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Article

# The *C. elegans* Dosage Compensation Complex Propagates Dynamically and Independently of X Chromosome Sequence

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## Summary

**Background:** The *C. elegans* dosage compensation complex (DCC) associates with both X chromosomes of XX animals to reduce X-linked transcript levels. Five DCC members are homologous to subunits of the evolutionarily conserved condensin complex, and two noncondensin subunits are required for DCC recruitment to X.
**Results:** We investigated the molecular mechanism of DCC recruitment and spreading along X by examining gene expression and the binding patterns of DCC subunits in different stages of development, and in strains harboring X;autosome (X;A) fusions. We show that DCC binding is dynamically specified according to gene activity during development and that the mechanism of DCC spreading is independent of X chromosome DNA sequence. Accordingly, in X;A fusion strains, DCC binding propagates from X-linked recruitment sites onto autosomal promoters as a function of distance. Quantitative analysis of spreading suggests that the condensin-like subunits spread from recruitment sites to promoters more readily than subunits involved in initial X targeting.
**Conclusions:** A highly conserved chromatin complex is appropriated to accomplish domain-scale transcriptional regulation during *C. elegans* development. Unlike X recognition, which is specified partly by DNA sequence, spreading is sequence independent and coupled to transcriptional activity. Similarities to the X recognition and spreading strategies used by the *Drosophila* DCC suggest mechanisms fundamental to chromosome-scale gene regulation.

## Introduction

In many animal species, sex is determined by how many copies of a particular chromosome are inherited from the parental gametes. One consequence of such a mechanism is that the two sexes will have a potentially lethal imbalance in the dosage of one chromosome. Mechanisms to correct for this imbalance have evolved and are referred to as "dosage compensation." Most dosage compensation mechanisms studied to date involve specific changes to the chromatin of the sex chromosome, which ultimately act to balance sex chromosome gene expression between males and females [1]. In *C. elegans*, XX hermaphrodites reduce transcript levels from each X chromosome by a factor of two to match the expression of XO males [2]. This is fascinating in many respects, among which is that the compensation must somehow be "tuned" to each locus so that genes expressed over a wide dynamic range are all subtly repressed by approximately 2-fold.

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The *C. elegans* dosage compensation complex (DCC) is composed of proteins encoded by the genes *sdc-1*, *sdc-2*, *sdc-3*, *dpy-21*, *dpy-26*, *dpy-27*, *dpy-28*, *capg-1*, and *mix-1* (Figure 1A) [3, 4]. DPY-30, a 13 kDa protein homologous to a subunit of a protein complex that methylates histone H3 at lysine 4 (H3K4), is also required for dosage compensation [5–8]. CAPG-1, DPY-26, DPY-28, DPY-27, and MIX-1 are homologous to the members of the condensin complex, which functions during chromosome condensation and segregation in organisms ranging from bacteria to humans [9]. Except for DPY-27, which is specific to the DCC, all of the condensin-like subunits also function as part of more typical mitotic and meiotic condensin complexes on all chromosomes [3].

During *C. elegans* embryogenesis, the DCC recognizes and associates specifically with each of the X chromosomes in XX embryos but does not bind to X in XO embryos [10–15]. Current hypotheses posit two distinct modes of DCC association with the X. The first involves initial recognition and recruitment of the DCC by discrete sites along the X called "*rex*" sites (for *r*ecruitment *e*lement on *X*). Immunofluorescence microscopy revealed at least 38 *rex* sites, which were defined by their ability to recruit the DCC onto multicopy extrachromosomal transgenic DNA [16–18]. The immunofluorescence studies [17, 18] and two genome-wide chromatin immunoprecipitation (ChIP)-chip studies [17, 19] identified a DNA sequence motif with a 10 bp core (TCGCGCAGGG) that occurs at many sites of DCC recruitment. Mutating the motif at a *rex* site reduces DCC binding, suggesting that the motif is critical for recruitment [17, 18]. However, the DNA sequence motifs do not fully account for X specificity, because many perfect matches to the motif occur on autosomes but are not bound by the DCC [17, 19]. The second mode of DCC association involves spreading of the DCC from the recruitment elements to adjacent chromatin.

Here, we test current hypotheses regarding DCC spreading and present two key findings. First, we show that DCC binding is dynamically specified according to gene activity during development, providing insight regarding how the process might be tuned to gene activity. Second, we show that the mechanism of DCC spreading is independent of X chromosome DNA sequence and that in X;A fusion strains spreading propagates from X-linked recruitment sites onto autosomal promoters as a function of distance. Additionally, quantitative comparison of binding data at *rex-1* and the nearby *dpy-23* promoter indicates that the condensin-like subunits of the DCC spread from recruitment sites to active promoters more readily than the SDC-2 and SDC-3 subunits involved in initial X targeting, suggesting a DCC subcomplex involved in spreading.

## Results

### Along the X, DCC Binding Is Dynamically Specified According to Gene Activity during Development

Previously published ChIP experiments performed in *C. elegans* embryos established two modes of binding on X [19]. The first mode is represented by high-amplitude signals termed "foci" (defined empirically as being more than two standard deviations greater than the mean peak amplitude). These foci were associated with a specific DNA motif and

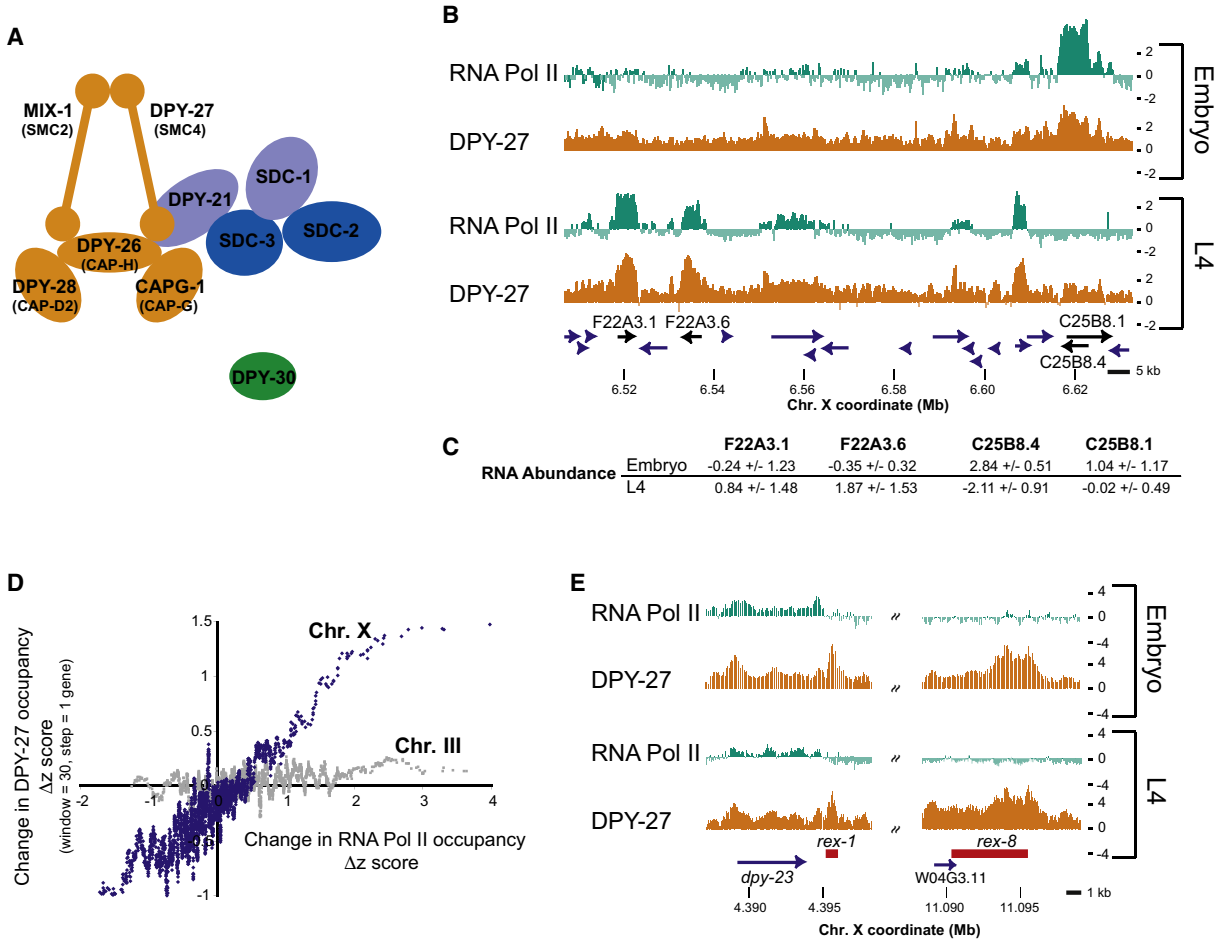


Figure 1. DCC Binding Is Dynamically Specified According to Transcriptional Activity during Development

(A) A schematic representation of the dosage compensation complex (DCC) inferred from condensin homology (in parentheses) and coimmunoprecipitation experiments. Members of the complex homologous to condensin subunits are shown in orange. SDC-2 and SDC-3 (darker blue) are involved in X-specific recruitment. DPY-30 is homologous to a subunit of an H3K4 methyltransferase complex.

(B) Average z scores of chromatin immunoprecipitations (ChIPs) performed in embryos or in L4 worms. F22A3.1 and F22A3.6 are expressed in L4s but not in embryos. Both RNA polymerase II (RNA Pol II) and DPY-27 binding are higher in L4s compared to embryos. In embryos, C25B8.4 and C25B8.1 are expressed and correspondingly are bound by high levels of DPY-27. In L4s, C25B8.4 and C25B8.1 transcription and DPY-27 binding are reduced.

(C) RNA abundance (average $\log_2$ expression ratio) for the four genes highlighted in (B) [33].

(D) Change in DPY-27 and RNA Pol II binding at promoters was calculated by subtracting the ChIP value in embryos from that of L4s. A moving average of values is plotted. Change in DPY-27 level (y axis) correlates positively with change in RNA Pol II binding (x axis) on the X chromosome (blue), but not on chromosome III (gray).

(E) Average DPY-27 and RNA Pol II binding in embryos and L4s at two distinct *rex* sites.

hypothesized to be involved in initial X recognition. The second mode of binding was a lower-amplitude accumulation of the DCC at gene promoters. Unlike foci, DCC accumulation at promoters was correlated with transcriptional activity and was not specified by a stereotypic DNA sequence motif. This led to the hypothesis that although recruitment is governed at least in part by DNA sequence, DCC association with promoters is specified chiefly by transcriptional activity [19]. The hypothesis predicts that the DCC would be redistributed to a new set of gene promoters in the context of a different transcriptional program.

To test this prediction, we performed DPY-27 and RNA polymerase II (RNA Pol II) ChIPs from animals in the fourth larval stage of development (L4) (Figure 1B; see also Figure S1A available online). Loci that are transcriptionally silent in embryos but expressed in L4 animals are bound by DPY-27 specifically in L4 (Figures 1B and 1C). The converse is also true: the DCC disengages from loci that are transcribed in embryos but silent in L4s (Figures 1B and 1C). DCC disengagement from repressed genes and recruitment to active genes during development occurs across the entire length of the X chromosome (Figure 1D).

In contrast to the dramatic changes in DPY-27 localization observed at gene promoters ($p = 5.7 \times 10^{-32}$), DPY-27 binding at *rex* sites [17] remains constant between the embryo and L4 stages of growth ($p = 0.474$; Figure 1E).

## The Condensin-like Members of the DCC Spread to Adjacent Chromatin More Efficiently than Noncondensin Members

The zinc-finger-containing protein SDC-3 functions during the early steps of DCC recruitment and is important for X