Replication-Coupled Chromatin Assembly Generates a Neuronal Bilateral Asymmetry in C. elegans

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Replication-Coupled Chromatin Assembly Generates a Neuronal Bilateral Asymmetry in C. elegans

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SUMMARY

Although replication-coupled chromatin assembly is known to be important for the maintenance of patterns of gene expression through sequential cell divisions, the role of replication-coupled chromatin assembly in controlling cell differentiation during animal development remains largely unexplored. Here we report that the CAF-1 protein complex, an evolutionarily conserved histone chaperone that deposits histone H3-H4 proteins onto replicating DNA, is required to generate a bilateral asymmetry in the C. elegans nervous system. A mutation in 1 of 24 C. elegans histone H3 genes specifically eliminates this aspect of neuronal asymmetry by causing a defect in the formation of a histone H3-H4 tetramer and the consequent inhibition of CAF-1-mediated nucleosome formation. Our results reveal that replication-coupled nucleosome assembly is necessary to generate a bilateral asymmetry in C. elegans neuroanatomy and suggest that left-right asymmetric epigenetic regulation can establish bilateral asymmetry in the nervous system.

INTRODUCTION

Anatomical and functional bilateral asymmetries of the brain are widespread features of humans and other animals and are thought to be important for behavior and cognitive functions (Toga and Thompson, 2003). Abnormal brain lateralization has been implicated in several neuropathologies, including autism and schizophrenia (Escalante-Mead et al., 2003; Li et al., 2007). Although the nodal signaling pathway has been shown to be central in establishing left-right asymmetry in visceral organs (Levin, 2005; Shiratori and Hamada, 2006), expression of the nodal signaling genes has not been detected in the developing mammalian brain (Hamada, 2008). Nonetheless, the mouse hippocampus has been shown to display molecular bilateral asymmetry (Kawakami et al., 2003). Thus, despite its importance, the molecular mechanisms that generate brain bilateral asymmetry remain largely elusive.

Although mostly bilaterally symmetric, the C. elegans nervous system displays a variety of bilateral asymmetries (Hobert et al., 2002). For example, the MI motor neuron is a single left-right unpaired neuron located in the pharynx (Albertson and Thomson, 1976). The MI neuron is generated from an invariant left-right asymmetric cell lineage in which the blastomere ABaraap divides and generates two daughter cells, ABarapa and ABaraapp, that give rise to identical sets of left-right paired cells, except for two cells, the left-right unpaired MI neuron on the right side of the cell lineage and the e3D pharyngeal epithelial cell on the left (Sulston et al., 1983) (Figure 1A). Thus, the generation of the single unpaired MI neuron results from the disruption of the bilateral symmetry in this cell lineage.

Here we report that chromatin assembly factor-1 (CAF-1), which deposits histone H3-H4 proteins onto replicating DNA (Smith and Stillman, 1989), is required to generate the MI-e3D asymmetry. We find that a mutation in 1 of 24 histone H3 genes can cause the loss of the MI-e3D bilateral asymmetry. This histone H3 mutation causes a defect in the formation of a histone H3-H4 tetramer and the consequent inhibition of CAF-1-mediated nucleosome formation. Our results indicate that replication-coupled de novo nucleosome assembly in the MI mother cell is required to generate a chromatin mark that is then transmitted to the postmitotic MI neuron. We suggest that left-right asymmetric epigenetic regulation is involved in the establishment of the neuronal bilateral asymmetry and that epigenetic regulation similarly acts to generate neuronal bilateral asymmetry in mammals through a mechanism independent of nodal signaling.
Figure 1. *n*5357 Is a Gain-of-Function Allele of a Histone H3 Gene and Causes the Loss of the MI-e3D Bilateral Asymmetry

(A) The ABaraap cell lineages in the wild-type and *n*5357 mutants.

(B) Expression of the D2096.6::pes-10::gfp and sams-5::gfp reporters in a wild-type and an *n*5357 animal. The arrowheads indicate e3D and the extra e3D-like cell. The arrow indicates the MI neuron. Scale bar, 5 μm.

(C and D) Percentage of animals showing the MI transformation defect. The MI transformation defects in (D) were determined at 15°C. We noted that the MI transformation defect of *n*5357/*n*5357 animals in (D) was less severe than that of *n*5357/*n*5357 animals in (C) and found that this difference was caused by a background mutation(s) present in the *n*5357/*n*5357 strain used in (D) (see Experimental Procedures).

(E) A physical map of the *n*5357 region and the location of a mutation in the *his-9* locus of *n*5357 animals are shown. The numbers indicate the chromosome coordinates to which we mapped the *n*5357 mutation.

(F) Germline transformation experiments using genomic *his-9* clones. The structure of each genomic clone and the percentage of animals showing the MI transformation defect at 15°C are shown.

See also Figure S1.
RESULTS

A Histone H3 Mutant Eliminates the MI-e3D Bilateral Asymmetry

To elucidate the mechanism that establishes the MI-e3D asymmetry, we performed genetic screens with an e3D cell-fate reporter, D2096.8::pes-10::gfp (Nakano et al., 2010), and sought mutants in which an extra e3D-like cell was present or e3D was absent (our unpublished data). Among the isolates we recovered was the mutation n5357, which caused the presence of an extra e3D-like cell (Figure 1B). We introduced an MI cell-fate reporter, sams-5::gfp (Nakano et al., 2010), into n5357 mutants and observed that MI was missing in n5357 animals (Figure 1B), indicating that MI is transformed into an e3D-like cell, thereby resulting in the loss of the MI-e3D bilateral asymmetry (Figure 1A). n5357 causes a cold-sensitive and semidominant phenotype (Figure 1C). To determine whether n5357 is a gain- or loss-of-function mutation, we performed a gene-dosage study and found that the presence of a free duplication chromosome covering the n5357 region neither enhanced nor suppressed the MI transformation defect caused by n5357 (Figure 1D and Experimental Procedures). This result indicates that n5357 is a gain-of-function mutation and is likely an altered-function mutation.

We mapped n5357 to a 55 kb interval of chromosome II that contains a cluster of 13 predicted histone genes (Figure 1E). We performed DNA sequence analysis of n5357 and identified a mutation in the gene his-9, which encodes a histone H3 protein (Pettitt et al., 2002). The sequence of the 135 amino acid HIS-9 protein displays a higher degree of identity to those of the human replication-dependent histones H3.1 and H3.2 than to that of the replication-independent histone H3.3 (Figure S1). The C. elegans genome contains 24 histone H3 loci, 14 of which are replication-dependent histone H3 genes that encode proteins with amino acid sequences identical to that of the HIS-9 protein (Pettitt et al., 2002). n5357 animals carry a transition mutation that is predicted to alter the glutamine 125 codon to an ochre stop codon (Figure 1E). To test whether this DNA lesion is responsible for the MI transformation of n5357 animals, we generated genomic clones carrying the wild-type his-9(+) or a mutant his-9(Q125sochre) gene and three wild-type histone genes (his-10, his-11, and his-12) located 5’ upstream of his-9 (Figure 1F). Because our gene-dosage study indicated that n5357 is a gain-of-function mutation, we introduced the his-9(Q125sochre) clone into wild-type animals. We observed that wild-type animals carrying the his-9(Q125sochre) clone displayed MI transformation (Figure 1F). By contrast, the MI neuron was not transformed in wild-type animals carrying an extrachromosomal array containing the his-9(Q125sochre) clone (Figure 1F). In addition, a frameshift mutation in the his-9 locus of the his-9(Q125sochre) clone prevented transformation of MI (Figure 1F). These results further indicate that n5357 is a gain-of-function allele of his-9.

Mutant H3 Lacking H3-H3 Interaction Sites Causes MI Transformation

During nucleosome assembly, a histone H3 protein binds to a histone H4 protein to form an H3-H4 dimer, and two H3-H4 dimers bind through an interaction between two H3 proteins to form an H3-H4 tetramer (Luger et al., 1997). Based on the crystal structure of the nucleosome core particle (Luger et al., 1997), we postulated that the his-9(Q125sochre) mutation eliminates two residues, leucine 126 and isoleucine 130, engaged in the H3-H3 interaction (Figure 2A). That the his-9(n5357) mutant phenotype is cold sensitive is consistent with the hypothesis that an abnormality in a protein-protein interaction causes the his-9 gain-of-function activity (Richards et al., 2000). To test this hypothesis, we introduced into wild-type animals his-9 genomic clones in which the his-9 coding sequences were truncated at the glutamine 125 codon (qQ125-A135), the arginine 116 codon (qR116-A135), or the aspartic acid 106 codon (JD106-A135) (Figure 2B). All of these mutant his-9 clones eliminated HIS-9 residues important for the H3-H3 interaction, and all caused MI transformation (Figure 2B).

Based on nucleosome crystal structure, H3-H3 interaction is mediated in part by a hydrogen bond between histidine 113 of one histone H3 protein and aspartic acid 123 of the second H3 molecule (Luger et al., 1997). To further test whether a lack of histone H3-H3 interaction sites causes the gain-of-function activity of his-9, we generated mutant his-9 genomic clones in which the histidine 113 codon or the aspartic acid 123 codon was altered to an aspartic acid codon (H113D) or a histidine codon (D123H), respectively (Figure 2B). Introduction of these mutant his-9 clones into wild-type animals caused MI transformation (Figure 2B). These results demonstrate that mutant HIS-9 proteins unable to form an H3-H4 tetramer through interaction with another H3 protein display a gain-of-function activity and cause the loss of the MI-e3D bilateral asymmetry.

his-9(gf) Acts in the MI Mother Cell to Cause the Loss of the MI-e3D Asymmetry

We next examined whether the his-9 gain-of-function activity affects the left-right asymmetric expression of CEH-36, NGN-1, and/or HLH-2. We integrated wild-type and mutant his-9 transgenes into C. elegans chromosomes and found that an integrated his-9(H113D) transgene caused the most severe MI transformation defect (Table S1). Expression of CEH-36, NGN-1, and HLH-2 remained left-right asymmetric in animals carrying the his-9(+) or his-9(H113D) transgene (Figures 3A–3C), indicating that the his-9 gain-of-function activity affects a process downstream of or in parallel to the left-right asymmetric expression of CEH-36, NGN-1, and HLH-2.

To identify when and where his-9 gain-of-function activity acts to cause the loss of the MI-e3D asymmetry, we performed a genetic mosaic analysis (Figure 3D). We generated animals carrying an extrachromosomal array containing the his-9(H113D) transgene marked with cell-autonomous gfp reporters sur-5::gfp and unc-119::gfp (Nakano et al., 2010). Extrachromosomal arrays in C. elegans are mitotically unstable, resulting in clones of cells that have lost the array. We used Nomarski optics and epifluorescence to examine animals carrying the his-9(H113D) transgene and determined in each animal (1) the fate of ABArappapa, i.e., an MI-neuronal fate or an e3D-like epithelial fate, judged by the size and morphology of its nucleus and (2) the cell division at which the his-9(H113D) transgene was lost, judged by the GFP fluorescence of cells derived from the ABAr cell lineage (Figure 3D). This analysis of GFP fluorescence
allowed us to determine the precise cell division at which an array loss occurred among the cell divisions of ABara descendants that give rise to the postmitotic ABaraappaaa. (We could not analyze the cell division of the MI mother cell ABaraappaa because the MI sister cell becomes the m1DR pharyngeal muscle cell, and m1DR fuses with five other m1 muscle cells later in development [Albertson and Thomson, 1976]. Thus, GFP fluorescence in m1DR might not represent the inheritance of the array to m1DR but rather result from the presence of the array in any of the five other m1 muscle cells derived from other cell lineages.) In our experiment, animals in which the array was lost at the division of the MI mother cell would have been counted as animals in which the array loss occurred at the previous cell division, that of ABaraapp. We observed that none of the 45 animals in which the array loss had occurred at or prior to the division of ABaraapp showed MI transformation (Figure 3D). By contrast, of 686 animals in which the his-9(H113D) array was present in the postmitotic ABaraappaaa cell, 293 animals (43%) displayed MI transformation (Figure 3D), suggesting that his-9 gain-of-function activity cell-autonomously acts in the MI mother cell to cause MI transformation. We concluded that his-9 gain-of-function activity cell-autonomously acts in the MI mother cell to cause MI transformation.

The MI Fate Specification Is Sensitive to Histone H3 Mutations

his-9(n5357) animals are grossly normal, and we did not identify defects in the generation of other pharyngeal cells in these mutant animals. Given this specific effect of his-9 gain-of-function activity, we asked whether expression of his-9 is restricted to the cell lineage that gives rise to MI. We generated wild-type his-9(+) and mutant his-9(H113D) translational mStrawberry fusion transgenes and found that his-9(H113D)::mStrawberry but not his-9(+)::mStrawberry caused MI transformation (Figure 4A), suggesting that these his-9 trangenes are functional. Both his-9(+):mStrawberry and his-9(H113D):mStrawberry localized to nuclei of many cells, including MI, e3D, and the extra e3D-like cell (Figure 4A), indicating that his-9 is broadly expressed.

Given the presence in the C. elegans genome of 13 additional replication-dependent histone H3 genes that encode proteins with amino acid sequences identical to that of HIS-9 (Figure 4B), we asked whether mutations in these histone H3 genes could...
also cause MI transformation. We generated wild-type (+) and mutant (H113D) transgenes of three additional histone H3 genes, his-2(V), his-13(II), and his-32(IV), and found that none of the wild-type transgenes caused MI transformation (Figure 4B). By contrast, all three of the mutant histone H3 transgenes caused MI transformation but no other gross abnormalities (Figure 4B). These observations suggest that MI cell-fate specification involves a mechanism that is markedly sensitive to gain-of-function mutations in histone H3 genes.

**CAF-1 Is Required for the MI-e3D Asymmetry**

The human CAF-1 protein complex deposits histones H3-H4 into nucleosomes for DNA-replication-dependent nucleosome assembly (Smith and Stillman, 1989). CAF-1 is composed of three subunits, p150, p60, and p48 (Kaufman et al., 1995; Verrreault et al., 1996), and preferentially binds to a histone H3-H4 complex that contains the replication-dependent histone H3.1 (Tagami et al., 2004). The *C. elegans* genome encodes proteins homologous to the CAF-1 subunits, including the p150 homolog T06D10.2, the p60 homolog Y71G12B.1, and the p48 homologs LIN-53 (Lu and Horvitz, 1998) and RBA-1 (Solari and Ahringer, 2000). We named T06D10.2 and Y71G12B.1 chaf-1 and chaf-2 (chromatin assembly factor), respectively. Inactivation of chaf-1, chaf-2, or rba-1 but not lin-53 by RNA interference (RNAi) caused MI transformation (Figure 5 and unpublished data); in addition, RNAi treatment of any of these four genes caused incompletely penetrant embryonic lethality (data not shown). We also isolated dele- tion alleles of chaf-1 and rba-1 (Figure S2). Both deletion mutants displayed maternal-effect embryonic lethality, but chaf-1(n5453D) and rba-1(n5418D) deletion homozygous animals derived from their respective heterozygous mothers did not show MI transformation (Table S2). Given that RNAi of chaf-1 and rba-1 caused MI transformation, these observations suggest that the MI transformation defect of chaf-1 and rba-1 mutants might be maternally rescued. We therefore tested whether the zygotic loss of chaf-1 and rba-1 activity sensitizes his-9(gf) mutants to the MI transformation defect. Indeed, we observed that chaf-1(n5453.D) and rba-1(n5418.D) each enhanced the MI transformation defect of his-9(n5357) animals (Table S2). We conclude that the *C. elegans* CAF-1 complex is required to establish the MI-e3D asymmetry.

Human CAF-1 has been shown to interact with the proliferating cell nuclear antigen PCNA (Shibahara and Stillman, 1999). PCNA associates with replicated DNA and helps mark newly replicated DNA for CAF-1-mediated chromatin assembly. We therefore asked whether the *C. elegans* ortholog of PCNA, pcn-1, is required to establish the MI-e3D asymmetry. We observed that RNAi treatment of pcn-1 caused MI transformation.
transformation (Figure 5). Thus, pcn-1 is also required to generate the MI-e3D asymmetry. We suggest that replication-dependent nucleosome formation mediated by CAF-1 and PCN-1 is necessary to generate the MI-e3D asymmetry.

Drosophila dASF1 was identified as a factor that stimulates CAF-1-mediated nucleosome formation (Tyler et al., 1999). ASF1 was shown to bind to an H3-H4 dimer (English et al., 2006; Natsume et al., 2007) and proposed to transfer an H3-H4 dimer to the CAF-1 complex (Loyola and Almouzni, 2004). We tested whether two C. elegans ASF1 homologs, unc-85 and asf-1 (Grigsby and Finger, 2008), are required for the MI-e3D asymmetry. We observed that MI and e3D dimer to the CAF-1 complex (Loyola and Almouzni, 2004). We tested whether two C. elegans ASF1 homologs, unc-85 and asf-1 (Grigsby and Finger, 2008), are required for the MI-e3D asymmetry. We observed that MI and e3D were correctly specified in his-9(n5357) and asf-1(ok2060) mutants (Figure S2 and Table S3), asf-1(ok2060); unc-85(ok2125) double mutants displayed sterility but did not show MI transformation (Table S3). These results indicate that the establishment of the MI-e3D asymmetry does not require the two C. elegans homologs of ASF1, and that CAF-1 functions independently of the ASF1 proteins to establish the MI-e3D asymmetry.

Mutant HIS-9 Proteins Inhibit Nucleosome Assembly

That a defect in any of the three CAF-1 subunits caused precisely the same highly specific abnormality as the his-9(n5357) mutation strongly suggests that CAF-1-mediated nucleosome formation is impaired by mutant HIS-9 proteins. To test this hypothesis, we performed replication-coupled chromatin assembly reactions with wild-type and mutant histone H3 genes that encode proteins with amino acid sequences identical to that of HIS-9 are shown. Numbers represent approximate genetic map positions. Percentages of animals with the MI transformation defect are shown.

Figure 4. MI Fate Specification Is Sensitive to Histone H3 Mutations

(A) Expression of translational his-9(+); mStrawberry (top panel) and his-9(H113D); mStrawberry (bottom panel) genes during embryonic (left panel) and larval (right panel) stages. Nomarski images (middle panel) of the same larvae and percentages of animals with the MI transformation defect are shown. Arrows indicate the MI neuron, and arrowheads indicate the e3D and the e3D-like epithelial cells. Scale bar, 5 μm.

(B) The genomic positions of his-9 and 13 additional replication-dependent histone H3 genes that encode proteins with amino acid sequences identical to that of HIS-9 are shown. Numbers represent approximate genetic map positions. Percentages of animals with the MI transformation defect are shown.
observed that addition of H3(H113D) to the MI-e3D bilateral asymmetry is disrupted. The causes an unexpectedly specific defect: the establishment of the gain-of-function allele of the his-9 gene from a genetic screen of a multicellular organism. This mutation, We report the isolation of the first mutant histone allele recovered from a genetic screen of a multicellular organism. This mutation, C. elegans

**DISCUSSION**

We report the isolation of the first mutant histone allele recovered from a genetic screen of a multicellular organism. This mutation, a gain-of-function allele of the C. elegans histone H3 gene his-9, causes an unexpectedly specific defect: the establishment of the MI-e3D bilateral asymmetry is disrupted. The his-9 gain-of-function activity acts cell-autonomously in the MI mother cell and affects a process downstream of or parallel to the left-right asymmetric expression of the transcription factors NGN-1 and HLH-2. The CAF-1 protein complex is required for the MI-e3D asymmetry, and the mutant HIS-9 protein acts by inhibiting CAF-1-mediated nucleosome formation. These results suggest
Thus, our results suggest that during normal replication-coupled chromatin assembly, CAF-1 binds to an H3-H4 dimer, and that two CAF-1-H3-H4 complexes assemble to form an H3-H4 tetramer, thereby depositing an H3-H4 tetramer that consists entirely of newly synthesized H3 and H4 proteins. During this process of DNA replication, a parental H3-H4 tetramer remains intact and is transferred to newly replicated DNA.

Our observations also reveal that the two C. elegans homologs of ASF1 are not required to establish the MI-e3D asymmetry, indicating that an ASF1-independent pathway exists that
leads to complex formation between CAF-1 and an H3-H4 dimer.

We previously showed that the specification of the MI-e3D asymmetry occurs at least three cell generations prior to the generation of MI and e3D within two sister cells, ABaraapa and ABaraapp, that first separate the left and right branches of the ABaraap cell lineage (Nakano et al., 2010). This initial asymmetry is transduced into the MI mother cell by a CEH-36/NGN-1/HLH-2 transcriptional cascade that acts through multiple rounds of cell divisions. The CEH-36 protein is present in the MI grandmother cell and promotes expression of two proneural bHLH proteins, NGN-1 and HLH-2. The NGN-1 and HLH-2 proteins form a dimer and are present in the MI mother cell. Given previous reports that mammalian homologs of these bHLH proteins regulate histone modifications (Beck et al., 2009; Pujadas et al., 2011), we suggest that the NGN-1/HLH-2 complex recruits histone-modifying enzymes that act on the nucleosomal arrays assembled by CAF-1 to generate epigenetic marks necessary for the specification of the MI neuron. Green circles represent a posttranslational modification. (See text for details.)

The effect of the gain-of-function mutations in histone H3 genes is strikingly specific to MI cell-fate specification, which suggests that a mechanism of specifying the MI neuron is particularly sensitive to histone H3 gain-of-function mutations. One possible mechanism could be the generation of differing nucleosome densities at a locus important for MI cell-fate specification between two sister chromatids during S phase of the MI mother cell (Figure 7B). Specifically, it has been proposed that human CAF-1 and PCNA can generate a difference in the densities of newly synthesized nucleosomes at a given locus between two sister chromatids (Shibahara and Stillman, 1999). Upon selective segregation of these epigenetically distinct chromatids marked with differing nucleosomal density results in the specification of the MI neuron.
propose that such newly synthesized nucleosomes are modified by the NGN-1/HL2 complex, leading to the generation of differing densities of modified histones between two sister chromatids. There would then be a selective inheritance of these distinct chromatin structures to the MI neuron and its sister m1DR muscle cell (Figure 7B). In this way, both NGN-1/HL2-mediated histone modifications and CAFC1-mediated specific nucleosome density would be required for expression of target genes necessary to specify the MI-neuronal fate.

An alternative possibility is that the NGN-1/HL2 complex and CAFC1 could act on distinct sets of target genes. In this case, the NNGN-1/HL2 complex might modify histones at one locus that are deposited by a replication-independent pathway, and CAFC1/PCCN1 might generate differing nucleosome densities at another locus between sister chromatids. Nonetheless, the simplest hypothesis is that both act on the same target genes to together control distinct aspects of a left-right asymmetric epigenetic regulation that specifies the MI bilateral asymmetry.

Such a mechanism of selective inheritance of sister chromatids might also be involved in left-right axis determination in mammals. *inversus viscerum* (iv) mutant mice were originally reported to show randomized directionality of their visceral asymmetries (Hummel and Chapman, 1959). The iv locus encodes a microtubule-based motor protein, called left-right dynein (LRD) (Supp et al., 1997), that is required for nodal cilia motility and nodal flow (Okada et al., 1999). Interestingly, LRD has been implicated in the biased chromatid segregation of mouse chromosome 7 in embryonic stem cells, endoderm, and neuroectoderm (Armakolas and Klar, 2007), and recently it was reported that the iv mutant hippocampus exhibits a loss of bilateral asymmetry rather than randomized directionality of the asymmetry (Kawakami et al., 2008). Our findings provide a link between these observations and suggest that LRD might promote a nervous system bilateral asymmetry through biased segregation of chromatids in a manner independent of nodal signaling. We suggest that selective segregation of chromatids marked with distinct chromatin states could be a conserved epigenetic mechanism that generates bilateral asymmetries in the nervous system in organisms as distant as nematodes and mammals.

**EXPERIMENTAL PROCEDURES**

**C. elegans Strains**

C. elegans strains were grown as described (Brenner, 1974). N2 (Bristol) was the wild-type strain. All strains were cultured at 20°C unless otherwise indicated. Germline transformation experiments were performed as described (Mello et al., 1991). A description of the plasmid constructs and concentrations used is available from the authors. The following extrachromosomal arrays, indicated: (Edgley et al., 2006), nEx1708[his-9(+), unc-76(+), tph-1::mStrawberry], nEx1711[his-9(H113D), unc-76(+), tph-1::mStrawberry], nEx1712[his-9(D123H), unc-76(+), sre-1::mStrawberry] (all this study).

Free duplications and extrachromosomal arrays: mnDp34[htf] (all this study).

**Isolation of his-9(n5357), chaf-1(n5453), and rba-1(n5418)**

We mutagenized wild-type animals carrying the D2096.6::pes-10::gfp reporter with ethyl methanesulfonate (EMS), and F2 progeny were observed using a fluorescence-equipped dissecting microscope. his-9(n5357) was isolated in an animal that contained an extra cell expressing the D2096.6::pes-10::gfp reporter. To isolate deletion alleles of chaf-1 and rba-1, we screened by PCR genomic DNA pools from EMS-mutagenized animals for deletion alleles of chaf-1 and rba-1, essentially as described (Liu et al., 1999). The chaf-1(n5453) and rba-1(n5418) alleles removed sequences between nucleotides 9937 and 10864 of cosmid T06D10 and between nucleotides 35977 and 37677 of cosmid K07A1, respectively.

**Gene-Dosage Study of n5357**

We allowed hermaphrodites of genotype rol-1(e91) n5357 unc-52(e444) to self-fertilize at 15°C and determined the MI transformation defect of their Rol Unc and non-Rol non-Unc progeny at 15°C. We noted that the MI transformation defect of animals of genotype rol-1(n5357) unc-52 rol-1(n5357) unc-52 shown in Figure 1D was less severe than that of animals of genotype n5357/n5357 shown in Figure 1C. We found that this difference in the penetrance of the MI transformation defects was caused by the presence of a mutation(s) that partially suppresses MI transformation and is derived from strains carrying unc-52(e444). The existence of this mutation, which was present in the two strains used in Figure 1D but was absent in all other strains used in this work, does not affect any of our conclusions. We did not further study this suppressor activity.

**Mapping of n5357**

We crossed rol-1(e91) n5357 unc-52(e444) animals with the wild-type polymorphic strain CB4856 and isolated F2 Rol non-Unc progeny, determined the presence or absence of n5357, and identified crossover sites, essentially as described (Wicks et al., 2001). We mapped n5357 to a 55 kb interval between nucleotides 13781415 and 13837046 of LGII.

**Supercoiling Assay**

Wild-type and mutant histone proteins were purified as described (Luger et al., 1997). We observed that the MI transformation defect of animals of genotype rol-1(n5357) unc-52 rol-1(n5357) unc-52 shown in Figure 1D was less severe than that of animals of genotype n5357/n5357 shown in Figure 1C. We found that this difference in the penetrance of the MI transformation defects was caused by the presence of a mutation(s) that partially suppresses MI transformation and is derived from strains carrying unc-52(e444). The existence of this mutation, which was present in the two strains used in Figure 1D but was absent in all other strains used in this work, does not affect any of our conclusions. We did not further study this suppressor activity.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes two figures and three tables and can be found with this article online at doi:10.1016/j.cell.2011.11.053.
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