Dosage compensation: **X-repress yourself**

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Dosage compensation in *Caenorhabditis elegans* involves the sex-specific recruitment to the X chromosome of a protein complex, the nature of which suggests that there are mechanistic links between chromosome segregation and global transcriptional regulation.

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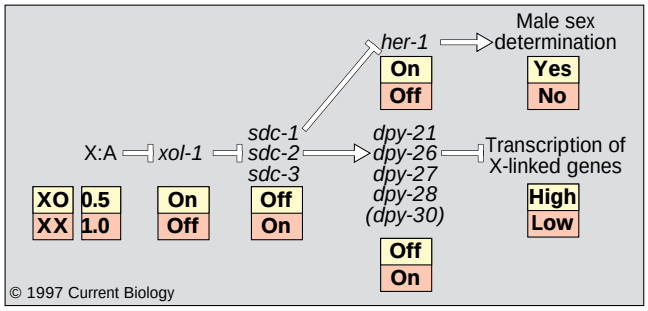
Animals whose sex is determined by different combinations of sex chromosomes face something of a paradox. The embryo must be able to distinguish between two alternative karyotypes in order to initiate the appropriate sexual differentiation. In most such animals, however, the differences between these karyotypes must be compensated for to ensure that non-sex-determining genes on the sex chromosomes are expressed at similar levels in the two sexes. Mammals such as ourselves, with XX female / XY male sex determination, solve this problem by separating it into two. Sex is determined independently of the number of X chromosomes by the presence or absence of a Y chromosome, which carries few genes with functions other than sex determination and male sexual differentiation. X chromosome dosage compensation is accomplished by effectively shutting down one or the other X chromosome in every cell of an early female embryo, by a mechanism that persists through subsequent cell generations and is irreversible in most tissues throughout life [1].

For invertebrates, such as the fruitfly *Drosophila* and the nematode *Caenorhabditis elegans*, in which sex is determined only by the number of X chromosomes — actually the X:A ratio of X chromosomes to sets of autosomes — the problem is trickier because sex must be determined by the same karyotypic difference that dosage compensation is designed to counteract. In flies, females are XX and males XY, but the Y chromosome plays no role in sex determination. In worms, hermaphrodites are XX and males XO. In each of these organisms, the problem posed above is solved by somewhat mind-boggling pathways of interacting regulatory genes, similar in complexity but different in mechanism, which coordinately control the effectors of sex determination and dosage compensation.

Figure 1



Proposed pathway of regulatory gene interactions for the control of sex determination and dosage compensation in *C. elegans* (some suggested regulatory loops have been omitted). The *sdc* genes act to repress *her-1* expression and activate *dpy* genes; *dpy-30* is now thought to act further upstream in the pathway (see Fig. 2).

In the fly and the worm, both sex determination and dosage compensation are under the control of a master regulatory gene on the X chromosome which is differentially activated in the two sexes according to the copy number of several other X-linked loci, known as numerator elements. Quite a lot is now known about the mechanism of this initial decision in flies, less in worms [2]. But now the logic, and some of the molecular functions, of the worm's regulatory apparatus, replete with branches, feed-forward and feedback loops, have become clear, due almost entirely to the work of Barbara Meyer and her colleagues, following earlier elucidation of the sex-determination pathway *per se* by Jonathan Hodgkin [3].

Dosage compensation in *C. elegans* is accomplished by down-regulating X chromosome gene expression in XX animals: not by inactivating one X, as in mammals, but rather by globally repressing expression of the genes on both X chromosomes by a factor of two relative to their expression level in XO animals [4,5]. This chromosome-wide modulation must be superimposed on the various gene-specific controls that regulate X-linked genes during development.

The model pathway for regulation of dosage compensation and its relationship to sex determination, shown in Figure 1, is based on a series of incisive genetic analyses by Meyer and colleagues between 1987 and 1995 (reviewed in [2]). The results suggested that *xol-1* (for XO lethal), which is activated specifically in XO animals, negatively regulates each of three *sdc* (sex determination and dosage compensation) genes. These in turn negatively regulate the first gene in the sex-determination pathway,

her-1, and also positively regulate a group of four 'dumpy' genes, *dpy-21*, *dpy-26*, *dpy-27* and *dpy-28*, which were thought to be effectors of dosage compensation; a fifth *dpy* gene, *dpy-30*, is regulated separately.

Loss-of-function mutations in *xol* or the *sdc* or *dpy* genes cause inappropriate dosage compensation, resulting in sex-specific lethality. As should be clear from Figure 1, *xol-1* mutations kill (and feminize) XO animals because the *sdc* genes are inappropriately activated, repressing *her-1* and X-linked genes; whereas *sdc* mutations kill (and masculinize) XX animals, because of the inappropriate lack of repression of *her-1* and X-linked genes. Mutations in most of the *dpy* genes also cause maternal-effect XX lethality (without sexual transformation), because of a failure of dosage compensation, or extreme 'dumpiness', a consequence of overexpression of X-linked genes, in the homozygous mutant XX progeny of a heterozygous hermaphrodite. Furthermore, *dpy-26* and *dpy-28* mutations cause a low level of generalized chromosome non-disjunction during meiosis.

What are the proteins encoded by these genes and how might they function? Earlier molecular analyses provided structural information, but little insight into their mechanisms of action. The *xol-1* gene, controlled at the level of transcript accumulation, is predicted to encode a novel protein of 425 amino acids, with an acidic carboxyl terminus, which is required for *sdc* regulation [6]. SDC-1 and SDC-3 are maternally and embryonically synthesized proteins that are present in both sexes. SDC-1, a large protein of 1203 amino acids, includes seven zinc-finger motifs [7], and SDC-3 has two mutationally separable domains near the carboxyl terminus: one includes a zinc-finger motif and is required for dosage compensation; the other includes a myosin-like putative ATP-binding region and is required for *her-1* regulation in sex determination. A third, more amino-terminal, domain of SDC-3 is also required for dosage compensation [8]. SDC-2, made only in XX animals, is a novel protein of 350 kD with no known sequence motifs [2,9]. DPY-30 is a small, novel and ubiquitously expressed nuclear protein that is thought to enhance SDC-3 function, as well as serving other, more general roles in development [10,11].

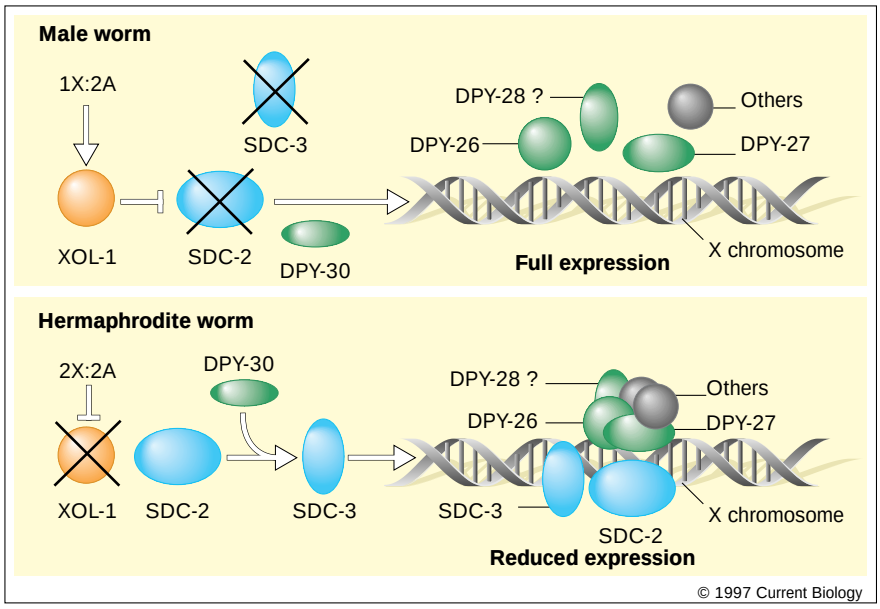
Recent molecular analyses of other *dpy* genes have provided clues to how the dosage-compensation process works. A breakthrough came in 1994, when the *dpy-27* gene was cloned and predicted to encode a protein of the SMC1 (structural maintenance of chromosomes) class [12], defined by chromosomal proteins from yeast [13] and vertebrates [14]. In both XX and XO pre-gastrulation embryos, DPY-27 was detected as diffuse immunostaining in all somatic nuclei. In XX embryos, after the onset of gastrulation, when dosage compensation is thought to be initiated, DPY-27 was seen to associate specifically with X chromosomes during all stages of the cell cycle. In XO embryos, DPY-27 staining remained diffuse; X-chromosome-specific association of DPY-27 was seen, however, in non-viable *xol-1* mutant XO embryos, which die because of inappropriate activation of the dosage-compensation machinery.

In two recent *Science* papers [15,16], Meyer and colleagues have reported analysis of another *dpy* gene, and evidence that the DPY proteins form a complex required for dosage compensation. DPY-26, a novel protein that is also made from early embryogenesis onward, differs from DPY-27 in that, before gastrulation, it associates with all chromosomes during mitosis. Thereafter, DPY-26 associates only with X chromosomes throughout the cell cycle, in XX embryos but not XO embryos unless they are mutant for *xol-1*. The X-chromosome-specific association of DPY-26 depends on DPY-27, and *vice versa*; for both proteins, the specific association also depends on the functions of *sdc-2*, *sdc-3*, *dpy-28* and *dpy-30*. The stabilities — or, less likely, the translation — of the two proteins are also mutually interdependent, and the function of the *dpy-28* gene, which has not yet been cloned, is required for the stability of both proteins. DPY-26 also differs from DPY-27 in that it is present in the adult hermaphrodite germ line, where it associates with all meiotic chromosomes, consistent with the above-mentioned requirement for *dpy-26* function to assure the fidelity of meiotic segregation. The stability of DPY-26 in the germ line depends on *dpy-28* function, but, in contrast to its role in embryonic dosage compensation, its chromosomal association does not depend on *sdc-2*, *sdc-3*, *dpy-27* or *dpy-30*.

These results suggested that DPY-26 might act in a complex with DPY-27, as well as DPY-28, on the X chromosome to mediate dosage compensation in the embryo, and possibly in a different complex, this time without DPY-27, to mediate meiotic chromosome segregation in the germ line. Direct evidence for the first complex was obtained by immunoprecipitating both DPY-26 and DPY-27 proteins from embryonic nuclear extracts with either anti-DPY-26 or anti-DPY-27 antibodies. At least two other proteins coprecipitated with DPY-26 and DPY-27, one of which was postulated to be the still uncharacterized DPY-28 [15]. Although no evidence for this was reported, the complex presumably also forms in the nuclei of XO animals, but in this case does not associate specifically with the X chromosome.

Davis and Meyer [11] have now found that the SDC-3 protein is also directly involved in chromosome modification. Like DPY-26 and DPY-27, SDC-3 also associates specifically with the X chromosome in XX embryos. This association requires the functions of *sdc-2*, *dpy-26*, *dpy-27*, *dpy-28* and *dpy*-30; the latter three genes are also required for the stability (or translation) of SDC-3. The authors

Figure 2



A model for sex-specific control of dosage compensation in *C. elegans* by the *sdc* and *dpy* gene products. (Modified from [2].)

present evidence that both the zinc-finger and amino-terminal dosage-compensation domains of SDC-3 are required for its association with the X chromosome: they suggest that the former may be involved in X-chromosome recognition and the latter in interactions with other components of the complex.

Interestingly, the functions of *sdc-1* and *dpy-21* are not required for the X-chromosome association or stability of either DPY-26, DPY-27 or SDC-3, suggesting they play other roles in dosage compensation. And finally, a piece of the puzzle missing so far has been the function of the novel protein SDC-2, which has been the prime candidate for dictating the sex specificity of X-chromosome association, because it is the only one of the required gene products that is produced only in XX animals. In recent work (cited in [2]), SDC-2 also has been found to associate with the X chromosomes in XX embryos, suggesting that it may initiate formation of the regulatory complex.

In summary, *C. elegans* appears to accomplish dosage compensation by a change in X chromosome structure that reduces expression of X-linked genes by a factor of two in XX, relative to XO, animals. To do so, *C. elegans* has evolved a sex-specific mechanism for targeting to the hermaphrodite X chromosomes a regulatory protein complex, which includes proteins that are also likely to play roles in chromosome segregation. Dosage compensation in *C. elegans* thus provides another example of a specific developmental process for which housekeeping proteins have been recruited and modified. Chromosomal proteins involved in both chromosome segregation and global transcriptional control are not new: such dual functions are exhibited, for example, by the heterochromatin-binding protein HP1 in *Drosophila* [17] and the Swi6 protein in fission yeast [18]. Not surprisingly, both these processes can be affected by changes in chromatin structure.

The emerging picture of the dosage-compensation machinery in *C. elegans* (Fig. 2) raises several questions that are now ripe for answering. First, what are the roles of *sdc-1* and *dpy-21*? Second, as known SMC1 proteins function as heterodimers with a related protein of the SMC2 class, could there be an SMC2 homologue that is also part of the complex, perhaps the product of *dpy-28* or an as yet unidentified gene? Third, does association of DPY-26 with all mitotic chromosomes in somatic nuclei of pregastrulation embryos play a role in directing mitotic chromosome segregation, or does this association simply serve to stabilize and distribute DPY-26 uniformly to all somatic cells in preparation for later dosage compensation? No defects in mitotic segregation resulting from mutations in *dpy* genes have been reported, and in XX embryos, at any rate, postgastrulation segregation of autosomes clearly must proceed without DPY-26. DPY-27 does not associate with mitotic chromosomes — could there be an additional SMC1 homologue that takes its place? Fourth, is there dosage compensation in the germ line, and if so, how is it accomplished in the absence of DPY-27? Fifth and last, how does DPY-26 act in its quite distinct role of directing meiotic segregation in the germ line? Does it have other partners, perhaps including other germ-line-specific homologues of the SMC proteins? The tools are available to address most of these questions, and more answers should soon be forthcoming.

Acknowledgements

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