Mechanism and Reduction: Decomposing Circadian Clocks

The Need to Decompose

- In order to explain the phenomenon in terms of a mechanism a researcher has to
  - Locate the mechanism within the larger system that exhibits the phenomenon
  - Decompose the mechanism into its parts and operations
- Decomposing involves differentiating the parts and operations
  - By physically detaching them from others
  - By distinguishing them conceptually and securing evidence of their individual characteristics
- Not all ways of cutting up a system differentiate parts and operations
  - Need to find the *working* parts—the parts that work in coordination to produce the phenomenon

Techniques for Decomposing Biological Mechanisms

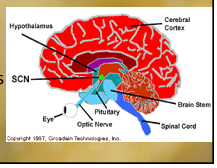
- Structurally
  - Physical dissection
  - Microscopy and other imaging techniques (CAT and MRI scans)
- Functionally
  - Lesion or knockout a part
    - To reveal what is lost without that part
  - Stimulate a part
    - To see what is behavior is increased
  - Manipulate an input and record changes in the behavior of a part
    - To see what parts are engaged in response to the input

Decomposition and Reduction

- Decomposing a mechanism into its parts and operations is often characterized as reductionistic
- For some philosophers, reduction has the connotation of taking something all the way down to its simplest parts and operations
  - But that often fails to serve the goal of explanation, which is to identify the parts and operations responsible for the phenomenon
- In going down further, one treats the operation performed by the part as the phenomenon of interest, and decomposes it into its parts and operations
  - But now one is asking a different question: how does the part perform its operation?

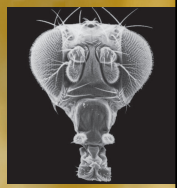
Localizing the Mammalian Circadian Mechanism

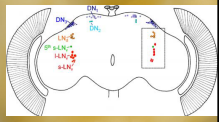
- Once it was accepted that circadian rhythms are maintained endogenously, the natural question to ask is where the mechanism is located
- In the early 1970s Robert Moore localized *the (?!)* circadian clock in a small nucleus known as the suprachiasmatic nucleus (SCN). Located above the optic chiasm in the anterior hypothalamus, the SCN consists of about 20,000 neurons in mice
  - Pathways from the retina project to the SCN
  - Lesions to the SCN disrupt rhythms
  - Much later: transplant of SCN into ventricle in lesioned rats restored circadian rhythms



Localizing the Fly Circadian Rhythm

- Localizing fly circadian clock in the head
  - Transplant head of flies with short cycle into abdomen of arrhythmic flies results in behavior with short cycle
  - Since no neuronal projections develop, must be attributed to diffusible factor
- Subsequently localized to specific neurons
  - Dorsal neurons (DNs) oscillate when LNs destroyed, but are insufficient to maintain locomotor rhythms
  - Oscillation in the lateral neurons (LNs) alone (achieved in transgenic flies) sufficient for locomotor rhythms





Localizing vs. Decomposing

- Localizing a phenomenon: Identifying where a phenomenon is controlled (locus of control)
  - The occurrence of the phenomenon may require other entities (your car requires the existence of oil fields), but typically there is a locus where the phenomenon is controlled)
- Localizing the phenomenon does not explain it
  - That requires decomposing the locus of control into its parts and operations and showing how they are organized to produce the phenomenon
- A second act of localization occurs in identifying the part responsible for the operation

The Task of Decomposing Circadian Rhythms

- Guiding metaphor: circadian rhythms are governed by a clock
  - A distinct mechanism that keeps time
  - This clock is located in the SCN in mammals and the lateral neurons in fruit flies
- Two possibilities:
  - Clock required the interaction of multiple neurons
  - Clock was contained within individual neurons
- The fact that single-cell organisms also exhibit circadian rhythms supported the latter possibility
- So the challenge became to figure out what inside cells generates regular 24-hour oscillations
  - Presumably proteins which are synthesized from genes

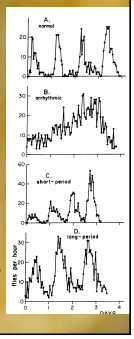
Fruit Flies as a Model Circadian Organism

- Eclosion occurs in the early morning hours before heat and dryness of the day

- In adults, many activities, such as feeding behavior (including the physiological processes of digestion) are restricted to daytime
- Since the work of Morgan, flies have been a highly productive model system for genetics
  - Relatively easy to induce mutations, identify their phenotypic effects, and localize one or more genes involved in generating the phenomenon—presumably via a protein for which it codes
- Strategy: induce mutations in fruit flies and screen for ones that exhibit unusual circadian rhythms

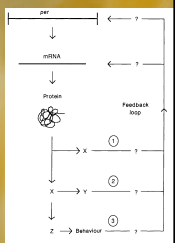
Seeking a Genetic Mechanism

- For his dissertation research, Ronald Konopka took up the challenge. In 1971 found mutations, all involving the same locus, that resulted in loss of rhythms or shortened or lengthened periods
  - Called the gene *period* (*per*)
  - In null mutations, normal rhythms could be restored by transplanting brain of WT into abdomen of fly with mutation
- Note: this does not *prove* that *per* (and presumably its protein) is part of the clock mechanism
  - It could be involved in something only tangentially related to the clock
- The more one can manipulate circadian rhythms by manipulating it, the more likely it seems that it is a part of the mechanism



Figuring Out What the *per* Gene Does

- Only with the advent of cloning technology was it possible to identify and measure concentrations of the protein PER and the mRNA transcript
  - Both were shown to oscillate with an approximately 24 hour period
- Hardin, Hall, and Rosbash (1990) proposed that *per, per* mRNA and PER protein are organized into a delayed feedback loop capable of oscillation—a clock
  - A mechanism sketch as there were several gaps or alternative pathways



Hardin et al., 1990

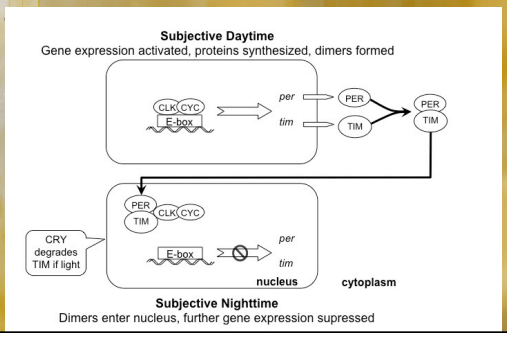
Next Challenge: How Does PER Inhibit *per*?

- PER lacks a domain (part of the protein) that is capable of binding to DNA and so serving to regulate transcription
  - Something else must be involved
- Seghal et al. (1994): PER has a partner TIMELESS (TIM) with which it formers a dimer
  - The PER:TIM dimer was transported back into the nucleus
- Discovery of TIM still left the mystery of how PER or TIM worked to inhibit transcription of *per* or *tim*
  - Discovery of a promoter site (E-box) on *per* and *tim* pointed to a regulatory protein that controlled *per* and *tim* transcription
  - Challenge: find this protein(s)

Mammals Provided the Clue

- Boldly trying to do in mice what Konopka had done in flies (since generation times are much longer, making it unlikely one would get a quick result), Vitaterna et al. (1995) discovered a gene whose mutants exhibited disrupted rhythms
  - Called the gene *Clock* (Circadian Locomotor Output Cycles Kaput)
  - Its dimerization partner was soon identified (BMAL1)
- Mouse CLOCK-BMAL1 dimers can bind with *Drosophila per* and *tim*
  - Proposal: PER:TIM in some way interacts with CLOCK:BMAL1 to interfere with its ability to bind to the *per* and *tim* promoters
  - Soon *Drosophila* homologs of *Clock* and *Bmal1 (cycle*) were found: dCLOCK and CYCLE (which, ironically, doesn't cycle)
- CLOCK-BMAL1 completed the transcription/translation feedback loop that constitutes the core oscillatory mechanism

Basic Schema of Circadian Mechanism in Fruit Flies



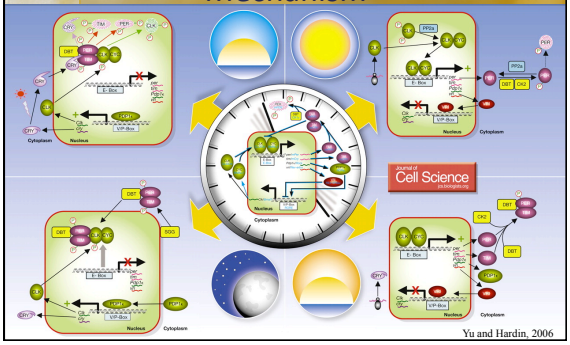
Keeping the Mechanism Entrained to our Planet

- Beyond endogenously maintaining approximately 24 hour oscillations, the circadian clock is also entrainable to the external light/dark cycle
- Researchers soon discovered cryptochrome, a blue light photoreceptor, that acts to degrade TIM when acted upon by light
- When CRY is affected by light, it stops PER:TIM from interrupting the ability of CYC:CLK to bind to promoter on *per* and *tim*
  - If this is in the early morning, the effect is to keep the clock in day condition and delaying the clock
  - If this is in the late night, the effect is to advance the clock to the conditions of the next day

More and More Clock Parts

- "It is tempting to speculate that the *Drosophila* four-component transcriptional feedback loop described here is sufficient to generate a rudimentary circadian rhythm. This oscillation would be amplified by other, unknown proteins that regulate RNA stability, protein stability, and phosphorylation of the oscillator components."
  - » Darlington et al., 1998
- Other components were soon to be discovered:
  - Kinases that phosphorylate PER and TIM, marking them for degradation
    - Doubletime (DBT) acts on PER
    - Shaggy (SGG) acts on TIM
  - Some of these involved a second feedback loop
    - PDP1ε binds to the promoter of *clock*
    - Vrille inhibits *clock* expression, and so *per* and *tim* transcription

Diagramming the Whole Mechanism



The Daily Cycle

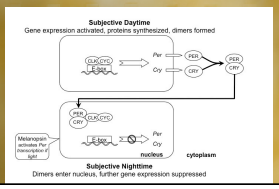
- Mid-day:
  - most PER and TIM is degraded
  - CLK::CYC bind to E-box on promoters of *per*, *tim*, and *vri*
- Afternoon:
  - VRI moves to the nucleus and suppresses *clk* expression
  - PER is phosphorylated by DBT and CK2 and degraded
- Late afternoon
  - Accumulation of TIM protects PER from degradation
  - SGG phosphorylates TIM, making it able to enter nucleus
- Early evening
  - PER and TIM heterodimerize and enter the nucleus
  - PER in nucleus interferes with CLK::CYC ability to bind *per* and *tim* promoter
- Late night
  - PER and TIM peak, causing increased CLK expression
  - PER::TIM dissociate, leading DBT to phosphorylate PER and subsequently its breakdown
- Dawn
  - PER drops to low levels while CLK peaks

Conserved but Modified Mechanism in Mammals

- *per* is conserved in mammals with 3 variants: *Per1*, *Per2*, and *Per3*
  - *Per1* and *Per2* play much the same role in the clock as *Drosophila per*
- The mammalian homologs of CRY, CRY1 and CRY2, act like *tim* in *Drosophila*
  - Form dimers with PER1 and PER2
  - Inhibit their own transcription by interacting with CLK:BMAL1
    - Also mammalian CLK doesn't cycle, but BMAL1 does
- The discovery that CRY1 and CRY2 figure in the central oscillator in the mammalian clock raised the question: what performs the entrainment role by responding to light?

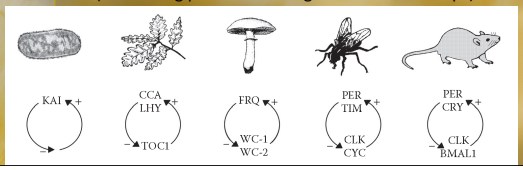
What Entrains the Mammalian Clock?

- Discovery of a new opsin compound, melanopsin, which is expressed in a distinct set of retinal ganglion cells from the visual photopigment rhodopsin,
  - intrinsically photosensitive Retinal Ganglion Cells
- Through a signaling pathway, melanopsin activates transcription of *Pers* when light is present at night.



Same Type of Mechanism in Other Orders

- Following the discovery of the core components of the fly and mouse clocks, researchers working on bacteria, fungi, and plants found similar transcription/translation feedback loops in model species in each order
- The central components of the clocks in the different orders of life are different, but the overall organization is very similar (interacting positive and negative feedback loops)

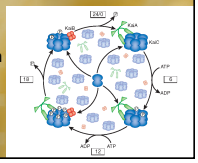


Across Orders: Conserved or Independently Evolved?

- The similar organization with different components has suggested to many that the clock mechanism has evolved independently several times
  - A case of convergent evolution
- But many of the ancillary components (e.g., kinases) are homologous (share a common descent)
  - Suggesting that the mechanism may have been conserved
- Possibly the organization has been conserved as well, with changes in some of the component parts (genes/proteins)
  - Functional conservation

A Clock Shock

- The transcription/translation feedback loop seemed to provide a universal account of circadian clocks even if the parts were different across species
- In 2005 Takao Kondo's laboratory demonstrated
  - That circadian rhythms in cyanobacteria could be maintained without transcription and translation
  - That just combining the proteins Kai A, Kai B, and Kai C together with ATP they could generate circadian rhythms
    - Kai C is both an autokinase and an autophosphorylase, with Kai A and Kai B helping to determining which process occurs



The Clock Shock Comes to Mammals

- In January 2011 O'Neill et al. report circadian rhythms in mammalian red blood cells
  - Which have no nucleus and hence no genes
- Mammals as well must have a mechanism that can work independently of the transcription/translation feedback loop
- Has the work on transcription/translation just been a mistake?
  - Or does it play a role (e.g., in making the clock robust)?