# Dosage Compensation Proteins Targeted to X Chromosomes by a Determinant of Hermaphrodite Fate

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**In many organisms, master control genes coordinately regulate sex-specific aspects of development. SDC-2 was shown to induce hermaphrodite sexual differentiation and activate X chromosome dosage compensation in *Caenorhabditis elegans*. To control these distinct processes, SDC-2 acts as a strong gene-specific repressor and a weaker chromosome-wide repressor. To initiate hermaphrodite development, SDC-2 associates with the promoter of the male sex-determining gene *her-1* to repress its transcription. To activate dosage compensation, SDC-2 triggers assembly of a specialized protein complex exclusively on hermaphrodite X chromosomes to reduce gene expression by half. SDC-2 can localize to X chromosomes without other components of the dosage compensation complex, suggesting that SDC-2 targets dosage compensation machinery to X chromosomes.**

In diverse organisms, the choice of sexual fate specifies not only the overt sexual characteristics evident in adults but also the amount of X chromosome gene expression in somatic cells throughout development. X chromosome–wide expression is controlled by the essential process of dosage compensation, which equalizes X chromosome expression between females (XX) and males (XY or XO). In mammals, flies, and nematodes, specialized dosage compensation complexes are targeted exclusively to the X chromosomes of one sex to modulate transcript levels (*1, 2*). Here we used *Caenorhabditis elegans* to understand how the dosage compensation machinery is targeted to X chromosomes of hermaphrodites (XX) and how dosage compensation is coordinately activated with the genetic pathway for sexual development.

Although dosage compensation is a sex-specific process, the nematode dosage compensation complex contains both dosage compensation-specific proteins such as DPY-27 (dumpy) (*3–5*) and chromatin-associated proteins that are also active in meiosis or mitosis such as DPY-26 (*6, 7*) and MIX-1 (mitosis and X) (*8*). These three proteins are similar to components of the frog 13*S* condensin complex that drives mitotic chromosome condensation in vitro (*4, 8, 9*), implying evolutionary recruitment of ancient mitotic proteins to the regulation of gene expression. All dosage compensation components, including the mitotic and meiotic proteins functional in both sexes, must be directed exclusively to X chromosomes of XX animals by a hermaphrodite-specific factor.

The sex-specific gene that induces hermaphrodite development, including dosage compensation, had not been determined. In males, the *xol-1* (XO lethal) gene initiates sexual development and represses dosage compensation by inactivating three hermaphrodite-specific *sdc* (sex and dosage compensation) genes (*10, 11*). In hermaphrodites, these genes activate dosage compensation and the genetic pathway for hermaphrodite sexual development (*12–15*). Both SDC-2 and SDC-3 are necessary for localization of dosage compensation proteins to X chromosomes (*5, 7, 8, 16*). XX animals that lack *sdc-2* activity develop as males and die from elevated X chromosome expression caused by the failure to dosage compensate (*14*). In contrast, *sdc-1* and *sdc-3* XX mutants exhibit less severe phenotypes (*13, 15*). Here we show that *sdc-2* encodes the pivotal sex-specific factor that triggers the hermaphrodite program of development.

## Hermaphrodite-Specific Expression of SDC-2

To elucidate the roles of *sdc-2* in dosage compensation and sex determination, we cloned *sdc-2* (*17*). We used germ line transformation assays to test cosmids (Fig. 1A) and subclones (Fig. 1B) from the *sdc-2* region for the ability to rescue *sdc-2* XX mutants. The smallest genomic region to confer rescue was a 12.6-kb DNA fragment (Fig. 1B) that corresponds to a single 9.5-kb transcript (Fig. 1D). Detection of a 4.8-kb deletion associated with the *sdc-2* allele *y74* confirmed the identity of the transcript as *sdc-2* (*18*). The deletion removes the first 606 codons of the transcript and 2.1 kb of upstream regulatory regions, consistent with *y74* eliminating gene function. *sdc-2* has 19 exons and is predicted to encode a highly

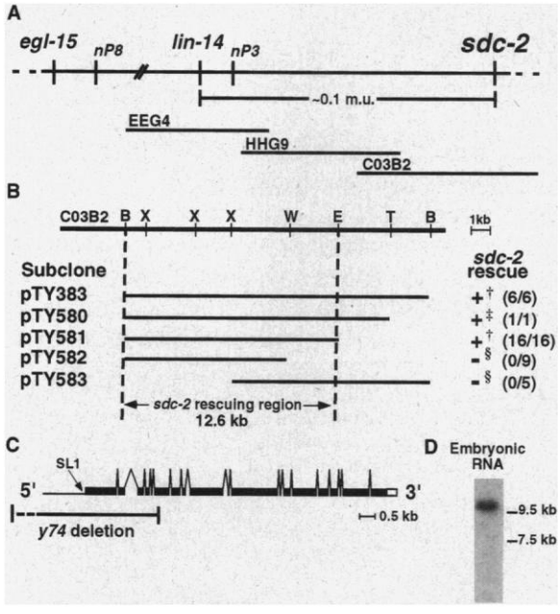


**Fig. 1.** Molecular analysis of *sdc-2*. (**A**) Corresponding genetic and physical maps of the X chromosome near *sdc-2*. Nearby genes (*egl-15* and *lin-14*), DNA polymorphisms (*nP8* and *nP3*), and overlapping cosmids (EEG4, HHG9, and C03B2) are shown. *sdc-2* resides on C03B2; m.u. indicates map unit. (**B**) DNA transformation rescue experiments to define *sdc-2*. A partial restriction map of C03B2 is shown. C03B2 subclones were tested for their ability to rescue *sdc-2(y46)* partial loss-of-function mutants (‡), *sdc-2(y74)* null mutants (§), or both (†). +, rescue; −, failure to rescue. The number of rescuing lines relative to total lines is indicated in parentheses. The minimal rescuing region (12.6 kb) is bounded by dashed vertical lines. B, Bgl II; X, Xba I; W, BsiW I; E, BstE II; T, Tth111 I. (**C**) *sdc-2* gene structure. Horizontal line, promoter; black boxes, exons; spaces, introns; open box, 3′ untranslated region. Approximate location of the *y74* deletion is shown. (**D**) Northern blot of poly(A)$^+$ RNA from wild-type XX embryos probed with *sdc-2* sequences. *sdc-2* encodes a highly charged 344-kD protein with a coiled-coil motif from amino acids 1226 to 1256.

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charged protein of 2962 amino acids with a coiled-coil motif (Fig. 1C).

Immunofluorescence experiments with SDC-2-specific antibodies (Fig. 2) (*19*) showed the SDC-2 expression pattern to be distinct from that of all other dosage compensation proteins. Dosage compensation proteins such as DPY-27 are diffusely distributed throughout nuclei of very young embryos (<30 cells) (Fig. 2A) in both sexes and only later become specifically localized to X chromosomes of XX (Fig. 2, D and F) but not XO animals (*4, 7, 8*). SDC-2 differs in three important ways. First, SDC-2 is not expressed in very young embryos (Fig. 2B). Its initial expression occurs around the 40-cell stage, a time that corresponds to the assembly of the dosage compensation machinery on X chromosomes (Fig. 2D) (*4*). Second, SDC-2 localizes to hermaphrodite X chromosomes from the onset of its expression (Fig. 2, E and F). Third, SDC-2 is not expressed in wild-type XO embryos (Fig. 3, A to C), which indicates that SDC-2 is sex-specifically regulated. *sdc-2* is repressed in males by the XO-specific gene *xol-1*: in *xol-1* XO mutants, SDC-2 is expressed and appears to be localized to X (Fig. 3, D to F). The observations that SDC-2 is expressed exclusively in XX embryos, that its initial expression coincides with its own X localization, and that *sdc-2* is required for X localization of other dosage compensation proteins (Fig. 2, G to I) (*5, 7, 8*) suggest that SDC-2 is the hermaphrodite-specific protein that triggers assembly of the dosage compensation machinery on X chromosomes.

## Dosage Compensation Activated by SDC-2

If *sdc-2* is the sex-specific switch that is both necessary and sufficient to activate dosage compensation, ectopic expression of SDC-2 in males should initiate dosage compensation, causing XO-specific lethality from underexpression of X chromosome–linked genes. SDC-2 was expressed in males from a chromosomally integrated transgene (*yIs30*) in which *sdc-2* transcription was controlled by the constitutively active *dpy-30* promoter (*20, 21*). Extensive XO-specific lethality resulted from the ectopic expression of *sdc-2*: 81% of *yIs30*/+ XO progeny were dead (Table 1). The male survivors were small, slow growing, and mating defective, further indications of inappropriate X chromosome expression. All mutant phenotypes, including lethality, were suppressed by mutations in hermaphrodite dosage compensation genes, either *sdc-3* or *dpy-27*, which indicates that ectopic expression of *sdc-2* activated the XX mode of dosage compensation in XO animals (Table 2).

The incomplete male lethality caused by SDC-2 suggested that another dosage compensation protein was limiting in males. SDC-3 was a likely candidate, because it associates with hermaphrodite X chromosomes and is required for X localization of other dosage compensation proteins. Moreover, SDC-3 is only weakly expressed in XO embryos (*16*). Whereas overexpression of SDC-3 [from *yIs3* (*16*)] caused only 2% male lethality, and expression of SDC-2 caused 83% lethality, overexpression of both *sdc-2* and *sdc-3* caused 99% male lethality (Table 1), which suggests that in XX animals SDC-3 assists SDC-2 in activating dosage compensation.

Direct demonstration that SDC-2 is sufficient to trigger assembly of the dosage compensation complex on X chromosomes was achieved by comparing the staining pattern of SDC-3 in both wild-type and SDC-2-expressing XO embryos. SDC-3 by itself does not associate with the male X chromosome: it is diffusely distributed in the nuclei of wild-type XO embryos (<100 cells) and in XO embryos engineered to overexpress SDC-3 (*16*). In contrast,

**Table 1.** Ectopic expression of *sdc-2* kills XO animals.

| sdc-2 *induces XO lethality** | | | | | |
|---|---|---|---|---|---|
| Maternal genotype† | Paternal genotype | XO lethality‡ | Sexual phenotype of XO survivors | No. of XO adults | No. of XX adults |
| *yIs30*[*dpy-30::sdc-2*] | Wild type | 81% | ♂ | 205 | 1077 |
| **sdc-3 *helps* sdc-2 *kill males*§** | | | | | |
| Arrays present¶ | Male lethality‖ | Hermaphrodite lethality‖ | No. of adult males | No. of adult hermaphrodites | No. of embryos (broods) |
| None | — | — | 701 (34%) | 1144 (56%) | 2051 (7) |
| *yIs30*[*dpy-30::sdc-2*] | 83% | 6% | 74 | 678 | 1287 (5) |
| *yIs3*[*sdc-3*(+)] | 2% | 1% | 679 | 1124 | 2031 (8) |
| *yIs3; yIs30*[*dpy-30::sdc-2*] | 99% | 0.5% | 6 | 1260 | 2261 (8) |

*Lethality of *yIs30*[*dpy-30::sdc-2*]/+ XO animals. †*rol-4*(*sc8*) *unc-76*(*e911*) *V*; *lon-2*(*e678*) *X*; *yIs30* hermaphrodites were crossed to wild-type males; XO (Lon non-Rol) and XX (non-Lon non-Rol) cross progeny were counted. The *unc-76* marker was used to indicate the presence of *yIs30*. ‡Calculated as 100 − [(no. of adult male progeny)/(no. of adult hermaphrodite progeny) × 100]. §Lethality of animals that overexpress *sdc-2*, *sdc-3*, or both from homozygous integrated arrays. ¶Strains contained homozygous integrated arrays and a *him-8* mutation, which increases X chromosome nondisjunction, raising the proportion of male progeny to 34%; 10% of *him-8* embryos are inviable. ‖Embryos and adults were counted from multiple self-progeny broods. Percent male lethality was calculated as 100 − {[(no. of adult male progeny)/(no. of embryos × 0.34)] × 100}. All XO animals that overexpress *sdc-3* are male. Percent hermaphrodite lethality was calculated as 100 − {[(no. of adult hermaphrodite progeny)/(no. of embryos × 0.56)] × 100}.

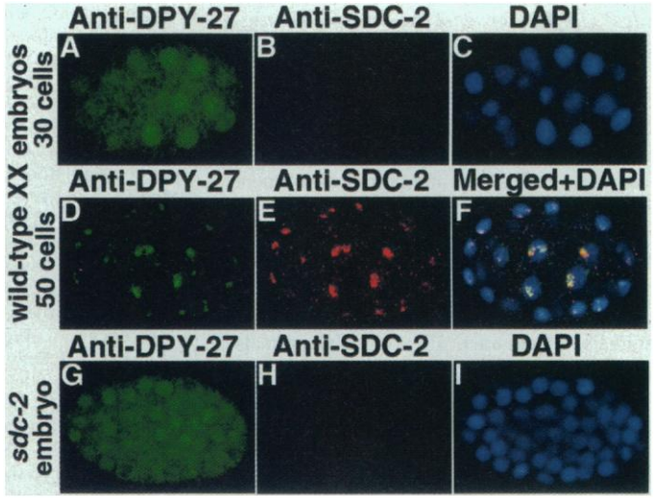


**Fig. 2.** SDC-2 localizes to X chromosomes of XX embryos. False color confocal immunofluorescence images of wild-type (**A** to **F**) and *sdc-2* mutant (**G** to **I**) XX embryos costained with anti-DPY-27 (green), anti-SDC-2 (red), and the DNA-intercalating dye 4′,6-diamidino-2-phenylindole (DAPI) (blue) are shown. Merged image in (F) superimposes images from the first two columns with DAPI. Yellow color indicates overlap between DPY-27 and SDC-2. (A to C) SDC-2 is not expressed in young XX embryos (<30 cells) (B), unlike the diffusely distributed DPY-27 protein (A). (D to F) SDC-2 is first expressed in 40- to 50-cell embryos, exhibiting a punctate pattern (E) coincident (F) with that of the X chromosome-localized DPY-27 (D). (G to I) Specificity of anti-SDC-2 is shown by the lack of SDC-2 staining in an *sdc-2* null embryo (>100 cells) (H). DPY-27 cannot localize to X in *sdc-2* mutants (G).

SDC-3 appears to be specifically localized to X chromosomes in SDC-2-expressing XO embryos (Fig. 3, G to I). Thus, SDC-2 is the hermaphrodite-specific factor that activates dosage compensation.

### Dosage Compensation Machinery Targeted to X Chromosomes by SDC-2

Does SDC-2 require other dosage compensation proteins for its association with X chromosomes? Evidence that SDC-2 can associate with X independently of other dosage compensation components would distinguish SDC-2 from all known dosage compensation proteins and implicate it in the recognition of X. The dosage compensation proteins DPY-26, DPY-27, MIX-1, and SDC-3 fail to associate with X chromosomes in certain dosage compensation mutants (*sdc-2*, *sdc-3*, and *dpy-30*) and are not stably expressed in other mutants (*dpy-26*, *dpy-27*, and *dpy-28*) (*5, 7, 8, 16*). In contrast, SDC-2 accumulates to significant quantities by midembryogenesis in *dpy-26*, *dpy-27*, *dpy-28*, *dpy-30*, and *sdc-3* mutants and exhibits a distinctly punctate nuclear pattern that is indistinguishable from the wild-type, X-localized pattern (Fig. 4) (*22*). Thus, despite a reduction in the amount of SDC-2 in dosage compensation mutants, SDC-2 appears to associate with X chromosomes without other components of the dosage compensation complex. This result implies that SDC-2 plays a central role in X chromosome recognition and confers chromosome specificity to dosage compensation.

### Hermaphrodite Sexual Development Induced by SDC-2

In addition to its pivotal role in dosage compensation, *sdc-2* plays a separate role in sex determination, promoting hermaphrodite sexual development in concert with *sdc-1* and *sdc-3* (*13–15*). Is *sdc-2* the sex-specific trigger for hermaphrodite development as it is for dosage compensation? If so, XO animals that express *sdc-2* should develop as hermaphrodites. Because such XO animals are dead, dosage compensation mutations were used to suppress the lethality and permit assessment of sexual fate. About 31% of *yIs30* XO animals rescued by a *dpy-27* null mutation were fertile hermaphrodites and 5% were intersexual (Table 2), which shows that *sdc-2* can trigger hermaphrodite development in XO animals. In contrast, all *yIs30* XO animals rescued by an *sdc-3* null mutation were male, consistent with the role of *sdc-3* in sex determination (Table 2). Because *sdc-2* feminized only 36% of XO animals, complete sexual transformation might require overexpression of *sdc-2* and another *sdc* gene, just as overexpression of *sdc-2* and *sdc-3* are needed to fully activate dosage compensation (Table 1).

Hermaphrodite sexual development requires transcriptional repression of the male autosomal gene *her-1* (hermaphrodite). Mutations in *sdc-1*, *sdc-2*, or the sex determination domain of *sdc-3* derepress *her-1* transcription in XX animals and cause masculinization, which suggests that these genes collaborate to turn off *her-1* (*15, 23*). To assess whether SDC-2 is a direct molecular repressor of *her-1*, we asked whether endogenous SDC-2 protein can associate with *her-1* regulatory sequences in vivo. We created transgenic strains in which extrachromosomal DNA arrays included multiple copies of either *her-1* regulatory regions (*24*) or control DNA. Arrays also included *lac* operator repeats (*lacO*) (*25*) and a transgene encoding a LacI::GFP

**Table 2.** Ectopic expression of *sdc-2* initiates hermaphrodite development in XO animals.

*Dosage compensation mutations rescue XO animals that express* sdc-2*

| Dosage compensation mutation (*m*) | *m/m* XO viability† | *m/+* XO viability† | *m/+* XX progeny | *m/m* XO progeny | *m/+* XO progeny |
|---|---|---|---|---|---|
| *sdc-3*‡ | 101% | 55% | 538 | 545 | 294 |
| *dpy-27*§ | 100% | 78% | 434 | 436 | 337 |

*Many* sdc-2*–expressing XO animals rescued by* dpy-27 *mutations develop as hermaphrodites*¶

| Maternal genotype‖ | Paternal genotype‖ | Extent of XO feminization** | No. of XO males | No. of XO intersexes†† | No. of XO hermaphrodites‡‡ |
|---|---|---|---|---|---|
| *dpy-27* | *dpy-27; yIs30 [dpy-30::sdc-2]* | 36% | 408 | 31 | 200 |

*Viabilities of *yIs30*/+ XO animals with an *sdc-3* or a *dpy-27* mutation. †Calculated as [(no. of XO cross progeny)/(no. of *m*/+ XX cross progeny)] × 100. ‡*rol-4 sdc-3(y126)/unc-76 V* males were crossed to maternally rescued *rol-4 sdc-3(y126) V; lon-2 X; yIs30* hermaphrodites. The number of *sdc-3/sdc-3* XO (Lon Rol) or *sdc-3*/+ XO (Lon non-Rol) progeny was compared with the number of *sdc-3*/+ XX (non-Lon non-Rol) progeny, all of which are viable. All *sdc-3/sdc-3* XX (Lon Rol) self progeny are inviable (*3*). All rescued XO animals were male. §*dpy-27(y167)/unc-32(e189) III* males were crossed to maternally rescued *dpy-27(y167) unc-32 III; lon-2 X; yIs30* hermaphrodites. The number of *dpy-27/dpy-27* XO (Lon non-Unc) or *dpy-27*/+ XO (Lon Unc) progeny was compared with the number of *dpy-27*/+ XX (Unc non-Lon) progeny, which are viable (*3*). ¶Shown are sexual phenotypes of *dpy-27; yIs30*/+ XO animals. ‖Maternally rescued *dpy-27 unc-32 III; lon-2 X* hermaphrodites were crossed to *dpy-27 III; him-8 IV; unc-76 V; yIs30* males. Lon non-Unc (XO) cross progeny were scored for sexual phenotype. **Calculated as [(no. of XO hermaphrodites + no. of XO intersexes)/(total no. of XO progeny)] × 100. †† Animals with both male and hermaphrodite characteristics. ‡‡Most XO hermaphrodite progeny were self-fertile and slightly egg-laying defective.

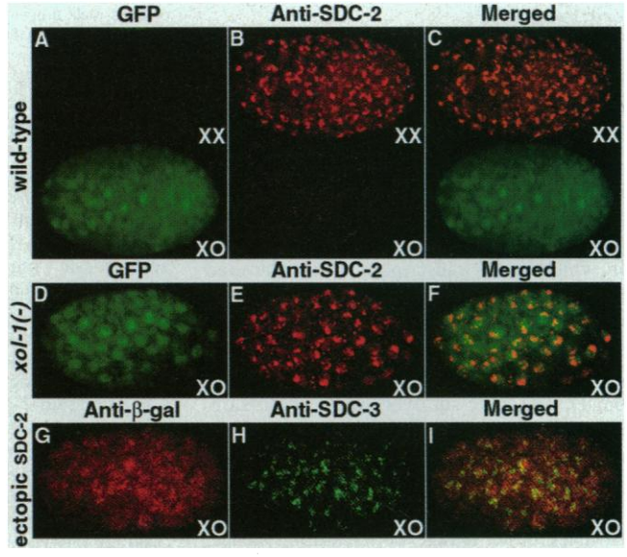


**Fig. 3.** SDC-2 protein is absent from wild-type XO embryos but assembles dosage compensation components on X in SDC-2–expressing XO embryos. (**A** to **F**) Confocal images of wild-type XO and XX (A to C) or *xol-1(y155)* mutant XO (D to F) embryos stained with anti-SDC-2 (red). All embryos carried the integrated *Pxol-1::gfp* reporter transgene (*yIs34*), in which the male-specific *xol-1* promoter drives expression of the *gfp* gene, which encodes the green fluorescence protein (GFP) (*29*). (A) Only XO animals produce nuclearly restricted GFP. (B and C) The wild-type XO embryo does not express SDC-2, but the XX embryo shows punctate staining that persists throughout development. (E and F) In the *xol-1(y155)* XO mutant, SDC-2 is expressed and localized to X. (**G** to **I**) Confocal images of an SDC-2–expressing XO embryo costained with anti–β-galactosidase (red) and anti-SDC-3 (green). The embryo carries an integrated X chromosome–linked array (*yIs29*) with *Pdpy-30::sdc-2* transgenes and an integrated array (*yIs2*) with the male-specific *Pxol-1::lacZ* reporter gene, which is similar to *yIs34* (*11*). SDC-3 is absent from wild-type XO embryos older than 100 cells (*16*), but in SDC-2–expressing XO embryos, SDC-3 accumulates and becomes localized to X.

fusion protein (*26*). LacI::GFP binds to *lacO* sequences, which allows arrays to be detected by GFP autofluorescence (*26, 27*). Of 400 embryos from two strains with independent *her-1* arrays, >90% showed colocalization of SDC-2 and GFP (Fig. 5, A to F). In contrast, of 200 embryos with control arrays, none showed any colocalization (Fig. 5, G to L). SDC-2 also localized to X chromosomes in all experimental and control embryos (Fig. 5, A to L). Thus, SDC-2 associates with *her-1* promoters in vivo.

If *her-1* is a functional target of SDC-2 in vivo, many copies of *her-1* regulatory regions on arrays might titrate some SDC-2 from X, thereby impairing dosage compensation. Indeed, two different genetic assays, both sensitive indicators of X chromosome expression, showed that dosage compensation was compromised in animals with *her-1* arrays. First, arrays containing *her-1* promoter sequences enhanced the mutant phenotypes of XX animals with reduced *sdc-3* activity. Without *her-1* arrays, *sdc-3(y126)*/+ XX animals are wild type, and *sdc-3/sdc-3* XX animals (from *sdc-3*/+ mothers) are fully viable but exhibit weak dosage compensation phenotypes (*3, 15*). However, with *her-1*-containing arrays, 29% of *sdc-3*/+ XX animals showed dosage compensation-specific defects and 40% of *sdc-3/sdc-3* XX animals were dead. Second, *xol-1* XO animals, normally dead from inappropriately activated dosage compensation (*10*), were rescued by *her-1* arrays. Thus, association of SDC-2 with multiple *her-1* regulatory regions has functional consequences for dosage compensation. These genetic assays, together with the *her-1* array assays, indicate that SDC-2 acts directly to repress *her-1* transcription, thus initiating hermaphrodite sexual development.

*sdc-3* is also required to repress *her-1*, but it does not trigger hermaphrodite development: XO animals engineered to overexpress SDC-3 develop as males (Table 1). To characterize the interaction between SDC-2 and

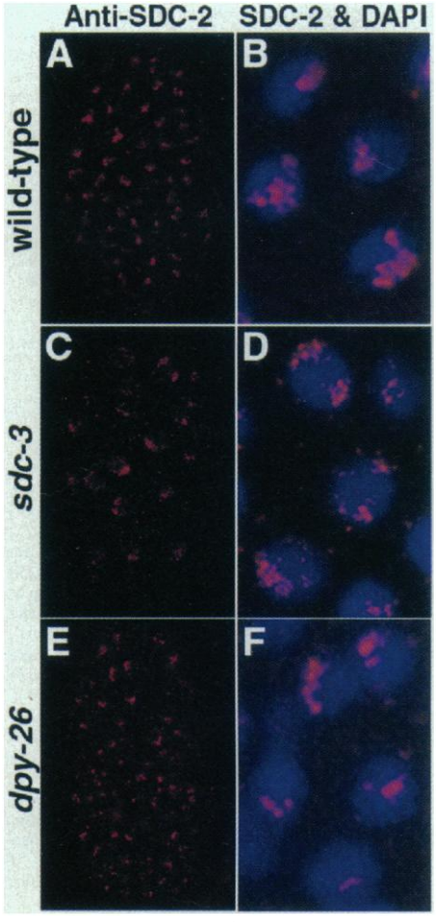


**Fig. 4.** SDC-2 exhibits a punctate staining pattern in dosage compensation mutants. Confocal images of wild-type (**A**), *sdc-3* (**C**), and *dpy-26* (**E**) mutant embryos stained with anti-SDC-2 (red). (**B**, **D**, and **F**) Enlarged sections of mutant embryos costained with anti–SDC-2 (red) and DAPI (blue). Regions of overlap are fuchsia. SDC-2 protein amounts are reduced in the mutants but SDC-2 appears to be X-localized. A similar SDC-2 pattern occurs in *dpy-27*, *dpy-28*, and *dpy-30* mutants, and a wild-type pattern occurs in *sdc-1* and *dpy-21* mutants, which exhibit mild dosage compensation defects (*3, 12, 22*). *dpy-30* participates in dosage compensation by activating *sdc-3* (*16*). These results implicate SDC-2 in X chromosome recognition. Because SDC-2 staining was faint in the mutants, the brightness was enhanced during imaging to show the punctate pattern.

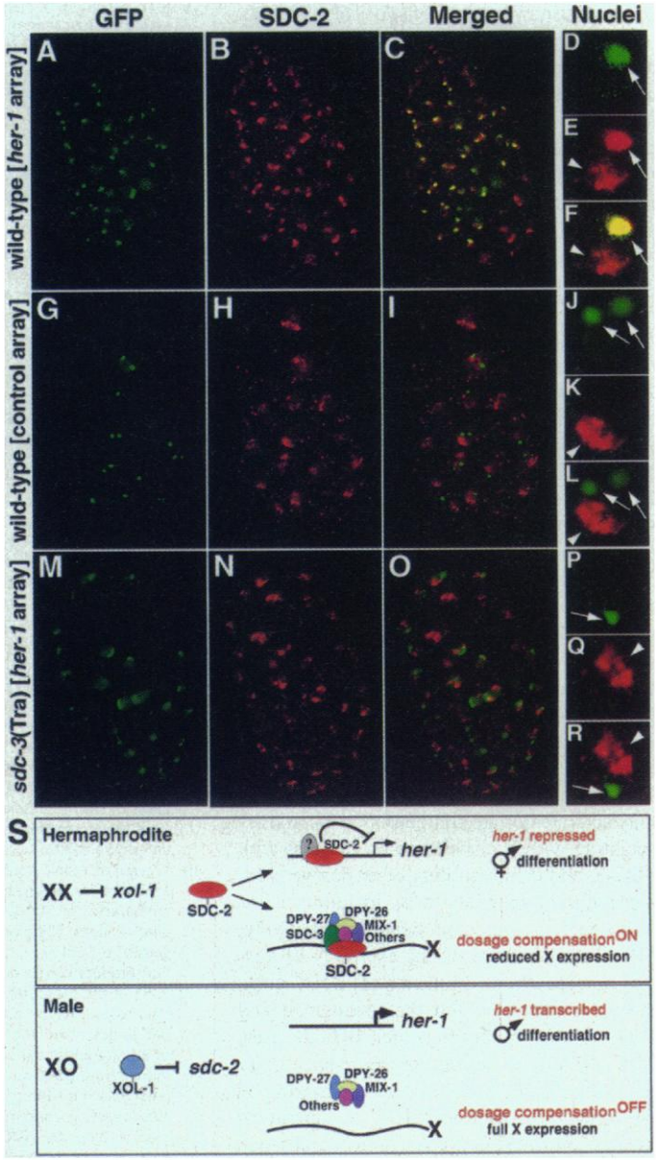


**Fig. 5.** SDC-2 associates with *her-1* promoter sequences. Confocal images of wild-type XX embryos carrying arrays (green), with (**A** to **C**) or without (**G** to **I**) *her-1* promoters, stained with anti-SDC-2 (red). All arrays also contain *lacO* sequences and express a transgene encoding a *lac* repressor::GFP fusion protein (LacI::GFP). LacI::GFP binds to *lacO* sequences within the DNA arrays, which allows them to be detected by green autofluorescence (*26, 27*). Colocalization (yellow) of GFP and SDC-2 indicates that SDC-2 binds to *her-1*-containing arrays but not to control arrays. Enlargement of single nuclei from wild-type embryos bearing *her-1* arrays (**D** to **F**) or control arrays (**J** to **L**). Arrows indicate arrays tagged by LacI::GFP, and arrowheads indicate X chromosomes. (**M** to **O**) Confocal images of an *sdc-3*(Tra) XX embryo bearing *her-1* promoter arrays that bound SDC-2 in wild-type embryos. *sdc-3*(Tra) prevents SDC-2 from associating with *her-1* but not X. (**P** to **R**) Enlargement of a single nucleus from the *sdc-3*(Tra) strain. (**S**) Model. *sdc-2* encodes the sex-specific factor that induces hermaphrodite development in XX animals by repressing the male sex-determining gene *her-1* and by triggering assembly of dosage compensation machinery on X, including proteins (DPY-26 and MIX-1) active in meiosis or mitosis. In XO embryos *sdc-2* is repressed by the male-specific XOL-1 protein. *xol-1* is the direct molecular target of the X chromosome counting mechanism that determines sex (*27*). Repression is indicated by —|.

SDC-3 in repressing *her-1*, we assessed the effect on SDC-2 of an *sdc-3*(Tra) mutation, which disrupts sex determination by derepressing *her-1*. This mutation has no effect on dosage compensation (*15*). SDC-2 failed to associate with *her-1* arrays in *sdc-3*(Tra) mutants but did associate with X chromosomes (Fig. 5, M to R). Thus, *sdc-3*(Tra) mutations derepress *her-1* by preventing the association of SDC-2 with *her-1* regulatory regions, which demonstrates the interdependence of SDC-2 and SDC-3 and validates use of the assay to identify repressors of *her-1*. These results show that SDC-2 has different requirements for its association with *her-1* and X, and they reveal the basis for the separation in sex determination and dosage compensation functions of *sdc-3* (*15, 16, 28*).

SDC-2 is the pivotal sex-specific factor that initiates the hermaphrodite program of sexual development and activates dosage compensation (Fig. 5S). It participates directly in both processes through an association with chromatin, acting in one case as a strong gene-specific repressor and in the other as a weaker chromosome-wide repressor. The distinct modes of SDC-2 repression are consistent with SDC-2 being a transcriptional repressor that resembles no known transcription factors. SDC-2 triggers sexual development by inactivating the male sex-determining gene *her-1*, which is repressed at least 20-fold (*23*). In contrast, SDC-2 achieves dosage compensation by reducing X chromosome expression twofold. The extent of repression conferred by SDC-2 is likely specified by interactions with its protein partners. Repression of *her-1* requires interplay between SDC-2 and the sex determination domain of SDC-3. In contrast, modulation of X expression requires SDC-2 to collaborate with the dosage compensation machinery and the dosage compensation-specific domains of SDC-3, which includes a pair of zinc fingers (*16*).

How can a robust transcriptional repressor also trigger assembly of the dosage compensation complex on X chromosomes? SDC-2 may activate dosage compensation by first associating with X chromosomes, perhaps with SDC-3, and then recruiting other dosage compensation components to X, including chromosome segregation proteins. Alternatively, SDC-2 may coordinate the assembly of dosage compensation complexes off DNA; complete complexes would then recognize and associate with X chromosomes. In both cases, SDC-2 could confer chromosome specificity to dosage compensation by recognizing X chromosomes, as implied by the apparent association of SDC-2 with X in the absence of intact dosage compensation complexes.

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17. *sdc-2* was previously positioned on the X chromosome physical map to the right of DNA polymorphism *nP3*, within 0.08 map unit of *lin-14* [D. L. DeVore, H. R. Horvitz, M. J. Stern, *Cell* **83**, 611 (1995)] (Fig. 1). We refined the location of *sdc-2* by identifying four *sdc-2* allele-specific DNA polymorphisms in Southern hybridization experiments with cosmid HHG9 probes. DNA transformation rescue experiments (Fig. 1) narrowed the *sdc-2* gene to the 12.6-kb region of genomic DNA in pTY581. We obtained a DNA sequence for both strands of this clone by using overlapping exonuclease III deletion derivatives [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989)]. We obtained partial confirmation of the gene structure from DNA sequence analysis of three cDNA clones corresponding to the 3′ 5 kb of genomic DNA. Additional *sdc-2* transcript sequence was confirmed by analysis of cloned reverse transcriptase–polymerase chain reaction (RT-PCR) products from wild-type embryonic polyadenylated [poly(A)$^+$] RNA. The 5′ end of the *sdc-2* transcript was amplified with an SL1-specific primer but not an SL2-specific primer, which indicates trans-splicing of the transcript to the SL1 leader. Using a 3.1-kb Xba I fragment from pTY581 as probe, we identified the *sdc-2* transcript. The accession number for the *sdc-2* transcript sequence is AF111934.
18. We first detected the *sdc-2(y74)* deletion by Southern hybridization experiments in which genomic DNA from *her-1(e1520); sdc-2(y74) XO* animals was probed with sequences from pTY81, a 3.1-kb subclone of cosmid HHG9. We then detected the deletion by PCR with primers DML-3 (CTGTGAACACTCGGGAAATTAG) and DB-41 (GAACTCCCGATTCCATGTAATC) on DNA from single Unc male segregants of the strain *sdc-2(y74) unc-3(e151)/szT1*. We used DNA sequence analysis of PCR products to determine deletion breakpoints.
19. We raised rabbit antibodies to SDC-2 (anti-SDC-2) against a fusion protein composed of the first 455 amino acids of SDC-2 tagged with six tandem histidine residues. The plasmid used to express the antigen (pTY1068) was constructed by subcloning a RT-PCR product corresponding to base pairs (bp) 27 to 1391 of the *sdc-2* transcript into the pRSET-A vector (Invitrogen). Fusion protein was expressed and purified as described (*16*). We used the pTY1068 antigen to affinity purify antibodies as described (*16*). Preimmune sera showed no immunoreactivity in *C. elegans* embryos. The affinity-purified antiserum contained cross-reacting contaminants that were removed by preabsorbing anti-SDC-2 antibodies to fixed *sdc-2* (*y74*) mutant embryos. Affinity-purified antibodies were diluted to twice their working concentration and incubated with fixed *her-1(hv1y101); xol-1(y9) sdc-2(y74) unc-9(e101)* embryos overnight with nutation. Preabsorbed antibodies were separated from *y74* embryos and cellular debris by centrifugation (three times for 15 min at 16,000*g*) and were used the same day. The SDC-2 specificity of the antibodies was demonstrated by the lack of staining in *sdc-2(y74)* embryos (Fig. 2, G to I). Embryos were collected from gravid hermaphrodites and fixed as described (*16*), except that the initial (4°C) fixation was eliminated. Antibody staining of embryos was done as described (*4*), except that primary antibody incubations were for 1 to 4 hours. Laser confocal microscopy was performed on a Leica TCS-NT confocal microscope. To assess the effect of dosage compensation mutations on the SDC-2 staining pattern, we used XO animals that had been converted to the XX mode of development by an *xol-1* mutation and were rescued by the *dpy* or *sdc* mutation being characterized, as described (*16*). Dosage compensation mutations used in antibody staining experiments are molecular or genetic nulls. We viewed at least 1000 stained embryos of each genotype in multiple experiments.
20. To create the *dpy-30::sdc-2* transgene, we introduced a Bgl II site at the fourth codon of *sdc-2* and subcloned an 11-kb Bgl II–Kpn I *sdc-2* fragment into the Bcl I and Kpn I sites of pTY647, a plasmid containing the *dpy-30* minimal rescuing region (*21*). The resulting transgene, pTY975, included the *dpy-30* promoter, the first three codons of *dpy-30*, a leucine codon, and the entire *sdc-2* structural gene beginning with its fifth codon. We then injected pTY975 and the transformation marker p76-16B [*unc-76*(+)]into *him-8*(*e1489*); *unc-76* hermaphrodites and established transmitting lines. Extrachromosomal arrays bearing pTY975 were stably integrated into the genome by γ-irradiation for 15 min as described [C. Mello and A. Fire, *Methods Cell Biol.* **48**, 451 (1995)]. Five independent integrated lines were isolated, two of which (*yIs29* on X and *yIs30*) were used in this study.
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24. The *her-1* gene contains a 1.6-kb primary promoter, P1, which produces the functional sex-specific transcript, and an internal 3.5-kb promoter, P2, which drives expression of a smaller transcript of unknown function [M. D. Perry *et al.*, *Genes Dev.* **7**, 216 (1993)]. To examine SDC-2 binding to *her-1* promoters, we constructed plasmid pTY1768, which includes 1253 bp of P1, the first two exons of *her-1*, and 2408 bp of P2. We subcloned a 3880-bp Bgl II fragment from the *her-1* gene into the Bam HI site of pBluescript (SK+). Stable lines of *her-1* array-bearing animals were made by coinjecting wild-type hermaphrodites with pTY1768 (50 μg/ml); pSV2-dhFr8.32 (50 μg/ml), which encodes 256 tandem repeats of the *lacO* sequence (*25*); pPD49-78 (50 μg/ml); which encodes a heat shock–inducible Lac I::GFP fusion protein (*26*); and pRF4 (100 μg/ml), a *rol-6*(d) transformation marker. We generated animals bearing arrays lacking *her-1* sequence by substituting pBluescript (SK+) for pTY1768 in the injection mix. Analysis was performed as described (*27*).
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