HTS NMR Probe Tracks Metabolism Cycles During Insect Dormancy

Chao Chen[1], **Rohit Mahar[2]**, **Matthew E. Merritt[2]**, David L. Denlinger[3], and Daniel A. Hahn[1]

[1]Department of Entomology and Nematology and [2]Department of Biochemistry and Molecular Biology, University of Florida, [3]Department of Entomology, Ohio State University, USA

Funding: MagLab (G.S. Boebinger, NSF DMR-1644779); ME Merritt (NIH P41-122698); DA Hahn (NSF IOS 1257298 and DEB 1639005, and United Nations FAO/IAEA Coordinated Research in Dormancy Management)

Metabolic changes during dormant periods experienced by insects and mammals offer clues to the critical control mechanisms related to suppression of metabolic functions during organ transplantation. The nutrient and waste cycles which occur during insect diapause are quite similar to mammalian torpor-arousal cycles.

Surviving long periods without eating is a challenge which insects confront during pupation. *The flesh fly, Sarcophaga crassipalpis, is a model known to cycle metabolic products effectively during this dormant stage*. It can survive the diverse changes in its environment through hormonal regulation and metabolic homeostasis. Intermediary metabolism and respiration is naturally diminished within a hypoxic state of burning glucose via glycolysis during this period, but *its metabolism also periodically cycles to enable aerobic respiration in order to engage mitochondria to replenish the pupal nutrient stores and clear anaerobic byproducts*.

^{13}C-nuclear magnetic resonance (NMR) spectroscopy is chemically selective, allowing direct assessment of how ^{13}C is incorporated into downstream metabolites. Positional ^{13}C, spin-spin multiplets, and [U-^{13}C]alanine tracers measure changes occurring through multiple pathways including anaplerotic and pyruvate oxidative fluxes, pyruvate cycling, and trehalose synthesis during the metabolic arousal stage (Figure 1). *The outstanding sensitivity of the ^{13}C HTS probe at the AMRIS Facility allows acquisition from a single pupa, enabling this study using multiple tracers in a time efficient manner*.

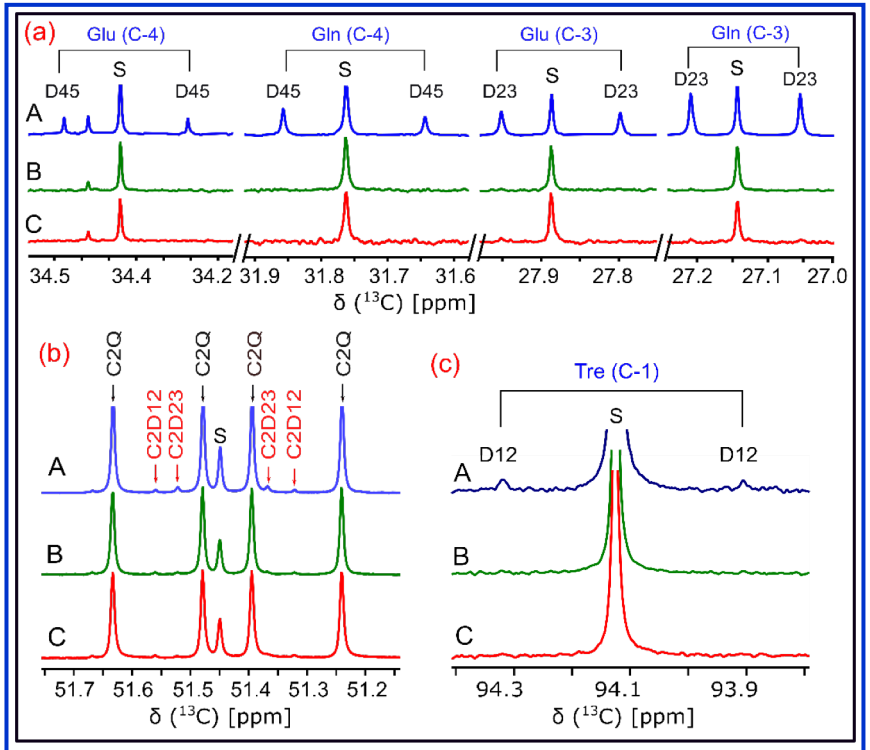

Figure 1. (a) *^{13}C NMR spectra of glutamate-C3, glutamine-C3, glutamate-C4, and glutamine-C4 in pupae injected with [U-^{13}C]alanine* ***(b)*** *Alanine recycled through pyruvate kinase (PK) flux: C2D12 and C2D23 represent [1,2-^{13}C]alanine and [2,3-^{13}C]alanine, whereas C2Q signals represent [U-^{13}C]alanine.* ***(c)*** *^{13}C NMR spectra of labeled trehalose C-1. S, singlet; D12, D23 and D45, doublet; Q, quartet.*

The representative stacked spectra in each set are from metabolic stages of pupae during the dormancy period: A) interbout of metabolic arousal (IBA), B) early metabolic depression, and C) late metabolic depression.

Facilities and instrumentation used: *AMRIS Facility, AMRIS 14.1 T NMR equipped with a HTS CryoProbe and Agilent Console*

Citation: Chen, C.; Mahar, R.; Merritt, M.E.; Denlinger, D.L.; Hahn, D.A., *ROS and hypoxia signaling regulate periodic metabolic arousal during insect dormancy to coordinate glucose, amino acid, and lipid metabolism,* **Proceedings of the National Academy of Sciences of the USA (PNAS)**, **118** (1), 603118 (2021) doi.org/10.1073/pnas.2017603118