ngn-1 and *hlh-2* Are Required for a Left-Right Asymmetric Neurogenesis Decision

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The body plan of *C. elegans* is mostly bilaterally symmetric. Much of this symmetry arises from analogous blastomeres, which through bilaterally symmetric cell lineages generate sets of left-right paired cells. To create either asymmetric cells or three-fold symmetry, this bilateral symmetry must be broken. The e3 pharyngeal epithelial cells form a three-fold symmetric structure that is composed of three cells, located on the ventral left (VL), ventral right (VR) and dorsal (D) region of the pharynx. e3VL and e3VR are generated as left-right lineal homologs, whereas e3D is generated by breaking left-right symmetry in a specific cell lineage. Specifically, 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 seeking mutants abnormal in pharyngeal anatomy, we identified two genes, *ngn-1 (neurogenin-1)* and *hlh-2 (helix-loop-helix-2)*, that when mutated cause the absence of MI and the presence of an extra e3-like cell. Laser ablation of the grandmother of the presumptive MI neuron in the *ngn-1* mutant results in the absence of the extra e3-like cell. These and other results suggest that MI is transformed into an e3-like cell in the mutants.

ngn-1 encodes a basic helix-loop-helix (bHLH) protein. The *ngn-1* homologs of vertebrates are required for neurogenesis. *hlh-2* is the *C. elegans* ortholog of *E2A/Daughterless*, a bHLH protein known to dimerize with other bHLH proteins, including those within the neurogenin subfamily. Mosaic analysis of *ngn-1* suggests that *ngn-1* acts cell-autonomously to specify the MI fate. We are currently examining the expression and localization of NGN-1 and HLH-2 to test whether these genes are expressed or localized asymmetrically to break the left-right symmetry in this cell lineage.

In short, we have identified two bHLH genes required for breaking symmetry in a left-right asymmetric cell lineage. We hypothesize that NGN-1 and HLH-2 form a transcriptional heterodimer that acts cell autonomously to induce left-right asymmetric neurogenesis. We hope that further analysis of these genes will uncover a mechanism by which *ngn-1* and *hlh-2* are differentially regulated to result in an invariant determination of this aspect of left-right asymmetry.

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