Sex and X-Chromosome-wide Repression in *Caenorhabditis elegans*

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In many organisms, sex is determined by a precise chromosome counting mechanism that distinguishes one sex chromosome from two. In flies and worms, for example, XX embryos become females (or hermaphrodites), while XO or XY embryos become males (Fig. 1a) (Bridges 1916; Nigon 1951; Madl and Herman 1979). Sex can also be specified by the mere presence of a particular sex chromosome, such as the Y chromosome of mammals: XY or XXY embryos are males, and XX embryos females (Gubbay et al. 1990; Sinclair et al. 1990; Koopman et al. 1991). These sex-determining mechanisms cause the two sexes to differ in their dose of X chromosomes, yet both sexes require equivalent levels of X-chromosome gene products. A

chromosome-wide regulatory process called dosage compensation neutralizes the difference in X-linked gene dose between males and females by equalizing X-chromosome transcript levels.

The strategies for dosage compensation are diverse (Fig. 2), but in all known cases, specialized dosage compensation complexes are targeted exclusively to the X chromosome(s) of one sex to modulate gene expression in only that sex (Fig. 1b,c). This selective recruitment of the dosage compensation machinery establishes the epigenetic regulation of X chromosomes that is maintained throughout the lifetime of the animal. Female mammals randomly inactivate one X chromosome (Huynh and Lee, this volume). Male flies double the transcription rate of

Figure 1. The dosage compensation process. (a) In *C. elegans*, sex is determined by an X-chromosome counting mechanism that distinguishes one X chromosome from two. XX animals are hermaphrodites, and XO animals are males. Shown is a male and hermaphrodite mating pair that harbors a mutation in the dosage compensation gene *dpy-28*. XX animals that escape lethality are dumpy in phenotype and retain their embryos; XO animals appear wild type. (b) The difference in X chromosome dose between sexes is neutralized by the process of dosage compensation. A dosage compensation complex (multicolored shapes) binds to both X chromosomes (blue) of hermaphrodites to repress transcript levels by half, thus equalizing the overall level of X chromosome gene expression between males and hermaphrodites. (c) The dosage compensation protein SDC-2 binds to both X chromosomes of XX embryos starting around the 40-cell stage. Shown is a multicell XX embryo stained with DAPI (blue) to identify nuclear DNA and SDC-2 antibodies (red) to monitor the dosage compensation complex. (d) Once bound, the dosage compensation complex remains localized to X chromosomes throughout the cell cycle. Shown is an XX L1 larva stained with an antibody to the dosage compensation protein DPY-26 (green) and one to histone H3 phosphorylated on serine 10 (red), which associates only with mitotic chromosomes. DPY-26 remains bound to both X chromosomes of the two mitotic cells in the larva.

Figure 2. Diverse strategies for dosage compensation. Organisms use different strategies to equalize X-linked gene expression between males (XY or XO) and females or hermaphrodites (XX). Female mammals randomly inactivate one X chromosome. Male fruit flies double the transcription rate of their single X chromosome. Hermaphrodite worms halve the expression of both X chromosomes.

their single X chromosome (Oh et al., this volume). Hermaphrodite worms keep both X chromosomes active, but repress transcript levels from each X chromosome by half (Meyer and Casson 1986). In flies and mammals, this gene regulation is accompanied by the sex-specific modification of histones on the regulated X chromosomes (Bone et al. 1994; Hilfiker et al. 1997; Heard et al. 2001; Mermoud et al. 2002). In all three species, dosage compensation is essential, and failure to accomplish this global regulation causes either male- or female-specific lethality.

Fundamental questions are relevant to all forms of dosage compensation. First, what is the composition of the machinery that implements dosage compensation? Second, what are the sex-specific factors that activate the dosage compensation machinery in only one sex? Third, what are the *cis*-acting recruitment sites that target X chromosomes for regulation by the dosage compensation complex? Fourth, what is the molecular mechanism that underlies the coordinate regulation of gene expression along an entire X chromosome? Fifth, what is the explicit mechanism for fine-tuning X-linked gene expression by only twofold? We have addressed these basic questions in the nematode *Caenorhabditis elegans*, using integrated genetic, biochemical, and cell-biological approaches to dissect this regulatory process.

THE *C. ELEGANS* DOSAGE COMPENSATION MACHINERY

In *C. elegans*, the discovery of sex-specific lethal mutations that preferentially killed XX hermaphrodites was pivotal for the identification of dosage compensation proteins (Hodgkin 1983; Plenefisch et al. 1989). Mutations in eight genes—*sdc-1*, *sdc-2*, *sdc-3*, *dpy-21*, *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-30*—reduced the viability of XX but not XO animals. All mutations disrupted dosage compensation, causing a twofold elevation in X-linked transcript

levels in XX but not XO animals (Meyer and Casson 1986; Villeneuve and Meyer 1987; Nusbaum and Meyer 1989; DeLong et al. 1993; Hsu and Meyer 1994).

The *sdc* mutations not only interfere with X-chromosome gene regulation, they also disrupt sex determination, causing XX embryos to develop as males. The *sdc* genes coordinately control the hermaphrodite mode of both sex determination and dosage compensation, acting at a common step in the genetic regulatory hierarchy prior to the divergence of sex determination and dosage compensation genes into two separate pathways (Villeneuve and Meyer 1987; Nusbaum and Meyer 1989; DeLong et al. 1993).

The hermaphrodite-specific elevation in X-linked transcript levels caused by all dosage compensation mutations was consistent with either of two mechanisms for dosage compensation: random inactivation of a single hermaphrodite X chromosome or repression of both hermaphrodite X chromosomes by half. X-inactivation was unlikely since neither of the two genetic phenomena it would cause had been observed: First, hermaphrodites are not mosaic in phenotype for cell-autonomous X-linked markers; second, most X-linked loss-of-function mutations fail to behave as dominant alleles with variable penetrance and expressivity. The molecular architecture of the dosage compensation proteins and their localization to the two hermaphrodite X chromosomes (see below) confirmed that dosage compensation involves the repression of both X chromosomes by half.

Molecular analysis of the dosage compensation genes showed the similarity of DPY-26, DPY-27, and DPY-28 proteins to components of 13S condensin, a complex essential for the resolution and compaction of mitotic and meiotic chromosomes from yeast to man (Fig. 3a) (Chuang et al. 1994; Lieb et al. 1996; Swedlow and Hirano 2003; C. Tsai et al., in prep.). The prototypical condensin complex contains at least five subunits, including a pair of SMC (Structural Maintenance of Chromosomes) proteins (SMC2 and SMC4) and three non-SMC ancillary factors, which belong to the Chromosome-Associated Polypeptide CAP-D2, CAP-G, and CAP-H/Barren families (Hirano 2002; Swedlow and Hirano 2003). DPY-26 resembles CAP-H, DPY-28 resembles CAP-D2, and DPY-27 resembles SMC4. These three dosage compensation proteins form a complex that associates with both X chromosomes of hermaphrodites (Fig. 3a,b).

Genetic analysis failed to reveal the *C. elegans* homolog of SMC2, but biochemical analysis of the dosage compensation complex did (Lieb et al. 1996). MIX-1, the SMC2 homolog, immunoprecipitated with the complex and was identified by immunoreactivity to antibodies specific for the single *C. elegans* SMC2 protein. Mutations in *mix-1* disrupt not only dosage compensation, but also mitotic chromosome resolution, condensation, and segregation, causing the death of both XX and XO animals (Lieb et al. 1996; Hagstrom et al. 2002; Chan et al. 2004). The role of MIX-1 in this vital cellular process precluded its identification in genetic screens that exploited the hermaphrodite-specific action of dosage compensation genes.

Figure 3. Two condensin complexes in *C. elegans*. (a) *C. elegans* has one condensin complex specialized for dosage compensation (middle) and one specialized for the resolution, compaction, and segregation of both mitotic and meiotic chromosomes (left). The canonical 13S condensin complex (right) is conserved from yeast to humans. Proteins in all three complexes are color-coded to denote homologous proteins. In addition to the condensin-like proteins, the dosage compensation complex contains proteins (SDC-2, SDC-3, and DPY-30) that are essential for the recruitment of the entire complex to X. The SMC2-like protein called MIX-1 is shared between the dosage compensation and mitotic condensin complexes. Preliminary biochemical analysis of the dosage compensation complex has identified an XCAP-G homolog (G. Csankovszki and B.J. Meyer, unpubl.). Preliminary biochemical analysis of the mitotic complex has identified an XCAP-H homolog and a different XCAP-G homolog (K.A. Hagstrom and B.J. Meyer, unpubl.). (b) The dosage compensation complex localizes to both X chromosomes of hermaphrodites. Shown is a multicell embryo stained with the DNA intercalating dye DAPI (blue) and an antibody to the dosage compensation protein SDC-2 (red). (c) The *C. elegans* mitotic condensin complex colocalizes with centromere proteins on the holocentric mitotic chromosomes. Shown is a two-celled embryo stained with the DNA dye DAPI (red) and antibodies to SMC-4 (green) and to tubulin (blue). One cell (left) is in metaphase and the other (right) is in prometaphase, where individual condensed chromosomes are readily apparent, and the centromeres appear as parallel tracts on the poleward sides of the sister chromatids. (c, Reprinted, with permission, from Hagstrom et al. 2002.)

MIX-1 binds to X chromosomes of hermaphrodites when it colocalizes with the dosage compensation protein DPY-27, and it colocalizes with the centromeres of mitosis chromosomes in both sexes when it associates with the *C. elegans* mitosis-specific SMC4 protein called SMC-4 (Fig. 3c) (Lieb et al. 1996; Hagstrom et al. 2002). That is, MIX-1 partitions its roles in two separate biological processes, gene expression and chromosome segregation, through its participation in two separate condensin-like complexes, a dosage compensation-specific complex and a mitotic complex (Fig. 3a) (Hagstrom et al. 2002; Chan et al. 2004). Together, these results suggest that the *C. elegans* dosage compensation process evolved by recruiting components used in other chromosome behaviors to the new task of regulating gene expression. The similarity of the dosage compensation complex to condensin and the participation of MIX-1 in both complexes suggest a common mechanism for repressing X-chromosome gene expression during dosage compensation and for establishing chromosome resolution and higher-order chromosome structure during mitotic chromosome segregation.

Other components of the dosage compensation complex have also retained their roles in conserved cellular processes. DPY-28 figures prominently in meiosis, where it regulates the number and distribution of crossovers between homologous chromosomes (C. Tsai et al., in prep.). DPY-30 not only binds X chromosomes to down-regulate

gene expression, it associates with the COMPASS complex to methylate histones (C. Hassig and B. Meyer, unpubl.). DPY-30 is the *C. elegans* homolog of the *Saccharomyces cerevisiae* protein Sdc1 (genetic locus *YDR469W*) (Hsu and Meyer 1994; Nagy et al. 2002).

Recruitment of the DPY and MIX proteins to X chromosomes requires the activities of SDC-2 (a novel protein), SDC-3 (a protein with two zinc fingers), and DPY-30 (Klein and Meyer 1993; Chuang et al. 1996; Lieb et al. 1996; Davis and Meyer 1997; Lieb et al. 1998; Yonker and Meyer 2003). These three proteins recruit all other dosage compensation proteins to X, including DPY-21 (a novel protein) and SDC-1 (a protein with seven zinc fingers), through their own association with X (Davis and Meyer 1997; Dawes et al. 1999; Chu et al. 2002; Yonker and Meyer 2003). SDC-3 requires both SDC-2 and DPY-30 for its localization to X (Davis and Meyer 1997), and DPY-30 requires both SDC-2 and SDC-3 to bind X (C. Hassig and B. Meyer, unpubl.). SDC-2 is unique in that it can localize to X independently from all other dosage compensation proteins, suggesting that SDC-2 is pivotal for X-chromosome recognition and confers chromosome specificity to dosage compensation (Dawes et al. 1999). Once the dosage compensation machinery assembles on X chromosomes, it appears to remain localized to X throughout the entire cell cycle, including mitosis (Fig. 1d) (Lieb et al. 1998).

SEX-SPECIFIC TARGETING OF THE DOSAGE COMPENSATION COMPLEX TO HERMAPHRODITE X CHROMOSOMES

SDC-2 also confers hermaphrodite specificity to dosage compensation. All properties of SDC-2 are consistent with its role as the sex-specific switch that activates dosage compensation in hermaphrodites (Dawes et al. 1999). All other dosage compensation proteins are supplied maternally and are diffusely distributed throughout the nuclei of very young embryos (<30 cells) in both sexes and only later become specifically localized to X chromosomes of XX but not XO animals. SDC-2 differs in four important ways. First, SDC-2 is not maternally contributed and not expressed in very young embryos. Its initial expression occurs around the 40-cell stage, the stage in which the dosage compensation machinery assembles on X. Second, SDC-2 localizes to hermaphrodite X chromosomes from the onset of its expression. Third, SDC-2 is not expressed in wild-type XO embryos, indicating that SDC-2, unlike other dosage compensation proteins, is sex-specifically regulated. *sdc-2* is repressed in males by the male-specific gene *xol-1*, the master sex-determination switch gene and direct target of the pri-

mary sex-determination signal (Fig. 4) (Miller et al. 1988). When *xol-1* is active (in XO embryos), male development ensues; when *xol-1* is inactive (in XX embryos), hermaphrodite development ensues, including the activation of dosage compensation (Rhind et al. 1995). Finally, ectopic expression of *sdc-2* in XO animals using a ubiquitously expressed promoter triggers assembly of the dosage compensation complex on the single X, causing death. Thus, SDC-2 is the hermaphrodite-specific switch that is both necessary and sufficient to activate dosage compensation.

The mechanism by which *xol-1* controls *sdc-2* remains a mystery. Whether it acts directly or indirectly is also not known. Unexpectedly, the crystal structure of XOL-1 revealed it to be a GHMP kinase family member, despite having sequence identity of <10% (Luz et al. 2003). Small molecule kinases such as galactokinase and homoserine kinase are prototypic members of the GHMP kinase family. XOL-1 does not bind ATP under standard conditions, suggesting that XOL-1 may act by a mechanism distinct from that of GHMP small molecule kinases. However, possible clues to XOL-1 function come from the identity of GHMP kinase family members as regulators of gene expression. *S. cerevisiae* GAL3p, a protein

Figure 4. Genetic control of sex determination and dosage compensation in *C. elegans*. (a) *xol-1* is the master sex-determination switch gene that controls both sex determination and dosage compensation. It is the direct molecular target of the X-chromosome counting mechanism that determines sex. The twofold difference in X-chromosome dose between males and hermaphrodites is translated into the ON/OFF state of *xol-1*. In XX hermaphrodites, two doses of the X-signal elements (XSEs) repress *xol-1* by overcoming the *xol-1* activation achieved by autosomal signal elements (ASEs). Two mechanisms of *xol-1* repression are used: repression at the level of transcription and pre-mRNA splicing. When *xol-1* is repressed, the XX-specific gene SDC-2 is active and stabilizes SDC-3. SDC-2 acts with SDC-3 to target dosage compensation proteins to X, thereby repressing gene expression by half. SDC-2 plays the lead role in recognizing X-specific sequences. It is the only dosage compensation protein expressed solely in XX animals. SDC-2 also activates the hermaphrodite program of sexual development by repressing the male-specific sex-determination gene *her-1* by 20-fold. SDC-2 acts with SDC-3 to recruit dosage compensation proteins to *her-1*. In this case, SDC-3 plays the lead role in recognizing *her-1* DNA targets. The *her-1* and X complexes differ by one component: DPY-21 is present on X but not on *her-1*. (b) In XO males, the single dose of XSEs is insufficient to overcome the activating influence of ASEs. *xol-1* is active and promotes the male fate by repressing the activities of *sdc* genes. *her-1* is transcribed, and the single X is not repressed. In *xol-1* XO mutants, the dosage compensation complex localizes to the single X chromosome, killing XO animals by reducing X-linked gene expression.

that is structurally similar to galactokinase and can be converted to a galactokinase by the substitution of two amino acids, is a galactose- and ATP-dependent transcriptional inducer of *GAL* genes (Platt et al. 2000). Moreover, *GAL1* of *Kluyveromyces lactis* is a bifunctional protein that acts as both a galactokinase and a transcriptional inducer of *GAL* genes (Meyer et al. 1991). XOL-1 might also act with a small effector molecule to repress transcription of a downstream target gene such as *sdc-2*.

xol-1, in turn, is regulated by the primary sex-determination signal, the X:A signal, the ratio of X chromosomes to sets of autosomes (the ploidy) (Fig. 4) (Nigon 1951; Madl and Herman 1979; Miller et al. 1988; Rhind et al. 1995). *xol-1* is repressed by a group of dose-sensitive, X-linked genes called X-signal elements (XSEs) (Akerib and Meyer 1994; Nicoll et al. 1997; Carmi et al. 1998; Carmi and Meyer 1999) and activated by a set of dose-sensitive autosomal-linked genes called Autosomal-signal elements (ASEs) (J. Powell and B. Meyer, in prep.). In XX animals, the double dose of XSEs opposes the double dose of ASEs, causing *xol-1* to be repressed. In XO animals, the single dose of XSEs cannot oppose the double dose of ASEs, allowing *xol-1* to be active (Fig. 4).

RECRUITMENT OF THE DOSAGE COMPENSATION COMPLEX FOR GENE-SPECIFIC VERSUS CHROMOSOME-WIDE REPRESSION

Not only does SDC-2 activate dosage compensation, it also induces hermaphrodite sexual differentiation in concert with SDC-1 and SDC-3 (Dawes et al. 1999; Chu et al. 2002). Proof that SDC-2 acts as the pivotal sex-specific factor to initiate the hermaphrodite program of sexual development came in part by the observation that overexpression of SDC-2 and SDC-1 in XO animals, made viable by a dosage compensation mutation, resulted in XO animals developing as hermaphrodites. SDC-2 activates hermaphrodite sexual development by binding directly to regulatory regions of the male sex-determination gene *her-1*, repressing its transcription 20-fold, thereby directing the sex-determination pathway to the hermaphrodite mode (Dawes et al. 1999). SDC-2 thus acts both as a strong gene-specific repressor and as a weaker chromosome-wide repressor. Unexpectedly, SDC-2, together with SDC-1 and SDC-3, recruits other dosage compensation components to *her-1*, directing this chromosome repression machinery to silence an individual, autosomal gene (Chu et al. 2002).

Functional dissection of *her-1* in vivo revealed three DNA recognition elements required for SDC binding, recruitment of the DCC, and transcriptional repression (Chu et al. 2002). These *her-1* elements differed in location (promoter and second intron), sequence, and strength of repression. Though no consensus sequence was found in the three *her-1* recognition elements, a 15-nucleotide (nt) repeat was shared between the two binding sites in the second intron. Scrambling of the 15-nt sequence prevented binding of the complex to the two recognition sites in vivo, causing derepression of *her-1* and thus mas-

culinization of XX animals. The *her-1* binding sites bear no obvious resemblance to any sequences on X, leading to the speculation that the chromosome-wide repression complex can achieve different degrees of repression in part by associating with different DNA sequences. These results also imply that the dosage compensation complex regulates transcription along X chromosomes using diverse DNA recognition elements.

Further analysis revealed important molecular differences in the composition and targeting of the repression complex to *her-1* versus X (Yonker and Meyer 2003). The dosage compensation protein DPY-21, a novel protein, localizes to X but not to *her-1*. Furthermore, within the complex, different proteins play the lead role in recognizing DNA targets. SDC-2 recognizes X-chromosome targets, while SDC-3 recognizes *her-1* targets. These results begin to explain how closely related complexes can achieve uniformly weak repression of many genes in one context and strong repression of a specific gene in another.

RECRUITMENT AND SPREADING OF THE DOSAGE COMPENSATION COMPLEX ALONG HERMAPHRODITE X CHROMOSOMES

While most components of the dosage compensation complex have been identified, the *cis*-acting X-chromosome recognition elements that recruit the complex have been elusive. To define these recognition elements on X (*rex*), a chromosome-wide search was conducted for regions of X sufficient to recruit the complex when detached from X (Csankovszki et al. 2004). Regions were analyzed in 32-ploid intestinal cell nuclei of XX hermaphrodite strains carrying either free or autosome-attached X-chromosome duplications (Fig. 5). X-chromosome territories were marked by fluorescent in situ hybridization (FISH) probes; one identified the duplicated region (red), and a second identified the rest of X (blue). Localization of the dosage compensation complex was visualized by antibodies to a dosage compensation protein (green).

Provided that *C. elegans* X chromosomes contained discrete X-recognition elements that recruit the complex, four possibilities existed for how the complex might be targeted to X. First, a single site on X could recruit the complex and nucleate long-range spreading of the dosage compensation complex across the entire chromosome. Second, a limited number of recognition sites could recruit the complex, and some or all sites could nucleate short-range spreading. Third, a limited number of recognition sites could recruit the complex but the complex would not spread. It would occupy only sites that autonomously recruited it, influencing gene expression from a long distance, perhaps by altering chromosome structure. Fourth, a high density of X-recognition sites could recruit the complex, but no spreading would occur, implying direct, short-range regulation by the complex.

The first possibility, the presence of a single recruitment site, was eliminated by analysis of hermaphrodites carrying one copy of the X duplication *mnDp1* and two chromosomes deleted for the corresponding region (Fig.

Figure 5. Multiple X regions recruit the dosage compensation complex and nucleate spreading. (a–d) Confocal images of individual 32-ploid intestinal cell nuclei with (a–c) and without (d) X chromosome duplications detached from X. Gut cells were stained with FISH probes to the duplicated region (red), FISH probes to the rest of X (blue), and antibodies to a dosage compensation protein (green). Cartoons on left represent the genotype of each nucleus, showing the copy number of each X duplication (red) and the location on X (blue) of the duplicated region (red). Shown are regions of X that strongly recruited the complex (a), weakly recruited the complex (b), or failed to recruit the complex (c). Regions that did not recruit the complex when detached from X exhibit robust recruitment of the dosage compensation when attached to the native X (d), indicating that the complexes spread to these regions from neighboring regions that contain X-recognition elements. Arrows mark duplications. (Reprinted, with permission, from Csankovszki et al. 2004 [©AAAS].)

5a and Fig. 6). The dosage compensation complex colocalized with both the duplication and the truncated X chromosomes, indicating that *mnDp1* harbors at least one X-recognition element, as does the rest of X. The interpretation of multiple, independent X-recognition elements was greatly reinforced by the identification of many other nonoverlapping, detached X regions that recruited the complex. Two classes of dosage compensation complex recruitment were found, strong recruitment typified by *mnDp1* and limited, but reproducible, recruitment typified by *mnDp57* (Fig. 5b and Fig. 6).

The fourth possibility, a high density of recruitment sites, was eliminated by the discovery of large segments

of X incapable of recruiting the complex when detached from X (Fig. 5c and Fig. 6). Despite the inability to autonomously recruit the complex, these regions, typified by *stDp2*, harbor dosage compensated genes when present on the native X chromosome (Fig. 6). These results indicated that the dosage compensation complex regulates gene expression in X regions that lack recruitment sites either by spreading to the regions from neighboring X-recognition elements (second possibility) or by acting over a distance (third possibility). The second possibility predicts binding of the dosage compensation complex to the region on the native X corresponding to *stDp2*, and the third possibility predicts no binding. Ro-

Figure 6. Map of X regions that recruit the dosage compensation complex when detached from the native X. Location of X regions that strongly (dark green), weakly (light green), or fail to (red) recruit the dosage compensation complex are shown relative to the gene-rich clusters (yellow) on X (blue) and the few known dosage compensated genes (*uvt-4*, *lin-14*, *myo-2*, *lin-15*) and non-dosage compensated genes (tRNA encoding genes *sup-7* and *sup-21*). Regions that apparently lack recruitment sites contain dosage compensated genes. This map summarizes DCC recruitment data obtained through experiments similar to those shown in Fig. 5.

Figure 7. Spreading repression. The *C. elegans* X chromosome harbors multiple X-recognition elements (*rex* sites) that distinguish it from autosomes to recruit the dosage compensation complex. The complex binds to *rex* elements on both hermaphrodite X chromosomes and then spreads *cis* to neighboring X regions that lack recruitment sites, thus establishing X-chromosome-wide repression that is maintained throughout the lifetime of the animal.

bust localization of the dosage compensation complex was found on the *stDp2* region (Fig. 5d), on other native X regions corresponding to duplications with no recruitment ability, and on regions corresponding to duplications with limited, but reproducible, binding. Thus, the *C. elegans* X chromosomes have discrete X-recognition elements that recruit the dosage compensation complex and nucleate spreading of the complex over short or long distances (Fig. 7) (Csankovszki et al. 2004).

MOLECULAR IDENTIFICATION OF DISCRETE X-RECOGNITION ELEMENTS

A general strategy was devised to identify small, discrete X-recognition elements responsible for recruitment activity (Csankovszki et al. 2004). Transgenic nematode lines carrying extrachromosomal DNA arrays with tandem copies of cosmids from a recruitment region were made, and arrays were assayed for dosage compensation complex binding. The arrays also carried *lac* operator repeats and *lac* repressor tagged with GFP. *lac* repressor binding to its operator sequences would allow the array to be identified by GFP autofluorescence or antibodies. Colocalization of dosage compensation protein antibodies with the array would indicate the presence of an X-recognition element (Fig. 8a). This approach identified the element *rex-1* from a region of X with limited recruitment ability (Fig. 6) (Csankovszki et al. 2004). Using a similar array approach, the recruitment activity was at-

tributed to a single cosmid and then ultimately a 790-base-pair subfragment (Fig. 8b) (Csankovszki et al. 2004).

Multiple copies of a bona fide X-recognition element would be expected to titrate dosage compensation complexes from X and thereby impair dosage compensation. Genetic assays indicated that dosage compensation was compromised in animals carrying arrays with X-recognition elements. Furthermore, X chromosomes could be detected easily by dosage compensation protein antibodies only in XX cells that lost arrays because of mitotic instability (Csankovszki et al. 2004). Adjacent cells harboring arrays showed robust localization of the dosage compensation complex to the array but not the X chromosomes (Fig. 8b). These results indicate that X regions with limited recruitment ability harbor high-affinity recruitment sites capable of competing with other recruitment sites on X for the dosage compensation complex (Csankovszki et al. 2004).

Figure 8. Molecular identification of an X-recognition element. (a) Strategy for isolating X-recognition elements that recruit the dosage compensation complex. Transgenic nematodes were created that carried extrachromosomal arrays made of X DNA, *lac* operator sequences, and a gene encoding the *lac* repressor-GFP bifunctional fusion protein. *lac* repressor binds the *lac* operator sequences, allowing arrays to be detected by GFP autofluorescence or antibodies (green, arrow). The dosage compensation complex is identified by antibodies to a dosage compensation protein (red), and the nuclear DNA by DAPI (blue). If X sequences contain an X-recognition element, dosage compensation protein antibodies will colocalize with the array. Within each nucleus, binding of the complex to both Xs serves as a staining control. (b) Identification of *rex-1*. This strategy identified an X-recognition element (*rex-1*) from a region of X with limited ability to recruit the complex (see map in Fig. 6). Shown are two gut cells, one with an array carrying a 4.5-kb piece of X DNA and one without. The dosage compensation complex binds to the array and titrates complexes from X. X chromosomes are readily detectable only in the cell that lacks the array.

A total of ten X-recognition elements have thus far been identified by this approach (J. Jans et al., unpubl.). Further dissection of these sites will reveal the features of X-recognition elements essential for recruitment of the dosage compensation complex. Those features could be primary DNA sequence, DNA structure, or a combination of both.

In summary, the strategy by which *C. elegans* X chromosomes attract the condensin-like dosage compensation complex is now known. Discrete X-recognition elements serve as entry sites to recruit the dosage compensation complex and to nucleate spreading of the complex to X regions that lack recruitment sites (Fig. 7). In this manner, a repressed chromatin state can be spread in *cis* over short or long distances, thus establishing the global regulation of X chromosomes that is maintained throughout the lifetime of hermaphrodites (Csankovszki et al. 2004).

CONCLUSIONS

Future endeavors will reveal whether the primary X-recognition protein SDC-2 binds X DNA directly or instead requires other X-bound cellular proteins to reach its target. Such endeavors will also define the mechanistic basis by which the dosage compensation complex spreads along the X chromosome and whether such spreading involves histone modifications or noncoding RNAs, as do other forms of epigenetic regulation across whole chromosomes or chromosomal domains (Allshire; Cam and Grewal; Oh et al.; Huynh and Lee; Martienssen et al.; all this volume). Ultimately, a detailed biochemical understanding of the twofold X-chromosome repression will emerge.

ACKNOWLEDGMENTS

This work was supported by NIH grant R37-GM30702 to B.J.M., NIH NRSA postdoctoral fellowship F32-GM065007 to G.C., and NIH pre-doctoral training grant GM07232 to P.M. B.J.M. is an investigator of the Howard Hughes Medical Institute.

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Cold Spring Harb Symp Quant Biol 2004 69: 71-80

Access the most recent version at doi:[10.1101/sqb.2004.69.71](https://doi.org/10.1101/sqb.2004.69.71)

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