Modeling the Complexity of the Circadian Clock

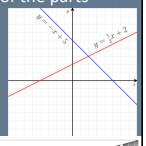
Beyond the Genome Project: Systems Biology

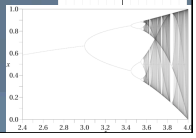
- The human genome project in the 1990s offered the promise that once the complete DNA sequence of the human genome was known, scientists would be able to solve many of the problems of biology and medicine
- The sequence of genomes of humans and many other species has provided biology with powerful new tools, but rather than solving the problems, it has revealed how difficult some problems are
  - The components of biological mechanisms have been found to interact with each other in a multitude of ways
  - Understanding these interactions requires the development of new tools to show how these interactions can give rise to the biological phenomena of interest
- Biologists are increasingly turning to mathematical/ computational modeling to understand the systems of interest

Complicated vs. Complex Systems

- Complicated systems are ones with lots of parts and operations
  - Biological systems certainly count as complicated
- Complex systems involve non-linear interactions that often give rise to behavior not anticipated from knowledge of the parts
  - May actually arise from just a few parts
- Linear equations such as
  - Ax + By = 0

  can be graphed as straight lines in Cartesian coordinates
- Equations with multiplicative relations, powers, etc., can generate much more complex graphs
  - Logistic map: $x_{t+1}=rx_t(1-x_t)$
    - For different values of x, fixed value, oscillations, chaos





From Basic to Dynamic Mechanism

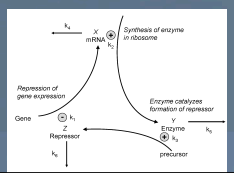
- Both mechanistic science and philosophical accounts of mechanism have emphasized decomposing mechanisms to identify the parts and operations that contribute to the phenomena of interest
- Biologists have tended to be skeptical of computational modeling, preferring laboratory research
  - Populations genetics the exception, but it mostly employed linear models
- But increasingly biologists are discovering systems which can only be represented in non-linear equations which generate complex (emergent) behavior
- Amending the characterization of mechanism to include dynamics
  - A mechanism is a structure performing a function in virtue of its component parts, component operations, and their organization. The orchestrated functioning of the mechanism, manifested in patterns of change over time in properties of its parts and operations, is responsible for one or more phenomena.

Reasons to Model Mechanisms Mathematically

- To understand how a complex mechanism will behave
- To suggest manipulations that can be made to test the proposed account experimentally
- To reveal how the mechanism might respond to altered conditions in the environment

Goodwin Oscillator (1950s)

- Following the discovery by Jacob and Monod of a feedback mechanism (the lac operon) whereby bacteria suppress transcription of specific genes except when they are needed, Brian Goodwin proposed a model of how such a control mechanism might generate oscillations
- Three differential equations, each of which has terms for the generation and degradation of one component
  - Goodwin showed that this system, with appropriate values for *n*, would generate oscillations in the quantities of the components
  - Provided an exemplar for modeling circadian rhythms



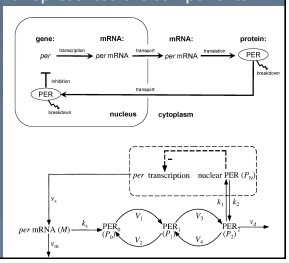
$$\frac{dX}{dt} = \frac{k_1}{Z^n + 1} - k_4 X$$

$$\frac{dY}{dt} = k_2 X - k_5 Y$$

$$\frac{dZ}{dt} = k_3 Y - k_6 Z$$

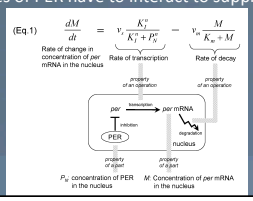
Modeling the Simple Per Loop

- 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. 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 (v_s= maximum rate of transcription



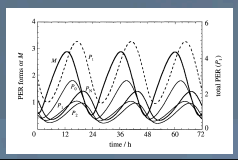
Modeling the Simple Per Loop

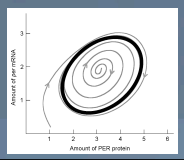
- The next step is to write equations to characterize how the values of each variable changes dependent upon other variables
- Eq. 1 has one term for the making of new *per* mRNA and one for its degradation
  - The equation introduces a non-linearity in the exponent *n* (which was taken to reflect the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



Modeling the Simple Per Loop

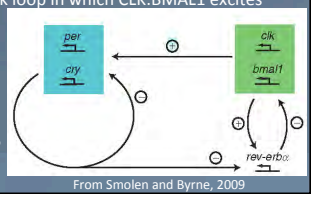
- Often, as in this case, it is not possible to derive a solution to multiple equations analytically and so modelers apply them iteratively to *simulate* the operation of the mechanism
- Goldbeter showed that the model generated sustained oscillations of
  - *per* mRNA
  - Total PER (P_t)
  - Nuclear 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





Taking Multiple Feedback Loops into Consideration

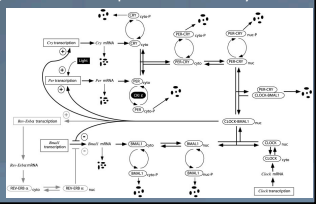
- In addition to the negative feedback loop whereby PER:CRY inhibits its own transcription (via removing the CLK:BMAL1 dimer from its promoter)
  - There is a second negative feedback loop in which CLK:BMAL1 excites production of REV-ERBα, which then inhibits production of BMAL1
  - And a positive feedback loop in which CLK:BMAL1 excites PER:CRY, which inhibits REV-ERBα and stops it from inhibiting BMAL1 expression
- Will multiple loops disrupt the 24-oscillations?



From Smolen and Byrne, 2009

Modeling the More Complicated Mechanism

- As more components of the mechanism were discovered, Goldbeter expanded his model (Goldbeter and Leloup, 2003)
  - Adding variables for the additional parts
  - Parameters for the additional operations
  - And many more equations (73 in the latest)
- Challenge: models with large numbers of equations and many parameters can be fitted to data and may not reveal how the mechanism works
  - Thus, some modelers prefer abstracting and employ *reduced models.*

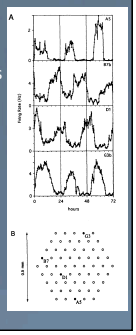


"Experimenting" with Computational Models

- Modelers often speak of conducting experiments with their models
  - Changing the values of variables or, more typically, parameters, and determining their effects
- One use of such use of models is to determine whether, on the account proposed, experimental manipulations of the actual mechanism would be expected to have the effect they have
  - Can manipulation of appropriate parameters reproduce the effects of various induced mutations (e.g., Konopka's original results)

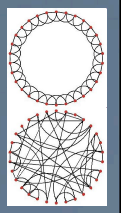
Modeling Dynamics at Large-Scales

- We saw that individual SCN neurons exhibit considerable variability in period and phase when cultured
  - This variability is radically reduced when neurons interact in a whole network—neurons synchronize their activity
- What is the organization of the network that facilitates synchronization?
  - Researchers have not yet been able to directly observe the network organization of the SCN
  - Instead they have worked by constructing hypotheses, representing them in computational models, and evaluating how well these models could explain the observed behavior



Analyzing the Behavior of Networks

- Most mathematical analysis of networks in the 20th century focused on
  - Regular lattices: High clustering, long characteristic path length
  - Random networks: Low clustering, short characteristic path length
- These lent themselves to mathematical analysis
  - Random networks achieve synchronized behavior quickly
  - Regular lattices support regular waves of behavior



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A First Network Model: Assume Network Totally Connected

- Gonze et al. (2005) developed a mean field model by assuming that VIP from individual neurons accumulated and distributed equally.
- Adopted Goodwin's oscillator by adding expressions for
  - *V* = neurotransmitter whose synthesis is induced by *X*
  - *F* = mean field or average concentration of neurotransmitter
  - *K* = sensitivity of individual oscillators to the VIP neurotransmitter/ coupling strength

$$\frac{dX_i}{dt} = v_1 \frac{k_1^n}{Z_i^n + k_1^n} - v_4 \frac{X_i}{k_4 + X_i} + v_c \frac{KF}{K_c + KF} + L$$

$$\frac{dY_i}{dt} = k_2 X_i - v_5 \frac{Y_i}{k_5 + Y_i}$$

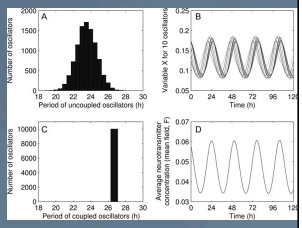
$$\frac{dZ_i}{dt} = k_3 Y_i - v_6 \frac{Z_i}{k_6 + Z_i}$$

$$\frac{dV_i}{dt} = k_7 X_i - v_8 \frac{V_i}{k_8 + V_i}$$

$$F = \frac{1}{N} \sum_{i=1}^{N} V_i$$

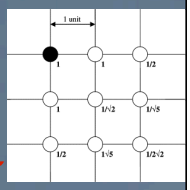
Totally Connected Network Achieves Synchronization

- Gonze et al. employed 1000 oscillators
- Set *K*=0 to simulate no VIP release
  - Periods were normally distributed with a mean of 23.5 h and an SD of 1.17 h
- Sett *K*=0.5 to simulate VIP release
  - All cells synchronized to a period of 26.5 h



Making Synchronization Depend on Distance

- To et al. 2007 modeled diffusion based on distance
- Started with the Leloup and Goldbetter mammalian model and added random perturbations in the basal *Per* transcription rate (v_{sp0}) so that ~40% of neurons oscillated
- Other parameters randomly varied to create range of oscillatory periods from 18 to 30h



$$\rho_i(t) = a\frac{M_{P,i}(t)}{M_{P,i}(t)+b}$$

$$\gamma_i(t) = \frac{1}{\varepsilon}\sum_{j=1}^{N} a_{ij}\rho_j(t)$$

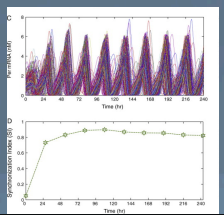
ρ_i is extracellular concentration of VIP produced by neuron *i*

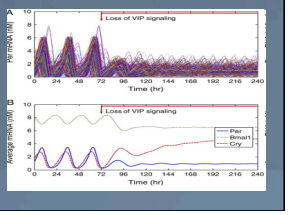
M_P is the *Per* mRNA concentration

γ is the local VIP concentration observed by neuron *i*

Local Neighborhood Structure Also Generates Synchronization

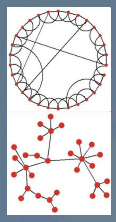
- Introduction of VIP resulted in rapid synchronization in *Per* mRNA oscillations
  - Results parallel those for SCN after prolonged blockage of action potentials with tetrodotoxin (TTX)
- Loss of VIP resulted in desynchronization





Small-world Networks and Their Dynamics

- What happens if most connections are local, but a few are long-distant?
  - High clustering and short characteristic path length
- In the 1990s Duncan Watts appealed to these characteristics to define small-world networks
  - Showed that they are highly suited for information processing
    - Local regions can specialize
    - Whole network can remain coordinated
- Many networks in the real world turn out to have a small-world structure: airline route systems, the internet, gene networks, protein networks, the brain



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What Good Are Small World Networks?

- Watts and Strogatz speculated that small world networks "would display enhanced signal propagation speed, synchronizability and computational power, as compared with regular lattices of the same size. **The intuition is that the short paths could provide high-speed communication channels between distant parts of the system, thereby facilitating any dynamical process (like synchronization or computation) that requires global coordination and information flow**" (Strogatz, 2001)
- Potential advantage over random networks: enable different clusters to specialize in different ways
  - Without sacrificing the ability to rapidly adapt to activity elsewhere

Could the SCN be a Small-World?

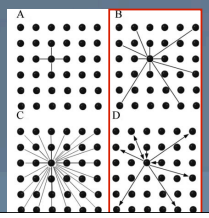
- Vasalou et al. (2009) set out to explore this question in a model. They replaced

$$\gamma_i(t) = \frac{1}{\varepsilon}\sum_{j=1}^{N} a_{ij}\rho_j(t)$$

- from the To et al. model with

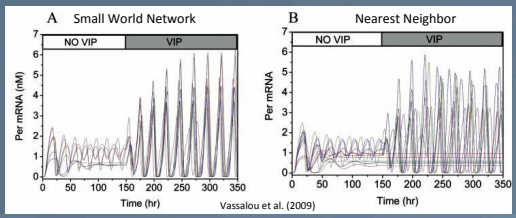
$$\gamma_i(t) = \frac{1}{k_i}\sum_{j=1}^{N} a_{ij}\rho_j(t)$$

  - Where k_i is number of synaptic inputs received by neuron i and
  - $a_{ij} = 1$ if there is a connection between i and j and 0 otherwise
- Network architectures:
  - A. Nearest neighbor, VIP expressed in all neurons
  - B. Small world: Additional connections added with prob p
  - C. Mean field or totally connected net
  - D. Small world with only some neurons producing VIP
- Small world when $0.01 < p < 0.1$



Small Worlds Outperform Nearest Neighbor

- *Per* mRNA concentration of ten randomly selected cells

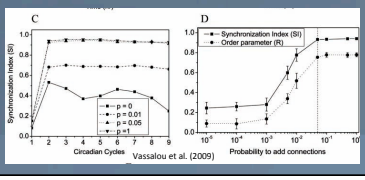


Vassalou et al. (2009)

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)

Small-World Model Explains Additional Phenomena

- Without VIP, only 30% to 40% of SCN neurons oscillate, but with VIP all do
  - A. When coupling increased from nearest neighbor to small-world, percentage of non-oscillating neurons dropped
- Without VIP, mean period is approximately 22 hours, whereas with VIP it approximates 24 hours
  - B. When coupling increased to small-world levels, period extends to approximately 24 hours
- Without VIP, there is large variability in periodicity, whereas with VIP oscillators are largely synchronized
  - C. When coupling increases to small-world levels, variability drops dramatically