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Targeting the Sex Chromosome for Repression

Researchers analyzing the machinery the roundworm *C. elegans* uses to repress genes on an entire chromosome have found surprising principles that may apply widely to this type of wholesale gene regulation. The researchers' findings are noteworthy because cells use this kind of coordinated gene regulation, which resembles a rheostat dimming electric lights, for a variety of critical functions.

"Large-scale gene regulation across entire chromosomes is vital to the life of an organism, yet we have virtually no understanding of how it occurs," said Barbara J. Meyer, a Howard Hughes Medical Institute investigator at the University of California, Berkeley, and the lead author of the new study.

In the study, the researchers focused on a phenomenon called dosage compensation, which ensures that developing roundworm embryos have the correct level of X-chromosome gene expression, whether they have one or two X chromosomes. To achieve the proper balance, roundworm embryos with two X chromosomes repress the expression of X-chromosome genes by half, to prevent their excessive activity, which would lead to death. Meyer and her colleagues reported the most detailed analysis yet of the sites on the X chromosome that recruit the dosage compensation machinery in a paper published online November 18, 2006, in the journal *Nature*, in advance of print publication. Meyer and her co-authors, Patrick McDonel, Judith Jans, and Brant Peterson are at the University of California at Berkeley.

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In *C. elegans*, X-chromosome dosage compensation is activated when a worm inherits two X chromosomes, which cause the embryo to develop into

a hermaphrodite. Males have only one X chromosome, but both sexes require the same level of X-chromosome products. In hermaphrodite worms, a large complex of proteins called the dosage compensation complex (DCC) targets the entire X chromosome for wholesale gene repression. The sites on the chromosome that attract the complex are called “recruit elements on X” (*rex*).

In earlier work, Meyer and her colleagues discovered the structure of the *C. elegans* DCC, which they found resembled a complex that all higher organisms, from yeast to humans, use to control chromosomal processes critical for cell division. That complex is known as condensin. The researchers had also identified large regions of the X chromosome that they knew must contain recruitment sites, but the actual sites had not been identified. In their new experiments, the scientists defined and dissected the structure of *rex* sites so they could better understand how those sites recruit the DCC.

“Everyone thought there had to be something unique about the X chromosome that made it a target for dosage compensation,” said Meyer. “The question was whether that uniqueness was specified by the primary DNA sequence, the higher-order DNA structure, or some other special property.”

To find out, the team created genetically altered worms carrying extra pieces of X chromosome, which they used to zero in on the *rex* sites by analyzing how the pieces bound to DCC. As they analyzed smaller and smaller segments of X-chromosome DNA, they discovered that the *rex* sites had an unusually high occurrence of at least two short DNA sequences, or motifs. To their surprise, however, these DNA motifs were not unique to X chromosomes; they existed on all *C. elegans* chromosomes. However, the clustering of the motifs on X chromosomes rendered them targets for the DCC.

“This is an important finding, we believe, because it shows that you can shuffle around common, short segments of DNA and by changing their distribution or abundance, create a property in one chromosome that doesn't exist in another,” Meyer said.

In further experiments with mutant worms, the researchers found that by altering the number of DNA motifs, they could dial DCC recruitment up or down.

Meyer noted that other researchers' studies of the fruitfly *Drosophila melanogaster*, in which dosage compensation is accomplished by increasing expression of genes on the male's single X-chromosome, seem to be revealing a similar recruitment strategy, using combinations of distinctive DNA motifs. “If this is true, it suggests for the first time that remarkably similar principles govern the X-specific binding of two evolutionarily unrelated dosage compensation complexes that regulate whole chromosomes

in opposite ways,” said Meyer.

However, she said, the experiments in her laboratory hinted that another very different mechanism was also at work influencing dosage compensation. That mechanism might involve regulating the overall structure of the chromatin - the complex of DNA and protein that constitutes chromosomes. “Our studies tell us that targeting a sex chromosome for dosage compensation involves more than what we have discovered,” said Meyer. She and her colleagues are now exploring those other steps, as well as further analyzing the details of how DCC binds to *rex* sites.