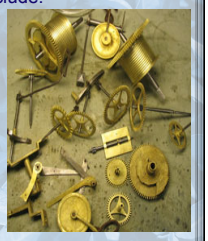
Mechanism and Levels of Organization: Recomposing and Situating Circadian Clocks

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
  - *Rora* and *Rev-erba* activator and inhibitor of *Bmal1*
  - Many chaperones
  - Various acetylases/methylases
  - etc., etc., etc.

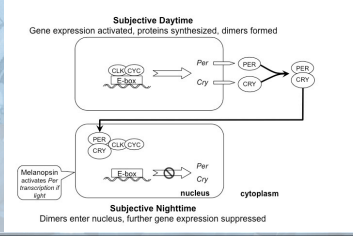


Putting the Mechanism Back Together

- A pile of separated parts won't produce the phenomenon
- To understand how they contribute to the phenomenon, 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

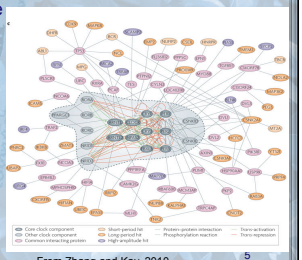
First Step: Transcription/ Translation Feedback Loop

- The proposal of the transcription/translation feedback loop and its linking to entrainment is a first step of recomposition



The Continuing Challenge of Recomposing the Intracellular Clock

- Genome-wide screen using small interfering RNA (siRNA) has identified more than 200 genes that affect clock period and amplitude
- Includes genes from four signaling pathways
  - Insulin pathway
  - Cell cycle
  - Hedgehog signaling
  - Folic acid biosynthesis
- The clock mechanism increasingly appears to be highly intertwined with the rest of the cell



From Zhang and Kay, 2010

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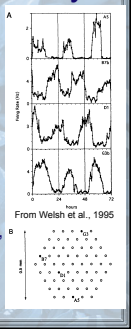
Recomposing Beyond the Individual Cell

- 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 in the SCN
  - Naïve hypothesis: The neurons in the SCN are all doing the same thing

Dibner et al., 2010

Individual SCN Neurons Oscillate, But Not in Synchrony

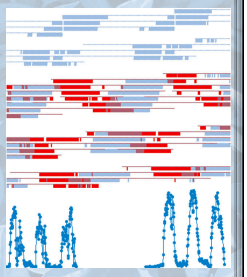
- Welsh et al. (1995) studied SCN neurons in a dissociated culture system
  - Despite "abundant functional synapses," neurons exhibited rhythms of widely different phases and periods
    - the four cells shown spike far out of phase with each other
      - Some exhibit peak spiking while others are exhibiting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



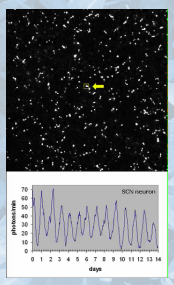
Welsh et al, 1995

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

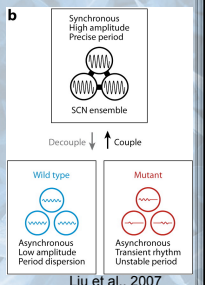
Visualizing Individual SCN Neurons

- 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



Synchronized Behavior in Intact SCN

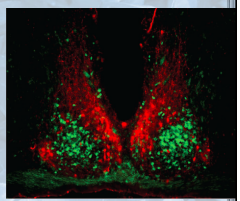
- In contrast to the dissociated SCN, the intact SCN exhibits highly regular oscillations with high frequency rhythms
- The interconnection of neurons with the SCN must be functionally significant
- Increased amplitude presumably due to neurons reacting in part to the activity of other neurons



Liu et al., 2007

Parts of the SCN

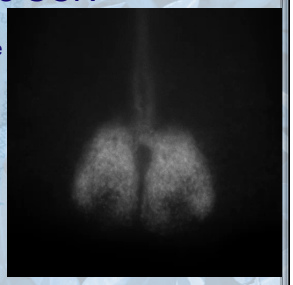
- Paired structure, each side containing ~10,000 neurons in mouse
- Each side has two regions
  - Core (green fluorescent)
    - Receives external input
    - ~1100 neurons express vasoactive intestinal polypeptide (VIP)
  - Shell (red labeling of AVP)
    - Partially envelop core
    - Receives its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



From Karatsoreos et al, 2004

Coordinated Behavior in Whole SCN

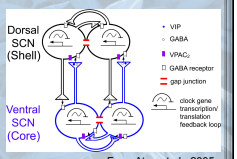
- Using a PER2::LUC knockin, one can visualize the behavior of a whole coronal slice of mouse SCN (over 7 days)
- PER expression begins in shell (dorsomedial) SCN and progresses to the shell
- Complex pattern of activity



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

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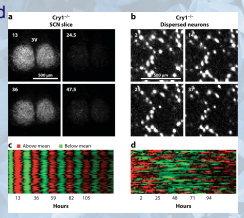
Effects of Organization Clearest in Mutants

- In mutant mice in which rhythms are already disrupted, contribution of organization to maintaining synchronization is rendered even apparent
- When organization is preserved *Cry1*$^{-/-}$ mutants still exhibit synchronized rhythms
  - Upper figure shows luminescence at different times
  - In lower plot, each row is a neuron
- When organization is lost, synchronization is lost



Liu et al, 2007

SCN Organization: Jet Lag and Photoperiod

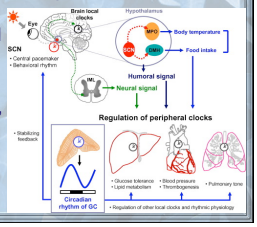
- Only the core of the SCN receives direct light signal, and so it is first to shift with shift in light-dark cycle
  - With east-bound travel, the core is shifted fairly rapidly
    - Causing it to oscillate in advance of the shell (reversing the normal order)
    - Several days are required to reset 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

Why Make a Clock Out of Sloppy Timekeepers?

- Note the kind of question this is
  - It seems to be asking us to speculate about evolution
  - We could put the question differently: What is the advantage to the organism of making the clock out of sloppy timekeepers
- Some proposals
  - Different oscillators could control different tissues with different time demands
  - Could be useful in tracking length of daylight in different seasons
  - Make clock robust against genetic perturbations

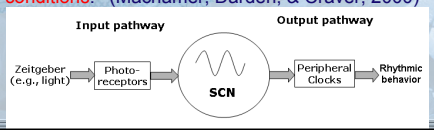
Clocks Everywhere

- 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)



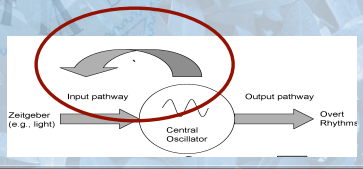
Sequentially Organized Mechanisms

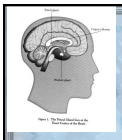
- The simplest way to put multiple components together is to assume that they interact sequentially
  - The output of one operation is the input for another
- "Mechanisms are entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination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



The SCN Regulates its own Inputs

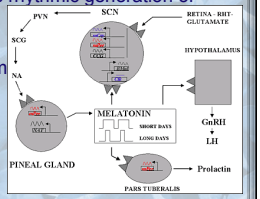
- Production of melanopsin in the retina itself affected by circadian rhythms
- Only when light is received during subjective night does the pathway from the retina transmit signal to the clock
- Roennenberg, Daan, & Mann, 2003: "the clock changes the properties of the input"





The Pineal Gland Loop

- In other vertebrates, the Pineal gland serves as a major Zeitgeber to the clock
- In mammals, major activity is rhythmic generation of melatonin—increased during darkness
- Part of the output mechanism for generating seasonal changes (growth of coat, appetite changes)
- Feeds back on the central oscillator in the SCN



From Endotext.com

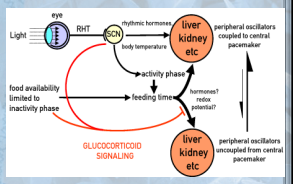
Are Peripheral Clocks Slaves?

- 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

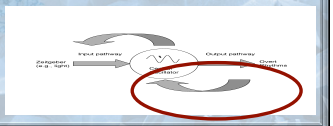
Independence of Peripheral Clocks

- 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



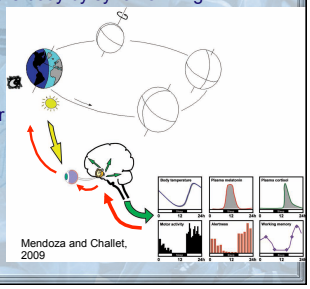
Feedback of Peripheral Oscillators on the SCN

- Clock regulation of redox potential in mitochondrial oxidative phosphorylation pathway
- 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

Decomposing and Recomposing

- To develop a mechanistic explanation of a phenomenon researchers must
  - Localize the mechanism
  - Decompose it into its parts and operations
- But localizing and decomposing is only one part of the endeavor
- Researchers must also
  - Recompose the mechanism to show how the parts and operations work together to generate the phenomenon
  - Situate the mechanism within the whole system

Integrated Systems: A New Holism?

- As researchers have put the circadian mechanism and other biological mechanisms back together, they discover 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