Myf5 and MyoD flex different muscles in transcription

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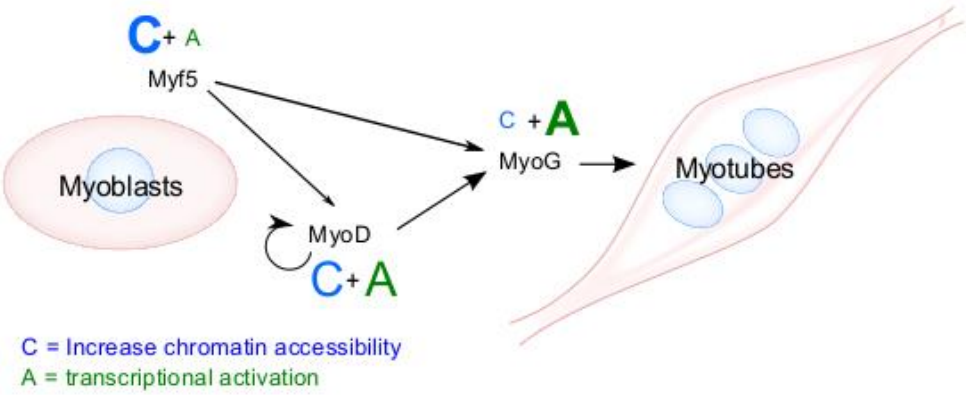

Subfunctionalization of transcription factors separates improved chromatin accessibility from transcriptional activation during differentiation.

Image provided by Dr. Melissa Conerly.

The astonishing diversity of cell types in our bodies is accomplished by activation of a specific set of genes in each cell type. For example, genes required for muscle development and differentiation are turned on in muscle but not neuronal precursors. Cell-type specific transcription is accomplished in part by a class of DNA-binding proteins known as transcription factors. Most transcription factors exist as part of large families and in some of these families it is unclear how functional specificity is achieved between structurally similar members. One such case involves Myf5 and MyoD, transcription factors of the basic helix-loop-helix family involved in skeletal muscle development. These related family members are both required for muscle development. Nevertheless they have distinct functions, as genetic studies showed that one cannot fully compensate for the other in some muscle groups. Previous studies that have focused on family members that function in different cell types have coalesced on a model wherein factor-specific functions are achieved through differences in DNA binding site preferences. A new Fred Hutch study led by Dr. Melissa Conerly, a joint post-doctoral fellow in the Groudine (Basic Sciences Division) and Tapscott Laboratories (Clinical Research and Human Biology Divisions), presents an alternative model wherein the separate roles of Myf5 and MyoD are due to inherent differences in their ability to activate transcription of target genes rather than divergence in their DNA binding site preferences. This study was recently published in *Developmental Cell*.

The authors first established a system to study the ability of either MyoD or Myf5 to induce muscle cell development and transcriptionally activate muscle-specific genes. To this end, they used lentiviruses to express either factor in mouse embryonic fibroblasts that lack both MyoD and Myf5 (M&M MEFs). These experiments revealed that MyoD, but not Myf5, could both robustly induce M&M MEFs to differentiate into myotubes and activate muscle development genes such as *Myog* and *Cdh15*. Genome-wide analyses using next-generation sequencing of RNA (RNA-seq), as well as chromatin immunoprecipitation followed by sequencing of bound DNA (ChIP-seq) of M&M MEFs expressing either MyoD or Myf5, revealed that these factors activated a similar set of genes and bound to the same DNA sites, but that Myf5 activation of myogenic genes was weaker than MyoD activity. Because both proteins bound DNA with similar affinity and histone H4 acetylation levels were equivalent, the investigators hypothesized that Myf5 lacks a strong transcriptional activation domain. To test this directly, portions of either MyoD or Myf5 outside of the DNA binding domain were fused to a heterologous DNA-binding domain (Gal4) and expression of a reporter gene containing Gal4-binding sites was assayed for the various chimeras. In this system, the N-terminal activation domain of MyoD was more than 10-fold more potent than any of the Myf5 domains tested. Importantly, a chimeric protein composed by the MyoD activation domain fused to full-length Myf5 (Myf5 chimera) rescued the ability of Myf5 to both upregulate gene expression and induce muscle differentiation. Finally, the researchers tested the ability of MyoD, Myf5, and the Myf5 chimera to recruit RNA polymerase II and found that of these, only Myf5 could not robustly recruit RNA polymerase II.

Overall, this study showed that MyoD and Myf5 have separate roles in cell type specification with Myf5 acting first mostly as chromatin modifier rather than a transcriptional activator, whereas MyoD retained both chromatin modifying and robust transcriptional activation during muscle differentiation. Said Dr. Conerly "Though Myf5 is classified as a transcription factor, we found that the primary function appears to be the modulation of chromatin structure rather than transcriptional activity. In contrast, MyoG, a late factor has little ability to modulate chromatin structure, but when presented with accessible sites, can robustly activate transcription. Thus the early events in lineage specification appear to be focused on 'setting the stage' for later factors to act".

Conerly ML,Yao Z,Zhong JW,Groudine M,Tapscott SJ. 2016. Distinct activities of Myf5 and MyoD indicate separate roles in skeletal muscle lineage specification and differentiation. *Dev Cell*. 36(4): 375-85.

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