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# Brains and brawn: Stress-induced myokine abates nervous system aging

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**Skeletal muscle secretes numerous systemic factors, termed myokines, which can regulate homeostasis of distal tissues. In this issue, Rai et al. (2021) identify and characterize a novel myokine, Amyrel, which is secreted under muscle proteasome stress and protects central nervous system health and function by enhancing protein quality control during aging.**

During aging, many organisms face declines in cellular homeostasis across tissues. In particular, disruptions to proteostasis, the ability for cells to manage unneeded or misfolded proteins, play a causal role in age-related diseases like neurodegeneration (Douglas and Dillin, 2010; Schmidt and Finley, 2014). Interestingly, a growing body of evidence suggests that cellular stress in a single tissue can be communicated systemically, so that multiple tissues can coordinate and respond to homeostatic perturbations.

An often-overlooked tissue that modulates systemic physiological and metabolic homeostasis is skeletal muscle. Besides its obvious role in physical movements, skeletal muscle is a major endocrine organ that secretes many cytokines and growth factors. These muscle-derived factors, termed myokines, have been causally linked to adaptive changes across multiple tissues, including enhanced neurogenesis in the central nervous system (CNS) and improved cognitive function (Pedersen, 2019). Until recently, however, the relationship between cellular stress in muscles, myokine release, and nervous system health during aging has remained poorly understood. In their recent work, Rai and colleagues delineate the mechanism by which proteasome stress in skeletal muscles causes the release of an amylase myokine called Amyrel, which ultimately drives metabolic changes and enhanced proteostatic function in the CNS during aging (Rai et al., 2021).

The ubiquitin-proteasome system is responsible for degradation and turnover of the majority of intracellular proteins, and is thus critical in maintaining cellular proteostasis (Kitajima et al., 2020). Misfolded and unneeded proteins are poly-ubiquitinated and targeted to proteasomes for degradation. Similar to other proteostasis pathways, proteasome function has been shown to decline with age in a number of tissues, including skeletal muscle (Fernando et al., 2019). To understand how proteasome stress in skeletal muscle affects tissues both locally and systemically, Rai et al. developed a model of muscle proteasome stress in *Drosophila melanogaster* by knocking down proteasome subunits via RNA interference (RNAi). RNA sequencing (RNA-seq) of muscle tissue with these proteasome disruptions revealed local compensatory transcriptional upregulation of chaperones, proteases, and peptidases. More interestingly, however, was their finding that muscle-specific proteasome stress did not alter the amount of insoluble ubiquitinated proteins that accumulates with age in muscles, but did lead to a significant decrease of such aggregates in CNS tissues.

Next, to understand how proteasome-stressed muscle signals to the CNS to promote protein degradation, Rai et al. tested several myokines regulated by proteasome stress, uncovered through RNA-seq analysis. They found that the amylase Amyrel was consistently upregulated during proteasome disruption, and that genetic overexpression of Amyrel in thoracic muscle tissue was sufficient to reduce insoluble proteasome substrates, as well

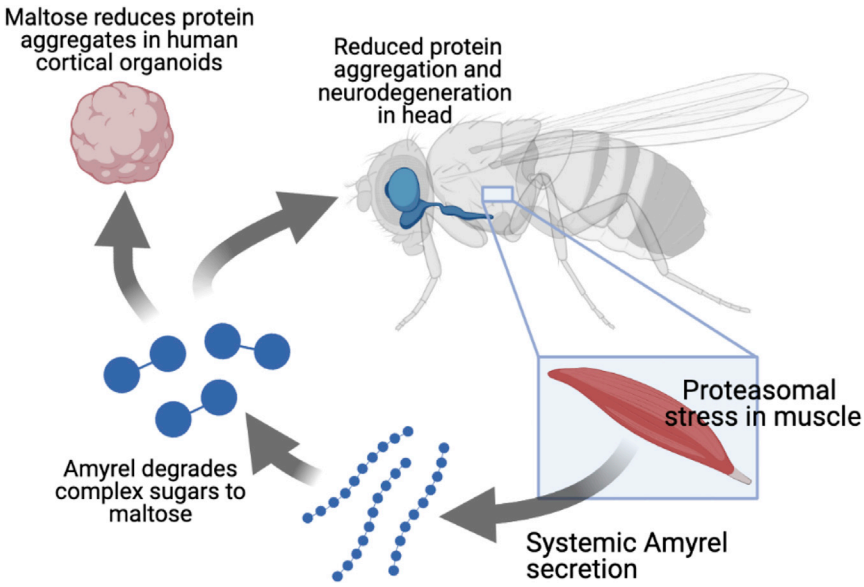


**Figure 1. Under proteasome stress, the myokine Amyrel elevates systemic maltose levels, which protect the CNS against age and stress-associated protein aggregation**

RNAi to proteasome subunits in thoracic muscle drives systemic secretion of an amylase myokine called Amyrel. Amyrel degrades polysaccharides into maltose, which is transported into central nervous system tissues by a sugar solute carrier protein (Slc45). In these tissues, maltose induces expression of chaperones and proteases, which abate the age-associated accumulation of insoluble proteins, and protects against Tau-induced neurodegeneration in *Drosophila melanogaster*. Finally, Amyrel and maltose reduce heat-shock-induced aggregation of proteins in human cell culture and cortical organoid models, and preserve neuronal function in cortical organoids.

as aggregates of the pathogenic mutant Huntingtin protein, in the CNS (Figure 1). Fascinatingly, Rai et al. also show that muscle-specific Amyrel overexpression protects retinal tissue from age-associated neurodegeneration in flies expressing pathogenic human Tau protein.

To determine how Amyrel functions to confer its benefits on the aging CNS, Rai et al. conducted RNA-seq on CNS tissue from muscle-specific Amyrel over-expressors versus control flies, and found an enrichment in chaperone genes, which are important in responding to misfolded and aggregated proteins. Notably, they found that knocking down these chaperone genes abated the neuroprotective effects of Amyrel overexpression, suggesting that Amyrel's protective effects likely act through this chaperone network.

In flies, Amyrel is a freely circulating amylase that breaks down complex sugars into the disaccharide maltose, so the authors posited that its enzymatic activity should be of importance for its function in the CNS. Indeed, they found that increased Amyrel levels led to increases in maltose levels in the body and head, and that addition of maltose on cultured fly cells led to a decrease in heat-shock-induced poly-ubiquitinated proteins. Further, they found that Amyrel's protective effect against aging, as well as the expression of chaperones in CNS tissue, depends upon expression of a maltose transport protein (Slc45).

As maltose is readily found in humans and other mammals, the authors further tested whether maltose supplement can promote proteostasis in cultured human cells and organoids. Remarkably, they found that human cortical organoids treated with maltose exhibit reduced insoluble protein aggregates following heat-shock, and improved neuronal function, as measured by neuronal action potentials, in a dose-dependent manner. These results suggest that maltose-mediated proteostasis might be an evolutionarily conserved mechanism in preserving CNS function.

Interestingly, while amylases are generally thought to be restricted to the digestive system, they are found to be enriched in human plasma with age (Lehallier et al., 2019) and secreted by mouse skeletal muscles (Deshmukh et al., 2015). Furthermore, this study found that amylase can be induced with proteasome stress in human cultured cells and brain cortical organoids, suggesting that amylase may act as a stress-induced signaling molecule for regulating systemic proteostasis. An obvious and exciting question is how amylase might be upregulated *in vivo*. Human and animal studies have shown that physical exercise can protect and restore brain function through a number of myokines (Pedersen, 2019). Are amylases also upregulated under exercise? One caveat is that previous studies in healthy mice show that exercise upregulates skeletal proteasome function instead of showing signatures of proteasome stress (VerPlank et al., 2019). However, given that proteasome function declines with age, it is possible that exercise might induce proteasome stress, and thus amylase secretion, in older animals.

Finally, more studies need to be dedicated to uncovering how maltose modulates distal cellular proteostasis. Rai et al. found that maltose promotes proteostasis in cell culture models, likely through its chemical-chaperone activity and/or inducing expression of chaperone and proteases. However, it is unclear whether the concentration of maltose used in these *in vitro* studies is physiologically relevant. Although maltose can be found in human serum (Darst et al., 2019), it is unclear how long it stays in circulation and whether it will accumulate at concentrations high enough to affect proteostasis in the CNS and other tissues. Future studies on how aging, exercise, and even diet can alter maltose levels will reveal how amylase and maltose regulate organismal proteostasis and health, and can potentially provide new therapeutic strategies for tackling neurological decline with age.

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## DECLARATION OF INTERESTS

The authors declare no competing interests.

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# Mitochondrial quality control: Just walk away

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**The repair and removal of damaged mitochondria is essential for sustaining cellular and tissue homeostasis. Now in *Cell*, Jiao et al. (2021) describe a novel mechanism of such quality control in which damaged mitochondria move to the plasma membrane where they are "packaged" and left behind the trailing edge of migrating cells.**

Damaged mitochondria are removed by a process of selective autophagy called mitophagy (Moehlman and Youle, 2020) or repaired by the extrusion of mitochondria-derived vesicles (MDVs) containing damaged or toxic mitochondrial proteins (Sugiura et al., 2014). Such quality control is important in sustaining the cell and its progeny, as well as in preventing the consequences of defective mitochondria, such as the production of reactive oxygen species (ROS). Now, a third mechanism of mitochondrial quality control has been discovered in migrating cells (Jiao et al., 2021). Defective mitochondria move to the plasma membrane at the trailing edge, where they move into small vesicular structures that form in the retraction fibers. Like breadcrumbs left by wandering siblings, these structures are deposited behind the migration path of the cell, in a process the authors call "mitocytosis."

Vesicular structures deposited during migration are generally known as "migrasomes" (Ma et al., 2015). Their formation is driven by the tetraspanin family of proteins, which can also be used to visualize these structures. Migrasomes in developing zebrafish embryos are enriched for morphogens and chemokines, and are important for normal organogenesis (Jiang et al., 2019), as they are perhaps required to create developmental gradients for proper chemotaxis.

In the context of mitocytosis, Jiao et al. (2021) present compelling evidence that defective mitochondria are placed into migrasomes as a mechanism for their disposal. In one particularly elegant experiment, they utilized cells that are heteroplasmic for mitochondria with mutant mitochondrial DNA and found that the migrasomes were enriched for this mutation. The authors observed mitocytosis in a variety of cell lines and in bone marrow-derived macrophages (miscalled as monocyte-derived), as well as in neutrophils monitored *in vivo*. Strikingly, macrophages and neutrophils defective in mitocytosis due to ablation of tetraspanin-9 accumulated defective mitochondria, as measured by a loss of mitochondrial transmembrane potential ($\Delta\Psi m$).

To induce mitocytosis, the authors employed several strategies to mimic mitochondrial damage. These included mild reduction of $\Delta\Psi m$ with the uncoupler, CCCP, inhibition of the $F_0F_1$ ATPase with oligomycin, treatment with the iron chelator deferiprone, or with the complex III inhibitor antimycin. It is difficult to identify a mitochondrial feature that is common to all of these treatments and might represent a signal to identify a defective mitochondrion. While the authors suggest that ROS production might be such a signal, and indeed, they show that the sequestered mitochondria in antimycin-treated cells stain with a probe for ROS, these reactive species are not generated by CCCP, oligomycin, or deferiprone. CCCP and antimycin cause a reduction in $\Delta\Psi m$. Oligomycin also causes such loss of $\Delta\Psi m$ in mitochondria that are defective for electron transport, which would be in keeping with this being a representative signal for mitocytosis—as it is for mitophagy and MDV