## **Supplemental Material**

# An MLL/COMPASS subunit functions in the *C. elegans* dosage compensation complex to target X chromosomes for transcriptional regulation of gene expression

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### **Table of Contents**

Supplemental Figure 11 DPY-30 and ASH-2 antibody specificity.
Supplemental Figure 2
Supplemental Figure 3
Like the DCC, DPY-30 and ASH-2 are found preferentially in promoters of expressed genes on both X and autosomes.
Supplemental Figure 4
DCC binding on X in DCC mutants resembles that on autosomes in wild-type embryos.
Supplemental Figure 5
DCC binding at <i>rex</i> sites is nearly eliminated in <i>sdc-2</i> , <i>sdc-3</i> and <i>dpy-30</i> mutants, whereas <i>dox</i> -site binding is greatly reduced but present.
Supplemental Figure 6
Quantification of levels of DCC subunits in wild-type and mutant strains.
Supplemental Figure 7
Autosomal binding of DPY-27 and SDC-3 is unchanged in DCC mutants and similar to that of SMC-4.
Supplemental Figure 8
COMPASS components localize to DCC binding sites throughout the genome, with the
exception of intergenic rex sites.

Supplemental Figure 9
The relative X versus Autosomal DPY-30 and ASH-2 binding is unchanged in an <i>sdc-2</i> mutant.
Supplemental Figure 10
<i>rex</i> sites can be predicted by the combination of ChIP-chip profiles of DCC components in dosage compensation mutants and of H3K4me3 in wild-type embryos.
Supplemental Figure 11
Supplemental Figure 12
Supplemental Figure 13
Supplemental Figure 14
Changes in gene expression positively correlate with changes in DPY-27 binding.
Supplemental Figure 15
Polymerase binding is correlated with gene expression.
Supplemental Figure 16
RNA Polymerase II binding is increased on X but not autosomes in dosage compensation mutants.
Supplemental Table 1
Supplemental Materials and methods



### DPY-30 and ASH-2 antibody specificity.

Antibodies raised against DPY-30 ((A) amino acids 24-123 from SDIX and

(B) N-LREKDRYNAENQNPAGQQ-C) and ASH-2 (N-LDHPPRVKREDDDDVKDIKKEIKQEI-C) were tested for specificity by western blots using extracts from wild-type and RNAi-treated animals. Embryos were hatched without food and placed on plates containing bacteria harboring either an empty vector control or double-stranded RNA to *ash-2* or *dpy-30*. Gravid hermaphrodites were bleached, their embryos were boiled in Laemmli sample buffer, and the extracts run on SDS-PAGE gels.

(A) Both DPY-30 antibodies recognize a product that is reduced in abundance compared to the  $\alpha$ -tubulin control in *dpy-30*(RNAi) embryos.

(B) The ASH-2 antibody recognizes a product that is reduced in abundance compared to the  $\alpha$ -tubulin control in *ash-2*(RNAi) embryos.



**The binding profile of DPY-30 is similar to that of other DCC components on the X and autosomes.** Representative ChIP-chip binding profiles of five DCC components (DPY-30, DPY-27, DPY-26, SDC-3, SDC-2) and one MLL/COMPASS component (ASH-2) on X and an autosome. The location of genes is indicated below the ChIP profiles, and the direction of transcription is indicated by arrows. DPY-30 binds to all previously indentified DCC binding sites on X and autosomes. The ASH-2 binding profile is very similar to the DCC profile across X and autosomes, with the exception of intergenic *rex* sites, which lack ASH-2 binding. (A) At the intergenic *rex-1* site, DPY-30 binding resembles that of other DCC components. ASH-2 does not bind to *rex-1*, but does bind the *dox-7* site in the neighboring promoter.

(B) The autosomal binding profiles of DPY-30 and ASH-2 are similar to that of known DCC components.



# Supplemental Figure S3. Like the DCC, DPY-30 and ASH-2 are found preferentially in promoters of expressed genes on both X and autosomes.

(A,B) Shown is the distribution of DPY-30 or ASH-2 peaks relative to the distance from the transcriptional start site (TSS) of expressed (blue line) or non-expressed (red line) genes. For each gene, DPY-30 or ASH-2 peaks with centers within +/- 5 kb of a TSS were mapped to the nearest TSS. Genes with peaks were then grouped in 250-bp bins relative to the TSS-to-peak distance. The percentage of genes in each bin was calculated relative to all genes in that class (expressed or non-expressed) within the +/- 5 kb interval. TSS locations were obtained from Wormbase release WS180. ASH-2 peaks were found in 7577 expressed and 716 non-expressed genes. DPY-30 peaks were found in 7914 expressed and 502 non-expressed genes. Both DPY-30 and ASH-2, like DCC components, preferentially bind to the promoters of expressed genes versus non-expressed genes.

(C,D) Shown is the distribution of DPY-30 or ASH-2 peaks relative to the distance from the TSS of all genes on X or autosomes. Peaks were mapped to the nearest TSS, within a +/- 5 kb interval around the TSS. Peaks were then grouped in 250-bp bins relative to the TSS-to-peak distance, and the percentage of peaks within each bin was calculated relative to all peaks within in the +/- 5 kb interval. DPY-30 and ASH-2 binding is enriched at promoters of genes on both X and autosomes. Some DPY-30-independent ASH-2 peaks were identified within the coding regions of genes, suggesting that ASH-2 may participate in protein complexes without DPY-30. This DPY-30-independent ASH-2 binding is demonstrated by the shoulder present 3' of the TSS in the ASH-2 binding distribution, but absent in the DPY-30 distribution.





wild type sdc-2 mutant



### DCC binding on X in DCC mutants resembles that on autosomes in wild-type embryos.

(A-C) Histograms showing the distribution of DPY-27 ChIP-chip probe scores across the X chromosome and autosomes in wild-type embryos compared to *sdc-3*, *sdc-2*, or *dpy-30* mutant embryos. Probe scores were grouped in bins with a step size of 0.06 (log2 scale), and the percentage of total probes in each bin was graphed on the Y-axis. In each mutant, the probe score distribution of the X chromosome more closely resembles that of autosomes than X chromosomes in wild-type embryos. These results support the model that low-level intrinsic binding to *dox* sites in the absence of DCC binding to *rex* sites mimics DCC binding to autosomes, and proximity in *cis* to *rex* sites is required for full occupancy of *dox* sites.



### В

### DPY-27 profile in wild type



### DPY-27 profile in dpy-30 mutant







### DPY-27 profile in wild type



### DPY-27 profile in dpy-30 mutant



### DPY-27 profile in sdc-3 mutant





# DCC binding at rex sites is nearly eliminated in *sdc-2*, *sdc-3* and *dpy-30* mutants, whereas *dox*-site binding is greatly reduced but present.

(A) Shown are ChIP-chip profiles of DPY-27 at representative *rex* and *dox* sites in wild-type embryos versus *sdc-2*, *sdc-3* or *dpy-30* mutant embryos. *rex*-site binding is nearly eliminated in these mutants, whereas *dox*-site binding is greatly reduced but present.

(B) Shown are graphical representations of DPY-27 ChIP-chip probe intensities along 5 kb regions centered on representative *rex* and *dox* sites (green line) in wild-type versus *sdc-3* or *dpy-30* mutant embryos.

(C) Shown are graphical representations of SDC-3 ChIP-chip probe intensities along 5 kb regions centered on representative *rex* and *dox* sites (green line) in wild-type versus *sdc-2*, *dpy-30* or *dpy-27* mutant embryos.



### Quantification of levels of DCC subunits in wild-type and mutant strains.

(A) SDC-3 and DPY-27 accumulate in *sdc*-2 null mutant embryos. Shown are western blots probing levels of SDC-3 and DPY-27, relative to  $\alpha$ -tubulin loading controls, in wild-type versus *sdc*-2 null mutant embryo extracts. SDC-3 is present at reduced levels in *sdc*-2 mutant embryos, while DPY-27 is present at comparable levels in both genotypes.

(B) SDC-2 is present at wild-type levels in a strain expressing rescuing FLAG-tagged SDC-2 off an extrachromosomal array. Shown is a western blot of equivalent amounts of either wild-type embryo extract, or extract from embryos expressing FLAG-tagged SDC-2 in an *sdc-2* null mutant background. SDC-2 protein levels are comparable in the two genotypes, indicating the extent of binding to *rex* and *dox* sites observed in FLAG-SDC-2 ChIP-chip is not due to over-expression of SDC-2.





Autosomal binding of DPY-27 and SDC-3 is unchanged in DCC mutants and similar to that of SMC-4. (A) Representative ChIP-chip profiles of DPY-27, SDC-3 and SMC-4 binding on chromosome I in wild-type and DCC mutant strains. Compared to DPY-27 and SDC-3 binding on X chromosomes (Figs. 3 and 4 and Supplemental Fig. 5), the binding on autosomes is relatively unchanged in DCC mutants. In addition, the binding profile of the condensin component SMC-4 is very similar to that of the DCC on autosomes, suggesting common binding sites for condensin-like complexes. The location of genes is indicated below the profiles, and the direction of transcription is indicated by arrows.

(B) Representative ChIP-chip profiles of SMC-4 in wild-type embryos and DPY-27 in wild-type and *sdc-2* mutant embryos at *dox* sites and one *rex* site on X. The binding profile of SMC-4 in wild-type embryos on X is very similar to the low-level intrinsic DPY-27 binding in *sdc-2* mutant embryos, suggesting these sites may have an affinity for binding of condensin-like proteins. The location of genes is indicated below the profiles, and the direction of transcription is indicated by arrows.





# Supplemental Figure S8. COMPASS components localize to DCC binding sites throughout the genome, with the exception of intergenic *rex* sites.

(A-D) Additional ChIP-chip profiles of DPY-30, ASH-2 or DPY-27 in wild-type or DCC mutant embryos. The location of genes is indicated below the profiles, and the direction of transcription is indicated by arrows.

(A) At the intergenic *rex* site *rex*-2, DPY-30 binding is eliminated in an *sdc*-2 mutant but not in an *sdc*-3 mutant. ASH-2 is not bound in embryos of either genotype, suggesting that DPY-30 binds to *rex*-2 through its association with the DCC rather than its association with MLL/COMPASS.

(B) While DPY-27 binding is reduced at *dox* sites in *sdc-2* mutant embryos, binding of DPY-30 and ASH-2 is unaffected, suggesting that DPY-30 binding to *dox* sites in *sdc-2* mutants occurs through its association with the MLL/COMPASS complex.

(C,D) Binding of DPY-30, ASH-2, and DPY-27 to autosomes is relatively unchanged in *sdc-2* and *sdc-3* mutants.



### **Distribution of ChIP-chip probe scores**

### The relative X versus Autosomal DPY-30 and ASH-2 binding is unchanged in an *sdc-2* mutant.

Histograms showing the distribution of ASH-2 or DPY-30 ChIP-chip probe scores across the X chromosome and autosomes in wild-type embryos compared to sdc-2 mutants. Probe scores were grouped in bins with a step size of 0.06 (log2 scale) and the percentage of total probes in each bin was graphed on the Y-axis. The probe score distribution for DPY-30 is slightly higher on the X chromosome than it is on autosomes, while the opposite is true for ASH-2. These distributions are consistent with ASH-2's participation in the COMPASS complex, and a role for DPY-30 in both COMPASS and the DCC. Neither distribution is significantly altered in sdc-2 mutants.





Name	% Recruitmen (total nuclei)	t Site Position	Location	MEX Motif P-Values In(P) < -10	DPY-27 peak score in wild type	DPY-27 peak score in <i>sdc-2</i> mutant	H3K4me3 peak score in wild type
dox-50	0% (102)	X:1410088914103427	Promoter	none	1.97	none	2.87
rex-39	96% (56)	X:1481209114814257	Coding	none	1.56	none	none
rex-40	99% (78)	X:804487807978	Intergenic	-10.01, -12.83, -13.27, -14.7	8 2.57	none	none
rex-41	98% (105)	X:1754317117546379	Intergenic	-10.07, -12.92	2.35	none	none
rex-42	99% (68)	X:1718017117182852	Intergenic	-12.92	1.81	none	none

# Supplemental Figure S10. *rex* sites can be predicted by the combination of ChIP-chip profiles of DCC components in dosage compensation mutants and of H3K4me3 in wild-type embryos.

(A) Large DPY-27 peaks that are significantly reduced in *sdc-2* mutants and lack H3K4 trimethylation are predicted to be *rex* sites. Shown are ChIP-chip profiles of DPY-27, DPY-30 and ASH-2 in wild-type and DCC mutants at the newly predicted *rex site, rex-42*.
(B) Confocal images showing two intestinal cell nuclei, one carrying an extrachromosomal array containing multiple copies of *rex-42* (labeled with a FISH probe, green) and one lacking the array, co-stained with DAPI (blue) and antibodies to DPY-27 (red). Extrachromosomal recruitment assays were performed as previously described (Jans et al. 2009). The *rex-42* array recruits the DCC and titrates it off the X chromosome, as seen by DPY-27 co-localization with the array, but not with X. In the adjacent nucleus not carrying an array, DPY-27 localizes to the X chromosomes.

(C) Table shows the percent of DCC recruitment, genomic location, MEX motif values, and H3K4me3 ChIP-chip results for four predicted *rex* sites and a large peak that does not fit our prediction and is a *dox* site. These criteria allow us to distinguish *rex* sites from *dox* sites.



# Supplemental Figure S11. Fusion of chromosome V to X increases DCC binding up to 2 Mb from the point of fusion, but does not alter DCC distribution on X or the rest of V.

(A-D) Histograms depicting the distribution of DPY-27 ChIP-chip peaks along either chromosomes V or X in wild-type embryos compared to those in the X:V fusion strain ypT28. Peaks were mapped along each chromosome and then grouped into 200 kb bins based on location on the chromosome. The percentage of total peaks within each bin was calculated relative to all peaks on that chromosome and plotted on the Y axis, with position along the chromosome (in Megabases) on the X axis. Overall peak distribution along the X chromosome remains unchanged in the X:V fusion strain. Binding on chromosome V is increased from the point of fusion (left-hand side of V) to approximately 2 Mb into the chromosome.





# Supplemental Figure S12. The new autosomal binding seen by Ercan *et al.* (2009) in an X:Autosomal fusion strain is similar to the binding we observe in wild-type embryos.

Studies from others (Ercan et al. 2009) have previously reported that fusion of the X chromosome to an autosome increases autosomal binding. However, that study (and the earlier Ercan et al. 2007 study) did not find many autosomal DCC binding sites in wild-type embryos and therefore argued that the majority of autosomal binding sites in the X:A fusion did not exist in wild-type embryos. As we detect significant autosomal binding in wild-type embryos, we compared our wild-type DPY-27 ChIP-chip dataset with DPY-27 ChIP-chip data in X:II fusion embryos from Ercan et al. (2009) and wild-type DPY-27 ChIP-chip data from Ercan et al. (2007). In each case, two replicates were analyzed using the same methods as in Ercan et al. (2009). Each individual replicate was normalized using ChIPOTle 2.0

(http://sourceforge.net/projects/chipotle-2/). Normalized replicates were then merged using ChIPOTle 2.0, and peaks were called using the same conditions outlined in Ercan et al. (2009) (ChIPOTle peak calling algorithm. Window size of 500. Step size of 100.  $p = 10^{-40}$ . Peaks are underlined in orange).

(A,B) Within the first 2.5Mb of the X:II fusion point, 65 peaks are called in the Ercan et al. (2009) X:II fusion experiments. Of these 65 DPY-27 peaks, 13 were called as peaks in our wild-type chromosome II. Of those not called as peaks, 71% showed appreciable binding by visual inspection in our wild-type chromosome II profiles. The peaks in common are not simply the result of a high background binding in our wild-type data sets, as we only observe 17 total peaks within the same 2.5Mb.

(C,D) Distant from the point of fusion between II and X, we observe higher levels of DPY-27 binding on II in our wild-type embryos (A and B) than seen on II by Ercan et al. (2009) on the X:II fusion chromosome or on the wild-type chromosome II by Ercan et al. (2007). Some of the peaks called in our data correspond to a small amount of binding in the Ercan et al. (2009) fusion and the Ercan et al. (2007) wild-type data set. The reason for the difference in the levels of autosomal DCC binding seen between our two groups is unknown. The significant DPY-27 binding we see along chromosome II plays an important role in our model of how the DCC binds to *rex* and *dox* sites.

22



**DPY-27 Profile in Embryos** 



### DPY-27 binding to rex sites is unchanged between embryos and fed L1 larvae.

Shown are graphical representations of DPY-27 ChIP-chip probe intensities along 5 kb regions centered on representative rex sites (green line) in embryos and fed L1 larvae. The profiles and levels of DPY-27 binding in both fed L1 replicates closely resemble that of embryos.





F

Chromosome	Pearson's Correlation Coefficient	p-value	Spearman's Rank Correlation Coefficient	p-value	gene no.
I	0.23	<10-6	0.56	<10 <sup>-6</sup>	1662
Ш	0.30	<10 <sup>-6</sup>	0.32	<10-6	1811
III	0.30	<10-6	0.35	<10-6	1592
IV	0.33	<10-6	0.36	<10 <sup>-6</sup>	1656
V	0.33	<10-6	0.36	<10-6	2129
X	0.47	<10-6	0.49	<10-6	1591

# Supplemental Figure S14. Changes in gene expression positively correlate with changes in DPY-27 binding.

(A-C) Plots showing the positive correlation between changes in DPY-27 promoter occupancy and changes in gene expression using moving averages as in Figure 6C. The positive quadrants of the axes correspond to higher binding or expression in embryos compared to fed L1s.

(A) Analysis of a second replicate of DPY-27 ChIP-chip in fed L1 larvae was performed as in Figure 6C. Similar to the other replicate, changes in DPY-27 binding from embryos to fed L1 larvae is positively correlated with changes in gene expression on both X and autosomes.

(B) Analysis of DPY-27 ChIP-chip in embryos vs. fed L1 larvae was performed as above. Here the X is compared to chromosome III genes.

(C) Analysis of DPY-27 ChIP-chip in embryos vs. fed L1 larvae was performed as above. Here the X is compared to all autosomal genes.

(D-F) Raw data showing the correlation between the transcriptional activity of a gene and the level of DPY-27 binding within its promoter. Values were calculated as above and plotted before a moving average was taken. The positive quadrants of the axes correspond to higher binding or expression in embryos compared to fed L1s.

(D) Scatterplot of the differences in DPY-27 binding and transcript levels in embryos and fed L1 larvae for genes on the X chromosome.

(E) Scatterplot of the differences in DPY-27 binding and transcriptional level in embryos and fed L1 larvae for genes on chromosome II.

(F) Statistical analyses to demonstrate the positive correlation between the differences in gene expression and the differences in DPY-27 binding within the promoter of a gene. The Pearson's correlation coefficient and Spearman's rank correlation coefficient were calculated for genes within each chromosome. The corresponding p-value for each correlation coefficient is listed. These analyses show a positive correlation between changes in DPY-27 occupancy and changes in gene expression.



### Supplemental Figure S15. Polymerase binding is correlated with gene expression.

(A) Scatterplots depicting the correlation of RNA Pol II binding in coding regions with level of gene expression for each Pol II antibody. For each gene, the median Pol II ChIP-chip probe score within the coding region (+500 to STOP) is graphed on the X axis, and the log2 gene expression level by microarray is plotted along the Y axis.

The Pearson's correlation coefficient (r) calculated for each set of probe scores and expression values showed a relatively strong positive correlation between Pol II occupancy and level of gene expression for all three antibodies. As expected, the ChIP-chip data for the elongating form of Pol II (phosphorylation of S2 in the CTD, labeled Phospho-S2 ) in the coding region of genes correlates most strongly with gene expression level.

(B) Scatterplots depicting the correlation of RNA Pol II binding in promoter regions with level of gene expression for each Pol II antibody. For each gene, the third highest probe score within the promoter (-2 kb to TSS) is graphed on the X axis, and the log2 gene expression level by microarray is plotted along the Y axis.

The Pearson's correlation coefficient (r) calculated for each set of probe scores and expression values showed a relatively strong positive correlation between Pol II occupancy and level of gene expression for all three antibodies. As expected, the ChIP-chip data for the initiating form of Pol II (phosphorylation of S5 in the CTD, labeled Phospho-S5 ) in the promoter region of genes correlates most strongly with gene expression level.





# Supplemental Figure S16. RNA Polymerase II binding is increased on X but not autosomes in dosage compensation mutants.

(A) ChIP-chip profiles using antibodies directed to three different forms of RNA Pol II: unphosphorlyated Pol II CTD (8WG16 and Bentley), initiating Pol II (Phospho-S5 CTD [S5]) or elongating/terminating Pol II (Phospho-S2 CTD [S2]) on chromosome III. Binding of all Pol II forms remains relatively unchanged in *sdc-2* mutants on autosomes. Binding of all 3 forms of Pol II is relatively unchanged on chromosome II in wild-type embryos compared to *sdc-2* mutant embryos, unlike the case for X (Fig. 7A and B).

(B) Histograms showing distribution of RNA Pol II ChIP-chip probe scores for the initiating form of Pol II (phosphorylation on S5 of the CTD) across the X chromosome and autosomes in wild-type embryos compared to *sdc-2* mutants. Probe scores were grouped in bins with a step size of 0.06 (log2 scale), and the percentage of total probes in each bin was graphed on the Y-axis. In wild-type embryos, the probe score distribution on X is slightly lower than on autosomes. Conversely, in *sdc-2* mutant embryos probe scores on X are generally higher than an autosomes, indicative of increased Pol II initiation across X.

(C) Histograms showing distribution of RNA Pol II ChIP-chip probe scores for the elongating form of Pol II (phosphorylation on S2 of the CTD) across the X chromosome and autosomes in wild-type embryos compared to *sdc-2* mutants. Probe scores were grouped in bins with a step size of 0.06 (log2 scale), and the percentage of total probes in each bin was graphed on the Y-axis. In wild-type embryos, the probe score distribution on X is slightly lower than on autosomes. Conversely, in *sdc-2* mutant embryos probe scores on X are generally higher than an autosomes, indicative of increased Pol II elongation across X.

# **Supplemental Table 1**

Name	Position on Chromosome V	Wild type DPY-27 Fold Enrichment	ChIP: SEM	X:V fusion DPY-27 Fold Enrichment	ChIP: SEM	P Value	Wild type DPY-27 ChIP-chip peak score
peak 1	240,444241,058	0.95	0.32	2.58	0.41	0.02	none
peak 2	665,330665,821	1.32	0.28	2.58	0.45	0.06	none
peak 3	28,72929,933	1.16	0.30	2.78	0.42	0.03	<0.75
peak 4	65,56467,418	1.07	0.30	2.15	0.44	0.05	<0.75
peak 5	443,729444,577	1.56	0.39	3.88	0.43	0.03	>0.75
peak 6	154,833155,820	1.89	0.28	4.26	0.38	0.02	>0.75

### Enhancement in DCC binding to autosomal sites is confirmed by quantitative real-time PCR.

Levels of DPY-27 binding by ChIP at select autosomal sites on the left-hand side of chromosome V were measured by qRT-PCR in wild-type or X:V fusion (ypT28) embryos. The DPY-27 ChIP binding levels for each primer set are expressed as the fold enrichment compared to ChIP input DNA normalized to the levels of the non-DCC-bound gene *him-1*. Experimental error is expressed as the standard deviation of the mean. qRT-PCR confirms the presence of autosomal DCC binding sites on wild-type chromosomes and also shows significant enrichment in DCC binding to V in the X:V fusion for three classes of sites: those showing strong (score  $\ge 0.75$ ), weak (score < 0.75), or no DCC binding on wild-type chromosome V.

## **Supplemental Materials and methods**

### Nematode culture

All worms for embryo ChIP-chip analysis were grown on NG agar plates with concentrated HB101 at 20°C, except for *dpy-30(y130ts)*, which was grown at the permissive temperature of 15°C, then shifted to the restrictive temperature of 20°C for the final 72 h before collecting by bleaching. Fed L1 worms were obtained by growing N2 animals on NG agar plates as above, then gravid hermaphrodites were bleached and their embryos were hatched off in M9 for 24 h at 20°C. Synchronous L1s were then placed in S medium at a density of 10 worms/ $\mu$ L and fed with 10 mg/mL HB101 for 3 h at 20°C in liquid culture.

*sdc-2* mutant embryos were prepared for Polymerase ChIP-chip experiments by growing *sdc-2(y93)* XX animals on Ahringer feeding library bacteria (Kamath et al. 2003) bearing an *sdc-2* plasmid. Feeding bacteria were prepared by seeding 1 L LB cultures with a single colony, growth overnight at 37°C, induction of RNA synthesis for 2 h with 5 mM IPTG, pelleting, and resuspension in 1 vol (w/v) of LB with 20% glycerol.

### Antibodies

Rabbit polyclonal antibodies against DPY-27 (rb699), MIX-1 (rb1822 and rb1951), SDC-3 (rb1079), SDC-2 (rb3778), DPY-26 (rb1450) and SMC-4 (rb2655) were described previously (Chuang et al. 1994; Lieb et al. 1996; Davis and Meyer 1997; Hagstrom et al. 2002; McDonel et al. 2006; Jans et al. 2009). Rabbit polyclonal DPY-30 antibodies were from SDIX (4511.00.02) or were raised against peptide N-LREKDRYNAENQNPAGQQ-C (rb106). Rabbit polyclonal ASH-2 antibodies were raised against peptide N-LDHPPRVKREDDDDVKDIKKEIKQEI-C (rb590). Other antibodies included anti-RNA Polymerase II (CTD), both Clone 8WG16 (Covance MMS-126R) and a rabbit polyclonal (David Bentley), anti-Phospho Ser2 (Bethyl Lab

A300-654A) or anti-Phospho Ser5 RNA Pol II antibodies (Bethyl Lab A300-655A), and mouse monoclonal antibodies to HA (Covance, Clone HA.11; MMS-101R) or α-tubulin (Sigma, Clone DM1a; T6199).

### Immunoprecipitation reactions and Western blots

Embryo extracts for immunoprecipitation (IP) reactions were made as for ChIP, but without a crosslinking step. IP experiments were performed by incubating 3 µg of the indicated antibody with approximately 3 mg of embryo extract from *dpy-30* mutants carrying a transgene encoding a rescuing HA-DPY-30 protein (TY2095) for 2 h at 4°C. Antibodies were collected for 30 min with Protein G Dynabeads (Invitrogen), and samples were washed 4 times with 50 mM HEPES-KOH (pH 7.6), 150 mM KCl, 0.05% NP-40, 1 mM EDTA, 1 mM DTT, 1 mM PMSF. Immunoblots were performed using mouse anti-HA (1:1000), rabbit anti-SDC-3 (1:2000), rabbit anti-SDC-2 (1:1000) and rabbit anti-ASH-2 (1:4000).

To determine H3K4me3 levels and antibody specificity, synchronous L1 larvae were placed on Ahringer feeding library bacteria bearing a plasmid to the indicated gene. Gravid hermaphrodites were then bleached and embryos were boiled in 2X Laemmli buffer and run on SDS-PAGE gels. Immunoblots were performed using mouse anti- $\alpha$ -tubulin (1:1000), rabbit anti-ASH-2 (1:4000), and two rabbit anti-DPY-30 antibodies (both at 1:1000).

### Gene expression arrays and data analysis

Embryo microarray expression data were previously published (Jans et al. 2009). For fed L1 expression data, three samples were collected and frozen in 100 µl aliquots. RNA was prepared and hybridized to Affymetrix *C. elegans* Genome Microarrays as previously described (Jans et

al. 2009). All array normalization, analysis, and determination of expressed genes by MAS5 analysis were performed as previously published (Jans et al. 2009).

### Comparison of DPY-27 binding and expression in embryos and fed L1s

For comparison with gene expression, the dynamic range of embryo and fed L1 ChIP-chip experiments were normalized using STATA SE 9.2 (StataCorp LP). After normalization, the third largest ChIP-chip probe score for the promoter (-2 kb to the TSS) was determined for every gene in both developmental states. Only genes that were larger than 500 bp and determined to be expressed (through MAS5 analysis of microarray data) in either embryos or fed L1s were examined further. For each of these genes, the fed L1 promoter score was subtracted from the embryo promoter score, and the fed L1 expression value was subtracted from the embryo expression value. Genes were arranged by increasing difference in the promoter scores and a moving average of 100 genes with a step size of 1 was determined and plotted. In Supplemental Fig. S14D,E, no moving average was taken and the data is displayed as scatterplots.

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