Metal Regulatory Transcription Factor 1 (MTF1) Interacts with SWI/SNF Chromatin Remodelers to Promote Myogenesis

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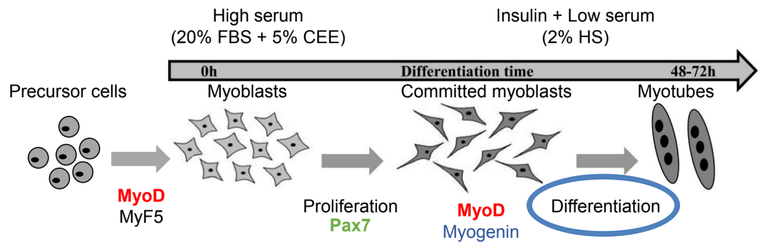
Introduction

Myogenesis is the biological process that leads to the development of skeletal muscle from embryonic stages through post-natal growth. We use primary myoblasts as a model of myogenesis (Fig. 1).

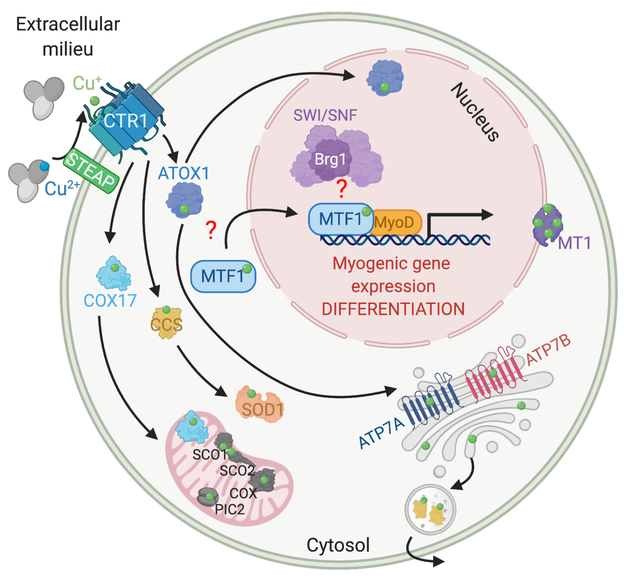
Fig. 1: Primary myoblasts culture



MTF1 is required for myogenesis[1]

- **Metal regulatory transcription factor 1 (MTF1)** maintains metals and redox homeostasis
- MTF1 and **copper** contribute to expression of several myogenic genes[1,2]
- MTF1 is essential for primary myoblasts, as deletion leads to apoptosis

Fig. 2: Schematic representation of copper network in cells during myogenesis



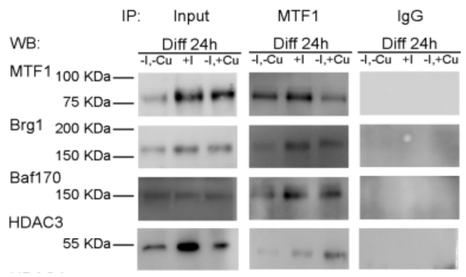
MTF1 may interact with the SWI/SNF complex at myogenic gene promoters

- **SWI/SNF** (SWItch/Sucrose Non-Fermentable) chromatin remodeler complex uses ATP hydrolysis to modify nucleosome structure
- The catalytic subunit **Brg1** is essential for myogenesis

Table 1: Immunoprecipitation and Mass Spec analysis of MTF1 interacting partners

MTF1 interacting proteins	Differentiating myoblasts + Ins	Differentiating myoblasts + Cu
Chromatin remodelers	SMARCA5, **Brg1**, Baf60, Baf57, Baf170	SMARCA5, Baf57, Baf170, **Brg1**, Baf60, EIF-5
Histones	H10-H15, H31, H2AZ, H2B1M	H12-15
Histone modifiers	HDAC1-3, Sap18b	HDAC2-3, RBBP7

Fig. 3: Representative WB validating interacting candidates identified by mass spec



Goal

To identify convergent transcriptional regulatory mechanisms between a classic metal responsive transcription factor and the SWI/SNF complex by identifying loci that are potentially coregulated by MTF1 and Brg1.

Results

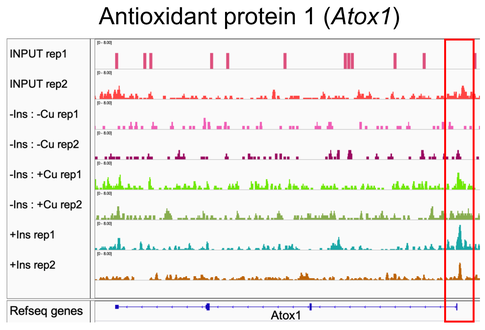
Comparative Bioinformatic Analyses of MTF1 ChIP-Seq and *Brg1* knockdown RNA-Seq

Fig. 4: A potential interaction between MTF1 and Brg1 may contribute to the regulation of myogenic gene expression but not for genes involved in metal homeostasis or mitochondrial biogenesis

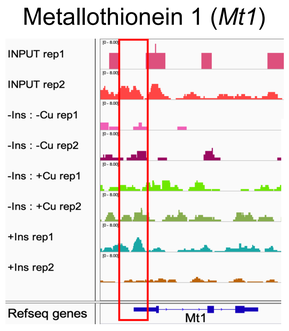
Fig. 4A: MTF1 ChIP-Seq peak tracks for representative target genes[1], visualized using IGV software. Promoter regions are in red boxes.

Representative Metal Homeostasis Genes

Antioxidant protein 1 (*Atox1*)

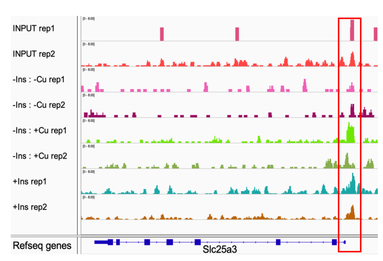


Metallothionein 1 (*Mt1*)

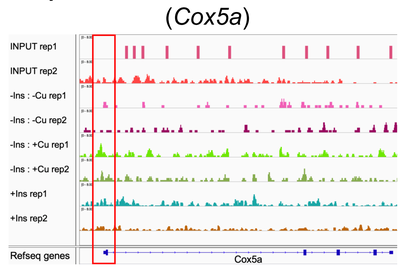


Representative Mitochondrial Biogenesis Genes

Pic2 (*Slc25a3*)

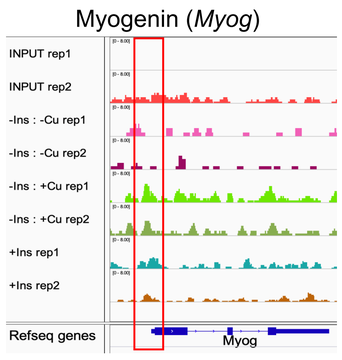


Cytochrome c oxidase subunit 5a (*Cox5a*)

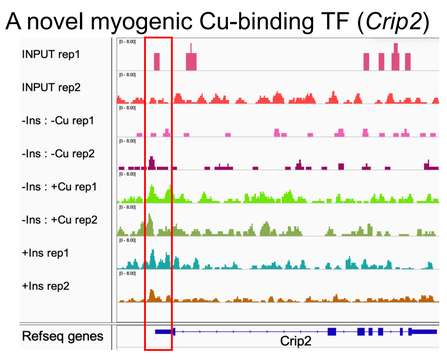


Representative Myogenic Genes

Myogenin (*Myog*)



A novel myogenic Cu-binding TF (*Crip2*)



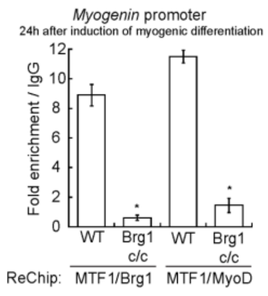
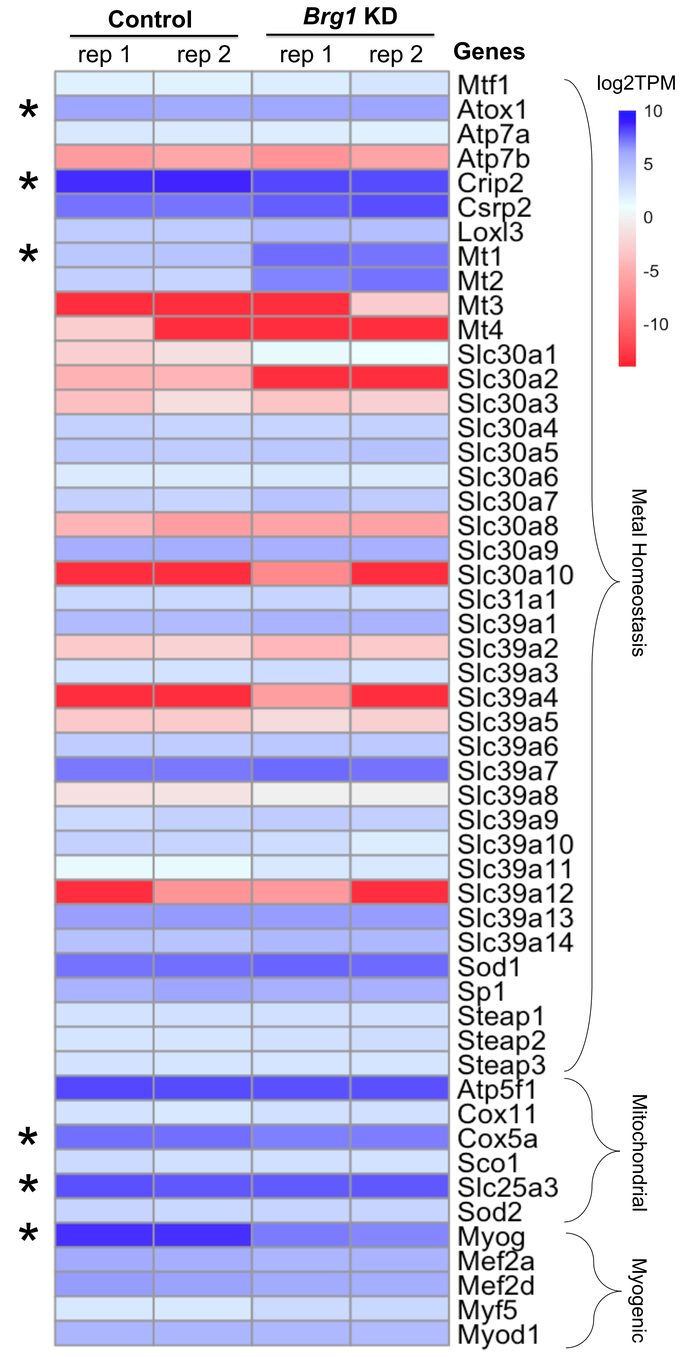


Fig. 5: Sequential ChIP-qPCR validating co-binding of MTF1 and Brg1 at the *Myogenin* promoter

Fig. 4B: Heat map showing changes in gene expression in differentiating C2C12 cells lacking Brg1. The genes shown are representative targets of MTF1. log2TPM values are shown. Overexpressed genes are in blue and downregulated genes are in red. Representative genes from Fig. 4A are marked with an asterisk (*).



Conclusions

- MTF1-binding to myogenic genes correlated with a decreased expression of these genes in *Brg1* depleted myoblasts.
- No correlation was found with genes involved in metal homeostasis or mitochondrial biogenesis.
- Our data suggests a potential mechanism where MTF1 and SWI/SNF act cooperatively to promote myogenesis. A different mechanism of gene activation may contribute to the maintenance of metal homeostasis and mitochondrial biogenesis.

Future Directions

- Investigate the myogenic transcriptional regulatory mechanism dependent on MTF1 and SWI/SNF. We will perform Brg1 ChIP-Seq and MTF1 KD RNA-Seq experiments to integrate with the analyses presented here.

Acknowledgements

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- MB&B Department

Materials and Methods

Primary myoblast culture: Mouse satellite cells were isolated from leg muscle of 3-6-wk-old WT C57B1/6 mice. To test the effect of Cu and MTF1 in myogenesis, insulin was eliminated from the differentiation medium from the Cu-treated cells because this condition partially inhibits myogenesis[1,2]. **ChIP-Seq**: Libraries of ChIP-enriched DNA were prepared from 2 biological replicates following the Illumina strategy (Illumina, San Diego, CA, USA)[1]. **Bioinformatic analyses**: The MTF1 ChIP-Seq from WT myoblasts and RNA-Seq dataset from C2C12 cells (another tissue culture model for myogenesis) knockdown for Brg1 undergoing differentiation were comparatively analyzed using IGV 2.8.4, Excel, and R Studio. GEO Accession numbers GSE116331 and GSE141407, respectively[1,3].

References

[1] Tavera-Montañez *et al*. 2019. Faseb J

[2] Vest, *et al*. 2018. Metallomics

[3] Zhu, *et al*. 2020. Nucleic Acids Res