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Foxp1 Binds to DNA Independently from Hox Genes in Upper & Lower Limb Motor Neurons (MNs)

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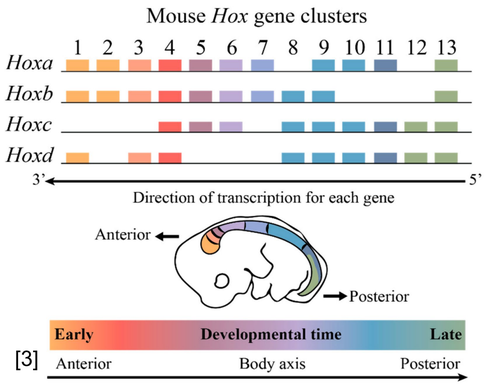
Introduction

Our cells contain thousands of different transcription factors (TFs) that are responsible for binding to DNA and turning our genes "on" or "off". The pattern of expressed vs not expressed genes in a cell determines how it differentiates into a specific role. **Foxp1 is a TF that regulates the differentiation of lateral motor column (LMC) motor neurons (MNs),** which control the movement of our upper and lower limbs. But before Foxp1 can function, it must first be activated by either the TF Hoxc6 or Hoxc10 [1].

Fig. 1: Hox Genes Selectively Activated to Map Body Structure



[3]

Hox genes are organized collinearly with the body. When they are selectively activated, they begin cascading events to form a specific structural region.

Hoxc6 in upper limbs and Hoxc10 in lower limbs activate Foxp1 to create LMC MNs. However, Hoxc6 & Hoxc10 have different binding patterns [2], and upper and lower limb MNs exhibit differences. This research aimed to answer the question:

RESEARCH QUESTION: **Does Foxp1 bind to DNA differently when it is activated by Hoxc6 vs Hoxc10?**

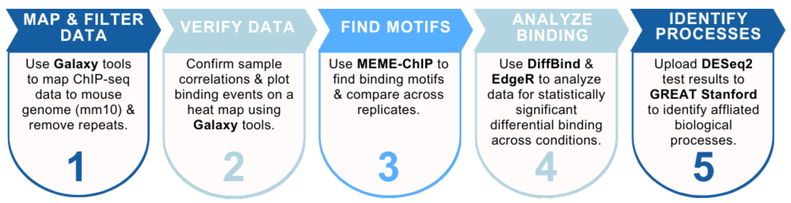
Motivations

- Continue collaborative work between Dr. Shaun Mahony's lab of PSU & Dr. Esteban Mazzoni's lab of NYU for understanding the role of TFs in determining different neural cellular identities
- Investigate if Hoxc6 & Hoxc10 differential binding influences Foxp1 to differentially bind as well in upper & lower limb LMC MNs

Methods

ChIP-seq data collected by the collaboration between Mahony's and Mazzoni's labs was analyzed using the methods outlined below. **5 data samples** were collected from lab-grown mouse embryotic stem cells:

- **2 replicates of iHoxc6 (cells with induced Hoxc6 expression)**
- **2 replicates of iHoxc10 (cells with induced Hoxc10 expression)**
- **1 input (control) without Hox gene manipulation**



iHox6 & iHox10 Replicates Correlated

Fig. 2: All Hox Replicates are Closely Correlated with Each Other but Not the Input

- **ChIP-seq successful:** input and Hox replicates are not closely correlated
- **iHoxc10 & iHoxc6 replicates tightly correlated**, supporting Foxp1 does not bind differently in upper & lower limb MNs

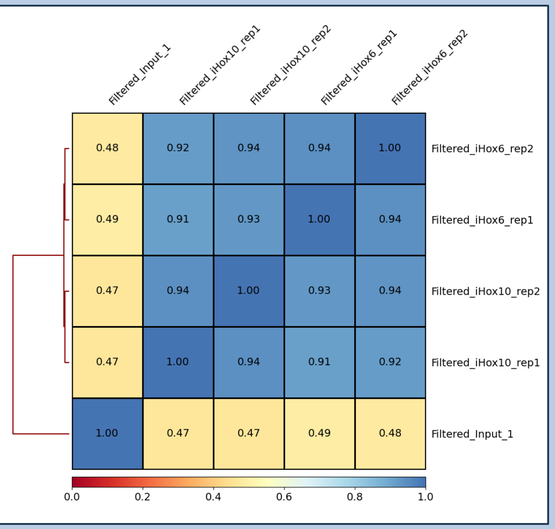
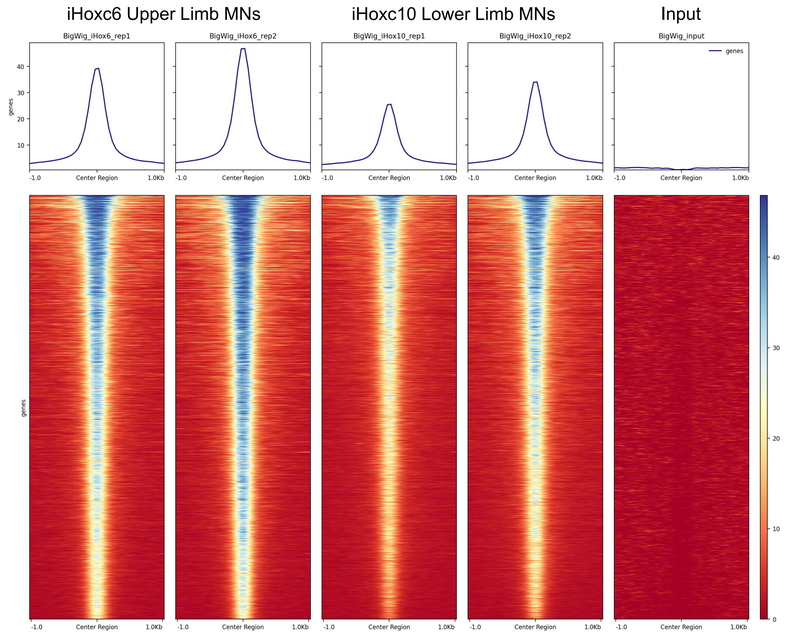


Fig. 3: Heatmap Verifies Foxp1 Binds DNA in Same Pattern Regardless of Hoxc6 or Hoxc10



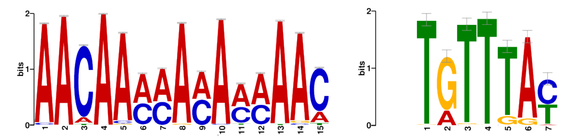
- **ChIP-seq successful**: iHoxc6 & iHox10 replicates have regions of strong protein binding (blue) whereas the input has no evident protein binding (red)
- **Consistent binding pattern across all 4 replicates** indicates Foxp1 binding is comparable regardless of active Hox gene

Citations: **[1]** Rousso, D. L., Gaber, Z. B., Wellik, D., Morrisey, E. E., & Novitch, B. G. (2008). Coordinated actions of the forkhead protein foxp1 and Hox proteins in the columnar organization of spinal motor neurons. *Neuron*, *59*(4), 674–675. https://doi.org/10.1016/j.neuron.2008.08.002 **[2]** Bulajić, M., Srivastava, D., Dasen, J. S., Wichterle, H., Mahony, S., & Mazzoni, E. O. (2020). Differential abilities to engage inaccessible chromatin diversify vertebrate Hox binding patterns. *Development*. https://doi.org/10.1242/dev.194761 **[3]** Afzal, Z., & Krumlauf, R. (2022). Transcriptional regulation and implications for controlling Hox gene expression. *Journal of Developmental Biology*, *10*(1), 4. https://doi.org/10.3390/jdb10010004 **[4]** Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B. A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., & Blankenberg, D. (2018). The Galaxy Platform for Accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research*, *46*(W1). https://doi.org/10.1093/nar/gky379 **[5]** Philip Machanick and Timothy L. Bailey, "MEME-ChIP: motif analysis of large DNA datasets", *Bioinformatics* 27(12):1696-1697, 2011. **[6]** Cory Y McLean, Dave Bristor, Michael Hiller, Shoa L Clarke, Bruce T Schaar, Craig B Lowe, Aaron M Wenger, and Gill Bejerano. "GREAT improves functional interpretation of *cis*-regulatory regions". *Nat. Biotechnol.* 28(5):495-501, 2010. PMID 20436461 **[7]** Yosuke Tanigawa, Ethan S Dyer, Gill Bejerano. "WhichTF is functionally important in your open chromatin data?" *PLoS Comput Biol*. 2022 Aug 30;18(8):e1010378, 2022. PMID 36040971

Same Motifs for Foxp1 in All Replicates

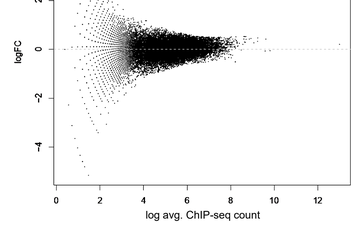
Fig. 4: Foxp1 Motifs Match in iHoxc6 & iHoxc10 Replicates

Same motifs in all replicates suggests Foxp1 has the **same function** regardless of active Hox gene.



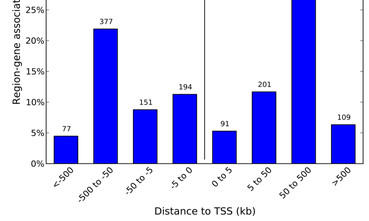
Insights on Where Foxp1 Binds

Fig. 5: No Significant Differences in Enrichment Across Replicates



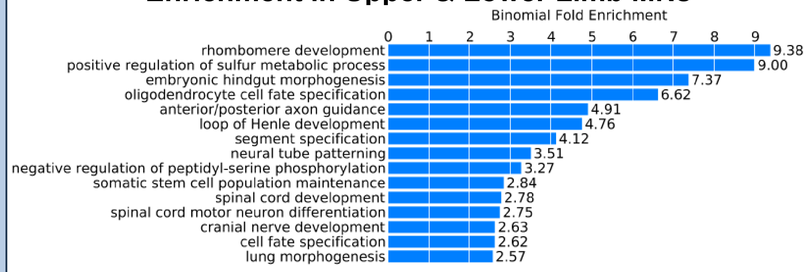
- **Black** statistically similar
- Both **Red** & **Blue** statistically different enrichment, but clustered closely to black indicates no biologically significant differences

Fig. 6: Foxp1 Influences Gene Expression by Binding Gene Enhancer Sites



- 63% Foxp1 gene enrichment events >50kp from transcription start site (TSS)
- Suggests Foxp1 primarily binds to gene enhancers, not promoters

Fig. 7: Biological Processes Associated with Foxp1 Enrichment in Upper & Lower Limb MNs



Top 15 biological processes associated with enrichment reinforce Foxp1 plays a vital part in motor neuron cell fate specification.

Conclusions & Future Research

- High correlation between all Hox cell replicates & heatmap with rich binding (blue) in replicates only indicate ChIP-seq experiment successful
- Foxp1 binds to the same motifs and locations in the genome of all LMC MNs despite the TFs that activate Foxp1 – Hoxc6 & Hoxc10 – binding differently
- To better understand MN differentiation, future research should focus on later steps in the pathways of biological processes linked to Foxp1 enrichment.

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