The Rhythms of Metabolism: A Backwards Tale of Translational Chronobiology Metabolomics in Three Acts

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Background and significance: Life has evolved in the context of rhythmic daily cycles rooted in the earth's rotation. Almost all life forms have thus adapted to circadian cycles of light/dark, temperature and nutrient availability (chronobiology). Rhythms in metabolism have been described in humans and model systems[1]. Characterization of these small molecule oscillations is challenging due to the need for quantitative, multiplexed data. NMR metabolomics is well suited for this application due to the unbiased and inherently quantitative nature of the technique[2,3]. This presentation will be a tale in three acts describing the interaction of metabolism with chronobiology. Counterintuitively, we will work backwards by first breaking the clock (cancer) [4], then slightly disrupting the clock in human sleep disorders (insomnia) [5] to finally understanding normal nutrient processing by metabolite tracing in Drosophila lipids by 2D NMR.

Experimental approaches: All NMR experiments were performed using a 700 MHz Bruker Avance III HD NMR spectrometer equiped with a 3 mm TXI probe and samplejet for high throughput capabilities. Polar metabolites were analyzed using the conventional NOESYPR1D type experiments with water suppression by presaturation during relaxation delay (1 second) and mixing time. Total acquisition time was 5 seconds. Polar metabolomics data was analyzed using either targeted profiling technique[2] or conventional spectral binning using Chenomx NMR suite v 8.0, depending on sample sizes. Metabolite identification was performed using conventional 2D NMR (TOCSY and HSQC), database search and/or Chenomx library based identification.

For NMR based lipidomics analysis, two dimensional ^{1}H-^{13}C correlation spectra were recorded using Perfect-HSQC pulse scheme[6]. This scheme improves conventional HSQC by suppressing J_{HH} interferences using Perfect-INEPT periods. Such manipulation results in pure in-phase cross peaks and peak volumes are not affected by J_{HH}, thereby leading to more efficient phase correction and signal integration. Lipidomics data was integrated using rNMR software by a RoI based approach followed by analysis of circadian oscillation.

Results: Act I: Breaking the clock to hijack metabolism. The premise of our story is that the molecular clock provides a metabolic advantage in normal biology by providing advantageous metabolic cycles. It follows then that dramatic disruption of the clock would be advantageous for disease processes. This is in fact demonstrated by overexpression of the MYC oncogene in a cell autonomous system with an intact clock (U2OS). We observe metabolic rhythms in normal U2OS cells which are severely abrogated by overexpression of MYC using 1D NMR.

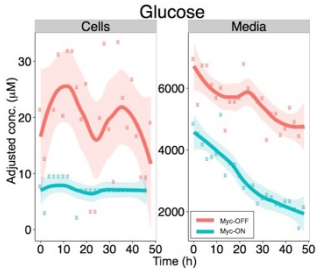

Figure 1: Quantitative loss of glucose cycling in cells and media upon MYC over-expression.

Act II: The metabolic insults of insomnia. Insomnia is a sleep disorder which is typically diagnosed subjectively and lacking quantitative indicators. Analysis of serum from controls and insomniacs demonstrates altered metabolism both globally across the day in discriminant analysis, as well as alterations in metabolite rhythms. These include shifts in peak phase of key metabolites such as lactate and certain amino acids, as well as altered amplitudes. Insomniacs display metabolic phenotypes reminiscent of pre-diabetic metabolic profiles with altered branched chain amino acid catabolism and increases night-time carbohydrates.

Act III: Using quantitative 2D NMR to understand nutrient fate: Given the circadian nature of nutrient availability and rhythms associated with carbohydrate processing, this final act will present preliminary results tracking the fate of glucose metabolism using an in vivo Drosophilia model to understand circadian metabolism. Using 2D NMR, we show that glucose is incorporated into bulk lipids in a time-of-day dependent manner.

Conclusions and future work: Coordination of the circadian clock with metabolism appears to play an evolutionary advantageous role in defense against disease and reparative metabolic cycles. Feeding and fasting cycles have profound impacts on bulk lipid changes through the circadian day *in vivo*.

(1) Krishnaiah, S. Y.; Wu, G.; Altman, B. J.; Growe, J.; Rhoades, S. D.; Coldren, F.; Venkataraman, A.; Olarerin-George, A. O.; Francey, L. J.; Mukherjee, S.; Girish, S.; Selby, C. P.; Cal, S.; Er, U.; Sianati, B.; Sengupta, A.; Anafi, R. C.; Kavakli, I. H.; Sancar, A.; Baur, J. A.; Dang Chi, V.; Hogenesch, J. B.; Weljie, A. M. *Cell Metab* **2017**, *25* (5), 1206.
(2) Weljie, A. M.; Newton, J.; Mercier, P.; Carlson, E.; Slupsky, C. M. *Anal Chem* **2006**, *78* (13), 4430–4442.
(3) Rhoades, S. D.; Sengupta, A.; Weljie, A. M. *Curr Opin Biotechnol* **2016**, *43*, 70–76.
(4) Altman, B. J.; Hsieh, A. L.; Sengupta, A.; Krishnanaiah, S. Y.; Stine, Z. E.; Walton, Z. E.; Gouw, A. M.; Venkataraman, A.; Li, B.; Goraksha-Hicks, P.; Diskin, S. J.; Bellovin, D. I.; Simon M, C.; Rathmell, J. C.; Lazar, M. A.; Maris, J. M.; Felsher, D. W.; Hogenesch, J. B.; Weljie, A. M.; Dang Chi, V. *Cell Metabolism* **2015**, *22* (6), 1009–1019.
(5) Gehrman, P.; Sengupta, A.; Harders, E.; Ubeydullah, E.; Pack, A. I.; Weljie, A. *Sleep* **2018**, *69* (6), 592.
(6) Castañar, L.; Sistaré, E.; Virgili, A.; Williamson, R. T.; Parella, T. *Magn Reson Chem* **2015**, *53* (2), 115–119.