ENERGY METABOLISM

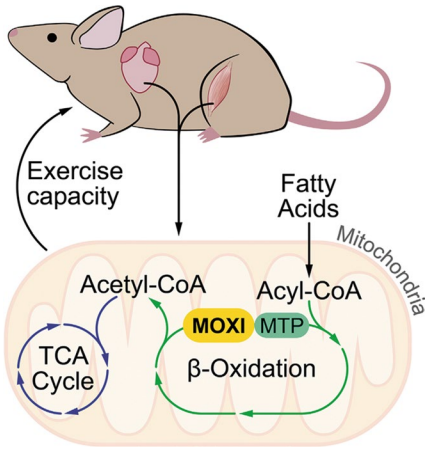
Small but important

Makarewich, C.A. et al. *Cell Rep.* **23**, 3701–3709 (2018).

A micropeptide is typically defined as a polypeptide of less than 100 amino acids and is the product of a short open reading frame (sORF). Many of these smallish proteins were not initially predicted as proteins. Rather, early passes through the relevant genomic sequences labeled them as long noncoding RNAs (lncRNAs); however, improved algorithms now recognize these lncRNAs as containing sORFs, with the cognate polypeptide validated by experimental data. Micropeptides have been identified in diverse taxa including fungi, animals, and plants. Additionally, they have been implicated in myriad of cellular processes such as: metabolic regulation, DNA maintenance, stress response, transcript decapping, apoptosis, and calcium homeostasis.

Eric Olson's lab at the University of Texas Southwestern Medical Center has been on the forefront of the field of micropeptide discovery, commented Catherine Makarewich, a lab member and first author of a new paper in *Cell Reports*, by email. In the manuscript, authors describe a new sORF product, Micropeptide regulator of β-oxidation (MOXI), and its role in mitochondrial metabolism in skeletal muscle and heart tissue.

Using *in silico* screening to look at muscle-enriched transcripts from internal and published RNA-seq datasets, investigators initially identified the sORF as coding for a 56 amino acid polypeptide that is highly conserved across multiple vertebrate classes. Furthermore, sequence analysis predicted a single transmembrane domain with a basic residue rich carboxyl terminus. *In vitro* transcription and translation assays confirmed generation of an appropriately sized polypeptide. Investigators validated the microprotein's *in vivo* presence in mice via Western blot of heart and skeletal muscle tissues as well as proteomic analysis of quadriceps samples. Authors then used immunohistochemistry and proteolysis susceptibility of mitochondrial membrane preparations in order to classify MOXI as having inner mitochondrial membrane (IMM) localization. Immunoprecipitation of a transgenic (TG) epitope-tagged MOXI, which was overexpressing in striated muscle, showed an association with Mitochondrial Trifunctional Protein (MTP), the enzymatic complex responsible for long-chain fatty acid β-oxidation that is also present at the IMM, in several different muscle tissues.



Physiology of MOXI in striated muscle of mice. Credit: Adapted from *Cell Rep.* **23**, 3701-3709 (2018)

In order to characterize MOXI's function, authors generated knockout (KO) mice as a compliment to overexpressing TG animals. KO and TG lines were then compared to wild type (WT) through a series of structural, biochemical, and physiological assays in order to characterize the novel micropeptide. To begin, heart function appeared normal in TG and KO mice relative to WT when assessed by echocardiography. And when whole body composition was compared via EchoMRI, the investigators saw no differences between the modified lines and WT.

Differences became apparent when authors took a closer look for structural differences. A pronounced myopathic phenotype was evident in histological preparations from TG animals in addition to abnormalities in mitochondrial structure seen by electron microscopy (EM) in both quadriceps and heart tissue. While no histological differences were apparent in KO animals, EM data of KO mice mitochondria revealed abnormalities in mitochondrial shape, appearing abnormally large with damaged christae.

Next, investigators assessed biochemical function by looking at substrate consumption preferences. Isolated mitochondria sourced from heart and skeletal muscle of KO mice presented a diminished capacity for palmitate consumption as compared to WT; alternatively, TG mice had enhanced fatty acid consumption. When investigators compared carbon source use in perfused hearts from WT and KO mice using isotopically-labeled substrates and nuclear magnetic resonance, KO preparations showed a reduction for long-chain fatty acid oxidation with a compensatory increase in carbohydrate consumption. This shift in preference for carbohydrates in KO hearts was very interesting according to Makarewich, given that the heart prefers fatty acid substrates.

Authors also looked at whole animal physiology by assessing performance of animals on a forced treadmill run. KO mice fatigued after going for only half the distance that WT animals went.

In total, the results point towards a role for MOXI in utilization of fatty acids, which are an important source of energy in skeletal muscle and heart tissues, as demonstrated by its ability to increase fatty acid oxidation in these tissues. Many of the experimental results are reminiscent of fatty acid oxidation disorder symptoms, including muscle myopathy and an inability to maintain extended aerobic exercise. Olson and colleagues speculate that MOXI may represent a possible regulatory mechanism for coordinating metabolism. More work is necessary to identify the details and players involved in this potentially novel layer of control.

The group is pursuing their work further. Makarewich stated, "Our initial follow up studies will be geared towards understanding the exact mechanism by which MOXI exerts its effect on the MTP. Central to this will be to determine exactly which subunit of the MTP MOXI binds to and also to identify the exact enzymatic reaction that MOXI enhances." More long term goals she added are to look at how MOXI expression and activity are affected in different disorders associated with fatty acid metabolism and to over express MOXI in order to increase β-oxidation in these pathological conditions where it could be therapeutic.

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