

## Two bHLH Transcription Factors Are Required for Breaking Symmetry in a Left-Right Asymmetric Cell Lineage

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The body plan of *C. elegans* is mostly bilaterally symmetric. Much of this symmetry arises from pairs of homologous blastomeres, which through bilaterally symmetric cell lineages give rise to sets of left-right paired cells. To create either asymmetry or three-fold symmetry, the bilateral symmetry in cell lineages must be broken.

The pharyngeal epithelial cells form a three-fold symmetric structure that consists of nine cells. The nine cells are divided into three groups of three cells, e1, e2, and e3, on the ventral left (VL), ventral right (VR), and dorsal (D) regions of the pharynx. e3VL and e3VR are generated as lineal homologs, whereas e3D is generated by breaking left-right symmetry in a specific cell lineage. The blastomeres ABaraapa and ABaraapp are sister cells that give rise to identical sets of left-right paired cells, except that the ABaraapaaaa cell becomes the e3D cell and its lineally homologous cell ABaraappaaa becomes the MI neuron. The differential determination of cell fate by these two cells breaks left-right symmetry in these cell lineages.

We sought mutations that transform MI into an e3-like cell or e3D into an MI-like cell, thereby regenerating symmetry in these cell lineages. From screens of 10,000 mutagenized haploid genomes and unrelated screens performed previously seeking mutants abnormal in pharyngeal anatomy, we identified four independent mutations that cause transformation of MI into an e3-like cell fate. Three of the four mutations, *n1921*, *n5020*, and *n5052*, define a single complementation group. The other mutation, *n5053*, semidominantly transforms MI into an e3-like cell fate and causes recessive embryonic lethality.

*n1921*, *n5020*, and *n5052* are alleles of *ngn-1* (*neurogenin-1*), which encodes a basic helix-loop-helix (bHLH) protein. *n5053* is an allele of *hlh-2* (*helix-loop-helix-2*), the *C. elegans* ortholog of *Drosophila* E/ Daughterless. Daughterless encodes a bHLH protein known to dimerize with other bHLH proteins, including those within the neurogenin subfamily. RNAi of either *ngn-1* or *hlh-2* resulted in transformation of MI into an e3-like fate.

Thus, we have identified two genes required for breaking symmetry in a left-right asymmetric cell lineage. We hypothesize that NGN-1 and HLH-2 form a transcriptional heterodimer required for establishing the asymmetry. We hope that further analysis of these genes will uncover a mechanism by which the invariant determination of left-right asymmetry is specified.

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