defects, which the authors correlated with H3K4me3 levels and levels of SET-2, a core component of the COMPASS complex that controls H3K4me3 (Demoinet et al., 2017). The authors do not know whether regulation of SET-2 by AMPK is direct, and whether that regulation is conserved across evolution remains to be seen.

Paralleling this work are recent reports that AMPK can control histone 3 lysine 27 methylation via direct phosphorylation of EZH2, the catalytic subunit of the PRC2 complex, which helps maintain stem cell pluripotency in mammals (Tang et al., 2018; Wan et al., 2018). As with the DNMT1 connection, the data supporting EZH2 as a direct substrate of AMPK *in vivo* need further study, but they raise another facet of epigenetic control that AMPK may engage. Whether AMPK in mammals coordinates histone 3 lysine 4 methylation and histone 3 lysine 27 methylation remains to be examined.

AMPK generally functions as a biological switch that responds to changes in energy state to maintain metabolic homeostasis (Garcia and Shaw, 2017). The finding that AMPK can directly regulate the epigenome suggests that it may function not only to restore energy balance in the present, but also to change the way cells respond to their environment in the future. AMPK regulation of DNA methylation provides a very conceptual example of how starvation could control gene expression to provide a "metabolic memory" to an organism or cell population.

The AMPK-TET2-5hmC pathway described in this study illustrates the need for more research into the role AMPK and other metabolic regulators play in altering the epigenome, as a better understanding of these interactions may allow us to leverage them for improved prevention and treatment of cancer and metabolic disease.

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Shipping Calpastatin to the Rescue: Prevention of Neuromuscular Degeneration through Mitofusin 2

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How neuromuscular junctions (NMJs) are lost in disease and aging is unclear. Recently in *Cell Metabolism*, Wang et al. (2018) discovered that endoplasmic reticulum-mitochondria tethering by Mitofusin 2 is required to organize a cleft between these two organelles, which, like a lorry, traffics down the axon to distribute calpastatin to terminals where it blocks NMJ degradation.

Loss of neuromuscular synaptic junctions (NMJs) is a common hallmark of neuromuscular disorders and aging, ultimately leading to skeletal muscle atrophy. The exact mechanisms leading to a decreased communication between motor neurons and muscle at NMJs are, however, unknown, limiting the possibility of targeted treatments. Recently in *Cell Metabolism*, Wang et al. highlighted a new function of mitochondria-associated membranes (MAMs) and their resident protein Mitofusin 2 (Mfn2) as mediators of axonal protein transport (Wang et al., 2018).

An association between Mfn2 and neuromuscular pathologies had been noted in the past: Mfn2 is decreased in

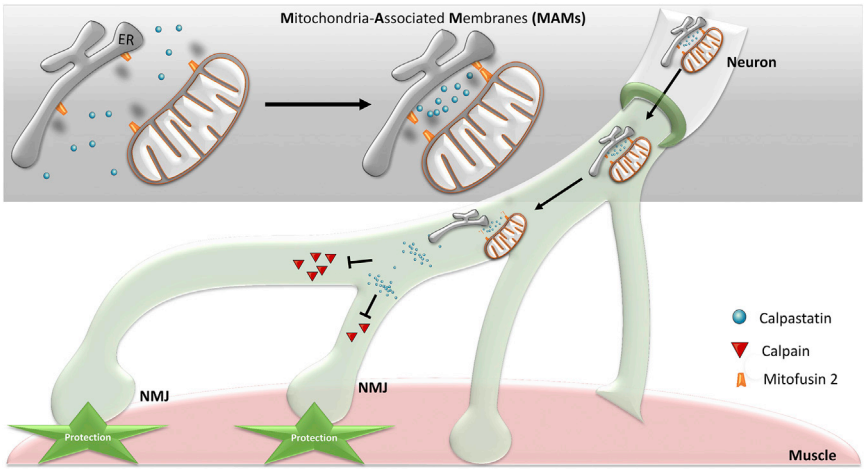

Figure 1. Mitofusin 2-Dependent ER-Mitochondria Tethering Ships Calpastatin to NMJs
The cartoon depicts the main finding of Wang and colleagues. Mfn2 allows co-delivery of calpastatin localized at the interface between ER and mitochondria to NMJ.

amyotrophic lateral sclerosis (ALS) and aging, and mutated in a form of inherited peripheral neuropathy called Charcot-Marie-Tooth type 2A (CMT2A). The genetic link between CMT2A and Mfn2 drove the hypothesis that changes in Mfn2 function can potentially associate with the onset and progression of neuromuscular degeneration. However, the idea of a possible role of Mfn2 in the maintenance of NMJ had never been explored until Wang and colleagues selectively ablated Mfn2 in spinal cord motor neurons in mice. This deletion led to the onset of motor and muscular symptoms, due to NMJ denervation and muscle atrophy. These neuromuscular abnormalities were detected in motor neurons and muscle of zebrafish embryos with reduced/mutated levels of Mfn2 where neuromuscular junctions were also reduced, to the level that they were basically absent at axonal terminals (Vettori et al., 2011). Since these features are common hallmarks of ALS, the authors investigated whether Mfn2 levels were reduced in spinal cords samples of transgenic $SOD1^{G93A}$ mice (a commonly used ALS mouse model) and ALS patients. Not only was this the case, but the authors were also able to delay skeletal muscle atrophy by partial preservation of NMJs throughout the lifespan of the mice. Reinforcing these data, they also showed that Mfn2 expression was reduced during aging, in parallel with NMJ degeneration, and that Mfn2 upregulation in aged mice could alleviate the phenotype. The authors wondered what the specific Mfn2 function mediating NMJ protection was. To address this question, they turned their attention to the key NMJ player calpastatin, previously shown to regulate NMJ function and axon survival (Spencer and Mellgren, 2002). Mfn2 loss hampered calpastatin distribution to sciatic nerves, leading to its accumulation in the spinal cord. Because degradation of axonal proteins such as βIII-tubulin and NF-L was unchanged in Mfn2-deficient mice, the authors hypothesized that a specific Mfn2-calpastatin signaling checkpoint controlled NMJ function and stability. The surprise came when they addressed the molecular mechanism by which this function was exerted: Mfn2 inhibits localized protein degradation at the NMJ because it allowed mitochondrial co-transport of calpastatin. However, the co-transport did not depend on direct association between the two, or on the profusion activity of Mfn2, but on its ability to form ER-mitochondria tethers (de Brito and Scorrano, 2008), as demonstrated by elegant genetic experiments and further corroborated by the fast calpastatin axonal transport observed in mice expressing an inducible synthetic ER-mitochondria linker (Figure 1). It is known that axonal mitochondrial transport depends on Mfn2 (Misko et al., 2010), but the work by Wang and colleagues introduces the new concept that via Mfn2, mitochondria can deliver MAMs and MAM-residing proteins like calpastatin to axonal terminals. Whether this depends on the co-transport of ER with mitochondria, or on the formation of specific contact sites at the NMJ, as might be suggested by the finding that the axonal ER is a continuous ramification of the somatic ER (Wu et al., 2017), remains unclear.

Calpastatin upregulation is protective in models of Parkinson's disease (PD) (Diepenbroek et al., 2014; Yang et al., 2013). This work places Mfn2 in the picture: by organizing the mitochondria-ER physical contact area, it can allow calpastatin delivery along the axon. It would be interesting to explore if upregulation of Mfn2, perhaps achieved pharmacologically (Miret-Casals et al., 2018), can similarly protect against PD. For certain, Mfn2 is a main target of Parkin in familial PD forms (Ziviani et al., 2010).

Like all important works, the Wang et al. paper not only extends our knowledge of the function of Mfn2, but it also opens several new exciting questions: is the "lorry" function of MAMs restricted only to calpastatin, or can this distribution mechanism be generalized to other proteins essential at the axonal end? Is this mechanism typical only of mitochondria-ER contacts, or is it a new function common to other inter-organelle contact sites? How does calpastatin dissociate from MAMs at axonal terminals? Which are the proteins that retain it at MAMs, since it does not interact directly with Mfn2? Is this process dependent on Ca^{2+} signaling?

These exciting results not only pave the way for future research, but also extend the function of Mfn2-dependent MAM formation to the maintenance of distinct neural districts.

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Fatty Acid Oxidation in Macrophages and T Cells: Time for Reassessment?

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Previous immunometabolism research using the CPT1 inhibitor etomoxir suggests that long-chain fatty acid oxidation (LC-FAO) supports IL-4-driven alternative macrophage activation (M(IL-4)) and regulates memory and regulatory T cell formation. Divakaruni et al. (2018) and Raud et al. (2018) now report that LC-FAO is largely dispensable for these processes.

Metabolic rewiring is a key hallmark of immune cell activation. Inflammatory macrophages, effector T (Teff) cells, and activated dendritic cells require increased aerobic glycolysis to support their phenotype and to fulfill their functions. Conversely, anti-inflammatory M(IL-4) macrophages, memory T (Tmem) cells, and regulatory T (Treg) cells are characterized by increased mitochondrial oxidative phosphorylation (OXPHOS), which is thought to energetically support their long-term survival and functions (O'Neill et al., 2016).

The reported increased long-chain fatty acid oxidation (LC-FAO) in those cells can certainly aid OXPHOS, but studies interrogating the functional importance of LC-FAO to support M(IL-4) phenotypes have yielded conflicting results (Van den Bossche et al., 2017). The need for increased LC-FAO in M(IL-4) macrophages was first suggested by Vats et al. (2006) in a study using 50 μM etomoxir, an inhibitor of carnitine palmitoyl transferase 1 (CPT1). This mitochondrial membrane enzyme, together with CPT2, facilitates the transport of LC-FAs into the mitochondrial matrix for subsequent oxidation (Figure 1). Follow-up studies with etomoxir concentrations ranging from 10 to 100 μM observed no effect on IL-4-induced activation of mouse and human macrophages, and thus the field awaited genetic models to resolve the debate surrounding whether LC-FAO is obligatory for alternative macrophage activation or merely associated with it (Van den Bossche et al., 2017). Nomura et al. (2016) took the first step and applied CPT2-deficient macrophages to demonstrate that M(IL-4) activation does not require LC-FAO. While this suggested off-target effects of etomoxir, these results could not rule out that CPT1 may have functions independent of LC-FAO. Therefore, experiments with CPT1-deficient macrophages were still needed to unequivocally clarify its role in M(IL-4) cells.

Employing distinct genetic and pharmacological approaches, Divakaruni et al. now demonstrate that macrophages still acquire their IL-4-elicited phenotype in the absence of CPT1a (considered the main isoform in leukocytes) or CPT2, and highlight that the increased LC-FAO in M(IL-4) cells is less crucial than previously considered. They also observe that a high concentration of etomoxir