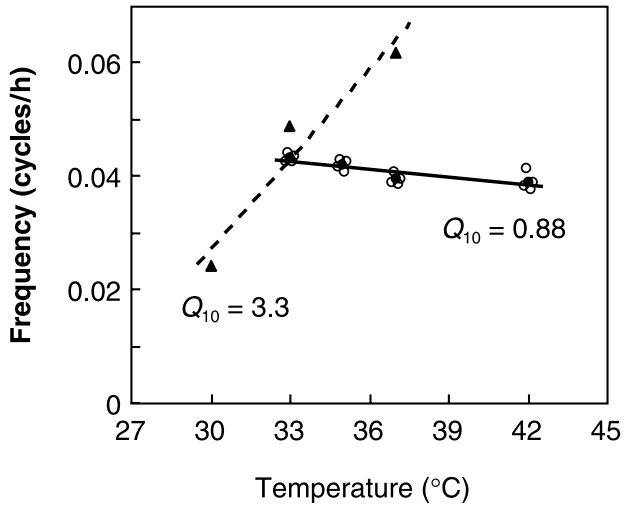
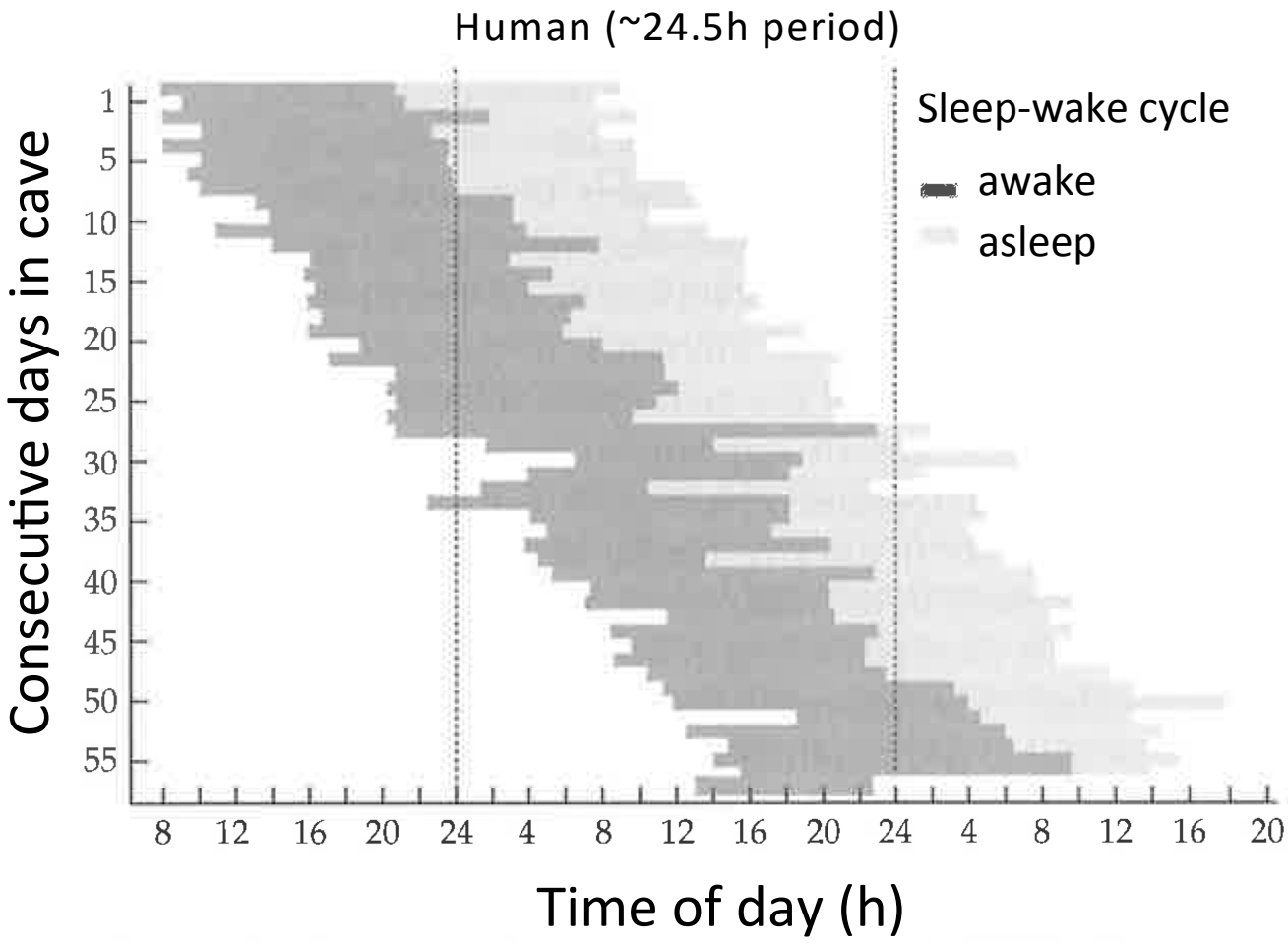
Mathematical study on the mystery of circadian rhythms

Gen Kurosawa
Theoretical Biology Lab, RIKEN
iTHEMS, RIKEN



Tsuchiya,..Nishida (2003)

We have autonomous daily rhythms in our body



Palmer (2002)

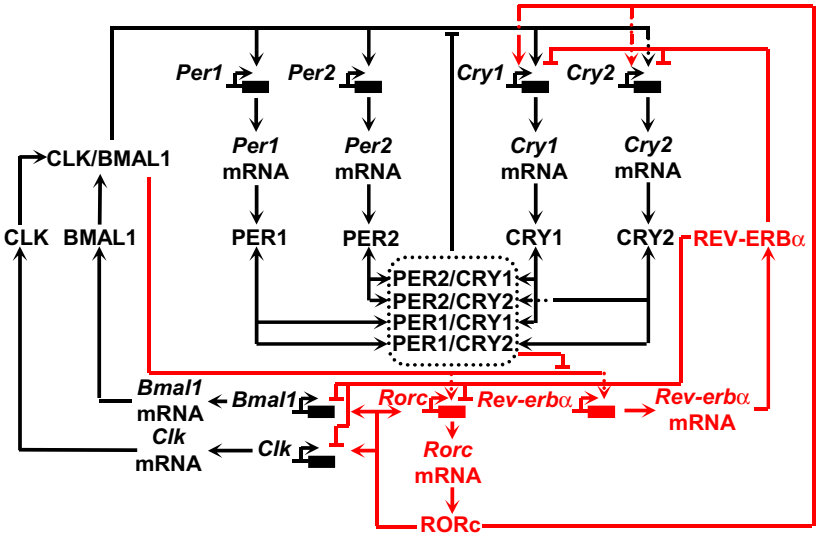
Gene-activity rhythms drive behavioral rhythms

(A movie of synchronized gene expression rhythms in brain)

Yamaguchi,.., Okamura (2003) *Science* 302, 1408-12

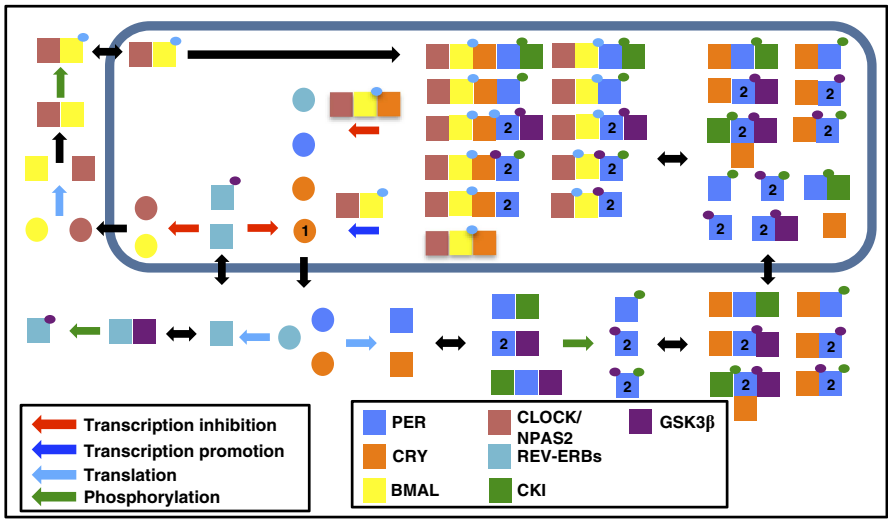
Regulatory network for circadian rhythms

21 variable-model



Mirsky,..Kay, Doyle (2009) *PNAS*

180 variable model



2012 Kim and Forger
Molecular Systems Biology

Negative feedback of gene expression
is necessary for circadian rhythms

"For the greatest benefit to mankind"
Alfred Nobel

The Nobel Assembly at Karolinska Institutet has today decided to award the

2017 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

to:

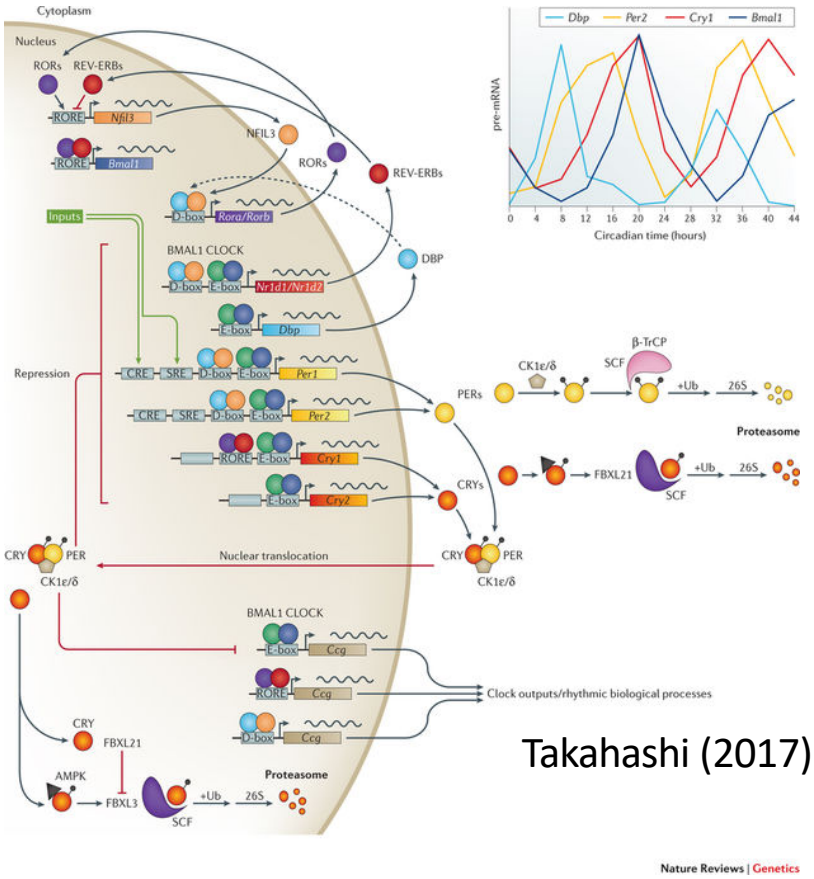
Illustrations: Niklas Elmehed. Nobel Prize Medal: © Nobel Foundation. Photo: Lovisa Engblom.

Jeffrey C. Hall
Michael Rosbash
Michael W. Young

"for their discoveries of molecular mechanisms controlling the circadian rhythm"

Nobelprize.org

Regulatory networks underlie circadian rhythms



Takahashi (2017) *Nat Rev Genet*

Based on experimental knowledge, we theoretically study fundamental questions in circadian rhythms. We make testable predictions from model.

Contents

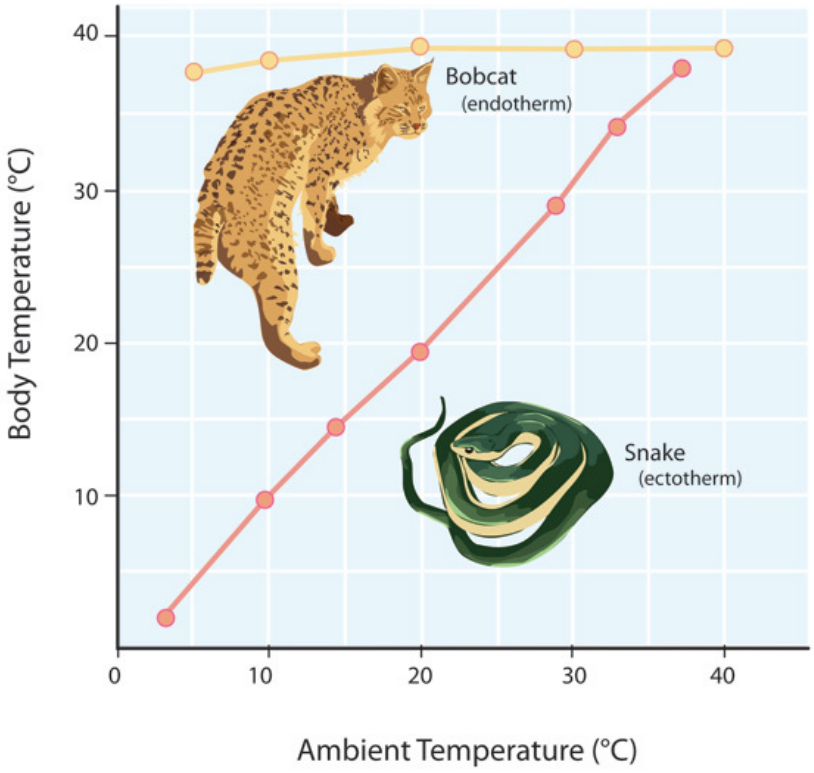
- **Mechanism of temperature compensation in circadian rhythms**

  Kurosawa, Fujioka, Koinuma, Mochizuki, Shigeyoshi (2017) *PLOS Computational Biology*

- **Regulation of circadian rhythms by RNA methylation**

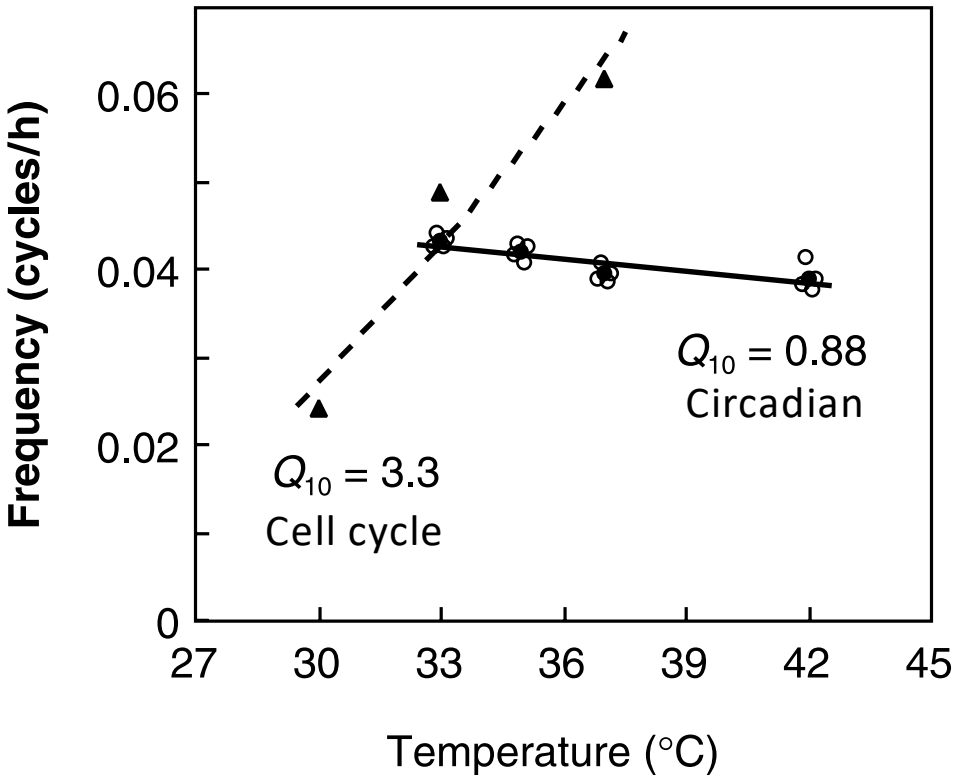
  Fustin,.., Gibo, Kurosawa,.., Okamura (2018) *PNAS*

Circadian period is stable to temperature (”temperature compensation”)



Akin (2011)

(Ex) Mammalian NIH3T3 cells

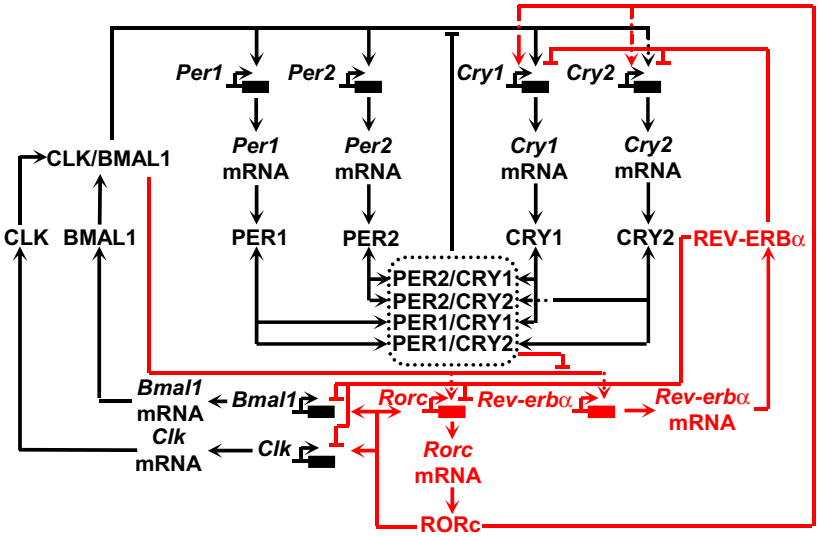


Tsuchiya,..Nishida (2003)

All the species which has circadian rhythm shows temperature compensation in period.

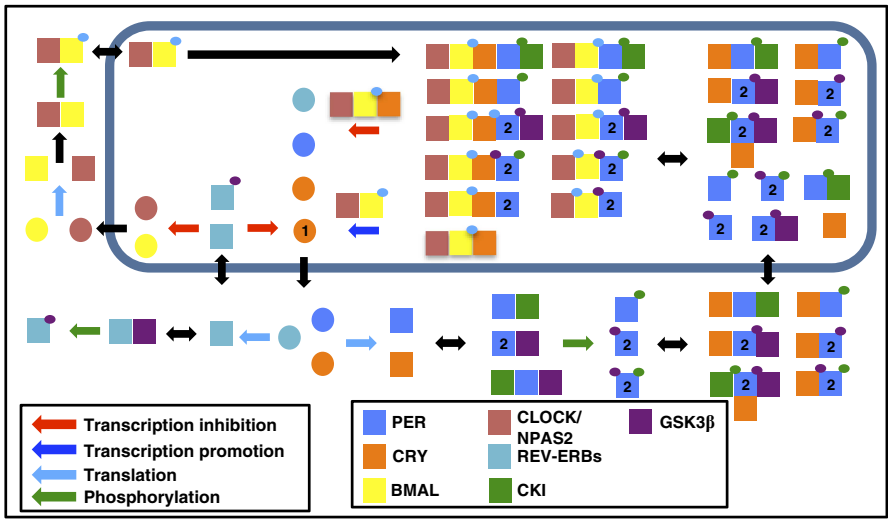
Regulatory network for circadian rhythms

21 variable-model



Mirsky,..Kay, Doyle (2009) *PNAS*

180 variable model



2012 Kim and Forger
Molecular Systems Biology

Negative feedback of gene expression
is necessary for circadian rhythms

We propose a simpler model for circadian rhythm

Model

activator change (ex. mRNA)

$$\varepsilon \frac{dX}{dt} = \left(k + \frac{vX^{\gamma}}{K_v^{\gamma} + X^{\gamma}} \right) \frac{1}{h + Y} - aX$$

gene-activation: $\frac{vX^{\gamma}}{K_v^{\gamma} + X^{\gamma}}$

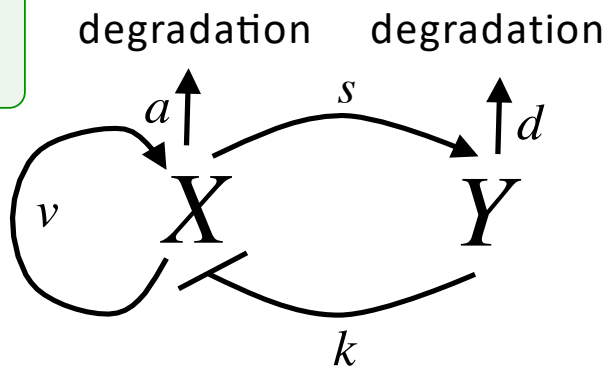
gene-inactivation: $\frac{1}{h+Y}$

inhibitor change (ex.protein)

$$\frac{dY}{dt} = \frac{sX^{\alpha}}{K_s^{\alpha} + X^{\alpha}} - dY$$

translation: $\frac{sX^{\alpha}}{K_s^{\alpha} + X^{\alpha}}$

degradation: dY



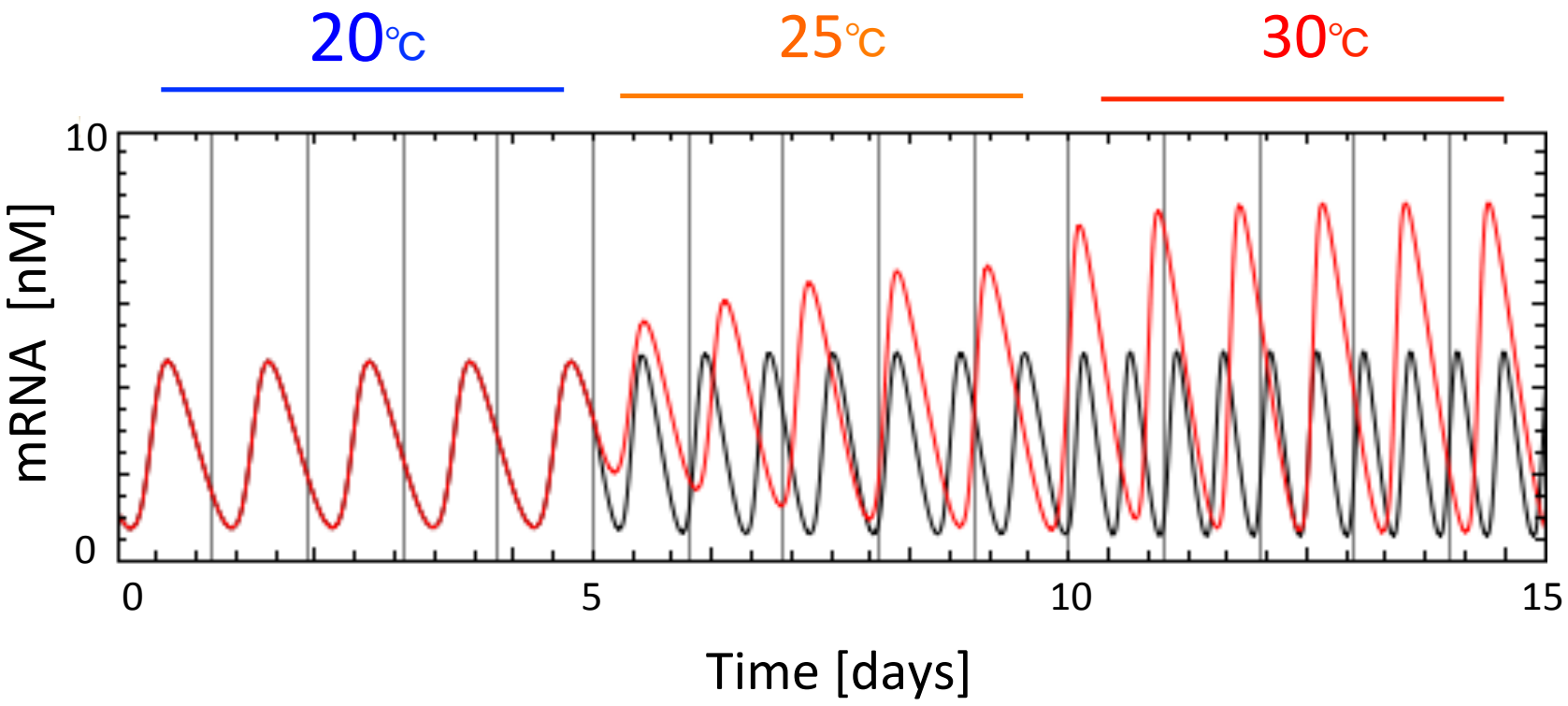
Kurosawa *et al* (2017) *PLOS Comp Biol*

Question: Suppose that reaction becomes faster with temperature.

$$\left(\text{i.e. } \frac{dk}{dT} \equiv s_k, \frac{da}{dT} \equiv s_a, \frac{dv}{dT} \equiv s_v, \frac{ds}{dT} \equiv s_s, \frac{dd}{dT} \equiv s_d \text{ and } s_k, s_a, s_v, s_s, s_d > 0 \right)$$

When period can be maintained as temperature increases?

Simulation of gene-protein dynamics with temperature change



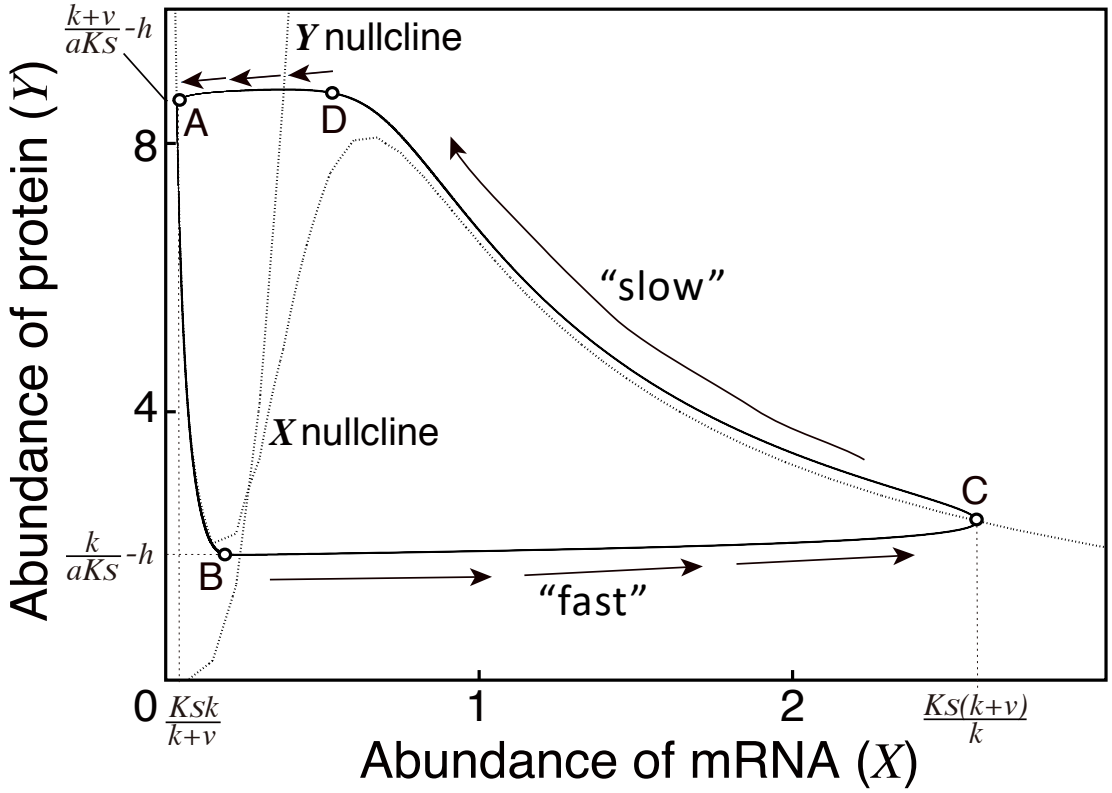
When period is stable, amplitude increases as temperature increases.

Method

Model

$$\varepsilon\, dX/dt = \left(k + vX^{\gamma}\Big/\left(K_V^{\gamma} + X^{\gamma}\right)\right)\Big/\left(h + Y\right) - aX$$

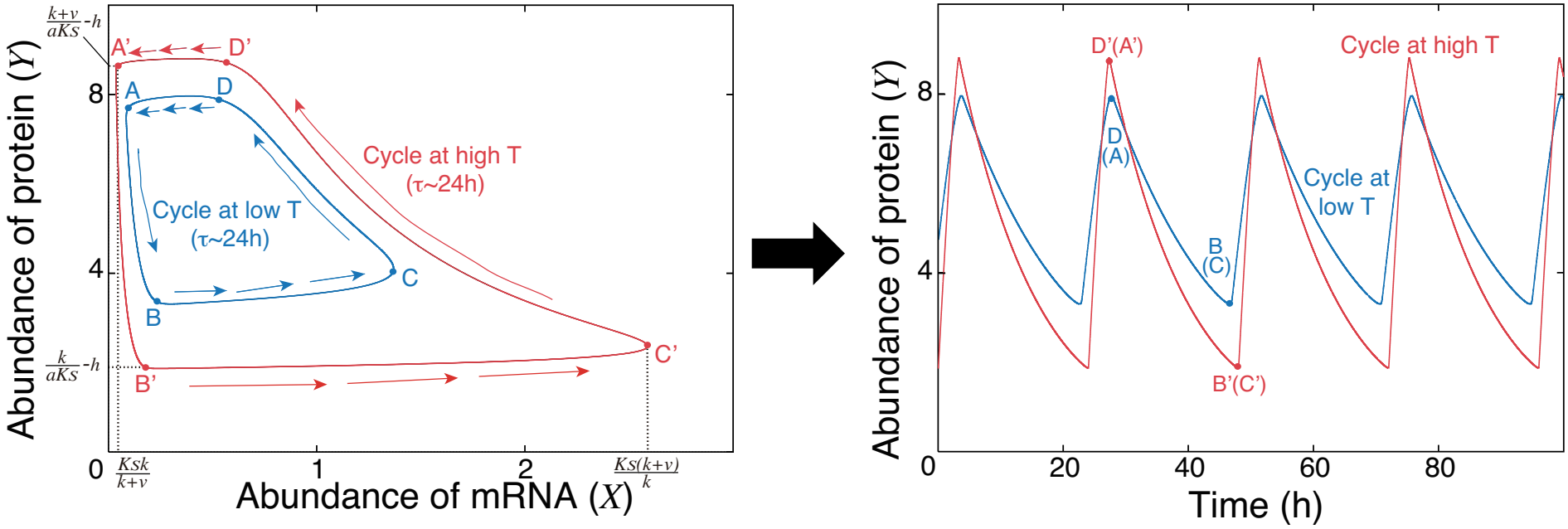
$$dY/dt = sX^{\alpha}\Big/\left(K_S^{\alpha} + X^{\alpha}\right) - dY$$



By assuming that time scale of mRNA dynamics and protein dynamics is different (i.e. ε very small), we can derive approximated form of period:

$$\begin{bmatrix}\text{Approximated}\\ \text{period}\end{bmatrix} = \frac{1}{d}\begin{bmatrix} log\left[\left(s + dh - dk/\left(aK_S\right)\right)\Big/\left(s + dh - d\left(k+v\right)/\left(aK_S\right)\right)\right] \\ +log\left[\left(\left(k+v\right)/\left(aK_S\right) - h\right)\Big/\left(k/\left(aK_S\right) - h\right)\right]\end{bmatrix}$$

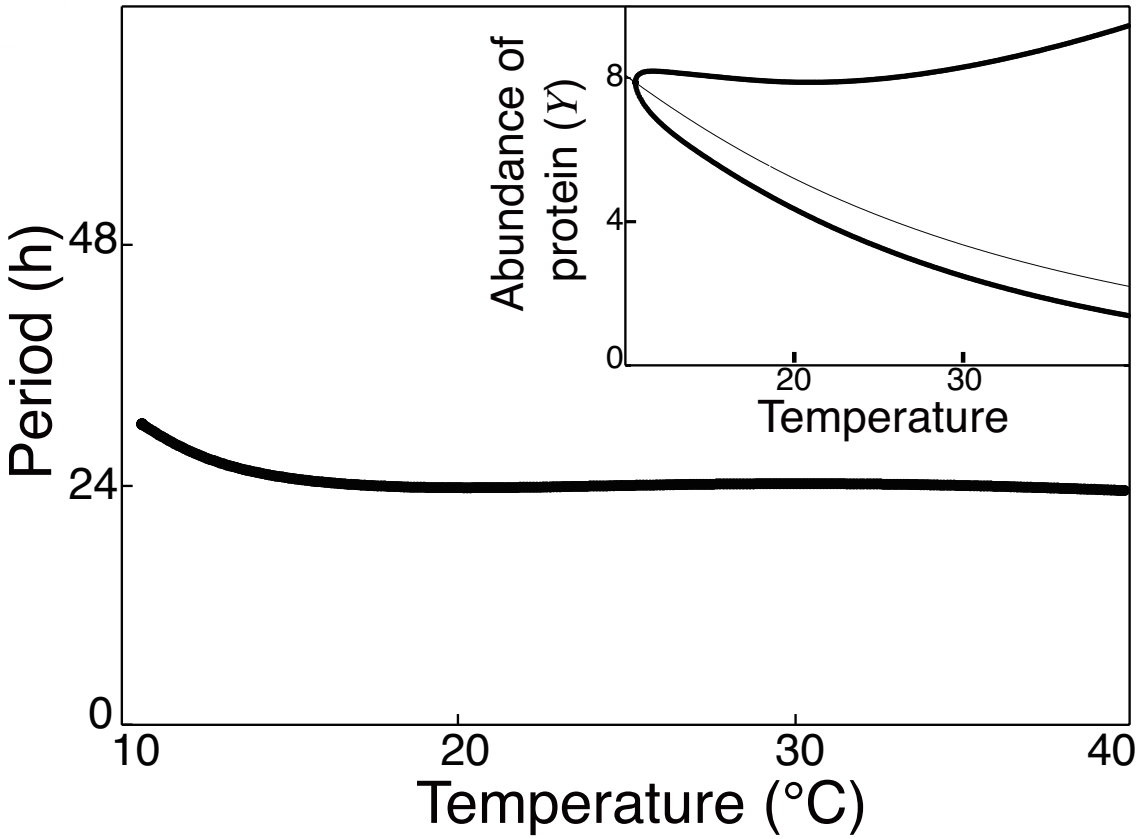
What is mechanisms for the stability of the daily rhythms against temperature?



We prove (for biologically plausible parameter condition (ds/dT>dd/dT)):

"it is impossible to maintain stable period if maximums of variables are smaller at higher temperature and, minimums of variables are larger at higher temperature".

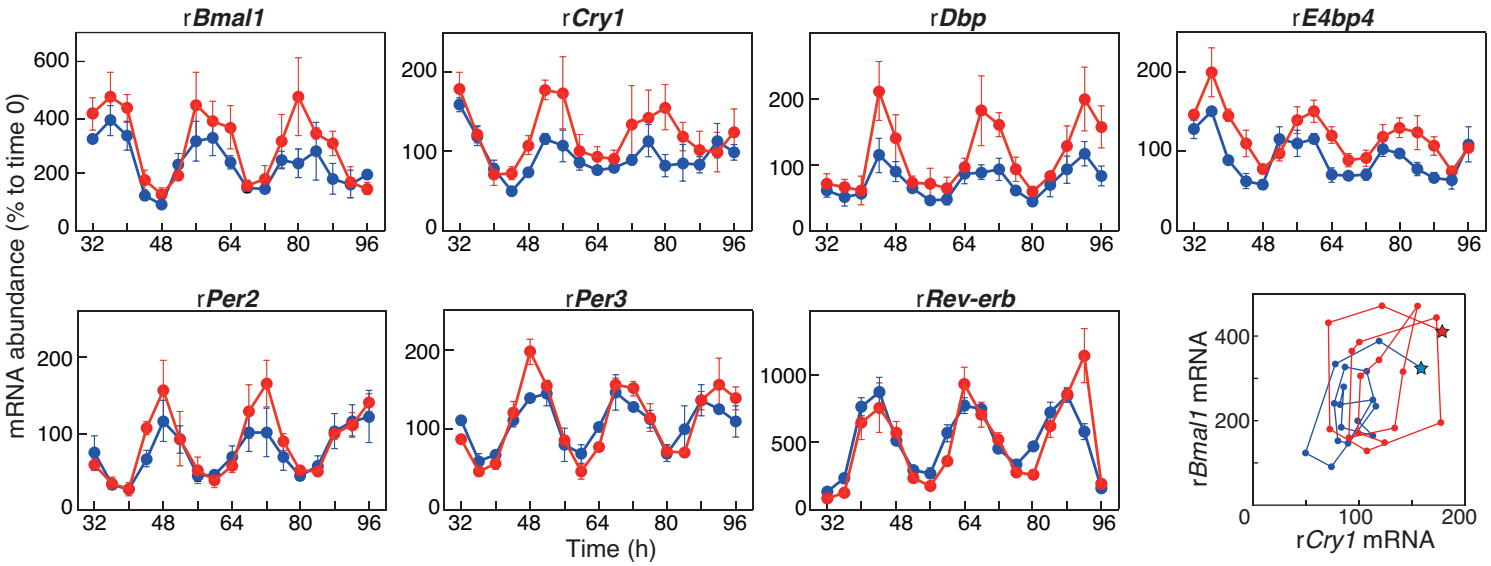
Prediction



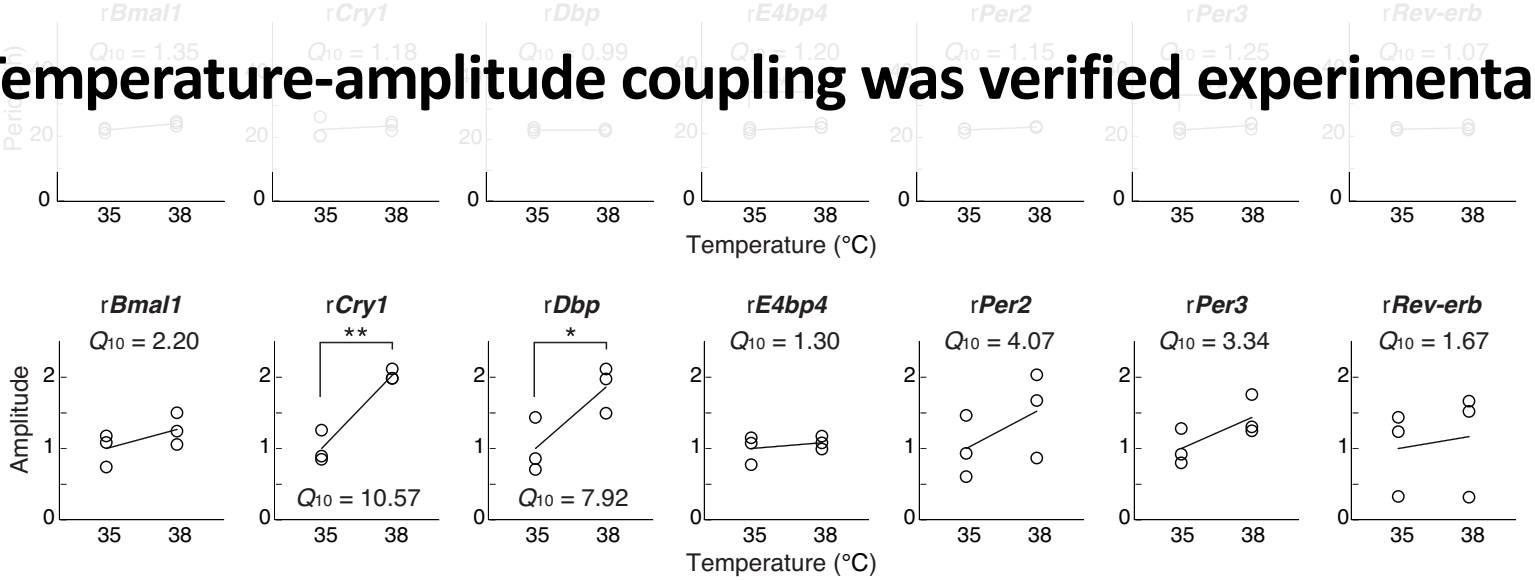
High temperature dependency of amplitude should be observed at daily rhythms.
(We call it as “temperature-amplitude coupling”.)

(Verification1) Can temperature-amplitude coupling be observed in reality (mammalian cultured cells) ?

By courtesy of Prof. Yasufumi Shigeyoshi, Kindai University (Kurosawa *et al* (2017) *PLOS Comp Biol*)



Temperature-amplitude coupling was verified experimentally.



2017年6月9日
理化学研究所

体内時計が温度に影響されない仕組み
－温度と振れ幅のカップリングが鍵－

この発表資料を分かりやすく解説した「60秒でわかるプレスリリース」もぜひご覧ください。

要旨

理化学研究所（理研）望月理論生物学研究室の黒澤元研究員と望月敦史主任研究員らの共同研究チーム※は、体内時計が温度に影響されない仕組みに関する新たな仮説「温度-振幅カップリング」を提唱しました。

2017年9月8日
理化学研究所

体内時計が温度に依存しない仕組みを原子レベルで解明
―リン酸化酵素内に温度依存的なブレーキを発見―

この発表資料を分かりやすく解説した「60秒でわかるプレスリリース」もぜひご覧ください。

要旨

理化学研究所（理研）生命システム研究センター合成生物研究グループの上田泰己グループディレクター、篠原雄太特別研究員、小山洋平研究員らの共同研究グループ※は、哺乳類の概日時計[1]（体内時計）の周期長を決定しているリン酸化酵素の"基質との結合"および"生成物との結合"の二つの結合の強弱が温度によって変化し、高温でのリン酸化反応速度の上昇にブレーキをかけることが、概日時計の温度補償性[2]に重要であることを解明しました。

Conclusion

- We theoretically showed that higher amplitude at higher temperature can stabilize the period to temperature (temperature-amplitude coupling).

- Our collaborators verified it experimentally.

Contents

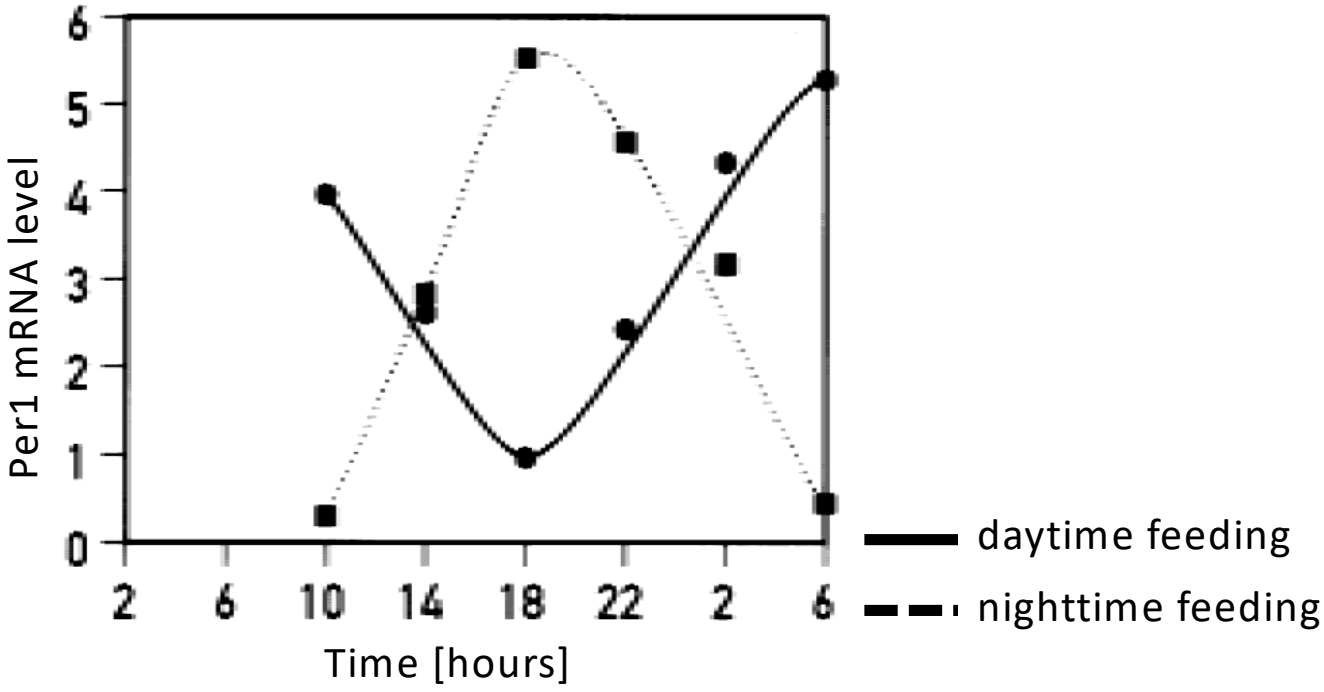
- **Mechanism of temperature compensation in circadian rhythms**

  Kurosawa, Fujioka, Koinuma, Mochizuki, Shigeyoshi (2017) *PLOS Computational Biology*

- **Regulation of circadian rhythms by RNA methylation**

  Fustin,.., Gibo, Kurosawa,.., Okamura (2018) *under review*

Introduction: Food timing and Per1 gene expression

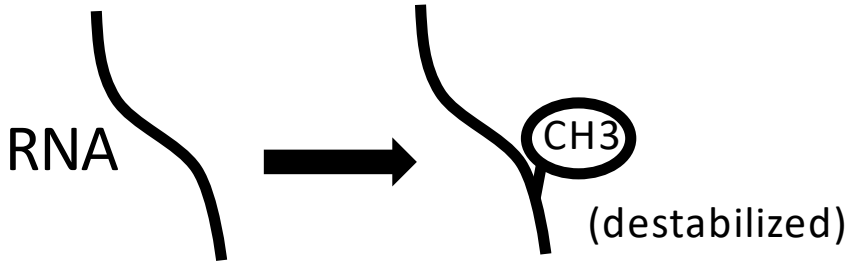


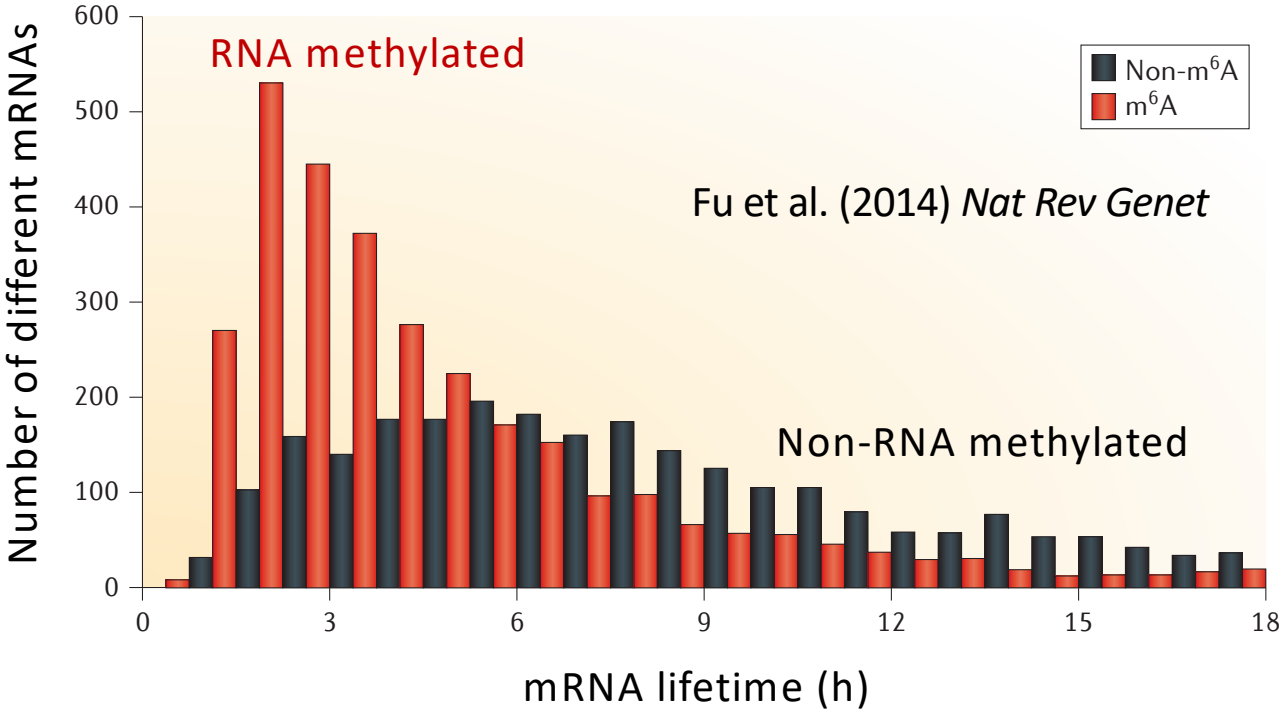
(Damiola et al., Genes & Dev., 2000)

How does food and/or metabolic state regulate the clock?

RNA methylation: a possible link between metabolic and genetic system

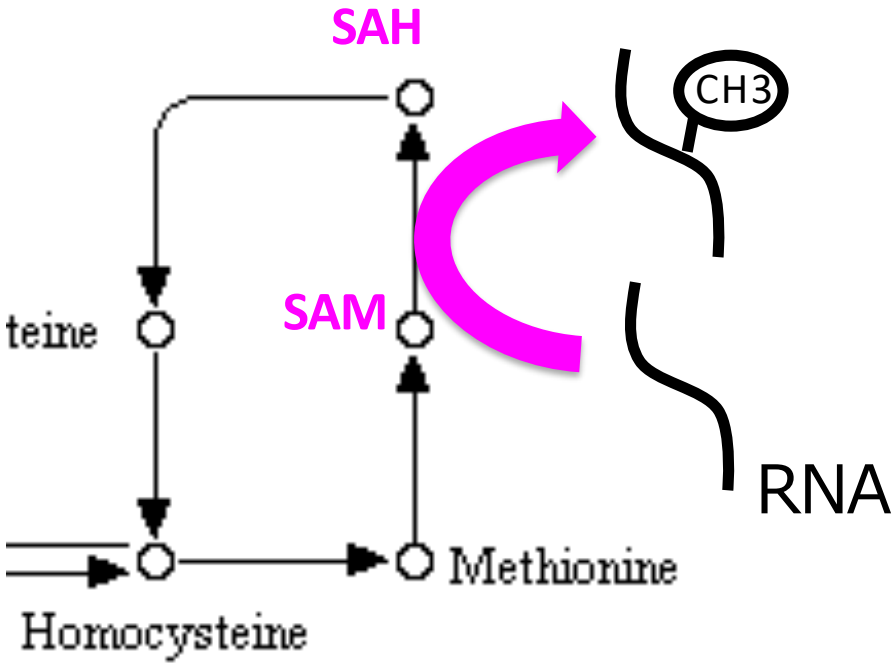
m6A RNA methylation





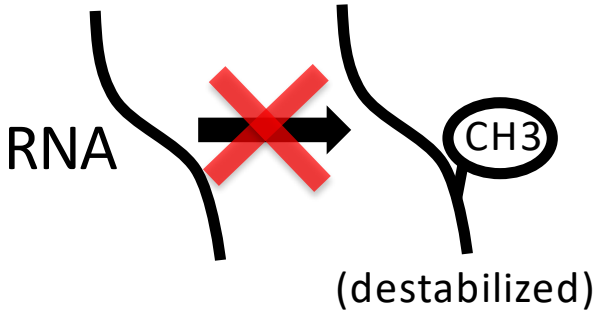
"RNA methylation may control gene-protein dynamics by RNA destabilization."

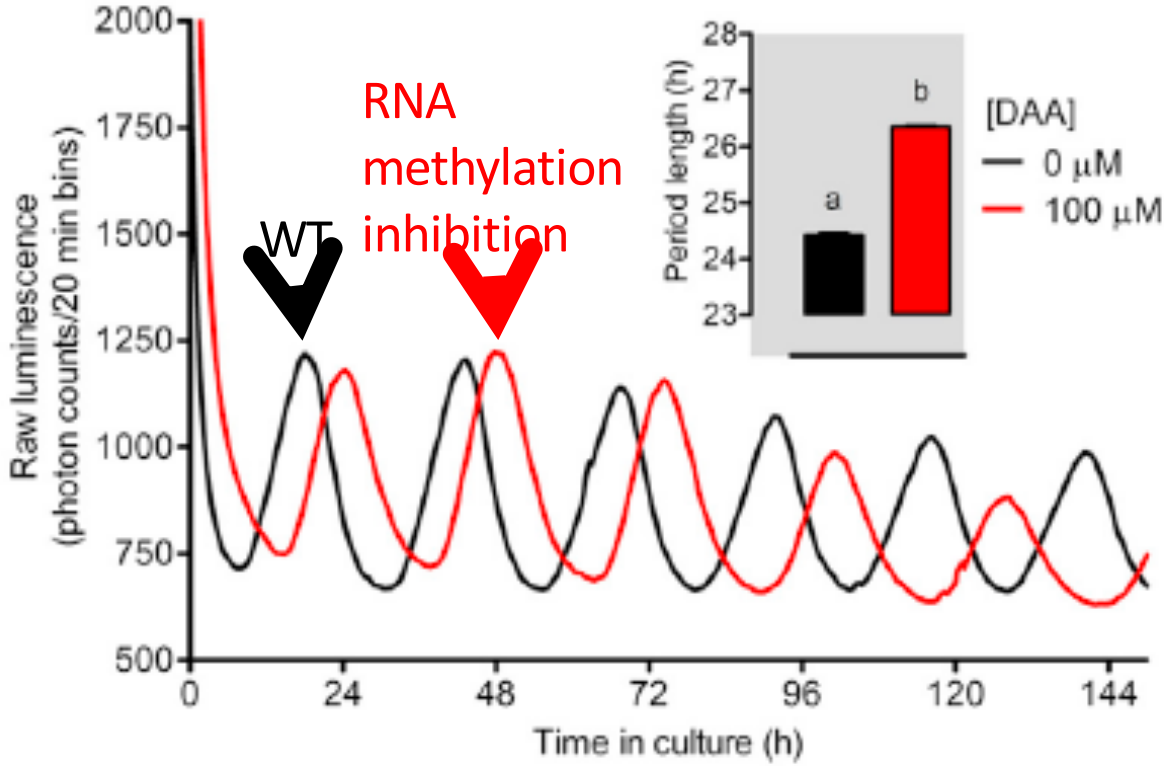
RNA methylation can be important output from biosynthesis network



RNA methylation inhibition elongates circadian period.

m6A RNA methylation

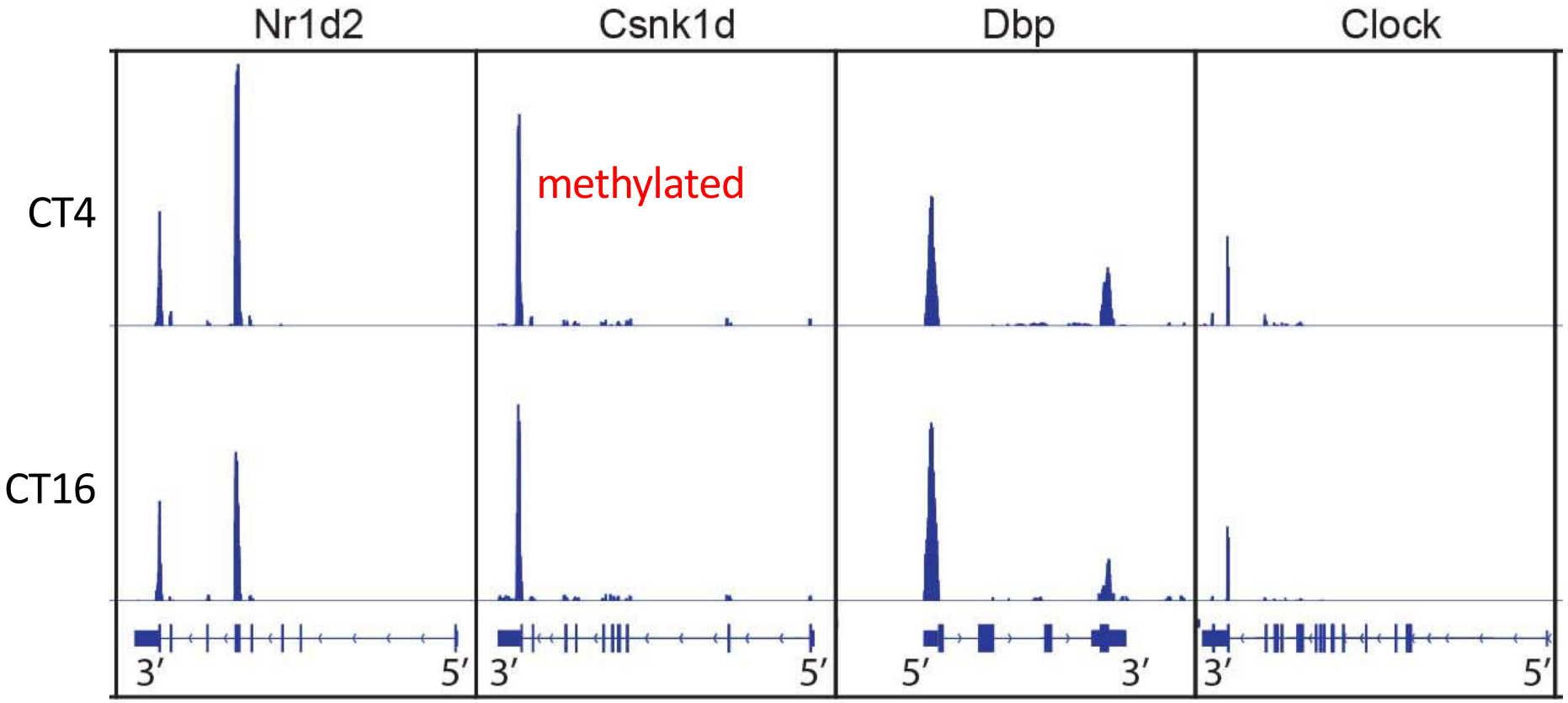




Fustin,.., Okamura, *Cell*, 2013

"How is the period elongated? "

circadian mRNAs are methylated



(thick segment: exon, thin line: intron)

We focused on Ck1d.

"Why Ck1d (Casein Kinase 1d)?"

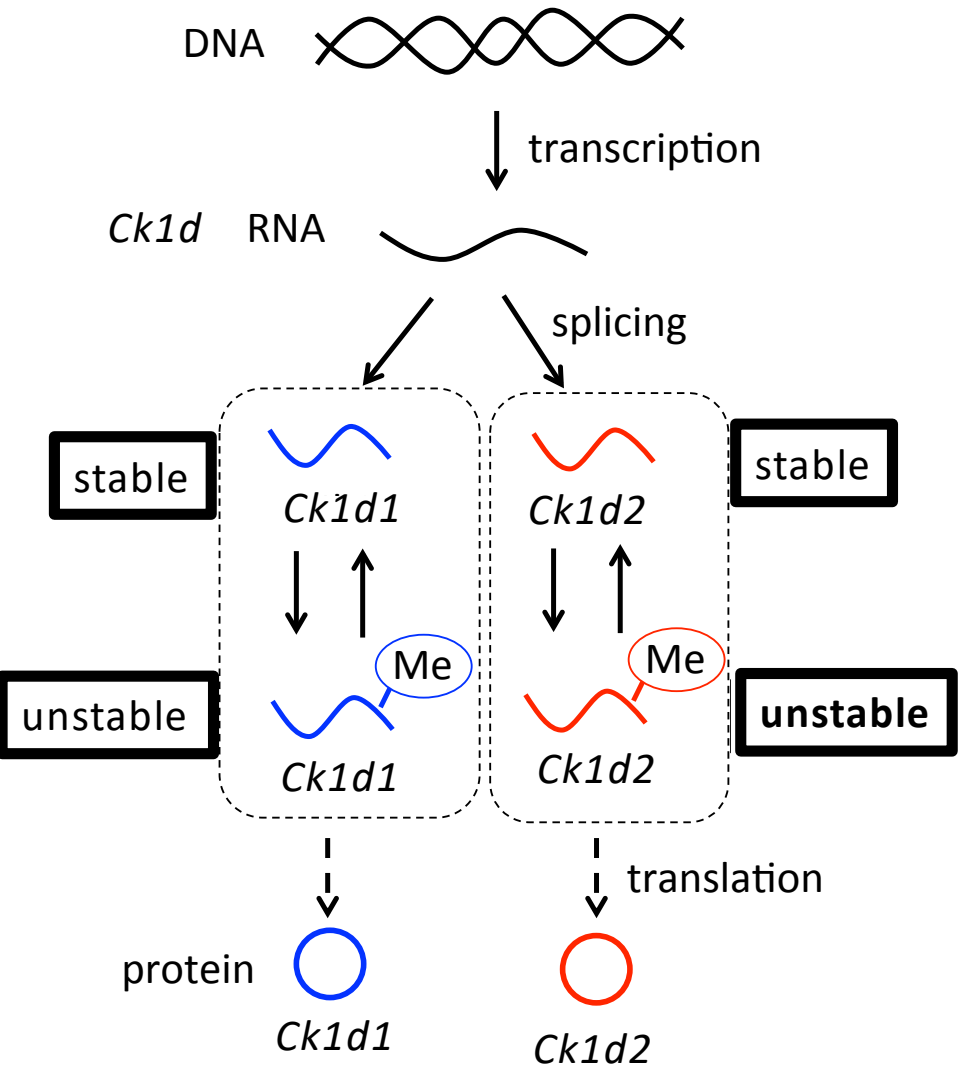
Familial Advanced Sleep Phase Syndrome originates from a mutated *Ck1d*.

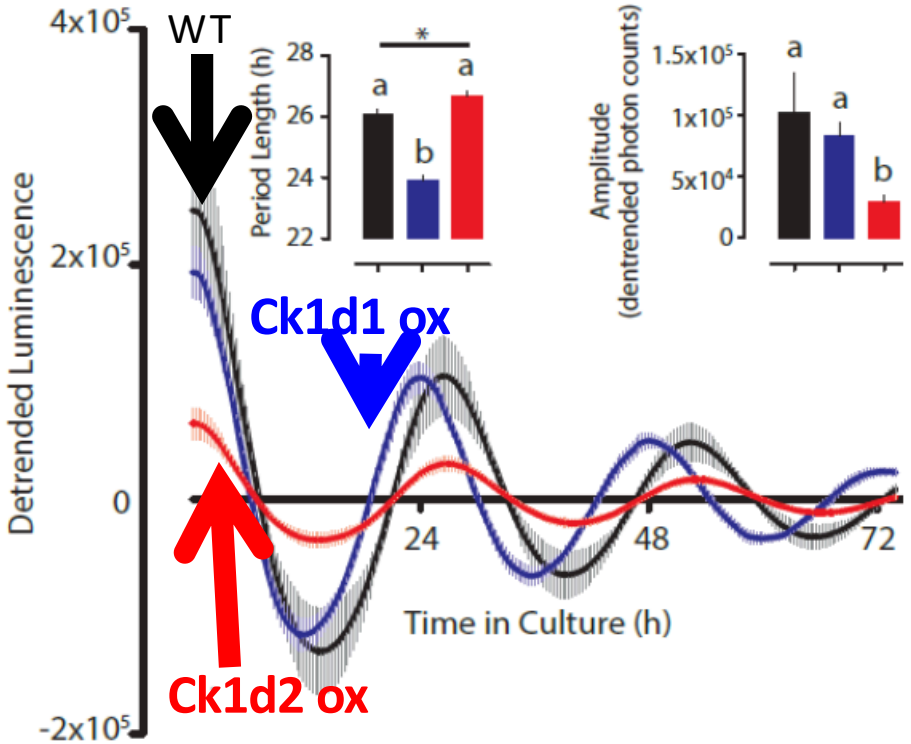
(Jones et al (1999) *Nat Med,* Toh et al. (2001) *Science*; Xu et al. (2005) *Nature*)

	Control ($n = 6$)	FASPS($n = 6$) Mean ± s.d
Sleep Onset	23:10 ± 0:40	19:25 ± 1:44
Sleep Offset[a]	07:44 ± 1:13	04:18 ± 2:00

splice variants of kinase gene (*Ck1d*)

(Fustin,.., Okamura 2018 *PNAS*)

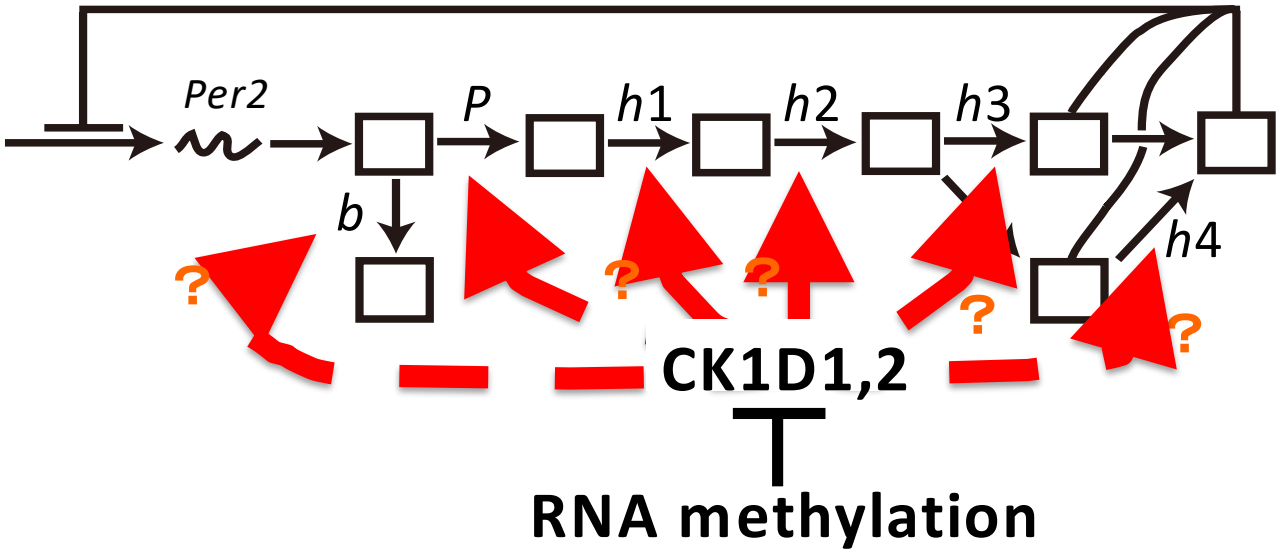




Effects of the two splice variants on period are opposite.

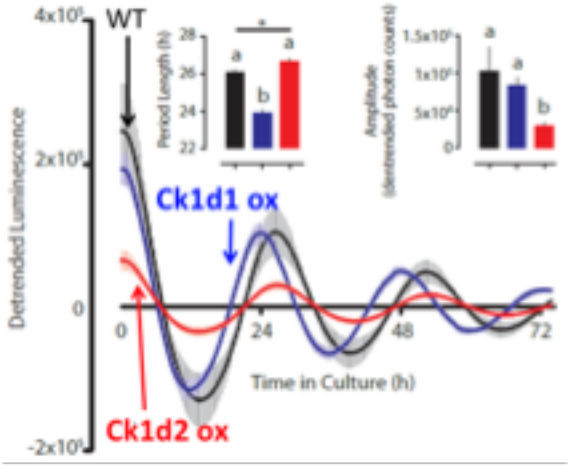
How circadian rhythms are regulated by RNA methylation through the kinase (CK1D1,2)?

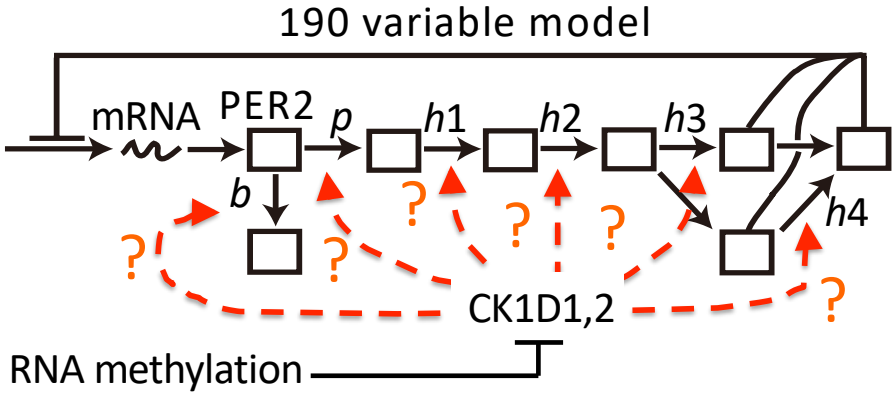
cf. Zhou et al. (2015) *Mol Cell*



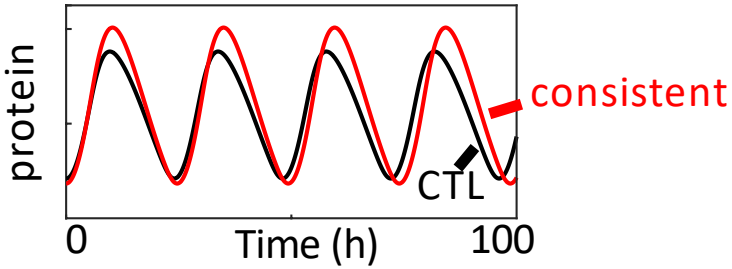
- Casein Kinase 1D (CK1D) is known to phosphorylate circadian proteins.
- CK1D possibly activates six phosphorylation processes, above.
- We didn't know which processes are actually activated by CK1D1,2.

Predicting the regulation of circadian rhythm by RNA methylation

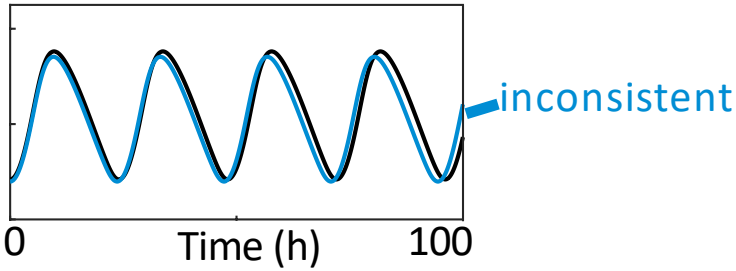




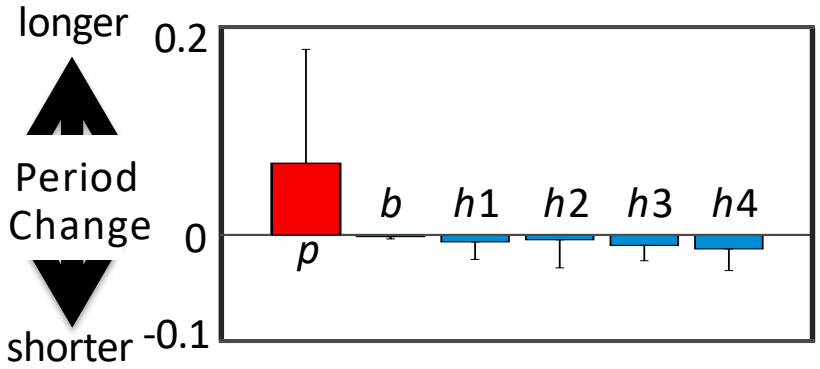
If CK1D2 activates *p*..



If CK1D2 activates *h3*..



Identification of the reaction activated by CK1D2



"CK1D2 should activate *p*."

(verified by Okamura Lab (Kyoto))

Fustin,..,Gibo, Kurosawa,..,Okamura 2018 *PNAS*

Contents

- **Mechanism of temperature compensation in circadian rhythms**

  ➡ **We predict that larger amplitude at higher temperature.**

  Kurosawa, Fujioka, Koinuma, Mochizuki, Shigeyoshi (2017) *PLOS Computational Biology*

- **Regulation of circadian rhythms by RNA methylation**

  ➡ **We predict the PER2 phosphorylation process *(*p) which is regulated by RNA methylation via Ck1d2.**

  Fustin,.., Gibo, Kurosawa,.., Okamura (2018) *PNAS*

Collaborators

Atsushi Mochizuki (RIKEN)
Shingo Gibo (RIKEN)

Yasufumi Shigeyoshi（Kindai Univ）
Atsuko Fujioka (Kindai Univ)
Satoshi Koinuma （Kindai Univ)

Hitoshi Okamura (Kyoto Univ)
Jean-Michel Fustin (Kyoto Univ)

Funding: CREST “Chrono-metabolism” project
(PI: Hitoshi Okamura, Kyoto Univ)

Can we simulate food effect on gene expression?

Assuming..