Hibernation in a dish: cell-autonomous response of mammalian cells to low temperatures

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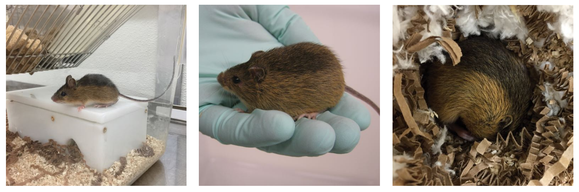
ABSTRACT

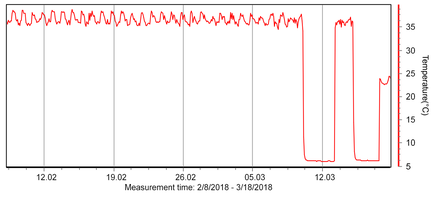
Hibernating mammals can reduce body temperature to near freezing for weeks at a time. This reduction in body temperature corresponds to physiological changes such as a change in energy source to stored fat and a significant reduction of metabolic rate. The underlying mechanism of how hypothermic hibernating mammals thrive at cold temperatures, while non-hibernators perish, remains a mystery. One main difficulty in fully understanding hibernation physiology is an incomplete understanding of how cold temperature affects processes at the cellular level. Using cultured cells from hibernating and non-hibernating species, we sought to develop a basic understanding of the cell-autonomous response to the cold. Contrary to expectation, we found that cultured cells retain viability for weeks at a hibernation-like temperature of 6 °C, and that optimum viability requires temperature-dependent adjustment of gas phase carbon dioxide to control growth medium pH. Cold exposure inhibits protein translation and cell cycle progression, and results in changes in a broad range of metabolic pathways. Rewarming allows rapid recovery and resumption of cell proliferation. Cold-exposed cells from hibernating (meadow jumping mouse) and non-hibernating (laboratory mouse) organisms exhibit similar changes in common pathways, including reductions in the metabolite pools of energetically costly pathways such as nucleotide and amino acid biosynthesis. These changes, together with the cessation of protein translation, may help to explain the greatly reduced metabolic rates observed in hibernating mammals.

1. Hibernation in the Meadow Jumping Mouse (Zapus hudsonius)

Summer Breeding *Fall Obese* *Winter Hibernating*



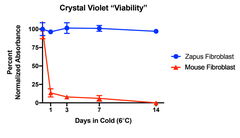
Core Body Temperature Falls to 6 ºC



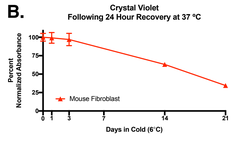
2. What Happens to Cells in the Cold?

Using cold-treated mouse fibroblasts (NIH 3T3) and meadow jumping mouse (*Zapus)* skin fibroblasts, we observe (**A**) a substrate attachment defect at 6 ºC in mouse cells that is (**B**) overcome following a 24 hour recovery at 37 ºC.

A.

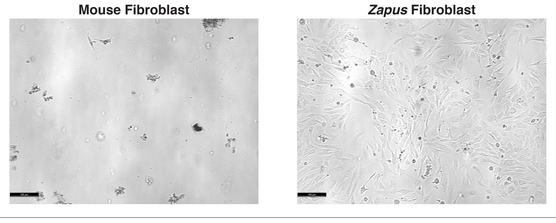


B.



C.

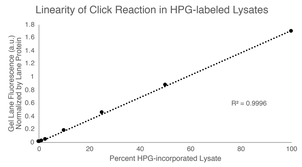
5 Weeks at 6 ºC + 2 Days 37 ºC Recovery

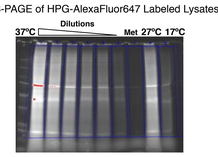


3. Protein Translation Stops at Hibernation Temperature

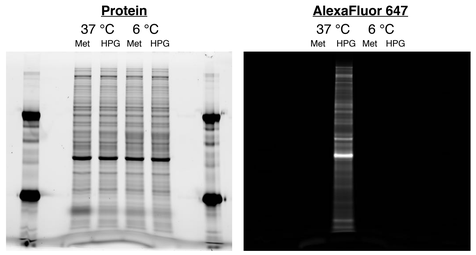
Protein translation consumes 25-30% of the total cellular energy budget. We sought to understand the temperature dependence of protein translation at hibernation-like temperatures by metabolic labeling of nascent proteins using a methionine analog, homopropargylglycine (HPG). Proteins labeled with HPG can be visualized and quantified following click chemistry addition of a fluorophore.

A. The click addition of azide-conjugated fluorophore to HPG-labeled cell lysates allows quantification of labeled protein.

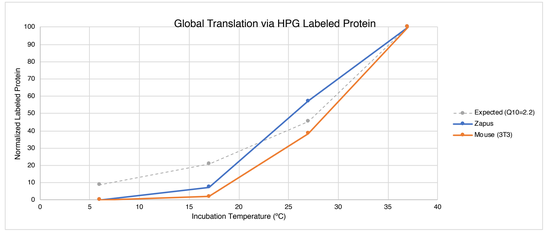




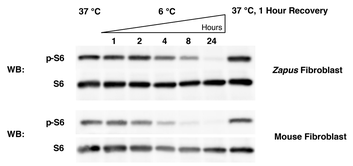
B. No protein labeling is detected when cells are incubated at 6 ºC.



C. Global translation is greatly reduced when cells are incubated at 17 ºC or lower temperatures.

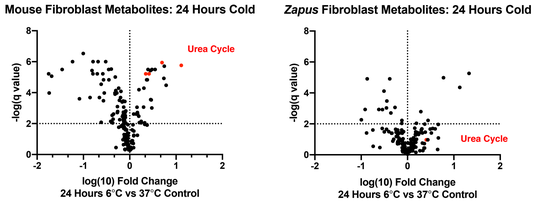


D. Inhibition of global translation by cold temperature occurs before loss of S6 phosphorylation.



4. Cold Temperature Globally Alters Metabolite Pool Sizes

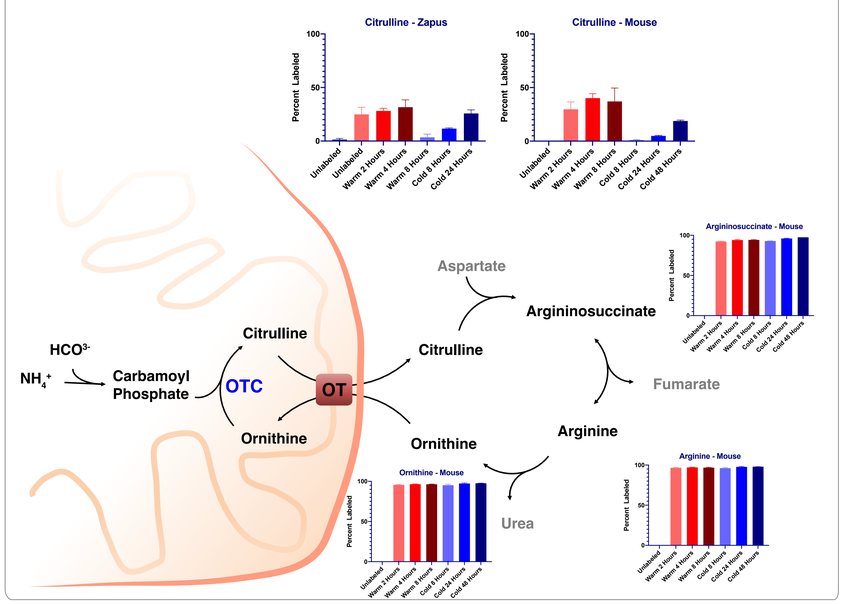
Mouse and *Zapus* fibroblasts experience changes in metabolite pool sizes follwing 24 hours in the cold (6 ºC). Affected pathways include the urea cycle, de novo pyrimidine biosynthesis, and phosphatidylcholine synthesis. The buildup of urea cycle intermediates observed in cold mouse fibroblasts mirrors the effects of Ornithine Translocase Deficiency, a heritable human metabolic disease also known as hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome.



Does cold temperature cause a defect in the urea cycle?

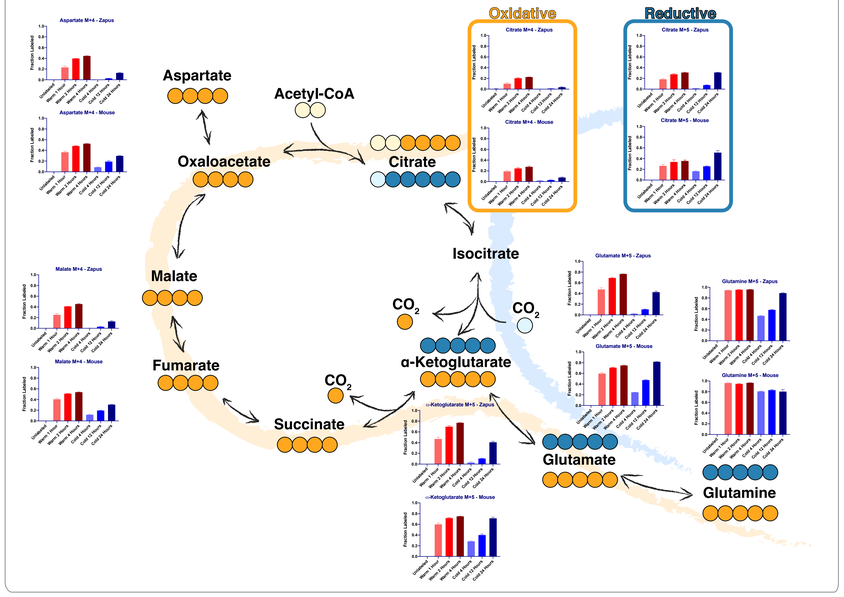
5. Urea Cycle: Reduced Ornithine-Citrulline Conversion in the Cold

Mouse and *Zapus* fibroblasts were labeled with fully-labeled ^{13}C-arginine in warm (37 ºC) or cold (6 ºC) culture conditions. Metabolites were extracted and analyzed by LC-MS. Conversion of ornithine to citrulline is reduced in the cold, consistent with impaired mitochondrial ornithine import via the ornithine translocase (OT) and/or reduced enzymatic activity of ornithine transcarbamoylase (OTC).



6. Cold Temperature Impairs Glutamine Oxidation in the TCA Cycle

Mouse and *Zapus* fibroblasts were labeled with fully-labeled ^{13}C-glutamine in warm (37 ºC) or cold (6 ºC) culture conditions. Metabolites were extracted and analyzed by GC-MS. Glutamine oxidation in the TCA cycle is impaired to a much greater extent than is reductive flux to citrate.



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