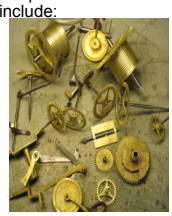
Mechanism and Levels of Organization: Recomposing and Situating Circadian Clocks

The Success of Decomposition

- Moving beyond *per*, researchers in the 1990s and early 2000s identified many clock components. Focusing just on mammals, these include:
  - Multiple forms of *Per* in mammals
  - Two form of cryptochrome dimerization partner of *Per*
  - Melanopsin entrainment
  - *Clock* activator of *Per*
  - *Bmal1* activator of *Per*
  - CASEIN KINASE 1ε (and other kinases) degradation
  - *Rorα* and *Rev-erbα* activator and inhibitor of *Bmal1*
  - Many chaperones
  - Various acetylases/methylases
  - etc., etc., etc.

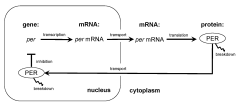


Putting the Mechanism Back Together

- A pile of separated parts won't produce the phenomenon
- To understand how they contribute to the phenomenon, researchers need to recompose the mechanism
  - Very hard to do so in reality (but new promise in synthetic biology)
  - Much of the focus is on recomposing a mechanism conceptually
    - Identifying how the parts are related and affect each other

Recomposing the Transcription/ Translation Feedback Loop

- The proposal of the transcription/translation feedback loop and its linking to entrainment is a first step of recomposition
- But what will such a feedback do? Will it actually generate sustained oscillations?
  - A first step is to mentally simulate the mechanism's behavior
    - First *per* will cause the production of PER, so its quantity will increase
    - As it increases, it will cause more inhibition of *per* transcription
    - Eventually PER breaks down and *per* will once again begin to produce more PER again
  - But will the quantities eventually reach equilibrium so that oscillation stops?



Clicker Question

What is a major objective of computational modeling of mechanisms?

To facilitate identifying new parts and operations

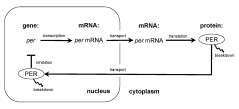
To associate operations with specific parts

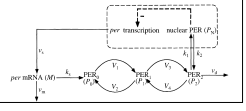
To determine whether the parts really perform the operations assigned to them

To show what the parts, when organized into a whole mechanism, will do on their own or in response to changes in inputs

Modeling the Simple Per Loop

- With non-sequential operations and non-linear reactions, mental simulation breaks down and mathematical modeling is required
- The first step in developing a mathematical model of a mechanism is to represent it in terms of quantities
- Goldbeter took the 1990 Hardin et al.'s model in which PER inhibits its own transcription and represented the components of the system in terms of
  - variables identifying concentrations of various parts (M=per mRNA concentration, etc.)
  - parameters specifying the rates governing various reactions (vs= maximum rate of transcription



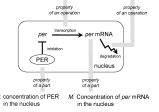


Modeling the Simple Per Loop

- The next step is to write equations to characterize how the values of each variable changes dependent upon other variables
- Eq. 1 has one term for the making of new per mRNA and one for its degradation
  - The equation introduces a non-linearity in the exponent *n* (which was taken to reflect the assumption that multiple molecules of PER have to interact to suppress transcription)

(Eq.1) $$\frac{dM}{dt} = \underbrace{v_s \frac{K_I^n}{K_I^n + P_N^n}}_{\text{Rate of transcription}} - \underbrace{v_m \frac{M}{K_m + M}}_{\text{Rate of decay}}$$

Rate of change in concentration of per mRNA in the nucleus

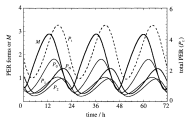


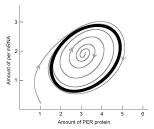
P_N: concentration of PER in the nucleus

M: Concentration of *per* mRNA in the nucleus

Modeling the Simple Per Loop

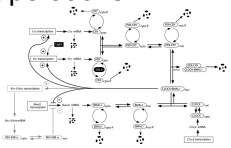
- Often, as in this case, it is not possible to derive a solution to multiple equations analytically and so modelers apply them iteratively to simulate the operation of the mechanism
- Goldbeter showed that the model generated sustained oscillations of
  - per mRNA
  - Total PER (P_t)
  - Nuclear PER (P_N)
  - Cytoplasmic PER whether phosphorylated (P_1 & P_2) or not (P_0)
- When plotted in phase space, the results showed a limit cycle

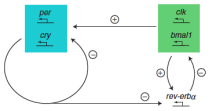




Strategies for Dealing with more Parts and Operations

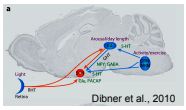
- Sometimes researchers try to represent all the hypothesized parts and operations
  - Leloup and Goldbeter (2003) required 73 equations
- Sometimes researchers simplify in the attempt to figure out what is most relevant to the behavior in question





Recomposing Beyond the Individual Cell

- We saw how researchers first localized circadian rhythms in mammals in the suprachiasmatic nucleus and then focused on finding the responsible mechanism within individual cells of the SCN
- But the SCN could only provide time information to the rest of the organisms if it is properly hooked up to it
- Before we get to that, there is the question of how the individual neurons are connected within the SCN



Dibner et al., 2010

Clicker Question

The readings refer to cells as *autonomous* oscillators. What does this mean?

- The individual cells work alone and are never affected by each other
- Individual cells can on their own maintain a rhythm of approximately 24-hours through internal processes
- Individual cells must be linked together in a network to maintain oscillations
- Cells actively resist any external forces applied to them

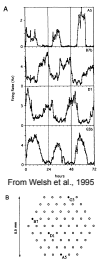
Clicker Question

What is meant by cells synchronizing the activity of cells?

- Cells share their resources with one another
- Each cell has the same period and amplitude in its oscillation
- The activity of individual cells is in phase with that of other cells
- Cells join together to form one unified cell structure

Individual SCN Neurons are Autonomous Oscillators, But Not in Synchrony

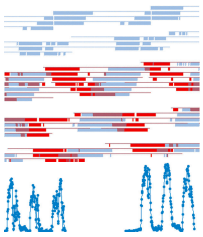
- Welsh et al. (1995) studied SCN neurons in a dissociated culture system
  - Despite "abundant functional synapses," neurons exhibited rhythms of widely different phases and periods
    - the four cells shown spike far out of phase with each other
      - Some exhibit peak spiking while others are exhibiting minimal activity
    - Periods range from 21.25 to 26.25 hours, with SD = 1.25 hours



From Welsh et al., 1995

Cell Autonomous Oscillators

- By recording from two individual neurons (blue and red hash marks) when their firing rate exceeds their daily mean, Welsh clearly showed that they had different periods
- Inhibiting action potentials with TTX temporarily blocked action potentials, but when released, they returned with same phase
  - Oscillation is maintained while firing is blocked
  - Cells are autonomous oscillators

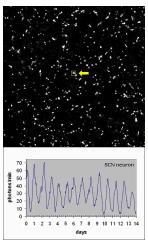

Welsh et al, 1995

Making Oscillations Visible

- A major challenge in answering any biological question is how to get evidence
  - Researchers can measure the concentrations of proteins such as PER, but not quickly or in real time
  - Challenge: how to visualize and record oscillations inside the mechanism in real time
- Fire flies exhibit periodic light emission that depends upon a luciferase protein
- Taking the luciferase gene from fireflies and conjoining it to the *Per* gene researches developed a system in which oscillations could be recorded in real time

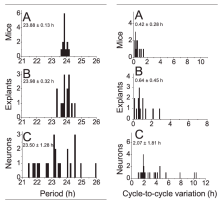
Visualizing Individual SCN Neurons

- PER2::Luc bioluminescence recorded from mouse SCN neurons in dispersed culture over two weeks
- If the number of cells is further reduced, few, but still some, remain rhythmic
- Thus:
  - Some individual SCN cells maintain rhythms
  - But these are out of phase and of varying periods



Robust Rhythms from Noisy Oscillators

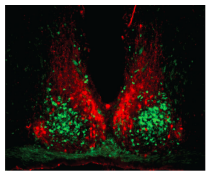
- Individual mice show little variation in their period or in the variation in their cycle
- Individual neurons, however, are quite variable
- Slices form the SCN, in which the connections between neurons are maintained, exhibit much less variability
- The network as a whole accomplishes what the parts do not



Herzog et al., 2004 17

Parts of the SCN

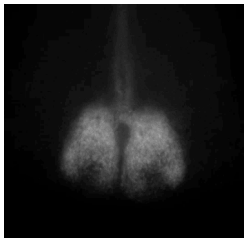
- Paired structure, each side containing ~10,000 neurons in mouse
- Each side has two regions
  - Core (green fluorescent)
    - Receives external input
    - ~1100 neurons express vasoactive intestinal polypeptide (VIP)
  - Shell (red labeling of AVP)
    - Partially envelop core
    - Receives its inputs from core
    - ~2100 neurons express arginine vasopressin



From Karatsoreos et al, 2004

Coordinated Behavior in Whole SCN

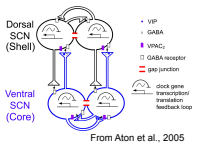
- Using a PER2::LUC knock-in, one can visualize the behavior of a whole coronal slice of mouse SCN (over 7 days)
- PER expression begins in shell (dorsomedial) SCN and progresses to the shell
- Complex pattern of activity



From Welsh et al., 2010

Organization with the SCN

- Only neurons in the core exhibit sustained oscillations
  - They release vasoactive intestinal protein (VIP)
  - $VPAC_2$ receptors for VIP are found in both core and shell SCN neurons

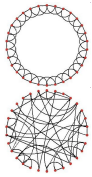


From Aton et al., 2005

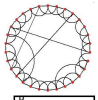
- Studies isolating shell neurons reveal low amplitude rhythms with shorter period than when coupled to the SCN
- Core seems to be crucial for coordinated SCN function—maintaining synchrony within the core and maintaining oscillation at all in the shell

How is the SCN Organized?

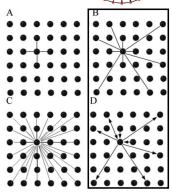
- Experimental research has yet to reveal the detailed functional wiring diagram of the SCN
- Alternative strategy: build computational models employing plausible wiring architectures and see which produces effects comparable to the SCN
- Two well-studied modes of organization:
  - Regular lattices: High clustering, long characteristic path length
    - Support generation of waves of activity
  - Random networks: Low clustering, short characteristic path length
    - Yield rapid synchronization across the network



The Small-World Alternative



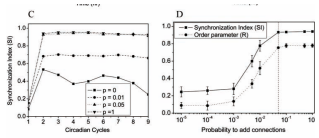
- Partly inspired to understand the synchronized firing of fireflies, Duncan Watts explored an alternative organization with mostly local connections but a few long-distance
- Various network designs have been employed in computational models of the SCN
  - A. Nearest neighbor, VIP expressed in all neurons
  - B. Small world: Additional connections added with prob p
  - C. Mean field or totally connected ne
  - D. Small world with only some neurons producing VIP



Behavior of Small-World Network

- Two measures computed from Per mRNA concentrations:
  - SI: Synchronization Index—compares instantaneous phase angle of each oscillator relative to a reference cycle, thereby quantifying the ability of the system to produce a coherent signal [in slice SI = 0.93]
  - R: order parameter represents the overall degree of synchrony over a specified time period.

Small world and totally-connected networks are comparable on these measures



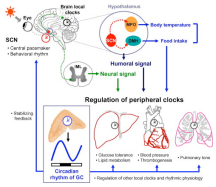
Vassalou et al. (2009)

SCN Organization: Jet Lag and Photoperiod

- Only the core of the SCN receives direct light signal, and so it is first to shift with shift in light-dark cycle
  - With east-bound travel, the core is shifted fairly rapidly
    - Causing it to oscillate in advance of the shell (reversing the normal order)
    - Several days are required to reset the shell
- In long (summer) days, the period of SCN activity is more spread out or even bimodal
  - Individual cells have a narrow period of peak firing
  - Photoperiod seems to be encoded in the distribution in the SCN population
  - Caudal (posterior) SCN neurons track dawn, rostral (anterior) track dusk

Clocks Everywhere

- Once clock proteins were identified, researchers could investigate whether they might be expressed in other tissues of the body
  - In fruit flies, *per* is expressed not just in lateral and dorsal neurons but in prothoracic gland, antenna, proboscis, Malphigian tubules, ovaries, testis, and gut
  - Likewise, mammalian clock genes are expressed, and cycle in many brain regions and most tissues of the body (liver, heart, lung, kidney, thyroid gland)



Discussion Question

If you had many clocks, each with a slightly different period (1 lost a minute a day, another gained 2.5 minutes per day) and you looked at the mean activity several months later, what would you expect to find?

A nice, regular oscillation of high amplitude

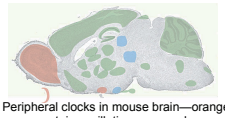
A regular oscillation, but of low amplitude

An essentially flat pattern

Other (be prepared to specify)

Are Peripheral Clocks Slaves?

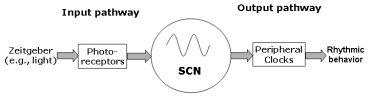
- The fact that peripheral clocks seem to dampen in circadian expression of clock genes after impairment of the SCN suggested that they could not sustain oscillations on their own
- Luciferase knock-ins permitted observation of sustained by not synchronized oscillators
  - If a population of oscillators is desynchronized it will appear that they are not oscillating
  - Peripheral clocks need a conductor, not a slave master



Peripheral clocks in mouse brain—orange areas sustain oscillations, green dampen. From Dibner et al., 2010

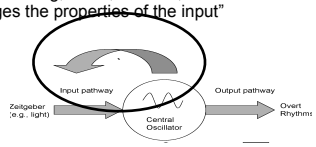
Sequentially Organized Mechanisms

- The simplest way to put multiple components together is to assume that they interact sequentially
  - The output of one operation is the input for another
- "Mechanisms are entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions." (Machamer, Darden, & Craver, 2000)



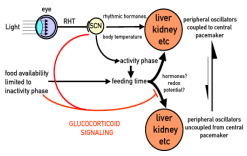
The SCN Regulates its own Inputs

- Production of melanopsin in the retina itself affected by circadian rhythms
- Only when light is received during subjective night does the pathway from the retina transmit signal to the clock
- Roennenberg, Daan, & Mann, 2003: "the clock changes the properties of the input"



Independence of Peripheral Clocks

- Traditional view was that entrainment by light is mediated by the SCN
- But light is not the only entrainment source
- If animals are fed only at a time outside of the usual feeding period, peripheral oscillators in the kidney and liver can be entrained to the alternative feeding time
- Location of the food-entrainable oscillator (FEO) is unclear

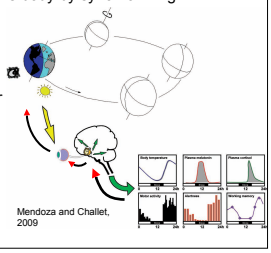


Feedback of Peripheral Oscillators on the SCN

- The circadian clock regulates redox potential in mitochondrial oxidative phosphorylation pathway
  - But, Hifialpha, transcription factor with bHLH-PAS domain, is regulated by redox potential
  - Likewise, clock constituents CLOCK, MOP3, NPAS2, are modulated by redox potential
- "Collectively, these observations may indicate that genes involved in redox regulation are both outputs of the clock as well as feedback on clock function."
  Panda & Hogenesch, 2004)

SCN's Inputs and Outputs

- SCN is entrained by light (and other Zeitgebers)
- The SCN is the central but not the only clock—it regulates functions in other parts of the body by synchronizing their clocks
- But there is also feedback:
  - Other clocks affect the SCN
  - SCN regulates behavior of retinal cells
  - People alter their environments
- Result: A highly integrated system



Mendoza and Challet, 2009

Integrated Systems: A New Holism?

- As researchers started putting the circadian mechanism and other biological mechanisms back together, they have discovered that the mechanism on which they have focused is enmeshed with other mechanisms
  - The "clock" no longer seems to be segregated from everything else
  - It regulates physiological and behavioral activities but it is also regulated by them
- Does such holistic integration undermine mechanism?
  - Or is it a triumph of mechanism?
    - Generating explanation that only mechanistic research could yield