MIRAGE DNA TRANSPOSON SILENCING

BY

C. ELEGANS CONDENSIN II SUBUNIT HCP-6

A Masters Thesis Presented

By

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ABSTRACT

Mobile genetic elements represent a large portion of the genome in many species. Posing a danger to the integrity of genetic information, silencing and structural machinery has evolved to suppress the mobility of foreign and transposable elements within the genome. Condensin proteins – which regulate chromosome structure to promote chromosome segregation – have been demonstrated to function in repetitive gene regulation and transposon silencing in several species. In model system *Caenorhabditis elegans*, microarray analysis studies have implicated Condensin II subunit HCP-6 in the silencing of multiple loci, including DNA transposon MIRAGE. To address the hypothesis that HCP-6 has a direct function in transcriptional gene silencing of the MIRAGE transposon, we queried MIRAGE expression and chromatin profiles in wild-type and *hcp-6* mutant animals. Our evidence confirms that HCP-6 does indeed function during silencing of MIRAGE. However, we found no significant indication that HCP-6 binds to MIRAGE, nor that HCP-6 mediates MIRAGE enrichment of H3K9me3, the repressive heterochromatin mark observed at regions undergoing transcriptional silencing. We suggest that the silencing of MIRAGE, a newly evolved transposon and the only tested mobile element considerably de-repressed upon loss of HCP-6, is managed by HCP-6 indirectly.

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CHAPTER I

Introduction

Maintenance of genome integrity is essential to the successful propagation and proliferation of any species. In all organisms, the abundant presence of repetitive and mobile elements within the genome presents a danger to the stability and fidelity of genetic information, as well as a potential reduction in fitness for the organism as a whole. Consequently, molecular machinery has evolved in order to suppress repeat sequence expression and transposable element (TE) mobility within the genome. Regulation of gene expression by the condensin complex of structural proteins has been observed in several species, and in model system *Caenorhabditis elegans*, Condensin II subunit HCP-6 has been implicated in silencing transposon MIRAGE, as well as genic loci.

The nematode small interfering RNA (siRNA) pathway is likewise responsible for the silencing of endogenous mobile elements and protein coding loci, as well as exogenously introduced double-stranded RNA (dsRNA). Silencing occurs via both transcriptional and post-transcriptional mechanisms, and has suggested the action of chromatin remodeling and structural proteins within the pathway. Serving as an essential structural component of chromatin, Condensin II may function as a key constituent in transposon silencing. This

study aims to elucidate the molecular mechanism by which *C. elegans* DNA transposon MIRAGE is controlled, and what function HCP-6 contributes to its silencing.

1.1 Mobile genetic elements pose a threat to the genome

The genomes of eukaryotes are largely composed of repetitive sequences. In addition to coding and functionally repetitive regions such as ribosomal DNA loci, operative gene duplications, telomeres and centromeres, a majority of the repetitive sequence pool is composed of non-coding or "junk" DNA – tandem arrays and satellite repeats, pseudogenes, and transposable elements. Set apart from the many non-coding RNAs that supply a variety of gene-regulatory roles, "junk" sequences of this nature serve no currently known function. On the converse, such repeat elements are considered molecular parasites, with overall detrimental effects on an organism's fitness.

An estimated 45% of the human genome is recognized as being derived from transposable elements (TEs)[58]. Characterized as mobile genetic elements, TEs are DNA sequences that are capable of being reproduced and integrated into new locations within the host genome[3,50]. Eukaryotic TEs are broken into two basic classes, and can be either autonomous or non-autonomous in variety. Class I Retrotransposons are mobilized through an RNA intermediate – they are expressed within the host, their messenger RNA (mRNA) reverse transcribed, and the resulting complimentary DNA (cDNA) introduced back into the

genome[50,81]. Class II DNA Transposons mobilize through a “cut-and-paste” mechanism – these elements encode and express a transposase enzyme, which excises the element’s DNA by binding at its termini; the excised sequence is re-integrated into the genome at preferred element-specific nucleotide sequences or genome hot-spots. Excision is generally mediated by transposase recognition of Terminal Inverted Repeats (TIRs) – inverted sequence of 10-400 bp flanking the transpose gene – although some known active Class II elements exhibit imperfect TIRs or lack them entirely[50,100].

As implied, autonomous elements are those that encode all of the enzyme(s) directly necessary for transposition. Alternatively, non-autonomous elements have lost the ability to transpose under their own expression, largely through mutation or degeneration of element sequence. Lack of self-sufficiency in non-autonomous elements does not keep them immobile; these deteriorated relic sequences may be recognized and excised by the active transposase of a related autonomous family-member element[81]. Additionally, the transposase of DNA elements is not *cis*-acting. Following expression, transposase mRNA must still be transported to the cytoplasm for production of the enzyme; upon re-entry to the nucleus, the enzyme is unable to discriminate between active and inactive elements[58].

Transposable elements are damaging both to an organism’s viability and the viability of its offspring. On the smallest scale, excision and insertion of TEs results in double strand breaks and nicks in DNA. Such lesions trigger DNA

damage-response and repair pathways, and may ultimately lead to cell-cycle checkpoint activation and cell death. On a grander scale, TE mobilization can trigger genome instability in the form of large deletions or rearrangements of the genome, precipitated by altered recombination via repetitive regions as well as the simple uptake and redistribution of neighboring sequence as an element mobilizes. Excision and insertion of an element may disrupt the gene coding sequence, or modify gene function by altering upstream or downstream regulatory regions. Any mutations sustained in the germline will be passed down to offspring, and have the potential to affect the fecundity of further progeny.

Mobile elements pose an additional danger to the genome when considering the ecology of an organism as a whole. Selfish genetic elements incite a manner of genetic conflict within an individual, as they are capable of "enhancing [their] own transmission relative to the rest of an [organism's] genome"[96]. Such competition is disadvantageous, as these elements impose increased energy demands on an organism by appropriating its transcriptional and translational resources and usurping cellular machinery.

Transposable elements in *Caenorhabditis elegans*. Roughly one-fifth of the genome of *C. elegans* is estimated to be composed of transposable elements and their derivatives[20,92]. While such representation is relatively moderate in comparison with other organisms, the nematode stands apart in that

95% of this sequence is of the Class II DNA Transposon variety – significantly higher than Class II representation in any other species tested thus far[25,58].

The remaining small fraction of mobile elements is comprised of LTR and non-LTR retrotransposons. Together consisting of 23 currently delineated families, they account for more than one thousand detected full-length and degenerate sequences[8,11]. However, retrotransposition in the nematode genome has yet to be reported[8].

The Tc1 DNA Transposon. The most abundant and characteristic species of DNA transposon within the nematode is Tc1; it is represented by 32 individually active copies within the *C. elegans* Bristol N2 strain, and upwards of 300 copies within the permissive strain Bergerac BO[20]. The founding member of the Tc1/*mariner* superfamily, in nematodes Tc1 is 1,610 base pair (bp) long and contains two 54 base pair terminal inverted repeats[71,72]. Extremely widespread in nature, Tc1 elements of varying lengths have been noted throughout the three domains of life[8].

In *C. elegans*, Tc1 is reported to be transcribed as a single long transcript containing two open reading frames (ORFs), and producing two polypeptides from different translational reading frames; the transposase enzyme only has been attributed to the transcript[71,97]. Transcription of Tc1 is entirely dependent on read-through from neighboring genes however, as the element does not have its own internal promoter[73,91]. Fully silenced in the germline of wild-type Bristol

N2 worms by siRNA mechanisms, Tc1 is active in the soma and requires only its single transposase for mobility; the intense activity of Tc1 in the germline of the Bergerac strain is hypothesized to be the main cause of spontaneous mutations in that strain[38,52]. Tc1 transposition favors integration into TA dinucleotide sequence, and the staggered double strand break (DSB) which remaining following excision generally reveal a TIR footprint[71].

The MIRAGE DNA Transposon. Unlike the widespread Tc1 element, the MIRAGE family of DNA transposon has been identified in the nematode genome only[50]. Discovered only recently based on computational studies, it is considered to be a very young autonomous transposable element[50]. Currently, there are twelve annotated copies of MIRAGE fixed in the genome – six full-length copies, and six partial or degraded copies. A full length MIRAGE element is represented by a 5,619 base pair sequence, composed of two open reading frames flanked by 53 base pair terminal inverted repeats. The novel transposase encoded by MIRAGE is not similar to those encoded by any known mobile element superfamily; however, discovery of a ribonuclease H (RNase H)-like fold containing the catalytic amino acid triad DDE/D – two aspartic acid (D) residues followed by a glutamic or aspartic acid (E/D) residue – strongly suggests that the transposase is functionally active[50,67,100]. Excision of MIRAGE generates 2 base pair target site duplications (TSDs).

Like Tc1, the transposase of MIRAGE does not possess an internal promoter, and expression may be attributed to read-through transcription; DNA transposons in many species exhibit a propensity for insertion within introns[25,26]. Indeed, several MIRAGE elements are located within large introns of protein-coding genes expressed throughout development[15,20,97]. Transcribed at significantly lower levels than the surrounding genic material, expression of MIRAGE mRNA nevertheless appears to peak at the young adult stage[97]. This is consistent with the paradoxical idea of low-level, baseline transcription of the entire genome, and the necessity of transcription from heterochromatic loci in order to induce their silencing[68,72,90].

Cer Family Retrotransposons. While comprising only a small fraction of the *C. elegans* TE pool, retroelements are represented within the organism by nineteen LTR families and four non-LTR families[8,29]. Like their viral precursors, the Cer (*C. elegans* retrotransposon) LTR elements contain Gag (group specific antigen) and Pol (polymerase) regions, which encode a zinc-coordinating motif and the reverse transcriptase, RNase H, and integrase (INT) enzymes, respectively[11]. In addition to LRTs, Cer elements begin and end with family-specific dinucleotide inverted repeats (DIRs), and terminal-most flanking repeats caused by repair of integration events[11].

1.2 Small interfering RNA pathways combat repetitive sequences

In order to counteract the adverse fitness effects presented by parasitic genetic elements, organisms throughout the three domains of life have evolved machinery to suppress transposon mobilization within the genome. In model system *Caenorhabditis elegans*, transposable element inhibition is mediated concurrently through the Piwi-interacting (piRNA) pathway and the endogenous WAGO/*mutator* associated siRNA pathway. Broadly, silencing pathways in *C. elegans* operate by initially producing low-abundance primary siRNAs; these are then used as triggers to amplify the small RNA silencing signal by generating – through an RNA-dependent RNA polymerase (RdRp) mechanism – highly abundant effector secondary siRNAs[5,70,99]. Secondary siRNAs interact with a subset of Argonaute proteins, and mediate transcriptional and post-transcriptional gene silencing (TGS and PTGS, respectively) via complimentary binding to nascent (TGS) and mature (PTGS) RNA transcripts[31,67].

Primary 21URNAs

Primary small interfering RNAs belonging to the piRNA pathway are directly transcribed by RNA Polymerase II from two regions of chromosome IV, and rely on two upstream sequence motifs to regulate expression[6,75]. Mature *C. elegans* piRNAs are 21 nucleotides in length with a 5' monophosphorylated uridine – consequently termed 21U RNAs – and interact with Argonautes PRG-1 and PRG-2 (Piwi-related gene-1 and -2)[6,75]. 21U RNA transcripts are highly

expressed in the germline and early embryo, and enriched for transposon sequence both in the sense and antisense orientation[5,6,21]. Paradoxically, many transposable elements currently annotated – including DNA Transposon MIRAGE – do not themselves exhibit mapping 21U RNAs, and may TEs escape 21U recognition[6]. 21U RNAs do not show sense/antisense bias[75].

Additionally, while PRG Argonautes do contain the catalytic motif indicative of endonuclease "slicer" activity, this is reported to be dispensable for 21U RNA silencing[5]. As such, primary 21U RNAs feed into the RdRp-driven secondary siRNA amplification cycle, producing the WAGO class 22G siRNAs responsible for the bulk of transposon silencing[2,5].

Secondary siRNAs

Secondary siRNAs are predominantly antisense to their targets, and exist in two flavors – the WAGO class 22G RNAs and the CSR-1 ("caesar" – chromosome-segregation and RNAi deficient-1) associated 22G RNAs. As implied, these siRNAs are most often 22 nucleotides in length, and predominantly start with a 5' guanosine; they posses a triphosphorylated 5' terminus as a result of RdRp amplification[31]. Dicer-related helicase-3 (*drh-3*) is a key component of the core RdRp module responsible for secondary siRNA amplification, and loss of this factor dramatically reduces both WAGO and CSR-1 22G RNA formation ,and desilences 22G RNA target loci[37].

WAGO group 22G RNAs and transcriptional silencing. The twelve WAGO group Argonautes can also be subdivided into two factions – nuclear and cytoplasmic. Nuclear WAGOs HRDE-1 (heritable RNAi defective-1) and NRDE-3 (nuclear RNAi defective-3) operate in nuclear silencing pathways of the germline and soma, respectively, and depend upon non-Argonaute core nuclear silencing factors NRDE-1/2/4 for effective transcriptional gene silencing[12,13,35]. The remaining cytoplasmic WAGOs act semi-redundantly to mediate post-transcriptional silencing[99]. However, WAGO Argonautes lack the catalytic residues required for an active endonuclease domain, and thus are unable to mediate silencing through "slicer" activity; precisely how WAGO 22G RNAs achieve post-transcriptional gene silencing remains under investigation[43,99].

The mechanisms of transcriptional gene silencing, long described in plants, have only recently been elucidated in the nematode[67,101]. In the present model, 22G RNAs associate with nuclear WAGOs HRDE-1 or NRDE-3, which recognize nascent pre-mRNA targets and engage downstream nuclear silencing factors NRDE-1/2/4[12,13]. As currently understood, the NRDE factors mediate transcriptional silencing via hierarchical assembly to both the nascent transcript and chromatin: upon target recognition, NRDE-2 is recruited to nascent pre-mRNA, followed by NRDE-1; NRDE-1 is then also recruited to chromatin, in a NRDE-4 dependent manner[13,36]. Association of NRDE factors with the targeted nascent transcript and chromatin serves to concurrently block elongation of transcribing RNA Polymerase II and recruit the repressive histone H3 lysine 9

trimethylation (H3K9me3) mark to the target site[12,13,36,37]. This manner of transcriptional silencing in *C. elegans* is referred to alternatively as “nuclear RNAi” and “NRDE-mediated” silencing.

CSR-1 group 22G RNAs and chromosome organization. The 22G RNAs associated with Argonaute CSR-1 primarily target germline expressed protein coding genes; rather than silence, CSR-1 binds chromatin at its target loci[17]. The function of CSR-1 siRNAs is to promote accurate chromosome segregation, and CSR-1 appears to establish distinct chromatin domains[17]. Loss of CSR-1 results in disorganized centromeres and mis-loading of kinetochore proteins[17]. Interestingly, loss of 22G RNA biogenesis factor DRH-3, and thus depletion of CSR-1 22G RNAs, likewise results in highly disordered mitotic localization of Condensin II subunit KLE-2[17].

1.3 Chromatin remodeling and structural components in small-RNA mediated silencing

The accumulation of a repressive histone mark to target sites during transcriptional gene silencing suggests the supplementary recruitment of chromatin remodeling and structural components. Heterochromatin proteins, several histone methyltransferases (HMT), and select Polycomb Repressive Complex (PRC) proteins have been linked to changes in chromatin domains during small-RNA mediated silencing[2,74]. Additionally, condensin proteins have

been broadly implicated in chromatin organization during silencing events, and specifically in repression of transcription in several species[57,76,98]. This function has been reported to be direct and local, through binding and altering of silencing-target loci, as well as indirect or from a distance (reviewed in Hirano 2012).

Heterochromatin domains at siRNA target loci

Heterochromatic marks established upon siRNA transcriptional gene silencing necessitate the function of HMTs upon target recognition. Studies in *S. pombe* have revealed that HMT Clr4 is recruited to transcribed regions upon Argonaute binding of nascent transcripts during RNAi mediated heterochromatin domain formation[4]. Likewise, heterochromatin formation upon heritable nuclear siRNA silencing in *C. elegans* is reported to be dependent on predicted HMT SET-25[2].

Similarly to SET-25 and related protein SET-32, piRNA silencing of endogenous repetitive loci is defective upon loss of heterochromatin protein homolog HP2, an essential component of heterochromatin[2,60]. The *Drosophila* HP1 homolog Rhino is also necessary in piRNA production, whereas repetitive loci in *S. cerevisiae* are silenced by its single condensin complex[39,56]. This lends evidence that chromatin modifiers and structural proteins – such as condensins – work in concert to effect heterochromatin domain formation upon small RNA silencing.

The condensin complex, transcriptional domains and transposons

Condensin proteins are conserved pentameric complexes able to bind and supercoil DNA, and are essential for the proper organization of chromatin structure, the faithful segregation of genetic information in both mitosis and meiosis, and the regulation of gene expression during interphase[22,40,45,55]. In *C. elegans*, three condensin complexes exist: Condensin I and Condensin II are required for the proper condensation and segregation of chromosomes in mitosis and meiosis, whereas the specialized dosage compensation complex Condensin I^{DC} functions in down-regulating hermaphrodite X-linked gene expression to equal that of males[18,19]. The subunit composition of these complexes is almost identical: Condensin I and II incorporate the same core Structural Maintenance of Chromosome (SMC) subunits but differ in their complement of Chromosome Associated Proteins (CAPs), while Condensin I^{DC} mimics Condensin I with the exception of only its SMC-4-type protein, DPY-27[18,40]. Each pentameric condensin complex is composed of two core SMC subunits and three CAPs. The SMC subunit MIX-1 is shared among all *C. elegans* condensins, and is coordinated with SMC-4 in Condensin I and II; SMC-4 is replaced by DPY-27 in Condensin I^{DC}[19]. Condensin I and I^{DC} share a CAP contingent of proteins DPY-26, DPY-28 and CAPG-1, while Condensin II is specific for CAPs KLE-2, HCP-6 and CAPG-2[40].

In addition to the maintenance and manipulation of chromatin architecture, condensin proteins from various organisms play a role in gene silencing at the level of transcription. Transcriptional silencing of tRNA and mating-type loci in *S. cerevisiae* depends upon the single yeast Condensin, and in *Drosophila*, the transcriptionally repressive state of heterochromatin depends upon Condensin I[22]. The CAP-G2 homolog of mouse Condensin II is antagonistic to gene activation through interaction with transcription factors, while binding of *S. pombe* condensin with RNA Polymerase III transcription machinery contributes generally to genome architecture, and specifically to repeat-associated centromere composition[41,47,64,98]. On a slightly extended scale, *C. elegans* hermaphrodite-specific gene regulation is achieved through motif-specific X chromosome binding and subsequent spreading of Condensin I[DC 10,49].

Drosophila Condensin II subunits have been reported to directly regulate localized clusters of genes, binding around active chromatin territories to potentially maintain a primed, poised-for-activity state[41,62]. Condensin II activity in *Drosophila* is also reported to compartmentalize the genome into distinct regions within the nucleus, and loss of the single *S. cerevisiae* condensin results in disruption of nucleolar tRNA clustering[7,39]. Studies in yeast suggest also that heterochromatin-euchromatin boundary elements created by tRNA genes are maintained and mediated by condensin proteins[48].

Condensin proteins have in addition been directly linked to transposon silencing. The *Drosophila* HCP-6 counterpart dCAP-D3 binds both retroelements

and the surrounding genomic environs to restrict transposition[7,76]. Transposon mobilization is similarly repressed by *Drosophila* dCAP-H2, the *C. elegans* Condensin II equivalent of KLE-2[76]. Nematode Condensin II binds to a subset of promoters, tRNA and long non-coding RNA regions, and the binding of these regions by KLE-2 is transcriptionally repressive[57].

Likewise, condensin proteins hint at association with small RNA silencing pathways. In *S. pombe*, loss of RNAi machinery abrogates accurate segregation of chromosomes, telomere clustering and centromere cohesion[42]. Loss of *C. elegans* DRH-3 – and thus 22G RNAs – results in anaphase bridging and chromosome segregation defects reminiscent of Condensin II depletion[23]. In *Drosophila,* HCP-6-type Condensin II subunit dCAP-D3 localizes within the body of repressed genes, potentially functioning as a boundary or insulation element – comparable to both the establishment of H3K9me3 marks during nuclear RNAi, and the establishment of chromosome organization by the CSR-1 22G RNA pathway[14,17,63]. The piRNA pathway of *Drosophila* is further linked to condensins as RNA helicase Vasa is reported to promote mitotic chromosome condensation and directly interact with Condensin I DPY-26-like subunit Barren[69].

1.4 Silencing of the MIRAGE element

With increasing evidence for a chromatin structural component in transcriptional silencing and condensin function in gene repression, the broad question becomes: do *C. elegans* condensins play a direct role in gene

silencing? Studies from the Hagstrom Lab initially addressed this question through comparison of gene expression in wild-type and Condensin II *hcp-6* mutant animals by microarray, and observed up-regulation of transposon-related loci. This finding introduced the consideration that Condensin II may have a general role in transposon silencing. Particularly, the nematode-specific DNA transposon MIRAGE shows evidence of drastic de-silencing upon loss of HCP-6. These observations propose that chromatin structural component HCP-6 may directly function in silencing of endogenous transposon loci, and may specifically regulate the young autonomous element MIRAGE. The molecular mechanism by which MIRAGE is controlled, and what function HCP-6 contributed to its silencing, are investigated using expression analysis and chromatin immunoprecipitation techniques.

CHAPTER II

Condensin II Subunit HCP-6 Functions in MIRAGE Silencing

2.1 Introduction

Endogenous small RNA silencing pathways in the worm *C. elegans* operate to repress expression of genic and non-genic sequences and the mobilization of transposable elements, preserving both soma and germline against hazards to the genome. In the germline, endogenous secondary 22G siRNAs load onto select worm-specific Argonautes of the WAGO clade, to mediate target repression via transcriptional gene silencing[12,37,102]. Silencing at the transcript level involves target site recruitment of heterochromatin marks and inhibition of RNA Polymerase II, and strongly suggests action of chromatin remodeling and structural components.

Chromatin mitotic and meiotic structural component Condensin II has been implicated in silencing endogenous germline targets. Condensin II shares core protein MIX-1 (mitosis and X-associated-1) with two other condensin complexes of *C. elegans*, but shares core protein SMC-4 only with Condensin I[18]. Subunits HCP-6 (holocentric chromosome binding protein-6), KLE-2 (kleisin family-2), and CAPG-2 (capg condensin subunit homolog-2) comprise the CAP complement of Condensin II, and are unique to the complex[18]. The SMC and

CAP subunits are able to localize to condensed chromosomes independently of each other, and the Condensin II complex as a whole localizes to chromatin domains enriched for centromeric heterochromatin protein CENP-A[16]. On the holocentric chromosomes of *C. elegans*, mitotic and meiotic localization of Condensin II mimics the discrete kinetochore/centromere pattern, which is distinct from the broad chromosomal localization of Condensin I[18]. Loss of both CENP-A paralogs disrupts localization of Condensin II CAP subunit HCP-6, but not SMC core subunit MIX-1[16]. Proper centromere assembly is required for HCP-6 organization on chromosomes, and disruption of CENP-A organization in CSR-1 Argonaut mutants mislocalizes Condensin II subunit KLE-2[17,83].

In the temperature sensitive point mutant *hcp-6(mr17)*, chromosomes fail to fully condense – they lack the rigidity of wild type chromosomes, and are prone to twisting[33,83]. Loss of Condensin II subunits results in chromosomal anaphase bridges and mitotic segregation defects; this phenotype is also displayed upon loss of 22G RNA biogenesis factor DRH-3[23,83]. DRH-3 is localized to perinuclear P granules with effector Argonaute CSR-1, and disruption of P granule assembly leads to up-regulation of a subset of genes also derepressed upon loss of HCP-6[17,82].

This small subset of genes also map small RNAs co-immunoprecipitating with germline nuclear Argonaute HRDE-1, suggesting that they undergo transcriptional repression via heritable nuclear RNAi[12]. MIRAGE DNA Transposon, RNase H protein *rnh-1.3* and W09B7.2 – which is located in a

region dense with retrotransposon sequence – are all upregulated in *hcp-6(mr17)*, and exhibit NRDE-pathway dependent H3K9me3 enrichment[12]. Derepression of transposon and transposon-associated loci, along with depletion phenotypes shared by siRNA pathway factors, implicates Condensin II subunit HCP-6 in silencing transposable element loci.

In interphase, HCP-6 is generally localized to the nucleus and, in *Drosophila*, is reported to sit within the body of genes that are being repressed[62]. With the above, this evidence predicts that Condensin II may operate to repress a subset of transposon loci subject to germline nuclear RNAi and marked by enrichment of heterochromatin.

2.2 Results

DNA transposon MIRAGE is de-repressed in a Condensin II mutant

Previous results from the Hagstrom Lab indicate that Condensin II subunit HCP-6 may play a role in gene silencing. Part of those initial studies, microarray analysis of gene expression in wild type and Condensin II *hcp-6(mr17)* mutant animals exhibited consistent mis-regulation of transposon-related loci. This holds with recently reported findings of condensin-dependent transposon regulation in other organisms[57,62,76].

Microarray results also implicated that loss of HCP-6 function also leads to de-repression explicitly of DNA transposon MIRAGE. MIRAGE derepression was exhibited specifically in adult hermaphrodite samples, which contain both

soma and germline tissues, and not L1 stage larval worms, which consist largely of somatic tissues. Additionally, MIRAGE element mis-regulation appears independent to that of "parent genes." A representative example of such is MIRAGE element WBTransposon00000002; composed of two open reading frames, R09E12.6 and R09E12.5, this element sits within the large first intron of parent gene Seven Trans-membrane Receptor *str-238*, which constitutes ORF R09E12.4. While fold change for this particular MIRAGE locus probe set is not drastic, expression mis-regulation of transposon versus parent gene is clearly detectable, and this specificity is maintained among separate biological replicates (Figure 2.1A). The high overall signal level of general MIRAGE element derepression is generated from the collection of probes mapping to all twelve MIRAGE loci, which together eclipse the low fold change signal generated from probes mapping specifically to MIRAGE ORFs R09E12.6 and R09E12.5.

Several families of both Class I and Class II transposons were represented on the array with a minimum cutoff of 10 probes; however, MIRAGE appeared as the sole element exhibiting increase in expression for the mutant condition (Figure 2.1B). Conversely, significant up-regulation was also observed for RNase H ribonuclease *rnh-1.3* and TATA-binding protein (TBP) associated factor *taf-7.2*, which has a potential gene regulatory role via its association with promoter and transactivator regions[95]. Additionally, *hcp-6(mr17)* mutant down-regulation was notable for half-molecule ATP-binding cassette (ABC) transporter *haf-6*, which is required for RNA interference and also displays transposon

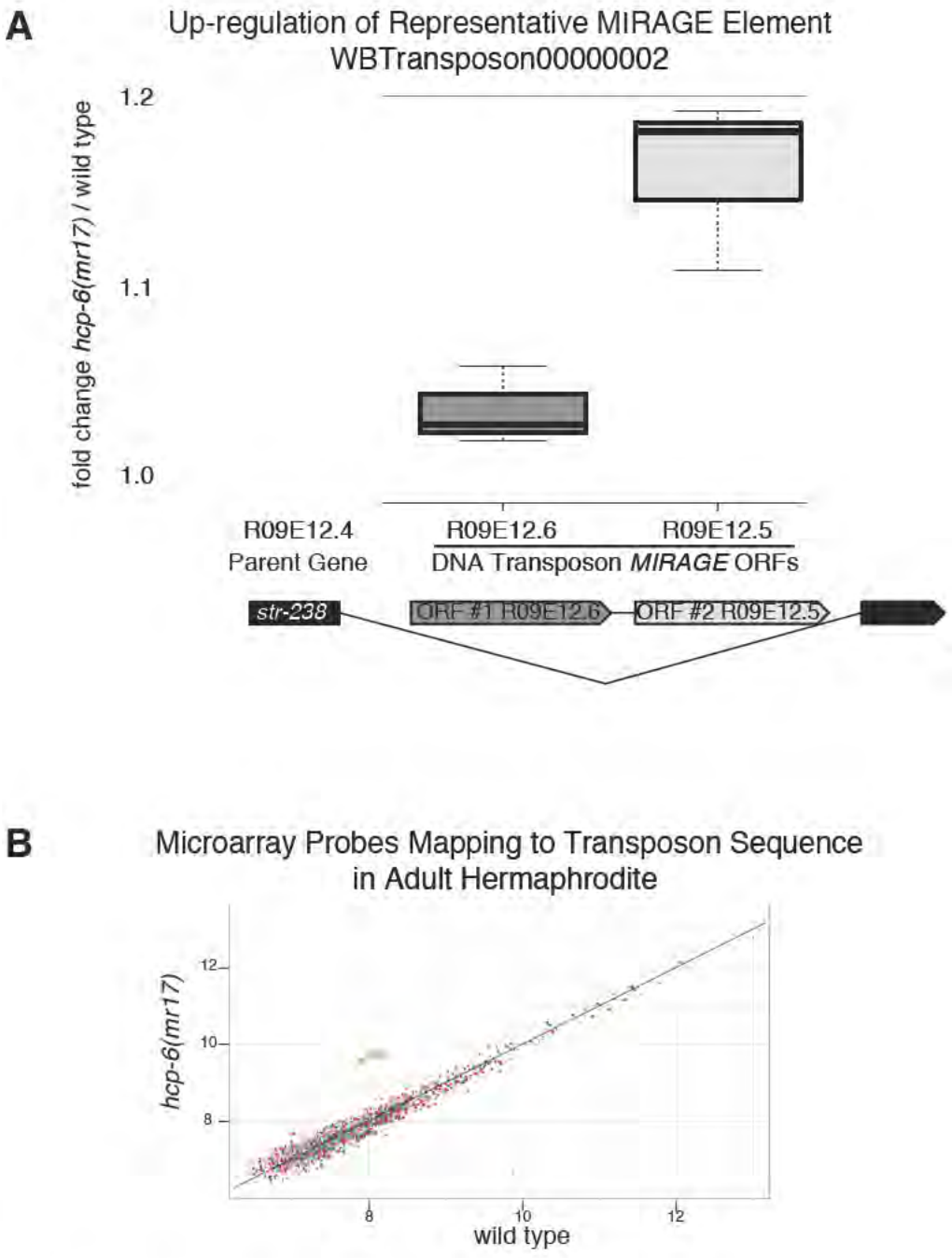

Figure 2.1 *(see next page for legend)*

Figure 2.1: Condensin II mutant shows derepression of MIRAGE by microarray

(A) Fold change and specific derepression of representative MIRAGE DNA transposon locus WBTransposon00000002, as observed by microarray. The two open reading frames forming MIRAGE transposon (R09E12.6 and R09E12.5) are contained within the large first intron of gene *str-238* (ORF R09E12.4). Condensin II mutant animals showed consistent and specific derepression of MIRAGE loci among biological replicates; at this locus, MIRAGE is mildly derepressed while parent gene *str-238* expression is unaffected. Cartoon at bottom depicts locus organization. Box plot courtesy of Jia Xu, UMMS Bioinformatics Core.

(B) Transposable element fold change in Condensin II mutant *hcp-6(mr17)* compared to wild type, representative biological replicate. Of transposons represented on the microarray, MIRAGE (yellow) is the sole element significantly mis-regulated in *hcp-6(mr17)*. Scatter plot courtesy of Jia Xu, UMMS Bioinformatics Core.

silencing defects upon its depletion[85,86]. This evidence raised the question of whether Condensin II had a general role in silencing transposons.

In order to address whether Condensin II functions broadly in transposon silencing – as well as corroborate the observed microarray data and confirm that results could be replicated – expression analysis of select transposable elements was carried out in wild-type and Condensin II mutant animals using quantitative reverse transcriptase PCR (qRT-PCR). Transposable elements tested comprised both Class I and Class II species, and were selected to represent different subclasses, structures and phylogenetic clades where possible.

Phylogenetic grouping of Cer family LTR retrotransposons is based upon the evolutionary relationship of RT domain proteins, and the specific tRNA primers utilized by these enzymes for transcription[11,27]. Cer1 elements fall into the *gypsy*/Ty3 clade, while Cer7 and Cer10 belong to the *Tas/Bel/Pao* group; Cer7 stands apart however, as it encodes its own 71 base pair tRNA primer[27,60]. Line2A is the only chosen non-LTR retrotransposon and, like the MIRAGE DNA transposon, contains a *C. elegans*-specific coding region; it is predicted to have been active very recently[97].

The Tc1/*mariner* superfamily of DNA transposons was represented by Tc1 and Tc3; following Tc1, Tc3 is the second-most abundant DNA transposon in *C. elegans*. Tc3 is particularly notable in that two distinct 21U RNA sequences have been discovered which map to the element; 21U RNAs mapping to Tc1 have yet to be revealed[21]. The element Tc4 exhibits a unique fold-back structure of near-

identical 774 base pair TIRs flanking a small internal sequence of only 57 base pairs[8]. Similarly, Turmoil2 is considered a palindrome; part of the HARBINGER superfamily, it exhibits extremely long and almost perfect TIRs[8]. The MudR2 element is part of a superfamily of highly aggressive plant and bacterial transposons[54].

Expression of transposon mRNA was assayed in three biological replicates of adult hermaphrodite wild type and *hcp-6(mr17)* animals, and fold change of mRNA was calculated using the ΔΔCt (Livak) method (Figure 2.2). Samples assayed were independent of those used in the initial microarray studies, and focused on synchronized adult hermaphrodite worms aged 24 hours post L3/L4 stage molt. The normalization control utilized was *ama-1*, encoding the large subunit of RNA Polymerase II. Of the Class I elements assayed, Cer10 appears to exhibit a moderate 2.6-fold average increase in expression upon loss of HCP-6 function; however, statistical analysis using Students T-Test proved this to be insignificant (p value = .056). Of the Class II elements assayed, MIRAGE again exhibits a striking increase in expression in the *hcp-6(mr17)* mutant. The average 8.3-fold change in expression was found to be statistically significant (p value = 3.27×10^{-5}), and confirmed microarray data reporting a 6.3-10.4 fold increase in expression from two annotated loci of MIRAGE (data not shown).

This data suggests that expression of the MIRAGE element is indeed moderated in a pathway or system dependent on Condensin II subunit HCP-6, in

Figure 2.2: Condensin II plays a role in silencing DNA Transposon MIRAGE.

Fold change in mRNA expression from transposable elements of both Class I and Class II transposon families was assayed by qRT-PCR in wild type and Condensin II subunit HCP-6 mutant worms. Mutant animals exhibited consistent and significant de-silencing of the MIRAGE DNA transposon ($p < .0005$, Students T-Test), which was not observed for any other transposable elements tested. Error bars represent standard deviation in three biological replicates.

a consistent and reproducible manner. Independent analysis by both microarray and qRT-PCR confirms MIRAGE DNA transposon mRNA expression levels are increased in *hcp-6(mr17)* mutants. However, transcript levels of several other transposable elements, representing different subclasses, structures and phylogenetic clades, were not appreciably altered in the condensin mutant. This indicates that Condensin II does not serve a broad function in general transposon silencing, but may rather play a specific role in silencing the young autonomous element MIRAGE.

As an integral component of chromatin structural machinery, HCP-6 localizes to the nucleus throughout the cell cycle, underscoring the likelihood of non-mitotic roles in gene regulation. Potential mechanisms by which Condensin II may silence MIRAGE were next addressed in the context of known transposon silencing processes.

Condensin II regulates targets of the germline siRNA pathway

Transposons, pseudogenes and cryptic loci – as well as some protein coding genes – are silenced in the germline to maintain genome stability and protect genome integrity. Targeting of these loci occurs in part through select secondary WAGO Argonautes and their associated 22G RNAs, collectively comprising the "nuclear RNAi pathway"[14,38]. Specifically, such "NRDE germline targets" rely upon WAGO HRDE-1 and factors NRDE-1/2/4, and exhibit loss of silencing upon loss of these components[12]. Transcriptional silencing via the

germline nuclear RNAi pathway is marked by accumulation of heterochromatin mark H3K9me3 at target loci[12,13,37]. As transposon silencing in *C. elegans* and other organisms occurs via germline nuclear small RNAs, and Condensin II subunit HCP-6 is observed to silence MIRAGE DNA transposon in the germline, two questions emerge[81,90,94]. First, does Condensin II also regulate expression of loci known to be targets of the germline NRDE pathway? And second, does Condensin II silence MIRAGE through the same H3K9me3-dependent transcriptional silencing mechanism utilized by the nuclear RNAi machinery?

In order to determine if Condensin II plays a role in the nuclear silencing of additional germline targets, expression analysis of identified NRDE germline targets was carried out in wild type and *hcp-6(mr17)* mutant animals using qRT-PCR. Also analyzed were the mutant *nrde-2(gg91)*, which compromises the nuclear RNAi pathway, and the mutant *drh-3(ne4253)*, which compromises formation of secondary 22G RNAs.

In addition to the MIRAGE DNA transposon, reported NRDE germline targets *bath-45*, B0250.8 and F15D4.5 were chosen for analysis as they have previously been observed to increase expression upon loss of both nuclear RNAi pathway and DRH-3 function[12,38]. Assayed as well were RNase H ribonuclease *rnh-1.3* and the uncharacterized protein coding locus W09B7.2, which have recently been reported to map siRNAs immunoprecipitating with nuclear RNAi WAGO HRDE-1, directly implicating them as NRDE germline targets[12]. These two loci had also exhibited up-regulation in the initial Condensin II mutant

microarray studies, strengthening the potential link of Condensin II action in the nuclear RNAi pathway.

Quantitative RT-PCR of target loci expression exhibits an interesting overlap in the features moderated by Condensin II (Figure 2.3A). Derepression of MIRAGE, and loci *rnh-1.3* and W09B7.2, is observed the Condensin II mutant and both siRNA pathway mutants, potentiating a shared silencing mechanism for these targets. Conversely, the loci *bath-45* and B0250.8 show no derepression upon loss of Condensin II, though as expected they are desilenced upon loss of *nrde-2* and *drh-3*. Interestingly, F15D4.5 presents an expression profile more similar to targets MIRAGE, *rnh-1.3*, and W09B7.2 in the high level of desilencing exhibited at this locus in *nrde-2* and *drh-3* mutants. However, the modest 2.5-fold increase of F15D4.5 mRNA in the Condensin II mutant did not prove statistically significant (p value = .14).

This data suggests a potential overlap in the subsets of loci whose expression is mediated by Condensin II and the nuclear RNAi pathway. *nrde-2* mutant overexpression of *rnh-1.3* and W09B7.2 displayed them to be targets of the NRDE pathway; overexpression of MIRAGE in *nrde-2* suggests that it too is silenced via the NRDE pathway. Results propose that the full complement of six tested loci may be considered targets of nuclear RNAi machinery, and potentially "true" endogenous germline target genes. However, Condensin II plays a role in silencing expression only in the subset of MIRAGE, *rnh-1.3* and W09B7.2 loci. Overexpression of these loci in mutant *drh-3(ne4253)*, an integral component of

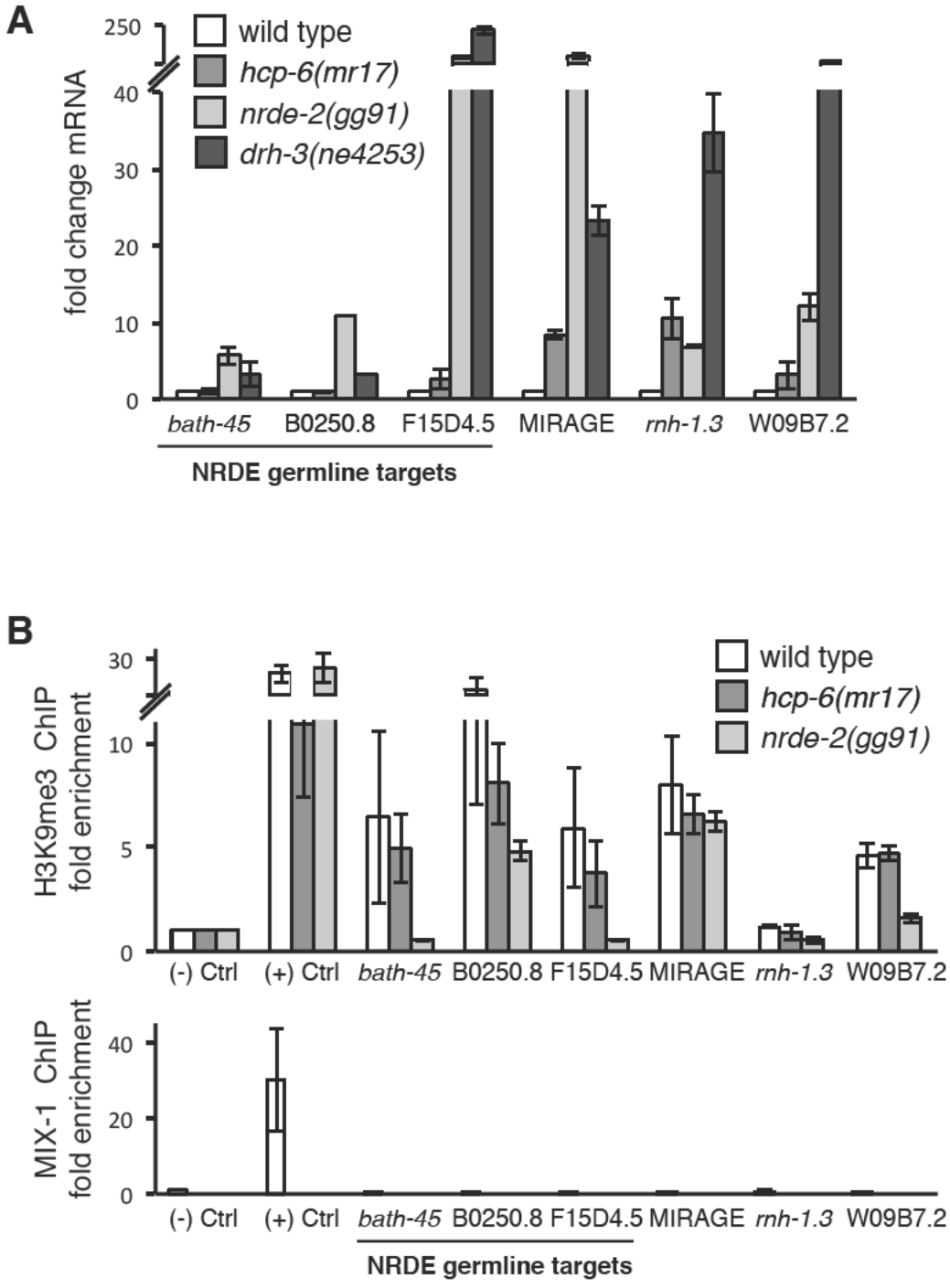

Figure 2.3 *(see next page for legend)*

Figure 2.3: Condensin II is involved in silencing several germline targets regulated by the nuclear RNAi pathway.

(A) Fold change in mRNA expression of targets regulated by the nuclear RNAi pathway and Condensin II. A subset of targets silenced by NRDE factors overlaps with those regulated by Condensin II. Early disruption of the NRDE germline RNAi pathway in the *nrde-2(gg91)* mutant, as well as disruption of germline effector 22G RNA production in the *drh-3(ne4253)* mutant, released mRNA silencing from all six target loci. Condensin II mutant *hcp-6(mr17)* showed notable increase of mRNA from MIRAGE, *rnh-1.3* and W09B7.2 loci, and modest 2.5 fold increase of F15D4.5 mRNA. Error bars represent range and standard deviation from two (*nrde-2* and *drh-3*) and three (wild type and *hcp-6*) biological replicates, respectively.

(B) Fold enrichment of repressive H3K9me3 mark (top) and Condensin II subunit MIX-1 (bottom) at loci targeted by germline siRNAs in the NRDE pathway. H3K9me3 was lost at germline target loci, but not at MIRAGE, upon nuclear RNAi pathway disruption (*nrde-2* mutant); Condensin II mutant *hcp-6(mr17)* showed no effect on H3K9me3 depletion at any of the target loci. MIX-1 did not show direct condensin enrichment at any of the tested loci. Error bars represent range from two (*nrde-2*) and three (wild type and *hcp-6*) biological replicates.

the RdRp core complex responsible for secondary 22G RNA formation, likewise confirms that the “Condensin II subset” of loci are indeed targeted by siRNAs. Taken together, this evidence suggests that at minimum, Condensin II subunit HCP-6 and the NRDE nuclear RNAi pathway silence expression of select common target loci.

Condensin II mutation does not alter MIRAGE heterochromatin state

The nuclear RNAi pathway operates primarily via transcriptional silencing of target loci, although HRDE-1 dependent silencing implicates a post-transcriptional component as well[2]. NRDE pathway nuclear WAGOs loaded with 22G RNAs bind nascent pre-mRNA, and promote accumulation of heterochromatic mark H3K9me3 at target loci; association of NRDE factors with target loci chromatin inhibits elongation of RNA Polymerase II downstream of the target site[13].

In order to determine if Condensin II plays a role in silencing endogenous nuclear RNAi targets via formation of repressive H3K9me3 heterochromatin, chromatin immunoprecipitation (ChIP) was performed to assay heterochromatin formation at target sites. Additionally, as “endogenous germline target genes” are specifically defined by a two-fold loss of H3K9me3 in *nrde-2* mutants, the ChIP assay will test if the “Condensin II subset” of loci may be truly classified as such.

Positive and negative controls for heterochromatin deposition were derived from ModENCODE regions locally enriched (*cpt-3* region III: 2084036-2084159) and depleted (*cpt-3* region III: 2089625-2089708) of H3K9me3, respectively. Accumulation of H3K9me3 in wild type and *hcp-6(mr17)* animals was tested at six NRDE-dependent loci – the three previously characterized NRDE germline targets *bath-45*, B0250.8 and F15D4.5, as well as the experimentally verified NRDE-dependent targets MIRAGE, *rnh-1.3* and W09B7.2. Compromise of the NRDE nuclear RNAi pathway, as in mutant *nrde-2(gg91)*, is expected to releases H3K9me3 accumulation and relieves transcriptional silencing at target loci.

As expected, fold enrichment of H3K9me3 at previously characterized NRDE germline targets is drastically reduced in the *nrde-2(ne4253)* mutant (Figure 2.3B *top*). Loss of H3K9me3 by more than two-fold in *nrde-2(ne4253)* is also demonstrated in the experimentally verified NRDE-dependent targets *rnh-1.3* and W09B7.2 – allowing them to be classified as true “endogenous germline targets” (*rnh-1.3* = 2.24 fold depletion). However, observed H3K9me3 enrichment at MIRAGE is unaffected; based on current knowledge, it cannot thus be classified as a true “endogenous germline target” of the NRDE pathway. Accumulation of H3K9me3 in the *hcp-6(mr17)* mutant is statistically unaffected at any tested locus, regardless of “endogenous germline target” classification.

To address whether condensin itself binds to chromatin at MIRAGE and at target loci of the germline nuclear RNAi pathway, ChIP for shared condensin

subunit MIX-1 was performed in wild-type adult hermaphrodites (Figure 2.3B *bottom*). As core SMC subunit MIX-1 is the only component common to all three *C. elegans* condensins, ChIP with antibody against MIX-1 isolates all three condensin complexes indiscriminately[18]. The positive control for MIX1 ChIP was the Rex-2 (recruitment element on X) consensus motif, one of several motif loci on the X chromosome reported to be bound by MIX-1; the negative control was Basal-X (basal binding to X chromosome) sequence, an X chromosome region reported to be free of MIX-1 binding[10,49]. ChIP with non-specific condensin subunit MIX-1 reveals that Condensin II is not observed at MIRAGE or the tested NRDE target loci (Figure 2.3B *bottom*).

While Condensin II mutant *hcp-6(mr17)* exhibits consistent and reproducible desilencing of transposon MIRAGE and NRDE target-like loci *rnh-1.3* and W09B7.2, these loci do not demonstrate altered H3K9me3 chromatin state as a result of HCP-6 mutation. Additionally, binding of condensin was not observed at any of the loci tested, as evidenced by ChIP with common condensin subunit MIX-1. Results suggest that Condensin II action in silencing of MIRAGE and related loci may proceed indirectly, through a mechanism independent of H3K9me3 heterochromatin signaling, and likely distinct from that used by the NRDE nuclear silencing pathway.

2.3 Conclusions

Condensin complex proteins have been implicated in gene regulation in several species, and the *C. elegans* specialized dosage compensation condensin is known to transcriptionally silence genes on the X chromosome[19,22,39]. When this study began, it was not known whether related worm condensins I and II likewise had roles in the silencing of gene expression. Quantitative RT-PCR and ChIP analysis show that DNA transposon MIRAGE, and retrotransposon related loci *rnh-1.3* and W09B7.2, are derepressed upon loss of Condensin II subunit HCP-6. With the exception of MIRAGE, these loci also prove to be true endogenous germline targets, based upon H3K9me3 depletion.

Loci *rnh-1.3* and W09B7.2 appear to be silenced by both HCP-6 and the NRDE pathway, while silencing of the remaining nuclear RNAi targets is unaffected by HCP-6. The transposon-type loci derepressed in *hcp-6(mr17)* thus appear to represent only a subset of the endogenous loci targeted by NRDE factors, as the HCP-6 dependent derepression they show does not extent to previously characterized endogenous targets *bath-45*, B0250.8 and F15D4.5. All target loci tested do exhibit enrichment of repressive mark H3K9me3, yet this enrichment remains unaffected upon loss of HCP-6 at both transposon-like "Condensin II subset" loci and the non-transposon target group, suggesting that HCP-6 is dispensable for heterochromatin accumulation at the assayed targets. Furthermore, MIX-1 ChIP evidence shows that Condensin II does not associate with target site chromatin. This suggests that Condensin II is able to mediate

silencing of select loci, but that this function may be indirect, through mechanisms independent of heterochromatin H3K9me3 formation.

CHAPTER III

Discussion

Fidelity of genetic information passed through the germline is paramount to the successful propagation of any species. Repetitive and mobile elements pose hazards to genome integrity, and the expression of these sequences reduces fitness for the organism as a whole. Molecular mechanisms have thus evolved to combat repetitive sequence expression and suppress transposable element mobility. Inhibition of threatening elements occurs at both the transcriptional and post-transcriptional level, through small RNA-mediated silencing networks.

Silencing is also mediated through the action of chromosome architecture and organization proteins. Chromosome condensation and segregation protein complex condensin has been broadly implicated in transcriptional silencing of repetitive genes and broad chromosomal domains. In nematode *C. elegans*, Condensin I^{DC} acts specifically to transcriptionally silence X chromosome gene expression, and gene regulatory roles for Condensin I and II have been strongly suggested through work in other organisms. Investigating the role of *C. elegans* Condensin II in gene regulation, initial microarray studies in the Hagstrom Lab

implicated Condensin II subunit HCP-6 in specifically silencing DNA transposon MIRAGE.

In order to query if general transposon silencing was a broad function of Condensin II, this study used independent qRT-PCR analysis to assay expression of MIRAGE and a variety of other nematode transposable elements upon loss of HCP-6. To determine the molecular mechanism by which MIRAGE silencing is mediated, chromatin immunoprecipitation techniques were used to assay heterochromatin enrichment and condensin binding at MIRAGE and related endogenous silencing targets in Condensin II mutant worms.

Condensin II proved to be dispensable for the silencing of both Class I Retrotransposons and Class II DNA Transposons, with exception only of DNA transposon MIRAGE. Transposon-related loci *rnh-1.3* and W09B7.2, up-regulated in the original microarray studies, were also confirmed to increase expression upon loss of HCP-6. Loss of HCP-6 did not appear to affect abundance or maintenance of local heterochromatin mark H3K9me3 at these targets. This, along with the observation that Condensin II is not bound to local target chromatin, suggests that the silencing function of Condensin II is specific to a small subset of tested loci, and that this silencing is indirect.

Notably, silencing of MIRAGE was also drastically relieved upon loss of transcriptional silencing factor NRDE-2, hinting that MIRAGE is regulated in a NRDE-dependent manner. However, this observation was not enough to classify MIRAGE as a true target of the NRDE germline pathway. “Endogenous germline

target loci" are defined as genes which exhibit at least a two-fold reduction in heterochromatin mark H3K9me3 upon loss of NRDE-2/4[12]. While MIRAGE mRNA exhibited extensive NRDE-dependent derepression, no change in H3K9me3 occupancy at the locus was observed in *nrde-2(ne4253)*. MIRAGE is thus not officially classified as an endogenous germline target of the NRDE pathway, underscoring the observation that silencing of this element appears to occur through indirect methods.

3.1 The Role of HCP-6

HCP-6 dependent silencing of MIRAGE mRNA is consistent and reproducible, and independent of the heterochromatin accumulation suggestive of transcriptional silencing pathways. This phenomenon is mimicked for the transposon-like targets *rnh-1.3* and W09B7.2 which, with MIRAGE, appear to be managed by HCP-6 as a "Condensin II subset" of NRDE-dependent loci. Expression at these loci in mutant *dhr-3(ne4253)* far exceeded expression in *hcp-6(mr17)* – directly implying a 22G RNA component to targeting of this subset. HCP-6 and the 22G RNA biogenesis factor DRH-3 also share chromosome segregation and anaphase bridge depletion phenotypes, strengthening a potential link between Condensin II and secondary siRNAs.

Argonaute CSR-1 and its associated 22G RNAs target genic loci for the formation and maintenance of chromatin structure. Targeted domains established in this way are excluded from domains of centromeric

heterochromatin CENP-A, yet loss of CSR-1 targeting disrupts CENP-A chromatin organization. Condensin II localizes to chromatin domains enriched for CENP-A, depletion of which abrogates proper localization of HCP-6. Likewise, depletion of CSR-1 results in disrupted and improper localization of Condensin II CAP subunit KLE-21[17]. *C. elegans* chromosomes are holocentric, and as condensin has been observed to establish active and repressive chromatin domains, it is conceivable that the Condensin II complex works with CSR-1 to balance the interplay between centromeric and non-centromeric chromatin.

As an HCP-6 dependent transcriptional silencing mechanism was not observed at any of the loci tested, it becomes likely that HCP-6 acts in an indirect manner to silence transposon-related loci. Condensin proteins are able to supercoil DNA, and Condensin II appears to be associated with chromosomes throughout the cell cycle. Loss of HCP-6 during interphase could relax repressive chromatin architecture, and allow H3K9me3 heterochromatin to move away from the repressive nuclear periphery. This concept is underscored by current models of chromatin looping, which rely upon condensin proteins as both boundary and structural elements[7,22,48,62].

3.2 MIRAGE and the Nuclear Silencing Pathway

Loss of silencing as measured by mRNA expression does not differentiate between transcriptional and post-transcriptional silencing mechanisms, and the

presence of heterochromatin does entirely negate the possibility of transcription through a region. Heterochromatin protein HP1 and H3K9me3 both bind actively transcribed genes, and while heterochromatin is a marking feature of repetitive loci, these sequences are basally transcribed at a low level in most eukaryotic genomes[32,68]. As genetic parasites, transposable elements are ultimately dependent the viability of their host, and as such must rely on only a dampened level of activity[71]. DNA transposon MIRAGE is transcribed at very low level in L4 and young adult worms, concurrent in development with gametogenesis and a large general expansion of germ cell number[97]. Developing oocytes must be maternally provisioned with protein coding genes and silencing signals. It is possible that the low basal level of MIRAGE transcription during these stages provides the necessary template – through intra-molecular pairing of TIRs – for amplification of silencing signals.

The maintenance of H3K9me3 at the MIRAGE locus upon de-repression implies lack of a transcriptional silencing component, but preserves the possibility of a post-transcriptional silencing mechanism. And indeed, germline silencing of Tc1 is reported to be post-transcriptional and heterochromatin independent[80]. However, in order to more fully determine the contribution of transcriptional and post-transcriptional pathways, transcription activity through the region should be assayed via nuclear run-on or RNA Polymerase II ChIP.

Additionally, the fact that MIRAGE mRNA expression appears extremely dependent on a nuclear silencing factor strongly links this locus to the nuclear

germline silencing pathway. Binding of NRDE-2 to nascent transcripts is thought to recruit histone methyltransferases, and thus accumulate H3K9me3. Interestingly, proclaimed endogenous NRDE germline target B0250.8 does not itself entirely conform to the "H3K9me3 two-fold depletion" rule – previous studies did not show two-fold depletion of H3K9me3 upon loss of NRDE-2; the classification remained as two-fold depletion of H3K9me3 was observed upon loss of NRDE-4[12]. Similarly, statistical analysis of matched ChIP data sets reveals that the observed fold change in H3K9me3 occupancy is not quite significant (p value = 0.056), replicating previous observations; this distinction is obscured when data is handled as one larger sample set (as shown) due to increased variability. The possibility is thus presented that heterochromatin formation at nuclear germline targets may not consistently depend on NRDE-2.

Furthermore, nuclear RNAi factor NRDE-2 does not itself associate with chromatin, but rather with the nascent pre-mRNA transcript[13]. This becomes relevant in light of the fact that several MIRAGE elements are located within the large introns of protein coding genes. Without an observed promoter, any transcription of MIRAGE over background/basal levels depends on read through from these genes – heterochromatin accumulation targeted to MIRAGE through NRDE-2 association with pre-mRNA could result in detrimental spread of H3K9me3 into these genic sequences, causing repression. It therefore becomes conceivable that, as observed, NRDE-2 is dispensable for H3K9me3 maintenance at MIRAGE.

The question of MIRAGE silencing through the transcriptional pathway therefore remains. As NRDE-2 is strictly a nuclear-localized factor, the possibility that it plays a direct role in cytoplasmic post-transcriptional silencing appears slim. In order to parse out the potential nuclear silencing aspect of MIRAGE, we must look to other components of the nuclear germline silencing pathway. Factor NRDE-1 likewise is recruited to and associates with pre-mRNA, but also associates directly with chromatin; NRDE-1 deposition to chromatin is NRDE-4 dependent. Upstream of these factors is the nuclear WAGO HRDE-1, which is loaded with the targeting 22G RNA and responsible for direct recognition of complimentary loci. Perhaps most telling, 22G RNAs mapping to MIRAGE transposon sequence K02B7.2 have been reported to co-immunoprecipitate with HRDE-1, directly implying germline transcriptional silencing of the element[12]. Depletion of H3K9me3 accumulation at this locus upon loss of factors NRDE-1 and NRDE-4, in concordance with RNA Polymerase II accumulation, would indicate that MIRAGE is in fact mediated by transcriptional gene silencing.

While the current role of Condensin II in MIRAGE silencing appears indirect, the transposon silencing activity of HCP-6 has immediate consequences for germline integrity and genome stability. Condensin function in gene silencing at a subset of transposon-related loci adds to the growing evidence of condensin protein action in non-mitotic roles.

CHAPTER IV

Materials and Methods

C. elegans strains

All strains were maintained at 15°C on NG plates using standard techniques. The temperature-sensitive (ts) mutant *hcp-6(mr17)* was shifted to 25°C to compromise HCP-6 function prior to assays; all strains being assayed concurrently were simultaneously shifted to maintain consistency in protocol.

Strain	Genotype	Lesion	Reference
TY2386	wild type Bristol N2		
AR1	*hcp-6 (mr17) I*	Missense	Chan R, et al. 2004
YY186	*nrde-2 (gg91) II*	Exon 2 Stop	Guang S, et al. 2010
WM206	*drh-3 (ne4253) I*	Missense	Gu W, et al. 2009

Oligonucleotide Primers

Except where noted, all primer sets were based upon sequence information in WormBase versions WB227 – WB232. Primers were designed using Primer3 Plus and synthesized by IDT. Primer sets *bath-45*, B0250.8 and F15D4.5 originally reported in Gu W, et al. 2009. Rex-2 and Basal-X sites reported in Blauwkamp TA and Csankovszki G, 2009.

Purpose	Target/Name		Sequence
Normalization Ctrl	ama-1	Forward	5’ CCAACGTACTCTCCAACATCTC 3’
Normalization Ctrl	ama-1	Reverse	5’ CCACCTCCACTTTCATAACTTG 3’

H3K9me3 Neg Ctrl*	cpt-3	Forward	5’ TCTTTGGTTGTCTGCGTCTC 3’
H3K9me3 Neg Ctrl*	cpt-3	Reverse	5’ AAGTTGGTGGTGTGTGTTGG 3’

**Based on ModENCODE data; H3K9me3 poor genome region III: 2089625-2089708*

H3K9me3 Pos Ctrl*	cpt-3	Forward	5’ CAACCCCGTTTAGTCCAATG 3’
H3K9me3 Pos Ctrl*	cpt-3	Reverse	5’ AATGCTCTGGGGCTAGATTG 3’

**Based on ModENCODE data; H3K9me3 rich genome region III: 2084036-2084159*

MIX-1 Neg Ctrl	Basal-X	Forward	5’ GCCAGTACCAACACCAACCA 3’
MIX-1 Neg Ctrl	Basal-X	Reverse	5’ GAAACACCCGAAACATGCTG 3’
MIX-1 Pos Ctrl	Rex-2	Forward	5’ GCCCCTGCATTCTTTTTAGG 3’
MIX-1 Pos Ctrl	Rex-2	Reverse	5’ TTTTCGCTCTCTTCCCCTCT 3’
	MIRAGE	Forward	5’ GCCTTCGTCAAAGAAACCAG 3’
	MIRAGE	Reverse	5’ AACAAGTCAACCGGGCATAG 3’
	bath-45	Forward	5’ ACGATGGTTGATGCTTTCCT 3’
	bath-45	Reverse	5’ CACCACAACAATCCCATCAG 3’
	B0250.8	Forward	5’ ATGCAGTGGACAATGGCAAG 3’
	B0250.8	Reverse	5’ CTGCCAACGTGCATTTGTTT 3’
	F15D4.5	Forward	5’ ACGAGAGAAGACCCCCAATTC 3’
	F15D4.5	Reverse	5’ TCGTAGTGACGAGCCGTTTC 3’
	Rnh-1.3	Forward	5’ CCACACTGATTCCAAGAACG 3’
	Rnh-1.3	Reverse	5’ TGCGGTCGATATCTCTGATG 3’
	W09B7.2	Forward	5’ GAAAACCAATCGTCCCACAC 3’
	W09B7.2	Reverse	5’ TCCTCGGATGTTCTCATTCC 3’
	Cer1	Forward	5’ AAAGGAGCAAATCCGTCAGC 3’
	Cer1	Reverse	5’ TCATTGCCCAGGAATCACTC 3’
	Cer7	Forward	5’ GGTCCAAACCATTGGAGATG 3’
	Cer7	Reverse	5’ GGAGCGTGCATTATCGTTTC 3’
	Cer10	Forward	5’ GGGACCAACTCCACAAAATG 3’
	Cer10	Reverse	5’ TGCTTCTGGCTGTTGTATGG 3’
	Line2A	Forward	5’ TGGTTTCGCTCTTTCCTCAG 3’
	Line2A	Reverse	5’ AAAACAGGAACGGTCCACAG 3’
	MudR2	Forward	5’ GGACGGTGAAATGAGAAAGC 3’
	MudR2	Reverse	5’ TGCGTCACCAAGGTTATACG 3’
	Tc1	Forward	5’ AACCGTTAAGCATGGAGGTG 3’
	Tc1	Reverse	5’ CACATGACGACGTTGAAACC 3’
	Tc3	Forward	5’ GAGCGTTCACGGAGAAGAAG 3’
	Tc3	Reverse	5’ AATAGTCGCGGGTTGAGTTG 3’

Tc4	Forward	5’ TCGTTTGGCACAGCTTACAC 3’
Tc4	Reverse	5’ TTTCCAGTGCCATAGCCATC 3’
Turmoil2	Forward	5’ ACACCCAAATCAGCAGAGG 3’
Turmoil2	Reverse	5’ AACAGAGAACCGGAATGTGG 3’

RNA Extraction

RNA extraction was performed using RNAzol RT (MRC Gene). Age-synchronous worms were collected and washed 3x in 1X M9 to remove any residual bacteria; large samples were split to 1.5mL tubes holding roughly 100uL pelleted worms. Supernatant was removed and 250uL of RNAzol RT was added to the worm pellet; worms were vortexed for approximately 30 seconds then flash frozen to facilitate breaking the cuticle. Samples were thawed rapidly by a second addition of 250uL RNAzol RT, and vortexed for a further 30 seconds. Extraction then proceeded as described in the RNAzol RT product protocol.

Chromatin Immunoprecipitation

Assay was done on gravid adult worms. Wild-type and mutant worms were synchronized by bleach preparation to release embryos, which were then raised at 15°C from L1 to L4 on SNG plates seeded with *E. coli* HB101. At L4 stage, worms were transferred to 25°C and grown until gravid adult. Worms were collected and washed 3x with 1X M9, and treated for 30 minutes with 10mM of long-arm crosslinker 3,3’-dithiobispropionimidate (DTBP) in 1X M9 while nutating.

Crosslinking was quenched with the addition of glycine to a 250mM final concentration, and nutating 10 minutes. Quenched worms were washed once with 1X M9 and once with crosslinking buffer (1% formaldehyde in 1X M9), then transferred to a 7mL glass dounce homogenizer; worms were lysed on ice by douncing approximately 30 strokes. The worm suspension was further crosslinked with 1% formaldehyde in 1X M9 for 30 minutes at with nutating, and again quenched by adding 250mM final concentration of glycine, and nutating for 10 minutes.

Samples were washed 3x with 1X M9 and once with FA Buffer (50mM HEPES/KOH pH 7.5, 1mM EDTA, 1% Triton X-100, 0.1% Sodium Deoxycholate, 150mM NaCl, 1X Roche Complete Protease Inhibitor); samples were pelleted and supernatant removed before aliquoting and flash freezing. Prior to sonication, thawed pellets were resuspended in three volumes of FA Buffer; sonication was performed for 12 cycles of 30 seconds ON and 30 seconds OFF using the Diagenode BioRuptor NextGen (UCD-300) set at “high.”

Immunoprecipitation against H3K9me3 used 10ug of antibody Abcam #8898. pRb α MIX-1 antibody was custom made (Covance) against C-terminal peptides and affinity purified in-house; approximately 40ug were used. 3.3mg of extract in a final volume of 500uL was incubated with antibody overnight at 4°C; 60uL of a 50% Protein A/G agarose slurry was used to recover material bound by antibody.

Under nutation, complexed material was washed as follows: FA Buffer (2x 5 minutes), FA Buffer with 1M NaCl (1x 5 minutes), FA Buffer with 500mM NaCl (1x 10 minutes), TEL Buffer (0.25M LiCl, 1% NP-40, 1% Sodium Deoxycholate in TE) (1x 10 minutes), and TE (2x 5 minutes). Complexes were eluted by two rounds of 15 minute incubation with agitation at 65°C in 150uL elution buffer (1% SDS, 250mM NaCl in TE). Eluates were combined and digested with Proteinase K for two hours at 55°C, and treated with RNase A for 30 minutes at 37°C. Crosslinks were reversed by overnight incubation at 65°C, and DNA recovered by phenol:chloroform extraction and ethanol precipitation.

Quantitative Reverse Transcription PCR

Expression analysis qRT-PCR was carried out on cDNA prepared from total purified RNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen). ChIP qRT-PCR was carried out directly on undiluted immunoprecipitated DNA and diluted input DNA samples. All primers were tested for amplification efficiency and generation of single product by standard curve analysis of serial dilutions and gel electrophoresis.

Amplification reactions were prepared using Power SYBR Green PCR Master Mix (ABI) in a final reaction volume of 15uL, with triplicate technical replicates. Cycling was performed using the StepOne Plus Real-Time PCR System (ABI), and reaction verified for presence of single PCR product by dissociation curve analysis and gel electrophoresis. Relative quantification for

target enrichment was calculated using the Livak method (comparative ΔΔCT). Students T-Test was used for statistical analysis.

REFERENCES

1. Ahn SH, Kim M, Buratowski S. (2004). Phosphorylation of Serine 2 within the RNA Polymerase II C-terminal domain couples transcription with 3' end processing. *Mol Cell* 13:67-76.

2. Ashe A, Sapetschnig A, Weick EM, Mitchell J, Bagjin MP, Cording AC, Doebley AL, Goldstein LD, Lehrback MJ, Le Pen J, et al. (2012). piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell* 150:1-12.

3. Ayarpadikannan S, Heui-Soo K. (2014). The impact of transposable elements in genome evolution and genetic instability and their implications in various diseases. *Genomics Inform* 12:98-104.

4. Bayne EH, White SA, Kagansky A, Bijos DA, Sanchez-Pulidio L, Hoe KL, Kim DU, Park HO, Ponting CP, Rappsilber J, Allshire RC. (2010). Stc1: a critical link between RNAi and chromatin modification required for heterochromatin integrity. *Cell* 140:666-667.

5. Bagijn MP, Goldstein LD, Sapetschnig A, Weick EM, Bouasker S, Lehrbach NJ, Simard MJ, Miska EA. (2012). Function, targets, and evolution of *Caenorhabditis elegans* piRNAs. *Science* 337:574-578.

6. Batista PJ, Ruby JG, Claycomb MH, Chiang R, Fahlgren N, Kasschau KD, Chaves DA, Gu W, Vasale JJ, Duan S, et al. (2008). PRG-1 and 21U-RNAs interact to form the piRNA complex required for fertility in *C. elegans*. *Mol Cell* 31:67-78.

7. Bauer CR, Hartl TA, Bosco G. (2012). Condensin II promotes the formation of chromosome territories by inducing axial compaction of polyploidy interphase chromosomes. *PLoS Gen* 8:e1002873.

8. Bessereau JL. Transposons in *C. elegans* (January 18, 2006). WormBook, ed. The *C. elegans* Research Community, WormBook. doi/10.1895/ wormbook.1.70.1, http://www.wormbook.org.

9. Bhalla N, Biggins S, Murray AW. (2002). Mutation of YCS4, a budding yeast condensin subunit, affects mitotic and nonmitotic chromosome behavior. *Mol Biol Cell* 13:632-645.

10. Blauwkamp TA, Csankovszki G. (2009). Two classes of dosage compensation complex binding elements along *Caenorhabditis elegans* X chromosomes. *Mol Cell Biol* 29:2023-2031.

11. Bowen NJ, McDonald JF. (1999). Genomic analysis of *Caenorhabditis elegans* reveals ancient families of retroviral-like elements. *Genome Res* 9:924-935.

12. Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, Kimble J, Fire A, Kenedy S. (2012). A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. *Nature* 489:447-451.

13. Burkhart KB, Guang S, Buckley BA, Wong L, Bochner AF, Kennedy S. (2011). A pre-mRNA-associating factor links endogenous siRNAs to chromatin regulation. *PLoS Genetics* 7:e1002249.

14. Burton NO, Burkhart KB, Kennedy S. (2011). Nuclear RNAi maintains heritable gene silencing in *Caenorhabditis elegans*. *PNAS* 108:19683-19688.

15. *C. elegans* Sequencing Consortium. (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282:2012-2018.

16. Chan RC, Severson AF, Meyer BJ. (2004). Condensin restructures chromosomes in preparation for meiotic divisions. *JCB* 167:613-625.

17. Claycomb JM, Batista PJ, Pang KM, Gu W, Vasale JJ, van Wolfswinkel JC, Chaves DA, Shirayama M, Mitani S, Ketting RF, Conte D, Mello CC. (2009). The argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. *Cell* 139:123-134.

18. Csankovszki G, Collette K, Spahl K, Carey J, Snyder M, Petty E, Patel U, Tabuchi T, Liu H, McLeod I, et al. (2009). Three distinct condensin complexes control *C. elegans* chromosome dynamics. *Curr Biol* 19:9-19.

19. Csankovszki G, Petty EL, Collette K. (2009). The worm solution: a chromosome-full of condensin helps gene expression go down. *Chrom Research* 17:621-635.

20. Cutter AD, Dey A, Murray RL. (2009). Evolution of the *Caenorhabditis elegans* genome. *Mol Biol Evol* 26:1199-1234.

21. Das PP, Bagjin MP, Goldstein LD, Woolford JR, Lehrbach NJ, Sapetschnig A, Buhecha HR, Gilchrist MJ, Howe KL, Stark R, et al. (2008). Piwi and piRNAs act upstream of an endogenous siRNA pathway to suppress Tc3 transposon mobility in the *Caenorhabditis elegans* germline. *Mol Cell* 31:79-90.

22. Dej KJ, Ahn C, Orr-Weaver T. (2004). Mutations in the *Drosophila* condensin subunit dCAP-G: defining the role of condensin for chromosome condensation in mitosis and gene expression in interphase. *Genetics* 168:895-906.

23. Duchaine TF, Wohlschlegel JA, Kennedy S, Bei Y, Conte D, Pang KM, Brownell DR, Harding S, Mitani S, Ruvkin G, et al. (2006). Functional proteomics reveals the biochemical niche of C. elegans DCR-1 in multiple small-RNA-mediated pathways. *Cell* 124:343-354.

24. Duret L, Marais G, Biemont C. (2000). Transposons but not retrotransposons are located preferentially in regions of high recombination rate in *Caenorhabditis elegans*. *Genetics* 156:1661-1669.

25. Feschotte C, Pritham EJ. (2007). DNA transposons and the evolution of eukaryotic genomes. *Annu Rev Genet* 41:331-368.

26. Fischer SEJ. (2010). Small RNA-mediated gene silencing pathways in *C. elegans*. *Int J Biochem Cell Biol* 42:1306-1315.

27. Ganko EW, Fielman KT, McDonald JF. (2001). Evolutionary history of *Cer* elements and their impact on the *C. elegans* genome. *Genome Res* 11:2066-2074.

28. Garrido-Lecca A, Blumenthal T. (2010). RNA Polymerase II C-terminal domain phosphorylation patterns in *Caenorhabditis elegans* operons, polycistronic gene clusters with only one promoter. *Mol Cell Biol* 30:3887-3893.

29. Gent JI, Lamm AT, Pavelec DM, Maniar JM, Parameswaran P, Tao L, Kennedy S, Fire AZ. (2010). Distinct phases of siRNA synthesis in an endogenous RNAi pathway in *C. elegans* soma. *Mol Cell* 37:679-689.

30. Gerstein MB, Lu ZJ, Van Nostrand EL, Cheng C, Ashinoff BI, Liu T, Yip KY, Robilotto R, Rechtsteiner A, Ikegami K, et al. (2010). Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project. *Science* 330:1775-1787.

31. Ghildiyal M, Zamore PD. (2009). Small silencing RNAs: an expanding universe. *Nature* 10: 94-108.

32. Grewal SI, Jia S. (2007). Heterochromatin revisited. *Nat Rev Gen* 8:35-46.

33. Green LC, Kalitsis P, Chang TM, Cipetic M, Kim JH, Marshall O, Turnbull L, Whitchurch CB, Vagnarelli P, Samejima K, et al. (2012). Contrasting roles of condensin I and condensin II in mitotic chromosome formation. *J Cell Sci* 125:1591-1604.

34. Grishok A, Sinskey JL, Sharp PA. (2005). Transcriptional silencing of a transgene by RNAi in the soma of C. elegans. *Genes Dev* 19:683-696.

35. Guang S, Bochner AF, Pavelec DM, Burkhart KB, Harding S, Lachoweic J, Kennedy S. (2008). An Argonaute transports siRNAs from the cytoplasm to the nucleus. *Science* 321:537-541.

36. Guang S, Bochner AF, Burkhart KB, Burton N, Pavelec DM, Kennedy S. (2010). Small regulatory RNAs inhibit RNA polymerase II during the elongation phase of transcription. *Nature* 465:1097-1101.

37. Gu SG, Pak J, Guang S, Maniar JM, Kennedy S, Fire A. (2012). Amplification of siRNA is *Caenorhabditis elegans* generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint. *Nat Genet* 44:157-164.

38. Gu W, Shirayama M, Conte D, Vasale J, Batista PJ, Claycomb JM, Moresco JJ, Youngman EM, Keys J, Stoltz MJ, et al. (2009). Distinct Argonaute-mediated 22G-RNA pathways direct genome surveillance in the *C. elegans* germline. *Mol Cell* 3:231-244.

39. Haeusler RA, Pratt-Hyatt M, Good PD, Gipson TA, Engelke DR. (2008). Clustering of yeast tRNA genes is mediated by specific association of condensin with tRNA gene transcription complexes. *Genes Dev* 22:2204-2214.

40. Hagstrom KA, Holmes VF, Cozzarelli NR, Meyer BJ. (2002). *C. elegans* condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis. *Genes Dev* 16:729-742.

41. Hagstrom KA, Meyer BJ. (2003). Condensin and cohesion: more than chromosome compactor and glue. *Nature Rev Gen* 4:520-534.

42. Hall IM, Noma K, Grewal SIS. (2007). RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *PNAS* 100:193-198.

43. Hammond SM. (2005). Dicing and slicing: the core machinery of the RNA interference pathway. *FEBS* 579:5822-5829.

44. Han T, Manoharan AP, Harkins TT, Bouffard P, Fitzpatrick C, Chu DS, Thierry-Mieg D, Thierry-Mieg J, Kim JK. (2009). 26G endo-siRNAs regulate spermatogenic and zygotic gene expression in *Caenorhabditis elegans*. *PNAS* 106:18674-18679.

45. Hirano T. (2005). Condensins: organizing and segregating the genome. *Curr Biol* 15:R265-R275.

** Hirano, T. (2012). Condensins: universal organizers of chromosomes with diverse functions. *Genes Dev* 26:1659-1678

46. Hutvagner G, Simard MJ. (2008). Argonaute proteins: key players in RNA silencing. *Nature* 9:22-32.

47. Iwasaki O, Tanaka A, Tanizawa H, Grewal SIS, Noma K. (2010). Centromeric localization of dispersed PolIII genes in fission yeast. *Mol Biol Cell* 21:254-265.

48. Iwasaki O, Noma K. (2012). Global genome organization mediated by RNA polymerase III-transcribed genes in fission yeast. *Gene* 493:195-200.

49. Jans J, Gladden JM, Ralston EJ, Pickle CS, Michel AH, Pferdehirt RR, Eisen MB, Meyer BJ. (2009). A condensin-like dosage compensation complex acts at a distance to control expression throughout the genome. *Genes Dev* 23:602-618.

50. Jurka J, Kapitonov VV, Kohany O, Jurka MV. (2007). Repetitive sequences in complex genomes: structure and evolution. *Annu Rev Genomics Hum Genet* 8:241-259.

51. Kapitonov VV, Jurka J. Molecular paleontology of DNA transposons in eukaryotic genomes [abstract]. In: Proceedings of the Fourth International Conference on Bioinformatics of Genome Regulation and Structure (BGRS). 2004 Jul 25-30; Novosibirsk, Russia. 1:83-86.

52. Ketting RF, Haverkamp THA, van Luenen HGAM, Plasterk RHA. (1999). *mut-7* of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner Syndrome Helicase and RNaseD. *Cell* 99:133-141.

53. Kim JH, Zhang T, Wong NC, Davidson N, Maksimovic J, Oshlack A, Earnshaw WC, Kalitsis P, Hudson DF. (2013). Condensin I associates with structural and gene regulatory regions in vertebrate chromosomes. *Nature Comm* 4:2538.

54. Kim SH, Walbot V. (2003). Deletion derivatives of the MuDR regulatory transposon of maize encode antisense transcripts but are not dominant-negative regulators of mutator activities. *Plant Cell* 15:2430-2447.

55. Kimura K, Hirano M, Kobayashi R, Hirano T. (1998). Phosphorylation and activation of 13S condensin by Cdc2 in vitro. *Science* 282:487-490.

56. Klattenhoff C, Xi H, Li C, Lee S, Xu J, Khurana JS, Zhang F, Schultz N, Koppetsch B, Nowesielska A, et al. (2009). The *Drosophila* HP1 homolog Rhino is required for transposon silencing and piRNA production by dual strand clusters. *Cell* 138:1137-1149.

57. Kranz AL, Jiao CY, Winterkorn LH, Albritton SE, Kramer M, Ercan S. (2013). Genome-wide analysis of condensin binding in *Caenorhabditis elegans*. *Gen Biology* 14:R112.

58. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, et al. (2001). Initial sequencing and analysis of the human genome. *Nature* 409:860-921.

59. Liu T, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre , Cheung MS, Ercan S, Ikegami K, Jensen M, Kolasinska-Zwierz P, et al. (2011). Broad chromosomal domains of histone modification patterns in *C. elegans*. *Genome Res* 21:227-236.

60. Llorens C, Futami R, Covelli L, Dominguez-Escriba L, Viu JM, Tamarit D, Aguilar-Rodriguez J, Vicente-Ripolles M, Fuster, G, Bernet GP, et al. (2011). The *gypsy* database (GyDB) of mobile genetic elements: release 2.0. *Nucl Acids Res* 39:D70-D74 doi: 10.1093/nar/gkq1061

61. Lomberk G, Wallrath L, Urrutia R. (2006). The heterochromatin protein 1 family. *Genome Biol* 7:228.

62. Longworth MS, Walker JA, Anderssen E, Moon NS, Gladden A, Heck MMS, Ramaswamy S, Dyson NJ. (2012). A shared role for RBF1 and dCAP-D3 in the regulation of transcription with consequences for innate immunity. *PLoS Gen* 8:e1002618.

63. Luteijn MJ, van Bergeijk P, Kaaij LJT, Vasconcelos-Almeida M, Roovers EF, Berezikov E, Ketting RF. (2012). Extremely stable Piwi-induced gene silencing in *Caenorhabditis elegans*. *EMBO* J 31:3422-3430.

64. Machin F, Paschos K, Jarmuz A, Torres-Rosell J, Pade C, Aragon L. (2004). Condensin regulates rDNA silencing by modulating nucleolar Sir2p. *Curr Biol* 14:125-130.

65. Majorek KA, Dunin-Horkawicz S, Steczkiewicz K, Muszewska A, Nowotny M, Ginalski K, Bujnicki JM. (2014). The RNase H-like superfamily: new members, comparative structural analysis and evolutionary classification. *Nucl Acids Res* 42:4160-4179.

66. Malone CD, Hannon GJ. (2009). Molecular evolution of piRNA and transposon control pathways in *Drosophila*. *Cold Spring Harb Symp Quant Biol* 74:225-234.

67. Moazed D. (2009). Small RNAs in transcriptional gene silencing and genome defense. *Nature* 457:413-420.

68. Palazzo AG, Gregory TR. (2014). The case for junk DNA. *PLoS Genetics* 10:e1004351.

69. Pek JW, Kai T. (2011). A role for Vasa in regulating mitotic chromosome condensation in *Drosophila*. *Curr Biol* 21:38-44.

70. Phillips CM, Montgomery TA, Breen PC, Ruvkin G. (2012). MUT-16 promotes formation of perinuclear Mutator foci required for RNA silencing in the *C. elegans* germline. *Genes Dev* 26:1433-1444.

71. Plasterk RHA, Izsvak Z, Ivics Z. (1999). Resident aliens: the Tc1/*mariner* superfamily of transposable elements. *Trends Genet* 15:326-332.

72. Plasterk RHA, van Luenen HGAM. (1997). Chapter 5: Transposons. In *C. elegans* II, DL Riddle, ed. (Cold Spring Harbor, NY; Cold Spring Harbor Laboratory Press), pp. 97-116.

73. Rebollo R, Romanish MT, Mager DL. (2012). Transposable elements: an abundant and natural source of regulator sequences for host genes. *Annu Rev Genet* 46:21-42.

74. Robert VJP, Sijen T, van Wolfswinkel J, Plasterk RHA. (2005). Chromatin and RNAi factors protect the *C. elegans* germline against repetitive sequences. *Genes Dev* 19:782-787.

75. Ruby JG, Jan C, Player C, Axtell MJ, Lee W, Nusbaum C, Ge H, Bartel DP. (2006). Large-scale sequencing reveals 21U-RNAs and additional micro-RNAs and endogenous siRNAs in *C. elegans*. *Cell* 127:1193-1207.

76. Schuster AT, Sarvepalli K, Murphy EA, Longworth MS. (2013). Condensin II subunit dCAP-D3 restricts retrotransposon mobilization in *Drosophila* somatic cells. *PLoS Gen* 9:1003879.

77. Sela N, Kim E, Ast G. (2010). The role of transposable elements in the evolution of non-mammalian vertebrates and invertebrates. *Genome Biology* 11:R59.

78. She X, Xu X, Fedotov A, Kelley W, Maine EM. (2009). Regulation of heterochromatin assembly on unpaired chromosomes during *Caenorhabiditis elegans* meiosis by components of a small RNA-mediated pathway. *PLoS Gen* 5:e1000624.

79. Sienski G, Donertas D, Brennecke J. (2012). Transcriptional silencing of transposons by Piwi and Maelstrom and its impact on chromatin state and gene expression. *Cell* 151:964-980.

80. Slijen T, Plasterk RHA. (2003). Transposon silencing in the *Caenorhabditis elegans* germ line by natural RNAi. *Nature* 426:310-314.

81. Slotkin RK, Martienssen R. (2007). Transposable elements and the epigenetic regulation of the genome. *Nature Rev Gen* 8:272-285.

82. Spike CA, Bader J, Reinke V, Strome S. (2008). DEPS-1 promotes P-granule assembly and RNA interference in *C. elegans* germ cells. *Development* 135:983-993.

83. Stear JH, Roth MB. (2002). Characterization of HCP-6, a *C. elegans* protein required to prevent chromosome twisting and merotelic attachment. *Genes Dev* 16:1498-1508.

84. Studencka M, Konzer A, Moneron G, Wenzel D, Opitz L, Salinas-Riester G, Bedet C, Kruger M, Hell SW, et al. (2012). Novel roles of *Caenorhabditis elegans* heterochromatin protein HP1 and linker histone in the regulation of innate immune gene expression. *Mol Cell Biol* 32:251-65.

85. Sundaram P, Echalier B, Han W, Hull D, Timmons L. (2006). ATP-binding cassette transporters are required for efficient RNA interference in *Caenorhabditis elegans*. *Mol Biol Cell* 17:3678-3688.

86. Sundaram P, Han W, Cohen N, Echalier B, Albin J, Timmons L. (2007). *Caenorhabditis elegans* ABCRNAi-Transporters interact genetically with *rde-2* and *mut-7*. *Genetics* 178:801-814.

87. Tabara H, Sarkissian M, Kelley MG, Fleenor J, Grishok A, Timmons L, Fire A, Mello CC. (1999). The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* 99:123-132.

88. Towbin BD, Meister P, Pike BL, Gasser SM. (2010). Repetitive transgenes in *C. elegans* accumulate heterochromatic marks and are sequestered at the nuclear envelope in a copy-number- and lamin-dependent manner. *Cold Spring Harb Symp Quant Biol* 75:555-565.

89. Towbin BD, Gonzalez-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, Askjaer P, Gasser SM. (2012). Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. *Cell* 150:934-947.

90. van Wolfswinkel JC, Ketting R. (2010). The role of small non-coding RNAs in genome stability and chromatin organization. *J Cell Science* 123:1825-1839.

91. Vastenhouw NL, Fisher SEJ, Robert VJP, Thijssen KL, Fraser AG, Kamath RS, Ahringer J, Plasterk RHA. (2003). A genome-wide screen identifies 27 genes involved in transposon silencing in *C. elegans*. *Curr Biol* 13:1311-1316.

92. Vastenhouw NL, Plasterk RHA. (2004). RNAi protects the *Caenorhabditis elegans* germline against transposition. *Trends Genet* 20:314-319.

93. Vasale JJ, Gu W, Thivierge C, Batista PJ, Claycob JM, Yougnman EM, Duchaine TF, Melloc CC, Conte D. (2010). Sequential rounds of RNA-dependent RNA transcription drive endogenous small-RNA biogenesis in the ERGO-1/Argonaute pathway. *PNAS* 107:3582-3587.

94. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SIS, Martienssen RA. (2002). Regulation of heterochromatic silencing and histone H3 lysine 9 methylation by RNAi. *Science* 297:1833-1837.

95. Walker AK, Rothman JH, Shi Y, Blackwell TK. (2001). Distinct requirements for *C. elegans* $TAF_{II}S$ in early embryonic transcription. *EMBO* 20:5269-5279.

96. Werren JH. (2011). Selfish genetic elements, genetic conflict, and evolutionary innovation. *PNAS* 108:10863-10870.

97. WormBase Web Site. http://www.wormbase.org Release WS245, 31 October 2014.

98. Xu Y, Leung CG, Lee DC, Kennedy BK, Crispino JD. (2006). MTB, the murine homolog of condensin II subunit CAP-G2, represses transcription and promotes erythroid cell differentiation. *Leukemia* 20:1261-1269.

99. Yigit E, Batista PJ, Bei Y, Pang KM, Chen CCG, Tolia NH, Joshua-Tor L, Mitani S, Simard MJ, Mello CC. (2006). Analysis of the *C. elegans* Argonaute family reveals that distinct Argonautes act sequentially during RNAi. *Cell* 127:747-757.

100. Yuan YW, Wessler SR. (2011). The catalytic domain of all eukarotic cut-and-paste transposase superfamilies. *PNAS* 108:7884-7889.

101. Zamore PD, Haley B. (2005). Ribo-genome: the big world of small RNAs. *Science* 309:1519-1524.

102. Zhang C, Montgomery TA, Gabel HW, Fischer SE, Phillips CM, Fahlgren N, Sullivan CM, Carrington JC, Ruvkin G. (2011). *mut-16* and other mutator class genes modulate 22G and 26G siRNA pathways in *Caenorhabditis elegans*. *PNAS* 108:1201-1208.