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Generally Physiological

A mitochondrial medley

This month's installment of *Generally Physiological* focuses on mitochondria, exploring circadian regulation of mitochondrial oxidative metabolism, what happens when you eliminate the mitochondrial calcium uniporter, and Ca^{2+} transport by the inner mitochondrial membrane protein Letm1.

Circadian regulation of mitochondrial bioenergetics

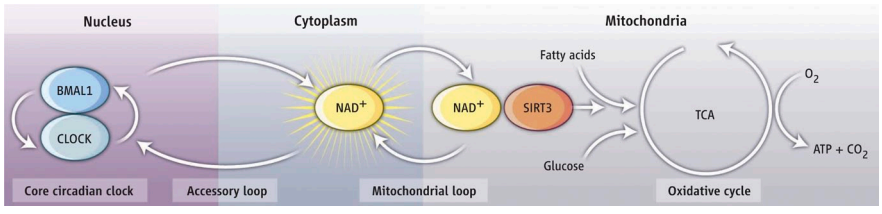
Circadian clocks synchronize physiological processes with the day/night cycle, enabling coordination of feeding/fasting and activity/rest cycles with the appropriate time of day. The coenzyme nicotinamide adenine dinucleotide (NAD^+), which plays a crucial role in metabolic redox reactions, participates in a feedback loop with the core clock machinery: NAD^+ regulates the transcription of clock genes through the NAD^+-dependent deacetylase SIRT1, while expression of the rate-limiting enzyme in NAD^+ biosynthesis is controlled by the clock (see Rey and Reddy, 2013). Noting that NAD^+ regulates the mitochondrial deacetylase SIRT3, Peek et al. (2013) explored the possibility that oscillations in NAD^+ could also provide a mechanism for the circadian regulation of mitochondrial oxidative metabolism. Liver fatty acid oxidation and NAD^+ abundance showed synchronized oscillations in fasted mice maintained in darkness, with peaks occurring toward the end of the rest cycle. Similarly, cultured mouse myoblasts showed rhythmic oscillations in NAD^+ accumulation, fatty acid oxidation, oxygen consumption, and glucose oxidation. Livers from circadian mutant mice lacking the clock gene *Bmal1* showed decreased fatty acid oxidation and total and mitochondrial NAD^+ compared with that from wild-type mice, and isolated mitochondria from the $Bmal1^{-/-}$ mice showed decreased oxygen consumption when supplied with fatty acids or pyruvate. Moreover, $Bmal1^{-/-}$ fibroblasts produced less ATP than wild-type fibroblasts under conditions favoring oxidative metabolism. Acetylation of various mitochondrial oxidative enzymes was increased in $Bmal1^{-/-}$ mice, whereas their activity was decreased, and a mitochondrial SIRT3 target showed rhythmic oscillations in acetylation—although the abundance of SIRT3 itself remained constant. Increasing NAD^+ rescued deacetylation of a SIRT3 substrate, activity of medium-chain acyl dehydrogenase (a mitochondrial enzyme that showed circadian oscillations in acetylation in wild-type mice as well as increased acetylation and decreased activity in mutants), and mitochondrial oxidative defects in circadian mutants. The authors thus conclude that circadian oscillations in NAD^+ regulate mitochondrial function through the SIRT3-dependent deacetylation of oxidative enzymes, to coordinate mitochondrial bioenergetics with daily rhythms in feeding and activity.

Circadian regulation of mitochondrial oxidative metabolism, and what happens when you eliminate the mitochondrial calcium uniporter

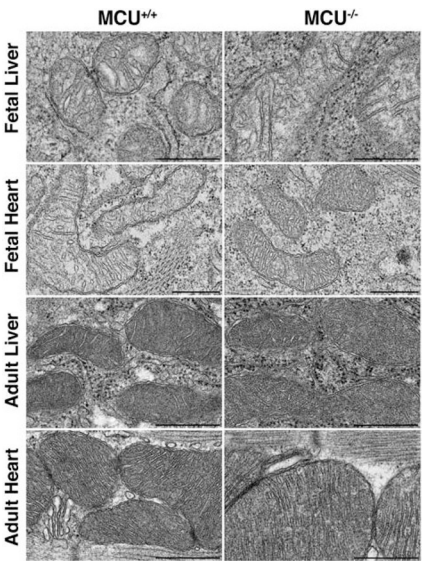
Exploring the roles of mitochondrial calcium

Energized mitochondria can rapidly sequester cytosolic calcium, which crosses the outer and inner mitochondrial membranes to enter the mitochondrial matrix, traversing the latter by way of the mitochondrial calcium uniporter (MCU)—a process driven by the mitochondrial membrane potential generated by electron transport. Matrix calcium, in turn, is thought to regulate various mitochondrial functions and—when present in excess—to contribute to cell death through a mechanism involving opening of the permeability transition pore (PTP) and the consequent collapse of the mitochondrial membrane potential. Pan et al. (2013) used a gene trap strategy to create mice lacking the MCU as a model system with which to explore MCU function and the physiological roles of matrix calcium.

Although slightly smaller than wild-type mice, $MCU^{-/-}$ mice had no other gross phenotypic abnormalities. However, calcium imaging of embryonic

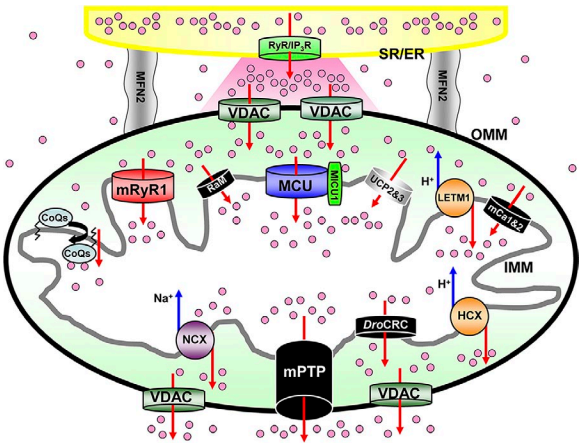


Circadian oscillations in NAD^+ abundance regulate mitochondrial oxidative metabolism by means of the deacetylase SIRT3. TCA, tricarboxylic acid cycle. (From Rey and Reddy. 2013. *Science*. 342:570–571. Reprinted with permission from AAAS.)



The abundance and morphology of mitochondria is similar in wild-type and *MCU*$^{-/-}$ mutant mice. (Reprinted by permission from Macmillan Publishers, Ltd. X. Pan, J. Liu, T. Nguyen, C. Liu, J. Sun, Y. Teng, M.M. Fergusson, I.I. Rovira, M. Allen, D.A. Springer, A.M. Aponte, M. Gucek, R.S. Balaban, E. Murphy, T. Finkel. 2013. *Nat. Cell Biol.* 15:1464, copyright 2013.)

fibroblasts, cardiac myocytes, and isolated mitochondria from wild-type or mutant mice indicated that *MCU*$^{-/-}$ mitochondria failed to rapidly accumulate calcium, and various functional tests revealed impaired skeletal muscle peak performance. Although basal oxygen consumption was comparable in wild-type and *MCU*$^{-/-}$ embryonic fibroblasts, isolated mitochondria, and mice, the robust calcium-dependent stimulation of oxygen consumption in mitochondria isolated from wild-type mice was absent in *MCU*$^{-/-}$ mitochondria. There was a marked decrease in basal matrix calcium in *MCU*$^{-/-}$ skeletal muscle mitochondria and, consistent with a role for the calcium-sensitive phosphatase in regulating pyruvate dehydrogenase (PDH) activity, PDH phosphorylation was increased in skeletal muscle of starved *MCU*$^{-/-}$ mice compared with wild-type mice, whereas PDH activity was decreased. Whereas exposure to 500 µM calcium stimulated opening of the PTP in wild-type mitochondria, it failed to do so with *MCU*$^{-/-}$ mitochondria. However, *MCU*$^{-/-}$ embryonic fibroblasts failed to show resistance to cell death in response to various stimuli (oxidative stress from hydrogen peroxide, ER stress from tunicamycin, the antineoplastic agent doxorubicin, C2-ceramide—which activates apoptotic and necrotic pathways—or thapsigargin). Similarly, *MCU*$^{-/-}$ mice showed no evidence of protection against cardiac ischemia-reperfusion injury, although, intriguingly, unlike wild-type mice, the PTP inhibitor cyclosporine A failed to protect them against cardiac damage.



Proteins involved in mitochondrial Ca^{2+} dynamics. MCU is shown in blue, the mitochondrial PTP is black, and Letm1is orange. Letm1 is depicted as mediating Ca^{2+} influx into the mitochondrial matrix; Tsai et al. (2014) argue that, under physiological conditions, Letm1would export Ca^{2+} from energized mitochondria. (From O-Uchi et al. 2012. *J. Gen. Physiol.* 139:435–443.)

Defining Letm1 function

The functions of Letm1 (leucine zipper, EF hand–containing transmembrane protein 1), another inner mitochondrial membrane protein, have been controversial. Initially implicated in mitochondrial volume regulation and proposed to play a role in K^+/H^+ exchange, Letm1 was later found to be involved in mitochondrial Ca^{2+} homeostasis and in mediating Ca^{2+}/H^+ exchange. Here, Tsai et al. (2014) reconstituted human Letm1 (purified from an *Escherichia coli* expression system) in liposomes to rigorously investigate its transport function. Whereas a flux assay using $^{86}Rb^+$ as a K^+ surrogate failed to reveal Letm1-dependent $^{86}Rb^+$ uptake, assays using either $^{45}Ca^{2+}$ or a fluorescent Ca^{2+} indicator indicated that Letm1 mediates Ca^{2+} transport. Mn^{2+} inhibited Ca^{2+} transport, with an apparent K_i of 32 µM; moreover, Letm1 could mediate Ca^{2+}-Mn^{2+} exchange. The lanthanides Gd^{3+} and La^{3+} also inhibited Ca^{2+} transport, whereas Mg^{2+} did not. Letm1-mediated Ca^{2+} transport was independent of membrane potential, indicating that it was electroneutral. Investigation of possible transport partners eliminated K^+, Na^+, and Cl^-: Ca^{2+} transport was similar in the presence of 120 mM KCl or 80 mM Na_2SO_4 and was unaffected by manipulation of the K^+, Na^{2+}, or Cl^- gradients. In contrast, acidification of the extraliposomal medium promoted rapid Ca^{2+} efflux, leading the authors to conclude that Letm1-functions as a Ca^{2+}/H^+ antiporter, with a 2:1 H^+/Ca^{2+} exchange ratio.

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