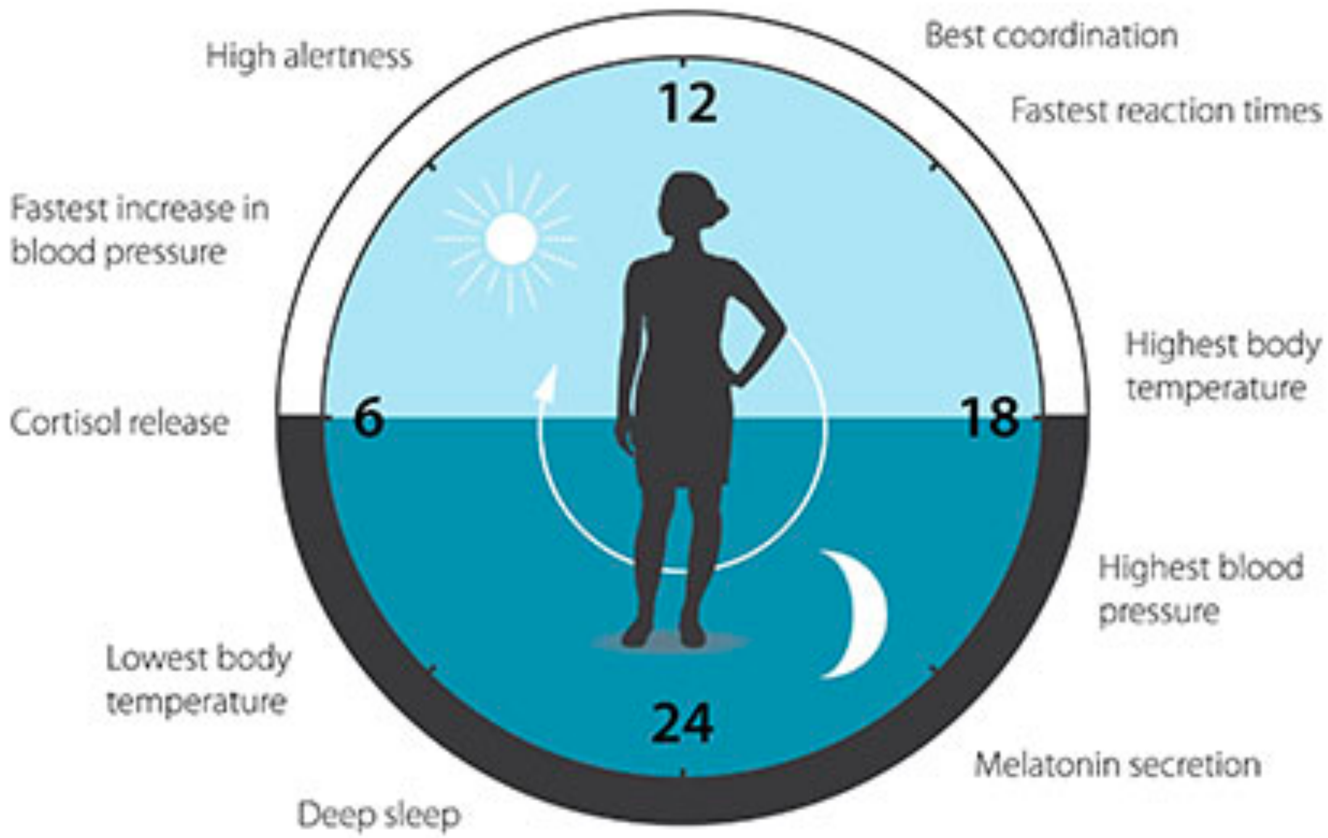
Circadian Rhythm: Molecular Mechanisms and Pharmacology



Scott Pedersen

MacMillan Group Meeting

January 10th, 2023

The Circadian Rhythm: The Clock of Life

A circadian rhythm or circadian cycle, is a natural, internal process that regulates the sleep–wake cycle and repeats roughly every 24 hours.

- Responsive to external stimuli (e.g. light, feeding, exercise)
- Widely observed in plants, animals, fungi, and cyanobacteria

But how does this work?

Outline

- Discovery of circadian rhythm
- Molecular mechanisms of mammalian circadian rhythms
- Overview of associated diseases and treatment landscape
- Case studies in modulating the circadian machinery

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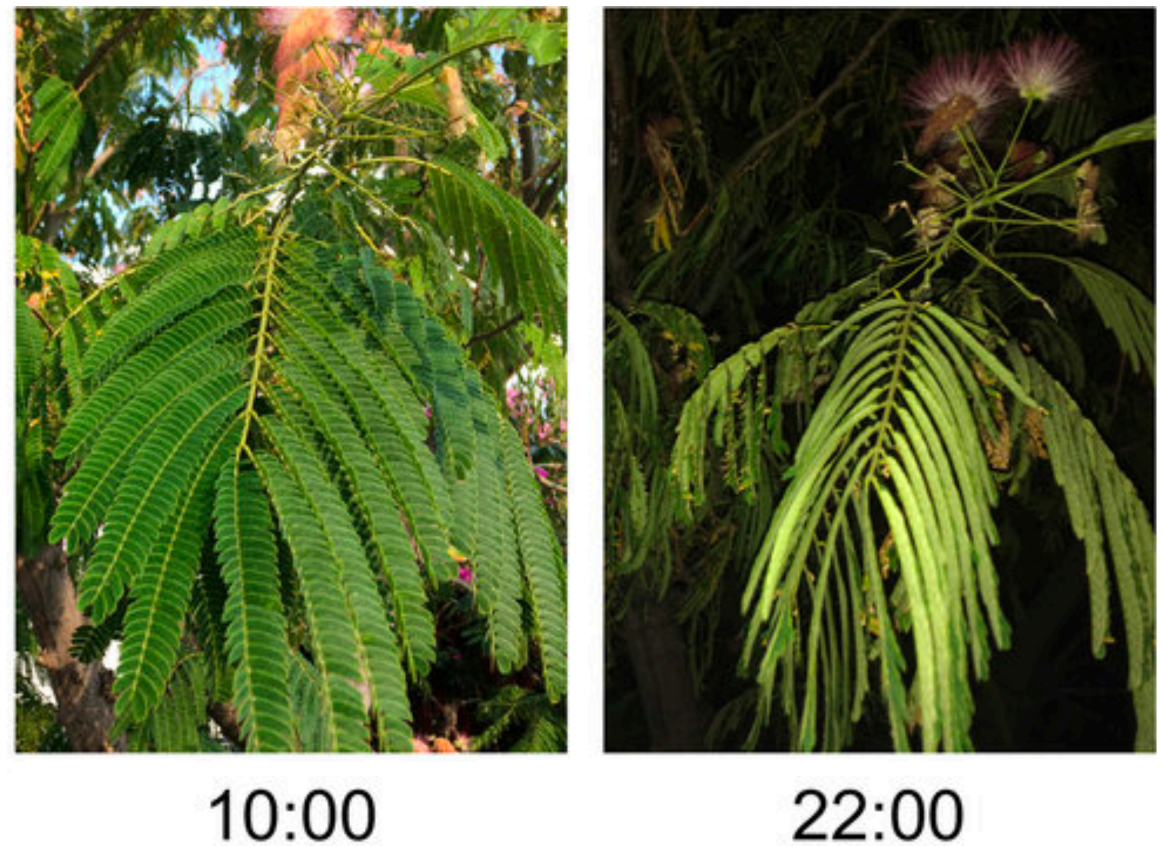
Circadian rhythms: a timeline

371 BC - First written account of a circadian rhythm

Theophrastus
Greek philosopher + father of botany
371 BC – 287 BC



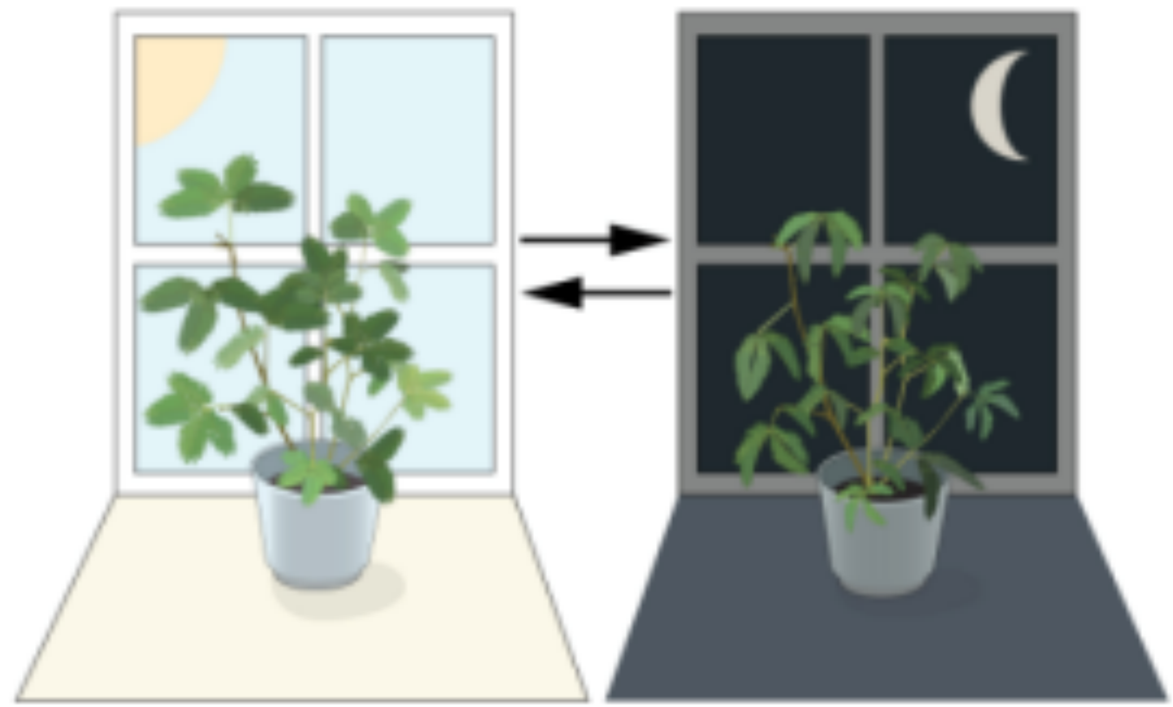
"… tree with many leaves like the rose, and that this closes at night, but opens at sunrise, and by noon is completely unfolded; and at evening again it closes by degrees and remains shut at night, and the natives say that it goes to sleep."

Theophrastus, 'Περὶ φυτῶν ἱστορία' (1916) Enquiry into plants. Translation by Sir Arthur Hort. *London W. Heinemann.*

Circadian rhythms: a timeline

1729 - first controlled experiment implicating a "biological clock"

Jean-Jeaque de Mairan
French scientist
1678–1771



Mimosa pudica

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Circadian rhythms: a timeline

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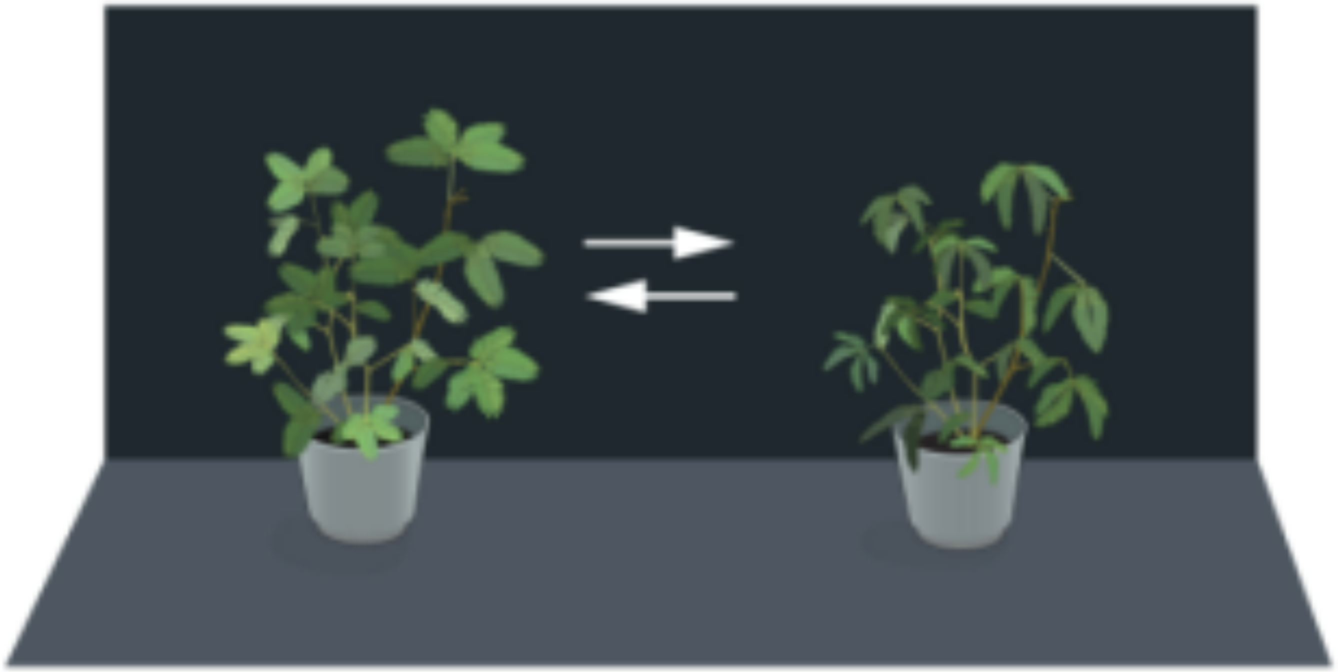


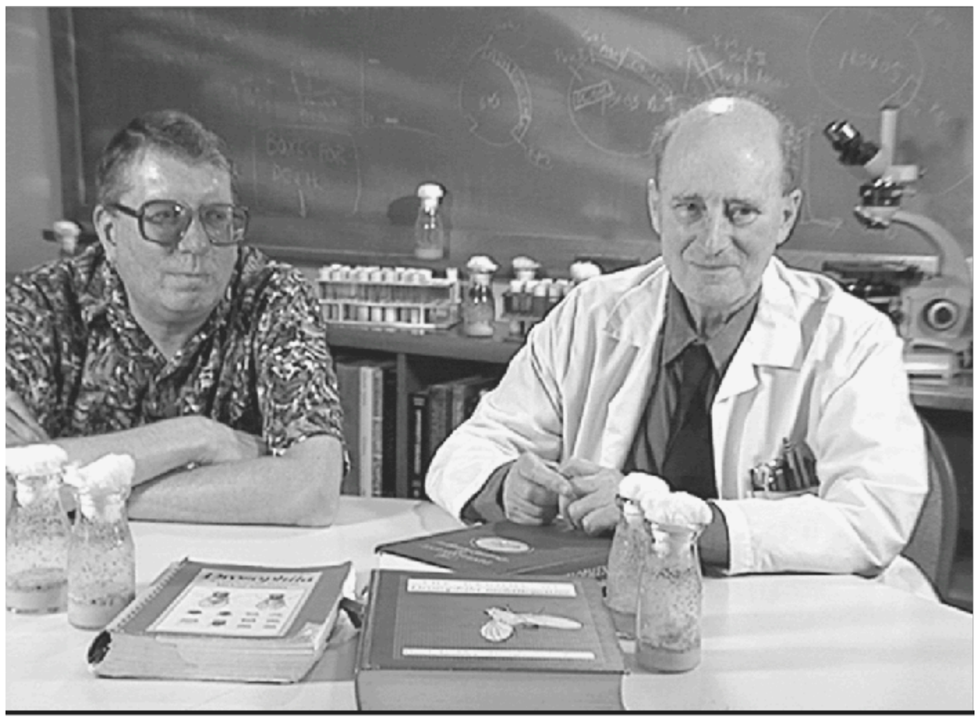
Mimosa pudica

Continued adherence to the daily rhythm in an unlit environment suggested a free running, intrinsic biological timer

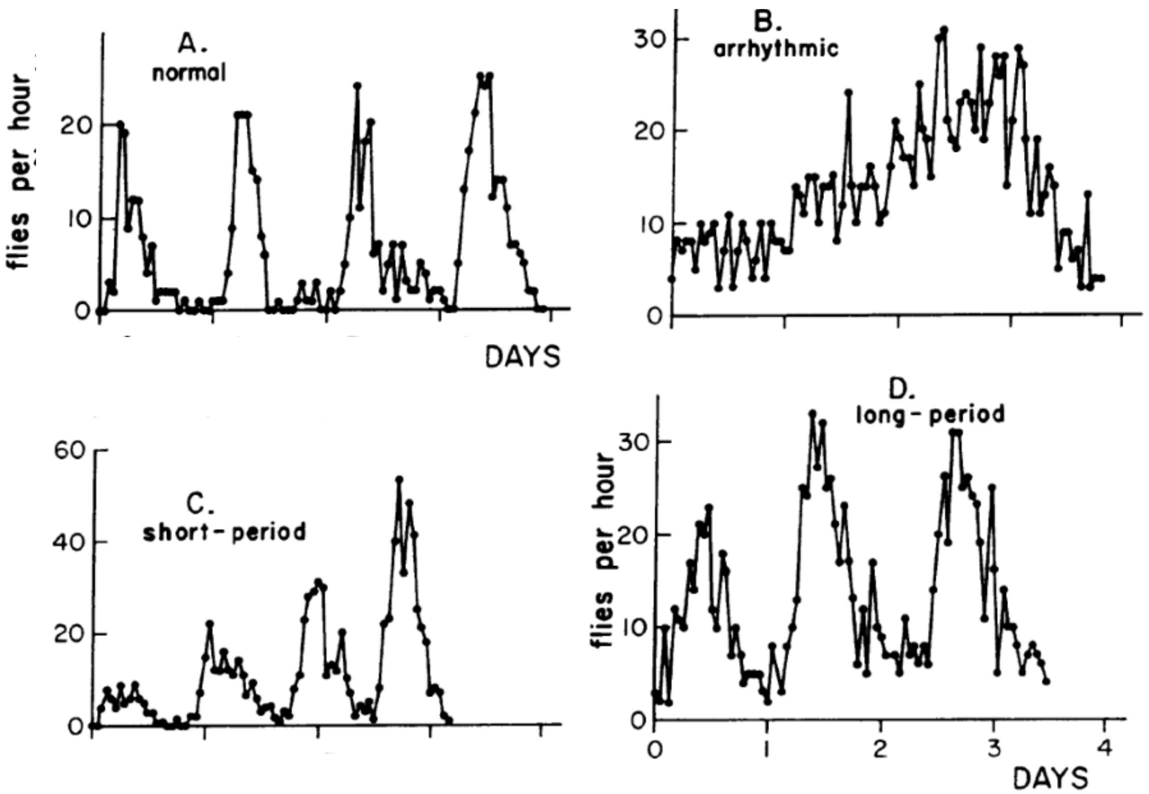
Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Circadian rhythms: a timeline

1971 - genetic link discovered for the first time



Ron Konopka (left) **Seymore Benzer** (right)



- 1971 Konopka and Benzer discover three genetic mutants of *drosophila* with altered circadian rhythms
- Different rhythms in eclosion (hatching) and locomotion were observed
- Mutations mapped to the same locus, subsequently named *period*

Konopka, R., J.; Benzer, S. *Proc. Nat. Acad. Sci.* **1971**, *68*, 2112.

Circadian rhythms: a timeline

1984 - drosophila period gene isolated

Jeffrey Hall

Michael Rosbash

Michael Young

Key finding: certain subsegments of the per region would restore rhythmicity in

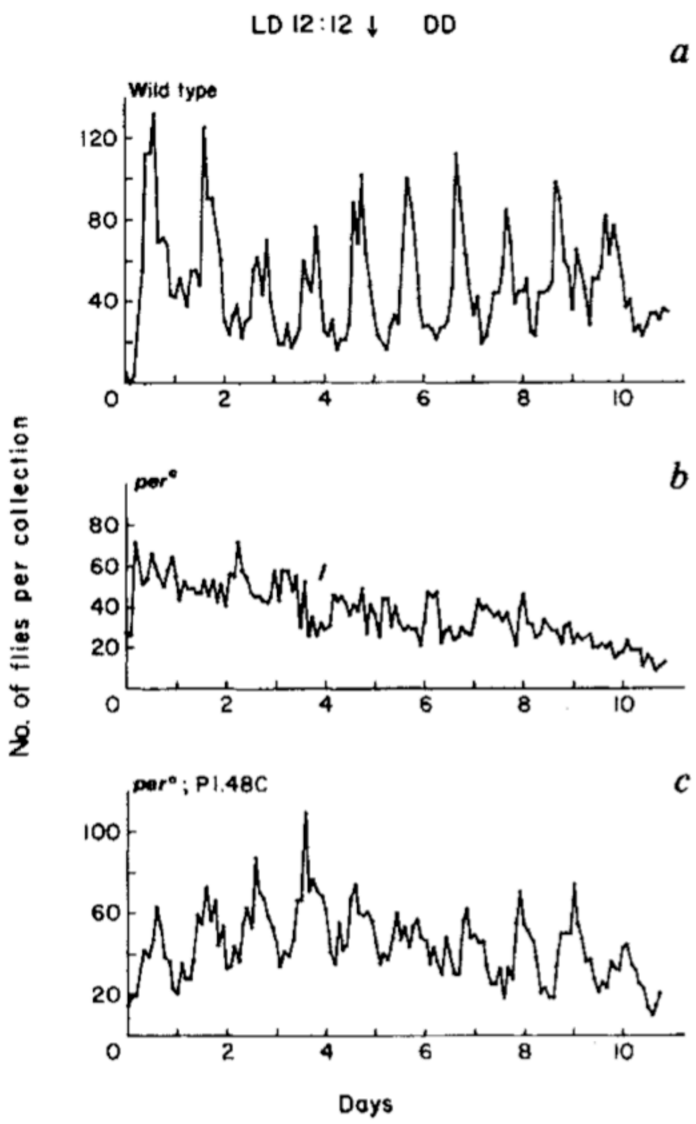
circadian locomotor behavior transduced into the genome of arrhythmic flies

Zehring, W. A. et al. *Cell*. **1984**, *39*, 369.

Bargiello, T. A.; Jackson, F. R.; Young, M. W. *Nature*. **1984**, *312*, 752.

Circadian rhythms: a timeline

1984 - drosophila period gene isolated



Wild type: eclosion occurs with rhythmicity

Per mutant: no rhythmicity in eclosion

Rhythmicity restored when *per*

gene transduced into mutant flies

Implication of a transcription/translation feedback loop

Zehring, W. A. et al. *Cell*. **1984**, *39*, 369.

Bargiello, T. A.; Jackson, F. R.; Young, M. W. *Nature*. **1984**, *312*, 752.

Circadian rhythms: a timeline

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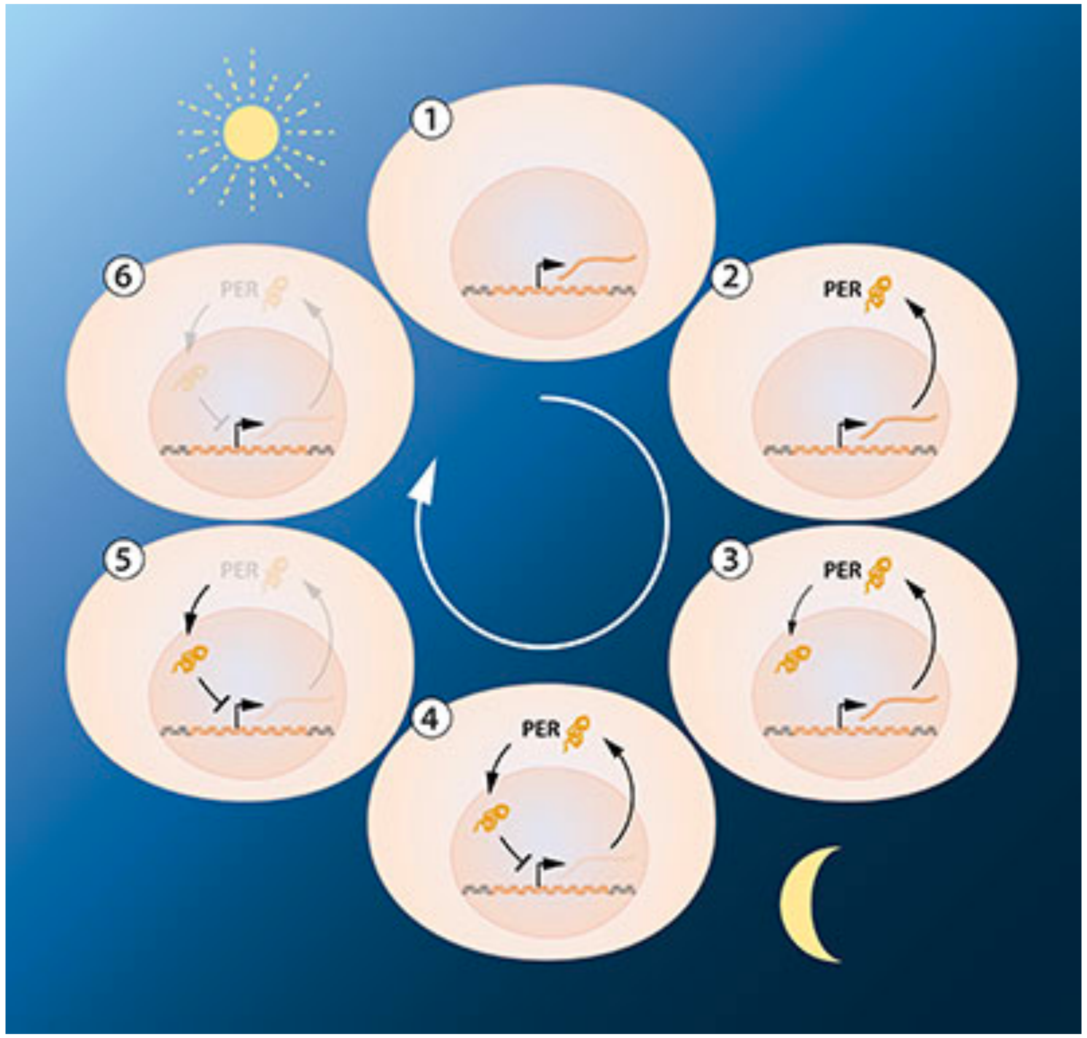


Per and the circadian clock

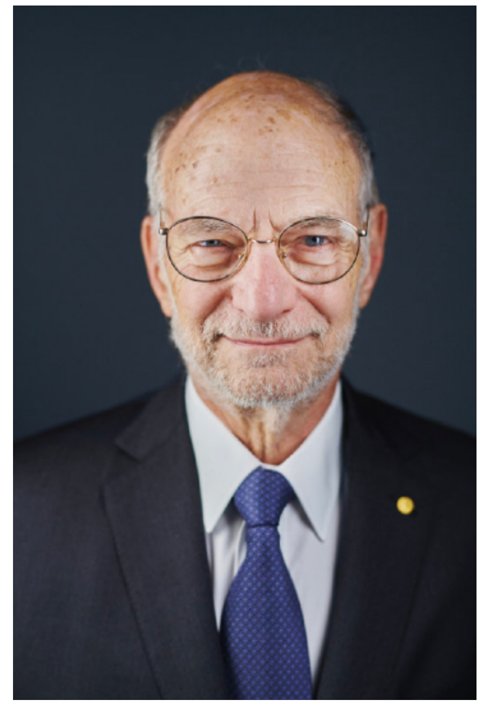
- *period* DNA transcribed into *period* mRNA
- *period* mRNA is transported into cytoplasm
- *period* RNA translated into *PER* protein
- *PER* protein accumulates in cell's nucleus
- *PER* protein inhibits transcription of *period* gene
- PER protein cleared by cell, resetting cycle

Implication of a transcription/translation feedback loop

The Nobel Prize in Physiology and Medicine 2017. The Nobel Prize. https://www.nobelprize.org/prizes/medicine/2017/press-release/ (accessed Jan 6 2023)

Circadian rhythms: a timeline

Jeffrey Hall



Michael Rosbash

Michael Young

The Nobel Prize in Physiology and Medicine 2017 was awarded jointly to
Jeffrey C. Hall, Michael Rosbash, and Michael W. Young

“for their discoveries of molecular mechanisms controlling the circadian rhythm”

The Nobel Prize in Physiology and Medicine 2017. The Nobel Prize. https://www.nobelprize.org/prizes/medicine/2017/press-release/ (accessed Jan 6 2023)

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- Discovery of circadian rhythm
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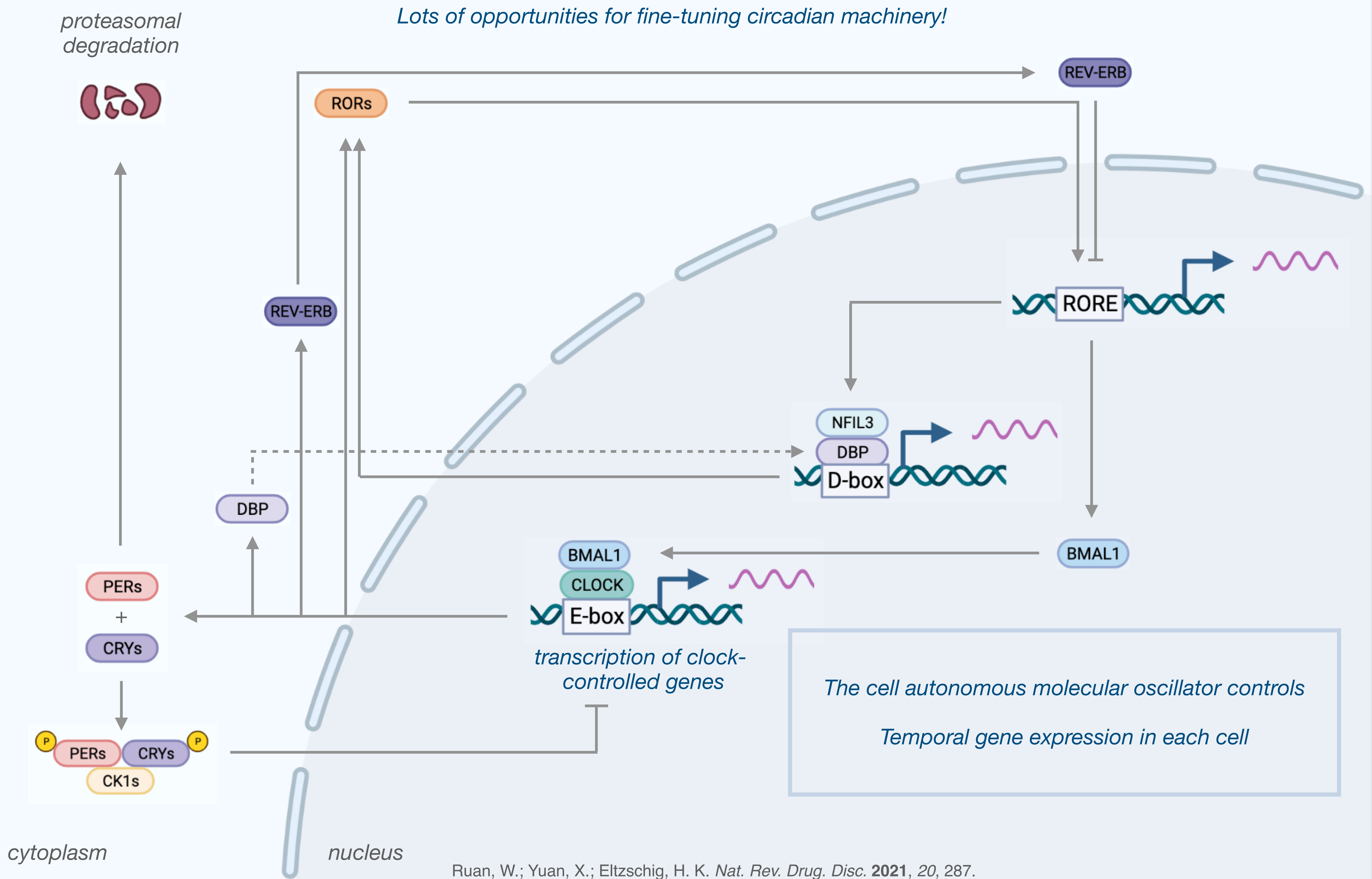
The Ubiquitous, Cell-Autonomous Molecular Oscillator

Lots of opportunities for fine-tuning circadian machinery!

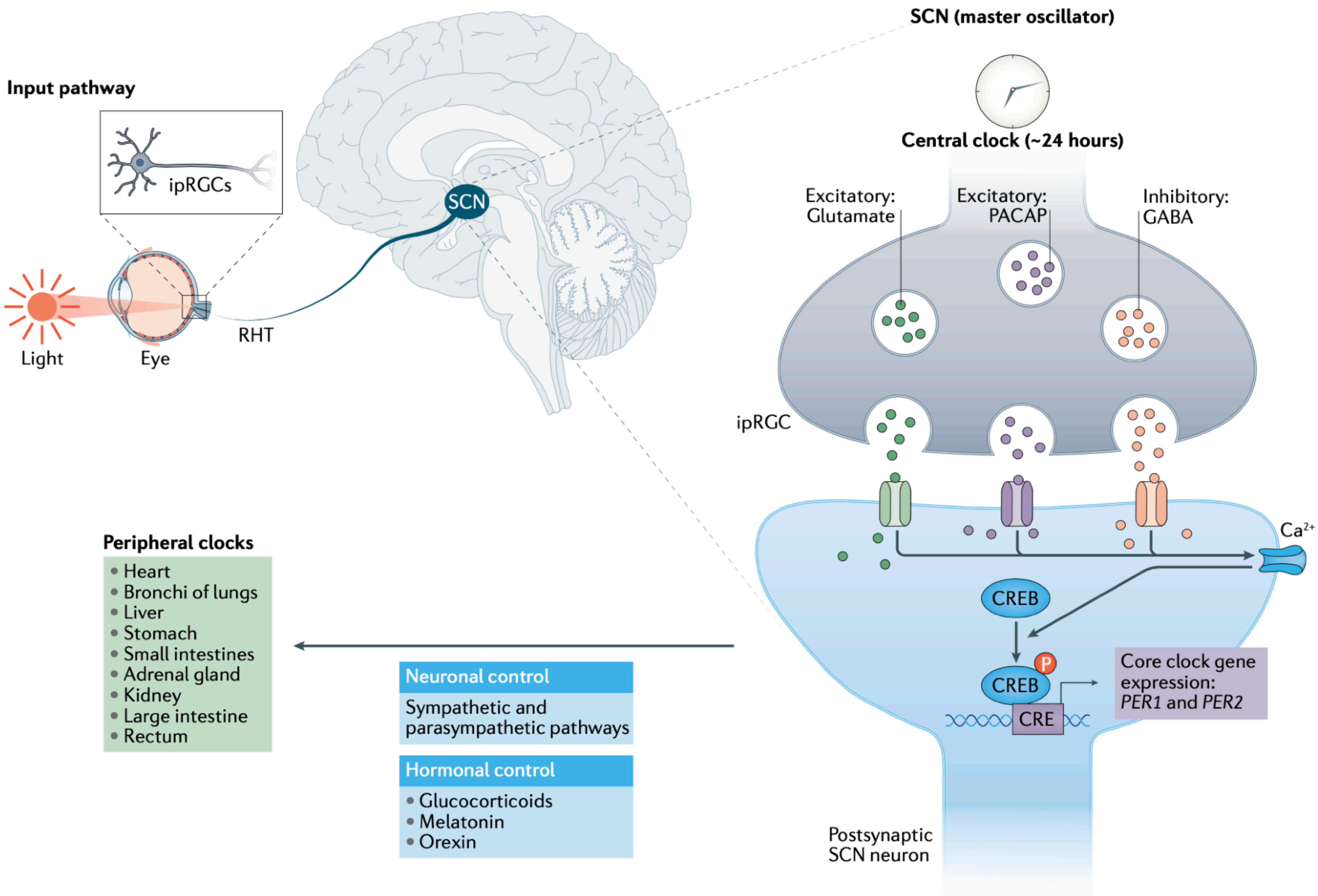


The cell autonomous molecular oscillator controls

Temporal gene expression in each cell

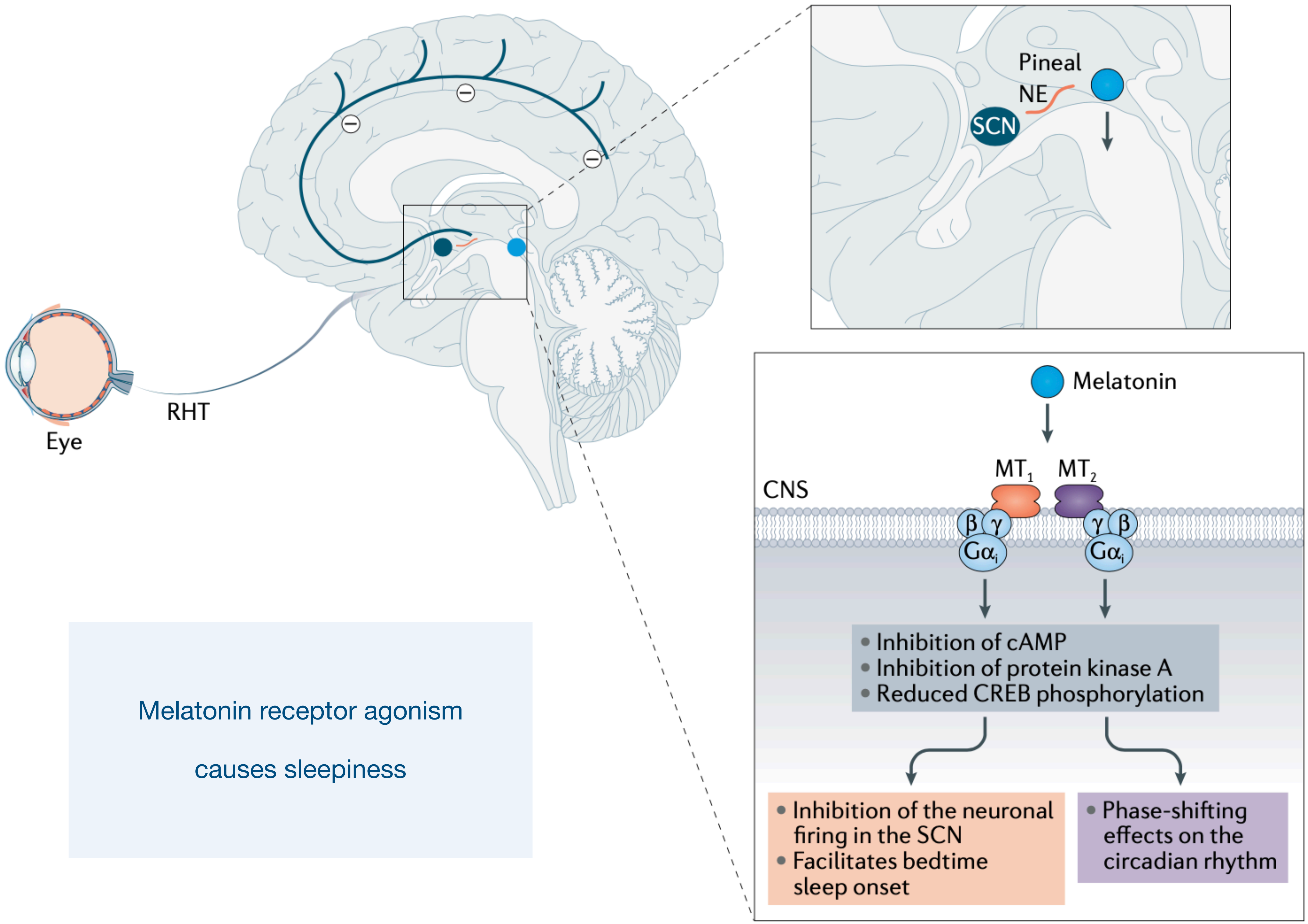
Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Suprachiasmatic Nucleus (SCN): The Master Oscillator



Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Hormonal signaling from the SCN: melatonin

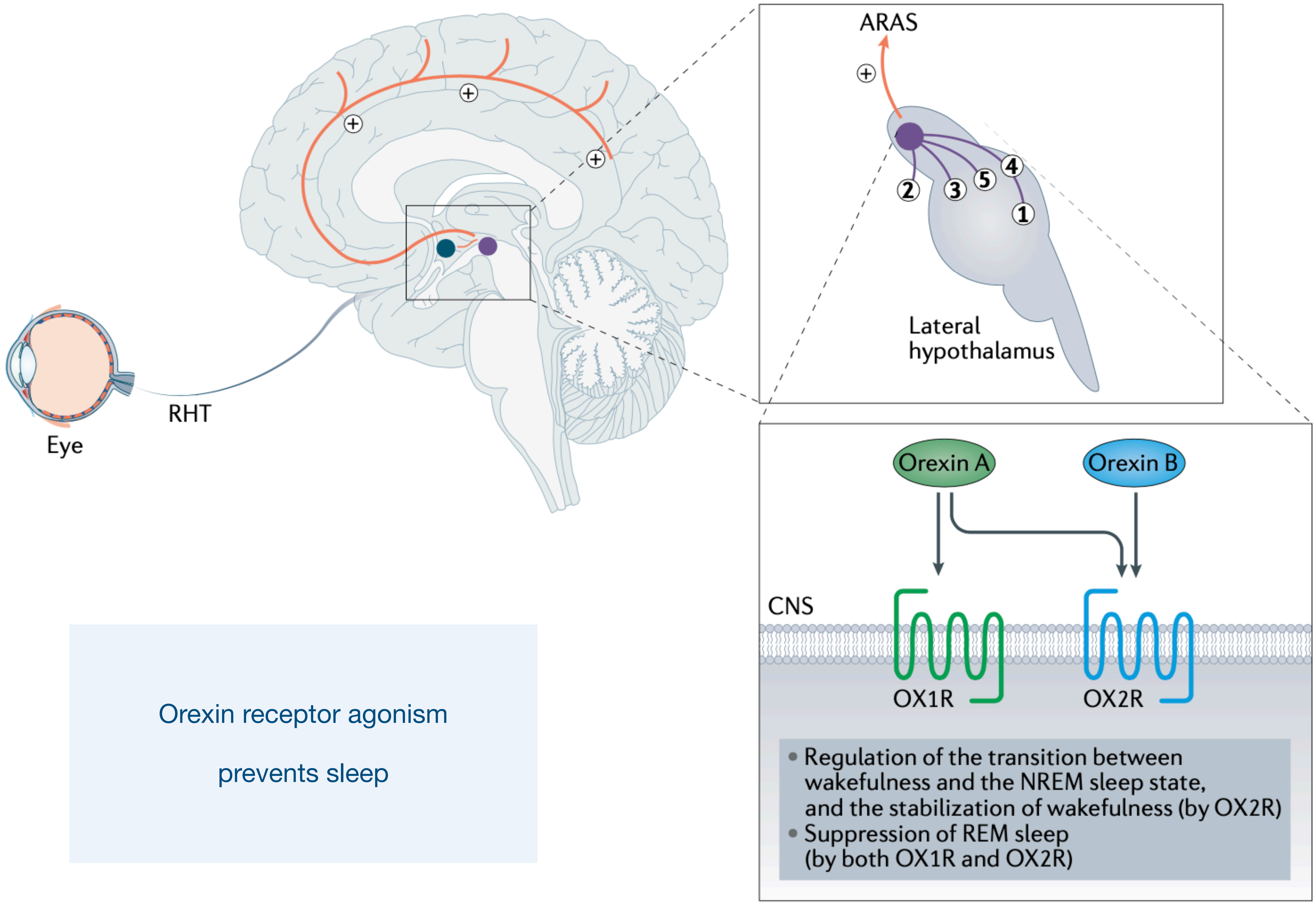


Melatonin receptor agonism

causes sleepiness

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Hormonal signaling from the SCN: orexin



Orexin receptor agonism

prevents sleep

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Outline

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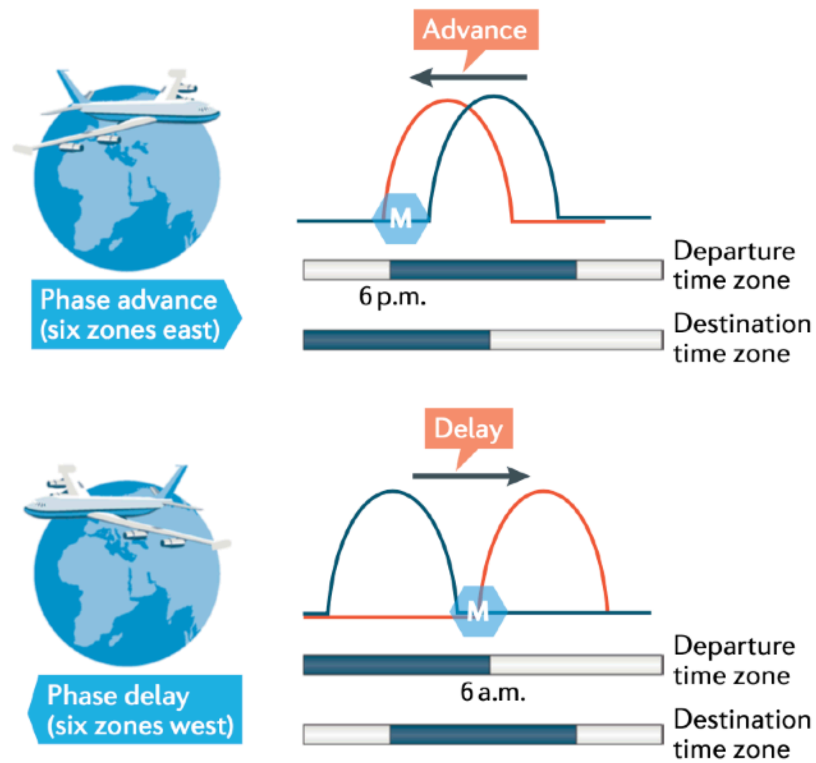
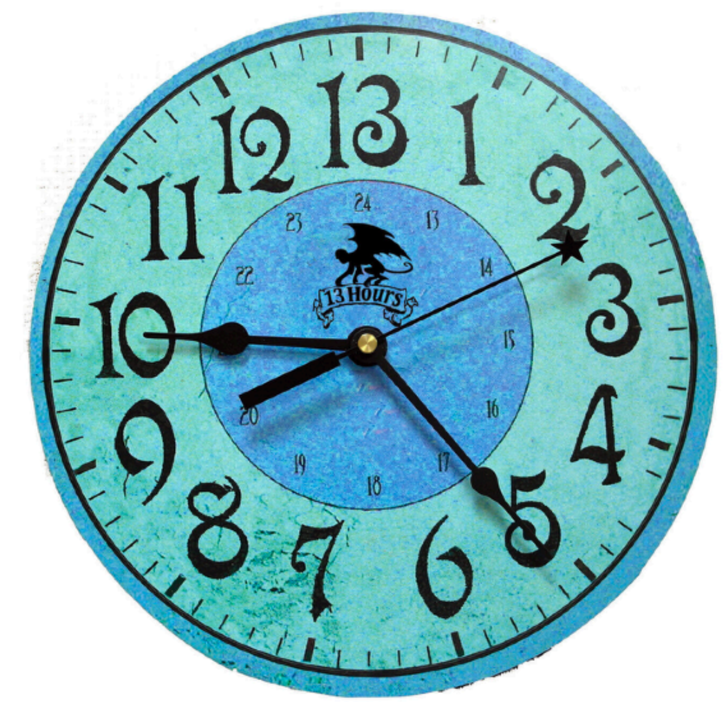
Some common sleep disorders

Insomnia



Jet Lag or Shiftwork

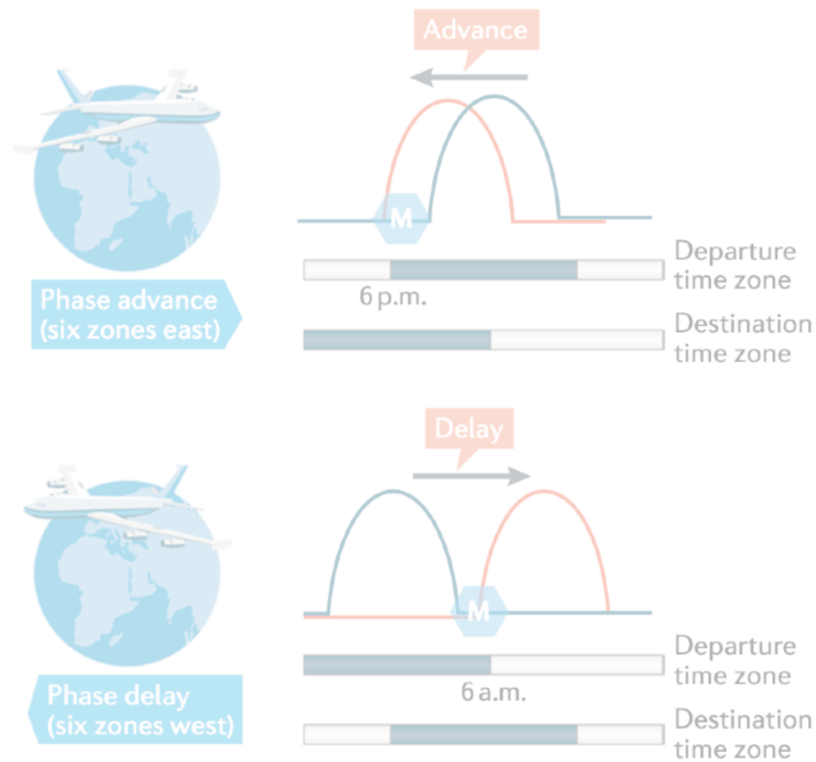


Non-24

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Some common sleep disorders

Insomnia



Jet Lag or Shiftwork

Non-24

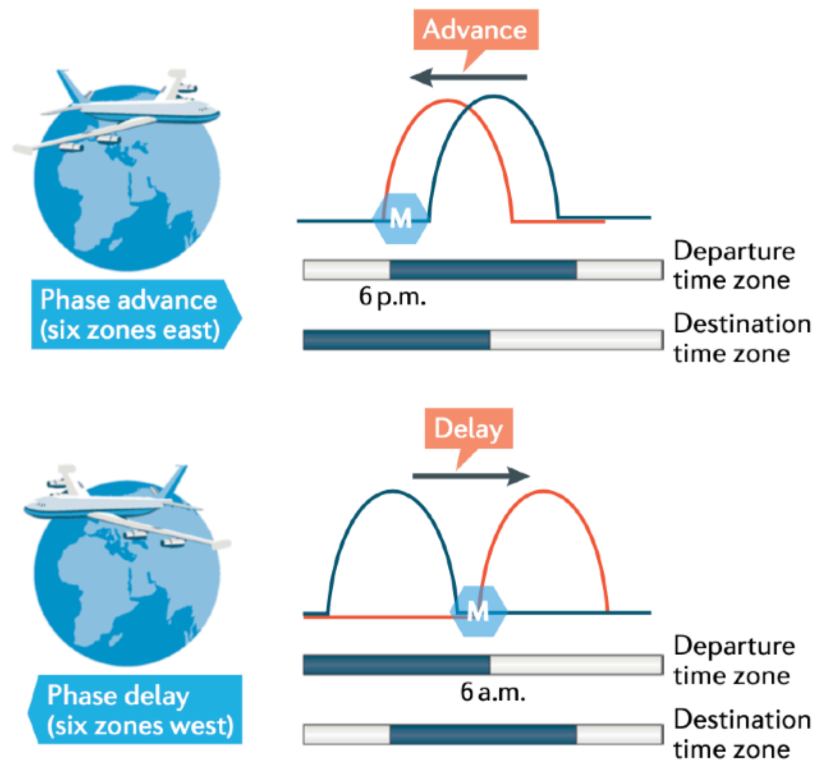
25% of Americans develop insomnia each year;
10% progress to chronic insomnia

Frequently associated with anxiety, depression,
risk of cardiovascular disease, and poor quality of life

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Some common sleep disorders

Insomnia



Jet Lag or Shiftwork

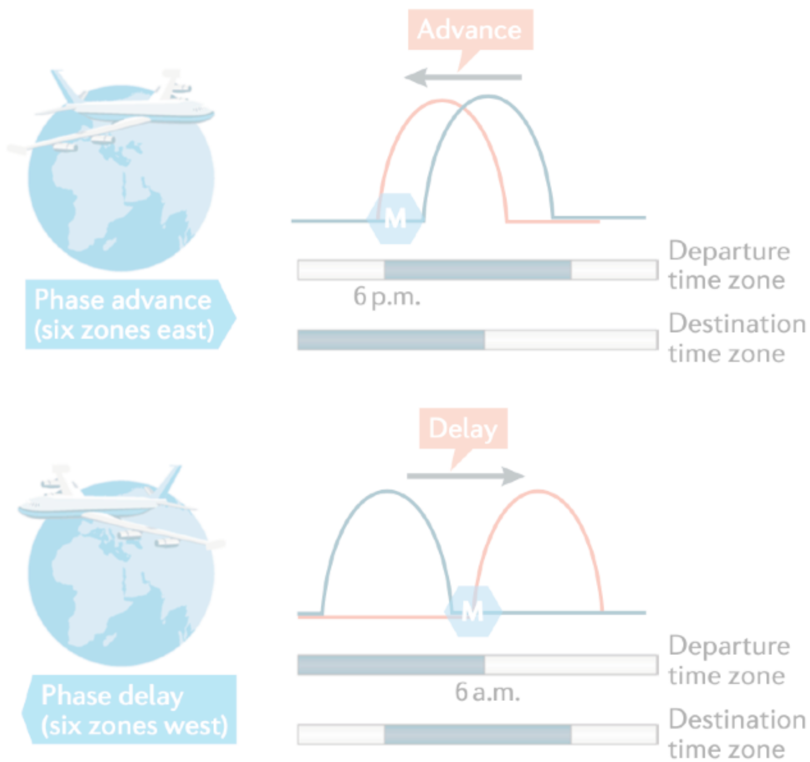
Non-24

Mice subjected to a chronic jet lag paradigm (8-hour phase shift)
Showed significantly reduced lifespan with disease development, including neurodegeneration, severe ulcerative dermatitis, aging, cystic renal dysplasia, and cancer

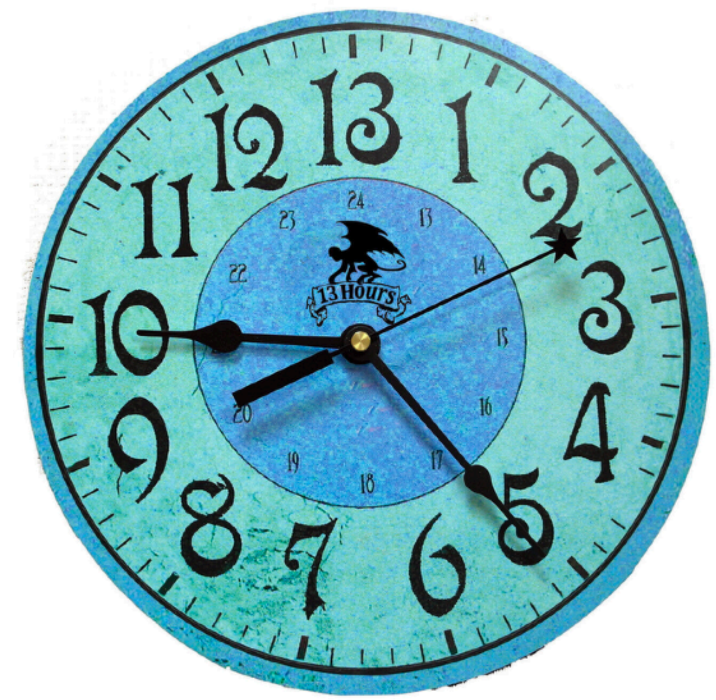
Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Some common sleep disorders

Insomnia



Jet Lag or Shiftwork



Non-24

chronic steady pattern comprising daily delays in sleep onset and wake times in an individual living in a society

>50% of the totally blind suffer from this affliction, rarely occurs in sighted people

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Circadian rhythm signaling in disease

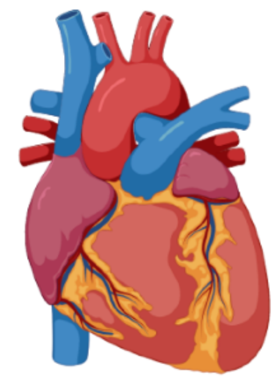
Circadian rhythm aberrations are implicated

in many chronic and acute illnesses

Rescuing or repairing circadian rhythm could

Minimize the progression of or outright

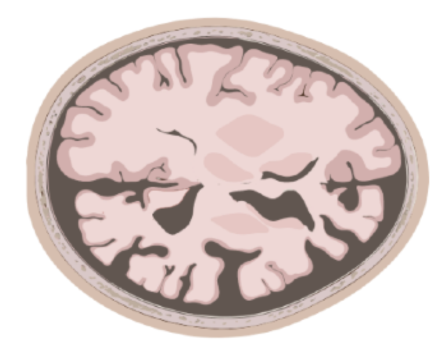
prevent a wide range of diseases



Heart attack/ stroke

Daily headaches

Aging

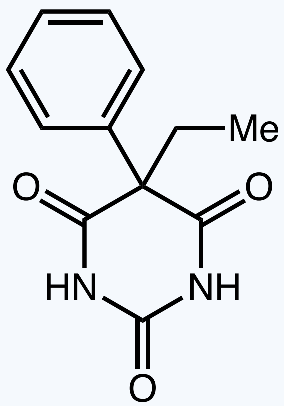


Alzheimer's

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

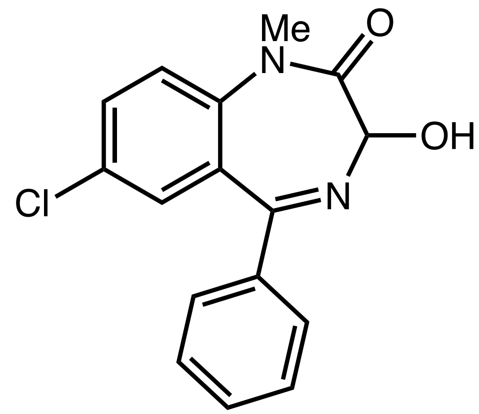
Current landscape of sleep medicine

barbiturates (1903)

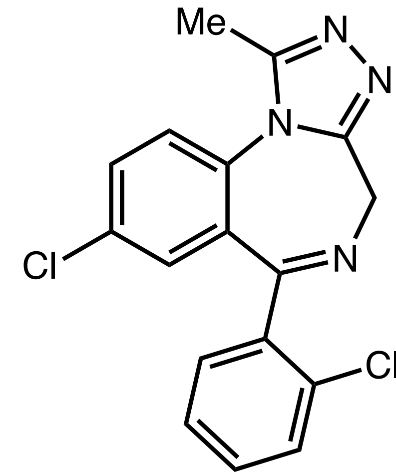


Phenobarbital
(Luminal)

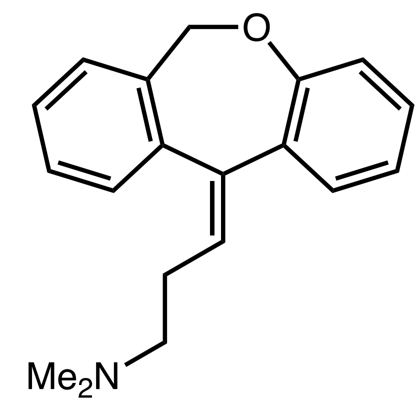
benzodiazepines (1960s)



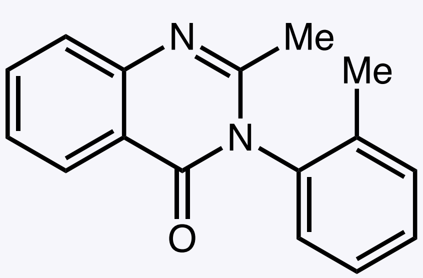
temazepam
(Restoril)



triazolam
(Halcion)

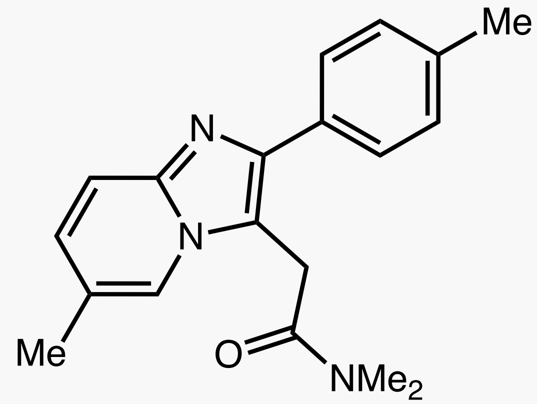


doxepin

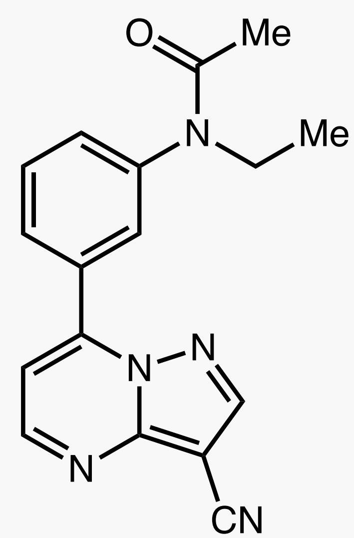


methaqualone (1961)
(Quaalude)

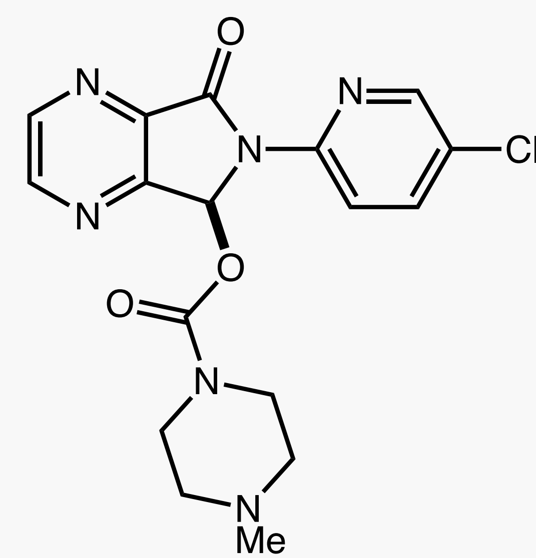
non-benzodiazepines (1980s)



zolpidem
(Ambien)



zaleplon
(Sonada)



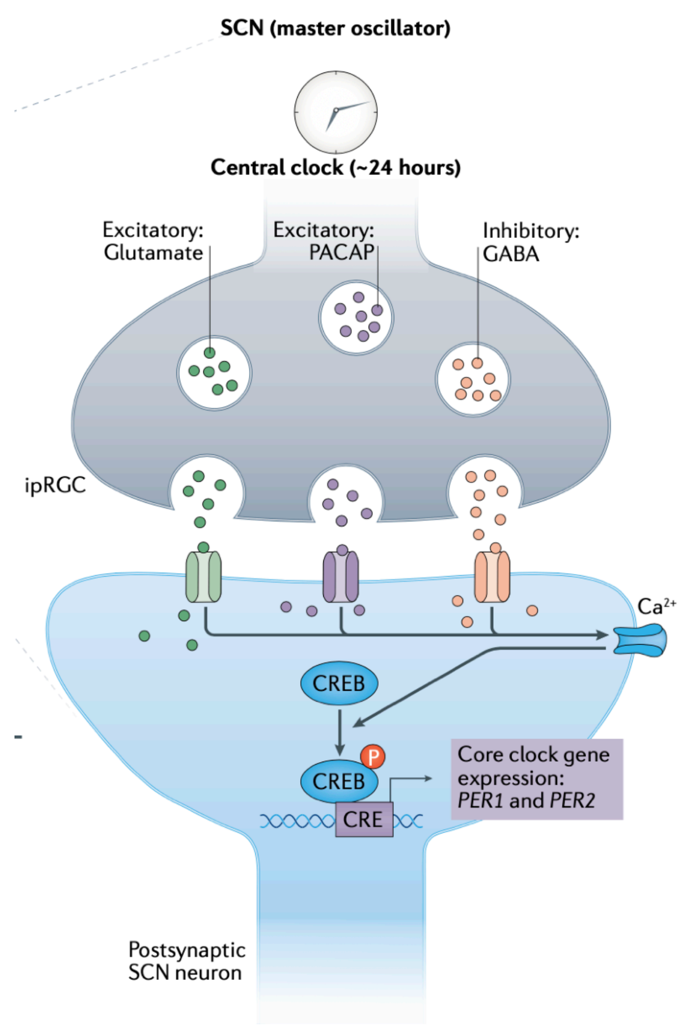
eszopiclone
(Lunesta)

Coleman, P.J. *et al. Annu. Rev. Pharmacol. Toxicol.* **2017**, *57*, 509.

Current landscape of sleep medicine

Why do all of these medicines exhibit similar negative effects?

All either act as $GABA_A$ receptor agonists or positive allosteric modulators



Miller, P.S.; Aricescu A. R. *Nature* **2014**, *512*, 270.

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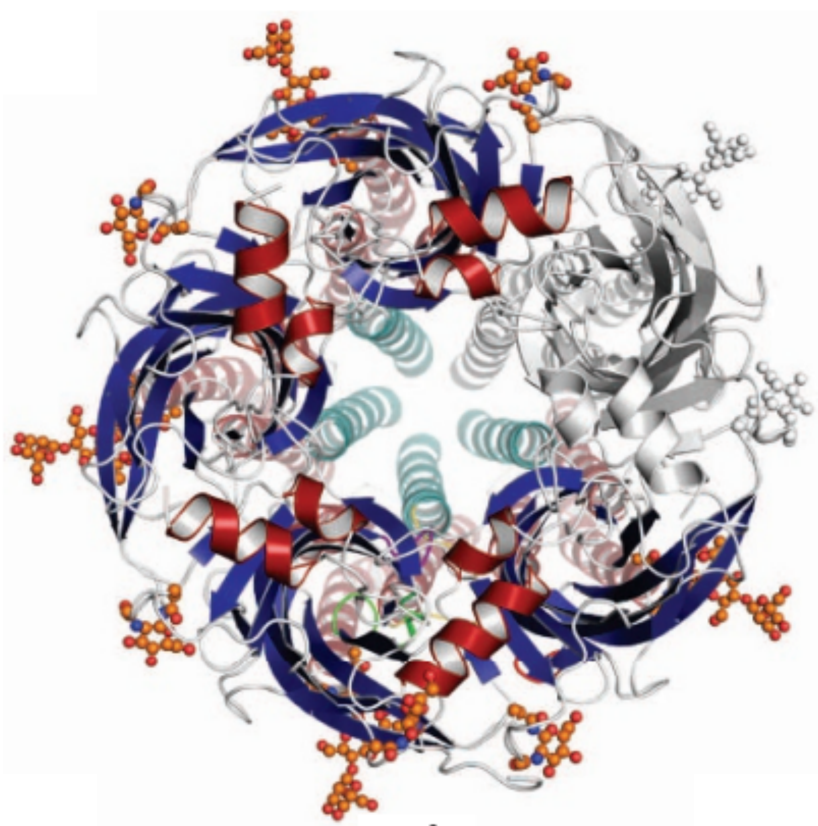


- Activates chloride ion flux in neurons
- inhibit the propensity of neurons containing $GABA_A$ to propagate action potentials
- change in mood, slowed reaction time, motor deficits, amnestic effects, and respiratory effects

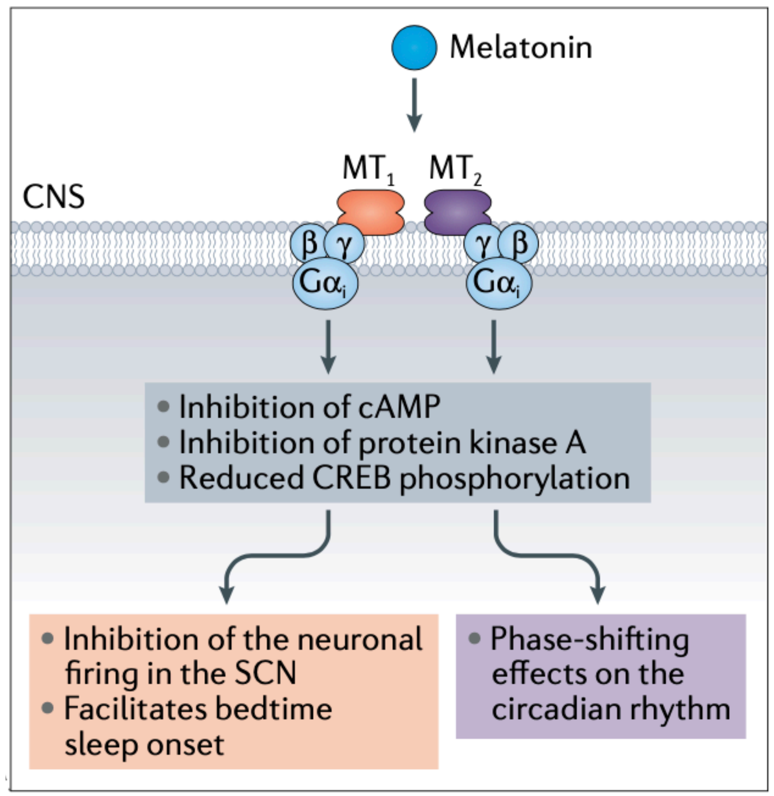
Miller, P.S.; Aricescu A. R. *Nature* **2014**, *512*, 270.

Coleman, P.J. *et al. Annu. Rev. Pharmacol. Toxicol.* **2017**, *57*, 509.

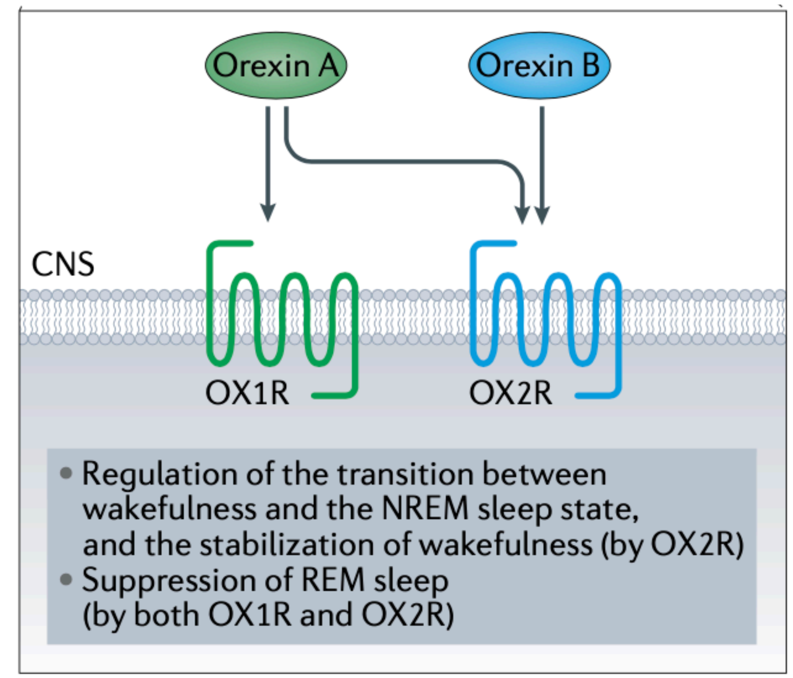
Hormonal signaling opportunities

Melatonin and orexin – two complementary hormones

Melatonin signalling pathway



Orexin signalling pathway



Agonism via melatonin or melatonin analogues

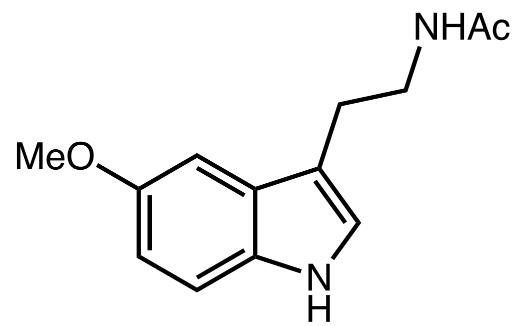
- Accelerate sleep onset
- Improve sleep duration
- Minimally impact other processes

Therapeutic goals

Antagonism via novel inhibitors

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

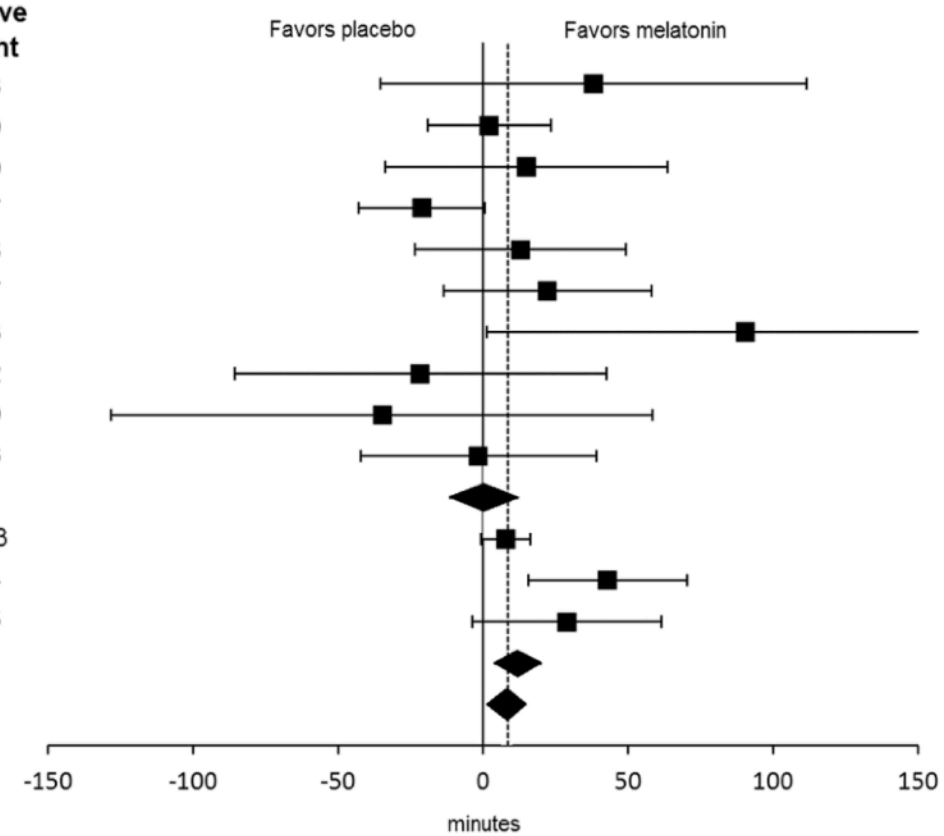
Melatonin supplementation as a sleep aid



Melatonin

- Approved for medical use in Europe, considered a dietary supplement in the US
- Found to decrease sleep latency by 7.1 minutes and increase sleep duration by 8.3 minutes
- Conclusion: safe, but minimally effective

Study	WMD (95% CI)	Relative weight
Kunz D, 2010 [23]	38.2 (-35.34 to 111.73)	0.78
Luthringer R, 2009 [24]	2.2 (-19.13 to 23.53)	9.29
Mundey K, 2005 [28]	15 (-33.56 to 63.56)	1.79
Almeida Montes LG, 2002 [	-21 (-42.84 to 0.84)	8.87
Zhadanova IV, 2001 [31]	13 (-23.47 to 49.47)	3.18
Kayumov L, 2001 [33]	22.3 (-13.65 to 58.24)	3.27
Dawson D, 1998 [35]	90.5 (1.39 to 179.6)	0.53
Ellis CM, 1996 [36]	-21.6 (-85.86 to 42.66)	1.02
Dahlitz M, 1991 [38]	-34.8 (-128.15 to 58.55)	0.49
James SP, 1989 [39]	-1.5 (-42.18 to 39.18)	2.56
Objective	*0.34 (-11.2 to 11.87)*	
Wade AG, 2011 [21]	7.8 (-0.69 to 16.29)	58.63
Smits MG, 2003 [29]	43 (15.62 to 70.37)	5.64
Smits MG, 2001 [32]	29 (-3.74 to 61.74)	3.95
Subjective	*11.93 (4.06 to 19.8)*	
Overall	**8.25 (1.75 to 14.75)**	



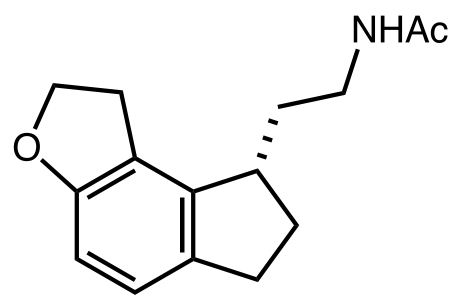
Fixed-effect model *Heterogeneity*
Z=2.48 p=0.013 I²=44% p=0.044
Random-effect model
WMD=8.48 minutes [95% CI: -4.02 to 20.98], Z=1.33, p=0.184

Note: melatonin in the US is not well quality controlled. Melatonin content has been found to range from -83% to +478% the listed amount

Ferracioli-Oda, E.; Qawasami, A.; Bloch, M. H. *PLOS One.* **2013**, *8*, e63773.

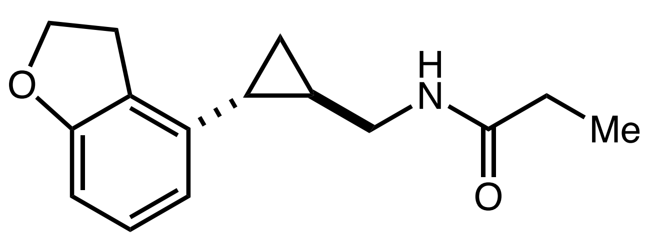
Other melatonin receptor agonists (late 2000s)

Goal: improve pharmacokinetics and half-life of melatonin (20–50 minutes)



ramelteon
(Rozerem)

- Half life of 1–2.6 hours
- sleep latency decreased by 4–7 minutes
- meta analyses show mixed impact on total sleep time
- approved for treatment of insomnia

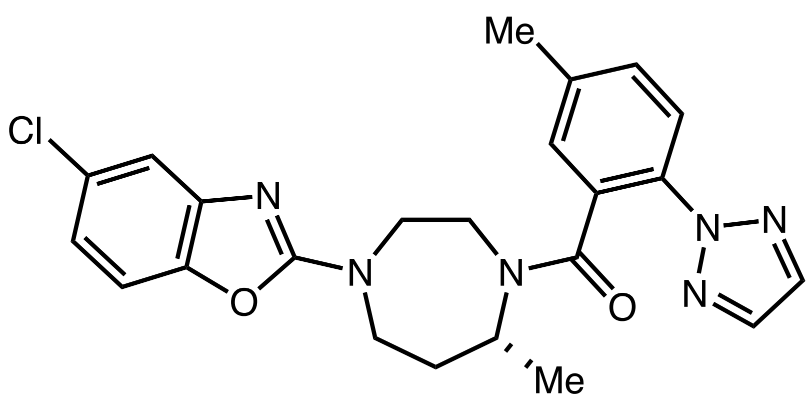


tasimelteon
(Hetlioz)

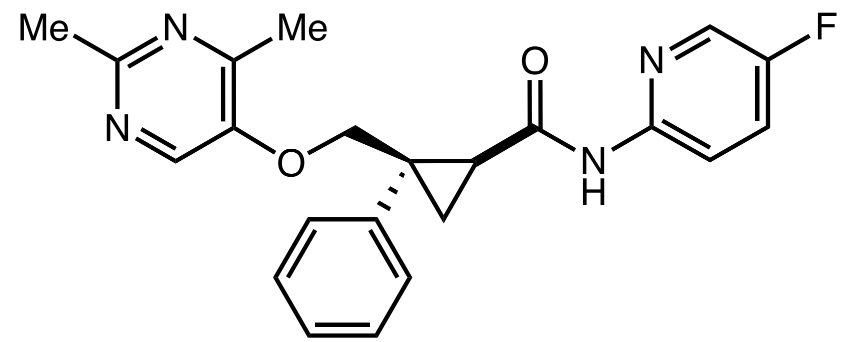
- Half life of 55–100 minutes
- Approved as orphan drug for N24SWD in blind patients
- Found to increase total sleep time by 60 minutes in patients with 8 hour jet lag from eastward travel, and improve time to sleep
- rejected by FDA 3 times between 2014 and 2022 for this indication.

Polymerpoulos, C. M. et al. *Front. Neurol.* **2020**, *11*, 611.
Liu, J.; Wang, L. N. *Int. J. Clin. Prac.* **2012**, *66*, 867.

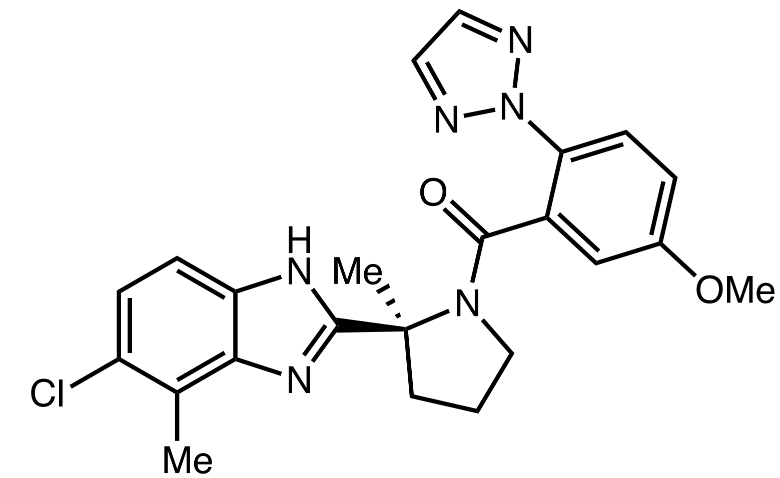
Orexin receptor antagonists (2010s)



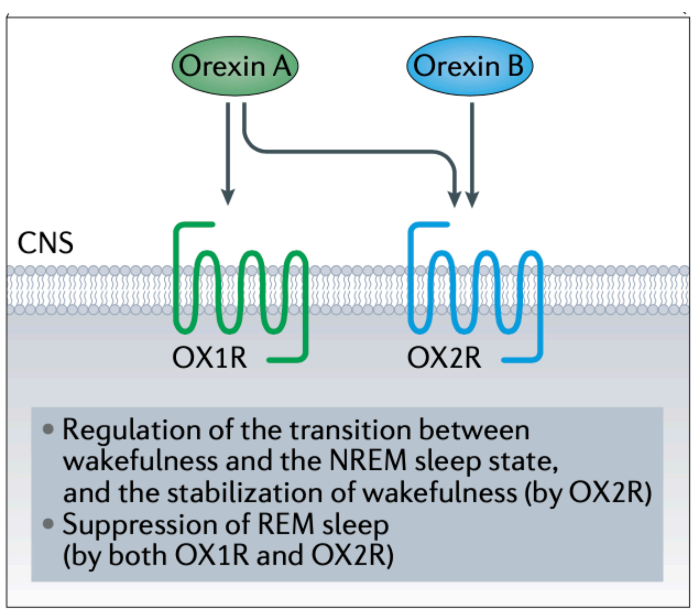
Suvorexant



Lemborexant



Daridorexant



Mechanism of action: prevent binding of orexin to promote sleep

- Preserve night time arousability (monkey)
- Improved daytime cognitive performance (Compared to other sleep aids, rats)
- Beneficial effects in total sleep time, decreases in waking after sleep onset, subjective sleep latency
- No evidence of addiction potential!
- Not as effective as benzodiazepines/ nonbenzodiazepines

Monkemeyer, N. M.; Thomas, S. V.; Hilleman, D. E.; Malesker, M. A. *U. S. Pharmacist* **2022**, *47(5)*, 43.

A universal first line of treatment

Sleep Hygiene	Stimulus Control	Sleep Restriction	Relaxation	Cognitive	Wrap-Up
appropriate bedroom environment avoiding screen-based devices before bedtime avoiding coffee or alcohol consumption	using bedroom only to sleep leaving bedroom- when cannot fall asleep	restricting sleep times increasing in-bed sleep times	taking short and long relaxations during the day	restructuring undesired thinking patterns	going over each component to prevent the relapse of insomnia

Cognitive behavioral therapy for insomnia (CBT-I) is considered the gold standard

Behavioral treatment by American and European guidelines

Uyumaz, B. E.; Fejis, L. Hu, J. *Int. J. Environ. Res. Public Health.* **2021**, *18*, 2929.

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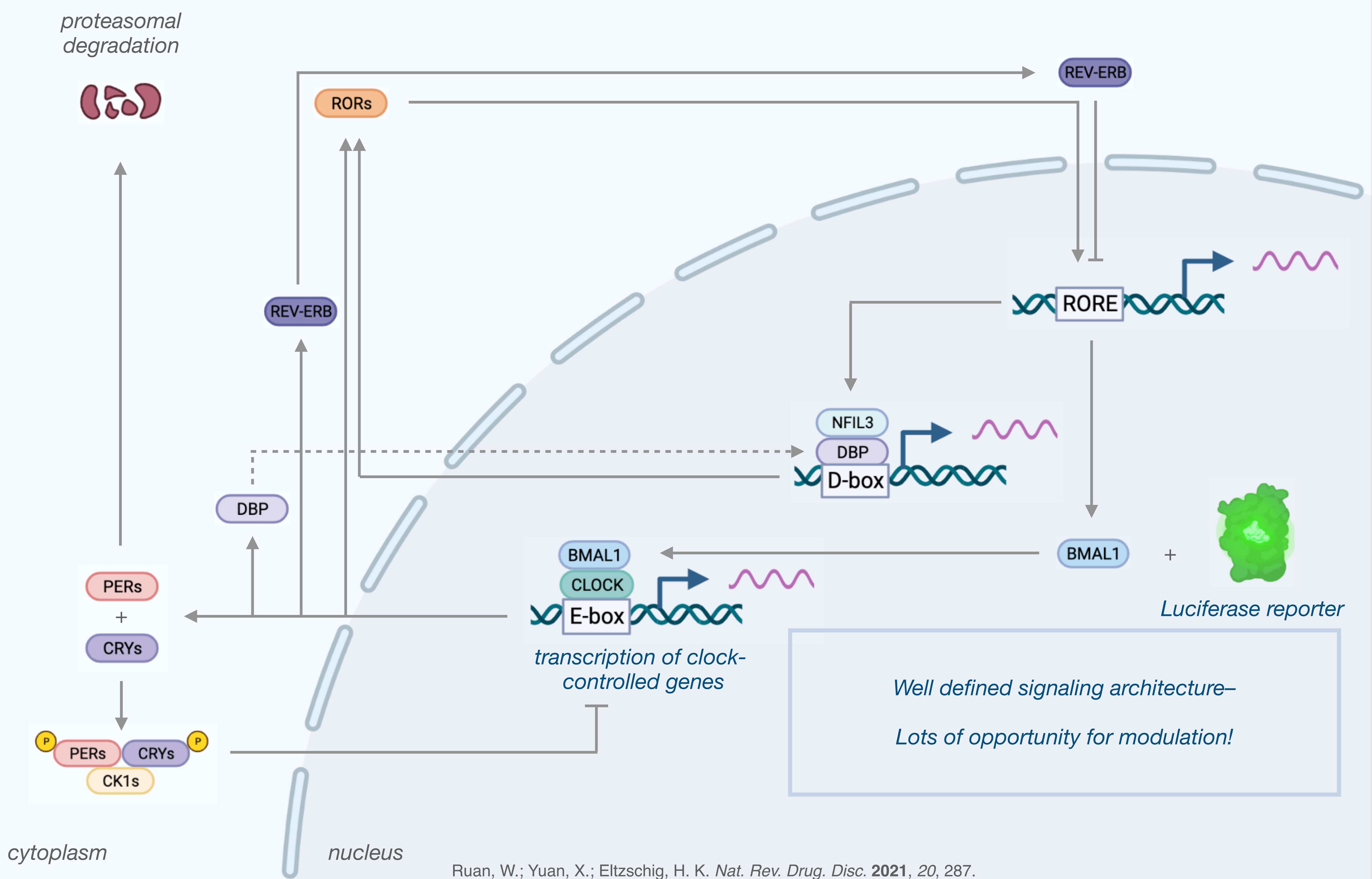
- Case studies in modulating the circadian machinery

The Ubiquitous, Cell-Autonomous Molecular Oscillator

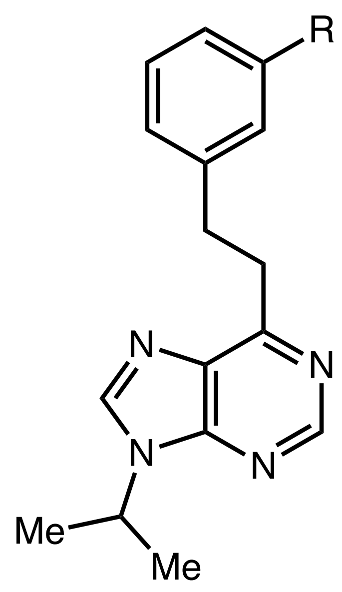


Well defined signaling architecture–

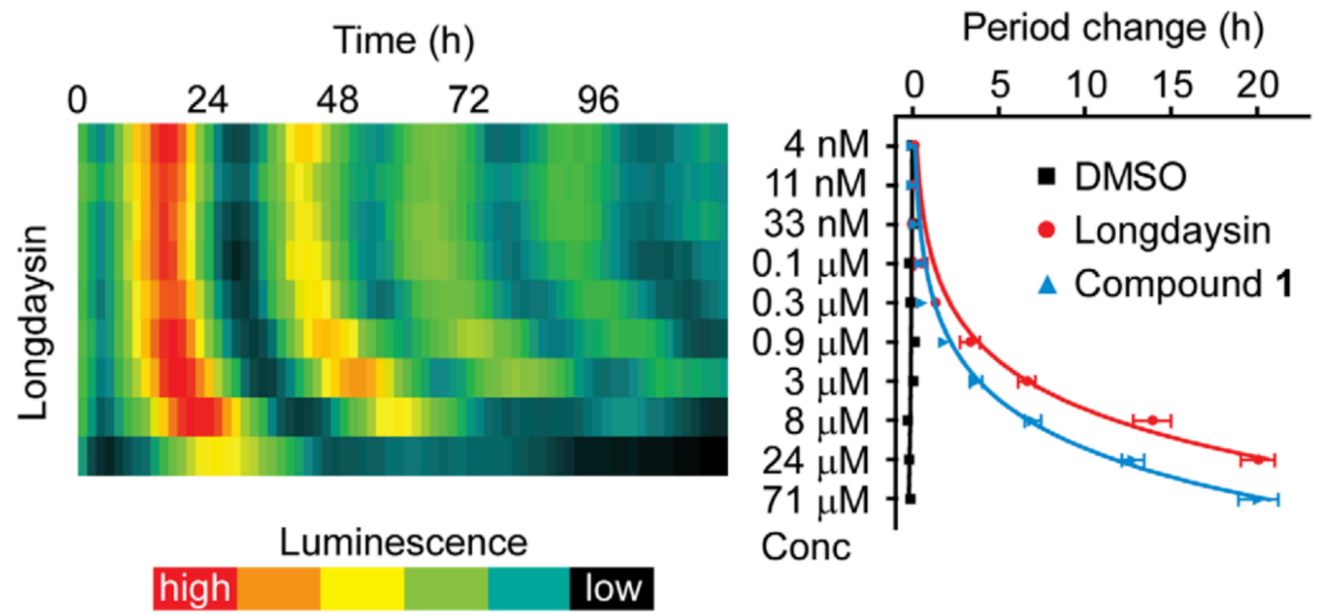
Lots of opportunity for modulation!

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

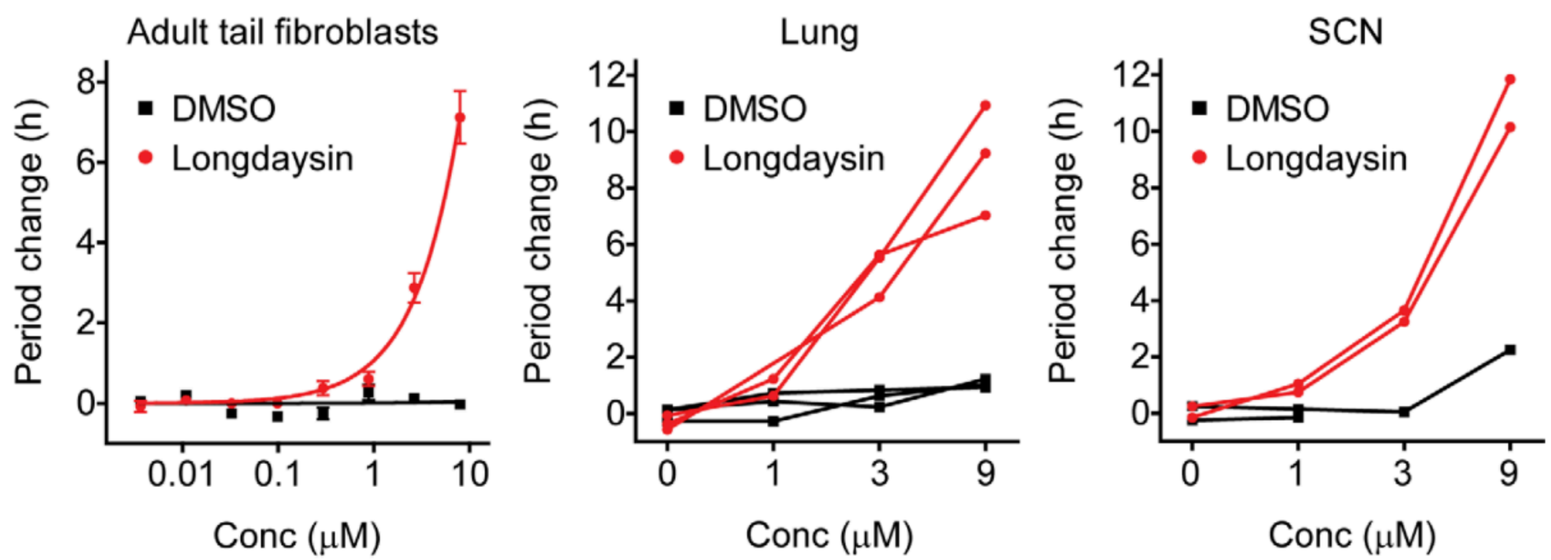
Phenotypic screening for circadian rhythm perturbation



Compound 1 (R = H)
Longdaysin (R = CF_3)



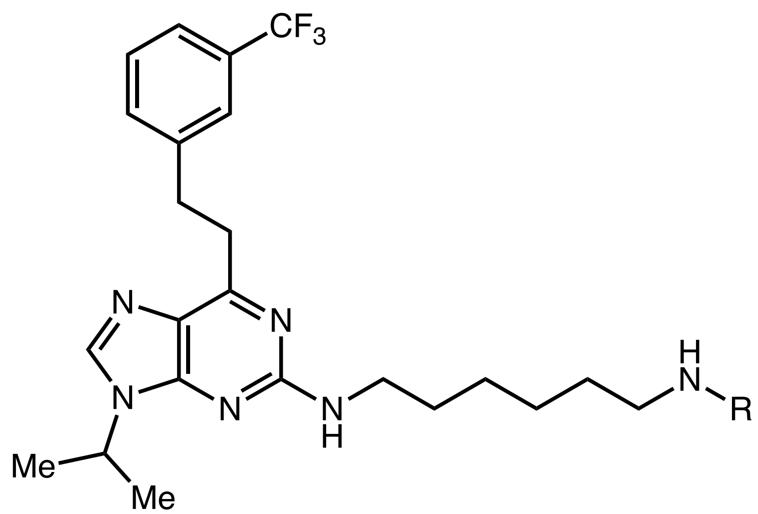
Longdaysin found to shift the period of circadian rhythm gene expression in a dose dependent manner in human U2OS cells



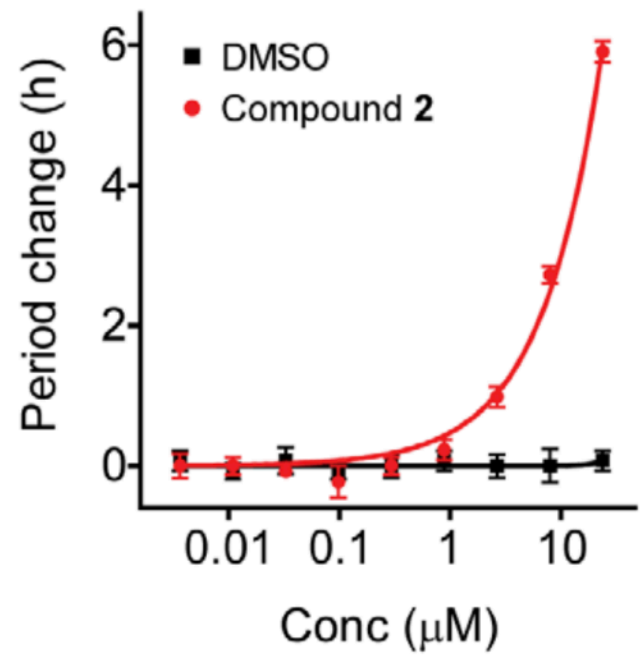
Longdaysin induced period change observed in diverse cell cultures

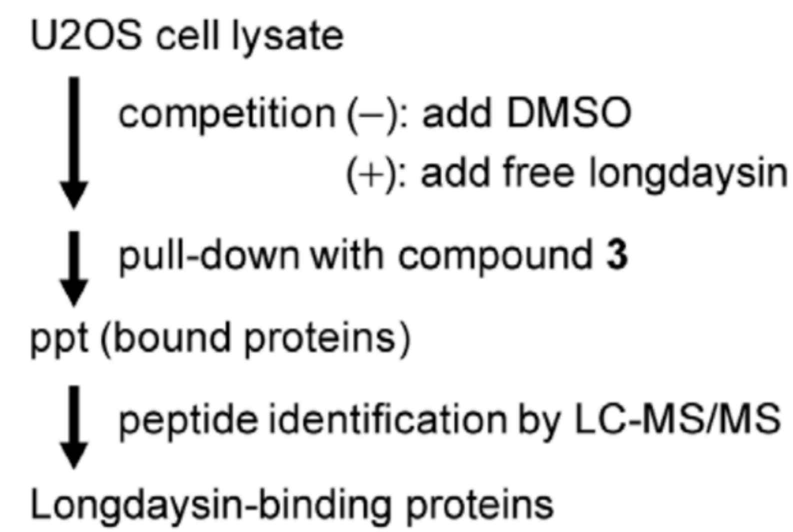
Ruan, W.; Yuan, X.; Eltzschig, H. K. *PLOS Biol.* **2010**, *8(12)*, e1000559.

What proteins does longdaysin target?



Compound 2 (R = Ac)
Compound 3 (R = bead)



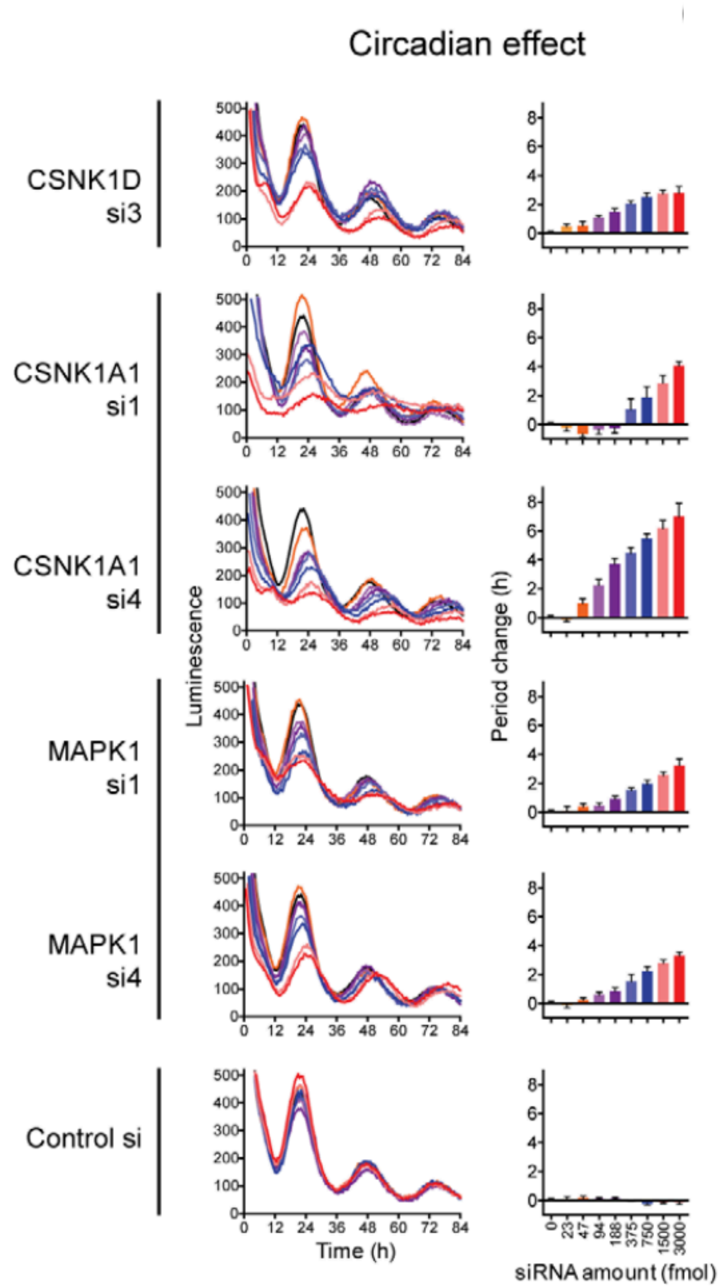


	U2OS Cell-Based Circadian Assay (Concentrations for Period Change, μM)[a]			In Vitro Kinase Assay (IC_{50}, μM)[b]			
Compound	**5 h**	**10 h**	**15 h**	**CKIδ**	**CKIα**	**ERK2**	**CDK7**
Longdaysin	1.5	5.7	13	8.8	5.6	52	29
Compound 1	4.4	17	38	21	23	160	29

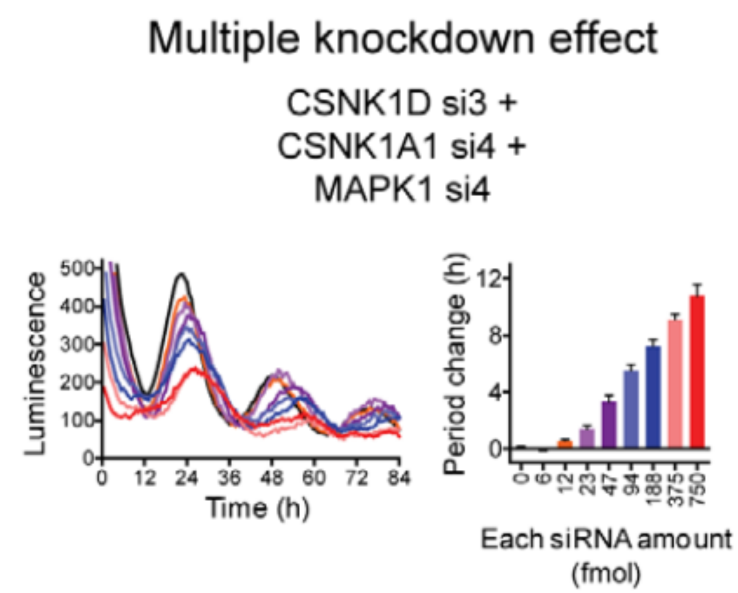
targets identified to be ~10 kinases with unknown connection to clock mechanisms

Ruan, W.; Yuan, X.; Eltzschig, H. K. *PLOS Biol.* **2010**, *8(12)*, e1000559.

Which of Longdaysin's targets causes phase delay?



Knockout of individual genes coding for longdaysin did not induce phase delay



Knocking out CK1δ, CK1α, and ERK together recapitulated phase delay of Longdaysin

Key point: no one kinase solely responsible for maintaining circadian rhythm

Ruan, W.; Yuan, X.; Eltzschig, H. K. *PLOS Biol.* **2010**, *8(12)*, e1000559.

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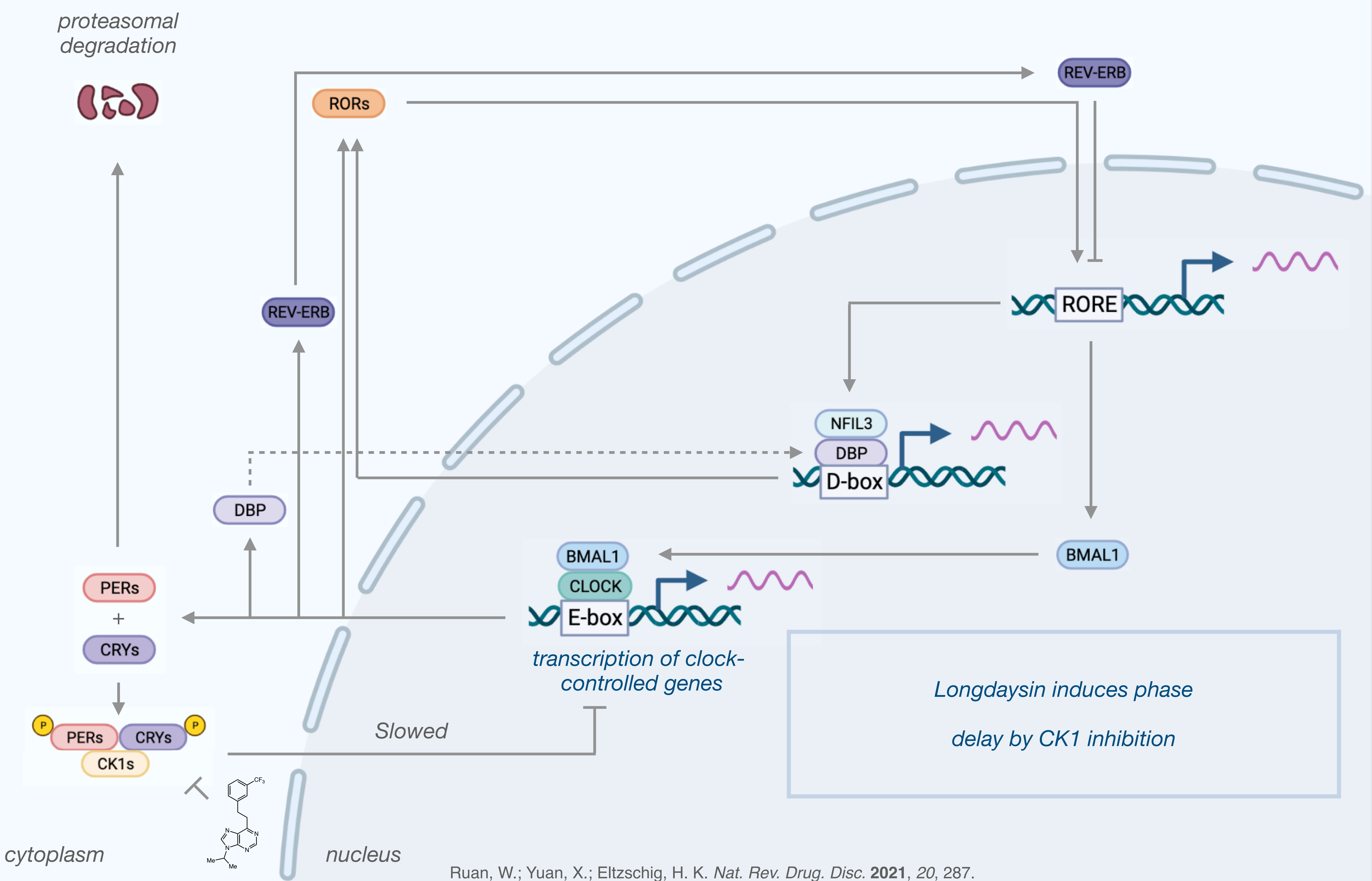
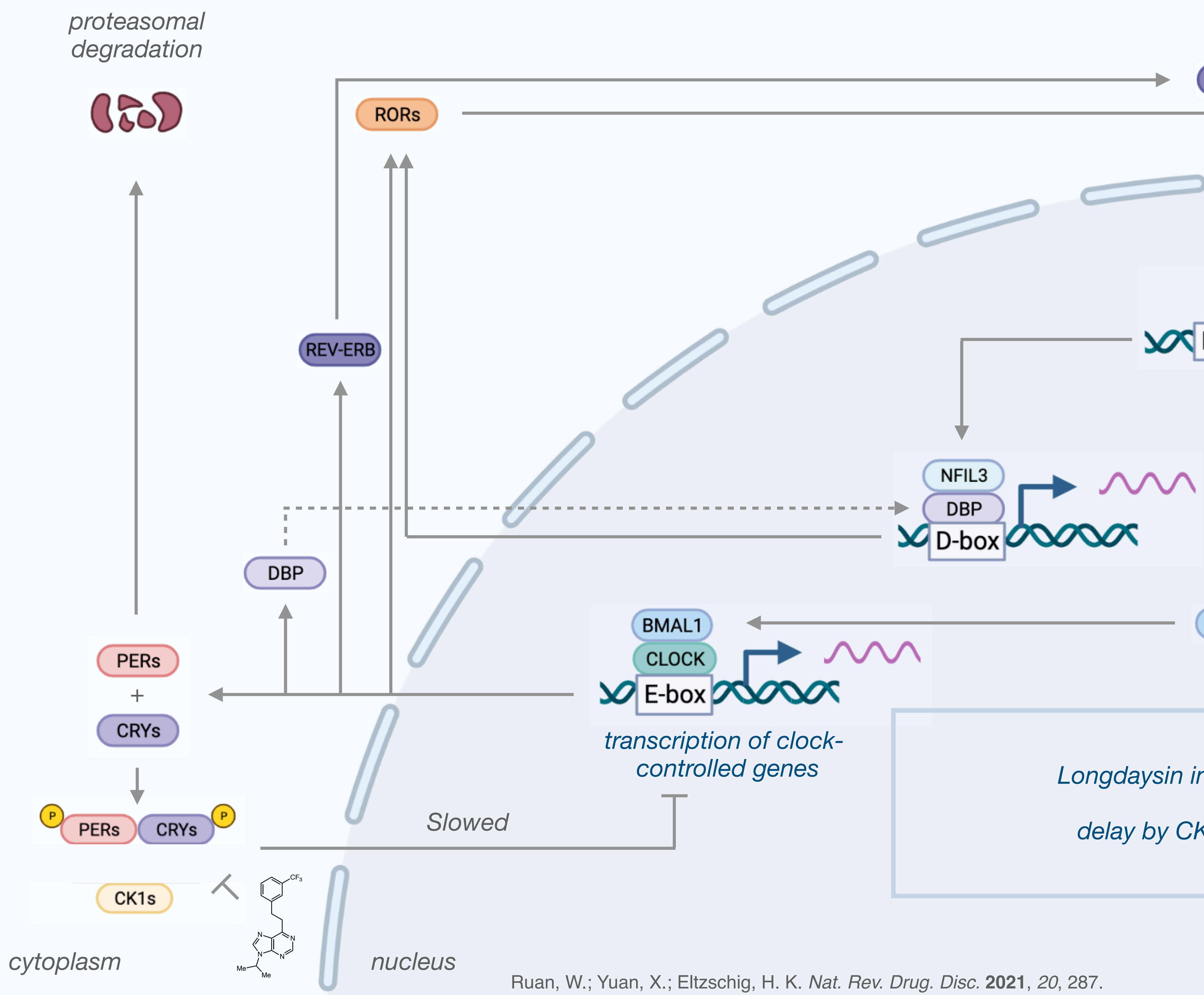


Longdaysin induces phase

delay by CK1 inhibition

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

The Ubiquitous, Cell-Autonomous Molecular Oscillator



Longdaysin induces phase

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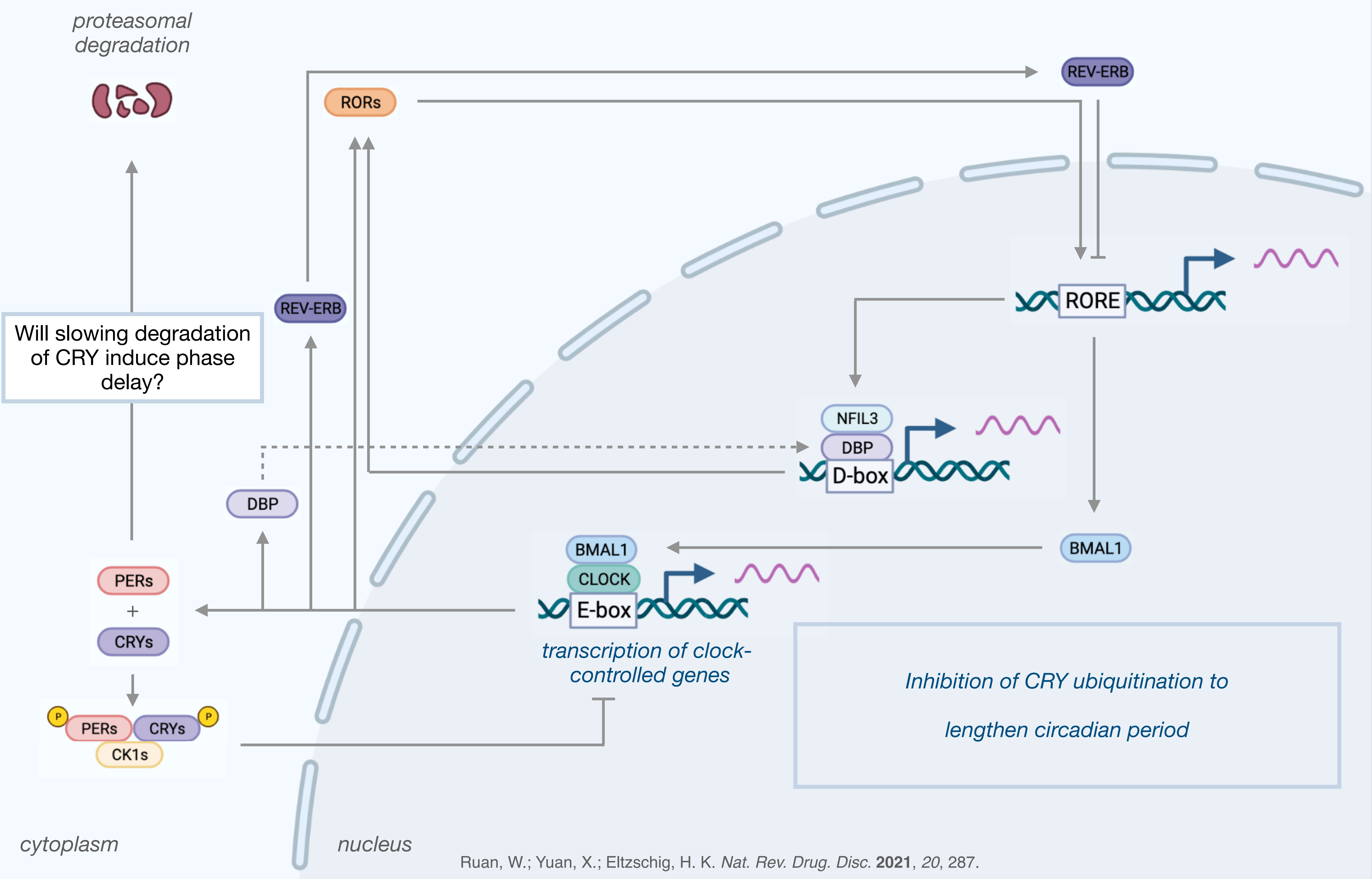


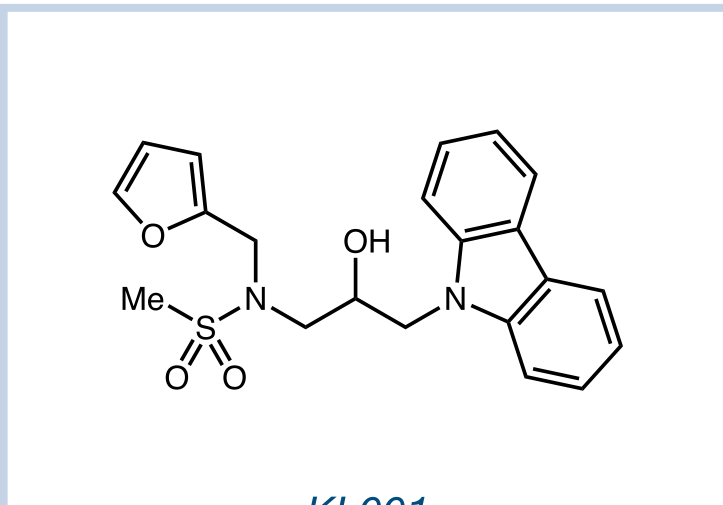
Will slowing degradation of CRY induce phase delay?

Inhibition of CRY ubiquitination to lengthen circadian period

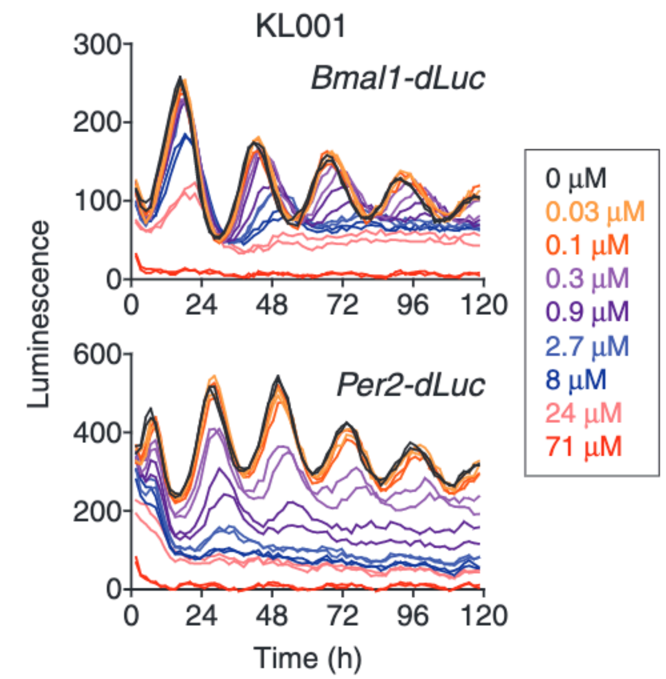
Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Cryptochrome ubiquitination inhibition leads to period lengthening

KL001 found to induce phase delay by inhibiting CRY ubiquitination

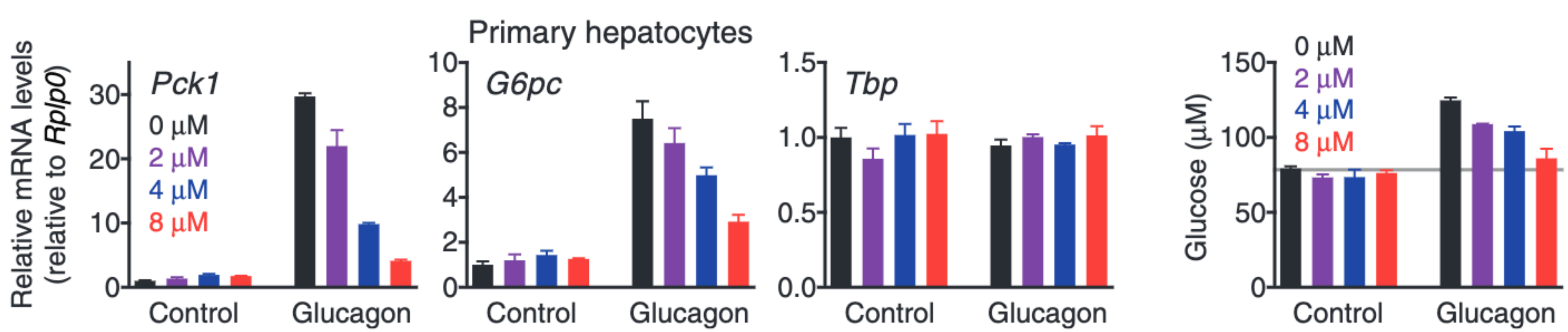


KL001



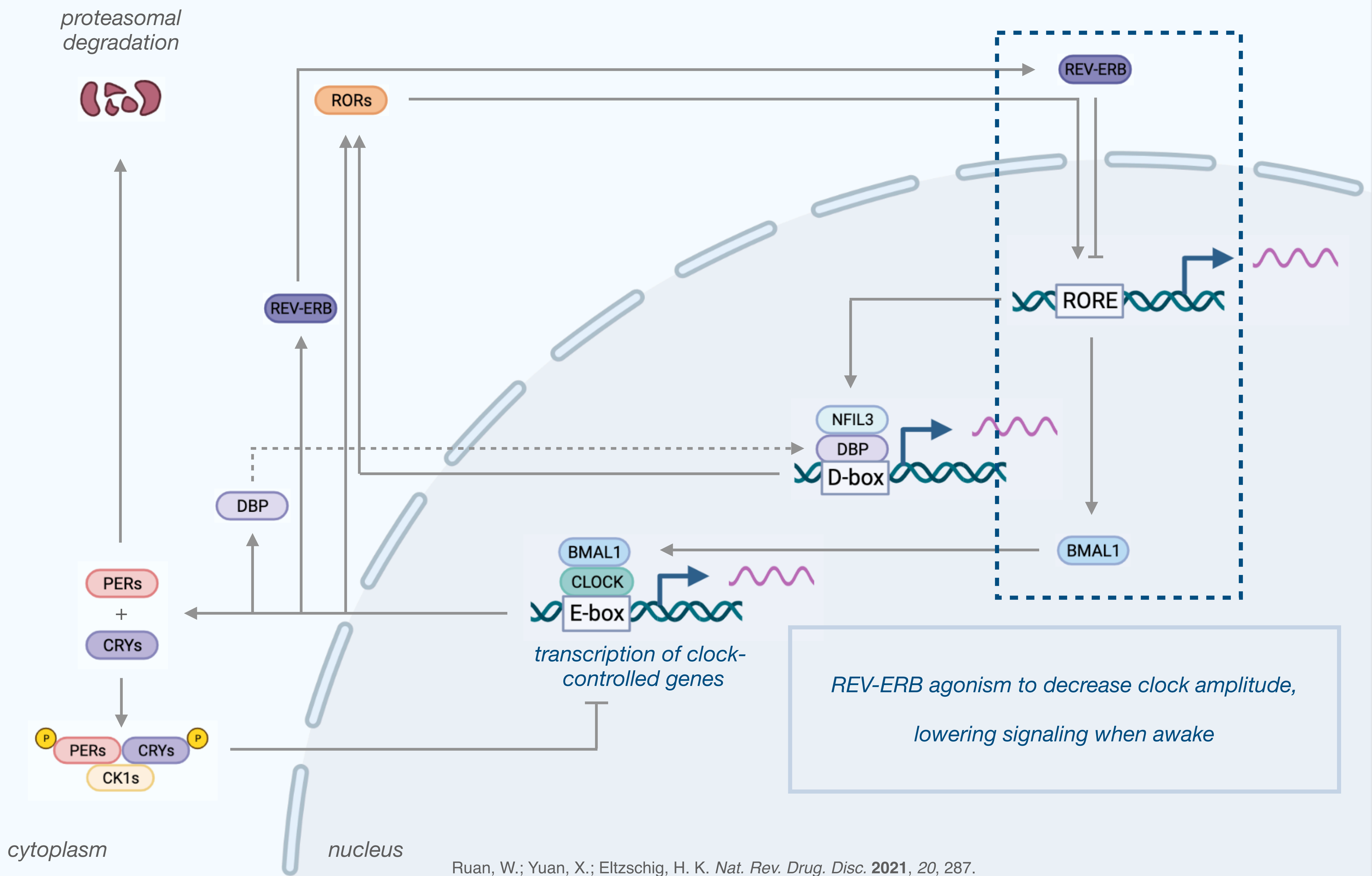
CRY proteins negatively regulate regulate genes encoding rate-limiting enzymes of gluconeogenesis

Opportunity for development of therapeutics for diabetes treatment



Hirota, T. et al. *Science* **2012**, *337*, 1094.

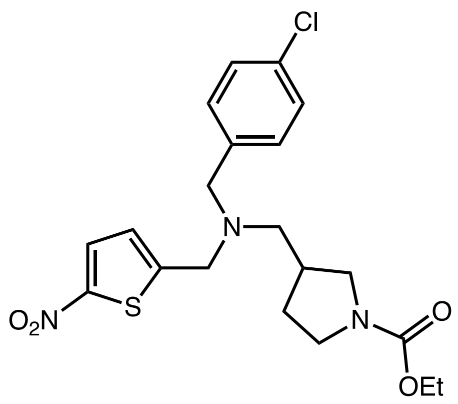
The Ubiquitous, Cell-Autonomous Molecular Oscillator



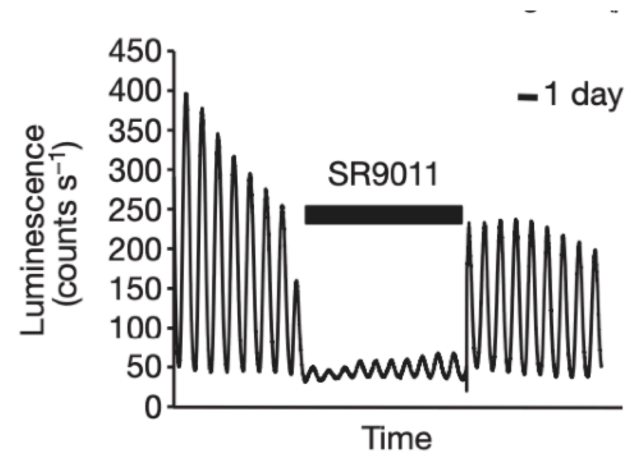
REV-ERB agonism to decrease clock amplitude, lowering signaling when awake

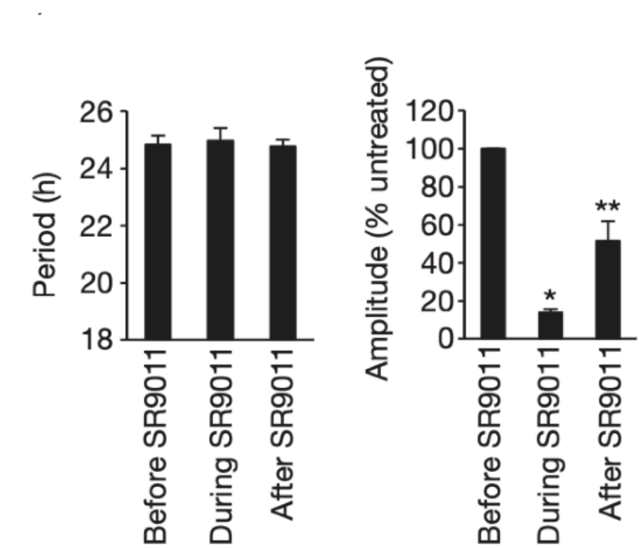
Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Can diminished expression of BMAL1 lower clock amplitude?

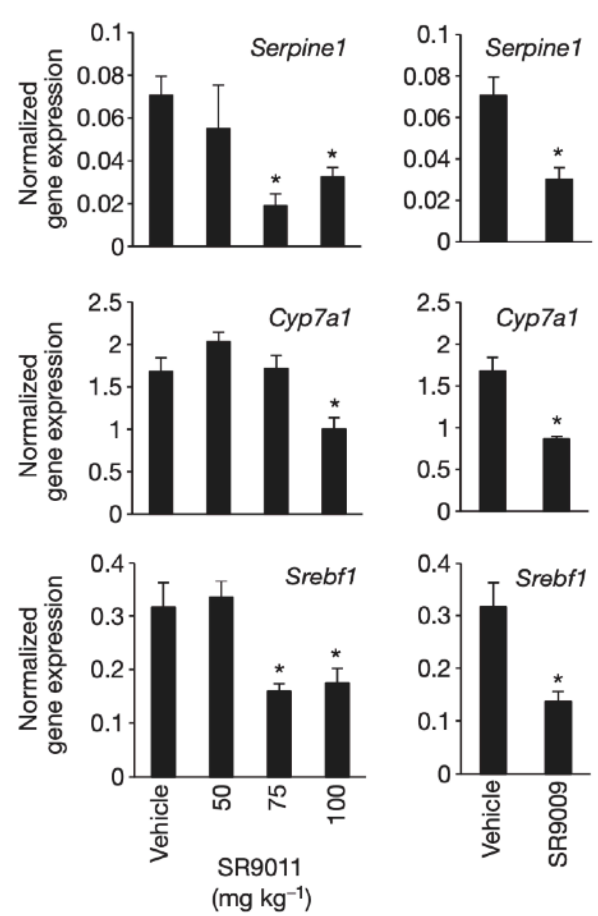


SR9011





REV-ERB agonism successfully shrinks amplitude of circadian signaling without modifying period



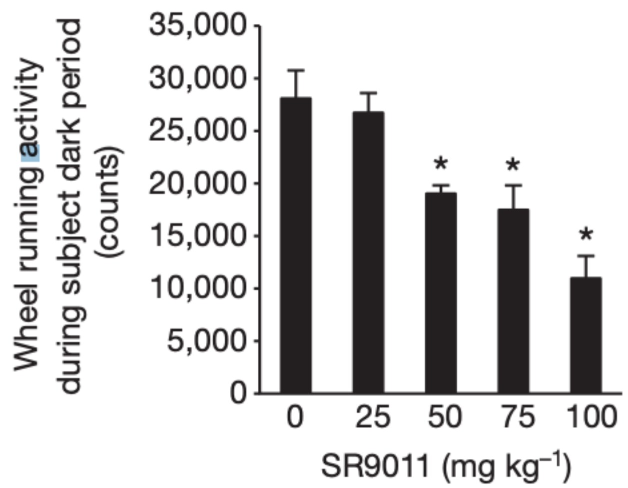
Expression of clock dependent genes

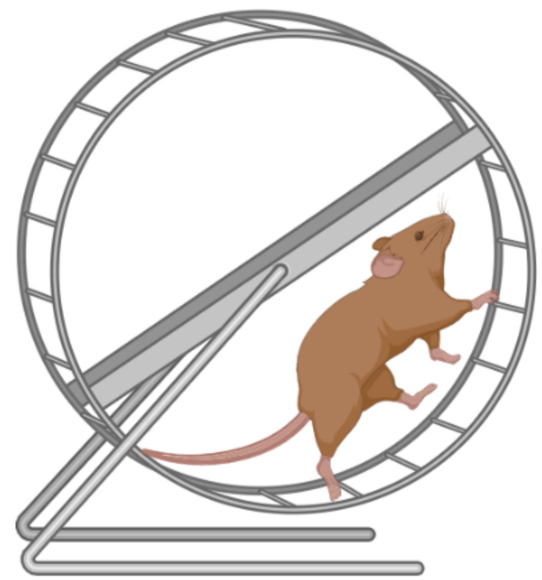
accordingly down-regulated

Solt, L. A. et al. *Nature* **2012**, *485*, 62.

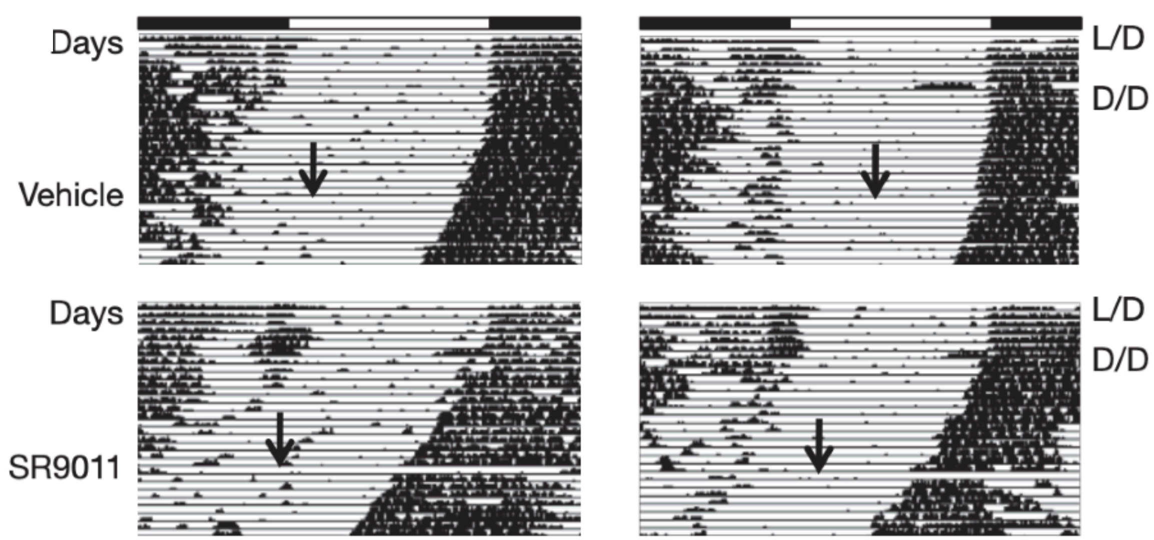
Diminished expression of BMAL1 in mice

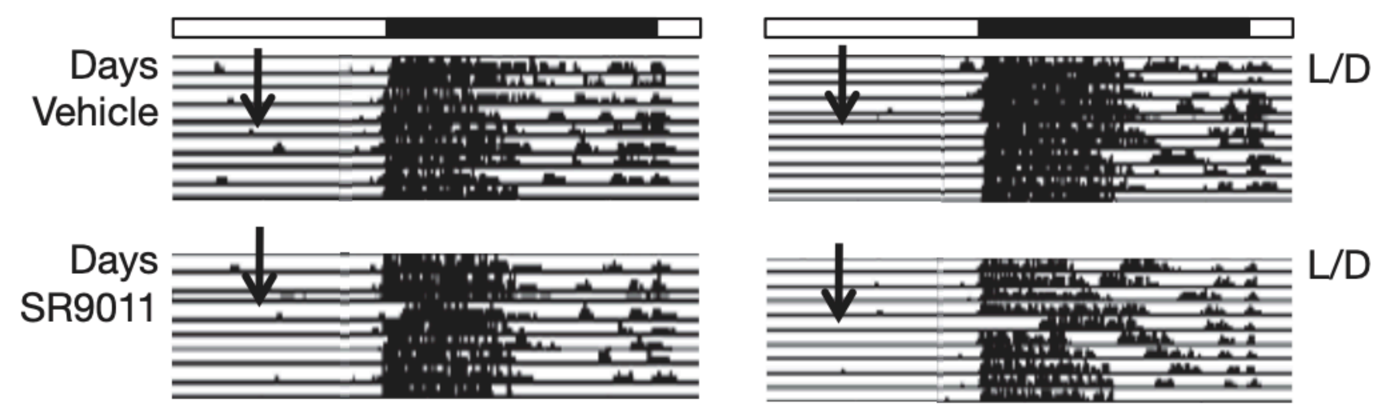
How does diminished expression of BMAL1 effect the activity of mice?





Mouse activity levels drop when BMAL1 is suppressed





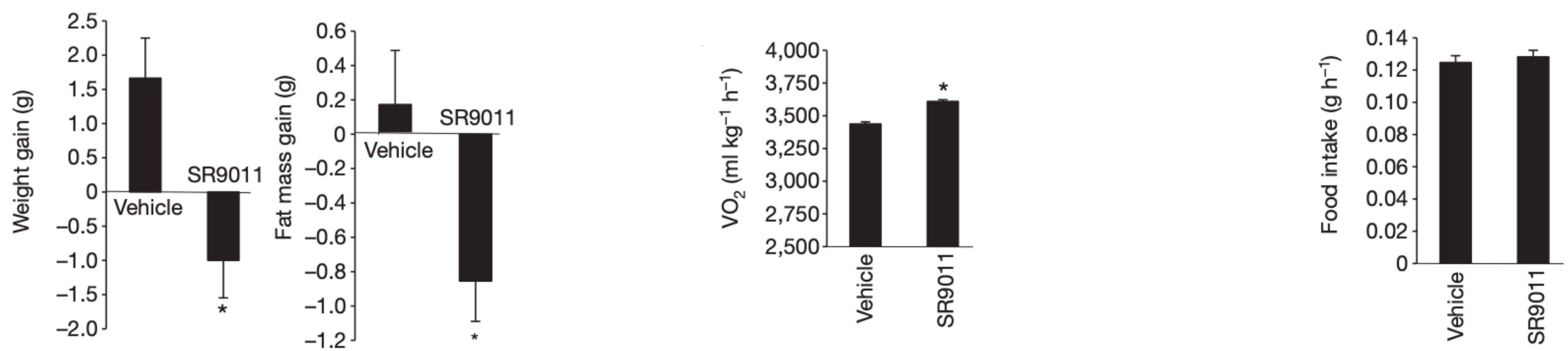
SR9011 lowered activity significantly in mice kept solely in dark

SR9011 delayed circadian activity by 3 h in mice kept in 12h light/dark cycles

Solt, L. A. et al. *Nature* **2012**, *485*, 62.

SR9011 interacts with metabolism

Despite lowered activity, mice were observed to lose weight following dosing with SR9011



Elevated energy expenditure (by VO_2) coupled with conserved food intake led to weight loss

Decreased fat mass, plasma triglycerides, and cholesterol levels

REV-ERB agonists as a treatment for metabolic diseases?

Solt, L. A. et al. *Nature* **2012**, *485*, 62.

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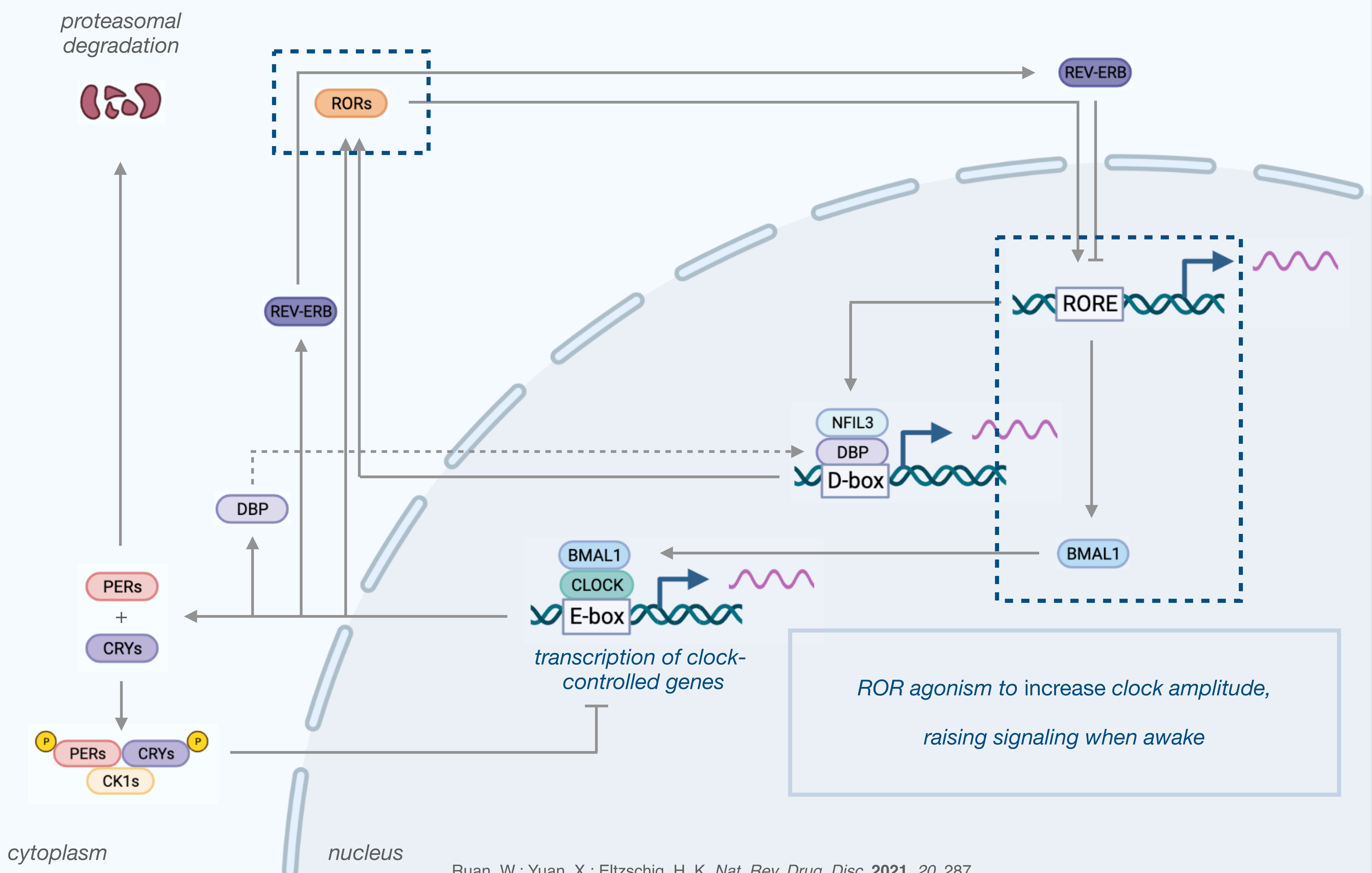
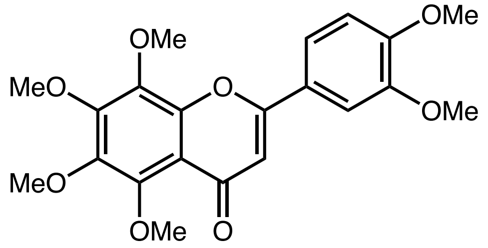


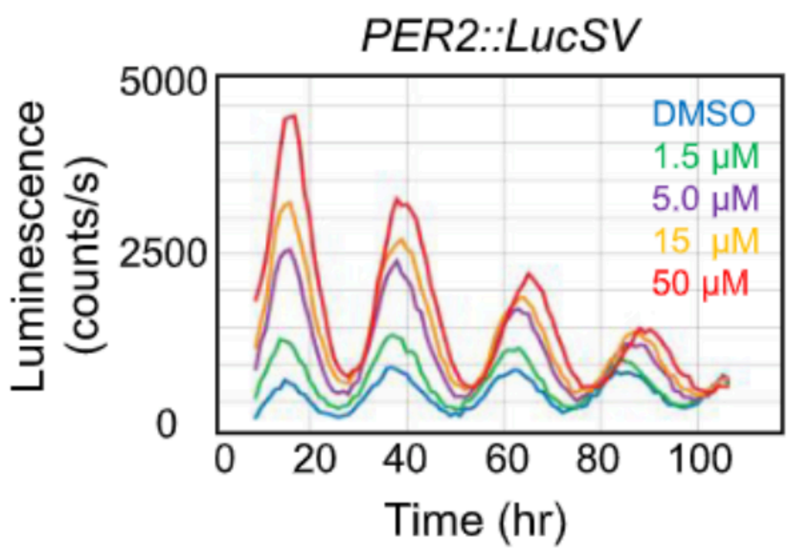
ROR agonism to increase clock amplitude, raising signaling when awake

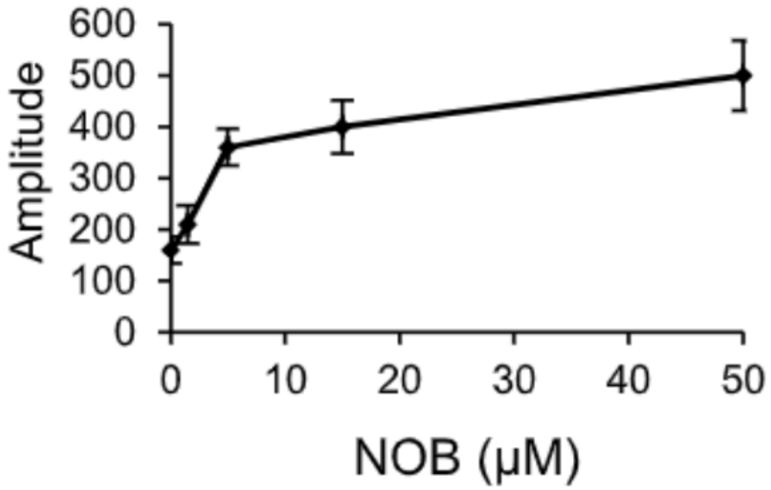
Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

Nobiletin as a circadian amplitude enhancer via ROR agonism

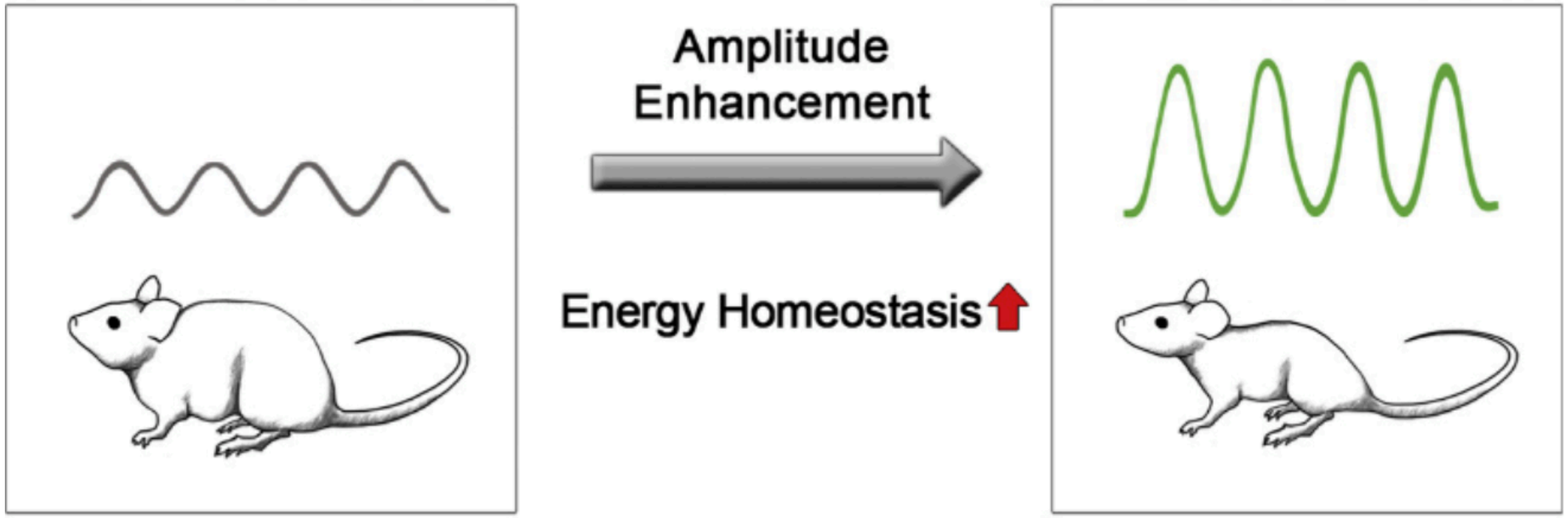


Nobiletin



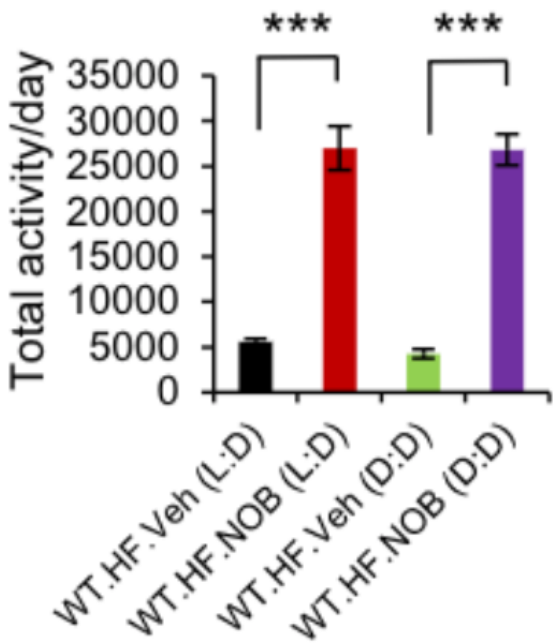


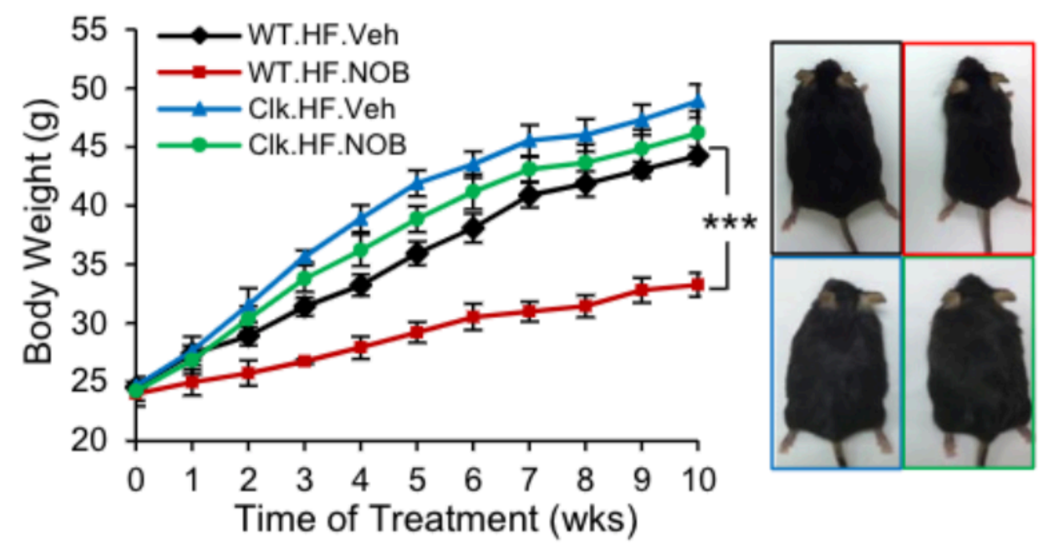
Nobiletin treatment increased the amplitude of circadian rhythm gene expression



He, B. et al. *Cell Metabolism* **2016**, *23*, 610.

Nobiletin treatment led to weight loss in mice via increased activity





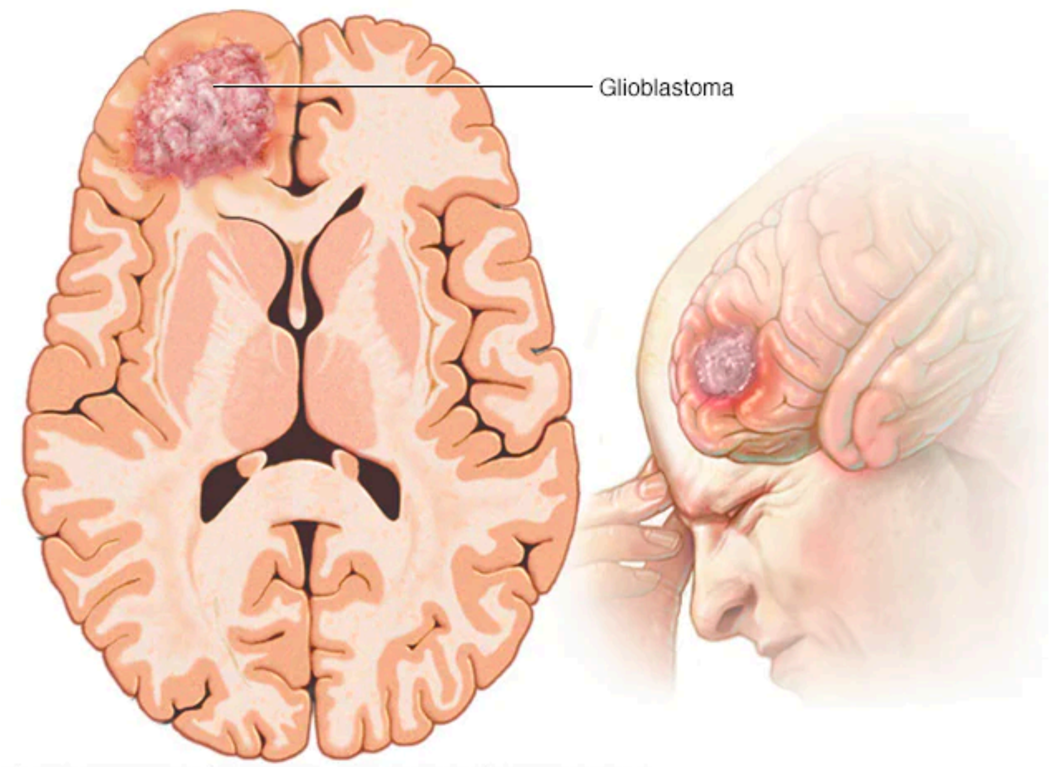
Nobiletin treated mice were more active regardless of light schedule

Nobiletin treated mice on a high fat diet gained less weight than controls – in a clock dependent manner

Circadian rhythm amplification as a treatment for metabolic disease and/or age related decline?

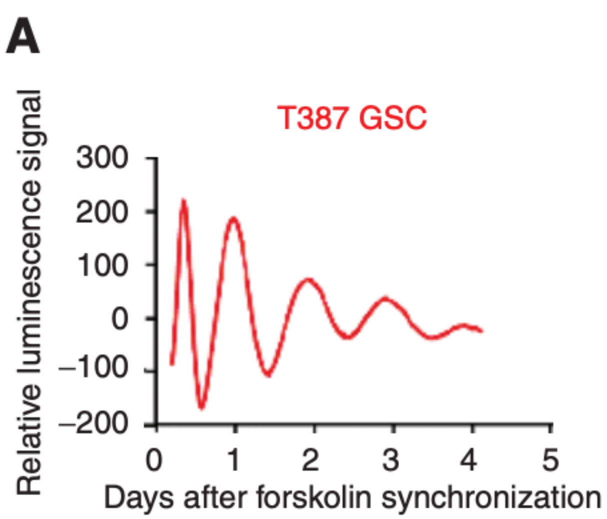
He, B. et al. *Cell Metabolism* **2016**, *23*, 610.

Circadian rhythm modulators in the treatment of glioblastoma



5–10% survive >5 years after diagnosis

Standard of care is maximal surgical resection, radiation therapy, and chemotherapy



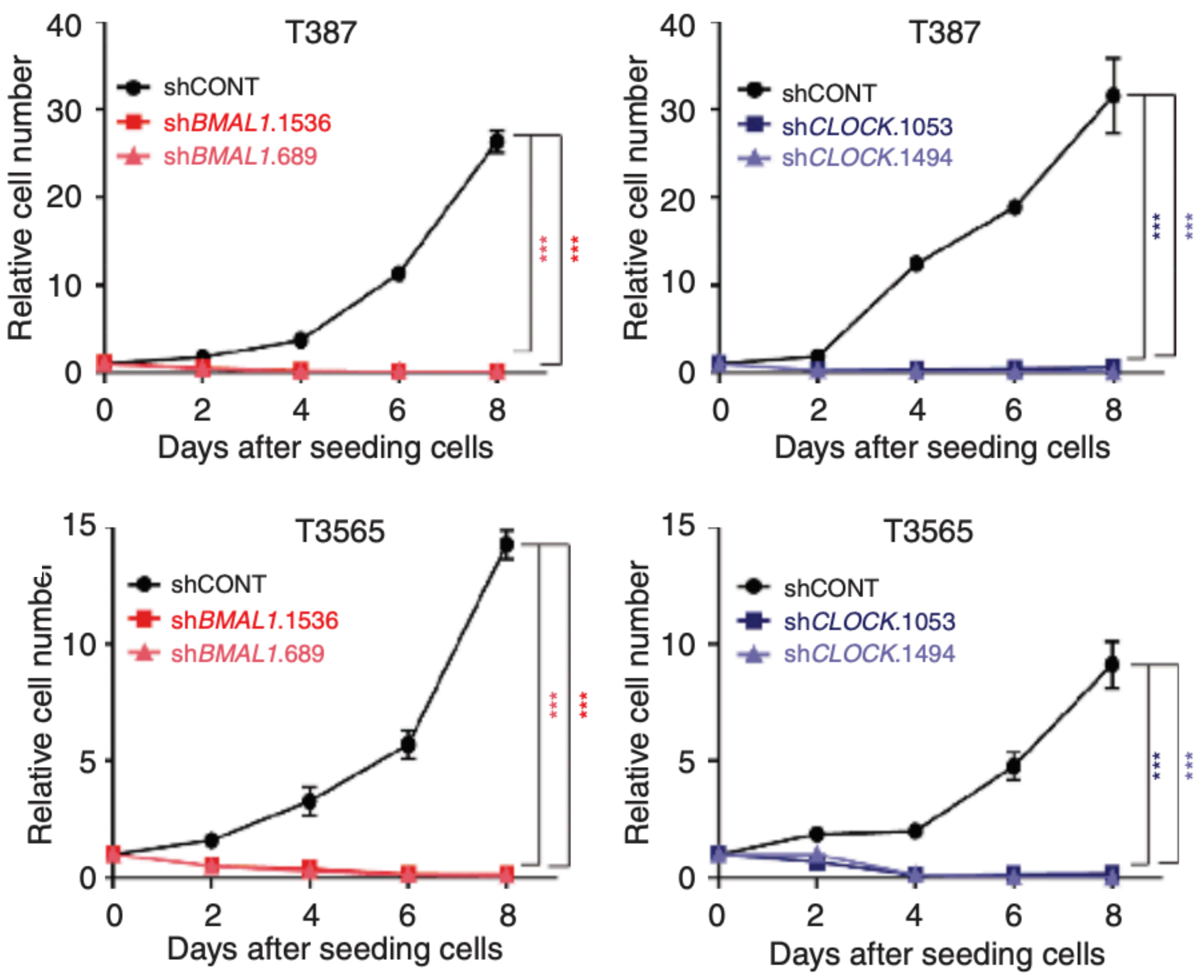
Glioblastoma stem cells found to exhibit Strong circadian signaling–

A therapeutic opportunity?

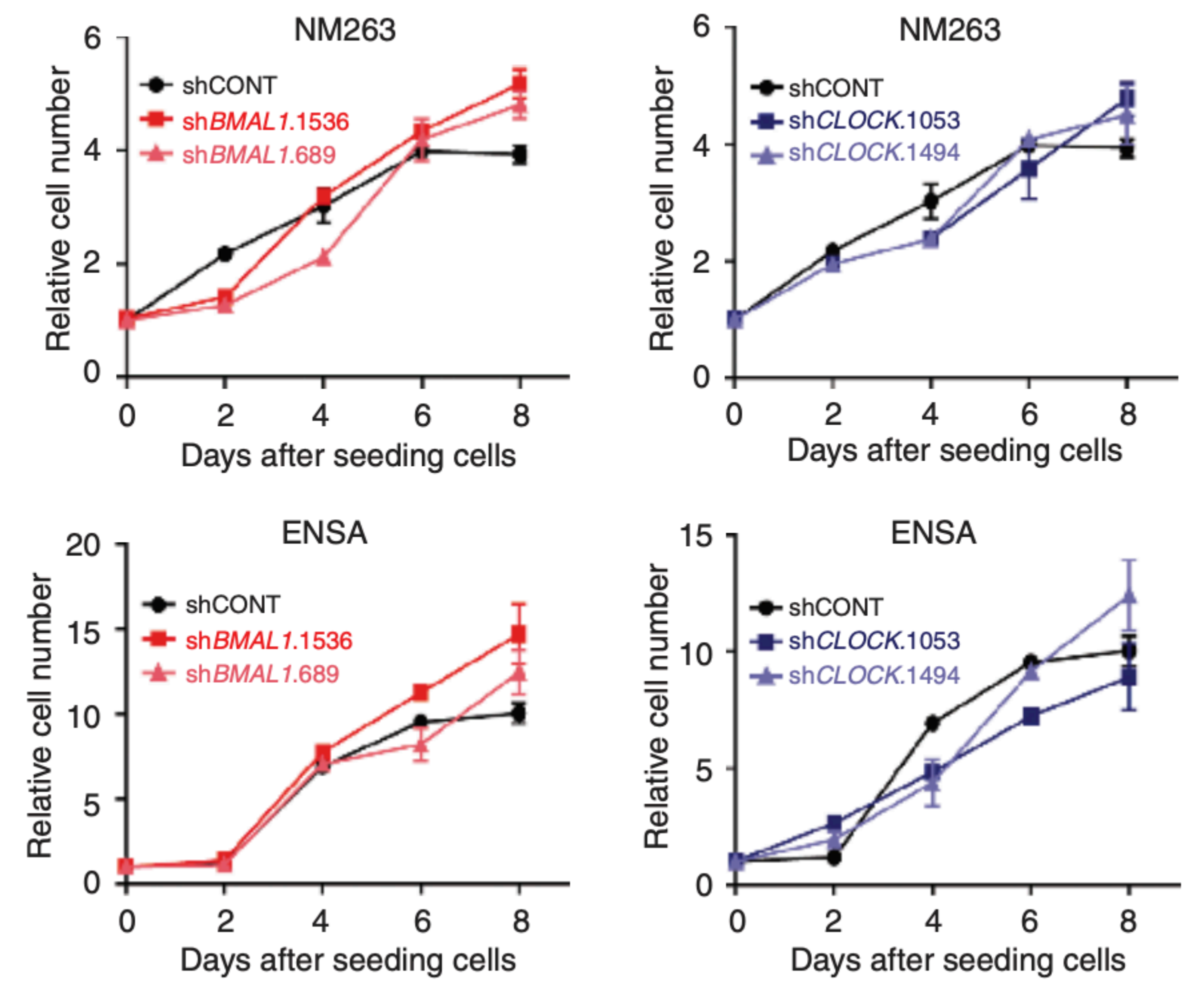
Dong, Z. et al. *Cancer Discov.* **2019**, *9*, 1556.

Will knocking out key clock transcription factors impact cell growth?

Proof of concept: knockout core clock transcription factors to inhibit glioblastoma growth



Growth in glioblastoma stem cells mitigated when BMAL1 and CLOCK genes were knocked out



Growth in other brain cell cultures maintained upon BMAL1 and CLOCK knockout

Glioblastoma growth uniquely sensitive to clock activity

Dong, Z. et al. *Cancer Discov.* **2019**, *9*, 1556.

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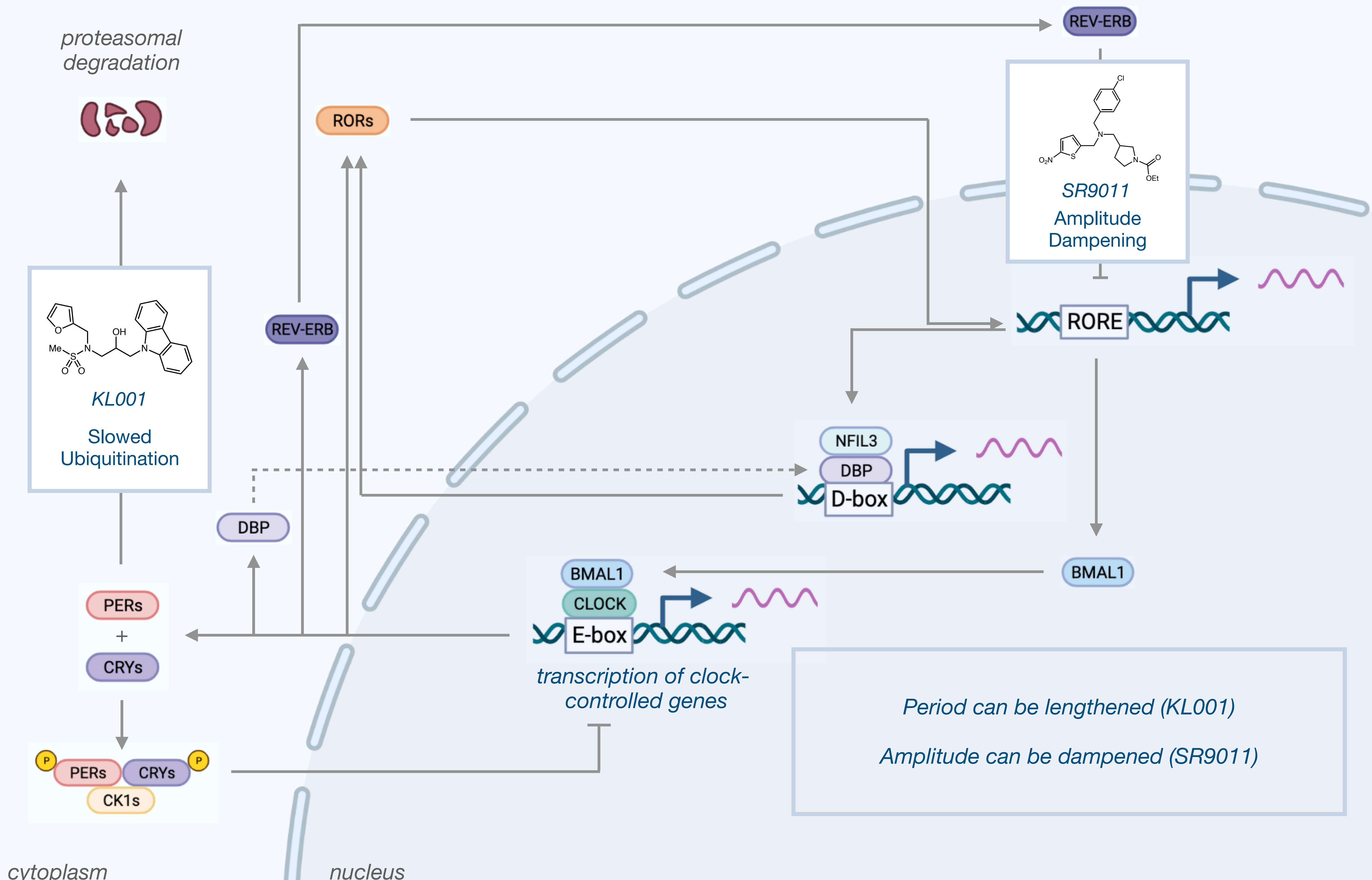
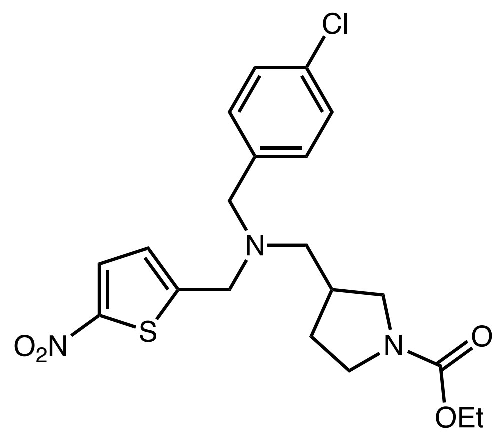


Period can be lengthened (KL001)

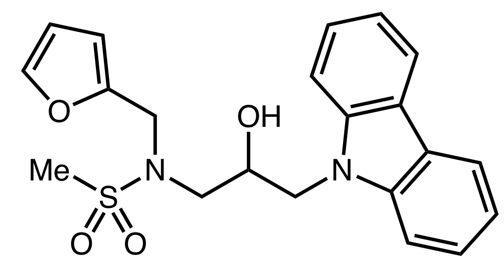
Amplitude can be dampened (SR9011)

Ruan, W.; Yuan, X.; Eltzschig, H. K. *Nat. Rev. Drug. Disc.* **2021**, *20*, 287.

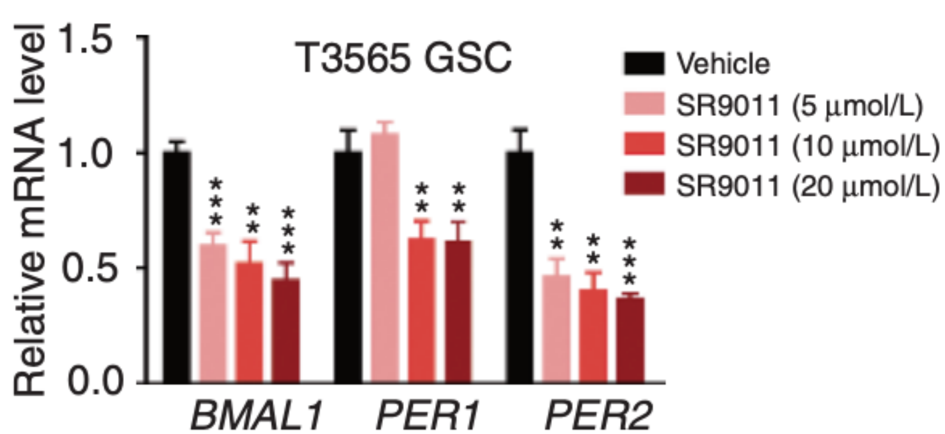
Pharmacological intervention in glioblastoma stem cell antagonism

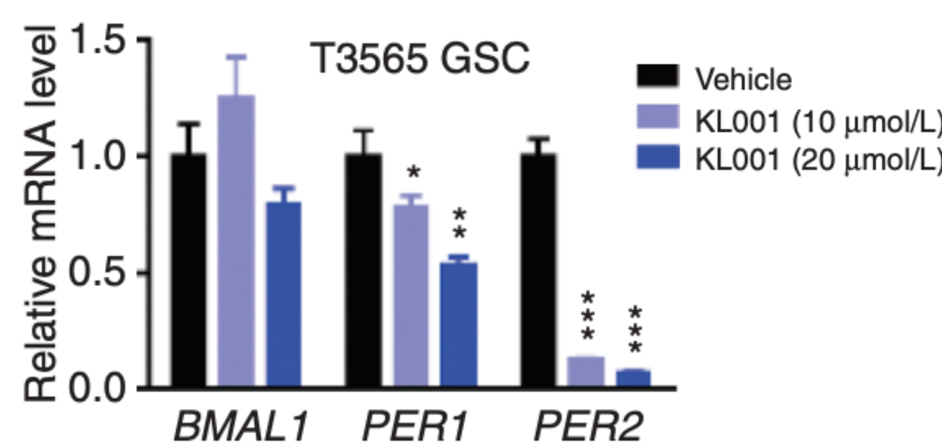


SR9011



KL001

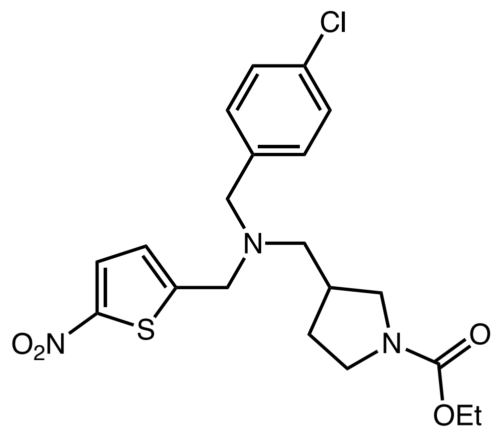




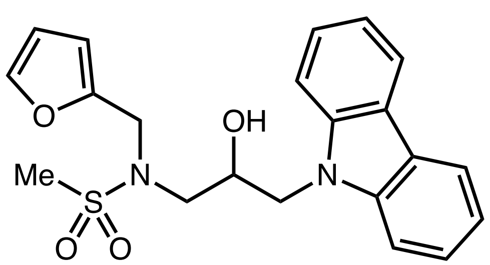
Clock interactions are maintained in glioblastoma cells through dose dependent gene suppression

Dong, Z. et al. *Cancer Discov.* **2019**, *9*, 1556.

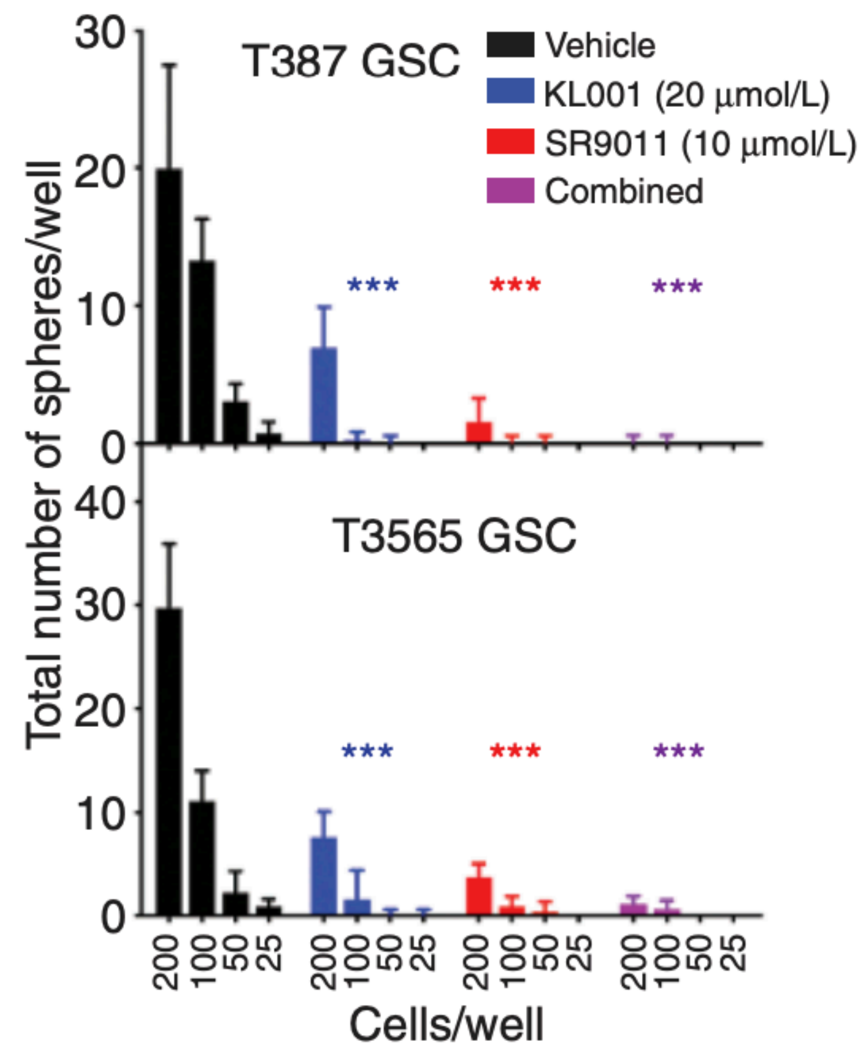
Pharmacological intervention in glioblastoma stem cell antagonism



SR9011



KL001



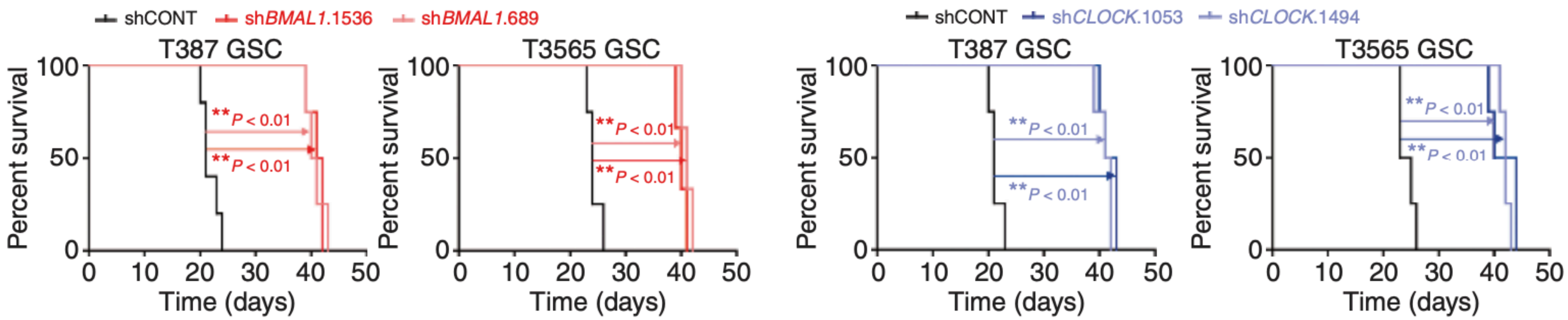
Both compounds inhibited GSC growth

Combination approach proved more effective

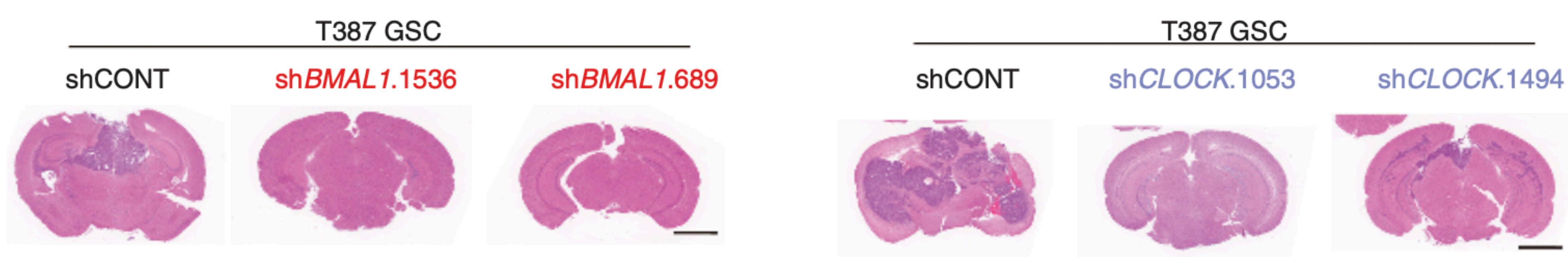
Dong, Z. et al. *Cancer Discov.* **2019**, *9*, 1556.

Clock transcription factor knockout in glioblastoma bearing mice

Core clock transcription factor knockout experiments performed in mice bearing glioblastoma stem cells



Survival of mice with core clock transcription factors BMAL1 and CLOCK knocked out exhibited greatly improved lifespan



Visual examination of mouse brain slices reveals minimal glioblastoma signs in knockout mice compared to control

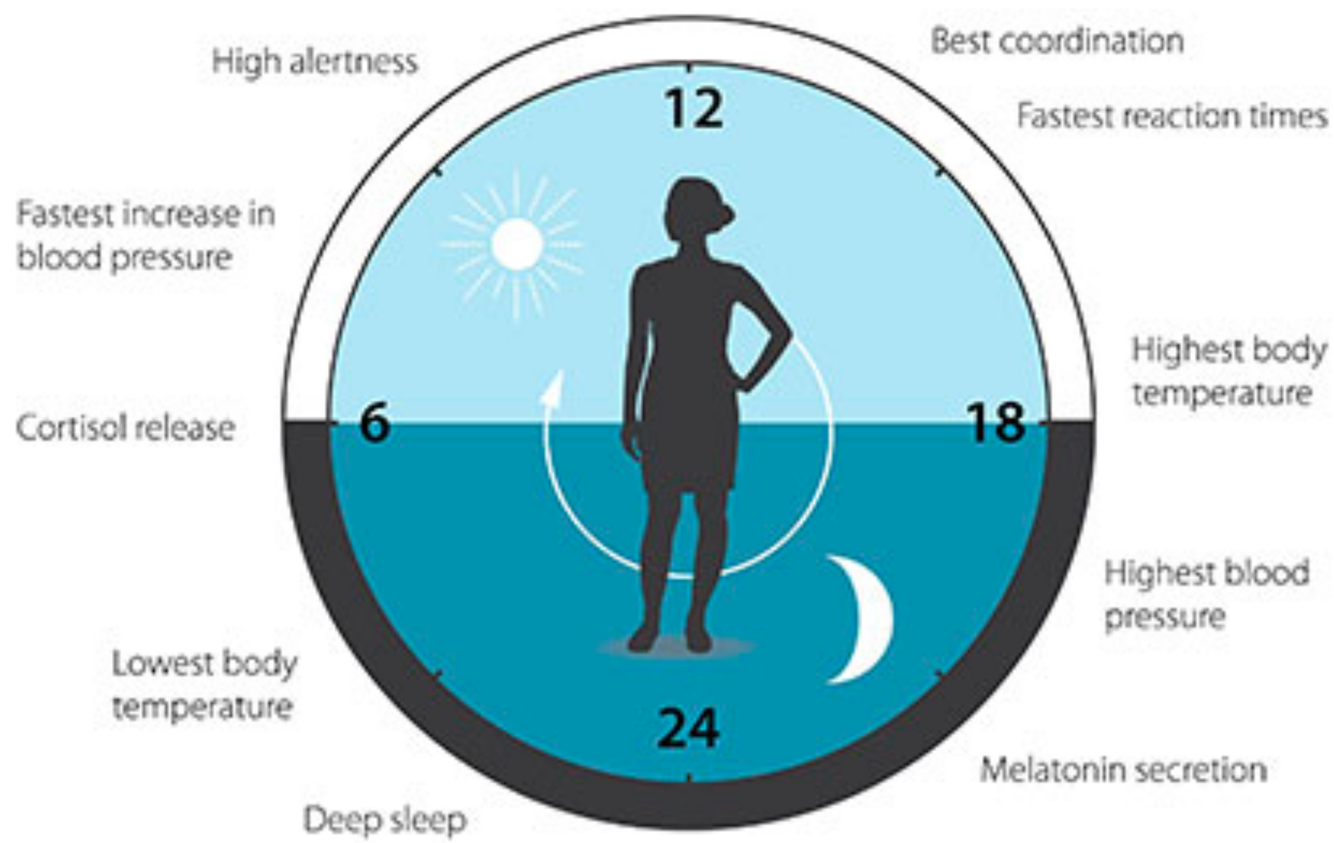
Dong, Z. et al. *Cancer Discov.* **2019**, *9*, 1556.

Perspective and Outlook

Circadian rhythm modulation offers an opportunity for treatment that is mechanistically distinct from classic approaches

Regulating the circadian machinery is a noteworthy approach for the treatment of a broad range of non-sleep related disorders

No period-shortening interventions have been published to date



Questions?