

Replication-Coupled Nucleosome Assembly Is Required to Generate a Bilateral Asymmetry in the *C. elegans* Nervous System

Shunji Nakano¹, Bruce Stillman² and Bob Horvitz¹

¹HHMI, Dept. Biology, MIT, Cambridge, MA 02139, USA

²Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11742, USA

Bilateral asymmetry in *C. elegans* can arise from left-right asymmetric cell lineages. The single left-right unpaired MI neuron descends from the right side of an otherwise left-right symmetric cell lineage that generates the MI neuron on the right and the e3D pharyngeal epithelial cell on the left.

We sought mutations that transform MI into an e3D-like cell or e3D into an MI-like cell, thereby generating left-right symmetry in these cell lineages. We recovered 16 mutations in seven genes. All of these mutations transform MI into an e3D-like cell. We previously showed that the establishment of the MI-e3D asymmetry requires asymmetric expression of a transcriptional cascade in which the *Otx* homeodomain protein CEH-36 is expressed in the MI grandmother cell but not in the e3D grandmother cell and that CEH-36 promotes asymmetric expression of two bHLH proteins, NGN-1 and HLH-2, in the MI mother cell but not in the e3D mother cell (Nakano et al., *Development* 137, 4017, 2010). Here we show that another isolate, *n5357*, is a gain-of-function allele of the gene *his-9*, which encodes a replication-dependent histone H3 protein. This *his-9(gf)* mutation acts cell-autonomously in the MI mother cell and affects a process downstream of or in parallel to the expression of CEH-36, NGN-1 and HLH-2.

Replication-dependent histone H3-H4 proteins are deposited onto nucleosomes by the CAF-1 (chromatin assembly factor-1) complex during DNA replication. The human CAF-1 complex is composed of three subunits, p150, p60 and p48. Inactivation of *T06D10.2*, *Y71G12B.1* and *rba-1*, which encode *C. elegans* proteins homologous to p150, p60 and p48, respectively, caused transformation of MI into an e3D-like cell, indicating that the *C. elegans* CAF-1 complex is required to generate the MI-e3D asymmetry. That a gain-of-function mutation in a histone H3 gene phenocopied the loss of CAF-1 function suggests that the mutant H3 protein inhibits CAF-1-mediated nucleosome formation. We performed a replication-coupled nucleosome assembly reaction to monitor CAF-1 activity *in vitro* and observed that corresponding mutant histone H3 proteins indeed inhibited CAF-1-mediated nucleosome formation. Our results reveal that replication-coupled nucleosome assembly is required to generate the MI-e3D asymmetry. We suggest that during S phase of the MI mother cell CAF-1 assembles nucleosomes on which the NGN-1/HLH-2 complex recruits histone-modifying enzymes to generate a chromatin state necessary to specify MI. We propose that CAF-1-mediated nucleosome formation and the asymmetric localization of the NGN-1/HLH-2 complex drive left-right asymmetric epigenetic regulation, leading to the establishment of the MI-e3D asymmetry.

Talk

Session topic: Development and Evolution; Cell fate patterning

Second session topic: Neurobiology; Neural development

Key word: bilateral asymmetry, MI, histone H3, CAF-1, chromatin assembly

Word Count: 2,994