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Cyclin C is Sufficient for Myoblast Differentiation-Induced Mitochondrial Fragmentation

Alicia N. Campbell
Rowan University

Randy Strich
Rowan University

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Alicia N Campbell, Dr. Randy Strich

Department of Molecular Biology, Rowan University GSBS, Stratford, NJ

Abstract

One of the largest and most dynamic tissues in the body, skeletal muscle, requires constant regeneration and upkeep. Dysregulation of this regeneration process has been implicated in many neuromuscular diseases and myotonic dystrophies. Regeneration requires the differentiation of myogenic lineages including exiting the cell cycle, gene expression changes, and fusing of myoblasts into multinucleate myotubes. Part of this reconstruction requires the breakdown and repopulation of mitochondrial networks. At the early onset of myoblast differentiation, there is an upregulation of dynamin-related protein, Drp1, and an increase in mitophagy mediated by sequestosome (SQSTM1) removal of mitochondria. Previously, our lab has shown that mitochondrial fragmentation following stress requires the transcriptional regulator cyclin C, the regulatory subunit for cyclin-dependent kinase 8 (Cdk8). Preliminary data indicate that cyclin C is required for mitochondrial fragmentation during myoblast differentiation. At the early onset, cyclin C co-localizes with the mitochondria, as visualized with indirect immunofluorescence. Cells were additionally treated with PFTμ, a cytosolic chaperone inhibitor that blocks translocation of cyclin C to the mitochondria, and in turn inhibition of cyclin C-mediated mitochondrial fragmentation. This treatment resulted in lack of mitochondrial fragmentation typically seen during the differentiation process. In addition, efficiency of differentiation was quantified using gene expression of myogenic regulatory factors (MRFs) MyoD and Myosin Heavy Chain (MyHC), which are normally expressed in a temporal manner throughout differentiation. PFTμ treatment significantly delayed the onset of MyoD. Our lab has previously identified a peptide S-HAD, that causes continual mitochondrial fragmentation via the release of cyclin C by targeting of the binding domain for nuclear retention. When treated with S-HAD, cells experienced impaired differentiation as seen through extensively fragmented mitochondria and lack of reticularity, as well as irregular expression of both MRFs via RT-qPCR. Based on these findings, it was determined that cyclin C is sufficient to induce mitochondrial fragmentation associated with myogenic differentiation

Differentiation-Induced Mitochondrial Dynamics

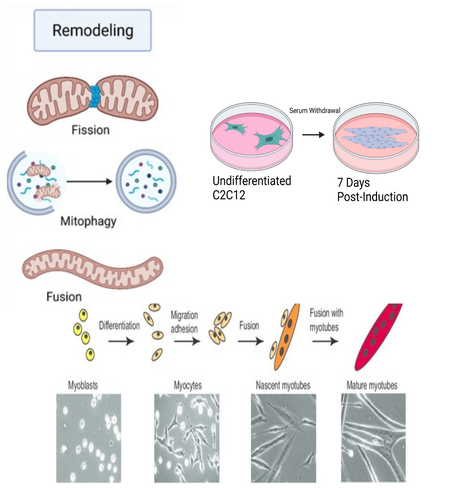

Figure 1. Mitochondrial dynamics in relation to myoblast differentiation. Constant fission and fusion are required for cellular homeostasis and response to environmental cues. Fission is required at the onset of differentiation to become mature myotubes, and fusion follows, building an enhanced mitochondrial network.

Cyclin C-Drp1-Mediated Mitochondrial Fission

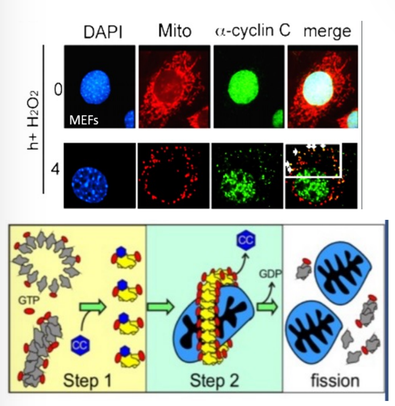

Figure 2. Cyclin C & Dynamin-related protein (Drp1) mediated mitochondrial fragmentation following stress. Following oxidative stress in MEFs, atypical cyclin C is released from the nucleus where it interacts with GTPase domain of Drp1. Drp1 forms concentric oligomeric rings around mitochondria, and cyclin C is released. Through successive rounds of hydrolysis, mitochondria become fragmented.

Cyclin C-Mediated Mitochondrial Fragmentation throughout Differentiation

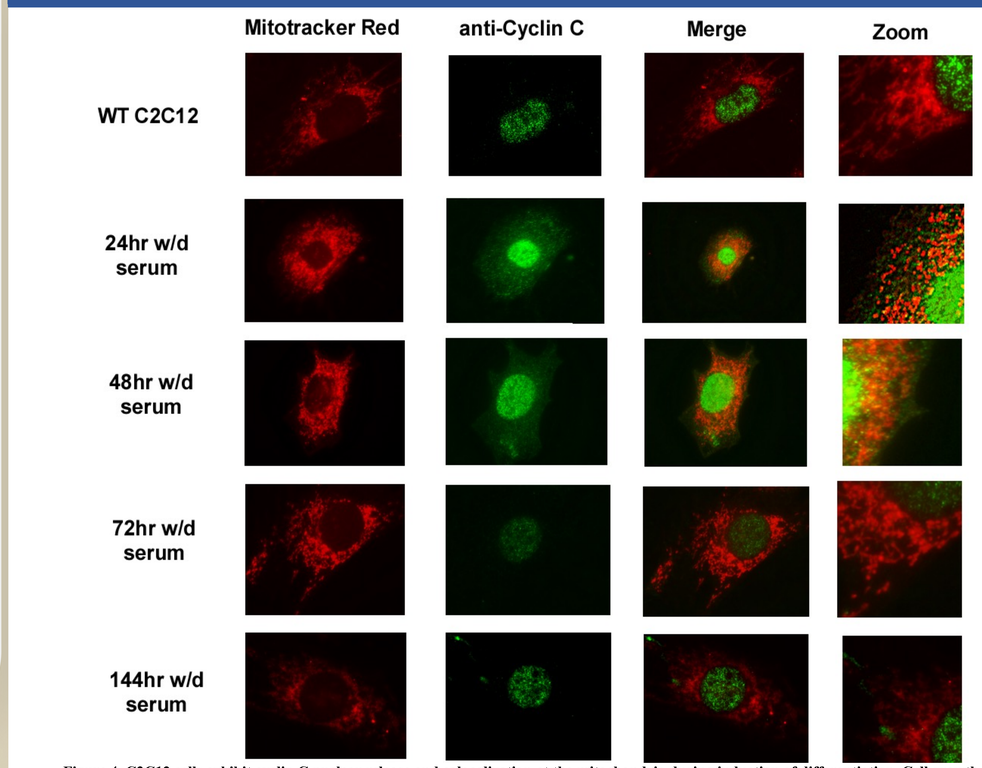

Figure 4. C2C12 cells exhibit cyclin C nuclear release and colocalization at the mitochondria during induction of differentiation. Cell growth media was switched to 2% horse serum (serum reduction), and time points were taken at indicated times. Indirect immunofluorescence was used to visualize cyclin C localization with respect to mitochondria.

Effects of PFTμ and S-HAD on Mitochondrial Morphology

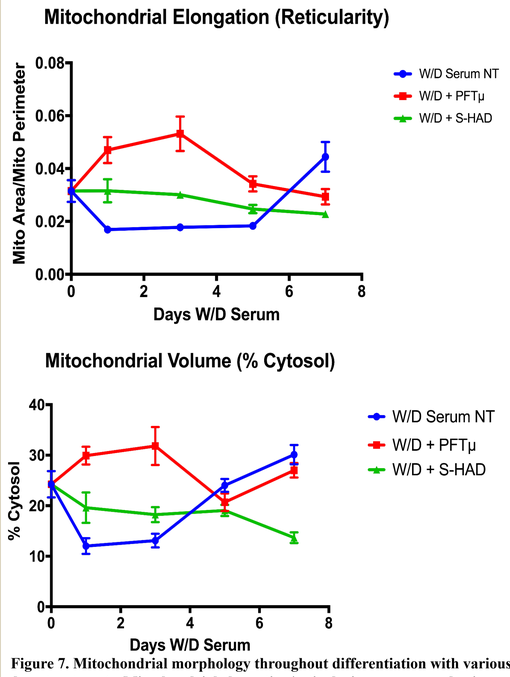

Figure 7. Mitochondrial morphology throughout differentiation with various drug treatments. Mitochondrial elongation/reticularity was assessed using an ImageJ macro that determined individual mitochondrion particles and analyzed sizes compared to average cell size. Mitochondrial volume was calculated as a percent of measured cytosol. Tukey's multiple analyses following two-way ANOVAs revealed statistically significant differences at T=1, T=3, T=5, and T=7 for both Mitochondrial Volume and Mitochondrial Elongation compared to W/D Serum NT.

Working Model & Drug Treatments

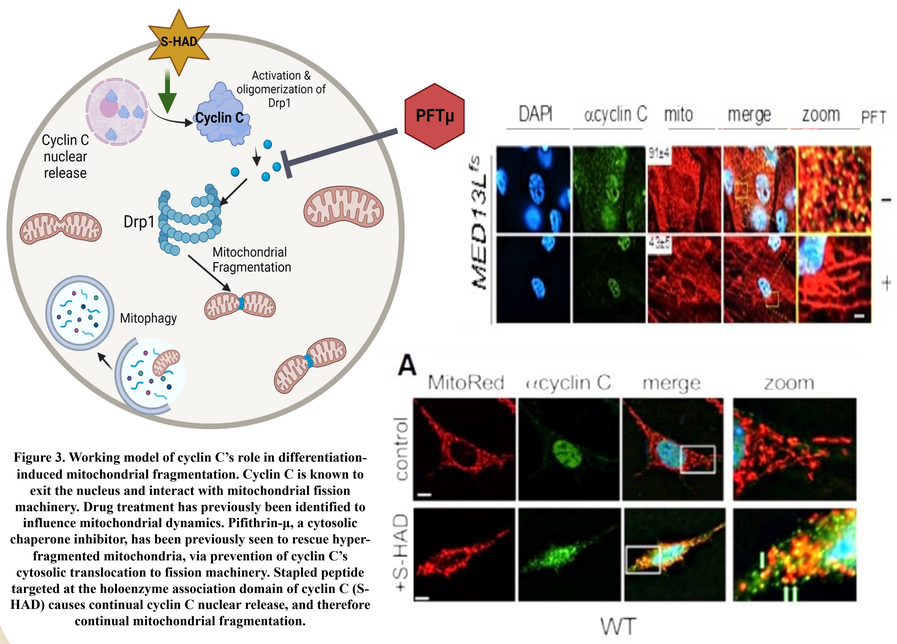

Figure 3. Working model of cyclin C's role in differentiation-induced mitochondrial fragmentation. Cyclin C is known to exit the nucleus and interact with mitochondrial fission machinery. Drug treatment has previously been identified to influence mitochondrial dynamics. Pifithrin-μ, a cytosolic chaperone inhibitor, has been previously seen to rescue hyper-fragmented mitochondria, via prevention of cyclin C's cytosolic translocation to fission machinery. Stapled peptide targeted at the holoenzyme association domain of cyclin C (S-HAD) causes continual cyclin C nuclear release, and therefore continual mitochondrial fragmentation.

Treatment with PFTμ or S-HAD alters C2C12 Differentiation

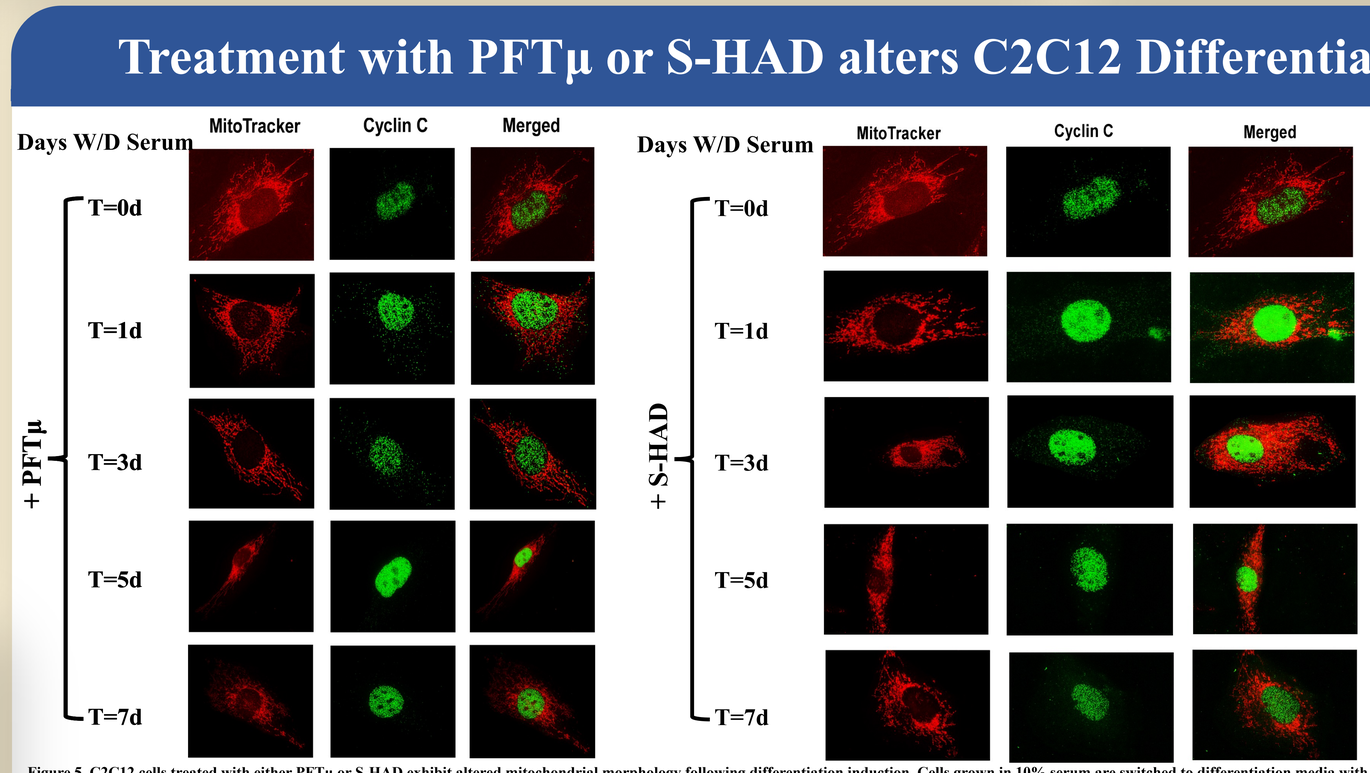

Figure 5. C2C12 cells treated with either PFTμ or S-HAD exhibit altered mitochondrial morphology following differentiation induction. Cells grown in 10% serum are switched to differentiation media with lowered serum concentration (2%). Cells treated each day with 1 μM PFTμ were fixed and subjected to indirect immunofluorescence against cyclin C. Cells were also treated with 10 μM S-HAD peptide each day, and analyzed via the same indirect immunofluorescence methods.

Differential Expression of Myogenic Regulatory Factors

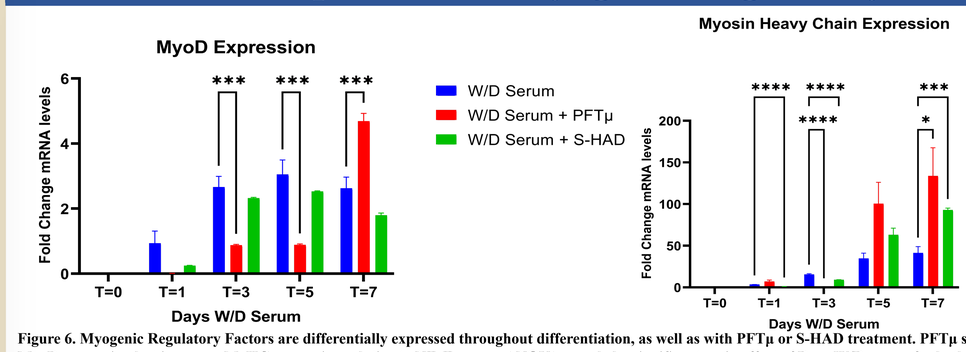

Figure 6. Myogenic Regulatory Factors are differentially expressed throughout differentiation, as well as with PFTμ or S-HAD treatment. PFTμ significantly delays MyoD expression but increases MyHC expression relative to NT. Two-way ANOVA revealed a significant main effect of Days W/D serum for both MyoD Expression and MyHC Expression((F(2.782, 56.33)=35.32, P<0.0001) (F(1.046,20.66)=22.61, P<0.0001) respectively). Analysis also revealed a significant interaction between treatment and days serum w/d for both MyoD and MyHC expression ((F(8,81)=9.067, P<0.0001),(F(8,79)=4.311, P=0.0002) respectively). Dunnett's post-hoc analysis revealed statistical differences between treatment conditions and no treatment (serum w/d only) (denoted above, ***=P<0.0001).

Conclusions & Future Directions

- **Cyclin C is sufficient to induce mitochondrial fragmentation during differentiation**
- **PFTμ treatment causes elongation of mitochondria, however it remains unclear whether this effect is direct**
  - **This elongation alters the gene expression pattern of differentiating cells, indicating that cyclin C-based mitochondrial fragmentation is required**
- **S-HAD peptide causes continual mitochondrial fragmentation via nuclear release of cyclin C**
  - **This continual release alters the expression of two different Myogenic Regulatory Factors (MRFs), indicating that cyclin C mediated fragmentation is sufficient**
- **CCNC-/- cell lines are in the works to determine if cells are still able to efficiently differentiate without cyclin C mediating activation of Drp1 for mitochondrial fission**