Multi-scale Modeling of Circadian Rhythms: From Metabolism to Regulation and Back in 24 hours

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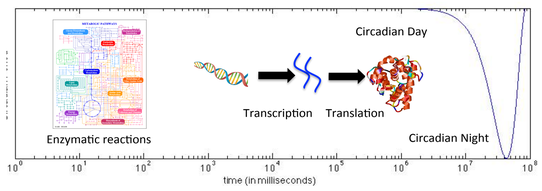
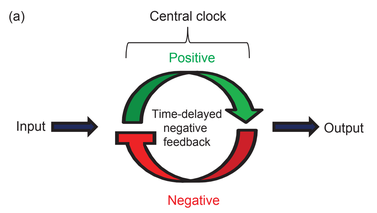
Overview

Goals: The goal of this research is to develop and implement a new computational and theoretical method for modeling biological systems that fills a gap in modeling mass action dynamics. Based on statistical thermodynamics, the method bridges data-poor scales (parameters for mass action kinetics) and data-rich scales (chemical potentials of metabolites, and metabolite, protein & transcript data) to enable predictive modeling from enzymatic reactions (10^{-3} to 10^{0} s^{-1}) to gene and protein regulation (~20 minutes) to circadian rhythms (24 hours). We are:

- Implementing a simulation approach that uses chemical potentials rather than rate constants. This approach involves a rescaling of the fast degrees of freedom, resulting in a compression of the time-dependence to fewer relative scales.
- Understand the relationship between central metabolism and circadian rhythms by using a multi-scale model that includes regulation of the clock.



Circadian Clocks



Circadian clocks lie at the epicenter of cellular physiology for both fungal and mammalian cells, both of which share clocks with equivalent regulatory architecture. At the core of these clocks, a heterodimeric transcription factor (TF) drives expression of genes whose protein products feed back, physically interact with, and depress the activity of their heterodimeric activator.

This negative feedback loop, yielding oscillatory TF activity, is the basis of fungal and animal circadian rhythms. Output from the clock occurs when these TFs regulate genes whose products do not impact the core feedback loop. For organisms having a circadian clock, nearly all genes are effectively clock-regulated, yielding the profoundly rhythmic metabolism that has a major impact on adaptation, optimal efficiency of the cell, enzyme production and both normal and disease physiology. *Neurospora crassa* is the best studied cellular circadian system and is a well-established model for eukaryotic including mammalian clocks. *Neurospora* provides an extremely tractable system in which to pioneer modeling of these cellular clocks and their influence on metabolism.

Modeling and Simulation

The new approach to the law of mass action does not require rate parameters but instead uses chemical potentials (1). Due to the statistical formulation of the theory, the approach can directly integrate metabolomics and proteomics data. We are using these methods to fundamentally understand the relationship between metabolism (2,3) and molecular circadian clocks with regard to the role of the circadian clock in increasing the metabolic efficiency of the cell (4).

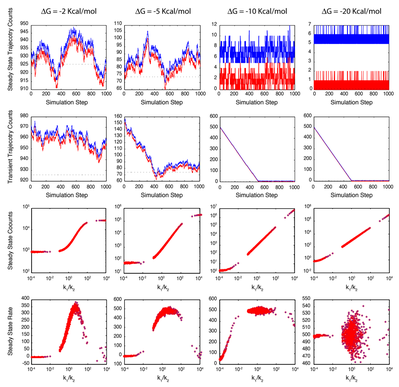
Simulation Scenarios

◆ Case 1. Steady state metabolite concentrations available

$$\gamma_{A,1}A \underset{-1}{\overset{+1}{\rightleftharpoons}} \gamma_{B,1}B$$

$$\gamma_{B,2}B \underset{-2}{\overset{+2}{\rightleftharpoons}} \gamma_{C,2}C$$

Red: stochastic kinetic simulations.
Blue: simulations using chemical potentials.



(A) Steady state trajectories (offset by +5). (B) Non-equilibrium transient trajectories. (C) Steady state counts of the intermediate *B* from 100,000 simulations. (D) Steady state net reaction rate (flux) values over the same set of simulations as in (C).

◆ Case 2. Missing steady state concentrations.

Given the constraints of the network and known steady-state concentrations, we use the least biased probability distribution to infer missing metabolite steady state concentrations and fluxes by assuming a maximum entropy production production principle.

Maximum ENTropy production Of the Stoichiometric matrix (MENTOS)

$$\begin{aligned}
\underset{\vec{\mathbf{a}},\vec{\mathbf{r}}_+,\vec{\mathbf{r}}_-}{\text{maximize}} \quad & -\sum_i \mathscr{P}_{+i} \log \mathscr{P}_{+i} - \sum_i \mathscr{P}_{-i} \log \mathscr{P}_{-i} \\
\text{subject to} \quad & S \cdot \vec{\mathbf{r}}_+ = S \cdot \vec{\mathbf{r}}_- \\
& \log \vec{\mathbf{r}}_+ - \log \vec{\mathbf{r}}_- = -\tfrac{1}{RT} S^T \cdot \vec{\mu}^0 - S^T \cdot \log \vec{\mathbf{a}} \\
& \vec{v}_{lower} \le \vec{\mathbf{r}}_+ - \vec{\mathbf{r}}_- \le \vec{v}_{upper} \\
& \vec{a}_{lower} \le \vec{\mathbf{a}} \le \vec{a}_{upper} \\
& \vec{\mathbf{r}}_+ \ge 0 \\
& \vec{\mathbf{r}}_- \ge 0
\end{aligned}$$

Where

$\vec{\mathbf{r}}_+, \vec{\mathbf{r}}_-$ are decision variables representing the forward and backward rates, respectively.

$\mathscr{P}_{+i}, \mathscr{P}_{-i}$ are the normalized forward and backward thermodynamic driving forces $\frac{r_{+i}}{r_{-i}}\left(\sum_j \frac{r_{+j}}{r_{-j}} + \frac{r_{-j}}{r_{+j}}\right)^{-1}$ and $\frac{r_{-i}}{r_{+i}}\left(\sum_j \frac{r_{+j}}{r_{-j}} + \frac{r_{-j}}{r_{+j}}\right)^{-1}$

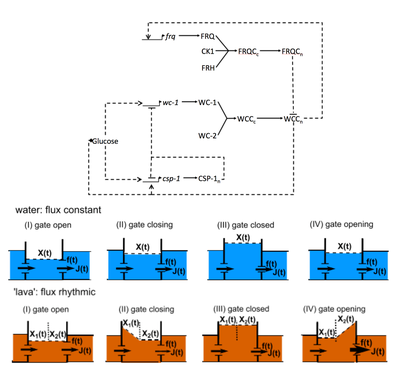
$\vec{\mathbf{a}}$ is a decision variable representing the chemical activity of each metabolite

S is the $m \times n$ stoichiometric matrix of representing m metabolites and n reactions of the model

$\vec{\mu}^0$ is the vector of standard chemical potentials

Thermokinetic circadian response analysis

We will extend our genome-scale[2] thermokinetic[1] model of *Neurospora* to predict how circadian rhythms propagate to metabolite concentrations and fluxes and to investigate how the clock regulatory network is influenced by and compensates for changes in metabolite levels[6] (right, top). Predictions and data will be compared using circadian response analysis[5] and Kullback-Leibler divergence. Different circadian responses can be understood by a water flow (right, middle) or lava flow analogy (right, bottom).



Experimental Design

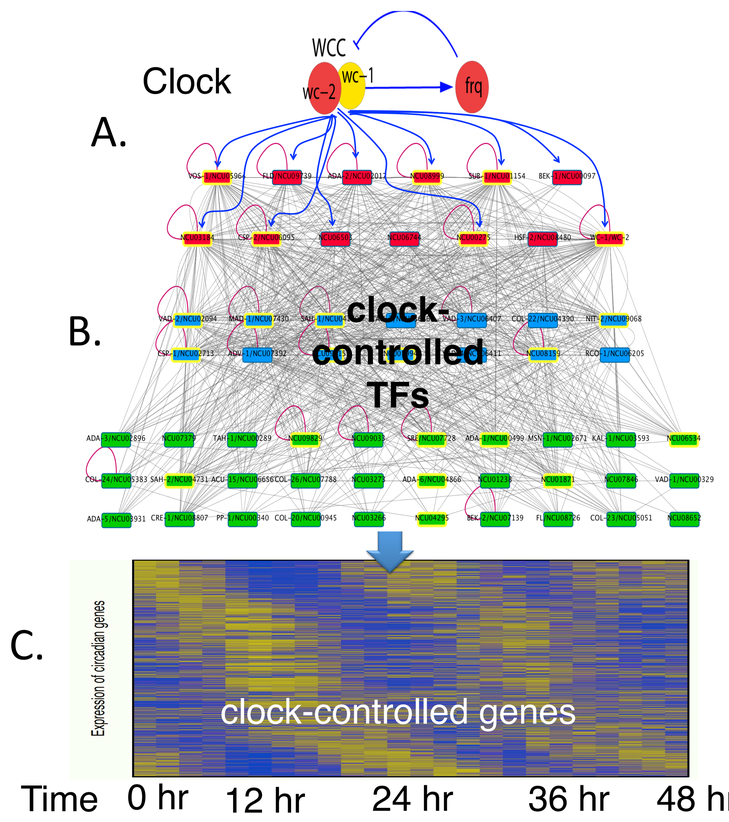
In a data set well beyond anything available in any other circadian system, the entire assemblage of clock-controlled genes has been described, and data are in-hand to delineate all clock-controlled proteins, including enzymes, and the clock-controlled metabolites to which they give rise (4). Each step from gene to protein to metabolite is regulated and the entire assemblage can be modeled using this unparalleled data set.

The circadian cycle is approximated in (**A**) by the negative feedback loop in which the heterodimer WC-1/WC-2 drives expression of *frq* which feeds back with other proteins (not shown) to depress WC-1/WC-2 activity. In (**B**), WC-1/WC-2, in turn, activate clock-controlled TFs (curved blue arrows) and these in turn regulate additional TFs, in all comprising a hierarchical network downstream from the clock. This transcriptional network, now largely described from ChIP-seq data for over 50 TFs, acts as a dynamic filter for time information generated by the circadian oscillator in (**A**). In the aggregate the TFs within this transcriptional network act on downstream genes in a combinatorial manner to regulate their expression. Shown in (**C**) is the heat map showing rhythmic expression of the *Neurospora* genome as determined by RNA-seq of samples collected every 2 hrs over 48 hrs in constant darkness.

A. The Neurospora circadian oscillator, and transcription–translation negative feedback loop.

B. The circadian transcriptional output network as determined by ChIP-seq.

C. Heat map showing rhythmic expression of the Neurospora genome over 48 hrs in constant darkness.



References

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