Myod Crashes Neurod2's Private Party

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The human body consists of hundreds of different cell types, each with a different form and function. A large body of evidence, including Nobel-prize winning work on generation of pluripotent stem cells, suggests that proteins known as transcription factors are master regulators of cell type identity that, by directly binding to DNA, function to switch on specific sets of genes in each cell type. One such transcription factor, MyoD, which was first discovered here at the Fred Hutch in the laboratory of the late Dr. Harold Weintraub almost 30 years ago, is a master regulator of muscle cells as it can convert non-muscle cells such as fibroblasts into skeletal muscle, in a process known as transdifferentiation. Since its discovery, other transcription factors with similar cell fate-determining functions have been identified. For instance, NeuroD2 can transdifferentiate cells into neurons. Both MyoD and NeuroD2 are part of a family of transcription factors that contain a basic-helix-loop-helix (bHLH) domain that is required for DNA binding and both bind to highly similar target sequences. Previous studies in Dr. Stephen Tapscott's Laboratory (Clinical Research and Human Biology Divisions) used chromatin immunoprecipitation followed by next-generation DNA sequencing (ChIP-seq) to show that while MyoD and NeuroD2 can bind to shared DNA sites, they also bind DNA sites that are specific to each factor, termed private sites, that are highly correlated with cell type specific gene activation (Fong *et al.,*2012).

However, it remained unclear which domains of MyoD and NeuroD2 were important for cell type determination. A new study by the Tapscott lab, led by research associate Dr. Abraham Fong and published in *Cell Reports*, addressed this question by generating a chimeric transcription factor wherein the bHLH domain of MyoD was replaced with the bHLH domain of NeuroD2. This chimera, MyoD (NeuroD2-bHLH), exhibited strong gene activation of a reporter containing a pair of NeuroD2 private sites and weak activation of a reporter containing a pair of either one of the shared sites or of the MyoD private sites, similar to NeuroD2 itself. This gene activation pattern correlated with DNA-binding affinity, as demonstrated by *in vitro* gel shift assays.

Next, to assay the DNA-binding properties of the chimera in cells, the authors performed ChIP-seq experiments with NeuroD2, MyoD and MyoD (NeuroD2-bHLH) in a mouse embryonal carcinoma cell line (P19). Indeed, discriminative motif enrichment analysis showed that MyoD (NeuroD2-bHLH) acquired the DNA-binding properties of NeuroD2. Next-generation sequencing of RNA (RNA-seq) of

P19 cells transduced with either NeuroD2, MyoD or MyoD (NeuroD2-bHLH), revealed that the chimera activated both neuronal and muscle genes. These studies were corroborated by examining both neuronal and muscle markers either in P19 cells or in zebrafish embyros that had been transduced or injected with the chimera, respectively.

Finally, the authors addressed the question of why the chimera still bound to a subset of muscle genes. To this end, they performed discriminative motif enrichment analysis of the DNA region surrounding sites bound both by the chimera and MyoD, but not NeuroD2, which revealed an enrichment for binding sites for homeodomain transcription factors of the PBX/MEIS family. Intriguingly, a PBX/MEIS complex had been previously shown by the Tapscott lab to be required for full activation of 10% of MyoD target genes. Strikingly, P19 cells transduced with a modified chimera in which the PBX/MEIS interaction domain was mutated essentially behaved as a dedicated neurogenic factor and lost the ability to activate key muscle genes such as myogenin.

"It was initially surprising to us that retargeting MyoD to the NeuroD2 binding sites was sufficient to convert it to a master regulator of neurogenesis, but now this helps us understand recent reports showing that spindle cell rhabdomyosarcomas have a mutation in MyoD that changes its binding specificity to Myc sites and makes it oncogenic. So by changing the MyoD DNA binding specificity we can change it from a myogenic factor to either a neurogenic factor or an oncogenic factor. This suggests a direct approach to tissue engineering as well as an understanding of how new transcriptional programs can arise during evolution", said Dr. Stephen Tapscott. In conclusion, this report showed that the DNA binding specificity of bHLH transcription factors largely determines their transcriptional output, which warrants similar studies of other transcription factor families to establish the generality of this concept.

Fong AP, Yao Z, Zhong JW, Johnson NM, Farr GHr, Maves L, Tapscott SJ. 2015. Conversion of MyoD to a Neurogenic Factor: Binding Site Specificity Determines Lineage. *Cell Rep*. 10,1937-1946.

See also:

Fong AP, Yao Z, Zhong JW, Cao Y, Ruzzo WL, Gentleman RC, Tapscott SJ. 2012. Genetic and Epigenetic Determinants of Neurogenesis and Myogenesis. *Dev Cell*. *22*, 721-35.

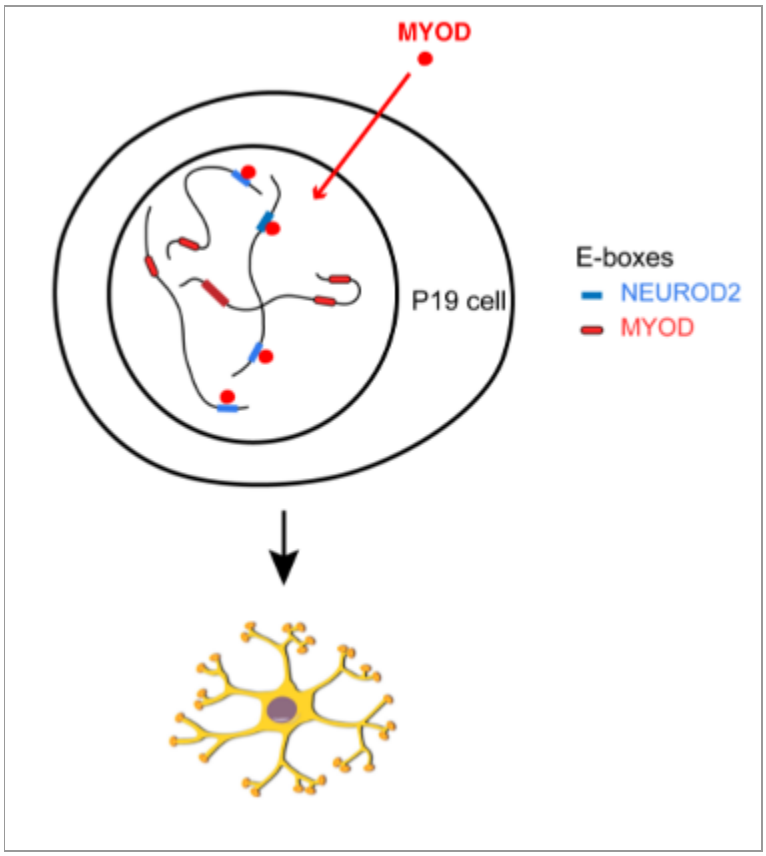

Image provided by Dr. Stephen Tapscott.

The transcription factor MyoD normally determines muscle fate by binding to its private DNA sites (red). Swapping MyoD's DNA-binding domain with a similar yet distinct domain in NeuroD2 converts MyoD into a neuron-determining factor, by retargeting the MyoD-NeuroD2 chimera to NeuroD2's private DNA sites (blue).