

The Alternative Splicing of NF-YA Regulates rouse Embryonic Stem Cells differentiation

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Embryogenesis depends on a delicate balance between pluripotency and differentiation, regulated by a restricted set of transcription factors (TFs). Among them, NF-Y is a heterotrimeric pioneer TF that promotes chromatin accessibility and gene expression. Its regulatory subunit, NF-YA, binds the CCAAT box motif, one of the most enriched DNA elements in enhancers and promoters of genes specifically active in embryonic stem cells (ESCs).NF-YA exists in two isoforms generated by alternative splicing (AS) of exon 3: the short isoform (NF-YAs) and the long isoform (NF-YAl), which differ by a 28–29 amino acid stretch in the N-terminal Q-rich activation domain. Although these isoforms share identical DNA-binding properties *in vitro*, they exert distinct functions in biological contexts, such as epithelial-to-mesenchymal transition in cancer. In embryogenesis, NF-YAs is predominant in undifferentiated ESCs and is crucial for maintaining pluripotency, whereas NF-YAl becomes prevalent during differentiation.

To dissect the functional contribution of NF-YA isoforms in this process, we forced NF-YAs expression by CRISPR/Cas9-mediated exon 3 deletion. The differentiation potential of NF-YAl-deficient clones was assessed using embryoid bodies (EBs), gastruloids, and BMP4-driven monolayer differentiation. In addition, NF-YA common and isoform-specific targets were identified through CUT&RUN to uncover their mechanisms of action.

The stable expression of NF-YAs and absence of NF-YAl during ESC differentiation led to the persistence of pluripotent-like cells and loss of mesodermal subpopulations. These findings reinforce the stemness-associated role of NF-YAs and reveal a novel requirement for NF-YAl in mesoderm formation.

Our results highlight the complementarity of NF-YA isoforms in balancing pluripotency and differentiation and NF-YA Alternative Splicing emerges as a potential regulatory node for embryonic differentiation.