To understand the gene regulatory network (GRN) governing caudal hindbrain formation in embryonic zebrafish, several early expressed factors have been manipulated, and multiple genetic mutants have been characterized. Such analyses have identified morphogens such as Retinoic Acid (RA) and Fibroblast growth factors (FGFs), as well as transcription factors like hoxb1b, hoxb1a, hnf1ba, and valentino as being required for rhombomere (r) r4-r6 formation in zebrafish. Considering that the caudal hindbrain is relatively complex – for instance, unique sets of neurons are formed in each rhombomere segment – it is likely that additional essential genes remain to be identified and integrated into the caudal hindbrain GRN.

Our results reveal that r4 gene expression is unaffected by the individual loss of hoxb1b, hoxb1a or RA, but is under the combinatorial regulation of RA together with hoxb1b. In contrast, r5/r6 gene expression is dependent on RA, FGF, hnf1ba and valentino – as individual loss of these factors abolishes r5/r6 gene expression. Analysis of six mutant lines (gas6, gbx1, sall4, eglf6, celf2, and greb1l) did not reveal rhombomere or neuronal defects, but transcriptome analysis of one line (gas6 mutant) identified expression changes for genes involved in several developmental processes – suggesting that these genes may have subtle roles in hindbrain development.

We conclude that r4-r6 formation is relatively robust, such that very few genes are absolutely required for this process. However, there are mechanistic differences in r4 versus r5/r6, such that no single factor is required for r4 development while several genes are individually required for r5/r6 formation.