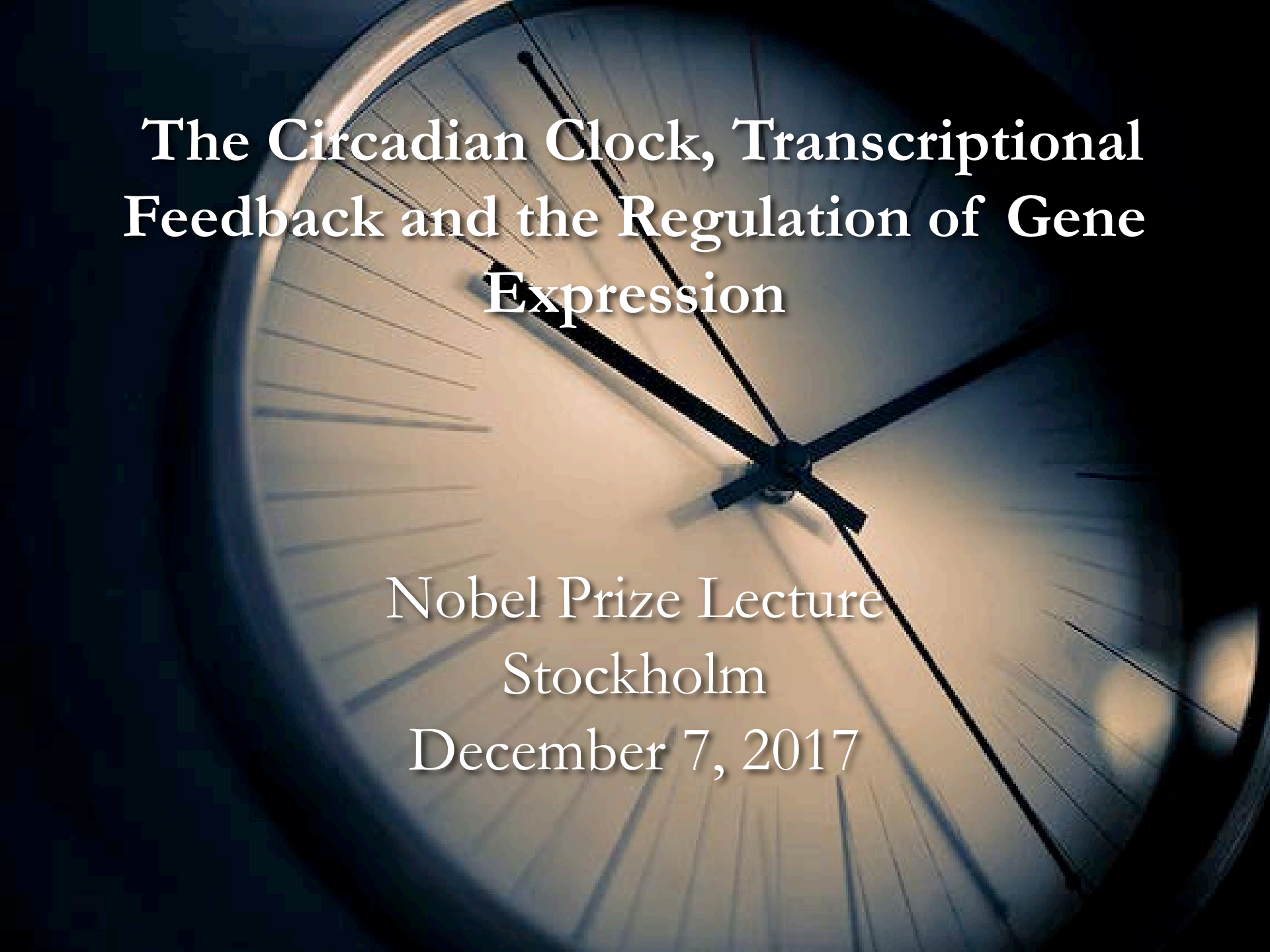


The Circadian Clock, Transcriptional Feedback and the Regulation of Gene Expression

Nobel Prize Lecture
Stockholm
December 7, 2017

Current Lab Members

- Kate Abruzzi
- Madelen Diaz
- Fang Guo
- Meghana Holla
- Hua Jin
- Qunlong Li
- Weifei Luo
- Dylan Ma
- Reazur Rahman
- Jenn Sherk
- Matthias Schlichting
- Patrick Weidner
- Jason Xi
- Weijin Xu
- Albert Yu

Current Lab Members

- **Kate Abruzzi**
- Madelen Diaz
- Fang Guo
- Meghana Holla
- Hua Jin
- Qunlong Li
- Weifei Luo
- Dylan Ma
- Reazur Rahman
- Jenn Sherk
- Matthias Schlichting
- Patrick Weidner
- Jason Xi
- Weijin Xu
- Albert Yu

CIRCADIAN RHYTHMS

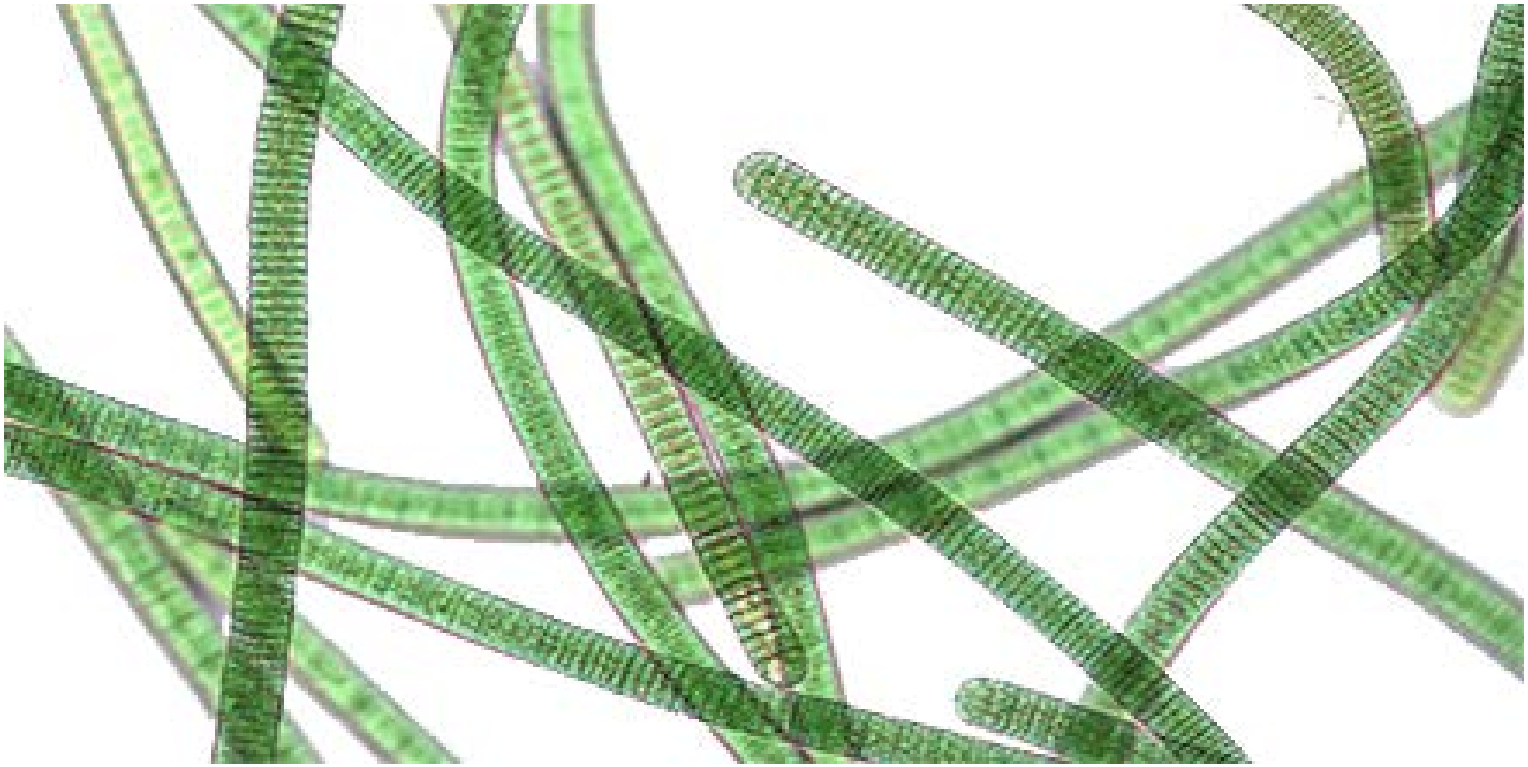
- Biochemical, physiological, behavioral adaptations to external daily oscillations - rotation of the earth.

- Purpose #1: anticipation of daily environmental changes.

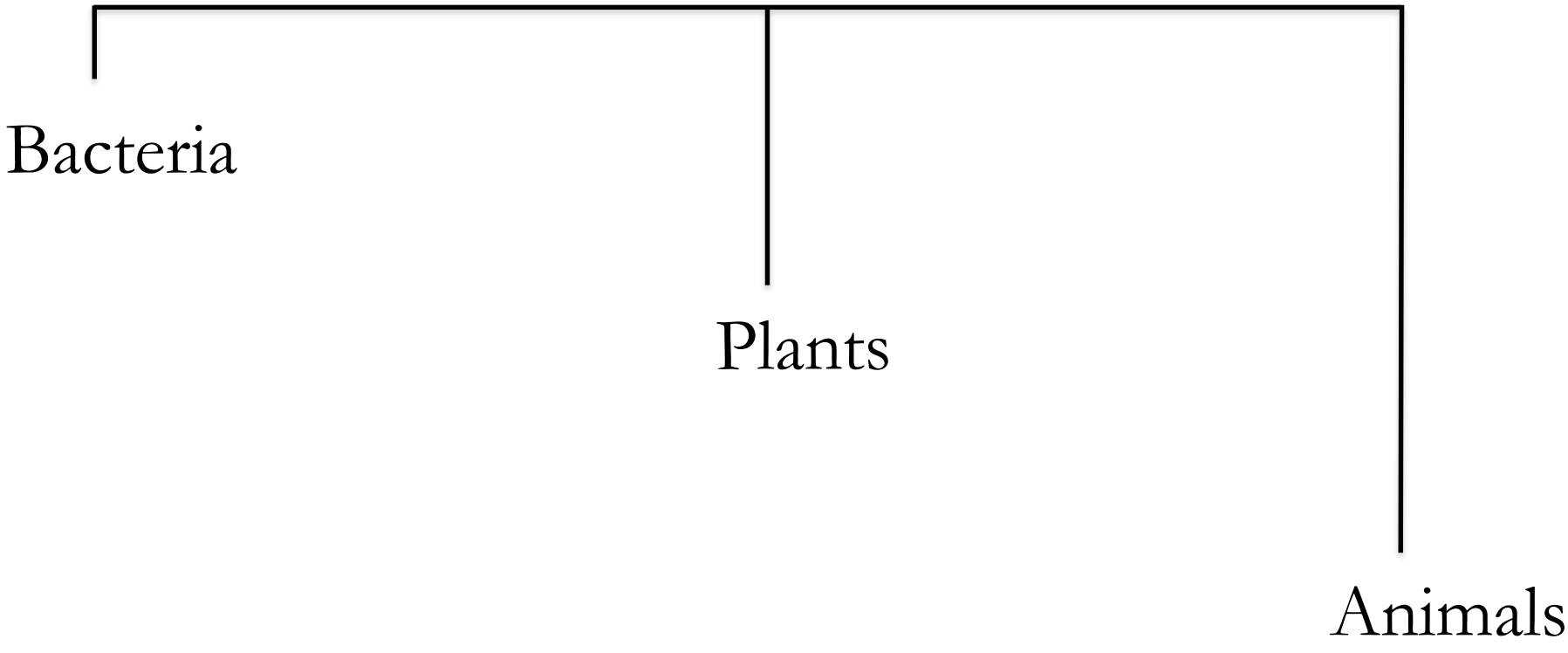
- Purpose #2: coherence of internal processes.

Circadian Rhythms are Ancient and Nearly Ubiquitous

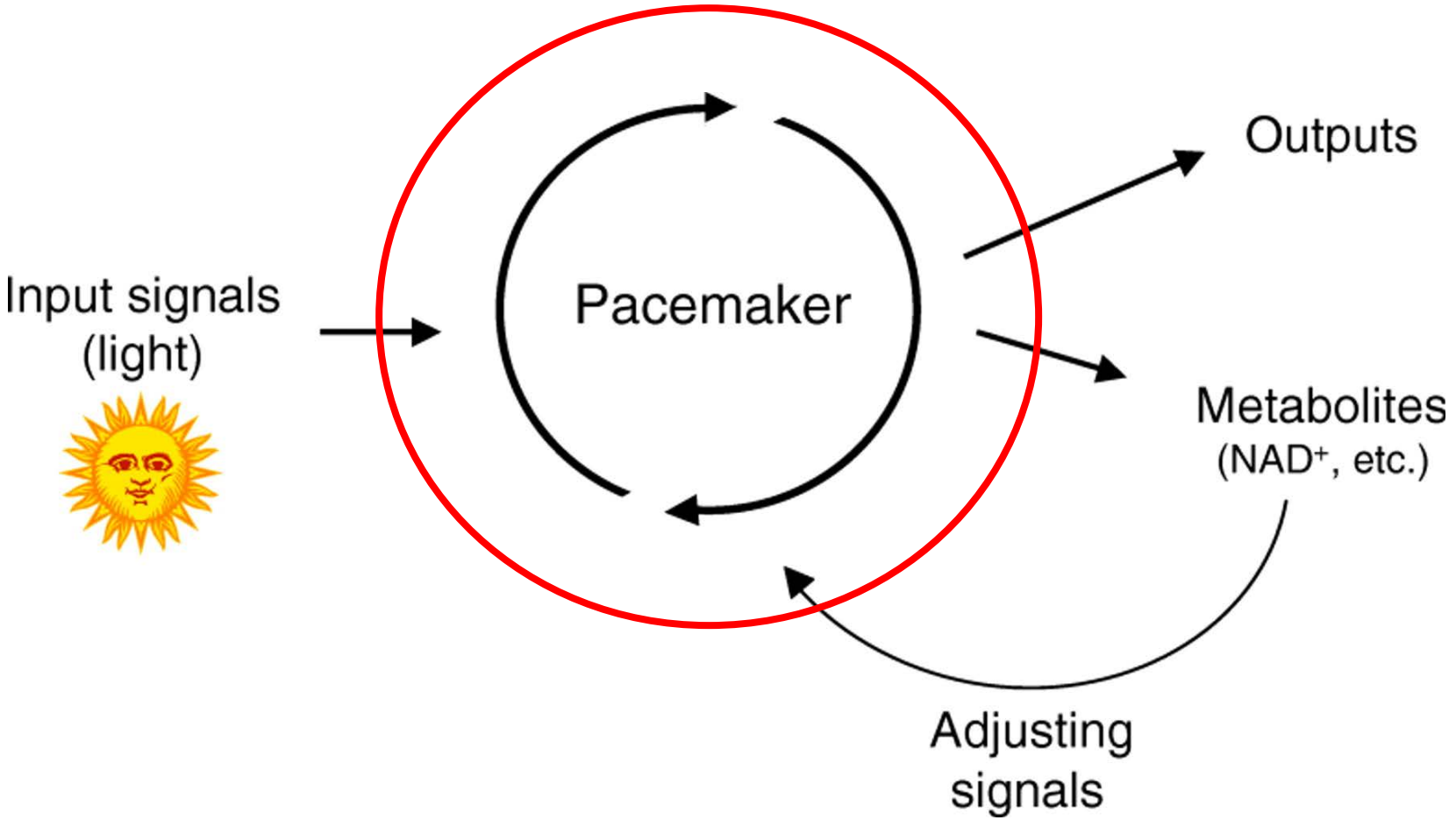
Oldest Known Clock is in Cyanobacteria: Oxygenation of the Atmosphere > 2 Billion Years ago



Circadian Clocks Exist in Different Kingdoms and Probably Evolved Multiple Times in Evolution

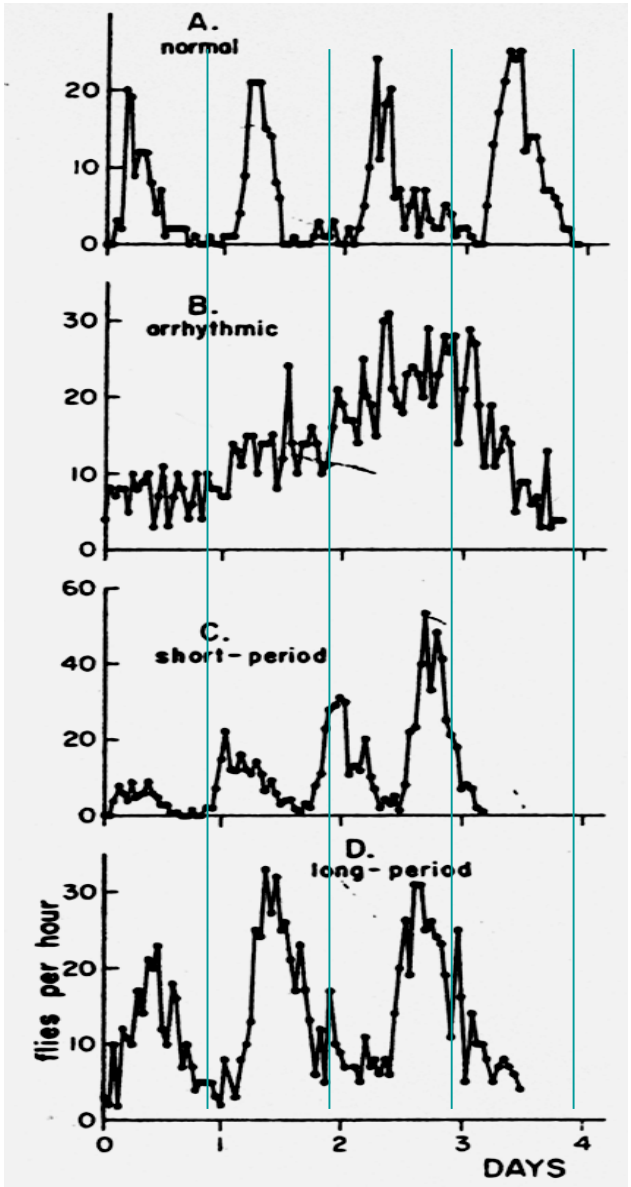


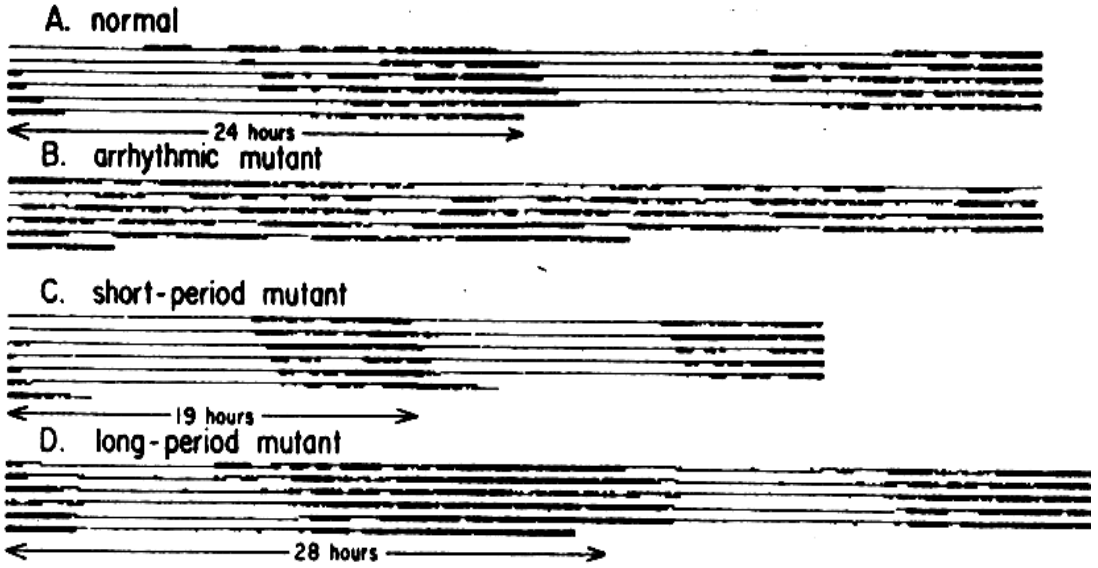
A Simple Systems View of Circadian Clocks



The Beginning (*period* mutants)

Konopka and Benzer (1971)





Early Brandeis Cloning Crew

Cloning, Identifying and Sequencing *period* Gene and Protein in the early-mid 80s

Rosbash Lab: Pranitha Reddy, Qiang Yu, Xin Liu, Yoav Citri

Pioneer Protein!

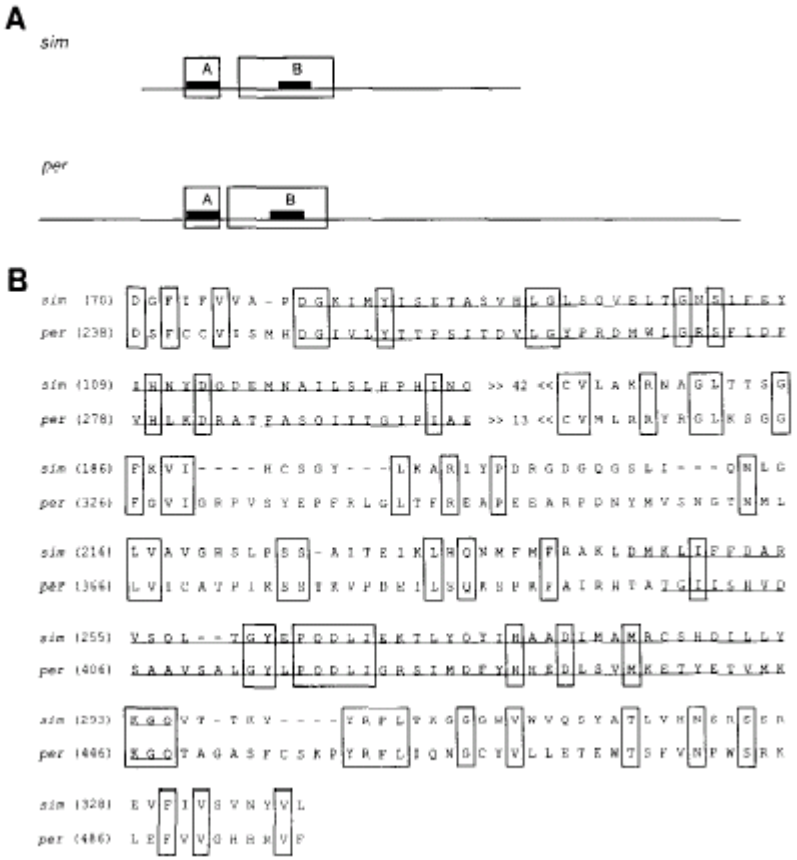
Yu et al., 1987

- PER has an intriguing repeat region, but it is not necessary for circadian function.

Cell. 1988 Jan 15;52(1):143-51.

The Drosophila single-minded gene encodes a nuclear protein with sequence similarity to the per gene product.

Crews ST, Thomas JB, Goodman CS.



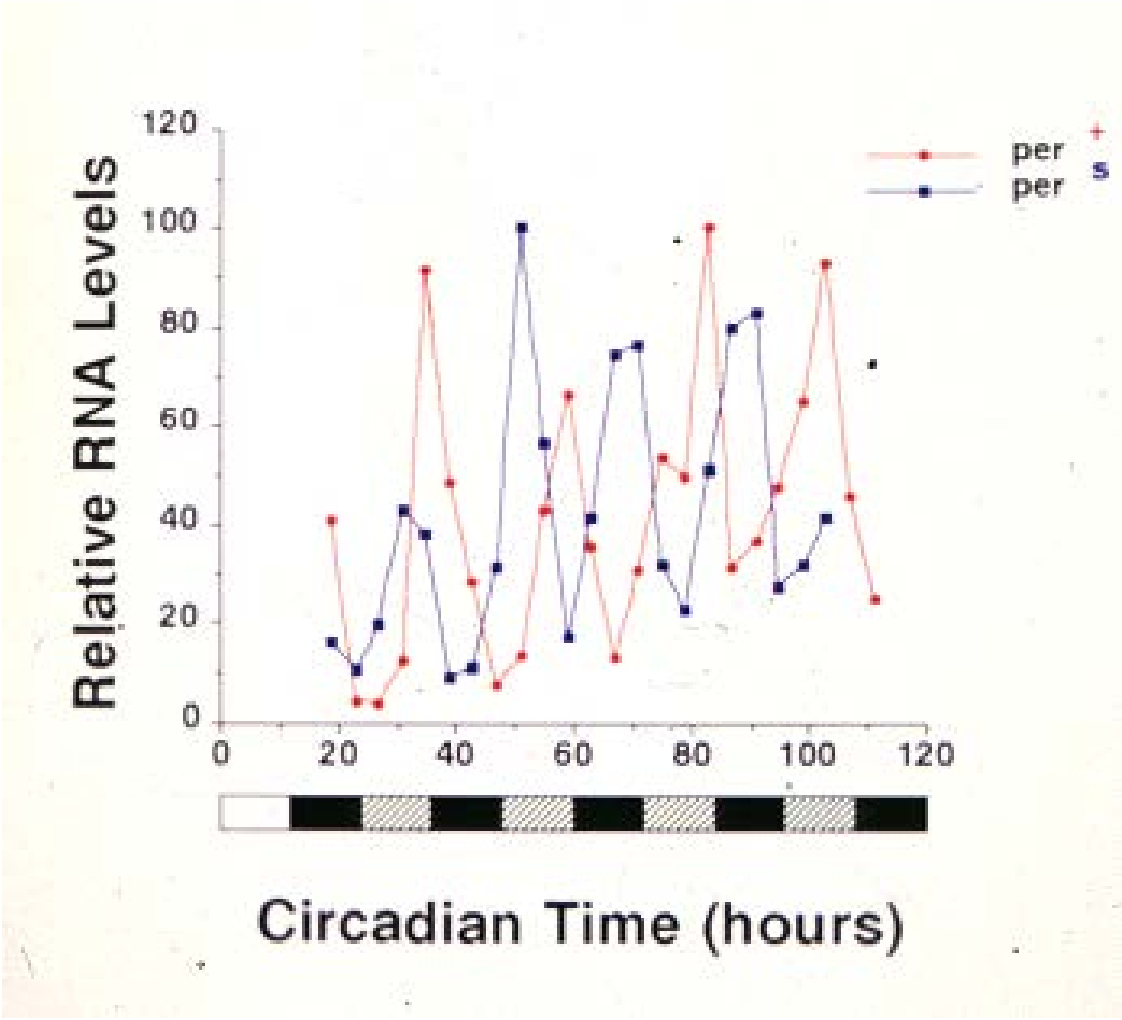
Two Decades Post-Konopka and Benzer…

Nature, 1990

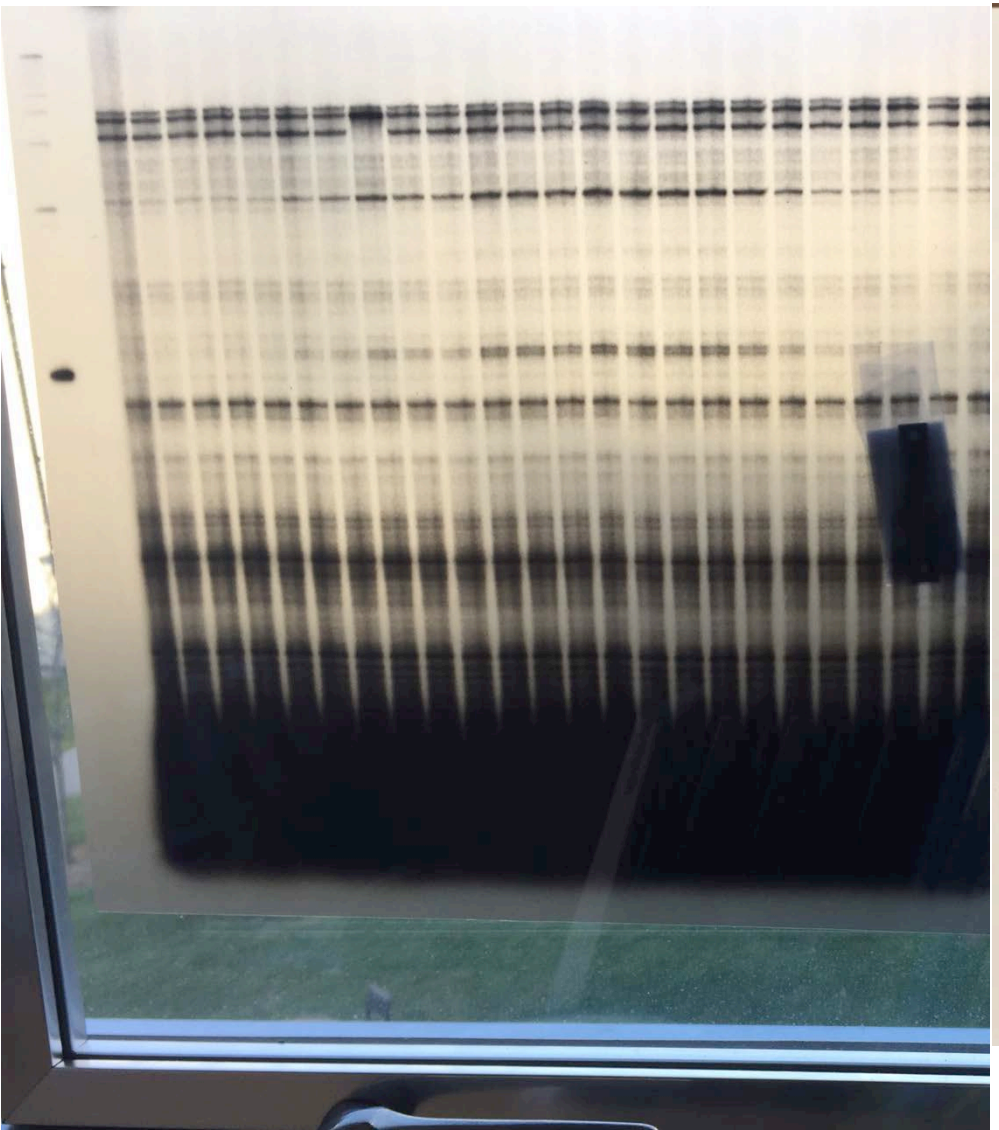
Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels

Paul E. Hardin*†, Jeffrey C. Hall† & Michael Rosbash*†

* Howard Hughes Medical Institute and † Department of Biology, Brandeis University, Waltham, Massachusetts 02254, USA


Relative RNA Levels
120
100
80
60
40
20
0
0
20
40
60
80
100
120
per +
per s
Circadian Time (hours)

Original Gel -- under Glass (A Recent Gift from Paul Hardin)



Reprinted from Nature, Vol. 343, No. 6258, pp. 536–540, 8th February, 1990

Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels

Dear Michael,
To a fantastic mentor.
Congratulations!

Paul E. Hardin*†, Jeffrey C. Hall† & Michael Rosbash*†

* Howard Hughes Medical Institute and † Department of Biology, Brandeis University, Waltham, Massachusetts 02254, USA

Mutations in the *period* (*per*) gene of *Drosophila melanogaster* affect both circadian and ultradian rhythms. Levels of *per* gene product undergo circadian oscillation, and it is now shown that there is an underlying oscillation in the level of *per* RNA. The observations indicate that the cycling of *per*-encoded protein could result from *per* RNA cycling, and that there is a feedback loop through which the activity of *per*-encoded protein causes cycling of its own RNA.

CIRCADIAN rhythms influence many behaviours and physiological processes. These rhythms are generated by an endogenous circadian 'clock', persist ('free-run') under constant environmental conditions, and respond to environmental time cues. In *Drosophila melanogaster*, two well-studied phenomena—eclosion and adult locomotor activity—are under the control of the circadian clock.

The product of the *period* (*per*) gene in *D. melanogaster* is a candidate clock molecule[1–3]. The original three mutations described at the *per* locus either shorten (*per*^{s}) or lengthen (*per*^{L}) or essentially abolish (*per*^{01}) circadian activity[4]. The *per* gene has been cloned and extensively analysed[5]. *In situ* hybridization and immunohistochemical analyses indicate that *per* is expressed in numerous adult tissues, including the eyes, antennae, lateral brain neurons and putative glia in heads, and the salivary gland, ovaries and gut in bodies[5,6,7]. Because the circadian oscillator has been mapped to the head[8], we consider that the *per* expression most relevant to clock function is located here.

In the adult visual system, the immunoreactivity of *per* gene product (Per protein) fluctuates[5]; intense staining is readily detectable in photoreceptor nuclei in the middle of the night, but essentially no staining is detectable in the middle of the day. The staining fluctuations persist in constant darkness. These

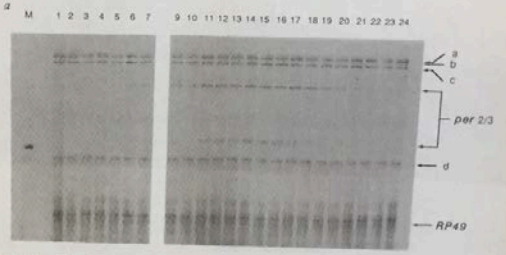
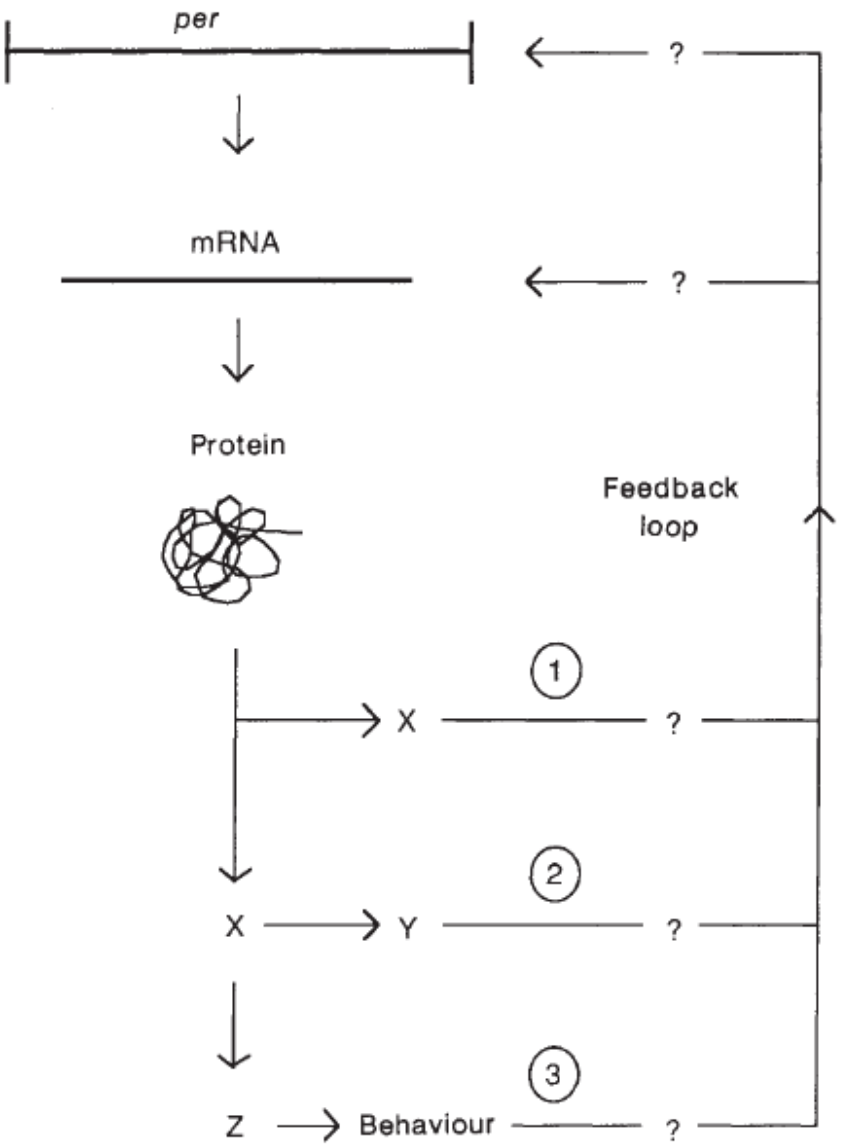


FIG. 1 Levels of *per*$^{+}$ cycle in flies under LD cycle conditions. *a*, RNase protections of RNA from flies collected during LD cycles. The number of hours after lights were turned on that the flies were collected are indicated above each RNase protection lane; lights were turned off after 12 h. M denotes the lane containing labelled 123-base pair markers (BRL). *per* 2/3 denotes the protected fragments from *per* RNA covering parts of exon 3 (upper) and exon 2 (lower). Bands a–d are due to incomplete digestions and full-length protections of the two probes by DNA. *b*, Quantitation of data shown in a. Relative RNA abundance refers to the values of *per*/RP49, where the peak value was adjusted to 100. The white and black bars represent lights on or off, respectively.

METHODS. Wild-type (Canton-S) adults (3–5-days-old) were exposed for 3 days to LD cycles before being collected as 6–8-day-old flies. Flies were then collected (in complete darkness for dark-phase collections) and immediately frozen on dry ice. Heads and bodies were separated[22], and total RNA was extracted from the heads[22]. The *per* 2/3 probe contains RNA from the *Bgl*II site 259 nucleotides from the start of exon 3, to the *Spe*I site, 134 nucleotides from the end of exon 2. The RP49 probe contains RNA from the *Hin*dIII site to the *Pvu*II site and protects a 58-nucleotide fragment[23]. The RP49 probe was transcribed using 20-fold more unlabelled UTP than the *per*-specific probes. RNA hybridizations were performed as described[22]. Quantitation was done by either densitometrically scanning or directly counting the *per* exon 3 and RP49 bands using a Bio-Rad model 620 video densitometer or an AMBIS radioanalytic imaging system, respectively.

Cautious Model: No mention of Transcription in the Title or Abstract



Four Important Follow-up Papers

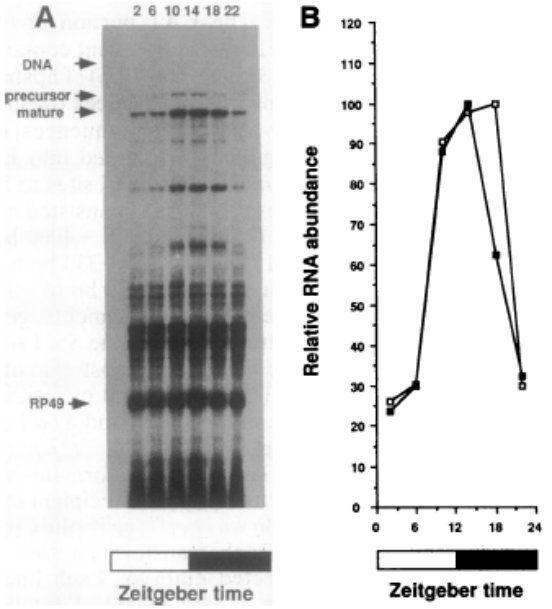
- 1992: Circadian oscillations in *period* gene mRNA levels are transcriptionally regulated. Hardin, P.E., Hall, J. C., and Rosbash, M. Proc.Natl.Acad.Sci. U.S.A 89:11711-11715.
- 1992: The *period* gene encodes a predominantly nuclear protein in adult *Drosophila*. Liu, X., Zwiebel, L. J., Hinton, D., Benzer, S., Hall, J. C., and Rosbash, M. J Neurosci. 12:2735-2744.
- 1994: Constitutive overexpression of the *Drosophila period* protein inhibits *period* mRNA cycling. Zeng, H., Hardin, P. E., and Rosbash, M. EMBO J 13:3590-3598.
- 1995: PAS is a dimerization domain common to *Drosophila period* and several transcription factors. Huang, Z. J., Edery, I., and Rosbash, M. Nature 364:259-262.

Four Important Follow-up Papers

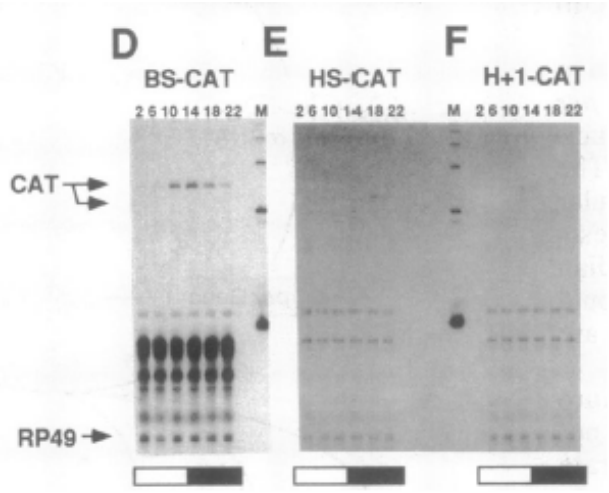
- **1992: Circadian oscillations in *period* gene mRNA levels are transcriptionally regulated. Hardin, P.E., Hall, J. C., and Rosbash, M. Proc.Natl.Acad.Sci. U.S.A 89:11711-11715.**
- 1992: The *period* gene encodes a predominantly nuclear protein in adult *Drosophila*. Liu, X., Zwiebel, L. J., Hinton, D., Benzer, S., Hall, J. C., and Rosbash, M. J Neurosci. 12:2735-2744.
- 1994: Constitutive overexpression of the *Drosophila period* protein inhibits *period* mRNA cycling. Zeng, H., Hardin, P. E., and Rosbash, M. EMBO J 13:3590-3598.
- 1995: PAS is a dimerization domain common to *Drosophila period* and several transcription factors. Huang, Z. J., Edery, I., and Rosbash, M. Nature 364:259-262.

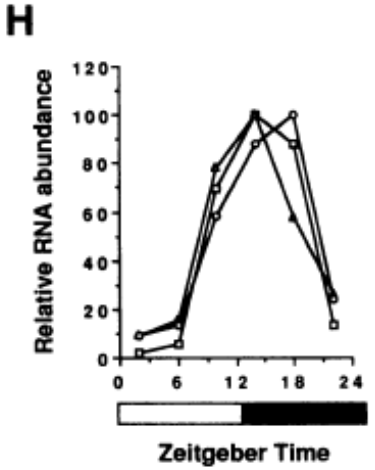
per mRNA Oscillations are Transcriptionally Regulated

Cycling of *per* pre-RNA

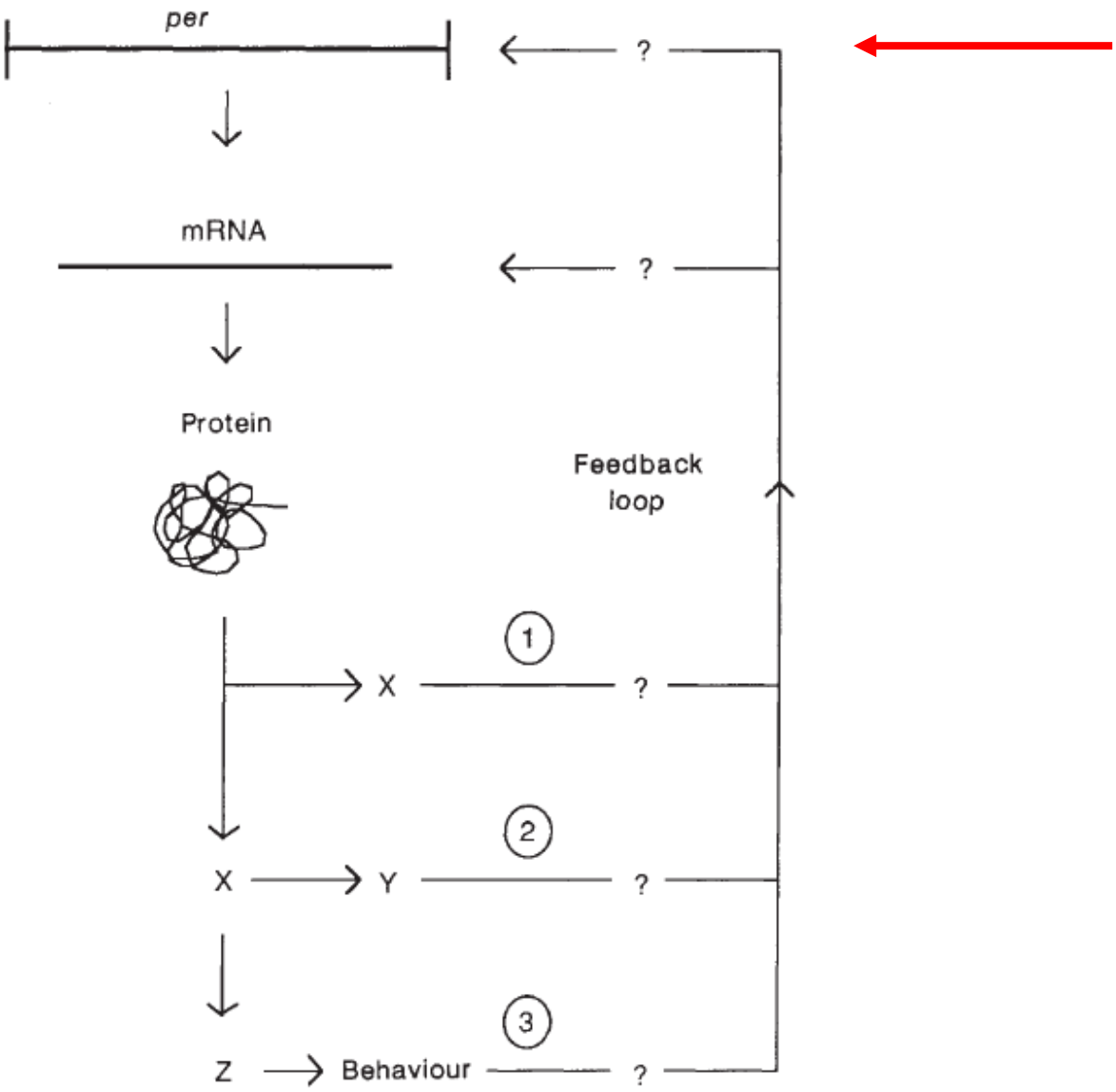


Cycling of *per* promoter CAT fusion mRNA





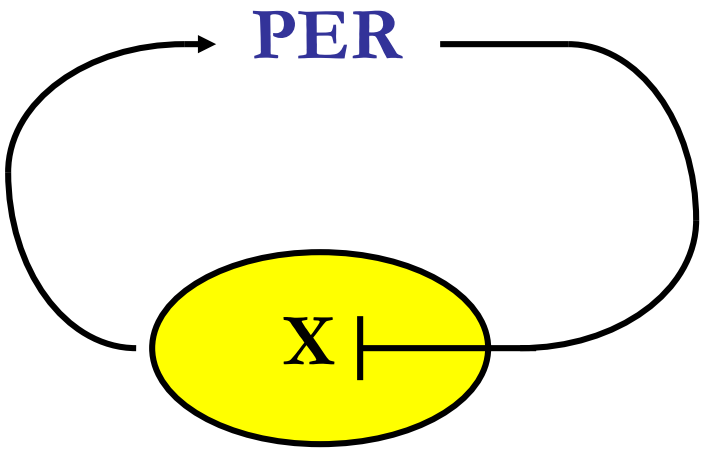
Strongly Favored Transcription



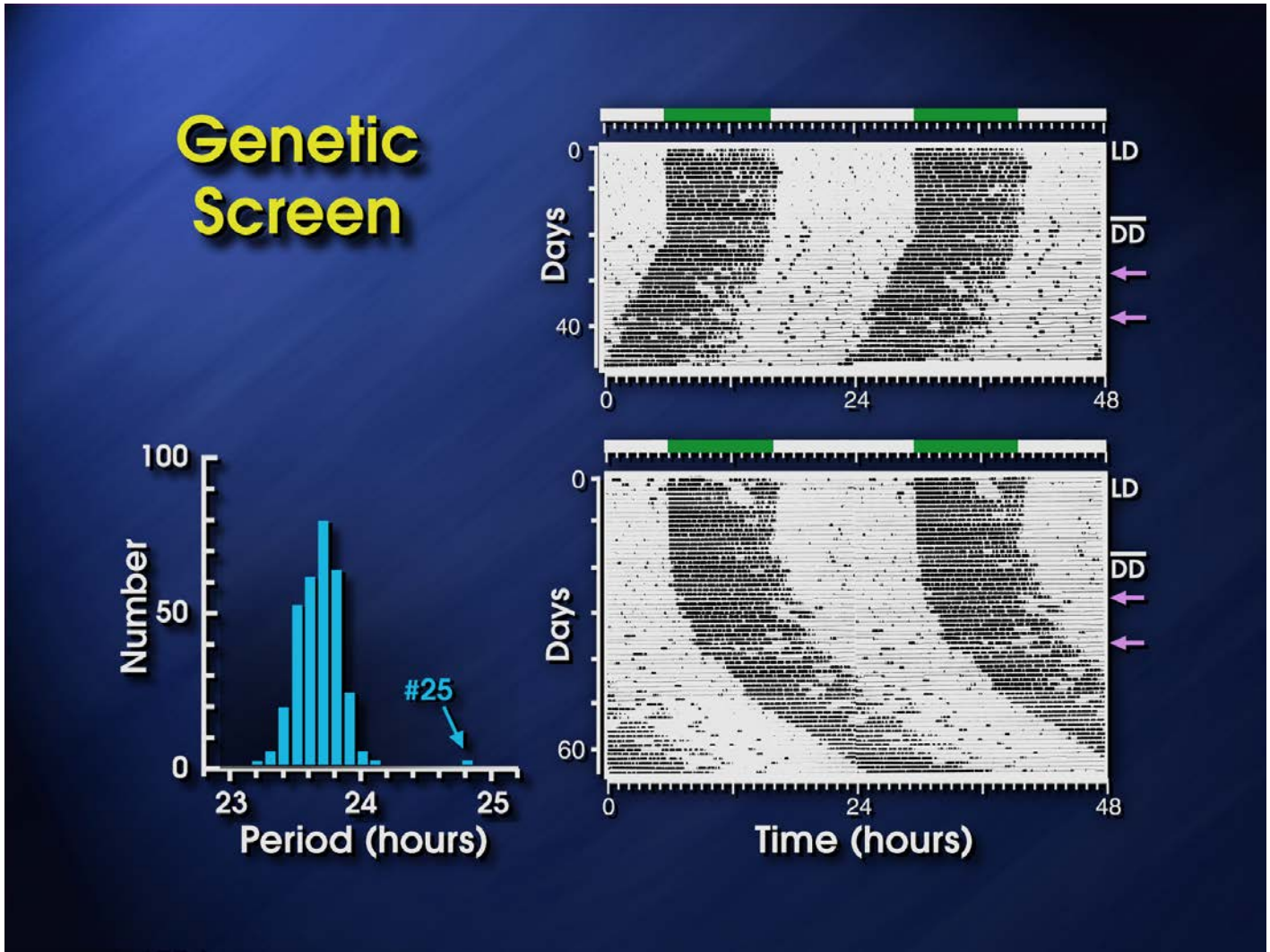
Four Important Follow-up Papers

- 1992: Circadian oscillations in *period* gene mRNA levels are transcriptionally regulated. Hardin, P.E., Hall, J. C., and Rosbash, M. Proc.Natl.Acad.Sci. U.S.A 89:11711-11715.
- **1992: The *period* gene encodes a predominantly nuclear protein in adult *Drosophila*. Liu, X., Zwiebel, L. J., Hinton, D., Benzer, S., Hall, J. C., and Rosbash, M. J Neurosci. 12:2735-2744.**
- **1994: Constitutive overexpression of the *Drosophila period* protein inhibits *period* mRNA cycling. Zeng, H., Hardin, P. E., and Rosbash, M. EMBO J 13:3590-3598.**
- **1995: PAS is a dimerization domain common to *Drosophila period* and several transcription factors. Huang, Z. J., Edery, I., and Rosbash, M. Nature 364:259-262.**

The *Drosophila* Transcriptional Negative Feedback Loop Model



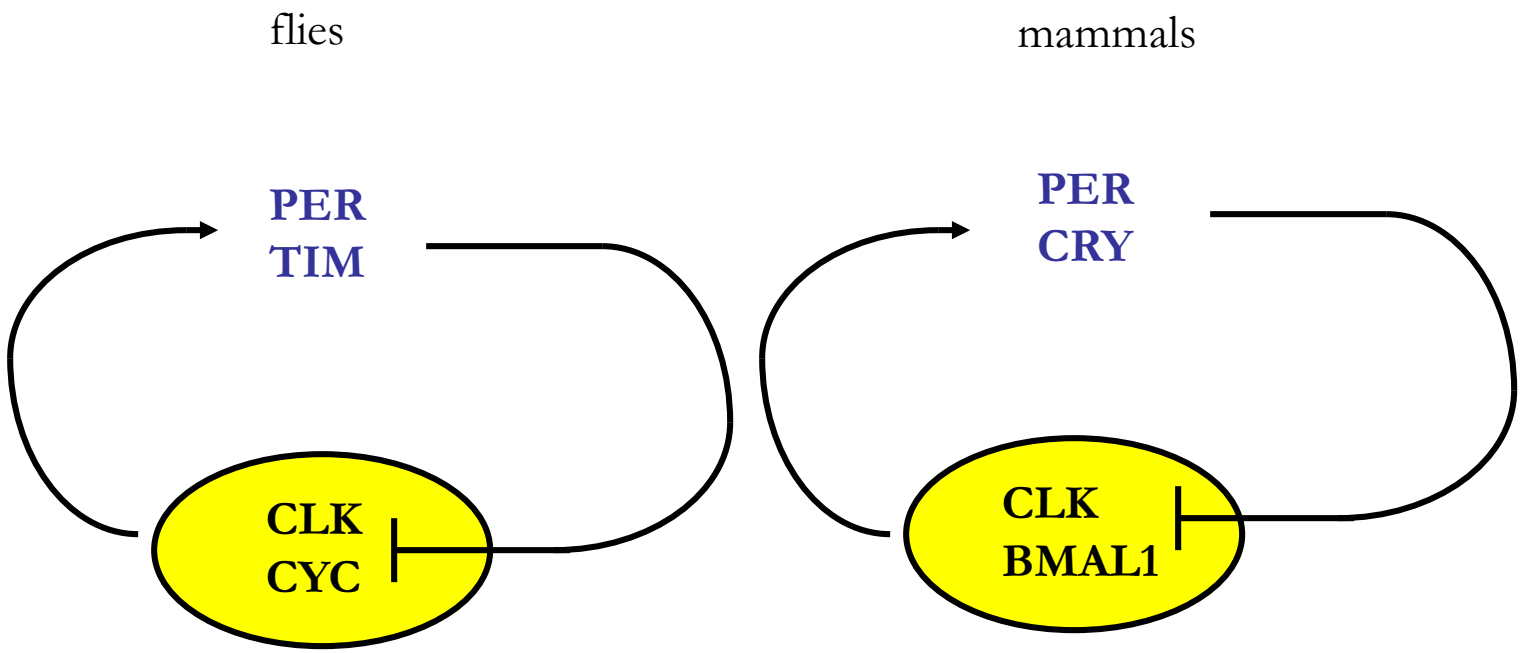
Takahashi Genetic Screen and Cloning Identified Mouse *Clock*



CLK and CYC (1998) (Ravi Allada and Joan Rutila)

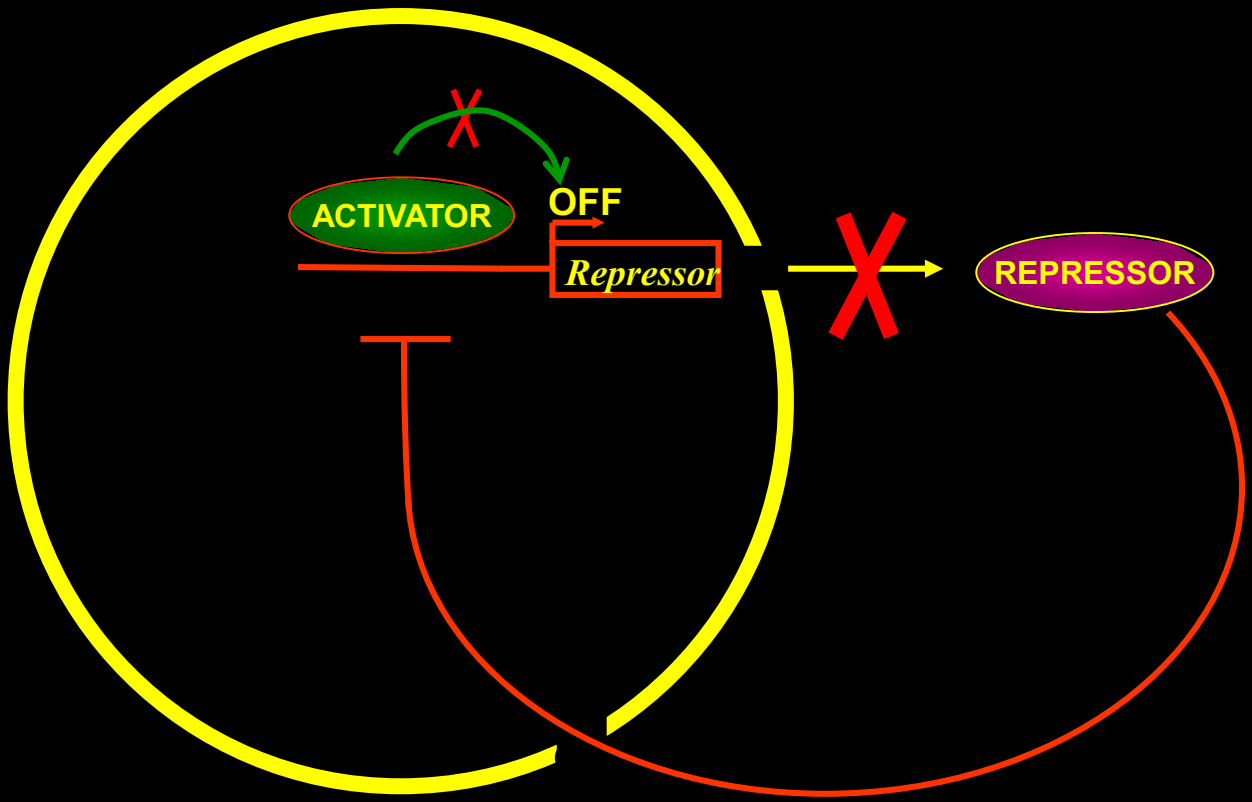
- Genetic Screen for Arrhythmic Mutant Strains (>40).
- Biochemical subscreen for non-cycling and low *per* and *tim* mRNA levels (3).
- Two alleles of *cyc*, one of *Clk.*

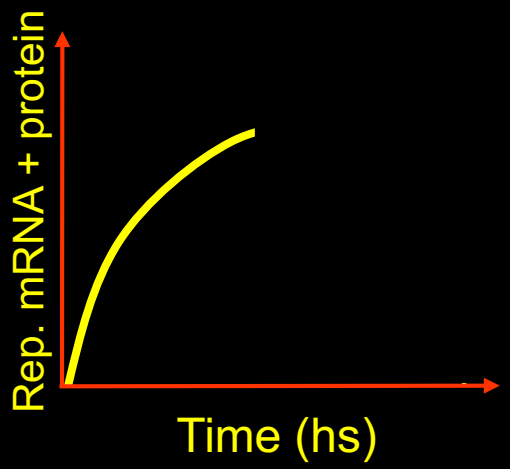
The Conserved Core of the Negative Feedback Loop in Metazoan Animals (1998-2017)



Ignoring Post-transcriptional Regulation, e.g., *tim*, *dbt*, etc.

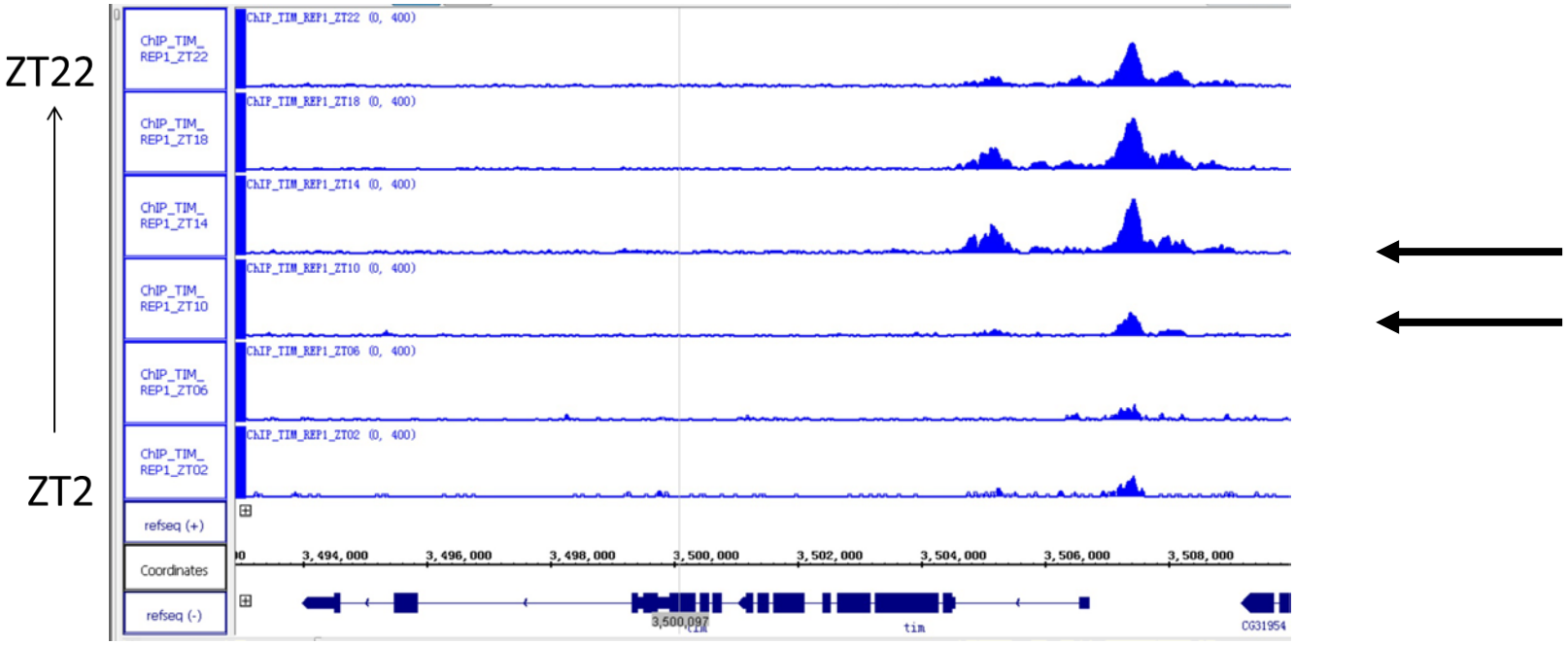
Common Theme among Circadian Oscillators





Neurospora, Drosophila, mice, plants etc.

TIM and PER ChIP-seq Binding to clock gene E-boxes Follows CLK Binding



PER and TIM associate strongly with CLK on chromatin in a more temporally discrete manner by Mass Spec

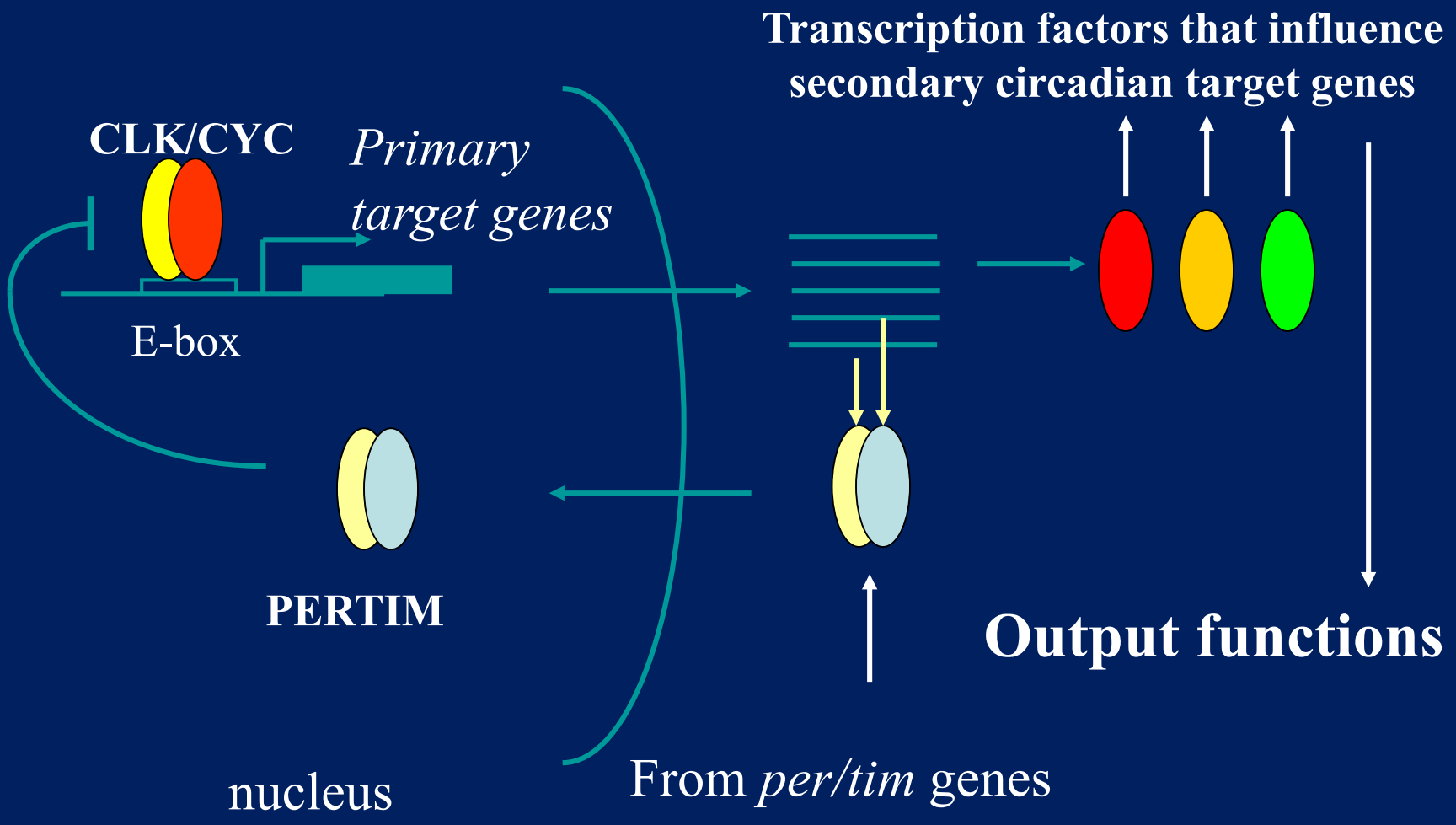
Peptide number	ZT10	ZT14	ZT18	ZT02
CLK	29	34	78	44
CYC	8	10	55	22
PER	2	1	102	54
TIM	0	0	102	2
DBT	3	0	53	13
NonA	21	41	15	8

The peptide number is the average of 2 experiments.

Beyond the Feedback Loop…

- McDonald and Rosbash, 2001
- There are a large numbers of cycling RNAs in fly heads.
- There are no bona fide cycling RNAs without functional *Clk*, i.e., everything is under CLK-CYC control.

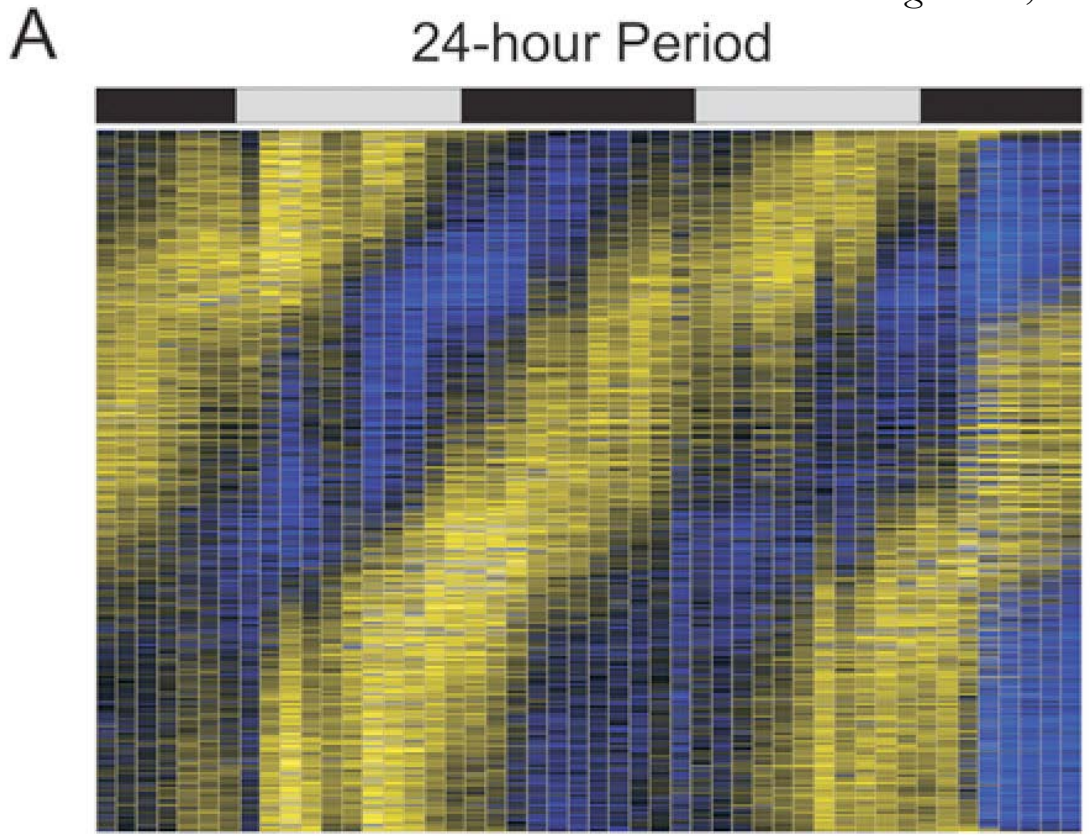
Current View: Core Clock Direct Target Genes Lead to the Cycling of Large Numbers of mRNAs

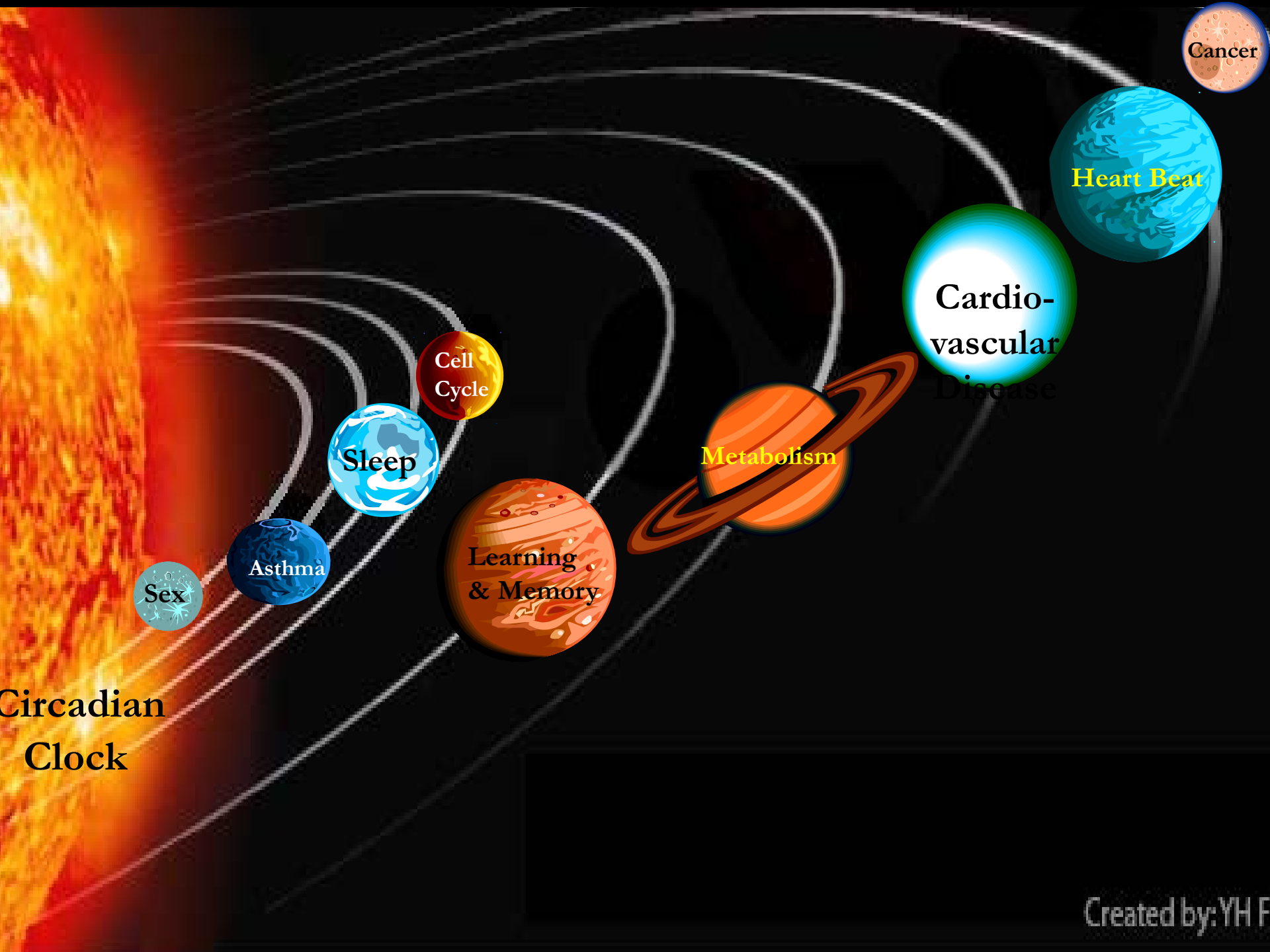


Mammals: Molecular Clocks are Present in Almost all Tissues…

Oscillating Gene Expression: ≥ 50% of all Gene Expression is Rhythmic

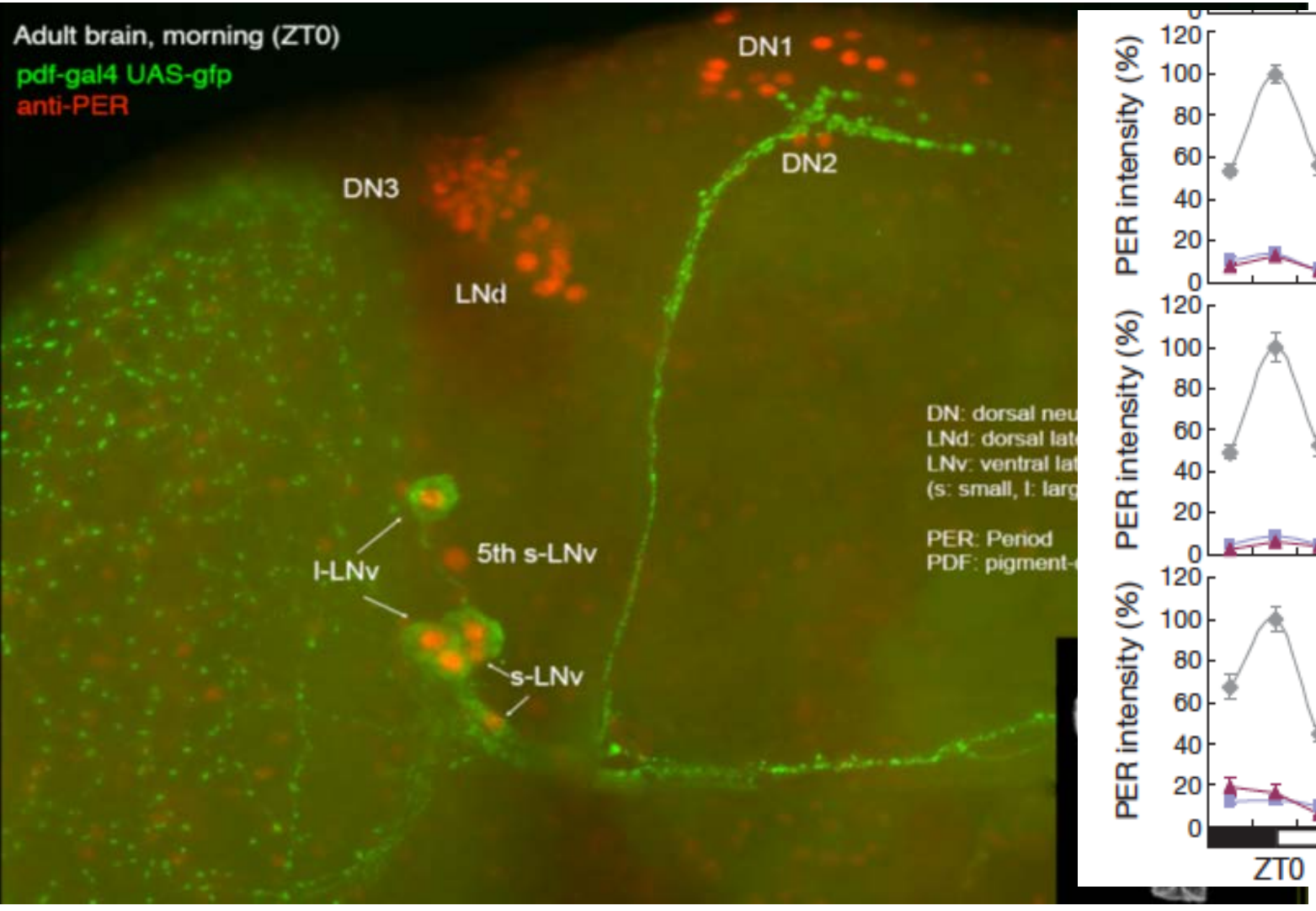
Hughes et l., 2009



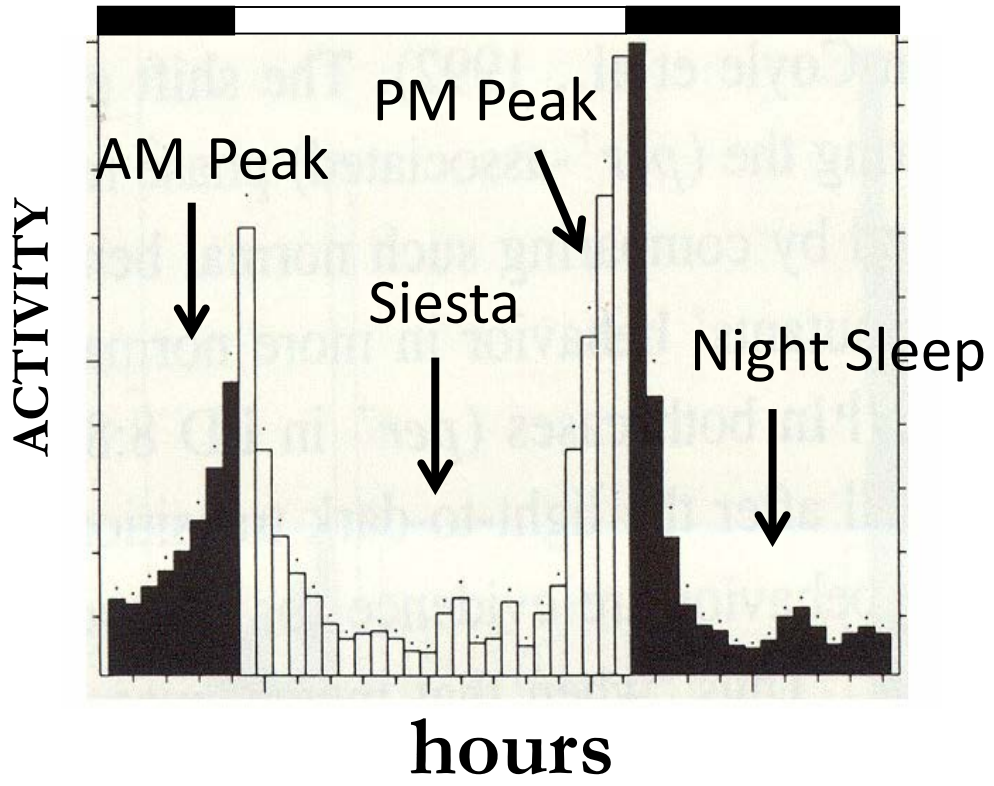

Cancer
Heart Beat
Cardio-
vascular
Disease
Metabolism
Cell
Cycle
Sleep
Learning
& Memory
Asthma
Sex
Circadian
Clock
Created by:YH F

Contemporary Interests

The Core Circadian Gene Expression Cycle Takes Place in Synch within ~75 *Drosophila* Central Brain Neurons



How do these Clock Neurons Organize (Affect? Control? Dictate?) the Classic *Drosophila* Locomotor Activity Pattern?



Also: How to Assay Firing Patterns of Discrete Neurons in Wake-Behaving Flies

Why am I Receiving this Prize? What did I Contribute to this Story (1982-2001)?

- Persistence
- Nucleic acid (RNA) expertise of my yeast lab.
- My training: RNA and protein half-lives make sense in light of ca. 24 hr timing.
- Stayed out of the way and evidently didn't screw things up too badly.

Luck (Good Fortune)

An Attaché Named Per here in Stockholm?

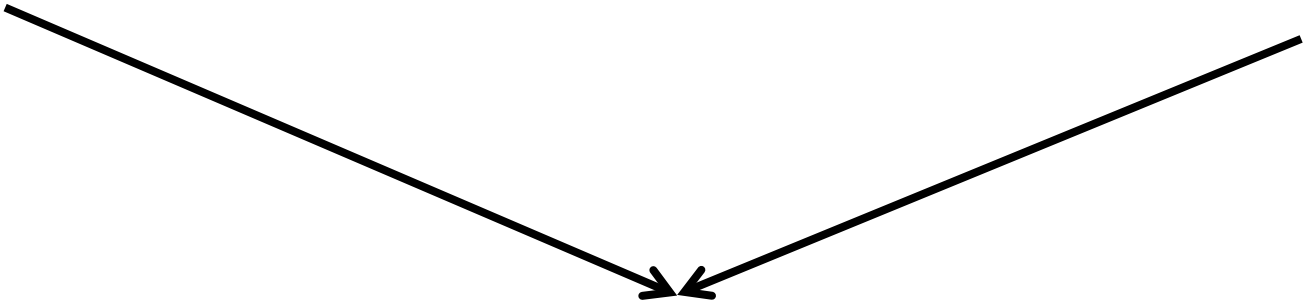
- Came to Brandeis and encountered genetics.
- Recombinant DNA came along at just the right time.
- Feedback Loop was correct and general.
- There are tons of cycling RNAs: Much (Most? All?) of Animal Physiology is Under Circadian Control.
- HHMI, NIH and Brandeis: Meritocracies, environment
- Wonderful Trainees and Collaborators

Jeff Hall

Classical Fly Genetics and Neurogenetics

Michael Rosbash

Molecular Biology and Gene Expression




Festsaal

Luck and Success: Rhythm and Fly People (1982…)

- Jose Agosto
- Ravi Allada
- Maisa Araujo
- Sean Bradley
- Xiao Chen
- Yoav Citri
- Hildur Colot
- Kathy Curtin
- Marina Dembinska
- Isaac Edery
- Patrick Emery
- Marta Frisardi
- Paul Hardin
- Gaiti Hasan
- Toni Hsu
- Josh Huang
- Sebastian Kadener
- Rachna Kaushik
- Carolyn Kotarski
- Ela Kula-Eversole
- Anthony James
- Myai Le
- Gaiti Hasan
- Yue Li
- Li Liu
- Xin Liu
- Lori Lorenz
- Scott Marrus
- Terri McCarthy
- Mike McDonald
- Aoife McMahon
- Jerome Menet
- Emi Nagoshi
- Pipat Nawathean
- Ying Peng
- Gabriel Peterson
- Zuwei Qian
- Pranitha Reddy
- Joe Rodriguez
- Francois Rouyer
- Joan Rutila
- Lea Sarov-Blat
- Rebecca Schoer
- Yuhua Shang
- Anna Sivachenko
- Wei-Qing (Venus) So
- Dan Stoleru
- Vipin Suri
- Anthony Tang
- Charles Vaslet
- Julie Vienne
- Alain Vincent
- Anand Vodola
- Qiang Yu
- Abby Zadina
- Hongkui Zeng
- Jie Zhao
- Larry Zwiebel

Luck and Success: Rhythm and Fly People

- Jose Agosto
- **Ravi Allada**
- Maisa Araujo
- Sean Bradley
- Xiao Chen
- **Yoav Citri**
- **Hildur Colot**
- Kathy Curtin
- Marina Dembinska
- **Isaac Edery**
- **Patrick Emery**
- Marta Frisardi
- **Paul Hardin**
- Gaiti Hasan
- Toni Hsu
- **Josh Huang**
- Sebastian Kadener
- Rachna Kaushik
- Carolyn Kotarski
- Ela Kula-Eversole
- Anthony James
- Myai Le
- Yue Li
- Li Liu
- **Xin Liu**
- Lori Lorenz
- Scott Marrus
- Terri McCarthy
- Mike McDonald
- Aoife McMahon
- Jerome Menet
- Emi Nagoshi
- Pipat Nawathean
- Ying Peng
- Gabriel Peterson
- Zuwei Qian
- **Pranitha Reddy**
- Joe Rodriguez
- Francois Rouyer
- **Joan Rutila**
- Lea Sarov-Blat
- Rebecca Schoer
- Yuhua Shang
- Anna Sivachenko
- **Wei-Qing (Venus) So**
- Dan Stoleru
- Vipin Suri
- Anthony Tang
- Charles Vaslet
- Julie Vienne
- Alain Vincent
- Anand Vodola
- **Qiang Yu**
- Abby Zadina
- **Hongkui Zeng**
- Jie Zhao
- **Larry Zwiebel**

Even More Luck: Yeast and RNA People

- Nadja Abovich
- Andrew Berglund
- Saverio Brogna
- Bruno Charpentier
- Simon Delgrave
- Sylvia Denome
- Jen Desrochers
- Lynn Dolnick
- Ken Dower
- Hansen Du
- David Elliot
- Margaret Fleming
- Noreen Francis
- Valerie Goguel
- Mark Gray
- Alain Jacquier
- Shawn Jennings
- Torben Heick Jensen
- Yevgenia Khodor
- Leo Kretzner
- Nick Kuperwasser
- Scott Lacadie
- Pierre LeGrain
- Alain Lescure
- Xioling Liao
- Domenico Libri
- Pablo Marina
- Houra Merrikh
- Megan Goodwin
- Yoav Nudell
- Kent Nybakken
- Peter O'Connell
- Mary Ann Osley
- Claudio Pikielny
- Jose Rodriguez Medina
- Brian Rymond
- Ulrich Schaefer
- Bertrand Seraphin
- Francoise Stutz
- Jie Tang
- Dan Tardiff
- John Teem
- Dan Treacy
- Irina Vainberg
- John Woolford
- Dong Zhang
- Yevgenia Khodor

Awesome Luck

- **Nadja Abovich**
- Andrew Berglund
- Saverio Brogna
- Bruno Charpentier
- Simon Delgrave
- Sylvia Denome
- Jen Desrochers
- Lynn Dolnick
- Ken Dower
- Hansen Du
- David Elliot
- Margaret Fleming
- Noreen Francis
- Valerie Goguel
- Mark Gray
- Alain Jacquier
- Shawn Jennings
- Torben Heick Jensen
- Yevgenia Khodor
- Leo Kretzner
- Nick Kuperwasser
- Scott Lacadie
- Pierre LeGrain
- Alain Lescure
- Xioling Liao
- Domenico Libri
- Pablo Marina
- Houra Merrikh
- Megan Goodwin
- Yoav Nudell
- Kent Nybakken
- Peter O'Connell
- Mary Ann Osley
- Claudio Pikielny
- Jose Rodriguez Medina
- Brian Rymond
- Ulrich Schaefer
- Bertrand Seraphin
- Francoise Stutz
- Jie Tang
- Dan Tardiff
- John Teem
- Dan Treacy
- Irina Vainberg
- John Woolford
- Dong Zhang
- Yevgenia Khodor

Family

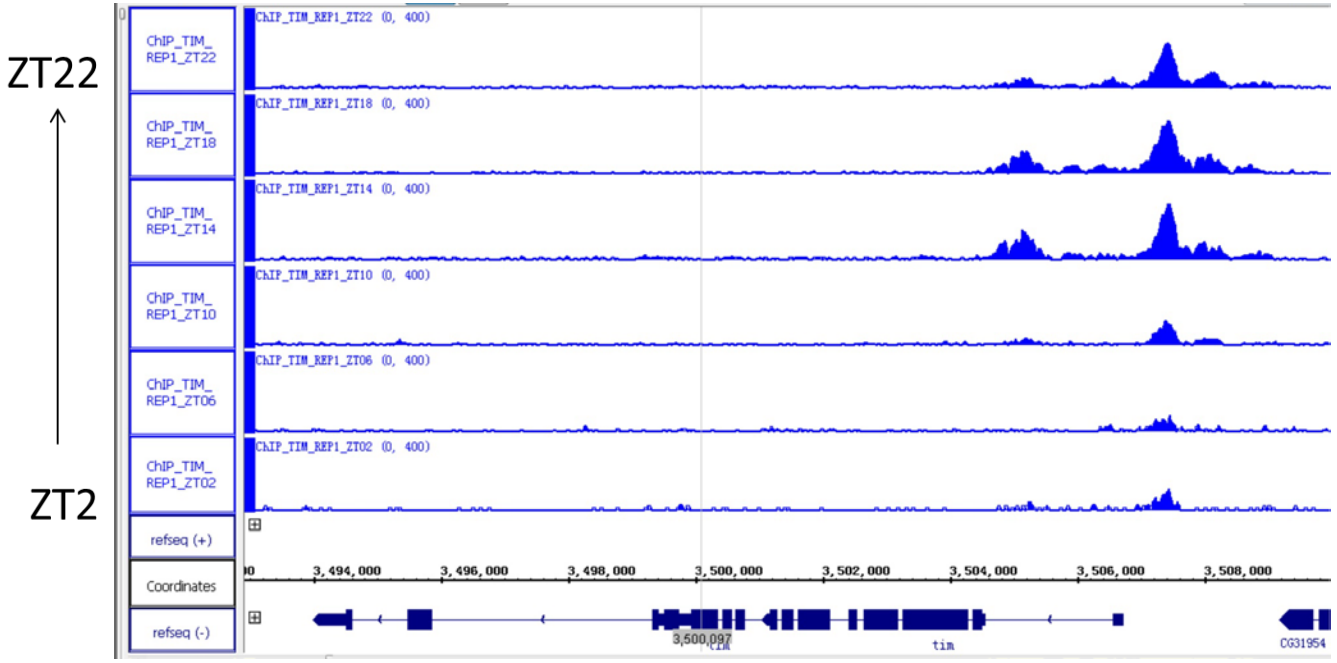
O Genotype, O Phenotype
This kiss had better last her;
He's off to see his other love,
Drosophila melanogaster.

From *Love on the Fly*
By Dani S. Grady

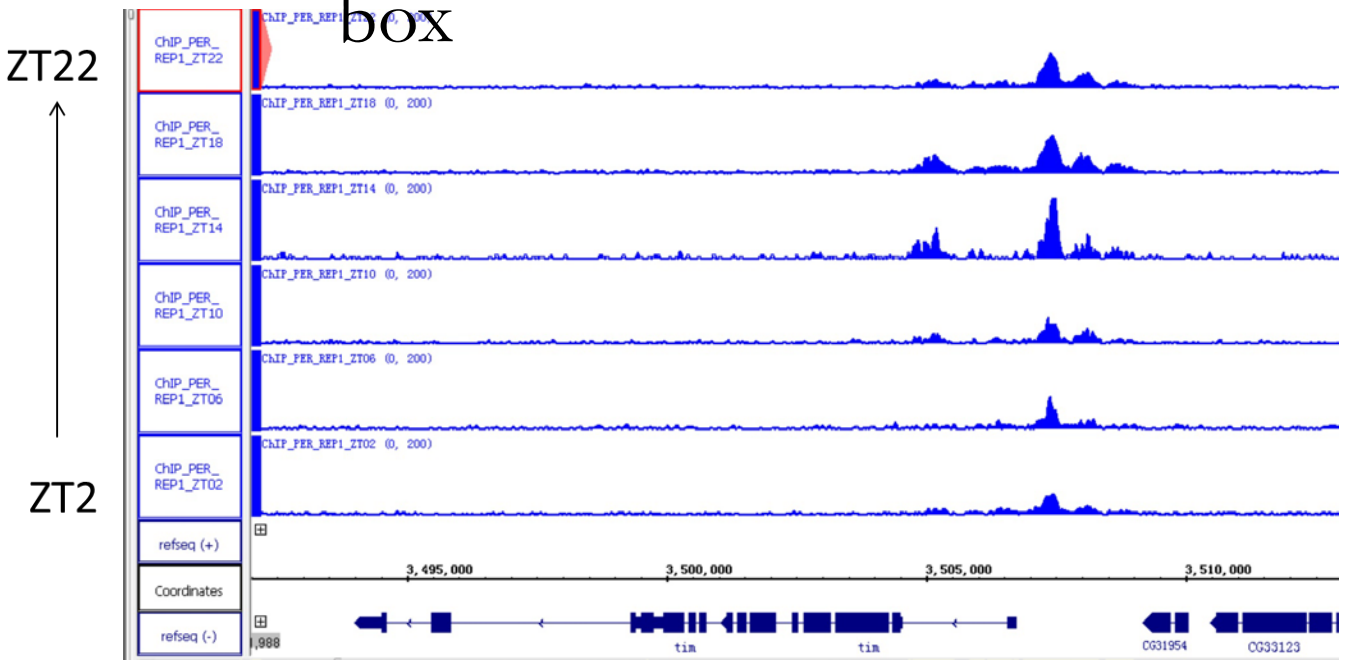
- Tei, H., Okamura, H., Shigeyoshi, Y.,
- et al
- . (1997) Circadian
- oscillation of a mammalian homologue of the
- Drosophila period
- gene.
- Nature
- 389

- Sun, Z.S., Albrecht, U., Zhuchenko, O.,
- et al
- . (1997)
- RIGUI
- ,a
- putative mammalian ortholog of the Drosophila
- period
- gene.

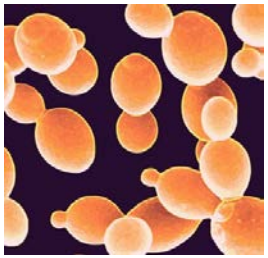
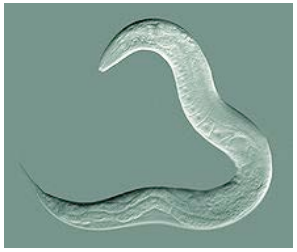
TIM ChIP_seq at tim E-box is Similar if not Identical to PER

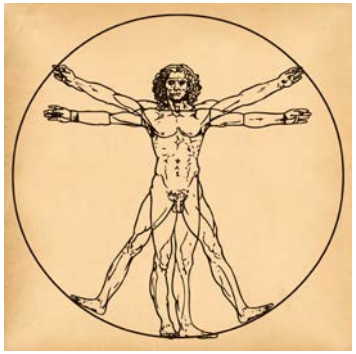


PER ChIP-seq at tim E-box



Genes associated with human diseases are conserved

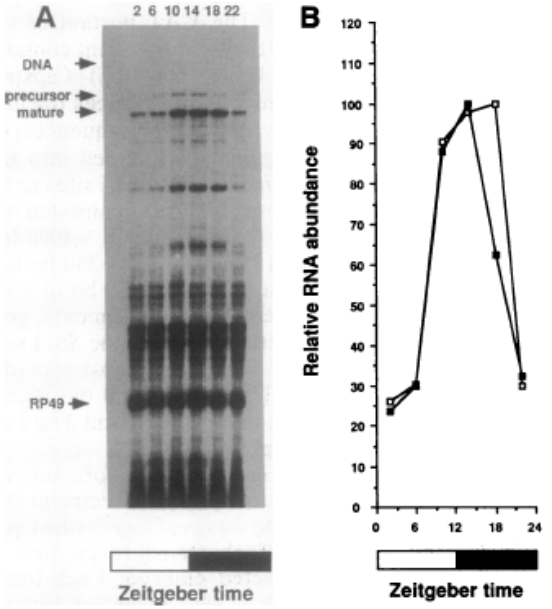



4554	8116	8516	10222	10495
43%	76%	80%	96%	99%

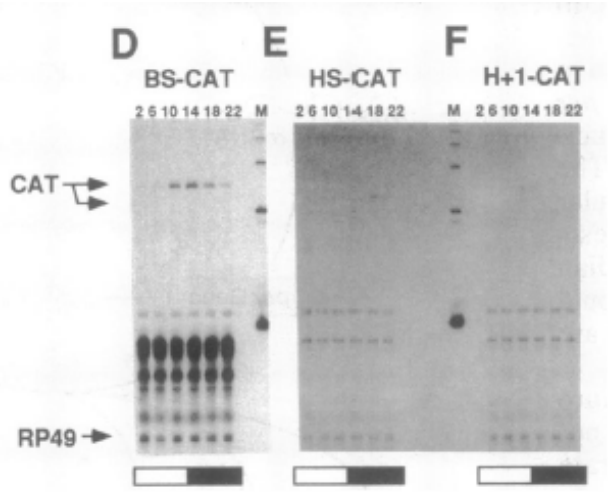
10636 protein-coding-genes associated with disease based

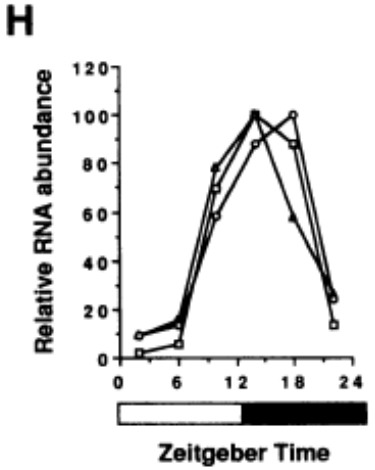
per mRNA Oscillations are Transcriptionally Regulated

Cycling of *per* pre-RNA



Cycling of *per* promoter CAT fusion mRNA





The Beginning (*period* mutants)

Konopka and Benzer (1971)