

# Investigating Neuromesodermal Progenitor Development and Differentiation Using *Danio rerio* Single Cell Sequencing Data

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Traditional views of embryonic development hold that germ layer differentiation begins and ends with gastrulation. However, recent evidence suggests mesoderm and ectoderm induction occurs post-gastrulation in neuromesodermal progenitors (NMPs). NMPs are a population of multipotent cells in the posterior-most portion of the embryo—the tailbud. Currently, mechanisms of NMP maintenance and differentiation are unclear. Single cell RNA-sequencing (scRNA-seq) data of zebrafish (*Danio rerio*) embryos can provide insight into the transcriptomes of individual cells from different lineages and embryonic stages, revealing how gene expression in NMPs changes during embryogenesis. Here, scRNA-seq data of 38,731 embryonic zebrafish cells spanning the high to 6 somite stages were downloaded and analyzed in R using the URD package. Gene expression patterns observed from the scRNA-seq data were validated by visualizing mRNA expression in zebrafish embryos using in situ hybridization chain reaction (HCR). Analysis of NMP gene expression over time revealed that NMPs are established during gastrulation and that a switch in NMP *soxB1* gene usage occurs at gastrulation's end. Likewise, the analysis indicated timepoints when genes in crucial pathways for NMP establishment are activated during gastrulation. Differential expression and gene ontology analyses of NMP versus mesodermal and ectodermal progenitors revealed distinct changes in the expression of key marker genes and signaling pathways. Furthermore, HCR analysis of the canonical Wnt signaling pathway and its target genes revealed potential mechanisms for how Wnt can both promote mesodermal differentiation and maintain the undifferentiated NMP state. These insights into NMP development enhance understanding of molecular mechanisms behind vertebrate embryogenesis.