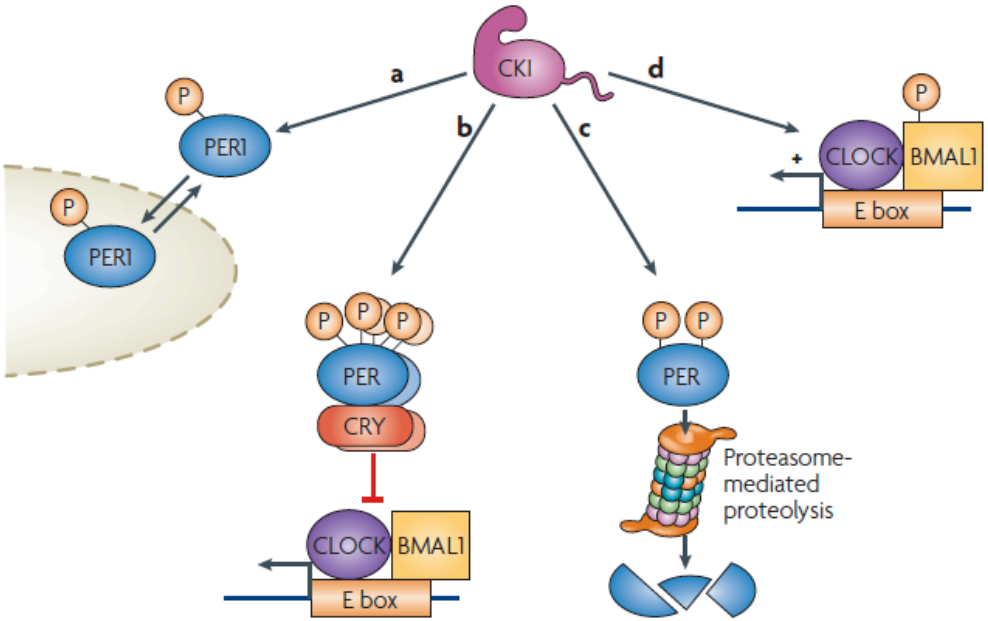


Figure 3 | **Multiple roles of casein kinase I in the mammalian circadian clock.** Casein kinase I (CKI) has many roles in the circadian clock. **a** | It has a confusing role in regulating the nuclear localization of the circadian repression protein period (in this example, PER1). In some cell types, CKI activity promotes the cytoplasmic accumulation of PER1, whereas in others it mediates the nuclear translocation of PER1 (REF. 84). **b** | Time-course studies have shown that the phosphorylation of PER proteins increases over the course of the circadian day, peaking when the repression of the positive transcription factors CLOCK and BMAL1 is maximal. Mapping studies indicate that there are many CKI sites on PER proteins[45], but the function of only a subset of these sites is known. Phosphorylation of PER proteins (and the associated transcription repressor cryptochrome (CRY)) might be linked to the inhibition of transcriptional activity[19]. **c** | One clear function of the phosphorylation of PER proteins is the regulation of protein stability. Phosphorylation of one or two distinct sites on PER1 and PER2 target these proteins for ubiquitin-mediated degradation by the 26S proteasome. Degradation of PER proteins can reset the clock, allowing the CLOCK–BMAL1 complex to become active[12,83–86]. **d** | PER and CRY proteins are not the only substrates of CKI in the clock. CKIε-mediated phosphorylation of the circadian regulator BMAL1 increases its transcriptional activity[44]. P, phosphate.

Circadian clock & phosphorylations

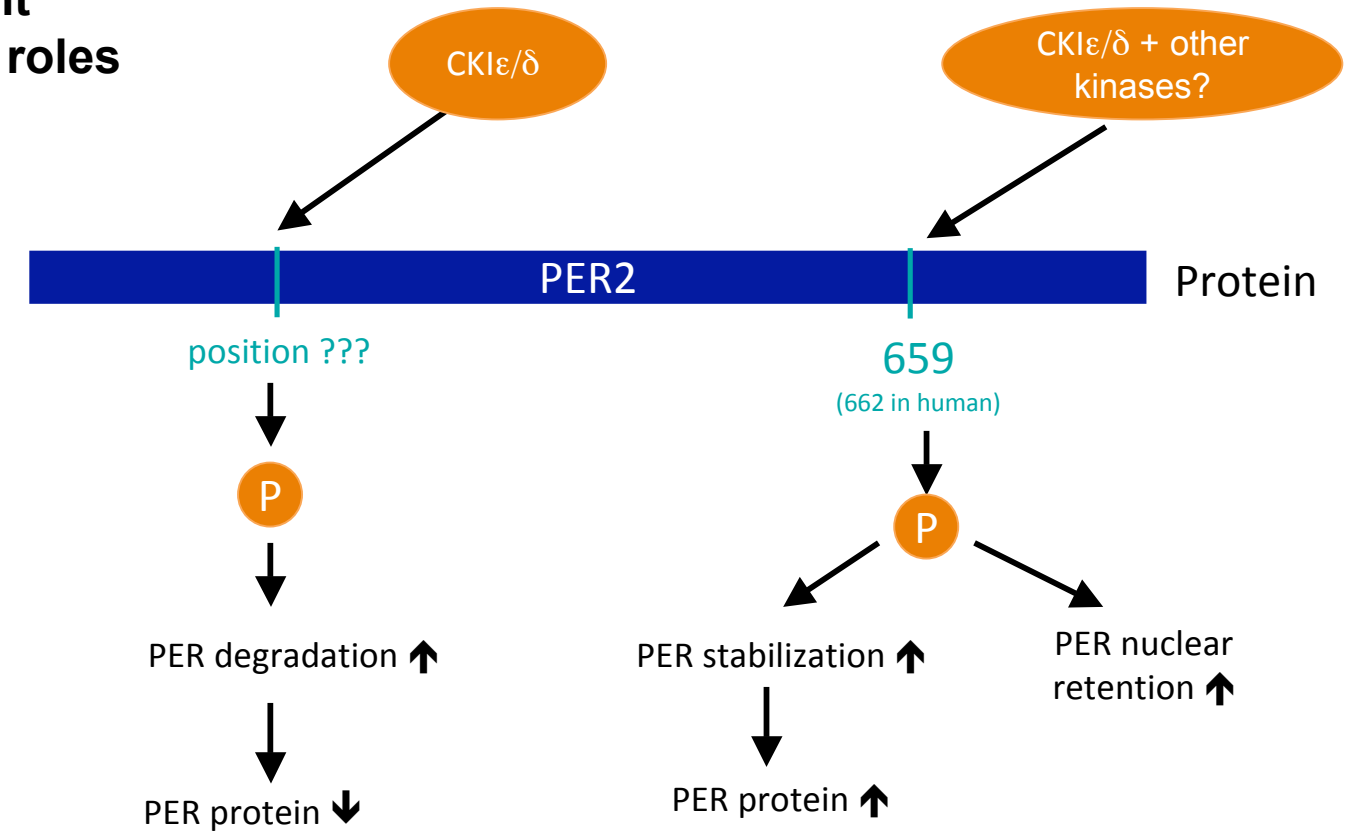
Differential effects of PER2 phosphorylation: molecular basis for the human familial advanced sleep phase syndrome (FASPS)

Katja Vanselow,[1] Jens T. Vanselow,[1] Pål O. Westermark,[2] Silke Reischl,[1] Bert Maier,[1] Thomas Korte,[3] Andreas Herrmann,[3] Hanspeter Herzel,[2] Andreas Schlosser,[1] and Achim Kramer[1,4]

PERIOD (PER) proteins are central components within the mammalian circadian oscillator, and are believed to form a negative feedback complex that inhibits their own transcription at a particular circadian phase. Phosphorylation of PER proteins regulates their stability as well as their subcellular localization. In a systematic screen, we have identified 21 phosphorylated residues of mPER2 including Ser 659, which is mutated in patients suffering from familial advanced sleep phase syndrome (FASPS). When expressing FASPS-mutated mPER2 in oscillating fibroblasts, we can phenocopy the short period and advanced phase of FASPS patients' behavior. We show that phosphorylation at Ser 659 results in nuclear retention and stabilization of mPER2, whereas phosphorylation at other sites leads to mPER2 degradation. To conceptualize our findings, we use mathematical modeling and predict that differential PER phosphorylation events can result in opposite period phenotypes. Indeed, interference with specific aspects of mPER2 phosphorylation leads to either short or long periods in oscillating fibroblasts. This concept explains not only the FASPS phenotype, but also the effect of the *tau* mutation in hamster as well as the *doubletime* mutants (dbt^S and dbt^L) in *Drosophila*.

Circadian clock & phosphorylations

Multiple phosphorylation sites and different phosphorylation roles



Adapted from Xu et al, 2007
(Source: JC Leloup)

Circadian clock & phosphorylations

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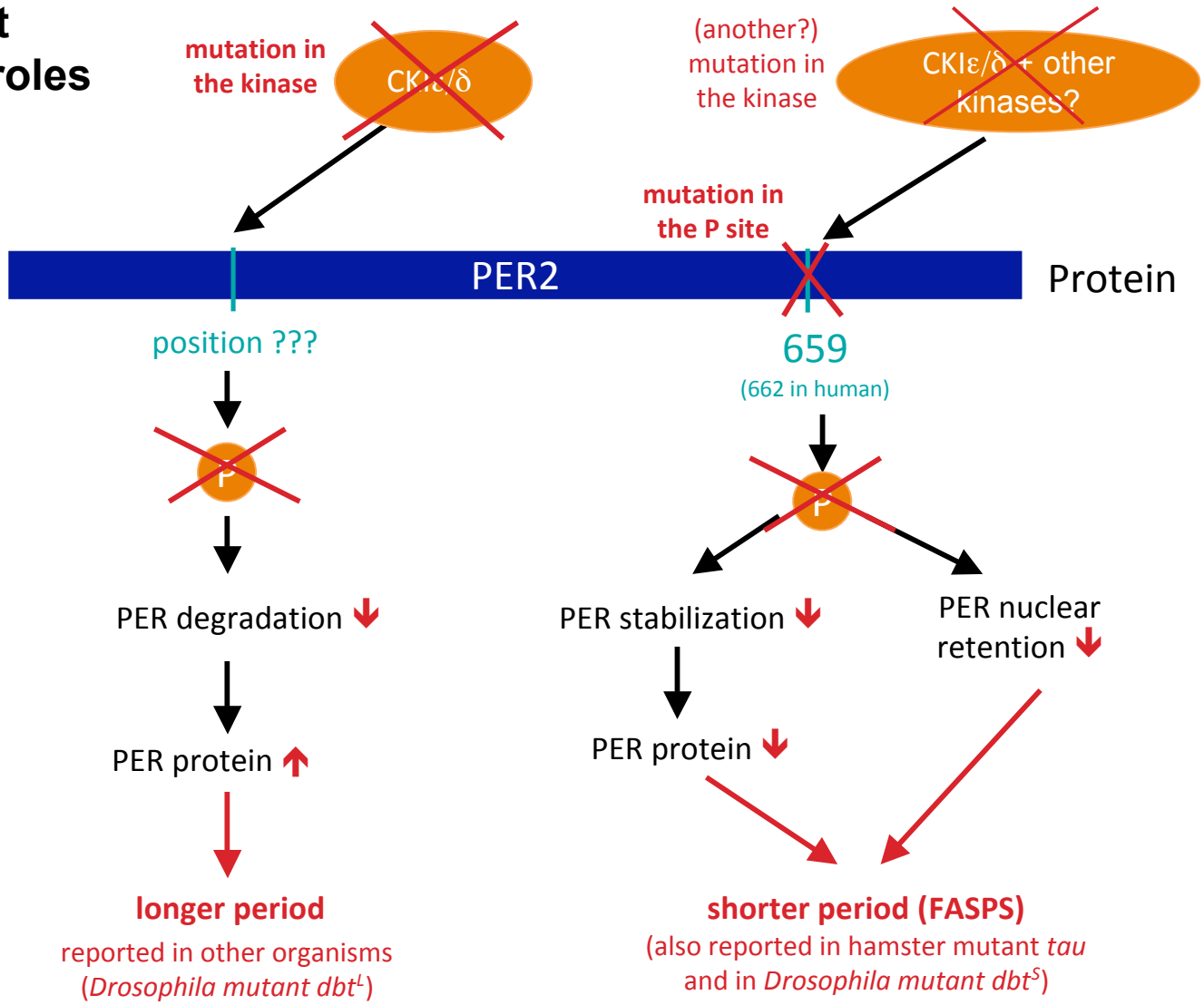
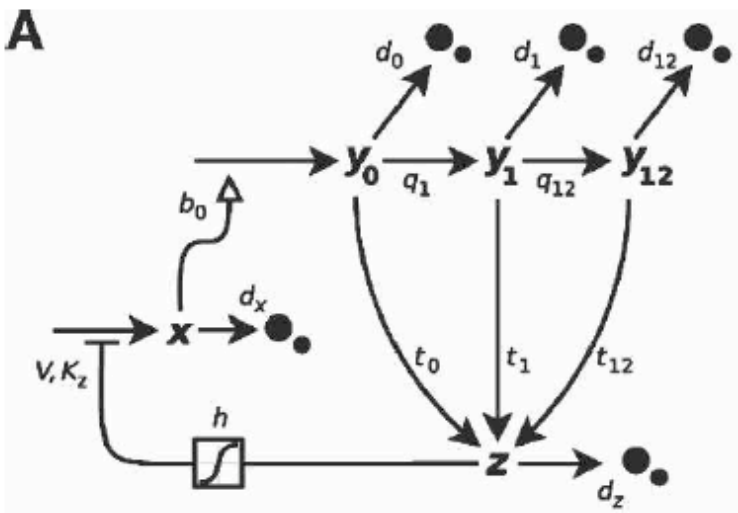


Circadian clock & phosphorylations



Goodwin-like model

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

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

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

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

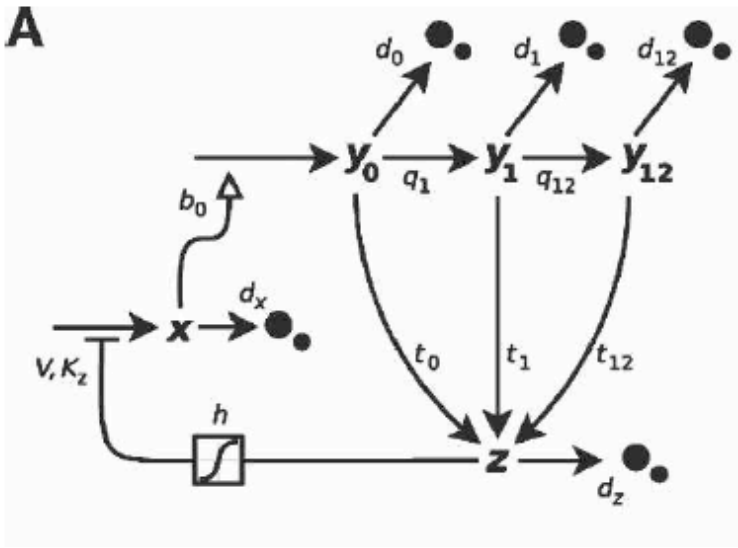
$$\dot{z} = t_0 y_0 + t_1 y_1 + t_{12} y_{12} - d_z z$$

Hypotheses:

- The phosphorylations are sequential (y_0 -> y_1 -> y_{12}) and characterized by the rate constants q_1 and q_{12}.
- The different states of the protein have different stability (i.e. different degradation rates, d_0, d_1, d_{12}) and different rates of nuclear transport (t_0, t_1, t_{12}).
- The phosphorylation state of the nuclear protein do not affect its degradation (d_z) or regulatory activity (K).
- Mutations in the kinase binding site affect the phosphorylation rates, q_1 or q_{12}.

Vanselow et al, 2006

Circadian clock & phosphorylations



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

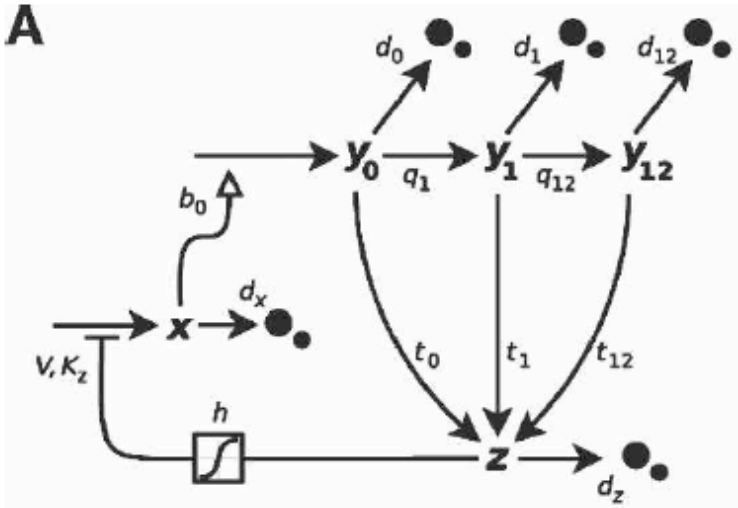
Parametrization:

- In the FASPS mutant (modeled here by a reduction of the phosphorylation rate q_{12}), the degradation of the protein is increased. The value of the degradation rates are thus chosen so that $d_1 > d_{12}$.
- In addition in FASPS mutants there is an accelerated nuclear clearance. This is taken into account by chosing $t_{12} > t_1$

Parameter	Value [a]
b_0	0.4 hour^{-1}
d_x	0.12 hour^{-1}
d_0	0.06 hour^{-1}
d_1	1.1 hour^{-1}
d_{12}	0.06 hour^{-1}
d_z	0.12 hour^{-1}
t_0	0.06 hour^{-1}
t_1	0.18 hour^{-1}
t_{12}	0.25 hour^{-1}
q_1	2 hour^{-1}
q_{12}	2 hour^{-1}
V	1 U × hour^{-1}
K	1 U
h	12

[a] U is an arbitrary concentration unit.

Circadian clock & phosphorylations



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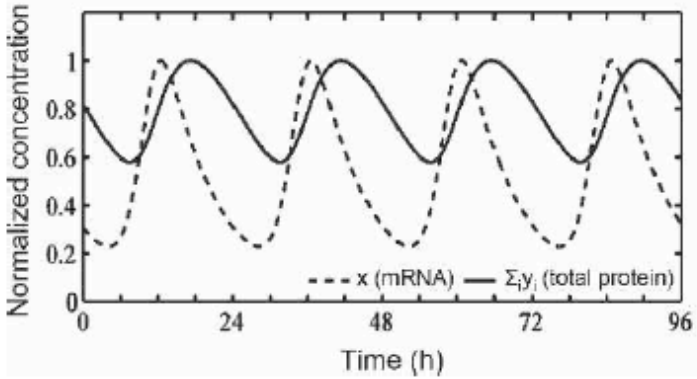
$$\dot{z} = t_0 y_0 + t_1 y_1 + t_{12} y_{12} - d_z z$$

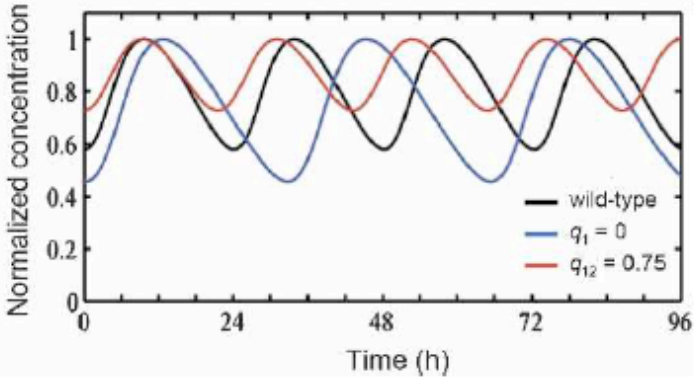
Blue = interference on the first P site (q_1=0)
=> longer period
Red = interference in the second P site (q_{12}=0.75)
=> shorter period (FASPS)

B

Default parameter set (q_1= q_{12} =2)
=> τ = 24h



C



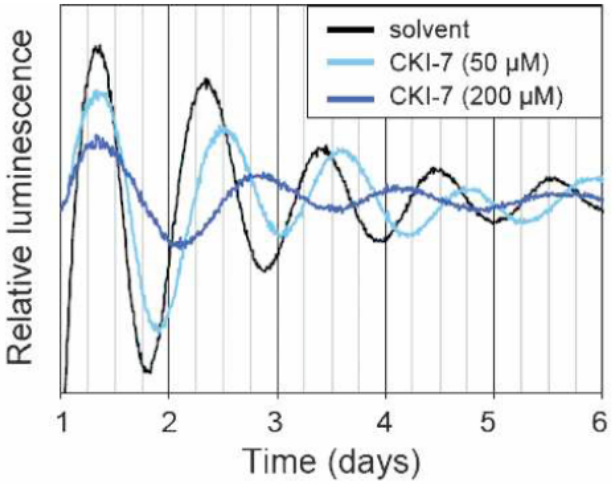
Vanselow et al, 2006

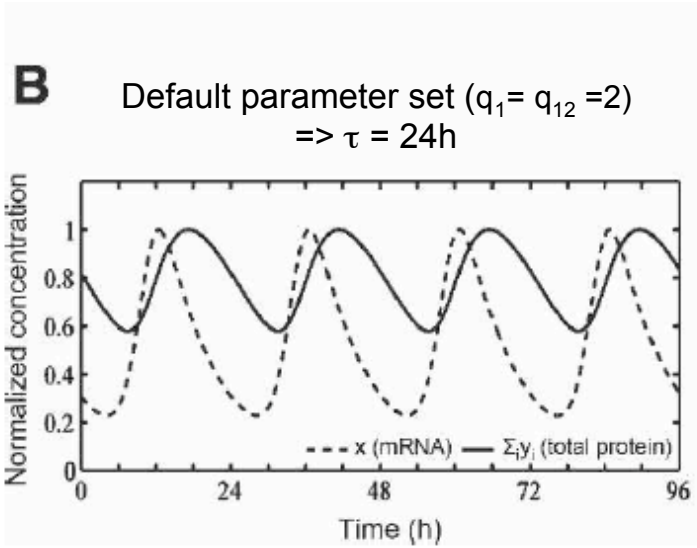
Circadian clock & phosphorylations

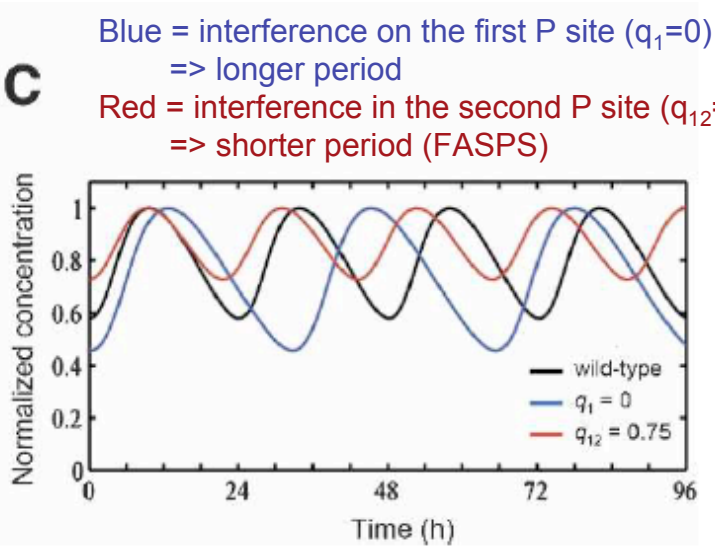
Prediction

The model accounts for the phenotype of the FASPS mutant (shorter period) and predicts a longer period for the alteration of the phosphorylation of the other site.

This prediction was experimentally verified using NIH3T3 cells treated with CKI inhibitor (CKI-7). A dose-dependent period lengthening is observed.







Vanselow et al, 2006

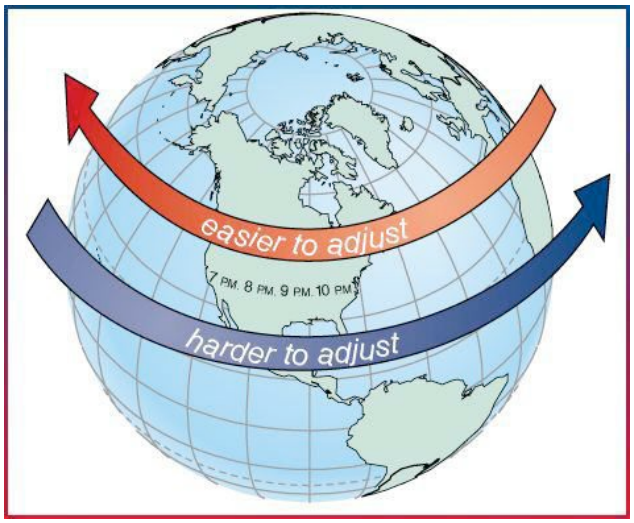
Jet lag

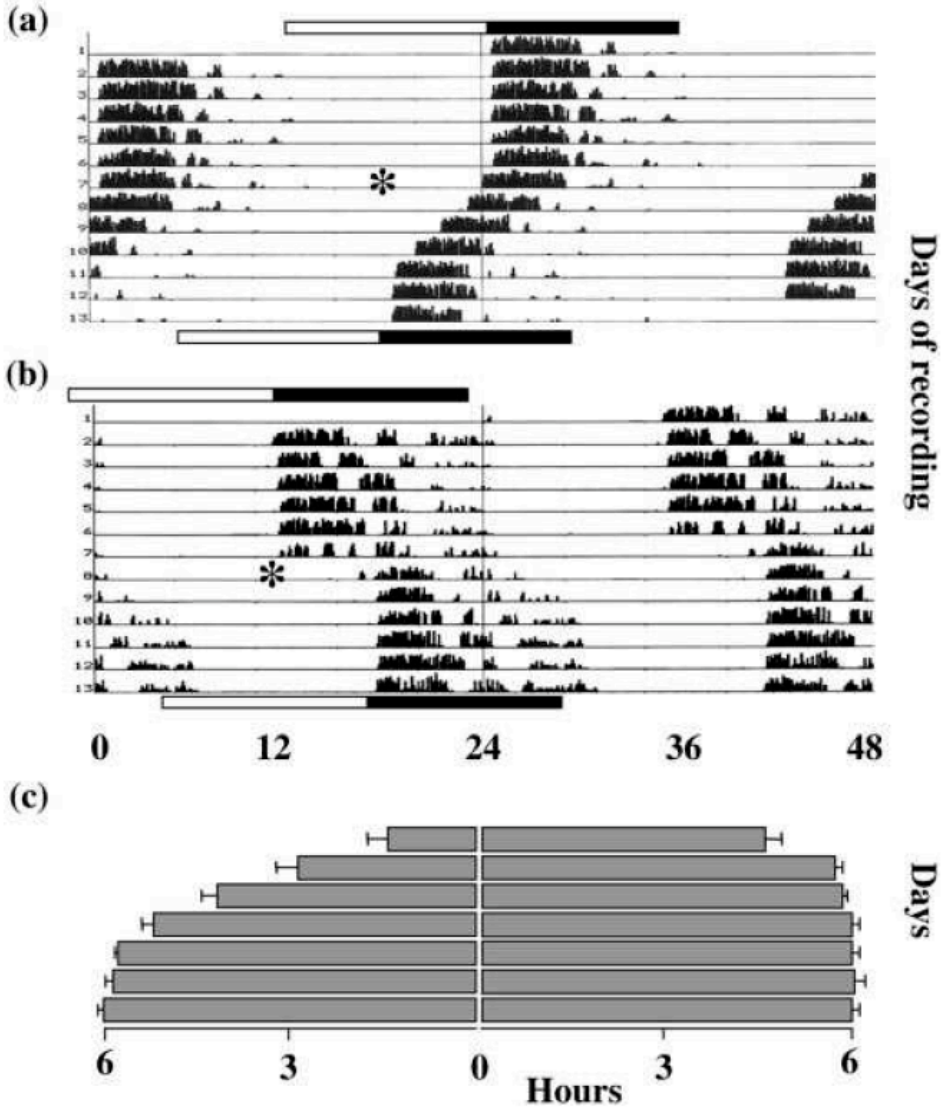
Jet lag

Jet lag

Resynchronization after a jet lag

Differential resynchronization of circadian clock gene expression within the suprachiasmatic nuclei of mice subject to a jet lag.





Reddy AB *et al.* (2002) *J Neurosci* 22:7326-30.

Jet lag

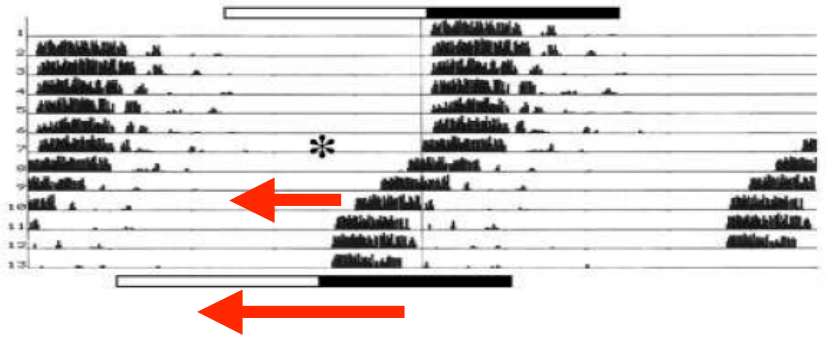
Orthodromic vs antidromic adjustment

Orthodromic re-entrainment occurs when circadian rhythms re-entrain by phase shifting in the **same direction** to the shift in external time.

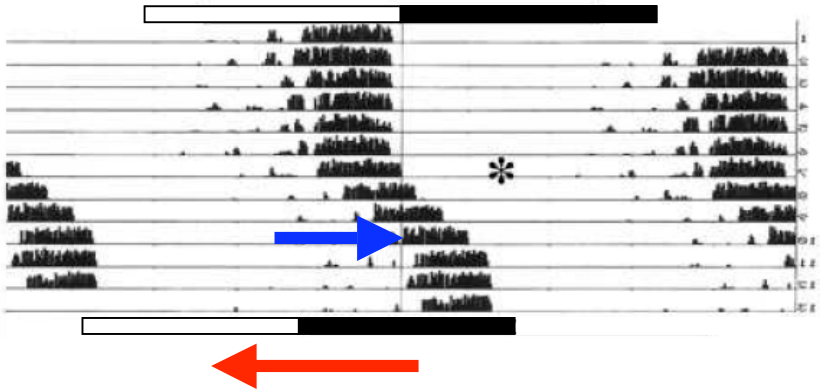
Antidromic re-entrainment occurs when circadian rhythms re-entrain by phase shifting in the **opposite direction** to the shift in external time, such as a phase delay instead of a phase advance after eastward travel.

(Burgess *et al.*, 2003, *J Biol Rhythms* 18:318-28)

Orthodromic adjustment



Antidromic adjustment



Antidromic shifts are quite common :

- **1 of 7 people** following an 8-h eastward flight.
  (Arendt *et al.*, 1987, *Ergonomics* 30:1379-93)

- **4 of 8 people** following a 9-h eastward flight.
  (Klein *et al.*, 1977, RR Mackie ed, 111-31, Plenum, NY)

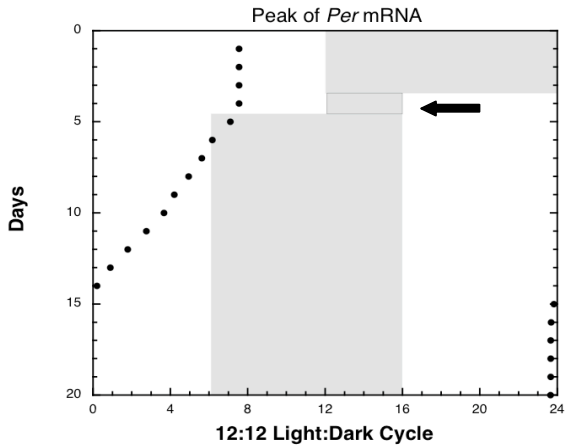
Source: JC Leloup

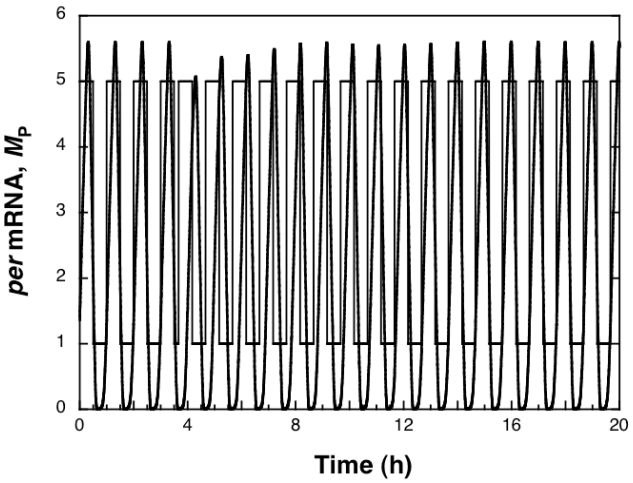
Jet lag

Orthodromic vs antidromic adjustment

Results obtained with the Leloup-Goldbeter model

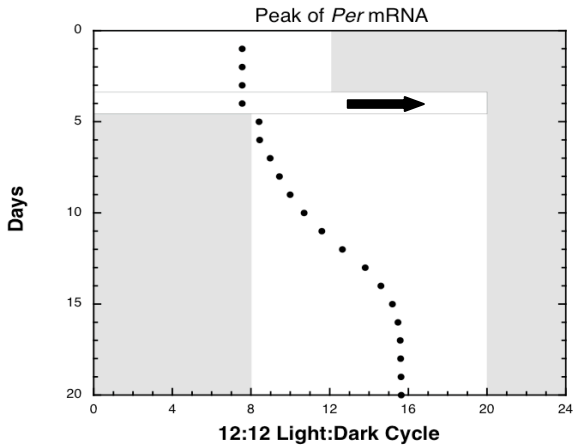
Phase Advance

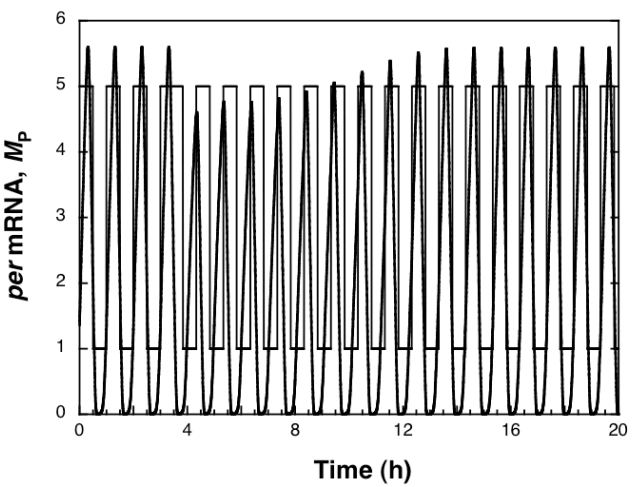




Orthodromic adjustment

Phase Delay





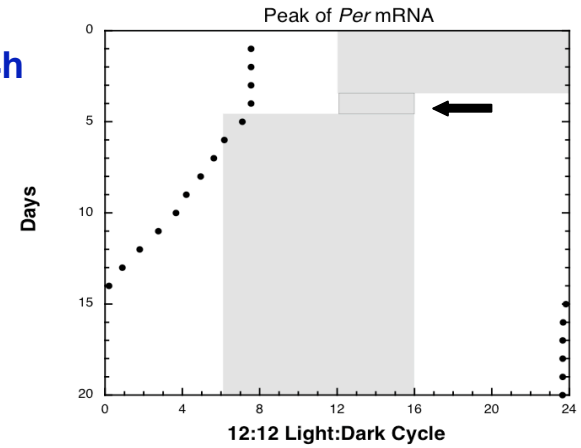
Jet lag

Orthodromic vs antidromic adjustment

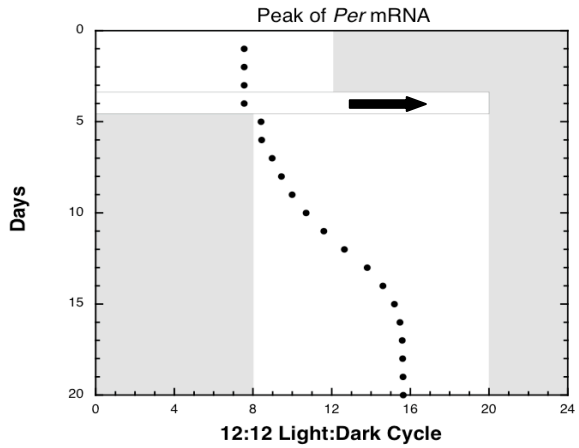
Phase Advance

Phase Delay

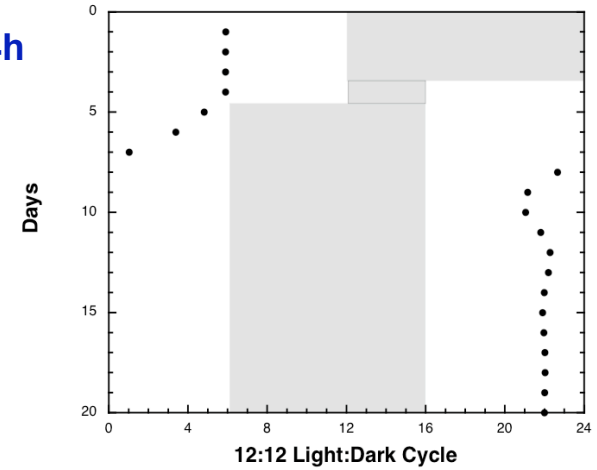
$\tau < 24h$

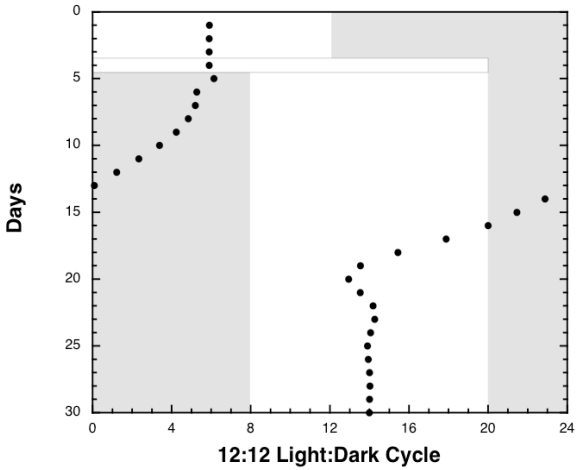


Orthodromic adjustment



$\tau > 24h$





Antidromic adjustment

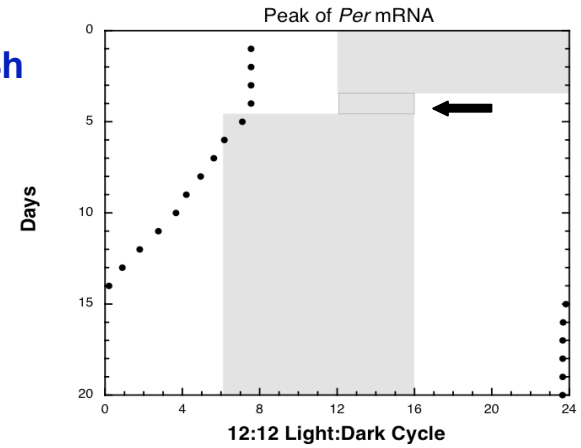
Jet lag

Orthodromic vs antidromic adjustment

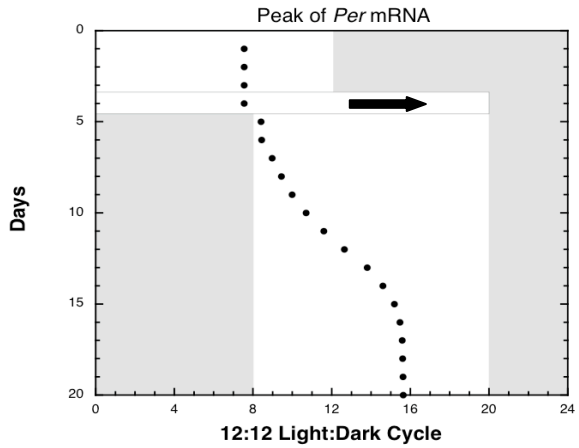
Phase Advance

Phase Delay

$\tau < 24h$



Orthodromic adjustment



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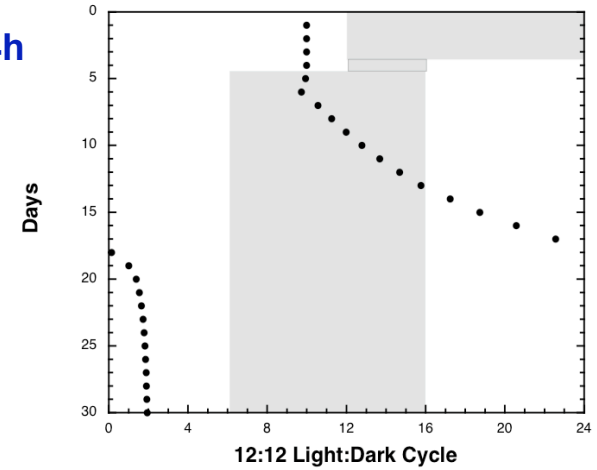
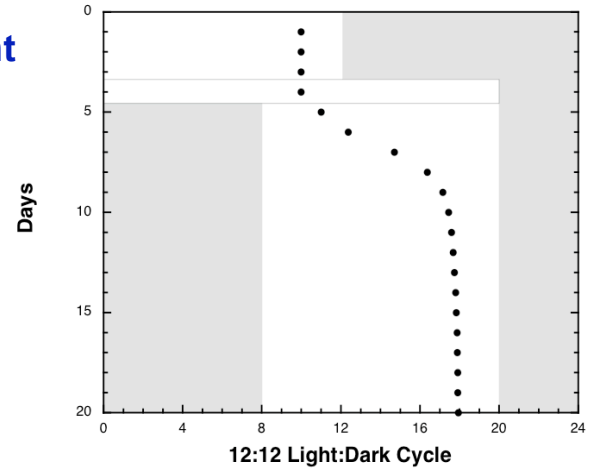
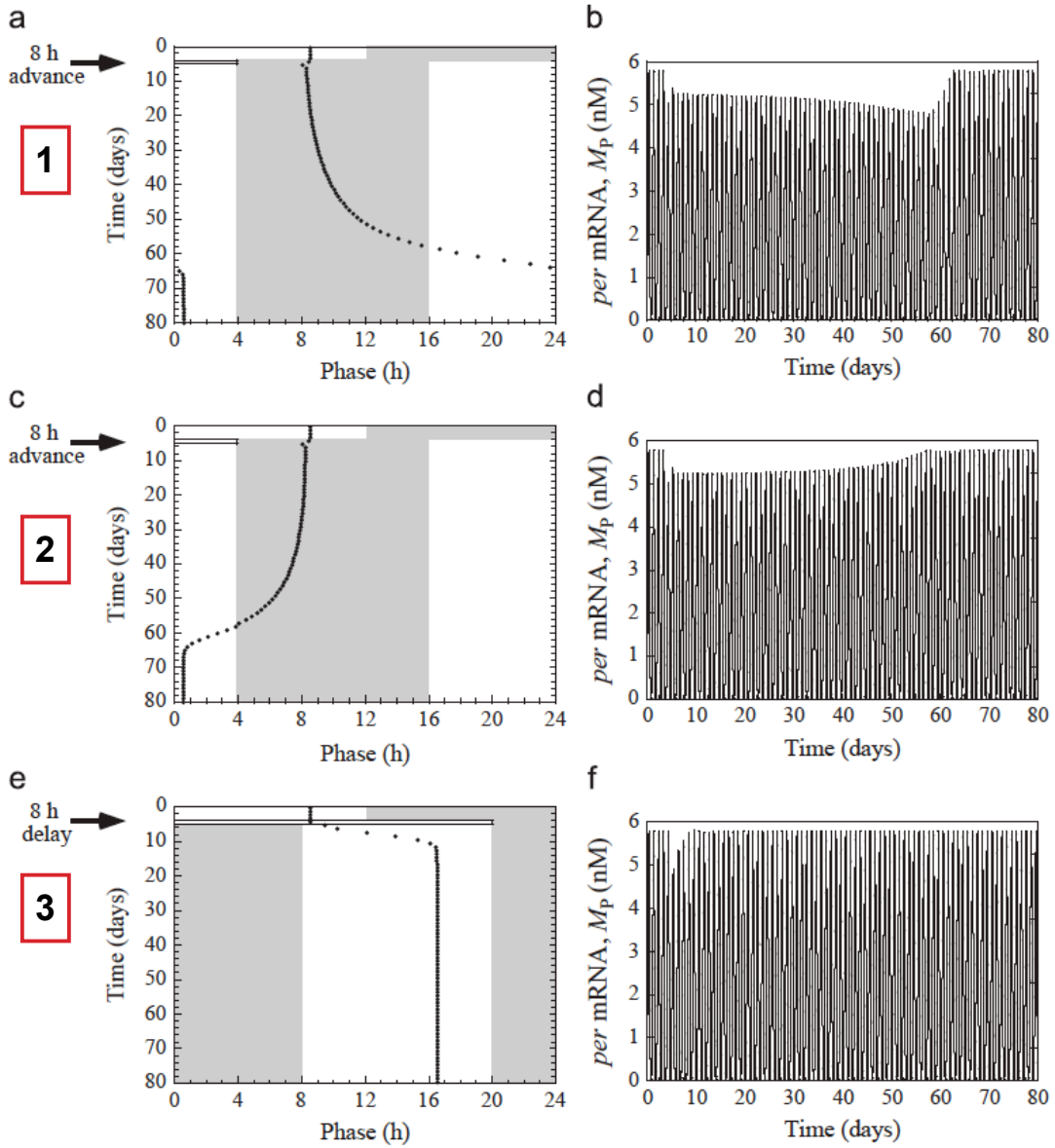


increased light intensity

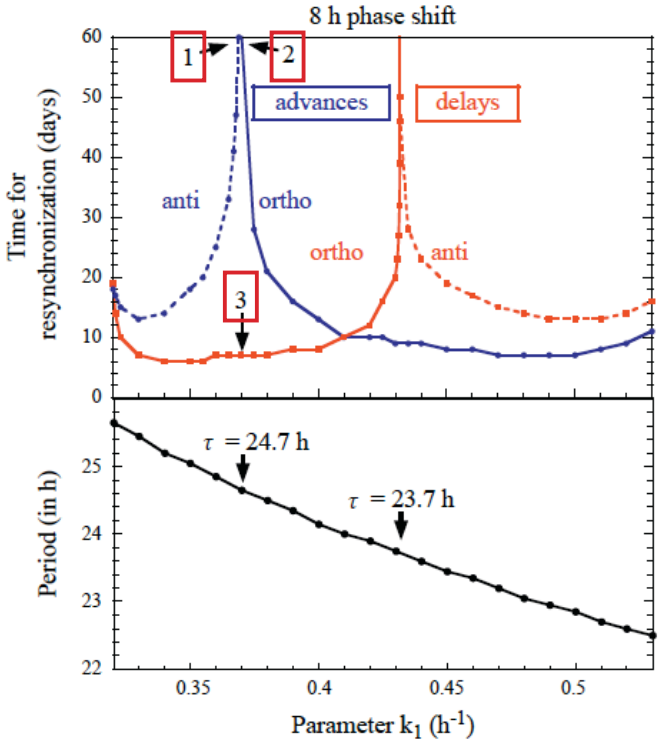
Antidromic adjustment



Jet lag



Slow resynchronization



The model predicts that in some particular conditions the resynchronization time can be very long

Leloup & goldbeter, JTB 2013

Jet lag

- The model shows that the resynchronization time (as well as the type of resynchronization) depends on various parameters(*):

  - Direction of the jet lag (advance vs delay)
  - Light intensity
  - Period of the endogeneous clock
  - Key kinetics parameters

- The model allows a systematic exploration of the effect of these factors.

- The model can then be used to predict protocols (i.e. when to apply a pulse of light, timing of melatonin treatment) in order to accelerate the resynchronization.

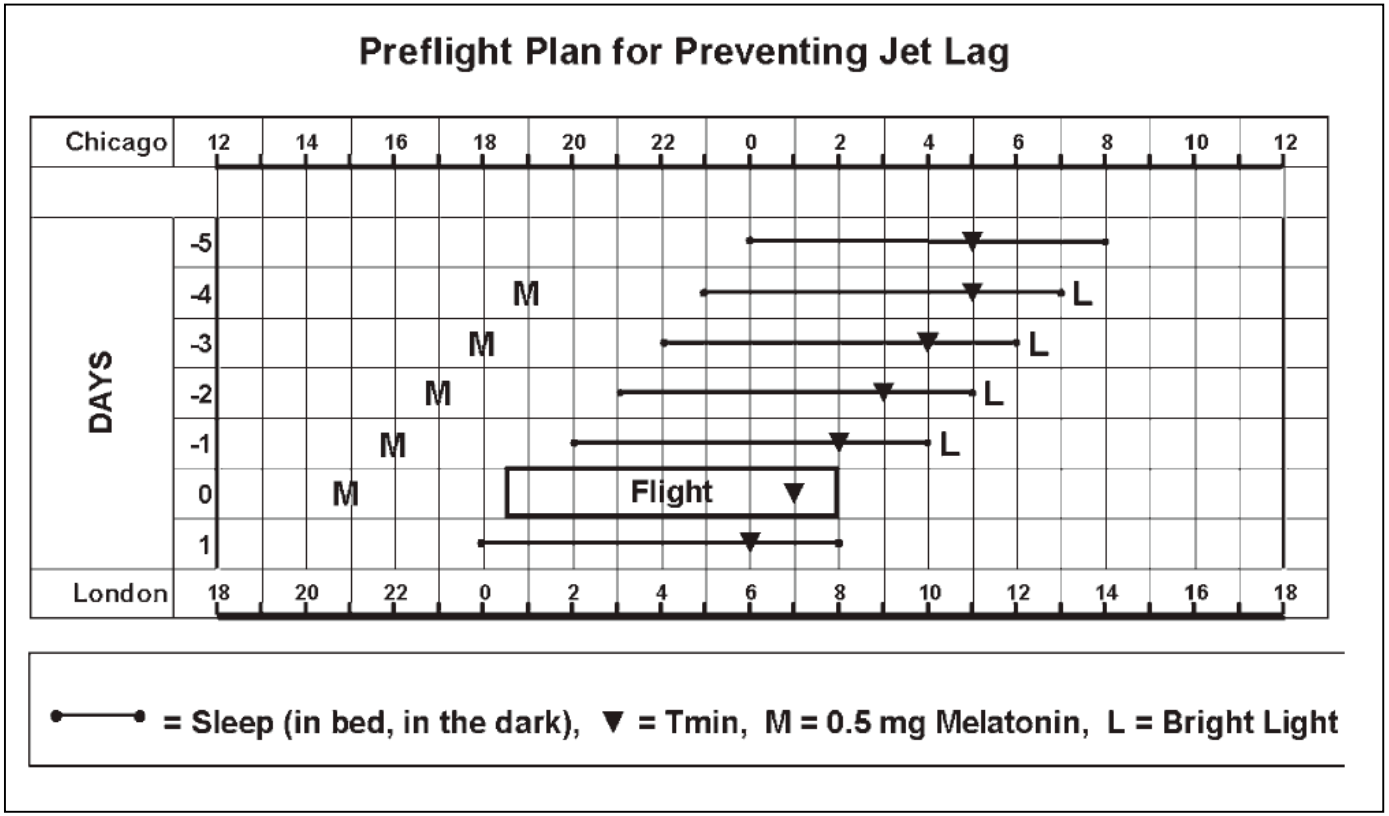
(*) Note that other factors influence the resynchronization: organ (SCN, lung, liver, kidney etc do not resynchronize at the same speed), age, season, gender, etc.

Jet lag

How to Trick Mother Nature into Letting You Fly Around or Stay Up All Night

Victoria L. Revell and Charmane I. Eastman[1]
Biological Rhythms Research Laboratory, Rush University Medical Center, Chicago, IL 60612

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 20 No. 4, August 2005 353-365



Jet lag

J Biol Rhythms. 2003 August ; 18(4): 318–328.

Preflight Adjustment to Eastward Travel: 3 Days of Advancing Sleep with and without Morning Bright Light

Helen J. Burgess[1], **Stephanie J. Crowley**, **Clifford J. Gazda**, **Louis F. Fogg**, and **Charmane I. Eastman**
Biological Rhythms Research Laboratory, Rush-Presbyterian-St. Luke's Medical Center, Chicago

Advancing Circadian Rhythms Before Eastward Flight: a Strategy to Prevent or Reduce Jet Lag

Charmane I. Eastman, Ph.D.[1]; Clifford J Gazda, B.A.[1]; Helen J. Burgess, Ph.D.[1]; Stephanie J. Crowley, B.A.[2]; Louis F. Fogg, Ph.D.[1]

[1]Biological Rhythms Research Laboratory, Psychology Department, Rush University Medical Center, Chicago, IL; [2]E.P. Bradley Hospital Sleep and Chronobiology Research Laboratory, Department of Psychology, Brown University, Providence, RI

Mammalian circadian clock: summary

Insights from the circadian clock models:

- Analysing and understanding complex situations that become difficult to describe in verbal terms and for which sheer intuition becomes unreliable.

  => Role of the balance PER/CRY for good entrainment

  => Suppression of rhythms by a light pulse

  => Complex regulation by multiple phosphorylations

- Rapid exploration of different mechanims and large ranges of conditions.

  => Systematic analysis of the LD condition to have entrainment

  => Systematic analysis of the resynchronization time after a jet lag

- Identification of key interactions and parameters, and their qualitative or quantitative influence for the system's behaviour.

  => Role of nuclear transport rate in *per* mutants in *Drosophila*

  => Role of PER phosphorylation in FASPS

- To address questions that are difficult or impossible to approach experimentally.

  => Clock design and robustness to noise

  => Mechanism of synchronization