Mechanism and Reduction: Decomposing Circadian Clocks

Clicker Question

On the Deductive-Nomological (DN) model of reduction, which of the following does not figure in providing the explanation (i.e., is not used in the deduction)

Higher-level laws (e.g., biological laws)
Lower-level laws (e.g., chemical laws)
Bridge principles
Boundary conditions

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 mechanism differentiate parts and operations
  - Need to find the working parts—the parts that work in coordination to produce the phenomenon

Discussion Question

What strategies can researchers use to figure out how parts of an organism are involved in some activity of the organism?

Remove the part to see if it has any effect on the activity

Excite or increase the activity of the part to see if it has any effect on the activity

Record the activity of the part and see if it varies as the activity varies

Any of the above

Techniques for Decomposing Biological Mechanisms

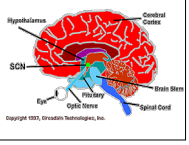
- Structurally
  - Physical dissection
  - Microscopy and other imaging techniques (CAT and MRI scans)
- Functionally
  - Lesion or knockout a part
    - To reveal what is lost without that part
  - Stimulate a part
    - To see what is behavior is increased
  - Record changes in the behavior of a part as the mechanism is operating
    - To see what parts are active at different stages of the operation

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?

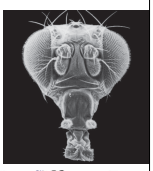
Localizing the Mammalian Circadian Mechanism

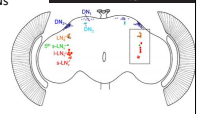
- Once it was accepted that circadian rhythms are maintained endogenously, a natural question to ask is where the mechanism is located
- In the early 1970s Robert Moore localized *the (?!)* circadian clock in a small nucleus known as the suprachiasmatic nucleus (SCN). Located above the optic chiasm in 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

- At first it was only possible to localize the 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 it was 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) proved 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 critical activities are performed
- 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 after decomposition in identifying the part responsible for an 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 requires the interaction of multiple neurons
  - Clock is 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

Clicker Question

What sort of investigation did Konopka undertake with fruit flies that provided the first information about parts of the clock mechanism?

He altered the timing of light exposure to determine which would lead to shortening or lengthening of the circadian rhythm

He used mutagens to produce mutations and screened to find mutants with shortened or lengthen rhythms

He moved neurons from the brain of the fly until he found ones that were required to maintain circadian rhythmicity

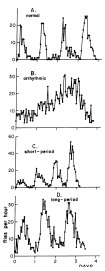
He shortened or lengthened the day-night cycle in the flies environment and recorded gene behavior

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

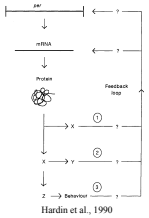
Seeking a Genetic Mechanism

- For his dissertation research, Ronald Konopka took up the challenge. In 1971 he 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 normal fly into the abdomen of a 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 in the 1980s 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
  - Note the question marks: this is a mechanism sketch in which there were several gaps and alternative possible pathways



Hardin et al., 1990

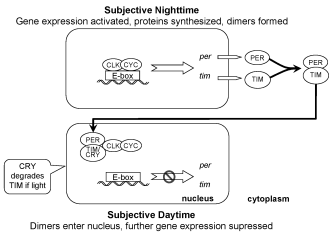
Next Challenge: How Does PER Inhibit *per*?

- 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) made a potentially relevant discovery: 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, it was very unlikely one would get a quick result), Vitaterna et al. (1995) discovered a gene whose mutants exhibited disrupted rhythms
  - They 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: CLOCK:BMAL1 acts as an activator of transcription of *per* and *tim* and 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

Complete Translation-Transcription Feedback Loop



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
- Mammalian forms 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
- Clock, together with either Cycle or Bmal1, serves to activate transcription of per, tim, and cry.
  - Mammalian CLK doesn't cycle, but BMAL1 does

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

Basic Schema of Circadian Mechanism

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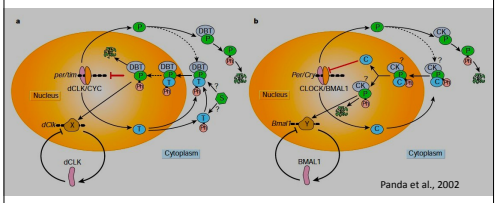
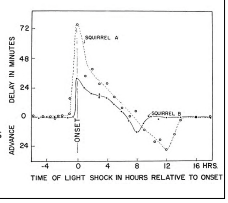


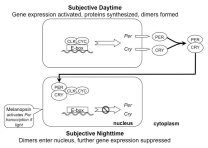
Keeping the Mechanism Entrained to our Planet

- Beyond endogenously maintaining approximately 24 hour oscillations, the circadian clock is also entrainable to the external light/dark cycle
- Fly 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 causes TIM to break down, thereby stopping PER:TIM from interrupting the ability of CYC:CLK to bind to promoter on *per* and *tim*
  - If this is in the early evenning, 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

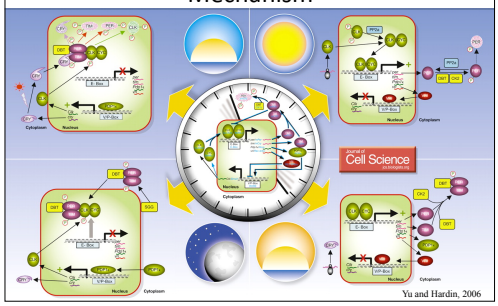


What Entrains the Mammalian Clock?

- Cry doesn't serve entrainment in mammals (a different form of Cry is the dimerization partner with Per, replacing Tim)
  - Leaves the question of what performs entrainment in mammals
- The search led to the discovery of a new opsin compound, melanopsin, distinct from the visual photopigment rhodopsin
  - It is expressed in a distinct set of retinal ganglion cells—intrinsically photosensitive Retinal Ganglion Cells
- Through a signaling pathway, melanopsin activates transcription of *Pers* when light is present at night.



Diagramming the Whole Mechanism

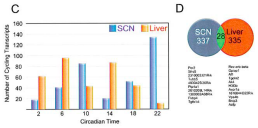


The Daily Cycle

- 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

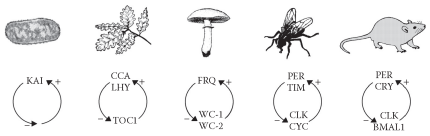
Hands of the Clock

- Although researchers now think they know a great deal about the core clock mechanism, very little is known about how the clock affects other functions (digestion, cell division) or behaviors (locomotion, sleep, reaction times)
  - Some clues have come from identifying cycling components in the mechanism responsible for the behavior (*lark* in ecolosion and *pdf* in motor activity in flies
- Genetic screens of different tissues have identified hundreds of genes that are expressed in a circadian fashion (not all in phase with each other)
  - Different genes in different tissues



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)

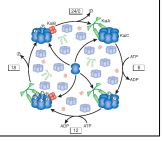


Across Orders: Conserved or Independently Evolved?

- 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
  - By 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?
  - It could be that the TTFL is the central mechanism in most tissues in most higher organisms and blood cells are an exception
  - Maybe it only plays a role (e.g., in making the clock robust)