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Article

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

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Summary

Background: 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.

Results: 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, whereas 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.

Conclusions: We demonstrate that condensin subunits in *C. elegans* form three complexes, one that functions in dosage compensation and two that function in mitosis and meiosis. 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.

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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 [1, 2]). 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). Although yeasts have a single condensin complex, higher eukaryotes have two: condensin I and condensin II [3–5]. 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 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 [3–5]. Condensin II is required for proper kinetics of chromosome condensation at prophase, whereas condensin I appears to stabilize chromosome rigidity [3–11]. Depletion of each complex individually leads to distinct and characteristic defects in chromosome morphology, and simultaneous depletion of both leads to more severe defects [3–6]. Each complex is required for sister-chromatid segregation at anaphase in all organisms tested (reviewed in [1, 2]). 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 1) [7, 9, 12, 13]. Depleting members of this complex impairs prophase condensation, anaphase segregation, and centromere organization in mitosis [7, 9, 11], as well as the restructuring and segregation of chromosomes during meiosis [12]. 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

A

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

B

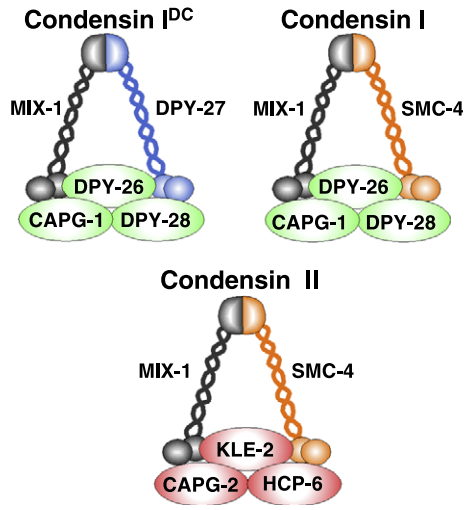


Figure 1. The Three-Condensin Model

(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.

2-fold, leading to equal X-linked gene expression levels between XX hermaphrodites and XO males (reviewed in [14]). Condensin I^{DC} shares the SMC2 subunit MIX-1 [13] but contains a unique SMC4 subunit DPY-27 [15] and the condensin I class CAP proteins DPY-28 [16] and DPY-26 [17, 18] (Figure 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 although hermaphrodites die because of inappropriately high X-linked gene expression, males carrying these mutations are viable [13, 15–17, 19, 20]. The apparent lack of a mitotic condensin I in *C. elegans* is puzzling, because 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 [2].

Although 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 nondisjunction of the X in meiosis [17, 19, 20], and *dpy-28* is required for germline mitosis and proper crossover number and distribution during meiotic recombination [16]. 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 [16, 17, 19, 20]. Condensin I is shown elsewhere (D. Mets and B.J.M., unpublished data) to control crossover number and distribution in meiosis. Here we describe different localization and phenotypes of condensin I and II, suggesting 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

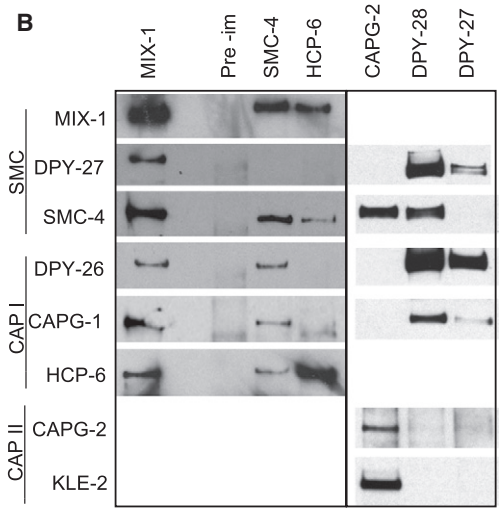
To identify all *C. elegans* condensin subunits, we performed large-scale immunoprecipitation (IP) from embryo extracts with antibodies against DPY-27, the SMC4 subunit of condensin I^{DC}, or against SMC-4, the SMC4 subunit of condensin II. Four independent IP experiments were analyzed by MudPIT (see Experimental Procedures and Supplemental Experimental Procedures available online) [21]. To eliminate nonspecific interactors, we subtracted proteins immunoprecipitated in controls with preimmune IgG or with an antibody against KLP-7, a mitotic protein that does not associate with SMC-4 or DPY-27 (data not shown). SMC-4 and DPY-27 IPs each recovered a set of interacting condensin subunits that appeared reproducibly and with high total spectrum counts (Figure 2A).

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 [5] or genetic screens [14]. Proteomics analysis of DPY-27

A

Identity	Gene name	Gene	DPY-27	SMC-4	KLE-2	CAPG-1	KLP-7
SMC							
SMC2	*mix-1*	M106.1	95	456	99	1034	0
SMC4	*smc-4*	F35G12.8	0	351	77	684	0
SMC4	*dpy-27*	R13G10.1	128	0	0	616	0
CAP I							
CAP-D2	*dpy-28*	Y39A1B.3	47	222	0	1133	0
CAP-G	*capg-1*	F29D11.2	39	131	0	1020	0
CAP-H	*dpy-26*	C25G4.5	30	209	0	895	0
CAP II							
CAP-D3	*hcp-6*	Y110A7A.1	0	203	127	3	0
CAP-G2	*capg-2*	F55C5.4	0	111	79	0	0
CAP-H2	*kle-2*	C29E4.2	0	131	156	0	0

B



C

	SMC			CAP I			CAP II		
	MIX-1	SMC-4	DPY-27	DPY-28	CAPG-1	DPY-26	HCP-6	CAPG-2	KLE-2
SMC									
MIX-1	+	+	+		+		+		
SMC-4	+	+	-	+	+	+	+	+	+
DPY-27	+	-	+	+	+	+	-	-	-
CAP I									
DPY-28		+	+	+	+	+	-	-	-
CAP-G1	+	+	+	+	+	+	-	-	-
DPY-26	+	+	+	+	+	+	-	-	-
CAP II									
HCP-6	+	+	-	-		-	+	+	+
CAP-G2		+	-	-	-	-	+	+	+
KLE-2		+	-	-	-	-	+	+	+

Figure 2. Condensin Subunit Interactions Identified by Immunoprecipitation

(A) 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. Numbers indicate the total mass spectra collected in three (KLE-2) or four (all others) IP samples corresponding to each protein indicated on the left.

(B and C) IP reactions from embryo extracts with antibodies against condensin subunits were performed and analyzed on western blots probed with an array of antibodies. Preimmune IP (Pre-im) is shown as control. Selected western blots are shown in (B); summary of all IPs analyzed is shown in (C). Plus sign indicates subunit was detected in the IP; minus sign indicates subunit was not detected in the IP; blank cell indicates interaction was not scored.

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) (Figure 2A). F29D11.2 contains ARM/HEAT motifs, and PSI-BLAST and phylogenetic analyses showed that F29D11.2 shares homology with CAP-G class subunits (Figure S1). F29D11.2 appears to encode the missing CAP-G subunit of condensin I^{DC} (see data below) and we named it *capg-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 (Figure 2A). F55C5.4 was shown by PSI-BLAST and phylogenetic analyses (Figure S1) to be a CAPG-2 class subunit, consistent with a previous prediction [5]. Based on data below, we conclude that F55C5.4 is the CAP-G2 subunit of condensin II and named it *capg-2*. KLE-2 was previously categorized as a CAP-H2 homolog [5] and a member of the kleisin ("closure") protein family and shown to affect chromosome segregation [18]. 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 (Figure 2A). Thus, we reconsidered the composition of *C. elegans* condensin complexes and hypothesized a three-condensin model (Figure 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 (Figure 2A). 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 (Figure 2A). 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 (Figures 2B and 2C). 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. 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 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 3) [15]. In contrast, CAPG-1 associates with mitotic chromosomes in a discontinuous coating pattern (Figure 3), similar to DPY-26 and DPY-28 [16, 17]. The three class I CAPs share this pattern and they colocalize on mitotic chromosomes (Figure S3). This class I CAP pattern differs from the "outlining" of condensed chromosomes by KLE-2 (Figure 3), a pattern observed for other condensin II subunits and coincident with centromere proteins along the outer face of *C. elegans* holocentric chromosomes [7, 10–12, 16]. The three class II CAPs share this pattern and they colocalize, although there is little overlap between class I and class II CAP patterns (Figure S3).

In mid-stage hermaphrodite embryos undergoing dosage compensation, condensin I^{DC} localizes to two nuclear subregions, the X chromosomes [13, 15–17]. Both DPY-27 and CAPG-1 localized to X (Figure 3; Figure S3). However, during mitosis, CAPG-1 localized not only in two bright foci, but also along all condensed chromosomes, an additional localization not shared by DPY-27 (Figure 3). We observed this pattern for all class I CAPs and suggest that it reflects their dual roles: they 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. Consistent with this interpretation, "mitotic coating" but not the two bright foci of CAPG-1 localization were observed in male embryos (Figure 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 of both sexes (Figure 3) [7, 10–12]. The distinct localization patterns observed for DPY-27, class I CAPs, and class II CAPs are consistent with the existence of three separate complexes.

SMC-4 and MIX-1 participate in both condensin I and II, so they should exhibit a hybrid localization pattern. These proteins show the bright condensin II "outlining" pattern, as reported [7, 12, 16], 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.

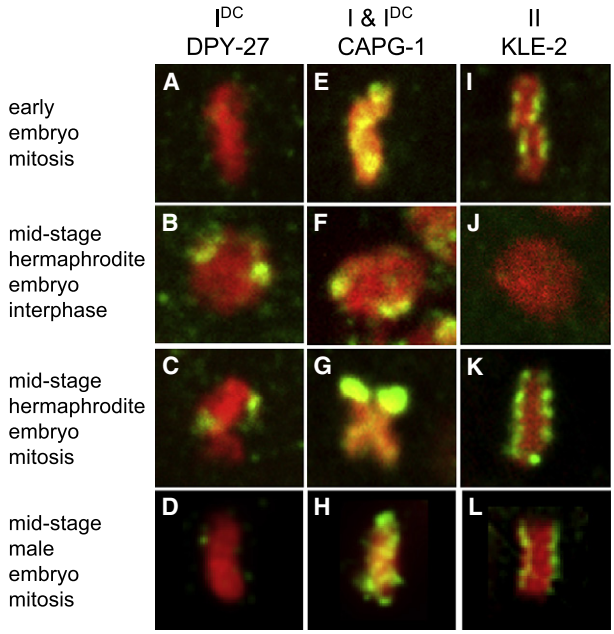


Figure 3. Condensin I Localizes to Mitotic Chromosomes in a Pattern Distinct from Condensin I^{DC} or Condensin II

DPY-27 antibody was used to indicate the subcellular localization of condensin 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.

Alternatively, the three class I CAP subunits might form a subcomplex lacking SMC subunits, comparable to the 11S subcomplex in *Xenopus* egg extracts [22]. However, during meiosis we clearly observed SMC-4 and MIX-1 in a hybrid pattern (see below and Figure 4), as predicted if they were shared between two different holocomplexes.

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 [14, 15, 23]. Like DPY-27, CAPG-1 accumulated in the oocyte nucleoplasm (Figure 4A). Unlike DPY-27, CAPG-1 is present throughout the germline (Figure 4 and data not shown), as are DPY-26 and DPY-28 [16, 17], presumably reflecting condensin I localization. In contrast to the condensin I^{DC} or condensin I patterns, KLE-2 localizes within each sister chromatid in oocytes (Figure 4A), as do other condensin II subunits [12].

We examined later meiotic stages in spermatocytes and fertilized oocytes undergoing meiotic divisions (Figure 4A). DPY-27 was not detected at this stage of meiosis. Class II CAPs appeared within each sister chromatid during meiosis I

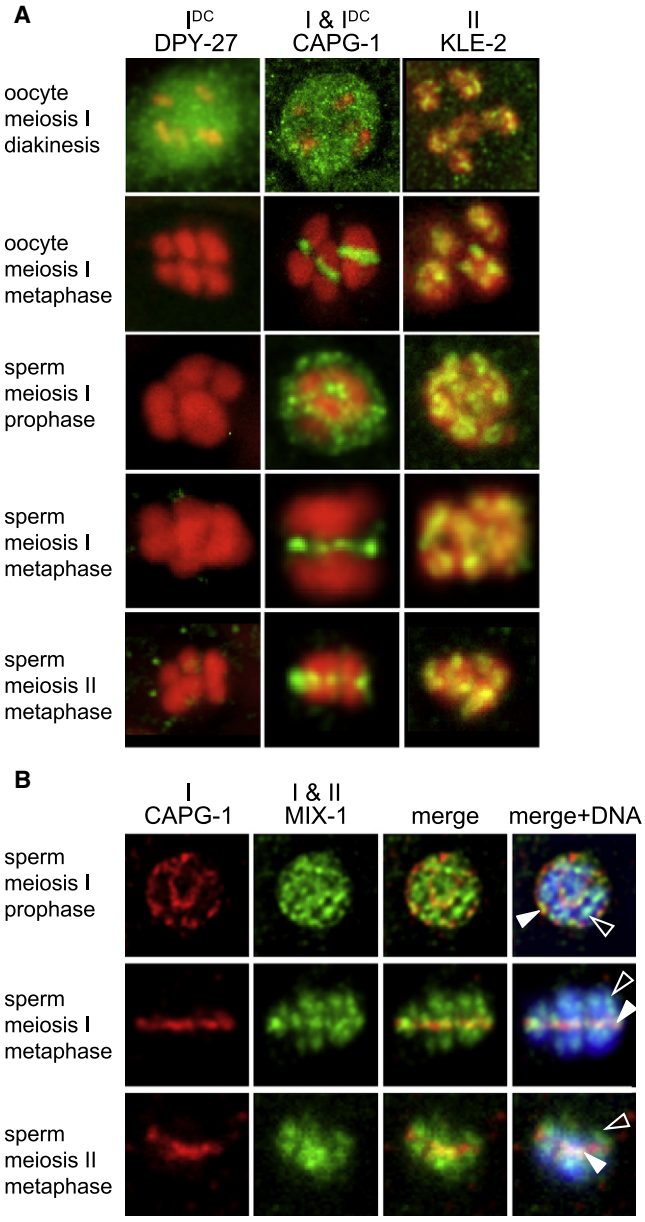


Figure 4. Condensin I Localizes to Meiotic Chromosomes in a Pattern Distinct from Condensin I^{DC} or Condensin II

(A) DPY-27 antibody was used to indicate the localization of condesin I^{DC}, anti-CAPG-1 to indicate condensin I and condensin I^{DC}, and anti-KLE-2 to indicate condensin II. DPY-27 was detected in the nucleoplasm of mature oocytes, but not at other stages of meiosis. CAPG-1 staining indicates that condensin I surrounds chromosomes in prophase then localizes to the interface between homologs (meiosis I) or sister chromatids (meiosis II). In contrast, KLE-2 staining indicates that condensin II localizes to the core of each sister chromatid. Antibody in green, DNA in red, merge in yellow.

(B) MIX-1 localization is a hybrid of condensin I and condensin II patterns. MIX-1 (green) partially colocalizes with CAPG-1 (red, representing condensin I) on sperm chromosomes (blue). MIX-1 and CAPG-1 colocalize surrounding chromosomes in meiosis prophase I, between homologs in metaphase I, and between sisters at metaphase II (filled arrows). In addition, MIX-1 localizes to the chromosome core at each stage (open arrows), a pattern resembling condensin II and not shared by CAPG-1.

prophase and metaphase and during meiosis II metaphase. In contrast, class I CAPs surrounded chromosomes at prophase of meiosis I. 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 4A and data not shown). 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 [9, 24]. This localization pattern raises the possibility that condensin I contributes to the separation of chromosomes during meiosis.

MIX-1 and SMC-4 showed a hybrid pattern of chromosomal association corresponding to class I and class II CAP patterns (Figure 4B). Part of the MIX-1 pattern overlaps with the pattern of CAPG-1 (class I CAP), surrounding chromosomes at meiotic prophase and localizing between them at metaphase of meiosis I and II. 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 (Figure 4B). These results are consistent with SMC-4 and MIX-1 being core subunits in both condensin I and condensin II.

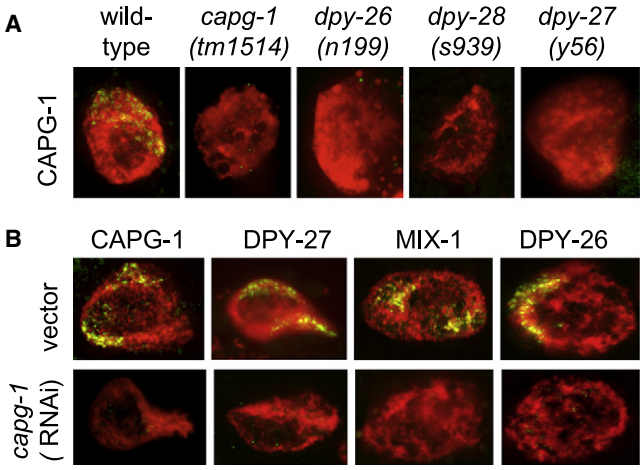
As a Member of Condensin I^{DC}, CAPG-1 Mediates X Dosage Compensation

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 [13, 16, 17, 25]. Indeed, CAPG-1 association with hermaphrodite X chromosomes was not detected in *capg-1, dpy-26, dpy-28*, or *dpy-27* mutant animals (Figure 5A). Reciprocally, association of DPY-27, MIX-1, or DPY-26 with X chromosomes was not observed in *capg-1* mutant or RNAi-depleted animals (Figure 5B). Like other condensin I^{DC} subunits, CAPG-1 localization to X depended on *sdc-2* and *sdc-3*, and SDC-3 localization to X was reduced in *capg-1* RNAi animals (Figure S4). SDC-2 and SDC-3 are non-condensin proteins that associate with condensin I^{DC} to confer sex specificity to dosage compensation [14, 23, 26].

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 [27, 28] and low embryonic lethality, which can be enhanced by depleting genes required for dosage compensation [29]. 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* (Figure 5C). 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 [28, 30]. 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 (Figure 5C). 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



C

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 5. CAPG-1 Functions in Dosage Compensation

(A) CAPG-1 (green) localizes to X chromosomes in wild-type hermphrodites, but not in animals carrying mutated subunits of condensin I^{DC}.

(B) Condensin I^{DC} subunits (green) localize to hermaphrodite X chromosomes in control vector RNAi animals, but not in animals fed RNAi food targeting *capg-1*. Shown are intestinal nuclei (red) of adult hermaphrodites.

(C) 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 because of 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. Percent lethality was calculated as dead progeny/total progeny × 100. Male rescue was calculated as number of males/expected number of males × 100. At least 200 animals were scored for RNAi of each gene.

by injection plus feeding RNAi and live-imaged chromosomes visualized by GFP::histone H2B (see Experimental Procedures). CAPG-2-depleted chromosomes failed to condense and individualize at mitotic prophase, as with depletion of any other condensin II subunit (Figure 6; Figure S5A) [7, 9–12, 18]. CAPG-2 and KLE-2 depletion also impaired chromosome segregation during meiosis (Figure 6A; Figure S5B). These phenotypes are consistent with CAPG-2 and KLE-2 being subunits of condensin II and with a more limited previous analysis [18].

To determine how condensin II impacts development, we characterized deletion mutants *smc-4(tm1868), capg-2(tm1833), kle-2(tm1823),* and *kle-2(ok1151).* They have a common homozygous phenotype, becoming uncoordinated, sick, sterile adults, with protruding vulvae and withered tails (data not shown). Similar phenotypes are observed in *mix-1(mn29)* homozygotes [13] or when animals carrying the temperature-sensitive mutation *hcp-6(mr17)* are shifted to nonpermissive temperature at early larval stages [11]. Such defects are typical of mutated cell-division genes. Early cell divisions are allowed by maternal-origin product, but as it diminishes, mitoses fail in late-dividing tissues. Indeed, homozygous adults mutant for each condensin II subunit exhibited abnormal connections between nuclei in late-dividing cell types such as ventral nerve cord (Figure S5C), gut, and germline (Figure 6B), which likely reflect failed mitotic chromosome segregation. Condensin II also appears to be required for proper germline formation, because in mutants the germline was extremely underproliferated and contained only a small patch of abnormal nuclei (Figure 6B).

These studies extend previous analysis of condensin II, show common developmental abnormalities caused by mutating members of this complex, and provide new data supporting the fact that CAPG-2 and KLE-2 are condensin II subunits.

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 all three subunits shared between condensin I and I^{DC} (DPY-28, CAPG-1, and DPY-26), but not observed upon depleting the condensin I^{DC}-specific subunit DPY-27.

In several different settings, depletion of DPY-28, CAPG-1, and DPY-26 caused chromosome segregation defects, whereas DPY-27 depletion did not. Worms grown to adulthood on RNAi food targeting *dpy-28, capg-1*, or *dpy-26* 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. Although most gut nuclei in control or *dpy-27* RNAi fed worms were completely separated, many gut nuclei in *dpy-28, capg-1*, or *dpy-26* RNAi-fed worms appear connected by a thin "bridge" of DNA (Figures 7A and 7B). Segregation defects caused by class I CAP depletion are unlikely to result from impaired dosage compensation or karyotype-specific defects, as indicated by the fact that they are observed in XX hermaphrodites (dosage compensation active) as well as XO males and XO hermaphrodites (dosage compensation inactive) (Figure 7B; Figure S6B).

To follow mitosis in real time, we extended RNAi feeding for two generations in a strain carrying GFP::histone H2B and filmed F2 embryos. Control and *dpy-27*-depleted chromosomes separated fully at anaphase, whereas chromosomes depleted of *dpy-28, capg-1*, or *dpy-26* remained connected by DNA strands (Figure 7C; Figure S6A). The anaphase segregation defect caused by depleting condensin I subunits was weaker, but similar to that caused by condensin II depletion (Figure S5). We confirmed that condensin II localizes normally to mitotic chromosomes in these condensin I-depleted F2 embryos (Figure S7A). 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* by using two generation RNAi feeding and analyzed adult and embryonic nuclei by FISH with a 5S rDNA probe (Figure 7D). Most nuclei in controls had two fluorescent signals (indicating diploidy) or one signal (presumably representing two unresolvable foci). However, several nuclei in *capg-1* RNAi-treated embryos or adult nerve cord had more than two signals, indicating aneuploidy (Figure 7D). Earlier

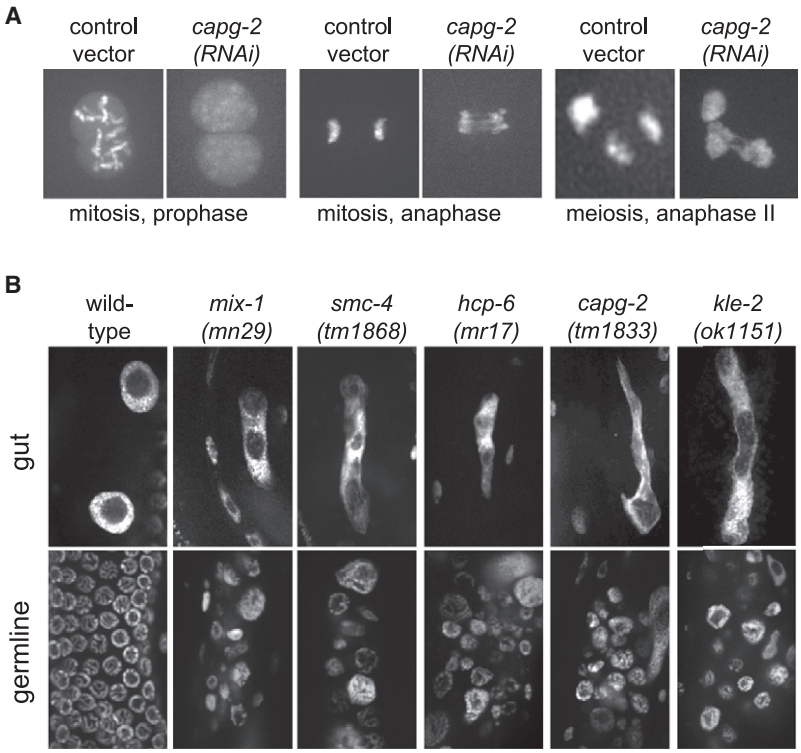


Figure 6. Depleting Each Condensin II Subunit Results in Common Chromosomal and Developmental Defects

(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 wild-type, but often connected in condensin II subunit mutants. Germline nuclei (bottom row) are uniformly sized and evenly spaced in wild-type. Condensin II subunit mutants have fewer germline nuclei, which are abnormally sized and unevenly distributed.

FISH analysis of SMC-4-depleted nuclei uncovered similar, albeit more severe, defects [7].

Meiotic chromosome segregation defects also occurred after RNAi of *dpy-28, capg-1*, or *dpy-26*, but not *dpy-27*. Time-lapse analysis of animals carrying GFP::histone H2B after two generations of feeding control or *dpy-27* RNAi showed normal completion of two meiotic divisions, producing two polar bodies and a separate oocyte pronucleus. But in animals depleted of *dpy-28, capg-1*, or *dpy-26*, the oocyte pronucleus often remained connected to the second polar body (Figure 7E), reflecting meiosis II chromosome segregation failure. We could not determine whether condensin I is required for meiosis I: we failed to observe meiosis I defects but we suspect that this could be attributed to incomplete depletion of the complex by RNAi (Figure S7B).

Finally, we analyzed phenotypes caused by mutating genes encoding condensin I subunits. Mutations in *dpy-26* and *dpy-28* cause near-complete hermaphrodite-specific maternal-effect lethality, but males are viable [19, 20]. The deletion mutant *capg-1(tm1514)* is more severe, and both sexes of homozygous mutant progeny from heterozygous mothers arrest as uncoordinated, sick, sterile adults with numerous bridged nuclei, unusually large or small nuclei, and underproliferated germlines (data not shown). These phenotypes resemble those caused by condensin II subunit mutations. Why RNAi depletion of each class I CAP subunit produces equivalent phenotypes but mutations show differing severities is unclear and may require additional alleles to resolve. Nevertheless, the surviving hermaphrodite and male progeny of *dpy-26, capg-1,* or *dpy-28* mutant mothers showed chromosome segregation defects in many tissues, whereas *dpy-27* mutants did not (scored for gut nuclei; Figure S6B), confirming that condensin I promotes mitotic chromosome segregation. Moreover, combining condensin I and II hypomorphic mutations caused more severe nuclear defects than either single mutant alone (Figure S8), as observed in other organisms [3–6], suggesting that both condensin I and II contribute to chromosome segregation. Because condensin I RNAi depletion produces weaker mitotic defects than does condensin II depletion, and because *dpy-26* and *dpy-28* males are viable, whereas condensin II subunit mutants are lethal, it seems that condensin II function predominates.

Discussion

Evolution of Diverse Condensin Complexes

Condensins I and II are conserved complexes that ensure the accurate segregation of chromosomes during cell division [1, 2]. In *C. elegans*, incomplete condensin I-like and condensin II-like complexes had been identified [7, 12, 14]. Previous studies, extended here with RNAi and mutant analysis of all subunits, indicated that *C. elegans* condensin II is required for prophase chromosome condensation and anaphase segregation, conserved condensin II activities [7, 12]. 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 [14]. 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 [2].

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

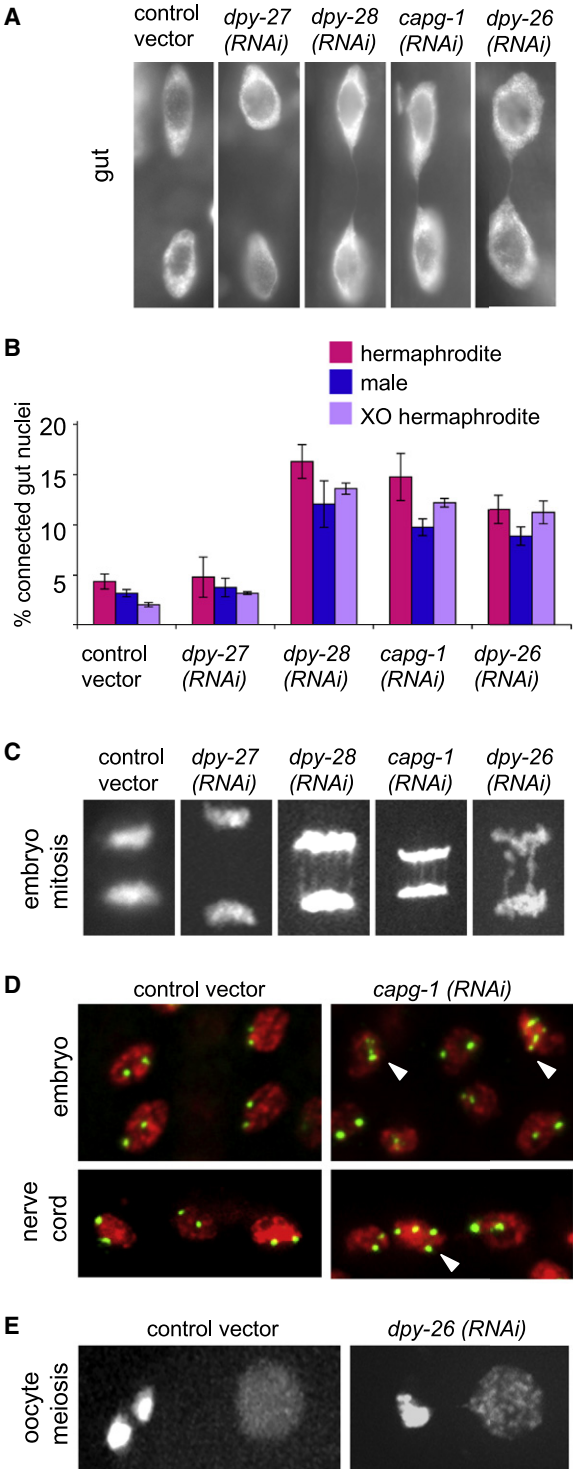


Figure 7. Condensin I Is Required for Mitotic and Meiotic Chromosome Segregation

(A) Gut nuclei from adults raised since hatching on bacteria expressing control vector or dsRNA against the subunit indicated. Gut 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) Quantification of defects shown in (A), scored in hermaphrodites (magenta), males (blue), and a mutant situation with half XO hermaphrodites and half XX hermaphrodites (*tra-2(e2531)*, purple). Percentage calculated as the number of gut nuclei with an obvious connection/total gut nuclei × 100. Standard deviation from three experiments is shown. In comparison to control vector by Fisher's exact test, *dpy-27* $p > 0.2$ in each sex (not significant); for *dpy-28*, *capg-1*, and *dpy-26* each $p < 0.0001$ (significant).

(C) 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.

(D) FISH analysis of nuclei from mid-stage (about 100-cell) embryos (top) or adult nerve cord cells (bottom) with 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 (arrow).

(E) Meiosis II after two generations of RNAi feeding in a GFP:histone H2B strain. The two meiotic divisions produce two small polar bodies and a separated oocyte pronucleus in control-treated animals. When condensin I subunit DPY-26 is depleted, the second polar body often fails to segregate from the oocyte pronucleus.

compensation. A related example is subunit paralogs that distinguish mitotic from meiotic versions of the cohesin SMC complex [31].

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 in multiple tissues and times in development. Differences in chromosomal distributions and depletion phenotypes suggest that 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 [3, 6]. Condensin I depletion generally caused less severe phenotypes than did condensin II depletion, with the exception of the strong *capg-1(tm1514)* deletion allele. It is conceivable that CAPG-1 has some functions independent of condensin I and I^{DC}.

We observed enhanced phenotypes when hypomorphic alleles of condensin I and II CAP subunits were combined, as seen in other systems [5, 6]. Yet a deletion allele of the SMC-4 subunit, which is shared between condensin I and II, appears no more severe than deletion alleles of condensin II CAP subunits alone (Figure 6; Figure S5). Together, these results suggest that condensin II plays a more critical role in mitosis and development than does condensin I in *C. elegans*, and 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 [2, 5]. 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 [3–5]. 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 [7, 10–12, 16, 17]. Perhaps the different pattern of condensins on holocentric chromosomes indicates an underlying difference in the placement or structure of the holocentric chromatid core, thought to be the scaffold around which loop domains are arranged. Despite major differences, the nonoverlapping 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 holocentrics 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. Whereas condensin II localizes within the core of meiotic sister chromatids ([12] 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 [32, 33]. *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 [34] and releasing cohesin [3, 35]. 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 [9, 24].

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 [36–38], it is currently unknown whether these activities are shared by condensin II. We previously demonstrated supercoiling activity in SMC-4 immunoprecipitates [7]. 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 [13, 15].

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. Condensin I subunits in *S. cerevisiae* function in silencing at the mating type locus [39], rDNA silencing [40], and tRNA gene-mediated silencing [41]. In *Drosophila*, condensin I subunits participate in repression of transgenes inserted in heterochromatin [42, 43]. A murine condensin II (CAP-G2) subunit antagonizes activation of reporter genes [44]. Even the bacterial SMC complex has roles in both chromosome partitioning and gene regulation, implying that these are ancient linked roles [45]. A common alteration of chromosome structure could conceivably underlie both chromosome preparation for mitosis and downregulation of gene expression. Continued comparative studies of condensin complexes are likely to shed light on the links between chromosome segregation and gene regulation.

Experimental Procedures

See Supplemental Data for MudPIT, phylogenetic analysis, strains, genetic assays, antibody information, and image collection.

Protein Analysis

Small-scale IPs and western blotting were performed as described [12]. Large-scale protein extract preparation and IPs for MudPIT were scaled-up versions of this protocol.

Microscopy

Antibody staining of embryos and dissected adults was performed as described in protocol #21 in [46]. To distinguish between older male and hermaphrodite embryos, we costained embryos with antibodies to SDC-3, which localizes only to the X chromosomes of hermaphrodites. DNA staining of whole worms was performed by treating with Carnoy's fixative, rehydrating in 1× M9 buffer, and mounting in ProLong antifade containing DAPI. FISH was performed as in protocol #25 in [46]. Time-lapse imaging 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 1× M9 buffer and covered gently with a coverslip. Z stacks were acquired with a Perkin Elmer spinning disk confocal microscope every 20 s with ~0.75 s exposure and 2 × 2 binning. For fluorescent image-collection details, see Supplemental Data.

RNA Interference

RNA interference by feeding was performed with the Ahringer lab RNAi feeding library [47].

One Generation RNAi Feeding

L1-stage larvae were placed on plates seeded with bacteria expressing dsRNA corresponding to the gene being knocked down and grown to adulthood ("PO" generation adults). See Figures 7A and 7B and Figures S4B and S4C.

Two Generation RNAi Feeding

PO adults from above were transferred to fresh RNAi plates and allowed to lay eggs, and these progeny ("F1" generation) were grown to adulthood and examined. Where stated, embryos from mothers of this F1 generation ("F2" generation embryos) were examined. See Figures 7C and 7D and Figure S5A.

RNAi Injection Plus Feeding

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 hr later. See Figure 6A and Figure S4A.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and eight figures and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(08)01627-8.

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