DOSAGE COMPENSATION

How Males and Females Achieve X Equality

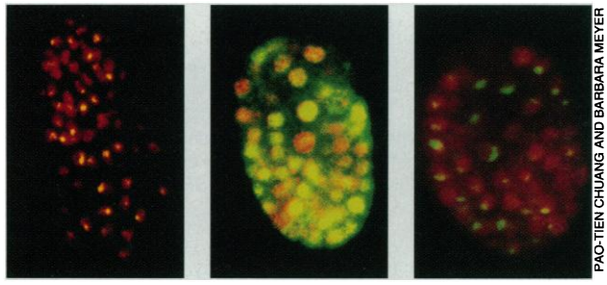
Sex has lots of advantages, as the number of species that indulge in it shows. But it also poses a potentially lethal problem. Most species use distinct X and Y sex chromosomes to determine who develops as female and who as male—and the female generally has more copies of the X chromosome than the male. But the X chromosome contains many genes needed equally by males and females, threatening females with what could be a lethal excess of X-chromosome gene products—or males with an equally serious deficiency. Researchers have known for decades that humans and other sexually reproducing species survive because of a correcting mechanism known as "dosage compensation" that equalizes the expression of X-linked genes between the sexes. But only now are they beginning to figure out how it works at the molecular level.

Within the past few years, they've cloned several of the genes needed for dosage compensation in species ranging from the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* to mice and humans. And even though these organisms use different general strategies to equalize X-chromosome gene expression in males and females, molecular and biochemical analysis of the dosage compensation genes and the proteins they make suggests that they all work by altering the structure of chromatin, the complex of DNA and proteins that gives chromosomes their higher order coiled structure. That makes dosage compensation a prime example of a gene-regulating mechanism that has become a hot new area of research. "More and more people are becoming interested in the function of chromatin," says John Lucchesi, a researcher on dosage compensation at Emory University in Atlanta.

But dosage compensation also displays some novel features. Not only must it be activated in just one sex, but it must affect genes only on the X chromosome. Indeed, says molecular biologist Bruce Baker of Stanford University, "the chromosome specificity is unique to dosage compensation." What's more, dosage compensation achieves a twofold change in gene expression, either doubling it in males to match that of females or halving it in females, depending on the species. "This is a much finer level of control than other mechanisms of controlling gene expression," says Lucchesi. Such fine control, he adds, provides "a window on a new level of gene regulation."

The dosage compensation window was originally pried open in the early 1930s, when the late geneticist Hermann Muller noted that certain X-linked genes in the fruit fly were expressed at similar levels in males and females, even though females have two X chromosomes. It was Muller who gave this phenomenon its name: dosage compensation. By the mid-1960s, researchers learned that fruit flies equalize X-chromosome gene expression between the sexes by doubling the activity of genes on the single X chromosome of males to match the levels occurring in females.

Over the years, it became clear that other species also perform dosage compensation—and have evolved different strategies for achieving it. Studies by Mary Lyons at Harwell Radiobiology Unit in the United Kingdom on the inheritance of X-linked coat color genes in mice in the early 1960s led her to propose that early in development, female mammals take care of the problem by randomly inactivating almost all the genes on one X chromosome per cell.



PAO-TIEN CHUANG AND BARBARA MEYER

X marks the spot. The dosage compensation protein DPY-1 *(yellow stain)* localizes to the X chromosome in a female *C. elegans* embryo *(left)* and mutant male embryo *(right)*, but in a normal male *(center)*, it is distributed throughout the cell nuclei.

And more recently, yet a third strategy has been discovered in *C. elegans* by Bill Wood's group at the University of Colorado, Boulder, and Barbara Meyer's laboratory at University of California, Berkeley. In this species, dosage compensation halves gene expression from both X chromosomes in the hermaphrodite form—which serves as the worm female—bringing the level down to that found in males. "Dosage compensation is a good example of how nature can achieve the same ends by very different means," says Neil Brockdorff, who works on X inactivation at London's Hammersmith Hospital.

All species face the same initial problem, however: how to turn on dosage compensation in one sex only. How mammals achieve this is still unknown, but *Drosophila* and *C. elegans* link activation of dosage compensation to genes in the pathways that make the initial decision to develop as male or female (see p. 1822).

Turning it on. Lucchesi and his colleagues and Kugao Oishi's team at Kobe University in Japan took the first steps toward uncovering the fly's strategy in the mid-1970s, when they set out to identify gene mutations that kill males only. They reasoned that such mutations might reflect a defect in dosage compensation. And indeed, that proved to be the case. The researchers found that fatal mutations in the genes called *maleless* (*mle*) and *male-specific lethal 1*, *2*, and *3* (*msl-1*, *-2*, *-3*) were associated with a reduction in the activity of genes on the male X chromosome. The result indicated that males could no longer boost the expression of their X chromosome genes to match the levels in females. At the time, the researchers did not know how these dosage compensation genes are controlled or exactly how they work, but a few years later, Tom Cline's team, then at Princeton University but now at Berkeley, provided a big clue to the control mystery.

In the early 1970s, Cline was studying the basis for a female-specific lethal effect of a gene called *daughterless* and was led to another gene discovered by Muller late in his career. Cline found that this gene had male-specific and female-specific lethal alleles, and he renamed it *Sex-lethal* (*Sxl*). By the early 1980s he had shown that *Sxl* is the master regulator in the sex determination pathway. It produces a functional protein only in females, and this protein sets in motion a series of events leading to development of the female organs.

But *Sxl* proved to have another function as well. Work by Cline and Lucchesi showed that it's also needed for dosage compensation. More recent evidence about how it's involved comes from Lucchesi, Baker, and Mitzi Kuroda at Baylor College of Medicine in Houston, who have shown that *Sxl* blocks the *mle* and *msl* genes' ability to up-regulate X-chromosome genes. That's part of the reason why female embryos die when *Sxl* is inactivated: Males need the MLE and MSL proteins to turn up their X-chromosome gene expression, but those same proteins will give females a fatal overdose of the X gene products.

A clue to how *Sxl* works came when a group led by Cline and his Princeton collaborator Paul Schedl showed that it regulates how the noncoding sequences called introns are spliced out of mRNAs before they are translated into protein structure. New work by the teams of Kuroda, Lucchesi, and Baker now points to the *msl-2* transcript as the key target in controlling dosage compen-

sation. By studying the RNA transcript of *msl-2*, which was cloned earlier this year, the researchers found a striking difference between males and females. "An intron in the upstream untranslated region of the gene transcript is removed in males but retained in females," Lucchesi says.

On further study, the researchers found that the transcript contains binding sites for the Sxl protein within the intron and at other sites. These results suggest, Lucchesi says, that by binding to the *msl-2* transcript, *Sxl* blocks removal of the intron in females and thus production of a functional MSL-2 protein. In contrast, in males, where *Sxl* is absent, the *msl-2* transcript is processed correctly and a functional protein produced. "It looks as if *msl-2* may be the target of the regulatory *Sxl* gene and the key to activation of dosage compensation in males," Lucchesi concludes.

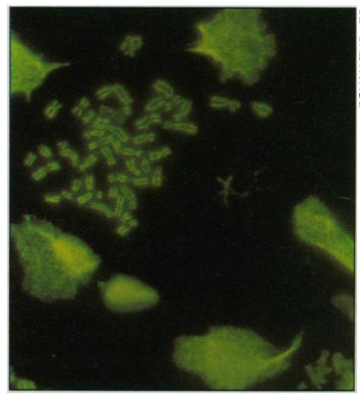
While boosting the expression of X-chromosome genes in the male is the major theme in dosage in the fruit fly, *C. elegans* takes the opposite tack: lowering X-chromosome expression in the hermaphrodite. Recent work, mainly from Meyer's team, has been clarifying just how this down-regulation is achieved.

Meyer and her colleagues have shown that in the worm, a gene called *xol-1* is the master sex switch and controls both sex determination and dosage compensation. Expression of this gene at high levels leads to the development of males. In males, XOL-1 inhibits the activities of *sdc-1*, *sdc-2*, and *sdc-3*, the next genes in the sex-determining pathway that controls the hermaphrodite mode of sex determination and dosage compensation. But in hermaphrodites, where *xol-1* activity is low, activity of the *sdc* genes is high, and they in turn lead to high activity of five further genes (*dpy-21*, *dpy-26*, *dpy-27*, *dpy-28*, and *dpy-30*) that together act to reduce females' X-chromosome gene expression.

Molecular mechanisms explored. Once these dosage compensation genes start making proteins in the appropriate sex, they must somehow modulate expression of genes along the entire X chromosome. Molecular evidence of how they do this is now emerging.

In both *Drosophila* and *C. elegans*, the first step is binding of the proteins to the X chromosome. Antibody studies have shown, Baker says, that in the fly, MLE and the three MSL proteins each bind to hundreds of specific sites along the X chromosome of male fruit flies. What's more, all four proteins apparently bind to the same sites. "These results suggest the MSLs and MLE are forming a multiprotein complex distributed along the chromosome," says Baker, whose group has carried out some of the key studies.

The MSLs may work by altering chromatin structure. A group of proteins, known as histones, plays a key role in maintaining chromosomal architecture, and Bryan Turner and his colleagues at the University of Birmingham, U.K., have detected an intriguing sex-dependent chemical difference in one histone. Histone H4 on the male X chromosome, but not the female X or any nonsex chromosome, is modified by the addition of an acetyl group to the amino acid lysine at position 16. Work by Turner and Kuroda showed that the distribution of this acetylated histone along the male X chromosome closely matches that of MSL proteins. Studies on other organisms suggest that histone acetylation and the resulting changes in chromosome structure may affect the activity of genes; in the case of the fruit fly male, Kuroda suggests, it might boost gene activity along the entire X chromosome.



BRYAN TURNER

Washout. All the chromosomes but the inactive X in these human cells carry a modified histone *(green stain)*.

Dosage compensation in *C. elegans* may also involve a multiprotein complex that binds all along the X chromosome and changes its chromatin—in this case lowering rather than raising gene expression. Last year, Meyer's group cloned the *dpy-27* gene, allowing the researchers to make the protein and then raise antibodies to it. Used as tracers of DPY-27's distribution in the cell nucleus, the antibodies revealed that it is in the right place to halve the expression of X-chromosome genes in the hermaphrodites. "It is localized on both X chromosomes in XX hermaphrodites, but not on the X chromosome in XO males," Meyer says. Other, as yet unpublished work from Meyer's team suggests that DPY-26, as well as the protein product of the *sdc-3* gene, binds to the X chromosome along with DPY-27.

Evidence that DPY-containing complexes modulate chromosome structure came from analysis of the sequence of DPY-27. "There are substantial similarities with a family of proteins, called SMC, involved in chromosome condensation in other organisms," says Meyer. Because alterations in chromosome structure lead to repression of gene transcription, this finding suggests that DPY-27 halves expression of the X-linked genes by condensing the X chromosomes of *C. elegans*.

In mammals, too, changes in chromatin structure may be the key to dosage compensation. Turner, with Peter Jeppesen at the Medical Research Council's Human Genetics Unit in Edinburgh, U.K., has found that the inactive X chromosomes of humans and mice are marked by a striking lack of histone H4 acetylation compared to other chromosomes. This fits neatly with the need for dosage compensation in mammals to repress gene expression from the X chromosome, he says.

But so far, there is no evidence that mammals use a protein complex to achieve X inactivation. Indeed, the only candidate gene linked to mammalian dosage compensation is the *Xist* gene, which was discovered in humans by Andrea Ballabio of the Telethon Institute of Genetics and Medicine in Milan, Italy, in collaboration with Huntington Willard's team at Case Western Reserve University in Cleveland, and in mice by Sohaila Rastans's group at the former Clinical Research Center in London. *Xist* apparently produces no protein, but does make a 15-kilobase RNA transcript.

Nevertheless, several lines of evidence suggest that *Xist* helps inactivate one of the two X chromosomes of females. The gene is unique in that it is expressed from the chromosome that undergoes inactivation, but not from the one that remains active. In addition, Willard has shown that the *Xist* RNA localizes along the length of the X chromosome just before it becomes inactive. Exactly what it does to shut down gene expression is unclear, however.

While researchers make progress on the mechanisms of dosage compensation, one key issue still raises more questions than answers: how the regulator molecules home in on the X chromosome while ignoring the others. Says Baker, "We don't know what the [recognition] sequences are on the X chromosome in *Drosophila* and how they function." Researchers also want a better picture of how the binding of the protein complexes or the RNA to the X chromosome alters gene expression but are optimistic they will get it. "As the molecular studies come out, the next experiments soon become obvious," says Baker.

Meanwhile, they are buoyant about what they have already learned. The research is panning out much faster than anyone imagined 5 years ago, Meyer says: "The pathways make sense, and the molecules are making sense. We're getting a great story."

–Nigel Williams

Additional Reading

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