SUMOylation is essential for sex-specific assembly and function of the *Caenorhabditis elegans* dosage compensation complex on X chromosomes

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The essential process of dosage compensation equalizes X-chromosome gene expression between *Caenorhabditis elegans* XO males and XX hermaphrodites through a dosage compensation complex (DCC) that is homologous to condensin. The DCC binds to both X chromosomes of hermaphrodites to repress transcription by half. Here, we show that posttranslational modification by the SUMO (small ubiquitin-like modifier) conjugation pathway is essential for sex-specific assembly and function of the DCC on X. Depletion of SUMO in vivo severely disrupts binding of particular DCC subunits and causes changes in X-linked gene expression similar to those caused by deleting genes encoding DCC subunits. Three DCC subunits are SUMOylated, and SUMO depletion preferentially reduces their binding to X, suggesting that SUMOylation of DCC subunits is essential for robust association with X. DCC SUMOylation is triggered by the signal that initiates DCC assembly onto X. The initial step of assembly—binding of X-targeting factors to recruitment sites on X—is independent of SUMOylation, but robust binding of the complete complex requires SUMOylation. SUMOylated DCC subunits are enriched at recruitment sites, and SUMOylation likely enhances interactions between X-targeting factors and condensin subunits that facilitate DCC binding beyond the low level achieved without SUMOylation. DCC subunits also participate in condensin complexes essential for chromosome segregation, but their SUMOylation occurs only in the context of the DCC. Our results reinforce a newly emerging theme in which multiple proteins of a complex are collectively SUMOylated in response to a specific stimulus, leading to accelerated complex formation and enhanced function.

epigenetics | chromatin | sexual dimorphism

The X chromosome-wide regulatory process called dosage compensation ensures that males (XO or XY) and females (or hermaphrodites) (XX) produce equivalent levels of X-chromosome products despite having different doses of X chromosomes. The failure to dosage-compensate is lethal. Dosage-compensation strategies differ from worms to humans, but typically a regulatory complex is targeted to the X chromosomes of one sex to regulate transcription along the entire chromosome (1–4). The molecular mechanisms by which these complexes assemble specifically onto X are not well understood. Here, we explore the role of posttranslational modification in the sex-specific assembly and function of the *Caenorhabditis elegans* dosage-compensation complex (DCC) on X chromosomes.

The nematode DCC binds to both X chromosomes of hermaphrodites to halve transcription of X-linked genes by reducing recruitment of RNA polymerase II (2, 5, 6). A separate regulatory mechanism acts in both sexes to increase X-linked gene transcription so that genes on X are expressed equivalently to genes on autosomes after dosage compensation (6, 7).

Five of the 10 DCC subunits are homologous to subunits of condensin, a protein complex required for the compaction, resolution, and segregation of chromosomes, suggesting that the DCC regulates gene expression by changing X-chromosome structure (2, 8). Of the five DCC condensin subunits, four participate in other *C. elegans* condensin complexes, permitting some DCC subunits (DPY-26, DPY-28, MIX-1, XCAPG-1) to engage in other chromosome behaviors, including chromosome segregation and the regulation of crossovers between homologous chromosomes (8–13).

To function in dosage compensation, the condensin subunits must be recruited selectively to hermaphrodite X chromosomes by three X-chromosome targeting factors, which also participate in the DCC. SDC-2 (sex determination and dosage compensation), a 350-kDa protein with a coiled-coil domain, is the key hermaphrodite-specific factor that activates dosage compensation by triggering assembly of all DCC subunits onto X chromosomes (14). DPY-30, a 13.5-kDa protein, is essential for the recruitment of all DCC subunits to X except SDC-2 (5). DPY-30 also participates in the MLL/COMPASS complex to promote local gene activation by facilitating the trimethylation of lysine 4 of histone H3 at the 5′ ends of genes on all chromosomes (5, 15). SDC-3, a 250-kDa zinc-finger protein, is essential for the X recruitment of all DCC subunits except SDC-2 and DPY-30 (5). Before our current work, no factors had been found that strengthen protein interactions between condensin subunits of the DCC and X-targeting proteins, thereby facilitating high-affinity DCC binding to X chromosomes.

The DCC binds to two classes of sites on X. *rex* (recruitment element on X) sites recruit the DCC in an autonomous, sequence-dependent manner using DNA motifs enriched on X (16, 17). In contrast, *dox* (dependent on X) sites lack a specific DNA

Significance

Dosage compensation equalizes X-chromosome transcription between nematode males (1X) and hermaphrodites (2X) via a dosage compensation complex (DCC) that binds hermaphrodite X chromosomes to repress transcription by half. We show that several DCC subunits are modified by the small ubiquitin-like modifier SUMO in response to the signal that triggers DCC assembly onto X. DCC assembly and function require SUMOylation. DCC subunit DUMPY-28 also acts in condensin complexes essential for chromosome segregation, but its SUMOylation is DCC-specific. We propose that specific signals trigger DCC protein SUMOylation, stimulating robust complex formation. SUMOylation facilitates distinct activities of proteins that function in multiple complexes.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo [accession nos. GSE48347 (gene expression studies) and GSE48413 (ChIP-chip studies)].

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motif and bind the DCC robustly only when attached to X (17). *dox* sites are more prevalent (5- to 10-fold) than *rex* sites and, unlike *rex* sites, occur preferentially in promoters of expressed genes. The higher the expression level of the gene, the higher the probability of DCC binding to its promoter. Sex-specific DCC recruitment to *rex* sites enhances DCC binding to *dox* sites *in cis* (5). Without SDC-2, SDC-3, or DPY-30, *dox* sites lack robust DCC binding, but retain low-level intrinsic binding (5).

Although DCC binding is strongly enriched on X chromosomes, DCC binding also occurs at infrequent sites on autosomes, in promoters of expressed genes (17). *dox* and autosomal sites have equivalent low-level intrinsic DCC binding ability, and the higher level of DCC occupancy at *dox* sites compared with autosomal sites is due to their physical linkage to *rex* sites (5). Attachment of *rex* sites to an autosome increases autosomal DCC binding near the site of fusion (5, 18).

We assessed whether posttranslational modification (PTM) of DCC subunits is critical for their assembly and high-affinity binding to X chromosomes, knowing that formation of protein complexes is often enhanced by PTMs of constituent subunits. We focused on the small ubiquitin-like modifier SUMO, because SUMOylation is essential for the viability of most eukaryotes and is central to such cellular processes as DNA repair, transcription, replication, and chromosome segregation (19). Covalent attachment of the ∼100-amino acid SUMO peptide to lysine residues on its target substrates occurs through an enzymatic pathway related to the ubiquitin pathway but of greater simplicity and promiscuity. Unlike the ubiquitin pathway, which uses a diverse set of ligases to achieve specificity in substrate modification, the SUMO pathway uses a small set of ligases with limited specificity in vitro (20). Higher eukaryotic genomes encode several SUMO paralogs with different functions, but *C. elegans* encodes only a single SUMO protein (21). Although a minimal consensus sequence often surrounds the target lysine residue, the sequence is ubiquitous and insufficient to direct SUMOylation, making it difficult to predict targets of SUMOyation with accuracy (22). Recognition of the SUMO modification occurs in part through a short hydrophobic SUMO-interacting motif (SIM) (23, 24). The SIMs mediate both intramolecular SUMOylation and intermolecular protein interactions. SUMOylation can be reversed by SUMO-specific proteases, and substrate proteins exist in dynamic balance between modified and unmodified forms (25).

In general, PTMs are highly specific for individual substrates and endow the substrates with new properties, such as a change in subcellular location or activity state. Although the SUMOylation machinery often targets a single protein and alters its binding partners and function (26), a few examples exist in which the machinery targets several proteins of a specific complex to alter its overall function. For example, SUMOylation of septins in the bud neck of yeast promotes the disassembly of septin rings after cytokinesis (27). SUMOylation of homologous recombination (HR) proteins upon their localization to DNA double-strand breaks (DSBs) enhances HR complex formation and accelerates DSB repair (28, 29). The additive benefit of the concurrent SUMOylation of multiple subunits in a complex helps explain why knockdown of the SUMO pathway is more deleterious than eliminating SUMOylation of a single subunit.

We discovered that SUMOylation is essential for proper dosage compensation in *C. elegans*. Depletion of SUMO preferentially increases the expression of genes on X in XX embryos, resulting in strong overlap between the X-linked genes having elevated expression in dosage compensation-defective embryos and those having elevated expression in SUMO-depleted embryos. Moreover, depletion of SUMO drastically reduces X-chromosome binding of the DCC condensin subunits and the X-targeting factor SDC-3. X binding in SUMO-depleted embryos is reduced to the low level of DCC binding typical for autosomes of wild-type embryos. In contrast, binding of the X-targeting factors SDC-2 and DPY-30 to X is not significantly altered by SUMO depletion, indicating that the first steps of DCC assembly onto X are independent of SUMOylation. The X-targeting factor SDC-3 and the condensin subunits DPY-27 and DPY-28 are then SUMOylated, and their SUMOylation is dependent on all three X-targeting factors. SUMOylated DCC subunits are enriched on X chromosomes, particularly at *rex* sites, and DCC subunits that participate in other condensin complexes are SUMOylated only in the context of the DCC.

We propose a model in which the initial steps of dosage compensation, the binding of X-targeting factors SDC-2 and DPY-30 to X chromosomes, occur independently of SUMOylation. DCC subunits are SUMOylated in response to the expression and X binding of SDC-2, the trigger that initiates DCC assembly onto X. Although low-level X-chromosome binding of DCC subunits can occur without SUMOylation, the SUMOylation of DCC subunits appears to enhance interactions between X-targeting factors and condensin subunits that promote robust DCC binding to X. Our results strengthen an emerging theme in which multiple proteins of a complex are collectively SUMOylated in response to a specific stimulus, leading to accelerated complex formation, stability, and enhanced function.

Results

SUMOylation Is Required for *C. elegans* Dosage Compensation. To determine whether posttranslational SUMOylation of DCC subunits is important for their sex-specific assembly onto X chromosomes, we first performed RNAi-mediated knockdown of *smo-1*, the sole *C. elegans* gene encoding a SUMO protein, and assessed defects in DCC localization by immunofluorescence (IF). Reduction of *smo-1* mRNA caused complete embryonic lethality of both sexes and disrupted DCC association with X. In control embryos, DCC proteins have a punctate subnuclear localization pattern, reflecting their enriched binding to X chromosomes. In SUMO-depleted XX embryos, the DCC components assayed (SDC-3, DPY-27, DPY-26, and MIX-1) had a diffuse nuclear localization pattern (Fig. 1*C* and Fig. S1). X-binding defects appeared more severe for the condensin subunits DPY-26, DPY-27, and MIX-1 than for the X-targeting protein SDC-3 (Fig. 1*C* and Fig. S1). This diffuse nuclear IF pattern in SUMO-depleted embryos suggested that proper DCC assembly onto X chromosomes requires SUMOylation of one or more cellular proteins.

If SUMOylation is required for X-chromosome gene expression, as implied by the *smo-1(RNAi)* experiments, significant overlap should exist between the genes exhibiting altered expression in SUMO-depleted embryos and those exhibiting altered expression in dosage compensation-defective embryos. Using Affymetrix microarrays, we compared genome-wide changes in gene expression in *smo-1* RNAi-treated XX embryos, control RNAi XX embryos, and *sdc-2* mutant XX embryos. As would be expected for a dosage-compensation defect, *smo-1(RNAi)* caused a disproportionate increase in the number of X-linked genes compared with autosomal genes having elevated expression. Of the 2,471 X-linked genes on the array, 37% (924 genes) were significantly overexpressed ($P < 0.05$) in SUMO-depleted embryos whereas only 17% (431 genes) were underexpressed. Conversely, more autosomal genes were underexpressed (27% of 15,466 genes) than overexpressed (10% of 15,466), a result mirroring the changes in gene expression caused by depletion of SDC-2. These changes in gene expression were unlikely caused by reduced expression of DCC-encoding genes because those genes were expressed at 0.5- to 1.0-fold their normal levels, and none is haploinsufficient.

Changes in gene expression caused by disrupting dosage compensation are positively correlated with changes caused by SUMO depletion. Of the 728 X-linked genes statistically overexpressed ($P < 0.05$) in *sdc-2* mutant embryos, 66% were also statistically overexpressed ($P < 0.05$) in *smo-1(RNAi)* embryos

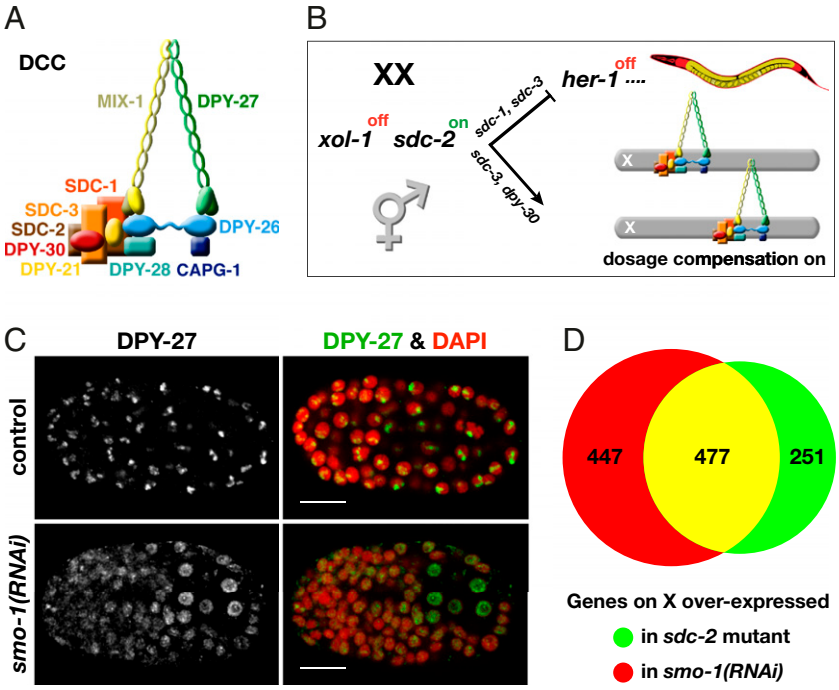

Fig. 1. Sumoylation is required for dosage compensation. (*A*) Diagram of the *C. elegans* dosage-compensation complex (DCC). The DCC includes five subunits (MIX-1, DPY-27, DPY-26, DPY-28, and CAPG-1) that are homologous to the five condensin subunits, three subunits (SDC-2, SDC-3, and DPY-30) critical for assembly of DCC subunits onto X, and two subunits (DPY-21 and SDC-1) critical for DCC activity. MIX-1 and DPY-27 are SMC proteins (structural maintenance of chromosomes) with long coiled-coil and ATPase domains. MIX-1, DPY-26, DPY-28, and CAPG-1 act in other *C. elegans* condensin complexes essential for chromosome segregation. DPY-30 also acts in the MLL/COMPASS complex that produces the H3K4me3 chromatin mark characteristic of activated genes. SDC-2 is the sole DCC subunit expressed exclusively in XX embryos. It confers sex specificity and X specificity to dosage compensation. (*B*) Sex-specific assembly of the DCC onto X chromosomes of XX embryos is controlled by a genetic pathway that regulates both sex determination and dosage compensation. *sdc-2* acts with the zinc finger proteins *sdc-1* and *sdc-3* to induce hermaphrodite sexual development by repressing transcription of the male sex-determining gene *her-1*. *sdc-2* acts with *sdc-3* and *dpy-30* to assemble the DCC onto X and thereby activate dosage compensation. Without *sdc-2*, the DCC cannot bind to X, causing the death of all XX embryos, and *her-1* is expressed, causing masculinization of XX embryos. (*C*) DCC subunit DPY-27 fails to localize to the X chromosomes of XX embryos depleted of the SUMO peptide. Confocal images of XX embryos treated with control RNAi or RNAi directed against *smo-1*, costained with DAPI (4,6-diamidino-2-phenylindole) (red in merge) and DPY-27 antibodies (green in merge). In control XX embryos, DPY-27 exhibits a punctate localization pattern in nuclei, indicating enrichment on X chromosomes. In SUMO-depleted embryos, DPY-27 is diffusely localized throughout nuclei, suggesting incomplete DCC assembly on X. (Scale bar, 5 μm.) (*D*) SUMO depletion causes elevated expression of X-linked genes, like SDC-2 depletion. Venn diagram shows the overlap (yellow) in X-linked genes with elevated expression in both *smo-1(RNAi)* and *sdc-2(y93, RNAi)* XX embryos.

(Fig. 1*D*). A significant overlap was also found between X-linked genes with decreased expression in *sdc-2* mutant embryos and in *smo-1(RNAi)* embryos. Of 64 X-linked genes with decreased expression in *sdc-2* mutant embryos ($P < 0.05$), 83% were also significantly underexpressed in SUMO-depleted embryos.

Of 2,672 autosomal genes with reduced expression in *sdc-2* mutants, 75% also had reduced expression in *smo-1(RNAi)* embryos. Of 642 autosomal genes with increased expression in *sdc-2* mutants, 70% had increased expression in *smo-1(RNAi)* embryos. The significant overlap in gene-expression changes caused by depletion of SUMO or DCC subunit SDC-2 further supports an important role for SUMOylation in *C. elegans* dosage compensation.

In prior studies, we classified a limited set of genes on X as dosage-compensated by the stringent criteria that their total transcript level was not different between XX and XO embryos, and their expression was significantly higher in dosage compensation-defective XX embryos than in wild-type XX embryos (17). Of 374 dosage-compensated genes, 64% were overexpressed in SUMO-depleted XX embryos, significantly more than the 37% expected by random overlap ($P < 0.0001$). Conversely, a limited set of genes was classified as escaping dosage compensation by the criteria that expression in XX embryos was higher than in XO embryos, and expression was not increased in dosage compensation-defective XX embryos (17). Of 290 noncompensated genes, only 18% were significantly overexpressed in SUMO-depleted XX embryos, less than the amount expected by random overlap. Thus, reduction of SUMOylation not only disrupts the localization of the DCC to X, it also disrupts expression of genes normally regulated by the dosage-compensation process.

X-Chromosome Binding of DCC Condensin Subunits Is Severely Reduced by Depletion of SUMO. To assess the defects in DCC binding caused by depleting SUMO, we first determined the genome-wide binding profiles of DCC condensin subunits (DPY-26, DPY-27, DPY-28, MIX-1) using chromatin immunoprecipitation reactions (IPs) with extracts from *smo-1(RNAi)* and control RNAi embryos followed by hybridization to tiling arrays (ChIP-chip). As predicted by our IF data, quantification of ChIP-chip experiments showed that binding of several DCC condensin subunits was severely reduced along the X chromosome, confirming that SUMOylation is essential for DCC assembly onto X (Fig. 2 *A*–*C*). For example, 70% of X-chromosome binding sites with high DPY-27 occupancy (peak score ≥ 75) in control embryos had no or low occupancy in *smo-1(RNAi)* embryos. In fact, binding of DPY-27 to X in the SUMO-depleted embryos was reduced to the low level of binding typical for autosomes in control embryos (Fig. 3*A*). Similar results were obtained for DPY-26 and DPY-28 (Fig. S2*A*). MIX-1 binding was affected less severely. These results indicate that robust binding of some DCC condensin subunits to X requires SUMOylation and that DCC subunits have different requirements for SUMOylation.

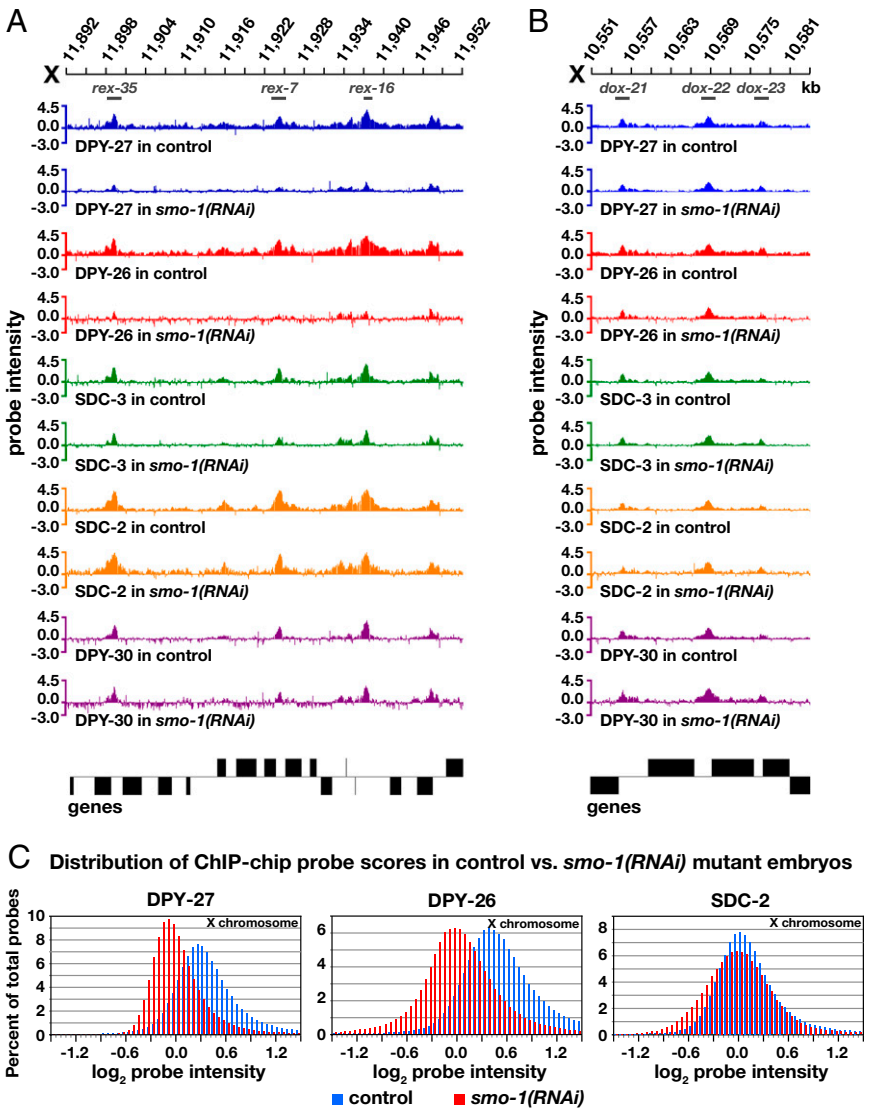

Fig. 2. SUMOylation is essential for proper assembly of the DCC onto X. (*A* and *B*) Representative ChIP-chip profiles of five DCC subunits in control and *smo-1(RNAi)* XX embryos at representative *rex* (*A*) and *dox* (*B*) sites on the X chromosome. Gene locations are indicated below the ChIP profiles. Genes transcribed to the right are above the line and those transcribed to the left are below the line. Binding of the condensin DCC subunits (DPY-27 and DPY-26) is severely reduced at *rex* sites, but only moderately reduced at *dox* sites. Binding of X-targeting factor SDC-3 is moderately reduced at *rex* sites, but binding of DCC X-targeting factors SDC-2 and DPY-30 is not significantly reduced at either *rex* or *dox* sites. (*C*) Histograms depicting the binding distribution of DCC ChIP-chip probe scores across the X chromosome in control versus *smo-1(RNAi)* XX embryos. Probe scores were grouped into bins with a step size of 0.06 ($\log_2$ scale), and the percentage of total probes in each bin was graphed on the *y* axis. In general, DCC components bind more strongly to the X chromosome of control embryos than of those SUMO-depleted embryos. The defect in binding is much more severe for condensin subunits (DPY-26 and DPY-27) than for the DCC recruitment protein SDC-2.

Although depletion of SUMO reduced DCC binding at both *rex* and *dox* sites, *rex*-site binding was more strongly affected than *dox*-site binding for all DCC condensin subunits tested (Figs. 2 *A* and *B* and 3 *B* and *C* and Fig. S3). For example, DPY-26 binding was nearly eliminated at 51% of *rex* sites and only 25% of *dox* sites. *rex* and *dox* sites that retained some DPY-26 binding showed substantially reduced binding (Figs. 2 *A* and *B* and 3 *B* and *C* and Fig. S3). The average DPY-26 ChIP-chip score at *rex* sites decreased from 2.25 in control embryos to 0.88 in *smo-1 (RNAi)* embryos, and the average score at *dox* sites decreased from 1.68 to 1.07. Similarly, DPY-28 and DPY-27 binding was reduced at both *rex* and *dox* sites, but more severely at *rex* sites (Figs. 2 *A* and *B* and 3*B* and Fig. S3). Thus, SUMOylation is most important for DCC recruitment to *rex* sites. Reduced binding at *dox* sites may be a secondary consequence of reduced binding at *rex* sites. Depletion of SUMO by RNAi treatment is incomplete and may contribute to some of the residual DCC binding at *rex* and *dox* sites.

Binding of Two X-Targeting Factors That Trigger Assembly of DCC Condensin Subunits onto X Is Not Significantly Changed by Depletion of SUMO. We assessed whether SUMOyation is critical for the X-chromosome association of factors that confer X specificity to DCC binding and initiate the assembly of DCC subunits onto X. Of the three X-targeting factors, SDC-2 is the key hermaphrodite-specific factor that triggers assembly of the DCC onto X. Unlike all other DCC subunits, which are maternally supplied to young embryos regardless of their future sex, SDC-2 is expressed exclusively in XX embryos after the hermaphrodite fate is specified (14). SDC-2 can associate with X chromosomes without other DCC components. Once produced, SDC-2 acts in collaboration with SDC-3 and DPY-30 to initiate the sex-specific, sequence-dependent recruitment of all DCC components to *rex* sites (5, 16). In contrast, DPY-30 requires SDC-2 but not other DCC subunits for its association with X, and SDC-3 requires both SDC-2 and DPY-30 (5).

Binding of the X-targeting factors SDC-2 and DPY-30 to X chromosomes was not significantly changed in response to depletion of SUMO (Figs. 2 *A*–*C* and 3 *B, E*, and *F* and Fig. S2*B*). Only small quantitative changes were detected in overall binding of SDC-2 and DPY-30 to X chromosomes in SUMO-depleted embryos, and binding to *rex* and *dox* sites was barely altered (Figs. 2 *A* and *B* and 3 *B, E*, and *F*). For SDC-2, the binding on X is greater than the binding on autosomes in *smo-1(RNAi)* embryos (Fig. 2*B*), as is true for all DCC subunits in control embryos. Ninety-four percent of *rex* sites with high SDC-2 occupancy (peak score > 0.75) in control embryos had high occupancy in SUMO-depleted embryos. The average SDC-2 ChIP-chip peak

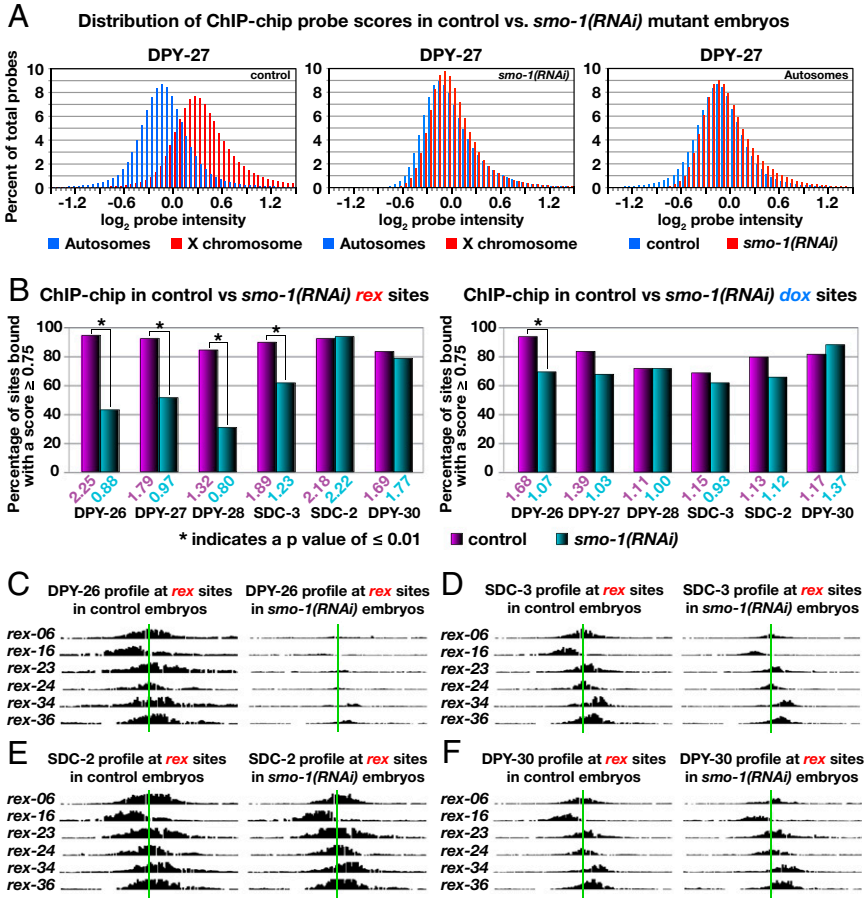

Fig. 3. DCC binding at both *rex* and *dox* sites is depleted by *smo-1* RNAi. (*A*) Histograms show the distribution of DPY-27 ChIP-chip probe scores across autosomes and X chromosomes in control or SUMO-depleted XX embryos. Probe scores were grouped into bins as in Fig. 2*C*. In *smo-1(RNAi)* embryos, X-chromosome binding of DPY-27 was reduced to the low level of binding on autosomes. Consistent with new DCC binding sites on autosomes, the average DCC ChIP-chip probe scores on autosomes is higher in *smo-1(RNAi)* embryos than in control embryos. (*B*) Histograms show quantification of DCC subunit binding at *rex* and *dox* sites in control versus *smo-1(RNAi)* XX embryos. The height of each bar shows the percentage of *rex* and *dox* sites with DCC peak scores of 0.75 or greater in DCC mutants. An asterisk indicates a *P* value of ≤0.01. The average peak scores shown below the histograms were calculated from the scores of all called peaks at *rex* and *dox* sites. Binding of condensin subunits DPY-26, DPY-27, and DPY-28 and X-targeting protein SDC-3 is severely affected. Binding at *rex* sites is more affected by *smo-1* RNAi than binding at *dox* sites. Binding of the X-targeting proteins SDC-2 and DPY-30 is not significantly affected. (*C*–*F*) Graphical representations of DCC ChIP-chip probe intensities along 5-kb regions centered (green line) on representative *rex* and *dox* sites in control versus *smo-1 (RNAi)* XX embryos. Binding of the condensin DCC subunit DPY-26 is severely reduced at *rex* sites, but only moderately reduced at *dox* sites. Binding of the X-targeting protein SDC-3 is also preferentially reduced at *rex* sites, but SDC-3 binding is less affected than DPY-26 binding. Binding of X-targeting proteins SDC-2 and DPY-30 is not significantly reduced by SUMO depletion.

score at *rex* sites was 2.18 in control embryos and 2.22 in *smo-1 (RNAi)*. *dox*-site binding was only minimally reduced (Fig. 3*B* and Fig. S3). Similarly, for DPY-30, 95% of *rex* sites with high DPY-30 occupancy (peak score ≥ 0.75) in control embryos had high DPY-30 occupancy in SUMO-depleted embryos, and the average peak score at *rex* sites was 1.69 in control embryos and 1.77 in *smo-1(RNAi)* (Fig. 3 *B* and *F*). Thus, the initial binding events that trigger recruitment of DCC subunits to X can occur independently of SUMOylation.

Curiously, the intensity of DPY-30 binding at *dox* sites increased in *smo-1* RNAi-treated embryos (Figs. 2*B* and 3*B*); the average score increased from 1.17 to 1.37. This increase in DPY-30 occupancy is likely due to DPY-30's dual participation in the DCC and in the MLL/COMPASS complex. The increased occupancy was not directly correlated with a change in expression of the promoters bound by DPY-30.

Binding of X-Targeting Factor SDC-3 Is Reduced by Depletion of SUMO. In contrast to SDC-2 and DPY-30, binding of SDC-3 to X chromosomes was reduced in SUMO-depleted embryos, as judged by IF and ChIP-chip experiments, although the SDC-3 binding defect was less severe than that of the DCC condensin subunits (Figs. 2 *A* and *B* and 3 *B* and *D* and Fig. S2). Only 30% of X-binding sites with high SDC-3 occupancy (peak score ≥ 0.75) in control embryos had low or no SDC-3 occupancy in SUMO-depleted embryos compared with 70% with high. The average score of sites with residual SDC-3 binding was reduced from 0.97 in control embryos to 0.71 in *smo-1(RNAi)* embryos. SDC-3 binding was nearly eliminated at 28% of *rex* sites and 7% of *dox* sites although *rex* and *dox* sites that retained SDC-3 binding had reduced average peak scores: from 1.89 to 1.23 at *rex* sites, and from 1.15 to 0.93 at *dox* sites (Fig. 3*B*). For SDC-3, SUMOylation is more important for optimal binding at *rex* sites than *dox* sites (Figs. 2 *A* and *B* and 3 *B* and *D* and Fig. S3).

Together, our results support a model in which the initial events of dosage compensation, the binding of SDC-2 and DPY-30 to *rex* sites, occur independently of SUMOylation. Maximal binding of SDC-3 to *rex* sites and almost all binding of DCC condensin subunits to *rex* sites subsequently require SUMOylation.

Depletion of SUMO Increases DCC Binding on Autosomes. Analysis of ChIP-chip profiles in SUMO-depleted embryos revealed new binding sites on autosomes for all DCC subunits, both condensin subunits and X-targeting factors, sites that were not present in control embryos (Fig. 4*A*). Across all autosomes, 1,535 new binding sites were detected in common between SDC-3 and DPY-27, representing a 38% increase in autosomal sites. Of these sites, 188 had high occupancy (peak score ≥ 0.75), reflecting a 23% increase in high-occupancy sites. Like *dox* sites on X and DCC binding sites on autosomes of control embryos, the new DCC binding sites on autosomes of SUMO-depleted embryos were strongly enriched in promoters of active genes (Fig. 4 *B* and *C*). The greater the increase in autosomal gene expression in *smo-1(RNAi)* embryos, the greater the increase in DCC occupancy of all DCC subunits on autosomes (Fig. 4*C* and Fig. S4). Whether these new DCC binding sites promote increased gene expression or result from increased gene expression is not known. Either way, SUMOylation of one or more proteins is essential not only for DCC assembly onto X chromosomes, but also for the wild-type pattern of autosomal DCC binding.

Multiple DCC Subunits Are SUMOylated. The observations that (*i*) the SUMO conjugation pathway is required for proper binding of several DCC subunits to X chromosomes and (*ii*) SUMOy-

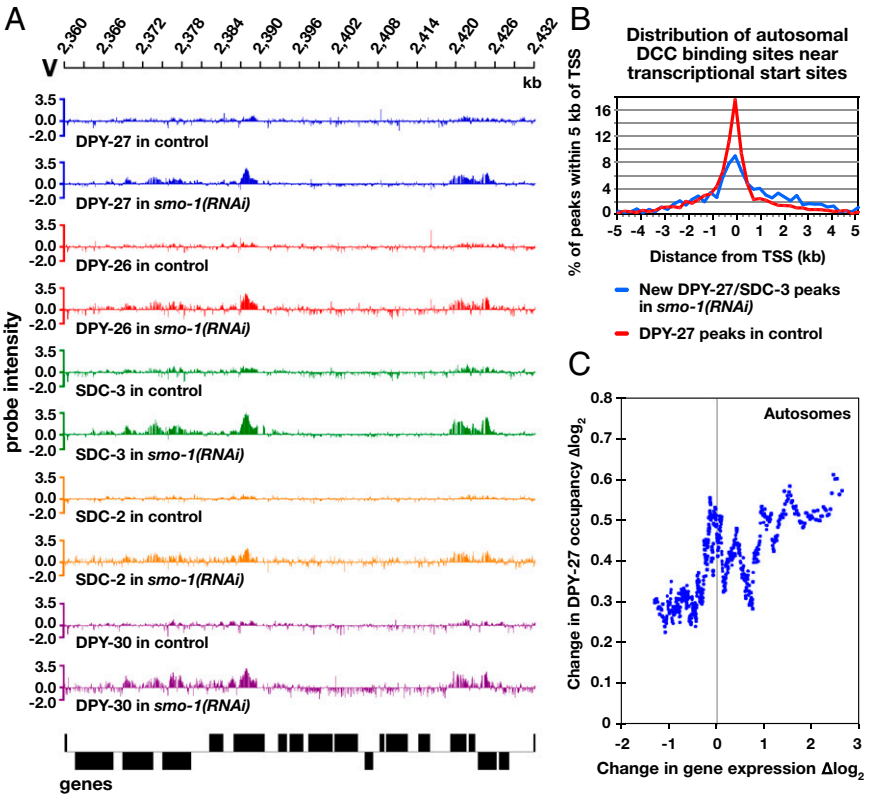

Fig. 4. *smo-1(RNAi)* results in new sites of DCC binding at the promoters of autosomal genes. (*A*) ChIP-chip profiles of five DCC subunits on chromosome V in control versus *smo-1(RNAi)* XX embryos. All DCC components analyzed bind to new autosomal sites in *smo-1(RNAi)* embryos. (*B*) New autosomal DCC binding sites in SUMO-depleted embryos are preferentially enriched in the promoters of genes. New binding sites were defined as those present in both SDC-3 and DPY-27 ChIP-chip experiments from *smo-1(RNAi)* embryos but not control embryos. For the 1,535 new DCC binding sites, the distance from the center of each peak to the nearest transcriptional start site (TSS) was determined. These distances were grouped into 250-bp bins, and the percentage of peaks within each bin was calculated for each category (negative *x* axis values correspond to peaks that lie upstream of the TSS). (*C*) New autosomal DCC binding sites in SUMO-depleted embryos are positively correlated with genes having increased expression. Shown is a moving average plot depicting the positive correlation between changes in DCC binding at autosomal promoters in *smo-1(RNAi)* embryos and changes in expression of the corresponding gene. For each gene with a new DCC promoter peak in *smo-1(RNAi)* embryos, the third highest ChIP-chip probe score within the promoter was used for the comparison of DCC binding in control versus *smo-1(RNAi)* embryos. The *smo-1 (RNAi)* ChIP-chip and gene expression values were subtracted from those of control embryos and ordered by the change in gene expression, from the smallest to the largest. A 50-gene average was calculated of a sliding window with a step size of 1 for the change in gene expression (*x* axis) and then the corresponding change in DCC binding (*y* axis). Positive quadrants of the axes correspond to higher expression or binding in *smo-1(RNAi)* versus control embryos. A statistically significant positive correlation exists between the new DCC peaks in promoters of *smo-1(RNAi)* embryos and increased expression of the cognate genes (Pearson's correlation coefficient $R = 0.625$, $P < 0.0001$).

lation of proteins often promotes protein–protein interactions caused us to assess whether DCC components are SUMOylated. Using antibodies to each of the 10 DCC proteins, we performed Western blots to determine whether control embryos had higher molecular weight variants of DCC subunits than SUMO-depleted embryos (Fig. 5 *A* and *B* and Fig. S5). Such higher molecular weight variants were found for three DCC subunits: SDC-3, DPY-27, and DPY-28 (Fig. 5*B*). For both DPY-27 and DPY-28, the increase in molecular weight was consistent with the addition of a single SUMO molecule. For SDC-3, one and often multiple higher molecular weight variants were detected.

Three lines of evidence confirmed that the higher molecular weight variants of DPY-27, DPY-28, and SDC-3 were caused by SUMO modification, rather than a SUMO-dependent modification such as ubiquitination. First, treatment of DPY-27, DPY-28, and SDC-3 immunoprecipitation reactions with a SUMO-specific protease before Western blot analysis greatly reduced the abundance of the higher molecular weight variants, indicating that DPY-27, DPY-28, and SDC-3 are SUMOylated (Fig. 5*C*). Second, FLAG antibodies detected the higher molecular weight DPY-27 variant in Western blot analysis of a *smo-1* deletion strain expressing an N-terminal FLAG-tagged SMO-1 molecule (Fig. 5*D*). Third, a high-throughput mass-spectrometry study identified DPY-27 and DPY-28 as potential targets of SUMOylation although the sites of modification were not mapped (30). Thus, SDC-3, DPY-27, and DPY-28 are bona fide targets of SUMOylation in XX embryos. The SUMOylated variants represented 40% (± 1.4% SEM) of total DPY-27 protein, 22% (± 5.0% SEM) of DPY-28 protein, and 31% (± 2.1% SEM) of SDC-3 protein.

Of importance, the DCC subunits that are modified by SUMO are the very components whose binding to X is most diminished by depletion of SUMO, supporting our hypothesis that SUMOylation of specific DCC components is essential for their recruitment to X. The only exception is DPY-26. The association of DPY-26 with X is dependent on DPY-27, making it likely that the severe defect in DPY-26 binding to X in SUMO-depleted embryos is a secondary consequence of reduced DPY-27 binding. The level of all 10 DCC proteins in SUMO-depleted embryos is at least 75% of the level in control embryos, indicating that reduction in protein levels is not responsible for the reduced recruitment of DCC subunits to X (Fig. S6*A*).

A Subunit Shared Between the DCC and Condensin I Is SUMOylated Only in the Context of the DCC. In embryos, DPY-28 participates in two chromosome-binding complexes, the DCC and condensin I; the latter modifies chromosome structure in preparation for mitotic chromosome segregation (11, 12). We therefore asked whether SUMOylation of DPY-28 occurs in connection with its participation in one or both complexes. To do so, we selectively immunoprecipitated DPY-28 from either the DCC or condensin I, using antibodies specific to only one complex: DPY-27 for the DCC and SMC-4 for condensin I. Only the DPY-28 protein that was immunoprecipitated from the DCC showed evidence of SUMOylation (Fig. 5*E*).

SUMOylated DCC Components Associate with X Chromosomes, Particularly at *rex* Sites. Our observation that depletion of SUMO severely reduced the X-chromosome binding of SUMOylated DCC subunits DPY-27, DPY-28, and SDC-3 raised the question of whether these SUMOylated DCC proteins are preferentially enriched on *rex* and *dox* sites compared with sites on autosomes. To assess the binding preference of SUMOylated DPY-27, we performed a series of sequential ChIP-chip experiments designed to capture first all SUMOylated proteins bound to chromatin

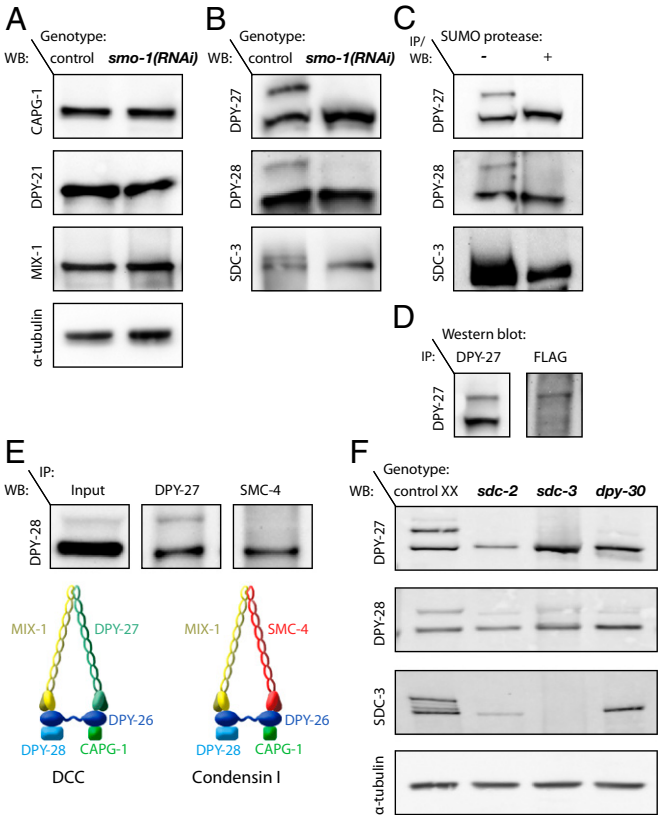

Fig. 5. Multiple components of the DCC are covalently modified by the small ubiquitin-like molecule SUMO. (*A*) Some DCC subunits lack SUMOylation. Western blots of MIX-1, DPY-21, and CAPG-1 showed similar protein bands in control and *smo-1(RNAi)* embryos. (*B*) DPY-27, DPY-28, and SDC-3 are modified. Western blots using DPY-27, DPY-28, and SDC-3 antibodies showed higher molecular weight variants in control versus *smo-1(RNAi)* embryos, implying SUMOylation. (*C*) Treatment with SUMO protease ULP-1 confirmed SUMOylation. IPs using SDC-3, DPY-27, or DPY-28 antibodies were treated with purified yeast SUMO-specific protease (ULP-1) and blotted with the same antibodies. ULP-1 treatment reduced or eliminated the higher molecular weight band for DPY-27 and DPY-28 and reduced SDC-3 to a narrower band. (*D*) Western blot of a FLAG-tagged SMO-1 strain further confirmed SUMOylation of DPY-27. DPY-27 IPs from FLAG-tagged SMO-1 embryo extract were blotted with antibodies to DPY-27 or FLAG. Anti-FLAG antibodies recognize only the upper band of DPY-27, showing that DPY-27 is SUMOylated. (*E*) DPY-28 is SUMOylated in the context of the DCC but not condensin I. Western blots probing DPY-28 that had been immunoprecipitated from either the DCC with DPY-27 antibodies or condensin I with SMC-4 antibodies show that the SUMOylated variant of DPY-28 is present in eluates of DPY-27 but not SMC-4 IPs. Diagram of DCC and condensin I subunits is below the blots. (*F*) SUMOylation of DPY-27, DPY-28, and SDC-3 is dependent on the X-targeting proteins SDC-2, SDC-3, and DPY-30. Western blots using antibodies against DPY-27, DPY-28, and SDC-3 showed that the SUMOylated variant of each protein is absent or greatly reduced in embryos depleted for X-targeting proteins. The protein loading control for *A*, *B*, and *F* is α-tubulin. Quantification is in Fig. S6*B*.

and then all DPY-27-chromatin complexes within the pool of SUMOylated protein–chromatin complexes. This regime should capture SUMOylated DPY-27-chromatin complexes. Using extracts from the *smo-1* deletion strain that expresses FLAG-tagged SMO-1 we performed four different ChIP-chip experiments: (*i*) ChIP using FLAG antibodies to determine the genome-wide binding sites for SUMOylated proteins, (*ii*) ChIP using FLAG antibodies followed by re-ChIP of eluted protein–chromatin complexes with DPY-27 antibodies to determine genome-wide binding sites for SUMOylated DPY-27 (referred to as DPY-27 re-ChIP experiments), (*iii*) ChIP using FLAG antibodies followed by re-ChIP of eluted protein–chromatin with IGG antibodies to determine background binding (referred to as IGG re-ChIP experiments, and (*iv*) ChIP using DPY-27 antibodies as a control to assess the efficiency of DPY-27 binding and detection in control vs. FLAG-tagged strains. The DPY-27 ChIP-chip profiles in control and FLAG-tagged SMO-1 embryos were virtually indistinguishable, indicating that the FLAG tag on SUMO did not impair DPY-27's ability to bind to X chromosomes or autosomes and be detected by ChIP.

Analysis of sequential ChIP-chip experiments showed that SUMOylated DPY-27 is enriched on X chromosomes, particularly at *rex* sites (Fig. 6*A* and Fig. S7). Eighty-two percent (33/40) of all *rex* sites showed strong occupancy of FLAG-tagged SUMO in both ChIP-chip replicates using FLAG antibodies (average peak score ≥ 0.75), and 65% (26/40) of all *rex* sites re-ChIPed with DPY-27 antibodies out of the pool of protein–chromatin complexes first ChIPed with FLAG antibodies. For a *rex* site to be considered positive in the DPY-27 re-ChIP experiments, the site had to be identified in both biological replicates of the DPY-27 re-ChIP experiments, and the average DPY-27 re-ChIP peak score had to exceed, by at least 0.25, the average peak score from the two biological replicates of IGG re-ChIP experiments. All *rex* sites that scored positive for the DPY-27 re-ChIP experiments showed either no or dramatically reduced DCC condensin binding in SUMO-depleted embryos, consistent with SUMOylation of DPY-27 being important for binding.

Using the same criteria, 80% (40/50) of *dox* sites showed strong occupancy of FLAG-tagged SUMO, but only 36% (18/50) of *dox* sites re-ChIPed with DPY-27 antibodies. The average DPY-27 re-ChIP score for the positive *rex* sites was 1.32 ± 0.21 whereas that for the positive *dox* sites is 0.88 ± 0.13. The two-tailed *t* test assuming unequal sample variances shows that the *rex* and *dox* sites behave statistically differently ($P = 0.0008$) with regard to the DPY-27 rechip experiment. These results indicate that the majority of *rex* sites are bound by SUMOylated DPY-27 protein, but *dox*-site binding by SUMOylated DPY-27 protein is less common. The high occupancy of *rex* sites with SUMOylated DPY-27 is consistent with *rex*-site binding being more strongly affected by *smo-1(RNAi)* than *dox*-site binding.

Of autosomal sites with strong FLAG-SUMO occupancy, 29.7% (121 of 408) were positive in the DPY-27 re-ChIP experiment. The average DPY-27 re-ChIP score for positive autosomal sites was 0.80 ± 0.018. For an autosomal site to be considered positive in the DPY-27 re-ChIP experiment, the site had to have strong FLAG-tagged SUMO occupancy in both ChIP-chip biological replicates (average peak score ≥ 0.75), the site had to be identified in both DPY-27 re-ChIP biological replicates, and the average DPY-27 re-ChIP peak score had to exceed, by at least 0.25, the average IGG re-ChIP peak score from both replicates.

Analysis using less stringent criteria for defining autosomal sites bound by FLAG-tagged SUMO proteins gave very similar results. Of autosomal sites identified in both FLAG ChIP replicates independent of peak score, 30% (178 of 593) were positive for DPY-27 re-ChIP using the criteria above. The average DPY-27 re-ChIP score for these positive autosomal sites was also 0.80 ± 0.02. Analysis by the two-tailed *t* test assuming unequal sample variances indicated that *rex* sites are preferentially enriched for binding of SUMO-modified DPY-27 compared with autosomal sites defined by either set of criteria ($P = 0.0001$), and that enrichment at *dox* sites is not different from that at autosomal sites ($P = 0.21$).

DCC SUMOylation Is Triggered by the X-Targeting Factors That Initiate Assembly of the DCC onto X. In principle, DCC subunits could be SUMOylated before their binding to X or in conjunction with their recruitment to X. If SUMOylation of DCC subunits occurs in conjunction with X recruitment, SUMOylation should be dependent on the sex-specific targeting factors—SDC-2, SDC-3, and DPY-30—that trigger DCC assembly onto X. We assayed the

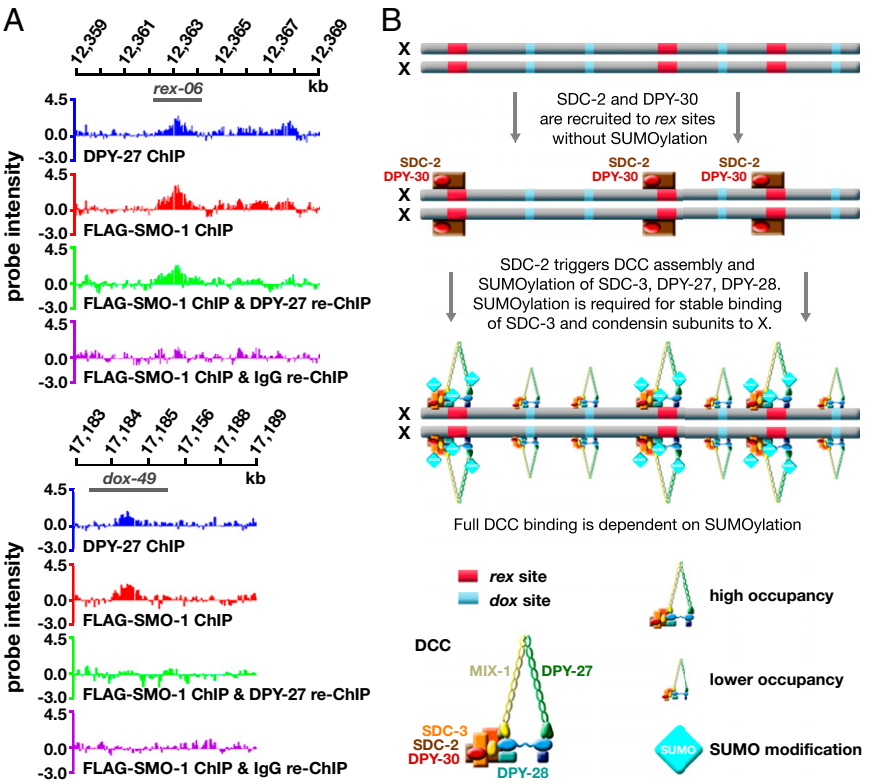

Fig. 6. SUMOylated DPY-27 is enriched at *rex* sites. (*A*) Representative profiles of *rex* and *dox* sites in the following ChIP-chip experiments using extracts from the FLAG-tagged SMO-1 strain: ChIP using DPY-27 antibodies; ChIP using FLAG antibodies; ChIP using FLAG antibodies followed by re-ChIP of eluted protein–chromatin complexes with DPY-27 antibodies; and ChIP using FLAG antibodies followed by re-ChIP of eluted protein–chromatin with IGG antibodies. SUMOylated DPY-27 is strongly enriched at *rex* sites but infrequently at *dox* sites. (*B*) Model for the role of SUMOylation in DCC assembly and function on X chromosomes. The initial step of DCC assembly, the binding of X-targeting factors to *rex* sites on X, occurs without SUMOylation and then triggers SUMOylation of DCC subunits. SUMOylation likely facilitates interactions between DCC subunits that leads to robust DCC assembly and function.

genetic requirements for SUMOylation of DCC subunits by comparing Western blots of DPY-27, DPY-28, and SDC-3 proteins from extracts of control embryos and XX embryos with partially depleted X-targeting factors in strains *sdc-2(y93, RNAi)*, *sdc-3(y129, RNAi)*, or *dpy-30(y130ts)*. The SUMOylated variants of DPY-27 and SDC-3 evident in control embryos were absent from the extracts made from X targeting-defective embryos (Fig. 5*F* and Fig. S6*B*). The SUMOylated variant of DPY-28 was reduced fourfold. The importance of X-targeting factors in establishing SUMOylation of DCC subunits is a strong indication that SUMOylation is triggered in response to DCC recruitment, by the signal that induces assembly of DCC subunits onto X chromosomes. The most plausible signal for SUMOylation of DCC subunits is the X-chromosome binding of SDC-2, the hermaphrodite-specific trigger of DCC assembly, which is expressed in the zygote only after the embryo has determined its sex.

Discussion

Here, we showed that the small ubiquitin-like modifier SUMO is essential for X-chromosome dosage compensation in *C. elegans*. Three subunits of the DCC are SUMOylated in response to the signal that triggers sex-specific assembly of the DCC onto X chromosomes. Depletion of SUMO preferentially reduces the binding of these specific DCC subunits on X chromosomes and causes changes in X-linked gene expression similar to those caused by genetic disruption of the dosage-compensation machinery. The initial step of DCC assembly, the binding of XX-specific X-targeting factors to *rex* sites, occurs independently of SUMOylation and triggers SUMO modifications that promote robust DCC assembly and binding, likely by enhancing interactions between X-targeting factors and condensin subunits. Our results provide a strong indication that posttranslational modification of the DCC is critical for its function and highlight an emerging principle that the SUMO conjugation pathway targets multiple proteins of a complex in response to a specific signal to stimulate robust complex assembly, stability, and function.

SUMOylation Promotes DCC Assembly but Confers Neither Sex Specificity nor X Specificity. Depletion of SUMO, like depletion of SDC-2, the X-targeting factor that confers X specificity and sex specificity, reduces binding of DCC condensin subunits on X to the low level of binding on autosomes in wild-type animals. Although DCC binding is reduced on X in SUMO-depleted embryos, it is increased on autosomes, unlike in *sdc-2* mutants, particularly at promoters of genes exhibiting elevated expression. Both condensin subunits and X-targeting proteins bind to the new autosomal sites, and these sites have the low level of DCC occupancy characteristic of autosomal sites in wild-type animals. The level of DCC binding is positively correlated with the expression level of the gene. These findings could be interpreted to mean that SUMOylation confers sex specificity and X-chromosome specificity to DCC binding. However, neither is true. In SUMO-depleted embryos, X-chromosome binding of the X-targeting factors SDC-2 and DPY-30 is nearly wild-type, indicating that X specificity and sex specificity is properly programmed. Furthermore, liberation of DCC subunits from X is not the cause of new autosomal binding because key X-targeting factors remain bound to X, while increasing their binding to autosomes.

Although the sex-specific signal for DCC assembly on X is nearly normal in SUMO-depleted embryos, DCC assembly and stability are adversely affected in a manner congruent with the genetic requirements for assembly of DCC subunits onto X chromosomes. In wild-type embryos, SDC-2 binds to X without other known DCC subunits; DPY-30 requires SDC-2; SDC-3 requires both SDC-2 and DPY-30; and condensin subunits require SDC-2, DPY-30, and SDC-3 (5, 14). In SUMO-depleted embryos, binding of SDC-2 and DPY-30 is relatively normal, binding of the X-targeting factor SDC-3, a subunit SUMOylated in wild-type embryos, is modestly reduced, and binding of condensin subunits, also SUMOylated in wild-type embryos, is severely reduced, particularly at *rex* sites. Binding of condensin subunits to X in SUMO-depleted embryos could be more severely impacted for two reasons: (*i*) SDC-3 binding to X is reduced; and (*ii*) interactions between condensin subunits and the residual SDC-3 bound to X are likely to be reduced.

Synergistic Action of SUMO Modification Facilitates DCC Assembly. SUMO modifications have been shown to act synergistically to promote stable protein interactions and protein complex assembly. A notable example is the SUMOylation of multiple DNA repair proteins in response to a DNA double strand break (28, 29). The SUMOylation facilitates the formation of a stable DNA repair complex. The importance of synergy was demonstrated by the fact that disrupting a single SUMO acceptor site on an individual protein had little or no effect on DNA repair, but blocking the SUMOylation of several repair proteins by disrupting the SUMO conjugation pathway had a strong effect on DNA repair. Functional synergy was attributed to the added effect of individual SUMO–SIM interations contributing to the stable formation of the repair complex.

The role of SUMOylation in the formation of the DCC appears to follow the paradigm of the DNA repair complex. DCC subunits have three important features that would permit SUMO modifications to act synergistically and thereby mediate robust protein interactions. The subunits have an affinity for each other without posttranslational modifications, multiple subunits of the complex are SUMOylated, and all but two subunits of the complex (SDC-1 and DPY-26) have multiple short hydrophobic SUMO-interacting motifs called SIMs that commonly mediate SUMO-dependent interactions. Similar to the repair complex, a specific signal initiates DCC SUMOylation. The production and X-chromosome binding of SDC-2 exclusively in young XX embryos triggers SUMOylation of multiple DCC subunits, likely potentiating physical interactions and DCC assembly. Although low-level X-chromosome binding of DCC condensin subunits can occur without SUMOylation, assembly of all DCC subunits onto X and robust association of the DCC with X requires SUMOylation, which only occurs once SDC-2 is expressed in the XX embryo. An important future test of this model would involve identifying the sites of SUMOylation, knocking them out, and analyzing binding of DCC subunits to X.

Specificity of SUMOylation and Effect on DCC Binding at *rex*, *dox*, and Autosomal Sites. Sequential genome-wide ChIP experiments that enriched first for chromatin bound to SUMOylated proteins and then for chromatin bound to SUMOylated DPY-27 protein showed that SUMOylated DPY-27 is preferentially enriched at *rex* sites compared with *dox* and autosomal sites. Consistent with this finding, SUMOylation appears to be of greater importance for DCC assembly and high-affinity binding at *rex* sites than the lower-affinity binding at *dox* sites because DCC binding is more severely reduced at *rex* sites than *dox* sites in SUMO-depleted embryos.

Several questions remain about the status and function of DCC SUMOylation at *rex*, *dox*, and autosomal sites. Not known is whether the preferential enrichment of SUMOylated DCC proteins at *rex* sites confers the higher DCC occupancy at *rex* sites than *dox* sites. Enhanced stability of SUMOylated protein complexes would contribute to better binding. Also unknown is whether *rex* sites, but not *dox* or autosomal sites, preferentially trigger SUMOylation because X-targeting factors critical for SUMOylation bind first to *rex* sites and initiate DCC assembly.

Psakhye and Jentsch (29) showed that specificity of the SUMOylation machinery for a protein complex is conferred by two factors: topological specificity and a highly specific trigger. Both of these conditions are met by *rex* sites but not *dox* or autosomal sites. *rex* sites have distinct DNA motifs that recruit the DCC in an autonomous manner, and the X-targeting factors that trigger SUMOylation have a strong preference for binding to *rex* sites. DCC binding at autosomal sites can occur without SDC-2 and is independent of DCC binding at *rex* sites (5). If SUMOylation is stimulated solely by DCC binding at *rex* sites, a trigger may not exist for DCC SUMOylation at autosomal or *dox* sites. Moreover, if DCC SUMOylation occurs only at *rex* sites, the reduced DCC binding at *dox* sites in SUMO-depleted embryos could be the consequence of reduced binding at *rex* sites.

Duration of DCC SUMOylation. Not all of the SDC-3, DPY-27, and DPY-28 proteins in the cell are SUMOylated. Two explanations are likely. First, SUMOylation is a dynamic process in which conjugation enzymes add SUMO, and SUMO-specific proteases remove SUMO. SUMOylation of DCC components might be of limited duration because SUMOylaton might be required for DCC establishment but not DCC maintenance and thus no mechanism exists to maintain SUMOylation. Second, DCC SUMOylation likely occurs only in the context of DNA binding in general, or *rex*-site binding in particular, and not all DCC proteins are DNA bound.

Specificity of DPY-28 SUMOylation. Our immunoprecipitation experiments showed that DPY-28 is SUMOylated in the context of the DCC but not condensin I. Two interpretations are plausible. SUMOyation of DPY-28 might create one or more sites for protein interactions with other DCC subunits but not condensin I subunits, thereby enhancing DPY-28's participation in dosage compensation but not chromosome segregation. Relevant to this possibility, SUMOylation of *Saccharomyces cerevisiae*'s condensin subunit Ycs4, the homolog of DPY-28, helps alter the subcellular location of the entire condensin complex and thereby change its functional activity. SUMOylation is essential for the anaphase-specific nucleolar localization of Ycs4 that is required for rDNA segregation. Robust ectopic induction of Ycs4 SUMOylation before anaphase is sufficient to cause premature Ycs4 nucleolar accumulation and concomitant segregation of rDNA markers (31). Thus, in yeast, SUMOylation of Ycs4 preferentially fosters one of its cellular activities. In contrast, the more likely reason for the preferential SUMOylaton of DPY-28 in the context of the DCC is that SUMOylation occurs in response to DPY-28's recruitment to X chromosomes as part of the DCC. Consistent with this interpretation, mutations that prevent DCC localization severely reduce DPY-28 SUMOylation.

Roles for SUMOylation in *C. elegans*. Loss of SUMO or the SUMO conjugating enzyme UBC-9 causes embryonic lethality in both sexes, showing that SUMOylation is essential for cellular functions beyond dosage compensation (21). Only a limited number of bona fide SUMO targets have been identified in *C. elegans*. SUMOylation of Polycomb group protein SOP-2, the first SUMO target identified, is required for its localization to nuclear bodies and its role in repression of Hox gene expression (32). A large-scale proteomic screen identified potential SUMO targets (30), and the study showed that SUMOylation of the cytoplasmic intermediate filament (cIF) protein named IFB-1 regulates cIF assembly through maintaining a cytoplasmic pool of nonpolymerized IFB-1.

Failure to do so causes defects in embryonic elongation and maintenance of muscle attachment to the cuticle. SUMOylation is also important for pharyngeal muscle development in the embryo (33) and development of the reproductive system, including morphogenesis of the vulva (34–36). DCC SUMOylation is unique among these examples in representing SUMO modification of a protein complex.

Conclusion. Our work demonstrates the fundamental role of SUMOylation in the formation and function of an essential chromosome-wide repression complex. SUMO modification of multiple subunits in response to a DNA-bound trigger appears to facilitate protein interactions essential for complex stability and sex-specific transcriptional repression. These discoveries transform the emerging theme (29) of protein SUMOylation to enhance complex formation into a more general paradigm.

Materials and Methods

Nematode Strains. The following nematode strains were used in this study: wild-type XX (N2) strain TY125; *sdc-2* partial loss-of-function strain *sdc-2(y93, RNAi)* XX; *sdc-3* partial loss-of-function strain *sdc-3(y129, RNAi)* XX; *dpy-30* partial loss-of-function strain TY1119, *dpy-30(y130ts)* XX; FLAG-tagged SUMO strain NX25, *smo-1(ok359)*; *Ex[FLAG-3xHIS-smo-1]* XX; FLAG-tagged SDC-2 strain TY4573, *sdc-2(y74)*; *yEx992[flag-sdc-2 + myo2::gfp]* XX.

ChIP Extracts and Reactions. Embryo extracts were prepared and ChIP experiments were performed as described previously (17) and noted in *SI Materials and Methods*, with the following changes. FLAG-tagged SDC-2 (TY4573) ChIP extracts were precleared with protein G Sepharose. Embryo ChIP experiments used 5 μg of antibody for anti-FLAG, DPY-26, and IGG ChIPs, and 10 μg of antibody for all other ChIPs. For sequential ChIP experiments (ChIP re-ChIP), twice the amount of embryo extract (6 mg) and antibody (10 μg) was used. The first round ChIP was eluted twice in 25 μL of 10 mM DTT for 15 min at 37 °C, for a combined volume of 50 μL. This eluate was diluted to 1 mL in ChIP Wash Buffer without DTT (100 mM NaCl, 1 mM PMSF, 10 mM N-Ethylmaleimide), and the second round ChIP used the standard ChIP protocol.

ChIP-Chip Platform and Data Analysis. The platform, hybridization conditions, and data analysis for ChIP-chip experiments were described previously (17). The reference genome was the WormBase WS180 release. Experiments were performed at least in duplicate.

Gene Expression Arrays and Data Analysis. Embryo microarray expression data from *sdc-2* mutants were published previously (17). For *smo-1(RNAi)* embryos, three samples were grown as for *smo-1(RNAi)* ChIP-chip, collected by bleaching, and frozen in 100-μL aliquots. RNA was prepared and hybridized to Affymetrix *C. elegans* Genome Microarrays as described previously (17). Array normalization, analysis, and determination of expressed genes by MAS5 analysis were performed as published (17).

SUMO Protease Experiments. For SUMO protease experiments, DCC components were immunoprecipitated as above. Before elution, IPs were washed three times with 1× SUMO protease buffer and resuspended in 40 μL of 1× SUMO protease buffer with or without 2 μL of SUMO protease (ULP1; Invitrogen; 12588–018). Reactions were performed at 20 °C for 1 h while nutating. IPs were eluted by boiling in SDS PAGE buffer.

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Supporting Information

Pferdehirt and Meyer 10.1073/pnas.1315793110

SI Materials and Methods

Nematode Culture. All worms for embryo ChIP-chip analysis and microarray expression studies were first grown to high density on nematode growth (NG) agar plates with concentrated HB101 bacteria at 20 °C. For RNAi control embryos, synchronous TY125 L1 larvae were spotted onto RNAi plates (NG agar with 100 μg/mL carbenicillin and 1 mM Isopropyl β-D-1-thiogalactopyranoside) with concentrated HT115 bacteria carrying an empty RNAi vector (L4440). Worms were grown for 96 h at 20 °C before collecting embryos by bleaching. For *smo-1(RNAi),* synchronous L1 larvae were first grown on HB101 bacteria for 24 h to reach the L2/L3 stage and then transferred to RNAi plates spotted with concentrated HT115 bacteria carrying an Ahringer feeding library bacteria plasmid expressing double-stranded RNA to *smo-1* (1). Worms were grown for 72 h at 20 °C on *smo-1(RNAi)* before harvesting embryos by bleaching.

Antibodies. Rabbit polyclonal antibodies against DUMPY (DPY)-27 (rb699), Sex determination and Dosage Compensation (SDC)-3 (rb1079), SDC-2 (rb3778), DPY-26 (rb1450), DPY-28 (rb1379), and Structural Maintenance of Chromosomes (SMC)-4 (rb2655) were used as described previously (2–6). Rabbit polyclonal antibodies against DPY-30 were from SDIX (4511.00.02). α-tubulin and α-FLAG antibodies were from Sigma (clone DM1a, cat. no. T6199 and clone M2, cat. no. F1804, respectively).

Immunofluorescence Microscopy. Embryos were fixed as described in Davis and Meyer (4) and stained as described in Chuang et al. (2), except that primary antibody staining was performed overnight and secondary antibody staining was performed for at least 3 h. All primary antibodies were used at a dilution of 1:200 in 1× PBST, and secondary antibodies (Alexa Fluor; Invitrogen) were diluted 1:500 in 1× PBST. Fixed and stained embryos were mounted in SlowFade Gold (Invitrogen) containing 0.5 μg/mL DNA intercalating dye 4′,6 diamidino-2-phenylindloe (DAPI). All images were captured on a Leica SP2 microscope.

ChIP Extract Preparation. In brief, embryos recovered by bleaching the adults were cross-linked in 2% (vol/vol) formaldehyde in M9 for 30 min at 20 °C and quenched with 100 mM Tris·HCl (pH 7.5). Samples were then washed twice with M9, once with Homogenization Buffer [50 mM Hepes-KOH (pH 7.6), 0.5% Nonidet P-40, 140 mM KCl, 1 mM EDTA, 5 mM DTT, 1 mM PMSF] and frozen in liquid nitrogen. To make extracts for sequential ChIP, 10 mM *N*-Ethylmaleimide (NEM) was added to the Homogenization Buffer. Samples were sonicated so that the DNA ranged from 200 bp to 1 kb using a Heat System XL2020 Sonicator with a Misonix 419 tip 10× for 30 s each at 10%, then centrifuged at 5,000 × *g* for 20 min at 4 °C. The supernatant was again sonicated for 10× for 30 s at 10% and spun at 20,000 × *g* for 20 min at 4 °C. The resulting supernatant was precleared with protein A Sepharose for 30 min and frozen in liquid nitrogen.

ChIP-Chip Platform and Data Analysis. ChIP-chip experiments were hybridized to 2.1 million feature high-density (HD2), isothermal (tm = 76 °C) tiling arrays from Nimblegen, covering the X chromosome and autosomes of WormBase release WS180 (Design ID 6737). Median probe spacing was 40 bp, and probe length varied in length from 50 to 75 bp, with repeat masking. ChIP hybridizations were performed at the Fred Hutchinson Cancer Research Center Genomics Facility. Replicates of the IGG and DPY-27 ChIPs were also hybridized by Nimblegen. Data were analyzed and peaks were called using NimbleScan software and viewed using SignalMap software.

Immunoprecipitations and Western Blots. Embryo extracts for immunoprecipitation (IP) reactions were made as for ChIP, but without a cross-linking step. IP experiments were performed by incubating 5 μg of the indicated antibody with ∼3 mg of embryo extract for 2 h at 4 °C. Antibodies were collected for 30 min with Protein G Dynabeads (Invitrogen) for mouse antibodies and Protein A Dynabeads (Invitrogen) for rabbit antibodies. Samples were washed four times with 50 mM Hepes-KOH (pH 7.6), 150 mM KCl, 0.05% Nonidet P-40, 1 mM EDTA, 1 mM DTT, and 1 mM PMSF and eluted by boiling in SDS/PAGE loading buffer.

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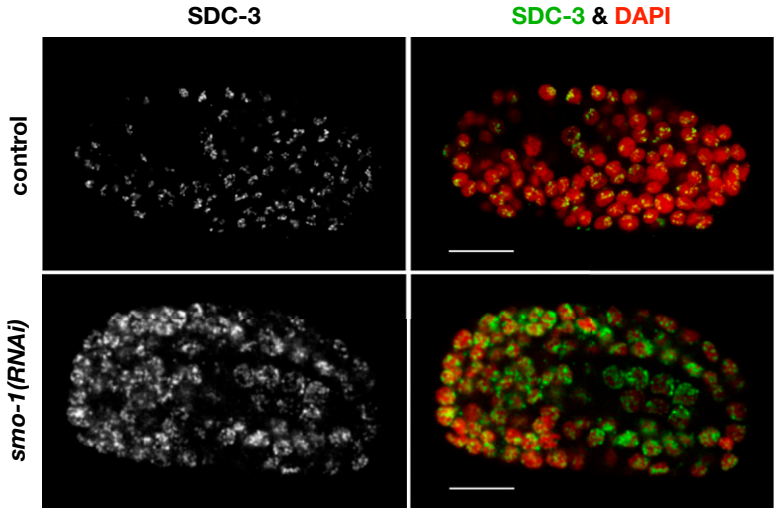

Fig. S1. SUMOylation is required for proper SDC-3 localization to X. Confocal images of XX embryos treated with either control (empty vector) RNAi or RNAi directed against *smo-1*, costained with DAPI (4,6-diamidino-2-phenylindole) (red in merge) and antibodies against SDC-3 (green in merge). In control XX embryos, SDC-3 exhibits a punctate pattern of nuclear localization, indicating proper enrichment on the X chromosome. In embryos depleted for the SUMO (small ubiquitin-like modifier) peptide, SDC-3 is more diffusely localized throughout the nucleus, indicating that it is not properly bound to X. (Scale bar, 5 μm.)

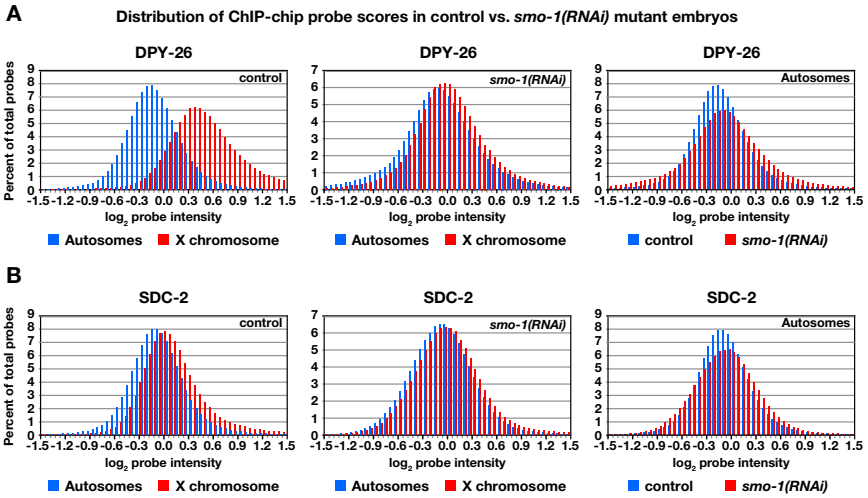

Fig. S2. X-chromosome binding of DPY-26 is more severely reduced than that of SDC-2 in *smo-1* mutant embryos, resulting in equivalent DPY-26 binding on X and autosomes. (*A* and *B*) Histograms depicting the distribution of DPY-26 or SDC-2 ChIP-chip probe scores across all autosomes or X chromosomes in control or SUMO-depleted XX embryos. Probe scores were grouped into bins as in Fig. 2*C*. In *smo-1(RNAi)* embryos, X-chromosome binding of DPY-26 was reduced to the low level of binding on autosomes. Consistent with new sites of autosomal DCC binding, the average DPY-26 ChIP-chip probe score on autosomes increased in *smo-1(RNAi)* embryos compared with control embryos. In contrast, only a slight effect on SDC-2 binding was seen on X or autosomes of *smo-1(RNAi)* versus control embryos.

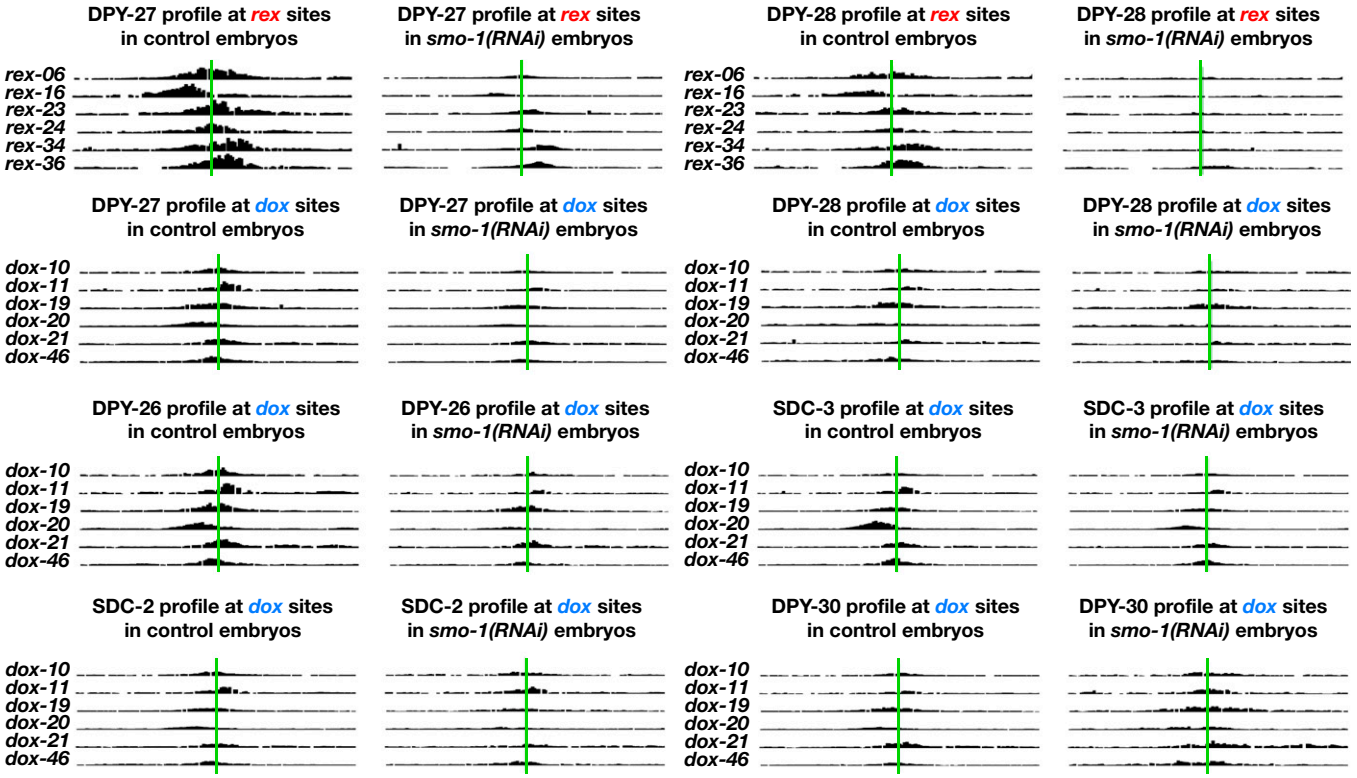

Fig. S3. DCC binding at both *rex* (recruitment sites on X) and *dox* (dependent on X) sites is depleted by *smo-1(RNAi)*. Graphical representations of DCC ChIP-chip probe intensities along 5-kb regions centered (green line) on representative *rex* and *dox* sites in control (empty RNAi vector) versus *smo-1(RNAi)* embryos. Binding of condensin DCC subunits (DPY-27, DPY-28, and DPY-26) was severely reduced at *rex* sites, but only moderately reduced at *dox* sites. Binding of the X-targeting protein SDC-3 was moderately reduced at *dox* sites, but binding of X-targeting proteins SDC-2 and DPY-30 was not reduced at *dox* sites.

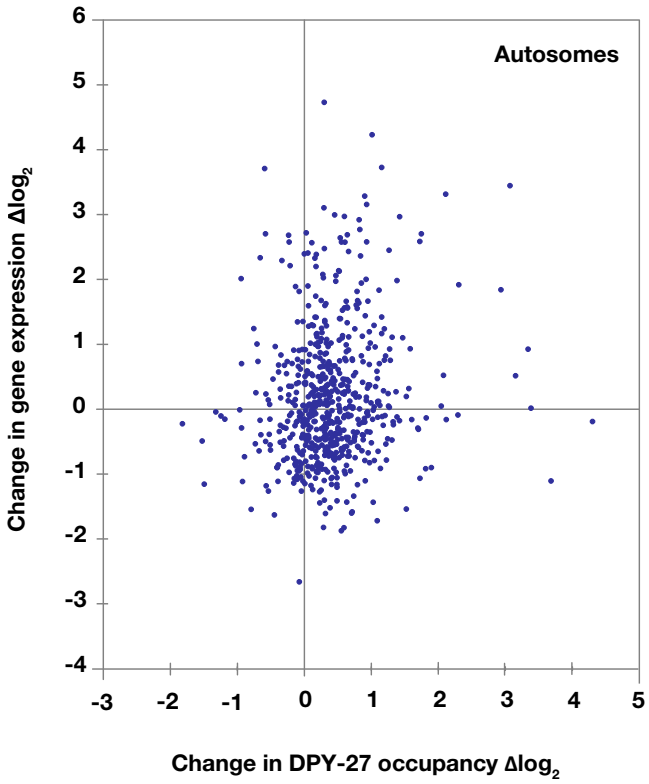

Fig. S4. New autosomal DCC binding sites in SUMO-depleted embryos are positively correlated with genes that increase in expression. Scatter plot depicting the positive correlation between changes in DCC binding at autosomal promoters in *smo-1(RNAi)* and changes in expression of the corresponding gene. For each gene with unique DCC promoter binding in *smo-1(RNAi)* (present in both SDC-3 and DPY-27 ChIP), the third highest ChIP-chip probe score within the promoter was calculated for both control and *smo-1(RNAi)*. The *smo-1(RNAi)* ChIP-chip and gene expression values were subtracted from those of control RNAi and plotted. The positive quadrants of the axes correspond to higher binding or expression in *smo-1(RNAi)* compared with control. A statistically significant positive correlation exists between unique DCC peaks in promoters of *smo-1(RNAi)* embryos and increased expression of the corresponding genes (Pearson's correlation coefficient $R = 0.136$, $P < 0.0006$).

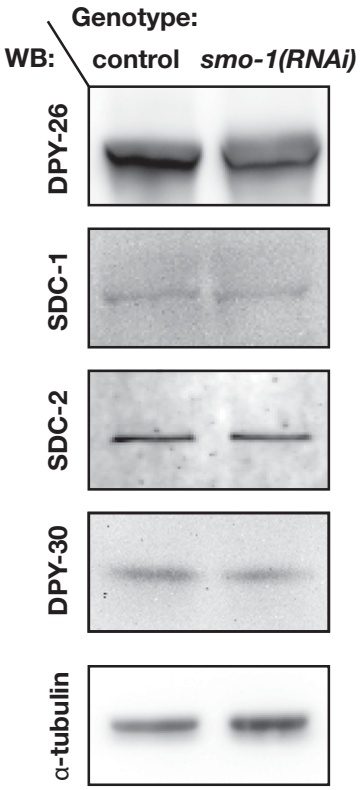

Fig. S5. Several DCC subunits are not SUMOylated. Shown are Western blots for four of the seven DCC subunits not showing signs of SUMOylation. No higher molecular variants were present in wild-type versus *smo-1(RNAi)* embryos. Western blots of three other subunits not SUMOylated (CAPG-1, DPY-21, and MIX-1) are shown in Fig. 5*A*. α-tubulin was the protein loading control. Quantification is in Fig. S6.

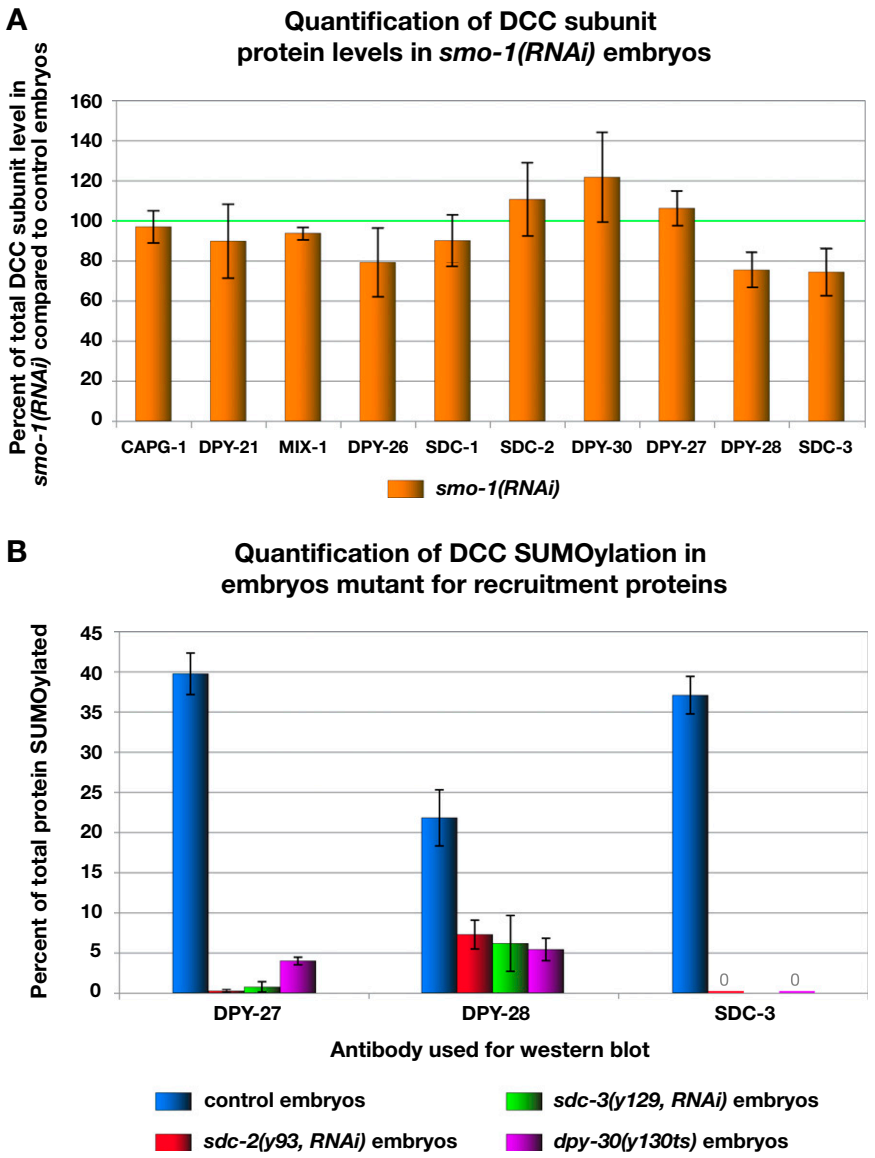

Fig. S6. Effect of *smo-1(RNAi)* on DCC subunit levels and effect of X-targeting factors on SUMOylation of DCC subunits. (*A*) Histogram shows the levels of DCC proteins in *smo-1(RNAi)* embryos as the percent (± SEM) of DCC protein levels in control embryos. Western blots (Fig. 5 *A* and *B* and Fig. S5) were performed and quantified in triplicate. (*B*) Disruption of X-targeting factors greatly diminishes the SUMOylation of DCC subunits. Histogram shows the percent (± SEM) of total DPY-27, DPY-28, and SDC-3 protein that is SUMOylated in X-targeting mutants relative to control embryos. Western blots (Fig. 5) were performed and quantified in triplicate.

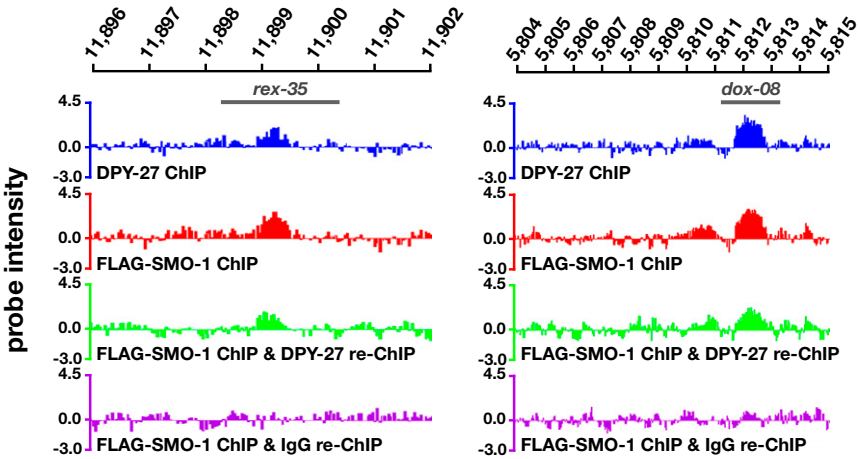

Fig. S7. SUMOylated DPY-27 is enriched at *rex* sites. Representative profiles showing *rex* and *dox* sites from ChIP-chip experiments of the following experimental regimes using extracts from the *smo-1* deletion strain expressing FLAG-tagged SMO-1: ChIP using DPY-27 antibodies as a control to assess the efficiency of DPY-27 binding and detection; ChIP using FLAG antibodies to determine the genome-wide binding sites for SUMOylated proteins; ChIP using FLAG antibodies followed by re-ChIP of eluted protein–chromatin complexes with DPY-27 antibodies to determine genome-wide binding sites for SUMOylated DPY-27; and ChIP using FLAG antibodies followed by re-ChIP of eluted protein–chromatin with IGG antibodies to determine background binding. SUMOylated DPY-27 is strongly enriched at most *rex* sites but mildly enriched at only some *dox* sites.