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Understanding the role of MRG-1 in *C. elegans* germline and somatic development

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An abstract of
A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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Abstract

Understanding the role of MRG-1 in *C. elegans* germline and somatic development
By Christine Abiva Doronio

The chromatin structure formed by the packaging of DNA into nucleosomes greatly influences the transcriptional regulation of genes. Histone modifications like acetylation and methylation alter how tightly DNA interacts with histones therefore changing gene accessibility to transcriptional machinery. Variations to histone modification patterns and chromatin structure can be detrimental to gene regulation and development. Therefore, it is necessary to understand the chromatin related proteins that modify and bind to histones because of their contribution to the overall chromatin landscape.

Human MRG15 (*MORF4*-Related Gene on Chromosome 15) is a chromatin associated protein that binds to methylated lysine 36 on histone H3 (H3K36me) through a conserved chromodomain. Similar to other chromatin proteins, MRG15 interacts with chromatin modifying proteins such as histone acetyltransferases (HAT) and histone deacetylases (HDACs) that contribute to transcriptional activation and repression, respectively. MRG-1, the *C. elegans* ortholog of MRG15, contains the conserved chromodomain and was recently shown to interact with similar HDAC complexes. However, the function of the MRG-1 chromodomain and its role in chromatin complexes has never been analyzed.

In this work, I mutated key residues in the conserved MRG-1 chromodomain and discovered novel phenotypes in *mrg-1* mutants. Null mutations of *mrg-1* lead to meiotic germline defects that produce sterile, but viable, phenotypically normal offspring. However, I demonstrate that point mutations expected to disrupt the conserved chromodomain (*mrg-1 CD)* cause embryonic lethality, suggesting a role for the MRG-1 chromodomain in somatic development. Additionally, the *mrg-1 CD* mutation produces a dominant RNAi resistance phenotype. The MRG-1 CD protein properly localizes in the germline but is excluded in somatic tissues at later stages. These distinct phenotypes in *mrg-1* null versus chromodomain mutants indicate separate roles for MRG-1 in the germline and soma. Interestingly, both *mrg-1* deletion and chromodomain mutants show signs of improper histone acetylation regulation in the germline, consistent with MRG-1's proposed role in a larger histone modifying complex. Through this research I identified novel phenotypes and distinct contributions of the MRG-1 chromodomain in germline and somatic tissue that will aid in understanding the influence of overall chromatin structure in development.

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CHAPTER 1. INTRODUCTION

The packaging of DNA into chromatin in the nucleus is a highly regulated, dynamic process. The chromatin structure formed by DNA wrapped around an octamer of the histone proteins (named histones H2A, H2B, H3, H4) to form a nucleosome greatly contributes to gene regulation. Covalent modifications to histone tails, such as acetylation and methylation, influence how tightly DNA interacts with histones regulating chromatin structure. The resulting chromatin landscape alters gene accessibility to RNA polymerase II and other transcriptional machinery, ultimately regulating gene transcription. The precise coordination of the expression of genes is required for successful development of a thriving, fertile organism. Therefore, multiple systems and pathways exist that contribute to proper gene expression including so-called "epigenetic" mechanisms, like histone modifications, that determine chromatin landscape and gene accessibility. Understanding histone modifications and the proteins that regulate them are essential to understand epigenetic contributions to development.

This chapter will introduce the main concepts of epigenetics, specifically the role of histone modifications, their associated histone modifying proteins, and the influence of these modifications on development. I will further introduce and focus on the MRG family of chromatin binding proteins, including the *C. elegans* homolog MRG-1, and what is currently known about their contributions to chromatin structure and development.

1.1 Introduction to Epigenetics

While mutations in DNA sequences of genes can affect their function, proper regulation of their expression is also required. Heritable mechanisms that control the proper function and expression of genes without altering DNA sequence are the basis of "epigenetics". Epigenetics

was first suggested by Waddington while trying to understand the development of physical traits in *Drosophila* that could not be described by standard Mendelian genetics (1). He suggested that differentiation and development was determined by the "epigenetic landscape" that controlled gene express (2, 3). Over the years, the study of epigenetics has greatly expanded to included various mechanisms. Molecular factors like DNA methylation, histone modifications, histone variants, and noncoding RNAs have been shown to influence gene expression and contribute to the epigenetic regulation of genes (4).

In the nucleus, DNA is wrapped around histone proteins to form the nucleosome that further organizes into more complex chromatin structures (5). Each nucleosome is made up of a histone octamer consisting of two copies each of H3, H4, H2A, and H2B with ~147 base pairs of DNA wrapped around it (5). The organization of nucleosomes in chromatin contributes to the accessibility of genes and ultimately their expression. Modifiable N-terminal histone tails protrude from the nucleosomes and interact with surrounding nucleosomes and chromatin-associated proteins. Therefore, the addition of post translational modifications to histone tails that alter how nucleosomes are packaged helps to determine the overall chromatin landscape (6, 7). Histone modifications also influence how other histone binding proteins are recruited to the chromatin which will be discussed in a later section. Common post-translational histone modifications include, but are not limited to, acetylation, methylation, phosphorylation, and ubiquitination. This chapter will introduce the effects of histone acetylation and methylation on chromatin and provide an emphasis on the methylation of lysine 36 on histone H3 (H3K36me).

1.2 Histone Modifications and Chromatin

Chromatin with an open and less condensed structure, defined as *euchromatin*, is either the result of ongoing gene activity or maintained as a structure more easily accessible to RNA polymerase II and other transcriptional machinery, thereby allowing for gene activation. When chromatin becomes tightly condensed, forming *heterochromatin*, transcriptional machinery can no longer easily access genes and expression is prevented (8). Different histone modifications and different degrees of modification are associated with specific types of chromatin conformation (9). Histone acetylation is often associated with euchromatin because of its ability to reduce the charge on positively charged histone residues, decreasing the interactions with negatively charged DNA and making the chromatin less condense and more accessible (6). Because histone acetylation increases gene accessibility, histone acetylation modifications are often found at the transcription start sites (TSS) of genes (10). For example, acetylated lysine 9 on histone H3 (H3K9ac) and acetylated lysine 27 on histone H3 (H3K27ac) are found near enhancers and promoters upstream of genes. Other histone residues like H3K4, H3K36, H3K79, and H4K20 can be acetylated and are often used in combination with each other or other histone modifications to determine chromatin status (10, 11).

Unlike histone acetylation that usually correlates with gene expression, different types of histone methylation are associated with heterochromatin and euchromatin (9). For example, tri-methylation at lysine residue 9 on histone H3 (H3K9me3) is associated with constitutive heterochromatin and marks transcriptionally inactive regions such as regions of repetitive DNA (e.g., telomeres and centromeres) (12-15). Tri-methylated lysine 27 on histone H3 (H3K27me3) contributes to facultative heterochromatin; i.e., genes that are quiescent but become differentially expressed during development (16). For example, the X-chromosome in female

mammalian cells is modified by the Polycomb Repressive Complex 2 (PRC2) which marks the inactive X with H3K27me3 (17, 18). Like H3K4ac, tri-methylation of H3K4 (H3K4me3) is also associated with euchromatin and is found near TSSs of actively transcribed genes (19, 20). Although all H3K4me is associated with euchromatin, the different degrees of methylation are associated with different regions of the gene. For example, H3K4me2 and H3K4me1 are located downstream of the TSS in the gene body. H3K4me1 also associates with active enhancers upstream of the TSS often with other modifications like H3K27ac (10, 19-21).The contributions of different histone modification combinations to the overall chromatin landscape will be discussed below.

1.3 Chromatin Modifiers: Writers, Erasers and Readers

Because post-translational modifications on histones play a major role in determining chromatin landscape and gene regulation, it is necessary to understand the different enzymes responsible for these modifications. Proteins involved in regulating histone modifications and their functions can be classified into three main groups: writers, erasers, and readers (Figure 1.3) (22). Writers, such as histone methyltransferases (HMTs) or histone acetyltransferases (HATs), covalently add their respective groups to specific residues such as H3K27 or H3K36. Histone demethylases (HDMs) and histone deacetylases (HDACs) remove post-translational modifications from histone tails and are characterized as “erasers" (23-25). HATs neutralize the charge of histone lysines by introducing an acetyl group using acetyl CoA which reduces the attraction between positively charged histone tails and negatively charged DNA. HDACs therefore increase the interaction between histones and DNA by removing acetyl groups and promoting repressive chromatin. HMTs facilitate the addition of methyl groups from S-

adenosylmethionine (SAM) through their enzymatic SET domain and are often amino acid position-specific and degree of methylation specific. For example, the H3K36-specific HMT NSD1 catalyzes mono and dimethylation of H3K36, while SETD2 is responsible for trimethylation (26). Similarly, histone demethylases act in a context and residue specific matter. The Lysine demethylase (LSD) family is limited to demethylation of mono and dimethyl substrates of H3K4 and H3K9, while the Jumonji C (Jmjc) family can demethylate trimethylated residues including H3K36 (27, 28). Because histone methylation does not affect histone tail charge, different histone methylations can be associated with both active and repressed chromatin as previously mentioned. It is therefore important to understand the relationship between the location and degrees of certain modifications and specificity between the modifications. The crosstalk between the different histone modifications will be elaborated in a following section.

In addition to the modifying enzymes, there are multiple proteins that recognize specific posttranslational modifications through specific binding domains. Like histone modification writers and erasers, histone modification interaction domains provide binding specificity to epigenetic reader proteins. Methylated residues are bound by specific domains including tudor domains, chromodomains, plant homeobox domains (PHD), and Pro-Trp-Trp-Pro (PWWP) domains (6). Many of these methyl binding domains have a specific Beta-barrel formation that allows them to interact with the methylated residues that are conserved between species, including the MRG family chromodomain which will be further described in a following section. Bromodomains, that recognize acetylated lysine residues are characterized by four helices with interhelical loops that interact with the acetyl group. These histone modification “readers” are important in regulating chromatin structure because they detect the modification status of the

surrounding chromatin landscape and recruit other chromatin modifying enzymes (7). For example, some chromodomain-containing readers that bind to H3K36me3 have been shown to recruit HATs and HDACs (29, 30). Additionally, histone binding proteins can be components of larger ATP-dependent chromatin remodeling complexes. The ISWI and CHD sub-families of chromatin remodelers contain PHD domains and chromodomains, respectively, that allow them to detect methylated histones and alter nucleosome spacing to allow or prevent transcriptional machinery from binding accordingly (31). Overall, these histone modifying enzymes and their associated readers play a major role in constructing and maintaining the chromatin landscape that contributes to the regulation of gene expression (11).

1.4 H3K36me

Similar to H3K4, methylation of lysine residue 36 on histone H3 (H3K36me) is associated with euchromatin and is found within gene bodies that are being, or have been actively transcribed. In yeast the histone methyltransferase (HMT) responsible for H3K36me, Set2, follows RNA Polymerase II and deposits H3K36me3 along gene bodies during elongation (26). The degree of methylation increases from mono-methylation at the promoters to trimethylation along the gene body (10). H3K36me2/3 has been shown to prevent aberrant transcription initiation within coding regions by recruiting the Reduced potassium dependency 3 Small deacetylase complex (Rpd3S) to prevent histone acetylation within the gene bodies (32, 33).The ability of Rpd3S to bind to both H3K36me2 and H3K36me3 suggests some redundancy for the marks.

In *C. elegans* H3K36me is added by two histone methyltransferases, MET-1 and MES-4. Both MET-1 and MES-4 are maternally loaded and contribute to H3K36me3 on the autosomes

(34, 35). However, they have distinct functions in adding and maintaining H3K36me3 in the germline such that depletion of H3K3me3 requires the loss of both activities (35, 36). Like yeast Set2, MET-1 activity is coupled with active transcription in germ cell chromatin as well as in somatic lineages. The establishment of H3K36me3 during gametogenesis provides the basis for transgenerational memory since MET-1 is quickly diminished after fertilization while the histone modification persists (35). MES-4 appears to function independently of transcription and RNA Pol II activity, and instead acts to maintain H3K36me3 modifications between generations (36). Maternally loaded MES-4 acts as the primary HMT during early embryogenesis when transcriptional activity is low. MES-4 detects H3K36me3 on gamete-inherited chromosomes and maintains the inherited modification early on in embryogenesis (35). This provides evidence for H3K36me3 as a mark of transgenerational inheritance.

In addition to its contributions to transcriptional activity and transgenerational inheritance, H3K36me has been associated with replication, DNA repair and maintaining genomic stability. In response to DNA damage, H3K36me2 plays a role in recruiting the DNA repair factors like KU70 to DNA DSBs in the non-homologous end joining (NHEJ) (37). The presence of H3K36me at DSBs influences the recruitment of other chromatin modifying enzymes and their associated histone modifications like H4K20 that recruits other DNA damage response factors (38). This further demonstrates the importance of different histone modifications and their potential influence on one another. The role of H3K36me in gene regulation and the overall contribution to development is further reviewed in Wagner and Carpenter, 2012 (26).

1.5 Histone Modification Cross Talk

As previously mentioned, the type and residue location of the modification determines the structure and type of chromatin that is formed (Figure 1.1). Additionally, some histone modifications correlate with the presence of others, while others are mutually exclusive and negatively affect the addition of other modifications (Figure 1.2) (39). For example, some proteins that interact with or "read" H3K4me can interact with different histone acetyl transferases (HATs) such that H3K4me3 at active promoters is thought to promote the acetylation of H3/H4 at active TSSs (40). The heterochromatic mark H3K9me occurs exclusively from H3K4me3 such that H3K9 must be demethylated before H3K4 is methylated. This occurs as the H3K9 demethylase JMJD2B interacts with the H3K4 methylase MLL complex before transcriptional activation (41). Interestingly, despite being another mark of active transcription, H3K36me3 recruits histone deacetylase complexes (HDAC) to gene bodies to prevent initiation of aberrant TSS sites following RNA pol II elongation (32, 33).

Additionally, H3K36me3 is mutually exclusive with H3K27me3, a repressive modification. Their exclusivity is due to the allosteric inhibition of PRC2, the H3K27me3 methylase, at the same histone tail in the presence of H3K36me3 (42). Therefore, when observing mutants that lack H3K36me3 methylases, an increase or redistribution of H3K27me3 modifications is seen across gene bodies (43, 44). The exclusivity between H3K36me and H3K27me plays a role in the silencing of X-linked and somatic genes and the expression of germline genes in *C. elegans* (43, 45, 46). It has been proposed that the presence of each mark prevents the aberrant spreading of the other such that H3K36me at genes prevents silencing by PRC2 and H3K27me3 prevents MES-4 from binding, thereby providing physical boundaries that contribute to proper gene expression. Recent studies have demonstrated that EZH2, a subunit of

PRC2, contains a binding pocket that senses the methylation status of H3K36 before methylating H3K27 (47). Interestingly, mutations in *jmjd-5*, the H3K36me2 demethylase, results in an increase of both H3K36me2 and H3K27me3 suggesting a more intricate balance between the marks may exist (48). This forms a complex system of histone modification combinations that control the structure of chromatin, the accessibility of the underlying DNA, and ultimately the controlled expression of genes during development (11).

1.6 Chromatin's Contribution to Development

Because the accessibility of chromatin structure influences the transcriptional outcome of genes, histone modifications and their related enzymes play major roles in development. Histone modifications and modifying enzymes have been associated with development, cellular processes, and disease (7, 49). For example, Polycomb Group proteins, a conserved set of proteins involved in gene silencing through its histone methylation (PRC2) and ubiquitination (PRC1) activity, were identified for its essential role in body segmentation in *Drosophila* (17). H3K27me, a silencing mark that is added by PRC2, is detected by the PRC1 which ubiquinates lysine 119 of H2A (H2AK119ub). This is results in further compaction and silencing of the chromatin. Disruption of this silencing mechanism leads to improper maintenance of pluripotency in stem cells and downstream developmental defects (50, 51). Similarly, the accumulation or loss of chromatin modifications and their associated modifiers have been associated with different cancers and disease progressions (17, 22, 52). The misregulation of genes targeted by chromatin modifiers, like PRC2 and lysine demethylase 1 (LSD1), in cancer have made them useful biomarkers and targets for therapeutics. In total, posttranslational

modifications of histones and their related modifying proteins are crucial in the proper regulation of development and health across species.

1.7 The MRG Family of Proteins

Because chromatin structure plays a crucial role in development and cellular progression, it is unsurprising that different chromatin binding proteins have been shown to influence development and related cellular processes. Among these proteins is MORF4-related gene on chromosome 15 (MRG15), which is a chromodomain-containing protein that binds to methylated lysine 36 on histone H3 (H3K36me3) (53). MRG15 is a member of the MORF4-related gene (MRG) family of proteins which includes MORF-4, MRG15, and MORF4-related gene on chromosome X (MRGX) (29). Members of the MRG family localize to the nucleus and contain an ATP/GTP binding region with a helix-loop-helix, a leucine zipper, and the MRG motif at the C-terminus (29). There are notable differences within the subfamily. While all members contain a nuclear localization signal (NLS), MORF4 and MRG15 have a divided NLS with a phosphorylation signal, and MRGX has a single NLS (54). Additionally, MRG15 is the only MRG protein to include an N-terminal chromodomain and known to interact directly with chromatin (Figure 1.3) (53, 54). The MRG family of proteins are known for roles in different biological processes. MORF4 was identified as a transcription factor involved in cellular senescence, while MRG15 and MRGX were shown to regulate transcription, cell cycle progression and proliferation (54-56).

1.8 MRG15 Chromodomain in chromatin regulation

The chromodomain of MRG15 provides a unique function compared to other MRG family members. Chromodomain containing proteins often function with other chromatin related complexes to regulate chromatin structure and gene expression. As mentioned, MRG15 is known to interact with H3K36me3 through its chromodomain (53, 57). The ability to bind to a specific chromatin modification suggests an important role for MRG15 in any chromatin remodeling complexes within which it acts. Previous studies have demonstrated that MRG15 acts in multiple complexes including both histone acetyltransferase (HAT) and histone deacetylase complexes (HDAC) (53, 58). Specifically, MRG15 was identified as a component of MRG15 Associated Factors 1(MAF1) where it functions in B-myb promoter activation, and MAF2 where it interacts with histone acetylase (HAT) proteins. Interestingly, the formation and histone acetylase activity of MAF2, and its activation of the B-myb promoter is dependent on MRG15's chromodomain (53). Pf1, a known component of the mSin3a HDAC complex that influences transcriptional repression, specifically interacts with MRG15 but not MRGX or MORF4 (58). This suggests that the presence of the chromodomain in MRG15 is essential for its function in different chromatin remodeling complexes and their roles in determining chromatin structure.

1.9 Conservation of the MRG15 Chromodomain

The function of MRG15 in chromatin remodeling complexes demonstrates its importance in chromatin structure and ultimately gene expression (53, 58). Because of this influence on gene regulation, MRG15 has been implicated in numerous biological processes including development, genomic repair, aging, and lipid metabolism (55, 59). The sequence and predicted structure of MRG15, including its chromodomain, is conserved across species and homologs of

MRG15 have been identified in similar processes (Figure 1.4) (57, 60). For example, in mice MRG15 is necessary for the proliferation and differentiation of neural precursor cells, and proper DNA damage repair (30, 61). Similarly, Mrg15 in *Drosophila* is a member of multiple complexes including the Tip60 complex that acetylates nucleosomes in response in DNA damage, and the Ash1 complex responsible for H3K36me histone methyltransferase activity at specific genes (62, 63).

However, the interactions of MRG15 homologs in chromatin complexes are not conserved across all species. MRG-1 in *C. elegans*, despite this organism having similar chromatin immunoprecipitation profiles of H3K36me and H3K36me histone methyltransferases, has not been identified in an H3K36 methyltransferase complex. Recent mass spectrometry data suggests that MRG-1 is a member of the conserved SIN3 HDAC complex with other chromatin proteins SIN-3 and HDA-1 (64-66). Interestingly, interaction with MRG-1 is dependent on the sumoylation status of HDA-1 (67). Like MRG15, MRG-1 functions in different biological processes. Maternally loaded MRG-1 in primordial germ cells (PGCs) is required for post-embryonic germ cell proliferation and the proper regulation of germline expressed genes (68-70). *mrg-1* mutants exhibit ectopic expression of somatic genes in the germline confirming its role in transcriptional regulation of germline identity (66, 71). Additionally, MRG-1 plays an important role in maintaining genomic integrity and meiosis (72, 73). Mutants that lack zygotically expressed MRG-1 exhibit a homolog alignment defect that prevents homologous chromosomes from fully aligning and synapsing after properly pairing (73). Interestingly, *mrg-1* mutants exhibit a maternal effect sterile (MES) phenotype similar to mutants that lack MES-4, an H3K36-specific methyltransferase, as well as members of the PRC2 complex in *C. elegans (35)*. Because MRG-1 has not been shown to interact with any of the MES related proteins, it suggests

that this phenotype is related to its predicted interaction with H3K36me3 through its conserved chromodomain and its ability to interact with other chromatin remodeling complexes (66).

1.10 *C. elegans* Germline and Maternally Loaded Proteins

The use of *C. elegans* as a model organism began with the work of Sydney Brenner in 1963 when he wanted to identify a system that would advance the study of development. Since then, *C. elegans* have been used in research laboratories to study different aspects of development and genetics. Their small size and transparent bodies, they are easily viewed with dissecting microscopes allowing for the easy study of meiosis and reproduction (74). Because *C. elegans* exists as hermaphrodites and males, the hermaphrodite germline provides a visualization of the complete process of meiosis from germ cell proliferation to sperm and oocyte production and finally fertilization. After fertilization, zygotic and somatic development can be easily followed in the next generation. This makes *C. elegans* an ideal model to study germline and somatic development.

An additional benefit of hermaphrodites in *C. elegans*, is their contribution to studying transgenerational inheritance. The development and future germline of an embryo is greatly influenced by proteins and genetic material contained in the oocyte from the mother (maternal load) despite their own genetic make-up (zygotic). In *C. elegans*, many chromatin related proteins are maternally loaded into the embryo and are necessary for successful for germline development. Mutants of these maternally loaded chromatin proteins produce a specific grandchildess phenotype and are described as Maternal Effect Sterile (MES) proteins (Figure 1.5). The MES proteins include MES-2/3/6 which function in H3K27 methylation, and MES-4 which methylates H3K36me as previously described (34, 45, 75). Similarly, the chromodomain

containing protein MRG-1 has a MES phenotype (68). The unique characteristics of *C. elegans* and their germline allow us to study the relationship between chromatin related proteins and their contributions within and between generations.

1.11 Scope of Dissertation

The following chapter of this dissertation examines the role of both maternal and zygotic roles of MRG-1 in the germline and soma. Using CRISPR editing, I created mutations in the conserved chromodomain and the mutant phenotypes suggest different functions for MRG-1 in germline and somatic development. Our data along with previous studies demonstrate a role for MRG-1 in meiotic progression such that mutants that lack any MRG-1 fail to fully align homologous chromosomes despite proper pairing center function and ultimately exhibit a delay in synapsis (73). Additionally, *mrg-1* null mutants have an aberrant number of crossover foci in late pachytene and an increase in univalents in oocytes suggesting improper crossover formation. However, *mrg-1* mutants that only have the chromodomain altered, exhibit distinct phenotypes. Unlike null *mrg-1* mutants, *mrg-1* chromodomain (CD) mutants are embryonic lethal and do not display a MES phenotype which produce sterile offspring. Similarly, *mrg-1 CD* mutants do not have meiotic defects such as signs of delayed synapsis. However, progeny with solely a maternal load of MRG-1 CD in the germline fail to develop a complete germline and grow up sterile, demonstrating that a maternal of contribution of MRG-1 CD is insufficient for germline development. Interestingly, a percentage of the progeny that only have maternal MRG-1 CD combined with either zygotic MRG-1 WT or MRG-1 CD can successfully develop a germline, suggesting zygotic expression of MRG-1 (either the CD mutant or WT) can rescue germline development. Additionally, a single copy of the *mrg-1 CD* allele causes a dominant RNAi

defect; i.e., these animals are resistant to dsRNA targeting multiple genes that were assayed. A similar phenotype has been reported in other chromatin modifying enzyme mutants like *hda-1,* a component of the HDAC complex SINS3. Because MRG-1 has been identified in similar complexes, this suggests a potential role for the chromodomain in localizing larger complexes to genomic regions that impact RNAi-based mechanisms.

Chapter 3 of this dissertation discusses MRG-1 functions within complexes and its potential interactors. MRG15 has been shown to interact with itself and other proteins in other complexes (30, 53, 57, 76). However, my data suggests that MRG-1 does not directly interact with itself. Differentially tagged versions of MRG-1 do not directly interact with one another despite visually colocalizing with each other in the germline. This suggests that in *C. elegans*, MRG-1 does not dimerize but probably exists as a component of complexes with other proteins. Recently, studies have identified MRG-1 in different chromatin modifying complexes such as the HDAC complex with HDA-1 and SIN-3(65-67). Interestingly, *mrg-1* CD mutants display similar embryonic lethal phenotypes to *sin-3*. Additionally, *mrg-1 CD* mutants have an increase in acetylation in the meiotic region of the germline. Recent studies have also shown that the interaction between HDA-1 and MRG-1 is dependent on the sumoylation status of HDA-1 (67). Interestingly, *hda-1* sumoylation mutants have an RNAi defect similar to *mrg-1* CD mutants. MRG-1 was also identified as an interactor with the SET/COMPASS complex containing CFP-1 and WDR-5 that methylates H3K4 (65). Single mutants provided with maternal protein but lacking zygotic *mrg-1* or *cfp-1* produce fertile progeny with a developed germlines. However when knocking down *cfp-1* in *mrg-1* M+Z- null mutants, double knockout progeny fail to develop a germline suggesting a synergistic interaction between the two. Although it is unclear

the exact function of MRG-1 in these complexes, possible roles will be discussed in later chapters.

Overall, the data presented provides further insight to the role MRG-1 plays in *C. elegans* development. It has been widely shown here and in previous studies that MRG-1 is necessary for properly meiotic progression and germline development. In this dissertation we provide evidence for a new, distinct role for the MRG-1 chromodomain influencing embryonic development and identify an essential role in somatic development. Additionally, preliminary data along with recent studies suggest MRG-1 functioning in larger chromatin binding complexes such that *mrg-1* mutants and HDAC mutants, *sin-3* and *hda-1*, have similar phenotypes. Although implications and suggestions for MRG-1 in development and chromatin binding still need to be further studied, the data presented in this dissertation provides new insights for the function of MRG-1 and its conserved chromodomain in *C. elegans* germline and somatic development.

1.12 Figures

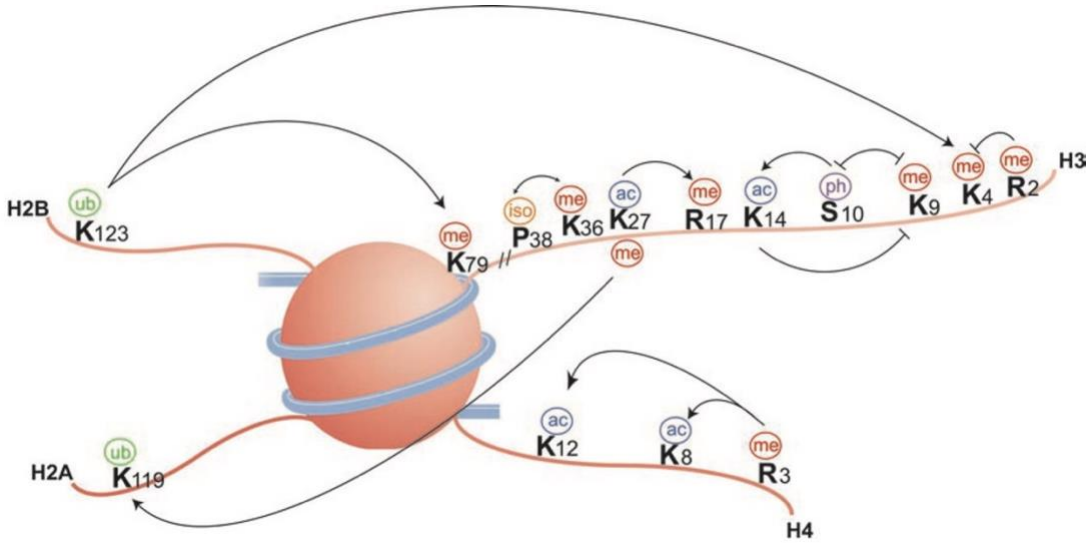


Figure 1.1 Histone Modification Coordination.

Different histone modifications can influence the presence of other histone modifications. Some modifications promote the addition of modifications (indicated by the arrow). Other modifications prevent or promote the removal of other modifications (indicated by straight bar). (Adapted from (6); Copyright © 2011, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences License: 5323200405190)

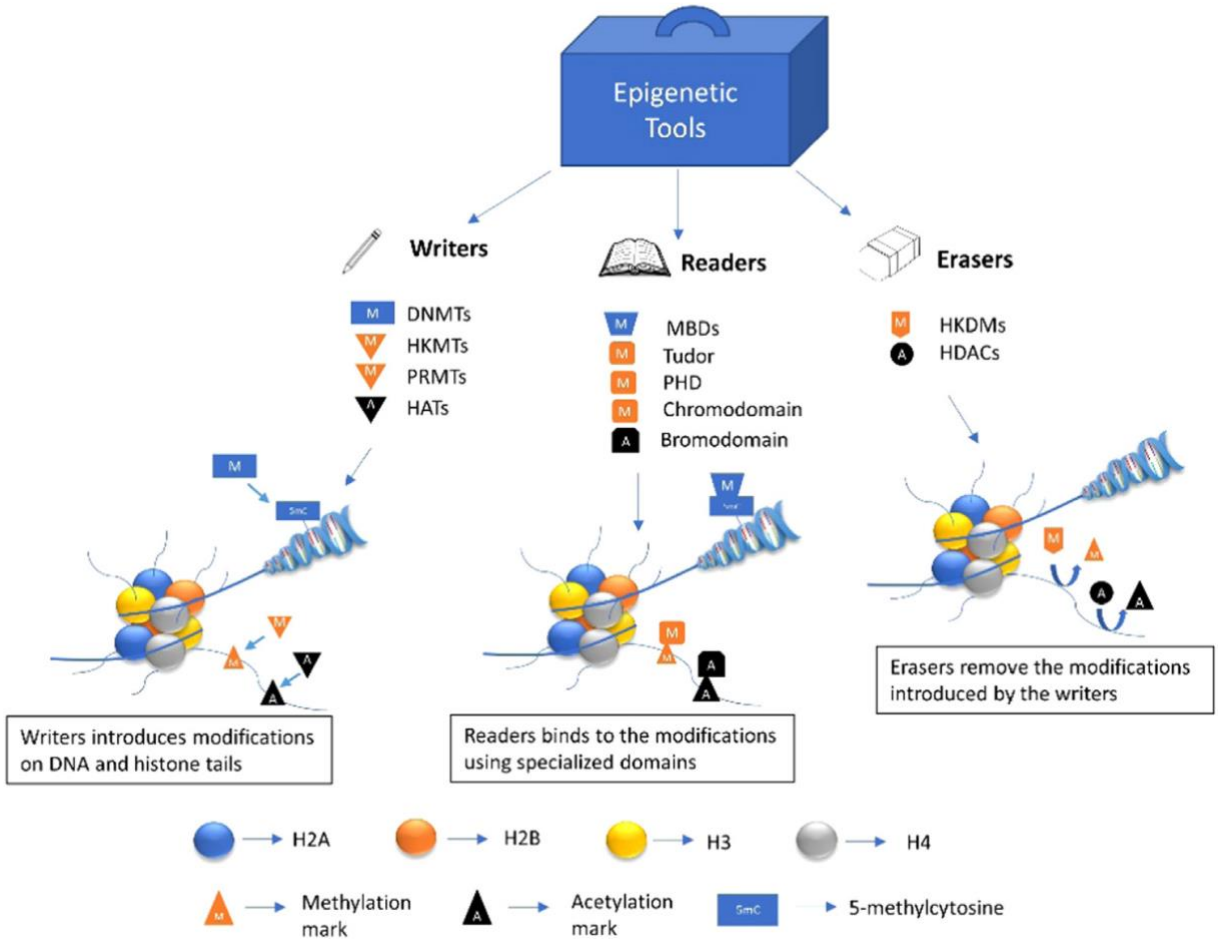


Figure 1.2 Chromatin Modifying Proteins.

Histone modifications are regulated by reader, writer, and eraser proteins. Histone modifications are ”written” by specific enzymes to histone tails (left), “read” by specific domains (middle), and “erased” by enzymes such as demethylases and deacetylases (right). (Adapted from (22), © 2018 Elsevier B.V. All rights reserved., license number: 5323170451648)

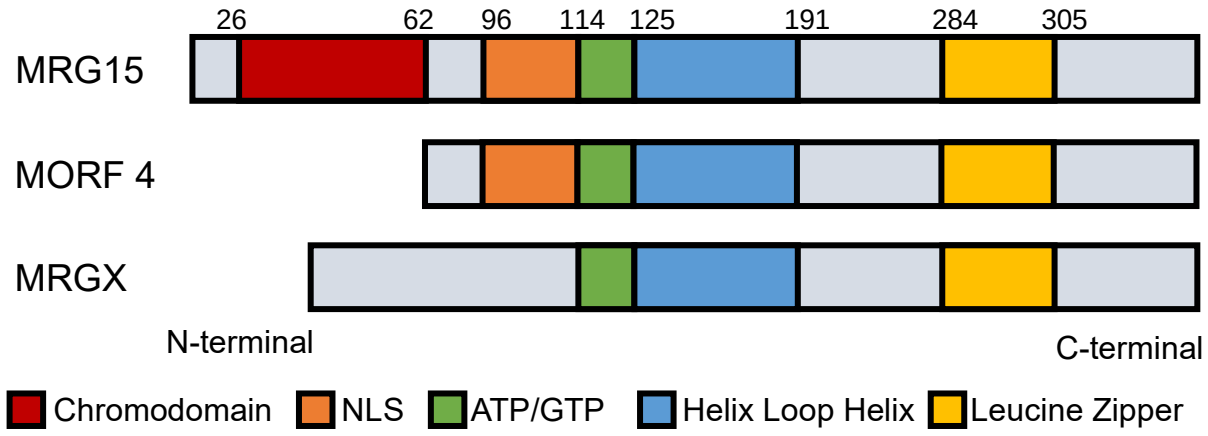


Figure 1.3 Schematic of the Structural Motifs in the MRG Family of Proteins.

MORF4, MRGX, and MRG15 are members of the MRG Family of proteins. They share similar structure with a ATP/GTP region, helix loop helix, and leucine zipper at the C-terminal. MORF4 and MRG15 contain an NLS site. MRG15 has a unique N-terminal chromodomain.

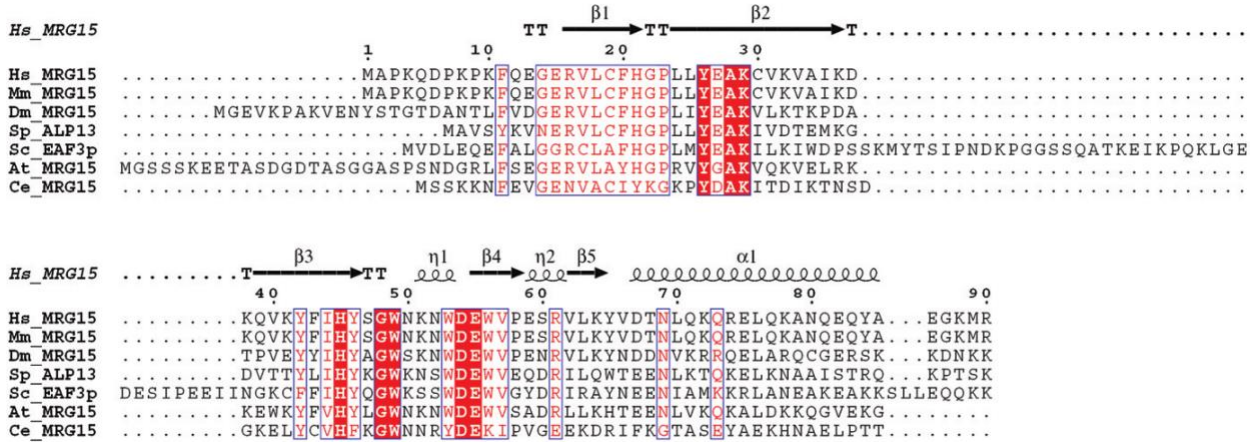


Figure 1.4 MRG15 Conserved Chromodomain Alignment.

The MRG15 The MRG15 chromodomain barrel is made up of conserved residues across different species: human, mouse, *Drosophila, S. pombe, S. cerevisiae, Arabidopsis, C. elegans*. Strictly conserved residues are filled in red and functionally conserved residues are shown in white boxes with red text. (Adapted from Zhang, et. al (57).)

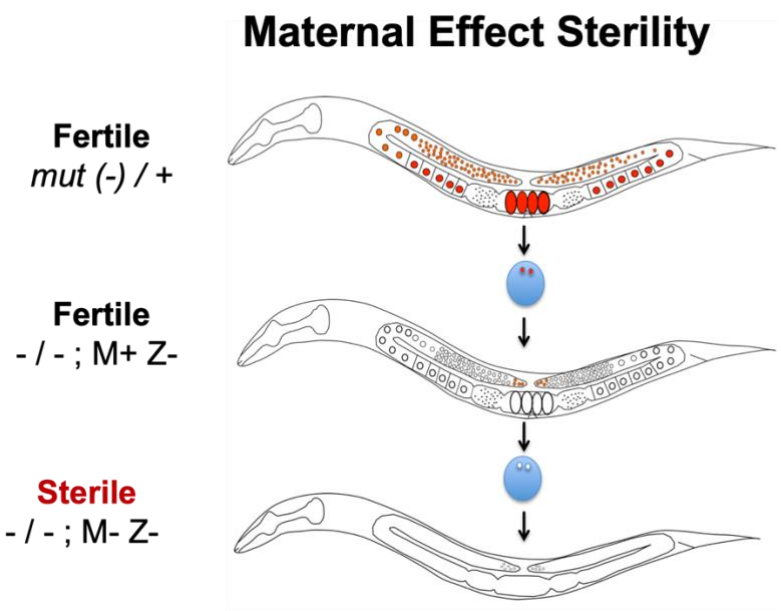


Figure 1.5 Maternal Effect Sterility

Maternal Effect Sterility (MES) phenotype occurs when a protein is maternally loaded from the previous generation. Mutants that have a maternal load of protein (M+) are fertile despite being zygotic minus (Z-). Progeny of zygotic minus mutants do not have a maternal load and become sterile (M-Z-).

CHAPTER 2. MRG-1 FUNCTIONS IN THE SOMA AND THE GERMLINE

The work in this chapter is adapted from "A Chromodomain Mutation Identifies Separable Roles for *C. elegans* MRG-1 in Germline and Somatic Development**"
Christine A. Doronio, Huiping Ling, Elizabeth J. Gleason, William G. Kelly
** Experiments and data analysis were performed by Christine Doronio. CRISPR guides were designed by Elizabeth J. Gleason and injected by Huiping Ling. This work is currently under review.

2.1 Abstract

The packaging of DNA into chromatin strongly influences gene regulation. Post-translational modifications of histones, and the proteins that bind to them, alter the accessibility of chromatin and contribute to the activation and repression of genes. The human MRG15 (*MORF4*- Related Gene on chromosome 15) protein is a conserved chromodomain-containing protein that binds to methylated lysine 36 on histone H3 (H3K36me) and plays important roles in development, genome integrity, and gene regulation. MRG15 affects transcriptional regulation through its interactions with both histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes. MRG-1, its *C. elegans* homolog, has similarly been shown to have important roles in genomic integrity and development, and has also been shown to co-purify with HDAC complexes. Like MRG15, MRG-1 is predicted to bind to H3K36me through its chromodomain, yet despite *mrg-1* mutants displaying developmental and germline phenotypes that overlap with H3K36 methyltransferase mutants, the role of the MRG-1 chromodomain has never been characterized. In this study, we examined meiotic cells lacking H3K36me3 to

compare to *mrg-1* mutant germ cell phenotypes, and mutated key residues in the MRG-1 chromodomain (CD) to assess its function. The CD mutations cause embryonic lethality but few post-embryonic germline defects, in contrast to *mrg-1* deletion mutants which are viable but sterile. The CD mutations therefore disrupt somatic development despite the apparent absence of a requirement for MRG-1 protein in embryogenesis. Furthermore, the CD mutants exhibit a dominant RNAi resistance phenotype that is not seen in other *mrg-1* mutant alleles. Interestingly, these phenotypes are similar to those seen in other chromatin related protein mutants, such as HDACs. This suggests that the function of MRG-1, and the chromatin modifying complexes with which it interacts, includes tissue-specific interactions involving different requirements for a functional chromodomain. We propose that the CD mutation disrupts proper guidance of complexes within which it acts, and this guidance defect results in improper HDAC and/or HAT regulation causing an indirect defect in RNAi machinery expression or targeting.

2.2 **Introduction**

The regulation of genes is largely controlled by their structural accessibility to transcription machinery within the nucleus. The structural consequences of the architecture within which DNA is packaged by nucleosomes in chromatin contributes to gene activation and repression, with different epigenetic modifications such as histone modifications and DNA methylation affecting that architecture. Consequently, proteins that alter and interact with chromatin play a major role in proper gene regulation and organismal development.

In humans, MRG15 (*MORF*4- Related Gene on Chromosome 15) interacts with the histone modification tri-methylated lysine 36 on Histone H3 (H3K36me3) through MRG15's

conserved chromodomain. Chromatin binding proteins like MRG15 that recognize specific histone modifications are referred to as epigenetic "readers" and are often associated with larger chromatin remodeling complexes that can alter transcription states of genes. For example, MRG15 is essential for the assembly of MAF1 and MAF2, HAT related complexes necessary for the activation of the B-myb promoter (53). Additionally, MRG15 is required for histone acetylation and subsequent RNA Polymerase II recruitment to lipid metabolism genes in mice (59). The presence of MRG15 at certain promoters can also prevent the binding of HDAC complexes during the cell cycle (63). Interestingly, MRG15 has been shown to play a role in both histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes that are associated with gene activation and repression respectively (30, 63). The requirement for MRG15 in various chromatin remodeling complexes suggests that its ability to “read” epigenetic modifications influences chromatin-regulated differentiation in specific cell types during embryonic development in mammals (30, 77).

Perhaps related to its role in chromatin binding and remodeling, MRG15 is known to contribute to DNA repair and genomic integrity. Through its interaction with the BRCA complex, MRG15 recruits different DNA damage response proteins, like RAD51 and PALB2, during homology directed DNA repair (78). Similar roles for MRG15 are seen in other mammalian species where MRG15 influences DNA repair, cellular differentiation, and pre-mRNA splicing in spermatogenesis (61, 79).

The structure of MRG15 consists of two conserved domains: an N-terminal chromodomain and a C-terminal MRG domain. The MRG15 chromodomain contains aromatic residues that form a barrel-like chromodomain pocket that interacts with modified histone tails, specifically H3K36me3 (57). When the chromodomain is mutated, MRG15 loses its

transcriptional regulation activity suggesting that the chromodomain is necessary for its role in HAT activity(53). The MRG15 chromodomain barrel is conserved across multiple species including, mice, *Drosophila*, and *C. elegans*. In *Drosophila*, Mrg15 interacts with Ash1 and stimulates its H3K36 methyltransferase activity(62, 80). However, this interaction with histone methyltransferases is not known to be conserved in *C. elegans(66).*

Like MRG15, its *C. elegans* homolog MRG-1 has various functions and has been found to co-purify with multiple chromatin modifying complexes. MRG-1 was recently identified as a member of the conserved HDAC complex, SINS3, that includes SIN-3 and HDA-1(65, 66). The SINS3 complex is thought to target histone deacetylation at different promoters, but the specific role of MRG-1 in this complex is unclear (65).

C. elegans MRG-1 is a maternally loaded protein that is essential for germline development. Maternally provided MRG-1 is both necessary and sufficient for the proper proliferation of primordial germ cells (PGCs) and the loss of MRG-1 results in improper regulation of germline-expressed genes (68-70). For example, MRG-1 is required for the suppression of numerous genes on the X-chromosome as well as the repression of germline-specific genes in somatic tissues (71). Conversely, MRG-1 also prevents ectopic expression of somatic genes in the germline, further confirming its role in transcriptional regulation in the germline(66). In addition to its transcriptional regulation functions, MRG-1 has also been shown to play roles in genomic integrity and meiosis. Mutants lacking MRG-1 exhibit a meiotic defect in which homologs are able to pair properly, but are unable to fully align for normal synapsis (73). The maternal effect sterility of *mrg-1* is similar to that seen in *mes-4* mutants. MES-4 is an H3K36 methyltransferase that, like *mrg-1*, has a maternal requirement that is both necessary and sufficient for germline development. The similarity between *mes-4* and *mrg-1* mutant

phenotypes, and the known binding of H3K36me3 by MRG-1's homolog MRG15, have led to the assumption that MRG-1's essential germline role is through its interactions with MES-4-dependent H3K36me3 (81).

Here we further explore the role of MRG-1 in *C. elegans* meiosis and development. We confirm MRG-1's essential function in meiosis and further characterize the synapsis defects in *mrg-1* mutants, including a role in proper crossover formation and regulation. We also show that a lack of zygotic MRG-1 protein causes a variety of partially penetrant somatic developmental defects. Given the presumed role of MRG-1's chromodomain in H3K36me3 recognition, a modification with important germline functions, we investigated the specific roles of H3K36me3 and the chromodomain of MRG-1 in meiotic events. Depletion of H3K36me3 in the germline results in meiotic defects that are similar to absence of the MRG-1 protein. Surprisingly, we find that an intact chromodomain is not necessary for MRG-1's function in meiosis or germline proliferation but is instead required zygotically for embryonic development. Oddly, mutation of the chromodomain causes novel phenotypes, including embryonic lethality and a dominant RNAi defect that is not observed in the null allele. We also observe an increase in histone H3 acetylation in germ cell chromatin. This suggests that the roles of H3K36 methylation and MRG-1 in germline development and function are complex, and that MRG-1's presumed recognition of H3K36 methylation may not be essential for post-embryonic germline development and meiotic regulation.

2.3 **Results**

2.3.1 Zygotic Requirements for MRG-1 in Meiosis

The human homolog of MRG-1, MRG15, plays important roles in transcriptional regulation(30, 53, 59, 63, 77). In *C. elegans*, MRG-1 is maternally loaded into the germline and the maternal contributions is required for proper germline development. MRG-1 is maternally necessary and sufficient for fertility: mutants have a grand-daughterless/maternal effect sterile (MES) phenotype in which homozygous *mrg-1/mrg-1*mutants from heterozygous *mrg-1/+* mothers develop a germline, but the next generation is sterile. The fertile F1 progeny are termed M+Z-; i.e., they inherited maternal MRG-1 produced by the mother (M+) but lack a zygotically functional *mrg-1* gene (Z-). F2 progeny from M+Z- mutants are thus sterile because they do not inherit a maternal source required for germline development (M-Z-). Despite the *mrg-1* M+Z- offspring being fertile, they display a decreased brood size and increased embryonic lethality when compared to wild type or *mrg-1* heterozygotes (Supplemental 2.1). This suggests that there is also a zygotic requirement for MRG-1 in the post-embryonic germline and soma.

We further confirmed results from previous studies that showed that zygotic MRG-1 is necessary for proper meiotic progression. In addition to increased sterility and embryonic lethality (Supplemental 2.1), Carolyn et al. showed that *mrg-1* M+Z- offspring exhibit a homologous chromosome alignment defect: homologs can successfully pair at their pairing centers, but the chromosome ends opposite these centers fail to align (73). Homolog pairing and alignment occurs in the transition zone of *C. elegans* meiosis before progressing into pachytene and engaging in synapsis. The transition zone is characterized by nuclei with condensed DAPI-stained chromosomes grouped at one side of the nuclear periphery in a characteristic crescent shape. We compared the length of the transition zones in N2 wild type and *mrg-1(tm1227) M+Z-*

mutants. In wild type hermaphrodites, the transition zone consists of a short region encompassing roughly 25-30% of the ovary region measured (described in Materials and Methods) (Figure 2.1A, C). In *mrg-1 M+Z-* worms, the transition zone length was significantly extended to as much as 50% of the calculated length and nuclei with the crescent-shape chromatin were also apparent in more proximal regions of the expected pachytene germline (Figure 2.1B, C). An extended transition zone is characteristic of delayed progression into pachytene and synaptic delay, as might be expected from previous reports of MRG-1's role in pairing-center independent alignment.

In *C. elegans*, 6 pairs of homologous chromosomes must align, synapse, and initiate double-stranded breaks for recombination. One double strand break matures into a single crossover (CO) between each homolog that is marked by localization of the COSA-1 protein (82). In worms expressing GFP::COSA-1, 6 GFP foci are visible in late pachytene nuclei (region 6: n=102) where 6 COs have successfully formed between homologs (Figure 2.2 A, C). Upon *mrg-1* knockdown by RNAi, 6 COSA-1 foci were observed in the majority of nuclei (region 6: n=63), yet nuclei with less than 6(region 6: n=28) and some with more than 6 COSA-1 foci (region 6: n=8) were also often observed, indicating a defect in CO regulation (Figure 2.2 B, D). Additionally, CO formation appears to occur earlier in pachytene (Region 4) after *mrg-1* knockdown. As cross-over interference and maturation is regulated by proper synaptonemal complex formation between homologs, this is likely another downstream effect of defective homolog alignment and abnormal synapsis with MRG-1 knock-down (83).

To summarize, in addition to maternal provision of MRG-1 being necessary and sufficient for the initial stages of germline development, continued zygotic expression of MRG-1 is also necessary for normal post-embryonic meiotic progression (73).

2.3.2 MRG-1 is required for the regulation of crossovers during meiosis

Given the defects in synapsis progression, we further characterized crossover formation in *mrg-1(tm1227)* mutants. As expected for defects in synapsis and CO formation, we also observed an increased number of achiasmatic chromosomes in diakinetic oocytes. In wildtype oocytes, 6 attached pairs of homologs (bivalents) are normally observed in the oocytes (n=121)(Figure 2.3A). In contrast, *mrg-1(tm1227) M+Z-* mutants exhibit a variable number of univalent chromosomes(n=25), with as many as 12 DAPI bodies observed in some oocytes (Figure 2.3B). It is important to note, however, that at least 50% of the *mrg-1* oocytes successfully synapsed and recombined all homolog pairs(n=36), indicating that zygotic MRG-1 is only partially required for proper homolog alignment, synapsis, and recombination (Figure 2.3C).

2.3.3 H3K36me3 is Required for Normal Meiotic progression

As a homolog of MRG15, it is predicted that MRG-1 recognizes and binds to H3K36 methylation. Indeed, ChIP analyses of MRG-1 has shown its enrichment in the genome overlaps with enrichment of H3K36me3 and H3K4me3 (66). In order to determine if H3K36me3, and thus its recognition by MRG-1, were associated with the *mrg-1(tm1227)* phenotypes, we reduced H3K36 methylation in germ cells and assessed any meiotic consequences. MET-1 and MES-4 are the two H3K36 methyltransferases in *C. Elegans*. MET-1 dependent H3K36 methylation is associated with active transcription, whereas MES-4 activity can occur independently of transcription and is thought to play a role in maintaining an epigenetic memory of germline transcription across generations (35, 36, 75). Single RNAi knock downs, and/or single mutations of either *mes-4* or *met-1* do not substantially decrease H3K36me3 levels in germ cells (Figure 2.4

A, B, E). Previous reports demonstrated that the disruption of both *met-1* and *mes-4* depletes all H3K36me3 in embryos (75). We therefore performed *mes-4* RNAi in L1 *met-1* mutant larvae. In addition to having a decreased brood size, we analyzed their germline and saw a dramatic decrease in H3K36me3 (Supplemental Figure 2.2, Figure 2.4 F).

We first asked if reduced H3K36me3 results in an extended transition zone, as observed in *mrg-1* M+Z- ovaries. In wildtype germlines, after being treated with both the empty control vector and *mes-4* RNAi, normal lengths of synapsis were visible and no transition zone nuclei were present in the proximal pachytene region (Figure 2.4 C, D). A similar result was seen in *met-1(n4337)* and in control RNAi germlines (Figure 2.4 G) suggesting that loss of either MET-1 or MES-4 activities alone has little effect on meiotic progression. In contrast, *met-1(n4337); mes-4(RNAi*) worms exhibited crescent shaped nuclei visible throughout the germline extending into the proximal pachytene region (Figure 2.4 H), indicating a delay in synapsis. This extended transition zone phenotype suggests that H3K36me3, like MRG-1, plays an important role in meiotic progression.

2.3.4 M-Z- *mrg-1* animals Exhibit Zygotic Defects in Soma

Maternal deposition of MRG-1 is essential for fertility and M-Z- offspring from fertile M+Z- mothers are sterile but grow into adulthood. A previous study using targeting *mrg-1* by RNAi observed the expected sterility, but also reported a significant frequency of post-embryonic somatic defects in the RNAi-treated animals (84). The low frequencies of the defects observed were attributed to incomplete RNAi efficiency. However, we similarly found that the M-Z- generation of the null *tm1227* allele also showed an incomplete penetrance of somatic developmental defects in adult animals. *Mrg-1(tm1227)* M-Z- animals frequently displayed

somatic defects such as multiple vulvas (Muv), tail defects, and dumpy (Dpy) morphology (Supplemental Table 2.1, Suppplemental Figure 2.3). These somatic phenotypes in the M-Z- generation indicate that zygotic expression of MRG-1 is also important for normal somatic development, and that its absence leads to stochastic developmental defects.

2.3.5 Mutations in MRG-1's Chromodomain Cause Embryonic Lethality

Our results from depleting H3K36me3 from germ cells implied that the role of MRG-1 in meiosis is linked to its presumed role as a conserved "reader" of H3K36 methylation. In addition, the somatic defects observed in *mrg-1(tm1227)* M-Z- animals suggested a role for zygotic MRG-1 in somatic development, which may also be related to its presumed role in H3K36me3 recognition. To investigate this, we mutated amino acids in two conserved positions that in MRG15 form the aromatic cage of the chromodomain, Y17 and F43, to alanines and assessed their effects on germline and somatic phenotypes. We first generated both GFP and mCherry tagged versions of the endogenous wild-type (WT) *mrg-1* gene using CRISPR. Both strains carrying the WT endogenous tagged versions had normally fertility and exhibited no overt phenotypes (below). We then further replaced Y17 and F43 with alanines in the *mrg-1::mCherry* strain in another round of CRISPR, yielding a new allele, *ck43,* which we will hereafter refer to as *mrg-1*^{CD} (Materials and Methods).

As mentioned, maternal provision of MRG-1 protein is necessary for germline development, and the M+Z- generation of *mrg-1(tm1227)* is fertile. The M-Z- offspring of homozygous *mrg-1(tm1227)* animals are viable but fail to develop a germline. After verifying and outcrossing the *mrg-1* chromodomain mutant (*mrg-1*^{CD}), it was genetically balanced with the qC1 chromosome for reasons described below. Although the *mrg-1*^{CD}*/qC1* exhibited decreased brood sized when

compared to wildtype/*qC1*, they were fertile. Surprisingly, 100% of the *mrg-1^{CD}/mrg-1^{CD}* offspring from heterozygous mothers died as embryos (Figure 2.5). Therefore, whereas the *mrg-1(tm1227)* null allele causes maternal effect sterility but no lethality, the *mrg-1CD* mutation causes zygotic lethality despite inheriting maternal WT MRG-1 protein produced from the qC1 balancer chromosome. The CD mutation causes a mid-embryogenesis arrest, although the arrest point appeared somewhat variable with some embryos developing to where movement was observed, but others did not. The *mrg-1CD* mutant's embryonic lethal phenotype is therefore worse than the homozygous null phenotype (M-Z-), which survives embryogenesis in the absence of any MRG-1 protein but grows up sterile. The presence of one dose of maternal MRG-1 protein in the *mrg-1^{CD}/mrg-1^{CD}* offspring from heterozygous mothers doesn't overcome the effect of the CD mutation, suggesting this allele is acting dominantly, or that the maternal load produced from a single copy of the WT gene is insufficient to overcome the deleterious effects of the CD mutation (Supplemental 2.12). We did not observed any obvious difference in the level of maternal protein, or its perdurance, in the CD M+Z- embryos compared to embryos inheriting WT protein. In addition, no post-embryonic somatic defects were observed in *mrg-1^{CD}/+* animals suggesting that the phenotype is more complicated than a dominant or antimorphic situation. However, as we will describe below, a non-lethal yet dominant RNAi defective phenotype *is* observed in these animals.

2.3.6 Maternally provided CD mutant MRG-1 is not sufficient for normal germline development.

Because the homozygous *mrg-1^{CD}* mutants die as embryos, we utilized the Auxin Inducible Degradation (AID) System to further examine the role of the chromodomain in the post-embryonic germline. To bypass the embryonic lethal phenotype, we used CRISPR to add

the AID degron to the endogenous (WT) *mrg-1* locus tagged with GFP, producing an *mrg-1::degron::gfp* strain. No fertility or developmental defects were observed in this strain. We crossed the *mrg-1::degron::gfp* strain into a strain carrying TIR1 driven by the *sun-1* promoter for germline specific degradation of the wild type MRG-1 (*mrg-1*$^{WT::Degron}$). We confirmed that auxin induced degradation of the MRG-1::degron::GFP protein in adults produced sterile offspring. The sterility was similar to M-Z- *mrg-1(tm2337)*, indicating that the auxin treatment efficiently produced the null phenotype. We then crossed the *mrg-1*^{CD} */qC1* balanced line with the *mrg-1*$^{WT::Degron}$ to generate *mrg-1*$^{CD/WT::Degron}$ heterozygous animals. MRG-1^{CD} mCherry and MRG-1$^{WT::Degron}$ GFP were equally visible in *mrg-1*$^{CD/WT::Degron}$ heterozygous animals, and their localization showed overlap in the germline (Figure 3.3). However, some variation was seen in the somatic nuclei between MRG-1^{CD} mCherry and MRG-1$^{WT::Degron}$ GFP. The significance of the decreased MRG-1^{CD} mCherry in adult somatic nuclei is currently unclear as there are no clear somatic phenotypic differences in *mrg-1*$^{CD/WT::Degron}$ heterozygous animals but may indicate post-embryonic degradation of the mutant protein. When observing embryos from heterozygote mothers, both maternally loaded MRG-1^{CD} mCherry and MRG-1$^{WT::Degron}$ GFP are visible in early embryo stages (Supplemental Figure 2.4).

The addition of the AID degron allowed controlled degradation of the wildtype MRG-1$^{WT::Degron}$, leaving only the mutant protein present and allowing us to examine the functional consequences of the CD mutations. Because TIR1 is driven by the germline-specific *sun-1* promoter, exposing the heterozygous MRG-1$^{CD/WT::Degron}$ animals to auxin yielded germline-specific degradation of MRG-1$^{WT::Degron}$, leaving only the mCherry-tagged MRG-1^{CD} protein present in the germline. As auxin is reported to poorly penetrate the embryo's eggshell (85) and thus unlikely to activate WT degradation via any maternal TIR1 protein, the embryos bypass the

CD mutant's embryonic lethality through zygotic expression of the GFP-tagged WT protein in the embryos' soma (86). Further, when *mrg-1*$^{CD/WT::Degron}$ embryos hatch onto auxin-treated plates, the WT protein is quickly and continuously degraded in the post-embryonic germline, allowing separation of somatic and germline requirements for an intact chromodomain to be assessed (Supplemental Figure 2.5).

Degradation of the MRG-1$^{WT::Degron}$ by auxin treatment was confirmed visually by the absence of GFP in the germline. We could not verify its complete absence by western blot or by other similar means due to the continued presence of both WT and CD proteins in the soma. However, as mentioned above auxin treatment efficiently induced a null-like sterile phenotype in animals expressing only the degron tagged WT protein. Additionally, when observing embryos from *mrg-1*$^{CD/WT::Degron}$ + auxin hermaphrodites, only MRG-1^{CD} is visibly loaded in the primordial germ cells (PGC) (Supplemental Figure 2.6). Embryos with a maternal load of MRG-1$^{WT::degron}$ that were exposed to auxin upon hatching showed immediate degradation of maternal WT MRG-1$^{WT::Degron}$ and also grew up sterile mimicking the *tm1227* null phenotype (Figure 2.7). Previous studies have shown that the *mrg-1* promoter is not active in the embryonic germline (87), thus at the earliest post-hatching stages any early, zygotically expressed MRG-1 protein should be immediately susceptible to auxin degradation. It is important to note that no noticeable degradation of MRG-1$^{WT::Degron}$ was observed when animals were grown without auxin suggesting there was no nonspecific activation of TIR1.

To determine if a functional MRG-1 chromodomain was required for germline development, embryos from *mrg-1*$^{CD/WT::Degron}$ hermaphrodites continuously exposed to auxin were hatched on auxin plates. Any heterozygous offspring therefore lacked both maternal and zygotic WT MRG-1 specifically in their germ cells, with only maternal and zygotic MRG-1^{CD}

mutant protein present in their germline at all stages (M-Z- for WT MRG-1, M+Z+ for MRG-1^{CD}) (Figure 2.6). In addition, all *mrg-1*$^{WT::Degron}$ homozygous siblings had inherited only maternal MRG-1^{CD} protein, and any zygotically expressed WT protein was degraded continuously upon hatching.

Individual animals were observed under a microscope to determine fertility status and genotyped. 100% (n=24) of the homozygous MRG-$1^{WT::Degron}$ offspring from auxin-exposed mothers (M-Z- WT; M+Z- MRG-1^{CD}) were sterile. This suggests that the maternal load of MRG-1 CD provided by the heterozygous mothers is on its own insufficient for germline development. In contrast, 69% (35/51) of the MRG-$1^{CD/WT::Degron}$ (M-Z- WT; M+Z+ MRG-1^{CD}) were fertile suggesting that maternal MRG-1 CD, if combined with zygotic expression of *mrg-1*^{CD}, is able to partially rescue germline development. Note that the M and Z designations above only refer to the protein in the germline; as *mrg-1*^{CD} */ mrg-1*^{CD} homozygous offspring failed to hatch.

2.3.7 The MRG-1 Chromodomain is not required for normal meiotic progression

Because many of the *mrg-1*$^{CD/WT::Degron}$ embryos hatched on auxin were fertile and successfully bypassed the embryonic lethal phenotype, we were able to analyze germlines that only contained the mutated version of the MRG-1 chromodomain. Because synapsis defects were apparent after reducing levels of H3K36me3 in the germline, we wanted to determine if similar phenotypes were seen after mutating the MRG-1 chromodomain that correlated with its presumed binding specificity. We placed *mrg-1*$^{WT::Degron}$ and *mrg-1*$^{CD/WT::Degron}$ on auxin plates starting from various larval stages and analyzed them as adults. When placed on auxin starting from L2 larval stage, *mrg-1*$^{WT::Degron}$ worms exhibited meiotic defects similar to the M+Z- *mrg-*

1(tm1227) mutants, including delayed synapsis (Figure 2.7 C, D). The synapsis defects became less severe in *mrg-1*$^{WT::Degron}$ worms if auxin degradation started after the young adult stage, despite having complete degradation of wildtype MRG-1 for 36 hours (Figure 2.7 B*).* *mrg-1*$^{WT::Degron}$ M+*Z- e*mbryos that hatched on auxin plates grew up to be sterile. This we assume to be due to the maternal load being immediately degraded shortly after hatching and before post-embryonic germline development, confirming a continued requirement for maternal MRG-1 at the initiation of germline development.

Mrg-1$^{CD/WT::Degron}$ heterozygotes (Figure 2.7 E-I), were similarly exposed to auxin for various amounts of time from different larval stages. No synapsis defects were observed after any length of auxin exposure (Figure 2.7 F-I). Unlike the *mrg*-1$^{WT::Degron}$ germlines that showed signs of delayed synapsis after auxin exposure from L2 larval stage, germlines with only the CD mutant appeared to have normal synapsis. Additionally, many *mrg-1*$^{CD/WT::Degron}$ embryos that were hatched on auxin developed into fertile adults, unlike their homozygous *mrg*-1$^{WT::Degron}$ siblings that were sterile (Figure 2.7 I). The apparently normal synapsis in *mrg-1*$^{CD/WT::Degron}$ germlines after auxin exposure suggests that the chromodomain of MRG-1, in contrast to the MRG-1 protein itself, is not important for MRG-1's role in meiosis. Therefore, MRG-1's meiotic function may be dependent on a different region of the protein, its required presence in a complex that is not dependent on the chromodomain for its function in the germline, or a redundancy for its role in meiosis. Complexes containing MRG-1 may depend on MRG-1 protein for complex stability since the absence of MRG-1 protein causes significant germline defects.

2.3.8 Zygotic MRG-1 combined with Maternal MRG-1CD partially rescues germline development

The *mrg-1* promoter is not active in the embryonic germline, and thus all embryonic germline functions for MRG-1 must strictly rely on its maternal supply (87). To further characterize the maternal requirements for MRG-1 in the germline, we looked at embryos from *mrg-1*$^{CD/WT::Degron}$ auxin-treated mothers that were hatched on OP50 bacteria plates lacking auxin. The offspring from these animals therefore had only the CD mutant as their maternal load, but zygotically expressed either both the CD and WT versions, or only WT *mrg-1* in their post-hatching germlines. Homozygous *mrg-1*^{CD} embryos did not hatch, as described above (Figure 2.5). F1 offspring of the auxin treated mothers that were hatched without auxin were examined 24 hours after L4 for fertility and genotyped. Of the worms that were maternally supplied with the CD mutant and only zygotic WT expression (M+Z- CD; M-Z+ WT) 43% were fertile (n=9) and 57% were sterile (n=12). This result suggests that zygotic expression of WT MRG-1 can only partially rescue germline development when coupled with maternal CD (Figure 2.8). Similarly, 53% (n=26) of offspring that had maternal CD and expressed both WT and CD zygotically (M+Z+ CD; M-Z+ WT) were fertile, while 47% (n=23) were sterile, again suggesting that zygotic expression of CD mutant and/or WT MRG-1 only partially rescues germline development when the maternal supply is the CD mutant. We note that some sterile worms that were M+Z+ CD; M-Z+ WT had partially developed germlines while others completely lacked any germ cell proliferation, similar to the maternal effect sterile phenotype (Supplemental figure 2.7).

2.3.9 The MRG-1 CD Mutant Exhibits a Dominant RNAi Defect

We originally tried to use RNAi to target the GFP-tagged WT in *mrg-1::gfp/mrg-1CD::mCherry* animals to bypass the embryonic lethality of the CD mutation and examine CD mutant germlines. However, these experiments failed because we observed a striking RNAi resistant phenotype. As expected, GFP RNAi successfully eliminated MRG-1 in *mrg-1::gfp* homozygous worms, resulting in the absence of GFP in the germline and the expected MES phenotype in the next generation (Figure 2.9 F). However, we found we could not deplete the GFP signal by GFP RNAi in *mrg-1::gfp/mrg-1CD::mcherry* heterozygous animals, suggesting that the presence of the *mrg-1 CD* mutation created a dominant resistance to the GFP RNAi (Figure 2.9 H). Additionally, after performing a brood size assay and analyzing embryonic lethality after GFP RNAi, we saw that there was no change in brood size or embryonic lethality after GFP RNAi in *mrg-1::gfp/mrg-1CD::mcherry* heterozygous animals (Supplemental Figure 2.8)

To further confirm this was an RNAi defect, we performed RNAi knockdown of *ama-1* in heterozygotes for the chromodomain mutant as well the *mrg-1(tm1227)* deletion allele. RNAi targeting *ama-1* (the gene encoding the large subunit of RNA polymerase II) in wildtype L1 larvae causes an arrested growth phenotype, and the larvae do not develop past L1-L2 stage (Figure 2.9 N). This arrest phenotype is also seen in *mrg-1(tm1227) M+Z-* and *mrg-1 (tm1227) M-Z-* null mutants exposed to *ama-1(RNAi)* (Figure 2.9 O-Q). However, no arrest in larval development was observed in *mrg-1CD/mrg-1WT* L1's exposed to *ama-1(RNAi),* confirming the dominant RNAi-defective phenotype (Figure 2.9 R). Chromodomain mutants display a slight reduction in length when compared to the RNAi control, suggesting some level of RNAi function in soma, but otherwise developed into fertile adults (Supplemental Figure 2.9).

RNAi experiments targeting animals to *him-3(RNAi)* yielded similar results: the CD/WT animals exhibited resistance to RNAi, whereas the other genotypes did not (Supplemental figure 2.10). We then examined whether CD/CD animals were RNAi defective using the AID system described above. Animals in which the WT MRG-1 had been degraded in their germlines with only the CD mutant present were also defective in RNAi (Supplemental Figure 2.11).

These results indicate that the CD mutation confers a dominant, gain-of-function phenotype on the MRG-1 protein that leads to an RNAi defective phenotype in both somatic lineages and the post-embryonic germline. MRG-1's chromodomain is thus essential for somatic development in the embryo and its function is important for exogenous RNAi, but it is not essential for post-embryonic germline development. This indicates there are separable roles for MRG-1 and its chromodomain in somatic and germline development.

2.4 Discussion

2.4.1 Both maternal and zygotic MRG-1 are required for normal germline development and function

As a homolog of MRG15, it is expected and indeed has been shown that MRG-1 plays various roles in *C. elegans* development. Here, our goal was to further characterize MRG-1's role in germline development, its presumed connection with H3K36me3 in germline function, and the role of its conserved chromodomain in its germline function. Consistent with previous reports our data verifies that in addition to its maternal requirement, zygotically produced MRG-1 is also necessary for proper germline function and normal meiosis in *C. elegans(84).* Worms that have only a maternal load and no zygotic expression of *mrg-1* (M+Z-) display significant meiotic defects despite inheriting the maternal protein, including synaptic delay and defective

crossover formation. Zygotic MRG-1 is thus required for normal meiotic progression and synapsis, consistent with previous studies by Carolyn et al. showing that zygotic MRG-1 is necessary for pre-synaptic alignment (73).

As MRG-1 is a proposed reader of H3K36me3, we investigated whether depleting H3K36me3 in the germline would yield consequences similar to the depletion of zygotic MRG-1 in the *mrg-1(tm1227)* M+Z- animals. Germlines that lacked normal levels of both MET-1 and MES-4, the two histone methyltransferases responsible for H3K36me3, exhibited efficient depletion of H3K36me3 and delayed meiotic progression and synapsis similar to that observed in *mrg-1* M+Z- mutants, suggesting that normal levels of H3K36me3 in meiotic chromatin are required for meiotic processes. Indeed, MET-1 and MES-4, and thus presumably the H3K36 methylation they provide, have been shown to be required for checkpoint activation(34). This result is also consistent with a similar report that looked at the contributions of H3K79 methylation in meiotic progression(88). Although it is unclear how specific histone modifications are mechanistically linked to meiotic processes, it is not surprising that chromatin organization plays an important role, given the unique architecture of meiotic chromatin.

Our data also further emphasizes the importance of the maternal load of wildtype MRG-1 in the early, post-embryonic germline. The promoter for MRG-1 is not active in the embryonic germline, and thus all MRG-1 present in the primordial germ cells (Z2 and Z3) before hatching is maternally supplied (87). In MRG-1$^{WT::Degron}$ homozygote embryos that hatch on auxin, the immediate degradation of the maternal load in their germline yields 100% sterility, similar to the maternal effect sterility seen in the *mrg-1(tm1227)* deletion mutant. Importantly, in MRG-1$^{WT::Degron}$ embryos from *mrg-1*$^{CD/WT::Degron}$ + auxin mothers (M-Z+ WT; M+Z- CD) that hatch on OP-50, fertility is only partially rescued by maternal CD mutant MRG-1 when in combination

with early zygotic WT MRG-1. A percentage of the sterile MRG-1$^{WT::Degron}$ (M-Z+ WT; M+Z- CD) show germ cell proliferation, but do not fully rescue fertility. In contrast, homozygous *mrg*-1$^{WT::Degron}$ embryos from *mrg*-1$^{CD/WT::Degron}$ + auxin mothers that hatch on auxin, therefore only inheriting maternal CD mutant but lacking any zygotic expression of MRG-1 (M-Z- WT; M+Z- CD) appear to lack any germ cell proliferation, similar to the null mutant MES phenotype. Therefore unlike maternally supplied WT MRG-1, maternal CD mutant cannot rescue germ cell proliferation without further zygotic synthesis. The chromodomain is thus important for the early, maternally provided function of MRG-1, without which MRG-1 must be combined with early zygotic expression of either CD mutant or WT MRG-1 protein to at least partially support fertility. However, we found no obvious defects in post-embryonic germlines possessing only the CD mutant protein, suggesting that the need for the chromodomain's function in the germline is stage-specific and likely required during the earliest stages of post-hatching germ cell proliferation.

2.4.2 Zygotic expression of *mrg-1* is necessary for normal somatic development

M-Z- *mrg-1(tm1227)* mutants lacking maternal MRG-1 fail to produce a germline due to defective proliferation of the primordial germ cells (PGCs) (36, 69, 71). Here we describe a somatic role for MRG-1 in the sterile M-Z- *mrg-1* mutants. In addition to lacking a germline, a percentage of M-Z- mutants display severe somatic defects such stunted growth, tail defects, and multiple vulva development. This is similar to earlier reports using *mrg-1(RNAi)* (84)**.** This provides evidence that zygotically expressed MRG-1 plays an important role in somatic development. As a homolog of MRG15, that is known to influence developmentally regulated transcription, somatic defects in *mrg-1 M-Z-* mutant development are not surprising and are

likely due to defective transcriptional regulation of developmental genes, presumably through its role(s) in histone modifying complexes. It is important to note, however, that despite the lack of either maternal or zygotic MRG-1, the *mrg-1(tm1227)* null allele M-Z- embryos survive embryogenesis, so MRG-1 is thus either redundant with another somatic factor or MRG-1 is not essential for early somatic development.

2.4.3 The MRG-1 Chromodomain Mutations Cause Novel Phenotypes

The chromodomain of MRG-1 is highly conserved between species, so we characterized its role in *C. elegans* development by disrupting two of the five residues predicted to form a functional chromodomain aromatic "cage". Interestingly, we found that the mutations caused a worse phenotype than the *tm1227* null allele: homozygous *mrg-1CD* mutants arrested and died as embryos. Since these homozygous CD/CD mutants arose from CD/WT mothers, the embryos inherited maternal WT MRG-1 and yet the maternal protein failed to rescue somatic development. Genetically, this would indicate that the CD mutant has a dominant, possibly antimorphic character that renders the maternal WT unable to rescue. However, since the null mutant shows that MRG-1 is not essential for embryonic viability, antagonism by the CD mutant of a WT protein that has no requirement in embryogenesis would seem to have few consequences (Supplemental 2.12). Indeed, embryos that are of genotype *mrg-1*^{CD}*/+ are* viable and exhibit no obvious meiotic defects, which indicates that presence of zygotically produced wild-type protein can suppress—or titrate away—any negative effects of the CD mutant. This may be related to tissue specific functions of MRG-1 and the complexes in which it plays a role; the CD mutations may in effect induce a novel function to MRG-1 and its associated HDAC or HAT complex(es) important for embryonic gene regulation. Indeed, mutations in the *hda-1* and

other HDAC complex members show embryonic lethality phenotypes similar to what we observed in the CD mutant embryos (89). The developmental defects caused by the CD mutations may be caused by changing how these complexes are targeted to genomic loci. Disruption of this targeting may alter deacetylase activity and its roles in regulating embryonic transcription.

The requirement for a functional CD is not observed in the post-embryonic germline. Using the AID system and auxin exposure starting in early larval stages, we were able to examine animals that had only the CD mutant present in their post-embryonic germline. These animals were fertile without any obvious germline defects and produced 100% dead embryos, as expected from the zygotic phenotype. Additionally, a majority of mrg-1$^{CD/WT::Degron}$ embryos from *mrg*-1$^{CD/WT::Degron}$ + auxin mothers that hatch on auxin (M-Z-WT; M+Z+ CD) grow up to be fertile suggesting that a combination of maternal and zygotic CD mutant MRG-1 can support fertility. This suggests the chromodomain of MRG-1is not essential for its role(s) in germline development.

2.4.4 A Requirement for the MRG-1 Chromodomain in RNAi.

We observed a strong RNAi defect in both CD/ + and CD/CD mutant animals, indicating that the RNAi defect is dominant. We do not know the direct cause of the RNAi defect, but MRG-1 has been identified by mass-spectrometry as a component of numerous chromatin-modifying complexes in *C. elegans* that have also been implicated in RNAi-dependent germline silencing mechanisms (65-67). MRG-1 has also recently been implicated in the regulation of the formation of piRNA genomic nuclear foci, which may also be tied to its role in HDAC regulation (90). The chromodomain mutation could affect any role MRG-1 may have in the

targeting of these complexes to their genomic substrates, which in turn could result in defective transcriptional regulation of RNAi machinery. Alternatively, defective histone acetylation dynamics could lead to defective suppression of endogenous RNAi targets, thereby causing endogenous and exogenous RNAi systems to compete for shared factors in their pathways. These phenotypes could be the result of the altered H3 acetylation we observe in *mrg-1* mutants, and resulting transcriptional defects that are normally regulated by HDACs and/or HATs through MRG-1's chromodomain, or dominantly affected by any novel activity induced by the CD mutations. It is also interesting to note that *mes*-4 M+Z- animals were shown to have an RNAi defect, which may point to H3K36 methylation, and possibly its recognition by MRG-1 via its chromodomain, as important for normal expression of RNAi effectors (91, 92). Importantly, whatever role the chromodomain has in transcription regulation is not evident in the post-embryonic germline, either because it is not required or is redundant with other histone modification readers. Further analyses of the CD mutant and its effects are obviously warranted and are in progress.

Indeed, the molecular function of MRG-1's chromodomain remains an open question. MRG-1 null mutations cause a maternal effect sterility similar to that observed in the H3K36 methyltransferase MES-4 mutants, its localization in chromatin overlaps with H3K36me3, and the post-embryonic germline defects are similar to those caused by depletion of H3K36me3, and its mammalian ortholog has been shown to bind H3K36me3 (75). It is thus reasonable to predict that its function is through interaction with this histone modification, but this has not yet been directly shown. Indeed, we have been unable to show binding of a purified MRG-1 chromodomain protein to H3K36me3 peptides which will be further discussed in the following chapter (Figure 3.2). In any case, determining what the chromodomain binds, whether a histone

modification or other proteins, and the effect of the CD mutation on this binding will be required to understand the role of this protein in germ cell chromatin regulation and RNAi-dependent processes in these cells.

2.5 Materials and Methods

Strains and Strain Maintenance

The following strains were used for these experiments: Bristol N2, XA6227 (*mrg-1*[*tm1227*]/qC1 [*dpy-19*(*e1259*) *glp-1*(*q339*) qIs26] III), AV630 (meIs8 [pie-1p::GFP::cosa-1 + unc-119(+)] II), MT16973(*met-1*[n4337] I). Additional transgenic strains were generated using the CRISPR-Cas9 system described below. All strains were maintained at 20°C on nematode growth medium (NGM) plates seeded with *E. coli* OP50. For *mrg-1(tm1227)* mutants, all analyses were performed in the M+Z- (maternal +/ zygotic -) generation. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Any newly generated strains will be made available through the CGC.

CRISPR Cas9 Generated Strains

The endogenous *mrg-1* gene was tagged C-terminally tagged with mCherry, GFP, or AIDdegron::GFP using the CRISPR-Cas9 genome editing system (described below)(86). Specific guide RNAs were used to induce breaks in the last exon of *mrg-1* and were repaired using short DNA gene fragments (Integrated DNA Technologies) containing the specific protein label. The CRISPR-Cas9 system was also used to mutate chromodomain of *mrg-1* in a similar manner with specific guide RNAs (gRNAs) and a repair oligonucleotide (S Table 2- 3). Large gBlock gene fragments were amplified using Platinum SuperFi PCR Master Mix (Invitrogen

#12358010) and concentrated using the Zymo DNA Clean & Concetrator-5 kit (Zymo D4004) before injection young adult worms.

Strain	Description
KW1668	ck42(*mrg-1::mcherry)* III
KW1681	ck43☹*mrg-1::mcherry CD* mutation); mutated Y17A & F43A/ qC1[dpy-19(e1259) glp-1(q339) qIs26] III
KW1686	ck44(*mrg-1::GFP)* III
KW1682	ck45(*mrg-1::degron::GFP*) III; ieSi38 [sun-1p::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)] IV
KW1683	*mrg-1(ck43)/mrg-1(ck45)* III; ieSi38 IV

Table 2.1. List of Strains

RNAi Conditions

RNAi was performed by using HT115 cells transformed with the empty L4440 RNAi vector or L4440 vector containing the cDNA of the gene of interest. Bacteria was grown shaking overnight at 37°C. Bacteria cultures were induced with IPTG 1mM final concentration for 1 hour while shaking and plated on NGM plates with ampicillin (100ug/mL) and IPTG(1mM). Bacteria was induced on ampicillin-IPTG plates overnight at 37°C degrees. After at least 36 hours of induction, synchronized L1s were plated on the RNAi bacteria plates and then moved to 20°C until analyses.

Synchronization

To synchronize worm populations, worms were grown on NGM plates until gravid adults. Animals were rinsed off the plates with M9 buffer, collected in a 15ml conical tube, and spun down at 1800rpm for 3 min. Once pelleted, M9 was discarded leaving 6X of worm pellet volume (up to 3mL). The pellet was resuspended with 10% volume of 50% bleach solution and 10 N NaOH and incubated on a shaker until worms were dissolved. The released embryos were then washed with 15ml M9 three times. After the last wash, the embryo pellet was resuspended in M9 buffer and placed on a shaker overnight for the embryos to hatch. The synchronized L1 larvae were then plated on seeded NGM plates.

Immunofluorescence

Germlines were dissected from young adult (24 hours after L4 larval stage) hermaphrodite worms in dissection buffer (2X sperm salts, 2.5mm levamisole) on poly-lysine coated slides and fixed with 1% paraformaldehyde with ethanol or methanol acetone following freeze crack on a chilled aluminum block. After fixation, slides were washed in TBST (tris buffer saline, 1% Tween-20) and primary antibody was added for incubation at 4° overnight. Following washes with TBST, slides were incubated with secondary antibody at room temperature for 4 hours. Slides were then washed with TBST, and then counterstained with DAPI. Slides were sealed with Prolong Gold and clear nail polish. Imaging was done using a Leica DMRXA with a Retiga2000r camera using Hamamatsu Photonics software. Confocal images were taken with an Olympus FV1000 confocal microscope using Olympus Fluoview v4.2 acquisition software. This research project was supported in part by the Emory University Integrated Cellular Imaging Core. The content is solely the responsibility of the authors and does not necessarily reflect the

official views of the National Institute of Health. All image analysis was done using FIJI imaging software(93).

The following primary antibodies were used: goat anti-SYP-1 (1:1500), chicken anti-GFP (Aves Labs GFP-1020, 1:300), mouse anti H3K36me3 (Active Motif 61020, 1:20000), rat anti H3K9Ac (BioLegend 698402, 1:500). Secondary antibodies used in this study were: donkey anti-goat 488 (Invitrogen A11055, 1:500), donkey anti-mouse 594 (Invitrogen A21203, 1:500), donkey anti-chicken 594 (Jackson Immuno Research Laboratories 703586155, 1:500), donkey anti-rat 594 (Invitrogen A21209, 1:500).

Brood Size Assay

Individual L4 larvae were placed on NGM plates seeded with OP50. After 24 hours, worms were transferred to new plates. Embryos and L1s were then counted and the number of unhatched embryos were scored the following day to determine embryonic lethality. This was repeated until embryos were no longer produced. Brood size was calculated as the total number embryos laid. Embryonic lethality was determined by the percentage of embryos in each brood that failed to hatch after 24 hours.

Transition Zone Measurement

Transition zone (TZ) nuclei were identified in dissected, fixed and DAPI stained gonads as nuclei with characteristic crescent shaped and condensed chromosomes asymmetrically located to one side of the nuclear periphery. The length of the TZ was measured by counting the number of linear nuclei from the first distal crescent-shaped DAPI nucleus to the most proximal. TZ

length analysis was done by comparing the nuclear length of the TZ to the total nuclear lengths from the first nucleus in the distal mitotic region to the last nucleus at the end of pachytene before diplotene. The Mann-Whitney U-test was used for statistical analysis (p =2.677E-05).

Crossover Formation Characterization

After RNAi, dissected gonads from AV630 were stained for GFP and SYP-1. Maximum projections of Z-stacks of each gonad were analyzed for crossover formations. The number of crossovers formed in each nuclei were determined by the number of GFP foci in each nuclei. GFP::COSA-1 foci were only counted if they colocalized with DAPI staining. The length of the gonad was divided into six sections and the number of GFP::COSA-1 foci per nucleus were noted in each section. Significance was calculated using the Chi-Square test comparing the type of nuclei present in region (Region 5: p= 2.03E-04, Region 6 p= 2.01E-05).

For bivalent and univalent formation analysis, the number of DAPI bodies were counted in oocytes between wildtype (n=121) and *mrg-1 M+Z-* (n=61). The Chi Square test was used for statistical analysis between control and *mrg-1*(p = 3.40275E-14).

Auxin Induced Degradation

Auxin Induced Degradation (AID) experiments were performed by the method of (86). OP50 seeded NGM plates were supplemented with 1mM of auxin and the bacteria lawn was allowed to grow for at least 48 hours at room temperature before worms were added to plates. Worms were placed on auxin containing plates for the indicated amount of time before being analyzed further.

2.6 Figures

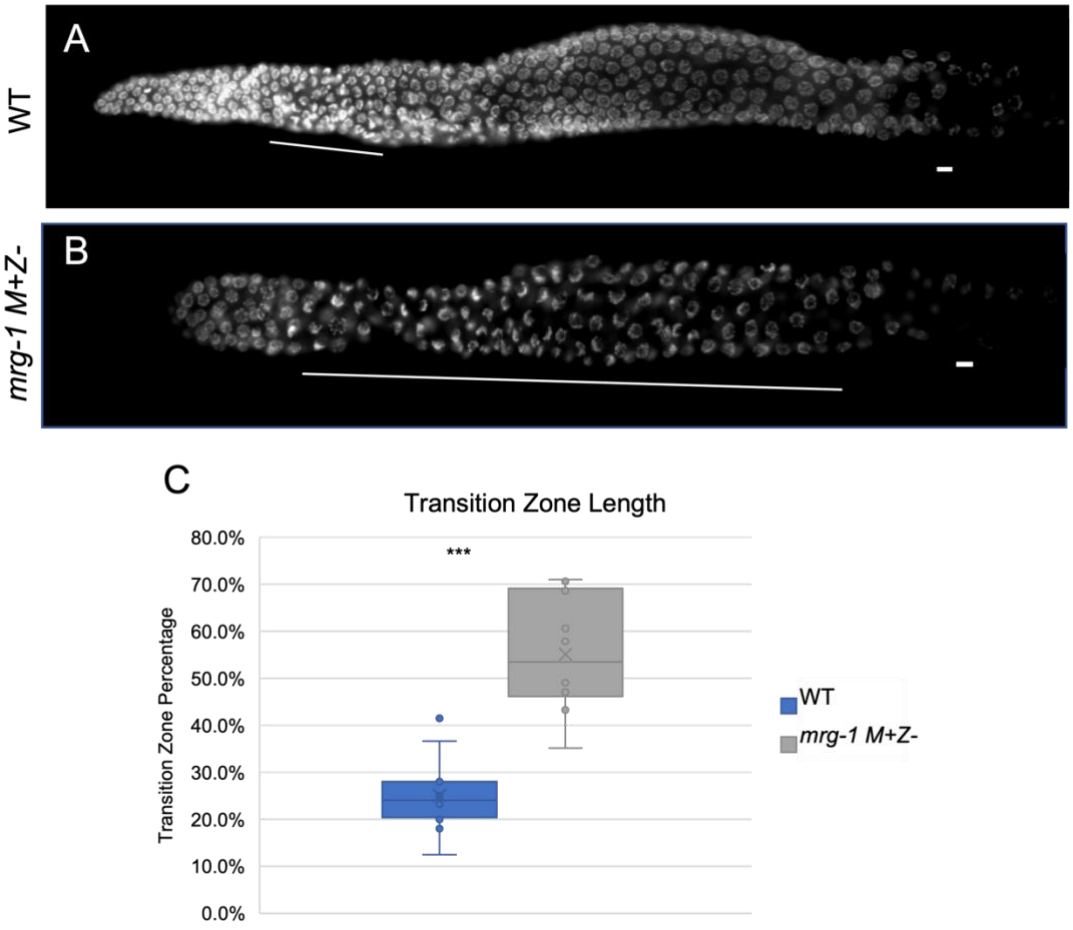


Figure 2.1. *mrg-1 M+Z-* mutants exhibit synaptic delay

Ovaries of WT and *mrg-1(tm1227) M+Z-* hermaphrodites stained with DAPI. The transition zone (white line) was measured in each germline and compared to the length from the distal tip to the end of pachytene as described in the Materials and Methods.. In comparison to the WT, *mrg-1* mutants display an extended TZ. The total length of the TZ is 25% in WT germlines. In the *mrg-1* mutants, the TZ is over 50% of the determined length. Germlines were dissected, fixed, and stained with DAPI 24 hrs post L4 larval stage (*** indicate $p \leq 0.001$).

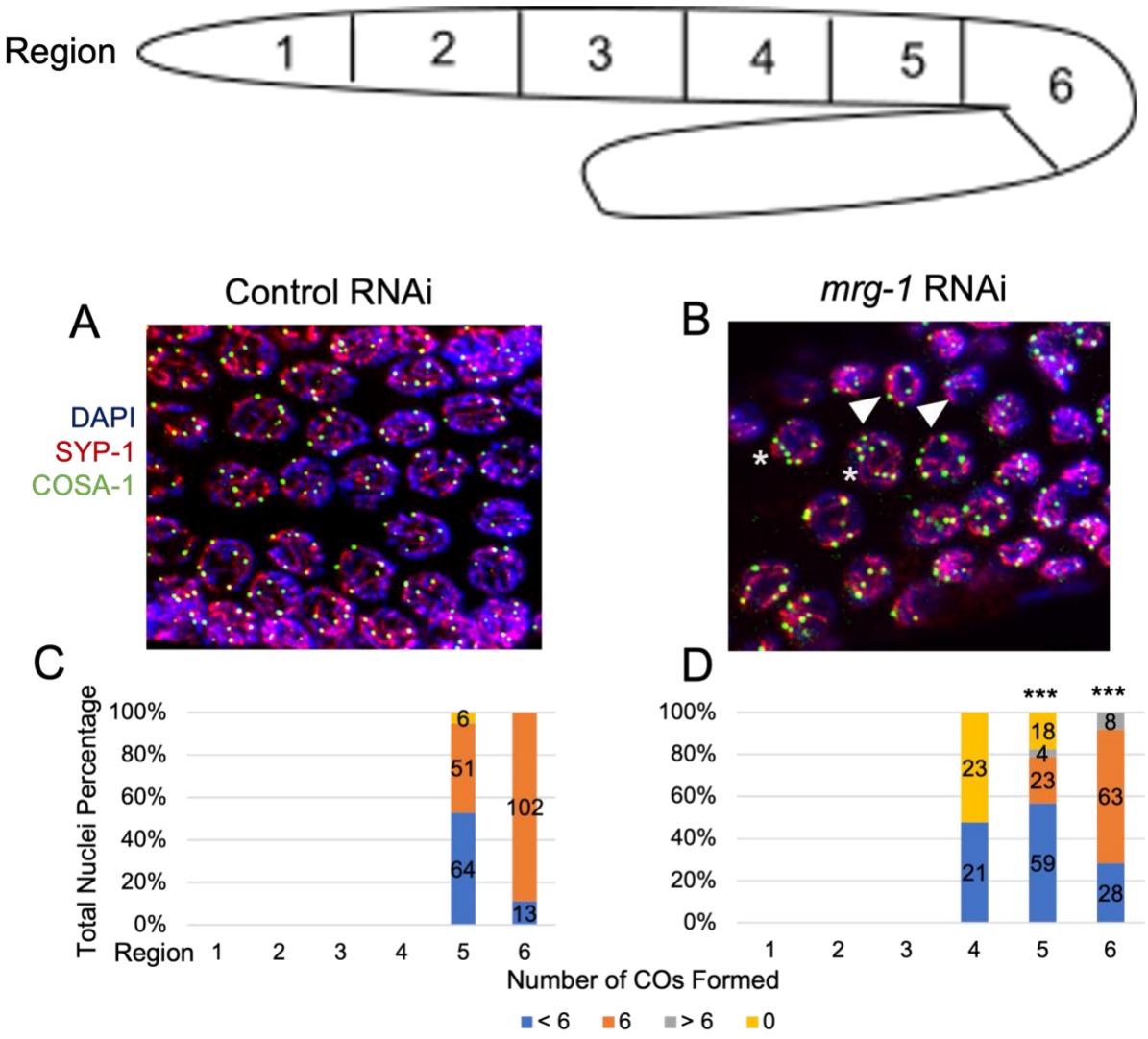


Figure 2.2. *mrg-1* M+Z- knockdown causes defective crossover regulation

Ovaries from control (A) or *mrg-1(RNAi)* (B) treated animals were dissected, fixed, and stained with anti-COSA-1, anti-SYP-1, and counterstained with DAPI. The total length of the gonad was divided into 6 regions and COSA-1 signals per nuclei were counted in each region (C, D). At the end of pachytene (Region 6), WT nuclei (C) have 6 mature crossovers (CO) marked by COSA-1 for each set of homologs. In *mrg-1* mutants (D), CO formation often occurs earlier in pachytene and have an abnormal number of COs (arrowhead <6, asterisk >6) (*** indicate $p \leq 0.001$).

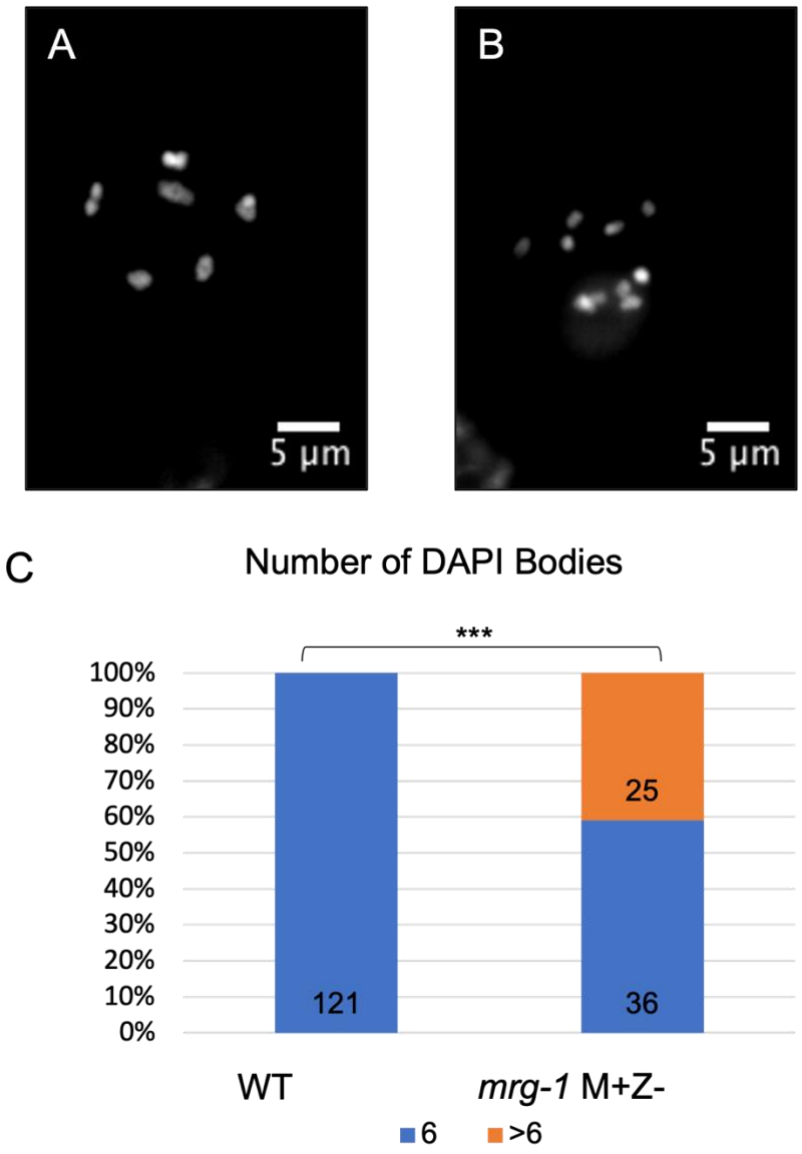


Figure 2.3. *mrg-1 M+Z-* mutants have an increased number of achiasmatic chromosomes.

(2) Oocytes in wildtype germlines have 6 bivalents that are generated after homologs have successfully recombined and formed productive crossovers. (B) Mutants lacking zygotic MRG-1 display a range in number of DAPI bodies suggesting that recombination is defective (C). Germlines were dissected, fixed, and stained with DAPI 24 hours after L4 larval stage (*** indicate $p \leq 0.001$).

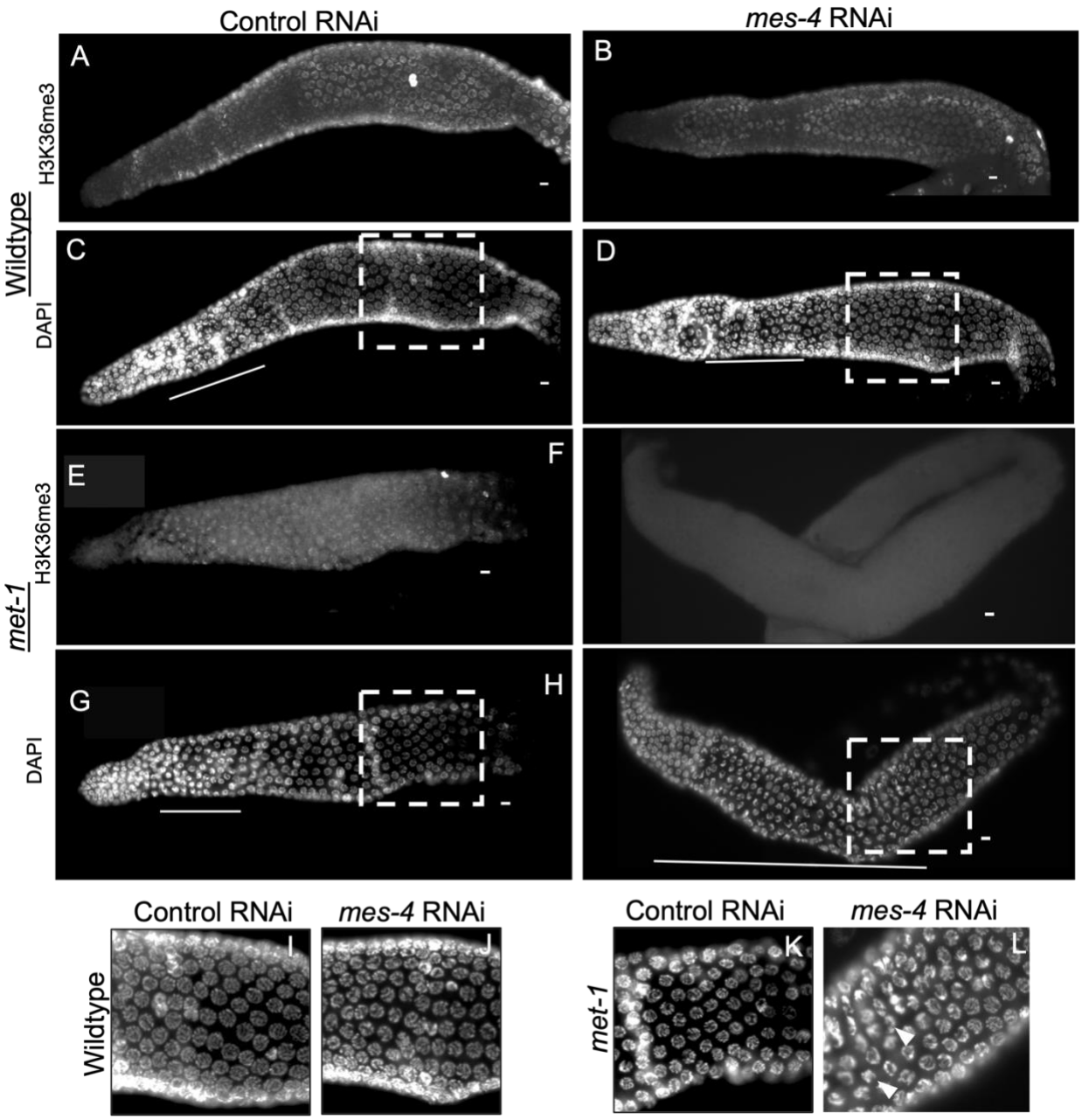


Figure 2.4. Depletion of H3K36me3 causes synaptic delay.

Ovaries from wildtype (A, C), *mes-4(RNAi)* (B, D), *met-1(n4337)* (E, F), and *met-1(n4337); mes-4(RNAi)* adult hermaphrodites were dissected, fixed, and probed with anti-H3K36me3 followed by DAPI staining. H3K36me3 is present in A, B, and E, but is reduced in F. The transition zone (indicated by white line) is restricted to the distal gonad in A, D, and G, but is

highly extended in H. Panels I-L are enlargements of regions outlined in panels C, D, G, and H. Arrowheads indicate nuclei with condensed chromatin located at the periphery, similar to those observed in the transition zone.

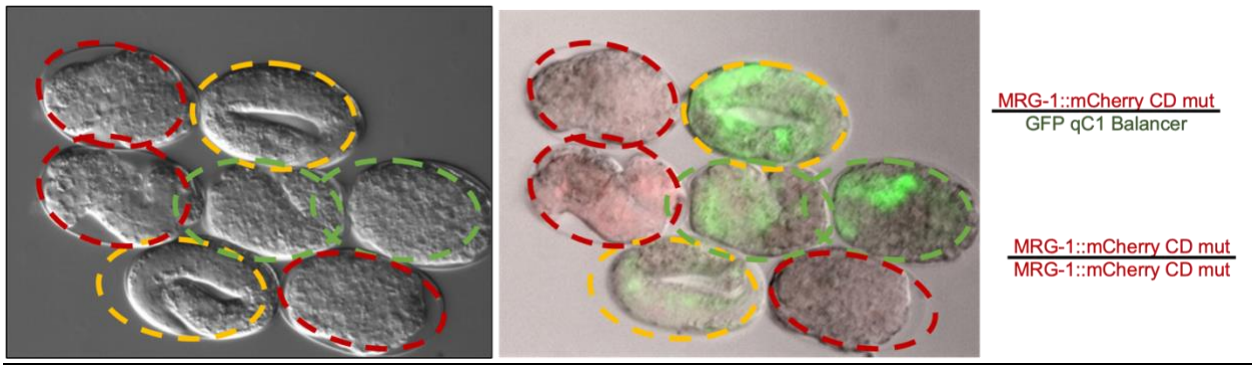


Figure 2.5. MRG-1 Chromodomain mutation causes embryonic arrest.

Embryos dissected from *mrg-1CD/qC1* balancer worms were dissected and allowed to develop. Embryos that were homozygous for *mrg-1CD::mCherrry* mutation lack GFP and arrested during embryogenesis (red outlines). Heterozygous siblings (express GFP and mCherry) complete embryogenesis, hatch and develop normally (yellow outlines). Embryos that are homozygous for the qC1 balancer also arrest (green outline)

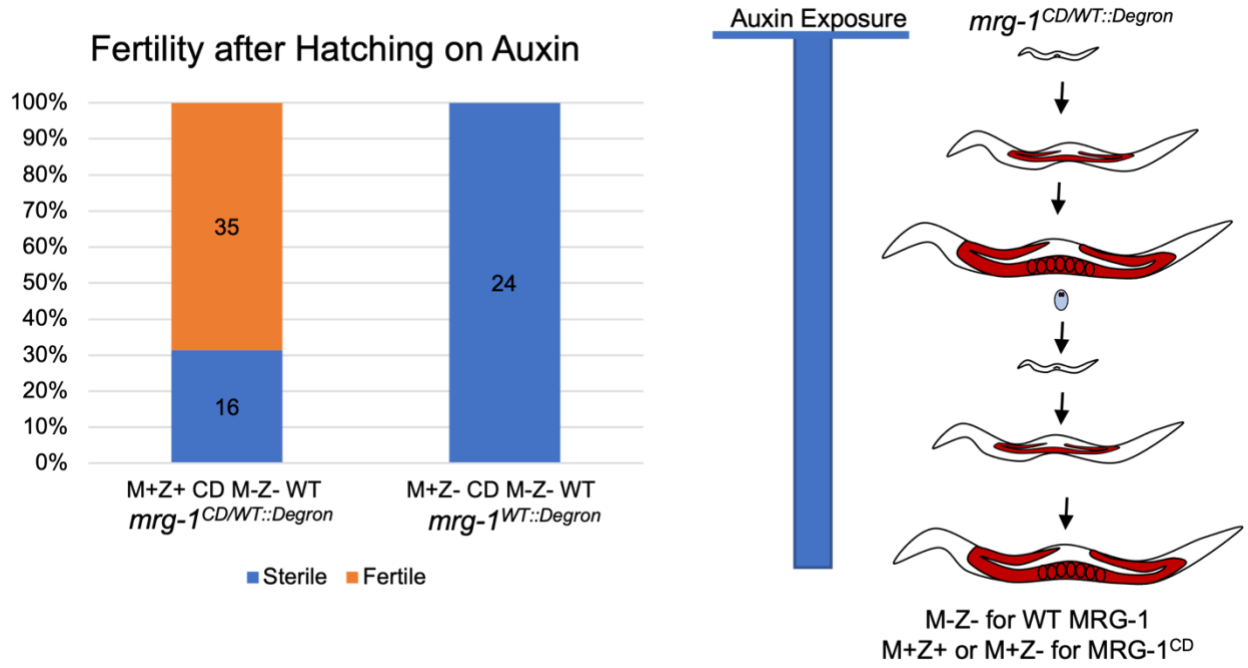


Figure 2.6. Maternal MRG-1 Chromodomain mutant alone is not sufficient for germline development.

Embryos dissected from MRG-1$^{CD/WT::Degron}$ hermaphrodites exposed to auxin during all of larval and adult development were hatched on auxin plates, yielding offspring that were M-Z- for WT MRG-1, all of which inherited maternal CD mutant, and 2/3 of which also expressed the CD mutant zygotically in their post-embryonic germline. All offspring M-Z- for WT MRG-1 grew up sterile. In contrast, 75% inheriting maternal *mrg-1*CD in combination with zygotic *mrg-1CD* mutant grew up fertile.

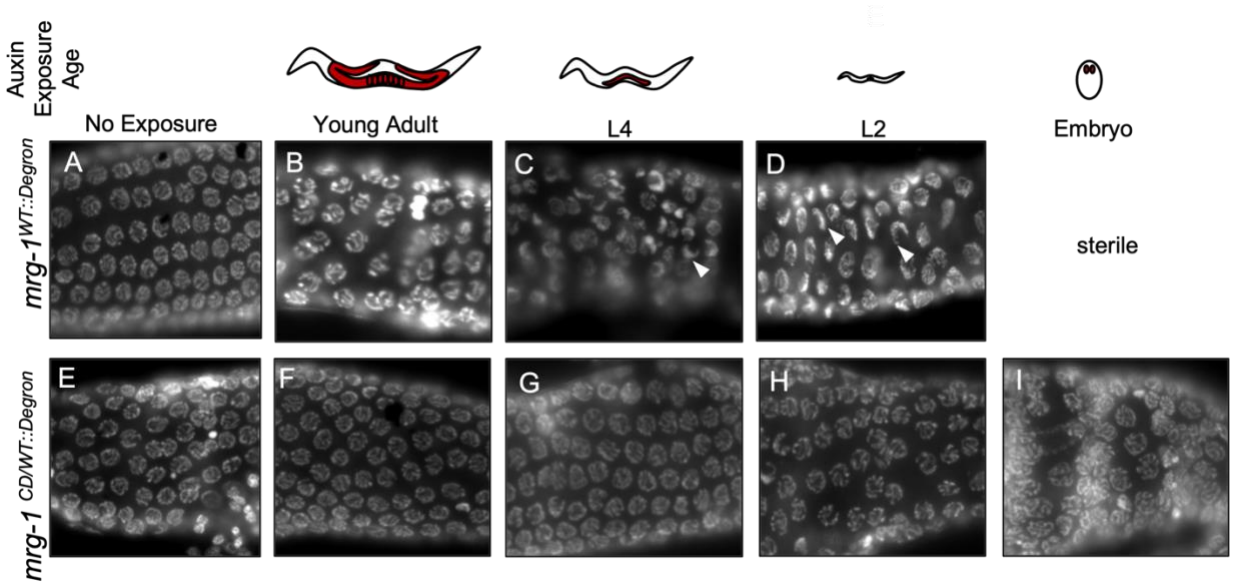


Figure 2.7. MRG-1 Chromodomain mutant supports normal meiosis.

Mrg-1$^{WT::Degron}$ (A-D) and *mrg-1*$^{CD/WT::Degron}$ (E-I) worms were placed on auxin (1mM) starting from various larval stages (indicated by blue bar). Gonads dissected from adult animals from each treatment were then fixed and stained with DAPI. The proximal region where synapsis normally occurs is shown. Nuclei exhibiting pre-synaptic delay are indicated by a crescent-like chromosome organization. Auxin-induced degradation of *mrg-1*$^{WT::Degron}$ starting from L2 larvae caused highly penetrant synaptic delay (arrowheads; C-D), whereas auxin exposure starting in adult animals caused chromosome morphology defects (B) but had minimal effect on synapsis. Earlier auxin degradation of WT MRG-1 begun at hatching caused sterility with no germline. In contrast, germlines with only the CD mutant present beginning at any stage showed no overt meiosis defects (E-I).

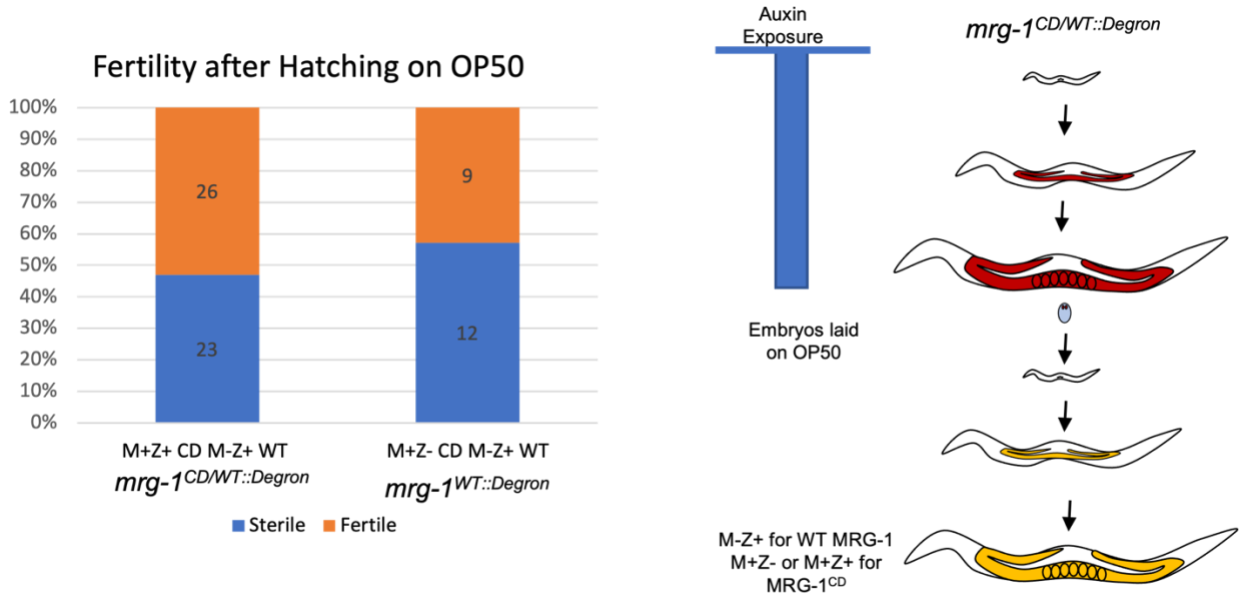


Figure 2.8. Zygotic expression of WT MRG-1 in combination with maternal MRG-1 CD can partially rescue fertility.

Embryos from *mrg-1*$^{CD/WT::Degron}$ hermaphrodites exposed throughout post-embryonic development were hatched on OP50 plates and scored for fertility as adults. Any adult animals exhibiting embryos in their uterus were scored as fertile; an absence of embryos was scored as sterile. These worms inherited only maternal CD mutant MRG-1 but could express either only zygotic WT MRG-1 (*mrg-1*$^{WT::Degron}$) or both WT and CD mutant MRG-1 (MRG-1$^{CD/WT::Degron}$) when hatched onto OP-50.

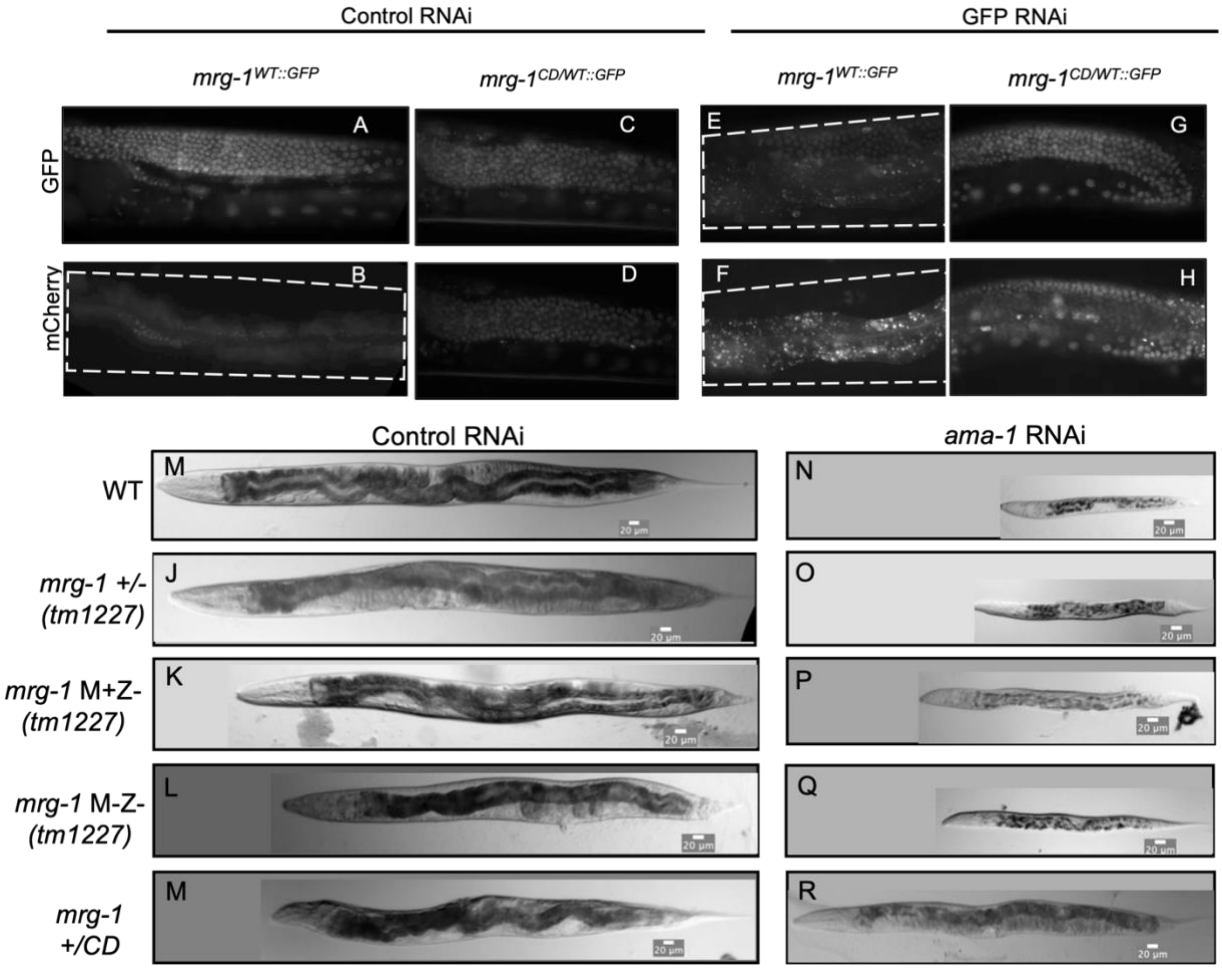
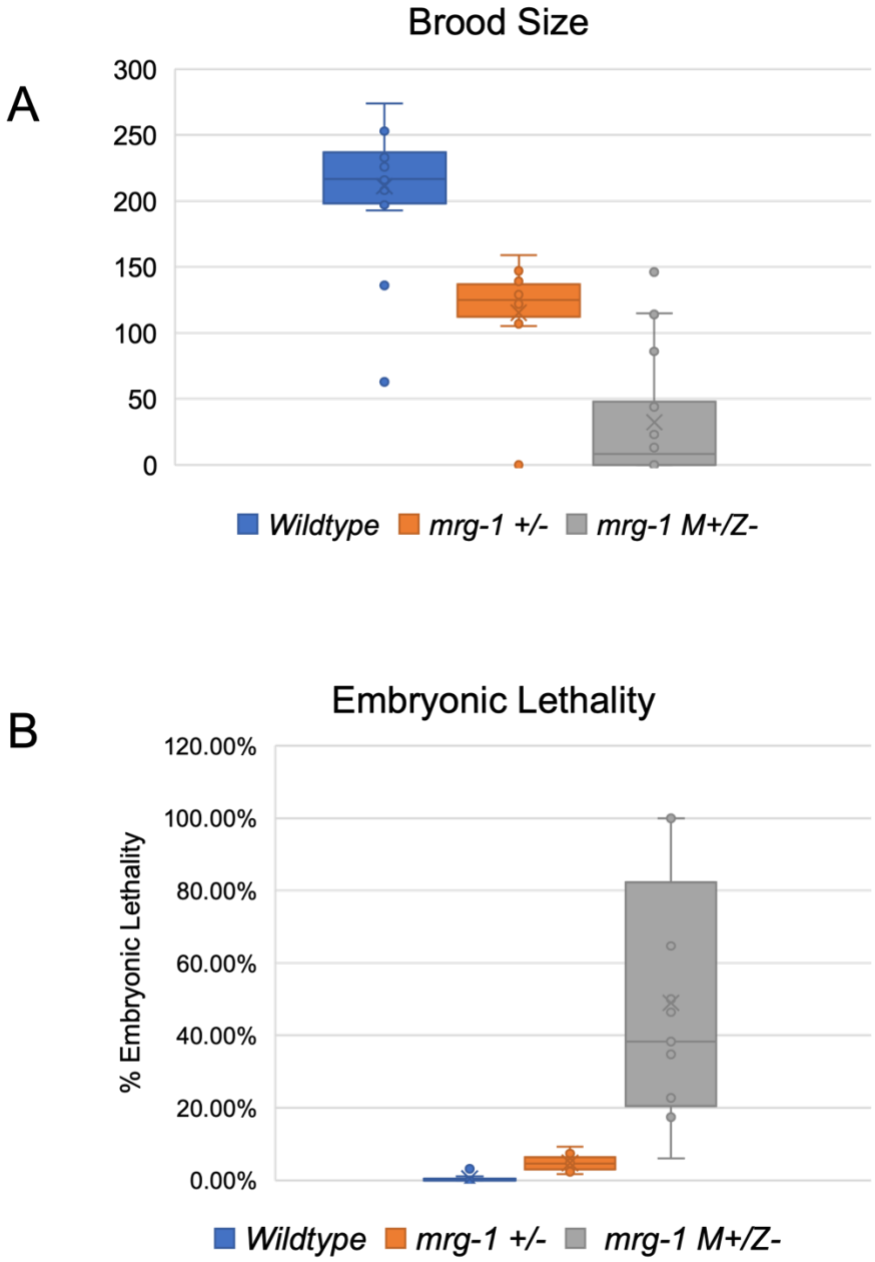


Figure 2.9. *mrg-1 CD* mutation causes a dominant RNAi resistance phenotype.

WT MRG-1$^{WT::GFP}$or heterozygous MRG-1$^{CD/WT::GFP}$were placed on control(L4440) (A-D) or GFP RNAi (E-H) plates as L1 larvae. GFP RNAi depleted the GFP signal in MRG-1$^{WT::GFP}$ animals, but was ineffective in animals carrying the CD mutant allele. (B) Wildtype, *mrg-1/+ (tm12227)*, *mrg-1 (tm1227) M+Z-*, *mrg-1 (tm1227) M-Z-*, and *mrg-1 CD/+* worms were placed on control (I-M) or *ama-1 RNAi* (N-R) plates as L1 larvae and grown for >48 hours. All strains except *mrg-1 CD/+* ® displayed L1 larval arrest.

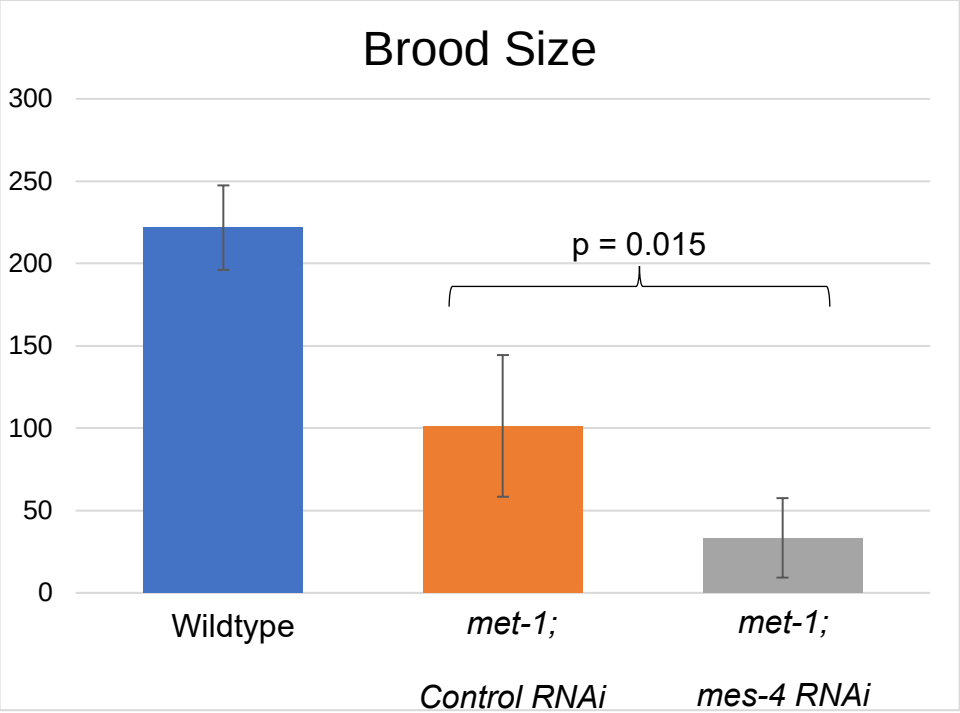
Supplemental Figures



Supplemental Figure 2.1. mrg-1 (tm1227) mutants have decreased brood size and increased embryonic lethality.

The total brood of wildtype, *mrg-1 (tm12227)* heterozygotes, and *mrg-1 M+Z-* worms were counted (A), and the percentage embryos failing to hatch was calculated (B). *mrg-1 M+Z-*

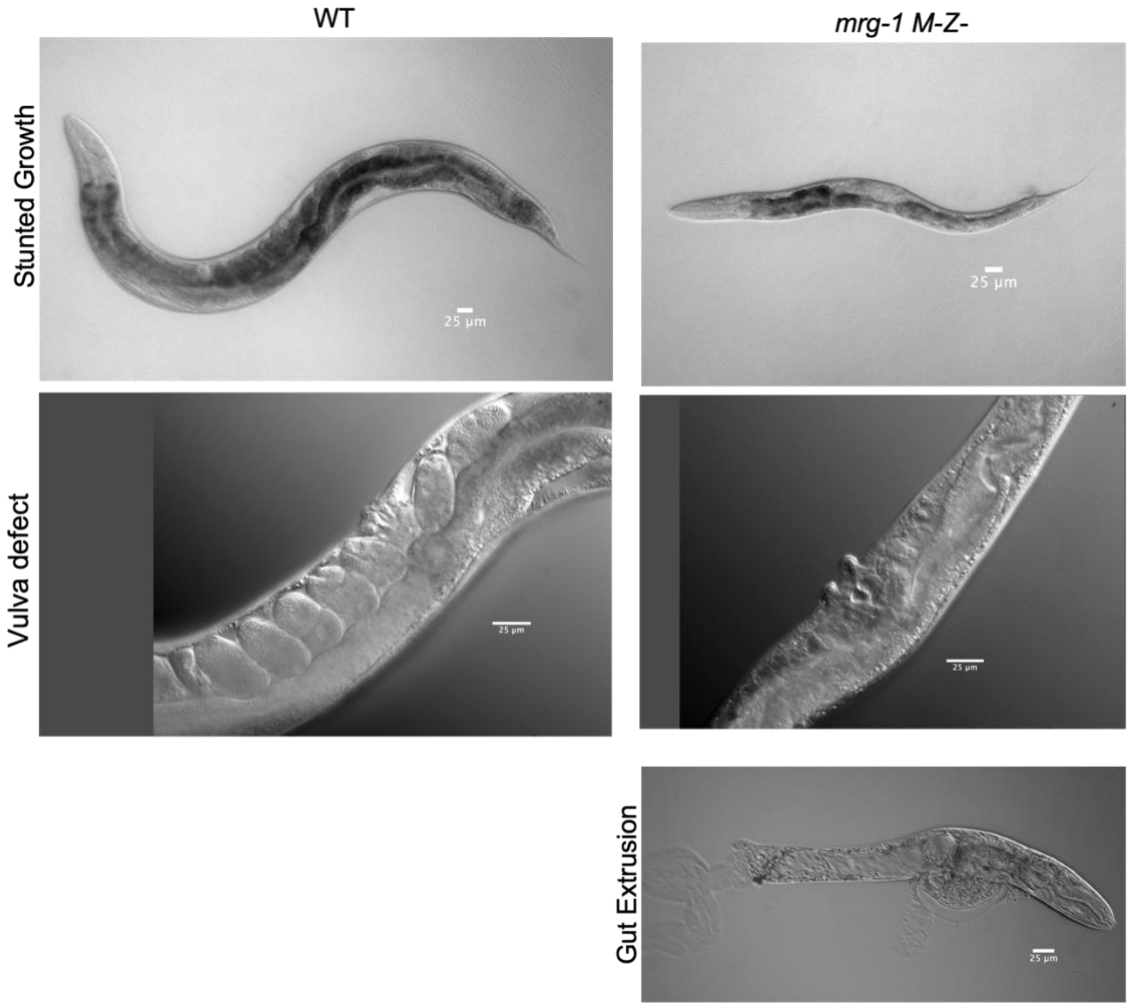
worms had a higher percentage of embryonic lethality compared to wildtype and *mrg-1* heterozygotes. The total broods of 25 animals were counted for each genotype.



Supplemental Figure 2.2 met-1; mes-4 RNAi mutants have a decreased brood size. Wildtype and *met-1* worms were placed on L4440 Control or *mes-4 RNAi* for brood size analysis. *met-1* mutants on control RNAi had a lower brood size than the wildtype, but higher than *met-1;mes-4* RNAi mutants. *met-1;mes-4* RNAi worms had a significantly lower brood size (p= 0.015, student's t-test)..

***mrg-1* M-Z- Somatic Defects**	
Phenotype	**Percentage (n = 51)**
Stunted Growth	21.6 % (11)
Protruding Vulva	51.0% (26)
Gut Extrusion	13.7%(7)
WT	13.7 %(7)

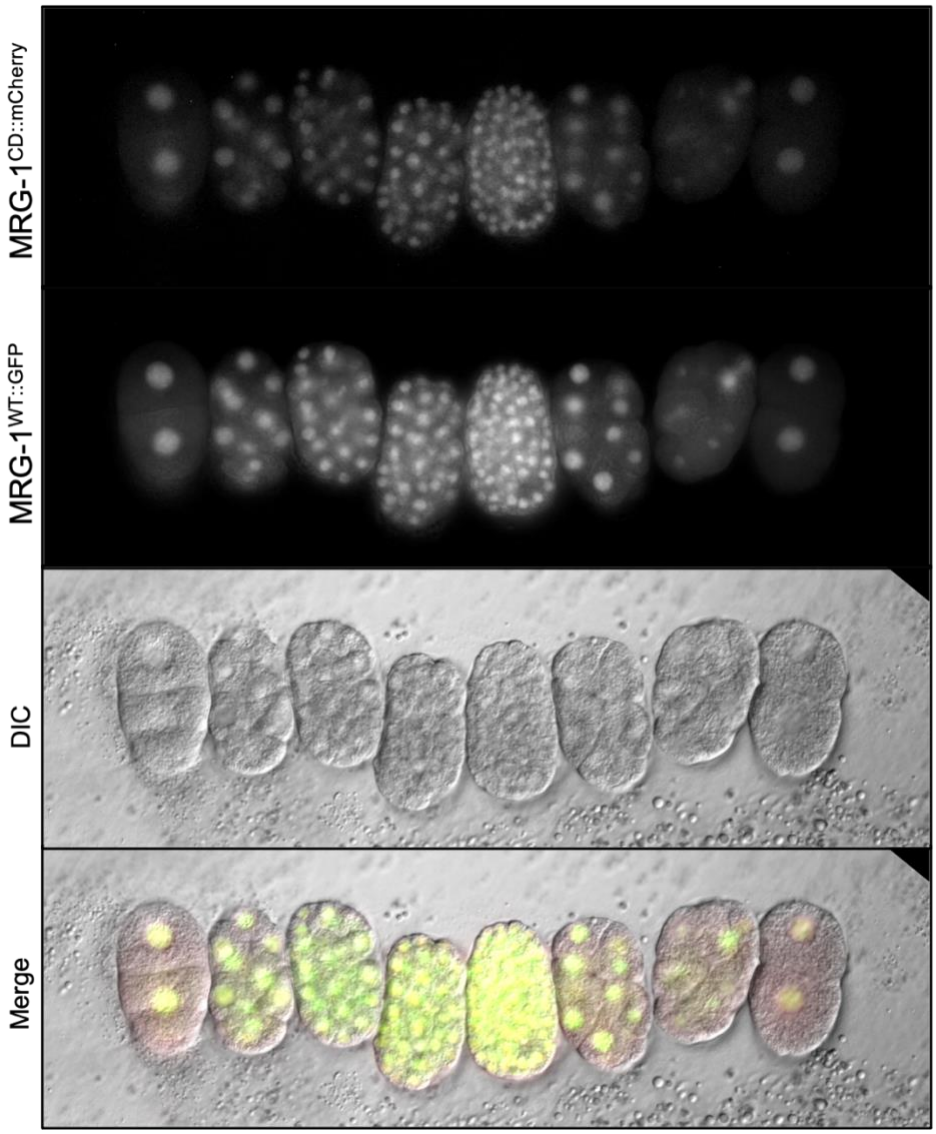
Supplementary Table 2.1. *mrg-1* M-Z- Somatic Defect



Supplemental 2.3. *mrg-1 M-Z-* mutants exhibit somatic defects.

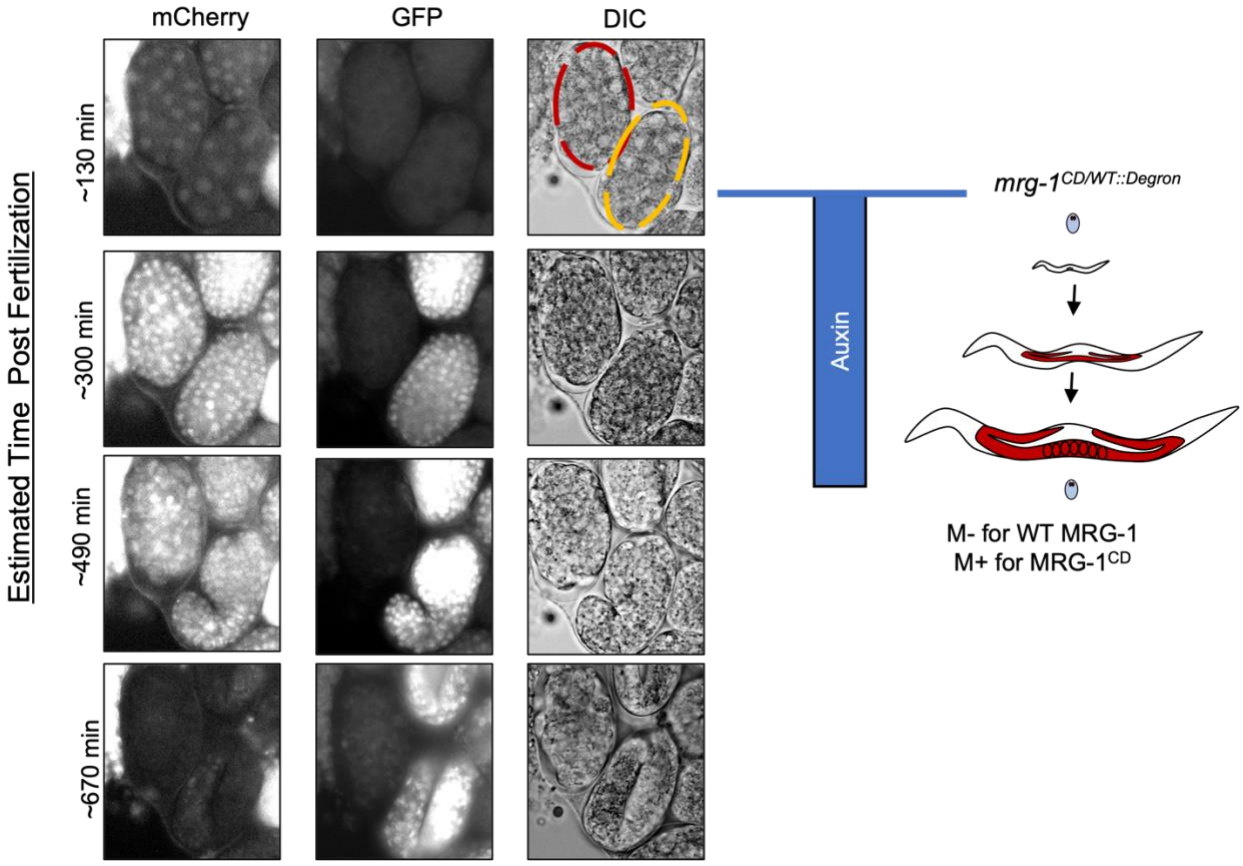
M-Z- progeny of M+Z- *mrg-1(tm1227)* mutants were imaged 24 hrs after the L4 stage. A majority of the worms displayed a variety of somatic defects including stunted growth,

protruding vulvae, and extrusion of the gut through the anus. The worms that did not display somatic defects were sterile as expected.



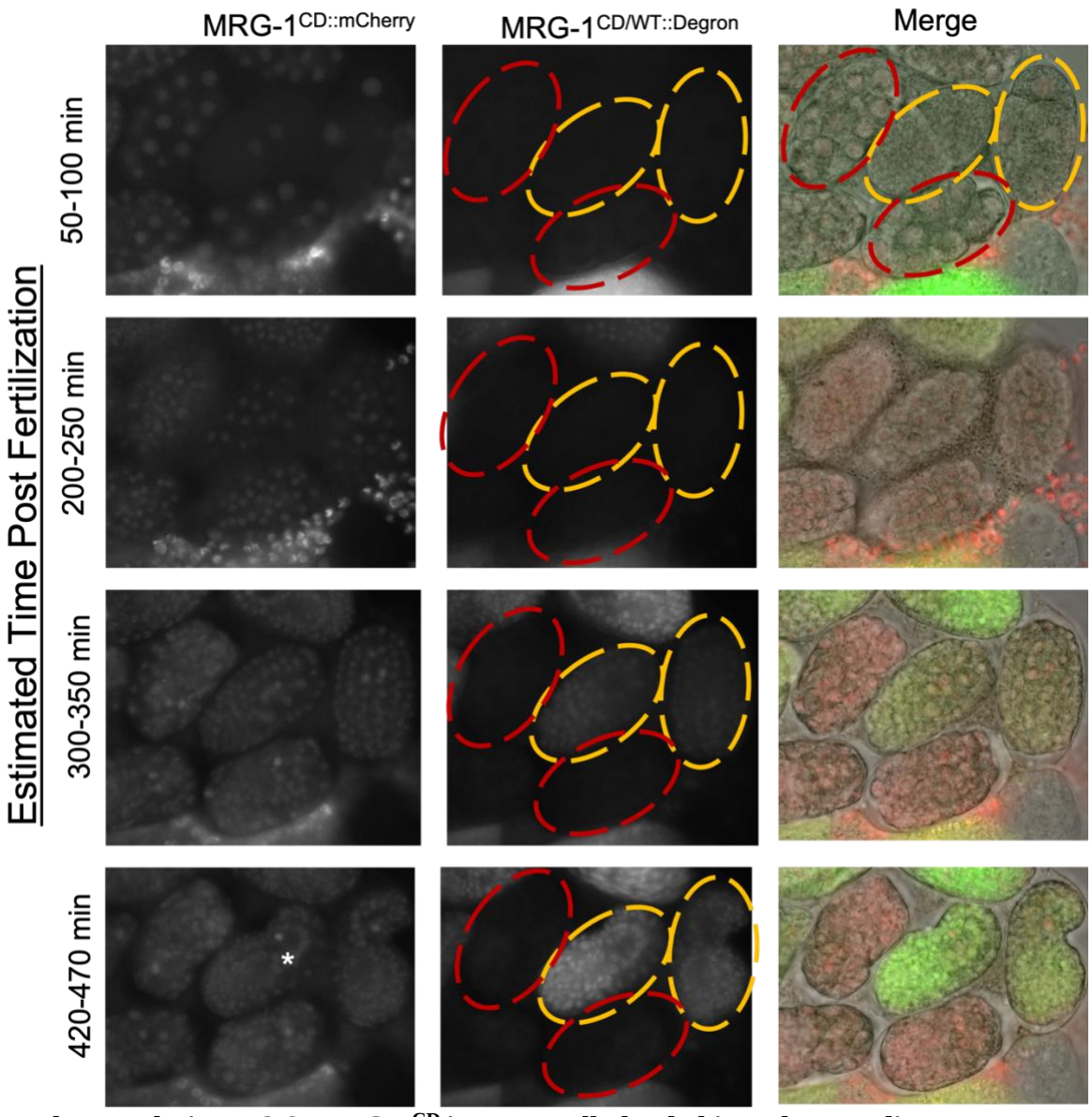
Supplemental Figure 2.4. Maternally loaded MRG-1 CD and MRG-1 WT are present in early embryos.

Embryos dissected from *mrg-1*$^{CD/WT::GFP}$ hermaphrodites were live imaged under DIC. Maternal loads of MRG-1 CD (mcherry) and the MRG-1 WT (GFP) are visible from the two-cell stage.



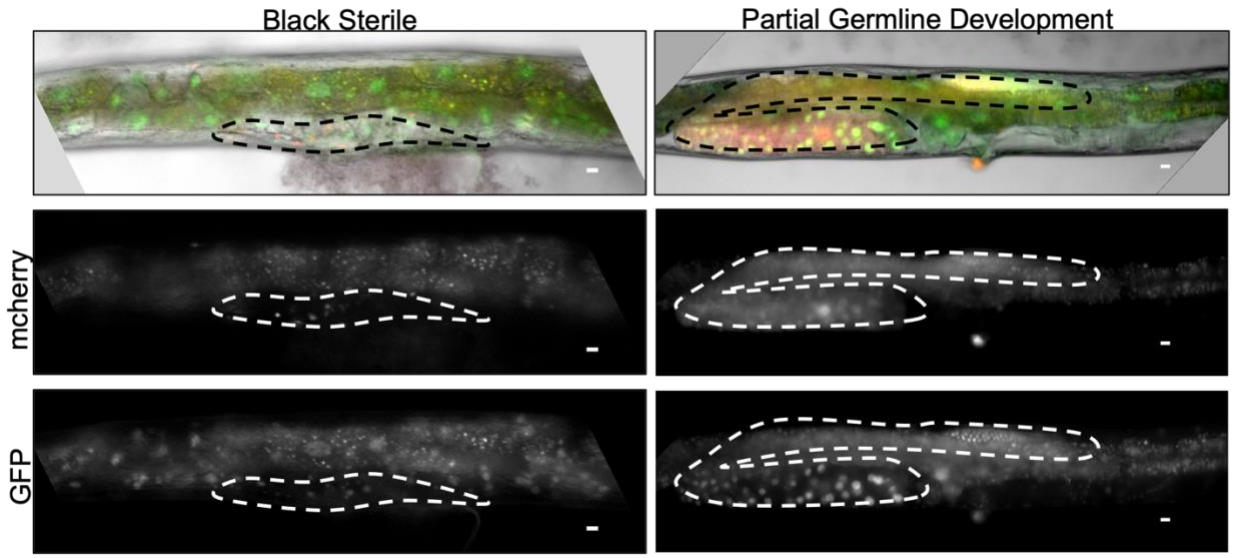
Supplemental 2.5. *mrg-1* Chromodomain Mutants arrest during embryogenesis.

Embryos were dissected from adult *mrg-1*$^{CD/WT::Degron}$ hermaphrodites that were exposed to auxin beginning at hatching and analyzed by fluorescence and phase microscopy over eleven hours. Embryos expressing zygotic GFP, either homozygous *mrg-1*$^{WT::Degron}$ or *mrg-1*$^{CD/WT::Degron}$ (yellow outline) developed normally. In contrast embryos expressing only *mrg-1*^{CD} (red outline) arrested in early- to mid-embryogenesis.



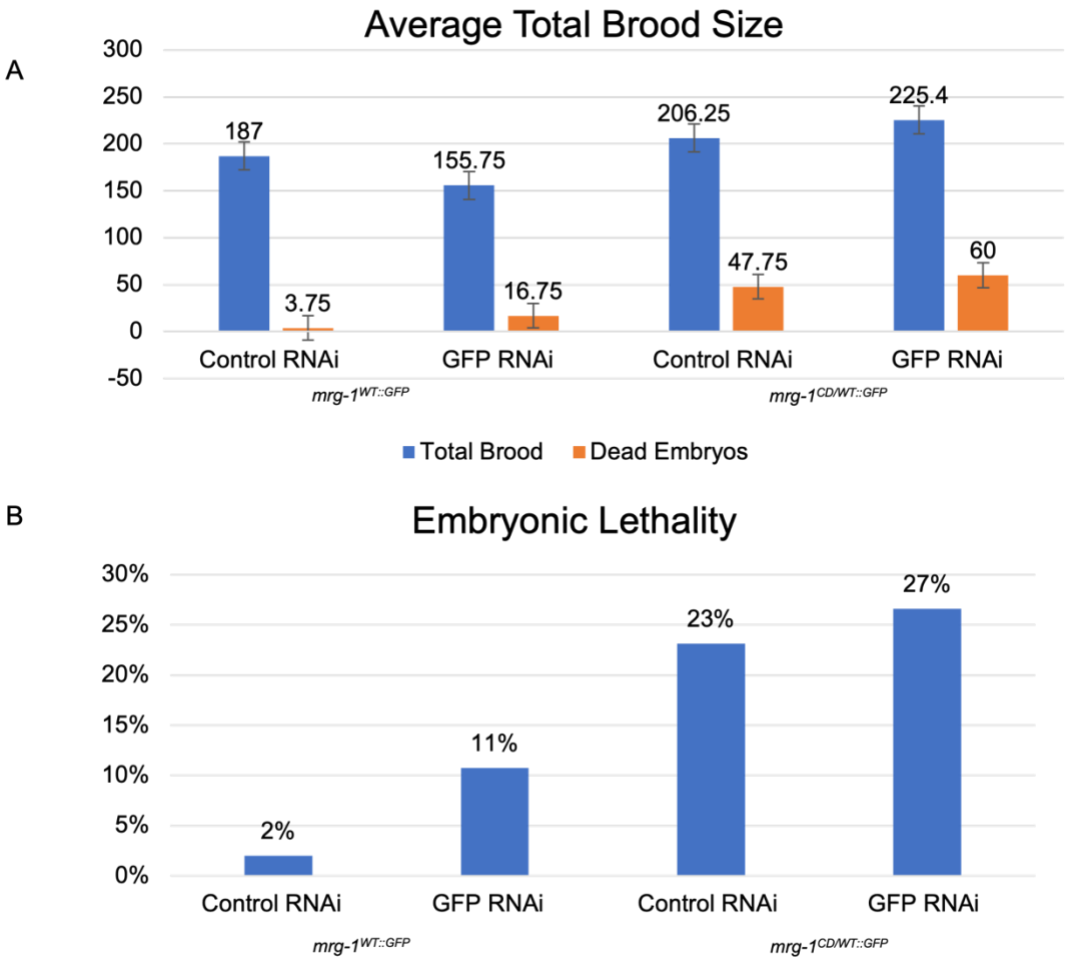
Supplemental Figure 2.6. MRG-1^{CD} is maternally loaded into the germline.

Embryos dissected from *mrg-1$^{CD/WT::Degron}$* + auxin hermaphrodites. *mrg-1^{CD}* and *mrg-1$^{CD/WT::Degron}$* are outlined in red and yellow respectively. Early in embryogenesis, only *mrg-1^{CD}* maternal load is present in embryos (top row). MRG-1$^{WT::Degron}$ begins to be zygotically expressed ~300 min post fertilization near the end of gastrulation (3rd row). Maternal loads of MRG-1^{CD} is visible in PGCS are seen in heterozygote embryos (asterisks).



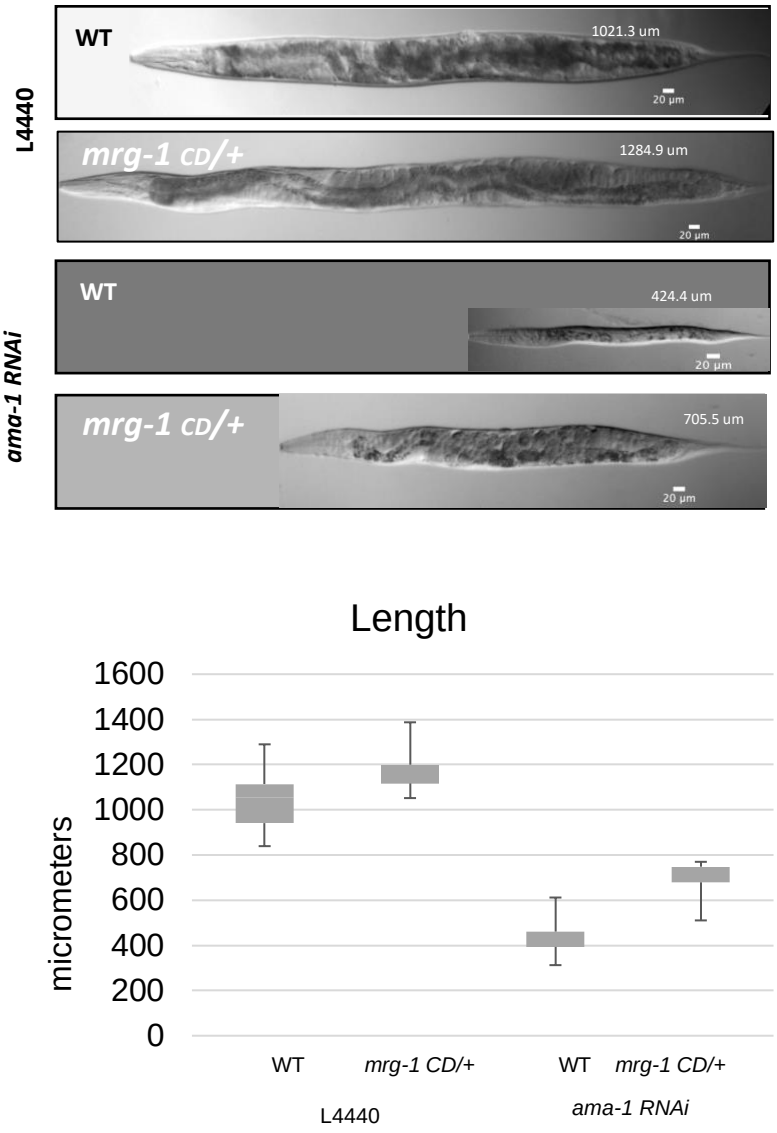
Supplemental 2.7. Zygotic expression of WT MRG-1 partially rescues germline development.

Sterile worms that had zygotic expression of WT or WT and CD mutant, had various levels of sterility. Some worms completely lacked germ cell proliferation and were black sterile (left), while other worms had expression of both WT and CD with germline development but still exhibited significant germline proliferation defects (Right).



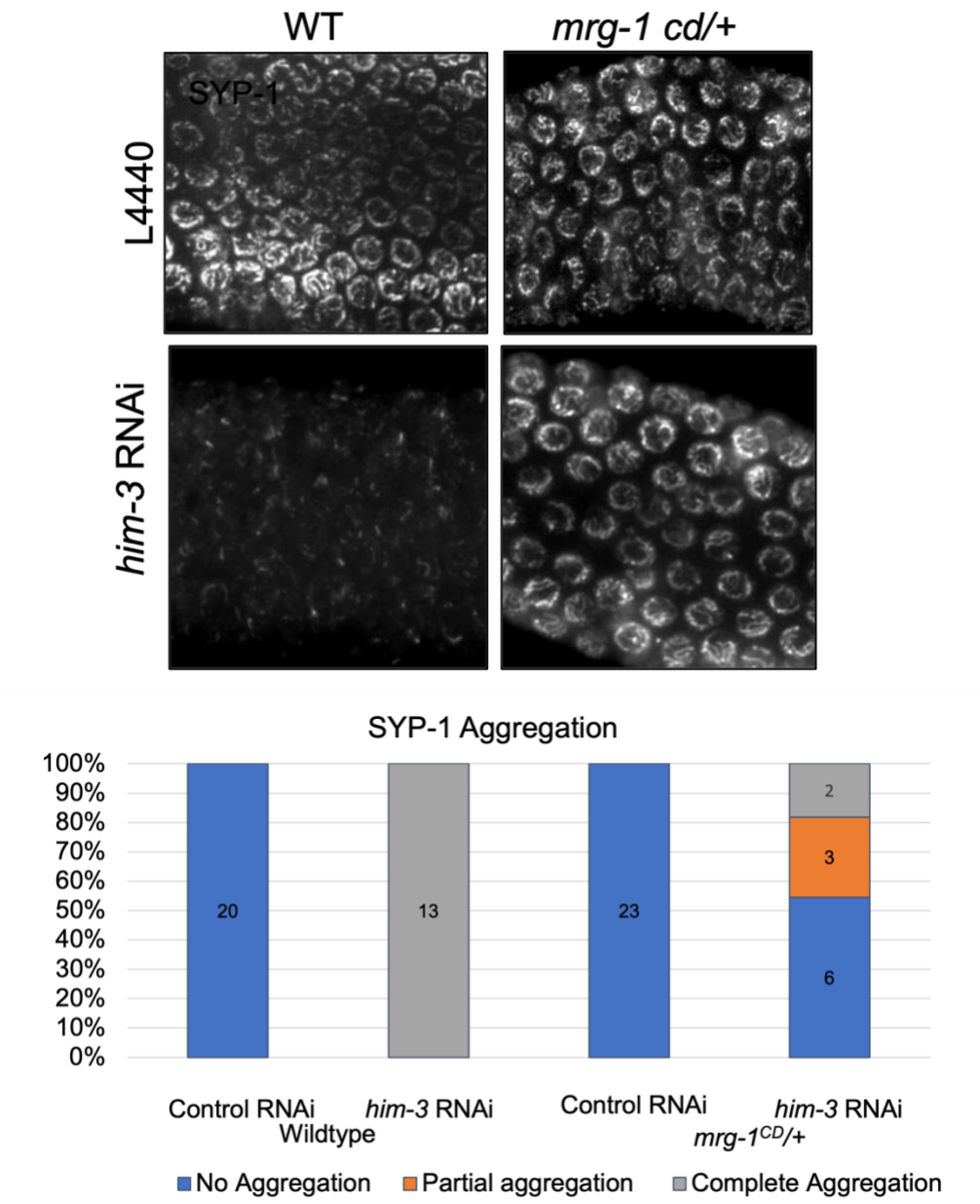
Supplemental Figure 2.8.Fertility of MRG-1$^{CD/WT::GFP}$ worms are not affected by GFP RNAi.

mrg-1$^{WT::GFP}$ and *mrg-1*$^{CD/WT::GFP}$ L1 larvae were placed on L4440 control or GFP RNAi and analyzed for fertility. Wildtype worms placed on GFP RNAi had a reduced brood size and increased embryonic lethality when compared to the control. *mrg-1*$^{CD/WT::GFP}$ worms had no decrease in brood size or embryonic lethality when compared to the control.



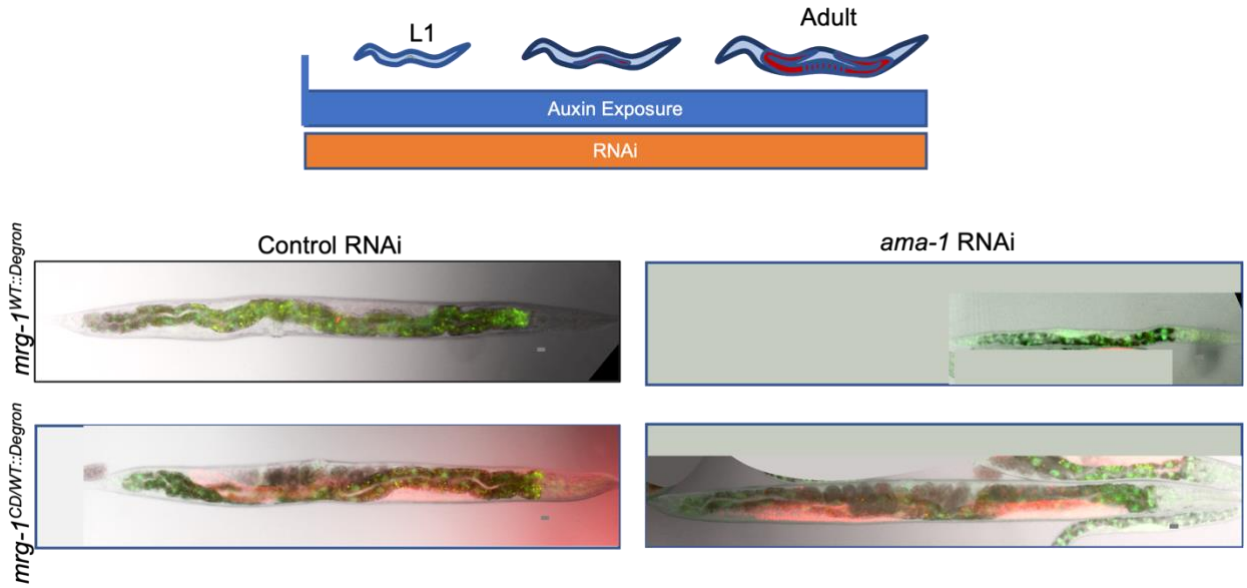
Supplemental Figure 2.9. *mrg-1*$^{CD/+}$ L1 Larvae do not arrest after *ama-1* RNAi.

Wildtype and *mrg-1*$^{CD/+}$ L1 larva were placed on L4440 control or *ama-1* RNAi and measured after 3 days (~24 hrs+ L4). Wildtype worms placed on *ama-1* RNAi arrest at L1 larva stage and do not develop a germline. *mrg-1*$^{CD/+}$ worms continued to develop and produced a germline. No significant difference was seen in worms placed on control RNAi.



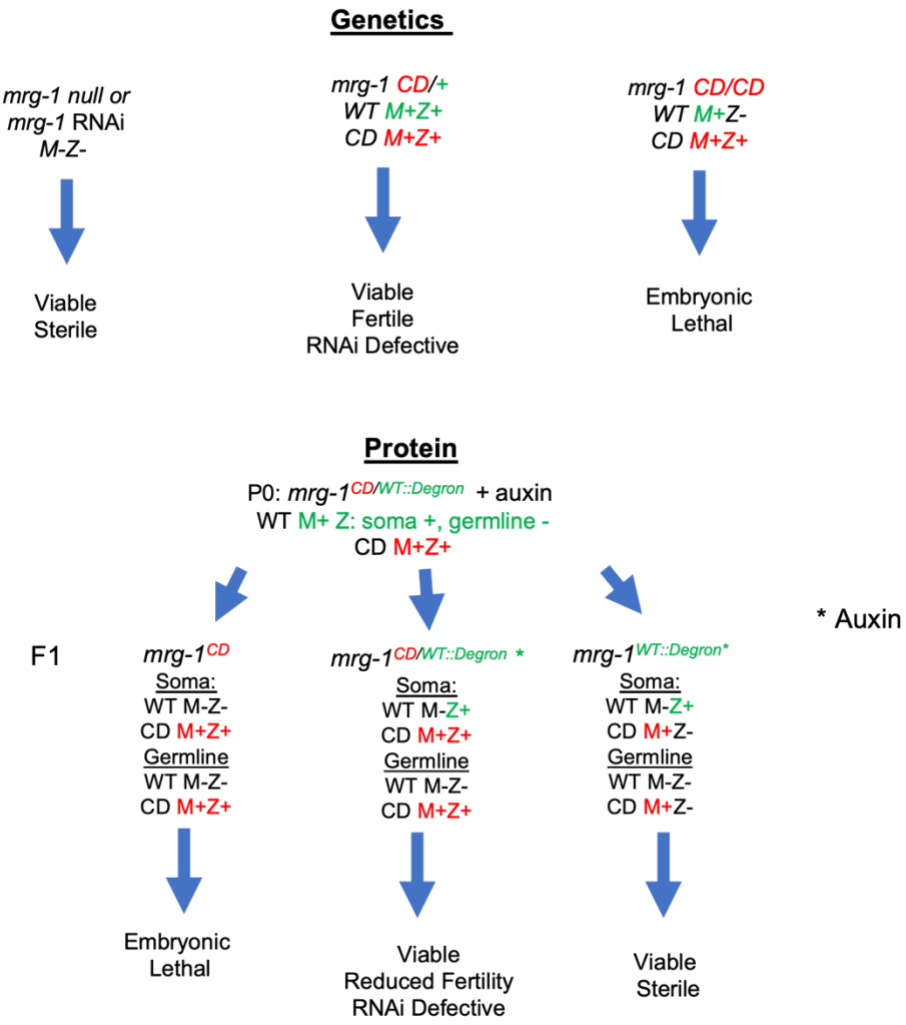
Supplemental Figure 2.10 *mrg-1 CD/+* hermaphrodites exhibit some SYP-1 aggregation after *him-3 RNAi.*

WT and *mrg-1 CD/+* hermaphrodites were placed on control or *him-3* RNAi from L1 Larval stage. SYP-1 aggregation was seen all WT germlines after *him-3* RNAi. In *mrg-1CD/+* germlines, a range of aggregation phenotypes were seen from zero to complete aggregation.



Supplemental Figure 2.11. *mrg-1 cd* mutants are resistant to *ama-1* RNAi.

mrg-1$^{WT::Degron}$ and *mrg-1*$^{CD/WT::Degron}$ worms were placed on L4440 control or *ama-1* RNAi + auxin plate starting from L1 larvae and analyzed at young adult stage. Both *mrg-1*$^{WT::Degron}$ and *mrg-1*$^{CD/WT::Degron}$ worms developed to adult on control RNAi. *mrg-1*$^{WT::Degron}$ worms on Control RNAi + auxin, were sterile. *mrg-1*$^{WT::Degron}$ on *ama-1* RNAi + auxin arrested at L1. *mrg-1*$^{CD/WT::Degron}$ worms on *ama-1* RNAi + auxin developed to fertile adults without MRG-1::degron::GFP in the germline.



Supplemental Figure 2.12 Summary of Genetic and Protein Phenotypes of the *mrg-1(tm1227)* and *mrg-1^{CD}* Mutant.

The phenotypes observed from the *tm1227* allele and those observed in the AID experiments with degron-tagged WT MRG-1. The presence or absence of maternally provided (M) or zygotically produced (Z) MRG-1 is indicated. The presence of GFP-tagged WT MRG-1 protein (Green) and/or mCherry tagged CD mutant MRG-1 protein (Red) are indicated.

S Table 2.2. Guide RNA Sequences.

Guide RNA sequences used to induce breaks at the endogenous *mrg-1* locus using the CRISPR-Cas9 system.

guide name	guide RNA sequence
MRG-1 guide 1	ATTCGCTCCAACTCCGTCGT
MRG-1 guide 2	TGGGATGGAACACATGATGA
MRG-1 guide 3	AACGTCGCGTGCATCTACAA
MRG-1 guide 4	ACTCTACTGCGTTCACTTCA
MRG-1 guide 5	GGAGTTGGAGCGAATCAAGG

	Repair Oligo Sequences
MRG-1 mCherry	CGATTTGATTGTGTTCCTGAATAAGAATCACGGGAAGTACTACAGAGGATCGT CTGATTATCAAGGTGCCTCCAACGACTACTATCGCCGATCTCTCGCCGCTGACG ACGGAGTTGGAGCGAATCAAGGAGGTGGAGGTGGAGCTATGGTCTCAAAGGG TGAAGAAGATAACATGGCAATTATTAAAGAGTTTATGCGTTTCAAGGTGCATA TGGAGGGATCTGTCAATGGGCATGAGTTTGAAATTGAAGGTGAAGGAGGAGG CCGACCATATGAGGGAACACAAACCGCAAAACTAAAGGTAAGTTTAAACATAT ATATACTAACTAACCCTGATTATTTAAATTTTCAGGTAACAAAGGGCGGACCAT TACCATTCGCCTGGGACATCCTCTCTCCACAGTTCATGTATGGAAGTAAAGCTT ATGTTAAACATCCGGCAGATATACCAGATTATTTGAAACTTTCATTCCCGGAGG GTTTTAAGTGGGAACGCGTAATGAATTTTGAAGACGGAGGAGTTGTTACAGTG ACGCAAGACTCAAGGTAAGTTTAAACAGTTCGGTACTAACTAACCATACATAT TTAAATTTTCAGCCTCCAAGATGGAGAATTTATTTATAAAGTCAAACTTCGAGG AACGAATTTCCCCTCGGATGGACCTGTTATGCAGAAGAAGACTATGGGATGGG AAGCTTCAAGTGAAAGAATGTACCCTGAAGACGGTGCTCTTAAGGGAGAGATT AAACAACGTCTTAAATTGAAAGATGGAGGACATTACGATGCTGAGGTAAGTTT AAACATGATTTTACTAACTAACTAATCTGATTTAAATTTTCAGGTGAAGACAAC TTACAAAGCCAAAAAACCAGTTCAGCTGCCAGGAGCGTACAATGTTAATATTA AACTGGATATCACCTCCCACAACGAGGATTACACTATCGTTGAGCAATATGAA AGAGCTGAAGGGCGGCACTCGACAGGTGGCATGGATGAATTGTATAAGTAAA CTGATCTGCTTCATCATGTGTTCCATCCCAGACGATCTTCAATACACCGATATA ACTTCAATAATTTTATACATTGTTCATTATTTTCCAAAGCTAGCCCCACCATTAT GTTTTTGTGA
MRG-1 Chromodomain Oligo	CAGATGTCTTCAAAGAAGAACTTCGAAGTCGGCGAGAACGTCGCGTGCATCGC TAAAGGCAAACCATACGATGCCAAAATTACTGATATCAAAACCAACAGCGATG GCAAGGAACTCTACTGCGTTCACGCTAAGGGCTGGAACAATCGATACGATGAG AAAATCCCAGTCGGCGAGG

MRG-1 GFP	CGATTTGATTGTGTTCCTGAATAAGAATCACGGGAAGTACTACAGAGGATCGT CTGATTATCAAGGTGCCTCCAACGACTACTATCGCCGATCTCTCGCCGCTGACG ACGGAGTTGGAGCGAATCAAGGAGGTGGAGGTGGAGCTATGAGTAAAGGAGA AGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAA TGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAGGGTGATGCAACATACGGAA AACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGGTAA GTTTAAACATATATATACTAACTAACCCTGATTATTTAAATTTTCAGCCAACAC TTGTCACTACTTTCTGTTATGGTGTTCAATGCTTCTCGAGATACCCAGATCATAT GAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAA GAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTAAGTTTAAACAG TTCGGTACTAACTAACCATACATATTTAAATTTTCAGGTGCTGAAGTCAAGTTT GAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGA AGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATG TATACATCATGGCGGACAAACAAAAGAATGGAATCAAAGTTGTAAGTTTAAAC ATGATTTTACTAACTAACTAATCTGATTTAAATTTTCAGAACTTCAAAATTAGA CACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATAC TCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACA ATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTG AGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA ACTGATCTGCTTCATCATGTGTTCCATCCCAGACGATCTTCAATACACCGATAT AACTTCAATAATTTTATACATTGTTCATTATTTTCCAAAGCTAGCCCCACCATTA TGTTTTTGTGA
MRG-1 Auxin Inducible Degron	TATCAAGGTGCCTCCAACGACTACTATCGCCGATCTCTCGCCGCtGACGACGGA GTTGGAGCGAATCAAGCTGGTGGGGGCGGAGGCGCGATGCCTAAAGATCCAG CCAAACCTCCGGCCAAGGCACAAGTTGTGGGATGGCCACCGGTGAGATCATAC CGGAAGAACGTGATGGTTTCCTGCCAAAAATCAAGCGGTGGCCCGGAGGCGGC GGCGTTCGTGAAGGGAGGTGGAGGTGGAGCTATGAGTAAAGGAGAAGAACTT TTCACTGGAGTTGTCCCAATTC

S Table 2.3 Oligonucleotide Repair Sequences.

Oligonucleotide sequences used as repair sequences to generate specified transgenic strains using the CRISPR-Cas9 System.

CHAPTER 3. MRG-1 IN A CHROMATIN INTERACTING COMPLEX

Some of the work in this chapter is adapted from "A Chromodomain Mutation Identifies Separable Roles for *C. elegans* MRG-1 in Germline and Somatic Development**"
Christine A. Doronio, Huiping Ling, Elizabeth J. Gleason, William G. Kelly
** Experiments were performed by Christine Doronio and William G. Kelly. Data analysis was performed by Christine Doronio.

3.1 Abstract

Post translational modifications of histones play a crucial role in the transcriptional regulation of genes by determining their accessibility in chromatin. Consequently, histone modifications are regulated by proteins that add the modifications and they are interpreted by proteins that interact with modified histones and ultimately influence chromatin structure. These proteins often work together in multiprotein complexes that can alter the overall chromatin landscape. Human MORF4-Related Gene 15 (MRG15) is a chromodomain-containing protein that binds to methylated lysine 36 on histone H3 (H3K36me) and interacts with itself and other chromatin modifying complexes including histone deacetylases (HDAC) and histone acetylases (HATs). This interaction allows MRG15 to play a role in both transcriptional repression and activation, respectively, and these roles are dependent on MRG15's chromodomain. MRG-1 is the *C. elegans* homolog of MRG15 that is predicted to interact with H3K6me3 through its conserved chromodomain. Like MRG15, MRG-1 has recently been identified as co-purifying with other chromatin modifying complexes including SINS3, a conserved HDAC complex. However, the exact role of MRG-1 in these chromatin modifying complexes is currently unknown. Utilizing endogenously tagged wildtype and chromodomain defective MRG-1 strains,

we demonstrate that wildtype or chromodomain defective MRG-1 does not interact directly with itself. Despite its inability to dimerize, MRG-1 CD still colocalizes with WT MRG-1 in the germline. However, this colocalization is diminished in the gut suggesting a separate role for the chromodomain in somatic nuclei. Additionally, we show that *mrg-1* null and chromodomain defective mutants have an increased level of acetylation on lysine 9 of histone H3 (H3K9ac) in the germline when compared to wildtype. A similar increase in H3K9 acetylation is seen in mutants that lack SIN-3, a component of the SINS3 complex. Changes in acetylation patterns in *mrg-1* mutants suggest a potential role for MRG-1 in regulation acetylation in the germline. Although further examination is needed, MRG-1 may have a potential role in histone acetylation regulation through its known interaction with HDAC complexes similar to MRG15.

3.2 Introduction

Chromatin organization within the nucleus is a major determining factor in the transcriptional regulation of genes. Histone modifications, like methylation and acetylation, influence the accessibility of chromatin to transcriptional machinery throughout the genome. The presence and absence of different histone modifications at genomic loci can work together to form a "histone code" that contributes to the overall chromatin landscape. Therefore, histone modifications are highly regulated by different chromatin related proteins characterized as writers, erasers and readers. These chromatin related proteins often work together in large, multiprotein complexes to further regulate the resulting chromatin structure. Consequently, to understand the role of chromatin in transcriptional regulation and development, we must understand the chromatin related proteins that regulate histone modifications which determine the overall chromatin landscape.

MRG15 (*MORF4*-Related Gene on Chromosome 15) is a chromodomain containing protein found in humans that binds to tri-methylated lysine 36 on Histone H3 (H3K36me3). As a chromatin binding protein that directly interacts with histone modifications, MRG15 functions with other chromatin remodeling complexes such HATs and HDACs. This association allows MRG15 to contribute to both gene activation and repression, respectively. It is important to note that interaction between MRG15 and other chromatin remodeling complexes is dependent on its dimerization and chromodomain binding abilities. MRG15 self-dimerizes through the C-terminal MRG domain, which is conserved in other MRG Family homologs. When dimerized, MRG15 binds to PAM14, a component of the MAF1 complex that regulates the B-myb promoter in retinoblastoma. However, when bound to Pf1, a component of the HDAC complex Rpd3/Sin3S, MRG15 dimerization is disrupted. In addition to the C-terminal MRG domain, MRG15 homologs have a conserved N-terminal chromodomain. This chromodomain interacts with H3K36me3 by forming a barrel-like pocket made of aromatic residues (57). When the chromodomain of MRG15 is mutated, MRG15 loses its HAT regulated transcriptional activity (53). Therefore MRG15's association with other chromatin related proteins and their functions is dependent on its conformation and binding capabilities of its specific domains. These complex interactions allow MRG15 to contribute different essential mechanisms such as gene regulation, genome integrity, DNA repair, and development.

The structure of the MRG15 chromodomain is conserved throughout several species such as mice, *Drosophila,* and *C. elegans.* Although MRG-1, the *C. elegans* homolog, is predicted to bind to H3K36me3 like MRG15, a direct interaction between H3K36me3 or its histone methyltransferase has never been identified (66). Similar to MRG15, MRG-1 functions in different processes and interacts with other chromatin modifying complexes. Recent data shows

that MRG-1 interacts with SIN-3 and HDA-1 which are members of SINS3, the conserved HDAC complex (65, 66). Mutations in components of the SINS3 complex lead to defects in development, embryogenesis, and dsRNA processing(65, 89, 94). The SINS3 complex is thought to influence gene expression by regulating histone deacetylation at different promoters (65). The interaction between HDA-1 and MRG-1 is dependent on its sumoylation status such that HDA-1 must be sumoylated in order to bind to MRG-1 (67). Interestingly, the sumoylated HDA-1 is only found in the germline demonstrating that a tissue specific mechanism dependent on post translational modifications (67). Although MRG-1 has been proposed as a component of the SINS3 complex in *C. elegans*, its exact function is currently unknown.

As a conserved homolog of MRG15, it is necessary to understand the dimerization and chromodomain interacting ability of MRG-1. In this chapter I characterize MRG-1's ability to self-dimerize using strains with differentially C-terminal tagged, endogenous wildtype and chromodomain defective MRG-1. My data suggests that unlike MRG15, wildtype MRG-1 or MRG-1 ^{CD} do not directly interact with one another *in vivo.* When visually observed in the germline, both wildtype MRG-1 and MRG-1 ^{CD} strongly colocalize with one another. Interestingly, this colocalization is diminished in gut somatic nuclei in adults, suggesting a role for the chromodomain in MRG-1 localization in the soma. Surprisingly, *in vitro* analysis of the MRG-1 chromodomain does not indicate binding with wildtype MRG-1. Data in this chapter also identify a potential role for MRG-1 in histone acetylation regulation by characterizing histone acetylation levels in the germline. Although more research needs to be done, the data in this chapter provide more evidence for MRG-1 functioning separately in the germline and soma. Perhaps MRG-1's localization is tissue specific and dependent on its interactions with other chromatin related proteins or the conserved chromodomain.

3.3 Results

3.3.1 Neither Wildtype MRG-1 or MRG-1 ^{CD} form detectable dimers *in vivo*

The MRG domain of the MRG family of proteins directly dimerizes with itself (95). However, this interaction has never been shown in the *C. elegans* homolog MRG-1. In this work, we used CRISPR editing to C-terminally tag the endogenous locus of MRG-1 with either a fluorescent GFP or mCherry tag. Both resulting strains did not exhibit any significant change in fertility. Similarly, CRISPR was used to further mutate the conserved chromodomain in the *mrg-1::mcherry* strain producing an addition strain called *mrg-1* ^{CD} that was characterized in the previous chapter.

In order to determine if MRG-1 can form dimers, worms that were wildtype MRG-1::GFP or heterozygous for wildtype MRG-1::GFP and wildtype MRG-1::mcherry were generated. Whole worm lysates were produced from these mutants and analyzed by immunoprecipitation using anti-GFP followed by Western Blot with anti-mcherry and anti-GFP. After immunoprecipitation with anti-GFP and Western blot with anti-GFP, MRG-1::GFP was detected in both the flow through and the immunoprecipitation samples of the MRG-1::GFP and heterozygous strains as expected. When analyzing the same samples with Western blot using anti-mCherry, MRG-1::mCherry was detected in the flowthrough but not in the immunoprecipitate (Figure 3.1A). The lack of MRG-1::mCherry co-precipitation with anti-GFP suggests that MRG-1::mcherry does not directly interact with MRG-1::GFP in the heterozygous animals. This suggests that unlike MRG15, MRG-1 does not directly dimerize with itself.

A similar experiment was performed with a population of worms that were heterozygous for wildtype MRG-1::GFP and the chromodomain mutated MRG-1::mCherry. After immunoprecipating with anti-GFP, samples were analyzed using Western blot with anti-GFP and

anti-mCherry. Like the previous experiment, MRG-1::GFP was detected by anti-GFP in all samples after anti-GFP immunoprecipitation in from the heterozygous population. However, after staining with anti-mCherry, the MRG-1::mCherry CD was detected in the input and flowthrough, but not the immunoprecipitation sample. This suggests that there is no direct interaction between wildtype MRG-1 and MRG-1 CD (Figure 3.1.B).

It is important to note that because the population of worms used were heterozygous for MRG-1::mcherry and MRG-1::GFP, there is a possibility of having a percentage of worms that were homozygous for either genotype and would therefore not have any interaction between MRG-1::GFP and MRG-1::mcherry *in vivo*. A sample of the population was examined under the microscope. Although some homozygotes were present for each genotype, a clear majority of the sample population were heterozygous indicating that the failure to detect co-precipitation was not due to overrepresentation of either homozygote.

3.3.2 The MRG-1 Chromodomain does not interact with H3K36me3 peptide

MRG15 contains an N-terminal chromodomain that binds with H3K36me. The chromodomain is made up of five residues and is conserved across species. Despite having a conserved chromodomain and similar chromatin immunoprecipitation patterns to H3K3K6me, a direct interaction has never been shown between MRG-1 and H3K36me. To determine if MRG-1 binds with H3K36me through the chromodomain, we collaborated with the lab of Xiaodong Cheng who expressed the first 100 amino acids of MRG-1, which includes the chromodomain, in a protein expression vector that adds an N-terminal GST tag. After expressing and purifying the GST-tagged MRG-1 wildtype chromodomain (Supplemental Figure 3.1), a pull down assay using histone peptides was performed (Figure 3.2). Immunoprecipitation pull down was

performed using modified and unmodified H3K4 and H3K36 peptide. After pulling down with H3K4me3 and H3K36me3, ZCWPW1, a control protein known to interact with both modifications, was detected (96). However, in the wildtype MRG-1 Chromodomain reaction, no GST-tagged protein was detected suggesting that the GST-MRG-1 Chromodomain peptide did not interact with either H3K4me3 or H3K36me3 peptide.

3.3.3 MRG-1 CD colocalizes with MRG-1 WT in the germline but is lost in the post-larval soma

MRG-1 is necessary for proper germline function: deletion mutants exhibit maternal effect sterility that shows a failure to proliferate embryonic germ cells (68-71, 73). After generating *mrg-1 CD*, we showed that altering the MRG-1 chromodomain does not disrupt its function in the post-embryonic germline (Figure 2.5). Although our immunoprecipitation experiments suggest that there is no direct interaction between MRG-1 CD and MRG-1 WT, the sub-cellular distribution of MRG-1 CD visualized in live heterozygote worms (Figure 3.3) substantially colocalizes with MRG-1 WT in the germline. Colocalization analysis shows a Pearson R value of 0.92, suggesting high levels of colocalization between MRG-1 CD and MRG-1 WT. Because our previous data suggest there is no direct interaction between MRG-1 CD and MRG-1 WT, this shows that both forms of the protein can independently target similar regions of the nucleus.

In addition to its localization in the germline, MRG-1 is also found in somatic tissue including gut and neuronal tissue (66). This is confirmed when examining both fluorescently tagged wildtype MRG-1 strains. When imaging live heterozygote MRG-1 $^{CD/WT}$ worms, MRG-1 WT is visible in both germline and somatic tissue, but MRG-1^{CD} was often observed to be absent in many somatic nuclei (Figure 3.3). Although it is found in the germline, there is a clear

absence of MRG-1^{CD} in somatic tissues where MRG-1 WT is present (Figure 3.4). When analyzing specific germline and somatic tissue regions, their Pearson scores for colocalization are 0.83 (Figure 3.4 ROI 1) and 0.13 (ROI 2), respectively. This demonstrates that there are high instances of overlap in the germline, but not in the gut somatic tissue. To confirm that this exclusion was specific to the chromodomain mutation, wildtype MRG-1::mcherry worms were imaged. Similar to the wildtype MRG-1::GFP strain, the wildtype MRG-1::mcherry is visible in both the germline and somatic tissue (Supplementary Figure 3.2). Early larval stages were also examined and both the MRG-1 CD and WT were observed in most if not all somatic nuclei, suggesting that the absence of the CD mutant protein in adult soma may be due to increased instability. Although further research needs to be done, this suggests that the chromodomain may be necessary for maintaining MRG-1 in somatic cells; e.g., alterations to the chromodomain may change its interactions with other proteins in a larger a complex that could affect its stability.

3.3.4 *mrg-1 CD* and *mrg-1 null* mutants have an increased level of H3K9 acetylation in the germline

MRG15 has been shown to interact with both HDAC and HAT complexes influencing gene repression and activation(30, 53). Additionally, the HAT activity of MAF2 and the localization of RPD3 HDAC complex is dependent on the chromodomain of MRG15 (53, 63). Like MRG15, MRG-1 has been identified as interactor with the HDAC complex SINS3. Further, mutations in members of the SIN3 complex, like *hda-1* and *sin-3,* have similar phenotypes to the *mrg-1 CD* mutant including RNAi resistance and embryonic lethality. However, the role of MRG-1 within this complex and its potential role in acetylation regulation is not known. To

further characterize MRG-1's potential role in acetylation regulation, germlines from wildtype, *mrg-1 CD* and *mrg-1 null (tm1227)* mutants were dissected and stained for acetylation of lysine 9 of Histone H3 (H3K9ac). In comparison to the wildtype germlines, both *mrg-1 CD* and *mrg-1 null (tm1227)* germlines had an increased level of H3K9 acetylation in specifically the meiotic region of the germline (Figure 3.5). This result suggests that MRG-1 and the MRG-1 chromodomain, play a role in histone acetylation regulation in the meiotic region of the germline.

Because of the changes acetylation and similar phenotypes to SINS3 complex mutants, we examined H3K9 acetylation patterns in *sin-3* mutants (Supplemental Figure 3.3). When compared to wildtype, *sin-3* mutants had an increase in acetylation. Interestingly, the increase in acetylation was restricted to the distal, pre-meiotic portion of the germline. When comparing the meiotic late pachytene region between wildtype and *sin-3* mutants, no significant difference in acetylation was seen. The difference in increase in acetylation in the premeiotic region in *sin-3* mutants and the meiotic region in *mrg-1 CD* mutants suggests that their roles in acetylation regulation do not overlap. Therefore, MRG-1 may be functioning in a different complex separate from SIN-3 when influencing histone acetylation in the germline.

3.4 Discussion

3.4.1 MRG-1 does not dimerize with itself

Many chromatin-binding proteins function in large, multiprotein complexes sometimes made up of homodimers or heterodimers of different proteins. Structural studies of the chromodomain containing protein MRG15 demonstrate that it dimerizes through its MRG domain (57, 97). The dimerization of the MRG15 appears to determine within which complex it

interacts. As a dimer, MRG15 interacts with PAM14, a component of the of the MAF1 complex that contributes to the regulation of the B-myb promoter in in retinoblastoma (53, 97-99). The ability dimerize through the MRG domain is conserved in in other MRG family homologs (95). However, *in vitro* studies show that the MRG15 dimer becomes disrupted and takes a conformational change when bound to Pf1. Pf1 is a main component of the conserved HDAC complex, Rpd3S/Sin3S, that plays a crucial role in the assembly and interaction of MRG15 to Sin3S complex (81, 100). Overall, this suggests that the function of MRG15 in different chromatin related complexes is dependent on its formation as self-interacting dimer.

As a conserved homolog of MRG15, here we sought to characterize the ability of MRG-1 to dimerize. By differentially tagging the endogenous MRG-1 with either a C-terminal GFP or mCherry, we were able to characterize the self-interaction *in vitro* (Figure 3.1). We collected protein samples from a population that was heterozygous for wildtype MRG-1::mcherry and wildtype MRG-1::GFP. After immunoprecipitating with anti-GFP and analyzing with anti-mCherry, we saw that there was no evidence of direct interaction between MRG-1::mCherry and MRG-1::GFP. A similar result is seen in mutants that are heterozygous for MRG-1::mcherry CD and wildtype MRG-1::GFP. This suggests that unlike MRG15, MRG-1 does not dimerize. Interestingly, when visually observing both heterozygote strains, wildtype MRG-1::mcherry and CD MRG-1::mcherry colocalize with wildtype MRG-1::GFP. The visual colocalization without evidence of direct interaction, suggests that differentially tagged MRG-1 are localized to the same region by possibly interacting with another protein or larger complex. It is important to note that the dimerization of MRG15 is through the C-terminal MRG domain (95). Although our C-terminal tag is located after MRG domain, the tag size of the fluorescent may disrupt its ability

to dimerize. However, we do not see any changes in fertility or sterility in these mutants and therefore suspect that MRG-1 is completely functional with the C-terminal tag.

It is also important to note that the conditions used to immunoprecipitate the tagged MRG-1 proteins were not harsh enough to disrupt interactions with other proteins within any complexes that MRG-1 interacts. Thus our results not only indicate MRG-1 does not exist as dimers *in vivo,* but may also suggest that the complexes within which MRG-1 associates contain a single MRG-1 molecule.

3.4.2 Wild Type MRG-1 Chromodomain does not interact with H3K36me3 peptide *in vitro*

MRG15 interacts with H3K36me3 through the conserved chromodomain (53, 57). The function of the chromodomain contributes to MRG15's HAT and HDAC interactions such that mutations in the chromodomain misregulates histone acetylation (53). The chromodomain is made up of five residues that form a binding pocket that interacts with H3K36me3 and are conserved across species, including MRG-1 (57). However, the interaction between MRG-1 and H3K36me3 has never been shown despite having similar localization and chromatin immunoprecipitation patterns. By using an *in vitro* system that tagged the first 100 amino acids of wildtype MRG-1, including the chromodomain, with GST our collaborators were able to perform an histone peptide binding assay with modified H3K4 and H3K36 peptides (Figure 3.2). After immunoprecipitating with methylated and unmethylated H3K34 and H3K36 peptides, purified protein samples were analyzed using anti-GST. ZCWPW1, a protein known to bind to both modified peptides, was detected in both samples (96). However, the GST::MRG-1 chromodomain peptide was not detected in immunoprecipitation samples. This suggests that *in vitro,* there was no interaction between the wildtype MRG-1 Chromodomain and H3K4me3 or

H3K36me3. It is important to note that the peptides used in the assay were partial peptides and may not fully support the binding ability between MRG-1 chromodomain and the H3K36me3. Therefore, it is necessary to perform a similar assay using complete proteins and histones.

3.4.3 The MRG-1 chromodomain is necessary for MRG-1's persistence in the soma

MRG-1 is normally expressed in both germline and somatic tissues (66, 68, 84).When lacking in the germline, *mrg-1* mutants that have a maternal load of MRG-1(M+Z-) exhibit meiotic defects. Without a maternal load of MRG-1 (M-Z-), a germline fails to produce and worms grow up sterile (68, 69, 73). Although some phenotypes exist in the M-Z- progeny, a majority of them do not exhibit somatic defects (Chapter 2). Interestingly, when mutating the MRG-1 chromodomain, no germline or obvious somatic defects are observed. However, unlike the null mutant, *mrg-1 CD* mutants are embryonic lethal and arrest during embryogenesis suggesting a potential role for the chromodomain in somatic development.

Visually, in the germline MRG-1 CD and MRG-1 WT directly overlap and colocalize with one another (Figure 3.3). However, MRG-1 with an altered chromodomain is absent from gut nuclei in adults (Figure 3.4). Currently the function of the MRG-1 chromodomain in somatic tissues is unclear. The exclusion of MRG-1 CD from gut nuclei suggests that chromodomain is necessary for its localization or function in the somatic tissue. This may explain why *mrg-1 CD* mutants are embryonic lethal and without a functioning chromodomain, the somatic tissues may not properly develop. Interestingly, when observed at a young larval stages, MRG-1 CD is present in somatic nuclei. Therefore, unlike MRG-1 CD in the germline, MRG-1 CD in the soma may become less stable in later stages. However, more research needs to be done. Because the worms are heterozygous for wildtype MRG-1, there are no clear defects in the somatic tissue.

3.4.4 MRG-1 contributes to histone acetylation regulation

As an interactor with HATs and HDACs, MRG15 plays in a role in regulating histone acetylation. Recently, MRG-1 was identified as a component of the similar HDAC complex, SINS3, that includes SIN-3 and HDA-1. Interestingly, the interaction between HDA-1 and MRG-1 is dependent on sumoylation status such that HDA-1 must be sumoylated when bound to MRG-1 (67). The sumoylation of HDA-1 is specific to the germline suggesting a tissue specific distinction that is dependent on post translational modifications. Additionally, *mrg-1 CD* mutants have similar phenotypes to *hda-1* and *sin-3* mutants including RNAi resistance and embryonic lethality (67, 89, 94). However, the exact role of MRG-1 in relation to the SINS3 complex and its functions are unclear.

To further understand the role of MRG-1 in histone acetylation regulation, we examined H3K9 acetylation in germlines from *mrg-1 (tm1227) M+Z-* and *mrg-1 CD* mutants. When compared to wildtype, both *mrg-1* mutants had an increase in H3K9 acetylation in the meiotic region of the germline suggesting some contribution of MRG-1 to histone acetylation regulation. Interestingly, unlike *mrg-1 null* mutants that exhibit meiotic defects, no other germline defects are seen in *mrg-1 CD* only mutants. This suggests that the acetylation regulation function of MRG-1 is separate from its function in meiosis. MRG-1 interacts with SIN-3, we also quantified H3K9 acetylation in germlines that lacked SIN-3. Similar to *mrg-1* mutants, mutants that lacked SIN-3 had an increase in H3K9 acetylation in the germline when compared to wildtype. However, the increase in acetylation was limited to the premeiotic, mitotic region unlike the *mrg-1* mutants that saw an increase in the meiotic region. This difference in increase location suggests that MRG-1 and SIN-3 may be interacting in different mechanisms regulating

acetylation. Although more analysis needs to be done, the similarities in acetylation and other phenotypes like embryonic lethality and RNAi resistance (previously described in chapter 2) suggest that MRG-1 and the chromodomain functions with other chromatin modifying proteins to regulate histone acetylation to contribute to the overall chromatin structure.

3.5 Materials and Methods

Strains and Strain Maintenance

The following strains were used for these experiments: Bristol N2, KW1668 (ck42(*mrg-1::mcherry)* III), KW1682 (ck45(*mrg-1::degron::GFP*) III; ieSi38 IV), KW1683 (*mrg-1(ck43)/mrg-1(ck45)* III; ieSi38 IV), KC565 (*sin-3* tm1276). For additional information on generation of these strains refer to Chapter 2. All strains were maintained at 20°C on nematode growth medium (NGM) plates seeded with *E. coli* OP50. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Any newly generated strains will be made available through the CGC.

Auxin Induced Degradation

Auxin Induced Degradation (AID) experiments were performed by the method of (86). OP50 seeded NGM plates were supplemented with 1mM of auxin and the bacteria lawn was allowed to grow for at least 48 hours at room temperature before worms were added to plates. Worms were placed on auxin containing plates for the indicated amount of time before being analyzed further.

Protein Co-Immunoprecipitation

Heterozygous, synchronized populations of indicated strains were grown on NGM plates seeded with OP50. At the young adult stage, worms were collected and rinsed with M9 buffer until pellet was free of bacteria. During the final rinse, worm pellet was washed and resuspended with lysis buffer (20mM HEPES pH 7.4 , 150 mM NaCl, 2 mM MgCl2 , 0.1% Tween 20, Protease inhibitors (1 tab /10ml)). Worm pellet in lysis buffer was then freeze thawed three times in liquid nitrogen to break down body walls tissues. Then sample was sonicated using a Biorupter (30 seconds on, 30 second off, 6 times) and then rocked at 4°C for 15 minutes. Sonication and rocking step were repeated. After centrifuging at maximum speed for 15 minutes at 4°, the supernatant was collected and measured. 50 ug of each sample were then incubated while rocking with magnetic anti-GFP beads (Chromotek, GTD) at 4° overnight. After incubation, flowthrough and immunoprecipitation samples were collected and analyzed using Western Blot.

Western Blot

Protein samples analyzed and run on a precast 4- 12% gradient Bis-Tris gel (Invitrogen NW04120BOX) and transferred onto a PVDF membrane using a Power Blotter (ThermoScientific 22834SPCL). Blots were blocked with 5% Bovine Serum Albumin in TBS + 0.1% Tween-20 (TBS-T) for three hours before incubation with primary antibody at 4° overnight. Blots were washed with TBS-T, incubated with secondary antibody at room temperature, and washed. Blots were then incubated with Clarity Western ECL substrate (BioRad 170-5060) for five minutes and visualized using the Bio-Rad ChemiDoc Imaging System. Primary antibodies used for this study were: chicken anti-GFP (1:2000, Aves Labs GFP-1020), rabbit anti-mcherry (1:500, Abcam ab183628)**.** Secondary antibodies used were donkey donkey anti-chicken peroxidase (1:2000, Jackson #703-035-153), donkey anti-rabbit HRP

(1:2000, Jackson 711-035-152). HRP visualized with Clarity Western ECL Substrate (Bio-Rad #170-5060).

Immunofluorescence and Live Imaging

Germlines were dissected from young adult (24 hours after L4 larval stage) hermaphrodite worms in dissection buffer (2X sperm salts, 2.5mm levamisole) on poly-lysine coated slides and fixed with 1% paraformaldehyde with ethanol or methanol acetone following freeze crack on a chilled aluminum block. After fixation, slides were washed in TBST (tris buffer saline, 1% Tween-20) and primary antibody was added for incubation at 4° overnight. Following washes with TBST, slides were incubated with secondary antibody at room temperature for 4 hours. Slides were then washed with TBST, and then counterstained with DAPI. Slides were sealed with Prolong Gold and clear nail polish. The following primary antibodies were used: rat anti H3K9Ac (BioLegend 698402, 1:500). Secondary antibodies used in this study were: donkey anti-rat 594 (Invitrogen A21209, 1:500).

Worms that were imaged live were placed on a 2% agarose pad and immobilized using 5mm Levamisole in Egg Buffer (1 M HEPES pH 7.3, 2 M NaCl, 2 M KCl, 1 M $CaCl_2$, 1 M $MgCl_2$). After immobilization, a coverslip was gently placed over the pad and imaged.

All imaging was done using a Leica DMRXA with a Retiga2000r camera using Hamamatsu Photonics software. All image analysis was done using FIJI imaging software(93).

Protein purification and histone peptide binding assay

The first 100 amino acids of wildtype MRG-1 were amplified and cloned into the pGEX-6p-1 vector containing an GST-tag using BamHI and XhoI restriction digest sites. After cloning the plasmid was transformed into transformed into E. coli BL21-Codon Plus (DE3)-RIL (Stratagene). Protein purification was done with a GST column. Peptide binding assay was performed as in materials methods in (96).

3.6 Figures

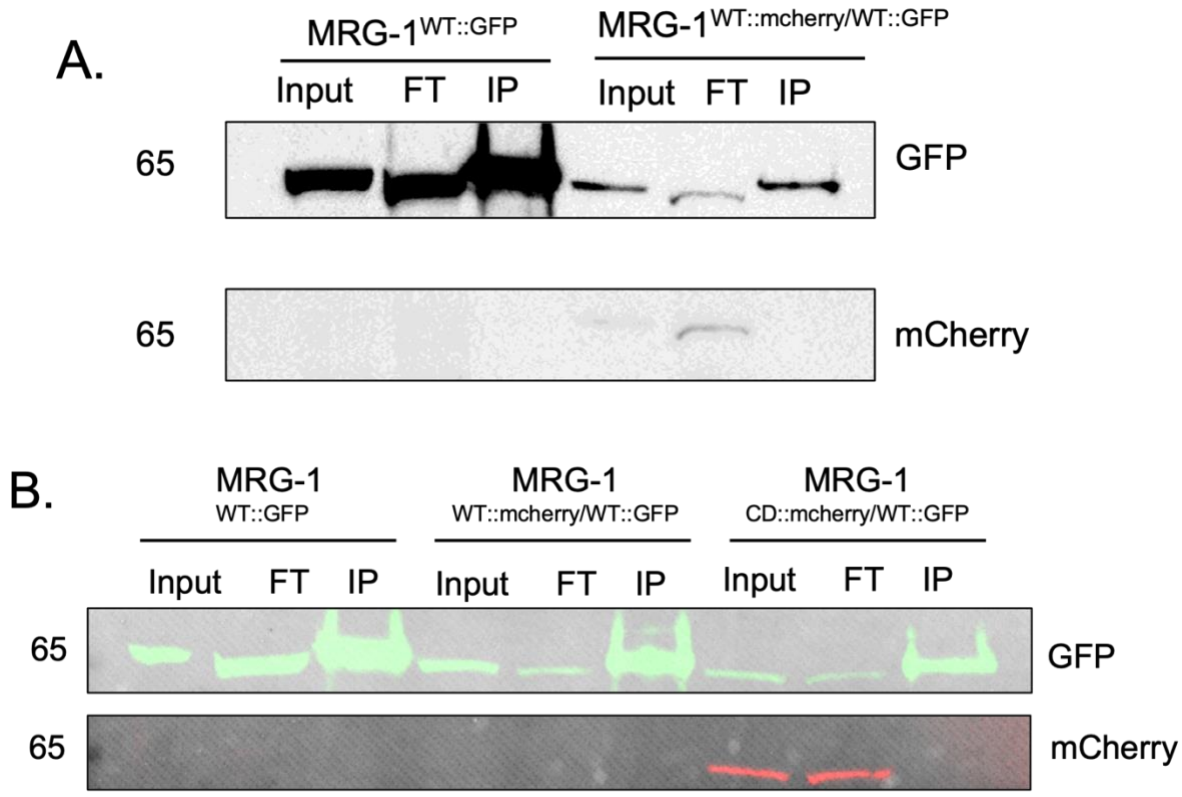


Figure 3.1. MRG-1 WT does not directly interact with itself or MRG-1 CD.

Lysates from MRG-1$^{WT::GFP}$, MRG-1$^{WT::mcherry/WT::GFP}$, and MRG-1$^{CD::mcherry/WT::GFP}$ adult were immunoprecipitated with anti-GFP beads and analyzed on Western Blot using anti-GFP or anti-mCherry antibodies. MRG-1$^{WT::GFP}$ was detected in the immunoprecipitation from all worms. MRG-1$^{WT::mcherry}$ and MRG-1$^{CD::mcherry}$ were detected in the input and flowthrough of the lysates but not the immunoprecipitation. Panel A analyzes lysate from MRG-1$^{WT::GFP}$ and MRG-1$^{WT::mcherry/WT::GFP}$. Panel B analyzes lysates from MRG-1$^{WT::GFP}$, MRG-1$^{WT::mcherry/WT::GFP}$, and MRG-1$^{CD::mcherry/WT::GFP}$. No WT MRG-1::mcherry was detected in pulldown from MRG-1$^{WT::mcherry/WT::GFP,}$ in Panel B possibly due to a skewed heterozygous population.

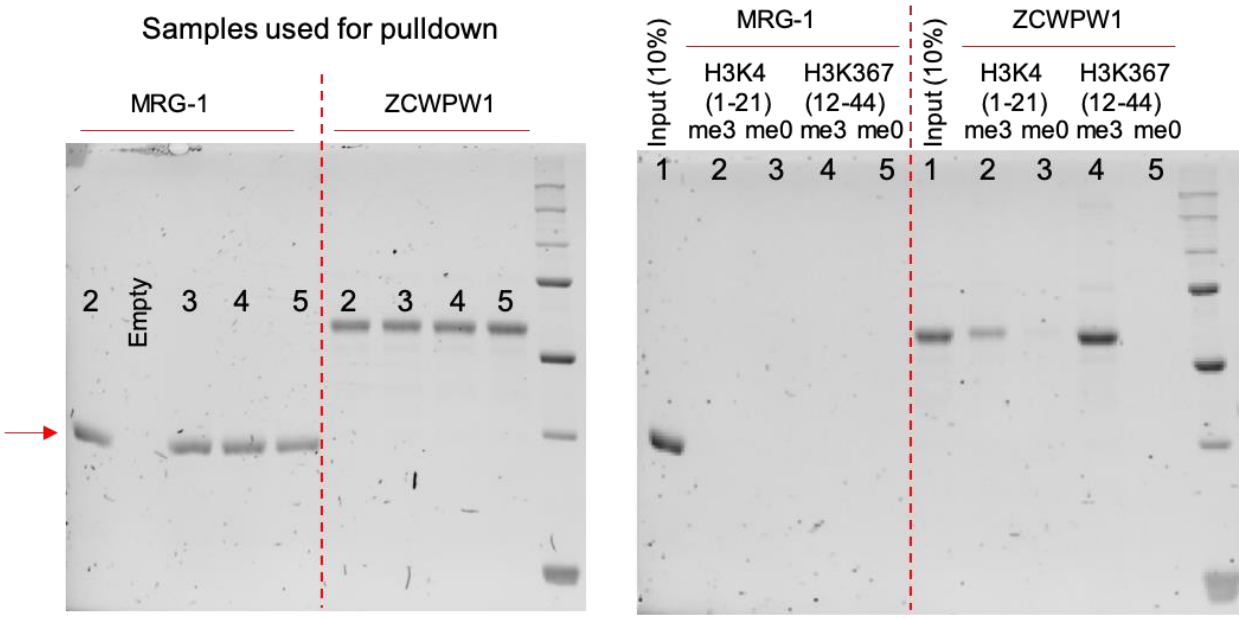


Figure 3.2. Wildtype MRG-1 Chromodomain peptide does not interact with H3K4me3 or H3K36me3.

The first 100 amino acids of wildtype MRG-1 were expressed in a GST-Tagged protein expression vector. After purifying GST::MRG-1, a histone peptide assay was performed. After pulling down with the listed modified histone peptides, samples were analyzed using Western Blot with anti-GST. The control, ZCWPW1, was detected after H3K4me3 and H3K36me3 immunoprecipitation. GST::MRG-1 was not detected in either samples.

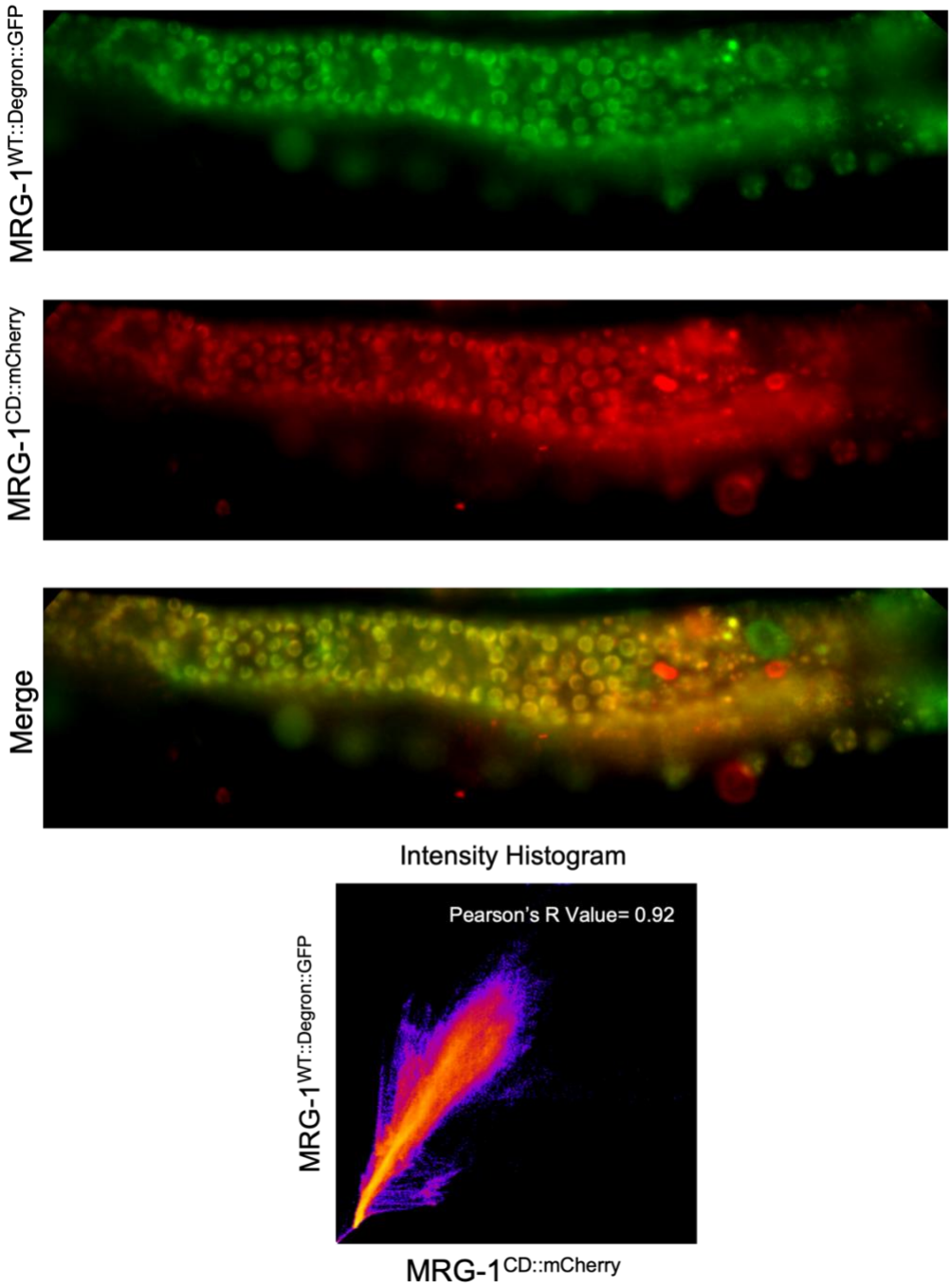


Figure 3.3 MRG-1CD::mcherry colocalizes with MRG-1WT::Degron::GFP in the germline.

Live images of *mrg-1*$^{CD/WT::Degron::GFP}$ worms were taken. MRG-1$^{CD:;mCherry}$ has the same localization pattern as MRG-1$^{WT::Degron::GFP}$ in the germline. Colocalization analysis was performed and resulted with a Pearson's R value of 0.92.

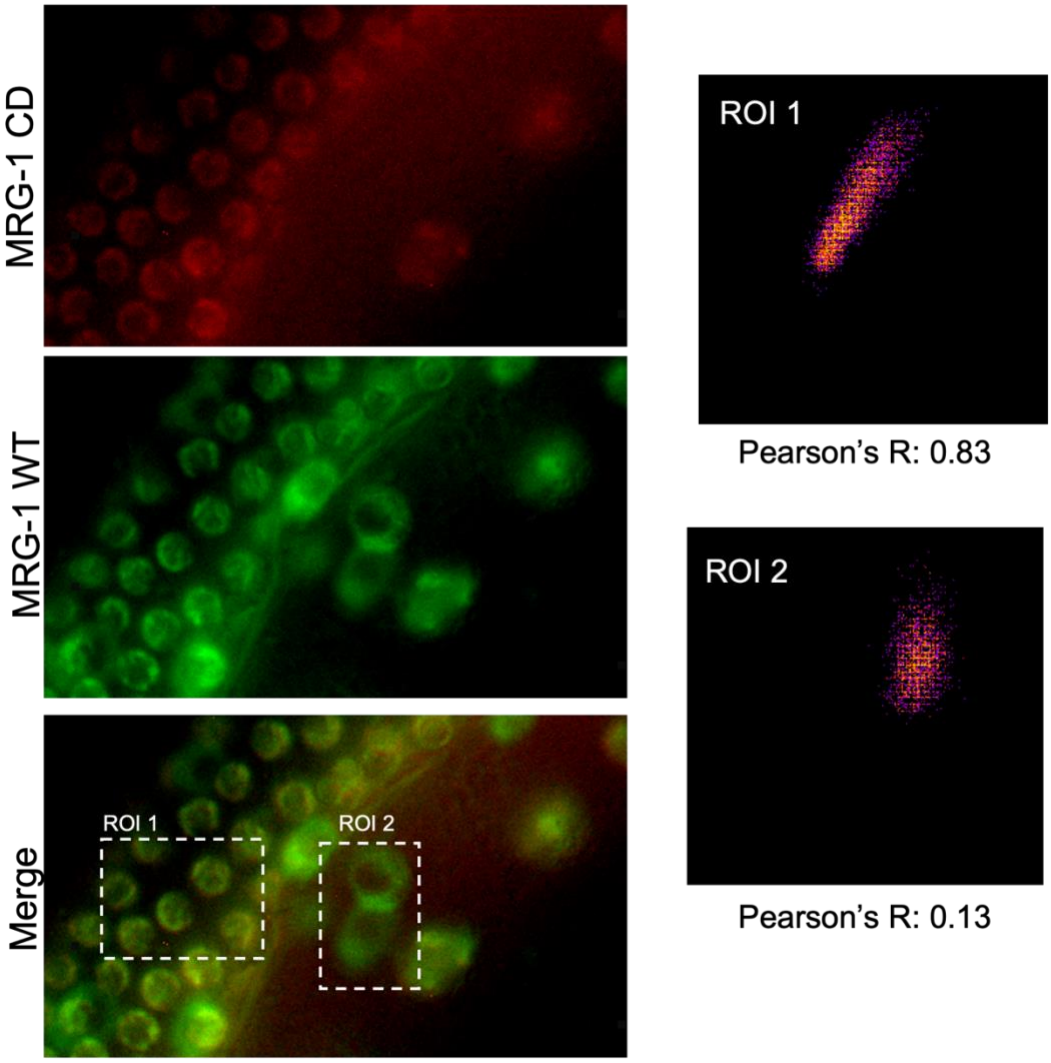


Figure 3.4 MRG-1$^{CD::mcherry}$ is excluded in gut somatic tissue.

Live images of *mrg-1*$^{CD/WT::Degron::GFP}$ worms were taken. MRG-1$^{CD:;mCherry}$ colocalizes with MRG-1$^{WT::Degron::GFP}$ in the germline with a Pearson's R value of 0.83 (ROI 1). MRG-1$^{CD:;mCherry}$ does not overlap with MRG-1$^{WT::Degron::GFP}$ in the somati gut nuclei (Pearson's R value: 0.13, ROI 2).

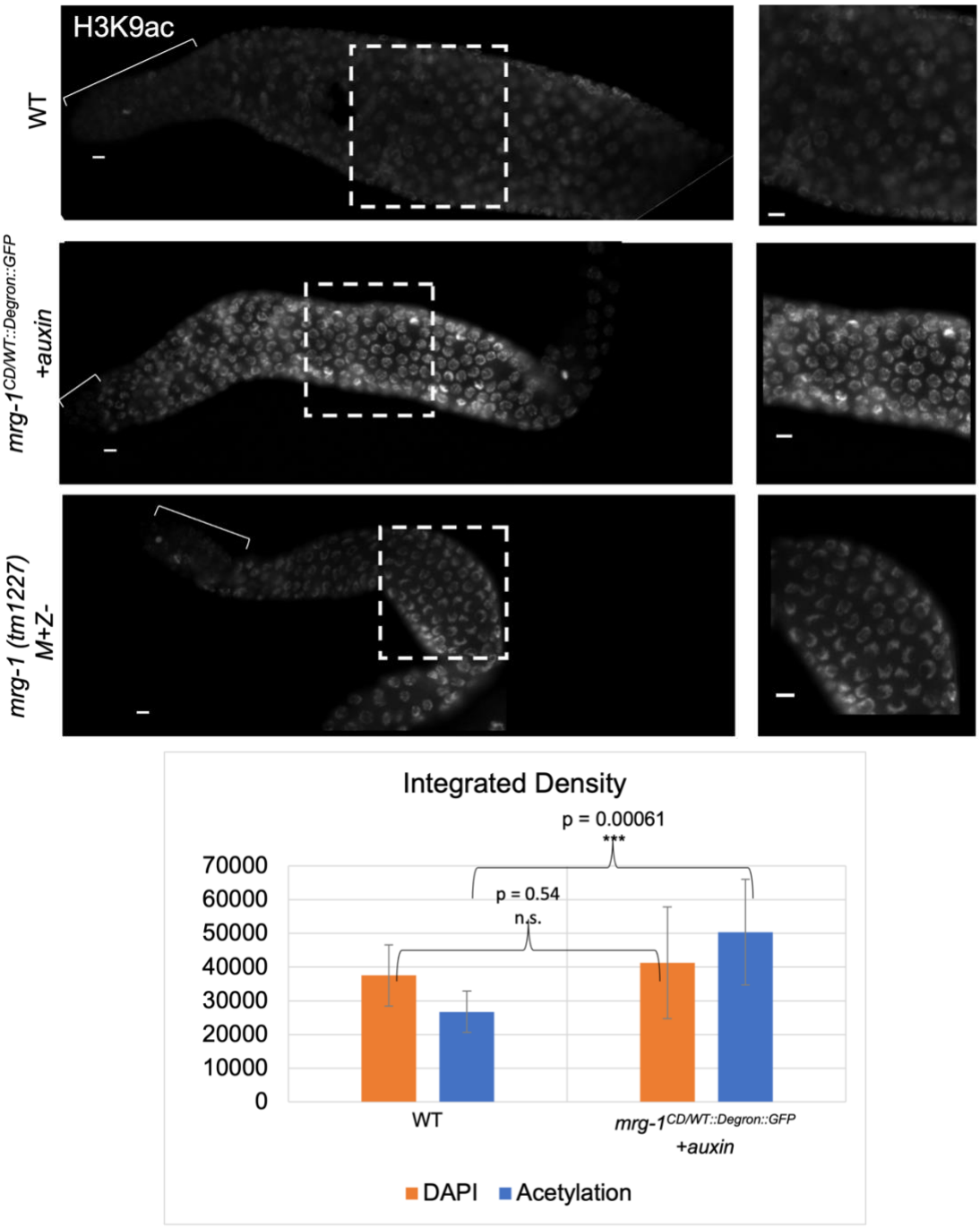
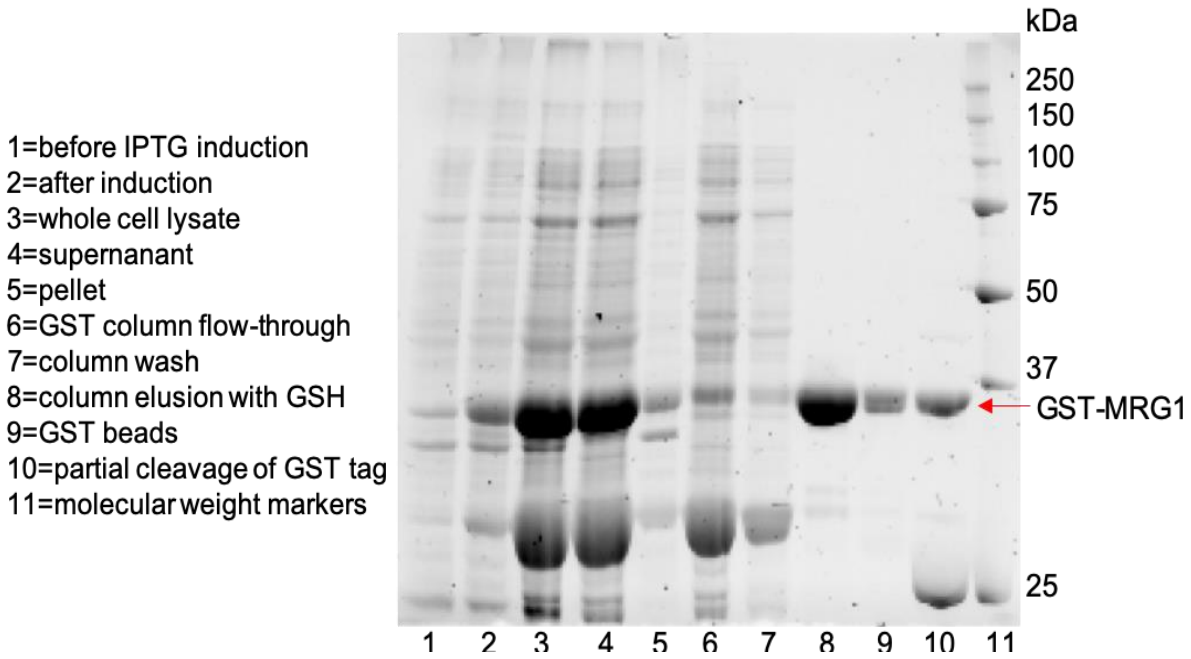
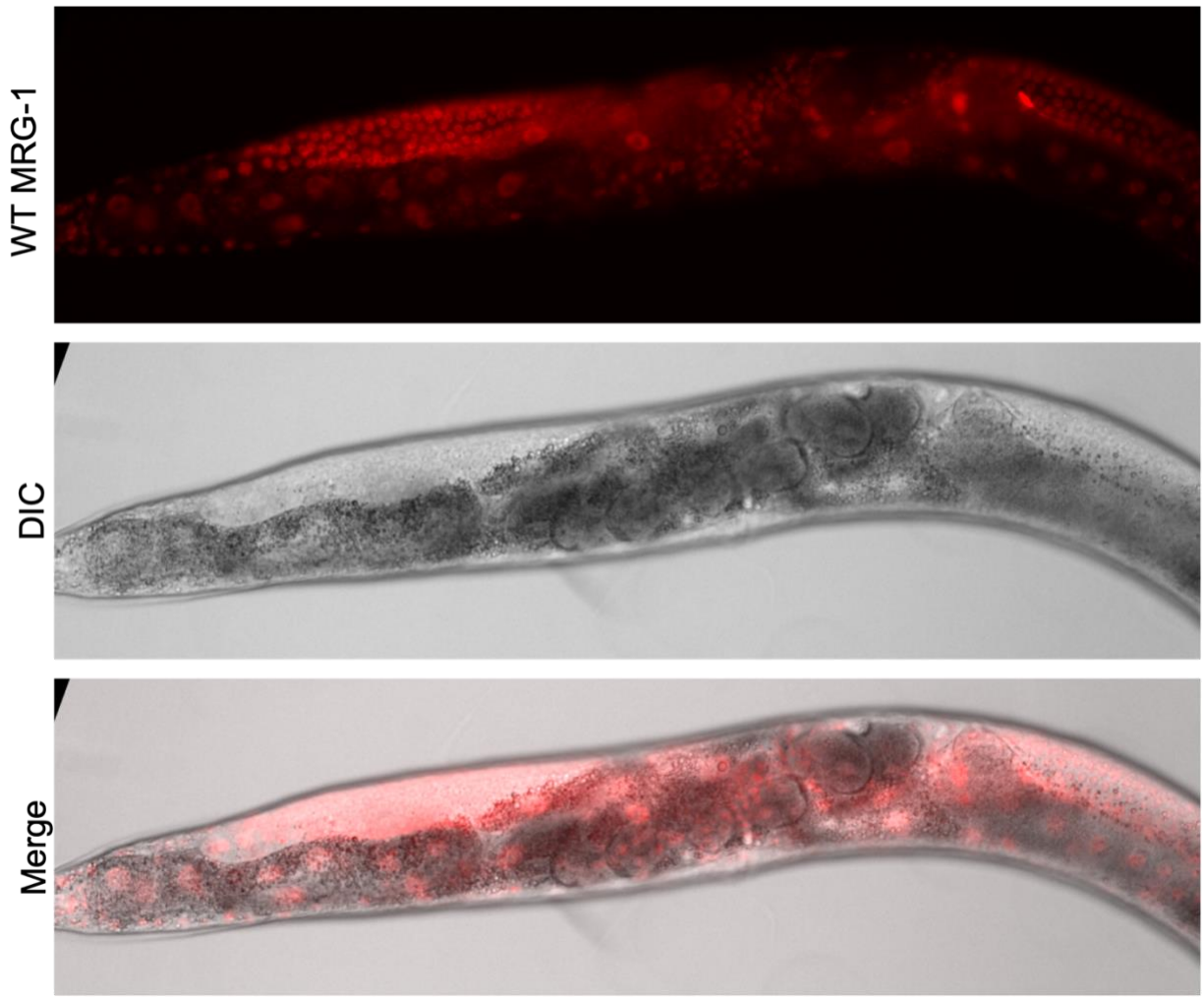


Figure 3.5. *mrg-1 CD* and *mrg-1* null mutants have increased levels of H3K9 acetylation. Wildtype, *mrg-1 CD,* and *mrg-1 (tm1227) M+Z-* adults were dissected and stained for H3K9acetylation. When compared to wildtype, *mrg-1 CD* and *mrg-1 (tm1227)*M+Z- germlines had increased levels of H3K9 acetylation in the meiotic region. Brightness was measured using integrated density in the late pachytene region (students t-test)



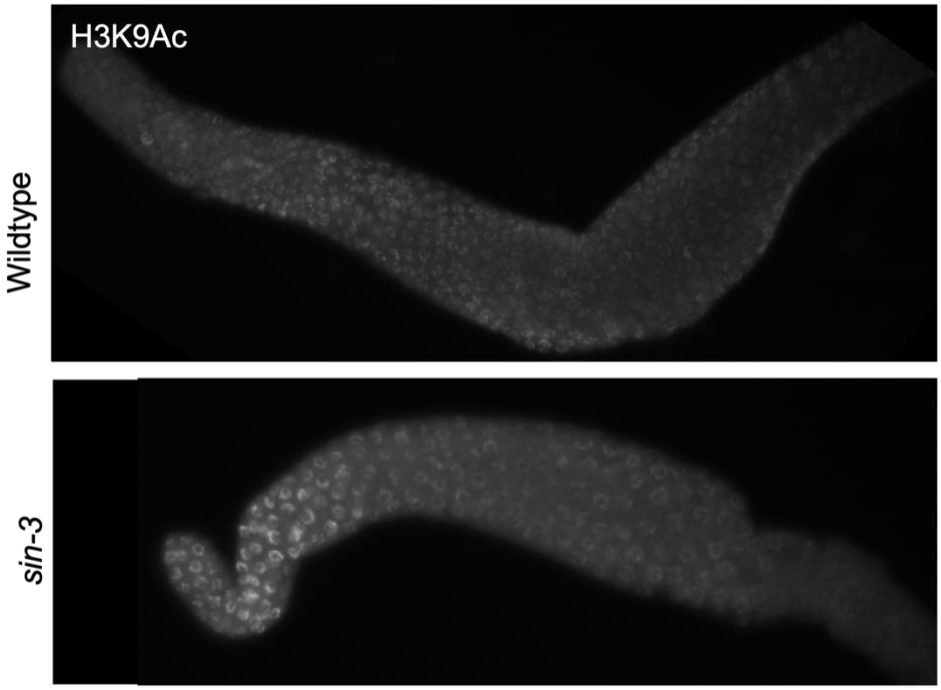
Supplemental Figure 3.1 Purification of N-terminally tagged GST MRG-1 chromodomain peptide.

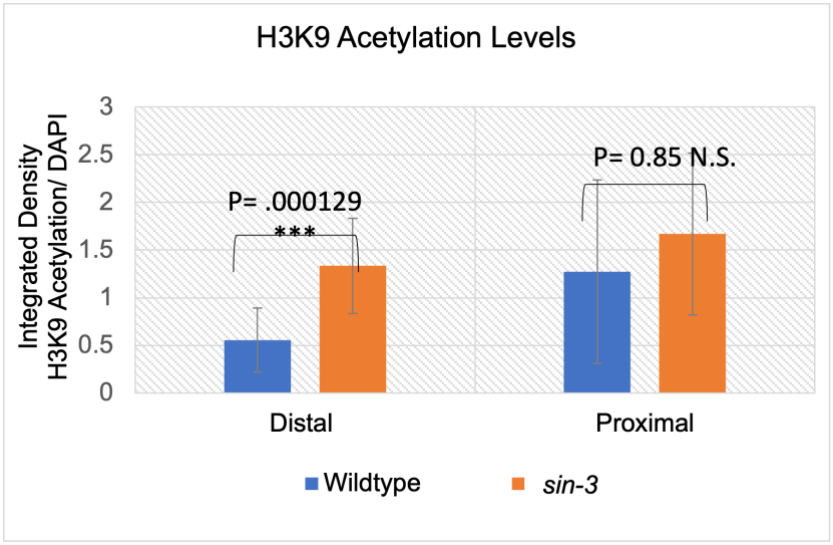
The first 100 amino acids of wildtype MRG-1 were cloned in to a protein expression vector that added an N-terminal GST tag. The GST::MRG-1 100 aa sequence was successfully expressed and purified using GST beads. The purified product was then used for histone peptide binding assay.



Supplemental Figure 3.2 Wildtype MRG-1::mcherry is found in the germline and soma.

Live images were taken of worms with the wildtype endogenous *mrg-1* tagged with mCherry. MRG-1::mcherry is clearly seen in both the germline and somatic tissues.





Supplemental Figure 3.3. sin-3 mutants have increased levels of H3K9 Acetylation in the distal region of the germline.

Wildtype and *mrg-1*$^{CD/WT::Degron::GFP}$ + auxin adults were dissected and stained for H3K9 acetylation in the germline. In comparison to the wildtype germlines, *mrg-1*$^{CD/WT::Degron::GFP}$ + auxin mutants had an increase in acetylation in the distal region of the germline. Brightness was quantified measuring integrated density (student's t-test).

CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Summary of Dissertation

In humans, MRG15 is plays an essential role in transcriptional regulation, genome integrity, and development (30, 53, 77-79, 97). Through its interactions with other chromatin modifying complexes such as HATs and HDACs, it contributes to both gene activation and repression (30, 53, 63). Some of these interactions and functions are dependent on the conserved chromodomain and its ability to bind to H3K36me3 (53). In *C. elegans*, MRG-1 is the homolog of MRG15 and contains a similar conserved chromodomain. Current research shows that MRG-1 functions in the germline and is necessary for successful meiotic progression and germline development (68, 71, 73, 84). However, the function of the MRG-1 chromodomain has never been examined. The goal of this dissertation was to further characterize the role of MRG-1 in the germline and identify the contributions of the MRG-1 conserved chromodomain in *C. elegans* development. With this research I provide evidence for novel, distinct roles for MRG-1 functioning in germline and somatic development.

In Chapter 2, I demonstrate that unlike *mrg-1* null mutants, mutations in the chromodomain result in embryonic lethality (Figure 2.5). Germlines that only have MRG-1 CD do not show any signs of meiotic defects or maternal effect sterility (Figures 2.6, 2.7). Additionally, a single copy of the *mrg-1 CD* allele produces a dominant RNAi resistance phenotype (Figure 2.9). These novel phenotypes are distinct from previously characterized phenotypes seen in *mrg-1* null mutants that are focused in the germline. By altering the MRG-1 chromodomain, I've shown that the MRG-1 chromodomain is essential for somatic development

during embryogenesis and proper RNAi-dependent gene silencing but does not function in the germline during meiosis.

In Chapter 3, I further characterize the interactions of MRG-1. MRG15 and its other homologs are known to dimerize and function in larger complexes (95). Using differentially tagged endogenous MRG-1 wildtype and altered chromodomain mutants, I show that wildtype MRG-1 does not appear to directly interact with itself *in vivo* despite strong subcellular colocalization in the germline and somatic tissues (Figures 3.1, 3.3). Therefore, unlike MRG15, MRG-1 does not appear to directly self-associate but may be a monomer within larger protein complexes. Similar to wildtype MRG-1, MRG-1 CD strongly colocalizes with wildtype MRG-1 in the germline despite showing no direct interaction *in vivo* (Figure 3.3). Interestingly, the colocalization between MRG-1 CD and wildtype MRG-1 disappears in the gut and other somatic nuclei in adult worms (Figure 3.4). This suggests that the MRG-1 chromodomain does not function in the localization or interaction of MRG-1 in the germline but is necessary in some somatic tissues. Additionally, I show that both *mrg-1 null* and *mrg-1 CD* mutants have an increased level of H3K9 acetylation in the meiotic region of the germline when compared to wildtype germlines (Figure 3.5). *sin-3* mutants have a similar increase in H3K9acetylation, but it is limited to the pre meiotic region of the germline (Supplemental Figure 3.2). Therefore, MRG-1 contributes to H3K9 acetylation regulation but possibly in a different mechanism separate from the SIN-3-containing HDAC complex in the germline.

4.2 MRG-1 potentially functions in various roles and complexes

Research has shown that human MRG15 functions in multiple chromatin related complexes including HATs and HDACs (30, 53). These interactions are dependent on

conformational changes that occur during MRG15 dimerization as well as its functioning chromodomain. The dimerization of ability of MRG15 is dependent on the MRG domain and is necessary for its interaction with PAM14, a component of the MAF1 complex (98). However, this dimer is disrupted when MRG15 interacts with Pf1, a component of the HDAC complex Rpd3S/Sin3S (81).

Although MRG-1 is highly conserved with MRG15, its chromodomain and dimerization capabilities have never been fully characterized. My data show that when mutating the MRG-1 chromodomain, novel phenotypes are produced that are distinct from mutants that lack any MRG-1 protein. Most notably, *mrg-1 CD* mutants are embryonic lethal and do not have any germline meiotic defects that are well characterized in *mrg-1 null* mutants. Visually, MRG-1 CD localization and its levels appear normal in the germline despite having an altered chromodomain. Therefore, MRG-1's function in the germline is not dependent on the chromodomain but possibly on other domains of MRG-1 that are still functional in the *mrg-1 cd* mutant. Interestingly, our data also demonstrate that neither wildtype nor MRG-1 CD directly interact with one another *in vivo.* This suggests that the MRG-1 is functioning a larger complex and this interaction is not dependent on the MRG-1 chromodomain and may explain why MRG-1 CD mutants do not exhibit any meiotic defects. If MRG-1 is a necessary component of larger chromatin complex, the complete lack of MRG-1 may disrupt multiple essential mechanisms required for meiotic progression in the germline. However, because MRG-1 CD is still present in the germline and has the potential to interact with other proteins through its MRG domain, proper meiotic progression may occur.

The embryonic lethal *mrg-1 CD* mutants demonstrate that a functioning chromodomain is essential for MRG-1 during embryogenesis and suggest a novel function for MRG-1 during

somatic development. My data also show that a single copy of the *mrg-1 CD* allele causes a dominant gain of function RNAi defect that is not seen in *mrg-1 null* mutants. Interestingly, these phenotypes are very similar to those seen in SINS3 complex mutants, such as *sin-3* and *hda-1* mutants (67, 89). Recently, MRG-1 was identified as an interactor of the SINS3 complex (65). The phenotype similarities between *mrg-1 CD* and SINS3 complex mutants suggest that the MRG-1 chromodomain is necessary for the function of the SINS3 complex in somatic tissues. Therefore, the MRG-1 chromodomain may be necessary for the localization of the SINS3 in somatic tissues such that when mutating the chromodomain the chromatin functions of the SINS3 complex are disrupted causing embryogenesis defects. Additionally, the lack of MRG-1 CD in somatic gut nuclei in adult worms may further suggest that the chromodomain is necessary for MRG-1's stability in somatic tissue. Sumoylation of HDA-1 alters its interaction with MRG-1 and the sumoylation status is dependent on its expression in the germline or soma (67). Although further examination needs to be done, perhaps the MRG-1 chromodomain regulates its interactions with other chromatin complexes in somatic tissue. Interestingly, both *mrg-1 null* and *mrg-1 CD* mutants show signs of defective histone acetylation in the germline that is distinct from *sin-3* H3K9 acetylation patterns. Therefore, MRG-1 may be contributing to a different histone acetylation mechanism in the germline separate of SIN-3.

Although this research discovered novel phenotypes for MRG-1 that are similar to other chromatin complexes, further research needs to be done characterize the function of the MRG-1 chromodomain and its role in chromatin remodeling complexes. When disrupting chromatin related proteins and consequently altering histone modification patterns, it is necessary to characterize expression profiles of different genes. One of the novel phenotypes observed in *mrg-1 CD* mutants was a dominant RNAi defect phenotype. By performing RNA-sequencing on

wildtype, *mrg-1 CD,* and *mrg-1 null* mutants, we would identify if there were any downstream transcriptional changes in the RNAi process pathway that would contribute to the dominant RNAi defect pathway. Additionally, we would be able to identify any differentially expressed genes related to development or other essential pathways. We would compare expression profiles to that of SINS3 complex mutants and identify any similarities or differences that would lead to the discovered phenotypes. To further characterize MRG-1 and the role of the MRG-1 chromodomain as an interactor of the SINS3 complex, mass spectrometry should be performed on proteins co-purifying with the MRG-1 CD and compared to wildtype. Previous mass-spec data have demonstrated that MRG-1 interacts with other chromatin related proteins in including SIN-3. By performing mass spectrometry on MRG-1 CD we would confirm which interactions are disrupted by altering the chromodomain. This would determine if the MRG-1 chromodomain is necessary for MRG-1's function with other chromatin related complexes.

4.3 Identifying specific histone residues that interact with MRG-1 Chromodomain

Studies have shown the MRG15 interacts with H3K36me through its conserved chromodomain (57). This binding of the H3K36me to the chromodomain is conserved in other MRG15 homologs. Other research has shown through Chromatin Immunoprecipitation Sequencing (ChIP Seq) analysis, that MRG-1 in *C. elegans* has a chromatin binding pattern that is identical to H3K36me3 (66). Therefore it has been predicted that MRG-1, like MRG15, binds directly to H3K36me3 through its conserved chromodomain. However, a direct physical interaction between the two has never been identified and characterized. During our examination of *mrg-1 CD* mutants, I sought to characterize the interaction between the MRG-1 chromodomain and H3K36me3. I cloned the first 100 amino acids of the MRG-1, including the

chromodomain, into a protein expression plasmid that tagged MRG-1 with GST. Our collaborators in the Cheng Lab then purified and expressed the GST::MRG-1 Chromodomain peptide and performed a histone peptide binding assay with H3K36me. They determined that a tagged wildtype MRG-1 Chromodomain peptide does not interact with an H3K36me3 peptide *in vitro*. This result may be due to improper binding conditions using partial peptides. Similar experiments with other chromatin binding proteins sometimes require a full nucleosome for proper interaction between the chromodomain and modified histone. Additionally, the MRG-1 chromodomain may interact with another histone modification that has a similar chromatin profile. In addition to H3K36me3, MRG-1 has a similar *C. elegans* genomic ChIP-Seq profile to H3K4me. Like H3K36me3, H3K4me and other histone modifications are associated with euchromatin and active transcription. However, similar to H3K36me, no binding between the MRG-1 chromodomain and H3K4me was detected. Therefore, the similar ChIP-Seq profiles between MRG-1 and H3K36me3 may be an indirect consequence of MRG-1 localizing to sites of overall active transcription through its interaction with other histone modifications associated with active transcription.

In order to gain a better understanding of the MRG-1 chromodomain and its histone modification interactions, a ChIP-Seq analysis of MRG-1 needs to be performed in *mrg-1 CD* mutants and compared to wildtype to determine if there is any mislocalization of MRG-1 after disrupting the chromodomain. A similar experiment for ChIP-Seq analysis for MRG-1 in *met-1;mes-4* RNAi mutants would be necessary to indicate if MRG-1 is mislocalized after depleting H3K36me3. With these experiments, we would be able to characterize the contributions of the MRG-1 chromodomain as well as the role H3K36me3 plays in the localization of MRG-1 to chromatin. Although ChIP-Seq analysis will indicate if MRG-1 localization is influenced by

alterations in the chromodomain, it does not demonstrate if there is a direct interaction between the chromodomain and H3K36me3. Therefore, an additional histone binding assay needs to be done. Our preliminary results suggest that complete MRG-1 or a whole modified nucleosome binding assay needs to be performed instead of partial peptides. A similar assay with MRG-1 CD would need to be completed to confirm if the modified histone interaction is dependent on the chromodomain. If binding is successful using whole protein MRG-1, then an additional histone binding assay using a modified histone peptide array could be performed to identify any other potential histone modification interactions. These results would give a comprehensive understanding on the contributions of the MRG-1 chromodomain and determine which specific histone modifications contribute to the localization of MRG-1 to chromatin.

4.4 Final thoughts and additional experiments

Through the research in this dissertation, I further characterized the role of chromodomain containing protein MRG-1. Specifically, I discovered novel phenotypes in mutants that have a disrupted chromodomain that are distinct from previously characterized *mrg-1 null* mutant phenotypes. Mutants that lack any MRG-1 protein, exhibit germline meiotic defects but still produce viable, sterile offspring. However, when mutating the MRG-1 chromodomain embryonic lethality occurs identifying a new function for MRG-1 in somatic embryogenesis. Additionally, I demonstrate that *mrg-1 CD* mutant germlines do not exhibit any meiotic defects suggesting that the chromodomain does not function in the germline. Mutations in the chromodomain also does not disrupt MRG-1's localization in the germline but excludes it from somatic gut nuclei. These data demonstrate that MRG-1 functions separately between the germline and somatic tissue. Preliminary data also suggest that MRG-1 may contribute to histone

acetylation regulation potentially through its interactions with SINS3 complex. These newly characterized phenotypes reveal that MRG-1's role in germline and somatic development is complex and dependent on the chromodomain.

Although additional research needs to be done to completely understand MRG-1 and its function in larger chromatin related complexes, this work in this dissertation is significant because of its identification of distinct roles for MRG-1 in germline and somatic development. By further characterizing the role of MRG-1 and the conserved chromodomain, we gain a better understanding of the role chromatin modifying proteins play in regulating chromatin structure and ultimately development.

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