The two faces of MyoD: repressor and activator of gene expression during myogenesis

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MyoD is well known for its ability to reprogram a broad range of cell types into myogenic cells and for its pioneer function in activating the myogenic program during muscle development and regeneration. The basic helix–loop–helix (bHLH) protein achieves this by directly binding to E-boxes in DNA and recruiting proteins like histone acetyltransferases and the SWI/SNF chromatin remodeling complex. Interestingly, Nicoletti and colleagues (doi:10.1101/gad.352708.125) report in this issue of *Genes & Development* an unexpected finding; namely, that MyoD can also act as a repressor. This repressive activity is E-box-independent, meaning that MyoD can be indirectly recruited to distinct sites in chromatin. Transcription factor motifs enriched at these sites correspond to E2F, NF-Y, and Jun/Fos motifs. The genes that are repressed by this noncanonical MyoD function control nonmyogenic fates and participate in cell cycle regulation as well as proliferation. At such sites, MyoD binding is associated with chromatin compaction and repression of transcription.

MyoD was the first transcription factor discovered to possess reprogramming activity and has since served as a paradigm for factors controlling cellular differentiation (Tapscott et al. 1988). MyoD is a basic helix–loop–helix protein, binds to DNA as either a homodimer or heterodimer, and recognizes the E-box, the canonical MyoD binding motif (Murre et al. 1989; Shklover et al. 2007). It is considered a true master regulator, positioned at the top of a regulatory hierarchy, and operates via a feed-forward mechanism to drive muscle differentiation. This involves MyoD activating its own expression as well as the expression of other muscle-specific genes, thereby establishing a positive feedback loop that reinforces skeletal muscle gene expression (Thayer et al. 1989). The myogenic activity of MyoD has been extensively studied. Its binding sites in the genome and its target genes have been well characterized, and substantial insights have been gained into how MyoD affects chromatin modifications and 3D chromatin architecture during myogenic differentiation (e.g., see Cao et al. 2010; Dall'Agnese et al. 2019; Zhang et al. 2020; for review, see also Dong and Cheung 2021). Much of this knowledge comes from reprogramming assays in which MyoD is (over)expressed in fibroblasts cultured in low growth factor conditions, conditions under which the cells undergo terminal myogenic differentiation. However, in vivo, MyoD is expressed not only during terminal differentiation but also in proliferating progenitors and myoblasts. In these proliferating cells, muscle gene expression is either not induced or only partially activated, suggesting that MyoD may regulate a distinct set of genes beyond the canonical myogenic program. This formed the rationale and starting point for the study by Nicoletti et al. (2025) They used MyoD-(over)expressing fibroblasts in high growth factor medium; that is, cells that express MyoD but do not undergo terminal differentiation. They analyzed MyoD binding, MyoD-dependent gene expression, and associated chromatin modifications. Additionally, their findings were validated in cultured primary muscle stem cells (MuSCs) or MuSCs obtained from regenerating muscle early after injury.

In the reprogramming experiments, MyoD was expressed in an inducible manner in fibroblasts. A comparison of the cells before and after MyoD induction revealed that a substantial portion (approximately one-third) of all deregulated genes was downregulated in the presence of MyoD. These included genes associated with fibroblast identity, such as *GATA4*, and transcription factors involved in growth control, such as *c-Fos*. Among the myogenic genes, early markers were weakly activated, whereas late markers remained uninduced. Motif analysis showed that the E-box sequence was most frequently associated with MyoD peaks at promoters of upregulated

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genes. In contrast, the most enriched motifs at promoters of repressed genes corresponded to those recognized by E2F family members E2F2, E2F3, and E2F7, as well as the NF-Y motif. Coimmunoprecipitation experiments demonstrated a direct interaction between MyoD and E2F2 but not with NF-Y. Notably, the promoter of the downregulated gene *c-Fos* contains two E2F2 binding sites, and mutation of these sites impaired MyoD's ability to repress c-Fos expression. These findings suggest that one mechanism—though likely not the only one—by which MyoD mediates gene repression is through indirect recruitment to promoters via interaction with E2F2.

Analysis of histone modifications enabled Nicoletti et al. (2025) to identify distal regulatory elements (namely, enhancers and superenhancers) during the reprogramming of fibroblasts. The results mirrored those observed at promoters. Specifically, MyoD-bound superenhancers that gained chromatin accessibility during reprogramming were highly enriched for E-box motifs, whereas those that lost accessibility were enriched for non-E-box motifs. Among these, motifs recognized by Jun/Fos family transcription factors were the most prominent. This analysis was integrated with previously generated Hi-C data that mapped the three-dimensional organization of chromatin, allowing for the identification of promoters physically associated with superenhancers. Superenhancers that emerged during reprogramming were found to associate with genes involved in cell differentiation and commitment to the myogenic lineage. In contrast, superenhancers that were lost during reprogramming were linked to genes involved in repressing growth factor-induced intracellular signaling and proliferative processes.

The distribution of H3K27ac, a histone modification typically associated with active enhancers and promoters (Rada-Iglesias et al. 2011), revealed an unexpected feature: MyoD-bound promoters that were downregulated and superenhancers that were lost during reprogramming retained the H3K27ac mark. This indicates that MyoD-mediated repression does not involve the removal of this chromatin modification.

The repressive function of MyoD was further validated in primary cells and in vivo. Cultured primary myogenic cells treated with or without siRNA targeting *MyoD* were compared, as were muscle stem cells (MuSCs) isolated at early (4 h) and later (60 h) stages of muscle regeneration, time points when MyoD is either absent or already expressed, respectively. In both experimental systems, MyoD-dependent genes were identified as either upregulated or downregulated. Notably, many of the repressed genes overlapped with those identified in the reprogramming experiments.

In summary, the findings of Nicoletti et al. (2025) demonstrate that MyoD functions not only as a transcriptional activator but also as a repressor both during myogenic reprogramming and in proliferating muscle progenitors. Notably, its repressive activity does not require the presence of an E-box motif, suggesting that MyoD is recruited to chromatin indirectly. Multiple mechanisms appear to be responsible for the recruitment, allowing MyoD to exert its repressive function at distinct genomic loci. This view is further supported by the experiments that show that different domains of the MyoD protein are required to repress distinct subsets of target genes.

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