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Targeting X Chromosomes for Repression

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Abstract

Dosage compensation is a chromosome-wide regulatory process that balances X-chromosome gene expression between males and females in species whose sex-determining mechanisms require each sex to have a different complement of X chromosomes. Recent advances have clarified the molecular nature of the *C. elegans* sex-determination signal, which tallies X chromosome number relative to the ploidy and controls both the choice of sexual fate and the process of dosage compensation. Dissecting the sex signal has revealed molecular mechanisms by which small quantitative differences in intracellular signals are translated into dramatically different developmental fates. Recent experiments have also revealed fundamental principles by which *C. elegans* dosage compensation proteins recognize and bind X chromosomes of XX embryos to reduce gene expression. Dosage compensation proteins function not only in a condensin complex specialized for regulating X-chromosome gene expression, but also in distinct condensin complexes that control other chromosome-wide processes: chromosome segregation and meiotic crossover recombination. The reshuffling of interchangeable molecular parts creates independent machines with similar architecture but distinct biological functions.

Introduction

Organisms that determine sex using chromosome-based mechanisms (e.g. XX female and XY or XO male), have evolved the essential, chromosome-wide regulatory process called dosage compensation to balance sex-chromosome gene expression between the sexes [1]. Strategies for dosage compensation differ, but invariably a regulatory complex is targeted to the sex chromosome of one sex to modulate transcript levels across the entire chromosome. Dosage compensation is exemplary for dissecting the coordinate regulation of gene expression over vast distances and the role of chromosome structure in controlling gene expression.

Mammals randomly inactivate one of the two female X chromosomes using non-coding RNAs that recruit the Polycomb complex [2,3]. Transient pairing of X chromosomes through the X-inactivation center heralds the onset of inactivation and helps specify the X to become inactivated [4,5]. A repressive nuclear compartment reliant on non-coding RNAs recruits the X genes to be silenced [6]. In contrast, flies increase expression of the single male X chromosome through a complex containing non-coding RNAs and MSL (Male Specific Lethal) proteins that binds the male X and acetylates histones [7]. Nematodes reduce expression of both X chromosomes in hermaphrodites by half through a dosage compensation complex (DCC) that binds the two X chromosomes [8]. The DCC resembles condensin, a protein

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complex conserved from yeast to humans to promote the compaction, resolution, and segregation of chromosomes during mitosis and meiosis [9-12]. In all three examples, selective recruitment of the dosage compensation machinery establishes the epigenetic regulation of X chromosomes that is maintained throughout the lifetime of the animal.

Fundamental issues relevant to all forms of dosage compensation include the regulatory pathway and sex-specific factors that activate dosage compensation in only one sex, the composition of the dosage compensation machinery, the *cis*-acting sites that selectively target the X chromosome for regulation, and the mechanism of fine tuning gene expression. This review focuses on *C. elegans* dosage compensation, with brief comparisons to *D. melanogaster*. Emphasis is placed on recent advances in understanding (1) the *C. elegans* regulatory hierarchy that controls dosage compensation, including the primary sex-determination signal, (2) fundamental principles by which the DCC recognizes and binds X chromosomes, and (3) the roles of dosage compensation proteins in controlling other chromosome-wide processes through association with distinct condensin complexes: crossover recombination during meiosis and chromosome segregation during mitosis and meiosis.

X:A signal: X and autosomal signal elements oppose each other to determine *C. elegans* sex

C. elegans determines sex by tallying X-chromosome number relative to the ploidy, the number of sets of autosomes (X:A signal) (Figure 1a) [13,14]. Ratios of 1X:2A and 2X:3A elicit male fate, while 2X:2A and 3X:4A elicit hermaphrodite fate. Not only does the X:A signal dictate sexual fate, it establishes the level of X-linked gene expression by controlling the process of dosage compensation [15,16]. Dissecting the sex determination signal in *C. elegans* has revealed molecular mechanisms by which small quantitative differences in intracellular signals can be translated into dramatically different developmental fates.

X-chromosome number is communicated by a set of *trans*-acting X-signal elements (XSEs) encoded on the X chromosome (Figure 1a) [17-20]. XSEs act in a cumulative, dose-dependent manner to repress the target gene called *xol-1* (XO lethal) in 2X:2A embryos. *xol-1* is earliest-acting sex-determining gene and encodes a GHMP kinase family member that specifies the male fate when active and the hermaphrodite fate when inactive [15,19,21,22]. *xol-1* also controls dosage compensation and hence viability. Inappropriate *xol-1* repression in 1X:2A embryos or inappropriate *xol-1* activation in 2X:2A embryos causes lethality due to incorrect levels of X-chromosome gene expression [22]. XSEs were discovered through genetic schemes that identified suppressors of the lethal effects of *xol-1* misregulation.

XSE-mediated repression of *xol-1* occurs at two distinct levels: transcriptional repression via the nuclear receptor SEX-1 and the ONECUT homeodomain protein CEH-39 and post-transcriptional repression via the RNA binding protein FOX-1 (Figure 1b) [17-20,23]. Both SEX-1 and CEH-39 repress *xol-1* directly, by binding to multiple sites in its promoter (B. Farboud, J. Gladden, and B. Meyer (unpublished). Direct RNA splicing control of residual *xol-1* transcripts by FOX-1 then enhances the fidelity of the counting process by creating an inactive *xol-1* mRNA splice variant in XX embryos (C. Pickle, M. Nicoll, and B. Meyer, unpublished). In summary, multiple repressors act through multiple sites to translate the two-fold difference in X dose between the sexes into the HIGH or LOW activity state of *xol-1*. Two tiers of *xol-1* regulation ensure the correct and stable choice of sexual fate.

Ploidy is communicated by an actual autosomal signal (Figure 1a). The autosomal signal contains discrete *trans*-acting autosomal signal elements (ASEs) that counter XSEs to coordinately regulate both sex determination and dosage compensation by activating *xol-1* [24]. ASEs were identified as suppressors of the XX-specific lethality caused by loss of XSEs.

SEA-1, a T-box transcription factor, and SEA-2, a zinc finger protein, act in a cumulative, dose-dependent manner to stimulate *xol-1* transcription [24] (P. Nix and B. Meyer, unpublished). Transcriptional activation is direct: ASEs bind to the *xol-1* promoter (Figure 1b) (M. Jow, P. Nix, and B. Meyer, unpublished). Thus, SEA-1 and SEA-2 engage in direct molecular warfare with XSEs to overcome their repressive effects, and *xol-1* integrates both X and autosomal signals to determine sexual fate. Antagonistic molecular interactions carried out on a single promoter explain how even tiny differences in the X:A ratio can signal different sexual fates: X:A of 0.67 signals male fate, while X:A of 0.75 signals hermaphrodite fate.

The concept of a sex signal composed of zygotic ASEs that oppose zygotic XSEs arose as an hypothesis for fruit flies in 1921 [25] and was soon thereafter presented in textbooks as fact. Ironically, while the worm sex signal fits this simple textbook paradigm, the fly sex signal does not: the effect of ploidy on fly X-chromosome counting is not through ASEs.

The target of the fly X:A signal is *Sxl* (Sex lethal), the feminizing sex-determination switch gene that induces female development when active and male development, including activation of the MSL dosage compensation complex, when inactive [26,27]. A set of XSEs serve as transcriptional activators of *Sxl* such that the double dose of XSEs in 2X:2A embryos turns *Sxl* on [28-32]. Extensive genetic screens to recover mutations in ASEs as suppressors of the XX-specific lethality caused by mutations in XSEs identified only a single ASE that acts as a weak transcriptional repressor of *Sxl* but cannot account for the effect of ploidy on sex determination [33,34]. Thus, autosomal factors in the form of *Sxl* repressors do not appear to serve as the monitor of ploidy. Instead, the ploidy affects the timing of cellularization and hence the interval of time during which XSEs can increase in concentration and activate the establishment promoter of *Sxl* [35]. The lower the ploidy, the longer the XSE promoters remain active and hence the greater the probability of activating *Sxl*. As a consequence, 1X: 1A embryos become females instead of males, and 2X:3A embryos become mosaic intersexes with less than 100% of cells stably activating *Sxl*.

The dosage compensation complex

Repression of *xol-1* in XX embryos permits the novel, XX-specific protein SDC-2 (Sex Determination and Dosage Compensation) to be active and thereby induce hermaphrodite sexual development and initiate dosage compensation (Figure 2a) [36,37]. SDC-2 acts with the zinc-finger proteins SDC-1 and SDC-3 to induce hermaphrodite development by repressing *her-1*, a sex-determination gene that elicits male development [37-39]. SDC proteins inactivate *her-1* directly by binding to three sites in the gene [38]. SDC-2 also triggers assembly of the DCC onto X through collaboration with SDC-3 and DPY-30 (DumPY) [37,40,41]. DPY-30 is a member of both the DCC and the transcription activation complex called COMPASS, which is responsible for trimethylation of histone H3 at lysine 4 (C. Hassig, R. Auty, W. Kruesi, B. Meyer unpublished) [42]. SDC-2 is the sole dosage compensation protein produced exclusively in XX embryos, and ectopic activation of SDC-2 in XO embryos causes the DCC to assemble onto the male X and the males to die [37]. SDC-2 confers both sex- and X-specificity to DCC binding.

The DCC includes ten proteins: the three SDC proteins, DPY-30, DPY-21 (a novel protein), and five proteins that are homologous to the members of condensin (Figures 2a and 3) [9,10, 37,38,40,43-46] (W. Kruesi, C. Hassig and B. Meyer, unpublished). The DCC and condensin each contain a pair of SMC proteins (Structural Maintenance of Chromosomes) and three non-SMC proteins [10,11,43,44,47]. The DCC SMC proteins DPY-27 and MIX-1, which are homologs of condensin SMC2 and SMC4, respectively, have nucleotide binding domains (NBDs) at their N- and C-termini that are linked by two long coiled coil domains separated by a hinge domain. Mutation of the NBDs disrupts dosage compensation [43,44]. SMC proteins

fold back on themselves to form a central region of two anti-parallel coiled coils flanked by the NBDs and the hinge. The two SMC proteins dimerize through interactions between their hinge domains and use their globular NBDs to bind the non-SMC proteins (DPY-26, DPY-28 and CAPG-1 in the DCC and CAP-H, CAP-D2, and CAP-G1, respectively, in condensin).

With the exception of MIX-1 and CAPG-1, which were identified through biochemical experiments, the DCC components were discovered through genetic screens that recovered either XX-specific lethal mutations or suppressors of the XO-specific lethality caused by *xol-1* null mutations. Dosage compensation proteins (DC proteins) function not only in a condensin complex specialized for dosage compensation, but also in two other biochemically distinct condensin complexes (condensin I and condensin II) that control other chromosome-wide processes (Figure 3) [10,48,49], as will be described subsequently.

Evidence that DC proteins form a complex on X came from multiple lines of experiments. (1) Immunofluorescence experiments using X-specific DNA probes and DC protein antibodies showed co-localization of all DC proteins with X [9,10,37,38,40,43-46,50] (W. Kruesi, C. Hassig and B. Meyer, unpublished). (2) Co-immunoprecipitation experiments using antibodies to each DC protein coupled with protein analysis using Western blots or mass spectrometry showed the association of all DC proteins [9,10,37,38,40,43-46,50] (W. Kruesi, C. Hassig and B. Meyer, unpublished). (3) Functional assays to identify X sites that recruit DC proteins *in vivo* when detached from X showed all components bind to recruitment sites [51-53]. (4) Chromatin immunoprecipitation experiments using antibodies to six different DC proteins followed by hybridization to genome-wide tiling arrays (ChIP-chip analysis) showed all subunits have similar binding peaks [53,54].

Targeting the DCC to X chromosomes: *cis*-acting sites

The combination of the genome-wide approach to identify DCC binding sites without regard to recruitment ability and the functional approach *in vivo* to assess DCC recruitment to sites detached from X showed the DCC binds to discrete, dispersed sites on X that partition into two classes (Figures 2a and 3c) [53]. *rex* sites (recruitment elements on X) recruit the DCC in an autonomous, DNA sequence-dependent manner using a 12 base pair MEX consensus motif that is enriched on X compared to autosomes (Figure 2d). MEX variants enriched on X by 3.8- to 25-fold are highly predictive (95%) for *rex* sites, and mutation of MEX motifs disrupts DCC binding (Figure 3a). MEX motifs are clustered in *rex* sites and collaborate to recruit the DCC [52]. *rex* sites confer X-chromosome specificity to dosage compensation and occur in only about 200 distinct locations on X.

dox sites (dependent on X) bind the DCC in their native context on X but fail to recruit the DCC when detached from X [53]. *dox* sites are ~7-fold more prevalent than *rex* sites. *dox* sites lack the MEX variants enriched on X, and motif searches have not identified a compelling motif that distinguishes *dox* sites from random X or autosomal DNA. *dox* sites per se are not the catalyst for DCC disbursement along X; rather *cis*-linkage to *rex* sites allows *dox* sites to become fully occupied by the DCC, as will be described subsequently. *dox* sites do not necessarily need sequences distinct from autosomal sequences, since *rex* sites mark X chromosomes for DCC binding and repression.

dox sites occur preferentially in highly transcribed promoters, whereas *rex* sites reside predominantly in intergenic regions [53]. Both the level of DCC binding at *dox* sites and the probability of a promoter having a *dox* site correlate directly with the expression level of the gene. Moreover, promoters dynamically regulated during development bind the DCC at higher levels during periods of transcriptional activity, further implicating transcription in binding to

dox sites [55] (W. Kruesi and B. Meyer, unpublished). In contrast, *rex*-site binding remains relatively constant throughout somatic development.

Although the MEX motif is central to the mechanism by which the DCC distinguishes X chromosomes from autosomes, it cannot be the sole source of X specificity [53]. Some *rex* sites have only MEX variants with consensus matches and distributions similar to those in *dox* sites, random autosomal sequences, and X sequences not bound by the DCC. A feature other than MEX must designate those sites for recruitment. Nonetheless, mutation of the weak motifs in such *rex* sites disrupts recruitment, showing that the MEX variants not enriched on X are important for DCC binding in the context of a *rex* site.

rex and *dox* sites are interspersed and separated by considerable distances (2-90 kb), implying that long-range communication might facilitate DCC loading onto X. The similarity of the DCC to condensin suggests models involving changes in chromosome structure, such as chromatin looping. Consistent with this view, in yeast, condensin complexes co-localize with widely dispersed tRNA genes to facilitate their clustering in the nucleolus [56]. In *C. elegans*, tRNA genes on X are highly correlated with DCC binding sites [53], suggesting the association of binding sites might occur.

DCC Loading onto X

Discovery of autonomous (*rex*) and dependent (*dox*) DCC binding sites suggests a model by which the DCC loads onto X chromosomes. The DCC binds to *rex* sites, which recruit additional complexes to bind along X [53]. This model is supported and refined by two additional sets of experiments that address the relationship between DCC binding at *rex* and *dox* sites. First, ChIP-chip analysis of DCC binding in mutants lacking any one of the recruitment proteins SDC-2, SDC-3 or DPY-30 showed that binding to *rex* sites was essentially abolished, binding to 65% of *dox* sites was eliminated, and binding to 35% of *dox* sites was reduced (R. Pferdehirt and B. Meyer, unpublished). These results show that DCC binding to *rex* sites requires all recruitment proteins and binding to *dox* sites is both dependent and independent of these proteins. That is, *dox* sites have an inherent, limited capacity to bind some DCC components, but full occupancy requires the recruitment proteins and presumably their binding to *rex* sites.

The dual roles of DPY-30 in recruiting the DCC to X and in activating gene expression by acting in COMPASS led to previous speculation that DPY-30 was the primary protein required for “spreading” of the DCC from *rex* sites to *dox* sites in active promoters [57]. However, the loss of binding to *rex* sites in *dpy-30* mutants contradicts a selective role for DPY-30 in “spreading” but instead indicates that DPY-30 acts similarly to SDCs in directing DCC binding to X (R. Pferdehirt and B. Meyer, unpublished). Moreover, the robust binding of SDC-2 to both *rex* and *dox* sites [53] contradicts the view that SDC-2 is required selectively for DCC binding to *rex* sites [55].

Second, if the proximity *of rex* sites to *dox* sites on X facilitates DCC binding to *dox* sites, then attaching *rex* sites to autosomes might enhance binding to autosomes. The DCC binds to autosomes, but at fewer sites (one-fifth) than on X [53]. Binding is not perturbed by disruption of *sdc-2*, *sdc-3* or *dpy-30*, consistent with the DCC having low, intrinsic binding capability at specific locations (R. Pferdehirt and B. Meyer, unpublished). The autosomal sites usually reside in promoters of expressed genes [53], and DCC binding correlates directly with the dynamic pattern of gene expression during development, as does binding to *dox* sites on X (W. Kruesi and B. Meyer, unpublished).

Two independent studies found enhancement of DCC binding to autosomal territories located adjacent to X in X-to-autosome fusion chromosomes. In one study [55], DCC binding to wild-

type autosomes was negligible and binding to the autosomal region adjacent to X in the fusion chromosome was interpreted as the establishment of new DCC binding sites. In the other study (R. Pferdehirt and B. Meyer, unpublished), DCC binding to the autosomal region adjacent to X generally represented an increase in occupancy at sites usually bound by the DCC on wild-type autosomes. While the mechanistic interpretations of the studies differ in significant ways, both support the view that *dox*-site binding on X is strengthened by proximity to *rex* sites. Thus, DCC binding to *rex* sites confers X specificity to dosage compensation and catalyzes increased DCC binding along X at *dox* sites (Figure 4).

Comparison of X-chromosome recognition and dosage compensation complex binding in flies and nematodes

Although the dosage compensation complexes of flies and nematodes are evolutionarily unrelated and regulate X chromosomes in opposite ways, similar principles appear to govern the X-chromosome targeting and binding of the two complexes. In flies, dosage compensation is achieved by the MSL (male-specific-lethal) complex, which binds to the single X chromosome of males to increase transcript levels [7]. As in worms, about 150 special chromatin entry sites recruit the MSL complex in a DNA-sequence dependent manner using a motif enriched on X [53,58,59]. For both flies and worms, a second mode of binding correlates with high levels of gene expression and appears sequence independent, suggesting that features common to transcribed genes facilitate additional binding on X [53,60-62]. Full binding to the transcription-associated class of sites requires attachment to the sequence-dependent recruitment sites [55,63] (R. Pferdehirt, W. Kruesi and B. Meyer, unpublished). Sequence-independent MSL binding favors the 3′ ends of active genes [64-66], while sequence-independent nematode DCC binding often favors 5′ ends of active genes [53,55].

The *C. elegans* DCC acts at a distance to repress genes on X

The mechanism of dosage compensation in *C. elegans* was explored by correlating the locations of DCC binding sites with the genes responsive to dosage compensation [53]. Several striking conclusions emerged. First, the DCC does not compensate all X genes, nor does it always achieve a precise 2-fold repression when it does. Second, although the DCC binds preferentially to more highly expressed genes on X, it can compensate X genes of all expression levels. Third and most unexpected, DCC binding to the promoter or body of a gene is neither necessary nor sufficient to elicit compensation of that gene. Compensated and non-compensated genes alike can have DCC binding sites or not. Thus, additional factors besides the proximity of DCC binding must help define whether a gene responds to the dosage compensation process. Although the DCC could potentially act locally at the sites it binds to modulate gene expression [54], it must act over long range to achieve the compensation of most genes [53]. The DCC likely alters higher-order chromosome structure to control interactions between dispersed regulatory elements that modulate gene expression.

Autosomal gene expression is affected by the DCC

DCC disruption causes opposite effects on X and autosomal genes: X genes have increased expression, and one-fourth of autosomal genes have reduced expression [53]. The DCC thus affects expression throughout the genome. The DCC binding sites on autosomes correlate infrequently with genes affected by dosage compensation mutations. The effect of DCC disruption on autosomal gene expression cannot simply be explained by an indirect effect on X-linked gene expression, because weak dosage compensation mutations have a similar effect on autosomal gene expression as strong mutations, even through strong mutations have a greater effect on X gene expression. However, an “indirect” effect on autosomal gene expression could result from a possible, albeit speculative model of DCC action in which the

DCC repels a rate-limiting activator from X, thus making more activators available to autosomes. In dosage compensation mutants, more activators would bind to X and activate X genes, leaving less activator available for autosomal genes.

DC proteins achieve diverse chromosome-wide functions through participation in distinct condensin complexes

DC proteins function not only in a condensin complex specialized for dosage compensation (condensin I^{DC}) but also in two other biochemically distinct condensin complexes in *C. elegans* (condensin I and condensin II) to carry out independent roles in chromosome segregation and meiotic crossover regulation (Figure 3) [10,12,48,49]. Condensin I differs from condensin I^{DC} by only one subunit: SMC-4 from condensin II replaces the SMC protein DPY-27 from condensin I^{DC} [10,48]. Condensin I controls the number and distribution of double strand breaks (DSBs) in meiosis and thereby the number and position of crossovers (COs) between homologous chromosomes by controlling the higher order structure of meiotic chromosomes (Figure 3b) [48]. Disrupting any condensin I subunit causes a dominant change in the distribution of DSBs, and hence COs, and dramatically lengthens the chromosome axis. Control of CO distribution was thought to be imposed only after DSB formation, affecting the decision of a DSB to become a crossover or noncrossover. Condensin I analysis showed instead that CO number and distribution can also be controlled on a chromosome-wide basis at the level of DSB formation by controlling chromosome structure.

Condensin I also affects mitotic and meiotic chromosome segregation, but in a manner distinct from condensin II, the primary condensin complex in *C. elegans* to control the compaction and resolution of mitotic and meiotic chromosomes in preparation for their separation (Figure 3c) [9,10,49]. Condensin II shares the SMC protein MIX-1 with condensin I^{DC} and both SMC proteins with condensin I [12,49]. Condensin II has non-SMC proteins that are distinct from, but homologous to, those shared by condensin I and condensin I^{DC} [10,12]. Condensin II binds to the centromeres of mitotic chromosomes and to meiotic chromosomes at pachytene exit to create the compact chromosome shape required for chromosome segregation [12,49]. The participation of dosage compensation proteins in diverse condensin complexes illustrates that reshuffling of homologous, interchangeable molecular parts can create independent machines with similar architecture but distinct biological functions.

Conclusions

Fundamental principles have emerged regarding the X-chromosome-specific targeting and loading of a condensin-like DCC to reduce gene expression from both X chromosomes of *C. elegans* hermaphrodites. Two distinct mechanisms govern DCC binding: sequence-dependent recruitment to autonomous binding sites that confer X-chromosome specificity (*rex*) and non-autonomous, sequence-independent binding to sites (*dox*) correlated with high levels of gene expression. The dosage compensation process is controlled by the primary signal that determines sexual fate, the X:A signal. Dissection of this signal has revealed molecular mechanisms by which small quantitative differences in intracellular signals are translated into alternative developmental fates.

The mechanism of *C. elegans* dosage compensation has not yet been determined, but given the homology of DCC components to condensin subunits, the long-range communication between *rex* and *dox* sites to facilitate DCC loading, the action of the DCC at a distance to regulate X gene expression, and the impact of the DCC on autosomal gene expression, the DCC likely functions by inducing changes in chromosome configuration. Such reconfiguration of chromosome architecture could bring regulatory DNA elements into proximity with genes to be regulated, could create sub-chromosomal territories within X for gene repression, could

create a specific sub-nuclear compartment for X to modulate its gene expression, or could even repel a rate-limiting, genome-wide activator from X to reduce gene expression. Precedent exists for such action of condensin. Condensin binds yeast tRNA genes and facilitates the clustering of these widely dispersed loci into the nucleolus [56,67,68]. Indeed, tRNA genes on X are highly correlated with DCC sites, raising the possibility that DCC changes X architecture through association with tRNA genes [53]. In general, condensin can also restrict interactions between chromosomes, thereby causing their isolation. For example, condensin is required for the programmed restructuring and disassembly of *Drosophila* polytene chromosomes, giant DNA structures with multiple paired chromatids formed by numerous rounds of DNA replication without intervening cell divisions [69]. The compartmentalization of chromatids may be exemplary for how the DC might restrict X chromosomes to domains of gene repression.

Future experiments will reveal the dosage compensation mechanism, a more precise understanding of how the DCC binds to X chromosomes, and the means by which condensin complexes that differ by only a single, homologous subunit can have distinct functions.

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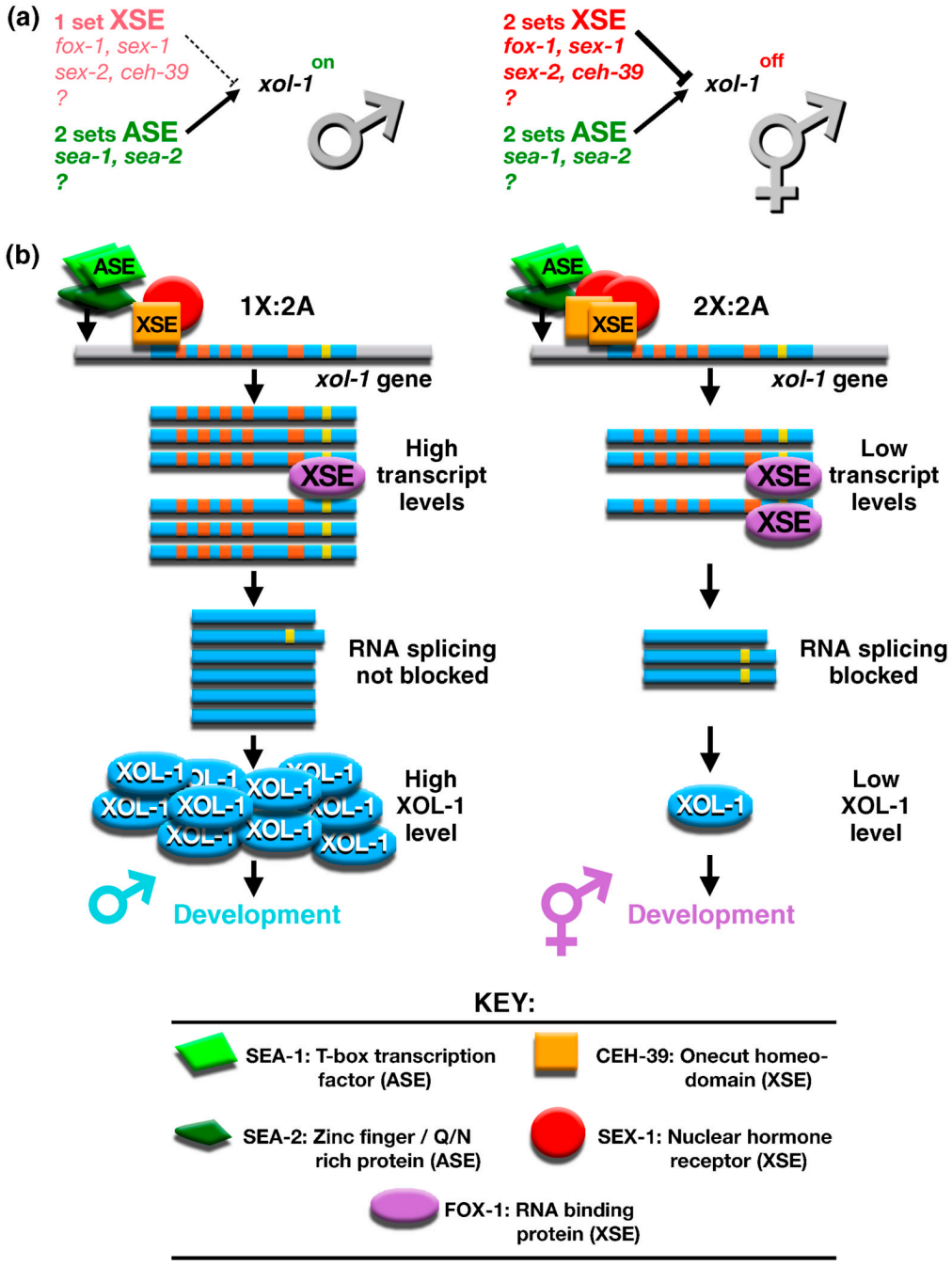

Figure 1. Primary sex determination: the X:A signal model

(a) In *C. elegans*, sex is determined by the number of X chromosomes relative to the ploidy, the number of sets of autosomes. This X:A signal is composed of X signal elements (XSEs) that activate the hermaphrodite program of development and Autosomal signal elements (ASEs) that oppose the XSEs to promote male development. The direct target of the X:A signal is the sex determination switch gene *xol-1*, which controls both the choice of sexual fate and the level of X-linked gene expression achieved through the process of dosage compensation. **(b)** X and autosomal signals antagonize each other directly at *xol-1* to determine *C. elegans* sex. Direct rivalry at the *xol-1* promoter between XSE transcriptional repressors (the ONECUT homeodomain protein CEH-39 and the nuclear hormone receptor SEX-1) and ASE

transcriptional activators (the T-box transcription factor SEA-1 and the zinc finger protein SEA-2) leads to high *xol-1* transcript levels in XO embryos and low levels in XX embryos. FOX-1, an RNA binding protein that acts as an XSE, then enhances the fidelity of signaling process by creating an inactive *xol-1* mRNA splice variant in a dose-dependent manner. High XOL-1 protein levels in XO animals cause male development, and low XOL-1 levels in XX animals cause hermaphrodite development, including loading of the DCC onto X. Decreasing XSE dose causes XX-specific lethality, while increasing it causes XO-specific lethality. The reciprocal is true for ASEs. Increasing ASE dose causes XX-specific lethality; decreasing it causes XO-specific lethality. (*xol-1* gene: promoter, gray; exons, blue; introns, orange and yellow).

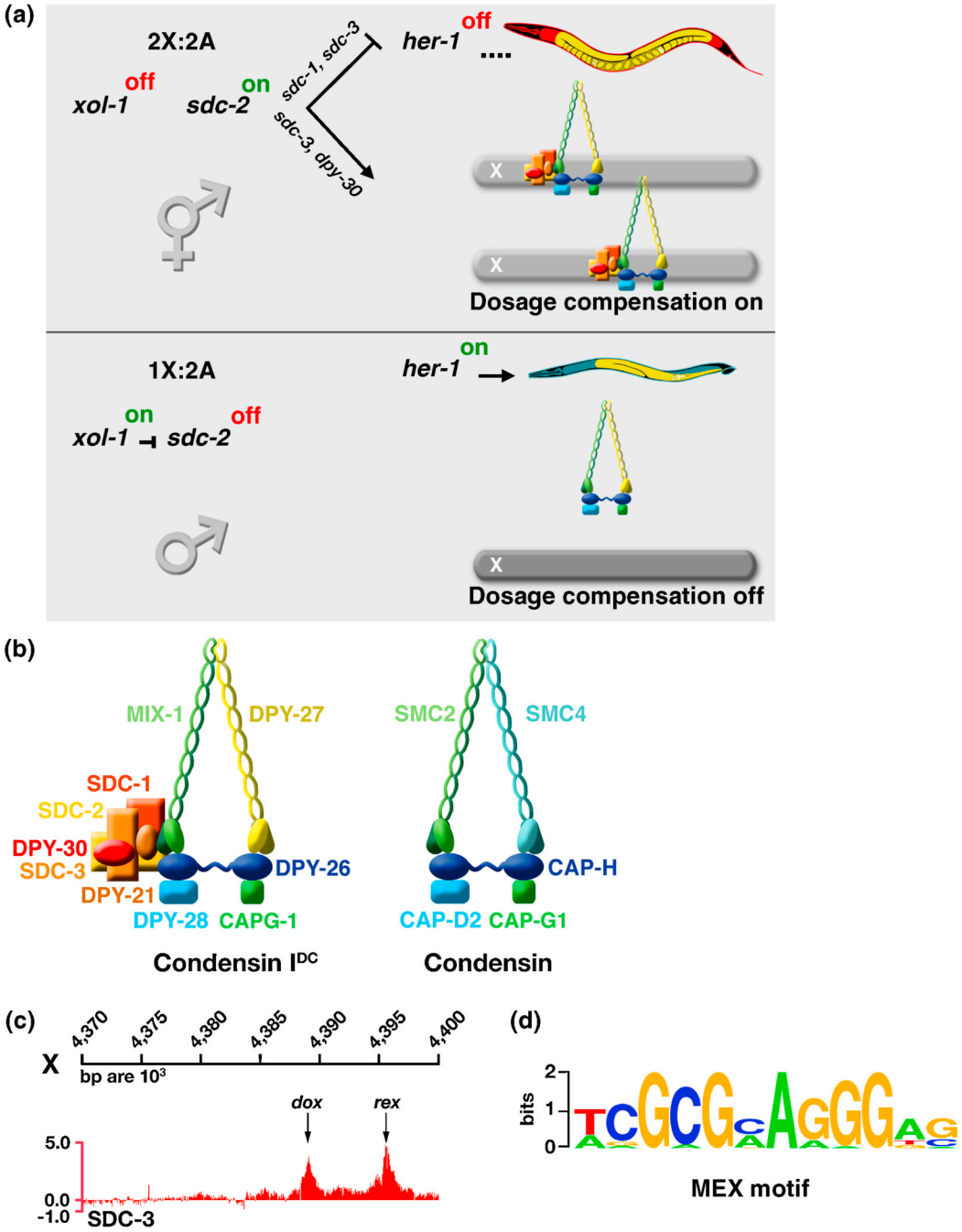

Figure 2. Regulation of Dosage Compensation in *C. elegans*

(a) Partial genetic pathway for sex determination and dosage compensation. In XX embryos, *xol-1* is repressed by the double dose of XSEs, permitting the XX-specific protein SDC-2 to activate dosage compensation and to repress *her-1*, a male-specific sex determination gene. SDC-2 acts with SDC-3 and DPY-30 to recruit the DCC condensin subunits to X. SDC-2 acts with SDC-1 and SDC-3 to repress *her-1*. SDC-2 plays the lead role in recognizing X sequences, while SDC-3 predominates in recognizing the SDC binding sites at *her-1*. In XO embryos, ASEs overcome XSEs to activate *xol-1*, resulting in *sdc-2* repression and *her-1* activation, thereby setting the male sexual fate. The DCC is not loaded onto X.

(b) The DCC consists of five condensin-like components (DPY-27, MIX-1, DPY-26, DPY-28, and CAPG-1) that are homologous to canonical condensin subunits SMC2, SMC4, CAP-H, CAP-D2, and CAP-G1, respectively The DCC also contains at least five additional factors that confer X- and sex-specificity (SDC-2, SDC-3, and DPY-30) or assist in repression (DPY-21 and SDC-1).

(c) DCC binding sites have been mapped by ChIP chip experiments and classified into two categories based on their ability to bind the complex when detached from the X chromosome. *rex* sites (recruitment elements on X) bind the complex robustly when they are detached from X and present either in multiple copies on extrachromosomal arrays (see Figure 3a) or in low copy number integrated onto an autosome. *dox* sites (dependent on X) fail to bind the DCC when detached; they depend on the broader X chromosomal context for their ability to associate with the DCC.

(d) Motif searches identified a twelve base pair consensus motif that is enriched at *rex* sites relative to *dox* sites and on X chromosomes relative to autosomes. Mutations within the motif disrupt the ability of *rex* sites to recruit the DCC (see Figure 3a).

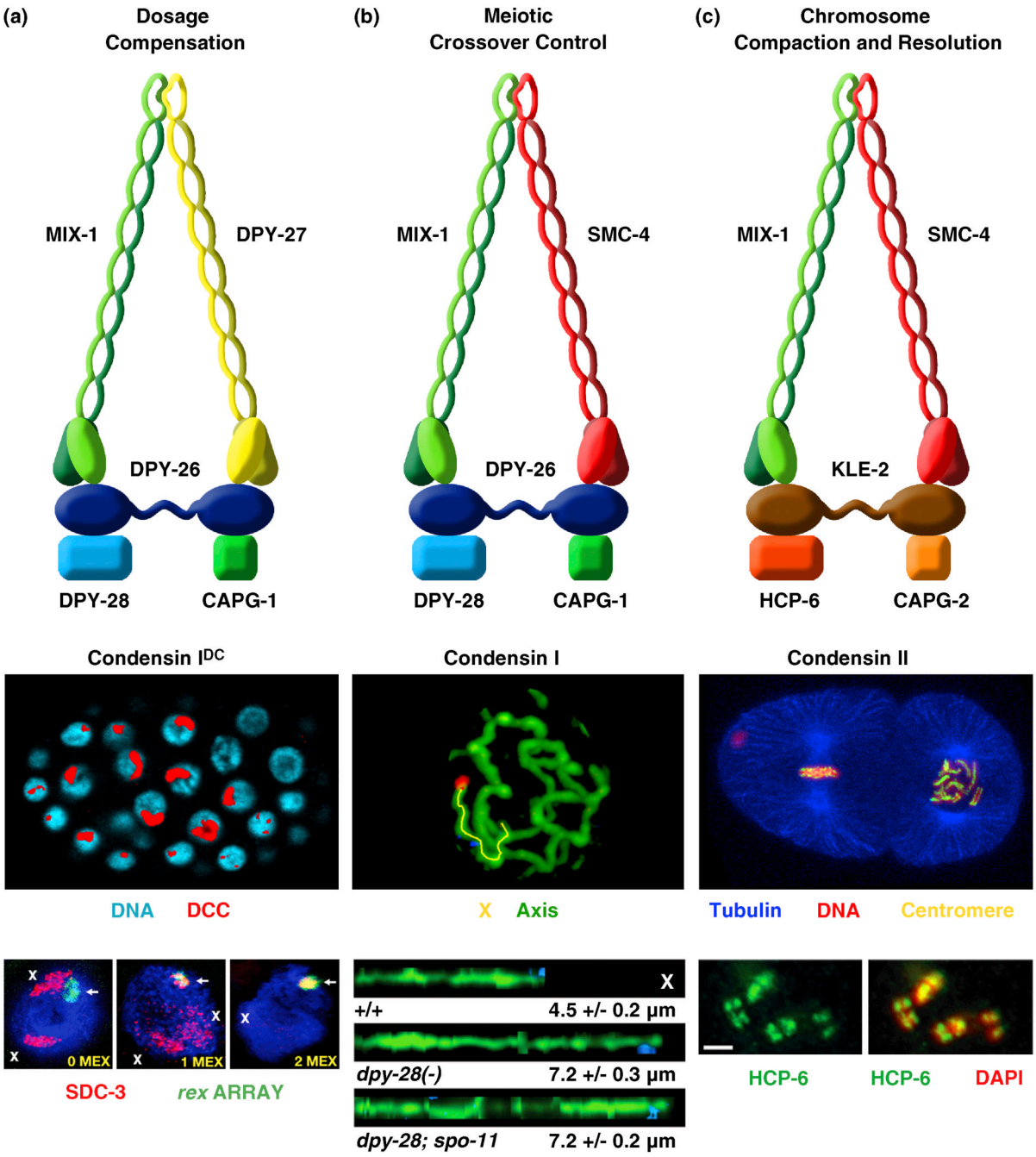

Figure 3. Three condensin complexes carry out distinct functions in *C. elegans*

(a) The dosage compensation complex (the DCC, also called condensin I^{DC}) (top) resembles condensin I. It equalizes X-linked expression between the sexes (XX hermaphrodites and XO males) by reducing transcript levels by half in hermaphrodites. Shown is an XX embryo stained with the DCC component SDC-2 (red) and DAPI (blue) (middle). The DCC binds to both X chromosomes. Shown also are images of gut cell nuclei carrying extrachromosomal arrays with multiple copies of *rex-1* derivatives stained with DAPI (blue), SDC-3 antibodies (red), and array FISH (green) (bottom). Arrays have *rex-1* fragments with different numbers of MEX motifs. Left, a 33 bp *rex-1* fragment in which its single MEX motif was mutated, thus abrogating DCC binding. X staining is apparent (red). Middle, a wild-type 33 bp *rex-1* fragment with 1

MEX motif. SDC-3 colocalizes with the array and X. Right, a 60 bp *rex-1* fragment with 2 MEX motifs. SDC-3 binds robustly to *rex-1* and is titrated from X, showing that MEX motifs collaborate to recruit the DCC. An array carrying a *rex-1* fragment that titrates the DCC from X can suppress the male lethality caused by mutation of *xol-1* in XO embryos.
(b) Condensin I. This complex differs from DCC condensin by a single subunit, SMC-4 (top). This complex controls meiotic DSB distribution through effects on chromosome structure. It also plays minor roles in chromosome segregation in mitosis and meiosis. Shown is a high resolution image of pachytene chromosomes in wild-type animals labeled with the axis protein HTP-3 and FISH probes (blue, red) to X (middle). X chromosomes from wild-type and *dpy-28* mutant animals were traced (yellow) in three dimensions and straightened computationally (bottom). Straightened chromosomes are shown horizontally. Genotypes, average total axis length, and standard error of the mean are below each axis. Disruption of *dpy-28* dramatically increases the X-chromosome axis length in a manner independent of DSBs made by SPO-11. In response, DSBs are increased in number and redistributed in *dpy-28* mutations, causing an increase in crossovers and their redistribution.
(c) Condensin II. This complex shares one subunit (MIX-1) with the DCC (condensin I^{DC}) and two subunits (MIX-1 and SMC-4) with condensin I (top). This complex is the prime condensin complex responsible for mitotic and meiotic chromosome compaction and resolution. Condensin II binds to the centromeres on the holocentric mitotic chromosomes. Shown is a two cell embryo (middle) with one cell in metaphase (left) and one in prometaphase (right). Centromeric proteins (yellow) and condensin II bind along the outer edge of each chromosome, adjacent to where the mitotic spindle (tubulin, blue) attaches. Condensin II also binds to meiotic chromosomes at pachytene exit to create the compact shape (diakinesis bivalents) required for chromosome segregation. Shown are the four sister chromatids of meiotic diakinesis bivalents (bottom) stained with HCP-6 antibodies (green) and DAPI (red). Merge is yellow.

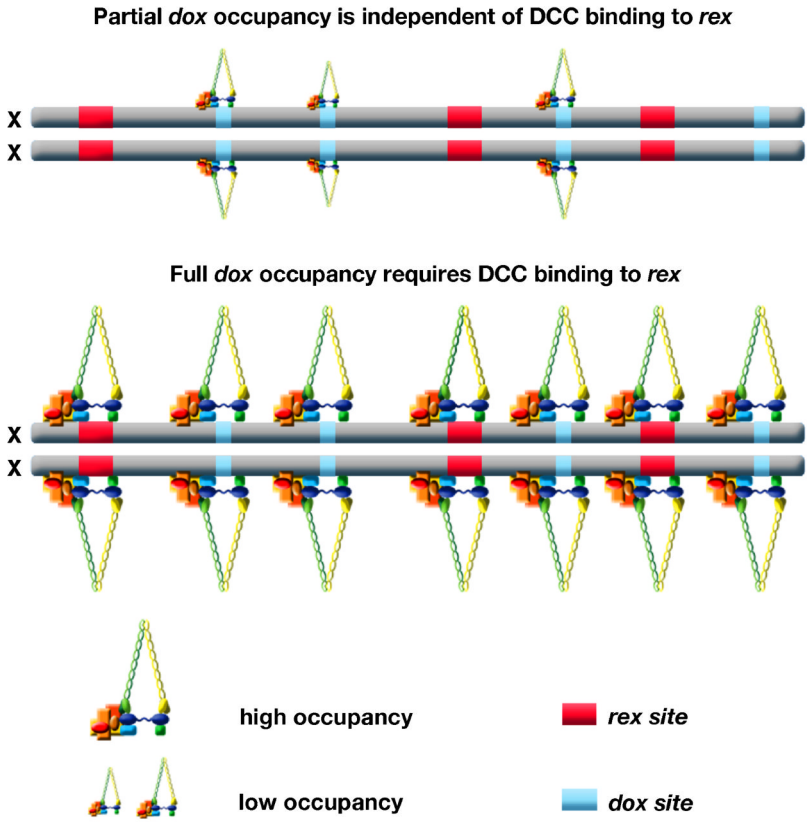

Figure 4. Model for loading of the DCC onto X

This model integrates current data. The DCC binds to *rex* sites in a sequence-dependent manner requiring SDC-2, SDC-3, and DPY-30. *rex* sites confer X-specificity to DCC binding and recruit additional complexes to bind along X at *dox* sites, which are unable to recruit the DCC when detached from X. Many *dox* sites on X have a small, intrinsic DCC binding capability; this binding is enhanced in response to DCC loading onto *rex* sites. *dox* sites are located preferentially in promoters of active genes, and positions of *dox* sites change as gene expression changes, implying that *dox*-site binding is transcription dependent.