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ECM degradation as a novel trigger for muscle atrophy mediated by mitochondrial dysfunction in C. elegans

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博士論文（要約）

ECM degradation as a novel trigger for

muscle atrophy mediated by mitochondrial dysfunction

in *C. elegans*

（線虫のミトコンドリア障害に伴う筋萎縮の主要因となる

細胞外マトリックスの分解）

平成 30 年度

東北大学大学院生命科学研究科

生態システム生命科学専攻

Surabhi Sudevan

Background: Muscle atrophy is the loss of muscle mass or activity and is a common phenomenon in aging, long duration bed rest, neuromuscular diseases as well as in spaceflight. Whilst several muscle degradation pathways such as the Ubiquitin-proteasome pathway, autophagy and calpain mediated pathway have been identified, no cure has been found yet. Mitochondrial dysfunction has been connected with muscle atrophy in several studies, including aging, diabetes, Duchenne muscular dystrophy (DMD) and spaceflight however the exact molecular pathway connecting the two is still unclear. Most of the muscle degradation pathways are activated as a result in intracellular cues but recent evidence about the importance of the integrin attachment complex for maintaining muscle cell integrity in *C. elegans* implicates the importance of the extracellular matrix (ECM) in muscle cell. Since there is no evidence for a clear role of ECM in muscle atrophy, this study explores the direct role of mitochondrial dysfunction as an intramuscular signal for muscle dystrophy through ECM degradation.
Objectives: In our quest for finding novel pathway of muscle atrophy we laid out our objectives as follows:

- To understand how mitochondrial dysfunction can lead to muscle atrophy
- To find if the ECM is one of the key factors connecting mitochondrial dysfunction with muscle atrophy

Results: I found that treatment of wild-type *C. elegans* with Antimycin A (an Electron Transport Chain inhibitor) leads to paralysis as well as muscle damage. The most common kind of damage observed was that of wavy myofilaments which were sometimes accompanied with breakage in myofilament structure (Figure 1). Surprisingly, the wavy myofilament phenotype was closely associated with EMB-9 (Collagen IV in *C. elegans*) degradation in Antimycin A treated wild-type worms. Similar wavy myofilaments were observed when the EMB-9 TS mutant (g34) was kept under restrictive temperature. On the other hand, overexpression of EMB-9 could suppress the motility defect as well as muscle damage. EMB-9 degradation is mediated by the matrix metalloproteinases (MMPs). MMP on the other hand, are activated by Furin. Furin is a calcium dependent proprotein convertase which undergoes structural modification and activation upon binding with calcium ion. I found that treatment with a Furin inhibitor or a MMP inhibitor could also rescue the muscle damage mediated by Antimycin A. I also observed an increase in muscle cytoplasmic Ca2+ concentration upon Antimycin A treatment, monitored through GCaMP3.35 imaging system. To confirm if Ca2+ overload is responsible for Antimycin A mediated muscle damage, I analyzed the effect of Antimycin

A on two calcium channel mutant strains. I found that both *unc-68* and *egl-19* mutant animals could suppress the muscle damage observed upon Antimycin A treatment. Duchenne muscular dystrophy (DMD) is a genetic disorder caused by mutation in dystrophin gene. Mitochondrial dysfunction and dysregulated calcium pathway have been implicated as possible causes of the disease. I found that unc-68 RNAi could suppress muscle damage in the *dys-1* (DMD model of *C. elegans)* mutant worms, as shown by some researchers previously. I also found that MMP inhibitor and Furin inhibitor could significantly reduce the muscle damage in the *dys-1* mutant worms as well. These results show that the MMP>Furin>ECM degradation axis is not only responsible for muscle damage in the Antimycin A model, but is also one of the key pathways activated in DMD.

Conclusion: This leads us to conclude that, in the case of mitochondrial dysfunction, as a first step there is an upregulation of intracellular calcium. The calcium overload activated the MMP>Furin>ECM axis, which leads to muscle atrophy (Figure 2). Preventing collagen degradation by inhibition of *unc-68,* MMP and Furin can delay muscle damage and keep the muscle structure intact for a longer time. Hence, we have established that ECM stability is crucial to prevent and delay muscle damage, and mitochondrial dysfunction is a key intramuscular signal of ECM degradation, which serves as a novel and important pathway of muscle atrophy.

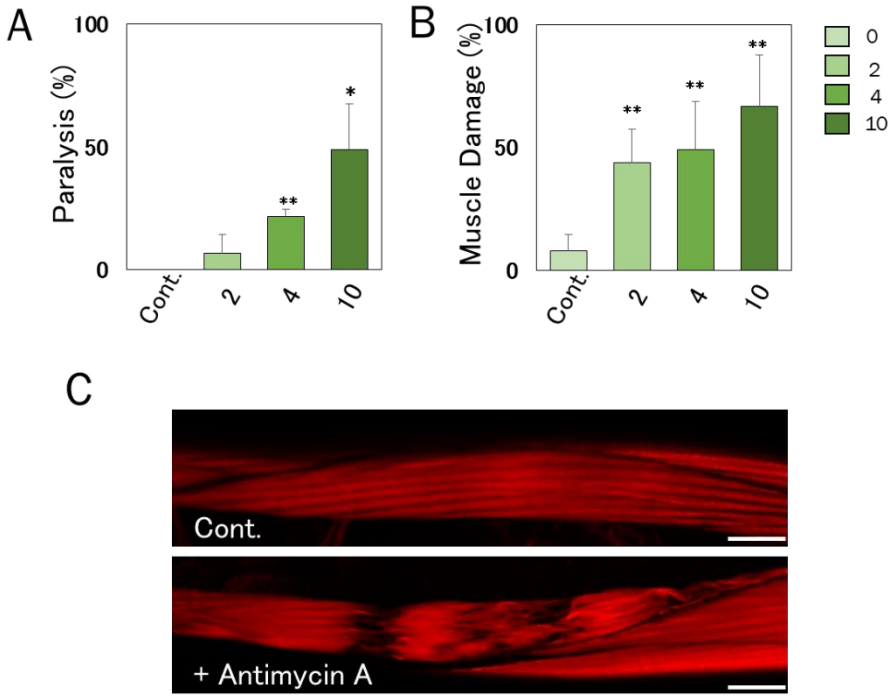

Figure 1: Mitochondrial dysfunction mediated by Antimycin A results in paralysis and muscle cell damage in *C. elegans*. Age synchronized wild-type animals were grown to young adulthood at 20°C. Adult animals were then treated with 0, 2, 4 and 10 µM Antimycin A for 36 h, after which: **(A)** the animals were analyzed with a platinum wire pick for movement defects/paralysis. Values indicate the percentage of animals paralyzed in each group. 20 animals were analyzed for each experiment with three independent repetitions (n = 60 animals). A significant increase in paralysis was observed in 4 µM ($p<0.01$) and 10 µM ($p<0.05$) concentrations. Number of worms from left to right: 59, 60, 60, and 59. **(B)** Muscle structure was visualized using Rhodamine Phalloidin after 36 h treatment with Antimycin A. Values indicate the average percentage of damaged muscle per animal. A significant increase in damaged muscle cells was observed in 2 µM ($p<0.01$), 4 µM ($p<0.01$) and 10 µM ($p<0.01$) Antimycin A. Number of muscle cells from left to right: 63, 54, 74, and 85. **(C)** Representative images of myofilament alignment in normal and Antimycin A-damaged muscle cells. The damaged muscle displays wavy myofilaments and large gaps in between, compared to normal muscle cells. Scale bar: 10 µM.

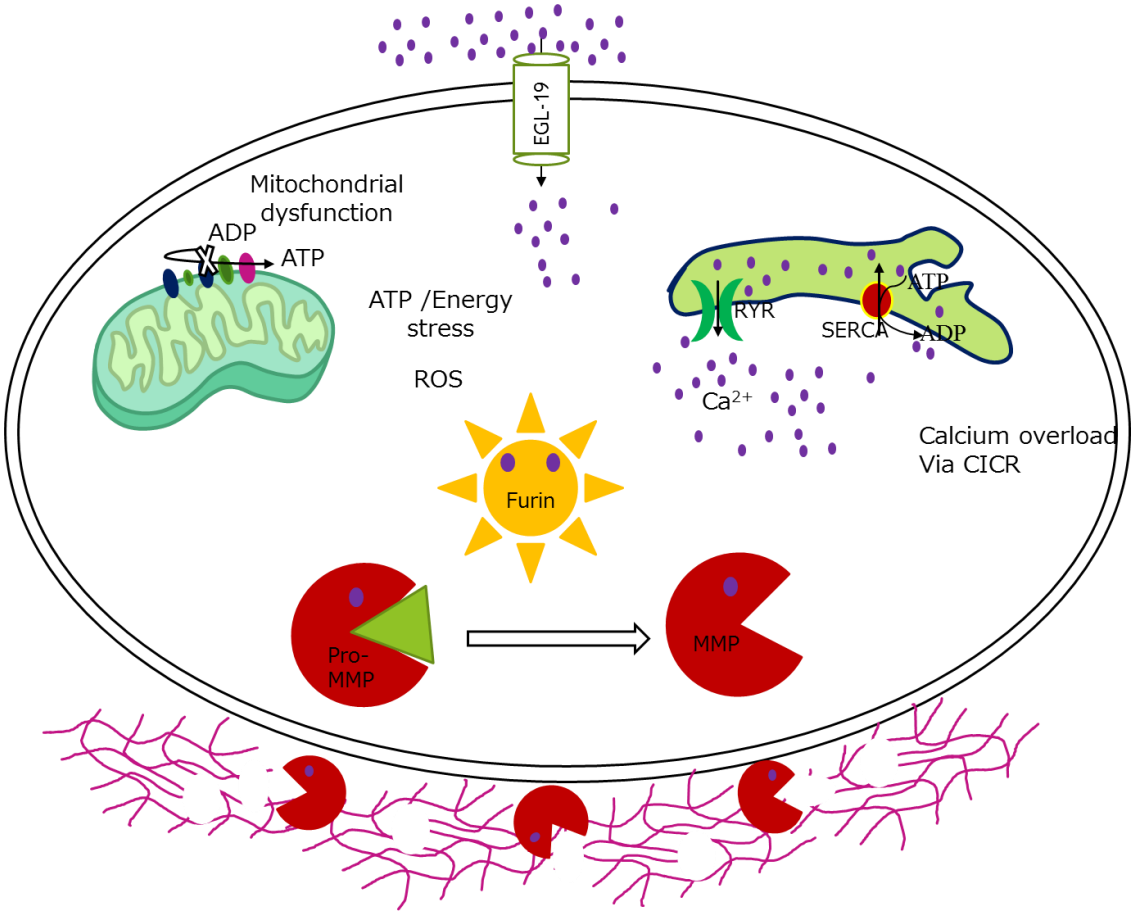

Figure 2: Collagen is essential for regulation of muscle cell activity and structure.

In the case of mitochondrial dysfunction, as a first step there is an upregulation of intracellular calcium (possibly due to SERCA loss of function) which leads to furin activation. Furin then further activates MMPs which degrade collagen, an important ECM component. This then leads to muscle dystrophy. Preventing collagen degradation by inhibition of RYR, MMP and Furin can delay muscle damage and keep the muscle structure intact for a longer time.