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
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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

- Bacteria
  - Plants
  - Animals
A Simple Systems View of Circadian Clocks

- Input signals (light)
- Metabolites (NAD$^+$, etc.)
- Outputs
- Adjusting signals
The Beginning (*period* mutants)

Konopka and Benzer (1971)
Early Brandeis Cloning Crew
Cloning, Identifying and Sequencing \textit{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.
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
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Abstract

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 behavioral and physiological processes. These rhythms are generated by an endogenous circadian clock, permit “thermoneutrality” under constant environmental conditions, and respond to environmental time cues. In *Drosophila melanogaster*, two well-studied phenomena—retina and circadian clock—are under the control of the circadian oscillator.

The product of *per* mRNA cycles in cells and organs of *Drosophila* described as the per torus either in a circadian oscillation or essentially abolish per protein**. The *per* gene has been cloned and characterized**. Site-directed mutagenesis and immunohistochemical analyses indicate that *per* is expressed in numerous adult tissues, including the eye, antenna, lateral brain neurons and posterior flight muscles, and the salivary gland, wings, and gut in larvae**. Because the circadian oscillator has been mapped to the brain, 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)** suggests that in photoreceptor nuclei in the middle of the night, but essentially no staining is demonstrable in the middle of the day. The staining fluctuations persist in constant darkness. These

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As a recent gift from Paul Hardin
Cautious Model: No mention of Transcription in the Title or Abstract
Four Important Follow-up Papers


Four Important Follow-up Papers


per mRNA Oscillations are Transcriptionally Regulated

Cycling of *per* pre-RNA

Cycling of *per* promoter CAT fusion mRNA
Strongly Favored Transcription

Diagram:

- `per`
  - mRNA
  - Protein
  - Feedback loop
    - X
      - Y
        - Z
          - Behaviour

Notes:
- Step 1
- Step 2
- Step 3
Four Important Follow-up Papers


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)

flies

mammals

PER
TIM

CLK
CYC

PER
CRY

CLK
BMAL1

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

<table>
<thead>
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<th>Peptide number</th>
<th>ZT10</th>
<th>ZT14</th>
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<td>NonA</td>
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<td>8</td>
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</tbody>
</table>

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

- **CLK/CYC**
- **E-box**
- **PERTIM**
- **nucleus**

**Primary target genes**

**From per/tim genes**

**Output functions**

Transcription factors that influence secondary circadian target genes.
Mammals: Molecular Clocks are Present in Almost all Tissues…
Oscillating Gene Expression: ≥ 50% of all Gene Expression is Rhythmic

Hughes et al., 2009
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
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
Even More Luck: Yeast and RNA People

- Nadja Abovich
- Andrew Berglund
- Saverio Brogna
- Bruno Charpentier
- Simon Delgrabe
- 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
- Dan Treacy
- Irina Vainberg
- John Woolford
- Dong Zhang
- Yevgenia Khodor
Awesome Luck

- Nadja Abovich
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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
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

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
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