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***C. elegans* dosage compensation: insights into condensin-mediated gene regulation**

Sarah Elizabeth Albritton¹ and Sevinç Ercan^{1,*}

¹Department of Biology, Center for Genomics and Systems Biology, New York University, New York, NY 10003

Abstract

Recent work demonstrating the role of chromosome organization in transcriptional regulation has sparked substantial interest in the molecular mechanisms that control chromosome structure. Condensin, an evolutionarily conserved multi-subunit protein complex, is essential for chromosome condensation during cell division and functions in regulating gene expression during interphase. In *Caenorhabditis elegans*, a specialized condensin forms the core of the dosage compensation complex (DCC) which specifically binds to and represses transcription from the hermaphrodite X chromosomes. DCC serves as a clear paradigm for addressing how condensins target large chromosomal domains and how they function to regulate chromosome structure and transcription. Here, we discuss recent research on *C. elegans* DCC in the context of canonical condensin mechanisms as have been studied in various organisms.

Keywords

condensin; gene regulation; X chromosome; dosage compensation; genome organization; cooperation

Mechanisms that control chromosome structure in gene regulation

A flurry of recent studies mapping genome-wide chromosomal interactions has inspired new work focused on understanding the mechanisms that control these interactions, especially as they relate to the regulation of gene expression. Across all organisms, members of the structural maintenance of chromosomes (SMC) family of proteins are key regulators of chromosome structure [1]. While prokaryotes contain only a single SMC complex that functions in chromosome organization and segregation, three classes of eukaryotic SMC complexes (cohesin, condensin, and SMC5/6) have diverged functions in chromosome segregation, transcription, and DNA recombination and repair. SMC complexes share an evolutionarily conserved ring structure capable of entrapping chromatin fiber [2], a mechanism thought to underlie SMC-mediated chromosomal interactions. Recent work on

Correspondence: se71@nyu.edu (S. Ercan).

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gene regulation by SMC complexes has largely focused on cohesin [3]. However, an increasing number of studies indicate that condensins, essential for chromosome condensation during cell division, are also critical for the regulation of gene expression during interphase [4]. Notably, in *C. elegans* we find a clear paradigm for condensin-mediated gene regulation: the *C. elegans* dosage compensation complex (DCC). At its core, DCC contains a specialized condensin complex, condensin DC, which binds specifically to the X chromosomes in hermaphrodite worms, resulting in two-fold repression of X-linked transcription [5]. In this review, we discuss recent insights into the mechanisms of DCC binding. Additionally, we summarize the role of DCC in regulating X chromosome transcription and structure.

Structure and function of metazoan condensins

The condensin ring structure is composed of two SMC and three chromosome-associated polypeptide (CAP) subunits. Unlike yeast, which contains a single condensin, metazoans have two condensins [6] (Figure 1A). Named condensins I and II, these complexes play partially redundant yet distinct roles in chromosome condensation and segregation during cell division [7–11]. Highlighting their critical role in these processes, condensins were recently shown to be part of a minimal set of factors sufficient to shape mitotic chromosomes *in vitro* [12, 13]. Based on two observed condensin activities (ATP-dependent encircling of DNA [14] and positive supercoiling of DNA [15]), several models posit that chromosome condensation is a consequence of condensin-mediated chromosomal looping [16]. Loop formation may be the result of a single condensin ring entrapping two chromatin fibers. Alternatively, condensin “handcuffing” (wherein two condensin rings interact independently with two distant DNA loci and also with each other) may underlie loop structure [17]. Condensin-dependent positive supercoiling may also result in chiral looping [18]. A series of tandem condensin-mediated loops may be made progressively larger by a process termed “loop extrusion,” wherein condensin actively pushes DNA through its ring [19, 20].

In addition to condensins I and II, *C. elegans* contains a third condensin, condensin I^{DC} (hereafter condensin DC), named for its essential role in dosage compensation [21]. Condensin DC is distinguished from condensin I by its SMC4 variant, DPY-27 (Figure 1A). Unlike condensins I and II, which associate with all chromosomes, condensin DC binds specifically to both X chromosomes in XX hermaphrodites [22]. Condensin DC interacts with at least five non-condensin proteins (SDC-1, SDC-2, SDC-3, DPY-30, and DPY-21) to form the *C. elegans* DCC [5]. All DCC subunits, less SDC-1 and DPY-21, are essential and are co-dependent on each other for robust binding.

Mechanisms and specificity of DCC binding

Condensin association with chromosomes depends on both DNA and chromatin interactions. Condensin ring entrapment of DNA is likely the most stable form of condensin binding [23]. Condensins may contact DNA via their SMC hinge domain [24, 25] and/or their HEAT domain-containing CAP subunits [26]. Condensins may also interact with chromatin by associating with histone proteins [27, 28]. Specific targeting of SMC complexes to

chromosomal sites is important for their function [29]. Recent work on X-specific binding of *C. elegans* condensin DC provides insight into mechanisms regulating condensin targeting.

Hermaphrodite and X-specific binding of the *C. elegans* DCC

DCC begins localizing to the X chromosomes at the ~40-cell stage of embryogenesis, following the onset of hermaphrodite-specific *sdC-2* expression [30]. The remaining DCC subunits (all of which are maternally loaded) show a diffuse pattern of nuclear localization prior to *sdC-2* expression [31]. Thus, SDC-2 is both necessary and sufficient to induce X-localization of the DCC [30]. Two additional DCC subunits, DPY-30 and SDC-3, are also required for DCC binding [32, 33], but only SDC-2 can specifically localize to the X in the absence of DPY-30 and SDC-3, suggesting that SDC-2 plays a critical role in initiating hermaphrodite and X-specific DCC binding [30].

Immunofluorescence and genome-wide ChIP analyses of DCC subunits indicated that DCC is distributed across the length of both hermaphrodite X chromosomes [5]. Low-level DCC association is punctuated by distinct sites of accumulation (Figure 1B), enriched at a subset of promoters, enhancers, and tRNA genes. These same genomic elements are also bound by canonical condensins in both *C. elegans* [22] and other eukaryotes [29], suggesting a conserved molecular mechanism governing condensin binding to eukaryotic chromosomes.

The “recruitment and spreading” model for X-specific DCC targeting

To understand X-specific DCC binding, early work used large chromosomal duplications (1–10Mb) to survey the ability of discrete X chromosome regions to independently recruit DCC when detached from the native chromosome [34]. While some were capable of autonomous recruitment, several X regions (bound by DCC in the context of the native X [35]) failed to independently recruit the complex. This X-dependency suggested a “recruitment and spreading” mechanism for DCC binding. Subsequent work identified DNA sequences capable of DCC recruitment onto multi-copy extrachromosomal arrays [36]. Named recruitment elements on the X (*rex*), these sequences were shown to coincide with DCC binding foci, which show exceptionally high ChIP-enrichment on the native X (Figure 2A) [35, 37, 38].

Hierarchy of DCC recruitment sites

Analysis of DNA sequence at DCC recruitment sites identified a 12-bp motif (Figure 2B). Motif mutation or deletion resulted in loci-specific elimination of DCC binding [38, 39], suggesting a critical role for motif sequence in DCC recruitment. However, instances of motif sequence can be found across all five autosomes, none of which recruit DCC [35, 38]. This specificity problem was recently addressed [39]. Briefly, ChIP-seq analyses of several DCC subunits revealed 64 recruitment sites that exhibited quantitative differences in DCC binding (Figure 2A). Importantly, these differences revealed innate recruitment efficiencies. When inserted into an ectopic autosomal locus, a strong recruitment site recruited DCC more effectively than a weaker site [39]. Furthermore, SDC-2 bound only to strong recruitment sites in the absence of full DCC binding, suggesting that these loci serve as initial recognition/entry sites on the X [39].

X-specific features of strong DCC recruitment sites

Identification of initial DCC entry sites allowed for determination of features that distinguish these sites from the autosomes. Most strong recruitment sites contain a motif cluster (two or more motifs within 200bp), and almost all overlap with high occupancy target (HOT) sites (genomic regions bound by a majority of mapped transcription factors [40, 41]). On the autosomes, only five motif clusters were identified, none of which overlap with a HOT-site. Combinatorially, motif clustering and HOT-site overlap fully distinguish the X from the autosomes for initial DCC recruitment (Figure 2B).

Along the X, strong recruitment sites are interspersed with weaker sites that contain a single 12-bp motif and/or overlap with tRNA genes [39]. Notably, the *C. elegans* X contains ~2.5 fold more tRNA genes compared to the autosomes. In yeast, TFIIC physically interacts with condensin, promoting recruitment of the latter to tRNA genes [42–44]. It is possible that, following DCC recruitment at strong sites, subsequent recruitment at weaker sites occurs via canonical condensin mechanisms. Highlighting the potential for shared mechanisms of recruitment, DNA motifs enriched at DCC and condensin II sites share sequence similarity (Figure 2C) [22].

Mechanisms of DCC loading onto the X

The molecular mechanisms governing recognition of 12-bp motif clusters at HOT-sites remain unclear. Surprisingly, a number of accessible 12-bp motifs on the X lack DCC binding [39]. Recent work in *Drosophila* highlighted the importance of DNA shape in X-recognition by fly dosage compensation machinery [45]. To date, the *C. elegans* subunit capable of recognizing the 12-bp motif remains unknown. Identification of this protein will be important for evaluating the role of DNA shape in DCC recruitment (Figure 3). It is also not clear how “HOTness” contributes to DCC recruitment. Across species, HOT-sites have been linked to transcription [46, 47]. In yeast, condensin is recruited to cell-cycle regulated genes via interaction with specific transcription factors [48]. Thus, either a yet-unknown feature of HOT-sites, or specific transcription factor interactions may promote DCC recruitment.

High nucleosome occupancy intrinsically encoded at strong DCC recruitment sites [39] may also contribute to sex-specificity of DCC recruitment. In early embryos (of either sex), nucleosome occupancy may initially prevent binding of maternally-loaded DCC subunits (Figure 3). In later embryogenesis, hermaphrodite-specific expression of SDC-2 may facilitate nucleosome removal at recruitment sites, allowing for DCC binding to the X chromosomes. Supporting a model in which SDC-2 mediates chromatin-opening upstream of DCC loading, nucleosome-removal was shown to be important for condensin binding in yeast [50]. In addition, DCC binding at a single motif within a cluster may compete with nucleosomes, locally displacing the latter and thereby increasing accessibility of nearby motifs, providing nucleosome-mediated synergy to DCC recruitment [49].

A recent study reported that DCC recruitment involves the small ubiquitin-like protein SUMO [51]. SUMO adds a bulky moiety that interacts with other proteins via SUMO interacting motifs (SIMs) [52]. Three DCC subunits (SDC-3, DPY-27, and DPY-28) are

SUMOylated, and all but two subunits (SDC-1 and DPY-26) contain multiple SIMs [51]. Thus, SUMO-SIM interactions within the DCC may stabilize complex recruitment to the X (Figure 3). SUMOylation was shown to be involved in condensin binding to rDNA in yeast, suggesting a conserved function in condensin recruitment [53, 54].

Both extrachromosomal-recruitment assays and *in vivo* DCC binding pattern suggest that recruitment sites on the X act as entry points for condensin DC loading. However, alternative models explaining these data have not been tested. For example, it may be that condensin DC loads across the length of the X, accumulating at recruitment sites which serve to block progression of complex spreading [55]. Such an indirect mechanism has been suggested for the accumulation of cohesin at CTCF binding sites [3]. Future work is needed to address whether SDC proteins act similarly to CTCF to regulate condensin DC distribution across the *C. elegans* X.

Long-distance cooperation between DCC recruitment sites

Robust targeting of DCC to the X involves long-distance cooperation between recruitment sites [39] (Figure 2D). Cooperation was demonstrated by the requirement for multiple copies of recruitment site sequence (inserted either in tandem or at distance (30–50kb)) for efficient ectopic recruitment of DCC to an autosome. While cooperation between genomic elements has been described over short distances (e.g. super enhancers [56]), *C. elegans* DCC recruitment represents the first clear demonstration of cooperative protein recruitment over long distances (0.1–1Mb on the X). One possible mechanism for long-distance cooperativity is direct interactions within three-dimensional space. Supporting this idea, genome-wide chromosome conformation capture (3C-seq) data in *C. elegans* found that *rex-to-rex* interactions are among the strongest and most frequent intrachromosomal interactions [57]. Alternatively, long-distance cooperativity may depend on DCC spreading. Here, recruitment at one site may increase local DCC concentration within a Mb-scale spreading domain (discussed below), allowing efficient recruitment at neighboring sites.

DCC spreading along the X

In strains bearing X-to-autosome fusion (X:A) chromosomes, DCC spreads 1–3 Mb into autosomal sequences attached to the end of the X [37]. Levels of ectopic autosomal DCC binding are inversely proportional to the distance from the end of the X, suggesting that spreading occurs linearly, becoming reduced with increased distance from X-linked recruitment sites (Figure 4A). Within the native X, deletion of a single recruitment site resulted in reduced DCC binding across Mb-sized domains flanked by topologically associating domain (TAD) boundaries, suggesting that spreading is influenced by the topology of underlying chromosomal interactions [39] (Figure 4B).

Chromosomal spreading of SMC complexes

Similar to DCC, other SMC complexes have been shown to spread both *in vitro* [58] and *in vivo* [42, 59–61]. It has been suggested that SMC rings may be pushed by RNA and DNA polymerases [62–64]. However, recent work in *B. subtilis* using time-point analysis of spreading by ChIP-seq measured bacterial SMC complex speed at ~50 kb/min [61]. This is approximately 10-fold faster than the estimated speed of elongating polymerases [65, 66],

suggesting that the ATPase-containing SMC complex may itself be a fast molecular motor [67, 68] or utilize additional non-linear mechanisms of spreading. Fluorescence recovery after photobleaching (FRAP) analyses indicated that ~80% of condensin I and ~15% of condensin II are dynamically bound to chromatin, with the rest remaining more stably associated [69, 70]. It is possible that, while stable condensin rings slide along chromatin, unstable condensins “hop” or “diffuse” to distant sites, utilizing three-dimensional organization of the chromosomes (Figure 4C).

Condensin-mediated regulation of transcription, chromosome structure and organization

Similar to DCC, studies in *C. elegans*, *D. melanogaster*, and mammalian cells suggest that canonical condensins are primarily repressive [22, 71–73]. However, not all studies have reported repressive functions [74–76], suggesting that condensin-mediated gene regulation is context-specific. Regulation of gene expression appears intertwined with canonical condensin functions; a recent study suggested that condensin-mediated transcriptional repression prepares chromosomes for segregation during cell division [73]. While an increasing number of studies assign a role for canonical condensins in transcriptional regulation, *C. elegans* condensin DC provides an unambiguous example of a condensin complex acting specifically to repress transcription across an entire chromosome. DCC binding results in ~2-fold reduction of X chromosome gene expression [38, 77]. Genome-wide ChIP analyses indicated that DCC mutation and/or knock down cause increased RNA Pol II binding on the X [33, 77]. Similarly, global run-on sequencing (GRO-seq) analyses showed increased levels of RNA Pol II at X-promoters in embryos lacking SDC-2 [78], indicating that DCC represses gene expression by specifically reducing transcription initiation on the X.

A chromosome-wide mechanism for DCC-mediated transcriptional repression

Both mRNA and GRO-seq data in DCC mutants indicated that DCC represses most active genes on the X, albeit to varying degrees [77, 78]. Early microarray experiments reported a group of genes that “escaped” dosage compensation in embryos [38]. However, recent work indicated that dosage compensation is established gradually during development. The escapers might have appeared as such due to incomplete dosage compensation in early embryos [77]. Indeed, analysis of several sex determination mutants failed to identify large groups of escapers in larval worms [77, 79]. Additionally, a reporter transgene integrated into several ectopic X sites was consistently repressed by the DCC, supporting a chromosome-wide mechanism of repression [80]. Notably, DCC-repressed genes (those that show increased expression in a DCC mutant) are not necessarily directly bound by DCC [38]. Experiments addressing indirect effects of DCC depletion will be required to evaluate this observed disconnect between DCC binding and transcriptional repression. In sum, current data indicate that the *C. elegans* DCC works at a distance across most genes on the X to achieve a chromosome-wide average of two-fold transcriptional repression (Figure 5A).

DCC regulates 3D-organization of the X

Genome-wide mapping of chromosomal interactions in *C. elegans* revealed TADs to be more prominent on the X compared to the autosomes [57, 81]. Interestingly, TAD organization on the X is DCC-dependent [57]; 8 out of 17 TAD boundaries overlap with DCC recruitment sites, and show reduced boundary strength upon SDC-2 depletion [57]. In turn, organization of TAD boundaries across the X affects DCC spreading along the chromosome (Figure 4B, C) [39]. In other organisms, TAD boundaries overlap with insulator elements that regulate specificity of gene-regulatory interactions and are often bound by CTCF, cohesin, and condensin [82]. While CTCF is conserved in most animals, no homolog has been identified in *C. elegans* [83, 84]. In addition to CTCF, TFIIC-bound sites can also act as insulators [85]. Given that DCC recruitment sites overlap with both TAD boundaries and tRNA genes, it is possible that one or several DCC subunits act as insulator proteins. Future work should address how mechanisms of DCC recruitment control TAD organization on the X.

DCC regulates nuclear X-compaction and localization

Recent work using 3D volumetric measurements (based on fluorescence *in situ* hybridization) demonstrated the hermaphrodite X chromosomes to be ~40% compacted compared to autosomes [86]. Notably, this compaction was shown to be DCC-dependent [86]. While the compacted X chromosomes tended to occupy distinct territories within the nucleus, X territories appeared “fuzzy” in DCC mutants [86, 87]. Increased X volume might thus reflect both the loss of condensin-mediated chromatin compaction and a disorganization of subnuclear X-localization, as discussed below.

By measuring the radial positioning of several X chromosome loci in *C. elegans* embryos, one study reported that the single male X is located more frequently at the nuclear periphery compared to both the autosomes and the hermaphrodite X chromosomes [88]. Subsequent analyses of X-positioning by other groups have generated conflicting results. In 32-ploid intestinal cells, the X appeared more centrally located in both males and DCC mutants [89]. Another group failed to demonstrate X-positioning preference in either sex [80]. Instead, periphery-proximity was dependent on which X locus was being observed [80]. Technical limitations may be one reason for the conflicting data. Each of these analyses relied on a three-zone-scoring assay, which requires that nuclei be roughly spherical; yet the protocols used chemical fixation which can alter nuclei shape [90].

Nevertheless, supporting a role for DCC in regulating X-localization, a recent study reported that mutations in histone methyltransferases, *met-2* and *set-25*, and the chromodomain protein *cec-4*, (all of which are involved in tethering chromatin to the nuclear membrane) resulted in release of the middle of the X into the radial center of the nucleus [89]. The left end of the X chromosome was largely unaffected, suggesting that lamina attachment [91] may preserve peripheral localization of this arm. Although “tethering” mutants affected subnuclear positioning of the X, they showed little effect on X-expression as measured by mRNA-seq [89].

DCC regulates X-enrichment of H4K20 methylation and H4K16 deacetylation

DCC activity has been linked to the regulation of two histone modifications: monomethylation of H4K20 (H4K20me1) and acetylation of H4K16 (H4K16ac) [31, 77, 92]. Following X-localization of DCC, H4K20me1 becomes gradually enriched on the hermaphrodite X chromosomes in a DCC-dependent manner [31, 77, 92]. Importantly, in an X;II fusion strain (in which DCC spreads into autosomal sequence), H4K20me1 becomes enriched on the autosome near the fusion boundary [31], indicating that DCC binding is sufficient to induce local enrichment of H4K20me1. The DCC subunit DPY-21, required for X-enrichment of H4K20me1, was shown to belong to a Jumonji C family of demethylases that convert H4K20me2 to me1 [93].

In *C. elegans*, mutation of H4K20 monomethylase *set-1* (homolog of human PR-SET-7), resulted in upregulation of X-expression [77]. A null mutation of *set-4* (the methyltransferase that converts H4K20me1 to me2 [31, 92, 94]) resulted in increased H4K20me1 across the genome, equalizing levels between the X and autosomes [31, 77]. Consistent with a role in repression, H4K20me1 increase correlated with decreased expression from all five autosomes [77]. However, as DCC begins repressing the X in early embryogenesis (before X-enrichment of H4K20me1), it is unlikely that H4K20me1 acts as the single downstream effector of transcriptional repression [77]. Instead, H4K20me1 may contribute to repression by increasing DCC binding to the X, as shown for condensin II in mammalian cells [27]. Alternatively, similar to the potential role of H4K20me1 on the inactivated X chromosome in mammals, H4K20me1 enrichment on the *C. elegans* X may play a role in the maintenance of dosage compensation [95]. Interestingly, X-enrichment of H4K20me1 results in SIR-2.1-mediated depletion of H4K16ac [92], a mark that is actively removed from mitotic chromosomes [96, 97]. Thus, high H4K20me1 and low H4K16ac may establish a mitotic-like chromatin structure that reinforces condensin DC binding to the X during interphase.

Causes and consequences of DCC-mediated transcriptional repression

DCC binding regulates specific histone modifications, TAD organization, chromosome compaction, and subnuclear positioning of the X chromosomes (Figure 5B). However, individual perturbations to these processes either do not produce strong dosage compensation defects or do not strongly correlate with observed DCC-mediated transcriptional repression [57, 77, 89, 98]. One possibility is that cumulatively these activities create a repressive territory within the nucleus that restricts and reinforces X repression. (A similarly complex picture involving multiple mechanisms of repression is also emerging for X chromosome silencing in mammals [99].) Alternatively, DCC-dependent genome organization might be a downstream consequence of transcriptional repression. Determining the molecular mechanism by which DCC prevents RNA Pol II binding to X chromosome promoters will be important for elucidating the causal relationships between DCC-mediated transcriptional repression and the regulation of X chromosome structure.

Concluding remarks

Over the past two decades, the remarkable structure and function of SMC complexes have made them a focus of intense research. Here, we discussed the molecular mechanisms of a specialized condensin that forms the core of the *C. elegans* DCC. Research on DCC binding suggests that specific targeting of condensins to large chromosomal domains involves two-steps: recruitment and spreading. DCC recruitment is initiated by a set of strong recruitment sites whose DNA-sequence and chromatin features are X-specific. Long-distance cooperation between recruitment sites allows for robust DCC binding to the X chromosomes. Following recruitment, DCC spreads across Mb-scale domains. DCC reduces RNA Pol II binding to promoters, resulting in an average two-fold repression across the X. DCC modulates levels of specific histone modifications and regulates chromosomal interactions, compaction, and nuclear localization of the hermaphrodite X chromosomes. Several important questions on DCC mechanisms remain unanswered (Outstanding Questions Box). Addressing these questions will be important to understand how a specialized condensin, co-opted by the *C. elegans* DCC, functions to target and transcriptionally repress an entire chromosome.

Outstanding Questions

1. What proteins recognize the 12-bp DNA sequence motif at the recruitment sites? What are the mechanisms that regulate condensin DC ring loading to the X chromosome?
2. How is DCC recruitment and spreading linked to chromosomal interactions and organization of TADs on the X chromosomes? What is the molecular mechanism by which the DCC spreads along chromatin?
3. Is DCC-dependent chromatin organization a cause or consequence of transcriptional repression? What is the role of nuclear position of the X chromosomes in dosage compensation? How does the DCC reduce RNA Pol II binding to X chromosome promoters?

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Trends box

1. The core of the *C. elegans* DCC is a specialized condensin that differs from the canonical condensin I by a single SMC-4 variant, DPY-27 [21].
2. Genome-wide analyses of DCC and canonical condensin binding in *C. elegans* indicate conservation in the genomic localization of different types of condensin complexes [22].
3. X-recognition by DCC occurs at a limited number of strong recruitment sites, which contain multiple copies of a 12-bp sequence motif and overlap with HOT-sites [35, 38, 39].
4. Long-distance cooperation between recruitment sites on the X establish and reinforce robust DCC binding [39].
5. Following recruitment, DCC spreads linearly across megabase distances flanked by TAD boundaries [37, 39].
6. DCC represses transcription by reducing RNA Pol II binding to X-chromosome promoters [33, 77, 78][77, 79, 80].
7. DCC regulates compaction, nuclear localization [86, 89], TAD organization [57], and levels of H4K20me1 and H4K16ac on the X chromosomes [31, 77, 92, 93].

Figure 1. Structure and binding of the different types of condensin complexes in *C. elegans*

A) Subunits of the three *C. elegans* condensin complexes and the DCC proteins are shown. Within a condensin, each SMC protein folds over to form a ~50nm anti-parallel coiled-coiled arm with a hinge and an ATPase domain at opposite ends. Two SMC proteins dimerize at their respective hinge domains, interacting with three CAP subunits at coiled-coil arms and near their ATPase domains. The CAPH (Kleisin) subunit links the two SMC proteins and interacts with two HEAT-domain containing CAP subunits (CAPD and CAPG). Condensins I and II share the same SMC heterodimer and are distinguished by their differing sets of CAP subunits. Condensin DC differs from condensin I by a single SMC4 variant, DPY-27. **B)** ChIP-seq binding profiles (from [22]) of condensin II (SMC-4 and KLE-2), condensin DC (DPY-27), condensin I/DC (DPY-26) subunits in *C. elegans* embryos are shown across a representative ~60 kb region on an autosome (left) and on the X (right). The condensin DC specific subunit, DPY-27, shows baseline enrichment on the X, punctuated with peaks previously shown to be enriched at a subset of gene promoters, enhancers, and other intergenic sites including tRNA and ncRNA genes [22].

Figure 2. Features of DCC recruitment sites on the *C. elegans* X chromosome

A) ChIP-seq binding profiles from embryos are shown for condensin DC (blue) and non-condensin (pink) subunits of the *C. elegans* DCC. This ~250kb region on the X contains a strong (pink) and a weaker recruitment site (grey). Data are from [39]. **B)** Features of strong recruitment sites (multiple motifs (shown in blue-green) and overlap with HOT-sites) are X-specific. Weaker sites tend to overlap with tRNA genes. **C)** The 12-bp DCC recruitment motif sequence and a 10-bp sequence motif enriched at autosomal condensin II binding sites. The two motif sequences share a "GCGC" core. **D)** Long-distance cooperation between recruitment sites located 0.1–1Mb apart may recruit DCC specifically and robustly to large chromosomal domains on the X chromosomes. Autosomes contain fewer potential recruitment sites and thus lack the benefit of cooperation between multiple recruitment sites.

Figure 3. A model for condensin DC ring loading onto the X chromosomes

The DCC subunits required for condensin DC binding are SDC-2, SDC-3, and DPY-30.

These recruiter proteins may recognize specific features of the recruitment sites including motif sequence within a nucleosomal context, DNA shape, and transcription factor binding at HOT-sites. High DNA-encoded nucleosome occupancy at the recruitment sites may be involved in nucleosome-mediated cooperativity of the motifs (small green bars), and may also prevent binding of maternally-loaded DCC subunits prior to *sdc-2* expression in early embryogenesis. The recruitment sites may be the actual sites of condensin DC ring loading upon removal of nucleosomes. Alternatively, condensin DC may load along the chromosome and then accumulate at the recruitment sites (not depicted). SUMO-SIM interactions within DCC subunits may stabilize complex binding at recruitment sites.

Figure 4. DCC spreading along the X chromosomes

A) DCC spreads into the autosomal sequence of X-to-autosome fusion (X:A) chromosomes. Spreading occurs over 1–3 Mb (dependent on X:A strain), and reduces with distance from the end of the X. **B)** On the X chromosomes, deletion of a single recruitment site reduces DCC binding across Mb-sized domains surrounded by TAD boundaries. The level of reduction in DCC binding correlates with the distance from the deleted recruitment site, suggesting existence of Mb-sized spreading domains. **C)** The possible molecular mechanisms by which stable and non-stable DCC complexes may spread along the X chromosomes. The mechanism of spreading may involve ring sliding, loop-extrusion, and/or diffusion/hopping.

Figure 5. The effect of DCC on X chromosome transcription and structure

A) The DCC reduces RNA Pol II binding to promoters, resulting in an average 2-fold reduction of gene expression across the hermaphrodite X chromosomes. **B)** During early embryogenesis, X chromosome targeting of the DCC is initiated by a set of strong DCC recruitment sites that are distributed across the ~17Mb X chromosomes. Long-range cooperation between multiple strong and weaker recruitment sites followed by local spreading establishes robust DCC binding across large chromosomal domains. DCC spreading is restricted by TAD boundaries (dotted circles), which are, in part, demarcated by DCC itself. During later embryogenesis, DCC binding results in increased levels of H4K20me1 and decreased levels of H4K16ac on both X chromosomes. As a result, the dosage compensated hermaphrodite X chromosomes become ~40% more compacted

compared to autosomes. Lastly, DCC binding influences nuclear localization of the X. Altogether, these observations suggest that a multitude of mechanisms are involved in establishing and maintaining a repressive environment across the hermaphrodite X chromosomes.

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