

Simulation and Experiment Uncover Complementary Regulation in the Circadian System *Neurospora crassa*

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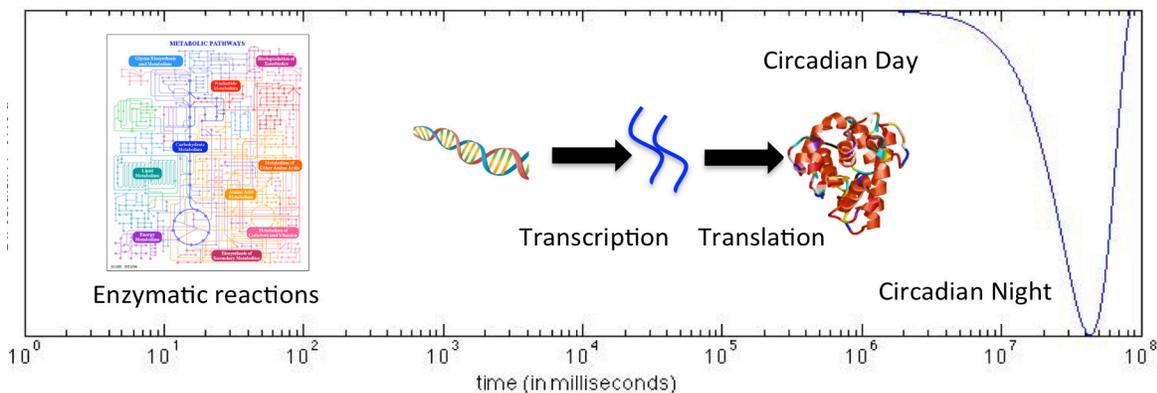
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Project Goals: The goal of this research is to develop and implement a new computational and theoretical method for modeling biological systems that fills a gap in modeling mass action dynamics. Based on statistical thermodynamics, the method bridges 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)



Timescales that the simulations using statistical thermodynamics will cover. Enzymatic reactions occur on the millisecond to second timescale while gene and protein expression occur on the minute to ~30-minute scale and the circadian rhythm occurs over a period of 24 hours.

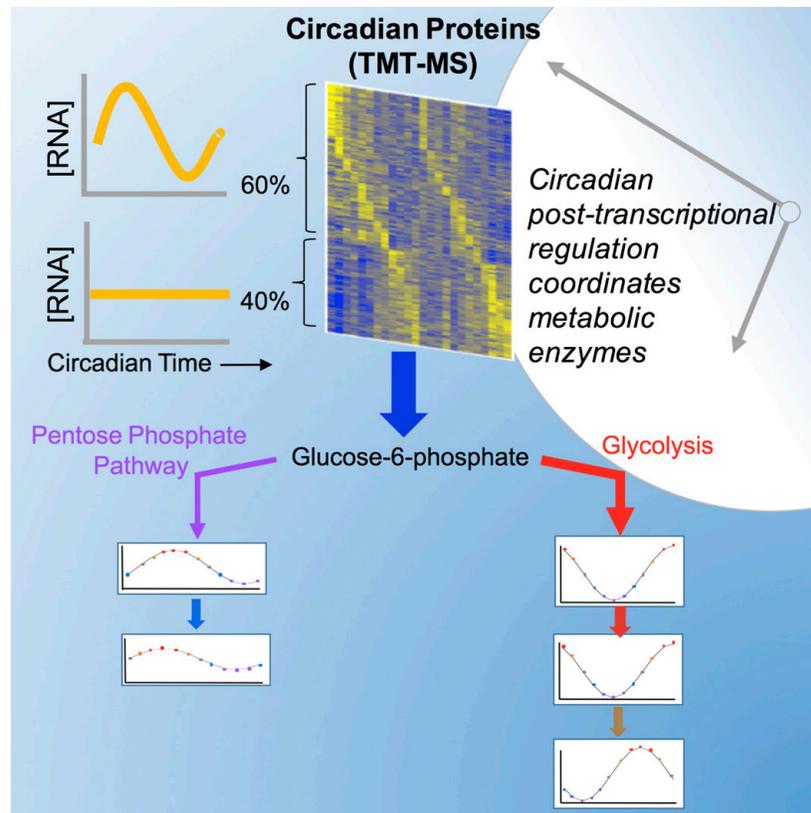
to gene and protein regulation (~20 minutes) to circadian rhythms (24 hours).

Progress: Nature selects those organisms that can reproduce the fastest while maintaining fitness and extracting the least amount of energy from their environment. This challenge makes regulation of natural systems critically important. We report the computational prediction of regulation, metabolite levels and rate constants using a maximum entropy method [1], and the experimental detection of circadian regulation of proteins and transcripts [2].

The computational method is applied in four steps: (1) a new constrained optimization approach based on Marcelin's 1910 mass action equation is used to obtain the maximum entropy distribution, (2) the predicted metabolite concentrations are compared to those generally expected from experiment using a loss function from which post-translational regulation of enzymes is inferred, (3) the system is re-optimized with the inferred regulation from which rate constants are determined from the metabolite concentrations and reaction fluxes, and finally (4) a

full ODE-based, mass action simulation with rate parameters and allosteric regulation is obtained. The method is applied to central metabolism and the flow of material through the three competing pathways of upper glycolysis, the non-oxidative pentose phosphate pathway, and the oxidative pentose phosphate pathway are evaluated as a function of the NADP/NADPH ratio.

The simulations complement experimental transcriptional and translational experiments performed over the circadian cycle of *Neurospora*. Transcriptional/translational feedback loops in fungi and animals drive circadian rhythms in transcript levels that provide output from the clock, but post-transcriptional mechanisms also contribute. To determine the extent and underlying source of this regulation, we applied novel analytical tools to a long-duration, deeply-



sampling, circadian proteomics time course comprising half of the proteome. We found a quarter of expressed proteins are clock-regulated, but >40% of these do not arise from clock-regulated transcripts. Contrary to predictions, rhythmic protein degradation plays little role in post-transcriptional regulation but instead rhythms arise from oscillations in translation. Our data highlighted the impact of the clock on metabolic regulation, with central carbon metabolism reflecting both transcriptional and post-transcriptional control and opposing metabolic pathways showing peak activities at different times of day. CSP-1, a transcription factor with a role

in metabolic regulation of the clock, contributes significantly to determining the rhythmicity and phase of clock-regulated proteins. The experimental data demonstrate that the rhythmic proteins within the Pentose-Phosphate pathway peak in the circadian morning, while conversely, in glycolysis and the TCA cycle, the rhythmic proteins peak in the circadian evening. That is, the rhythmic proteins of glycolysis are in anti-phase to the rhythmic proteins of the Pentose-Phosphate pathway.

References

1. Cannon, W.R., et al., *Prediction of metabolite concentrations, rate constants and post-translational regulation using maximum entropy-based simulations with application to central metabolism of *Neurospora crassa**. Processes, 2018. 6(6)<https://doi.org/10.3390/pr6060063>
2. Hurley, J.M., et al., *Circadian Proteomic Analysis Uncovers Mechanisms of Post-Transcriptional Regulation in Metabolic Pathways*. Cell Systems, 2018<https://doi.org/10.1016/j.cels.2018.10.014>

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