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The X chromosome in *C. elegans* sex determination and dosage compensation

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Abstract

Abnormalities in chromosome dose can reduce organismal fitness and viability by disrupting the balance of gene expression. Unlike imbalances in chromosome dose that cause pathologies, differences in X-chromosome dose that determine sex are well tolerated. Dosage compensation mechanisms have evolved in diverse species to balance X-chromosome gene expression between sexes. Mechanisms underlying nematode X-chromosome counting to determine sex revealed how small quantitative differences in molecular signals are translated into dramatically different developmental fates. Mechanisms underlying X-chromosome dosage compensation revealed the interplay between chromatin modification and three-dimensional chromosome structure imposed by an X-specific condensin complex to regulate gene expression over vast chromosomal territories. In a surprising twist of evolution, this dosage-compensation condensin complex also regulates lifespan and tolerance to proteotoxic stress.

Introduction

Anomalies in chromosome number can disrupt the balance of gene expression and thereby decrease organismal fitness and viability [1], but differences in X-chromosome dose that determine sex are well tolerated in diverse species. Dosage compensation mechanisms have evolved to balance X-chromosome gene expression between sexes, allowing them to accommodate these differences.

Strategies for dosage compensation differ, but invariably a regulatory complex is targeted to X chromosomes of one sex to modify histones, alter three-dimensional structure, and modulate transcription along the entire X [2–5] (Figure 1). Human and mouse XX females inactivate one of their two X chromosomes [2,6–8], *D. melanogaster* XY males double transcription from their single X chromosome [4,5,9], and *C. elegans* XX hermaphrodites reduce transcription from both X chromosomes by half [3] to balance X-chromosome gene expression between sexes (Figure 1). Studies of diverse dosage compensation mechanisms have enhanced our understanding of the spatial organization of chromatin during development and its central role in regulating gene expression [10]. Here,

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Nothing declared.

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mechanisms underlying X-chromosome counting and X-chromosome dosage compensation are reviewed for *C. elegans*.

X-chromosome counting mechanisms determine sex and regulate dosage compensation

In nematodes, as in flies, sex determination and dosage compensation are linked through a master sex-determination switch gene that regulates both processes and is itself controlled by an X-chromosome counting mechanism [3,11,12]. Failure to count X chromosomes accurately when determining sex aborts development in both organisms. In nematodes, the counting mechanism measures the number of X chromosomes relative to the sets of autosomes (X:A signal) to determine sex [3].

The X:A signal determines nematode sex by regulating its direct target, *xol-1*, the master sex-determination switch gene controlling sexual fate and X-chromosome dosage compensation [13–16] (Figure 2a,b). *xol-1* encodes a GHMP kinase that directs male development in XO embryos by repressing the XX-specific gene *sdc-2*, which encodes a 350 kDa protein lacking known homology [17,18]. *sdc-2* directs hermaphrodite development in XX embryos by repressing the male sex-determining gene *her-1* [19,20] and by triggering binding of a dosage compensation complex (DCC) to both X chromosomes, where it reduces transcription by approximately half, thereby balancing X expression between XX and XO embryos [18,21] (Figure 2a, b, f). XO animals become feminized and die if *xol-1* is deleted. XX animals become masculinized and die if *sdc-2* is deleted.

Dose-sensitive signals relay X-chromosome and autosome number to determine sex by regulating *xol-1* transcription and pre-mRNA splicing

X-linked genes called X-signal elements (XSEs) communicate X-chromosome dose by repressing *xol-1* in a cumulative, dose-dependent manner via two distinct mechanisms in XX embryos: transcriptional regulation by the nuclear hormone receptor SEX-1 and the ONECUT homeodomain protein CEH-39 [16,22,23]; and pre-mRNA splicing regulation by the RNA-binding protein FOX-1 [15,24,25] (Figures 2a, b, 3a). The single dose of XSEs in XO embryos is insufficient to repress *xol-1*. Regulation by XSE dose occurs in the context of autosomal genes called autosomal-signal elements (ASEs) that communicate ploidy by stimulating *xol-1* activity in a dose-dependent manner to counter XSEs [16,26] (Figure 2a–d).

SEX-1 and CEH-39 repress *xol-1* transcription in XX embryos by binding to multiple, distinct sites in the 5′ regulatory regions of *xol-1* [16] (Figure 3b). In contrast, two ASE transcriptional activators, the T-box transcription factor SEA-1 and the zinc-finger protein SEA-2, bind to multiple sites in *xol-1* to activate transcription (Figure 3b) [16]. XSE and ASE binding sites are non-overlapping, suggesting that direct competition for binding is not how XSEs counter ASEs. Instead, XSEs and ASEs likely antagonize each other by recruiting cofactors with reciprocal activities that induce opposite transcriptional states.

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This antagonism makes the sex-determination process sensitive to relative doses of X chromosomes and autosomes, permitting tiny changes to elicit different sexual fates.

Fidelity of X:A signaling is enhanced by a second tier of dose-dependent *xol-1* repression via the RNA-binding protein FOX-1, which regulates alternative pre-mRNA splicing of residual *xol-1* transcripts to inhibit formation of the 2.2 kb transcript essential for male-determining activity (Figure 3a,b) [15,24,25]. Dose-sensitivity of *xol-1* splicing regulation is achieved by combining multiple FOX-1 binding motifs in the alternatively spliced *xol-1* intron VI with the two-fold difference in FOX-1 concentration between XX and XO embryos [15] (Figure 3a, b).

FOX-1 binds to 2 GCAUG and 3 GCACG motifs in intron VI (Figure 3a). In mammals, both motifs are utilized for Rbfox-mediated splicing regulation, but only one motif copy is needed. Rbfox has a tyrosine-rich, low-complexity domain that nucleates its own aggregation to attain adequate concentrations of bound proteins [27,28]. In nematodes, FOX-1 binding to five motifs precludes the formation of male-determining XOL-1 protein in XX animals by causing either intron retention, and hence premature translational termination, or by promoting alternative 3′ splice-site usage to create a transcript lacking essential exon-coding sequences (Figure 3a) [15]. In hermaphrodites, mutating only one copy of *fox-1* or only GCACG motifs or only GCAUG motifs increases intron removal, allowing active 2.2 kb transcripts to predominate (Figure 3a). Thus, multiple high-affinity RNA-binding sites in *xol-1* intron VI permit FOX-1 produced from two *fox-1* doses in XX embryos to reach the threshold level necessary to block formation of properly spliced male-determining *xol-1* transcripts.

Dosage compensation machinery: a molecular motor controlling higher order chromosome structure and gene repression

Regulating X-chromosome gene expression is functionally related to a structural problem relevant to all chromosomes during cell division: achieving ordered compaction and resolution prior to segregation. Five DCC subunits (MIX-1, DPY-26, DPY-27, DPY-28, and CAPG-1) resemble subunits of condensin [29–34], an essential complex that controls the structure, resolution, and segregation of chromosomes during mitosis and meiosis from yeast to human [35,36] (Figure 2e).

DCC condensin subunits except DPY-27 also control the structure of mitotic and meiotic chromosomes by participating in distinct condensin complexes that perform independent roles in chromosome segregation [30,33,34,37] (Figure 2e, g, h). Participation of DCC proteins in diverse condensin complexes illustrates that reshuffling of homologous proteins can create independent machines with similar architecture but distinct biological functions.

Of five non-condensin DCC proteins (SDC-1, SDC-2, SDC-3, DPY-21, and DPY-30), subunits SDC-2 and SDC-3 recruit the DCC to X chromosomes and coordinately control both sex determination and dosage compensation. SDC-2 is the sole DCC protein expressed exclusively in hermaphrodites. It achieves both sex-specificity and X-specificity for dosage compensation [18]. SDC-3, a zinc-finger protein, assists SDC-2 in the X-recruitment process

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[38], as does DPY-30 [39,40] (Figure 4b). DPY-30 also participates in the MLL/COMPASS histone methyltransferase complex that trimethylates lysine 4 on histone H3 (H3K4me3) to facilitate gene activation in both sexes [39]. SDC-1, a zinc-finger protein, participates in X repression, but not X recruitment [20,41]. DPY-21, a Jumonji C H4K20me2 demethylase, enriches H4K20me1 on X by converting H4K20me2 to H4K20me1 [42].

Posttranslational modification of SDC-3 and condensin subunits DPY-27 and DPY-28 by sumoylation is essential for sex-specific assembly of the DCC onto X [43]. Reducing sumoylation elevated X expression.

Current evidence suggests the DCC represses X expression by reducing recruitment of RNA polymerase II to hermaphrodite X chromosomes [39,44]. X-linked genes are expressed at half the level as autosomal genes, because no chromosome-wide mechanism elevates X expression in both sexes prior to dosage compensation to enable equivalent expression of X and autosomal genes [44,45].

Dosage compensation complex recruitment to X

A chromosome-wide search to identify *cis*-acting sites on X that recruit the DCC defined megabase-sized regions sufficient to recruit the DCC when detached from X, indicating multiple independent recruitment sites [46]. Some detached regions failed to recruit the complex, yet these regions exhibited abundant DCC binding on native X chromosomes. These results suggested that a limited number of recruitment sites occur on X, and some or all nucleate DCC spreading (Figure 4a–c). These recruitment elements on X (*rex*) sites were defined precisely by functional assays *in vivo* monitoring DCC recruitment to extra-chromosomal arrays carrying multiple DNA copies [46–48] and by DCC chromatin immunoprecipitation studies [39,48,49] followed by analysis *in vivo* [39,48]. Sequence analysis of 31 *rex* sites revealed a 12-base-pair consensus motif named MEX (Motif Enriched on X) that is highly enriched on X compared to autosomes and expands motifs from initial *rex* studies [47,48] (Figure 4d). Mutational analysis *in vivo* established the importance of MEX for DCC binding [48,50,51]. Many *rex* sites have multiple MEX motifs of varying consensus similarity, suggesting motif clustering is important for DCC recruitment. Indeed, inserting individual or multiple *rex* sites onto autosomes showed that DCC binding at *rex* sites is aided by cooperation among recruitment motifs and sites [45,50,51].

MEX motifs cannot be the sole determinant of X specificity for DCC binding, since only 50% of *rex* sites have high-scoring MEX motifs that can recruit the DCC to X. The number of *rex* sites is unknown, but at least 100 sites are predicted, with 48 already verified.

DCC-binding peaks lacking autonomous DCC binding in recruitment assays *in vivo* were also defined on X [39,48]. These *dox* (dependent on X) sites are consistent with the model of DCC recruitment to X and spreading. *dox* sites uniformly lack MEX variants exhibiting high enrichment on X.

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Dosage compensation complex spreading along X

Maximal DCC condensin binding at *dox* sites requires SDC-2, SDC-3, and DPY-30 binding on X, but reduced DCC condensin binding can occur without them. Many *dox* sites have an inherent low-level ability to bind condensin subunits independently of the genetic hierarchy governing sex-specific DCC assembly onto X [39]. When SDC-2, SDC-3, and DPY-30 are bound to X, condensin binding at *dox* sites is greater than SDC binding, indicating a one-to-one stoichiometry between Sex determination and Dosage Compensation (SDC) and condensin subunits is not universal at all binding sites [39] (Gosh, R. Kruesi, W., and Meyer, B.J. unpublished). These results imply that condensin subunits are the predominant DCC subunits to spread along X (Figure 4b, c).

The relationship between DCC binding at *rex* and *dox* sites was further revealed by assessing DCC binding after deleting high-occupancy *rex* sites from X or inserting *rex* sites into ectopic X locations [50,51]. The eight highest occupancy *rex* sites across X that also drive higher order X-chromosome structure (discussed later) were deleted sequentially, and effects on DCC binding were evaluated by ChIP-seq [50]. Binding of DPY-27 condensin and SDC-3 non-condensin subunits was reduced up to 16-fold adjacent to each *rex* deletion; binding returned to wild-type levels by 20 kb on either side [50]. Reciprocally, inserting a strong *rex* site into ectopic locations on X caused 16-fold enrichment in DCC binding at each location. Binding decreased to wild-type levels by 15 kb on either side [50]. These results corroborate the model that DCC binds to autonomous recruitment sites and spreads to non-autonomous sites.

Condensin-driven remodeling of X-chromosome topology during dosage compensation

Interphase chromosomes are organized into a series of ordered structures ranging from kilobase-scale chromatin loops that join gene promoters with their distant DNA regulatory sequences to megabase-scale subchromosomal territories [10,52–54]. Intermediate-sized structures of about one megabase called topologically associated domains (TADs) also occur during interphase [55–57]. These structures, a common feature of mammalian chromosomes, enable loci in one TAD to interact predominantly with each other, while being insulated from interactions with loci in neighboring TADs. This insulating property confines the action of distant DNA regulatory regions to within a TAD, thereby preventing, for example, inappropriate activation of onco-genes [57–61]. However, mechanisms that define TAD boundaries and their biological importance are under debate [10,57,62]. Since the DCC acts at a distance [45,48], nematode dosage compensation provided an excellent model to explore TAD formation and the role of chromosome structure in gene expression.

X chromosomes undergo changes in conformation during dosage compensation. DCC binding increases compaction of X [50,63] and remodels hermaphrodite X chromosomes into a sex-specific spatial conformation distinct from that of autosomes or male X chromosomes [50,64]. The DCC creates eight self-interacting domains on X resembling mammalian TADs. The eight TAD boundaries coincide with the highest affinity *rex* sites and are lost in *sdc-2* mutants (Figure 4f, g). Formation of DCC-dependent TAD

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boundaries is enhanced by methylation of lysine 9 on histone H3 (H3K9me) from the methyltransferases MET-2 and SET-25 [65]. Thus, the DCC imposes a higher order structure onto X chromosomes while regulating gene expression chromosome-wide.

Deleting the *rex* site at each dosage compensation complex-dependent topologically associated domain boundary eliminated the boundary, while inserting a *rex* site at an ectopic location on X created a boundary

In mammalian cells, proteins that establish TADs, including the zinc-finger protein CTCF (CCCTC-binding factor) and the SMC (Structural Maintenance of Chromosomes) complex cohesin, localize at TAD boundaries [66]. These proteins also participate in essential cellular processes such as chromosome segregation. Thus, reducing protein levels could not distinguish TAD functions from other SMC-complex functions. Functions of nematode DCC-dependent TADs and mechanisms of TAD boundary formation were therefore analyzed by deleting DCC recruitment sites.

Individual *rex* sites were deleted sequentially at each of eight DCC-dependent TAD boundaries on endogenous X chromosomes (*8rex*Δ strain) [50]. Deleting the single *rex* site at each TAD boundary eliminated that boundary. Deleting all eight *rex* sites at DCC-dependent boundaries recapitulated the disrupted TAD structure of X chromosomes in *sdc-2* mutants, which lack DCC binding (Figure 4g, h). DCC-dependent TADs were eliminated in *8rex*Δ and *sdc-2* mutant strains but weaker DCC-independent TADs on X and autosomes remained. Deleting only eight *rex* sites was sufficient to disrupt TAD structure, even though DCC binding occurred at myriad remaining *rex* sites across X. Thus, a high-occupancy *rex* site is necessary to define each DCC-dependent TAD boundary. A single high-occupancy *rex* site is also sufficient to create a new TAD boundary on X but not on autosomes [50].

Current evidence supports a loop-extrusion model for DCC-dependent TAD boundary formation (Figure 4e) [50]. Accordingly, DCC condensin would bind X chromatin and extrude loops that would not extend beyond a high-occupancy *rex* site. Thus, *rex* sites could act as both recruitment centers and barriers, thereby promoting the insulation profiles defined by Hi-C interaction-probability maps of X. Different DCC components could extrude loops or block loop extrusion to create X TADs, analogous to roles played in mammals by cohesin, which extrudes loops, and CTCF, which forms a loop-extrusion barrier [66,67]. DCC condensin could extrude loops, and non-condensin subunits could halt extrusion when bound to high-occupancy *rex* sites that comprise TAD boundaries [50].

X-chromosome topologically associated domain structure is not required for dosage compensation

The *8rex*Δ XX worms had normal brood sizes and manifested no dosage compensation defects, such as XX-specific lethality, egg-laying failure, or dumpiness [50]. Moreover, *8rex*Δ mutations did not rescue dying *xol-1* mutant XO males. The *8rex*Δ XX mutants exhibited no expansion of X-chromosome volume or elevation in X expression, unlike

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DCC-defective XX mutants (Figure 4g, h). Therefore, DCC-dependent TADs are neither the cause nor consequence of transcriptional repression [50].

The *8rex*Δ strain revealed DCC-dependent architecture on X that persisted without TADs and has the potential to facilitate long-range gene repression [50]. High-frequency interactions occur between loci spanning 0.1–1 Mb on X, but not autosomes, of wild-type and *8rex*Δ embryos, but not DCC-defective embryos. These DCC-dependent interactions may create X segments with environments unfavorable for RNA polymerase II recruitment.

Eliminating dosage compensation complex-dependent topologically associated domains on X reduced thermotolerance, accelerated aging, and shortened lifespan

Although disrupting X structure in *8rex*Δ XX hermaphrodites did not elevate embryonic X-linked expression under normal growth conditions, it adversely affected the ability of adults to tolerate proteotoxic stress [50]. Accumulation of unfolded proteins in worms exposed to proteotoxic stress triggers activation of genes needed to refold proteins, restore homeostasis, and prevent death [68]. Viability of *8rex*Δ XX adults was reduced by the accumulation of unfolded proteins caused by heat stress (Figure 4h) [50]. However, *8rex*Δ XX animals were not more sensitive to paraquat-induced mitochondrial stress, which increases reactive oxygen species, or to tunicamycin-induced endoplasmic reticulum stress, which increases unfolded glycoproteins. Thus, DCC-dependent TADs promote thermotolerance but not tolerance to other proteotoxic stress.

The *8rex*Δ XX hermaphrodites also exhibited a 20% reduction in lifespan, which was not restored by inserting an ectopic *rex* site [50] (Figure 4f, h). Since male lifespans were not affected by *rex* deletions, the reduction reflects a DCC-dependent function of *rex* sites. Changes in behavior, including premature decline in speed and distance of backward movement after reaching adulthood, indicated that *8rex*Δ hermaphrodites died prematurely from accelerated aging rather than from general debilitation during development. Thus, although *rex* deletions that abrogated hermaphrodite TAD structure did not affect DCC-regulated gene expression during embryogenesis, they increased thermosensitivity, accelerated aging, and shortened adult lifespan, implying a role for chromosome architecture in regulating stress responses and aging [50].

The dosage compensation complex creates local negative supercoils at *rex* sites, but not large domains of supercoiling on X

The predominant form of nuclear DNA is a double-stranded, right-handed helix with 10.4 bp per helical turn. However, biological processes such as transcription and replication can overwind or underwind DNA, thereby creating DNA supercoils [69]. Supercoils have been proposed to regulate higher order chromosome structure and chromosome-wide gene expression [70]. While it is accepted that prokaryotic chromosomes are organized into supercoiled topological domains [71], the role of supercoils in eukaryotic chromosome structure, including TADs, has been controversial [70,72,73]. Dosage compensation enabled

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the relationship between 3-dimensional chromosome topology, condensin, and supercoiling to be explored *in vivo* [74]. Experiments demonstrated that X-chromosome repression is not achieved by regulating supercoiling at TSSs of X-linked genes. However, high-occupancy *rex* sites exhibited local DCC-dependent negative supercoils, with the level of supercoiling scaling with DCC-binding strength [74]. Although supercoils occurred at *rex* sites that trigger TAD boundary formation, supercoils did not propagate beyond 500 bp, a distance less than a TAD. Hence, the limited DCC-dependent supercoiling at *rex* sites cannot change DNA interaction frequencies on the mega-base scale needed to create TADs.

Control of hermaphrodite X-chromosome topology and repression by a dosage compensation complex subunit that functions as a histone H4K20 demethylase

The chromatin modification H4K20me1 is enriched on interphase X chromosomes of hermaphrodites in a DCC-dependent manner during dosage compensation (Figure 5a, b) [42,75–78]. H4K20me1 is also enriched on the inactive X chromosome of female mammals, revealing a common feature of diverse dosage compensation strategies [79]. Although H4K20 methylation had been implicated in many nuclear functions, the role of H4K20me1 in gene regulation, its impact on higher order chromosome structure beyond chromatin-fiber compaction, and the mechanisms that regulate different H4K20me states had been elusive [80–82]. Analyzing causes and consequences of H4K20me1 modification during dosage compensation elucidated these mechanisms.

A 1.8 Å resolution crystal structure of the DPY-21 carboxy-terminal domain revealed a Fe^{2+}- and α-ketoglutarate (α-KG)-containing jumonji (JmjC) demethylase domain (Figure 5a) [42]. JmjC demethylases are Fe^{2+}- and α-KG-dependent dioxygenases that demethylate lysines in histone and nonhistone proteins [83]. Biochemical assays of the DPY-21 domain and the homologous mouse ROSBIN domain revealed Fe^{2+}- and α-KG-dependent conversion of H4K20me2 to H4K20me1 *in vitro* [42].

DPY-21 acts in a cell-cycle-dependent manner to enrich H4K20me1 on hermaphrodite X chromosomes of embryonic somatic cells during interphase [42]. H4K20me1 enrichment is not evident on interphase X chromosomes before the 200-cell embryo stage, well after X recruitment of other DCC subunits (30–40-cell stage), implying a role in dosage compensation maintenance rather than initiation (Figure 5b). *dpy-21(JmjC)* mutations eliminated H4K20me1 enrichment on X, disrupted dosage compensation, and increased X volume by 30% (Figure 5a) [42,84]. The strength of X TAD boundaries was also reduced, showing that JmjC activity is important for both X compaction and DCC-driven remodeling of X topology, in addition to X repression. Compaction could be achieved by an H4K20me1-specific reader using different mechanisms (see Figure 5c–e).

DPY-21 also binds to autosomes (but not X) of meiotic germ cells in a DCC-independent manner to enrich H4K20me1 and compact chromosomes (Figure 5a) [42]. DPY-21 mutations caused a 20% increase in autosomal axis length. DPY-21 itself lacks obvious DNA and chromatin-binding domains to confer target specificity; hence, its demethylase

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activity can be harnessed during development for distinct biological functions by interacting proteins. In both somatic and germ cells, H4K20me1 modulates three-dimensional chromosome topology, showing a direct link between chromatin modification and higher order chromosome structure [42].

H4K20me1 is also enriched on the inactive X of female mice [79]. Knockout of mouse H4K20me1 methyltransferases diminished H4K20me1 enrichment on the inactive X and caused decondensation of X [85]. H4K20me1 enrichment requires the long non-coding RNA *Xist* that triggers mammalian X inactivation, but partial-loss-of-function *Xist* mutations that prevent H4K20me1 enrichment on X can nonetheless permit X inactivation, but with lower efficiency [86]. H4K20me1 appears to facilitate X-chromatin compaction characteristic of facultative heterochromatin on the inactive X rather than initiating early gene silencing [86]. Analysis of H4K20me1 in worms and mammals offers new directions for unraveling the interplay between chromatin modification and chromosome structure.

Future

Evolution of nematode dosage compensation required the recruitment of ancient condensin proteins that remodel chromosome structure to promote chromosome segregation for the new role of regulating chromosome-wide gene expression. Condensin proteins co-opted for dosage compensation retained their original roles in chromosome segregation by participating in other nematode condensin complexes. This co-option demonstrates that reshuffling of homologous interchangeable proteins can create independent molecular machines with similar architecture but distinct biological functions.

Future research will further reveal how different DCC subunits nucleate and spread on X chromosomes and how chromatin modification and chromosome structure at local and global scales can respond to and regulate nucleation, spreading, and transcription attenuation. Single-molecule imaging of individual DCC subunits and RNA polymerase in living embryos will reveal the dynamic nature of DCC assembly, how RNA polymerase recruitment to X is regulated, and the extent to which DCC subunits function in separate complexes to alter chromatin composition and topology, thereby regulating transcription attenuation.

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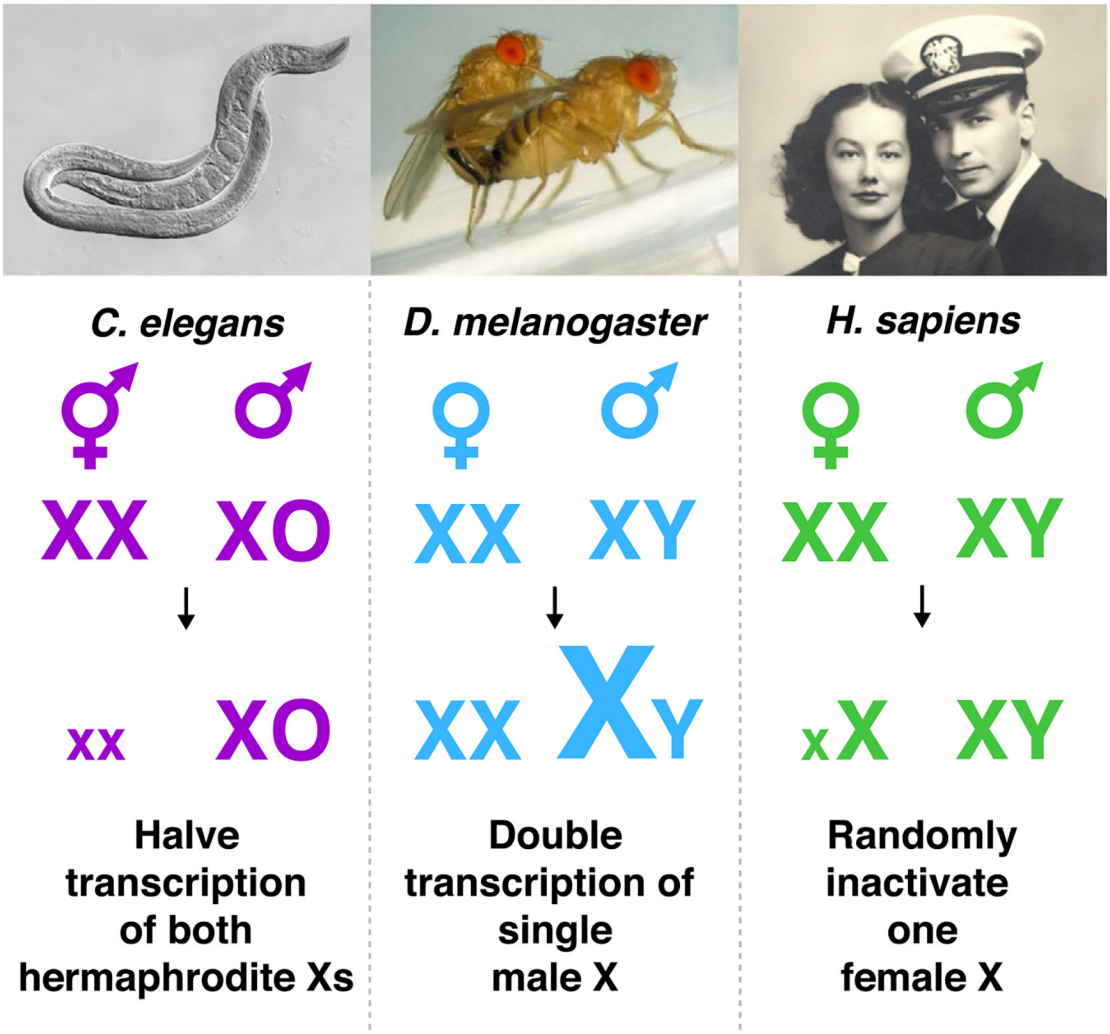

Figure 1.

Diverse strategies for X-chromosome dosage compensation. *C. elegans* XX hermaphrodite nematodes reduce transcription from both X chromosomes by half by recruiting a DCC that contains condensin and non-condensin subunits [3]. The DCC includes a Jumonji histone K4K20 demethylase that enriches H4k20me1 on both hermaphrodite X chromosomes. The DCC is recruited to X via sequence-specific recruitment elements (*rex* sites) that contain clustered X-enriched motifs. *rex* sites engage in DCC-dependent long-range interactions, and dosage-compensated X chromosomes have increased compaction and consist of self-interacting domains resembling mammalian TADs. Nematode hermaphrodites die without halving X transcription. *D. melanogaster* XY male fruit flies double transcription from their single X chromosome by recruiting a DCC called the male-specific lethal complex (MSL) that includes two long non-coding RNAs and deposits the activating histone modification histone H4K16ac [4,5]. Initial sites of MSL recruitment, called chromatin entry sites, reside in euchromatin and contain binding motifs that cluster in two-dimensional and three-dimensional space. Fruit fly males die without doubling their X transcription. XX female mice and humans randomly inactive one of their two X chromosomes [2]. Random expression of the X-linked long non-coding RNA called *Xist*

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from the X inactivation center triggers heterochromatin formation and X-chromosome inactivation on one X chromosome. In mice, coating of X by *Xist* results in a sequence of chromatin modifications linked to transcriptional silencing. First comes loss of most active histone modifications followed by accumulation of modifications associated with gene repression: PRC1-dependent H2AK119Ub, then PRC2-dependent H3K27me3, and Rosbin-dependent H4K20me1. Deposition of H3K9me2, MacroH2A, and CpG promoter methylation follow. The *Xist*-binding protein SPEN, a transcriptional repressor, is recruited to X upon upregulation of *Xist*. SPEN integrates transcriptional and epigenetic control of X-inactivation [87].

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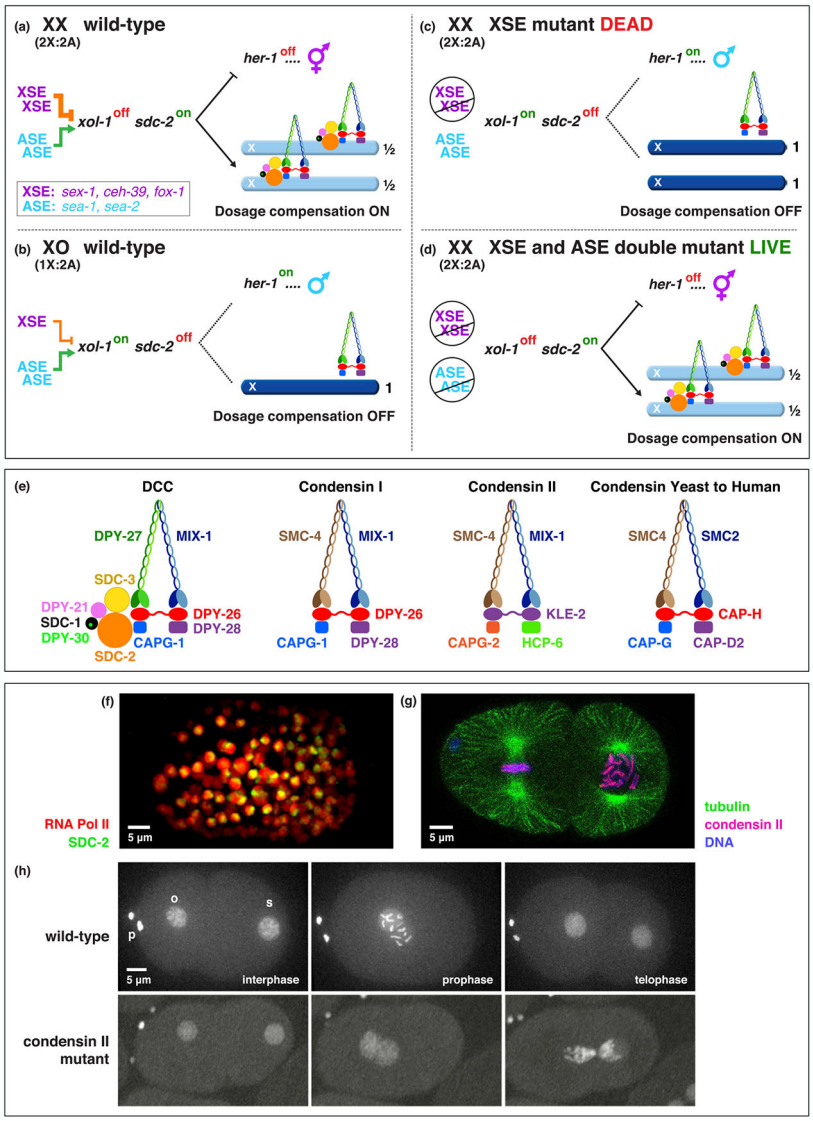

Figure 2.

Overview of the X:A signal and regulatory hierarchy that controls nematode sex determination and dosage compensation. **(a)** In wild-type animals, the X:A sex-determination signal is a competition between a set of genes on X called XSEs that repress their direct target *xol-1* (XO lethal) in a cumulative dose-dependent manner and a set of genes on autosomes called ASEs that stimulate *xol-1* transcription in a cumulative dose-dependent manner. XSEs include the nuclear hormone receptor SEX-1, the homeodomain protein CEH-39, and the RNA binding protein FOX-1. ASEs include the T-box transcription factor SEA-1 and the zinc-finger protein SEA-2. Two doses of XSEs in diploid XX animals win out and repress *xol-1*, permitting *sdc-2* (sex determination and dosage compensation), together with *sdc-1*, *sdc-3*, and *dpy-30*, to repress the male-determining gene *her-1* and to trigger binding of the condensin DCC to both X chromosomes. All DCC subunits require SDC-2 for their binding to X, but SDC-2 can bind X independently. Deleting the *sdc-2* gene in XX embryos masculinizes the embryos, prevents DCC binding, and kills all embryos by

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elevating X expression [3,18]. **(b)** In diploid XO embryos, the single dose of XSEs fails to turn *xol-1* off, permitting *sdc-2* to be repressed and *her-1* to be activated. Deleting the *xol-1* gene in XO animals activates *sdc-2*, causes the DCC to bind the single X, and kills males by reducing X expression [3,13,14]. *her-1* is repressed, and dying XO animals are feminized. **(c)** The distinguishing genetic feature of XSEs is the reciprocal, sex-specific phenotypes caused by changing their dose in XX versus XO diploid animals. Decreasing XSE dose kills XX hermaphrodites by inappropriately activating *xol-1* in XX embryos. Increasing XSE dose kills XO males by inappropriately repressing *xol-1* in XO embryos. **(d)** ASEs were discovered through loss-of-function mutations that suppressed the XX-specific lethality caused by loss-of-function mutations in XSEs [16,26]. **(e)** Three condensin complexes in *C. elegans*. DCC condensin resembles condensin complexes conserved from yeast to human and **(f)** binds to both X chromosomes of XX embryos to reduce X expression by half. DPY-27 and MIX-1 belong to the SMC family of chromosomal ATPases. The ATP binding domains at their N-termini and C-termini are linked by two long coiled coil domains separated by a hinge domain. DPY-27 and MIX-1 dimerize through interactions between their hinge domains and use their globular nucleotide binding domains to bind the three non-SMC condensin DCC proteins (DPY-26, DPY-28, and CAPG-1). Mutation of the NBDs in DPY-27 and MIX-1 disrupts dosage compensation. Condensin subunits also participate in other condensin complexes within *C. elegans* to carry out independent roles in chromosome segregation **(e)**. SMC subunit MIX-1 participates in both condensin I and II. Condensin I differs from the DCC condensin by only one subunit: SMC-4 replaces its SMC paralog DPY-27. Condensin II contains both MIX-1 and SMC-4 but differs from both condensin I and the DCC by including non-SMC proteins (KLE-2, CAPG-2, and HCP-6) that are distinct from, but homologous to those of the other two condensin complexes. **(g)** During mitosis, condensin II co-localizes at the outer edge of holocentric chromosomes where the spindle attaches. **(h)** Condensin II depletion disrupts mitotic prophase condensation, holocentromere organization, and chromosome segregation (p, polar bodies; o, oocyte pronucleus; s, sperm pronucleus). Chromosome segregation defects are severe, causing the developmental arrest of both XX and XO embryos. Condensin I depletion (not shown) causes chromatin bridges between anaphase chromosomes and results in a milder mitotic chromosome segregation defect than that caused by condensin II depletion.

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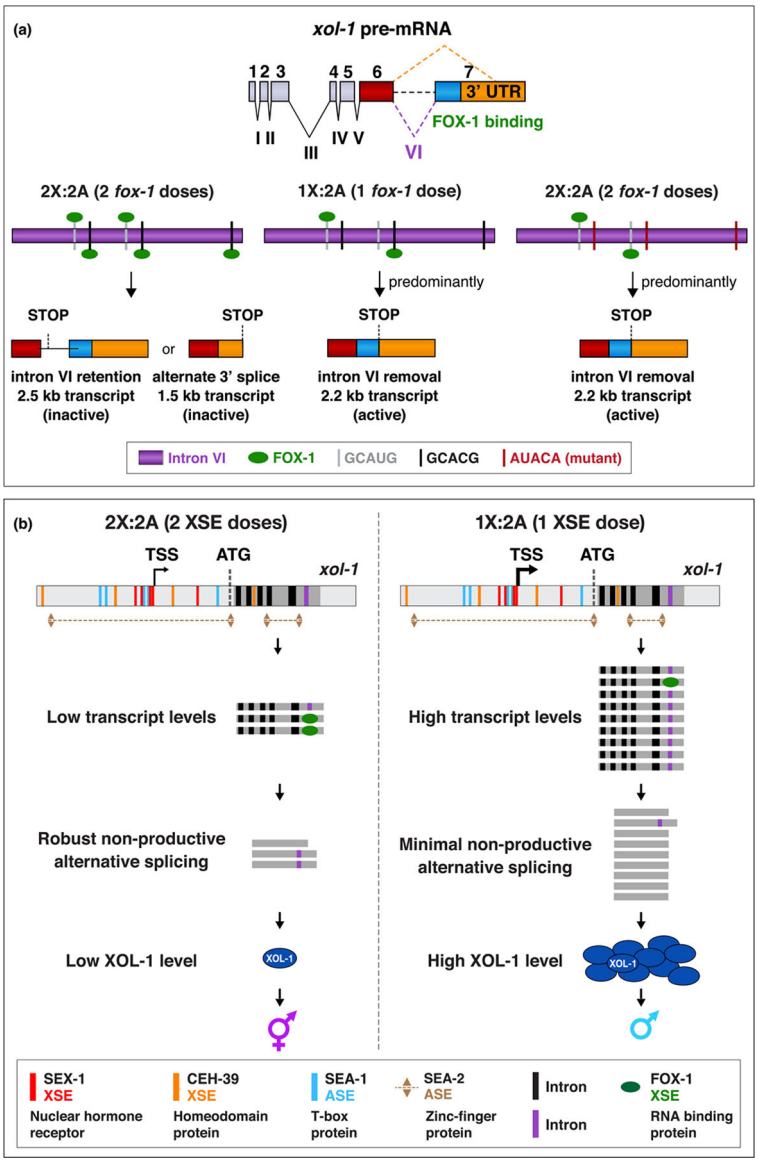

Figure 3.

Model for X:A signal assessment: two tiers of *xol-1* repression. **(a)** Dose-dependent pre-mRNA splicing regulation of *xol-1* by the RNA-binding protein FOX-1. By binding to two GCAUG (gray) and three GCACG (black) motifs in intron VI of *xol-1*, the XSE FOX-1 reduces formation of the male-determining 2.2 kb transcript by causing intron VI retention (2.5 kb transcript) or by directing use of an alternative 3′ splice acceptor site, causing deletion of essential exon 7 coding sequences (blue) and part of the 3′ UTR (orange) (1.5 kb transcript). In 1X:2A animals that have only one dose of FOX-1, insufficient FOX-1 is present to bind all the motifs and block intron VI removal. Hence, the predominant transcript is the 2.2 kb active transcript. The dose-sensitive action of FOX-1 binding motifs in XX animals was shown by experiments using a sensitized mutant background causing partially elevated *xol-1* transcripts and hence some XX lethality from reduced SEX-1 activity [15]. Mutating different combinations of endogenous GCAUG and GCACG motifs

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reduced non-productive splicing and enhanced XX-specific lethality. Mutating one *fox-1* allele or one copy of all five binding motifs killed almost all XX animals sensitized by reduced XSE activity. However, mutating only one copy of GCACG motifs (shown) or one copy of GCAUG motifs caused only intermediate XX-specific lethality. Changing both motifs to low-affinity GCUUG motifs blocked proper splicing and increased XX lethality. Thus, multiple high-affinity RNA binding sites in a *xol-1* intron permit the level of FOX-1 protein produced from two *fox-1* doses in XX embryos to reach the threshold necessary to block formation of properly spliced male-determining *xol-1* transcripts [15]. **(b)** XSEs and ASEs bind directly to numerous non-overlapping sites in the 5′ regulatory region of *xol-1* to antagonize each other's opposing transcriptional activities and thereby control *xol-1* transcription. Molecular rivalry at the *xol-1* promoter between the XSE transcriptional repressors and ASE transcriptional activators causes high *xol-1* transcript levels in 1X:2A embryos with one dose of XSEs and low levels in 2X:2A embryos with two doses of XSE. As an example of the rivalry, mutation of ASE genes suppresses the complete hermaphrodite lethality caused by deleting SEX-1 and CEH-39 binding sites in the *xol-1* promoter [16]. In a second tier of *xol-1* repression, the XSE RNA-binding protein FOX-1 then enhances the fidelity of X-chromosome counting by binding to numerous sites in intron VI (purple) of the residual *xol-1* pre-mRNA, thereby causing non-productive alternative splicing and hence *xol-1* mRNA variants that have in-frame stop codons or lack essential exons [15]. High XOL-1 protein induces the male fate, and low XOL-1 permits the hermaphrodite fate. Light gray rectangles represent 5′ and 3′ *xol-1* regulatory regions, dark gray rectangles represent *xol-1* exons, black rectangles represent unregulated *xol-1* introns, and the purple rectangle represents the alternatively spliced intron VI 3regulated by FOX-1. The orange rectangle that represents a CEH-39 binding site in the gene body resides in an exon.

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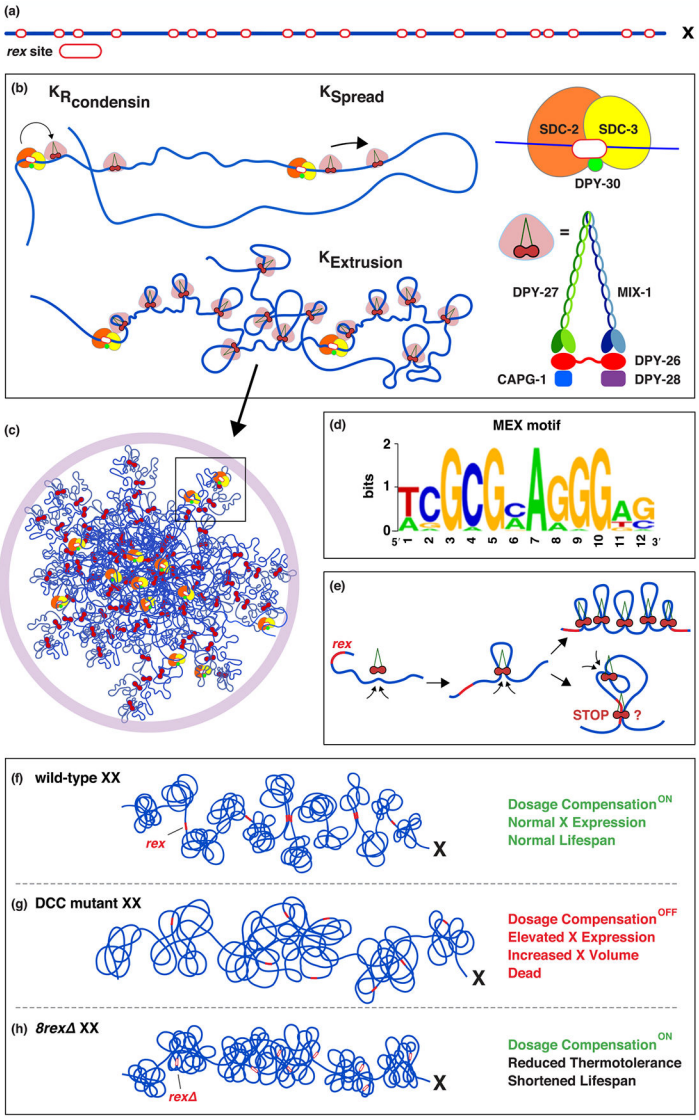

Figure 4.

Recruitment and spreading of the DCC on X. **(a)** *rex* sites across X recruit the DCC. This figure serves as a cartoon to indicate that *rex* sites are widely and randomly spaced. It is not a precise representation; not all *rex* sites have been defined. **(b, c)** SDC-2, SDC-3, and DPY-30 bind to *rex* sites on X and recruit DCC condensin to X, thereby initiating DCC condensin spreading. SDC and condensin subunits do not exhibit a one-to-one stoichiometry on X. DCC condensin is more abundant, implying that only DCC condensin spreads. **(d)** Consensus motif at *rex* sites necessary for DCC binding. **(e)** Bidirectional loop extrusion model for TAD formation by the DCC. In this model, DCC condensin complexes load onto X chromatin and extrude loops but cannot extend beyond a high-occupancy rex site with multiple X-enriched motifs. In one version, multiple independent looping events could occur between *rex* sites. In a second version, loops could form within loops, leading to nested looping events. **(f–h)** X-chromosome domain structure established by DCC binding to *rex* sites regulates *C. elegans* lifespan but not dosage compensation. **(f)** DCC binding at each of

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eight high-occupancy *rex* sites (red rectangles) results in a TAD boundary on hermaphrodite X chromosomes. Median lifespan of wild-type XX hermaphrodites is 23 days. **(g)** *sdc-2* XX mutant animals lack all DCC-dependent TAD boundaries on X, and embryos exhibit overexpression of X-linked genes and die. X-chromosome volume is expanded. **(h)** A single *rex* deletion at each TAD boundary disrupts the boundary. Deletion of all eight strong *rex* sites eliminates DCC-dependent TADs. In contrast to *sdc-2* mutant embryos, *8rex*Δ mutant embryos exhibited no changes in X volume or X expression, indicating that TADs are neither the cause nor consequence of X repression. Abrogating TAD structure did, however, did reduce thermotolerance of adult hermaphrodites (but not males), accelerate aging, and shorten lifespan by 20%.

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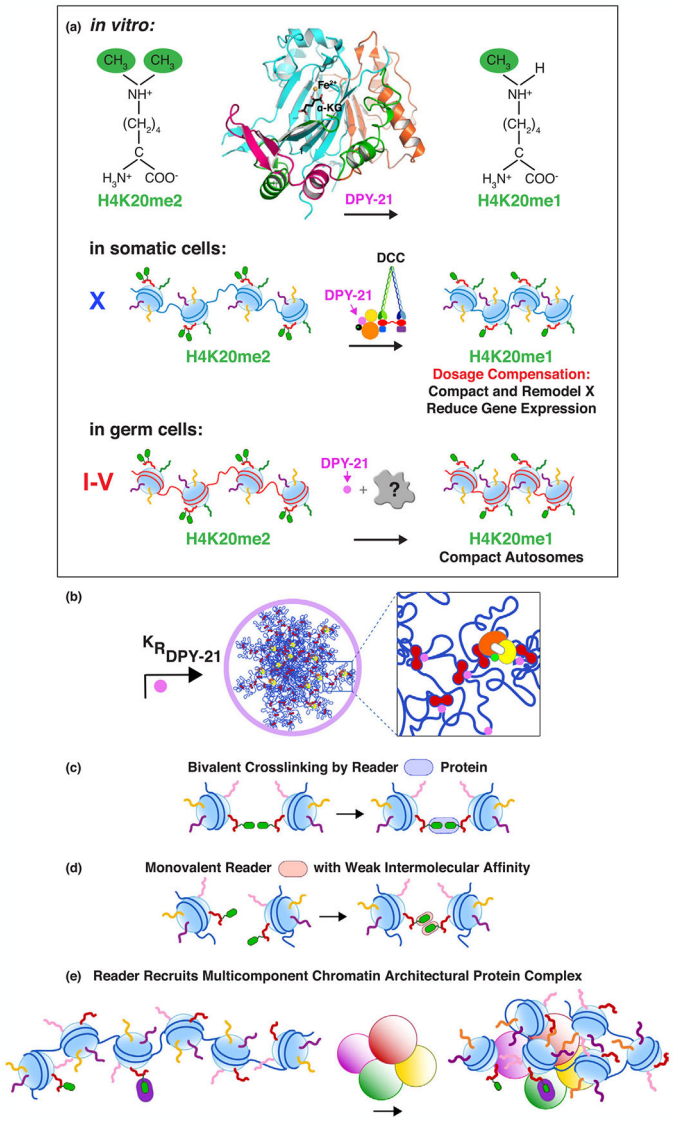

Figure 5.

Control of X-chromosome histone modification, topology, and repression by an H4K20me2 demethylase. **(a)** The DPY-21 H4K20me2 histone demethylase regulates three-dimensional X-chromosome structure and gene expression by catalyzing enrichment of H4K20me1. A 1.8 Å resolution crystal structure of DPY-21 identified a new, highly conserved H4K20me2 JmjC demethylase subfamily that converts H4K20me2 to H4K20me1 in an Fe^{2+}- and α-ketoglutarate-dependent manner. Substitutions of alanine for DPY-21 residues H1452 and D1454, predicted to coordinate α-KG and chelate Fe^{2+}, eliminated H4K20me2 demethylase activity *in vitro*. In somatic cells, DPY-21 binds to X chromosomes via the DCC **(a, b)** and enriches H4K20me1 to repress gene expression. **(a)** In germ cells, DPY-21 enriches HK20me1 on autosomes, but not X, in a DCC-independent manner to promote chromosome compaction. **(c, d)** Models exemplifying mechanisms by which an H4K20me1 reader might compact X chromatin. Compaction might be achieved by **(c)** an H4K20me1-specific reader that can simultaneously bind to H4K20me1 on two nucleosomes and constrain them; **(d)** a

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reader that can bind to H4K20me1 on separate nucleosomes and multimerize with itself to constrain chromatin; or **(e)** a reader that can recruit a multicomponent protein complex that condenses chromatin. For **(c)**, the degree of compaction will scale with the concentration of the reader and of H4K20me1. For **(d, e)**, the degree of compaction could be non-linear, and for **(e)**, non-stoichiometric interactions could cause such phenomena as phase separation.