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

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| 論文題目 | ECM degradation as a novel trigger for muscle atrophy mediated by mitochondrial dysfunction in *C. elegans*<br>（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） |
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論文内容の要旨

**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.

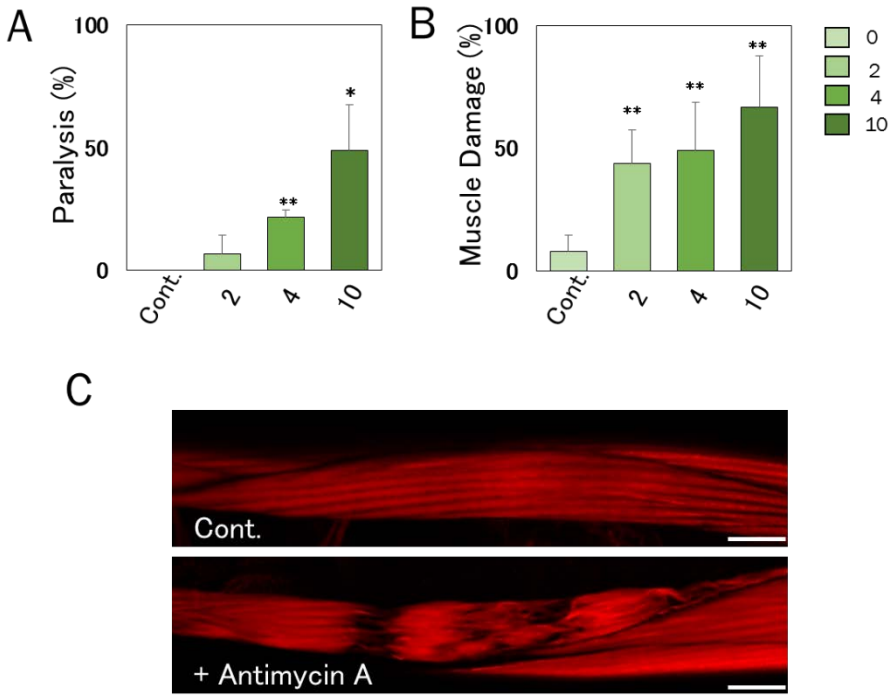


**Figure 1: Mitochondrial dysfunction mediated by Antimycin A results in paralysis and muscle cell damage in *C. elegans*.** Age synchronized wild-type animals were grown to young adulthood at 20°C. Adult animals were then treated with 0, 2, 4 and 10 µM Antimycin A for 36 h, after which: **(A)** the 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.

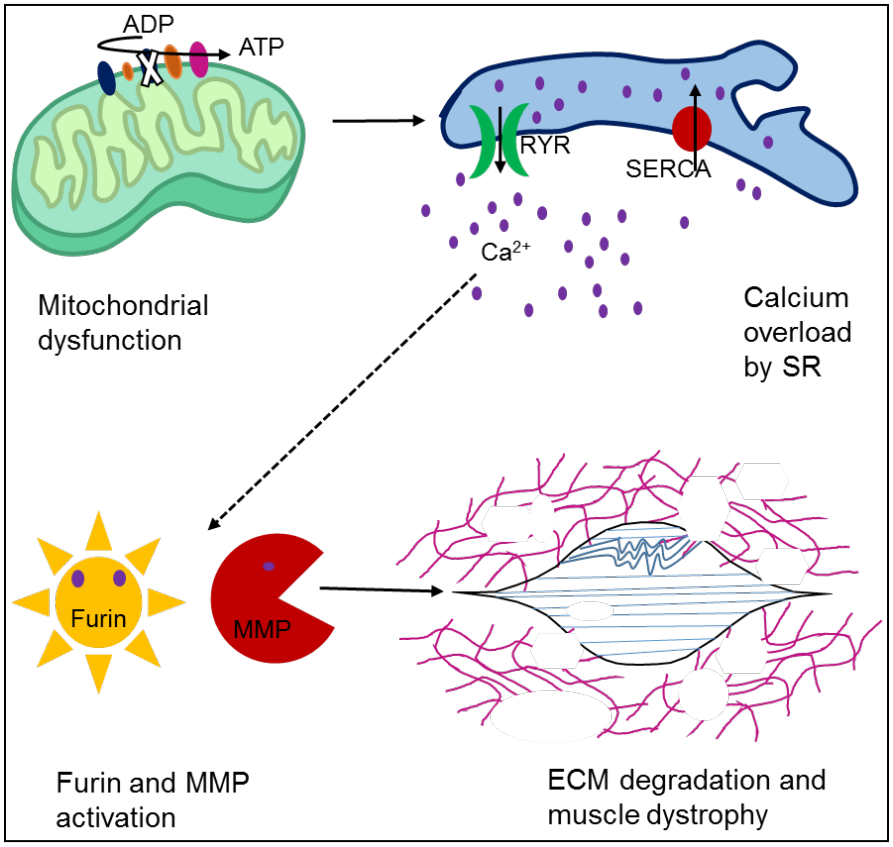


**Figure 2: Collagen is essential for regulation of muscle cell activity and structure.**
In the case of mitochondrial dysfunction, as a first step there is an upregulation of intracellular calcium (possibly due to SERCA loss of function) which leads to furin activation. Furin then further activates 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.

論文審査結果の要旨

ミトコンドリアの機能障害や低下は、 加齢や糖尿病、 筋ジストロフィーはじめ多くの疾患、さらにミトコンドリア病などにおいても広く観察される。また、その機能障害は、運動機能の低下のみならず筋肉の著しい萎縮が生じることも知られている。 しかしながら、ミトコンドリア障害から筋萎縮をつなぐ分子メカニズムについては不明な点が多く、 筋萎縮を抑えるにはその解明が不可欠である。 Surabhi Sudevan 女史は、 モデル生物の 1 つ線虫 Caenorhabditis el egans を実験材料に用いて、 ミトコンドリアの機能障害時に起因する筋萎縮メカニズムの解明を行った。

これまでの多くの知見は、 主に筋タンパク質の分解に関わる機構の解析から、 ユ ビキチンープロテアソームによる分解系、 アポトーシス、 カルパインなどの分子の関与が報告されてきたが、これらタンパク質分解系の抑制だけでは筋萎縮を抑えることができず、その鍵となる機構の存在の解明が不可欠であった。 今回、 同女史による線虫を用いた研究により、 新たに筋細胞の細胞外マトリックス（ECM）コラーゲンが、 ミトコンドリア機能障害に伴い速やかに分解されること、 また、 その分解にはマトリックスメタロプロテアーゼならびにその活性化酵素フリンが関わること、 そして、 これら酵素の活性化に必要なカルシウム濃度が上昇することを見出した。 すなわち、 カルシウム濃度の上昇を抑える方法、 また、 フリンの阻害、 マトリックスメトロプロテアーゼの阻害のいずれもが、 ミトコンドリア機能障害に伴う筋萎縮の抑制に効果的に作用することを世界に先駆けて発見した。 さらに、 これらの阻害は、 筋ジストロフィー疾患の線虫モデルにおいても有効に作用することを証明した。これらの研究成果は、多くの疾患の治療のみならず、高齢化社会で益々問題となるロコモティブシンドロームの治療や予防にも資する新たな可能性を明示し、 当該分野の基礎科学の発展のみならず応用に向けた大きなブレークスルーといえる。 また、線虫を用いたこれまでの宇宙実験サンプルを解析し、 今後の宇宙微小重力下における筋萎縮の抑制策についての提言も同学位論文に取り纏めている。 これらの内容は、 今後、 同女史が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。 したがって、 Surabhi Sudevan 女史の提出論文は、 博士（生命科学）の博士論文として合格と認める。