Roles for Condensin in *C. elegans* Chromosome Dynamics

by

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Dedicated to my family

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With the exception of figures and tables listed below, all work is this dissertation is my own.

Table 2.1: DPY-27 IP – Györgyi Csankovszki,
SMC-4, KLE-2 and KLP-7 IPs – Hagstrom Lab

Figure 2.3 and Table 2.2: Csankovszki Lab

Figure 2.9, 2.10B: Hagstrom Lab

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Abstract

Condensin complexes are essential for higher order organization of chromosome structure. Higher eukaryotes have two condensins (condensin I and II) dedicated to mitotic and meiotic chromosome dynamics. *C. elegans* was thought to be an anomaly, with only a single mitotic condensin (condensin II), and one specialized for dosage compensation (condensin I^{DC}). Condensin I^{DC} binds both X chromosomes in hermaphrodites to down regulate gene expression by half, equalizing X-linked gene product in males (XO) and hermaphrodites (XX), while condensin II is essential for efficient chromosome organization and segregation during mitosis and meiosis. It was proposed that the unusual holocentric chromosomes in *C. elegans* did not require condensin I and II to accomplish cell division, and therefore, condensin I was customized for X chromosome regulation. However, we showed that subunits from condensin I^{DC} and condensin II interact to form a second mitotic/meiotic condensin, the bonafide *C. elegans* condensin I. Our findings raise *C. elegans* to a unique status, with three distinct condensins controlling holocentric chromosome dynamics.

Condensin I and II have distinct localization patterns on mitotic and meiotic chromosomes, suggesting that their roles in chromosome organization may be distinct. During mitosis, condensin II colocalizes with the centromere while condensin I discontinuously coats chromosomes. Condensin I, but not II, colocalizes with aurora B kinase, AIR-2, and our data suggests that in mitosis, AIR-2 activity is required for the recruitment of condensin I, but not condensin II. In meiosis, condensin II localizes to the sister chromatid core, while condensin I localizes to the interface between homologous chromosomes. Condensin I and AIR-2 colocalize at this interface. Similar to mitosis, in AIR-2 depleted animals undergoing meiosis, condensin II is not affected but condensin I mislocalizes to the interface between sister chromatids, as well as homologous

chromosomes. This work indicates that AIR-2 provides important spatial cues for condensin I localization on meiotic chromosomes.

The contribution of condensin I to during mitosis and meiosis is not well-defined. A comparative analysis of chromosome organization and mitotic/ meiotic progression between wildtype and condensin I - depleted animals will provide a better understanding of condensin I function in chromosome dynamics during cell division.

Chapter 1

Introduction

During mitosis and meiosis, DNA, arranged into chromatin fibers by histone proteins, is transmitted from cell to cell and parent to offspring respectively. The condensin complex facilitates the accurate transfer of genetic information by reorganizing interphase chromatin into compact chromosomes at the onset of cell division. The focus of this dissertation is the discovery, regulation and function of a novel condensin complex in *C. elegans*, **condensin I**. This chapter introduces condensin and the Chromosome Passenger Complex (CPC), two key players in the regulation of mitotic/ meiotic events, and discusses their role in preserving the fidelity of cell division. Three condensin complexes control chromosome dynamics in *C. elegans*. Collaboration between the Csankovszki and Hagstrom (UMass Worcester) labs has provided a comprehensive description of condensin proteins in this organism, presented in Chapter 2. The chromosome passenger kinase, aurora B, controls the association of *C. elegans* condensin I with mitotic and meiotic chromosomes, strengthening a conserved link between two critical influences on chromosome behavior. This work is presented in Chapter 3. Chapter 4 provides concluding remarks and some ideas about the future direction of this project.

Mitosis and meiosis proceed through similar stages, but are fundamentally different. In both processes, chromosomes undergo condensation, capture and alignment by microtubules of the spindle, and segregation to daughter nuclei, followed by cytoplasmic division or cytokinesis. However, mitosis and meiosis accomplish very different goals. The purpose of mitosis is to increase cell number for growth, maintenance and repair of tissues and organs. Two identical daughter cells arise from the original parent cell. In mitosis, one round of DNA replication precedes one cell division. Each replicated

chromosome consists of a pair of identical sister chromatids, one chromatid of which segregates to each daughter nucleus, maintaining chromosome number. Meiosis, by contrast, halves the chromosome number of a diploid germ cell, and generates haploid gametes for fertilization. A single round of DNA replication is followed by two cell divisions. Homologous chromosomes separate during meiosis I and sister chromatids separate during meiosis II. A brief description of chromosome dynamics during mitosis and meiosis is presented next.

I. Chromosome dynamics during mitosis and meiosis

I. A. Mitotic chromosome dynamics

DNA replication and the establishment of cohesion between replicated DNA molecules are the initial events of cell proliferation (Watrin and Legagneux, 2003). Replication is simultaneously initiated at numerous chromatin locations called origins of replication. From each origin, two replication forks proceed in opposite directions along the chromatin fiber. As each chromosome is duplicated into identical sister chromatids, cohesion is established between sisters, which is essential for their accurate segregation to daughter nuclei in the following mitosis. Sister chromatids are held together by cohesin, a complex related to condensin (Hirano, 2002; Jessberger, 2002). Cohesin likely facilitates cohesion by creating a ring around two DNA molecules (Haering et al., 2002; Nasmyth, 2002). Replication and establishment of cohesion occur during interphase, immediately before mitosis. Condensin aids the faithful transfer of DNA into daughter cells by packaging interphase chromatin into compact, precisely organized chromosomes (Nasmyth, 2002; Swedlow and Hirano, 2003). Condensin-dependent compaction during prophase and prometaphase aids the decatenation of chromatin fibers that get intertwined during replication (Holmes and Cozzarelli, 2000). In comparison to dispersed chromatin fibers, compact chromosomes are more efficiently transported to opposite poles of a dividing cell, without incurring damage during cytokinesis (Swedlow and Hirano, 2003).

Correct metaphase orientation of chromosomes on the mitotic spindle and carefully regulated cohesin removal are crucial for error-free segregation. Orientation is controlled at the centromere-kinetochore region of chromosomes. The multisubunit kinetochore

assembles on the centromere, and interacts directly with spindle microtubules. An *amphitelic* attachment, with two sister kinetochores arranged back-to-back forming attachments to microtubules from opposite poles, is ideal. In this type of attachment, sister chromatids kinetochores face away from each other, and are said to be bioriented (Figure 1.1). Kinetochore- microtubule connections occur via trial and error, and three types of incorrect attachments may form as spindle fibers search for available kinetochore sites. A *monotelic* attachment is one in which a sister chromatid kinetochore becomes attached to microtubules from one pole while the other sister kinetochore remains unattached. In a *syntelic* attachment, both sister kinetochores are attached to microtubules from a single pole, and a *merotelic* attachment is one in which a single sister kinetochore is attached to microtubules from both spindle poles (Figure 1.2) (Cimini, 2007).

Two-step dissolution of sister chromatid cohesion (Waizenegger et al., 2000) is necessary for accurate chromosome orientation and segregation. The majority of cohesin is removed from sister chromatid arms during prophase and prometaphase, resulting in chromatid individualization. Centromeric cohesion is preserved at this time, to prevent untimely separation of sister chromatids. At metaphase, strong tension is exerted on the kinetochores of bioriented chromatids, which is necessary to stabilize the kinetochore-microtubule connection. Cohesin aids the accurate biorientation of chromatids by contributing to kinetochore tension, which is generated when kinetochore-associated microtubules pull sister chromatids toward opposite poles, but poleward forces are countered by cohesin- dependent cohesion between chromatids at the centromere.
At anaphase onset, the protease separase destroys cohesin linkages from centromeres, and triggers sister chromatid separation (Figure 1.3) (Nasmyth, 2002; Petronczki et al., 2003).

Cytokinesis is the final step in cell division. A contractile ring or cleavage furrow, composed of actin and myosin filaments, assembles at a location dictated by the anaphase spindle, and ingresses. When furrow ingression completes, the cells are still connected by a cytoplasmic remnant, the midbody. The resolution of the plasma membrane, called abscission, completes cytokinesis to give rise to two distinct cells (Glotzer, 2005).

The events described above pertain to monocentric chromosomes, on which the centromere is restricted to one specific location. On holocentric chromosomes like those of *C. elegans*, mitosis proceeds in a similar manner, but the mitotic centromere/ kinetochore region extends along the entire length of the chromosomes (Figure 1.4) and spindle microtubules attach to all sites on the diffuse kinetochore (Figure 1.6). Similar to monocentric sister kinetochores, the holocentric kinetochores of replicated sister chromatids are arranged in a back-to-back manner to facilitate biorientation and accurate chromosome segregation during mitosis (Maddox et al., 2004).

I. B. Meiotic chromosome dynamics

The purpose of meiosis is to generate haploid gametes from diploid germ cells (Petronczki et al., 2003). Similar to mitosis, meiosis is immediately preceded by DNA replication. A bivalent forms when replicated homologous chromosomes, each with two chromatids, associate. The four chromatids in each bivalent have to be distributed into four haploid nuclei. Homologous chromosomes are separated during the first meiotic division, which halves chromosome number and is referred to as the reductional division. The second or equational meiotic division, is analogous to mitosis, and separates sister chromatids.

In order to ensure accurate segregation of homologous chromosomes during the first division, physical connections must be created between the maternal and paternal chromosome in each pair of homologs. These linkages are key to the establishment of tension, which in turn is necessary for correct biorientation of homologous chromosomes on the metaphase I spindle. Conveniently, genetic information is reciprocally exchanged between non- sister chromatids of homologous chromosomes during meiosis I, and cohesin surrounding the site of exchange (crossover) creates linkages between homologous chromosomes, along chromosome arms. Before the maternal and paternal chromosomes exchange genetic material, they must pair and synapse. The Synaptonemal Complex (SC) is responsible for pairing and synapsis of homologous chromosomes, during the leptotene/ zygotene stage of meiotic prophase I. The recombination process starts at this stage with the production of double strand breaks (DSBs). During pachytene,

full (SC-dependent) synapsis and reciprocal exchange between maternal and paternal chromosomes has occurred. During diplotene, the recombination process is completed, the SC disassembles and chromosomes desynapse, but are now linked by cohesin, because it remains, for example, between the maternal chromatid that participated in genetic exchange, and the section of maternal chromatid that was exchanged and is now part of the paternal chromosome. Chromosome condensation, aided by condensin, initiates during diplotene, and continues into diakinesis.

In a perfect meiosis, homologous chromosomes biorient at metaphase I and separate at anaphase I while sister chromatids only biorient and separate at metaphase II and anaphase II respectively (Figure 1.1). Kinetochores on homologous chromosomes must be bioriented and sister kinetochores must be cooriented (point to the same pole) at metaphase I. Thus, tension must be generated between kinetochores of homologous chromosomes, rather than between sister chromatid kinetochores. Tension arises when homolog kinetochores are pulled in opposite directions by the spindle, countered by cross over –dependent linkages between homologous chromosomes. Sequential loss of cohesin also ensures that homologous chromosomes separate at meiosis I while sister chromatids remain together until meiosis II (Figure 1.5). At anaphase I, cohesin along chromosome arms is removed. This ensures that homologous chromosomes are no longer tethered by cohesin around the site of crossover, and can migrate to opposite poles. Conversely, cohesin at the centromere is preserved, keeping sisters together. During prophase II, sister chromatids undergo condensation, biorient on the metaphase II spindle, and when centromeric cohesin is cleaved by separase at the onset of anaphase II, sister chromatids migrate towards opposite spindle poles. On monocentric chromosomes, the critical meiotic events of orientation and cohesin removal are coordinated around the discrete centromere/ kinetochore (Figure 1.6), but on the holocentric chromosomes in *C. elegans*, these events have to be executed in a very different manner (discussed in the next section).

During oocyte meiosis, only one haploid nucleus (out of four) forms a gamete. Polar bodies are formed as by-products of oocyte meiosis. One set of homologous

chromosomes (first polar body, Meiosis I) and one set of sister chromatids (second polar body, Meiosis II) are discarded from the fertilized embryo as meiosis progresses. By contrast, during spermatogenesis, all four haploid progeny form sperm.

I.C. Chromosome dynamics in *C. elegans* meiosis

Reviewed by (Schvarzstein et al., 2010)

Similar to monocentric meioses, holocentric chromosomes in *C. elegans* undergo all the classic stages of meiotic prophase described above (Albertson et al., 1997). The phenomenon of crossover interference (each pair of homologous chromosomes participates in only one crossover event) is displayed in *C. elegans* meiosis (Hillers and Villeneuve, 2003). The single crossover occurs in an off-center position, typically in the terminal one-third of the chromosome (Barnes et al., 1995). The crossover drives asymmetric disassembly of the synaptonemal complex (SC), initiating a sequence of events that ultimately creates two distinct domains on the bivalent, the short and long arm (Chan et al., 2004; Nabeshima et al., 2005). The region extending from the site of the crossover to the nearer chromosome end is the future short arm of the bivalent, while the region from the crossover to the farther chromosome end is destined to be the long arm of the bivalent (Albertson et al., 1997).

The precise, crossover –dependent differentiation of *C. elegans* bivalents into short and long arm domains facilitates correct chromosome orientation and orderly loss of cohesin during meiosis (Figures 1.7 and 1.8). At metaphase I, the homologous chromosomes that comprise a bivalent are arranged in an end-to-end fashion, oriented in opposing directions, with the long arm of the bivalent parallel to spindle microtubules (Albertson and Thomson, 1993; Wignall and Villeneuve, 2009). Microtubule bundles run along the sides of the bivalent (Figure 1.6), and ensheathment of the bivalent in this manner likely places spatial restrictions on the chromosomes, thereby promoting their biorientation (homologs in meiosis I and chromatids in meiosis II) (Wignall and Villeneuve, 2009). During meiosis, kinetochore assembly is uncoupled from centromeric chromatin (Monen et al., 2005). Instead, the kinetochore assembles around the bivalent in an oval structure, interrupted by a gap at the short arm (Figure 1.6). This cup-like configuration of the

kinetochore envelops a pair of sister chromatids and likely aids their coorientation in meiosis I (Dumont et al., 2010).

Cohesin removal is regulated at the short and long arms of the bivalent. The long arm of the bivalent represents cohesion between sisters, akin to centromeric cohesion in monocentric organisms. Conversely, the short arm is the region of contact between homologs. At this interface, cohesion between sister chromatid segments that are reciprocally exchanged during crossover holds homologs together (Albertson et al., 1997; Chan et al., 2003; Pasierbek et al., 2001). The short arm will be aligned at the metaphase plate at meiosis I, and cohesin is removed from the short arm to allow efficient separation of homologous chromosomes (Kaitna et al., 2002; Rogers et al., 2002). Although the meiotic kinetochore in *C. elegans* oocytes is essential for orientation of chromosomes, once appropriate orientation is achieved, kinetochores become dispensable for chromosome segregation. The spindle forms between separating chromosomes, and likely provides the outward pushing force needed for segregation (Dumont et al., 2010). During meiosis II, inter-chromatid cohesin is degraded on the long arm, sister chromatids biorient, and separate.

II. Condensin

II. A. Condensin – discovery, subunits and architecture

The versatile Structural Maintenance of Chromosome (SMC) protein family wields a powerful influence on chromosome behavior during mitosis and meiosis. There are six SMC subcategories, SMC1 – 6. Condensin consists of an SMC2-SMC4 dimer, SMC1-SMC3 form the core of the related cohesin complex, which maintains cohesion between sister chromatids during mitosis and meiosis, while SMC5-SMC6 is important for DNA repair (Hirano, 2002; Jessberger, 2002; Wood et al., 2010). Altogether, the multifaceted SMC family regulates critical aspects of chromosome architecture. SMC proteins are structurally unique. The N and C termini together form an ATP binding and hydrolysis domain. Between the termini are two helical motifs separated by a flexible hinge domain. At the hinge domain, the protein folds back on itself and the helices associate in an

antiparallel manner creating a coiled - coil region. Eukaryotic SMC proteins dimerize via the hinge region (Hirano, 2006).

The SMC2-SMC4 dimer is essential for the formation and maintenance of compact mitotic and meiotic chromosomes. SMC2 and SMC4 were abundantly isolated from *Xenopus* mitotic chromosomes assembled *in vitro*. Depletion of SMC2 or SMC4 inhibited the formation of condensed mitotic chromosomes, and chromosome structure unraveled when SMC2/4 were depleted after condensation was complete (Hirano and Mitchison, 1994). Further studies showed that SMC2 and SMC4 exist as part of a larger complex with CAPs -D2, G and H (Chromosome Associated Polypeptide). The complex (1:1:1:1:1 stoichiometry) was named 'condensin' for its important role in DNA condensation (Figure 1.9) (Hirano et al., 1997). Studies in budding and fission yeast also identified SMC (Saka et al., 1994; Strunnikov et al., 1995) and CAP (Lavoie et al., 2002, 2004; Lavoie et al., 2000; Sutani et al., 1999) subunits of condensin. An increase in the distance between two loci (labeled by FISH) on chromosome arms was observed in condensin-depleted nuclei, indicating that these proteins are critical for chromosome condensation (Freeman et al., 2000; Lavoie et al., 2000; Saka et al., 1994; Strunnikov et al., 1995). DPY-27, an unusual SMC4 protein in *C. elegans,* was also discovered in the mid 90's. DPY-27 associates with X chromosomes in the hermaphrodite sex, and dampens their expression. This finding reveals a mechanistic link between chromosome-wide gene regulation and mitotic chromosome assembly (Chuang et al., 1994).

The condensin complex is conserved from yeast to humans (Hudson et al., 2009). In higher eukaryotes, two condensins, Condensin I and II, cooperate to organize and segregate the genome (Chan et al., 2004; Csankovszki et al., 2009; Hagstrom et al., 2002; Ono et al., 2003). Condensin I proteins are homologs of the condensin complex that was initially identified in *Xenopus*, and the single yeast condensin is a condensin I complex. Condensin I and II in any given organism have identical SMC proteins, and distinct, yet similar CAP proteins. CAP proteins form a subcomplex that regulates SMC activity (Kimura and Hirano, 2000). Condensin I CAP proteins are CAP-G, D2 and H; condensin II CAPs are CAP-G2, H2 and D3. CAP-G/ G2 and CAP-D2/D3 contain clusters of

HEAT repeats, motifs that are implicated in protein – protein interactions (Neuwald and Hirano, 2000), while CAP-H/H2 belongs to the recently described Kleisin (closure) family (Schleiffer et al., 2003).

Condensin assumes a 'pseudo symmetrical' organization (Figure 1.9). Each half of the complex consists of an SMC subunit, half a kleisin molecule, and one HEAT protein. SMC2 and 4 are tightly associated with each other, and both associate with the kleisin protein CAP-H/ H2. CAP-H/ H2 also interacts with both HEAT repeat-containing proteins, CAP-D2/D3 and CAP-G/G2. The interaction between CAP-D2/D3 and CAP-G/G2 is negligible in the absence of CAP-H/ H2. Thus, in accordance with its name, the CAP-H/ H2 kleisin creates a 'closed' condensin molecule (Onn et al., 2007).

Condensin I and II associate with chromosomes independently (Gerlich et al., 2006; Ono et al., 2003), but within each condensin complex, subunits are interdependent for stability and /or chromatin association [Chapter 3 and (Cobbe et al., 2006; Coelho et al., 2003; Hagstrom et al., 2002; Hirota et al., 2004; Oliveira et al., 2005; Ono et al., 2003; Savvidou et al., 2005; Steffensen et al., 2001; Vagnarelli et al., 2006; Watrin and Legagneux, 2005)]. Interestingly, *C. elegans* condensin II displays different loading requirements during mitosis and meiosis. The SMC2 protein (MIX-1) and CAP-D3 protein (HCP-6) are independent during mitosis, but in meiosis, MIX-1 requires HCP-6 for efficient binding to chromosomes (Chan et al., 2004).

II.B. Condensin complexes in *C. elegans*

Chapter 2 is a report of the comprehensive analysis of *C. elegans* condensin complexes performed in the Csankovszki and Hagstrom (UMass Worcester) labs. Two condensins were previously identified in *C. elegans*. Condensin II is essential for the accurate assembly and segregation of mitotic and meiotic chromosomes; condensin I^{DC} regulates X chromosome gene expression. As mentioned previously, the SMC4 subunit of condensin I^{DC}, DPY-27, was discovered around the same time as SMC proteins that facilitate mitotic chromosome formation and stability, suggesting that the mechanism of dosage compensation is similar to that of mitotic chromosome condensation (Chuang et al.,

1994). As further evidence for commonalities in the two processes, MIX-1, the SMC2 partner for DPY-27 in condensin I^{DC}, and condensin I^{DC} CAP proteins, DPY-26 and DPY-28, are required for accurate organization and segregation of chromosome during mitosis and meiosis, in addition to their contributions to X chromosome repression (Lieb et al., 1998; Lieb et al., 1996; Tsai et al., 2008). MIX-1 and the SMC4 protein, SMC-4, (Hagstrom et al., 2002), are part of the condensin II complex, together with the CAP-D3 protein HCP-6 (Chan et al., 2004; Stear and Roth, 2002). However, whether DPY-26 and DPY-28 implemented their cell division function as part of a typical five- subunit condensin complex, or as a two-member team, remained unknown. Additionally, the CAP-G subunit of condensin I^{DC} and CAP-G2 and H2 subunits of condensin II were unidentified. We discovered these missing subunits of condensin II and condensin I^{DC}. Remarkably, we also discovered that CAP subunits from condensin I^{DC} (DPY-26, DPY-28 and the novel protein CAPG-1) and SMC proteins of condensin II (MIX-1 and SMC-4) associate to form a novel condensin complex, condensin I. Condensin I collaborates with condensin II during mitosis and meiosis to efficiently organize and segregate the *C. elegans* genome (Csankovszki et al., 2009).

Our discovery of condensin I refines condensin evolution in *C. elegans*. Due to the apparent lack of a mitotic condensin I complex, *C. elegans* was considered an anomaly amongst higher eukaryotes. In an effort to explain this peculiarity, it was suggested that the unusual holocentric chromosomes in *C. elegans* (Figure 1.4) did not require both condensin I and II to accomplish cell division. As a result, Condensin II function was retained to fulfill mitotic/ meiotic duties, while the ancestral condensin I complex was customized for X chromosome regulation, and became condensin I^{DC}. We showed that SMC subunits from condensin II, and CAP proteins from condensin I^{DC} associate to form condensin I. Thus, like mitotic condensin I and II in other organisms, *C. elegans* also possesses a condensin I and II that share an SMC core, have distinct CAP proteins, and cooperate during mitosis and meiosis. Instead of losing a mitotic condensin I complex, *C. elegans* has gained a specialized dosage compensation condensin (Csankovszki et al., 2009).

II. C. Condensin is required for robust chromosome assembly

Condensin enables the formation of compact, strong chromosomes, whose accurate design facilitates error-free segregation. Defective chromosome partitioning in mitosis and meiosis is the hallmark of condensin depletion in all organisms (Bhat et al., 1996; Chan et al., 2004; Cobbe et al., 2006; Coelho et al., 2003; Csankovszki et al., 2009; Dej et al., 2004; Gerlich et al., 2006; Hagstrom et al., 2002; Hartl et al., 2008; Hirota et al., 2004; Hudson et al., 2003; Lieb et al., 1998; Oliveira et al., 2005; Ono et al., 2004; Ono et al., 2003; Samoshkin et al., 2009; Savvidou et al., 2005; Siddiqui et al., 2003; Stear and Roth, 2002; Steffensen et al., 2001; Vagnarelli et al., 2006; Watrin and Legagneux, 2005; Wignall et al., 2003; Yu and Koshland, 2005; Yu and Koshland, 2003). Condensin was designated as a chromatin condenser in *Xenopus* and reports from budding and fission yeast confirm its importance in this process (Hirano et al., 1997; Hirano and Mitchison, 1994; Lavoie et al., 2002, 2004; Lavoie et al., 2000; Saka et al., 1994; Strunnikov et al., 1995; Sutani et al., 1999). In *C. elegans, Drosophila*, and cultured cells, condensin - depleted chromosomes exhibit abnormal morphology and organization, showing that condensin is essential for accurate and robust chromosome architecture (Bhat et al., 1996; Chan et al., 2004; Cobbe et al., 2006; Coelho et al., 2003; Csankovszki et al., 2009; Dej et al., 2004; Gerlich et al., 2006; Hagstrom et al., 2002; Hartl et al., 2008; Hirota et al., 2004; Hudson et al., 2003; Lieb et al., 1998; Oliveira et al., 2005; Ono et al., 2004; Ono et al., 2003; Samoshkin et al., 2009; Savvidou et al., 2005; Siddiqui et al., 2003; Stear and Roth, 2002; Steffensen et al., 2001; Vagnarelli et al., 2006; Watrin and Legagneux, 2005; Wignall et al., 2003; Yu and Koshland, 2005; Yu and Koshland, 2003). Thus, when condensin function is disrupted, the defective compaction and aberrant structure of chromosomes gives rise to anomalies during anaphase segregation.

Condensin imparts structural integrity to mitotic chromosomes (Hirota et al., 2004; Hudson et al., 2003; Ono et al., 2003). When wildtype mitotic chromosomes are repeatedly transferred between a buffer that causes unfolding of compact chromosomes, and one that supports recompaction, they exhibit the resilience to retain their shape and morphology through many cycles. In contrast, condensin - depleted chromosomes lack this strength, and disintegrate after one or two cycles. In particular, condensin facilitates

robust centromere assembly, which enables accurate chromosome orientation and proper kinetochore function (Brito et al., 2010; Gerlich et al., 2006; Hagstrom et al., 2002; Oliveira et al., 2005; Ono et al., 2004; Ribeiro et al., 2009; Samoshkin et al., 2009; Stear and Roth, 2002; Tada et al., 2011; Wignall et al., 2003). Microtubule attachment defects arise when condensin is depleted, due to faulty centromere organization (Hagstrom et al., 2002; Stear and Roth, 2002; Tada et al., 2011) and reduced structural integrity, which makes the centromere-kinetochore region more susceptible to deformation (Brito et al., 2010; Gerlich et al., 2006; Oliveira et al., 2005; Ono et al., 2004; Ribeiro et al., 2009; Samoshkin et al., 2009; Stear and Roth, 2002; Wignall et al., 2003). Altogether, condensin loss disrupts centromere structure, and leads to dysfunction at the kinetochore.

Distinct phenotypes are obtained upon depletion of condensin I or condensin II, consistent with the complexes operating non-redundantly to shape mitotic chromosomes (Ono et al., 2003). Condensin II associates with chromosomes at prophase, and is required for efficient chromosome condensation at this stage (Chan et al., 2004; Csankovszki et al., 2009; Hirota et al., 2004; Ono et al., 2004). In contrast, condensin I is cytoplasmic in prophase and associates with chromosomes after nuclear envelope break down (NEBD) at prometaphase (Gerlich et al., 2006; Hirota et al., 2004; Ono et al., 2004). Chromosomes are fuzzy and lack individualization in the absence of condensin I (Cobbe et al., 2006; Coelho et al., 2003; Dej et al., 2004; Oliveira et al., 2005; Savvidou et al., 2005; Steffensen et al., 2001; Watrin and Legagneux, 2005; Wignall et al., 2003), and progression from prometaphase to anaphase is delayed (Hirota et al., 2004; Seipold et al., 2009; Watrin and Legagneux, 2005).

II.D. Condensin in meiosis

Condensin makes essential contributions to the fidelity of meiosis. Condensin facilitates accurate meiotic chromosome alignment by augmenting the localization of monopolin in budding yeast (Brito et al., 2010), a complex required for sister kinetochore coorientation during the first meiotic division (Marston and Amon, 2004). Budding yeast condensin is also required for chromosome compaction and individualization, efficient pairing of homologous chromosomes, and the resolution of recombination-dependent linkages

between chromosomes (Yu and Koshland, 2005; Yu and Koshland, 2003). In *C. elegans*, deficient crossover regulation arises in condensin - depleted chromosomes due to the increased length of chromosome axes compared to wildtype (Mets and Meyer, 2009; Tsai et al., 2008). Condensin is also necessary for the resolution of aberrant linkages between homologous chromosomes (Chan et al., 2004; Csankovszki et al., 2009). The compaction and resolution of meiotic chromosomes is severely disrupted when condensin II is depleted (Chan et al., 2004). In *Drosophila,* Condensin II function is essential for chromosome territory formation and prophase condensation, which are prerequisites for proper chromosome segregation. Therefore, depletion of condensin II results in persistent connections between homologous and non-homologous chromosomes during anaphase (Hartl et al., 2008).

Investigations of meiotic condensin regulation and function (this dissertation and work described in this section) represent an emerging and important area of condensin research, that has, for the most part, been focused on condensin's contribution to mitotic chromosome behavior.

II. E. Condensin's influence on cohesin

Condensin's influence on cohesin is unclear. In mitosis, condensin was found to indirectly facilitate the elimination of residual cohesin along chromatid arms at anaphase. Chromosomal regions stretch when cohesin opposes movement of chromatids towards opposite poles. Following this stretch, condensin-dependent chromosome recoiling enables cohesin removal to permit complete chromosome segregation (Renshaw et al., 2010). In HeLa cells also, condensin I function is necessary for the complete removal of cohesin from chromosome arms (Hirota et al., 2004). Similarly, during meiosis, condensin activity is required for the resolution of recombination-dependent linkages between chromosomes (Yu and Koshland, 2003). In condensin - depleted cells, these linkages remain due to persistent cohesin. Condensin facilitates the chromosomal association of polo kinase, and polo kinase-dependent phosphorylation of cohesin is primarily responsible for its dissociation from chromatin (Yu and Koshland, 2005). However, contradictory data from *Xenopus* states that cell cycle-regulated chromosomal

association and dissociation of condensin and cohesin occurs independently of each other (Losada et al., 1998).

III. Aurora B kinase and the Chromosome Passenger Complex

The chromosome passenger kinase, aurora B, recruits condensin to mitotic chromosomes in several model systems, including *C. elegans* (discussed in the following section). In addition to its role in condensin recruitment, the Chromosome Passenger Complex (CPC) facilitates accurate microtubule-kinetochore attachments and robust spindle checkpoint function, regulates cohesin removal from mitotic and meiotic chromosomes, and is required for the successful execution of cytokinesis. CPC proteins first associate with condensing chromatin at prophase, move to the centromere during prometaphase and metaphase, relocate to the central spindle/ spindle midzone at the metaphase - anaphase transition, and finally associate with the midbody (cytoplasmic bridge connecting two daughter cells) during telophase and cytokinesis (Vagnarelli and Earnshaw, 2004). Severe chromosome missegregation, highly aberrant microtubule organization, and cytokinesis failure leads to early embryonic lethality in CPC depleted mice, identifying the complex as an integral component of the regulatory networks that govern cell division (Cutts et al., 1999; Uren et al., 2000).

III.A. Subunits of the CPC

Aurora B kinase, inner centromere protein INCENP, survivin and borealin/ dasra B (referred to as borealin hereafter) are chromosome passenger proteins, conserved from yeast to humans (Vagnarelli and Earnshaw, 2004). CPC proteins are interdependent for stability (Honda et al., 2003; Klein et al., 2006; Nakajima et al., 2009; Sampath et al., 2004; Vader et al., 2006) and accurate positioning at the centromere and spindle midzone (Adams et al., 2000; Carvalho et al., 2003; Chen et al., 2003; Ditchfield et al., 2003; Gassmann et al., 2004; Hanson et al., 2005; Honda et al., 2003; Jeyaprakash et al., 2007; Kaitna et al., 2000; Klein et al., 2006; Lens et al., 2003; Romano et al., 2003; Sampath et al., 2004; Speliotes et al., 2000; Vader et al., 2006; Wheatley et al., 2001).

Aurora B, a serine/threonine kinase, is the enzymatic core of the CPC. The characteristically dynamic localization of the CPC reflects movement of aurora B to act on different cellular substrates (Figure 1.10). This ever-expanding list of aurora B targets include histone H3 (Adams et al., 2001; Hsu et al., 2000), myosin II regulatory light chain (Murata-Hori et al., 2000), CENP-A (Zeitlin et al., 2001), Topoisomerase II alpha (Morrison et al., 2002), MgcRac1GAP (Minoshima et al., 2003), ZEN-4/ MKLP-1(Guse et al., 2005), and MCAK (Andrews et al., 2004; Lan et al., 2004).

INCENP, survivin and borealin are regulatory subunits of the CPC. INCENP is a substrate and activator of aurora B. The conserved INBOX motif at the INCENP C terminal interacts with aurora B (Bolton et al., 2002; Kaitna et al., 2000; Klein et al., 2006; Leverson et al., 2002). The kinase is activated by autophosphorylation during the association with INCENP (Yasui et al., 2004) and then phosphorylates conserved serine residues in the INCENP INBOX to further boost its (own) kinase activity (Bishop and Schumacher, 2002; Honda et al., 2003; Kang et al., 2001; Kim et al., 1999). By slightly modifying its interaction with aurora B at distinct times during mitosis, INCENP prepares the CPC to perform its different functions by turning up or down kinase activity as needed (Xu et al., 2009).

Survivin is classified as an IAP (inhibitor of apoptosis) protein by virtue of its N terminal BIR domain, a zinc finger motif found in the IAP protein family, which regulates apoptosis via caspase inactivation (Vagnarelli and Earnshaw, 2004). In addition to its potential anti-apoptotic duties, survivin is an important regulator of mitotic events as part of the CPC (Lens et al., 2006b; Li et al., 1998; Li et al., 2000; Rajagopalan and Balasubramanian, 1999; Uren et al., 1999; Wheatley et al., 2001). An INCENP- Survivin interaction is necessary for centromere targeting of the CPC (Vader et al., 2006). Very little is known about borealin, the most recently described member of the CPC. Borealin has DNA binding ability *in vitro* (Klein et al., 2006), suggesting that it might serve as the link between chromatin and the CPC. In the *C. elegans* CPC, CSC-1 (chromosome segregation and cytokinesis defective-1) substitutes for borealin (Romano et al., 2003). The two proteins may be related, as they share an 8 amino acid stretch, but are otherwise

dissimilar (Ruchaud et al., 2007b). Borealin and CSC-1 facilitate the interaction between INCENP and Survivin (Nakajima et al., 2009; Romano et al., 2003; Vader et al., 2006).

III.B. Condensin recruitment by the CPC

Aurora B proteins are essential for the chromosomal association of condensin in several model systems. In cultured *Drosophila* cells, condensin recruitment (as judged by the CAP-H subunit) was dramatically diminished upon aurora B depletion (Giet and Glover, 2001). In HeLa cells, aurora B is required to recruit and maintain condensin I on mitotic chromosomes, and mitosis-specific phosphorylation of the condensin I CAP subcomplex also depends on aurora B kinase activity (Lipp et al., 2007). In fission yeast also, aurora B activity is necessary for condensin recruitment (Petersen and Hagan, 2003). However, in *Xenopus*, chromosomal association of condensin is unaltered by aurora B depletion (Losada et al., 2002; MacCallum et al., 2002).

Results from aurora B depletion in *C. elegans* have also been contradictory. Initial studies reported that aurora B depletion in mitosis hinders recruitment of SMC proteins, MIX-1 and SMC-4 (Hagstrom et al., 2002; Kaitna et al., 2002). However, another study found that loading of SMC-4 and condensin II specific subunit CAPG-2 was largely unaffected by aurora B depletion (Maddox et al., 2006). Condensin recruitment by aurora B has not been reevaluated in *C. elegans* after the discovery of condensin I in this organism. Chapter 3 provides a current analysis of aurora B's contribution to condensin targeting in *C. elegans*, and for the first time, addresses how meiotic condensin is regulated by aurora B. The temporal and spatial patterns of Condensin I and II are distinct on *C. elegans* mitotic and meiotic chromosomes. Aurora B in *C. elegans*, AIR-2, regulates condensin I localization during mitosis and meiosis, but does not influence the chromosome association of condensin II in either process. Thus, AIR-2, together with other unknown differential condensin regulators, is responsible for non- overlapping patterns, and perhaps, by extension, contributes to the unique roles of condensin I and II.

III.C. Aurora B promotes accurate chromosome orientation and a robust spindle attachment checkpoint

Aurora B depletion drastically disrupts chromosome orientation on the spindle, and subsequent segregation (Biggins and Murray, 2001; Biggins et al., 1999; Carvalho et al., 2003; Cheeseman et al., 2002; Cimini et al., 2006; Ditchfield et al., 2003; Hauf et al., 2007; Hauf et al., 2003; Kallio et al., 2002; Knowlton et al., 2006; Lampson et al., 2004; Lens et al., 2003; Liu et al., 2009; Monje-Casas et al., 2007; Tanaka et al., 2002). At prometaphase, aurora B is perfectly situated at the centromere (Ruchaud et al., 2007a; Tanaka, 2002; Vagnarelli and Earnshaw, 2004; Watanabe, 2010) to detect inappropriate or reduced tension at sites of incorrect attachment, and facilitate their release. Aurora B enhances microtubule turnover and disassembly, thereby destabilizing mis-attachments (Cimini et al., 2006; Lampson et al., 2004). An attractive model is that aurora B converts incorrect attachments into temporary monotelic attachments, so that freed kinetochores are available to reattach to microtubules from the correct pole (Hauf et al., 2003).

Kinetochore subunits that influence microtubule interactions are ideal candidates for aurora B regulation. The phosphorylation status of kinetochore substrates depends on the extent of spatial separation from aurora B at the centromere (Liu et al., 2009; Tanaka et al., 2002). Targets experiencing low tension (for example, in a syntelic connection) are physically closer to the kinase at the centromere, and undergo phosphorylation, whereas targets subjected to high tension (as seen in a bioriented attachment) are pulled away from aurora B and escape phosphorylation. Phosphorylation reduces the affinity of kinetochore proteins for microtubules (Cheeseman et al., 2006; Ciferri et al., 2008; DeLuca et al., 2006), resulting in a severed connection. Aurora B substrates at the centromere include Ndc80p and Dam1p (Cheeseman et al., 2002) and Ndc10p (Biggins et al., 1999). MCAK (mitotic centromere associated kinesin), a major regulator of microtubule dynamics, is also an aurora B substrate at the centromere (Andrews et al., 2004; Knowlton et al., 2006; Lan et al., 2004).

Aurora B activity also results in accurate chromosome alignment in meiosis. Aurora B is necessary for homolog biorientation on the meiosis I spindle in budding (Monje-Casas et

al., 2007; Yu and Koshland, 2007) and fission yeast (Hauf et al., 2007). The monopolin complex (Marston and Amon, 2004; Watanabe, 2004) in budding yeast aids aurora B in achieving accurate orientation by ensuring the coorientation of sister kinetochores. Monopolin fuses sister kinetochores together and conceals one of the two microtubule attachment sites, so that an attachment can only be made to a single pole (Monje-Casas et al., 2007). In fission yeast, aurora B promotes coorientation of sisters by removing merotelic attachments of unified sister kinetochores (Hauf et al., 2007).

In *C. elegans* meiosis, the precise organization of the bivalent promotes accurate chromosome alignment. The cohesin subunit REC-8 localizes to the long and short arms of the bivalent, and ensures sister chromatid coorientation during the first meiotic division (Severson et al., 2009). Sister coorientation is aided by the encompassment of chromatids by the meiotic kinetochore (Figure 1.6) (Dumont et al., 2010). Aurora B localizes to the short arm of bivalents, between homologous chromosomes (Rogers et al., 2002). Chromosomes on either side of aurora B must be bioriented to segregate away from each other at anaphase I, and microtubule bundles running along the sides of the bivalent play a critical role in this process (Figure 1.6) (Wignall and Villeneuve, 2009). Aurora B at the short arm is ideally located to promote accurate chromosome orientation, although no such role has been demonstrated. By forbidding microtubules to cross the zone of high aurora B activity, the kinase could ensure that homologous chromosomes never make stable attachments to microtubules from the incorrect pole.

Spindle Attachment Checkpoint (SAC) components are recruited to unattached/ mis-attached kinetochores, and postpone APC/C (Anaphase Promoting Complex/ Cyclosome) activity to delay anaphase until all kinetochores are properly positioned on the metaphase plate (Nasmyth, 2002). Cells with a dysfunctional CPC are unable to mount a robust SAC response. Aurora B augments the recruitment of checkpoint effectors (Ditchfield et al., 2003; Hauf et al., 2003; Lens et al., 2006a; Petersen and Hagan, 2003; Vigneron et al., 2004) and directly instigates the checkpoint in response to faulty tension on chromosomes (Biggins and Murray, 2001). In addition, its surveillance at the centromere creates free kinetochores that are also stimuli for SAC trigger (Biggins and Murray, 2001;

Carvalho et al., 2003; Ditchfield et al., 2003; Lens et al., 2003). Recruitment of APC/C components is also dependent on aurora B activity (Acquaviva et al., 2004; Vigneron et al., 2004). Kinetochore recruitment of APC/ C proteins may modify or sequester them, in order to delay anaphase. Altogether, aurora B and the CPC at the centromere protect the cell against catastrophic chromosome missegregation.

III.D. Cohesin Regulation by the CPC

In mitosis, sister chromatid cohesion is dissolved in two steps (Waizenegger et al., 2000). In prophase and prometaphase, Polo like kinase 1 (Plk1) and aurora B are responsible for the removal of the majority of cohesin from sister chromatid arms (Gimenez-Abian et al., 2004; Losada et al., 2002). Plk-1 phosphorylates cohesin (Losada et al., 2002) and likely diminishes its affinity for chromatin (Losada et al., 2002; Sumara et al., 2002). Aurora B driven phosphorylation of histones might enable easier dissociation of cohesin molecules from chromatin (Losada et al., 2002). Conversely, aurora B protects cohesin at the centromere, which must remain intact until mitotic anaphase to prevent untimely separation of sister chromatids. Shugoshins are protector proteins, essential for the preservation of centromeric cohesion during mitosis (Kitajima et al., 2005; Salic et al., 2004; Watanabe and Kitajima, 2005). Aurora B is required to properly target shugoshin to the centromere (Dai et al., 2006). Phosphorylation of centromeric cohesin molecules renders them more attractive to separase (Alexandru et al., 2001). Shugoshin recruits the phosphatase PP2A to the centromere to maintain cohesin in a dephosphorylated state, protecting it from degradation (Kitajima et al., 2006; Riedel et al., 2006).

Accurate meiotic chromosome segregation also depends on the preservation of cohesin at the centromere during meiosis I (Figure 1.5) (Petronczki et al., 2003). Similar to mitosis, aurora B aids cohesin retention at the centromere during meiosis. In budding yeast, aurora B maintains the centromeric localization of Rts1 (PP2A subunit) to protect cohesin by keeping it in a dephosphorylated state throughout meiosis I (Yu and Koshland, 2007). Shugoshin proteins also protect centromeric cohesion is meiosis (Kitajima et al., 2004; Lee and Orr-Weaver, 2001; Marston et al., 2004; Rabitsch et al., 2004) and during

Drosophila meiosis I, the chromosome passenger INCENP is necessary for the stable centromeric association of MEI-S332/ shugoshin (Resnick et al., 2006).

In *C. elegans*, meiotic sister chromatid cohesion relies on the non-redundant activity of three kleisin paralogs, REC-8, COH-3 and COH-4 (Severson et al., 2009). Aurora B facilitates the release, rather than the protection of meiotic cohesin. Inter – homolog cohesin at the short arm is potentially phosphorylated by aurora B for separase-mediated destruction (Kaitna et al., 2002; Rogers et al., 2002). Cohesin on the long arm of the bivalent keeps sister chromatids together through the first meiotic division, and in order to prevent the untimely loss of sister chromatid cohesion during meiosis I, aurora B must be restricted to the short arm. Meiotic cohesion protection in *C. elegans* relies on shugoshin-independent mechanisms. The proteins HTP-1 (Martinez-Perez et al., 2008) and LAB-1 (de Carvalho et al., 2008) associate with the long arm of the bivalent, shielding it from aurora B. LAB-1 executes this pro-cohesion function by directly or indirectly recruiting GSP-1 and GSP-2, PPI phosphatases that antagonize aurora B kinase activity (de Carvalho et al., 2008; Hsu et al., 2000). Together, these proteins ensure that the aurora B-occupied domain, and the zone of cohesin removal during meiosis I remains strictly restricted to the inter-homolog interface at the short arm (Figure 1.11). During meiosis II, aurora B activity on the long arm drives chromatid separation.

III. E. CPC function during cytokinesis

CPC proteins are necessary for the successful completion of cytokinesis, the final step of cell division. Cytokinesis defects (cleavage regression) and multinucleate cells are abundantly observed upon perturbation of CPC function (Vagnarelli and Earnshaw, 2004). Polar body extrusion, which is mechanistically similar to cytokinesis, albeit asymmetrical, is also inhibited by loss of CPC function (Fraser et al., 1999; Kaitna et al., 2000; Romano et al., 2003; Schumacher et al., 1998; Speliotes et al., 2000). The spindle midzone (overlapping antiparallel microtubules at the cell equator that get compacted into bundles and form the midbody) is in disarray upon CPC depletion (Giet and Glover, 2001; Schumacher et al., 1998; Uren et al., 2000; Zhu et al., 2005), and this disorder likely prevents the progression and stabilization of the cleavage furrow.

Aurora B phosphorylation of targets in the cleavage furrow and midzone facilitates their essential roles during cytokinesis. Aurora B promotes actomyosin contractility by phosphorylating the regulatory light chain of myosin (MRLC), augmenting myosin ATPase activity (Murata-Hori et al., 2000). Microtubule motor proteins belonging to the kinesin superfamily are important effectors of cytokinesis (Glotzer, 2005). CPC dependent recruitment of the kinesin ZEN-4/ MKLP-1 is critical for cleavage furrow stabilization and cytokinesis (Giet and Glover, 2001; Kaitna et al., 2000; Schumacher et al., 1998; Severson et al., 2000; Zhu et al., 2005). Aurora B – dependent phosphorylation of ZEN-4/ MKLP-1 is essential for its role in cytokinesis, which is the organization a functional spindle midzone (Glotzer, 2005; Guse et al., 2005). ZEN-4/ MKLP-1 collaborates with CYK-4 (Jantsch-Plunger et al., 2000; Mishima et al., 2002), a Rho GTPase activating molecule (Rho GAP), to fulfill its roles in central spindle assembly and cytokinesis. Aurora B phosphorylates CYK-4 to stimulate its activity (Minoshima et al., 2003). Together, the proteins form a tetrameric complex named *centralspindlin*. Centralspindlin depleted cells have a disorganized central spindle, fail to complete cell division and become multinucleate (Mishima et al., 2002).

Aurora B protects the cell from premature completion of cytokinesis. The presence of chromosome bridges in the path of the cleavage furrow triggers an aurora B - dependent checkpoint in HeLa cells and delays abscission. Aurora B activity (possibly via MKLP-1) stabilizes the cleavage furrow and prevents its regression, buying time for cells to correct errors in chromosome segregation (Steigemann et al., 2009). In yeast, aurora B is a key upstream component of NoCut, which inhibits abscission when midzone defects impede chromosomal movement to the poles. This delay in abscission prevents fragmentation of trapped chromosomes by the cleavage furrow (Norden et al., 2006).

In summary, the CPC is a major controller of mitotic and meiotic events. On chromosomes, it recruits the chromosome-shaping condensin complex, and carefully monitors chromosome orientation on the bipolar spindle. Cohesin regulation by the CPC ensures orderly segregation of homologous chromosomes during meiosis I, and sister

chromatids during meiosis II. Finally, at cytokinesis, the CPC promotes a stable spindle midzone, regulates aspects of actin-myosin contractility, and delays abscission until chromosomes are completely cleared from the path of the advancing cleavage furrow.

IV. Concluding Remarks

Condensin and aurora B kinase are essential contributors to the successful execution of cell division. Aurora B is directly involved in cohesin removal and chromosome orientation, two activities that primarily influence the fidelity of chromosome segregation. During mitosis in *C. elegans*, aurora B activity is essential for chromosomal recruitment of condensin I. Condensin I and II together ensure that chromosomes are accurately organized for equitable partitioning to daughter nuclei. Aurora B colocalizes with and spatially restricts condensin I to the short arm on *C. elegans* meiotic bivalents, and segregation of homologous chromosomes is aberrant when condensin I is depleted (Chapters 2 and 3). At the short arm of the bivalent, aurora B enforces cohesin removal. In addition to this role, we propose that aurora B also supports perfect chromosome alignment on the meiotic spindle, similar to its dual roles in cohesin regulation and chromosome orientation on monocentric chromosomes. Given condensin's role in the removal of cohesin (mitosis and meiosis) and its influence on kinetochore function (mitosis), we speculate that condensin I, via a manipulation of chromosome structure, collaborates with aurora B to facilitate accurate chromosome orientation and the timely removal of cohesin in *C. elegans* meiosis.

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Figure 1.1: Orientation of sister chromatids and homologous chromosomes during mitosis and meiosis. Arrowheads indicate direction of chromosome movement at anaphase

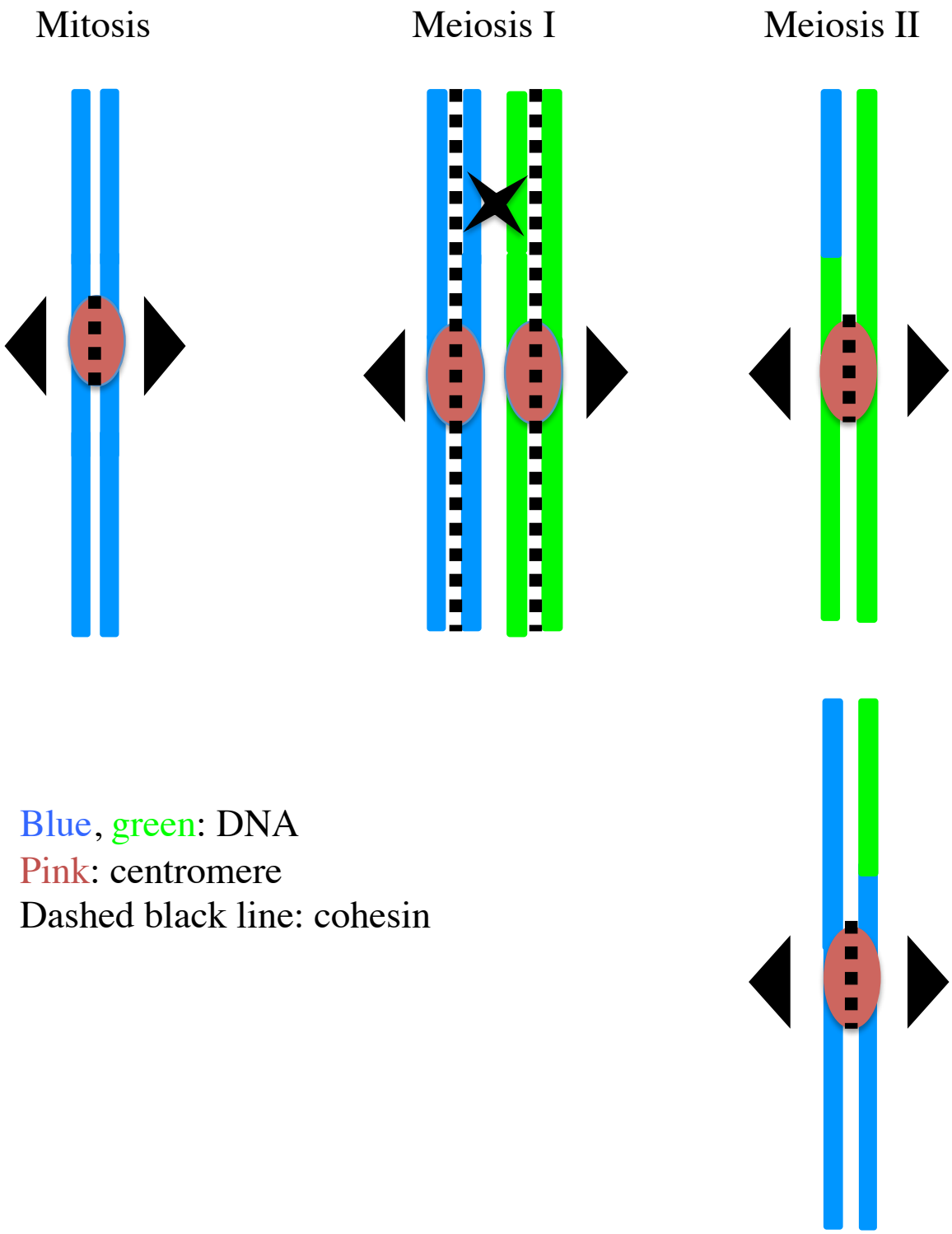


Figure 1.2: Kinetochore – microtubule attachments in mitosis

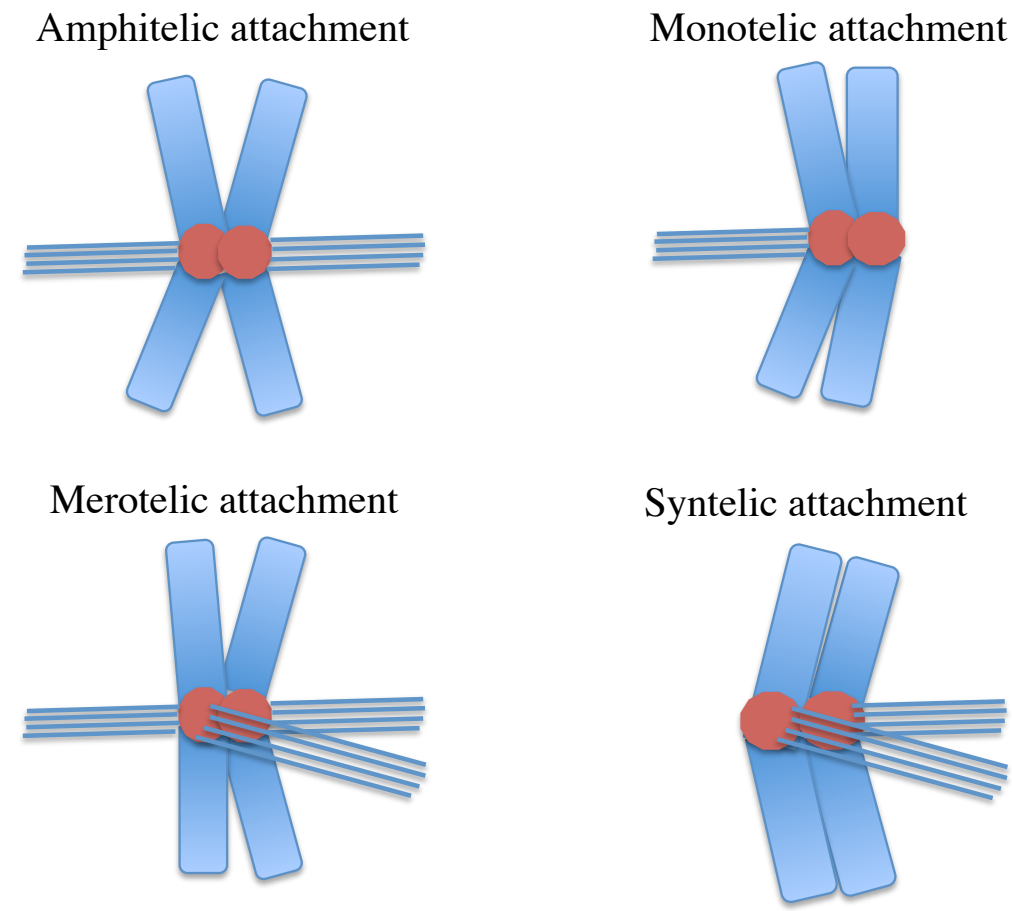
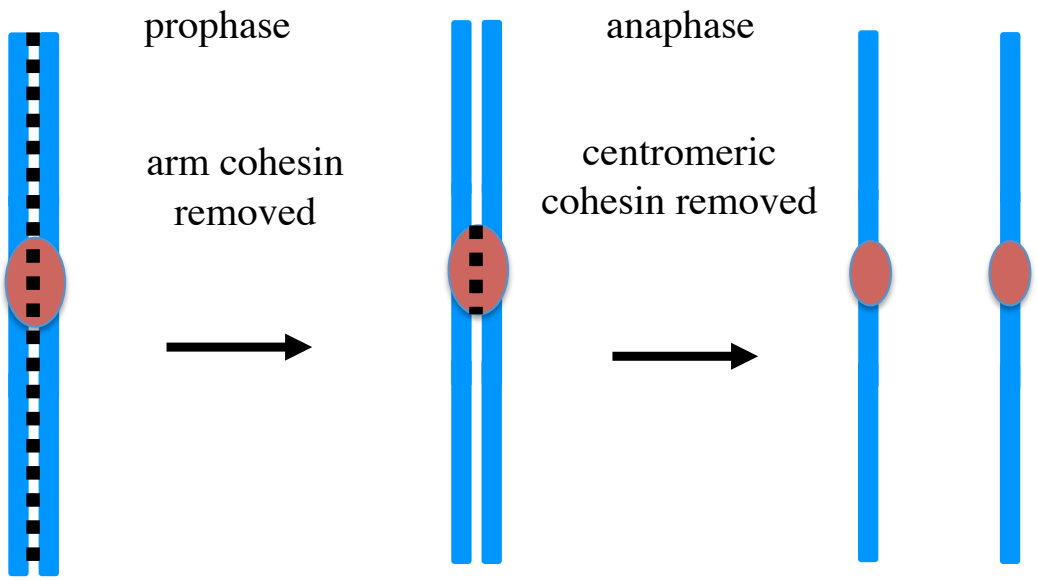


Figure 1.3: Cohesin removal in mitosis. Cohesin is removed along sister chromatid arms at prophase. Centromeric cohesin is intact before anaphase and contributes to kinetochore tension that is necessary for accurate biorientation of chromatids. Centromeric cohesin is removed at anaphase onset, and sister chromatids separate.



Blue: DNA
Pink: centromere
Dashed black line: cohesin

Figure 1.4: Centromere organization on monocentric and holocentric chromosomes. The centromere is restricted to a single location on monocentric chromosomes, but assembles along the entire length of holocentric chromosomes.

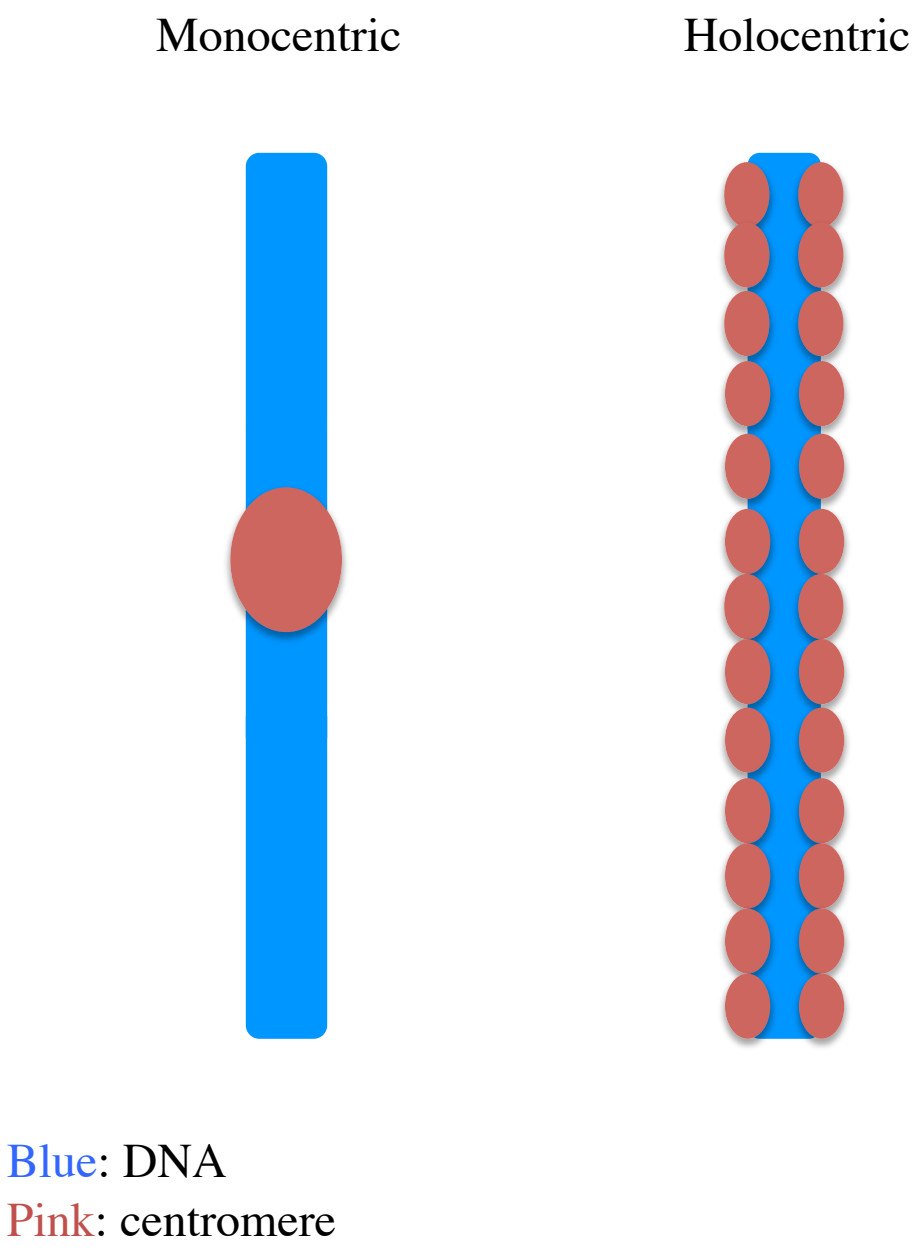
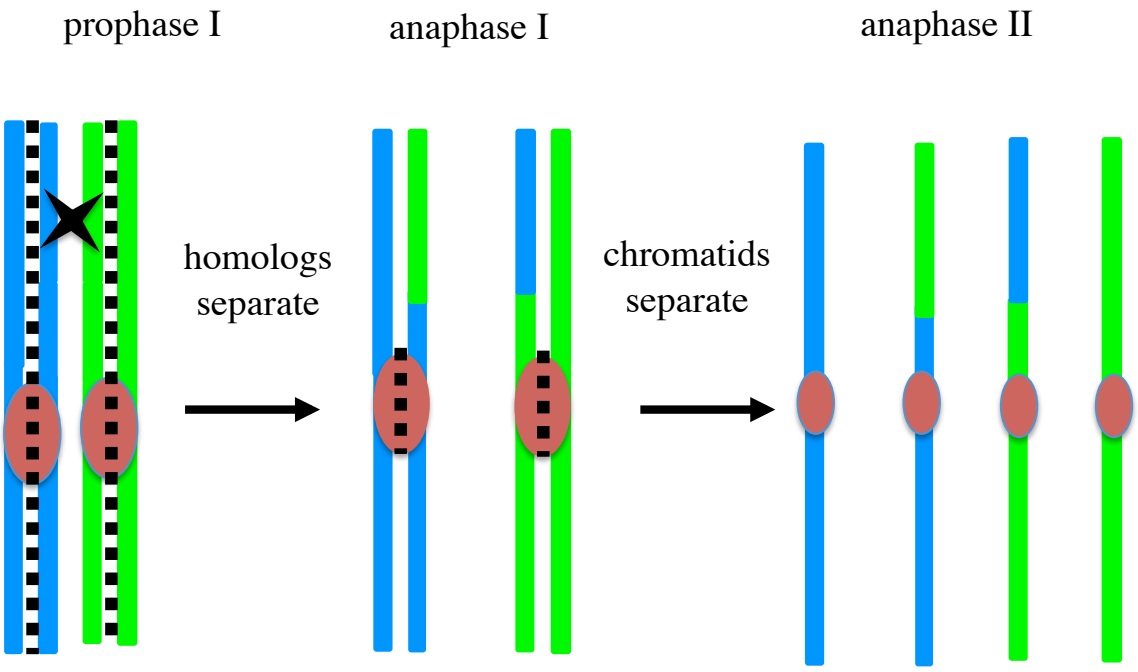


Figure 1.5: Two – step cohesin removal during meiosis in monocentric organisms. During anaphase I, cohesin is removed along chromosome arms, and homologous chromosomes separate. Cohesin is preserved at the centromere at this time, and sister chromatids remain together. Cohesin at the centromere is removed at anaphase II, and sister chromatids separate.



Blue, green: DNA
Pink: centromere
Dashed black line: cohesin

Figure 1.6: Kinetochore organization on monocentric and holocentric chromosomes during mitosis and meiosis. In mitosis, the centromere assembles on the kinetochore, which is located along the entire length on holocentric chromosomes. On monocentric chromosomes, centromere and kinetochore assembly is restricted to a single isolated site. In meiosis, the kinetochore cups homologous chromosomes on *C. elegans* bivalents.

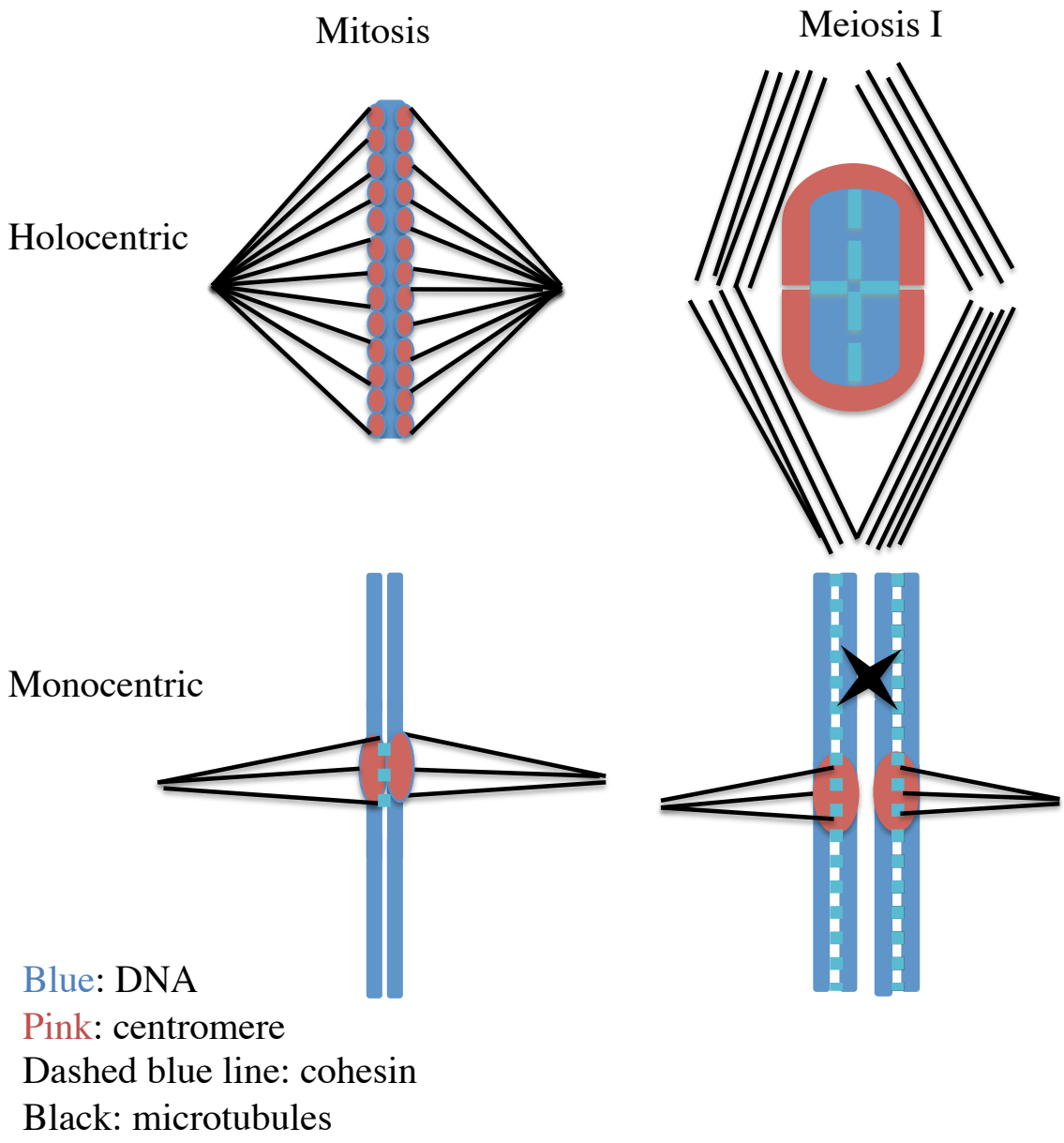


Figure 1.7: Two – step cohesin removal during meiosis in *C. elegans*. Compact bivalents have a short arm and a long arm. During anaphase I, cohesin is removed from the short arm, and homologous chromosomes separate. Long arm cohesin is preserved at this time, and sister chromatids remain together. Cohesion on the long arm is removed at anaphase II, and sister chromatids separate.

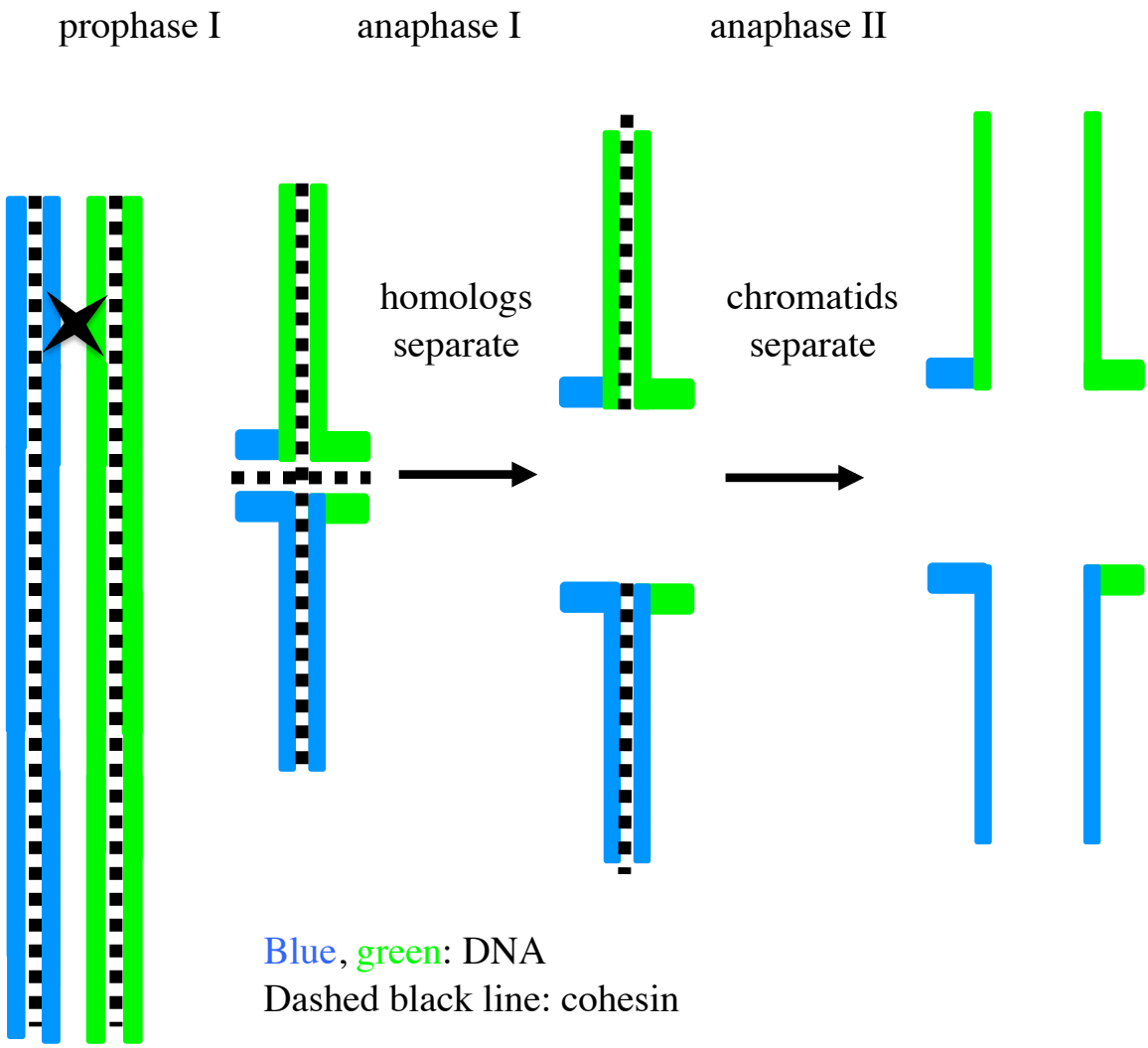


Figure 1.8: Orientation of sister chromatids and homologous chromosomes during mitosis and meiosis in *C. elegans*. Arrowheads indicate direction of chromosome movement at anaphase.

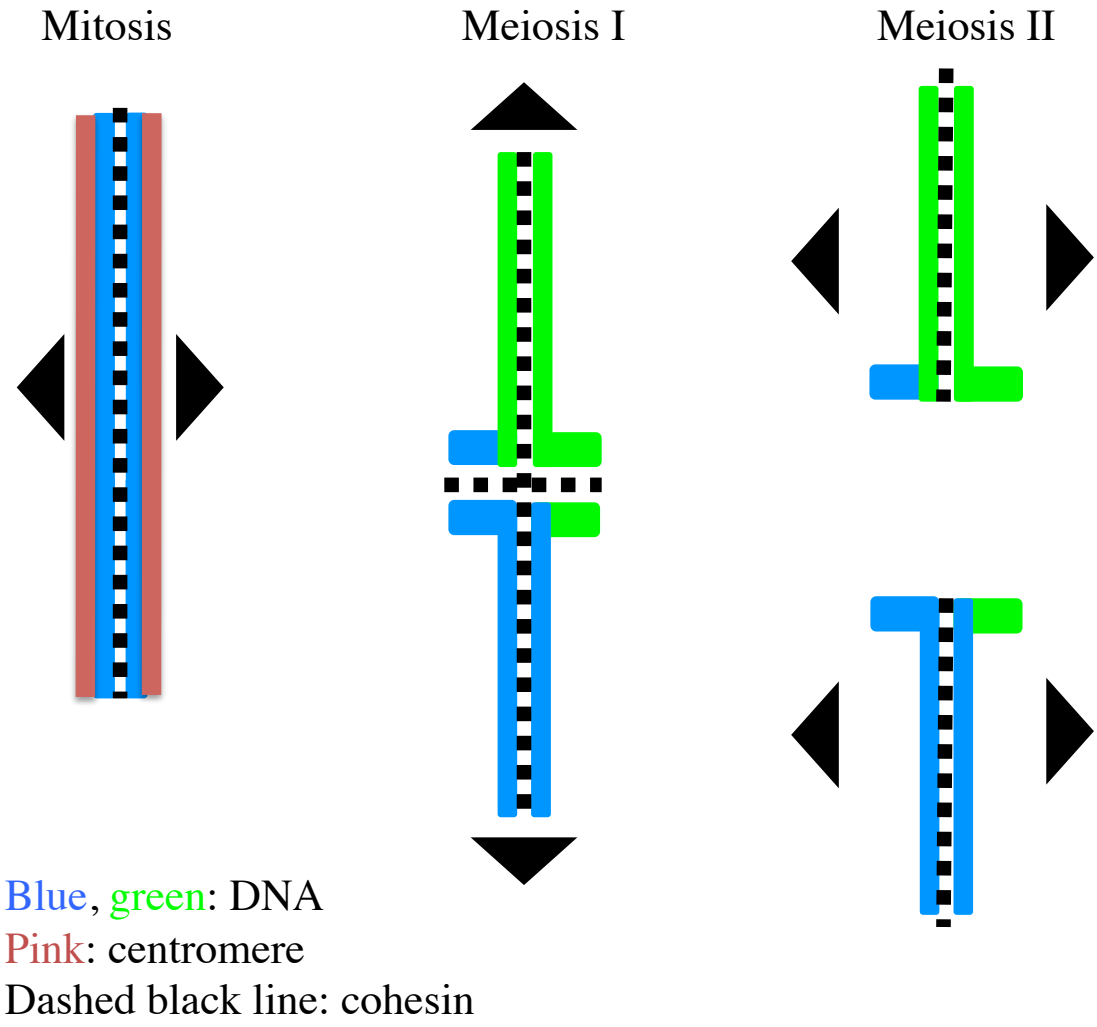


Figure 1.9: Condensin is a five – subunit complex. Structural Maintenance of Chromosome (SMC) family proteins, SMC2 and SMC4 are the ATPase subunits of condensin and Chromosome Associated Polypeptide (CAP) proteins regulate SMC activity. CAP – H, G and D2 are condensin I proteins, CAP – H2, G2 and D3 are condensin II subunits.

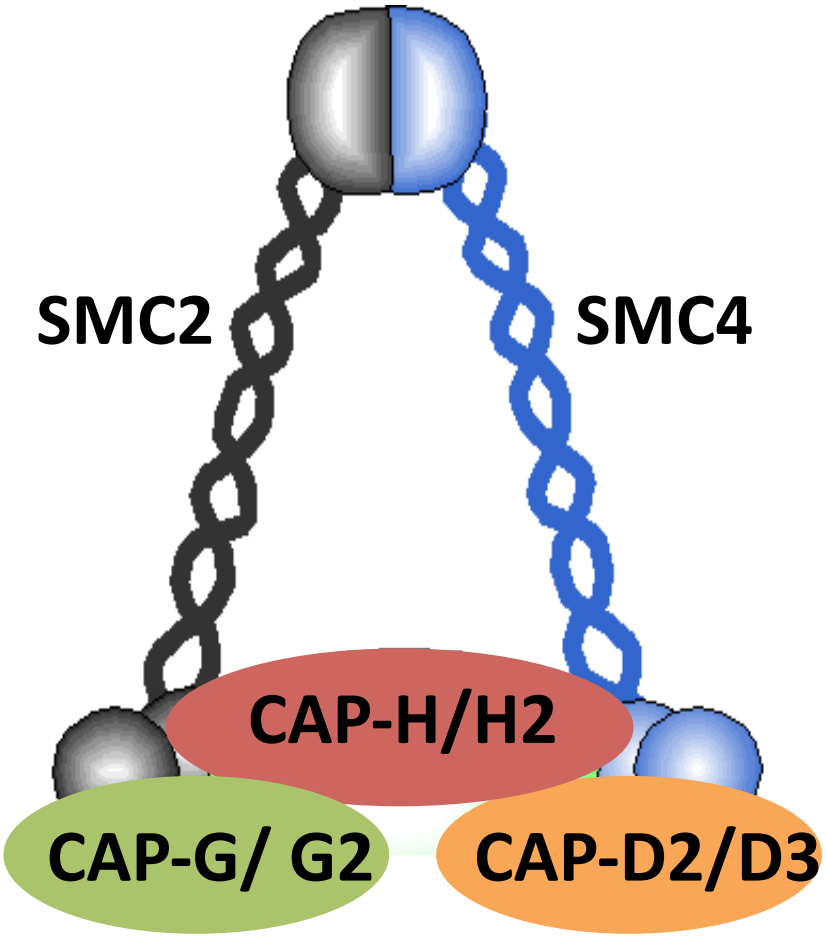


Figure 1.10: Dynamic localization of the Chromosome Passenger Complex. Aurora B kinase and CPC proteins are enriched at the centromere during prophase and prometaphase. At anaphase, CPC proteins transfer to the central spindle, between separating chromosomes.

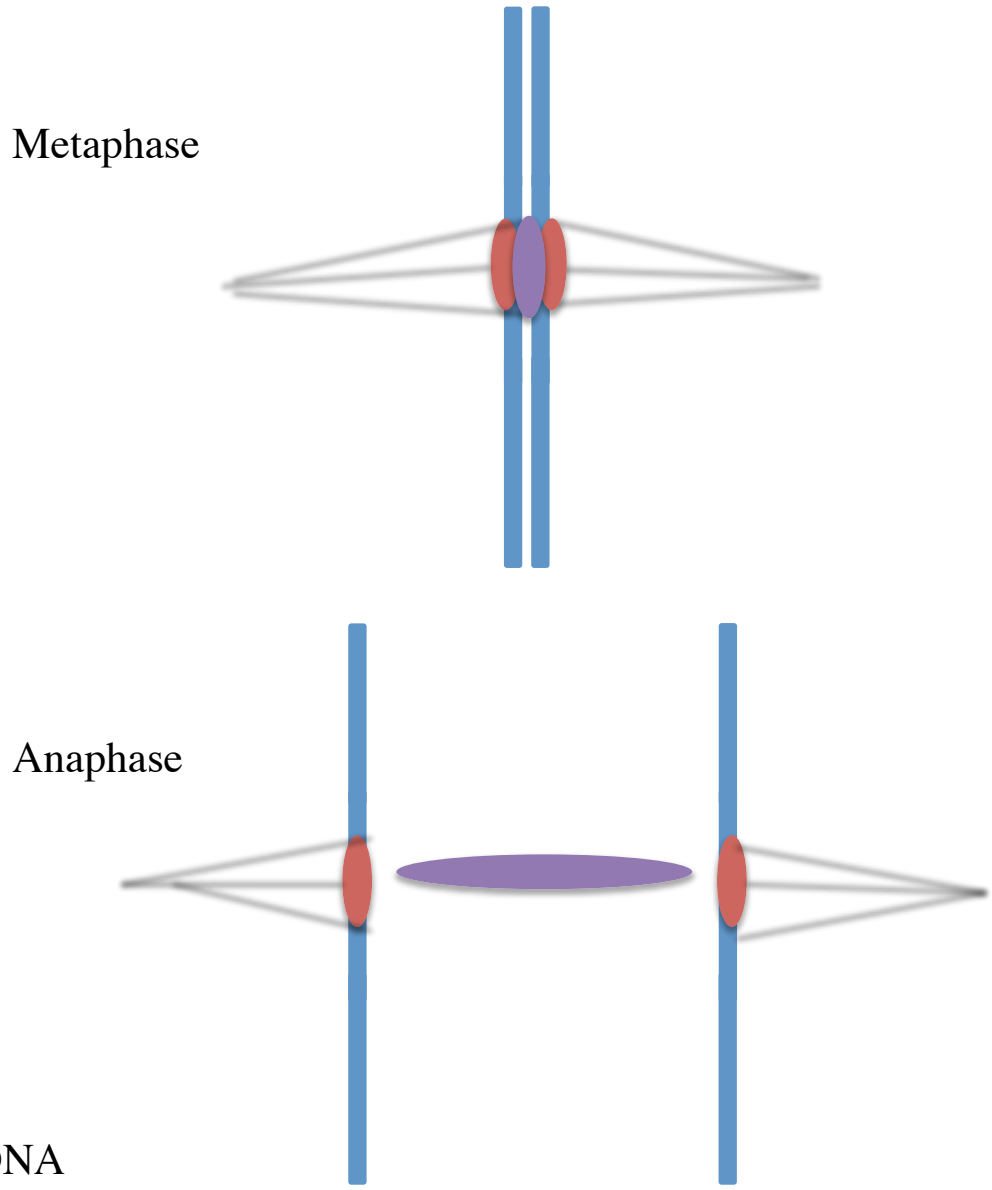
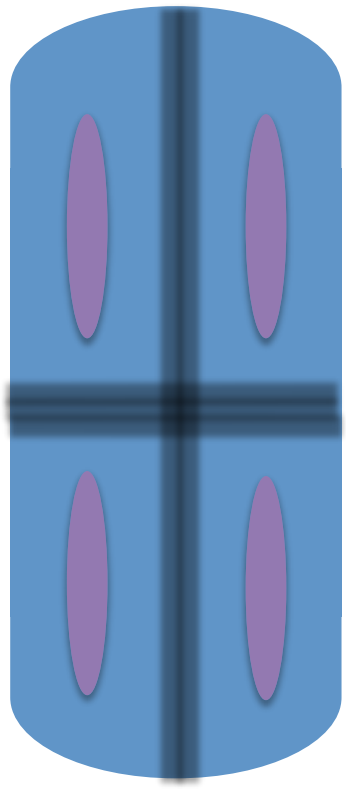


Figure 1.11: Molecular composition of a diakinesis bivalent in *C. elegans*. The compact bivalent has a defined long and short arm. Condensin I and the aurora B kinase, AIR-2, colocalize at the short arm. HTP-1 and LAB-1 are long arm proteins. Cohesin along the long arm maintains cohesion between sister chromatids, and crossover- dependent cohesin linkages at the short arm hold homologous chromosomes together. The condensin II complex associated with the core of sister chromatids.



Blue: DNA
Purple: Condensin II
Green: HTP-1/ LAB-1
Dark blue: Cohesin
Orange: AIR-2
Pink: Condensin I

Chapter 2

Three Distinct Condensin Complexes Control *C. elegans* Chromosome Dynamics

Summary

Condensin complexes organize chromosome structure and facilitate chromosome segregation. Higher eukaryotes have two complexes, condensin I and condensin II, each essential for chromosome segregation. The nematode *Caenorhabditis elegans* was considered an exception, because it has a mitotic condensin II complex but appeared to lack mitotic condensin I. Instead, its condensin I-like complex (here called condensin I^{DC}) dampens gene expression along hermaphrodite X chromosomes during dosage compensation. Here we report the discovery of a third condensin complex, condensin I, in *C. elegans*. We identify new condensin subunits and show that each complex has a conserved five-subunit composition. Condensin I differs from condensin I^{DC} by only a single subunit. Yet condensin I binds to autosomes and X chromosomes in both sexes to promote chromosome segregation, while condensin I^{DC} binds specifically to X chromosomes in hermaphrodites to regulate transcript levels. Both condensin I and II promote chromosome segregation, but associate with different chromosomal regions during mitosis and meiosis. Unexpectedly, condensin I also localizes to regions of cohesion between meiotic chromosomes before their segregation. These results highlight how the duplication and divergence of condensin subunits during evolution may facilitate their adaptation to specialized chromosomal roles, and illustrate the versatility of condensins to function in both gene regulation and chromosome segregation.

Introduction

Chromosomes must be structurally organized for the proper reading and propagation of genetic information. During interphase, chromatin structure can dictate whether genes are expressed or silenced. During mitosis, duplicated chromosomes must be precisely folded to ensure their accurate segregation into daughter cells. Gene regulation and mitotic chromosome condensation have historically been thought to involve different mechanisms. However, condensin proteins have emerged as important determinants in both processes.

Condensins are conserved protein complexes that bind chromosomes and facilitate chromosome segregation, DNA repair, and gene regulation (reviewed in (Belmont, 2006; Hirano, 2005)). Condensin complexes consist of a heterodimer of two SMC (Structural Maintenance of Chromosomes) ATPase subunits (SMC2 and SMC4) and three regulatory subunits referred to as CAPs (Chromosome Associated Polypeptides). While yeasts have a single condensin complex, higher eukaryotes have two: condensin I and condensin II (Hirota et al., 2004; Ono et al., 2004; Ono et al., 2003). Each contains the same SMC2/4 heterodimer but has a unique set of CAP subunits: CAP-D2, CAP-G, and CAP-H in condensin I, and CAP-D3, CAP-G2, and CAP-H2 in condensin II (Figure 2.1A).

Condensins I and II localize to different chromosome regions and make distinct contributions to chromosome segregation. Condensin II is nuclear, and concentrates on chromosomes when condensation initiates at prophase. In contrast, vertebrate condensin I does not access chromosomes until nuclear envelope breakdown, then becomes enriched within each chromatid in alternating regions from condensin II (Hirota et al., 2004; Ono et al., 2004; Ono et al., 2003). Condensin II is required for proper kinetics of chromosome condensation at prophase, while condensin I appears to stabilize chromosome rigidity (Gerlich et al., 2006; Hagstrom et al., 2002; Hirota et al., 2004; Hudson et al., 2003; Kaitna et al., 2002; Maddox et al., 2006; Ono et al., 2004; Ono et al., 2003; Stear and Roth, 2002). Depletion of each complex individually leads to distinct and characteristic defects in chromosome morphology, while simultaneous depletion of both leads to more severe defects (Gerlich et al., 2006; Hirota et al., 2004; Ono et al., 2004;

Ono et al., 2003). Each complex is required for sister chromatid segregation at anaphase in all organisms tested (reviewed in (Belmont, 2006; Hirano, 2005)). How condensins I and II achieve different chromosomal distribution and function is not well understood.

In *C. elegans*, two incomplete condensin complexes have been characterized. A condensin II-like complex performs chromosome segregation functions. Condensin II consists of the SMC4 subunit SMC-4, the SMC2 subunit MIX-1, and the CAP-D3 subunit HCP-6 (Figure 2.1) (Chan et al., 2004; Hagstrom et al., 2002; Kaitna et al., 2002; Lieb et al., 1998). Depleting members of this complex impairs prophase condensation, anaphase segregation, and centromere organization in mitosis (Hagstrom et al., 2002; Kaitna et al., 2002; Stear and Roth, 2002), as well as the restructuring and segregation of chromosomes during meiosis (Chan et al., 2004). By contrast, the known condensin I-like complex (condensin I^{DC}) in worms functions in X-chromosome dosage compensation. Condensin I^{DC} binds both hermaphrodite X-chromosomes to downregulate expression two-fold, leading to equal X-linked gene expression levels between XX hermaphrodites and XO males (reviewed in (Meyer, 2005)). Condensin I^{DC} shares the SMC2 subunit MIX-1 (Lieb et al., 1998), but contains a unique SMC4 subunit DPY-27 (Chuang et al., 1994), and the condensin I class CAP proteins DPY-28 (Tsai et al., 2008) and DPY-26 (Lieb et al., 1996; Schleiffer et al., 2003) (Figure 2.1).

C. elegans condensin I^{DC} appears to have no mitotic function. Except for the shared MIX-1 subunit, mutations in condensin I^{DC} genes were not reported to show mitotic defects in the soma, and while hermaphrodites die due to inappropriately high X-linked gene expression, males carrying these mutations are viable (Chuang et al., 1994; Hodgkin, 1983; Lieb et al., 1998; Lieb et al., 1996; Plenefisch et al., 1989; Tsai et al., 2008). The apparent lack of a mitotic condensin I in *C. elegans* is puzzling, since in all other systems examined, both condensin I and II are essential for mitosis. It has been suggested that during evolution an ancient condensin I lost its mitotic role and became adapted for the specialized function of dosage compensation (Hirano, 2005).

However, while the condensin I^{DC} complex per se appears not to function in chromosome segregation, roles for its CAP subunits in germline mitosis and meiosis have been reported. *Dpy-26* and *dpy-28* mutant alleles increase non-disjunction of the X in meiosis (Hodgkin, 1983; Lieb et al., 1996; Plenefisch et al., 1989), and DPY-28 is required for germline mitosis and proper crossover number and distribution during meiotic recombination (Tsai et al., 2008). These results raise the possibility that these proteins perform chromosome segregation functions independent of their role in condensin I^{DC}.

Here we provide evidence that *C. elegans* does have a mitotic condensin I complex. By identifying new subunits and re-examining existing subunits, we reveal three distinct complexes and characterize their different composition, localization, and function. We demonstrate that the newly identified condensin I complex binds mitotic and meiotic chromosomes and promotes chromosome segregation. Condensins I and I^{DC} share many subunits, clarifying previous indications that some condensin I^{DC} subunits function outside of gene regulation (Hodgkin, 1983; Lieb et al., 1996; Plenefisch et al., 1989; Tsai et al., 2008). Condensin I is shown elsewhere (Mets and Meyer 2009) to control crossover number and distribution in meiosis. Here we describe different localization and phenotypes of condensin I and II, suggestive of distinct functions, and we provide the first comparison in any system of these complexes along holocentric chromosomes and during meiosis. Despite sharing all but one subunit with condensin I, condensin I^{DC} is unique among known condensins, modulating sex- and chromosome-specific gene expression rather than promoting segregation. These results suggest that duplication and divergence of an SMC subunit facilitated the evolutionary adaptation of mitotic/meiotic condensin I for chromosome-specific gene silencing. This illustrates how chromosome architectural complexes may evolve and diversify their functions to meet the needs of more complex eukaryotic genomes.

Results

A proteomics approach identifies new condensin subunits and suggests three condensin complexes

To identify all *C. elegans* condensin subunits, we performed large-scale immunoprecipitation (IP) from embryo extracts using antibodies against DPY-27, the SMC4 subunit of condensin I^{DC}, or against SMC-4, the SMC4 subunit of condensin II. Four independent IP experiments were analyzed by MudPIT (see methods) (Delahunty and Yates, 2007). To eliminate non-specific interactors, we subtracted proteins immunoprecipitated in controls with pre-immune IgG or with an antibody against KLP-7, a mitotic protein that does not associate with SMC-4 or DPY-27. SMC-4 and DPY-27 IPs each recovered a set of interacting condensin subunits that appeared reproducibly and with high total spectrum count (Table 2.1).

C. elegans condensin I^{DC} resembles condensin I in other organisms, but functions uniquely in dosage compensation and no CAP-G subunit had been identified by bioinformatics (Ono et al., 2003) or genetic screens (Meyer, 2005). Proteomics analysis of DPY-27 IPs revealed a previously uncharacterized protein, F29D11.2 that was consistently recovered along with all four known condensin I^{DC} subunits (DPY-27, MIX-1, DPY-28, and DPY-26). F29D11.2 contains ARM/HEAT motifs, and PSI-BLAST and phylogenetic analyses showed that F29D11.2 shares homology with CAP-G class subunits (data not shown). F29D11.2 appears to encode the missing CAP-G subunit of condensin I^{DC} (see data below) and we named it *capg-1* (Table 2.1). Thus, despite its unusual function, condensin I^{DC} has the subunit composition of a bona fide condensin I complex.

SMC-4 IPs recovered SMC-4, MIX-1, and HCP-6, the known members of condensin II, as well as F55C5.4 and KLE-2. F55C5.4 was shown by PSI-BLAST and phylogenetic analyses (data not shown) to be a CAPG-2 class subunit, consistent with a previous prediction (Ono et al., 2003). Based on data below, we conclude that F55C5.4 is the CAP-G2 subunit of condensin II and named it *capg-2* (Table 2.1). KLE-2 was previously

categorized as a CAP-H2 homolog (Ono et al., 2003) and a member of the kleisin ("closure") protein family and shown to affect chromosome segregation (Schleiffer et al., 2003). Here we demonstrate that KLE-2 interacts with MIX-1/SMC-4, as previously speculated, and is the CAP-H2/kleisin subunit of condensin II. Thus, *C. elegans* has a conserved five-subunit condensin II complex.

To our surprise, the SMC-4 IP recovered not just the set of three CAP II class subunits, but also the three CAP I subunits DPY-26, DPY-28, and CAPG-1 (Table 2.1). Thus, we reconsidered the composition of *C. elegans* condensin complexes and hypothesized a three condensin model (Figure 2.1). In our model, *C. elegans* contains not only condensin I^{DC} and condensin II, but also a previously unrecognized condensin I complex, composed of a MIX-1/SMC-4 core and the condensin I CAPs DPY-28, CAPG-1, and DPY-26.

Subunit associations suggest three distinct complexes

To test this model, we raised antibodies against CAPG-1, CAPG-2, and KLE-2 and examined associations among all condensin subunits. We first performed IPs with CAPG-1 and KLE-2 antibodies and analyzed interacting proteins by MudPIT (Table 2.1). CAPG-1 IPs recovered all subunits of condensin I^{DC}, consistent with the prediction that it is the missing subunit of this complex. CAPG-1 IPs also recovered SMC-4, but not class II CAPs, consistent with CAPG-1 and SMC-4 forming a complex that is distinct from condensin II. The KLE-2 IPs recovered CAPG-2 and known subunits of condensin II, supporting the prediction that KLE-2 and CAPG-2 are subunits of this complex (Table 2.1). KLE-2 IPs did not pull down DPY-27 or class I CAPs, confirming that KLE-2 is a subunit of condensin II but not condensin I^{DC} or I.

To further test subunit interactions, we performed IPs of each of the nine condensin subunits and analyzed them on Western blots probed with a battery of condensin subunit antibodies (Figure 2.2 and data not shown). The SMC2 homolog MIX-1 interacted with each SMC4 class subunit and with both class I and II CAPs, consistent with MIX-1 participating in all complexes. SMC-4 interacted with both class I and class II CAPs,

consistent with SMC-4 being shared between condensin I and condensin II. DPY-27 interacted with MIX-1 and class I CAPs, but not SMC-4 or class II CAPs, consistent with it being unique to condensin I^{DC}. Finally, each CAP subunit interacted with the two others in its class, but CAP subunits of different classes never interacted. This result suggests that CAP subunits of different classes do not "swap," and that each condensin complex contains a complete set of either class I CAPs or class II CAPs.

These data support our three condensin model (Figure 2.1). In *C. elegans*, as in other metazoans, a single set of SMC2/4 core proteins (MIX-1/SMC-4) interacts with three class I CAPs to form condensin I or with three class II CAPs to form condensin II. Unlike other metazoans, *C. elegans* has an additional SMC4 class protein, DPY-27, which is used to form condensin I^{DC}, a complex highly related to condensin I but with a unique role in dosage compensation.

Three condensins localize to chromosomes in distinct patterns

We used immunofluorescence to compare chromosome association patterns of the condensin complexes throughout the cell cycle and development. DPY-27 was used to indicate condensin I^{DC}, KLE-2 or HCP-6 was used to indicate condensin II, and CAPG-1 patterns not shared by DPY-27 were presumed to represent condensin I localization.

In early hermaphrodite embryos, before dosage compensation initiates, DPY-27 appears diffusely nuclear in interphase and absent from mitotic chromosomes (Figure 2.3) (Chuang et al., 1994). In contrast, CAPG-1 associates with mitotic chromosomes in a discontinuous coating pattern (Figure 2.3 and 2.4), similar to DPY-26 and DPY-28 (see Fig 3.2) and (Lieb et al., 1996; Tsai et al., 2008). This class I CAP pattern differs from the "outlining" of condensed chromosomes by HCP-6 (Figure 2.3 and 2.4), a pattern observed for other condensin II subunits and coincident with centromere proteins along the outer face of *C. elegans* holocentric chromosomes (Hagstrom et al., 2002; Maddox et al., 2006; Stear and Roth, 2002; Chan et al., 2004; Tsai et al., 2008)

During interphase in older hermaphrodite embryos undergoing dosage compensation, condensin I^{DC} localizes to sub-nuclear territories of the X chromosomes (Chuang et al., 1994; Lieb et al., 1998; Lieb et al., 1996; Tsai et al., 2008). Both DPY-27 and CAPG-1 localized to X (Figure 2.3). However, during mitosis in older embryos after dosage compensation onset, CAPG-1 localized along all condensed chromosomes, a localization not shared by DPY-27, and specific enrichment of CAPG-1 to the X is observed. Thus, CAPG-1 associates associate with X chromosomes in hermaphrodites as part of condensin I^{DC} and with all mitotic chromosomes in both sexes as part of condensin I (Figure 2.3). In contrast, condensin II subunits appeared at low or background levels during interphase, showing bright "centromere-like outlining" of mitotic chromosomes in both early and late embryos (Figure 2.3 and 2.4) (Chan et al., 2004; Hagstrom et al., 2002; Maddox et al., 2006; Stear and Roth, 2002). The distinct localization patterns observed for DPY-27, class I CAPs and class II CAPs are consistent with the existence of three separate complexes.

Since SMC-4 and MIX-1 participate in both condensin I and II, they should exhibit a hybrid localization pattern. These proteins show the bright condensin II "outlining" pattern, as reported (Chan et al., 2004; Hagstrom et al., 2002; Tsai et al., 2008), but not a condensin I "coating" pattern distinguishable from background during mitosis (data not shown). The condensin I pattern may be difficult to visualize in the presence of intense condensin II localization. Alternatively, the three class I CAP subunits might form a sub-complex lacking SMC subunits, comparable to the 11S sub-complex in Xenopus egg extracts (Kimura and Hirano, 2000). However, during meiosis we clearly observed MIX-1 in a hybrid pattern (see below and Figure 2.7), as predicted if they were shared between two different holo-complexes.

Condensin I and II associate with meiotic chromosomes in distinct patterns

We next analyzed condensin localization during meiosis. Condensin I^{DC} proteins are maternally contributed to embryos and accumulate in oocytes, which are arrested at prophase of meiosis I (Chuang et al., 1994; Dawes et al., 1999; Meyer, 2005). Like DPY-27, CAPG-1 accumulated in the oocyte nucleoplasm (Figure 2.5). Unlike DPY-27,

CAPG-1 was present throughout the germline (Figure 2.5, 2.6, 2.7 and data not shown), as are DPY-26 and DPY-28 (Lieb et al., 1996; Tsai et al., 2008), presumably reflecting condensin I localization. Class I CAPs were not associated with chromosomes at prophase of meiosis I (Figure 2.5, data not shown). In contrast to the condensin I^{DC} or condensin I patterns, KLE-2 localizes within each sister chromatid in oocytes (Figure 2.5), as do other condensin II subunits (Chan et al., 2004). DPY-27 was not detected at this stage of meiosis (data not shown).

We examined later meiotic stages in spermatocytes and oocytes undergoing meiotic divisions. Strikingly, class I CAP immunostaining became restricted to the interface between aligned homologous chromosomes during metaphase of meiosis I and to the region between sister chromatids at metaphase of meiosis II (Figure 2.5, 2.6 and 2.7). These locations correspond to the last points of contact between homologs (meiosis I) and sister chromatids (meiosis II), where the mitotic/meiotic kinase AIR-2/AuroraB promotes the release of cohesion by phosphorylation of the meiotic cohesin subunit REC-8 (Kaitna et al., 2002; Rogers et al., 2002). This localization pattern raises the possibility that condensin I contributes to the separation of chromosomes during meiosis. Class II CAPs appeared within each sister chromatid during meiosis I prophase and metaphase, and meiosis II metaphase (Figure 2.5 and 2.7).

MIX-1 showed a hybrid pattern of chromosomal association corresponding to class I and class II CAP patterns (Figure 2.7). Part of the MIX-1 pattern overlaps with the pattern of CAPG-1 (class I CAP), localizing between homologs at metaphase of meiosis I. Another part of the MIX-1 pattern did not overlap with CAPG-1, localizing instead within sister chromatids in the pattern of class II CAP subunits. These results are consistent with MIX-1 (and SMC-4) being core subunits in both condensin I and condensin II.

As a member of condensin I^{DC}, CAPG-1 mediates X dosage compensation

CAPG-1 colocalizes with MIX-1 and DPY-28 on the X chromosomes in hermaphrodite somatic nuclei (Figure 2.8), and interacts with dosage compensation proteins (Table 2.1 and Figure 2.2). Next, we investigated the functional consequences of depleting

condensin subunits. Condensin I^{DC} binds X chromosomes in an all-or-none fashion so that inactivating one subunit disrupts localization of the others (Chuang et al., 1996; Lieb et al., 1998; Lieb et al., 1996; Tsai et al., 2008). Indeed, CAPG-1 association with hermaphrodite X chromosomes was not detected in *dpy-26 or dpy-27* RNAi animals (Figure 2.8), or in *dpy-26, dpy-27*, or *dpy-28* mutant animals (data not shown). Reciprocally, association of DPY-27, DPY-28 (Figure 2.8), DPY-26 and MIX-1 (data not shown) with X chromosomes was not observed in *capg-1* RNAi animals.

Next we asked whether CAPG-1 depletion influenced strains sensitized to perturbations in dosage compensation. Mutations in *sex-1* cause partial disruption of dosage compensation (Carmi et al., 1998; Miller et al., 1988) and low embryonic lethality, which can be enhanced by depleting genes required for dosage compensation (Gladden et al., 2007). Indeed, the 15% embryonic lethality in *sex-1*(y263) mutants fed control vector was increased to about 90% in those fed dsRNA targeting *dpy-27, dpy-28, dpy-26*, or *capg-1* (Table 2.2). A second assay used a *xol-1*(y9) *sex-1*(y263) mutant strain, in which loss of XOL-1 function inappropriately triggers dosage compensation in males, leading to male lethality that can be rescued by depleting dosage compensation genes (Miller et al., 1988; Rhind et al., 1995). We found that RNAi depletion of *dpy-27, dpy-28, dpy-26*, or *capg-1* in this background rescued a large proportion of male progeny (Table 2.2). Thus, in two genetic assays *capg-1* depletion appears to disrupt dosage compensation. Together, these results indicate that CAPG-1 functions as a member of condensin I^{DC} to regulate X-linked gene expression in hermaphrodites.

As components of condensin II, CAPG-2 and KLE-2 mediate chromosome condensation and segregation

We tested whether CAPG-2 and KLE-2 function like other subunits of condensin II. We depleted each subunit individually by injection plus feeding RNAi and live imaged chromosomes visualized by GFP::histone H2B (see methods). CAPG-2 depleted chromosomes failed to condense and individualize at mitotic prophase, as with depletion of any other condensin II subunit (Figure 2.9) (Chan et al., 2004; Hagstrom et al., 2002; Kaitna et al., 2002; Maddox et al., 2006; Schleiffer et al., 2003; Stear and Roth, 2002).

CAPG-2 depletion (Figure 2.9) and KLE-2 depletion (data not shown) also impaired chromosome segregation during meiosis. Germline formation was severely disrupted by CAPG-1 and KLE-2 depletion, like with depletion of other condensin II proteins. These phenotypes are consistent with CAPG-2 and KLE-2 being subunits of condensin II, and with a more limited previous analysis (Schleiffer et al., 2003).

Condensin I subunits promote mitotic and meiotic chromosome segregation

We next determined the functions of the newly identified condensin I complex. We reasoned that condensin I functions could be derived by determining depletion phenotypes observed for CAPG-1, DPY-26 or DPY-28 (condensin I + I^{DC}) but not observed upon depleting the condensin I^{DC}-specific subunit DPY-27. Depletion of CAPG-1, DPY-26 or DPY-28 caused chromosome segregation defects, while DPY-27 depletion did not. Worms grown to adulthood on RNAi food targeting *capg-1, dpy-26,* or *dpy-28* showed inappropriate chromatin bridges between nuclei in many tissues. This defect was most easily scored in gut cell nuclei, which become polyploid after their divisions. While most gut nuclei in control or *dpy-27* RNAi fed worms were completely separated, many gut nuclei in *capg-1*, *dpy-26,* or *dpy-28* RNAi fed worms appear connected by a thin "bridge" of DNA (Figure 2.10).

We confirmed that condensin II localizes normally to mitotic chromosomes in condensin I-depleted embryos (data not shown). Thus, the observed mitotic defects result from condensin I depletion and not from condensin II mislocalization. To test whether condensin I depletion leads to aneuploidy, we depleted CAPG-1 using RNAi feeding and analyzed adult and embryonic nuclei by FISH using a 5S rDNA probe (Figure 2.11). Most nuclei in controls had two fluorescent signals indicating diploidy, or one signal, presumably representing two un-resolvable foci. However, several nuclei in *capg-1* RNAi treated embryos or adult nerve cord had more than two signals, indicating aneuploidy. Earlier FISH analysis of SMC-4 depleted nuclei uncovered similar, albeit more severe, defects (Hagstrom et al., 2002).

In animals depleted of CAPG-1, connections between homologous chromosomes are not efficiently resolved (Figure 2.10), reflecting meiosis I chromosome segregation failure. Meiosis II segregation defects have been observed upon *dpy-26* RNAi (data not shown). Thus, condensin I is required for faithful partitioning of chromosomes during both meiosis I and II. However, due to incomplete depletion of the complex by RNAi and resistance of the germline to RNAi, meiotic segregation defects are infrequently observed.

Discussion

Evolution of diverse condensin complexes

Condensins I and II are conserved complexes that ensure the accurate segregation of chromosomes during cell division (Belmont, 2006; Hirano, 2005). In *C. elegans*, incomplete condensin I-like and condensin II-like complexes had been identified (Chan et al., 2004; Hagstrom et al., 2002; Meyer, 2005). Previous studies, extended here, indicated that *C. elegans* condensin II is required for prophase chromosome condensation and anaphase segregation, conserved condensin II activities (Chan et al., 2004; Hagstrom et al., 2002). In contrast, the previously identified condensin I-like complex, condensin I^{DC}, seemed not to function in chromosome segregation, but instead to modulate chromosome- and sex-specific gene expression (Meyer, 2005). Moreover, despite extensive genetic screens and homology searches, the CAP-G subunit of condensin I^{DC} was missing. It had therefore been suggested that *C. elegans* condensin I lost its mitotic function during the course of evolution and was converted to the specialized function of dosage compensation. One speculation was that holocentric chromosomes might not demand condensin I activity for segregation (Hirano, 2005).

Our discovery of condensin I changes the basis for this viewpoint. We show that *C. elegans* contains both conserved condensin I and II complexes, each with a typical suite of five subunits, and that both promote chromosome segregation. The main difference between *C. elegans* and other organisms is a second SMC4 class protein, DPY-27, the only subunit that distinguishes condensin I^{DC} from condensin I. Thus, our findings indicate that mitotic condensin I was not lost, but that the duplication and divergence of

an SMC4 subunit allowed the ancestral mitotic condensin I to be adapted for dosage compensation. A related example is subunit paralogs that distinguish mitotic from meiotic versions of the cohesin SMC complex (Revenkova and Jessberger, 2006).

Roles of condensin I and II in chromosome segregation

How condensin I and II contribute differently to chromosome structure and function remains unclear. We show that in worms, depletion of either complex causes defects in chromosome segregation. Differences in chromosomal distributions and depletion phenotypes suggest each complex has specialized functions. RNAi mediated depletion of condensin I or II each caused segregation defects, but only condensin II depletion caused prophase condensation defects, as in vertebrate cells (Gerlich et al., 2006; Hirota et al., 2004). Condensin I depletion generally caused less severe phenotypes than condensin II, suggesting that condensin II plays a more critical role in mitosis and development than condensin I in *C. elegans*. This may explain why condensin I had not been identified prior to this study.

Differential distribution of condensin I and II on holocentric chromosomes during mitosis and meiosis

The balance of condensin I and II distribution and activity has been speculated to influence the different chromosome shapes in different organisms (Hirano, 2005; Ono et al., 2003). Most model organisms have chromosomes with distinct arms and a discrete centromere (monocentric organization), and condensin I and II show alternating distribution within the core of each sister chromatid arm, with enrichment of condensin II at the centromere (Hirota et al., 2004; Ono et al., 2004; Ono et al., 2003). Our study is the first to compare condensin I and II on chromosomes with an extended centromere (holocentric organization). Condensin I is broadly distributed on mitotic chromosomes internal to condensin II, which is restricted to the outer face of each sister chromatid coincident with the extended centromere, consistent with previous studies of individual subunits (Chan et al., 2004; Hagstrom et al., 2002; Lieb et al., 1996; Maddox et al., 2006; Stear and Roth, 2002; Tsai et al., 2008). Despite major differences, the non-overlapping distribution of condensin I and II and the enrichment of condensin II at the centromere

are conserved. The more extreme difference in condensin I versus II localization on holocentric chromosomes makes an ideal setting in which to study how localization differences are dictated. Future genome-wide chromatin IP studies could yield further insight into differential functions by mapping DNA elements bound by each complex.

Our studies also provide the first evidence in any organism for differences in condensin I and condensin II localization during meiosis. While condensin II localizes within the core of meiotic sister chromatids ((Chan et al., 2004) and this work), *C. elegans* condensin I localizes to the region between paired homologs at meiosis I and between sister chromatids at meiosis II. This contrasts with its broad distribution along mitotic chromosomes and with the localization of condensin I in other species to the internal core of sister chromatids during meiosis (Viera et al., 2007; Yu and Koshland, 2003). *C. elegans* condensin I enrichment corresponds to regions where cohesion between homologs (meiosis I) or sisters (meiosis II) is maintained until anaphase. A speculation is that condensin I contributes to the proper formation and/or release of cohesion during the meiotic divisions. Previous studies have implicated condensin in mediating cohesion (Lam et al., 2006) and releasing cohesin (Hirota et al., 2004; Yu and Koshland, 2005). It is interesting to note that AIR-2, the *C. elegans* homolog of the Aurora B kinase, also localizes between homologs at meiosis I and between sisters at meiosis II, and is required for proper release of a meiotic subunit of cohesin (Kaitna et al., 2002; Rogers et al., 2002).

Condensin function in chromosome segregation and gene regulation

An unresolved question is how differences between condensin complexes are specified. In principle, functional differences could be due to differences in biochemical activity, regulation, or chromosomal targeting. Although condensin I is known to have ATP-dependent supercoiling and DNA compaction activities (Kimura and Hirano, 1997; Kimura et al., 1999; Strick et al., 2004), it is currently unknown whether these activities are shared by condensin II. We previously demonstrated supercoiling activity in SMC-4 immunoprecipitates (Hagstrom et al., 2002). That preparation, in light of the current study, contained both condensin I and II, making the source of supercoiling activity

ambiguous. Gene regulation by condensin I^{DC} may also require an ATP-dependent process, because lesions in the ATP binding domain of *dpy-27* and *mix-1* impair dosage compensation (Chuang et al., 1994; Lieb et al., 1998).

For condensins I and I^{DC}, a difference in one subunit has a profound effect on function, converting a mitotic complex into a gene regulatory complex. Interestingly, condensin I and II subunits in other organisms have been implicated in gene regulation (please see Introduction). A common alteration of chromosome structure could conceivably underlie both chromosome preparation for mitosis, and down-regulation of gene expression. Continued comparative studies of condensin complexes are likely to shed light on the links between chromosome segregation and gene regulation.

Materials and Methods

C. elegans strains

Animals were maintained on NG agar plates using standard methods. The following strains were used:

wild type (N2)

sex-1(y263) X

him-8(e1489) IV; *xol-1*(y9) *sex-1*(y263) X

dpy-26 (n199) IV /nT1 [qIs51] (III;IV)

dpy-28(s939) III/qC1 III

Dosage compensation mutations cause XX specific maternal effect lethality. *Dpy-26* and *dpy-28* mutants were maintained as balanced strains. Homozygous mutants (animals lacking the balancer chromosomes) were picked individually from the population for staining experiments.

RNAi assays to assess dosage compensation

The two RNAi assays used were described in (Gladden et al., 2007).

Enhancement of lethality in the *sex-1*(y263) background: Wild type (control) and sex-1(y263) worms were grown on RNAi plates from L1 to adulthood. Three adults were moved to fresh RNAi plates and allowed to lay eggs for 24 hours. Hermaphrodite

mothers were removed and total progeny counted. The next day, eggs not hatched were scored as dead progeny. % lethality was calculated as dead progeny/total progeny x100. At least 200 embryos were scored for each RNAi experiment.

Male rescue: *him-8*(e1489); *xol-1*(y9) *sex-1*(y263) hermaphrodites were grown from L1 to adulthood on RNAi plates. Two adults were moved to fresh plates and allowed to lay eggs for 24 hours. Total progeny was counted as soon as mothers were removed from the plates. In three days, progeny was scored as either male or hermaphrodite. Male rescue was calculated as # males/expected number of males x 100. The expected number of males was assumed to be 38% of total progeny, due to the presence of the *him-8* mutation (Phillips et al., 2005). At least 200 animals were scored for RNAi of each gene.

Antibodies

The following peptides were coupled to KLH and used for raising antibodies in rabbits (all proteins) and rats (CAPG-1):
DPY-27 (QPFKRRALTSDDDRPYADTDSMPEVDLDVDRRR)
KLE-2 (CFDDDEEDVRPRGERP)
CAPG-1 (PPKKRIRGPKLPALREEKSTGC)
DPY-26 (CKTTSDLGAIVEEEEMEE)
DPY-28 (CKSAVADDDSDSDEFMLDD)
Secondary antibodies were purchased from Jackson ImmunoResearch. Antibodies to SMC-4 (Hagstrom et al., 2002), MIX-1 (Lieb et al., 1998), DPY-26 (Lieb et al., 1996), and HCP-6 (Chan et al., 2004) have been described previously. In all cases, antibody-staining patterns described were reduced or absent in the corresponding RNAi background.

Analysis of interacting proteins by MudPIT

Sample Preparation: To collect embryos, worms were grown to high density on egg plates (Chu et al., 2006). Embryo extracts were prepared by sonicating in homogenization buffer (50mM HEPES-KOH pH7.6, 140 mM KCl, 1mM EDTA, 0.5% NP-40, 10% glycerol) with protease inhibitor cocktail, and the supernatant was collected after

centrifugation at 5,000 x g for 20'. For large-scale IPs, 100 mg of total protein extract and 500 ug of antibody were used. Immunocomplexes were pulled down using 500 ul protein A sepharose beads and washed extensively (3 times in homogenization buffer, 3 times in homogenization buffer without glycerol or detergent), and eluted in 100 mM glycine (pH 2.5). TCA precipitated pellets were analyzed by MudPIT mass spectrometry (see below) (Delahunty and Yates, 2007). To each of the four samples (which were all TCA precipitated protein pellets) 60 µL of 8 M Urea, 100 mM Tris, pH 8.5 were added to solubilize the protein. The subsequent mixture was then reduced by adding 0.3 µL of 1M TCEP (for a final concentration of 5 mM TCEP) and incubated at room temperature. To alkylate, 1.2 µL of Iodoacetamide (10 mM final concentration) were added and the samples were subsequently incubated at room temperature while in the dark for 15 minutes. Endoproteinase Lys-C (0.1 µg/µL) was then added in the amount of 1.0 µL and shaken for 4 hours while incubated in the dark at 37 oC. The addition of 180 µL of 100 mM Tris pH 8.5 diluted the solutions to 2 M Urea. Calcium chloride (100 mM) was then added (2.4 µL) for a final concentration of 1 mM CaCl2. Trypsin (0.5 µg/µL) was added in the amount of 4.0 µL. The resulting mixtures were then shaken for 18 hours and incubated in the dark at 37 oC. To neutralize 15.00 µL of Formic Acid (90%) was added for a final concentration of 5% Formic Acid. The tubes were centrifuged for 30 minutes at 2 ^{0}C on a table top centrifuge.

Multidimensional Protein Identification Technology (MudPIT):

Upon completion of the digestion, the proteins were pressure-loaded onto a fused silica capillary desalting column containing 3 cm of 5-µm strong cation exchange (SCX) followed by 3 cm of 5-µm C18 (reverse phase or RP material) packed into a undeactivated 250-µm i.d capillary. Using 1.5 mL of buffer A (95% water, 5% acetonitrile, and 0.1% formic acid) the desalting columns were washed overnight. Following the desalting process, a 100-µm i.d capillary consisting of a 10-µm laser pulled tip packed with 10 cm 3-µm Aqua C18 material (Phenomenex, Ventura, CA) was attached to the filter union (desalting column– filter union–analytical column). The resulting split-columns were placed inline with a ThermoFinnigan Surveyor MS Pump (Version 2.3; Palo Alto, CA) and analyzed using a customized 4-step separation method

(90, 110, 110, and 150 minutes respectively).

Step 1 utilized only buffer A (95% water, 5% acetonitrile, and 0.1% formic acid) and buffer B (80% acetonitrile, 20% water, and 0.1% formic acid). It began with 5 min of 100% Buffer A, followed by the following buffer B gradients: 5 min of 0- 10%, 40 min of 10-45%, and 10 min of 45-100%. Twenty minutes of 100% buffer B ensued and the gradient program ended with 10 min of 100% buffer A. Steps 2-4 utilized Buffers A, B, and C (500 mM ammonium acetate, 5% acetonitrile, and 0.1% formic acid). Steps 2 and 3 each began with: 3 min of 100% buffer A, 7 min of X% buffer C, a 5 min gradient from 0 – 10% buffer B, a 75 min gradient from 10-45% buffer B, and then 5 min of a 45-100% buffer B gradient. Five minutes of 100% buffer B followed and then the sequence ended with 10 min of 100% buffer A. The buffer C portions consisted of 20% for step 2 and 50% for step 3.

Step 4 began in a similar fashion (3 min of 100% buffer A, 7 min of 100% buffer C, and a 5 min gradient from 0 – 10% buffer B) yet its 10-45% buffer B gradient lasted for 85 minutes and the 45-100% buffer B gradient was for 10 min. Ten minutes of 100% and then a gradient of 0-100% buffer B ensued with the run ending with 10 min of 100% buffer A. By increasing the salt concentration (buffer C) peptides “bump” off of the SCX and then with a gradient of increasing hydrophobicity (buffer B) the peptides can elute from the RP into the ion source. To elute the peptides from the micro capillary column, a distal 2.5 kV spray voltage was applied. The applied voltage caused the peptides to directly electro spray into an LTQ 2-dimensional ion trap mass spectrometer (ThermoFinnigan, Palo Alto, CA). First a cycle of one full- scan mass spectrum (400-2000 m/z) and then 5 data-dependent MS/MS spectra at a 35% normalized collision energy was performed throughout each step of the multidimensional separation. The aforementioned HPLC solvent gradients and MS functions were all controlled by the Xcalibur data system (Version 1.4).

Analysis of Tandem Mass Spectra

As each step was executed, its spectra were recorded to a RAW file. This data was then converted into .ms2 format through the use of RawXtract (Version 1.9). From the .ms2 files, poor quality tandem mass spectra were removed using an automated spectral quality assessment algorithm known as a "PARC" filter (Bern et al., 2004). The remaining MS/MS spectra were searched with the SEQUESTTM algorithm (Eng et al., 1994) against the WormBase_C-elegans protein database (created on 12/17/06). So as to assess the false positive rate the protein sequences from the database were reversed (decoy database) (Peng et al., 2003). The number and quality of spectral matches to the decoy database were used to estimate the false positive rates. A computer cluster consisting of 100 1.2 GHz Athlon CPUs was used to perform the search (Sadygov et al., 2002). The SEQUEST search did not employ any enzyme specifity and the final data set was filtered using the DTASelectTM (version 2.0.9) program (Cociorva et al., 2007; Tabb et al., 2002). The digestion method employed was specified (--trypstat for tryptic digests) so as to specifically filter for peptides with trypsin specificity. A user-specified false positive rate was used to dynamically set XCorr and DeltaCN thresholds through quadratic discriminant analysis. This dataset was then further filtered to remove contaminants (i.e. keratin) while the default filter (minimum of 2 peptide and half tryptic status) was applied. Interacting proteins were ranked by estimated relative abundance using the total number of spectra observed for each (Liu et al., 2004).

Protein analysis

Small-scale IPs and western blotting were performed as described (Chan et al., 2004).Large-scale protein extract preparation and IPs for MudPIT were scaled-up versions of this protocol.

Immunostaining

Adult worms were dissected in 1X sperm salts (50 mM PIPES pH7, 25 mM KCl, 1 mM $MgSO_4$, 45 mM NaCl, 2 mM $CaCl_2$), fixed in 2% paraformaldehyde in 1X sperm salts for five minutes and frozen on dry ice for ten minutes. Slides were washed three times for ten minutes each in PBS/0.1% Triton (PBST) before incubation with 30μL of diluted primary

antibody in a humid chamber, overnight at room temperature (RT). Double labeling of samples was performed with all primary antibodies simultaneously during this overnight incubation. Slides were then washed three times, ten minutes each, with PBST, incubated for one hour with 30μL diluted secondary antibody at 37^0C, washed again twice for ten minutes each with PBST, and once for ten minutes with PBST plus DAPI. Slides were mounted with Vectashield (Vector Labs).

Embryos were obtained from hermaphrodites by bleaching, fixed with Finney fixative (2% paraformaldehyde, 18% methanol, 10mM PIPES pH 7.5, 60 mM KCl, 8 mM NaCl, 2.6 mM EGTA, 0.4 mM spermidine, 0.16 mM spermine, 0.4% b-mercaptoethanol), frozen at -80^0C for 20 minutes, thawed, fixed for 20 minutes at RT, and washed in PBST for 15 minutes. Samples were incubated with primary antibody overnight at RT. Embryos were washed three times (15 minutes each) in PBST, and incubated overnight with secondary antibody at RT. This incubation was followed by two PBST washes and a third wash in PBST plus DAPI. Embryos were mounted on slides with Vectashield. FISH was performed as in protocol #25 in (Shaham, 2006).

Images were captured using a Hamamatsu ORCA-ERGA CCD camera on an Olympus BX61 motorized X-drive microscope using a 60X PlanApo oil immersion objective with a NA of 1.42. Images were captured in Z stacks with planes at 0.2μm intervals and deconvolved and projected with 3i Slidebook software. Adobe Photoshop was used for image assembly.

Time-lapse imaging (performed in the Hagstrom lab) of mitotic and meiotic chromosome segregation was performed on animals carrying GFP::histone H2B. These were dissected with a syringe needle on a 2% agar pad in 1x M9 buffer and covered gently with a coverslip. Z stacks were acquired with a Perkin Elmer spinning disk confocal microscope every 20s with ~0.75s exposure and 2x2 binning.

RNA interference

RNA interference by feeding was performed using the Ahringer lab RNAi feeding library (Kamath and Ahringer, 2003). 50 mL bacterial cultures were grown at 37^0C for 20 hours, induced with IPTG for two hours, pelleted and resuspended in 500μL of fresh LB broth and plated as a concentrated bacterial lawn. RNAi was initiated at the L1 stage. L4 worms were transferred to fresh plates, and allowed to produce progeny (F1) for 24 hours. F1 worms were processed 24 hours post L4 for IF. Control experiments were performed using the same conditions as RNAi experiments.

RNAi injection plus feeding (as in Figure 2.9)

dsRNA corresponding to a region of each gene encoding a predicted condensin II subunit was prepared by in vitro transcription and injected into young hermaphrodites. These were recovered onto plates seeded with the corresponding RNAi food and examined 48 hours later.

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Figure 2.1: The three condensin model. (A) *C. elegans* condensin subunits and their human homologs. We propose that *C. elegans* has three condensin complexes of the composition shown. Condensin I and II share the same SMC2 and SMC4 subunits but associate with either class I or class II CAP subunits, respectively. Condensin I^{DC} differs from condensin I only by its unique SMC4 subunit, DPY-27. (B) Cartoon depicting the three proposed complexes.

A

Human condensin I	*C. elegans* condesin I^{DC}	*C. elegans* condensin I	Human condensin II	*C. elegans* condensin II
SMC2	MIX-1	MIX-1	SMC2	MIX-1
SMC4	DPY-27	SMC-4	SMC4	SMC-4
hCAP-D2	DPY-28	DPY-28	hCAP-D3	HCP-6
hCAP-G	CAPG-1	CAPG-1	hCAP-G2	CAPG-2
hCAP-H	DPY-26	DPY-26	hCAP-H2	KLE-2

B

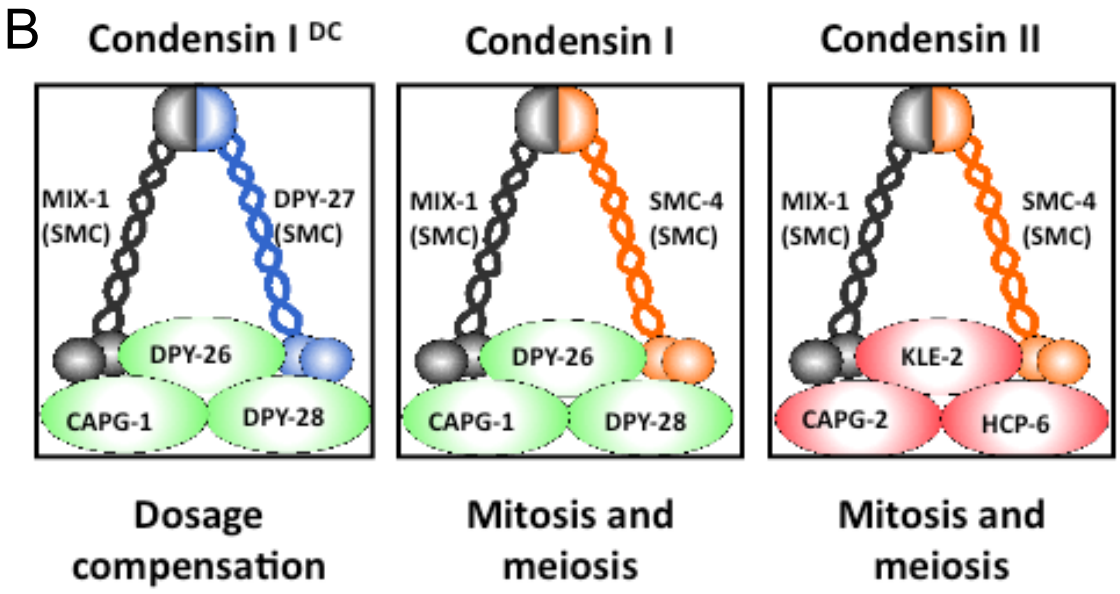


Table 2.1: Condensin subunit interactions identified by immunoprecipitation (IP) - MudPIT mass spectrometry. Immunoprecipitation (IP) from embryo extracts with antibodies against DPY-27, SMC-4, KLE-2, CAPG-1 and KLP-7 (control) were analyzed by MudPIT mass spectrometry. (+) and (-) symbols indicate whether an interaction was (+) or was not detected (-) between condensin subunits. Three (KLE-2) or four (all others) repetitions of the IP- MudPIT experiments were performed.

SMC		DPY-27 IP	SMC-4 IP	CAPG-1 IP	KLE-2 IP	KLP-7 IP
	MIX-1	✔	✔	✔	✔	✗
	SMC-4	✗	✔	✔	✔	✗
	DPY-27	✔	✗	✔	✗	✗
CAP I						
	DPY-28	✔	✔	✔	✗	✗
	CAPG-1	✔	✔	✔	✗	✗
	DPY-26	✔	✔	✔	✗	✗
CAP II						
	HCP-6	✗	✔	✗	✔	✗
	CAPG-2	✗	✔	✗	✔	✗
	KLE-2	✗	✔	✗	✔	✗

Figure 2.2: Immunoprecipitation (IP) reactions from embryo extracts using condensin antibodies were performed and analyzed on western blots. Pre-immune IP (Pre-im) is a negative control. Selected western blots are shown.

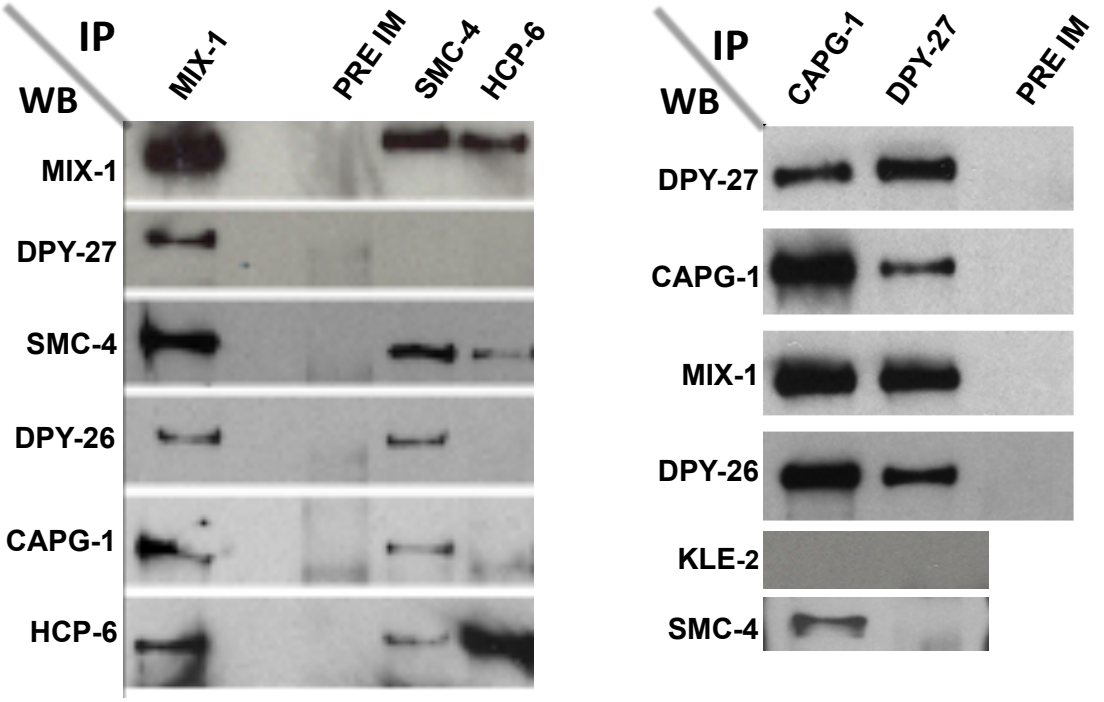


Figure 2.3: Condensin I localizes to mitotic chromosomes in a pattern distinct from condensin I^{DC} or condensin II. Anti-DPY-27 antibody was used to indicate the subcellular localization of condesin I^{DC}, anti-CAPG-1 to indicate both condensin I and condensin I^{DC}, and anti-KLE-2 to indicate condensin II. Condensin I^{DC} does not associate with mitotic chromosomes in early stage hermaphrodite embryos (A), or in male embryos (D), but associates with the two hermaphrodite X chromosomes after the onset of dosage compensation, both during interphase (B) and during mitosis (C). CAPG-1 staining not shared by DPY-27 indicates that condensin I localizes to mitotic chromosomes in both hermaphrodites (E) and males (H). Once dosage compensation initiates in hermaphrodite embryos CAPG-1 localizes to the X chromosomes in interphase, as part of condensin I^{DC} (F). During mitosis CAPG-1 localizes to two bright X foci (as part of condensin I^{DC}) and less intensely to other chromosomes (as part of condensin I) (G). Condensin II shows no distinct pattern during interphase (J), and a centromere-like pattern on mitotic chromosomes in both hermaphrodites (I, K) and males (L). Antibody in green, DNA in red, merge in yellow.

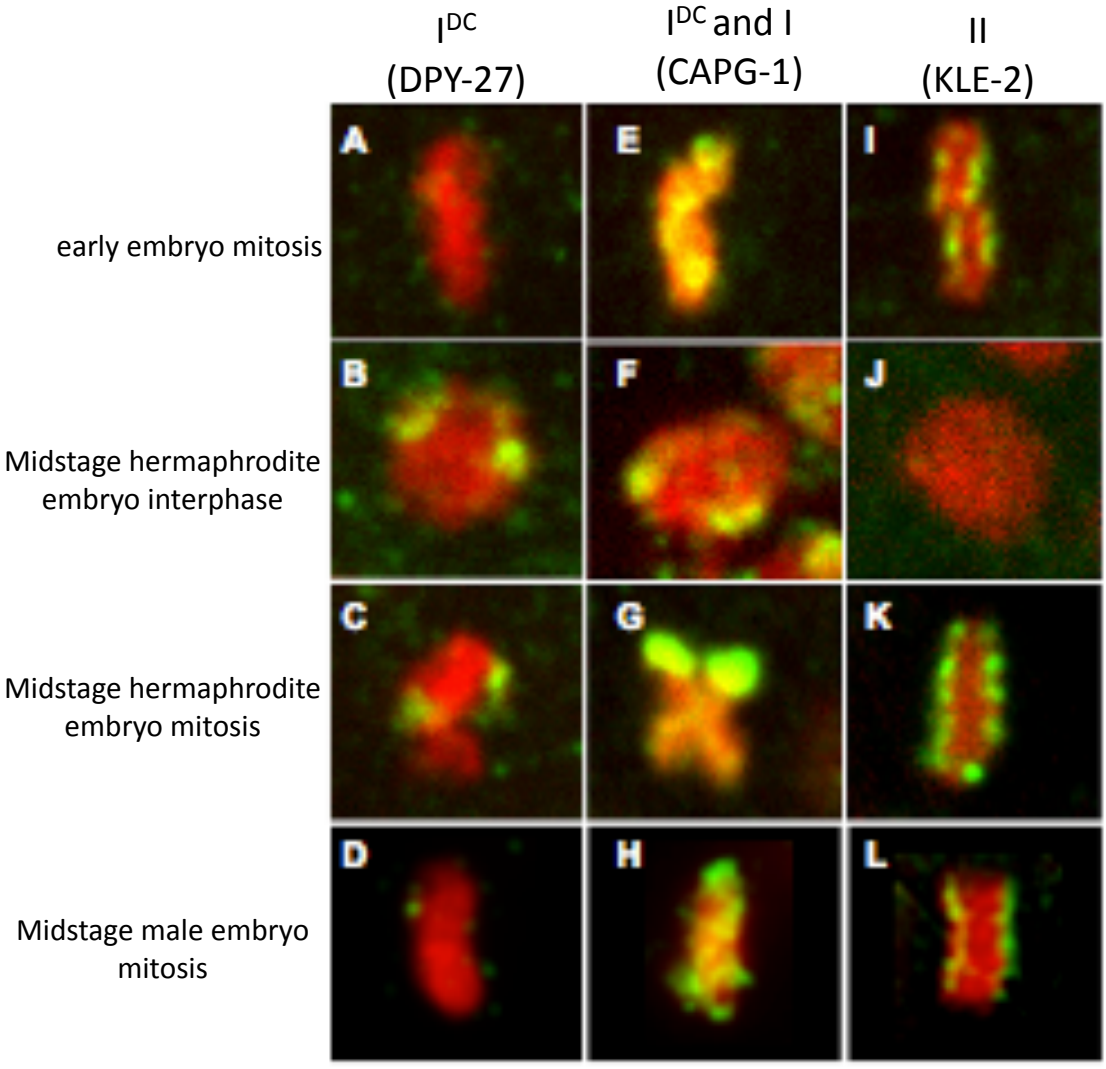


Figure 2.4: Costain of condensin I and condensin II on metaphase chromosomes in an early embryo. Condensin I antibody (CAPG-1) in green, condensin II (HCP-6) antibody in red, DNA in blue. Scale bar = 2μm

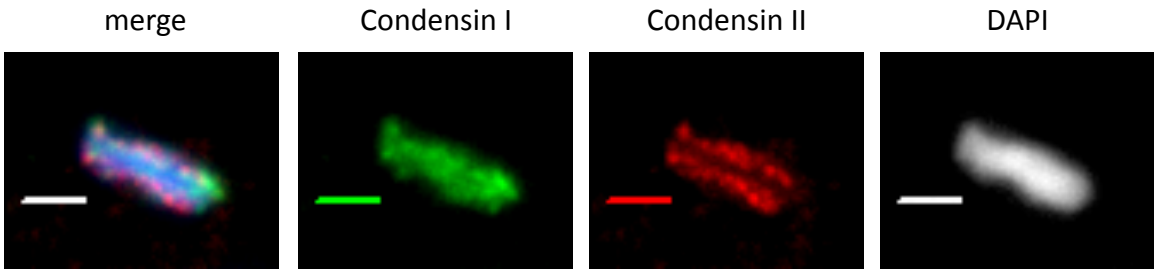


Figure 2.5: Condensin I localizes to meiotic chromosomes in a pattern distinct from condensin I^{DC} or condensin II. (A, B) Anti-DPY-27 antibody was used to indicate the localization of condensin I^{DC}, anti-CAPG-1 to indicate condensin I and condensin I^{DC} and anti-HCP-6 or anti KLE-2 to indicate condensin II. DPY-27 was detected in the nucleoplasm of mature oocytes, but not at other stages of meiosis (data not shown). CAPG-1 staining is nucleoplasmic and surrounds chromosomes in prophase then, as part of condensin I, localizes to the interface between homologs (meiosis I). In contrast, HCP-6 and KLE-2staining indicates that condensin II localizes to the core of each sister chromatid. Condensin I and condensin I^{DC} antibody in green, condensin II antibody in red, DNA in blue. Scale bar = 10μm (A), 5μm (B)

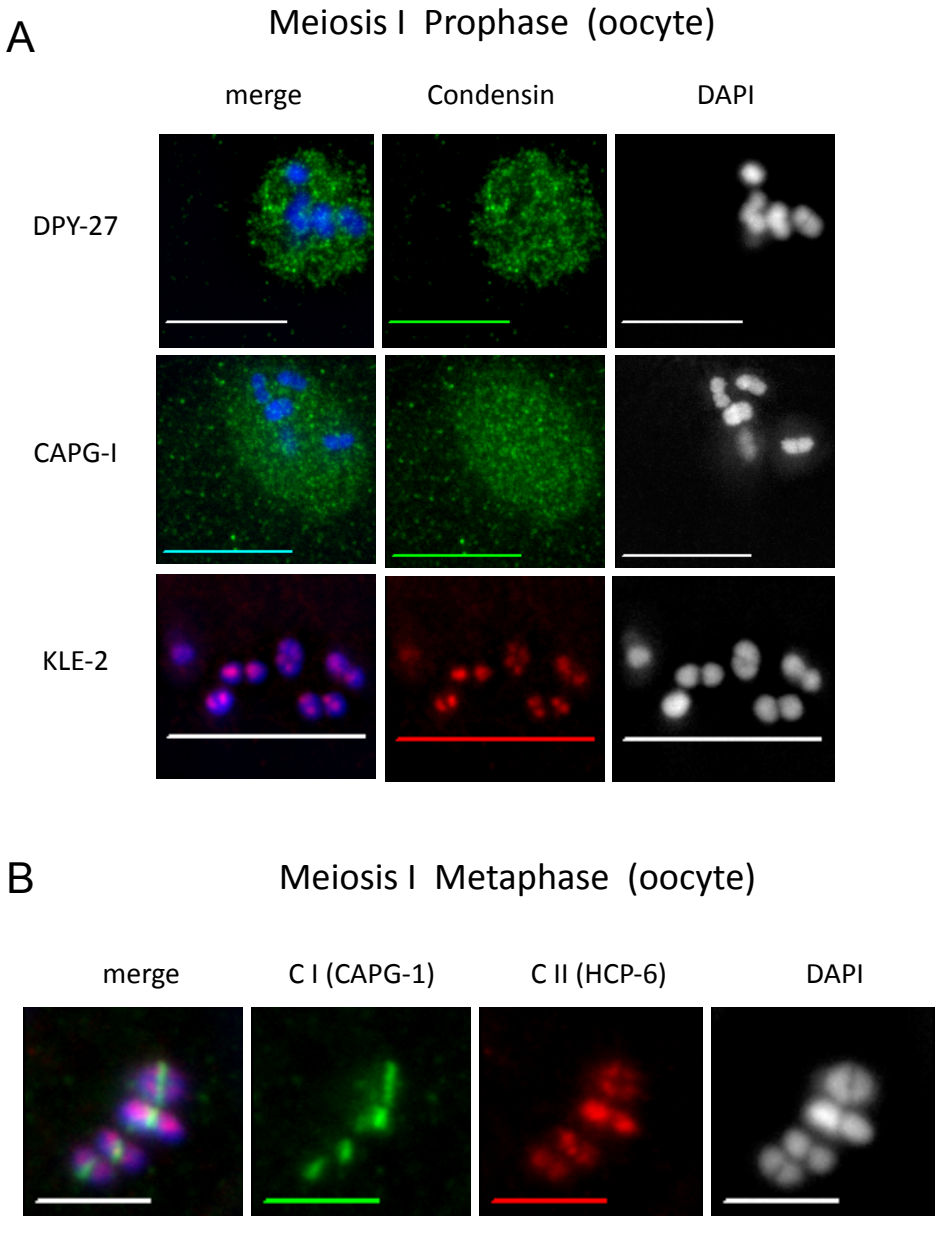


Figure 2.6: Condensin I localizes between homologous chromosomes during meiosis I and between sister chromatids during meiosis II in sperm. Inter-sister localization of CAPG-1 has also been observed in oocytes during meiosis II. Condensin II localizes to the core of sister chromatids throughout meiosis in oocyte and sperm. Condensin I (CAPG-1) in green, Condensin II (HCP-6) in red and DNA in blue. Scale bar = 2μm

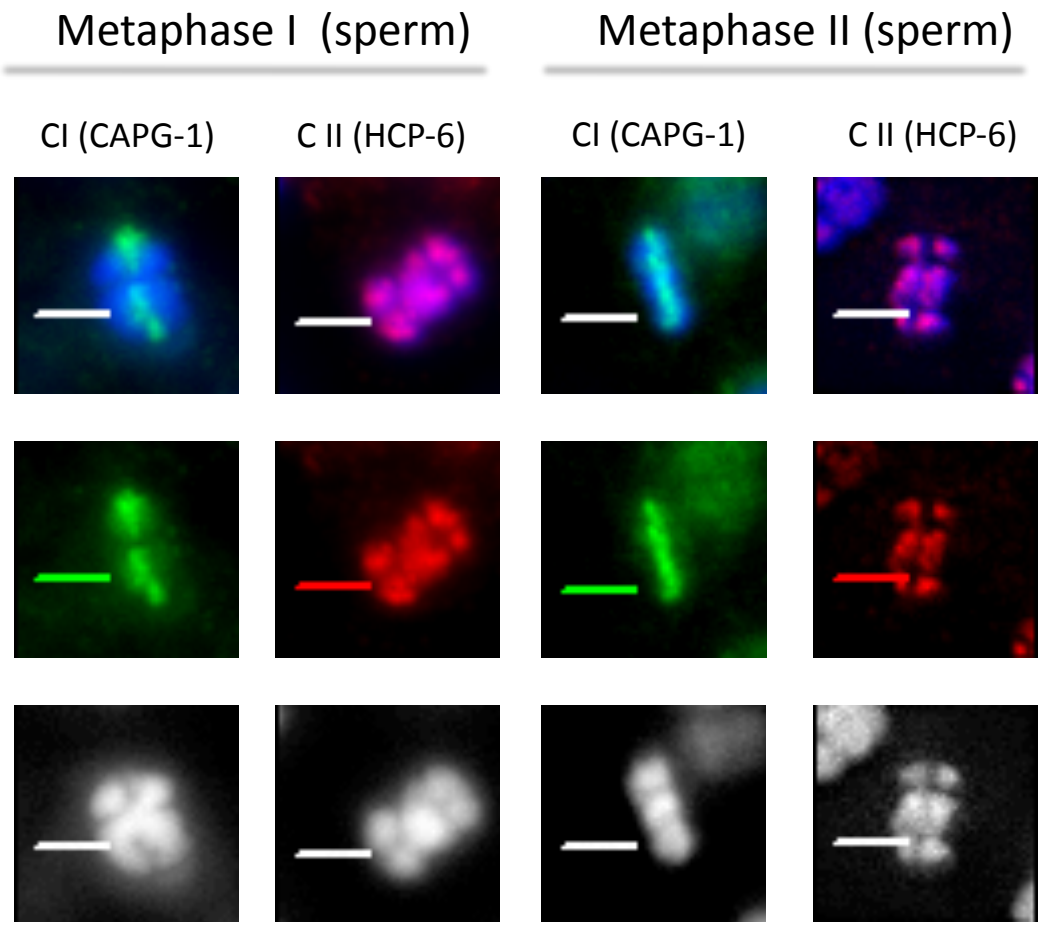


Figure 2.7: MIX-1 localization is a hybrid of condensin I (CAPG-1) and condensin II (KLE-2) patterns. MIX-1 (red) partially colocalizes with CAPG-1 between homologous chromosomes (green, representing condensin I) and KLE-2 on the core of sister chromatids (red, representing condensin II) on sperm chromosomes (blue). Scale bar = 2μm.

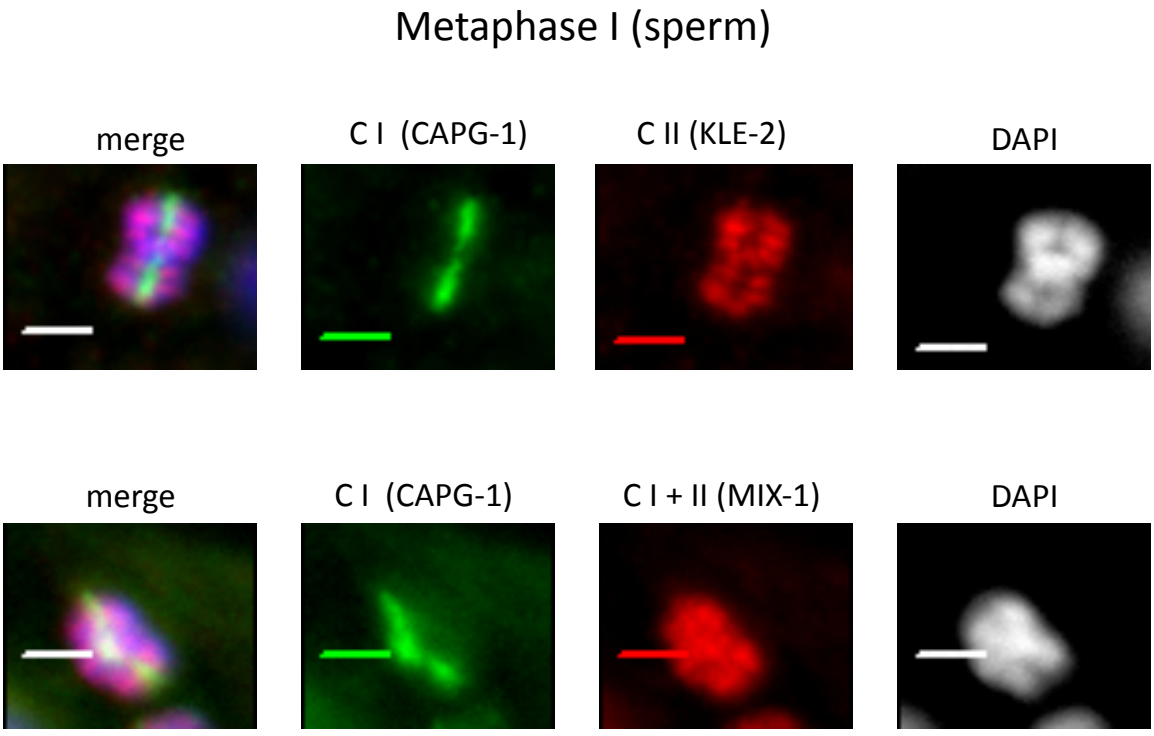


Figure 2.8: CAPG-1 functions in dosage compensation. (A) In vector-control RNAi animals, CAPG-1 (green) localizes to X chromosomes with condensin I^{DC} proteins DPY-28 and MIX-1 (red). (B) CAPG-1 (green) requires other condensin I^{DC} subunits for stable localization to the X. (C) In *capg-1* RNAi animals, DPY-27 and DPY-28 fail to localize to the X. Somatic intestinal nuclei in adult hermaphrodites are shown, with DNA in blue. Scale bar = 10μm.

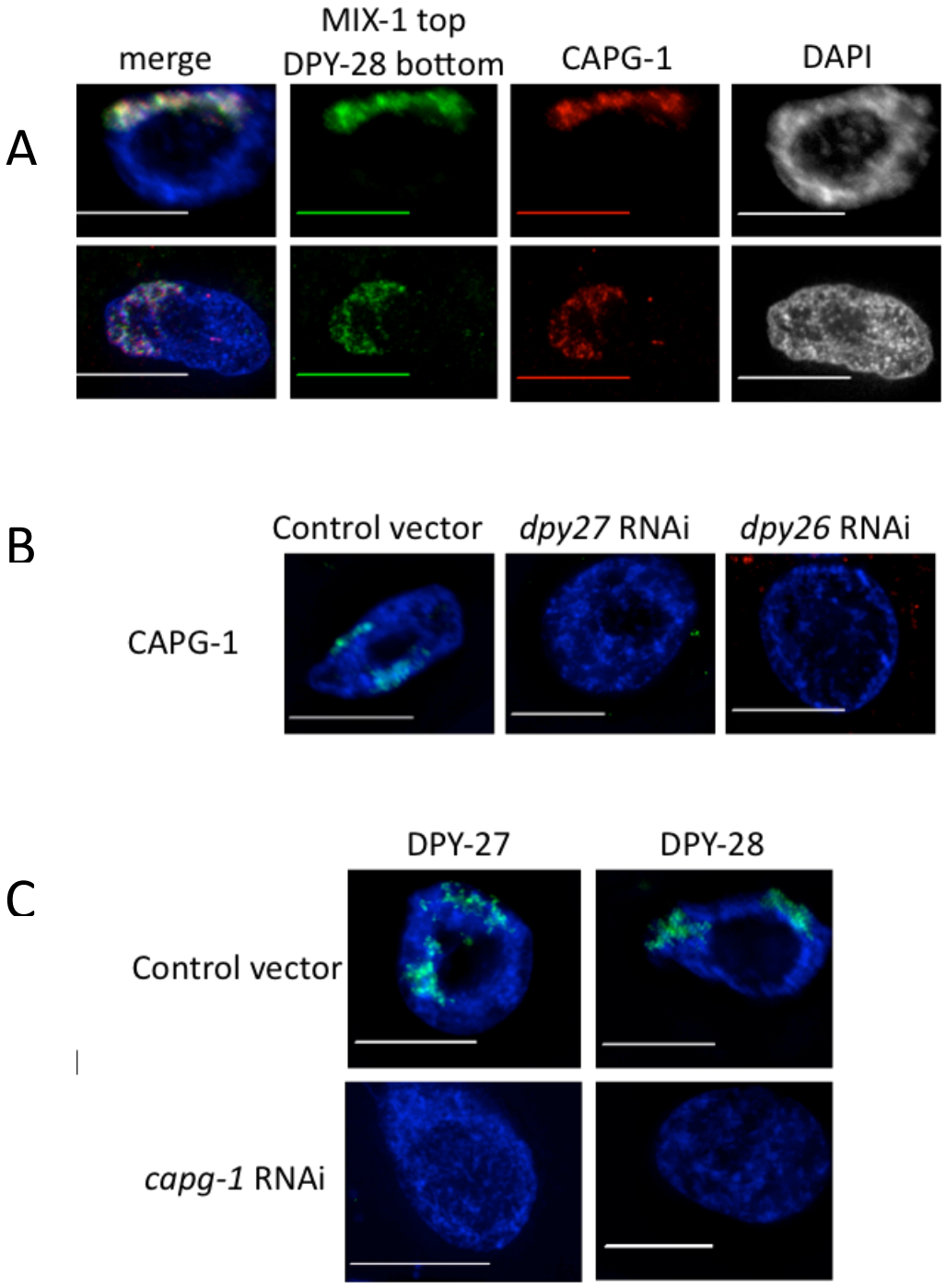


Table 2.2: Genetic assays testing dosage compensation function. Feeding RNAi targeting condensin I^{DC} subunits was minimally lethal in wild-type embryos, and enhanced lethality in the *sex-1* mutant background. By contrast, *xol-1 sex-1* males die due to inappropriate activation of dosage compensation, but can be rescued by RNAi depletion of a condensin I^{DC} subunit. In both assays, *capg-1* RNAi yields results similar to RNAi of other condensin I^{DC} subunits. % lethality was calculated as dead progeny/total progeny x100. Male rescue was calculated as # males/expected number of males x 100. At least 200 animals were scored for RNAi of each gene.

RNAi construct	wild-type % lethality	*sex-1(y263)* % lethality	*xol-1(y9) sex-1(y263)* %male rescue
vector	0.7	15	<1
dpy-27	1.7	92	40
dpy-28	0.7	89	60
dpy-26	0	93	33
capg-1	0.8	91	60

Figure 2.9. Depleting each condensin II subunit results in common chromosomal and developmental defects. (A) Images from time-lapse movies of control or capg-2 RNAi-depleted worms carrying a GFP::histone H2B transgene. At the first embryonic mitosis, capg-2 depleted chromosomes fail to condense into distinct rod shapes during prophase (left). At anaphase, CAPG-2 depleted chromosomes fail to separate completely and abnormal DNA connections are observed (middle). Separating chromatids during meiosis anaphase II also show abnormal DNA connections in CAPG-2 depleted animals (right). (B) Wild-type or homozygous condensin II subunit mutant adult hermaphrodites, stained with DNA dye. Gut nuclei (top row) are separated in wildtype, but often connected in condensin II subunit mutants. Germline nuclei (bottom row) are uniformly sized and evenly spaced in wildtype. Condensin II subunit mutants have fewer germline nuclei, which are abnormally sized and unevenly distributed.

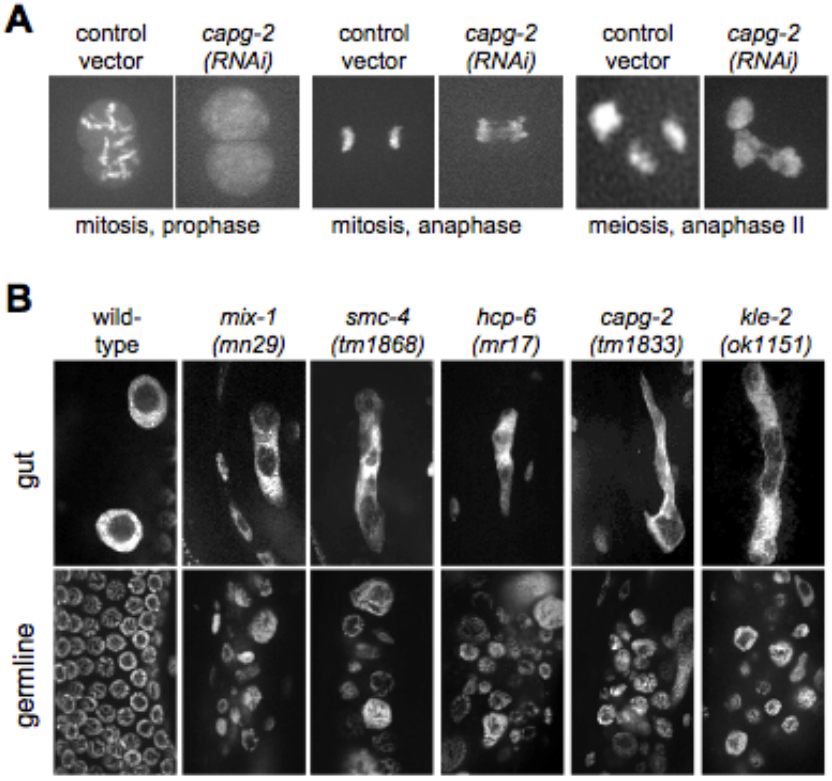


Figure 2.10: Condensin I is required for mitotic and meiotic chromosome segregation. (A) Intestinal nuclei from adults fed on bacteria expressing control vector or dsRNA against the subunit indicated. Intestinal nuclei (stained for DNA) are normally separated in control or *dpy-27* RNAi treated animals, but abnormally connected after depleting condensin I subunits DPY-28, CAPG-1, and DPY-26. (B) Still images from time-lapse movies of the first mitosis in F2 embryos after two generations of RNAi feeding in a GFP:histone H2B strain. Chromosome segregation errors characterized by DNA strands between separating sets of chromosomes were observed with *dpy-28, capg-1*, and *dpy-26* RNAi but not with control or *dpy-27* RNAi depletion. (C) Homologous chromosomes are completely separated at anaphase I in control vector treated animals. Error-prone chromosome segregation during meiosis I is exhibited when condensin I subunit CAPG-1 is depleted, and homologous chromosomes remain connected by unresolved chromatin bridges at anaphase I. Scale bar = 2μm.

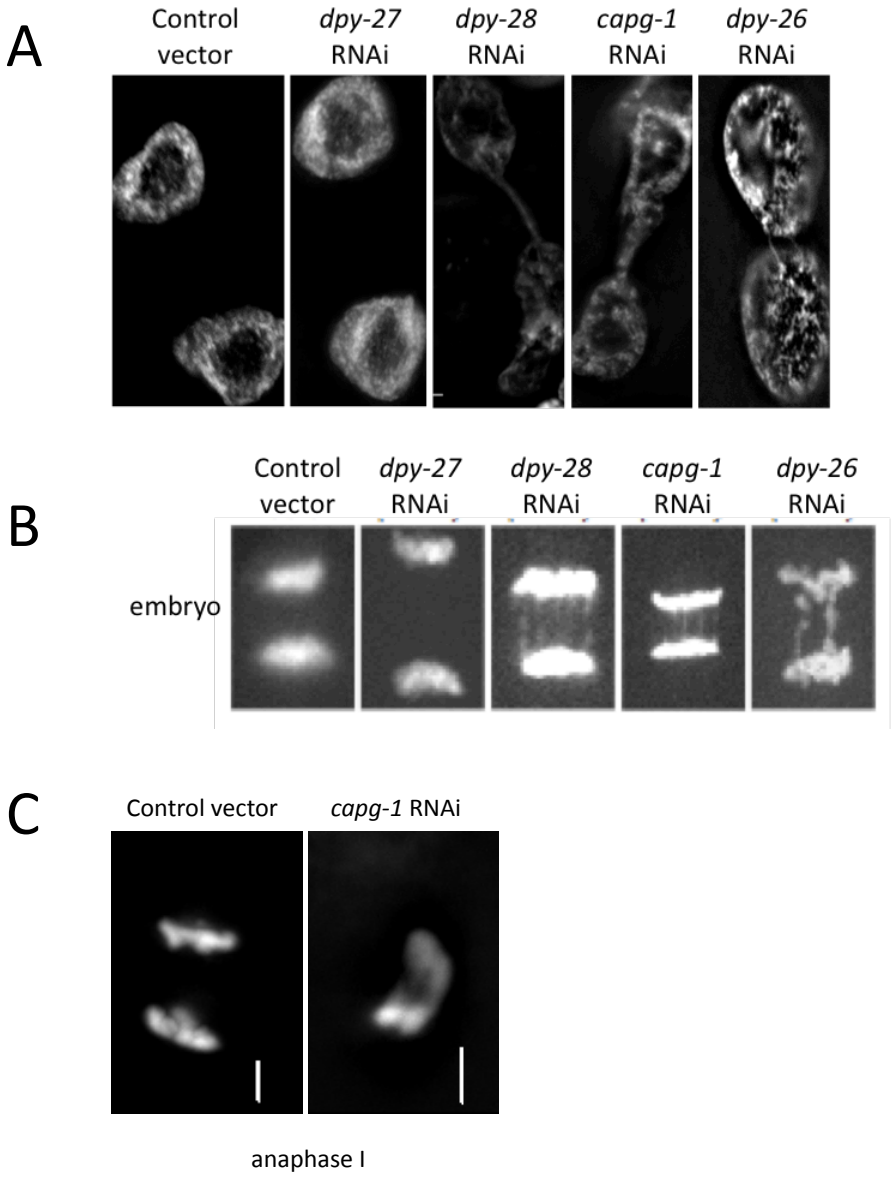
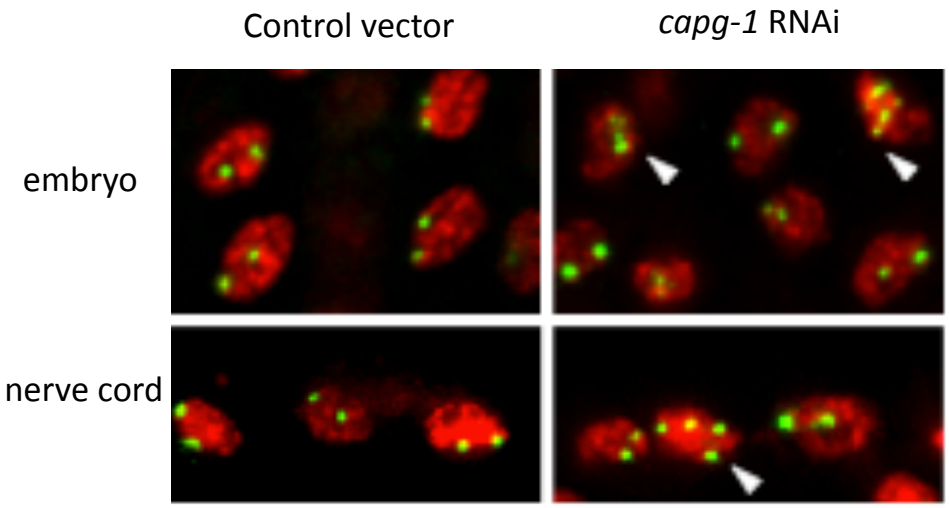


Figure 2.11: FISH analysis of nuclei from mid-stage (about 100-cell) embryos (top) or adult nerve cord cells (bottom) using a 5S rDNA probe. After two generations of RNAi feeding, progeny of animals fed control vector contain diploid nuclei with two signals. Only 3 of 15 vector treated embryos contained nuclei with more than two spots, and in each case, it was limited to a single nucleus within the embryo. By contrast, 14 of 16 capg-1 RNAi-fed progeny contained aneuploid nuclei with more than two signals (arrow). In adults, nuclei in control worms had two signals, indicating diploidy. Only 1 of 8 control treated animals had a nucleus with more than two signals. By contrast, 12 of 13 capg-1 RNAi treated worms had nuclei with multiple signals, indicating aneuploidy (arrowhead). 5S rDNA probe in yellow and DNA in red.



Chapter 3

Different Roles for Aurora B in Condensin Targeting during Mitosis and Meiosis

Summary

Condensin complexes are essential for mitotic and meiotic chromosome segregation. *C. elegans*, like other metazoans, has two distinct mitotic/meiotic condensin complexes (I and II), which occupy distinct chromosomal domains and perform non-redundant functions. Despite the differences in mitotic and meiotic chromosome behavior, we uncovered several conserved aspects of condensin targeting during these processes. During both mitosis and meiosis, condensin II loads onto chromosomes in early prophase and condensin I at entry into prometaphase. During both mitosis and meiosis, the localization of condensin I, but not condensin II, closely parallels the localization of the chromosomal passenger kinase Aurora B/AIR-2. Interestingly, condensin I and AIR-2 colocalize not only on chromosomes. They are also observed on the spindle midzone during anaphase of mitosis and between separating chromosomes during anaphase of meiosis. Consistently, Aurora B/AIR-2 affects the targeting of condensin I, but not condensin II. However, the role AIR-2 plays in condensin I targeting during these processes is different. In mitosis, AIR-2 activity is required for chromosomal association of condensin I. By contrast during meiosis, AIR-2 is not required for condensin I chromosomal association, but it provides cues for correct spatial targeting of the complex.

Introduction

Condensin is a five-subunit complex that functions in the formation, compaction and segregation of mitotic and meiotic chromosomes (Hirano, 2005; Hudson et al., 2009).

Condensin has been isolated in eukaryotic organisms ranging from yeast to humans. Two Structural Maintenance of Chromosome (SMC) subunits of the SMC2 and SMC4 classes form the enzymatic core of the complex. In addition, condensin contains three regulatory Chromosome Associated Polypeptide (CAP) proteins. While yeast has a single condensin complex, higher eukaryotes possess two, condensins I and II, with condensin I being more homologous to the single yeast condensin (Hirota et al., 2004; Ono et al., 2004; Ono et al., 2003). Condensins I and II have identical SMC proteins, and distinct, yet similar CAP components: CAP-G, -D2 and -H in condensin I, and CAP-G2, -D3 and -H2 in condensin II.

In mammalian cells, condensins I and II associate with chromosomes at different times in the cell cycle: condensin II early in prophase and condensin I after nuclear envelope breakdown (NEBD). The two complexes also occupy distinct domains on mitotic chromosomes and perform non-redundant functions (Hirota et al., 2004; Ono et al., 2004; Ono et al., 2003).

How the two condensin complexes are targeted to distinct chromosomal domains in mitosis is not known. Studies in yeast identified *cis*-acting sites that play a role in targeting the single yeast condensin to chromosomes (D'Ambrosio et al., 2008). If *cis*-acting elements also play a role in condensin targeting in metazoans, the elements must be different for the two condensin complexes.

Alternatively, differential targeting may be achieved by trans-acting factors differentially regulating the two complexes. One candidate for such a regulator is the mitotic kinase and chromosomal passenger complex (CPC) member Aurora B. In some experimental systems, Aurora B depletion had no effect on the chromosomal targeting of condensin(s) (Losada et al., 2002; MacCallum et al., 2002; Ono et al., 2004). However, in other studies depletion of Aurora B led to defects in loading of the single yeast condensin in fission yeast (Petersen and Hagan, 2003), and defects of condensin I loading in *Drosophila* (Giet and Glover, 2001), *Xenopus* (Takemoto et al., 2007), and HeLa cells (Lipp et al., 2007).

Interestingly, condensin II in HeLa cells was unaffected (Lipp et al., 2007), indicating that Aurora B may preferentially affect condensin I targeting in mitosis.

We are using *C. elegans* as a model to study the function and differential regulation of condensin complexes. *C. elegans* chromosomes are holocentric, and the kinetochores assemble along the entire length of chromosomes, rather than being localized to a single site as on monocentric chromosomes. Despite this difference, chromosomal proteins and their functions are conserved between worms and other eukaryotes (Maddox et al., 2004). *C. elegans* also has two mitotic/meiotic condensin complexes, and an additional, third condensin, condensin I^{DC}, which functions in the hermaphrodite and X-chromosome-specific process of dosage compensation (Csankovszki et al., 2009; Mets and Meyer, 2009). The two mitotic complexes have identical SMC subunits, SMC-4 and MIX-1, and distinct sets of CAP proteins. Condensin I and condensin I^{DC} only differ in their SMC4 subunits (Figure. 3.1A). While condensins I and II associate with all chromosomes, condensin I^{DC} binds only the X chromosomes in hermaphrodites to downregulate gene expression two-fold, equalizing X-linked product in XX hermaphrodites and XO males (Csankovszki et al., 2009).

Some aspects of condensin loading onto mitotic chromosomes are conserved between monocentric mammalian chromosomes and holocentric worm chromosomes. In both systems, condensin II is enriched at the centromeres (Hagstrom et al., 2002; Ono et al., 2004; Stear and Roth, 2002). *C. elegans* AIR-2/AuroraB was also reported to affect chromosomal association of MIX-1 and SMC-4, components of both condensins I and II (Hagstrom et al., 2002; Kaitna et al., 2002). However, in a different study, recruitment of SMC-4 and the condensin II subunit CAPG-2 appeared unaffected by depletion of AIR-2 (Maddox et al., 2006). These *C. elegans* studies were conducted before the identification of two distinct mitotic complexes. Since SMC proteins are common to condensins I and II, it remains to be determined whether AIR-2/Aurora B is needed for recruitment of one or both condensins.

Compared to mitosis, relatively little is known about condensin distribution and regulation in meiosis. In *C. elegans* meiosis, the two condensin complexes associate with chromosomal domains different from the domain they occupy in mitosis. During meiosis, condensin II localizes to an interior domain within sister chromatids, while condensin I is found between homologs in meiosis I and between sister chromatids in meiosis II (Csankovszki et al., 2009). The differences between mitotic and meiotic localization patterns likely reflect differences in chromosome behavior during these processes. These differences also raise the question whether recruitment mechanisms are comparable between mitosis and meiosis.

The differences between mitotic and meiotic chromosome behavior arise from the unique events during meiosis I, when homologs are separated while sister chromatids stay together. In monocentric organisms the centromere plays a central role in the coordination of these meiotic activities (reviewed in (Sakuno and Watanabe, 2009)). In meiosis I, cohesion between sister centromeres is preserved, while cohesion along chromosome arms is released to allow separation of homologs. In addition, cohesion at the centromeres ensures that microtubules attached to sister kinetochores connect to the same pole, while microtubules attached to kinetochores of homologs are attached to opposite poles to establish tension (Sakuno et al., 2009).

On the holocentric chromosomes of worms the lack of a localized centromere necessitates coordination of meiotic events in a different manner (reviewed in (Schvarzstein et al., 2010)). During worm meiosis, the site of the crossover, and not a localized region of centromeric CENP-A containing chromatin, ultimately determines the plane of chromosome orientation and the site of cohesion release (Monen et al., 2005; Nabeshima et al., 2005). Worm chromosomes typically have a single site of crossover, located in an off-center position. During meiosis I prophase, paired homologs (bivalents) are restructured into cross-shaped figures, in which the short arm corresponds to the region between the crossover and the closer chromosome end, and the long arm corresponds to the region between the crossover and the more distant chromosome end (Chan et al., 2004; Nabeshima et al., 2005). During metaphase, the short arms of

bivalents are lined up along the metaphase plate and the long arms point toward opposite poles. Cohesin along the short arm will be released during meiosis I to separate homologs, and the remaining cohesin will be released in meiosis II to separate sisters (see Figure. 3.4). Since the crossover can happen at either end of the chromosome, the identity of short and long arms is different for the same chromosome in different meioses.

During worm meiosis condensin I is restricted to the short arm of bivalents (Csankovszki et al., 2009). Since the short arm can correspond to either end of the chromosome, condensin I is targeted to different DNA sequences in different meioses. This observation makes it unlikely that *cis*-acting DNA elements provide the primary targeting cue. A more likely targeting signal originates from other chromosomal proteins localizing to the same region. Interestingly, AIR-2/AuroraB, the protein implicated in condensin I targeting during mitosis in various systems, also localizes to the short arm of bivalents (Kaitna et al., 2002; Rogers et al., 2002). How AIR-2/AuroraB activity influences condensin loading in meiosis has not been addressed.

In this study we investigated the timing and regulation of condensin recruitment in mitosis and meiosis in *C. elegans* with particular attention to the role of AIR-2/Aurora B. We found that the need for Aurora B for correct condensin I targeting is conserved between mitosis and meiosis, but the exact role AIR-2 plays differs between the two processes.

Results

Condensin complexes in mitosis

Since condensin I and II are loaded onto chromosomes at different times in mammalian cells (Hirota et al., 2004; Ono et al., 2004; Ono et al., 2003), we set out to determine the timing of condensin loading during *C. elegans* mitosis using immunofluorescence microscopy (IF). In mammalian cells, NEBD marks the entry into prometaphase. By contrast, in *C. elegans,* nuclear pore complexes (NPCs) break down in prometaphase, but the nuclear envelope does not fully disassemble until anaphase (Lee et al., 2000). We monitored the breakdown of NPCs using an antibody (mAb414) which recognizes a

subset of nucleoporins (Davis and Blobel, 1986). Using our fixation conditions, the NPC signal greatly diminishes by prometaphase in embryos of all stages, as judged by chromosome morphology and microtubule staining (Figure 3.1 and data not shown). To investigate the timing of condensin loading onto chromosomes, we used antibodies against CAPG-1, DPY-26, or DPY-28 to mark condensin I, and antibodies against HCP-6 or KLE-2 to mark condensin II (Figure 3.1A). CAPG-1, DPY-26 and DPY-28 are components of both condensins I and I^{DC}. Given that condensin I^{DC} is absent from mitotic chromosomes in early embryos prior to the onset of dosage compensation (Csankovszki et al., 2009), we performed all our analysis in 1-8 cell embryos to focus on the chromosomal targeting of condensin I.

In early prophase only condensin II associated with chromosomes. Condensin I loaded onto chromosomes after NPC disassembly in prometaphase (Figure 3.1B). These data indicate that the timing of condensin loading onto chromosomes is conserved between worm and mammalian mitotic cells. From prometaphase to anaphase, the spatial patterns of condensins I and II were different, with condensin II in a centromere-like pattern (Hagstrom et al., 2002; Stear and Roth, 2002), and condensin I diffusely coating all chromosomes (Figure 3.1B). During anaphase condensin I was also seen colocalizing with microtubules at the spindle midzone, using both IF and live imaging of GFP-tagged CAPG-1 (Figure 3.1C, D), in addition to the chromosomal signal. The other condensin I CAP subunits DPY-26 and DPY-28 associate with chromosomes at the same time and with similar patterns as CAPG-1 (Figure 3.2A). Furthermore, the chromosomal association of CAPG-1 is dependent on the presence of DPY-26 and DPY-28 (Figure 3. 2B), indicating that the condensin I CAP subunits associate with mitotic chromosomes as a complex.

AIR-2 is needed for mitotic recruitment of condensin I but not condensin II

The pattern of condensin I, but not condensin II, on mitotic chromosomes resembles the distribution of AIR-2, the Aurora B homolog in *C. elegans* (Schumacher et al., 1998). Indeed, during metaphase CAPG-1 colocalizes with AIR-2 on chromosome, and during anaphase, the spindle localization of condensin I is co-incident with AIR-2 (Figure 3.3A).

These observations prompted us to examine whether AIR-2 is needed for condensin recruitment in *C. elegans* mitosis.

To inactivate AIR-2 in mitotic cells, worms homozygous for a temperature-sensitive loss-of-function allele, *air-2(or207)* (Severson et al., 2000), were shifted to the restrictive temperature. Control wild type worms were subjected to the same temperature shift. We monitored levels of H3S10Ph, a mark deposited by AIR-2, to assess efficiency of AIR-2 inactivation, and used anti-tubulin as a staining control and to mark mitotic cells. We restricted our analysis to metaphase, at which point both condensin complexes are normally associated with chromosomes. 29/29 control wild type metaphases had condensin I staining, and 20/20 had condensin II staining (Figure 3.3B). In 14/18 metaphases from *air-2(or207)* embryos with no H3S10Ph staining, condensin I was lost or greatly reduced. We attribute the weak condensin I staining on some metaphase figures to residual AIR-2 activity which likely remains even after the temperature shift. By contrast, on most (25 out of 37) mitotic figures with no detectable H3S10Ph, condensin II levels were comparable to wild type controls (Figure 3.3B), while on the remaining figures condensin II levels were reduced, but not absent. We conclude that, similar to what was observed in mammalian cells (Lipp et al., 2007), Aurora B activity preferentially affects the mitotic recruitment of condensin I to chromosomes. Note that the *or207* allele at the restrictive temperature produces a catalytically inactive AIR-2 protein which associates with chromosomes (Severson et al., 2000). Our results indicate that the mutant protein is not sufficient for condensin I recruitment.

Condensin I and II during oocyte meiosis

We next investigated chromosomal targeting of condensin complexes during meiosis in worms. In the *C. elegans* germline, syncitial nuclei are organized in a temporal-spatial array of meiotic stages (Schedl, 1997) (Figure 3.4A). Mitotic nuclei in the distal germline are followed by a meiotic transition zone (leptotene/ zygotene) where homologs begin pairing and alignment. In pachytene, homologs are synapsed via the synaptonemal complex (SC). During late pachytene and diplotene, the SC disassembles, and chromosome pairs are condensed and restructured into compact bivalents (Figure 3.4B)

(Chan et al., 2004; de Carvalho et al., 2008; Martinez-Perez et al., 2008; Nabeshima et al., 2005). In hermaphrodites, oocytes in the proximal germline arrest at diakinesis with homolog pairs organized into six bivalents. The most proximal oocyte, referred to as -1, undergoes maturation followed by fertilization (McCarter et al., 1999). After fertilization, the oocyte-derived nucleus completes meiosis giving rise to two polar bodies and the haploid maternal pronucleus.

To characterize the timing of condensin loading onto chromosomes during oocyte meiosis, we compared CAPG-1 (condensin I) and HCP-6 (condensin II) patterns in the hermaphrodite germline and fertilized embryos. While HCP-6 staining was apparent by early diplotene, as reported previously (Chan et al., 2004), chromosomal association of CAPG-1 was not seen until late diakinesis. During meiosis, NEBD occurs in the -1 oocyte at the time of maturation, immediately preceding fertilization (McCarter et al., 1999). We observed strong CAPG-1 staining only after, but not before, NEBD in the -1 oocytes, while HCP-6 staining is apparent both before and after NEBD (Figure 3.5A). These results indicate that the timing of condensin loading is conserved between mitosis and meiosis in *C. elegans*. After NEBD, the two condensin complexes occupy distinct domains. Condensin I is found at the interface between homologs marked by reduced DAPI staining ("DAPI-free zone"), while condensin II localizes to sister chromatids throughout meiosis (Figure 3.5A). During anaphase, condensin I localized on the acentrosomal meiotic spindle between separating chromosomes (Figure 3.5 A-C). As in mitosis, condensin I co-localized with AIR-2 on chromosomes during prometaphase and metaphase of meiosis, and on the spindle during anaphase (Figure 3.5D).

Condensin I localizes to the short arms of bivalents, where cohesion between the exchanged parts of sister chromatids holds homologs together. Viewed from the side, it appears as a straight line intersecting the bivalent along its shorter axis. Viewed from the end, CAPG-1 appears as a ring around the center of the bivalent (Figure 3.5E), a pattern that has also been seen for AIR-2 and the chromokinesin KLP-19 (Dumont et al., 2010; Wignall and Villeneuve, 2009). This ring-like appearance was observed both in mature oocytes and in fertilized embryos in metaphase of meiosis I as well as at the sister

chromatid interface in meiosis II (Figure 3.5E). This ring-shaped midbivalent domain precedes the formation of anaphase linker structures between chromosomes that may function to drive chromosome separation (Dumont et al., 2010; Wignall and Villeneuve, 2009). The localization of condensin I on the meiotic anaphase spindle may be related to these previously observed central spindle structures (Figure 3.5C).

Before NEBD in oocytes, CAPG-1 is present in the nucleus, but it does not associate with chromosomes. The intensity of nucleoplasmic staining diminishes after NEBD, representing diffusion of the protein into the much larger volume of the oocyte cytoplasm (Figure 3.5A). The same pattern is also seen for the unique condensin I^{DC} subunit DPY-27 and therefore represents the loading of condensin I^{DC} into oocytes in preparation for dosage compensation in fertilized embryos (Chuang et al., 1994; Csankovszki et al., 2009). We used two methods to ensure that the diffuse nuclear staining is not obscuring chromosomal CAPG-1 association. First, we used detergent extraction to reduce nucleoplasmic CAPG-1 staining. In extracted oocytes, chromosomal association of CAPG-1 was still not observed before NEBD, even though chromosomal staining after NEBD remained comparable to unextracted nuclei (data not shown). Second, to reduce condensin I^{DC} levels in oocytes, we examined worms carrying a partial loss-of-function mutation in *dpy-27(y57)*. In these worms, nucleoplasmic staining of CAPG-1 in oocytes is greatly reduced, corresponding to a reduction in condensin I^{DC}, but chromosomal association is still only detected after NEBD (data not shown). Based on these results, we conclude that maximal enrichment of CAPG-1 on chromosomes occurs after NEBD in the -1 oocyte.

To investigate whether all condensin I CAP subunits associate with chromosomes in a similar pattern, we performed IF using anti-DPY-26 and anti-DPY-28 antibodies. We observed DPY-26 and DPY-28 at the DAPI-free zone at the short arms of diakinesis bivalents and also at the chromosome interface in fertilized embryos (Figure 3.6A and B). CAPG-1 localization depends on the presence of DPY-26 and DPY-28, since the short arm staining of CAPG-1 is undetectable in oocytes homozygous for strong loss-of-function alleles, *dpy-26(n199)* and in *dpy-28(s939)* (Figure 3.6 C, D), or upon RNAi

depletion of DPY-26 or DPY-28 (data not shown). Therefore, the condensin I CAP subunits appear to localize to the short arm as a complex.

Condensin localization in the male germline follows the same general pattern (Figure 3.7A). Both condensins I and II begin to accumulate in nuclei by late pachytene. Condensin II associates with chromosomes before NEBD, by the karyosome stage, a stage unique to the male germline in worms (Shakes et al., 2009), but condensin I chromosomal enrichment is not seen until after NEBD in late diakinesis (Figure 3.7A,B). Following NEBD, condensin I is between aligned homologs (meiosis I) and between sisters (meiosis II), while condensin II remains associated with sister chromatids throughout. Interestingly, condensin I behavior in sperm and oocytes differs at anaphase. In oocytes, condensin I colocalizes with microtubules and AIR-2 between separating chromosomes during anaphase (Figure 3.5 C and D). In sperm, AIR-2 and microtubules are not prominent between separating chromosomes, and condensin I is absent from this region. Instead, condensin I and AIR-2 colocalize on the inner edges of separating chromosomes (Figure 3.7C).

AIR-2 restricts condensin I to the short arm of the bivalent

The chromosomal association patterns of condensin complexes are dissimilar in mitosis and meiosis. However, in both cases, condensin I localization closely parallels that of AIR-2, while condensin II occupies a distinct domain (Figures 3.3 and 3.5). This observation, coupled with the finding that condensin I requires AIR-2 for mitotic chromosomal association, prompted us to investigate the potential role for AIR-2 in condensin I recruitment in meiosis.

To deplete AIR-2 levels, we used RNAi in the *air-2* temperature sensitive mutants shifted to the restrictive temperature. We needed to use this combination, since the mutation alone or RNAi alone did not completely eliminate the H3S10Ph signal in meiotic tissues. We limited our analysis to oocytes in which H3S10Ph levels were reduced to below the level of detection by IF. For control, wild type worms fed empty vector RNAi were shifted to the same temperature. In control oocytes after NEBD, we observed condensin I

at the short arm of the bivalent, while condensin II associated with the four sister chromatids (Figure 3.8A). In AIR-2 depleted oocytes, condensin II staining on the four sister chromatids was discernable, despite the somewhat disorganized structure of the bivalent. Similar results were obtained with condensin II subunits HCP-6 (Figure 3.8A) and KLE-2 (data not shown). By contrast, condensin I appeared mislocalized. In AIR-2-depleted oocytes, CAPG-1 occupied a cross shape as though localizing to both arms of the bivalents (Figure 3.8A). Co-staining with CAPG-1 and HCP-6 specific antibodies demonstrated that in AIR-2 depleted oocytes organization of the sister chromatids into four quadrants remained intact, as evidenced by four distinct HCP-6 signals. CAPG-1 occupied the domains between these chromatids in a cross shape (Figure 3.8B). Taken together, similar to mitosis, AIR-2 influences the chromosomal localization of condensin I, but not condensin II, during meiosis. However, unlike in mitosis, AIR-2 activity is not needed to load condensin I onto chromosomes in meiosis, indicating that AIR-2, or H3S10Ph, is unlikely to serve as a direct recruiter of condensin I.

AIR-2 provides spatial cues for Condensin I targeting in meiosis

In worms, crossovers divide bivalents into highly asymmetric structures, with AIR-2 targeted to the short arms. The AIR-2 occupied domain dictates not only the plane of cohesin release (Kaitna et al., 2002; Rogers et al., 2002), but also the plane of chromosome orientation. At metaphase I, the short arms are lined up at the metaphase plate, while the long arms are parallel to spindle microtubules (Albertson and Thomson, 1993; Wignall and Villeneuve, 2009). To further investigate how condensin I is targeted to the short arm, we analyzed mutant backgrounds in which the activity of AIR-2 is not restricted to this specialized domain or in which this domain does not exist due to lack of chiasma formation.

HTP-1, a HORMA domain protein, and LAB-1, a worm-specific chromosomal protein containing a PP1 phosphatase interaction domain, assume a reciprocal localization pattern with respect to AIR-2 and are restricted to the long arm of diakinesis bivalents (de Carvalho et al., 2008; Martinez-Perez et al., 2008) (Figure 3.4B). Complete depletion of these proteins lead to a defect in pairing and chiasma formation, and oocytes contain 12

univalents rather than 6 bivalents. However, in some *htp-1(gk174)* oocytes (homozygous for a deletion of the gene), the two X chromosomes form a bivalent. These rare bivalents lose their asymmetric features, many appear less elongated than wild type, and AIR-2 localizes in a cross shape on both bivalent arms (Martinez-Perez et al., 2008). Similarly, when LAB-1 is partially depleted using RNAi, such that most oocytes still contained six bivalents, AIR-2 localizes to both arms in a cross shape (de Carvalho et al., 2008). We observed AIR-2 spreading on some, but not all *htp-1(gk174)* and *lab-1(RNAi)* bivalents. On bivalents with a cross-shaped AIR-2 domain, CAPG-1 also spread onto both arms of the bivalent, while bivalents that do not show spread of AIR-2 also did not show spread of CAPG-1 (Figure 3.9A). Interestingly, the H3S10Ph domain spread out all over the bivalent, over a much larger domain than that occupied by AIR-2 on all bivalents, perhaps reflecting transient AIR-2 association at these regions (Figure 3.9B). However, condensin I spreading is only observed in the more restricted domain occupied by AIR-2. These data indicate that stable AIR-2 association with chromosomes may be sufficient to guide condensin I localization, but H3S10Ph is not.

We next analyzed mutant backgrounds in which homologs are not held together in meiosis I, and instead of six bivalents they form twelve univalents. Therefore, there is no homolog interface to which both AIR-2 and condensin I would be normally targeted. In the *spo-11(ok79)* (deletion allele) background, chiasmata do not form due to a defect in double strand break formation (Dernburg et al., 1998). Since crossovers are required for the orderly asymmetric organization of bivalents, in *spo-11* mutants, AIR-2 and HTP-1 are localized in a stochastic, rather than orderly manner. By diplotene/diakinesis they acquire mutually exclusive localization patterns, with some *spo-11* univalents staining only with HTP-1, and others only with AIR-2 (Martinez-Perez et al., 2008; Nabeshima et al., 2005). We observed AIR-2 localization on about half of the *spo-11(ok79)* univalents and CAPG-1 and AIR-2 always colocalized (Figure 3.10A). These results indicate that crossover formation is not necessary for condensin I targeting to chromosomes, and that AIR-2 is sufficient to dictate the spatial distribution of condensin I, even when AIR-2 localization is stochastic. Note that on some univalents, AIR-2 and condensin I colocalize along a faint DAPI-light zone intersecting the univalent. Most univalents had H3S10Ph

staining of varying intensity, but condensin I only localized to those with most intense staining, presumably reflecting stable AIR-2 association (Figure 3.10B).

In *rec-8(ok978)* (deletion) mutants, sister chromatids are held together by REC-8 paralogs COH-3 and COH-4 until anaphase I, forming 12 univalents. *rec-8* univalents biorient at metaphase I, and sisters will prematurely separate toward opposite spindle poles at anaphase I (Severson et al., 2009). Unlike on the co-oriented *spo-11* univalents, AIR-2 consistently localizes to a prominent DAPI-free zone between sisters on all twelve *rec-8* bioriented univalents. H3S10Ph intensity was also uniform among univalents. In these oocytes, condensin I colocalized with AIR-2 between sister chromatids, similar to what we observe in wild type meiosis II (Figure 3.10A). Taken together, our data suggest that although AIR-2 is not required for recruitment of condensin I, it provides spatial cues that determine the localization of condensin I on meiotic chromosomes. AIR-2, an important determinant of bivalent asymmetry and chromosome orientation, is also responsible for guiding condensin I to the chromosomal domain which will be aligned at the metaphase plate, whether on wild type meiosis I bivalents, wild type meiosis II sister chromatids, or *rec-8* mutant meiosis I univalents.

Discussion

Timing of condensin loading to chromosomes in mitosis and meiosis

Meiosis includes a prolonged prophase I during which homologous chromosomes pair, synapse and exchange genetic material. By contrast, prophase in mitosis is relatively brief. Despite these differences, condensin complexes load at analogous time points: condensin II as chromosomes begin to condense in early prophase and condensin I in prometaphase. This time point in worm oocytes coincides with maturation and fertilization. The timing of condensin I and II recruitment in mitosis is conserved between worms and mammals (Ono et al., 2004), raising the possibility that it is also conserved in meiosis in all metazoans. Consistent with that, condensin I loads onto chromosomes by prometaphase in mouse spermatocytes (Viera et al., 2007). However, condensin II or NEBD were not analyzed in this study.

What triggers condensin I loading at NEBD/prometaphase is unclear. All components of condensin I are present in the nucleoplasm prior to NEBD, yet they do not associate with chromosomes. This is clearly demonstrated in the male germline, where condensin CAP subunit staining cannot be attributed to the presence of condensin I^{DC}. Aurora B activity and H3S10Ph staining is also apparent on both mitotic and meiotic chromosomes prior to prometaphase, excluding the possibility that H3S10 phosphorylation triggers condensin I assembly. Future studies will be needed to determine how the timing of condensin I loading is coordinated with other cell cycle events.

Mitotic recruitment of condensin complexes

Similar to what was observed in mammalian cells (Lipp et al., 2007), AIR-2 inactivation in worms disrupts efficient recruitment of condensin I to mitotic chromosomes, but condensin II recruitment is unaffected. It remains unclear whether this reflects a direct recruitment by the kinase or its chromatin mark H3S10Ph, or alternatively, it reflects a need for an AIR-2 mediated change in chromatin structure. Our results also resolve previous conflicting data in the field. Previous studies concluded that AIR-2 is required for loading of SMC proteins MIX-1 and SMC-4 (shared between condensins I and II) onto mitotic chromosomes (Hagstrom et al., 2002; Kaitna et al., 2002). However, a different study failed to detect a noticeable change in SMC-4 and CAPG-2 (condensin II CAP subunit) recruitment (Maddox et al., 2006). We suggest that the observed reduced recruitment of SMC-4 and MIX-1 upon AIR-2 depletion reflects a loss of condensin I from chromosomes. Since condensin II recruitment is unaffected, its subunits remain on chromosomes. Our results also explain the findings that MIX-1 function before prometaphase is AIR-2 independent, yet its chromosomal association in metaphase is (at least partially) AIR-2 dependent (Kaitna et al., 2002). Prior to prometaphase, condensin II complexes containing MIX-1 associate with chromosomes in an AIR-2-independent manner and facilitate chromosome condensation. By contrast, after prometaphase, condensin I complexes also containing MIX-1 associate with chromosomes in an AIR-2-dependent manner.

Meiotic recruitment of condensin complexes

Despite the differences in mitotic and meiotic chromosome architecture, condensin I occupies the same domains as AIR-2 in both processes (this study), and condensin II colocalizes with centromeric protein CENP-A in both processes (Chan et al., 2004; Hagstrom et al., 2002; Stear and Roth, 2002). Consistently, AIR-2 is required for condensin I recruitment in mitosis and for correct condensin I localization in meiosis, but not for condensin II targeting in either process. By contrast, CENP-A is needed for recruitment of condensin II in mitosis, but not during meiosis (Chan et al., 2004; Stear and Roth, 2002). The fact that condensin II can load onto meiotic chromosomes in the absence of CENP-A, is consistent with CENP-A function being dispensable during *C. elegans* meiosis (Monen et al., 2005).

Our data is consistent with a wild type AIR-2 protein or its chromatin mark H3S10Ph serving as a direct recruiter for condensin I in mitosis. However, an inactive kinase is not sufficient for recruitment (see Figure 3.3). In meiosis, AIR-2 plays a different role. In meiosis, condensin I can associate with chromosome in the absence of AIR-2, but without targeting cues from AIR-2, it localizes to both bivalent arms. Interestingly, we observed similar spreading of condensin I in oocytes in which the AIR-2 domain is expanded, indicating that when present, AIR-2 is sufficient to dictate condensin I localization. Consistent with that, condensin I also colocalizes with AIR-2 on *spo-11* univalents (where AIR-2 distribution is stochastic), and at the sister chromatid interface on bioriented *rec-8* univalents.

The more limited localization of AIR-2 compared to the broader distribution of H3S10Ph in the *lab-1(RNAi)* and the *htp-1(gk174)* backgrounds is reminiscent of what was observed for some histone modifying enzymes and their modification in the context of gene silencing (Kahn et al., 2006; Papp and Muller, 2006; Schwartz et al., 2006) or activation (Gelbart et al., 2009; Parker et al., 2008). It is unclear whether it represents a transient spreading of AIR-2 to phosphorylate H3S10 in a broader region, or transient looping of other chromosomal territories into the AIR-2 occupied domain for

modification. In any case, H3S10Ph was not sufficient to mislocalize condensin I. Only where AIR-2 was detectable by IF, could we see a spreading of the condensin I occupied domain.

Condensin I and chromosomal passengers

It is intriguing that condensin I colocalizes with AIR-2 not just on chromosomes, but also on the anaphase spindle, both in mitosis and in meiosis. Localization on the anaphase spindle is most prominent during the acentrosomal oocyte meiotic anaphase (Figure 3.5A-C), when spindle microtubules are found predominantly between chromosomes (Dumont et al., 2010; Wignall and Villeneuve, 2009), but can also be detected during mitosis (Figure 3.1B-D). During centrosome-based sperm meiosis, midbody microtubules are not prominent, and cytokinesis is sometimes incomplete until spermatids bud off from the residual body (Shakes et al., 2009). Under these circumstances, condensin I and AIR-2 levels are also low between separating chromosomes. The spindle localization of condensin I mirrors Aurora B kinase and other CPC components (Rogers et al., 2002) and it will be interesting to determine whether condensin I contributes to Aurora B function at this stage.

Is the role of Aurora B in condensin I targeting during meiosis conserved?

Aurora B regulates many events to coordinate cell division, including kinetochore microtubule attachments, chromosome orientation, cohesion release and cytokinesis. Most of these functions are conserved between monocentric and holocentric organisms, with some important differences in meiosis (Figure 3.11). On monocentric chromosomes Aurora B is needed for coorientation of sister kinetochores and biorientation of kinetochores of homologs by destabilizing improper kinetochore-microtubule attachments at the centromeres (Hauf et al., 2007; Monje-Casas et al., 2007). In holocentric organisms like *C. elegans*, localized centromeres are lacking, and instead the location of crossover determines which end of the chromosome will form the short arm of the bivalent (Nabeshima et al., 2005), which in turn determines the plane of chromosome orientation (Albertson and Thomson, 1993; Wignall and Villeneuve, 2009). In both monocentric and in holocentric organisms, Aurora B is located in an ideal position to

monitor homolog biorientation and sister co-orientation: at the centromeres in monocentric organisms and at the bivalent short arm in holocentric organisms. However, the role of Aurora B in the regulation of sister chromatid cohesin during meiosis I is different in monocentric and holocentric organisms. During meiosis I, sister chromatid cohesion is preserved at centromeres of monocentric chromosomes and at the long arm of holocentric chromosomes, while cohesion is released along chromatid arms of monocentric chromosomes and the short arm of holocentric bivalents. In monocentric organisms Aurora B promotes preservation of cohesion at centromeres (Monje-Casas et al., 2007; Resnick et al., 2006; Yu and Koshland, 2007). By contrast, in worms, AIR-2/AuroraB functions to promote cohesion release at the short arm (Kaitna et al., 2002; Rogers et al., 2002).

In monocentric organisms the activities that orient chromosomes and those that maintain connections between sisters during meiosis are located at the same place, at the centromere. By contrast, in holocentric organisms these activities are located at opposite domains: chromosome orientation is achieved by activities along the short arms of bivalents, while preservation of connection between sisters is achieved along the long arm (de Carvalho et al., 2008; Martinez-Perez et al., 2008; Rogers et al., 2002). These spatial differences likely explain why Aurora B evolved different roles with respect to regulation of cohesion in these organisms. Given the similarities and differences in Aurora B functions in monocentric and holocentric organisms, it will be interesting to determine which aspects of condensin I regulation by Aurora B are conserved in meiosis in monocentric organisms.

Materials and Methods

C. elegans strains

All strains were maintained as described (Brenner, 1974) and grown at 20°C, unless indicated otherwise. For analysis of AIR-2 deficiency, *air-2 (or207ts*) L4 worms were shifted to 25 ^{0}C for 24 h. Strains include N2 Bristol strain (wild type), EKM28 *unc-119(ed3)* III; *cldEx4 [Ppie-1::CAPG-1::GFP unc-119(+)]*, EU630 *air-2(or207ts)*I, VC666 *rec-8*(*ok 978*) IV/ nT1[*qIs51*](IV; V), TY0420 *dpy-27*(y57)III, TY3837 *dpy-28(s939)*III/ qC1, and TY4341 *dpy-26*(n199) *unc-30(e191)*/ nT1(G)IV;V, EKM21 *spo-11(ok79)* IV/nT1(G) IV;V, EKM22 *htp-1(gk174)*IV/nT1(G) IV;V.

Antibodies

Primary antibodies: rabbit α-CAPG-1(Csankovszki et al., 2009); rabbit α-KLE-2, α-DPY-26 and αDPY-28 (Kirsten Hagstrom [U Mass Worchester] (Csankovszki et al., 2009)), rabbit α-HCP-6 (Raymond Chan [U Michigan] (Chan et al., 2004)), rabbit α-AIR-2 (Jill Schumacher [U Texas, MD Anderson Cancer Center] (Schumacher et al., 1998)), mouse α-H3S10Ph (6G3) (Cell Signaling Technology), mouse α-NPC [mab414] (Abcam), mouse α - alpha- tubulin (DM1A) (Sigma). Secondary antibodies: FITC or cy3 conjugated donkey anti-rabbit, and donkey anti-mouse (Jackson Immunoresearch).

RNA interference

RNAi by feeding was performed as described (Kamath et al., 2003). To generate an RNAi construct for *air-2*, a genomic region was PCR amplified (using primers catgctcgagtggacatttccatgtagcga and gatcaagcttggggttagacgattgggaa), digested with Xho I and Hind III cloned into the DT7 vector (Kamath et al., 2003). For *lab-1* RNAi, bacterial cultures were grown at 37^{0}C for 20 hours and induced with IPTG for two hours prior to plating; for *air-2* RNAi, 50 mL bacterial cultures were grown at 37^{0}C for 20 hours, induced with IPTG for two hours, pelleted and resuspended in 500μL of fresh LB broth and plated as a concentrated bacterial lawn. RNAi was initiated at the L1 stage. L4 worms were transferred to fresh plates, and allowed to produce progeny (F1) for 24 hours. F1 worms were processed 24 hours post L4 for IF. To deplete AIR-2 in meiosis, *air-2(or207ts)* hermaphrodites were grown on AIR-2 RNAi plates at 25^{0}C from L1 to

adulthood. Control experiments were performed using the same conditions as RNAi experiments.

Immunostaining

Adult worms were dissected in 1X sperm salts (50 mM PIPES pH7, 25 mM KCl, 1 mM $MgSO_4$, 45 mM NaCl, 2 mM $CaCl_2$), fixed in 2% paraformaldehyde in 1X sperm salts for five minutes and frozen on dry ice for ten minutes. Slides were washed three times for ten minutes each in PBS/0.1% Triton (PBST) before incubation with 30μL of diluted primary antibody in a humid chamber, overnight at room temperature (RT). Double labeling of samples was performed with all primary antibodies simultaneously during this overnight incubation. Slides were then washed three times, ten minutes each, with PBST, incubated for one hour with 30μL diluted secondary antibody at 37^0C, washed again twice for ten minutes each with PBST, and once for ten minutes with PBST plus DAPI. Slides were mounted with Vectashield (Vector Labs). For colocalization studies of CAPG-1 and AIR-2, or CAPG-1 and HCP-6, rabbit antibodies were directly labeled using the Zenon Rabbit IgG labeling kit (Molecular Probes) according to manufacturers instructions.
Detergent extraction: Nucleoplasmic proteins were extracted from oocytes by dissecting adults in 1X sperm salts plus 1 % Triton, and processed as above.
Methanol-acetone fixation (Fig 3.6A): Adult hermaphrodites were dissected in 1X sperm salts, and frozen on dry ice for ten minutes. The slides were fixed for one minute each in methanol followed by acetone at -20^0C. Slides were washed three times for ten minutes each in PBST, before incubation with primary antibody.

Embryos were obtained from hermaphrodites by bleaching, fixed with Finney fixative (2% paraformaldehyde, 18% methanol, 10mM PIPES pH 7.5, 60 mM KCl, 8 mM NaCl, 2.6 mM EGTA, 0.4 mM spermidine, 0.16 mM spermine, 0.4% b-mercaptoethanol), frozen at -80^0C for 20 minutes, thawed, fixed for 20 minutes at RT, and washed in PBST for 15 minutes. Samples were incubated with primary antibody overnight at RT. Embryos were washed three times (15 minutes each) in PBST, and incubated overnight with secondary antibody at RT. This incubation was followed by two PBST washes and a third wash in PBST plus DAPI. Embryos were mounted on slides with Vectashield.

Images were captured using a Hamamatsu ORCA-ERGA CCD camera on an Olympus BX61 motorized X-drive microscope using a 60X PlanApo oil immersion objective with a NA of 1.42. Images were captured in Z stacks with planes at 0.2μm intervals and deconvolved and projected with 3i Slidebook software. Adobe Photoshop was used for image assembly.

Live imaging

Embryos were dissected into blastomere culture medium (Shelton and Bowerman, 1996) and mounted in a hanging drop to alleviate osmotic and mechanical pressures. Imaging was performed on a PerkinElmer Spinning-disk confocal system with a Nipkow CSU10 scanner (Yokogawa), an EM-CCD camera (C9100-50, Hamamatsu Photonics), an inverted microscope (Axio Observer; Carl Zeiss, Inc.) and a 63X Plan Apochromat 1.4NA objective (Carl Zeiss, Inc.). Images were acquired with Volocity acquisition software (PerkinElmer) and collected every 5 seconds with 2 second exposure time and 2X binning. Images were processed with Gaussian blur and to reduce noise and levels adjustments using ImageJ (Abramoff et al., 2004).

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Figure 3.1: Condensin I and II during mitosis. (A) Subunit composition of human and C. elegans condensin complexes. (B) In early prophase, condensin I (CAPG-1, green) is not detected on chromosomes, while condensin II (HCP-6, green) localizes to the centromeres. From prometaphase on, condensin I discontinuously coats mitotic chromosomes and condensin II maintains its centromere-enriched localization. Nuclear pore complex staining is shown in red, DAPI in blue. All images are from 2-8 cell embryos. (C) Longer exposure of an anaphase figure from an 8-cell embryo reveals condensin I (CAPG-1, green) staining on spindle midzone microtubules (red). (D) Live imaging of CAPG-1::GFP in an 8-cell embryo reveals similar patterns of chromosomal association at metaphase and spindle localization during anaphase (arrowhead). Scale bar = 5μm

A

Human condensin I	*C. elegans* condesin I^{DC}	*C. elegans* condensin I	Human condensin II	*C. elegans* condensin II
SMC2	MIX-1	MIX-1	SMC2	MIX-1
SMC4	DPY-27	SMC-4	SMC4	SMC-4
hCAP-D2	DPY-28	DPY-28	hCAP-D3	HCP-6
hCAP-G	CAPG-1	CAPG-1	hCAP-G2	CAPG-2
hCAP-H	DPY-26	DPY-26	hCAP-H2	KLE-2

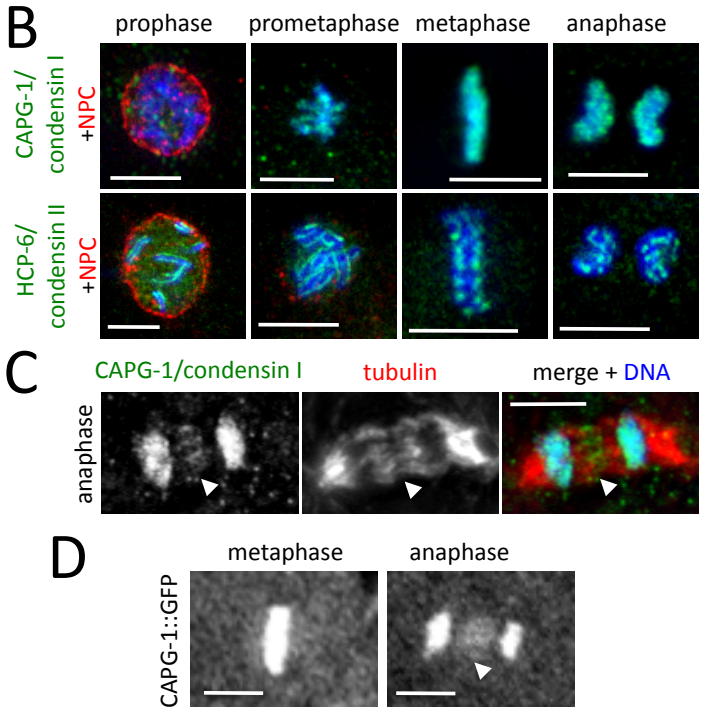


Figure 3.2: The CAP subunits of condensin I associate with mitotic chromosomes as a complex. (A) Similar to CAPG-1, DPY-26 and DPY-28 (green) also associate with mitotic chromosomes from prometaphase to anaphase, but not in early prophase. Nuclear pore complex is shown in red and DAPI in blue. (B) In the absence of DPY-26 or DPY-28, CAPG-1 no longer associates with mitotic chromosomes. Metaphase plates from young embryos are shown, CAPG-1 in green, tubulin in red. In DPY-28 depleted embryos, CAPG-1 binding to mitotic chromosomes is undetectable, while in DPY-26 depleted embryos, CAPG-1 staining is reduced (top row) or absent (bottom row). DAPI is shown in blue and gray. Scale bar = 2μm.

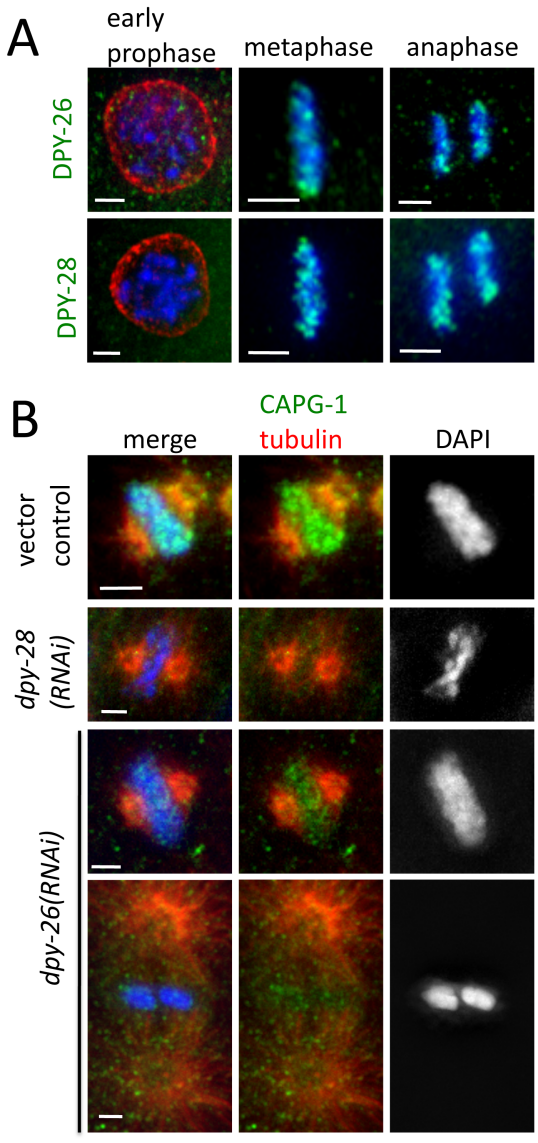


Figure 3.3: Condensin I, but not II, depends on AIR-2 for mitotic recruitment.
(A) Condensin I (CAPG-1, green) and AIR-2 (red) colocalize on mitotic chromosomes at metaphase. At anaphase, AIR-2 dissociates from DNA and localizes to the spindle midzone. Condensin I remains on DNA, but also colocalizes at the midzone with AIR-2. (B) Chromosomal association of condensin I (CAPG-1, green) depends on the activity of AIR-2. In air-2 mutant embryos, the H3S10Ph mark (red) is undetectable, condensin I is not recruited to metaphase chromosomes, but condensin II (HCP-6, green) recruitment less affected. All examples are from the 1st, 2nd, or 3rd mitotic division. Scale bar =2μm

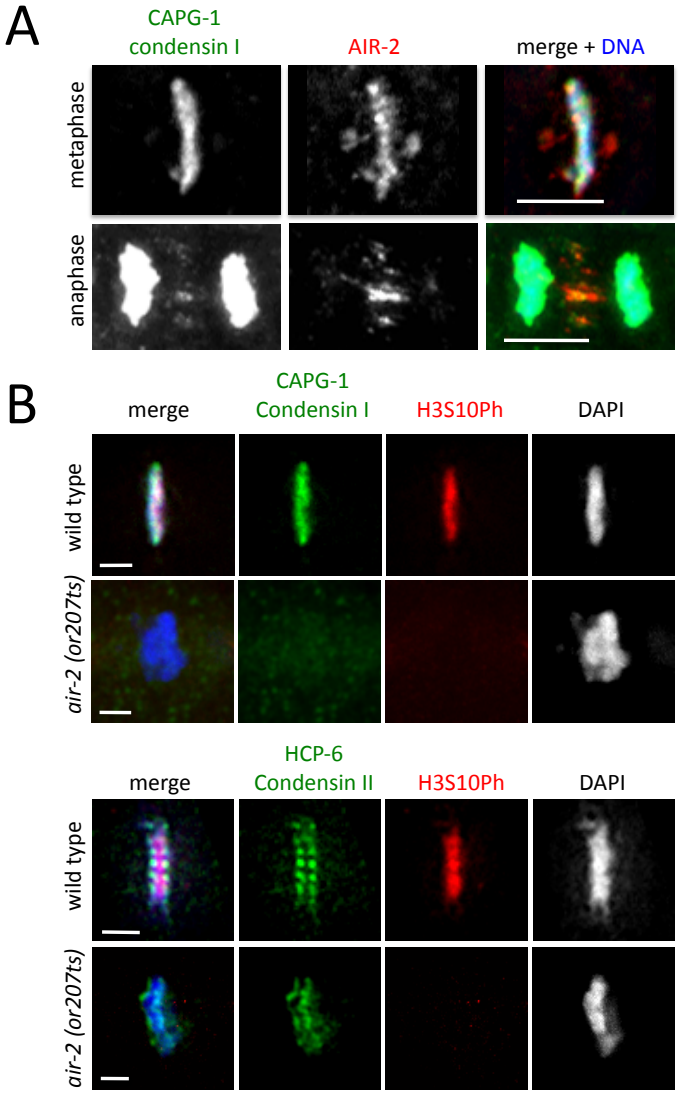


Figure 3.4: C. elegans meiosis. (A) A diagram of the adult hermaphrodite gonad. Nuclei enter meiosis in the transition zone (TZ, leptotene and zygotene), and proceed through pachytene, diplotene, and diakinesis prior to fertilization. Sperm are stored in the spermatheca (sp). The most proximal oocyte is designated -1. Oocytes move through the spermatheca, are fertilized and then complete meiotic divisions (MI and MII). A set of homologs is extruded as the first polar body (PB1) during MI, and a set of sister chromatids is extruded as the second polar body (PB2) during MII. Oocyte pronucleus (O), sperm-derived pronucleus (S). (B) Meiotic chromosomes are extensively restructured between pachytene and diakinesis. During pachytene, replicated chromosomes are held together by the SC (i). A single off-center crossover divides the paired homologs into two domains. At pachytene exit, HTP-1 and LAB-1 are retained between the crossover and the more distant chromosome end, and SYP-1 is retained between the crossover and the closer chromosome end. AIR-2 is recruited to the domain where SYP-1 is retained (ii). The AIR-2 bound domain becomes the short arm, and the HTP-1/LAB-1 bound domain becomes the long arm of the bivalent (iii). Bivalents undergo extensive condensation (iv and v). At metaphase of meiosis I, bioriented bivalents are aligned with their long arms parallel to spindle microtubules and the AIR-2 occupied domain at the metaphase plate (spindle pole axis indicated by arrows). In meiosis I, AIR-2 promotes cohesion loss at the short arm and homologs move away from each other (v). In meiosis II, the AIR-2 occupied domain at the sister chromatid interface is aligned at the metaphase plate and sister chromatids become bioriented and eventually separated (vi).

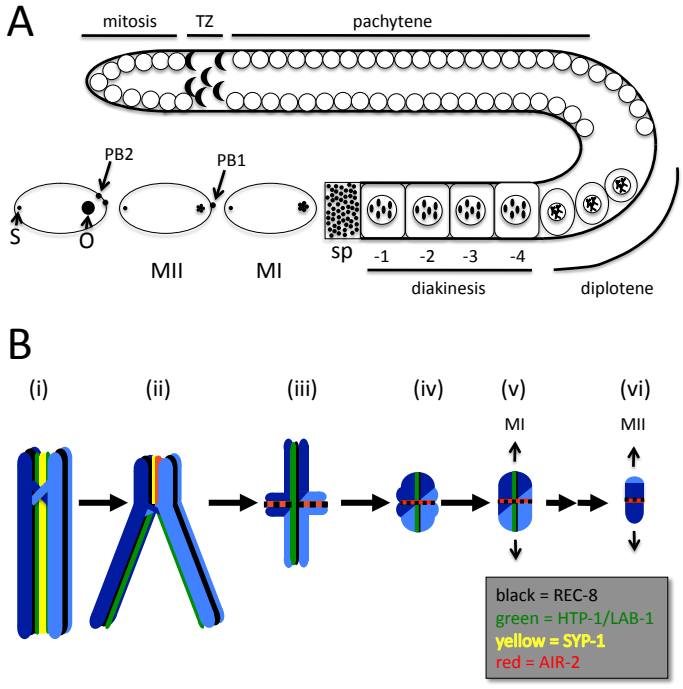


Figure 3.5: Condensin I and II in oocyte meiosis. (A) Condensin I (CAPG-1, green) associates with the short arm of bivalents only after NEBD in the -1 oocyte. During metaphase I, condensin I localizes between homologous chromosomes, at anaphase I between separating homologs, at metaphase II between sister chromatids and on the polar body (PB). Condensin II (HCP-6, green) associates with chromosomes prior to NEBD and remains at the core of sister chromatids throughout meiosis. Nuclear pore complex is shown in red, DAPI in blue. (B) Live imaging of CAPG-1::GFP showing condensin I on chromosomes and between separating chromosomes in a fertilized oocyte undergoing meiosis I. (C) Condensin I (CAPG-1, green) and tubulin (red) localization patterns. During acentrosomal meiosis, microtubules on the poleward side disassemble after metaphase and are seen primarily between separating chromosomes during anaphase. Condensin I colocalizes with microtubules during anaphase I and anaphase II. (D) Condensin I also colocalizes with AIR-2 at the metaphase plate, and on the anaphase I spindle. (E) Enlarged images of a diakinesis bivalent (meiosis I), and pair of sister chromatids during meiosis 2. Viewed from the side, condensin I (CAPG-1, green) and AIR-2 (red) appear as a line between chromosomes. Viewed from the end, they appear as a ring encircling the chromosomes. Scale bar = 2 μm (A-D) and 1 μm (E).

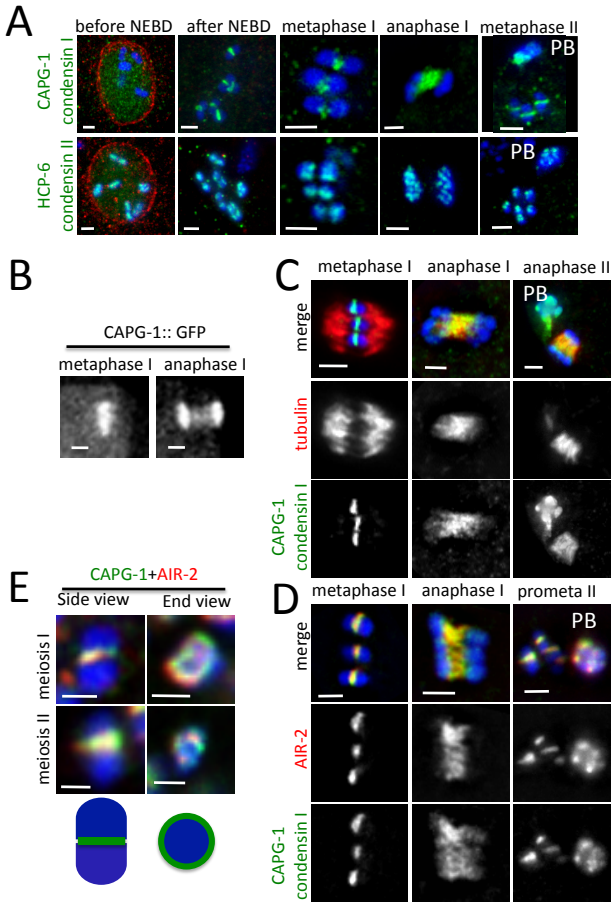


Figure 3.6: The CAP subunits of condensin I associate with meiotic chromosomes as a complex. (A) DPY-26 and DPY-28 (green) localize to the short arms of diakinesis bivalents after NEBD. Methanol/ acetone fixed oocytes are shown. (B) DPY-26 localizes to the interface between sister chromatids in metaphase of meiosis II. Methanol acetone fixed embryo is shown. (C and D) Short arm association of CAPG-1 (green) depends on the presence of DPY-26 and DPY-28. In *dpy-26 (n 199)* (C) and *dpy-28 (s939)* (D) mutants, CAPG-1 localization to the short arm is no longer detected. Oocytes after NEBD are shown, NPC staining is red, and DAPI is blue and gray. Heterozygotes are shown as controls. Scale bar = 5μm.

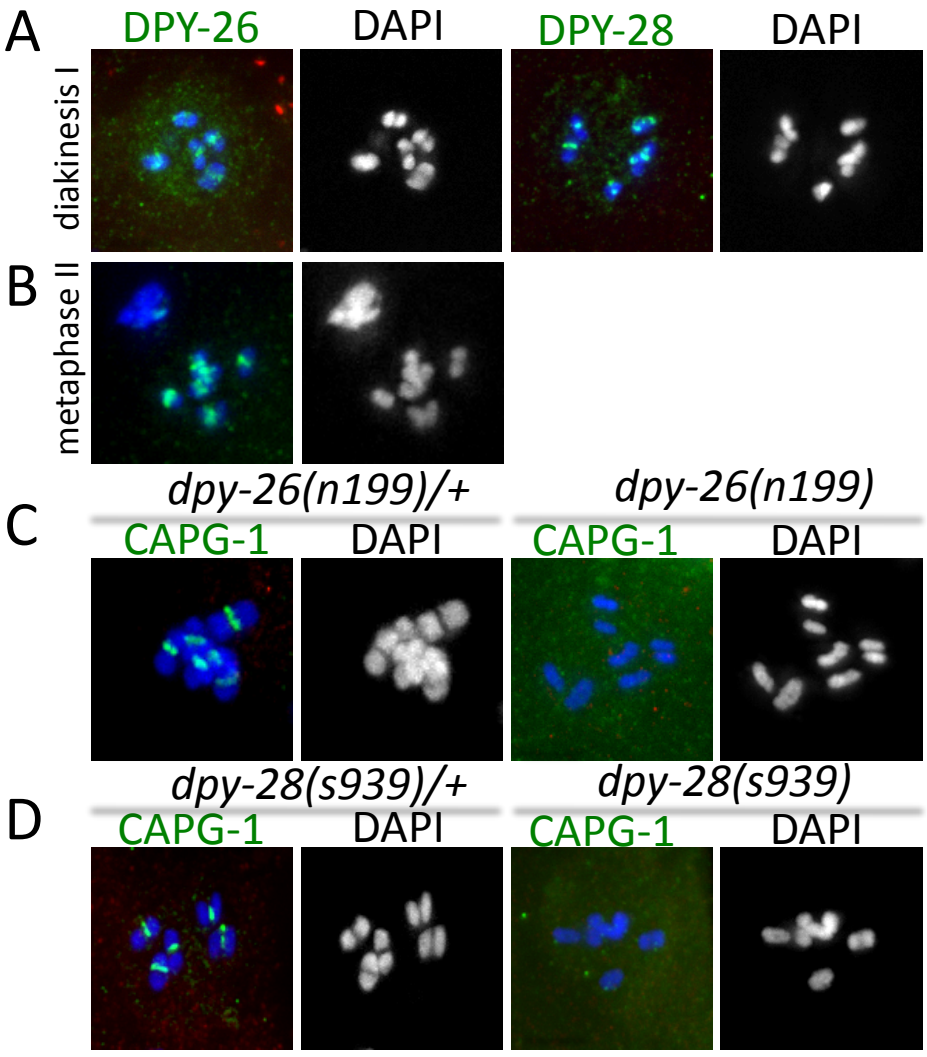


Figure 3.7: Condensin I and condensin II in sperm meiosis. (A) Images taken from dissected male gonads co-stained with antibodies specific to condensin I (CAPG-1, green) and condensin II (KLE-2, red). Condensin I is visible in pachytene, karyosome, and early diakinesis nuclei surrounding, but not on chromosomes, while condensin II associates with chromosomes by late pachytene/ early diplotene. Condensin I localized to the short arm of bivalents in late diakinesis and metaphase I, to the inner edges of separating chromosomes in anaphase I, between sister chromatids in metaphase II, and to the inner edges of separating chromatids in anaphase II. Throughout all stages, condensin II associates with sister chromatids. (B) Condensin I (CAPG-1, green) is seen inside the nucleus prior to NEBD, but does not associate with chromosomes until after NEBD. NPC is shown in red, DAPI in blue. (C) CAPG-1 (green), tubulin (red), and AIR-2 (red) are not prominent between separating chromosomes during anaphase I in sperm meiosis. Sperm meiosis in L4 larval hermaphrodite is shown for tubulin, and male for AIR-2 staining. Scale bar = 2μm.

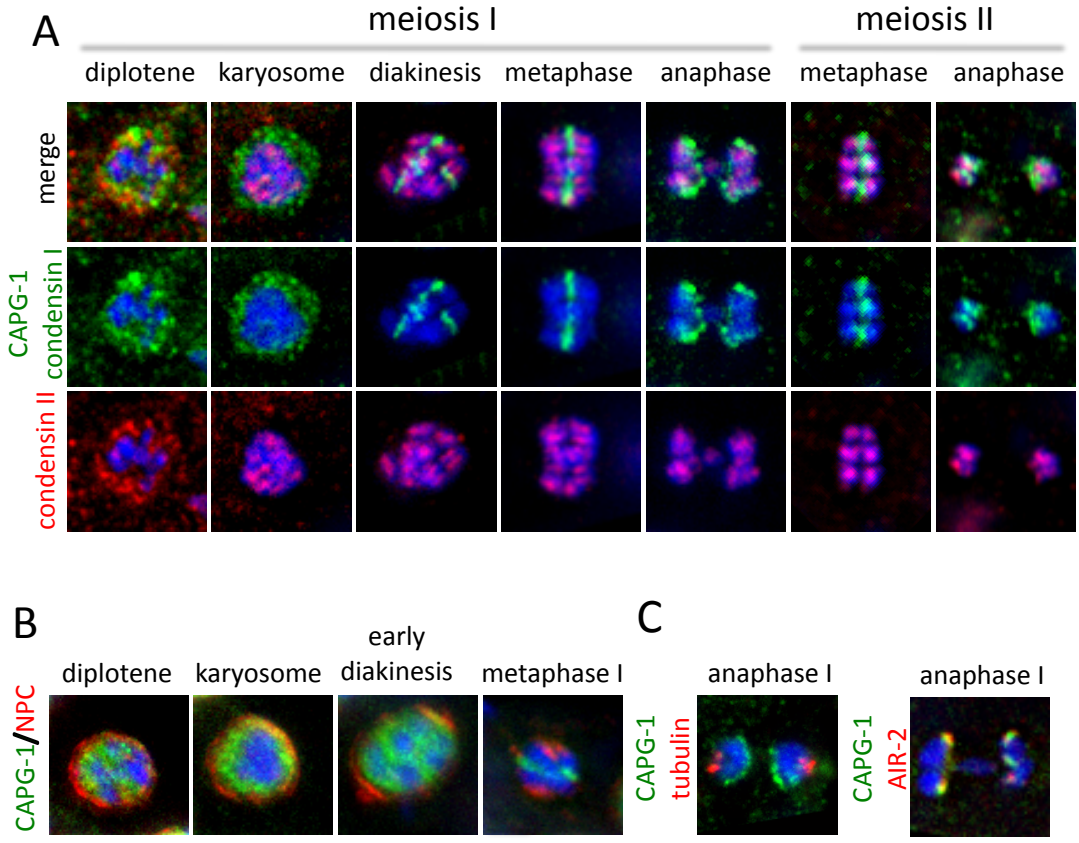


Figure 3.8: AIR-2 is needed for correct targeting of condensin I, but not condensin II, in meiosis. Enlarged bivalents from oocytes after NEBD. On control bivalents, condensin I (CAPG-1, green) is restricted to the short arm, and H3S10Ph (red) is seen on both sides of the condensin I domain. In AIR-2-depleted oocytes, the H3S10Ph signal is absent, and condensin I mislocalizes to both arms of the bivalents and appears in a cross shape. By contrast, chromosomal association of condensin II (HCP-6, green) appears similar on control and AIR-2-depleted bivalents. DAPI is shown in blue and gray. Scale bar = 1μm.

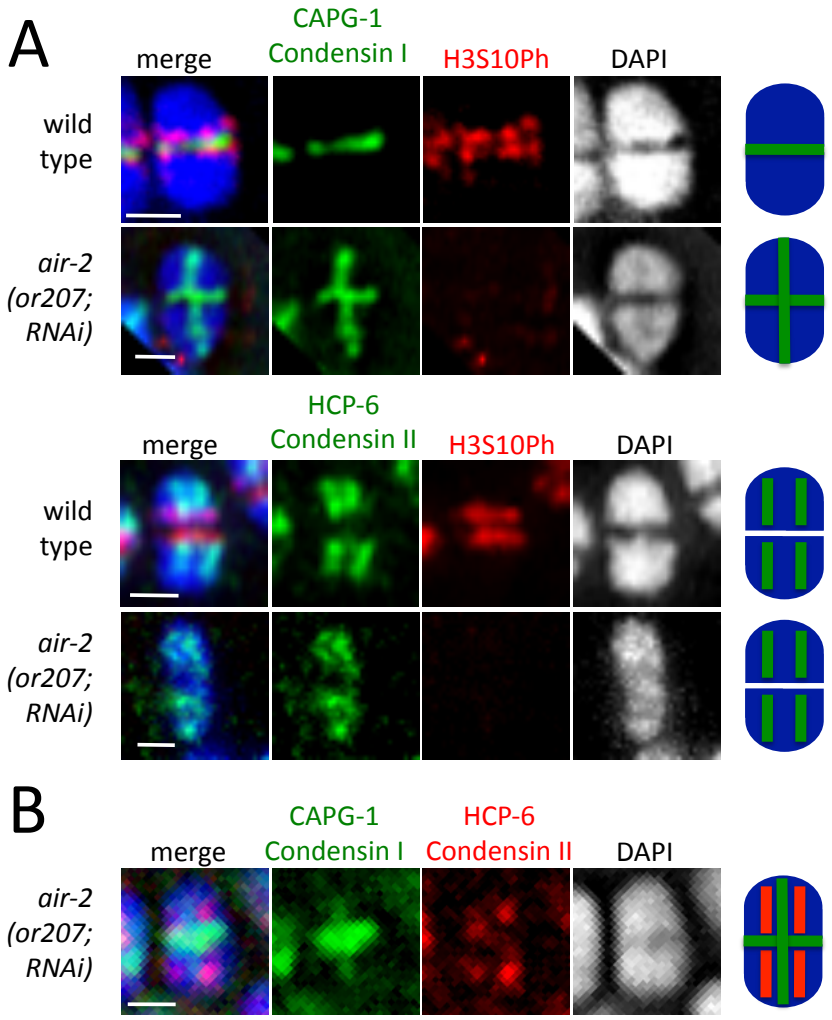


Figure 3.9: Spreading of condensin I and AIR-2 on meiotic bivalents. Enlarged bivalents from oocytes after NEBD, stained with antibodies specific to CAPG-1 (green) and either AIR-2 (red) (A) or H3S10Ph (red) (B). On wild type bivalents, condensin I, AIR-2 and H3S10Ph localize to the short arm. On some htp-1(gk174) and lab-1(RNAi) bivalents, H3S10Ph spreads to cover the bivalent surface, but AIR-2 either remains at the short arm or spreads to both arms. When AIR-2 spreads away from the short arm, condensin I follows AIR-2 and extends onto the long arm. When AIR-2 remains restricted to the short arm, condensin I also remains restricted to this region. Spreading of condensin I and AIR-2 is most pronounced on more rounded and less asymmetric bivalents.

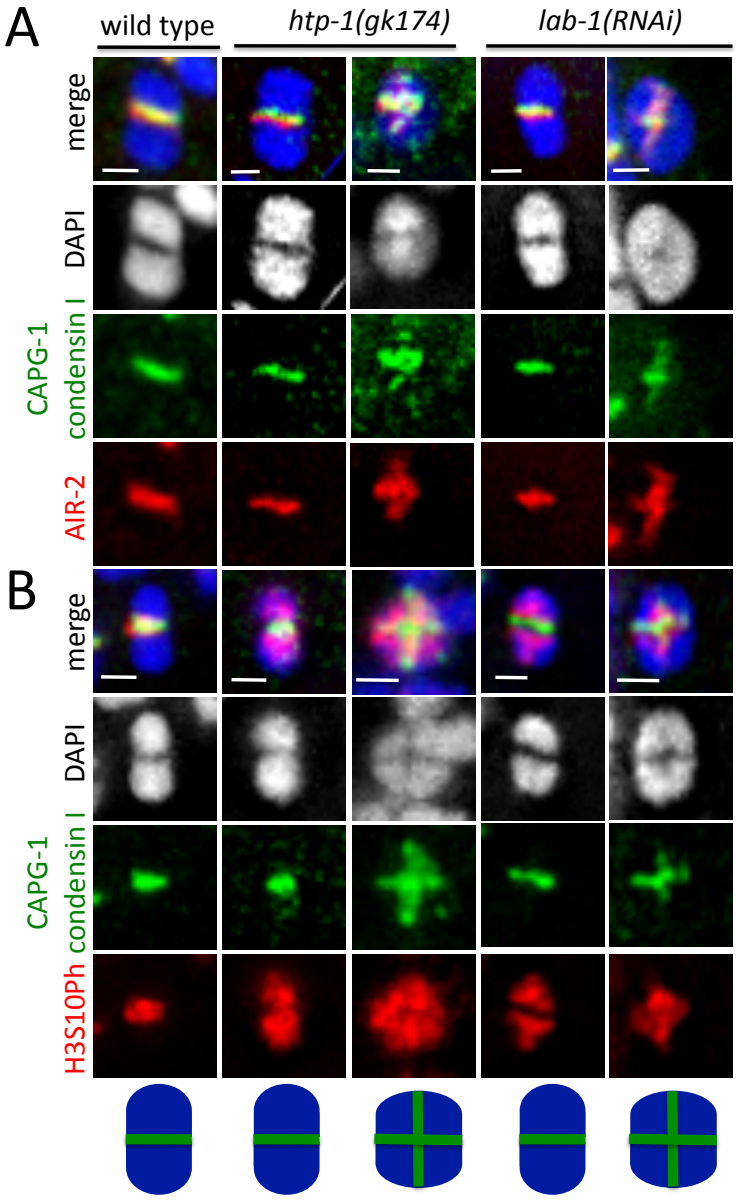


Figure 3.10: Condensin I localization on meiosis I univalents. Enlarged univalents from oocytes after NEBD, stained with antibodies specific to CAPG-1 (green) and either AIR-2 (red) (A) or H3S10Ph (red) (B). On some spo-11 univalents, condensin I (CAPG-1) and AIR-2 colocalize to a DAPI-light line, while on others neither condensin I nor AIR-2 are detected. Most spo-11 univalents have H3S10Ph staining of varying intensity, and condensin I only localizes to univalents with brighter H3S10Ph staining. On all rec-8 univalents, condensin I and AIR-2 colocalize at the interface between sister chromatids and H3S10Ph forms a boundary on either side of condensin I. Scale bar = 1 μm

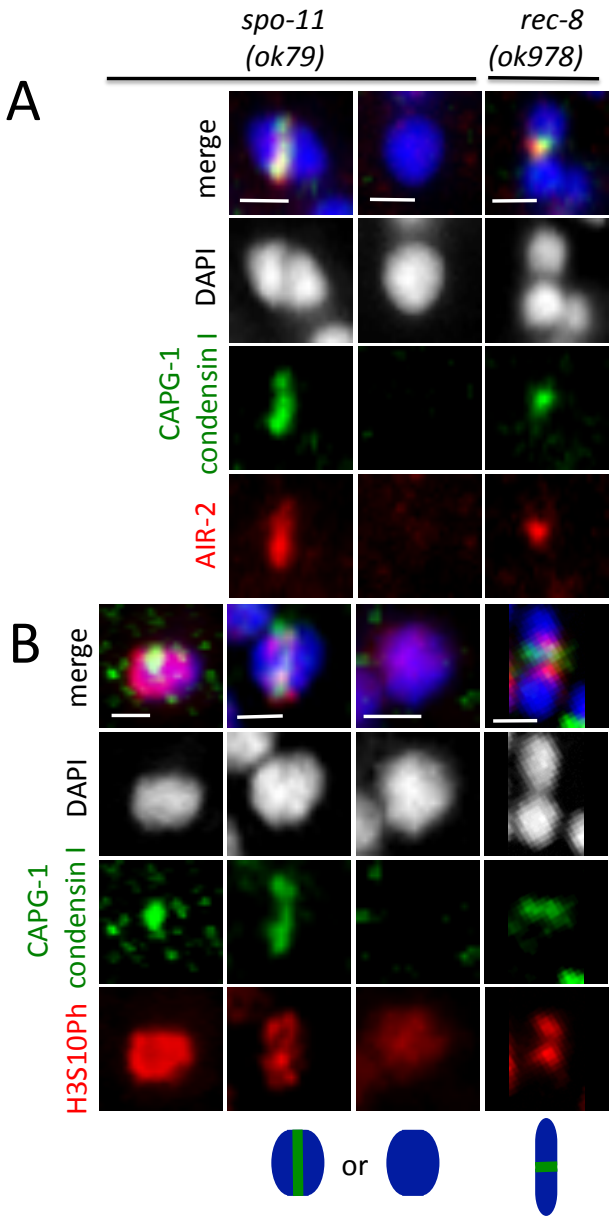
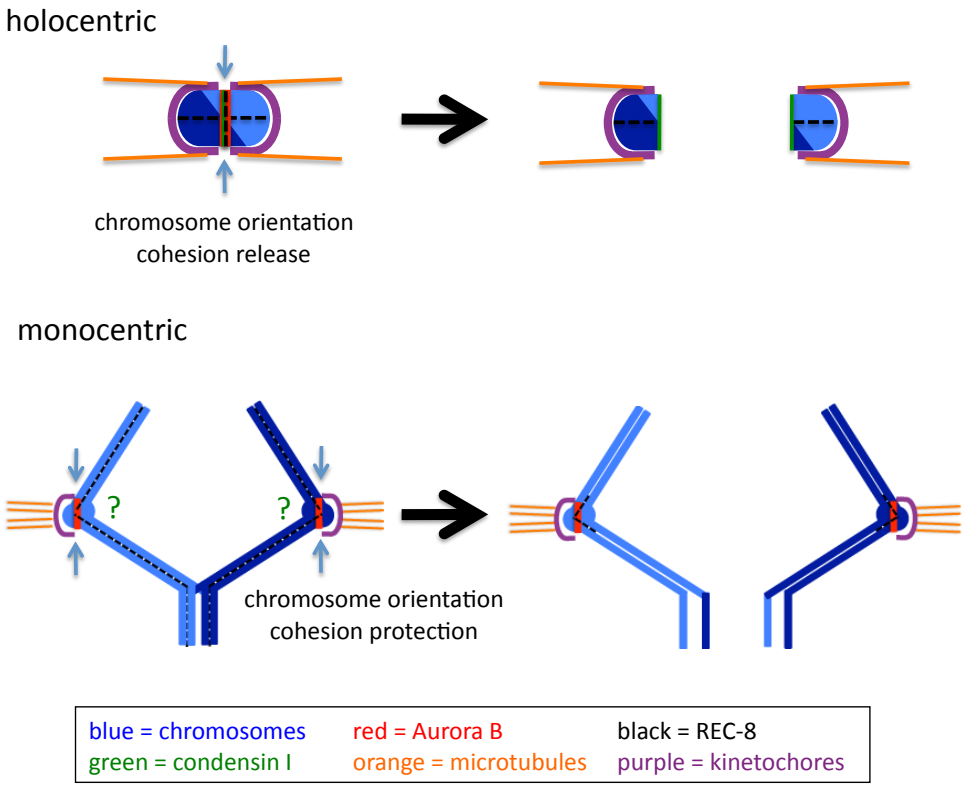


Figure 3.11: Model for AIR-2 activity on monocentric and holocentric chromosomes during meiosis I. On holocentric chromosomes, AIR-2 is in an ideal position at the short arm of the bivalent to promote both homolog biorientation and sister coorientation by ensuring that microtubules do not cross the AIR-2 zone, thereby keeping sisters together and homologs apart. The AIR-2 zone is also the region where sister chromatid cohesion must be released in meiosis I to allow homolog separation. At the short arm of bivalents, AIR-2 activity is also needed to restrict condensin I to the short arm of the bivalent. On monocentric chromosomes, Aurora B is enriched at the inner centromere and promotes homolog biorientation and sister coorientation. This zone of Aurora B activity is where centromeric cohesion must be protected in meiosis I. The role of Aurora B in condensin I and II targeting in meiosis in monocentric organisms is not known.



Chapter 4

Conclusion

The discovery of condensin I in *C. elegans*

Multiple condensins control chromosome dynamics in higher eukaryotes. Mitotic/ meiotic chromosomes built with condensin are precisely organized with an intrinsic structural integrity that enables correct alignment and segregation on the spindle apparatus. With the exception of yeast, eukaryotic organisms possess two condensins, I and II, which cooperate to beget perfect mitotic chromosome assembly and segregation. *C. elegans* was considered an outsider, with only one mitotic condensin (condensin II) and a condensin I complex with a unique role in X chromosome dosage compensation (condensin I^{DC}).

The comprehensive analysis of *C. elegans* condensin proteins presented in Chapter 2 has clarified this issue. We identified condensin subunits that were missing from condensin II and condensin I^{DC}. We were also able to definitively show that another condensin I complex exists, and is composed of CAP subunits from condensin I^{DC} and the SMC core from condensin II. The three complexes have distinct localization patterns on chromosomes during mitosis, meiosis and interphase. Finally, the new condensin I complex is a mitotic/ meiotic condensin, and collaborates with condensin II to organize and segregate holocentric chromosomes in *C. elegans*. The SMC4 protein DPY-27, unique to dosage compensation, likely arose through duplication and divergence of the SMC-4 gene (the SMC4 subunit of mitotic condensin I and II). DPY-27 is the foundation of condensin I^{DC}. Interaction with DPY-27 modifies the CAP I proteins and MIX-1 so that, all together, they stably associate with hermaphrodite X chromosomes and dampen their expression.

The utilization of a condensin complex to achieve dosage compensation implies that the processes of dosage compensation and mitotic chromosome condensation are likely to be mechanistically related. The ATPase activity of the SMC core of condensin I^{DC} (DPY-27 and MIX-1), is necessary for dosage compensation (Chuang et al., 1994; Lieb et al., 1998), and might be restrained in condensin I^{DC} to enable partial chromatin condensation that is just sufficient to facilitate a two-fold reduction in X-chromosome expression. A combination of *C. elegans* condensin I and II is capable of supercoiling DNA (Hagstrom et al., 2002), an *in vitro* activity of mitotic condensins (Kimura and Hirano, 1997). To gauge the extent of similarity between mitotic condensin function and that of condensin I^{DC}, it would be interesting to determine whether condensin I^{DC}, purified from embryonic protein extract with antibodies against DPY-27, also possesses this ability to manipulate DNA.

The relative contribution of condensin I versus condensin II during mitosis and meiosis varies between organisms, perhaps due to differences in chromosome structure. In *Xenopus*, condensin I function predominates, whereas in HeLa cells, condensin I and II contribute equally to chromosome compaction and assembly (Ono et al., 2003). In *C. elegans*, we find that Condensin II is the major contributor to holocentric chromosome organization while condensin I plays an important but secondary role.

C. elegans condensin I and II localize to mitotic and meiotic chromosomes at different times and places

The mitotic condensin I pattern is very different from its chromosome association pattern during meiosis, and the same holds true for condensin II. Condensin I is distributed all over mitotic chromosomes while condensin II localizes exclusively to the centromere (Chan et al., 2004; Hagstrom et al., 2002; Stear and Roth, 2002). During meiosis, condensin II associates with the core of sister chromatids (Chan et al., 2004) while condensin I is sharply restricted to the short arm (inter-homolog region) of the bivalent. The timing of condensin loading is conserved between mitosis and meiosis for each complex, despite their different localization patterns. Consistent with a role in facilitating the early stages of chromosome condensation, condensin II loads onto mitotic

chromosomes in prophase, before nuclear envelope break down (NEBD). During meiosis, condensin II also associates with chromosomes before NEBD, during diplotene-diakinesis of prophase I, and is vital for their reconfiguration into compact bivalents (Chan et al., 2004). In contrast, condensin I is observed on mitotic and meiotic chromosomes only after the nuclear envelope disintegrates at prometaphase. Condensin I and II do not overlap. Since condensin I and II occupy almost mutually exclusive domains in mitosis and meiosis, *C. elegans* chromosomes provide an ideal opportunity to elucidate how the two complexes might be differentially regulated.

Condensin recruitment by AIR-2

Aurora B's role in condensin I regulation is well conserved (see introduction). In *C. elegans*, the aurora B kinase, AIR-2, controls condensin I targeting in mitosis and meiosis, but does not affect the chromosomal association of condensin II in either process. During mitosis, AIR-2 activity recruits condensin I to chromosomes. During meiosis, condensin I is recruited to chromosomes independent of AIR-2, but then relies on AIR-2 for accurate localization.

An outstanding question is whether chromosome association of condensin is dependent on AIR-2 *per se* or an AIR-2 dependent modification; for example, H3S10Ph. Our analysis of condensin targeting by AIR-2 in meiosis affords some insight into this question. The chromosomal domain occupied by H3S10Ph dramatically expands when AIR-2 activity is augmented via depletion of its antagonistic proteins HTP-1 and LAB-1. Immunofluorescence analysis reveals that the spread of H3S10Ph on chromosomes is far more extensive than the spread of AIR-2 itself. Yet, the expanded condensin I domain is identical to that of AIR-2 and does not include H3S10Ph occupied regions without AIR-2. Thus, at least in meiosis, condensin I localization is likely regulated by AIR-2 and not by its chromatin signature, H3S10Ph.

Condensin I targeting in meiosis

Condensin I targeting in meiosis is not fully understood. Although AIR-2 is present on meiotic chromosomes from late pachytene onwards (Chan et al., 2004; Nabeshima et al.,

2005), and condensin I subunits are all present in the nucleoplasm before nuclear envelope break down (NEBD) in diakinesis oocytes, chromosomal association does not occur till after NEBD. This observation indicates that AIR-2 is not sufficient to drive initial chromosomal association of condensin I. The hypothetical meiotic condensin I recruitment factor 'Z' remains elusive. Various speculations can be made for how condensin I is loaded onto chromosomes. One possibility is that Z gains nuclear access after NEBD, and triggers the chromosomal loading of condensin I. Alternatively, NEBD might trigger a posttranslational modification of condensin I or (nuclear recruitment factor) Z, which then enables chromosome association of the complex.

When AIR-2 antagonists (long arm proteins HTP-1 and LAB-1) are depleted from meiotic chromosomes, AIR-2 (normally restricted to the short arm) extends onto the long arm of the bivalent, and condensin I always spreads to long arm regions ectopically occupied by AIR-2. This observation suggests that although AIR-2 cannot initially trigger chromosome loading, once condensin I is on DNA, AIR-2 is sufficient to guide its localization. Perhaps an initial 'priming' modification by factor Z makes condensin I competent at binding chromatin, and once primed, condensin I can go to chromosomal locations dictated by AIR-2. Following condensin I recruitment, if AIR-2 exclusively controlled condensin I localization, we would expect to see general mislocalization of condensin I on AIR-2 - depleted bivalents. Surprisingly, we have repeatedly observed that when AIR-2 is depleted, condensin I is retained at the short arm, and mislocalizes specifically onto the long arm of the bivalent, in a cross –shape. This is confusing, in light of our observation that long arm spread of condensin I also occurs in an AIR-2-dependent manner. Clearly, we lack important pieces in the puzzle of meiotic condensin I targeting, and additional targeting factors, like factor Z, that operate along with AIR-2, await discovery.

Does condensin I clear DNA?

The localization of AIR-2 and CAPG-1 on meiotic chromosomes is absolutely correlated with a clearing of DNA (for example, see *spo-11* univalents in Figure 3.10). At prometaphase/ metaphase AIR-2 and CAPG-1 always assemble at the DAPI free line that

is the boundary between homologous chromosomes (meiosis I) and sister chromatids (meiosis II). This line represents the region of cohesion release and the plane of biorientation on the metaphase plate. On *rec-8* univalents (sister chromatid pairs) that will prematurely separate at meiosis I, the DAPI free zone, along with AIR-2 and CAPG-1, is present between sisters. AIR-2 and CAPG-1 ectopically extend onto the long arm on HTP-1 and LAB-1 depleted chromosomes, and strikingly, reduced DAPI staining is obvious at every region where the two proteins spread. On *spo 11* univalents (at meiosis I) also, a reduced DAPI zone exists where AIR-2 and CAPG-1 colocalize. Reduced DAPI intensity accompanies the spread of CAPG-1 to the long arm upon AIR-2 depletion also, but this decrease is mild, and not as obvious as the DAPI reduction seen when CAPG-1 and AIR-2 spread together. We speculate that perhaps the difference lies in the proteins residing on the short arm. When AIR 2 is depleted, HTP-1 and LAB-1 are still present on the long arm, and may resist (partially) clearing of long arm chromatin by CAPG-1. AIR-2 and HTP-1/ LAB-1 are mutually exclusive on chromosomes, so when CAPG-1 mislocalizes along with AIR-2, HTP-1 and LAB-1 are absent, and cannot protect long arm chromatin from being cleared.

Condensin I on the central spindle

Condensin I dramatically relocates from the chromosomes to the spindle, where it colocalizes with AIR-2 during anaphase of meiosis. In contrast to mitosis, it appears as though the majority of meiotic condensin transfers onto the spindle. At the short arm of meiotic bivalents, AIR-2 recruits a hierarchy of proteins that are required for anaphase separation of chromosomes. During acentrosomal oocyte anaphase, these proteins form 'linker structures' between segregating chromosomes that likely provide the pushing force necessary for poleward movement (Dumont et al., 2010). Colocalization of condensin I with AIR-2 on the spindle is consistent with it being a component of these anaphase linker structures that facilitate efficient meiotic chromosome segregation. Initial attempts to address how spindle-associated condensin I is affected by AIR-2 depletion in meiosis failed because with our depletion regimen, AIR-2 depleted meiotic chromosomes do not make it to anaphase.

Summary

In conclusion, our discovery of condensin I in *C. elegans* has provided significant insight into condensin biology. Condensin proteins are versatile, and can be switched between functionally diverse condensin complexes. Four out of five subunits are shared between condensin I and condensin I^{DC}, revealing a potential mechanistic link between dosage compensation and chromosome condensation. The *C. elegans* chromosome passenger kinase, aurora B (AIR-2), regulates condensin I localization to chromosomes during mitosis and meiosis, where its function is required for accurate chromosome segregation. However, the specific contribution of condensin I to chromosome dynamics in *C. elegans,* or any model organism, is yet to be determined. Ongoing research in diverse systems is key to the elucidation of how condensin facilitates flawless chromosome design.

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